3-17-2016

Modeling the Extent of Virus Removal in Waste Stabilization Ponds to Support Reuse of Wastewater

Kelly James Vannoy

Modeling the Extent of Virus Removal in Waste Stabilization Ponds to Support Reuse of Wastewater

by

Kelly James Vannoy

A thesis submitted in partial fulfillment
of the requirements for the degree of
Master of Science in Environmental Engineering
Department of Civil and Environmental Engineering
College of Engineering
University of South Florida

Major Professor: James Mihelcic, Ph.D.
Jeffrey Cunningham, Ph.D.
Stewart Oakley, Ph.D.

Date of Approval:
March 7, 2016

Keywords: Lagoons, Mathematical modeling,
Water reuse, Pathogen, Virus removal rates

DEDICATION

This thesis is dedicated to all the people who have supported, encouraged, and inspired me throughout my personal and academic life thus far. My parents - Jim and Jacquie, thank you for providing me with the resources and unwavering support that has allowed me to pursue my passions and become the person that I am today. My brothers - Corey and Trevor, thank you for being great brothers, terrific role models, and a constant source of inspiration. My grandparents – Marge, Ben, and Gene, thank you for your unconditional love, support, and encouragement. The rest of my family, friends, and teachers – thank you the unique influence that all of you have had on me.

ACKNOWLEDGMENTS

First and foremost, I would like to acknowledge my advisor, Dr. James Mihelcic, and the other members of my thesis committee: Dr. Jeffrey Cunningham and Dr. Stewart Oakley. I wish to especially acknowledge Dr. Matthew Verbyla, who has provided a tremendous amount of assistance, knowledge, and encouragement throughout my thesis research.

This material is based upon work supported by the National Science Foundation under Grants 0965743 and 1243510. Any opinions, findings, conclusions, or recommendations expressed in this thesis are those of the author and do not necessarily reflect the views of the National Science Foundation.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABSTRACT

Waste stabilization ponds (WSPs) are one of the most prevalent types of domestic wastewater treatment technologies employed worldwide, and global stressors such as urbanization, population growth, climate change, and water scarcity have increased the demand for reusing treated wastewater. The safe reuse of treated wastewater in agriculture can ease water scarcity, aid in food production, and reduce environmental degradation from the discharge of wastewater effluent to surface waters. The ability to predict virus concentrations in wastewater effluent is an important criterion for determining whether wastewater is suitable for discharge to the environment or for reuse in agriculture. However, many uncertainties remain about virus removal efficiency in WSPs and there is currently no mechanistic or empirical model that reliably predicts virus removal in WSPs.

The overall objective of this thesis research was to model the extent of virus removal in individual waste stabilization ponds to support the reuse of wastewater. A literature review was used to create a database of estimated apparent virus removal rate coefficients ($K_{v,app}$) in three different WSP types (anaerobic, facultative, and maturation ponds). The database consisted of 249 paired influent and effluent concentrations of enteric viruses or bacteriophages from 44 unique WSP systems, comprised of 112 individual WSPs from 19 different countries. Apparent virus removal rate coefficients ($K_{v,app}$) were calculated for each individual WSP using the following three mathematical models from reactor theory: complete mix, plug flow, and dispersed flow. Pearson's correlation analysis was used to determine correlations between $K_{v,app}$

values and the following design, operational, and environmental parameters: solar radiation, air temperature, pond depth, hydraulic retention time (HRT), and virus loading rates. The median $K_{v,app}$ values were greater for anaerobic ponds than for facultative and maturation ponds; however, $K_{v,app}$ values in facultative and maturation ponds had more significant correlations with design, operational, and environmental parameters. Additionally, $K_{v,app}$ values appear to be significantly different for various types of enteric viruses and bacteriophages.

Alternative multiple linear regression equations were developed to predict $K_{v,app}$ values using the design, operational, and environmental parameters as explanatory variables. Analysis of variance (ANOVA) tests were used to select the most appropriate multiple linear regression equations with the least amount of explanatory variables. The most appropriate plug flow and dispersed flow multiple linear regression equations for predicting $K_{v,app}$ values included air temperature and HRT as explanatory variables. The results indicate that the plug flow regression equation was able to better predict $K_{v,app}$ values ($R^2 = 0.38$) than the dispersed flow regression equation ($R^2 = 0.24$) in facultative and maturation ponds based on the dataset. However, both the dispersed flow and plug flow models had R^2 values of approximately 0.84 when they were used to predict effluent virus concentrations in facultative and maturation ponds based on the dataset. According to this research, the plug flow regression equation is recommended for predicting apparent virus removal rate coefficients in WSPs. However, a multi-model approach that utilizes both the plug flow and dispersed flow models may yield a more robust mathematical model that can improve WSP design, reliably predict virus removal in WSPs, and ultimately be used to support wastewater reuse.

CHAPTER 1: INTRODUCTION

1.1 Global Occurrence of Waste Stabilization Ponds

Waste stabilization ponds (WSPs, lagoons, ponds) are one of the most common types of wastewater treatment technologies worldwide, predominantly found in rural areas, small communities, and developing communities, as well as some large cities (Mara, 2004; Oakley, 2005). Overall, WSP systems account for nearly half of all wastewater treatment facilities in Latin America, New Zealand, and the United States (Noyola et al., 2012; Mara, 2004; USEPA, 2011). For example, there are reportedly more than 8,000 WSP systems in the United States (USEPA, 2011), approximately 2,500 systems in France (Mara and Pearson, 1998), and at least 100 systems in Colombia. They are also the most commonly used technology in Mexico, the Dominican Republic, and Brazil (Noyola et al., 2012). WSP systems have proven to be an appropriate technology that are inexpensive and simple to construct, operate, and maintain, especially when compared to some mechanized wastewater treatment technologies (Muga and Mihelcic, 2008).

WSPs are shallow engineered basins (approximately 1-5 m in depth) that employ natural processes such as gravity settling, photosynthesis, microbial metabolism, and sunlight-mediated mechanisms to reduce the concentrations of organic matter (measured as biochemical oxygen demand, BOD), total suspended solids (TSS) and pathogens in wastewater (Mara, 2004). The principal types of WSPs are classified as either anaerobic, facultative, or maturation ponds, based on their depths, treatment objectives, and dissolved oxygen content. Table 1.1 summarizes the

key characteristics of each of these three types of WSPs. Depending on topography, gravity may be utilized to direct the wastewater through a series of ponds. A conventional pond system configuration consists of facultative ponds followed by maturation ponds, or anaerobic ponds followed by facultative and maturation ponds. Anaerobic and facultative ponds are typically designed for biochemical oxygen demand (BOD) and total suspended solids (TSS) removal, and maturation ponds are designed for pathogen removal and further removal of BOD and TSS (Mara, 2004). Maturation ponds can produce effluent with low concentrations of BOD, TSS, and pathogens if a series of ponds is properly designed.

Table 1.1: Characteristics of the principal types of waste stabilization ponds

Type of WSP	Characteristics	Typical Depth (m)	Hydraulic Retention Time (days)	Purpose
Anaerobic	• No oxygen, deep, non-aerated • Anaerobic digestion occurs in sludge layer (produces biogas).	2-5	1-7 [a]	• Primary function is BOD/TSS removal (around 60 %) • Treat high strength wastewaters • Recover biogas
Facultative	• Dissolved oxygen on top layer • No oxygen on bottom layer • Combination of aerobic, anoxic, and anaerobic processes	1.2 – 2.5	10-180	• Moderately effective at removing settleable solids, BOD, pathogens, fecal coliform, and ammonia
Maturation	• Dissolved oxygen throughout entire depth • Aerobic processes.	1 - 1.5	3-15	• Pathogen removal, such as pathogenic bacteria, viruses, protozoan cysts and helminth eggs • Polishing (further BOD/TSS removal)

Sources: Mara (2004); Mihelcic and Zimmerman (2014) ; [a] for wastewater with a BOD of ≤ 300 mg/l, a 1-day retention time is sufficient at a temperature of 20° C (Mara, 2004)

1.2 Significance and Motivation

Increasing global stressors such as urbanization, population growth, climate change, and water scarcity have placed strain on economic, social, and environmental well-being at a local to global scale (Zimmerman et al., 2008). Therefore, reuse of treated wastewater is becoming increasingly important for providing food and water security, though there are challenges to promote water reuse related to pathogen control (Verbyla et al., 2015). WSPs are often constructed in areas that may be favorable for reusing treated wastewater for irrigating crops (Verbyla et al., 2013a). Furthermore, approximately three-quarters of the world's irrigated agriculture (192 million hectares) is located in developing countries, and it is estimated that 10 percent of this land is irrigated with raw or partially treated wastewater (Raschid-Sally and Jayakody, 2008). Trends also suggest that the use of treated wastewater in urban areas is expected to grow in the future for irrigating trees, parks, and golf courses (United Nations, 2015). When used properly, wastewater reuse can aid in the production of food, increase income, improve nutrition and the quality of life in poor areas (Jiménez, 2006), and reduce the carbon footprint and eutrophication potential of wastewater treatment (Cornejo et al., 2013).

However, contact with treated, partially treated, or untreated wastewater that is discharged to the environment may negatively impact human health, as water is one of the main transmission routes for pathogenic diseases (Mihelcic et al., 2009). More than 150 known enteric pathogens may be present in untreated wastewater (Reynolds et al., 2008), and this may include more than 100 different species of enteric viruses (Melnick, 1984; Macler, 1995). Enteric viruses are specialized to exist in human hosts and, in most cases, enter the environment through excreted human fecal matter (Reynolds et al., 2008). They are typically transmitted via the fecal-oral route and replicate in the gastrointestinal tract of humans after ingestion or contact with

contaminated food or water. Enteric viruses are primarily associated with diarrhea and gastroenteritis in humans; however, they are also known to cause respiratory infections, conjunctivitis, hepatitis, polio, and other diseases with high mortality rates (Kocwa-Haluch, 2001). According to the World Health Organization (WHO), diarrhea kills approximately 800,000 children under the age of five per year, is the leading cause child malnutrition, and is the second leading cause of child mortality under five years of age (WHO, 2013). Gastroenteritis, which results in diarrhea, can be caused by a wide range of pathogens; however, enteric viruses are thought to be the leading cause (WHO-UNICEF, 2009). While not all cases of diarrhea can be linked to enteric viruses, it can be ascertained that a significant amount are.

Enteric virus outbreaks, especially from norovirus, rotavirus, and hepatitis A, associated with wastewater pollution in agriculture, aquaculture, drinking water, and recreational waters, have been documented in several studies (Shuval et al., 1986; Beuchat, 1998; Harris et al., 2003; WHO, 2006b; Drechsel et al., 2010). Particularly in developing countries, it is often a challenge to attribute enteric virus outbreaks to specific exposure routes due to limited resources for virus detection methods and other contributing factors that are a result of poor hygiene. Nevertheless, a significant proportion of enteric virus diseases can be prevented with adequate wastewater management.

One of the main advantages of WSPs is their ability to remove pathogenic organisms, such as protozoan cysts and oocysts, helminth eggs, and pathogenic bacteria (von Sperling, 2005). In fact, they are considered the most efficient form of wastewater treatment for pathogen removal without the addition of advanced disinfection treatment processes (Mara, 2004; Shilton, 2005). It is known that a well-designed WSP system can remove fecal coliforms to concentrations less than 1,000 fecal coliforms per 100 mL, which complies with the 1989 World

Health Organization (WHO) guidelines for unrestricted irrigation, although the 2006 WHO guidelines recommend a quantitative microbial risk assessment (QMRA) approach (Mara, 2004; WHO, 2006b). Mathematical models derived from reactor theory widely used in the process-engineering field have been proposed as one way to predict fecal coliform removal (and presumably *E. coli* removal) in WSP systems (Marais, 1974; von Sperling, 2005; Shilton, 2005). These models include the completely mixed flow reactor, plug flow reactor, and dispersed flow reactor. However, fecal coliforms have been shown to be poor indicators of the presence and removal of enteric viruses in WSPs (Maynard et al., 1999). This is likely because viruses are smaller than fecal bacteria (Bitton, 2005), are often more resistant to treatment and environmental conditions (Symonds et al., 2009), and have been shown to have different removal rate coefficients (Herrera and Castillo, 2000). Many uncertainties remain about the efficiency and prediction of virus removal in WSPs (Maynard et al., 1999; Mara, 2004), and the mechanisms responsible for virus removal in WSPs are still poorly understood (Symonds et al., 2014; Verbyla and Mihelcic, 2015).

There is currently no mechanistic or empirical model that reliably predicts virus removal in WSPs. The ability to predict and measure virus concentrations in wastewater effluent is an important criterion for determining whether the wastewater is suitable for discharge to the environment or for reuse in agriculture or aquaculture. In general, there are still many knowledge gaps in the literature about mechanisms responsible for removing viruses in WSPs, virus removal efficiency in WSPs, and the risks of enteric virus affliction directly associated with WSP effluent. Nevertheless, the ability to accurately model virus removal in WSPs is an important consideration for safeguarding public health. This leads to the conclusion that there is a need to develop a mathematical model for virus removal in WSPs that can be used for design purposes.

1.3 Research Objectives and Hypotheses

Based on the challenges and opportunities described previously, the objectives of this research are to: (1) compile a database of enteric virus and bacteriophage removal reported in the literature for individual WSPs; (2) estimate overall apparent virus removal rate coefficients ($K_{v,app}$) for each WSP type (anaerobic, facultative, maturation) using the complete-mix, plug flow, and dispersed flow models from reactor theory; (3) identify correlations and relationships between these virus removal rate coefficients and design, operational, and environmental parameters for WSPs; (4) recommend the mathematical model from reactor theory that best predicts virus removal in WSPs; and (5) determine if the recommended model can reliably be used for design purposes. This study addresses the following hypotheses:

1. The correlations between virus removal rate coefficients ($K_{v,app}$) and solar radiation and air temperature in WSPs will be positive, and the correlation between $K_{v,app}$ values and pond depth will be negative, and there will be no correlation between $K_{v,app}$ values and hydraulic retention time.
2. Virus removal rate coefficients ($K_{v,app}$) will differ based on the type of virus and type of WSP.
3. Virus removal rate coefficients derived from the dispersed flow model will be more representative of virus removal in WSPs than the complete-mix and plug flow models.

Specifically, the objectives and hypotheses of this study will be examined by: (1) obtaining influent and effluent virus concentration data for individual WSPs from data published in literature; (2) performing a correlation analysis between estimated virus removal rate coefficients ($K_{v,app}$) and design, operational, and environmental (DOE) parameters in WSPs, using Pearson's correlation coefficients, test statistics, and probability values based on a

Student's *t*-distribution; (3) performing a multiple linear regression analysis between $K_{v,app}$ values and DOE parameters to derive best fit regression equations for predicting $K_{v,app}$ values in WSPs with three mathematical models (CMM, PFM, DFM); (4) selecting the mathematical model with the best regression equation for predicting $K_{v,app}$ values and using it to predict effluent virus concentrations in WSPs; and (5) assessing the applicability of the selected mathematical model as a design equation that can be used for predicting virus removal in WSPs, and determining what implications this may have for wastewater reuse.

To the author's knowledge this is the first study that has modeled the global extent of enteric virus and bacteriophage removal in individual waste stabilization ponds. The results of this study will provide insight into the status of virus removal in WSP systems and may be used by engineering professionals and wastewater managers to make informed decisions about wastewater treatment and the potential for wastewater reuse in their communities. With safer reuse of wastewater that supports agriculture, environmental degradation from discharge of treated effluent to surface water can be lessened and economic and social benefits can also be achieved. In addition, the overall goal of promoting resource recovery from wastewater (in this case the water and embedded nutrients) can also be met (Guest et al., 2009; Mihelcic et al., 2011).

The following chapter (Chapter 2) includes a literature review that provides information on the health risks associated with exposure to enteric viruses, the removal of viruses in WSPs, and the use of bacteriophages as surrogates for enteric viruses in wastewater systems. In addition, three mathematical models derived from reactor theory from the process-engineering field (complete mix, plug flow, dispersed flow) are reviewed and compared. Chapter 3 provides details on the materials and methods used in this study. In Chapter 4 the results of the modeling

and statistical analyses are presented and discussed. Lastly, in Chapter 5, conclusions and recommendations for future research are provided to assist efforts to better design WSPs and better predict virus removal.

CHAPTER 2: LITERATURE REVIEW

2.1 Health Risks from Enteric Viruses

Viruses are ultramicroscopic (10-300 nm), metabolically inert, infectious agents that replicate only within the cells of living hosts (Cann, 2003). Each virus contains a single type of nucleic acid, either RNA or DNA, which is enclosed by a protein shell called a capsid (Flint et al., 2009). In general, viruses are extraordinarily diverse and pervasive, for example, at least one virus has evolved to infect every known organism on the planet (Flint et al., 2009).

Enteric viruses are viruses that are specialized to exist in human hosts and, in most cases, enter the environment through excreted human fecal matter (Reynolds et al., 2008). They are a common type of waterborne pathogen that are transmitted via the fecal-oral route and most replicate in the gastrointestinal tract of humans after ingestion or contact with contaminated food, water, soil, hands, or fomites. More than 150 known enteric pathogens may be present in untreated wastewater (Reynolds et al., 2008), and this may include more than 100 different species of enteric viruses (Melnick, 1984; Macler, 1995). Viruses from the common families *Picornaviridae, Adenoviridae, Caliciviridae,* and *Reoviridae* are classified as enteric viruses (Flint et al., 2009). These viruses are primarily associated with diarrhea and gastroenteritis in humans; however, they are known to cause other infections and diseases with high mortality rates (Kocwa-Haluch, 2001). Table 2.1 lists some of the specific types of enteric viruses commonly found in wastewater, along with their characteristics and associated illnesses.

Table 2.1: Common enteric viruses found in wastewater

Virus Family	Genera/Group/Species	Nucleic Acid	Size (nm)	Associated Illnesses
Adenoviridae	*Adenovirus*	dsDNA	94	Gastroenteritis, upper respiratory disease, eye infections, heart disease
Caliciviridae	*Norovirus (Norwalk virus)*	ssRNA	40	Gastroenteritis, flu-like symptoms, vomiting
	Calicivirus	ssRNA	41	
	Astrovirus	ssRNA	27-30	
Picomaviridae (Enteroviruses)	*Poliovirus*	ssRNA	32	Paralysis, meningitis
	Enterovirus (several types)	ssRNA	28-30	Meningitis, respiratory infection, gastroenteritis, myocarditis, nervous system disorders, birth defects
	Coxsackievirus A Coxsackievirus B	ssRNA	33	Hand, foot, and mouth disease, muscle injury, paralysis, organ damage
	Echovirus	ssRNA	32	Encephalitis, meningitis, nerve system disorders
	Hepatitis A virus	ssRNA	27	Hepatitis, liver damage
Reoviridae	*Reovirus*	dsRNA	75	Gastroenteritis, dysentery
	Rotavirus	dsRNA	80	

Source: Reynolds et al. (2008); WHO (2006a); Flint et al. (2009); Carrillo-Tripp et al. (2009)

According to the World Health Organization (WHO), diarrhea kills approximately 800,000 children under the age of five per year, is the leading cause of child malnutrition, and is the second leading cause of child mortality under five years of age (WHO, 2013). Gastroenteritis, which results in diarrhea, can be caused by a wide range of pathogens; however, enteric viruses are thought to be the leading cause (WHO-UNICEF, 2009). While not all cases of diarrhea can be linked to enteric viruses, it can be ascertained that a significant amount are. Specifically, rotaviruses and noroviruses have been determined to be the principal cause of viral diarrhea in both developing and industrialized countries (WHO-UNICEF, 2009). To quantify the impact, data from 1986 and 2000 suggests that rotaviruses caused between 352,000 – 592,000 deaths per year in children under five years old, with 82 percent of the casualties being in developing countries (Parashar et al., 2003). Since enteric viruses are transferred through fecal-

oral transmission, a significant amount of enteric virus diseases may be prevented with adequate wastewater management; because sanitation, hygiene, and safe drinking water all depend on the proper management of fecal matter.

The extent of enteric virus removal that can be achieved depends on the type of wastewater treatment process and the type of virus. The removal of enteric viruses in a WSP has been shown to be erratic, with removal efficiencies ranging from zero to 99 percent (Maynard et al., 1999; NRC, 2004), with rare instances resulting in high removal efficiencies. If wastewater is not further disinfected, effluent with potentially harmful quantities of enteric virus concentrations may be discharged to the environment. Enteric viruses in wastewater are a significant health risk due to their large initial concentrations in sewage, their resistance to certain types of treatment, their persistence in environmental media, and their low infective doses. For instance, enteric viruses are often shed in large quantities in feces on the order of 10^9 to 10^{10} viruses per gram of feces, so even an 8-$\log_{10}$ unit reduction in virus concentration may not be sufficient to eliminate risks of virus affliction (Fields et al., 1996). This is because small doses of a virus, on the order of tens to hundreds of virus particles, can cause an infection in a susceptible host (Melnick and Gerba, 1980). Furthermore, enteric viruses can survive for extended periods of time in nature (weeks to several months) under a wide range of temperatures and pH (Straub et al., 1993; Jansons et al., 1989). Due to their structures, some enteric viruses are resistant and many are not easily removed in current wastewater treatment processes (Fong and Lipp, 2005). For example, in 2009 ten types of enteric viruses were identified in wastewater effluent samples from twelve different cities throughout the United States (Symonds et al., 2009).

Unfortunately, a large amount of enteric virus affliction cases go undocumented, especially in the developing world. Three different groups of people are considered to be at risk

from wastewater effluent that is discharged to the environment or reused in agriculture, aquaculture, and for recreational purposes. These people are farm or pond workers and their families, local communities in close proximity to wastewater discharge or reuse operations, and product consumers (WHO, 2006b). Enteric virus outbreaks, especially from norovirus, rotavirus, and hepatitis A, associated with domestic wastewater pollution in agriculture, aquaculture, drinking water, and recreational waters, have been documented by many authors (Shuval et al., 1986; Beuchat, 1998; Harris et al., 2003; WHO, 2006b; Drechsel et al., 2010). Due to their small size and persistence, enteric viruses are the most probable form of human pathogens to contaminate groundwater. Enteric viruses can infiltrate through the soil into groundwater and can move considerable horizontal distances, with documented penetration depths of 67 meters and horizontal migration distances as far as 408 meters (Borchardt et al., 2003). Studies on the impact related to gastroenterintestinal diseases from consumption of contaminated vegetables have been reviewed extensively (Beuchat, 1998; Harris et al., 2003). Certain enteric viruses tend to persist for long periods of time on crops, in some instances up to 60 days (Drechsel et al., 2010). Enteric virus infections have also been found to be transmitted through the consumption of shellfish grown in sewage polluted marine environments (Okoh et al., 2010). This risk is also increased since shellfish are often consumed raw, or only slightly cooked (Sincero et al., 2006). It has also been suggested that viral pathogens are the leading causative agents of recreational waterborne illnesses (Jiang et al., 2007; Sinclair et al., 2009).

Although there may be little data on the direct association of enteric virus affliction directly from WSP effluent, it is probable that WSP effluent may be an important source of wastewater pollution and enteric virus transmission. It is important to note, that it is often easier to detect pathogenic bacteria and protozoa than it is to detect enteric viruses. There are

significantly more cases of illness reported in the literature due to bacteria (*E. coli, Salmonella*) and protozoa associated with food or water that was contaminated from WSP effluent than for enteric viruses (FAO/WHO, 2008; Drechsel et al., 2010); however, it is possible that enteric viruses were present in these cases but not detected. Finding the original contamination source for food and water is often difficult as well. Particularly in developing countries, it is often a challenge to attribute enteric virus outbreaks to specific exposure routes due to limited resources for virus detection methods and other contributing factors that are a result of poor hygiene. Nevertheless, a significant amount of enteric virus diseases can presumably be prevented with adequate wastewater management.

2.2 Use of Viral Indicator Organisms for Detecting Fecal Pollution in Water

Fecal indicator bacteria (i.e., total coliforms, fecal coliforms, *E. coli*, fecal streptococci, and enterococci) have been used for over a century to detect sewage contamination in water in order to protect the public from harmful diseases caused by fecal pathogens, such as cholera and typhoid fever (NRC, 2004). The use of fecal indicator bacteria was adopted to allow for timely and cost effective monitoring of water sources, since direct measurement of all known waterborne pathogens simply is not practical. In the monitoring of WSPs, fecal indicator bacteria are often used, perhaps inappropriately, as an indication of pathogen concentrations in treated wastewater effluent and to assess the microbiological quality of the water to ensure its suitability for discharge into the environment. The 1989 World Health Organization (WHO) guidelines for wastewater reuse in agriculture and aquaculture were based on fecal coliform concentrations in the wastewater effluent. However, the new 2006 WHO guidelines recommend the use of quantitative microbial risk assessment (QMRA) to estimate the risk from exposure to pathogenic microorganisms (WHO, 2006b).

Bacteria respond to environmental degradation and treatment processes differently than viruses, so traditional fecal indicator bacteria may not necessarily be the best indicators of enteric virus removal and persistence in water. While a number of researchers have reported correlations between fecal coliform bacteria and enteric viruses (Mara, 2004; Gersberg et al., 2006), current research supports the inadequacy of fecal coliform bacteria as a reliable indicator for enteric viruses. For example, several studies have detected the presence of enteric viruses in treated wastewater effluent even though traditional fecal indicator bacteria were at very low or non-detectable concentrations (Kageyama et al., 2003; da Silva et al., 2007; Haramoto et al., 2011; Kuo et al., 2010; Simmons et al., 2011).

2.2.1 Use of Bacteriophages as Viral Indicators for Fecal Pollution

In simple terms, bacteriophages (also known as phages) are viruses that can only infect bacterial cells (Calendar, 2004). As the largest known virus group, approximately 5,000 bacteriophage groups have been identified (Calendar, 2004). They exist naturally in the environment and many different bacteriophages are present in the feces of warm-blooded animals, while certain strains are more specific to humans (Bitton, 2005). Bacteriophages and enteric viruses have similarities in size, morphology, and survival in aquatic environments; therefore, bacteriophages have been investigated as viral indicators of fecal pollution in water sources (USEPA, 2015). The attributes for an ideal fecal contamination indicator include the following (NRC, 2004):

1. The indicator should be present in the intestinal microflora of warm-blooded animals
2. The indicator should only be present when pathogens are present
3. The indicator should be present in greater numbers than the pathogen
4. The indicator should be at least as resistant as the pathogen to environmental factors and

disinfection via wastewater treatment processes

5. The indicator should not multiply in the environment
6. The indicator should be detectable by easy, rapid, and inexpensive methods
7. The indicator should be nonpathogenic
8. The indicator should be correlated to health risk
9. The indicator should be specific to a fecal source or identifiable source of origin

Coliphages are a subset of bacteriophages that infect *E. coli* (Calendar, 2004). They are the most common type of bacteriophage that has been researched as a viral indicator for fecal contamination (USEPA, 2015). Due to the diverse number and behavior of viruses in the environment and water treatment systems, it may be concluded that no single organism will be able to fulfill all the necessary requirements for an ideal viral fecal indicator. However, coliphages fully meet half of the criteria listed above (1, 3, 6, and 7) and partially meet half of the criteria (2, 4, 5, and 8) (USEPA, 2015). As such, coliphages have been researched for several decades (Simkova and Cervenka, 1981; Havelaar et al., 1993; Sobsey et al., 1995; Hot et al., 2003; Wu et al., 2011) and have been considered for official use by the U.S. Environmental Protection Agency (USEPA) as a viral indicator of fecal contamination in ambient water (USEPA, 2015).

The three groups of bacteriophages that have commonly been used as viral surrogates in wastewater are somatic coliphages, F-specific coliphages (also known as male-specific or F+ phage), and *Bacteroides fragilis* phages (Bitton, 2005). These three groups of bacteriophages are used as viral surrogates because they share similarities to enteric viruses in their physical structure, composition, morphology, survivability in the environment, and resistance to treatment

processes (Havelaar et al., 1993; Grabow, 2001). The characteristics of each group of bacteriophages are provided in Table 2.2.

Table 2.2: Characteristics of bacteriophages used as surrogates for enteric viruses

Characteristic	Somatic Coliphage	F-specific Coliphage	*B. fragilis* phage
Common Strains	ΦX174	MS2, F2	-
Nucleic Acid	dsDNA	ssRNA, ssDNA, dsDNA	dsDNA
Host Strains	*E. coli CN 13*	*E. coli* F_{amp}	*Bacteroides fragilis HSP40*
Concentration in wastewater	$10^3 – 10^4$ / mL	$10^3 – 10^4$ / mL	$<1 – 10^3$ / mL
Concentration in human waste	Intermediate	Intermediate	Low
Probability of replication in the environment	Intermediate	Low	Very low
Resistance to removal	Intermediate	Low	High
Ease of detection	Easy	Somewhat easy	More labor intensive and expensive

Sources: Calendar (2004); Bitton (2005); Grabow (2001); Gerardi and Zimmerman (2005)

Somatic coliphages are a group of DNA bacteriophages that mostly infect *E. coli* (Calendar, 2004). Somatic coliphages share similarities with enteric viruses, but are found in higher numbers in wastewater and are easier and more rapid to detect (Bitton, 2005). The somatic coliphage strain ΦX174 is commonly found in wastewater and used in laboratory methods (USEPA Methods 1601, 1602). Studies indicate that somatic coliphages are excreted at higher levels than F-specific coliphages and that somatic coliphages are likely to be more persistent in water than F-specific coliphages (Grabow, 2001; Schaper et al., 2002; Lee and Sobsey, 2011). Additionally, some somatic coliphages have been shown to be morphologically similar to adenovirus (King et al., 2011).

F-specific coliphages are a group of bacteriophages that infect strains of *E. coli* and *Salmonella* by attaching to the F-pilus (Calendar, 2004). There are both RNA and DNA families

of F-specific coliphages; however, F-specific RNA coliphages from genotypes II and III are mainly associated with human waste and found in wastewater (Bitton, 2005). Specifically, the F-specific coliphage strains MS2 and F2 are commonly found in wastewater and used in laboratory methods (USEPA Methods 1601, 1602). F-specific RNA coliphages have been shown to be morphologically similar to enteroviruses, caliciviruses, astroviruses, and hepatitis A virus (King et al., 2011).

Lastly, bacteriophages that infect *Bacteroides fragilis* HSP 40 have been identified as indicators of enteric viruses in wastewater (Bitton, 2005). *B. fragilis* phages are commonly detected in human waste, wastewater, and polluted aquatic environments (Cornax et al., 1990; Tartera and Jofre, 1987). One study has shown that *B. fragilis* phages are more resistant to wastewater treatment processes than pathogenic bacteria, somatic coliphages, F-specific coliphages, and certain enteric viruses (Jofre et al., 1995). Other studies have shown positive correlations between *B. fragilis* phages and enteroviruses, rotaviruses, and hepatitis A virus in seawater and shellfish (Jofre et al., 1989; Lucena et al. 1994).

There are still shortcomings and limitations of using bacteriophages as indicators of enteric virus removal in wastewater treatment systems that should be noted. For example, unlike enteric viruses, bacteriophages may continue to replicate in surviving bacterial hosts after being shed in feces (Nasser and Oman, 1999). They also may be excreted by animals, and some phages (such as somatic coliphages) have low specificity for human feces (Harwood et al., 2013). Additionally, bacteriophages may significantly exceed the quantities of enteric viruses in a water source or may be absent despite the presence of enteric viruses. Nevertheless, the three groups of bacteriophages identified in this section have been used as surrogates for enteric viruses in wastewater. Other viral indicators of fecal pollution may emerge in the future, such as pepper

mild mottle virus, although its use for the study of WSPs has been limited (Symonds et al., 2014).

2.2.2 Bacteriophage and Enteric Virus Detection Methods

Sampling methods, analytical measures, and detection methods for enteric viruses in water are well documented (APHA et al., 2012; Fong and Lipp, 2005). Virus detection is primarily based on two principles, detection of viruses by propagation in cell culture (i.e., culture assays) or by molecular amplification techniques (i.e., molecular assays) such as polymerase chain reaction (PCR) or PCR with reverse transcription (Fong and Lipp, 2005). The various methods used to detect bacteriophages and enteric viruses are provided in Table 2.3, which includes different types of culture methods, molecular methods, or a combination of both (Fong and Lipp, 2005).

Table 2.3: Common methods for the detection of enteric viruses and bacteriophages

Bacteriophage Detection Methods	Enteric Virus Detection Methods
Culture USEPA Method 1601	Cell Culture
Culture USEPA Method 1602	PCR (RT-PCR)
SM9224F Membrane Filtration	Nested PCR (semi/heminested)
PCR / RT-PCR	Multiplex PCR and Multiplex RT-PCR
qPCR / RT-qPCR (quantitative)	qPCR/RT-qPCR
Mulitplex qPCR-RT-qPCR	ICC-PCR and ICC-RT-PCR
CLAT	
Culture Fast Phage	

Source: Fond and Lipp (2005); APHA et al. (2012); USEPA (2015); Abbreviations: PCR = polymerase chain reaction, RT = reverse-transcriptase, ICC = integrated cell culture, CLAT = Culture, Latex Agglutination, and Typing

Each method of detection has limitations, advantages, and disadvantages. For example, not all enteric virus samples can grow in cell culture (e.g., norovirus) (Fong and Lipp, 2005). There is also variability between molecular methods and culture methods, due in part to: (1) most molecular methods do not distinguish between infectious and noninfectious viruses; (2) the high sensitivity of polymerase chain reaction (PCR) may contribute to artifacts, which may result in false positives; and (3) natural inhibitors in the environment may reduce or block PCR amplification resulting in false negatives or under-representation of infectious viruses (Fong and Lipp, 2005; Mocé-Llivina et al., 2005; USEPA, 2015). A recent review on the suitability of coliphages as viral indicators for fecal pollution covers the advantages and disadvantages of each detection method for enteric viruses and bacteriophages in great detail (USEPA, 2015). Methods for detecting enteric viruses and bacteriophages are becoming more efficient, accurate, and cost effective; however, researchers should be cognizant that the differences between detection methods may greatly affect the presence, absence, and/or strength of correlations found between bacteriophages and enteric viruses.

An extensive review of virus removal in WSPs was recently completed by Verbyla and Mihelcic (2015), in which enteric virus and bacteriophage concentration data were gathered from 48 publications. This critical review demonstrated that a variety of different detection methods and cell hosts have been used to quantify enteric viruses and bacteriophages in WSP samples. The pie charts that were developed by Mihelcic and Verbyla (2015) are provided in Figure 2.1, and represent the different detection methods and cell hosts that were used for 71 different WSP systems from around the world.

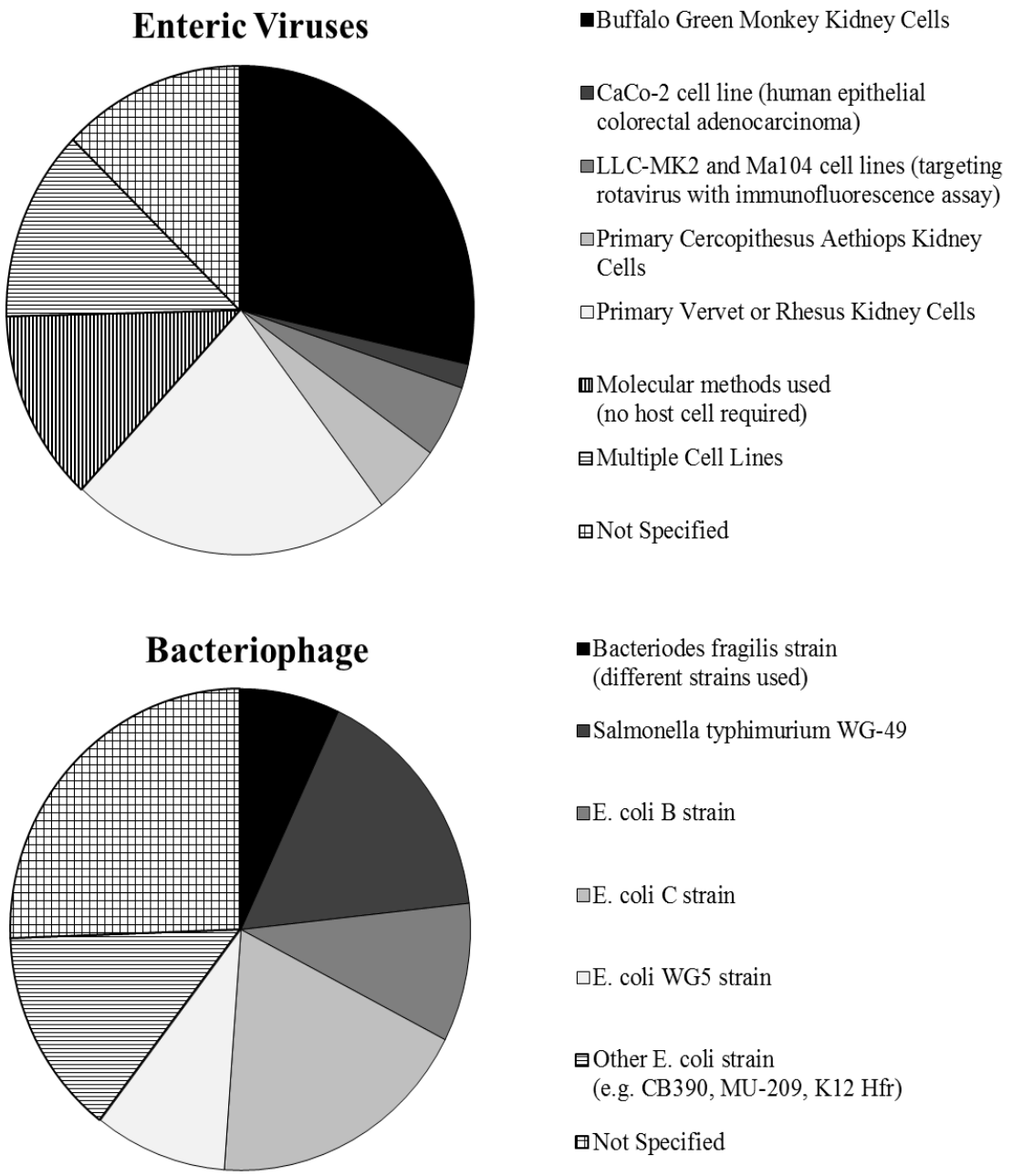

Figure 2.1: Different methods and host cell lines or bacteria strains used for enteric virus and bacteriophage assays from a systematic waste stabilization pond review (Reprinted from Water Research, Vol. 71, Verbyla, M.E. and Mihelcic, J.R., A review of virus removal in wastewater treatment pond systems, Pages 107 – 124, 2015, with permission from Elsevier)

2.3 Virus Removal Mechanisms in Waste Stabilization Ponds

One of the major advantages of a WSP is its ability to effectively remove pathogens via natural processes. In fact, they are considered the most efficient form of wastewater treatment for pathogen removal without the addition of advanced disinfection treatment processes (Mara, 2004; Shilton, 2005). While inactivation mechanisms of pathogenic bacteria and viruses in WSPs do share similarities, the removal rate coefficients differ and virus removal appears to be much more erratic than fecal coliform removal (Maynard, 1999). As previously mentioned in Section 2.2, fecal coliforms have been shown by several authors to be poor indicators of the presence and removal of enteric virus in WSPs (Maynard et al., 1999; Feachem and Mara, 1978; Herrera and Castillo, 2000; Symonds et al., 2009). Therefore, removal mechanisms of enteric viruses and bacteriophages in WSPs are the primary topic reviewed in this chapter.

Virus removal refers to the destruction, inactivation, elimination, or physical removal (e.g., sedimentation) of viruses via natural processes, and does not include additional sterilization processes (Macdonald and Ernst, 1986). The primary removal mechanisms recognized to contribute to the overall removal of viruses in WSPs include sedimentation, predation by organisms of higher trophic levels, and sunlight-mediated mechanisms (Mara, 2004; Shilton, 2005). These primary virus removal mechanisms are summarized in Table 2.4. It is also likely that the persistence of enteric viruses in WSPs are dependent on and influenced by several other factors, including virus type, environmental conditions, chemical and microbiological composition of water, design factors, and treatment processes (Sobsey and Mesche, 2003; Davies-Colley et al., 2000). Some of these factors are highlighted in Table 2.5.

Table 2.4: Primary mechanisms of virus removal in waste stabilization ponds

Removal Mechanism	Effect on virus inactivation or removal
Sunlight-mediated / UV Radiation	DNA damage by solar UV-B radiation (direct sunlight inactivation) Indirect damage to proteins or genome by reactive intermediates created by sunlight (UV and visible wavelengths) reacting with photosensitizers (indirect sunlight inactivation)
Sedimentation	Settlement of virus particles, may be increased by virus association with certain particles
Predation/Microbial Antagonism	Viruses are ingested by organisms of higher trophic levels

Source: Shilton (2005)

Table 2.5: Other factors that may influence virus removal in waste stabilization ponds

Factor	Influence on virus inactivation or virus removal
Design	
Pond geometry and configuration	Length to width ratio affects pond hydraulics and mixing, which may positively or negatively influence virus removal (e.g., for maturation ponds, a length to width ratio of 3:1 or greater is recommended)
Pond Depth	Shallower ponds (1 – 1.5 m) may positively influence virus inactivation by affecting sunlight exposure in the water column, while deeper ponds (>2 m) may negatively influence virus inactivation
Hydraulic Retention Time (HRT)	Affects extent of virus removal. Generally longer HRTs result in more virus removal
Inlet and outlet structures	Influence pond hydraulics; may promote plug flow or cause short circuiting
Baffling	Baffles may improve pond hydraulics by reducing short circuiting and sharpening the residence time distribution, which is expected to positively influence virus removal
Physical	
Temperature or thermal effects	Increasing virus inactivation at higher temperatures, decreasing at lower temperatures. Protein denaturation, RNA damage, interference with enzymatic activity.
Aggregation	Clumping may protect viruses from inactivating agents
Adsorption to particles or surfaces	Adsorption may protect viruses from inactivating agents or contribute to inactivation
Encapsulation or embedding	Viruses within membranes or larger particles may be protected from inactivation
Chemical	

Table 2.5: Continued

Factor	Influence on virus inactivation or virus removal
pH	Viruses survive best near neutral pH and worst at pH extremes
Dissolved Oxygen	High dissolved oxygen may improve light-mediated removal mechanisms
Organic matter	Viruses may be protected by dissolved, colloidal, and solid organic matter, including fecal organics and humic materials (alternatively, their proximity to organic matter may make them more vulnerable to indirect sunlight-mediated inactivation)
Ionic strength	Ionic strength may affect adsorption and elution of viruses from particles
Salts	Increased concentrations of salts (e.g. NaCl) are antiviral for many viruses; some viruses are destabilized and inactivated by water lacking stabilizing salts (e.g. NaCl)
Biological	
Microbial activity	Several contributing mechanisms; microbial activity and metabolism in soils, sediments, and water
Enzyme activity	Certain enzymes inactivate/denature virus proteins
Chemical	Capsid conformation change, opening of capsid
Biofilms	Virus adsorption to biofilms can be protective or microbial activity in biofilms and cause virus inactivation and degradation

Source: Sobsey and Meschke (2003); Shilton (2005)

2.3.1 Sunlight-Mediated Mechanisms

A substantial and increasing amount of evidence indicates that sunlight-mediated mechanisms are the single most important virus inactivation mechanism in WSPs (Mayo, 1995; Maynard et al., 1999; Davies-Colley et al., 2000; Verbyla and Mihelcic, 2015). Two different sunlight-mediated mechanisms operate simultaneously and contribute to virus inactivation in WSPs: direct inactivation and indirect exogenous inactivation (Mattle et al., 2015). Several factors are believed to influence the efficiency of sunlight-mediated virus inactivation mechanisms in WSPs, which include the strength of radiation, the optical and physiochemical characteristics of wastewater, and the properties of the virus (Davies-Colley et al., 2000; Romero et al., 2011). The review by Verbyla and Mihelcic (2015) demonstrated the variance of these factors by showing that S_{90} values (fluence required to achieve 90% inactivation) vary greatly with respect to virus type, water type, and experimental conditions. Another recent study has

recommended MS2 coliphage as a surrogate to study sunlight-mediated virus inactivation in WSPs (Mattle et al., 2015). Lastly, Davies-Colley et al. (2000) have suggested that virus removal in WSPs may be improved by increasing sunlight exposure, which can be achieved with shallower ponds or longer hydraulic retention times. All in all, a significant amount of research suggests that sunlight-mediated processes may be the most important virus inactivation mechanisms in WSPs.

2.3.2 Sedimentation

Sedimentation is believed to be the dominant mechanism for removal of larger pathogens in WSPs, such as helminth ova (Maynard et al., 1999; Verbyla, 2012; Verbyla et al., 2013); however, few studies have actually documented virus sedimentation in WSPs (Verbyla and Mihelcic, 2015). Several authors have suggested that virus-particle association and sedimentation is a primary virus removal mechanism in WSPs (Feachem et al., 1983; Mara, 2004; Shuval, 1990), but their results were not conclusive. One study by Ohgaki et al. (1986) found that F-specific RNA coliphages adsorbed onto particles in a facultative pond under aerobic conditions, which suggests the possibility of virus removal by sedimentation. In theory, viruses may be removed by sedimentation in WSPs if they adsorb onto larger, settleable particles (Shilton, 2005). While sedimentation is not thought to be the primary virus removal mechanism in WSPs, it may still be significant under some conditions.

2.3.3 Predation

Virus predation is considered to be a removal mechanism in WSPs, but to what extent is unknown. Predation occurs when viruses are ingested by antagonistic microbes, or higher trophic-level organisms. Shilton (2005) suggests that virus predation may be a removal mechanism that occurs in times of low sunlight exposure, at night, and deep in the water column

in WSPs. Studies have documented the internalization of enteric viruses by free-living protozoa (Danes and Cerva, 1984), nonflagellates (Manage et al., 2002), mites (Verbyla and Mihelcic, 2015), and ciliates (Battistini et al., 2013); however, at this time it is not completely understood whether internalization of viruses inactivates them or protects them from inactivation (Scheid and Schwarzenberger, 2012). Thus, more research is needed to determine if virus predation contributes to virus removal by direct inactivation or via sedimentation within other organisms, or if in some instances virus predation shields viruses.

2.3.4 Temperature

Pond water temperature, which is correlated with solar radiation, is likely to be a secondary factor that can influence the rate of other virus removal mechanisms in WSPs, particularly light-mediated mechanisms. In general, temperature plays a fundamental role in the attachment, penetration, multiplication, occurrence, and viability of viruses (Sobsey and Meschke, 2003). A study by Nasser et al. (1993) documented incremental increases in removal rates of adenovirus and poliovirus in raw wastewater when temperatures increased from 10°C to 20°C to 30°C. The Marais (1974) formula for the design of maturation ponds uses pond temperature, number of ponds in series, and hydraulic retention time to predict fecal coliform reduction in WSPs. Similar to fecal coliforms, enteric viruses and bacteriophages in WSPs have also been observed to generally have higher removal rates in hot or tropical climates with high average temperatures (>20 degrees Celsius) (Feachem et al., 1983; Herrera and Castillo, 2000; Mara, 2004; Davies-Colley et al., 2005). Conversely, lower virus removal rates are commonly observed in colder temperatures. Although it is currently unknown how suitable the Marais formula is for predicting virus removal, temperature is still a factor that plays a role in sunlight-mediated inactivation mechanisms of viruses in WSPs. For instance, the rate of indirect

exogenous sunlight inactivation for MS2 coliphage was determined to be more efficient with increasing temperatures (Romero et al., 2011).

2.3.5 Pond Hydraulics

The hydraulic efficiency of a WSP is an important factor that determines the overall performance of a pond. A pond is considered to be hydraulically efficient if the pond exhibits plug flow characteristics, does not have short circuiting, and has an actual hydraulic retention time (HRT) that is close to the theoretical HRT (Shilton, 2005). The hydraulic efficiency has been shown to influence the removal of bacteria (von Sperling, 2005), parasites (Verbyla, 2012; Verbyla et al., 2013b), and enteric viruses (Herrera and Castillo, 2000) in WSPs. The results of dye-tracer studies commonly reveal the existence and extent of short circuiting in WSPs (Herrera and Castillo, 2000), and actual HRTs have been found to be much shorter than theoretical HRTs. Also, differences in HRT distributions have been documented using computational fluid dynamics (CFD) modeling (Persson, 2000) and dye-tracer studies (Torres et al., 1999). Overall, pond water hydraulics are primarily governed by the original pond design, which includes pond configuration (length to width ratio), pond depth, and inlet and outlet structures. An additional way to improve pond hydraulics and promote plug flow is to install baffles or multiple evenly-spaced inlet and outlet structures (Mara, 2004).

2.3.6 Virus Removal Rate Coefficients in Waste Stabilization Ponds

Very few studies have reported virus removal rate coefficients (K_v) in WSPs. Rather, most studies have reported the overall virus removal based on percent removal either throughout an entire pond system or for individual ponds. An important distinction is the difference between intrinsic and apparent virus removal rate coefficients. Apparent virus removal rate coefficients ($K_{v,app}$) are dependent upon site-specific conditions and hydraulic regime (e.g., complete mix,

plug flow, dispersed flow), whereas intrinsic virus removal rate coefficients are independent of the hydraulic regime. For example, intrinsic fecal coliform removal rate coefficients (K_b) were determined by putting wastewater samples encapsulated in four plastic receptacles (presumably bags) in a facultative pond and sampling the receptacles daily (Yanez, 1984), thus eliminating the bias from pond hydraulics and flow regime. The average intrinsic K_b value reported by Yanez (1984) was 0.647 $days^{-1}$. For a comparison, apparent fecal coliform removal rate coefficients (K_b) in facultative and maturation ponds are reported to range from 0.26 $days^{-1}$ to 2.42 $days^{-1}$ (von Sperling, 1999). Intrinsic K_b values are more consistent than apparent K_b values in WSPs because apparent K_b values can increase or decrease based on site-specific conditions, hydraulic efficiency, and hydraulic regime.

Determining intrinsic or apparent virus removal rate coefficients for individual ponds are required to better predict virus removal and improve WSP design. However, all virus removal rate coefficients ($K_{v,app}$) discussed or reported in this thesis are apparent, as no intrinsic virus removal rate coefficients were found in the literature. Some apparent virus removal rate coefficients for coliphages and rotavirus in WSPs reported in literature are provided in Table 2.6. Table 2.6 shows that apparent virus removal rate coefficients have ranged from 0.3 $days^{-1}$ to 3.0 $days^{-1}$. It should be noted that apparent virus removal rate coefficients may vary widely depending on pond type, virus and bacteriophage type, pond depth, temperature, solar radiation, hydraulic retention time, organic loading, viral loading rates, the mathematical model (hydraulic regime) used for prediction, and other possible factors.

Enteric virus removal in WSPs has been assumed to follow pseudo first-order kinetics, but the rate of exogenous sunlight-mediated inactivation in WSPs has been found to follow second-order kinetics (Kohn and Nelson, 2007; Mattle et al., 2015). Some recent studies have

determined that enteric viruses and coliphages have faster inactivation rates under conditions of full sunlight as compared to dark conditions (Sinton et al., 2002; Romero et al., 2011). While these sunlight specific inactivation rates may be an important factor in overall virus removal rate coefficients, more research is still needed to reliably predict overall virus removal rate coefficients for individual WSPs.

Despite the many advances in the realm of knowledge about virus removal in WSPs, there are still vast knowledge gaps about important virus removal mechanisms that must be elucidated. The variable physiochemical conditions in WSPs and the influence of environmental factors on virus removal mechanisms must be considered to accurately model virus removal in WSPs. Modeling virus removal in WSPs will be discussed in Section 2.4.

Table 2.6: Apparent enteric virus and coliphage removal rate coefficients reported in waste stabilizations ponds

Pond Type	Enteric virus or coliphage type	Mean Water Temperature [°C]	Removal Rate Coefficient ($K_{v,app}$) [days^{-1}]	Source
Maturation	Somatic coliphage	12.9	0.30	Herrera and Castillo (2000)
Maturation	Somatic coliphage	25.4	2.34	Herrera and Castillo (2000)
Facultative	Somatic coliphage	25.9	2.38	Ceballos et al. (1995)
Facultative	Somatic coliphage	Winter	0.28	Ceballos et al. (1995)
Facultative	Somatic coliphage	Summer	0.50	Ceballos et al. (1995)
Facultative (lab scale)	MS2 Coliphage (F+RNA)	n/a	0.46	Benyahya et al. (1998)
Facultative (lab scale)	φX-174 Coliphage (Somatic)	n/a	0.37	Benyahya et al. (1998)
Anaerobic	Rotavirus	25.0	~ 3.0	Oragui et al. (1995) and Mara (2004)
Facultative	Rotavirus	25.0	~ 0.3	Oragui et al. (1995) and Mara (2004)
Maturation	Rotavirus	25.0	~ 0.5	Oragui et al. (1995) and Mara (2004)

2.4 The Use of Mathematical Models in the Design of Waste Stabilization Ponds

Mathematical models are an important tool to assist the development of the most suitable design criteria for a certain condition under analysis in a WSP. One common approach has been to apply reactor theory derived from the field of process engineering. Reactor analyses use a mass-balance approach to analyze constituents in a control volume that is either a chemical reactor or natural system modeled as a chemical reactor (Mihelcic and Zimmerman, 2014). The broad classes of reactors are ideal flow and non-ideal flow reactors. The ideal flow models are the completely mixed flow reactor (complete mix model, CMM) and plug flow reactor (plug flow model, PFM), and the non-ideal flow models include the dispersed flow model (DFM) and tanks-in-series model (TIS). These models, with the exception of TIS, have been previously used to model pathogen and BOD removal in WSPs (Mara, 2004; Shilton, 2005). The following paragraphs aim to describe each mathematical model in order to better understand the suitability and validity of each model for predicting virus removal in WSPs.

A completely mixed flow reactor is an ideal flow model in which complete mixing is assumed to occur instantaneously and uniformly throughout the reactor (Mihelcic and Zimmerman, 2014). Reactions proceed at an identical rate everywhere in the reactor, and the concentrations throughout the reactor are the same as the effluent concentration (Crittenden et al., 2012). CMMs tend to result in the lowest removal efficiencies out of all the models, representing the lower bound of ideal flow. A plug flow reactor is an ideal flow model in which fluid moves through the reactor as a plug and the fluid does not mix with fluid elements in front of or behind it. As a result, the reaction rate and concentrations of the reactants decrease as the fluid moves toward the exit of the plug flow reactor (Crittenden et al., 2012). Plug flow reactors have the highest removal efficiencies out of all models, representing the upper bound of ideal flow. In reality, the flow through ponds has been shown to be non-ideal (e.g., Verbyla et al.,

2013b) and the constituent removal efficiencies will always exist in between the bounds of complete mix and plug flow, which is represented by the dispersed flow model. The dispersed flow model (DFM) (Wehner-Wilhelm model) accounts for non-ideal flow conditions based on the extent of dispersion. Dispersion results from molecular diffusion as described by Fick's Law, and turbulent dispersion (Mihelcic and Zimmerman, 2014). Table 2.7 compares the three models and displays the steady-state first-order formulas used for predicting effluent concentrations for a particular reactant or constituent.

The complete mix, plug flow, and dispersed flow models have previously been used by researchers to predict BOD or fecal bacteria removal in WSPs (Mara, 2004; Shilton, 2005). The complete mix model is the most common model employed to design a WSP for BOD and fecal coliform removal because it yields lower estimated removal efficiencies, which corresponds to more conservative pond sizing and prevents inadequate design (Shilton, 2005). Additionally, the complete mix model been shown to model fecal coliform particularly well in slightly rectangular or square ponds (von Sperling, 2005) and anaerobic ponds (Mara, 2004). The plug flow model is not as commonly used for WSP design due to its tendency to overestimate removal efficiencies, but is most representative of elongated ponds (von Sperling, 2005). The dispersed flow model more closely represents the actual flow that occurs in a WSP and is more robust than the ideal flow models. The dispersed flow model accounts for dispersion, can be adjusted to account for a variety of different pond geometries, and has been commonly used to model fecal coliform removal in WSPs (Mara, 2004; von Sperling, 2005).

Table 2.7: First-order steady-state mathematical models used to estimate reactant or constituent concentrations in waste stabilization ponds

Hydraulic Model	Formula for effluent concentration (1st order)	Assumptions and limitations	Source
CMM	$C_e = \frac{C_i}{1+K \cdot t}$ (1)	• Ideal flow • Instantaneously mixed throughout reactor • Infinite dispersion, d = ∞ • Under estimates removal efficiency (lower bound) • Conservative approach	Marais (1974)
PFM	$C_e = C_i e^{-K \cdot t}$ (2)	• Ideal flow • No mixing • No dispersion, d = 0 • Over estimates removal efficiency (upper bound) • Aggressive approach	Thirumurthi (1974)
DFM[a]	$C_e = C_i \cdot \frac{4ae^{1/(2d)}}{(1+a)^2 e^{a/(2d)} - (1-a)^2 e^{-a/(2d)}}$ (3) $a = \sqrt{1+4K \cdot t \cdot d}$ (4)	• Non-ideal flow • Accounts for dispersion • Removal efficiency lies between CMM and PFM	Wehner and Wilhelm (1956)

C_e = effluent concentration (e.g., virus/L); C_i = influent concentration (e.g., virus/1L); d = dispersion number; d = D/(VL), where D = dispersion coefficient, V = flow velocity (m^2/s), L = reactor length (m); K = kinetic removal rate coefficient (days $^{-1}$); t = average hydraulic retention time (days); [a] The assumptions and boundary conditions for the Wehner and Wilhelm (1956) dispersed flow model equation are: steady state, first order reaction, constant cross-sectional area, constant flowrate, no short-circuiting, uniform temperature throughout reactor, continuity of concentration and flux at each boundary, applicable for reactive systems with open or closed entrance or exit conditions.

The complete mix model developed by Marais and Shaw (1961) and refined by Marais (1974) was the first model adopted for predicting BOD and fecal bacteria reduction in WSPs. Thirumurthi (1974) advocated for the use of the plug flow model in WSPs instead of the complete mix model, but it hasn't been as widely used since it overestimates removal efficiencies. The Wehner and Wilhelm (1956) equation for dispersed flow has been used by

several authors for predicting BOD and fecal bacteria reduction in WSPs (Thirumurthi, 1969; Polprasert and Bhattarai, 1985) and was found to more accurately predict fecal coliform removal in WSPs when compared with the complete mix model (Polprasert and Bhattarai, 1985). More recently von Sperling (1999, 2002, 2003, 2005) has verified both the complete mix and dispersed flow models for predicting fecal bacteria reduction in WSPs and they are now widely accepted to reliably and accurately predict *E. coli* removal in WSPs.

In contrast, there is currently no model that has been developed to accurately describe and predict virus removal in WSP systems. This is likely because viruses are smaller than fecal bacteria (Bitton, 2005), often more resistant to treatment and environmental conditions (Symonds et al., 2009), have been shown to have different removal rate coefficients (Herrera and Castillo, 2000), and virus removal appears to be much more erratic than fecal coliform removal in WSPs (Maynard, 1999). The absence of a model for virus removal may also be due to the lack of documented virus or coliphage concentration data from WSPs.

2.4.1 Kinetic Reaction Rate Coefficient in Mathematical Models

The kinetic reaction rate coefficient, K, included in all of the mathematical models previously discussed, is fundamental to reliably predicting virus concentrations. The kinetic reaction rate coefficient represents the physical, chemical, and biological processes that occur in a reactor, or waste stabilization pond in this instance. The removal of protozoan cysts, oocysts, helminth eggs, pathogenic bacteria, and BOD have generally been observed to have first-order kinetics in WSPs (Shilton, 2005), and viruses appear to follow pseudo first-order kinetics, although the rate of exogenous sunlight-mediated inactivation in WSPs has been found to follow second-order kinetics (Kohn and Nelson, 2007; Mattle et al., 2015). In regards to virus removal, the kinetic reaction rate coefficient (K) is referred to as the virus removal rate coefficient (K_v).

The virus removal rate coefficient has rarely been annotated in literature, so it was chosen to be symbolized as K_v.

The most common method for determining K values is to back-calculate the K from one of the mathematical models described previously using field data. In this case, a larger set of data is usually preferred. After this is accomplished, a regression analysis can be used to correlate K values with other parameters to produce an empirical input-output equation that can calculate K values based on input parameters such as temperature, pond depth, and retention time (Shilton, 2005). von Sperling (2005) acknowledged that several researchers have developed models that predict K_b (fecal coliform removal rate) as a function of additional variables such as pH, algal concentration, soluble BOD, applied COD load, solar radiation, and light extinction coefficient. However, many of those variables may not be known before the design phase and, therefore, should not be used as input variables. Equations for predicting K values that have previously been developed for fecal coliforms (K_b) and coliphages ($K_{v,app}$) that were adjusted for standard temperature (20°C) using the Arrhenius expression are provided in Table 2.8.

Table 2.8: Empirical first-order equations for bacteria and coliphage removal rate coefficients in waste stabilization ponds

Microorganism	**Removal rate coefficients equations**	**Source**
Fecal coliform	$K_b = 2.6(1.19)^{T-20}$	Marais (1974)
Fecal coliform	$K_b = 0.917H^{-0.877}t^{-0.329}1.07^{T-20}$	von Sperling (1999)
Fecal coliform	$K_b = 0.549H^{-1.456}1.07^{T-20}$	von Sperling (2005)
Somatic coliphage	$K_v = 0.439(1.044)^{T-20}$	Herrera and Castillo (2000)

K_b = fecal coliform removal rate coefficient at 20°C ($days^{-1}$); K_v = virus removal rate coefficients at 20°C ($days^{-1}$); t = average hydraulic retention time (days); H = pond depth; T = temperature (°C); 1.19, 1.07, and 1.044 are temperature adjustment coefficients

Herrera and Castillo (2000) are the only authors identified to date to report a virus removal rate equation for coliphages or viruses; however, their equation was derived based on the data from only one pond system. Although only one equation for $K_{v,app}$ has been identified in the literature, there is still potential for a reliable $K_{v,app}$ equation to be developed as long as influent and effluent virus concentration data are collected from a significant number of waste stabilization ponds. In order to yield robust and accurate predictions of virus removal and virus removal rate coefficients in WSPs, a large set of paired influent and effluent virus or bacteriophage concentrations from different ponds must be analyzed.

2.4.2 Dispersion Number

The dispersion number (d) is the dimensionless constant that is present in the dispersed flow model. In reactor theory, the dispersion number is equal to the inverse of the Peclet number (Crittenden et al., 2012). The dispersion number is also theoretically present in the complete mix and plug flow models. A dispersion number equal to infinity is assumed for the complete mix model, signifying complete and instantaneous mixing, and a dispersion number equal to zero is assumed for the plug flow model, signifying no longitudinal mixing (Mara, 2004). The dispersion number characterizes the flow in WSPs by quantifying the extent of longitudinal mixing as the water flows through the pond (Shilton, 2005). In a WSP the dispersion number accounts for several physical influences that may affect the flow in a pond, which include: the flowrate and its variation over time; the design of the inlets and outlets; wind shear and its variation over time; pond geometry; and temperature and density effects (Shilton, 2005). Equations for calculating dispersion numbers in a WSP are provided in Table 2.9.

Table 2.9: Methods for calculating the dispersion number in waste stabilization ponds

Method for calculating dispersion number	Source
$d = 0.102\left(\frac{3(W+2H)t\upsilon}{4LWH}\right)^{-0.410}\left(\frac{H}{L}\right)\left(\frac{H}{W}\right)^{-(0.981+\frac{1.385H}{W})}$	Agunwamba et al. (1992); von Sperling (1996)
$d = \frac{L/W}{-0.261+0.254\left(\frac{L}{W}\right)+1.014\left(\frac{L}{W}\right)^2}$	Yanez (1993)
$Pe= 0.1\left(\frac{L}{W}\right)+0.01\left(\frac{L}{H}\right)$	Nameche and Vasel (1998)
$d = W / L$	von Sperling (1999, 2003)

d = dispersion number, W = pond width (m), H = pond depth (m), L = pond length (m), t = average hydraulic retention time (days), υ = kinematic viscosity [m^2/day]; Pe = (1/d)

The dispersion number can by determined by dye-tracer studies, but it is impractical to always perform tracer studies in every WSP. Furthermore, a tracer study only elucidates the flow pattern and extent of dispersion after the pond has been designed, whereas it is more advantageous to predict the dispersion number mathematically in the design process of a WSP. However, tracer studies are particularly useful to determine whether a pond is performing how it was designed. For instance, Herrera and Castillo (2000) performed a tracer study and found that the dispersion number of the secondary pond resembled a complete mix reactor, while the two primary ponds resembled dispersed flow reactors.

There is clearly a lack of information about modeling virus removal with reactor theory in WSPs and thus a need for more research. Although fecal coliform removal has been shown to not be representative of virus removal in WSPs, many insights about modeling virus removal can still be formulated from the mathematical models used for modeling fecal coliform in WSPs. Following a similar methodology outlined by von Sperling (2005) for viruses in lieu of fecal

coliforms may have the potential to yield a model or models that can reliably predict virus removal in WSPs.

2.5 Knowledge Gaps Identified in Literature

Reliably predicting virus removal in WSPs is important for determining whether wastewater effluent is safe for discharge to the environment and for reuse in agriculture or aquaculture. When used properly, wastewater reuse can aid in the production of food, increase income, improve nutrition and the quality of life in poor areas, and can reduce the carbon footprint of wastewater treatment. Conversely, when not used properly, public health degradation is likely to occur through the affliction of enteric viruses and other pathogenic diseases present in wastewater.

The results from the literature review demonstrate the overall lack of knowledge pertaining to virus removal efficiency, virus inactivation and removal mechanisms, and virus removal rate coefficients ($K_{v,app}$) in WSPs. Virus removal efficiencies have been shown to be erratic in WSPs, the overall consensus on primary virus removal mechanisms has been very inconsistent throughout decades of research, and more information is available about coliphage removal rates than enteric virus removal rates. The limited amount of documented influent and effluent virus concentration data for WSPs from field studies is another limiting factor in this field of research. This can likely be attributed to the overall difficulty and costliness of detecting viruses in wastewater samples. While there are gaps in literature about the direct association between WSP effluent and enteric virus affliction in humans, enough evidence is available to consider it a burden to public health. The most notable research gap is that no mathematical model currently exists that has been shown to reliably predict virus removal in WSPs.

Based on the research needs and limitations discussed above, the present thesis will fill the identified knowledge gap by collecting available influent and effluent virus concentration

data for individual WSPs from literature and field studies, and based on these data, assess the applicability of existing mathematical models for predicting virus removal in WSPs. Throughout this process, more information about virus removal mechanisms and virus removal rate coefficients in WSPs is expected to be elucidated.

CHAPTER 3: MATERIALS AND METHODS

3.1 Waste Stabilization Pond Database

Influent and effluent concentrations for viruses and bacteriophages for individual WSPs were gathered from the literature. Verbyla and Mihelcic (2015) recently performed a systematic review of 48 publications on virus and bacteriophage removal in WSPs and compiled a database with removal data from 71 different WSP systems around the world. That review was conducted by searching for relevant keywords in English (virus, phage, coliphage, bacteriophage, pond, lagoon, stabilization, anaerobic, facultative, maturation, polishing), Spanish (laguna de estabilización, virus entérico, colifagos), and French (basin de stabilization, basin de lagunage, lagune de stabilization, virus entérique, bacteriophage) using the following search engines: ScienceDirect, Web of Science, ISI Web of Knowledge, PubMed, Academic Search Premier, JSTOR, Google Scholar, and Google. Peer-reviewed journals, reports from government agencies, conference proceedings, theses, dissertations, and field studies were all considered in that database.

The database compiled by Verbyla and Mihelcic (2015) was the primary source of data used for this study. Additional virus concentration data were sought using the same methodology as Verbyla and Mihelcic (2015) and one additional WSP was added to the initial database (Jurzik et al., 2015), resulting in a total of 50 publications (including Verbyla and Mihelcic (2015)) with virus or bacteriophage removal data from 72 WSP systems. However, whereas Verbyla and Mihelcic (2015) assessed virus removal in WSP systems, the purpose of the present study is to

assess virus removal in individual WSPs. Therefore, the data obtained were systematically reviewed to ensure that all the essential characteristics and data were reported for each individual WSP. A list of the 50 publications is provided in Table A1 in Appendix A. Figure 3.1 shows the decision making process that was followed to determine which data possessed the suitable requirements for mathematical modeling and statistical analysis and which data were determined to be outliers and thus removed from further evaluation.

Several additional measures, besides those identified from the process shown in Figure 3.1, were employed to determine additional outliers in the WSP database. For example, all virus concentrations reported as zero and non-detectable (i.e., C_e = "-" or "<") were excluded. There are statistical methods for analyzing non-detectable data (Wendelberger and Campbell, 1994); however, this was beyond the scope of this study and not critical because the magnitude and frequency of non-detectable data in the database was low. Also, reported virus concentrations that increased between influent and effluent samples were removed from the database because these suggested growth in enteric virus populations which are not capable of replicating in the environment outside of their host (Cann, 2003). Bacteriophages can theoretically replicate in the environment because they are viruses that infect bacteria, but data suggest that somatic and F-specific coliphages rarely, if ever, replicate in *E. coli* in aqueous environments (Grabow, 2001; Jofre, 2009). Additionally, virus concentrations reported as the same value for influent and effluent samples were excluded. Three censored effluent virus concentration data (Oragui et al., 1995; Pearson et al., 1995; Rao et al., 1981), six effluent virus concentration data that showed growth (Symonds et al., 2014; da Silva et al., 2008; Malherbe and Strickland-Cholmley, 1967b), and six effluent virus concentrations (Symonds et al., 2014; El-Deeb Ghazy et al., 2008; Oragui et al., 1995; Verbyla and Mihelcic, 2015; Zhenbin et al., 1993) that were the same as influent

concentrations were excluded, amounting to a small percentage of the total data (5.7 %). Other publications that described virus removal in stormwater ponds, aerated ponds, and laboratory scale experiments were also excluded.

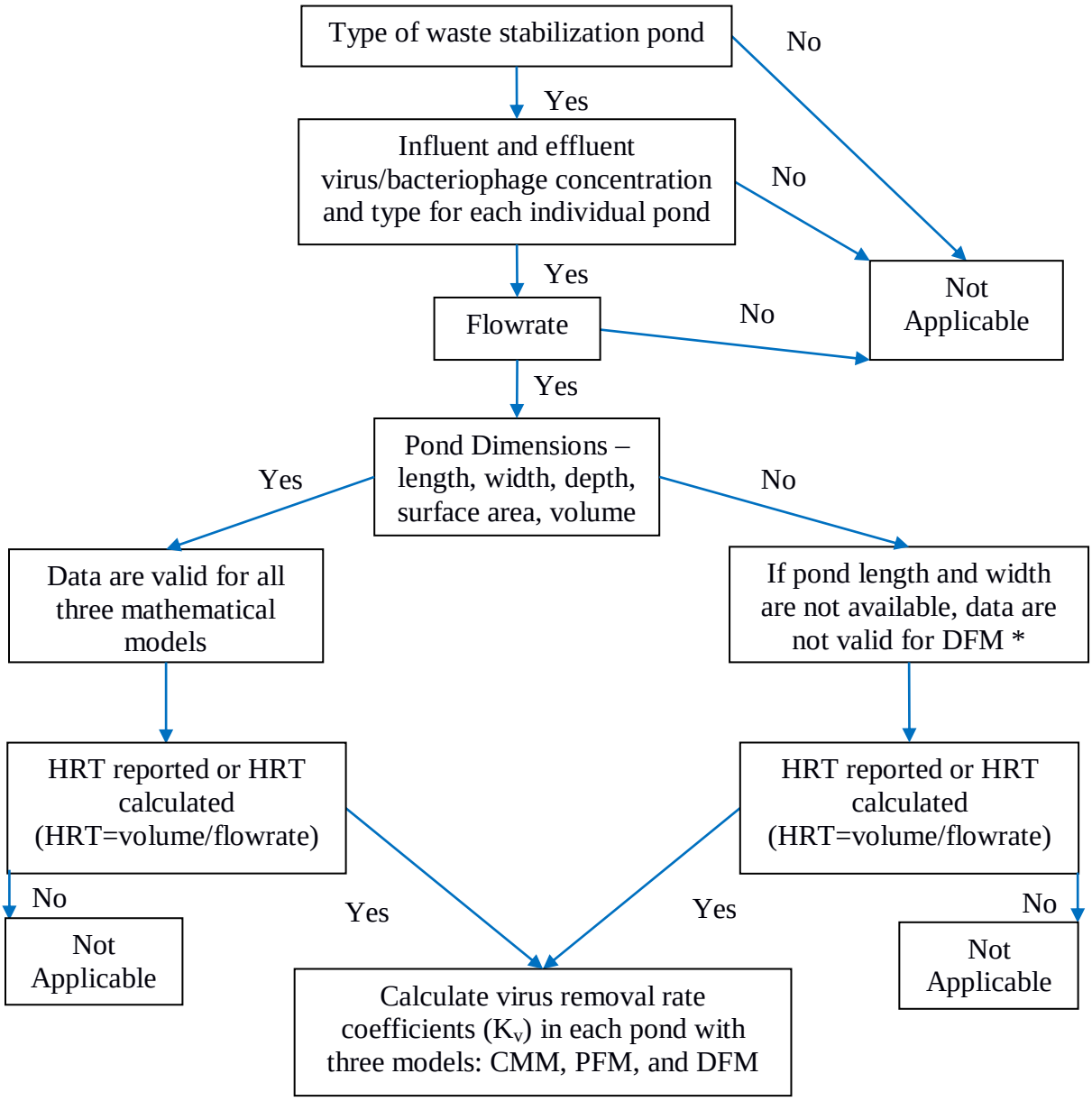

Figure 3.1: Method used to determine if reported waste stabilization pond characteristics and data were appropriate to be used for statistical analyses and mathematical modeling (*exceptions may be considered)

Based on theoretical considerations for virus removal mechanisms in WSPs, information about design, operational, and environmental (DOE) parameters were included in the database

for each individual pond to explore their correlations with virus removal rate coefficients ($K_{v,app}$). The design and operational parameters included pond type (anaerobic, facultative, maturation), pond depth, and hydraulic retention time (HRT). Virus type and concentration, bacteriophage type and concentration, and the following environmental parameters were also recorded in the database: water temperature and pH (when reported), air temperature, solar radiation, and viral loading rates.

The updated database included data from 34 publications and 44 WSP systems. These 44 WSP systems represented a total of 112 individual WSPs. Analyzing the data according to the selection criteria outlined in Figure 3.1 and discussed previously yielded a final data set that included 249 data points for influent and effluent virus or bacteriophage concentrations for individual WSPs. 332 data points were removed from the original set of 581 data points based on the selection criteria, yielding the final amount of 249 data points. Table A2 in Appendix A displays all the data points included in the final WSP database. There are more data points (n = 249) than ponds (p = 112) in the database because some authors reported multiple types of viruses for the same ponds and others reported virus concentrations under different operating conditions (i.e., different flows, time of year). The majority of ponds in the database are part of full-scale WSP systems, with the exception of two pilot-scale systems (Oragui et al., 1986; Oragui et al., 1995; Pearson et al., 1995). The pilot-scale WSP systems were located outdoors and had realistic dimensions, so they were considered to be representative of full-scale WSP systems and are included in the database.

The distribution of ponds in the database can be broken down according to pond type: (1) facultative: 51 ponds (147 data points); (2) maturation (includes 8 polishing): 47 ponds (78 data points); (3) anaerobic: 14 ponds (24 data points). The geographical distribution of the 44 unique

WSP systems by country is: USA: 8; Spain: 5; India: 4; Bolivia: 4; Brazil: 4; Israel: 2; Venezuela: 3; New Zealand: 2; Australia: 1; South Africa: 1; United Kingdom: 2; China: 1; Thailand: 1; Chile: 1; France: 1; Egypt: 1; Colombia: 1; Germany; 1; Uruguay: 1. Histograms showing the latitudes and hydraulic retention times of each WSP data point (n = 249) in the updated database are provided in Figures 3.2 and 3.3, respectively. The distribution and frequency of viruses and bacteriophages reported in the WSPs for each WSP data point are displayed in Table 3.1 and Figures 3.4, 3.5, and 3.6. The histogram of latitudes shows that the geographical distribution of the data points from the WSPs are widely dispersed with the majority being located in temperate regions. The HRTs for each WSP data point range widely from 0.4 days to 76 days, but approximately 80 percent of the data points came from ponds with HRTs of 20 days or less. Lastly, there are six different groups of viruses and four different groups of bacteriophages included in the database, with a total of more than twice as many viruses (v = 173) as bacteriophages (b = 76). These statistics represent the diversity of the physical, environmental, and operating conditions that exist in this WSP database.

Table 3.1: Overall distribution of virus and bacteriophage types among data points in the final waste stabilization pond database

Virus or Bacteriophage Group (strain)	**Frequency**
Culturable Enteric Virus	119
Rotavirus	46
Norovirus (GI)	5
Norovirus (GII)	2
Adenovirus	1
Somatic coliphage	32
F- specific coliphage	14
F- specific coliphage (MS2)	4
F- specific coliphage (RNA)	4
Coliphage (unspecified)	20
B. fragilis phage	2
Total viruses	173
Total bacteriophages	76

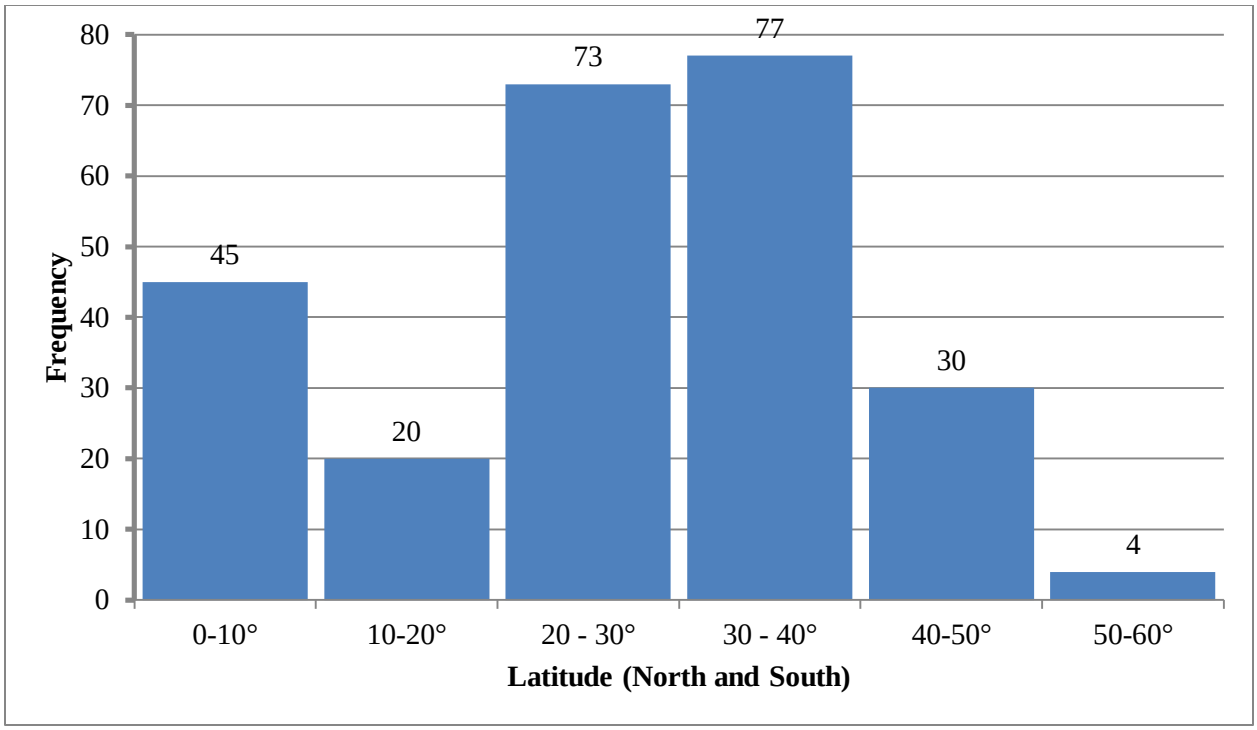

Figure 3.2: Frequency of data point latitudes for each waste stabilization pond in the final database

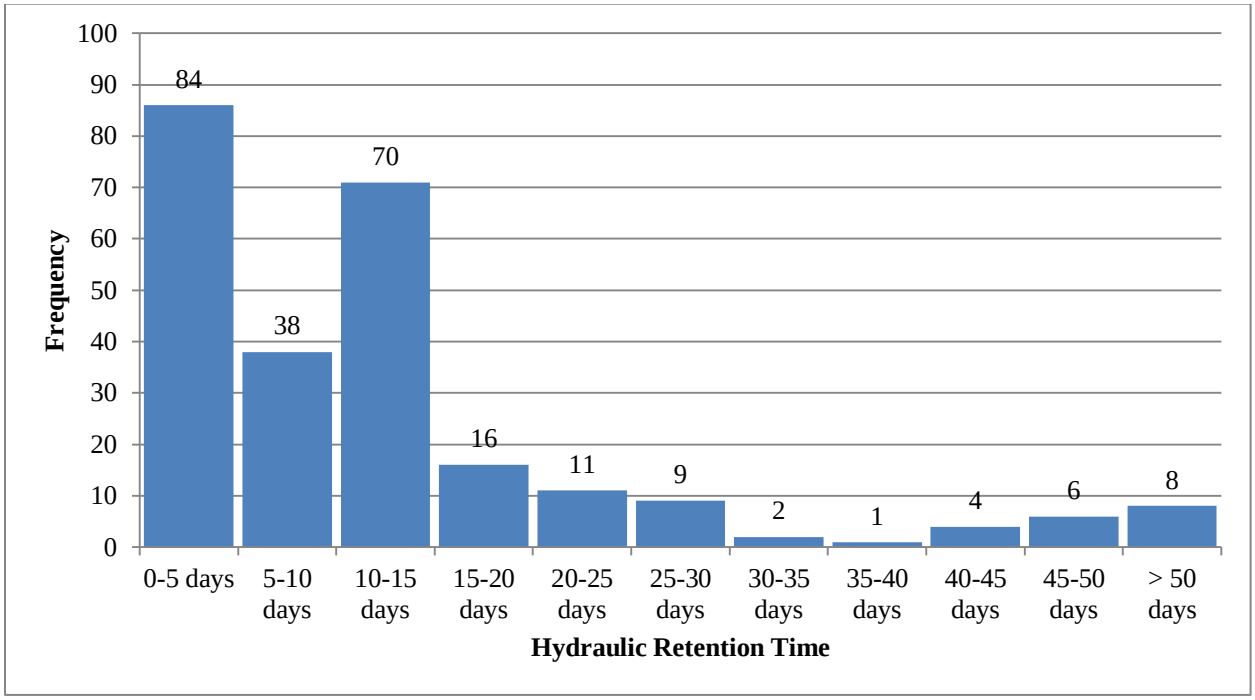

Figure 3.3: Frequency of data point hydraulic residence times (HRTs) for each waste stabilization pond in the final database

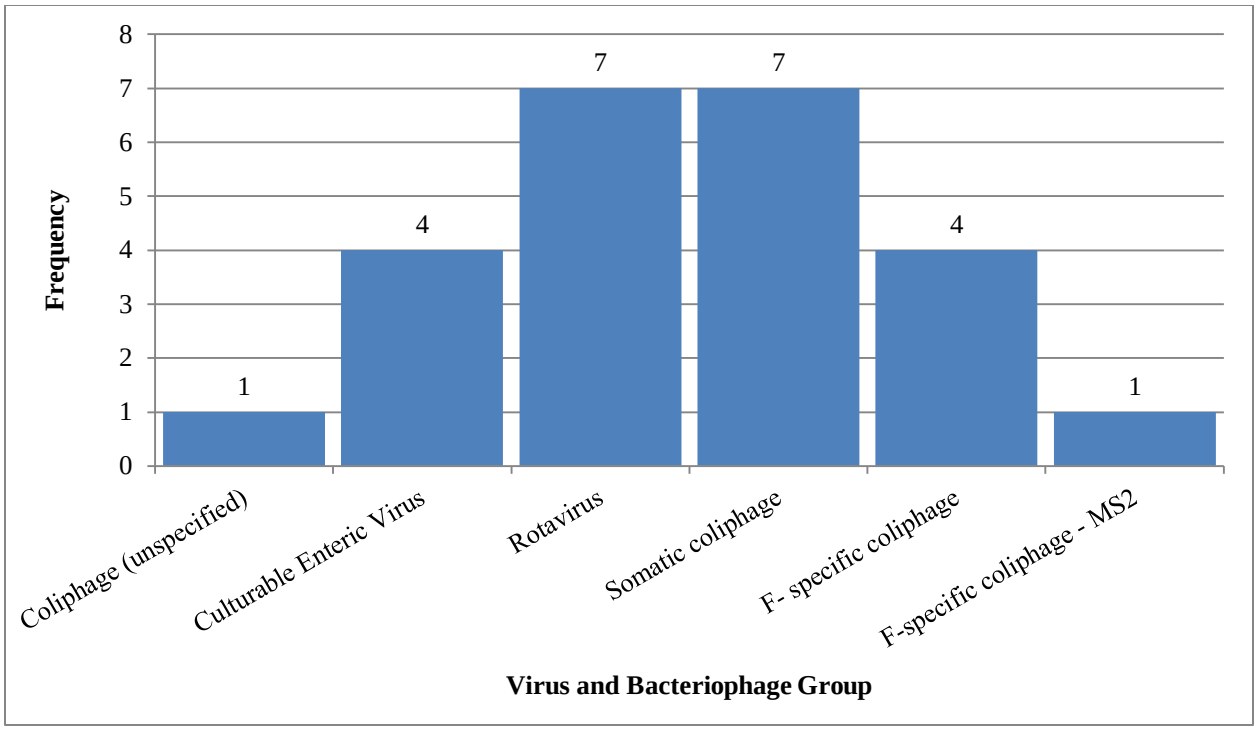

Figure 3.4: Groups of viruses and bacteriophages targeted in anaerobic ponds in the final database

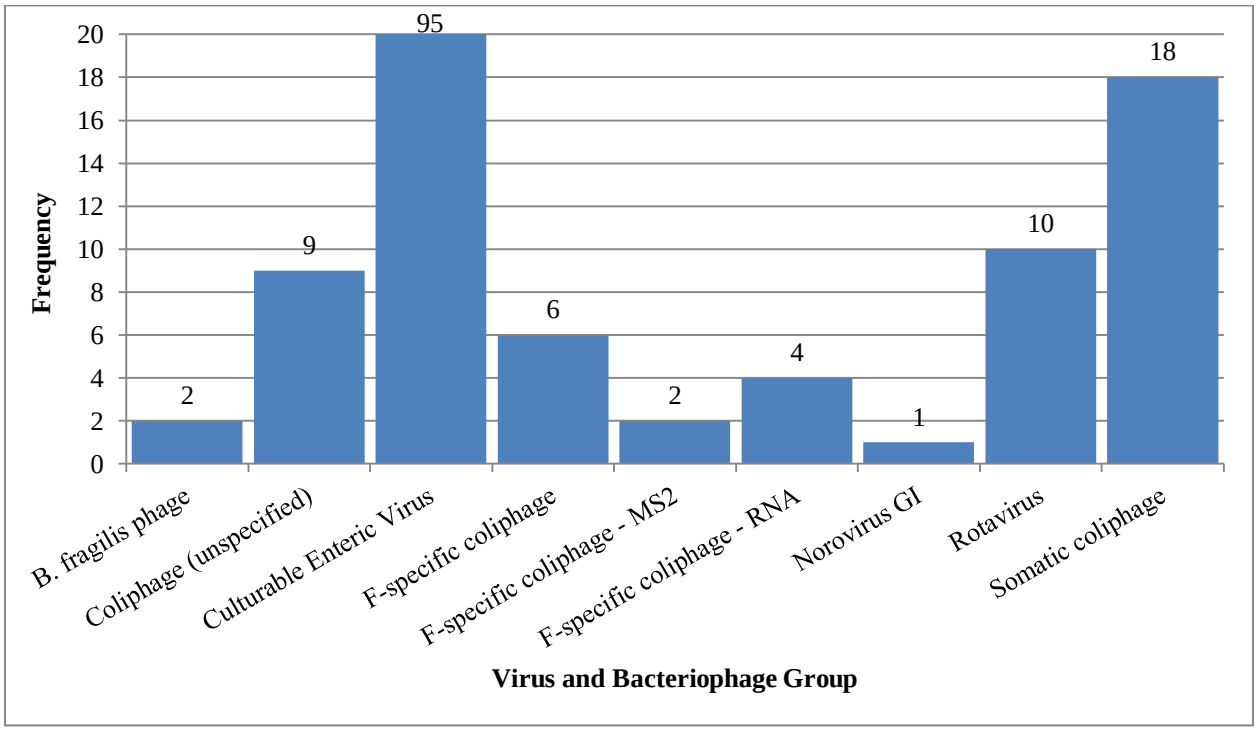

Figure 3.5: Groups of viruses and bacteriophages targeted in facultative ponds in the final database

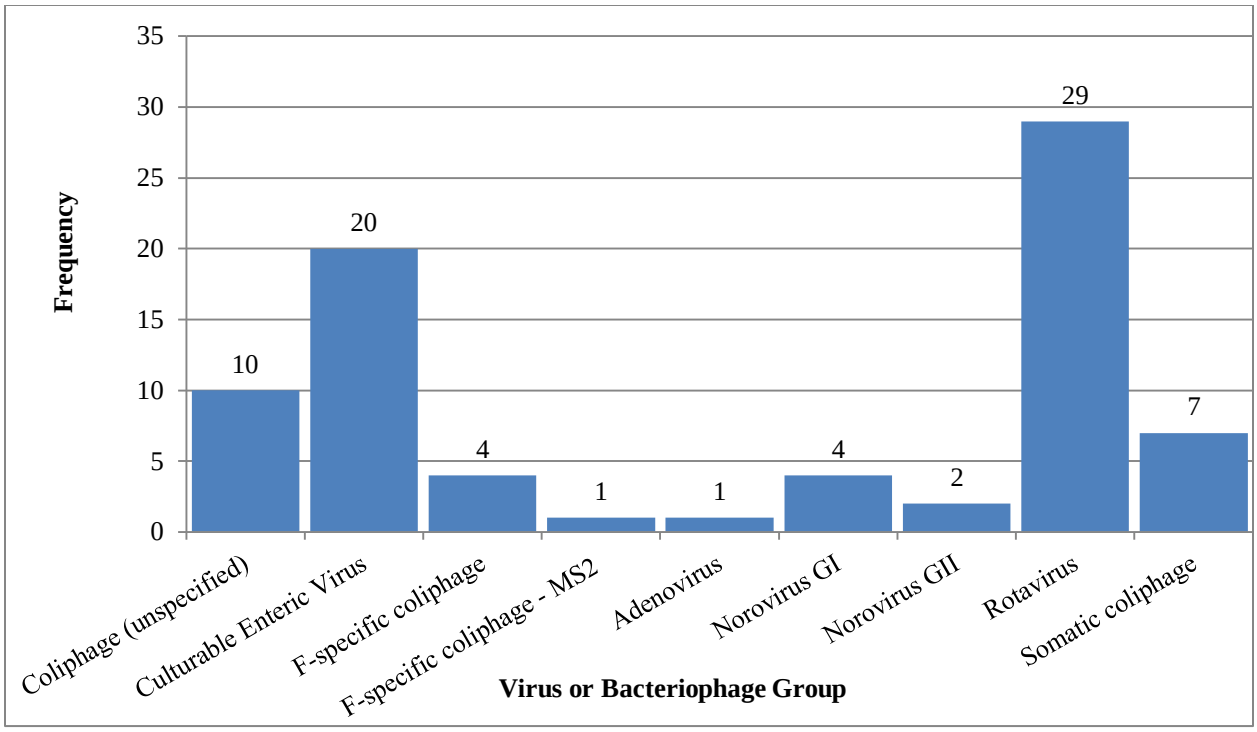

Figure 3.6: Groups of viruses and bacteriophages targeted in maturation ponds in the final database

3.1.1 Assumptions and Limitations

There are several limitations and assumptions associated with the virus concentrations and the environmental and operational parameters for the data used to develop this study's WSP database. In a few circumstances, some assumptions were applied to reported virus influent and effluent concentration data so the data could be included in mathematical modeling and statistical analyses. Seven of the data points in the database (7/249 or 2.8 percent) came from four publications (England et al., 1967; Omura et al., 1985; Malherbe and Strickland-Cholmley, 1967b; Macdonald and Ernst, 1986) that only used semi-quantitative methods, which means only the presence or absence of viruses in replicate samples was reported. For these data points, the most probable number statistics method was utilized to estimate the virus influent and effluent concentrations (Jarvis et al., 2010).

Some limitations of the data include: (1) several publications did not report the length, width, or depth of the WSP that was studied; (2) it was often not specified whether the wastewater flowrates were actually measured or whether the design flowrates were reported; (3) the mean theoretical HRT was often reported instead of the actual measured mean HRT; (4) the month of the year the data were collected was not always reported, which may affect virus removal rate coefficients due to differences in solar radiation and temperature; and (5) the bacterial host strain cultivated to measure coliphage plaque-forming units was not reported for 20 data points.

If the length and width of a pond were not reported by the study author(s), the Google Earth ruler tool was utilized to obtain these data. The accuracy of the Google Earth ruler tool was tested by measuring the length and width of 11 ponds from the database with known dimensions and comparing those measured dimensions to the reported pond dimensions (Betancour, 2013; Reinoso et al., 2011; El-Deeb Ghazy et al., 2008; Campos et al., 2002). The measured and reported length and width of the ponds were found to be within one to three meters when using this method (i.e., within 10 percent). If the length and width of a pond were not reported by the study author(s) and the pond could not be found using Google Earth, these data points were excluded from the DFM dataset for statistical analysis but were still included in the CMM and PFM datasets for statistical analyses. A total of eight data points from three authors (Zhenbin et al., 1993; Morris, 1984; Malherbe and Stickland-Cholmley, 1976b) were removed from the DFM dataset because pond length and width were not available. If the HRT wasn't reported by the study author(s), the theoretical HRT was calculated by dividing the pond volume by the wastewater flowrate. If the solar radiation and temperature were not reported, the latitude and longitude of each pond system were used to gather these data from the United States National

Aeronautics and Space Administration (NASA) (https://eosweb.larc.nasa.gov/cgi-bin/sse/retscreen.cgi?email=rets40nrcan.gc.ca). The surface viral loading rates (viruses/ha/day) and volumetric viral loading rates (viruses/m^3/day) for WSPs were not reported in the literature, but were calculated for all data points using the pond surface area (m^2), volume (m^3), flowrate (m^3/day), and virus influent concentration (viruses/L).

3.2 Mathematical Models Used to Calculate Virus Removal Rate Coefficients

The mathematical models from reactor theory that were discussed in Section 2.4 were used to back-calculate virus removal rate coefficients ($K_{v,app}$) for each WSP in the database. In order to calculate the virus removal rate coefficients for each set of data using the complete mix (CMM) and plug flow (PFM) models, the HRT and influent and effluent virus or bacteriophage concentrations had to be known for each WSP. For the dispersed flow model (DFM), the dispersion number had to be determined in order the back-calculate for the $K_{v,app}$ value. The first-order equations associated with each of the three models and the specific equations that were used to back-calculate the $K_{v,app}$ values are provided in Table 3.2.

The process for back-calculating the virus removal rate coefficient for the complete mix and plug flow models was straight forward and did not require the length and width of each pond, which are required to determine the dispersion number. The dispersed flow model, on the other hand, required a more robust process for back-calculating virus removal rate coefficients. The dispersion number equation (Equation 5) validated by von Sperling (1999, 2003) was used as an input in the DFM equation. To justify the reliability of this dispersion number, von Sperling (2003) conducted a sensitivity analysis on the dispersion number equation by performing Monte Carlo simulations (1,000 runs) and found that for design purposes, the simplified method for estimating the dispersion number is sufficient because the dispersion

number has a relatively small influence on the estimate of fecal indicator concentrations when compared to the high uncertainty of other WSP input variables, such as population, flowrate, wastewater volume, and HRT.

Table 3.2: First-order steady-state mathematical models used to estimate virus/bacteriophage concentrations and removal rate coefficients ($K_{v,app}$) in waste stabilization ponds

Mathematical Model	**Formula describing effluent concentration (1st order)**	**$K_{v,app}$ equation**
CMM (Marais, 1974)	$C_e = \frac{C_i}{1+K_v \cdot t}$ (1)	$K_v = \frac{(C_i/C_e)-1}{t}$ (6)
PFM (Thirumurthi, 1974)	$C_e = C_i e^{-K_v \cdot t}$ (2)	$K_v = \frac{\ln C_i - \ln C_e}{t}$ (7)
DFM (Wehner and Wilhelm, 1956)	$C_e = C_i \cdot \frac{4ae^{1/(2d)}}{(1+a)^2 e^{a/(2d)} - (1-a)^2 e^{-a/(2d)}}$ (3) $a = \sqrt{1+4K_v \cdot t \cdot d}$ (4) $d = W / L$ (5)	$K_{v,app}$ is calculated using an iterative process (Solver tool in Microsoft Excel)

C_e = effluent virus concentration (e.g., viruses/L); $C_{i\,=}$ influent virus concentration (e.g., viruses/L); d = dispersion number; $K_{v,app}$ = virus removal rate coefficient (days $^{-1}$); t = hydraulic retention time (days)

The virus removal rate coefficient ($K_{v,app}$) was back-calculated for the dispersed flow model using the Solver tool (Generalized Reduced Gradient Algorithm) in Microsoft Excel. First, the dispersion number was calculated for each WSP with the length and width dimensions that were reported or measured. Next, the *a* value in the dispersed flow model, which is a substitution variable, was calculated using the dispersion number, HRT, and an initial guess for the value of $K_{v,app}$. The initial guess for $K_{v,app}$ was estimated from the $K_{v,app}$ values that were calculated for the same ponds using the complete mix and plug flow equations. According to reactor theory, the removal efficiency and removal rate coefficient of the dispersed flow model has to be in between the complete mix and plug flow model removal efficiencies, which are the

lower and upper bounds, respectively (Crittenden et al., 2012). Therefore, the arithmetic mean of the complete mix and plug flow $K_{v,app}$ values was used as the initial guess $K_{v,app}$ value in the dispersed flow model equation, which was required for the iterative process to calculate the actual $K_{v,app}$ value. Next, the dispersed flow model concentration equation was used to calculate the effluent virus concentration based on the inputs and initial guess $K_{v,app}$. The dispersed flow model effluent concentration equation was rearranged to be set equal to zero (Equation 3) to make the iterative calculation process simpler. The Solver tool in Microsoft Excel was then used to solve for the actual $K_{v,app}$ value. This was performed by setting the rearranged DFM equation equal to zero, by changing the initial $K_{v,app}$ value.

3.3 Statistical Analysis of Data

This section describes the methodology used to address the third objective of this research, which was to identify correlations and relationships between the virus removal rate coefficients ($K_{v,app}$) and several design, operational, and environmental (DOE) parameters for the individual WSPs. Accordingly, correlation, multiple linear regression (MLR), and analysis of variance (ANOVA) tests were performed because they are common and appropriate statistical methods for this purpose. The statistical methods used in this study for each mathematical model (complete mix, plug flow, and dispersed flow) are summarized in Table 3.3.

Each statistical method will be discussed in the following sub-sections in more detail. The results of the statistical methods outlined in this chapter are also expected to address the fourth and fifth objectives of this research, which are to recommend the mathematical model from reactor theory that best predicts virus removal in WSPs, and to determine if the recommended model can reliably be used for WSP design purposes.

Table 3.3: Description of statistical methods used for data analysis

Statistical Method	Description	Software Used
Descriptive Statistics	• Used to describe the basic features of the derived virus removal rate coefficients ($K_{v,app}$) in the database (e.g., mean, standard deviation, median, standard error, kurtosis, and skewness).	Microsoft Excel
Correlation Analysis	• Pearson's correlation coefficients (*r*) were calculated between design, operational, and environmental (DOE) parameters and $K_{v,app}$ values to determine whether there was a correlation between the variables. • Test statistics (*t*) were calculated to determine whether each Pearson's *r* coefficient was significantly different than zero and to calculate probability values (p-values) using a Student's *t*-distribution. • A p-value that was less than a level of significance value (alpha, α) of 0.10 implied that a Pearson's *r* coefficient was significantly different than zero and a significant correlation exists between the variables.	Microsoft Excel
Multiple Linear Regression and Analysis of Variance (ANOVA)	• Alternative multiple linear regression (MLR) equations were used to characterize the relationship between $K_{v,app}$ values and DOE parameters by fitting linear equations to the observed data set. • ANOVA tables were created for each MLR equation to test the statistical significance of the explanatory variables using the F-ratios, and to decide whether to add or subtract explanatory variables from subsequent MLR equations. • The best MLR equations were used to predict $K_{v,app}$ values (response variables) based on significant explanatory variables (DOE parameters).	R (Version 3.2.2)

3.3.1 Descriptive Statistics

Descriptive statistics (i.e., number of data, mean, standard deviation, median, 25^{th} percentile, 75^{th} percentile, standard error, sample variance, kurtosis, skewness, minimum, and maximum) were calculated for the virus removal rate coefficients ($K_{v,app}$ values) from the

complete mix, plug flow, and dispersed flow models for all three WSP types (anaerobic, facultative, and maturation).

3.3.2 Correlation Analysis

The Pearson's correlation coefficient (*r*) is used to detect the degree of association that exists between two variables (Helsel and Hirsch, 2002). In this case, Pearson's correlation coefficients were calculated between the design, operational, and environmental (DOE) parameters (pond depth, HRT, air temperature, solar radiation, and surface and volumetric viral loading rates) and the virus removal rate coefficients ($K_{v,app}$) for each mathematical model (complete mix, plug flow, and dispersed flow). Pearson's correlation coefficients are numbers between 1 and -1, and the closer the absolute value of the coefficient is to 1, the greater the correlation between the two variables.

Pearson's *r* coefficients follow a Student's *t* distribution with $n - 2$ degrees of freedom. A test statistic is used for hypothesis testing to test whether Pearson's *r* coefficients are significantly different than zero (Helsel and Hirsch, 2002). Test statistics are also used for calculating probability values (p-values) using a Student's *t*-distribution. Test statistics for the Pearson's *r* coefficients were calculated using the following formula:

$$t = r\sqrt{\frac{n-2}{1-r^2}} \tag{8}$$

where *n* is the number of data points, $n - 2$ is the degrees of freedom, and *r* is the Pearson's correlation coefficient.

The p-value is used to determine the significance of the correlation between two variables. In order for a correlation to be significant, the Pearson's correlation coefficient must be significantly different than zero. If the p-value is less than a predetermined alpha value (α), or

level of significance, then the null hypothesis is rejected, which implies that Pearson's *r* coefficient is significantly different than zero and a significant correlation exists between the variables (McDonald, 2014). For this analysis an alpha value of 0.10 (10%) was used because samples from full-scale natural treatment systems (like WSPs) are likely have results that vary more than controlled laboratory scale experiments. An alpha value of 0.10 is commonly used in environmental studies and helps to avoid the potential misinterpretation of moderately extreme p-values that may generate false negatives and support the null hypothesis (FDEP, 2011). Right-tailed and left-tailed tests were performed based on the predetermined hypothesis of whether the correlation between the variables was positive or negative. If a positive correlation was expected a right-tailed test was used, if a negative correlation was expected a left-tailed test was used, and if the correlation between variables could hypothetically be positive or negative a two-tailed test was used. In Microsoft Excel the T.DIST.2T, T.DIST.RT, and T.DIST functions were used for two-tailed, right-tailed, and left-tailed tests, respectively. The inputs for the functions were the test statistics and the degrees of freedom and the outputs were the p-values.

3.3.3 Multiple Linear Regression Analysis

A multiple linear regression (MLR) analysis, also known as a multiple least square regression analysis, is used to characterize the relationship between a response variable and multiple explanatory variables in an experiment or model by fitting a linear equation to an observed data set (Wu and Hamada, 2000). A multiple linear regression analysis results in the following general form of a multiple linear regression equation:

$$Y_o = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 ..... \beta_n X_n + \varepsilon \quad (9)$$

where Y_o represents the modeled response variable, β_0 represents the intercept, β_1, β_2, β_3, and β_n are the least-square estimate constants (regression coefficients) for the explanatory variables, and ε is the remaining unexplained error.

In this analysis the significantly correlated explanatory variables (design, operational, and environmental parameters) and the response variable ($K_{v,app}$ value) for each mathematical model (complete-mix, plug flow, and dispersed flow) were fit and characterized by MLR equations. The explanatory variables (pond type, virus type, solar radiation, air temperature, pond depth, HRT, and surface and volumetric viral loading rates) were initially chosen based on theoretical considerations for virus removal mechanisms in WSPs and on the correlation analysis. Next, analysis of variance (ANOVA) statistic tables (explained in section 3.3.4) were generated for alternative MLR equations to test the statistical significance of included explanatory variables using the F-ratios, and to decide whether to keep or remove certain explanatory variables from subsequent MLR equations. The most appropriate MLR equations were then used to predict $K_{v,app}$ values based on the significant explanatory variables.

A measure of the strength of the regression relationship is the coefficient of determination (R^2 value), which represents the portion of the variance in the response variable that can be explained by the linear relationship with the explanatory variables. The higher the R^2 value, or the closer it is to 1, the stronger the linear relationship between the response variable and the explanatory variables. In general, a good model must have a simple structure and explain as much of the variance of the response variable as possible with a small number of explanatory variables (Helsel and Hirsch, 2002). For this study, alternative regression equations with the highest coefficients of determination (R^2 values) and the fewest explanatory variables were considered to be the best and most appropriate equations for design purposes.

There are several assumptions and factors that must be assessed in order to determine the validity of a regression equation (Helsel and Hirsch, 2002). The regression diagnostics used to validate the variables and assumptions in this MLR analysis and the variables used in this analysis were:

1. Homoscedasticity (constant variance of the errors)
2. Nonstochastic explanatory variables (explanatory variables are accurately measured)
3. Normality of the residual error distribution
4. Linearity (randomness of residuals with respect to the explanatory variables)
5. Multi-collinearity (no significant correlation between explanatory variables)
6. Independence of observations
7. Outliers

1. Homoscedasticity, or the constant variance of the errors of the residuals, is an important assumption for a linear regression analysis. This was tested by creating a scatterplot in "R" of the standardized residuals of the response variable ($K_{v,app}$) versus the fitted (predicted) values of the response variable ($K_{v,app}$). The scatterplot was analyzed to verify that the residuals varied randomly around zero and consistently throughout the plot with no systematic patterns. When plotted in "R", the residuals should vary randomly above and below the horizontal line that is generated at zero (Figure B3). This demonstrates that there are no major violations of homoscedasticity. If there was a violation of homoscedasticity, which is called heteroscedasticity, there would be a sloping red line (generated in "R") or residuals that get larger as the predicted values increase.
2. Multiple linear regression assumes that explanatory variables are nonstochastic (nonrandom), accurately measured, and that errors are uncorrelated with the individual explanatory

variables (Draper and Smith, 1998). However, there are some limitations with some of the explanatory variables included in the database, such as the way virus concentrations, HRTs, and flowrates were measured and reported in literature. For example, several different methods were used for measuring virus concentrations and a small portion of these data were censored or defined by a probability distribution. Additionally, it was often unspecified whether the wastewater flowrates were actually measured, estimated, or whether the design flowrates were reported, and the mean theoretical HRT was often reported instead of the actual measured mean HRT. These limitations were unavoidable due to the realities of limited parameter monitoring at many full scale WSPs. However, any errors caused from these limitations are likely reduced by the large amount and range of the data and because the only goal of this regression analysis is to estimate the response variable as a function of the explanatory variables and not vice versa (i.e., bi-directional regression) (Draper and Smith, 1998).

3. Normality of the residuals errors is an important assumption of linear regression. This was examined by plotting the residuals against predicted values using a Q-Q (quantile-quantile) plot (Chambers et al., 1983). Departures from a straight line suggest a non-normal distribution. For example, refer to the scatterplot in Figure B4. If the data generally fall along the straight line, this indicates that the normality assumption is not violated. Initial regression models constructed with the data for this study resulted in non-linear Q-Q plots. Therefore, the $K_{v,app}$ values (response variables) were logarithmically transformed (natural log) and used in all subsequent regressions, which produced normally-distributed residual plots. It is common practice to logarithmically transform variables in a regression model to adjust the

residuals so that they are more normally distributed to improve the overall MLR model (Helsel and Hirsch, 2002).

4. MLR assumes that there is a linear relationship between each explanatory variable and the response variable. To test this, each individual explanatory variable (design, operational, and environmental parameters) was plotted on the x-axis against the residuals of the response variable ($K_{v,app}$ values) on the y-axis. The graphs were analyzed to ensure that the residuals were randomly distributed and that the data followed a linear trend.
5. The non-existence of multi-collinearity is another assumption of MLR. Multi-collinearity is when one or more explanatory variables in a MLR equation are significantly correlated (i.e., the variables are not independent), which may artificially inflate the goodness of fit of a regression equation (Helsel and Hirsch, 2002). One diagnostic for measuring multi-collinearity is to calculate Pearson's correlation coefficients (r) between all explanatory variables. If there is a moderate to strong positive or negative correlation ($r > 0.6$ or $r < -0.6$) then the multi-collinearity among those variables is considered significant. Another diagnostic for measuring multi-collinearity is the variance inflation factor (VIF). Multi-collinearity is commonly considered to be significant when the VIF is in a range between 2.5 and 10 or greater (Helsel and Hirsch, 2002). Therefore, variables with VIFs less than 2.5 can likely be considered to not violate multi-collinearity. The VIF is calculated with the following equation

$$VIF_j = 1/(1- R_j^2) \quad (10)$$

where R_j^2 is the multiple coefficient of determination between the explanatory variables (Marquardt, 1970).

6. The data reported from the authors included in the database was reviewed to ensure there were no obvious biases in the authors' selection of WSPs to study. The data included in this database came from publications by 34 different authors from 44 WSP systems, which represented 112 individual WSPs from 19 different countries. Based on the wide geographic distribution and large amount of data, it was assumed that were no intentional biases and that the independence of observations assumption was not violated.
7. All variables were screened for outliers using Tukey's method (Tukey, 1977). This method was selected because it uses quartiles, which are resistant to extreme values. Additionally, Tukey's method is applicable to data that has been log-transformed and found to follow a log-normal distribution, such as the $K_{v,app}$ values from this study. Tukey's method, which is commonly used to construct boxplots, uses the median (50^{th} percentile), first quartile (25^{th} percentile), third quartile (75^{th} percentile), lower bound, and upper bound of a data set to determine outliers. Any values greater than the upper bound or less than the lower bound are considered strong outliers. The upper bound and lower bound are calculated by the following equation:

$$\text{Upper Bound} = Q_3 + (3 \cdot \text{IQR}) \tag{11}$$

$$\text{Lower Bound} = Q_1 - (3 \cdot \text{IQR}) \tag{12}$$

where Q_1 is the first quartile (25^{th} percentile), Q_3 is the third quartile (75^{th} percentile), and IQR is the inter quartile range ($Q_3 - Q_1$).

3.3.4 Analysis of Variance

Analysis of variance (ANOVA) is commonly used to analyze differences between several groups of data, to determine whether particular categories of variables have different effects or influences, and to test the statistical significance of explanatory variables in MLR equations (Wu

and Hamada, 2000). ANOVA statistics tables use the number of explanatory (independent) variables used in the model, the number of data points, sum of squares about the mean (SSY), sum of squares due to error (SSE), and the degrees of freedom (df) to calculate the coefficient of determination (R^2), the mean square error of regression variables (MSR), and the mean square error of the residuals (MSE) (Helsel and Hirsch, 2002). The F-ratio, which is computed from the mean square terms in the MLR regression equation, is commonly used to test the significance of the explanatory variables in the regression equation. ANOVA tables were created for each MLR equation to test the statistical significance of the explanatory variables using the F-ratios, and to decide whether to include explanatory variables from the MLR equations. Additionally, ANOVA tables were used to compare the alternative MLR equations that were developed for each mathematical model with varying amounts of explanatory values, using F-ratios. The F-ratio is given by (Helsel and Hirsch, 2002)

$$F = MSR / MSE \tag{13}$$

3.3.5 Linear Model Fitting in R

"R" is an integrated suite of software facilities that is used for data manipulation, calculation, statistics, and graphical display (Venables et al., 2014). "R" version 3.2.2 was used to run multiple linear regression analyses, ANOVA, and several regression diagnostics tests. To run these analyses in "R", the linear model function "lm" was used. For each mathematical model, the $K_{v,app}$ values and the explanatory variables were exported from the database in Microsoft Excel into "R". The two main resources that were used for running statistical analyses in "R" were Venables et al. (2014) and Fox and Weisberg (2010). An example of the script that was used to run the analyses in R is displayed in Figure 3.7.

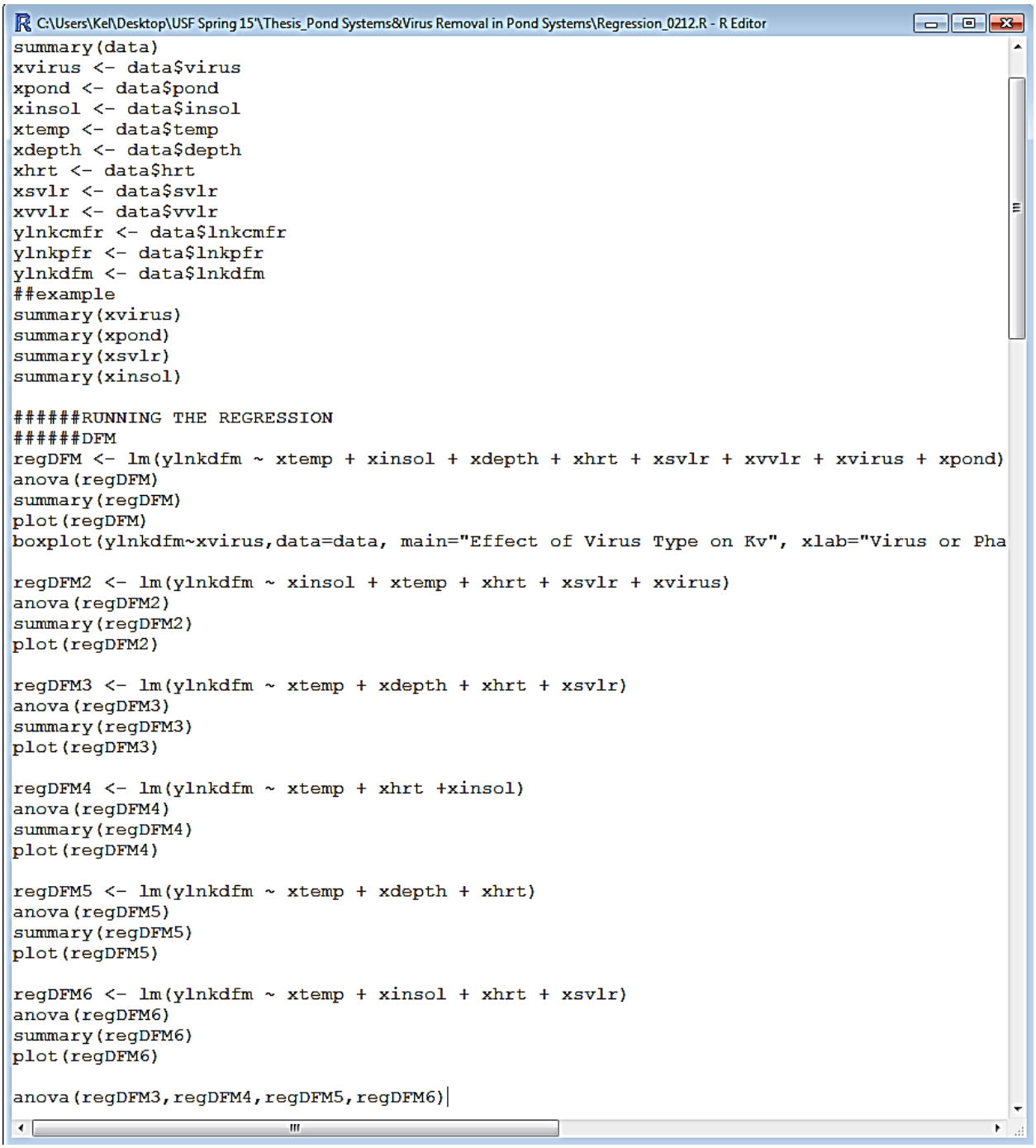

Figure 3.7: An example of script used to run statistical analyses in R (version 3.2.2)

CHAPTER 4: RESULTS AND DISCUSSION

4.1 Descriptive Statistics of Virus Removal Rate Coefficients

The descriptive statistics for the apparent virus removal rate coefficients ($K_{v,app}$) for all three mathematical models are provided in Table 4.1. There are fewer data for the dispersed flow model because there were eight WSPs where the lengths and widths were unknown; therefore, they were removed from the dispersed flow model because pond length and width are required for estimating the dispersion number (d = W/L) (von Sperling, 2003). The apparent $K_{v,app}$ values ranged from 0.07 $days^{-1}$ (PFM) to 17.3 $days^{-1}$ (CMM) for the anaerobic ponds, from 0.004 $days^{-1}$ (PFM) to 74.6 $days^{-1}$ (CMM) for the facultative ponds, and from 0.003 $days^{-1}$ (PFM) to 517 $days^{-1}$ (CMM) for the maturation ponds. The median $K_{v,app}$ values were the greatest for the anaerobic ponds and lowest for the maturation ponds. The distributions of the $K_{v,app}$ values for all pond types and all mathematical models were positively skewed (mean values were all greater than median values), and for the facultative and maturation ponds, the distributions also had very high kurtosis (> 12), meaning that they were heavy-tailed on the positive side of the median. The positively skewed data and the high kurtosis may indicate that the $K_{v,app}$ values do not follow a normal distribution. The standard errors of the $K_{v,app}$ values were much greater when the complete mix model was used compared to the plug flow model. When the dispersed flow model was used, the standard errors in the $K_{v,app}$ values were only slightly greater than they were when the plug flow model was used.

Table 4.1: Descriptive statistics of apparent virus removal rate coefficients ($K_{v,app}$) from three mathematical models for three different pond types. CMM = complete mix model, PFM = plug flow model, DFM = dispersed flow model, $K_{v,app}$ units = $days^{-1}$

Statistic	Anaerobic Ponds			Facultative Ponds			Maturation Ponds		
	CMM $K_{v,app}$	PFM $K_{v,app}$	DFM $K_{v,app}$	CMM $K_{v,app}$	PFM $K_{v,app}$	DFM $K_{v,app}$	CMM $K_{v,app}$	PFM $K_{v,app}$	DFM $K_{v,app}$
Number of data (*n*)	24	24	24	147	147	142	78	78	75
Mean	3.791	0.973	1.713	3.519	0.249	0.523	19.762	0.404	0.828
Standard Deviation	5.355	0.994	2.129	8.953	0.279	0.618	80.652	0.527	1.340
Q1 (25th percentile)	0.590	0.339	0.493	0.300	0.101	0.181	0.238	0.116	0.167
Median (50th percentile)	1.841	0.743	1.301	0.852	0.183	0.337	0.511	0.206	0.302
Q3 (75th percentile)	3.031	1.133	2.002	2.255	0.299	0.619	1.616	0.441	0.755
Minimum	0.086	0.069	0.080	0.004	0.004	0.004	0.003	0.003	0.003
Maximum	17.299	4.223	9.438	74.635	1.970	4.411	517.548	2.993	6.007
Standard Error	1.093	0.203	0.435	0.738	0.023	0.052	9.132	0.060	0.155
Sample Variance	28.673	0.988	4.534	80.153	0.078	0.382	6504.760	0.277	1.795
Kurtosis	2.258	5.249	7.918	33.278	15.578	14.456	24.199	7.766	6.241
Skewness	1.896	2.204	2.686	5.257	3.563	3.289	4.838	2.590	2.628

The $K_{v,app}$ values estimated in this study were much lower than pseudo-first-order sunlight-mediated inactivation coefficients reported in a laboratory study by Mattle et al. (2015) for MS2 coliphage, ΦX174 phage, and human adenovirus in WSP water, which were generally between 0.2 and 0.6 min^{-1}. Additionally, the $K_{v,app}$ values from the present study associated with F-specific coliphages (*n* = 22) ranged from 0.18 $days^{-1}$ for PFM to 1.81 $days^{-1}$ for CMM, with a median value of 0.56 $days^{-1}$ for the DFM (min. $K_{v,DFM}$ = 0.08; max. $K_{v,DFM}$ = 2.22). These are lower than the F-specific coliphage K values reported for a baffled open wetland cell by Silverman et al. (2015), which ranged from 1.4 $days^{-1}$ (winter) to 5.0 $days^{-1}$ (summer). However, while Silverman et al. (2015) used the DFM, they assumed a different dispersion number based on results from a dye tracer study.

The virus removal efficiencies (expressed as $\log_{10}$ units removed) for the anaerobic (24 data), facultative (147 data), and maturation (78 data) ponds in the database are displayed in a box-plot in Figure 4.1. The reported virus concentrations and removal efficiencies for each data point are provided in Table A2 in Appendix A. The reported data in the database suggests that facultative ponds provided the best virus removal efficiencies, followed by maturation ponds and anaerobic ponds. The median values for the removal efficiencies were 1.00 $\log_{10}$ units for facultative ponds, 0.60 $\log_{10}$ units for maturation ponds, and 0.58 $\log_{10}$ units for anaerobic ponds. The overall virus removal efficiency in $\log_{10}$ units for each pond type may be useful for estimating an approximate final effluent virus concentration in a WSP system since $\log_{10}$ unit removals can be added up for ponds in series to yield total $\log_{10}$ removal estimates for an entire system. However, the main purpose of this research is to assess virus removal in individual WSPs.

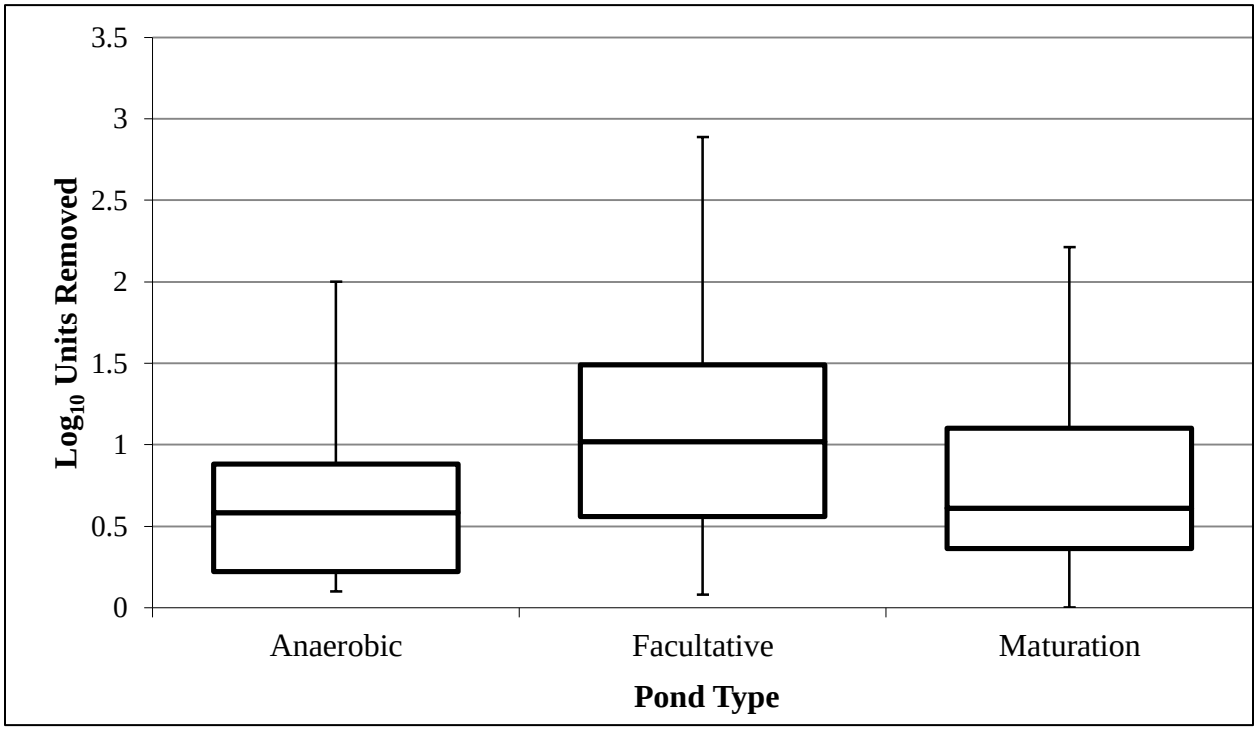

Figure 4.1: Box plots of observed virus removal efficiencies ($\log_{10}$ units) for anaerobic, facultative, and maturation ponds in the WSP database

4.2 Correlation Analysis

The Pearson's correlation coefficients for each WSP type are presented in Table 4.2. At the significance level (α) of 0.05, there was a significant positive correlation between $K_{v,app}$ and solar radiation and between $K_{v,app}$ and air temperature for facultative and maturation ponds for each mathematical model, with the exception of the CMM for facultative ponds. This means that higher temperatures and higher solar radiation values corresponded with higher apparent virus removal rates, which is consistent with a previous study on the rates of exogenous sunlight-mediated inactivation (Romero et al., 2011). There was a significant negative correlation between $K_{v,app}$ values and pond depth in facultative and maturation ponds for the PFM and DFM cases. This was expected, because overall virus removal rates should theoretically decrease as pond depth increases due to the fact that sunlight-mediated virus inactivation primarily occurs at the pond surface since sunlight is rapidly absorbed in WSPs (Davies-Colley et al., 2005; Kohn et al., 2016). The positive correlation between virus loading rates and $K_{v,app}$ values in maturation ponds for each mathematical model was also shown to be significant. This was expected because although virus inactivation in ponds has been assumed to follow pseudo first-order kinetics, the rate of some mechanisms (e.g., exogenous sunlight-mediated inactivation) is second-order (Kohn and Nelson, 2007; Mattle et al., 2015). Surprisingly, there was a significant negative correlation between theoretical HRTs and $K_{v,app}$ values in facultative and maturation ponds for the PFM and DFM cases. In reality, there should be no correlation between HRT and K_v in a flow reactor, as K_v should be the same throughout the entire reactor (e.g., WSP). One possible explanation for this negative correlation between HRT and $K_{v,app}$ may be the inadequacy of the mathematical models (i.e., PFM and DFM) to describe the actual flow hydraulics of the WSPs in this database. Another explanation could be that the overall kinetics of K_v are actually second-order instead of

pseudo first-order. Future research may want compare inactivation rate coefficients for exogenous sunlight-mediated mechanisms (second-order) with overall virus removal rate coefficients (pseudo first-order) to better understand the kinetics of virus removal in WSPs.

Table 4.2: Pearson's correlation coefficients between virus removal rate coefficients and selected design, operational, and environmental factors

	$K_{v,CMM}$ ($days^{-1}$)	$K_{v,PFM}$ ($days^{-1}$)	$K_{v,DFM}$ ($days^{-1}$)
Solar Radiation ($kWh/m^2 \cdot d$)	-0.20 (A) -0.02 (F) -0.05 (M)	0.05 (A) **0.23** (F)** 0.08 (M)	0.01 (A) **0.21** (F)** 0.06 (M)
Air Temperature (°C)	-0.23 (A) **0.10" (F)** 0.04 (M)	-0.10 (A) **0.30*** (F)** 0.06(M)	-0.15 (A) **0.32*** (F)** 0.06 (M)
Pond Depth (m)	-0.22' (A) -0.05 (F) -0.13' (M)	-0.22' (A) **-0.16* (F)** -0.12' (M)	-0.23' (A) **-0.18* (F)** **-0.18" (M)**
Theoretical HRT (days)	0.04 (A) -0.09 (F) -0.14 (M)	**-0.47* (A)** **-0.38*** (F)** **-0.42*** (M)**	-0.31' (A) **-0.30*** (F)** **-0.32** (M)**
Surface VLR (per ha·day)	-0.06 (A) -0.01 (F) **0.73*** (M)**	-0.01 (A) 0.02 (F) **0.27** (M)**	0.01 (A) 0.01 (F) **0.39*** (M)**
Volumetric VLR (per $m^3 \cdot$day)	-0.04 (A) -0.02 (F) **0.68*** (M)**	0.02 (A) -0.02 (F) **0.24* (M)**	0.05 (A) -0.03 (F) **0.35*** (M)**

Bold values indicate significant correlations, where: ' p –value <0.15," p-value < 0.10, * p-value < 0.05, ** p-value < 0.01, *** p-value < 0.001, VLR = viral loading rate, A = anaerobic pond, F = facultative pond, M = maturation pond

4.3 Multiple Linear Regression Analysis

The multiple linear regression (MLR) analysis was performed for the dispersed flow model first because the correlation analysis results (Table 4.2) indicated that the dispersed flow model had the most significant correlations between $K_{v,app}$ values (response variable) and design, environmental, and operational parameters (explanatory variables). For the first MLR analysis, all explanatory variables were used (solar radiation, air temperature, pond depth, HRT, and

surface and volumetric viral loading rates), including categorical explanatory variables (virus and pond type). Regression diagnostics were used to validate two important assumptions of MLR, which are the constant variance of the errors of the residuals (homoscedasticity) and normality of the residual error distribution. Figure B1 displays a scatterplot of the residuals against the fitted values that was used to analyze the homoscedasticity of the data and Figure B2 displays a quantile-quantile (Q-Q) plot that was used to analyze the normality of the residual error distribution (Appendix B).

The inconsistent variance in the errors of the residuals and the downward trend of the data in Figure B1, along with the trend and departure of the residuals away from the straight line in Figure B2, indicate that the residuals of the $K_{v,app}$ values deviate from the normal distribution. As a result, all of the $K_{v,app}$ values for each mathematical model (CMM, PFM, and DFM) were logarithmically transformed (natural log). Descriptive statistics were calculated for the $\log_e$-transformed $K_{v,app}$ values for each model and the results are provided in Table 4.3. The kurtosis and skewness of the $\log_e$-transformed $K_{v,app}$ values are significantly lower than the original $K_{v,app}$ values, indicating that the data more closely follows a log-normal distribution instead of a normal distribution.

A regression equation with the $\log_e$-transformed $K_{v,app}$ values for the dispersed flow model ($K_{v,DFM}$) and all of the explanatory variables was developed and the same regression diagnostic plots were analyzed again to assess the normal distribution of residual errors assumption of MLR (Appendix B). The residuals versus fitted values plot (Figure B3) shows that the residuals vary randomly around zero, which indicates there are no systematic patterns and no major violations of homoscedasticity. The Q-Q plot (Figure B4) shows that the residuals of the $\log_e$-transformed $K_{v,app}$ values generally fall along a linear line, which indicates that the normality

of residual errors assumption is not violated and that the residuals generally follow a log-normal distribution. Therefore, the $\log_e$-transformed $K_{v,app}$ values were used in all subsequent regressions.

Table 4.3: Descriptive statistics of $\log_e$-transformed apparent virus removal rate coefficients (ln $K_{v,app}$) from three mathematical models for three different pond types. CMM = complete mix model, PFM = plug flow model, DFM = dispersed flow model, $K_{v,app}$ units = $days^{-1}$

Statistic	Anaerobic Ponds			Facultative Ponds			Maturation Ponds		
	CMM ln $K_{v,app}$	PFM ln $K_{v,app}$	DFM ln $K_{v,app}$	CMM ln $K_{v,app}$	PFM ln $K_{v,app}$	DFM ln $K_{v,app}$	CMM ln $K_{v,app}$	PFM ln $K_{v,app}$	DFM ln $K_{v,app}$
Number of data (*n*)	24	24	24	147	147	142	78	78	75
Mean	0.451	-0.468	-0.060	-0.224	-1.809	-1.182	-0.329	-1.564	-1.119
Standard Deviation	1.440	1.012	1.185	1.758	0.970	1.139	2.176	1.253	1.467
Q1 (25th percentile)	-0.553	-1.085	-0.728	-1.205	-2.290	-1.707	-1.435	-2.156	-1.793
Median (50th percentile)	0.607	-0.297	0.261	-0.160	-1.696	-1.089	-0.671	-1.578	-1.199
Q3 (75th percentile)	1.109	0.123	0.692	0.813	-1.206	-0.480	0.477	-0.819	-0.281
Minimum	-2.449	-2.669	-2.522	-5.410	-5.503	-5.456	-5.857	-5.894	-5.866
Maximum	2.851	1.441	2.245	4.313	0.678	1.484	6.249	1.096	1.793
Standard Error	0.294	0.207	0.242	0.145	0.080	0.096	0.246	0.142	0.169
Sample Variance	2.072	1.024	1.404	3.091	0.941	1.298	4.733	1.570	2.151
Kurtosis	-0.373	-0.103	-0.234	0.214	1.959	1.506	2.175	1.703	1.285
Skewness	-0.087	-0.295	-0.256	-0.010	-0.688	-0.758	0.801	-0.661	-0.465

The purpose of this multiple linear regression analysis was to determine which explanatory variables contribute significantly to explaining the variability in the response variable. A regression equation that contains all potential explanatory variables will always yield a maximum R^2 value; however, some explanatory variables may not significantly contribute to explaining the variability in the response variable and can be removed to simplify the model without greatly reducing the R^2 value. Therefore, probability values (p-values) in analysis of variance (ANOVA) tables generated in R (version 3.2.2) were used to determine which

explanatory variables to keep in the regression equations. Table 4.4 displays the ANOVA table for the initial regression equation including $K_{v,DFM}$ values and all explanatory variables.

Table 4.4: ANOVA table for initial regression equation including $K_{v,DFM}$ values and all explanatory variables

Explanatory Variable	Degrees of Freedom	Sum of Squares	Mean Square Error	F ratio	P-value	Significance
Virus type	7	45.646	6.521	5.7302	4.09E-06	***
Pond type	2	14.547	7.273	6.3916	0.001996	**
Solar radiation	1	8.345	8.345	7.3328	0.007291	**
Temperature	1	52.709	52.709	46.3182	9.01E-11	***
Depth	1	3.242	3.242	2.8488	0.09283	”
HRT	1	17.105	17.105	15.0309	1.39E-04	***
SVLR	1	2.249	2.249	1.9759	0.161201	
VVLR	1	0.972	0.972	0.8543	0.356336	
Residuals	225	256.044	1.138			

Significance: ” p-value < 0.10, * p-value < 0.05, ** p-value < 0.01, *** p-value < 0.001

All of the explanatory variables were determined to have significant linear correlations with the $\log_e$-transformed $K_{v,DFM}$ values except for the surface (SVLR) and volumetric viral loading rates (VVLR). However, the p-values from the correlation analysis (Table 4.2) suggest that there are significant positive correlations between SVLRs and VVLRs and $K_{v,app}$ values. This discrepancy is because the multiple linear regression only denotes significant linear correlations, while significant Pearson’s correlation coefficients may result from nonlinear (i.e., exponential, logarithmic) trends as well. The difference between the significant p-values for SVLR and VVLR suggests that these parameters are not linearly distributed and may follow a different trend. To test this assumption the $K_{v,app}$ values were plotted against the SVLRs on a logarithmic scale as shown in Figure 4.2. The weak positive trend in Figure 4.2 justifies the $\log_e$ transformation of the SVLR variable. A similar plot was constructed for VVLRs which displayed

a weak positive trend as well. Therefore, the $\log_e$-transformed values for SVLRs and VVLRs were used for all subsequent MLR equations.

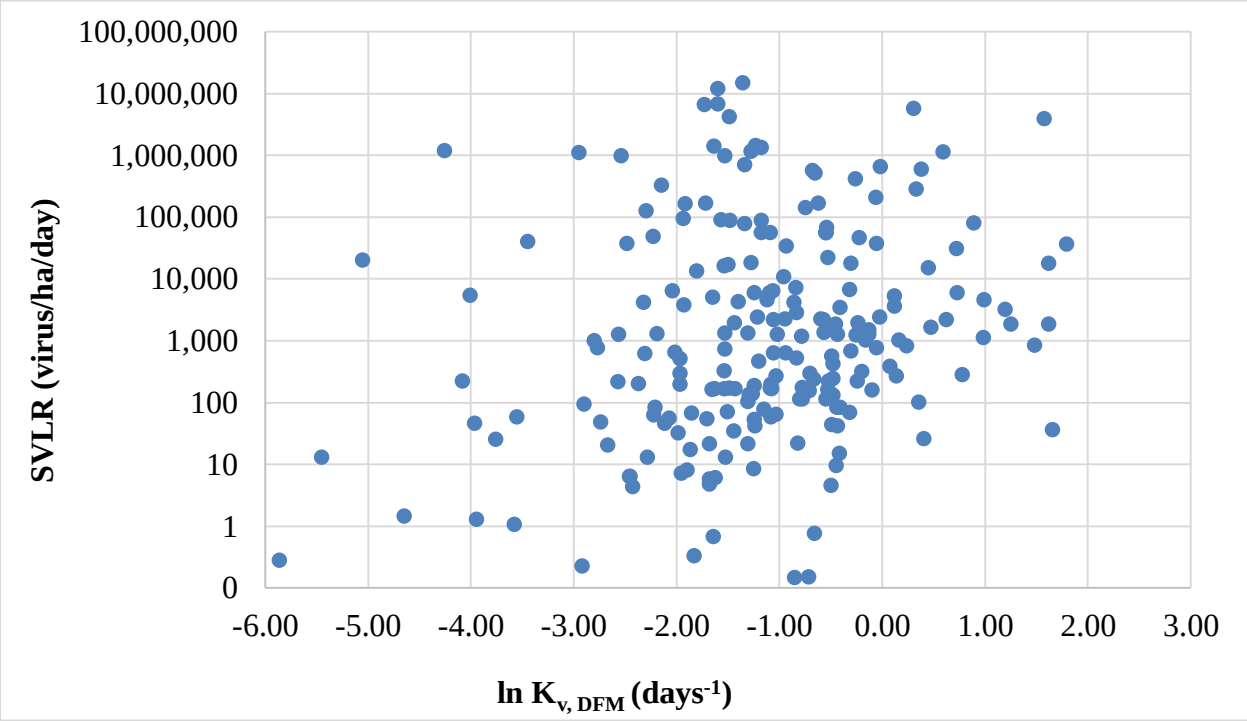

Figure 4.2: Surface viral loading rates (SVLRs) plotted on a log scale versus $\log_e$-transformed virus removal rate coefficients

An ANOVA table for the regression equation including $K_{v,DFM}$ with all the explanatory variables and $\log_e$-transformed virus loading rates is provided in Table 4.5. After the SVLRs were $\log_e$-transformed the ANOVA results yielded a p-value of 0.04, indicating that SLVR may significantly impact $K_{v,app}$ values. This aligns with the assumption that virus removal in WSPs is driven by sunlight-mediated mechanisms (Kohn and Nelson, 2007; Mattle et al., 2015), which depend on the surface area of the pond (not the volume).

Table 4.5 suggests that pond type has a statistically significant effect on $K_{v,app}$ values. A box plot of the $K_{v,app}$ values for each type of pond (anaerobic, facultative, and maturation) and mathematical model (CMM, PFM, DFM) is displayed in Figure 4.3. The results of the box plot illustrate that the $K_{v,app}$ values for anaerobic ponds for each model are significantly higher than

the $K_{v,app}$ values for facultative and maturation ponds. Therefore, anaerobic pond data were analysed separately and not included with facultative and maturation ponds in any of the subsequent multiple regression equations. This was expected as anaerobic ponds differ significantly from facultative and maturation ponds with regards to dissolved oxygen content, depth, and hydraulic retention time. In theory, virus removal in anaerobic ponds may also be governed by different virus removal mechanisms than facultative and maturation ponds. No other explanatory variables besides virus type explained a significant amount of the variance in the regression equations that were generated for anaerobic ponds. This is probably due to the small sample set of anaerobic ponds (n = 24) and because there were five different virus/phage types measured. Therefore, no regression equation is recommended for predicting virus removal in anaerobic ponds. However, the median $\log_{10}$ unit virus removal of the anaerobic ponds (Figure 4.1) from this database may still have implications for wastewater reuse (Section 4.4).

Table 4.5: ANOVA table for regression equation including ln $K_{v,DFM}$ with $\log_e$-transformed virus loading rates

Explanatory Variable	Degrees of Freedom	Sum of Squares	Mean Square Error	F ratio	P-value	Significance
Virus type	7	45.646	6.521	5.7911	3.49E-06	***
Pond type	2	14.547	7.273	6.4596	1.87E-03	**
Solar radiation	1	8.345	8.345	7.4108	0.006991	**
Temperature	1	52.709	52.709	46.8107	7.31E-11	***
Depth	1	3.242	3.242	2.879	0.091122	”
HRT	1	17.105	17.105	15.1907	1.28E-04	***
ln SVLR	1	4.735	4.735	4.2056	0.04145	*
ln VVLR	1	1.179	1.179	1.0469	0.307329	
Residuals	225	253.351	1.126			

Significance: ” p-value < 0.10, * p-value < 0.05, ** p-value < 0.01, *** p-value < 0.001

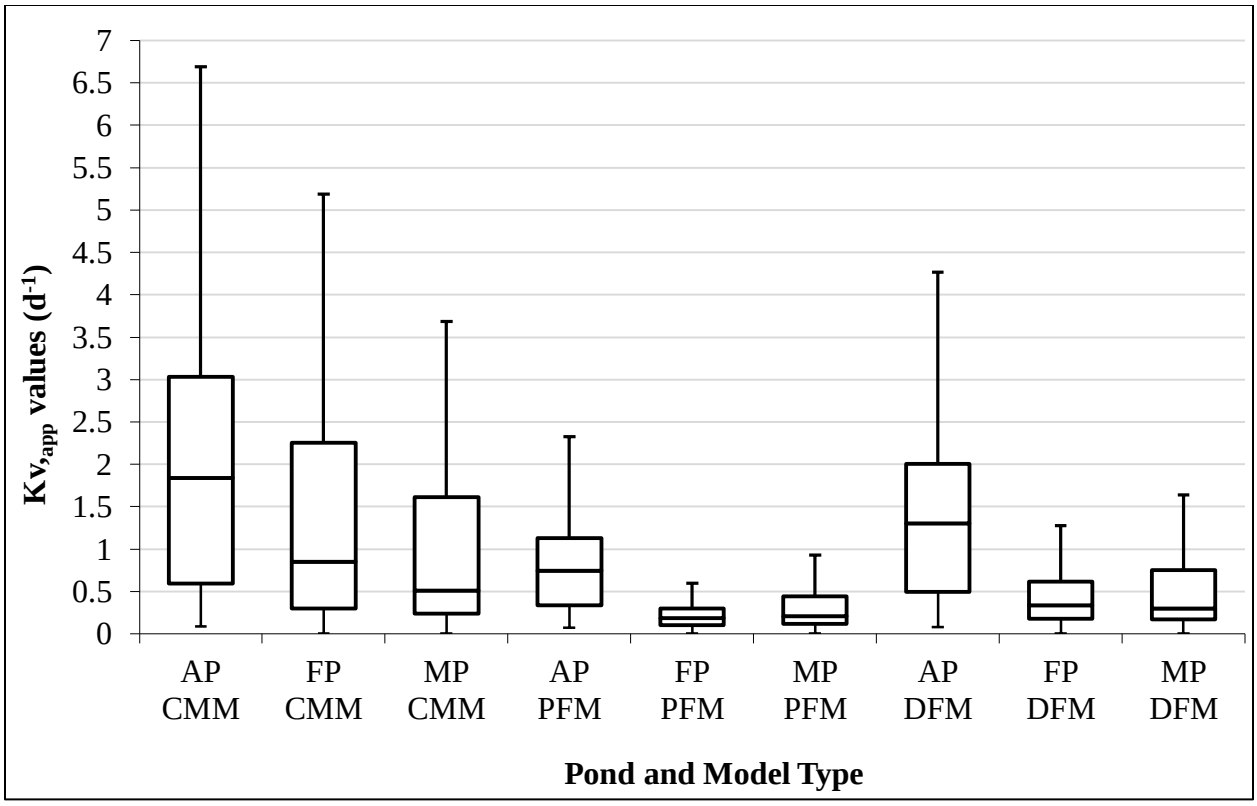

Figure 4.3: Virus removal rate coefficients for each pond type (AP = anaerobic pond, FP = facultative pond, MP = maturation pond) for each mathematical model

For the following regression analyses, facultative and maturation ponds were grouped together and anaerobic ponds were excluded. There are a total of eight different virus types (four enteric virus groups and four phage groups) that comprise the 'virus type' explanatory variable. Based on p-values from an ANOVA table, an improved MLR equation for $K_{v,DFM}$ was selected that included temperature, depth, HRT, SVLR, and virus type as explanatory variables. Table 4.6 displays a regression summary table that includes a representative regression equation, regression coefficients, the coefficient of determination (R^2), and the p-values that indicate the significance of each explanatory variable. The results in the regression summary table indicate that each virus type has a statistically significant influence on the $K_{v,app}$ values and likely accounts for a significant portion of the variance described by the R^2 value. This provides evidence to support the second hypothesis in this thesis, which is that different types of viruses

and phages have significantly different $K_{v,app}$ values in WSPs. The distribution of $K_{v,app}$ values according to virus and phage type are displayed in Figure 4.4.

Table 4.6: Regression summary table for ln $K_{v,DFM}$ that includes all virus/phage groups as explanatory variables

Explanatory Variables	Coefficients (β)	Std. Error	P-value	Significance
Temperature (T)	(β_1) 0.048301	0.012914	0.000239	***
Depth (D)	(β_2) -0.24194	0.145213	9.72E-02	”
HRT (t)	(β_3) -0.02439	0.005865	4.71E-05	***
ln SVLR (S)	(β_4) 0.043639	0.023767	0.067786	”
B. fragilis phage (b)	(β_5) 3.034587	1.315396	0.022055	*
Coliphage (c)	(β_6) 1.800613	1.119973	0.109434	
Culturable enteric virus (e)	(β_7) 2.573549	1.090542	1.92E-02	*
F-specific coliphage (f)	(β_8) 2.931576	1.10522	8.62E-03	**
Norovirus (n)	(β_9) 1.943732	1.157257	0.094558	”
Rotavirus (r)	(β_{10}) 2.483909	1.094176	0.02424	*
Somatic coliphage (s)	(β_{11}) 2.757009	1.096558	0.012698	*
Intercept (adenovirus)	(β_0) -4.23172	1.113476	0.00019	***

$\ln K_{v,DFM} = \beta_1 T - \beta_2 D - \beta_3 t + \beta_4 S + \beta_5 b \ldots\ldots\ldots \beta_{11} s - \beta_0$

$R^2 = 0.3209$

Significance: ” < 0.10, * < 0.05, ** < 0.01, *** < 0.001

For the the regression analysis in thesis, all virus and phage types were consolidated into two groups (i.e., all enteric virus groups = virus; all phage groups = phage). This was done because it was practical and expedient, and there were not enough data to treat each virus and phage type separately. Additionally, a regression equation with eight virus types as explanatory variables was considered to be too cumbersome to be used as a simple model to predict virus removal rate coefficients. In accordance with Figure 4.4, however, future research may want to develop separate models for each virus and phage type because they appear to have significantly different $K_{v,app}$ values in WSPs. Some possible explanations for the variations in $K_{v,app}$ values among different virus groups are: differences in nucleic acid type (DNA or RNA), particle size,

capsid structure, presence of an envelope, isoelectric points, particle charge, and the quantification method used to measure viruses (culturable versus molecular methods). In addition, more research should be done to assess the removal of adenovirus in particular, because there are very few data available, it may have a much lower $K_{v,app}$ value than other enteric viruses, and sunlight-mediated inactivation rates for adenovirus have been difficult to predict (Mattle et al., 2015). More extensive analyses are needed to elucidate the distinct reasons for the variability among apparent $K_{v,app}$ values for different virus groups recorded in this WSP database.

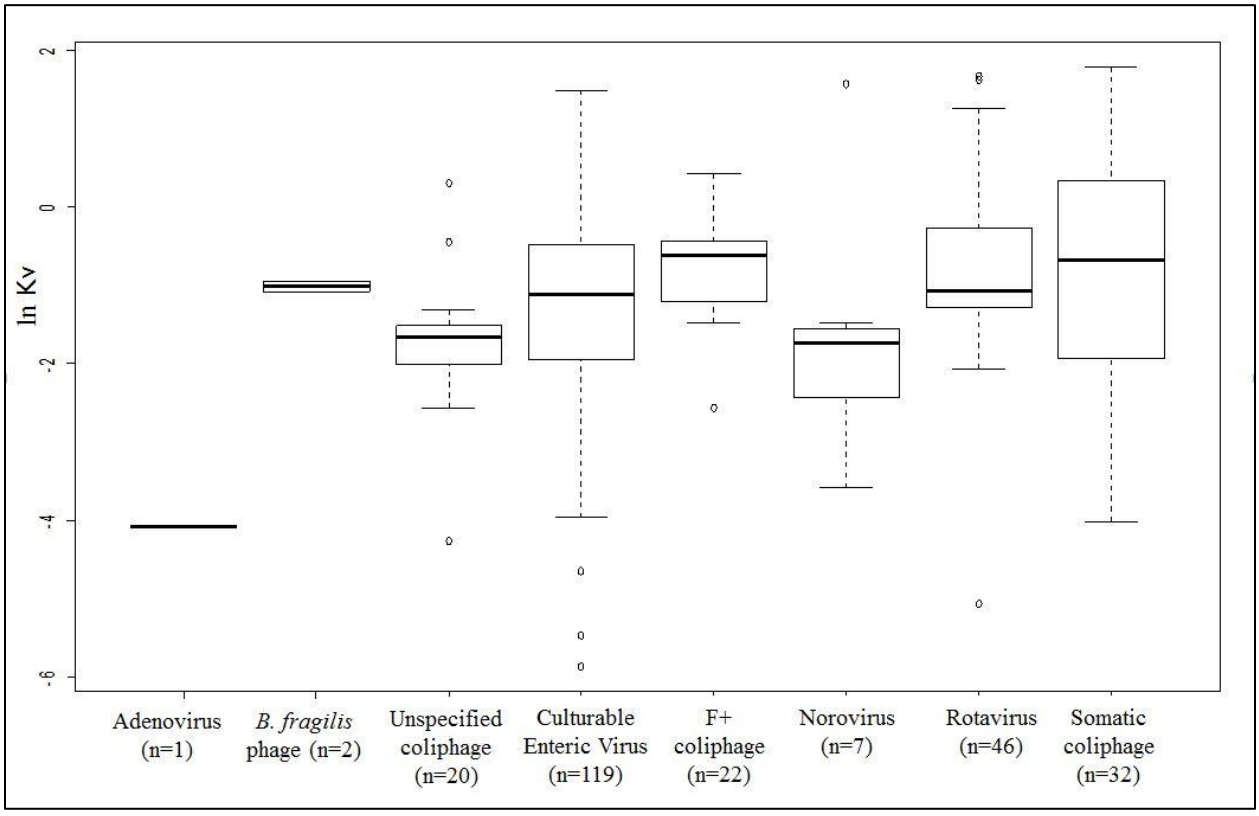

Figure 4.4: Box plots that display the variability of $K_{v,app}$ values for different virus and phage groups based on the dispersed flow model

Next, alternative regression equations for facultative and maturation ponds for each mathematical model were derived. After the virus and phage types were consolidated they no longer had a statistically significant effect on the R^2 value of subsequent alternative regression

equations, so virus type was excluded as an explanatory variable. Additionally, there was not a statistically significant difference between facultative and maturation ponds, so facultative and maturation ponds were grouped together and pond type was excluded as an explanatory variable. The best alternative regression equations for each mathematical model are provided in Table 4.7. All the regression diagnostics outlined in Section 3.3.3 were performed for each regression equation and are provided in Appendix B. The diagnostics tests verified that there were no major violations of MLR assumptions by any of the alternative regression equations.

Table 4.7: Alternative best fit multiple linear regression equations for predicting $K_{v,app}$ values in facultative (fp) and maturation ponds (mp) for each mathematical model

Alternative regression equations for predicting $K_{v,app}$	**R^2**	**Eqn ID**
$lnK_{v,PFM} = 0.033957*T - 0.16088*D - 0.03081*t + 0.052357*lnS - 2.07313$	0.4142	14
$lnK_{v,PFM} = 0.034284*T - 0.08993*D - 0.03634*t – 1.74242$	0.3850	15
$lnK_{v,PFM} = 0.034902*T - 0.03656*t – 1.89011$	**0.3830**	**16**
$lnK_{v,DFM} = 0.04902*T - 0.2166*D - 0.0186*t + 0.06771*lnS - 2.01693$	0.2757	17
$lnK_{v,DFM} = 0.048217*T - 0.128293*D - 0.026465*t – 1.539477$	0.2412	18
$lnK_{v,DFM} = 0.049236*T - 0.02676*t – 1.754246$	0.2380	19
$lnK_{v,CMM} = 0.07433*T - 0.49853*D + 0.13972*lnS - 1.95281$	0.1885	20

T = air temperature (C°), D = pond depth (m), t = hydraulic retention time (days), S = surface viral loading rate (viruses/ha/day)

The coefficient of determination (R^2), in addition to equation simplicity, was used to select the most appropriate regression equation for predicting $K_{v,app}$ values in facultative and maturation ponds. The best fit regression equations for the DFM and PFM included temperature, depth, HRT, and SVLR as explanatory variables. The significantly correlated explanatory variables in the DFM and PFM equations explained 24 to 28 percent and 38 to 41 percent of the variability in the $K_{v,app}$ values, respectively. The significantly correlated explanatory variables for

the complete mix model explained very little of the $K_{v,app}$ variability, so only one alternative complete mix model equation was assessed. Overall, the regression equations for the PFM were considered the best because they were able to explain the most amount of the variance in predicted $K_{v,app}$ values. The best fit regression equation for the PFM was not considered to be the most appropriate equation, however. The most appropriate regression equation for the PFM (Equation 16) only included temperature and HRT as explanatory variables. Depth was removed from the appropriate regression equation because of the small impact it had on R^2 and SVLR was removed because of the added complexity it presents for design and planning purposes. The regression equations for the DFM indicate that it may still be useful for predicting $K_{v,app}$ values and virus removal in WSPs, however, due to its added complexity (i.e., estimating dispersion) and its low R^2 it was not considered to be the best model according to this analysis. It should be noted that, in theory, the DFM is expected to yield as good or better results than the PFM because the PFM is just a special case (i.e., dispersion = 0) of the DFM. One explanation for the apparent superiority of the PFM in this research might be that the estimated dispersion numbers were too large or not representative enough of the actual dispersion for the WSPs in this database. To test this explanation, the same analysis should be performed with different dispersion numbers reported from literature (Table 2.9) to determine what affect different dispersion numbers have on predicting the $K_{v,app}$ values and effluent virus concentrations observed (estimated) in this database. However, this approach was not included in the scope of this thesis. Lastly, the CMM was considered to not be applicable for predicting $K_{v,app}$ values according to this research.

Figure 4.5 presents the comparison between the observed K_{vPFM} values and the predicted $K_{v,PFM}$ values for a total of 98 WSPs (225 data points), comprised of 51 facultative ponds (147

data points) and 47 maturation ponds (78 data points) from the WSP database. The observed $K_{v,app}$ values were calculated with the rearranged first-order equation for a PFM (Equation 7), using the influent and effluent virus concentrations; and the predicted $K_{v,app}$ values were calculated with the most appropriate PFM regression equation (Equation 16), using the ambient temperature and theoretical hydraulic retention time. Figure 4.6 presents the comparison between the observed virus effluent concentrations and the predicted virus effluent concentrations for the same set of WSPs.

Although only 38 percent ($R^2 = 0.383$) of the variance in $K_{v,app}$ values was accounted for by the most appropriate PFM regression equation (Equation 16), approximately 84 percent ($R^2 = 0.839$) of the variance in the effluent virus concentrations were accounted for when using the predicted $K_{v,app}$ values from Equation 16 to predict effluent virus concentrations with the first-order plug flow equation (Equation 2). The R^2 values for predicting $K_{v,app}$ and effluent virus concentrations in this analysis are comparable to predictive K_b values (fecal coliform removal rate coefficients) ($R^2 = 0.580$) and predicted $\log_{10}$ unit effluent coliform concentrations ($R^2 = 0.874$) for maturation and facultative ponds using the dispersed flow model (von Sperling 2005). The DFM equation for predicting K_b values and effluent coliform concentrations reported by von Sperling (2005) is widely accepted for designing facultative and maturation ponds to achieve effluent coliform concentrations that can meet guidelines for wastewater reuse. It is worth mentioning, however, that the dispersed flow model (Equations 19, 3, and 5) for virus removal derived in this study still had a high R^2 value of 0.842 when it was used to predict effluent virus concentrations.

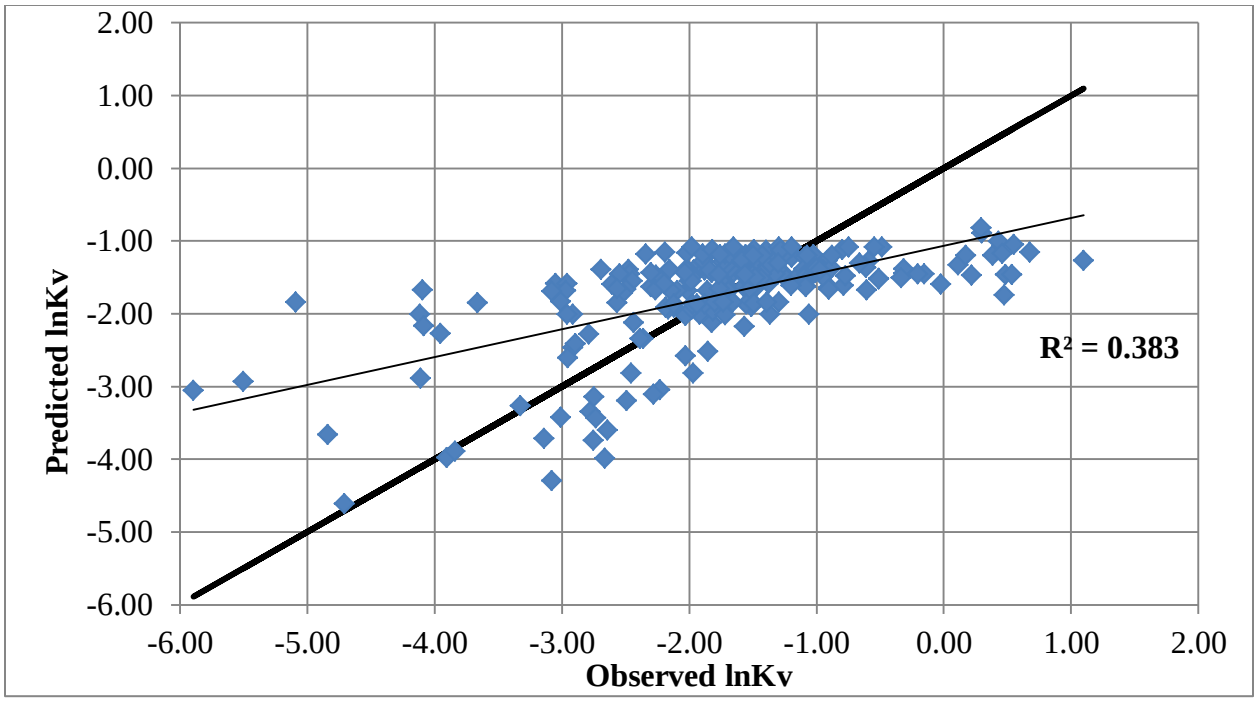

Figure 4.5: Observed $K_{v,app}$ values versus predicted $K_{v,app}$ values by regression equation 16 for the plug flow model. ($lnK_{v,PFM}$ = 0.034902*T - 0.03656*t – 1.89011)

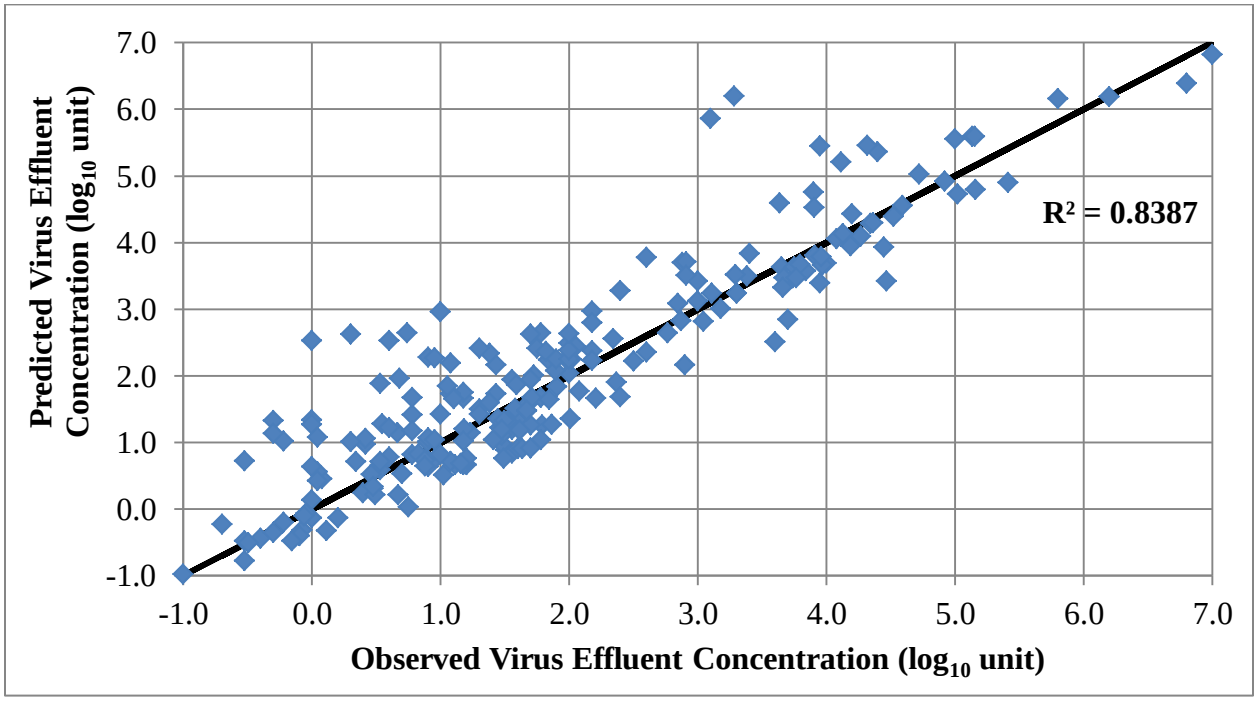

Figure 4.6: Observed virus effluent concentrations versus predicted virus effluent concentrations using plug model for facultative ponds and maturation ponds. ($K_{v,PFM}$ = exp(0.034902*T - 0.03656*t – 1.89011); $C_e = C_i e^{-K_v \cdot t}$)

The added complexity of including the surface viral loading rate (SVLR) in a regression equation would require the influent virus concentration to be known (measured or estimated) for each pond in series (facultative or maturation) before the regression equation could be used to predict $K_{v,app}$ values. This complex best fit PFM regression equation (Equation 14) could still be used for elucidating $K_{v,app}$ values in an existing pond or WSP system, but for WSP design purposes a simpler model in which all input variables can be easily measured or estimated is preferable.

The apparent goodness of the recommended plug flow model (Equation 16 and Equation 2) to predict effluent virus concentrations ($R^2 = 0.839$) as shown in Figure 4.6 has an important limitation. Equation 2 requires that the influent virus concentration is known (measured or estimated) in order to predict an effluent virus concentration. In many cases, the influent virus concentration may not be known in the design phase of a WSP. If this is the case, only the percent virus removal can be predicted with the plug flow model because it removes influent virus concentration as an input variable. Figure 4.7 presents the comparison between the observed percent virus removal and the predicted percent virus removal for all the data points in the WSP database for the PFM.

As displayed in Figure 4.7, the plug flow model is only capable of predicting 10 percent ($R^2 = 0.10$) of the variance in the observed percent virus removal for the data points in the WSP database if the influent virus concentration is not known. This suggests that in order to predict effluent virus concentrations using the recommended plug flow model the influent virus concentration must be measured or accurately estimated.

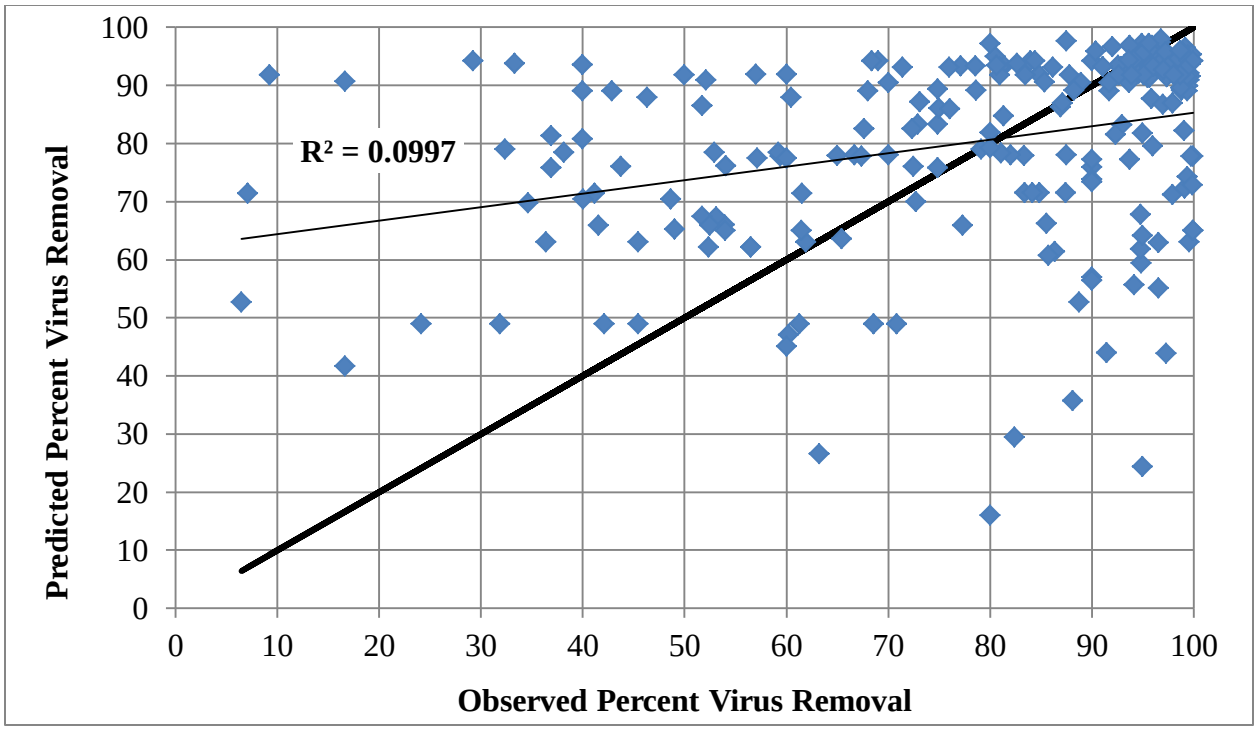

Figure 4.7: Observed percent virus removal versus predicted percent virus removal using plug model for facultative ponds and maturation ponds

4.4 Implications for WSP Design and Wastewater Reuse

Mathematical models from reactor theory may have important implications for improving the design of WSPs to achieve virus removal, for predicting virus removal in existing WSPs, and ultimately for supporting wastewater reuse. The recommended mathematical model based on the regression analysis in this thesis is the plug flow model. An appropriate (simpler) regression equation was selected for estimating virus removal rate coefficients ($K_{v,app}$) based on the principle of parsimony in regression models, which means a simple regression equation with the least amount of explanatory variables is preferred. For a model to be used for WSP design it is important that the input variables can easily be measured or estimated prior to design and operation. The selected PFM regression equation for predicting $K_{v,app}$ values (Equation 16) and the first-order PFM equation for predicting effluent virus concentrations (Equation 2) are reprinted below:

$$K_{v,\,PFM} = \exp\,(0.034902 \cdot T - 0.03656 \cdot t - 1.89011) \quad (R^2 = 0.383) \tag{16}$$

$$C_e = C_i e^{-K_v \cdot t} \quad (R^2 = 0.874) \tag{2}$$

where $K_{v,app}$ = virus removal rate coefficient ($days^{-1}$), T = air temperature (C°), t = hydraulic retention time (HRT) (days), C_i = influent virus concentration (viruses/L), and C_e = effluent virus concentration (viruses/L).

The estimation of effluent virus concentrations is the most important design variable of interest for WSPs in order to assess the viability of wastewater reuse. Theoretically, the recommended plug flow model could be suitable for estimating effluent virus concentrations because the input variables (temperature, HRT, influent virus concentration) can be estimated or measured prior to design and operation. However, one limitation might be measuring or estimating the influent virus concentration and selecting a virus or phage that is a good reference viral surrogate; as different enteric viruses have different removal rates, different infective doses, and there is not a widely accepted reference viral surrogate for which a threshold can be set to ensure the sufficient removal of enteric viruses. To assess virus removal requirements for water reuse, Mara et al. (2007, 2010) used quantitative microbial risk assessment (QMRA) to demonstrate that a 4-log_{10} reduction of viruses via treatment (with additional health protection measures implemented on the farm) would be sufficient for the irrigation of lettuce.

Table 4.8 displays predicted virus removals for different HRTs using the recommend plug flow model. Assuming a facultative or maturation pond with a HRT of 15 days, it is predicted that 1.14 log_{10} units of viruses can be removed per pond. This log_{10} unit removal can be summed for each pond in series to yield the total log_{10} removal for a WSP system. For example, four WSPs in series (combination of facultative and/or maturation) with an HRT of 15

days each (total HRT of 60 days) should achieve at least a 4 log_{10} unit removal of viruses, which would be sufficient for restricted irrigation (Symonds et al., 2014).

Table 4.8: Predicted effluent virus concentrations in a facultative or maturation pond using the recommended plug flow model

HRT (days)	K_v (d^{-1})	C_e (virus/L)	C_e (log_{10} units)	Log_{10} unit reduction per 1 WSP
3	0.2721	4.42E+06	6.65	0.35
5	0.2529	2.82E+06	6.45	0.55
10	0.2106	1.22E+06	6.09	0.91
15	0.1754	7.20E+05	5.86	1.14
20	0.1461	5.38E+05	5.73	1.27
25	0.1217	4.77E+05	5.68	1.32
30	0.1014	4.78E+05	5.68	1.32

Note: $K_{v,}$ = Equation 16; C_e = Equation 2; C_i = 1.00E+07 virus / L; air temperature = 20°C ; effluent virus target threshold = ~ <1,000 viruses per 100mL (3 log_{10} units) (adapted from Mara et al. 2010)

The log_{10} unit virus removal values displayed in Table 4.8 are comparable to the range of median log_{10} unit virus removal values in the WSP database compiled in this thesis. Virus removal in Table 4.8 ranges from 0.35 to 1.32 log_{10} units per pond, and virus removal in the WSP database was previously shown to range from 0.1 to 2.8 log_{10} units per pond (facultative and maturation) (Figure 4.1). For a similar assessment of overall virus removal, Verbyla and Mihelcic (2015) found that virus removal was more predictable in WSP systems with four or more ponds in series.

An additional recommendation for reusing treated wastewater from WSPs is to store the effluent in a storage reservoir. Storage reservoirs have two main benefits; the controlled discharge of water to maximize water efficiency for irrigation, and additional removal of pathogens. Storage reservoirs, depending on the type, have been shown to achieve an additional one to three log_{10} unit removal of pathogens (Mara et al., 2010). Storage reservoirs are a practical

and economical way to achieve additional pathogen reduction in WSP effluent and are highly recommended maximize water reuse efficiency.

The planning process of a WSP system should always include a detailed characterization of the influent wastewater parameters in order to properly design a system. Measuring for a reference viral surrogate (e.g., norovirus or somatic coliphage) may be a potential method for being able to better characterize wastewater and allow for a more robust regression equation capable of predicting $K_{v,app}$ values and overall virus removal to be developed. In reality, especially in the developing world, measuring viruses before the design or operation of a WSP may be a burden. Nevertheless, a simple model such as the plug flow model (Equations 16 and 2) introduced in this thesis may assist in predicting virus removal in WSPs if initial virus concentrations can be measured or reliably estimated prior to design.

4.5 Limitations

The results associated with this analysis have the following limitations: (1) the quality of the data is unknown. Full-scale WSPs are subject to more performance variability than controlled reactors, and aging WSPs without routine maintenance (e.g., desludging) do not perform according to design specifications; (2) it is likely that many theoretical hydraulic retention times were estimated based on design flowrates, and not actually based on measured flowrates at the time of the individual studies; (3) $K_{v,app}$ values have been shown to differ based on climate and/or time of year in WSPs and this was not accounted for. No temperature coefficient (θ) was used to standardize the $K_{v,app}$ values to 20°C, which is commonly done for other kinetic reaction rate coefficients using the Arrhenius equation; (4) water temperature in the WSPs often was not reported, so air temperature was used as an explanatory variable in regression equations instead; (5) several different types of viruses and bacteriophages were reported, using a variety of

methods for quantification, and the bacterial host strain cultivated to measure coliphage plaque-forming units was not reported for 20 data points; (6) the variation of $K_{v,app}$ values for different virus and bacteriophage types makes it difficult to predict effluent virus concentrations that represent all virus types; and (7) the plug flow model is empirical (not mechanistic) and should not be used for WSPs with greater than 27 day HRTs. Also, users of this equation should not extrapolate for temperatures or HRTs that are different from the ones included in this WSP database.

For practical design purposes, it is unlikely that a WSP system would be designed with four ponds in series with 15 day HRTs (60 days total) that only expects to receive approximately 4.5 $\log_{10}$ unit removal of viruses. According to the data reported, there are many WSPs in this database with shorter HRTs (< 15 days) that received similar or better $\log_{10}$ unit removal of viruses than predicted by the plug flow model. Possible explanations for the low prediction of the $\log_{10}$ unit virus removal by the plug flow model might the overall variability of virus removal in this database, and the potential existence of hydraulic inefficiencies (e.g., short circuiting) in many of the WSPs in this database.

It is well known that WSPs often have shorter mean HRTs than theoretical HRTs due to short circuiting and dead space, and four studies (Herrera and Castillo, 2000; Macdonald and Ernst, 1986; Pedahzur et al., 1993; Frederick and Lloyd, 1996) have specifically indicated that reduced hydraulic efficiency can decrease the efficiency of virus or phage removal in WSPs. This limitation was not assessed in this thesis, however, more research should be done on the hydraulic efficiency of the WSPs in this database, and the difference between virus removal efficiency in WSPs with good hydraulic efficiencies and poor hydraulic efficiencies should be compared.

CHAPTER 5: CONCLUSIONS AND RECOMMENDATIONS

To the author's knowledge, this is the first study that attempted to model the global extent of enteric virus and bacteriophage removal in individual waste stabilization ponds. While the removal of fecal indicator bacteria in WSPs has been well characterized, many uncertainties and knowledge gaps still remain about virus removal efficiency; which makes it difficult to estimate the viral risk associated with wastewater reuse. There is currently no mechanistic or empirical model that reliably predicts virus removal in WSPs, and the ability to predict virus concentrations in wastewater effluent is an important criterion for determining whether wastewater is suitable for discharge to the environment or for reuse in agriculture or aquaculture.

The overall objective of this thesis research was to model the global extent of virus removal in individual WSPs to support the reuse of wastewater. This was assessed by: (1) compiling a database of enteric virus and bacteriophage removal reported in the literature for individual WSPs; (2) deriving apparent virus removal rate coefficients ($K_{v,app}$) for each WSP type (anaerobic, facultative, and maturation ponds) using the complete mix, plug flow, and dispersed flow models; (3) identifying correlations and relationships between $K_{v,app}$ values and design, operational, and environmental parameters in WSPs; (4) developing alternative multiple linear regression equations to predict $K_{v,app}$ values and using mathematical models to predict effluent virus concentrations in WSPs; and (5) determining the best mathematical model and assessing its potential to aid in WSP design and support wastewater reuse. A summary of the key

findings and broader implications and recommendations for future research are discussed in the following sections.

5.1 Summary of Key Findings

A database was compiled that consists of 249 paired influent and effluent concentrations for enteric viruses and bacteriophages from 44 unique WSP systems. These 44 systems represent a total of 112 individual WSPs in 19 different countries. To the author's knowledge, this constitutes the largest database of individual WSPs from which virus removal has been assessed.

The first hypothesis of this study was that the correlations between virus removal rate coefficients ($K_{v,app}$) and solar radiation and air temperature in WSPs will be positive, the correlation between $K_{v,app}$ values and pond depth will be negative, and there will be no correlation between $K_{v,app}$ values and hydraulic retention time. The results from the correlation analysis (Table 4.2) confirmed that there was a significant positive correlation between $K_{v,app}$ and solar radiation and between $K_{v,app}$ and air temperature for facultative and maturation ponds for each mathematical model, with the exception of the CMM for facultative ponds. This means that higher temperatures and higher solar radiation values corresponded with higher virus removal rates, which is consistent with a previous study on the rates of exogenous sunlight-mediated inactivation (Romero et al., 2011). There was a significant negative correlation between $K_{v,app}$ values and pond depth in facultative and maturation ponds for the PFM and DFM cases, which was expected because sunlight-mediated virus inactivation primarily occurs at the pond surface in WSPs (Davies-Colley et al., 2005; Kohn et al., 2016). The significant negative correlation between $K_{v,app}$ values and hydraulic retention time was a surprise. This could be explained by the inadequacy of the mathematical models to describe the actual flow hydraulics in these WSPs,

or could indicate that the overall kinetics of K_v are actually second-order instead of pseudo first-order.

The second hypothesis of this research was that virus removal rate coefficients ($K_{v,app}$) will differ based on the type of virus and type of WSP. Multiple linear regression and ANOVA validated that $K_{v,app}$ values varied depending on enteric virus or bacteriophage type (Figure 4.4) and on WSP type (Figure 4.3). $K_{v,app}$ values were found to be significantly higher in anaerobic ponds than in facultative and maturation ponds. However, $K_{v,app}$ values were not found to be significantly different in facultative and maturation ponds. Although $K_{v,app}$ values varied depending on the type of enteric virus or bacteriophage, the significance and explanation of these variations were not determined.

The third hypothesis of this study was that virus removal rate coefficients derived from the dispersed flow model would be more representative of virus removal in WSPs than the complete mix and plug flow models. The plug flow model, however, was found to predict $K_{v,app}$ values with higher coefficients of determination (R^2) than the dispersed flow model. Comparatively, the best DFM regression equation for predicting $K_{v,app}$ with air temperature and HRT had a R^2 value of 0.238, while the best PFM regression equation for predicting $K_{v,app}$ with air temperature and HRT had a R^2 value of 0.383. Therefore, the plug flow model is recommended for predicting virus removal rate coefficients in facultative and maturation ponds. However, both the dispersed flow and plug flow models had R^2 values of approximately 0.84 when they were used to predict effluent virus concentrations in WSPs. This suggests that either model, or a combination of the two, may be adequate for predicting overall virus removal in WSPs.

A summary of the highlights from the regression analysis and examples of how the recommended plug flow model might be used to predict virus removal in WSPs are listed below:

1. The recommended plug flow equations for predicting virus removal rate coefficients ($K_{v,app}$) (Equation 16) and predicting effluent virus concentrations (Equation 2) are reprinted below.

Equation 16: $K_{v,\,PFM} = \exp(0.034902 \cdot T - 0.03656 \cdot t - 1.89011)$

Equation 2: $C_e = C_i e^{-K_v \cdot t}$

2. A multiple linear regression equation (Equation 16) was able to predict 38 percent (R^2 = 0.383) of the variance in $K_{v,app}$ values derived from the plug flow model in the WSP database using only two explanatory variables, air temperature (T) and HRT (t). Using Equation 16, the plug flow equation (Equation 2) was able to predict 84 percent (R^2) of the variance in effluent virus concentrations reported in the WSP database if the initial virus concentration is known.
3. The recommended plug flow model (Equation 16 + Equation 2) may be suitable for WSP design purposes (for ponds with HRT < 27 days) because the input variables (air temperature, HRT, influent virus concentration) can be estimated or measured prior to operation.
4. A theoretical example of how the plug flow model could be used to predict virus concentrations in WSP effluent was assessed. Using the plug flow model, it was predicted that a combination of four ponds in series (two facultative and two maturation ponds) would be necessary to yield a 4 $\log_{10}$ unit reduction in viruses.

The final two objectives of this thesis were to recommend a mathematical model from reactor theory that best predicts virus removal in WSPs and to determine if this model can

reliably be used for design purposes. A simple model such as the plug flow model (Equations 16 and 2) or the dispersed flow model (Equations 14, 3, and 5) that was introduced in this thesis may be an adequate way to predict virus removal in WSPs, and ultimately support wastewater reuse.

While the plug flow model was recommended, it is known to overestimate removal efficiencies; therefore, a factor of safety would have to be considered in order to use this model. Additionally, the plug flow model has generally only been used for designing maturation ponds when the organic loading has already been substantially reduced (Shilton, 2005). Primary facultative ponds are usually sized according to organic loading using the complete mix or dispersed flow model, and if the plug flow model was used it could lead to organic overloading (Shilton, 2005). Therefore, this may establish precedence for a multi-model approach for improving the design of WSPs to predict virus removal.

5.2 Broader Implications and Recommendations for Future Research

Overall, this thesis has accomplished the first step in filling the research knowledge gap of establishing a mathematical model that can be used to predict effluent virus concentrations in WSPs. The recommended plug flow regression equation for predicting $K_{v,app}$ values had a moderate R^2 value of 0.383 using only two explanatory variables (air temperature and HRT) and a more complicated plug flow equation had a better R^2 value of 0.414 when four explanatory variables were used (air temperature, HRT, pond depth, and SVLR). These results are encouraging for developing a simple mathematical model to predict virus removal rate coefficients and effluent virus concentrations in WSPs. The current recommended plug flow model is still a preliminary WSP design equation and needs to be assessed in greater detail, but it certainly establishes precedence for future research in this area. Thus, this research is considered to be the first step that can be built upon in an advancing field of future research.

There are still many knowledge gaps in the literature about the mechanisms responsible for removing viruses in WSPs and overall virus removal efficiency in WSPs that must be elucidated. The recommended model from this research is capable of predicting virus effluent concentrations in WSPs when the initial virus concentration is known, but the reliability of its ability to predict virus removal rate coefficients may need to further validation in order to be used for design purposes. Suggestions for further research include:

1. Derive an equation to convert the plug flow $K_{v,app}$ values to dispersed flow $K_{v,app}$ values to determine what implications this may have for WSP design. The dispersed flow model is most commonly used for design purposes, and this methodology for transforming K_b values has been developed for the design of WSPs for fecal coliform removal (von Sperling, 2002).
2. Assess the practicality of using a combination of the dispersed flow and plug flow model for WSP system design (multi-model approach). Consider deriving a regression equation with the dispersed flow model to predict $K_{v,app}$ values and effluent virus concentrations in facultative ponds, and deriving a regression equation with the plug flow model to predict $K_{v,app}$ values and effluent virus concentrations in maturation ponds.
3. In addition to this database, efforts to establish a larger database of paired influent and effluent concentrations for enteric viruses or bacteriophages in individual WSPs should be considered.
4. WSP system operators and/or nearby researchers should select a well-designed WSP system to continuously research and monitor. Dye-tracer studies should be performed to determine mean HRTs, several groups of enteric viruses or bacteriophages should be regularly measured, and apparent virus removal rate coefficients should be compared with intrinsic virus removal rate coefficients.

5. Because different groups of enteric viruses and bacteriophages presumably have different $K_{v,app}$ values, a reference viral surrogate for which a threshold can be set to ensure the sufficient removal of enteric viruses should be established.

The results of this study have provided several insights about virus removal rate coefficients and overall virus removal in WSPs. With the aid of future research, engineering professionals and wastewater managers should be able to make informed decisions about wastewater treatment and the potential for wastewater reuse in their communities. With safer reuse of wastewater that supports agriculture, environmental degradation from discharge of treated effluent to surface water can be lessened and economic and social benefits can also be achieved. In addition, the overall goal of promoting resource recovery from wastewater can also be met.

REFERENCES

Agunwamba J.C., Egbuniwe N., Ademiluyi J.O. (1992). Prediction of the dispersion number in waste stabilization ponds. Water Research, 26(1), 85–89.

Alcalde, L., Oron, G., Gillerman, L., Salgot, M., Manor, Y. (2003). Removal of fecal coliforms, somatic coliphages and F-specific bacteriophages in a stabilization pond and reservoir system in arid regions. Water Science and Technology, 3(4), 177-184.

APHA, AWWA, WEF. (2012). Standard Methods For The Examination Of Water and Wastewater, 22nd ed. Washington, D.C.

Battistini, R., Marcucci, E., Verani, M., Di Giuseppe, G., Dini, F., Carducci, A. (2013). Ciliate-adenovirus interactions in experimental co-cultures of Euplotes octocarinatus and in wastewater environment. European Journal of Protistology, 49(3), 381-388.

Bausum, H.T., Schaub, S.A., Rose, W.E., Gibbs, P.H. (1983). Enteric virus removal in wastewater treatment lagoon systems. PB 83-234-914. National Technical Information Service, Springfield VA, USA.

Benyahya, M., Bohatier, J., Laveran, H., Senaud, J., Ettayebi, M. (1998). Comparison of elimination of bacteriophages MS2 and ΦX-174 during sewage treatment by natural lagooning or activated sludge: A study on laboratory-scale pilot plants. Environmental Science and Technology, 19(5), 513-519.

Betancour, G. (2013). Deteccion y analisis molecular de rotavirus a partir de aguas provenientes de plantas de tratamiento de aguas. Licentiate Thesis. Department of Biochemistry, Universidad de la Republica, Uruguay.

Beuchat, L.R. (1998). Surface decontamination of fruits and vegetables eaten raw: A Review. Food Safety Unit, World Health Organization, WHO/FSF/98.2.

Bitton, G. (2005). Wastewater Microbiology. John Wiley and Sons, Hoboken, New Jersey.

Borchardt, M.A., Bertz, P.D., Spencer, S.K., Battigelli, D.A. (2003). Incidence of enteric viruses in groundwater from household wells in Wisconsin. Applied and Environmental Microbiology, 69, 1172-1180.

Botero, L., Montiel, M., Estrada, P., Villalobos, M., Herrera, L. (1997). Microorganism removal in wastewater stabilisation ponds in Maracaibo, Venezuela. Water Science and Technology, 35 (11-12), 205-209.

Calendar, R. (2004). Bacteriophages. Oxford University Press, Oxford, UK.

Campos, C., Guerrero, A., Cardenas, M. (2002). Removal of bacterial and viral faecal indicator organisms in a waste stabilization pond system in Choconta, Cundinamarca (Colombia). Water Science and Technology, 45(1), 61-66.

Cann, A. (2003). Principles of Molecular Virology, 4th Edition. Elsevier, London, UK.

Carrillo-Tripp, M., Shepherd, C.M., Borelli, I.A., Venkataraman, S., Lander, G., Natarajan, P., Johnson, J.E., Brooks, C.L., Reddy, V.S. (2009) VIPERdb2: an enhanced and web API enabled relational database for structural virology. Nucleic Acids Research, 37(1), D436-D442.

Castillo, G.C., Trumper, B.A. (1991). Coliphages and other microbial indicators in stabilization ponds. Environmental Toxicology and Water Quality, 6(2), 197-207.

Ceballos, B.O., Konig, A., Lomans, B., Athayde, A.B., Pearson, H.W. (1995). Evaluation of a tropical single-cell waste stabilization pond system for irrigation. Water Science and Technology, 31(12), 267-273.

Chambers, J.M., Cleveland, W.S., Kleiner, B., Tukey, P.A. (1983). Graphical Methods for Data Analysis. Wadsworth, Belmont, CA.

Cornax, R., Morinigo, M.A., Paez, I.G., Munoz, M.A., Borrego, J.J. (1990). Application of direct plaque assay for detection and enumeration of bacteriophages of *Bacteroides fragilis* from contaminated water samples. Applied and Environmental Microbiology, 56, 3170–3173.

Cornejo, P.K., Zhang, Q., Mihelcic, J.R. (2013). Quantifying benefits of resource recovery from sanitation provision in a developing world setting. Journal of Environmental Management, 131, 7-15.

Crittenden, J. C., Trussell, R.R., Hand, D.W., Howe, K.J., Tchobanoglous, G. (2012). MWH's Water Treatment: Principles and Design, 3rd ed. John Wiley and Sons, Hoboken, New Jersey.

da Silva, A.K., Le Saux, J.C., Parnaudeau, S., Pommepuy, M., Elimelech, M., Le Guyader, F.S. (2007). Evaluation of removal of noroviruses during wastewater treatment, using real-time reverse transcription-PCR: Different behaviors of genogroups I and II. Applied and Environmental Microbiology, 73(24), 7891-7897.

Danes, L., Cerva, L. (1983). Poliovirus and echovirus survival in Tetrahymena pyriformis culture in vivo. Journal of Hygiene, Epidemiology, Microbiology, and Immunology, 28(2), 193-200.

Davies-Colley, R.J., Donnison, A.M., Ross, C.M., Nagels, J.W. (1999). Inactivation of faecal indicator microorganisms in waste stabilisation ponds: Interactions of environmental factors with sunlight. Water Research, 33(5), 1220–1230.

Davies-Colley, R.J., Donnison, A.M., Speed, D.J. (2000). Towards a mechanistic understanding of pond disinfection. Water Science Technology, 42(10-11), 149–158.

Davies-Colley, R.J., Craggs, R.J., Park, J., Sukias, J.P.S., Nagels, J.W., Stott, R. (2005). Virus removal in a pilot-scale "advanced" pond system as indicated by somatic and F-RNA bacteriophages. Water Science and Technology, 51(12), 107–110.

Donnison, A.M., Ross, C.M. (1995). Somatic and F-specific coliphages in New Zealand waste treatment lagoons. Water Research, 29(4), 1105-1110.

Draper, N.R., Smith, H. (1998). Applied Regression Analysis, 3rd ed. John Wiley and Sons, New York, NY.

Drechsel, P., Scott, C., Raschid-Sally, L., Redwood, M., Bahri, A. (2010). Wastewater Irrigation and Health: Assessing and Mitigating Risk in Low-income Countries. Earthscan, London, UK.

El-Deeb Ghazy, M.M., El-Senousy, W.M., Abdel-Aatt, A.M., Kamel, M. (2008). Performance evaluation of a waste stabilization pond in a rural area in Egypt. American Journal of Environmental Sciences, 4(4), 316-325.

Emparanza-Knorr, A., Torrella, F. (1995). Microbiological performance and Salmonella dynamics in a wastewater depuration pond system of southeastern Spain. Water Science and Technology, 31(12), 239-248.

England, B., Leach, R.E., Adame, B., Shiosaki, R.. (1967). Virologic assessment of sewage treatment at Santee, California. In: Berg, G. (Ed.), Transmission of Viruses by the Water Route. John Wiley and Sons, New York, NY, 401-418.

FAO/WHO. (2008). Microbiological hazards in fresh leafy vegetables and herbs: Meeting Report. Microbiological Risk Assessment Series, 14.

Fattal, B., Berkovitz, A., Shuval, H.I. (1998). The efficiency of pilot plant stabilization pond treatment of wastewater. International Journal of Environmental Research and Public Health, 8(2), 153-156.

Feachem R.G., Mara D.D. (1978). A reappraisal of the role of faecal indicator organisms in tropical waste treatment processes. Public Health Engineering, 31-33.

Feachem, R.G., Bradley, D.J., Garelick, H., Mara, D.D. (1983). Sanitation and Disease: Health Aspects of Excreta and Wastewater Management. John Wiley and Sons, Chichester, UK.

Fields, B.N., Knipe, D.M., Howley, P.M., Chanock, R.M., Melnick, J.L. (1996). Fields Virology, Volume 1, 3rd ed. Lippincott-Raven, Philadelphia, PA.

Flint, S.J., Enquist, L.W., Racaniello, V.R., Skalka, A.M. (2009). Principles of Virology, Volume 1: Molecular Biology, 3rd ed. ASM Press, Washington, D.C.

FDEP (Florida Department of Environmental Protection). (2011). Development of type iii site specific alternative criteria for nutrients. FDEP Bureau of Assessment and Restoration Support. DEP-SAS-004/11

Fong, T.T., Lipp, E.K. (2005). Enteric viruses of humans and animals in aquatic environments: health risks, detection, and potential water quality assessment tools. Microbiology and Molecular Biology Reviews, 69(2), 357-371.

Fox, J., Weisberg, S. (2010). An R companion to applied regression. Sage Publications, Inc.

Frederick, G.L., Lloyd, B.J. (1996). An evaluation of retention time and short-circuiting in waste stabilisation ponds using Serratia marcescens bacteriophage as a tracer. Water Science and Technology, 33(7), 49-56.

Gerardi, M.H., Zimmerman, M.C. (2005). Wastewater Pathogens. John Wiley and Sons, Hoboken, NJ.

Gersberg, R.M., Rose, M.A., Robles-Sikisaka, R., Dhar, A.K. (2006). Quantitative detection of hepatitis A virus and enteroviruses near the United States-Mexico border and correlation with levels of fecal indicator bacteria. Applied and Environmental Microbiology, 72, 7438-7444.

Grabow, W.K. (2001). Bacteriophages, Update on application as models for viruses in water. Water SA, 27(2), 251 – 268.

Guest, J.S., Skerlos, S.J., Barnard, J.L., Beck, M.B., Daigger, G.T., Hilger, H., Jackson, S.J., Karvazy, K., Kelly, L., Macpherson, L., Mihelcic, J.R., Pramanik, A., Raskin, L., van Loosdrecht, M.C.M., Yeh, D.H., Love, N.G. (2009). A new planning and design paradigm to achieve sustainable resource recovery from wastewater. Environmental Science and Technology, 43(16), 6126–6130.

Hadley, S.N. (2013). Assessment of a Modified Double Agar Layer Method to Detect Bacteriophage for Assessing the Potential of Wastewater Reuse in Rural Bolivia. University of South Florida. Master's Thesis.

Haramoto, E., Yamada, K., Nishida, K. (2011). Prevalence of protozoa, viruses, coliphages and indicator bacteria in groundwater and river water in the Kathmandu Valley, Nepal. Transactions for the Royal Society of Tropical Medicine and Hygiene, 105(12), 711-716.

Harris, L.J., Farber, J.M., Beuchat, L.R., Parish, M.E., Suslow, T.V., Garrett, E.H., Busta, F.F. (2003). Outbreaks associated with fresh produce: incidence, growth, and survival of pathogens in fresh and fresh-cut produce. Comprehensive Reviews in Food Science and Food Safety, 2, 78–141.

Harwood, V.J., Boehm, A.B., Sassoubre, L.M., Vijayavel, K., Stewart, J.R., Fong, T.T., Caprais, M.P., Converse, R.R., Diston, D., Ebdon, J., Fuhrman, J.A., Gourmelon, M., Gentry Shields, J., Griffith, J.F., Kashian, D.R., Noble, R.T., Taylor, H., Wicki, M. (2013). Performance of viruses and bacteriophages for fecal source determination in a multi-laboratory, comparative study. Water Research, 47(18), 6929-6943.

Havelaar, A.H. (1993). Bacteriophages as models of human enteric viruses in the environment. American Society of Microbiology News, 59, 614-619.

Helsel, D.R., Hirsch R.M. (2002). Statistical Methods in Water Resources Techniques of Water Resources Investigations. U.S. Geological Survey.

Herrera, L., Castillo, G. (2000). Dynamics of a waste stabilization pond in Chile. Water Science and Technology, 42(10-11), 83-90.

Hodgson, B., Paspaliaris, P. (1996). Melbourne water's wastewater treatment lagoons: design modifications to reduce odours and enhance nutrient removal. Water Science and Technology, 33(7), 157-164.

Hot, D., Legeay, O., Jacques, J., Gantzer, C., Caudrelier, Y., Guyard, K., Lange, M., Andreoletti, L. (2003). Detection of somatic phages, infectious enteroviruses and enterovirus genomes as indicators of human enteric viral pollution in surface water. Water Research, 37(19), 4703-4710.

IAWPRC. (1991). Bacteriophages as model viruses in water quality control. Water Research, 25, 529–545.

Iriarte, M., Mercado, A., Verbyla, M.E., Fuentes, G., Rocha, J.P. Almanza, M. (2013). Monitoreo de microorganism indicadores y patogenos en dos sistemas de lagunas de estabilizacion en el Valle Alto de Cochabamba. In: Proceedings from the XV Congreso Bolivariano de Ingenieria Sanitaria Y Medio Ambiente, November 20-22, 2013. ABIS-AIDIS, Cochabamba, Bolivia.

Jansons, J., Edmonds, W., Speight, B., Bucens, M. (1989). Survival of viruses in groundwater. Water Research, 23, 301-306.

Jarvis, B., Wilrich, C., Wilrich, P.T. (2010). Reconsideration of the derivation of most probable numbers, their standard deviations, confidence bounds and rarity values. Journal of Applied Microbiology, 109(5), 1660-1667.

Jenner, G. (2009). Review of Pathogen Reduction in Waste Stabilisation Ponds. Masterton, New Zealand. http://resources.mstn.govt.nz/sewerageupgrade/nz1report.pdf (accessed 01.15.16.).

Jiang, S.C., Chu, W., He, J.W. (2007). Seasonal detection of human viruses and coliphage in Newport Bay, California. Applied and Environmental Microbiology, 73(20), 6468-6474.

Jiménez, B. (2006). Irrigation in developing countries using wastewater. International Review for Environmental Strategies, 6(2), 229-250.

Jofre, J., Blasi, M., Bosch, A., Lucena, F. (1989). Occurrence of bacteriophages infecting *Bacteroides fragilis* and other viruses in polluted marine sediments. Water Science and Technology, 21, 15–19.

Jofre, J. (2009). Is the replication of somatic coliphages in water environments significant? Journal of Applied Microbiology, 106(4), 1059-1069.

Jofre, J., Olle, E., Ribas, F., Vidal, A., Lucena, F. (1995). Potential usefulness of bacteriophages that infect *Bacteroides fragilis* as model organisms for monitoring virus removal in drinking water treatment plants. Applied and Environmental Microbiology, 61, 3227–3231.

Jurzik, L., Hamza, I.A., Wilhelm, M. (2015). Investigating the Reduction of human adenovirus (hadv) and human polyomavirus (hpyv) in a sewage treatment plant with a polishing pond as a tertiary treatment. Water, Air, and Soil Pollution, 226(9), 1-8.

Kageyama, T., Kojima, S., Shinohara, M., Uchida, K., Fukushi, F., Hoshino, F.B., Takeda, N., Katayama, K. (2003). Broadly reactive and highly sensitive assay for Norwalk-like viruses based on real-time quantitative reverse transcription-PCR. Journal of Clinical Microbiology, 41(4), 1548-1557.

King, A.Q., Adams, M.J., Carstens, E.B., Lefkowitz, E.J. (2011). Virus Taxonomy: Ninth report of the international committee on taxonomy of viruses. Elsevier, London, UK.

Klock, J.W. (1971). Survival of coliform bacteria in wastewater treatment lagoons. Journal of the Water Pollution Control Federation, 43(10), 2071-2083.

Kocwa-Haluch, R. (2001). Waterborne enteroviruses as a hazard for human health. Polish Journal of Environmental Studies, 10, 485–487.

Kohn, T., Nelson, K. (2007). Sunlight-mediated inactivation of MS2 coliphage via exogenous singlet oxygen produced by sensitizers in natural waters. Environmental Science and Technology, 41(1), 192-197.

Kohn, T., Mattle, M.J., Minella, M., Vione, D. (2016). A modeling approach to estimate the solar disinfection of viral indicator organisms in waste stabilization ponds and surface waters. Water Research, 88, 912-922.

Kott, Y., Roze, N., Sperber, S., Betzer, N. (1973). Bacteriophages as viral pollution indicators. Water Research, 8, 165-171.

Kott, Y., Ben-Ari, H., Betzer, N. (1978). Lagooned, secondary effluents as water source for extended agricultural purposes. Water Research, 12, 1101-1106.

Kuo, D., Simmons, F.J., Blair, S., Hart, E., Rose, J.B., Xagoraraki, I. (2010). Assessment of human adenovirus removal in a full-scale membrane bioreactor treating municipal wastewater. Water Research, 44(5), 1520-1530.

Lee, H.S., Sobsey, M.D. (2011). Survival of prototype strains of somatic coliphage families in environmental waters and when exposed to UV low-pressure monochromatic radiation or heat. Water Research, 45(12), 3723-3734.

Lewis, G.D., Austin, F.J., Loutit, M.W., Sharples, K. (1986). Enterovirus removal from sewage. Water Research, 20(10), 1291-1297.

Lijklema, L., Habekotte, B., Hooijmans, C., Aalderink, R.H., Havelaar, A.H. (1986). Survival of indicator organisms in a detention pond receiving combined sewer overflow. Water Science and Technology, 18(10), 9-17.

Lucena, F., Lasobras, J., McIntosh, D., Forcadell, M., Jofre, P. (1994). Effect of distance from the polluting focus on relative concentrations of *Bacteroides fragilis* phages and coliphages in mussels. Applied and Environmental Microbiology, 60, 2272–2277.

Lucena, F., Duran, A.E., Moron, A., Calderon, E., Campos, C., Gantzer, C., Skraber, S., Jofre, J. (2004). Reduction of bacterial indicators and bacteriophages infecting faecal bacteria in primary and secondary wastewater treatments. Journal of Applied Microbiology, 97(5), 1069-1076.

Macdonald, R.J., Ernst, A. (1986). Disinfection efficiency and problems associated with maturation ponds. Water Science and Technology, 18(10), 19–29.

Macler, B.A. (1995). Developing a national drinking water regulation for disinfection of groundwater. Ground Water Monitoring and Remediation, 15, 77-84.

Malherbe, H.H., Strickland-Cholmley, M. (1967a). Quantitative studies on viral survival in sewage purification processes. In: Berg, G. (Ed.), Transmission of Viruses by the Water Route. John Wiley and Sons, New York, NY.

Malherbe, H.H., Strickland-Cholmley, M. (1967b). Survival of viruses in the presence of algae. In: Berg, G. (Ed.), Transmission of Viruses by the Water Route. John Wiley and Sons, New York, NY.

Manage, P.M., Kawabata, Z., Nakano, S., Nishibe, Y. (2002). Effect of heterotrophic nanoflagellates on the loss of virus-like particles in pond water. Ecological Research, 17, 473-479.

Mara, D.D. (2004). Domestic wastewater treatment in developing countries, Earthscan/James & James, London, UK.

Mara, D.D., Pearson, H.W. (1998). Design Manual for Waste Stabilization Ponds in Mediterranean Countries, Lagoon Technology International, Ltd., Leeds, UK.

Mara, D.D., Sleigh, P.A., Blumenthal, U.J., Carr, R.M. (2007). Health risks in wastewater irrigation: comparing estimates from quantitative microbial risk analyses and epidemiological studies. Journal of Water and Health, 5(1), 39-50.

Mara, D.D., Hamilton, A., Sleigh, A., Karavarsamis, N. (2010). Discussion paper: options for updating the 2006 WHO guidelines. WHO, FAO, IDRC, IWMI.

Marais, G.V.R. (1974). Faecal bacterial kinetics in waste stabilization ponds. Journal of the Environmental Engineering Division ASCE, 10(1), 119-139.

Marais, G.V.R., Shaw, V.A. (1961). A rational theory for the design of sewage stabilization ponds in Central and South Africa. Transactions of the South African Institute of Civil Engineers, 3, 205-227.

Marquardt, D.W. (1970). Generalized inverses, ridge regression, biased linear estimation, and nonlinear estimation. Technometrics, 12, 591-612.

Mattle, M.J., Vione, D., Kohn, T. (2015). A conceptual model and experimental framework to determine the contributions of direct and indirect photoreactions to the solar disinfection of MS2, phiX174, and adenovirus. Environmental Science and Technology, 49(1), 334-342.

Maynard, H.E., Ouki, S.K., Williams, S.C. (1999). Tertiary lagoons: a review of removal mechanisms and performance. Water Research, 33(1), 1-13.

Mayo, A.W. (1995). Modeling coliform mortality in waste stabilisation ponds. Journal of the Environmental Engineering ASCE, 121, 140-152.

McDonald, J.H. (2014). Handbook of Biological Statistics, 3rd ed. Sparky House Publishing, Baltimore, Maryland.

Melnick, J.L. **(**1984). Etiologic agents and their potential for causing waterborne virus diseases. Enteric Viruses in Water, 15, 1-16.

Melnick, J.L., Gerba, C.P. (1980). Viruses in water and soil. Public Health Review. 9, 185-213.

Mihelcic, J.R., Myre, E.A., Fry, L.M., Phillips, L.D., Barkdoll, B.D. (2009). Field Guide in Environmental Engineering for Development Workers: Water, Sanitation, and Indoor Air. American Society of Civil Engineers (ASCE) Press, Reston, VA.

Mihelcic, J.R., Fry, L.M., Shaw, R. (2011). Global potential of phosphorus recovery from human urine and feces. Chemosphere, 84(6), 832-839.

Mihelcic, J.R., Zimmerman, J.B. (2014). Environmental Engineering: Fundamentals, Sustainability, Design, 2nd ed, John Wiley and Sons, Hoboken, NJ.

Mocé-Llivina, L., Lucena, F., Jofre, J. (2005). Enteroviruses and bacteriophages in bathing waters. Applied and Environmental Microbiology, 71(11), 6838-6844.

Morris, R. (1984). Reduction of naturally occurring enteroviruses by wastewater treatment processes. Journal of Hygiene, 92(1), 97-103.

Muga, H.E., Mihelcic, J.R. (2008). Sustainability of wastewater treatment technologies. Journal of Environmental Management, 88(3), 437–47.

Nameche, T., Vasel, J. (1998). Hydrodynamic studies and modelization for aerated lagoons and waste stabilization ponds. Water Research, 32(10), 3039-3045.

Nasser, A.M., Tchorch, Y., Fattal, B. (1993). Comparative survival of E. coli, F+ bacteriophages, HAV and poliovirus 1 in wastewaters and groundwaters. Water Science and Technology, 27(3-4), 401-407.

Nasser, A.M., Oman, S.D. (1999). Quantitative assessment of the inactivation of pathogenic and indicator viruses in natural water sources. Water Research, 33, 1748-1752.

Noyola, A., Padilla-Rivera, A., Morgan-Sagastume, J.M., Guereca, L.P., Hernandez-Padilla, F. (2012). Typology of municipal wastewater treatment technologies in Latin America. Clean. Soil, Air, Water, 40(9), 926–932.

NRC (National Research Council). (2004). Indicators for Waterborne Pathogens. The National Academic Press, Washington, D.C.

Nupen, E.M. (1970). Virus studies on the Windhoek waste-water reclamation plant (Southwest Africa). Water Research, 4, 661-672.

Nupen, E.M., Bateman, B.W., McKenny, N.C. (1974). The reduction of virus by the various unit processes used in the reclamation of sewage to potable waters. In: Malina, J.F., Sagik, B.P. (Eds.), Virus Survival in Water and Wastewater Systems. Center for Research in Water Resources. The University of Texas at Austin, Austin, TX, 107-114.

Oakley, S.M. (2005). The need for wastewater treatment in Latin America: a case study of the use of wastewater stabilization ponds in Honduras. Small Flows Quarterly, 6(2), 36-51.

Ohgaki, S., Ketratanukul, A., Praesertsom, U. (1986) Effect of sunlight on coliphages in an oxidation pond. Water Science and Technology, 18(10), 37–46.

Okoh, A.I., Sibanda, T., Gusha, S.S. (2010). Inadequately treated wastewater as a source of human enteric viruses in the environment. International Journal of Environmental Research and Public Health, 7(6), 2620–2637.

Omura, T., Shin, H.K., Ketratanakul, A. (1985). Behaviour of coliphages in oxidation ponds. Water Science and Technology, 17(10), 219-227.

Oragui, J.I., Curtis, T.P., Silva, S.A., Mara, D.D. (1986). The removal of excreted bacteria and viruses in deep waste stabilization ponds in Northeast Brazil. Water Science and Technology, 18(10), 31-35.

Oragui, J.I., Arridge, H., Mara, D.D., Pearson, H.W., Silva, S.A. (1995). Rotavirus removal in experimental waste stabilization pond systems with different geometries and configurations. Water Science and Technology, 31(12), 285-290.

Parashar, U.D., Hummelman, E.G., Bresee, J.S., Miller, M.A., Glass, R.I. (2003) Global illness and death by rotavirus disease in children. Emerging Infectious Diseases, 9(5), 565–572.

Pearson, H.W., Mara, D.D., Arridge, H.A. (1995). The influence of pond geometry and configuration on facultative and maturation waste stabilisation pond performance and efficiency. Water Science and Technology, 31(12), 129-139.

Pedahzur, R., Nasser, A.M., Dor, I., Fattal, B., Shuval, H.I. (1993). The effect of baffle installation on the performance of a single-cell stabilization pond. Water Science and Technology, 27(7-8), 45-52.

Persson, J. (2000). The hydraulic performance of ponds of various layouts. Urban Water, 2, 243-250.

Polprasert, C., Bhattarai, K. (1985). Dispersion model for waste stabilization ponds. Journal of the Environmental Engineering Division, ASCE, 111(1), 45-59.

Rao, V.C., Lakhe, S.B., Waghmare, S.V. (1981). Virus removal in waste stabilization ponds in India. Water Research, 15, 773-778.

Raschid-Sally, L., Jayakody, P. (2008). Drivers and characteristics of wastewater agriculture in developing countries: Results from a global assessment. International Water Management Institute Research Report 127. Colombo, Sri Lanka.

Reinoso, R., Torres, L.A., Becares, E. (2008). Efficiency of natural systems for removal of bacteria and pathogenic parasites from wastewater. Science of the Total Environment, 395(2-3), 80-86.

Reinoso, R., Blanco, S., Torres, L.A., Becares, E. (2011). Mechanisms for parasites removal in a waste stabilization pond. Microbial Ecology. 61(3), 684-692.

Reynolds, K.A., Mena, K.D., Gerba, C.P. (2008). Risk of waterborne illness via drinking water in the United States. Reviews of Environmental Contamination and Toxicology. 192, 117-158.

Romero, O.C., Straub, A.P., Kohn, T., Nguyen, T.H. (2011). Role of temperature and Suwannee River natural organic matter on inactivation kinetics of rotavirus and bacteriophage MS2 by solar irradiation. Environmental Science and Technology, 45(24), 1385–1393.

Salter, H.E., Boyle, L., Ouki, S.K., Quarmby, J., Willliams, S.C. (1999). The performance of tertiary lagoons in the United Kingdom. Water Research, 33(18), 3775-3781.

Schaper, M., Jofre, J., Uys, M., Grabow, W.O. (2002). Distribution of genotypes of F-specific RNA bacteriophages in human and non-human sources of faecal pollution in South Africa and Spain. Journal of Applied Microbiology, 92(4), 657-667.

Scheid, P., Schwarzenberger, R. (2012). Acanthamoeba spp. as vehicle and reservoir of adenoviruses. Parasitology Research, 111(1), 479-485.

Sheladia, V.L., Ellender, R.D., Johnson, R.A. (1982). Isolation of enteroviruses from oxidation pond waters. Applied and Environmental Microbiology. 43(4), 971-974.

Shilton, A. (2005). Pond Treatment Technology. IWA Publishing, London, UK.

Shuval, H.I. (1970). Detection and control of enteroviruses in the water environment. In: Shuval, H.I. (Ed.), Developments in Water Quality Research: Proceedings of the Jerusalem International Conference on Water Quality and Pollution Research. Ann Arbor-Humphrey Science Publishers, London, UK, 47-71.

Shuval, H.I. (1990). Wastewater irrigation in developing countries: Health effects and technical solutions. Summary of World Bank Technical Paper 51, Integrated Resource Recovery, Washington, D.C.

Shuval, H.I., Adin, A., Fattal, B., Rawitz, E., Yekutiel, P. (1986). Wastewater irrigation in developing countries: Health effects and technical solutions (Technical Paper Number 51). Integrated Resource Recovery, Washington, D.C.

Silverman, A.I., Akrong, M.O., Amoah, P., Drechsel, P., Nelson, K.L. (2013). Quantification of human norovirus GII, human adenovirus, and fecal indicator organisms in wastewater used for irrigation in Accra, Ghana. Journal of Water and Health, 11(3), 473-488.

Silverman, A.I., Nguyen, M.T., Schilling, I.E., Wenk, J., Nelson, K.L. (2015). Sunlight inactivation of viruses in open-water unit process treatment wetlands: Modeling endogenous and exogenous inactivation rates. Environmental Science and Technology, 49(5), 2757-2766.

Simkova, A., Cervenka, J. (1981). Coliphages as ecological indicators of enteroviruses in various water systems. Bulletin of the World Health Organization, 59(4), 611-618.

Simmons, F.J., Kuo, D.W., Xagoraraki, I. (2011). Removal of human enteric viruses by a full-scale membrane bioreactor during municipal wastewater processing. Water Research, 45(9), 2739-2750.

Sincero, T.M., Levin, D.B., Simões, C.O., Barardi, C.M. (2006). Detection of hepatitis A virus (HAV) in oysters (Crassostrea gigas). Water Research, 40, 895-902.

Sinclair, R.G., Jones, E.L., Gerba, C.P. (2009). Viruses in recreational water-borne disease outbreaks: A review. Journal of Applied Microbiology,107(6), 1769-1780.

Sinton, L.W., Hall, C.H., Lynch, P.A., Davies-Colley, R.J. (2002). Sunlight inactivation of fecal indicator bacteria and bacteriophages from waste stabilization pond effluent in fresh and saline waters. Applied and Environmental Microbiology, 68(3), 1122-1131.

Sobsey, M.D., Battigelli, D., Handzel, T., Schwab, K. (1995). Male-specific coliphages as indicators of viral contamination of drinking water. In Proceedings of the 1994 Water Quality Technology Conference. American Water Works Association, Denver, CO. 1847-1862.

Sobsey M.D., Meschke, J.S. (2003). Virus survival in the environment with special attention to survival in sewage droplets and other environmental media of fecal or respiratory origin. Report for the World Health Organization, Geneva, Switzerland, 70.

Soler, A., Torrella, F., Saez, J., Martınez, I., Nicolas, J., Llorens, M., Torres, J. (1995). Performance of two municipal sewage stabilization pond systems with high and low loading in south-eastern Spain. Water Science and Technology, 31(12), 81-90.

Straub, T.M., Pepper, I.L., Gerba, C.P. (1993). Virus survival in sewage sludge amended desert soil. Water Science and Technology, 27, 421-424.

Symonds, E.M., Griffin, D.W., Breitbart, M. (2009). Eukaryotic viruses in wastewater samples from the United States. Applied and Environmental Microbiology, 75(5), 1402–1409

Symonds, E.M., Verbyla, M.E., Lukasik, J.O., Kafle, R.C., Breitbart, M., Mihelcic, J.R. (2014). A case study of enteric virus removal and insights into the associated risk of water reuse for two wastewater treatment pond systems in Bolivia. Water Research, 65, 257–270.

Tartera, C., Jofre, J. (1987). Bacteriophage active against *Bacteroides fragilis* in sewage-polluted waters. Applied and Environmental Microbiology, 53,1632–1637.

Thirumurthi, D. (1969). A break-through in the tracer studies of sedimentation tanks. Journal of the Water Pollution Control Federation, 41(11), 405-418.

Thirumurthi, D. (1974). Design criteria for waste stabilization ponds. Journal of the Water Pollution Control Federation, 46(9), 2094- 2106.

Torres, J.J., Soler, A., Saez, J., Leal, L.M., Aguilar, M.I. (1999). Study of the internal hydrodynamics in three facultative ponds of two municipal WSPs in Spain. Water Research, 33(5), 1133-1140.

Tukey, J.W. (1977). Exploratory Data Analysis. Addison-Wesley Series in Behavioral Science: Quantitative Methods, Reading, Massachusetts.

Turner, S.J., Lewis, G.D. (1995). Comparison of F-specific bacteriophage, enterococci, and faecal coliform densities through a wastewater treatment process employing oxidation ponds. Water Science and Technology, 31(5-6), 85-89.

United Nations. (2015). The United Nations World Water Development Report 2015: Water for a Sustainable World. UNESCO, Paris.

USEPA (United States Environmental Protection Agency). (2001a). Method 1602: Male-specific (F+) and Somatic Coliphage in Water by Single Agar Layer (SAL) Procedure. EPA 821-R-01-029.

USEPA (United States Environmental Protection Agency). (2001b). Method 1601: Detection of Male-specific (F+) and Somatic Coliphage in Water by Two-Step Enrichment Procedure. EPA 821-R-01-030

USEPA (United States Environmental Protection Agency). (2011). Principles of Design and Operations of Wastewater Treatment Pond Systems for Plant Operators, Engineers, and Managers. EPA 600-R-11-088

USEPA (United States Environmental Protection Agency). (2015). Review of Coliphages as Possible Indicators of Fecal Contamination for Ambient Water Quality. EPA 820-R-15-098

Venables, W.N., Smith, D.M., R Core Team. (2014) Notes on R: A Programming Environment for Data Analysis and Graphics. Version 3.2.2.

Verbyla, M. E. (2012). Assessing the Reuse Potential of Wastewater for Irrigation: The Removal of Helminth Eggs from a UASB Reactor and Stabilization Ponds in Bolivia. University of South Florida. Master's Thesis.

Verbyla, M.E., Oakley, S.M., Mihelcic, J.R. (2013a). Wastewater infrastructure for small cities in an urbanizing world: Integrating protection for human health and the environment with resource recovery and food security. Environmental Science and Technology, 47(8), 3598-3605.

Verbyla, M.E., Oakley, S.M., Lizima, L.A., Zhang, J., Iriarte, M., Tejada-Martinez, A.E., Mihelcic, J.R. (2013b). Taenia eggs in a stabilization pond system with poor hydraulics: Concern for human cysticercosis? Water Science and Technology, 68(12), 2698–2703.

Verbyla, M.E., Mihelcic, J.R. (2015). A review of virus removal in wastewater treatment pond systems. Water Research, 71, 107-124.

Verbyla, M.E., Cairns, M.R., Gonzalez, P.A., Whiteford, L.M., Mihelcic, J.R. (2015) Emerging challenges for pathogen control and resource recovery in natural wastewater treatment systems. Wiley Interdisciplinary Reviews: Water, 2(6), 701-714.

von Sperling, M. (1999). Performance evaluation and mathematical modelling of coliform die-off in tropical and subtropical waste stabilization ponds. Water Research, 33(6), 1435–1448.

von Sperling, M. (2002). Relationship between first-order decay coefficients in ponds, according to plug flow, CSTR and dispersed flow regimens. Water Science and Technology, 45(1), 17–24.

von Sperling, M. (2003). Influence of the dispersion number on the estimation of faecal coliform removal in ponds. Water Science and Technology, 48(2), 181–188.

von Sperling, M. (2005). Modelling of coliform removal in 186 facultative and maturation ponds around the world. Water Research, 39(20), 5261-5273.

Wehner, J.F., Wilhelm, R.H. (1956). Boundary conditions of flow reactor. Chemical Engineering Science, 6(2), 89–93.

Wendelberger, J., Campbell, K. (1994). Non-detect data in environmental investigations. Los Alamos National Laboratory Report, LA-UR-94-1856, Los Alamos, NM.

WHO (World Health Organization). (2006a). Water Recreation and Disease. Plausibility of Associated Infections: Acute Effects, Sequelae and Mortality. World Health Association, Genevea, Switzerland.

WHO (World Health Organization). (2006b). Guidelines for the Safe Use of Wastewater, Excreta and Greywater, Volume 2: Wastewater Use in Agriculture, World Health Organization, Geneva, Switzerland.

WHO (World Health Organization). (2013). Diarrhoeal disease. Retrieved 12, August 2015 from www.who.int/mediacentre/factsheets/fs330/en/index.html

WHO-UNICEF. (2009). Diarrhoea: Why children are still dying and what can be done. World Health Organization, Geneva, Switzerland

Wu, J., Long, S.C., Das, D., Dorner, S.M. (2011). Are microbial indicators and pathogens correlated? A statistical analysis of 40 years of research. Journal of Water and Health, 9(2), 265-278.

Wu, C.J., Hamada, M.S. (2011). Experiments: Planning, Analysis, and Optimization. John Wiley and Sons, Hoboken, NJ.

Yanez, F. (1984). Reducción de organismos, patógenos, y diseño de lagunas de estabilización en países en desarrollo. XIX Congreso Interamericano de Ingeniería Sanitaria y Ambiental (AIDIS), Santiago, Chile.

Yanez, F. (1993). Lagunas de Estabilización: Teoria, Diseño, Evaluación, y Mantenimiento. Instituto Ecuatoriano de Obras Sanitarias, Ministerio de Salud Pública, Quito, Ecuador.

Zhenbin, W., Yicheng, X., Jiaqi, D., Qijun, K., Facui, Z., Xitao, C. (1993). Studies on wastewater treatment by means of integrated biological pond system: design and function of macrophytes. Water Science and Technology, 27(1), 97-105.

Zimmerman, J.B., Mihelcic, J.R., Smith, J.A. (2008). Global stressors on water quality and quantity: sustainability, technology selection, and governance in a dynamic world, Environmental Science and Technology, 42(4), 4247-4254.

APPENDIX A: WASTE STABILIZATION POND DATABASE

Table A1: All 50 publications that were collected for the original WSP database

1 Alcalde et al. 2003	25 Lijklema et al. 1986
2 Bausum et al. 1983	26 Lucena et al. 2004
3 Benyahya et al. 1998	27 Macdonald and Ernst 1986
4 Betancour 2013	28 Malherbe and Strickland-Cholmley 1967a
5 Botero et al. 1997	29 Malherbe and Strickland-Cholmley 1967b
6 Campos et al. 2002	30 Morris 1984
7 Ceballos et al. 1995	31 Nupen 1970
8 da Silva et al. 2008	32 Nupen et al. 1974
9 Davies-Colley et al. 2005	33 Ohgaki et al. 1986
10 Donnison and Ross 1995	34 Omura et al. 1985
11 El-Deeb Ghazy et al. 2008	35 Oragui et al. 1995
12 Emparanza- Knörr and Torrella 1995	36 Oragui et al. 1986
13 England et al. 1967	37 Pearson et al. 1995
14 Fattal et al. 1998	38 Pedahzur et al. 1993
15 Hadley 2013	39 Rao et al. 1981
16 Herrera and Castillo 2000	40 Reinoso et al. 2011
17 Hodgson and Paspaliaris 1996	41 Reinoso et al. 2008
18 Iriarte et al. 2013	42 Salter et al. 1999
19 Jenner 2009	43 Sheladia et al. 1982
20 Jurzik et al. 2015	44 Shuval 1970
21 Klock and John 1971	45 Silverman et al., 2013
22 Kott et al. 1973	46 Soler et al. 1995
23 Kott et al. 1978	47 Symonds et al. 2014
24 Lewis et al. 1986	48 Turner and Lewis 1995
	49 Verbyla and Mihelcic 2015
	50 Zhenbin et al.1993

Table A2: All data parameters that were included in the final WSP database and used for data analysis, divided by data point number and pond type

Data Point	Authors	Virus Type	Pond Type	Location	Latitude	Solar Radiation [kWh/m^2/d]	Air Temp	Surface Area (m^2)	L (m)	W(m)	D (m)	Vol. (m^3)	Q (m^3/d)	HRT (d)
1	El-Deeb Ghazy et al (2008)	Coliphage	Anaerobic	El-Mofti Kafr El-Sheikh, Egypt	31.3000	5.62	21.3	475	23.75	20.00	3.00	1,425.0	232.0	6.1
2	Oragui et al (1987)	Culturable Enteric Virus	Anaerobic	EXTRABES, Campina Grande, Brazil	-7.2306	5.58	25.1	7	6.00	1.10	3.40	22.4	22.4	1.0
3	Bausum et al. (1983)	Culturable Enteric Virus	Anaerobic	Kermit, TX, USA	31.8185	5.56	21.5	10,434	222	47	2.60	27,128	3,000.0	9.0
4	Bausum et al. (1983)	Culturable Enteric Virus	Anaerobic	Kermit, TX, USA	31.8185	4.23	9.0	10,434	222	47	2.60	27,128	3,000.0	9.0
5	Bausum et al. (1983)	Culturable Enteric Virus	Anaerobic	Kermit, TX, USA	31.8185	6.23	17.4	10,434	222	47	2.60	27,128	3,000.0	9.0
6	Iriarte et al. (2013)	F-specific coliphage	Anaerobic	Punata, Cochabamba, Bolivia	-17.5733	5.38	15.7	783	29	27	2.0	1566	2,730.2	0.6
7	Iriarte et al. (2013)	F-specific coliphage	Anaerobic	Arani, Cochabamba, Bolivia	-17.5668	5.38	15.7	210	14	15	2.00	420	747.9	0.6
8	Alcalde et al (2003)	F-specific coliphage	Anaerobic	Arad, Israel	31.2560	6.37	24.6	2,300	50	46	5.00	11,500	5,750.0	2.0
9	Alcalde et al (2003)	F-specific coliphage	Anaerobic	Arad, Israel	31.2560	3.52	14.5	2,300	50	46	5.00	11,500	5,750.0	2.0
10	Verbyla and Mihelcic (2015)	F-specific coliphage - MS2	Anaerobic	Belding, Michigan, USA	43.0804	5.32	18.4	4,371	93	47	3.00	13,113	6,434.5	2.0
11	Oragui et al (1987)	Rotavirus	Anaerobic	EXTRABES, Campina Grande, Brazil	-7.2306	5.58	25.1	7	6.0	1.1	3.40	22	22.4	1.0
12	Oragui et al (1995) andPearson et al. (1995)	Rotavirus	Anaerobic	Catingueira, Campina Grande, Brazil	-7.2306	5.34	25.0	8	4.90	1.65	2.50	20.2	20.2	1.0
13	Oragui et al (1995) and Pearson et al. (1995)	Rotavirus	Anaerobic	Catingueira, Campina Grande, Brazil	-7.2306	5.34	25.0	8	4.90	1.65	2.50	20.2	20.2	1.0

Table A2: Continued

Data Point	Authors	Virus Type	Pond Type	Location	Latitude	Solar Radiation [kWh/m2/d]	Air Temp	Surface Area (m2)	L (m)	W(m)	D (m)	Vol. (m3)	Q (m3/d)	HRT (d)
14	Oragui et al (1995) and Pearson et al. (1995)	Rotavirus	Anaerobic	Catingueira, Campina Grande, Brazil	-7.2306	5.34	25.0	2	1.80	1.20	1.50	3.2	3.2	1.0
15	El-Deeb Ghazy et al (2008)	Rotavirus	Anaerobic	El-Mofti Kafr El-Sheikh, Egypt	31.3000	2.80	16.4	475	24	20	3.00	1,425	232.0	6.1
16	El-Deeb Ghazy et al (2008)	Rotavirus	Anaerobic	El-Mofti Kafr El-Sheikh, Egypt	31.3000	3.04	14.8	475	24	20	3.00	1,425	232.0	6.1
17	El-Deeb Ghazy et al (2008)	Rotavirus	Anaerobic	El-Mofti Kafr El-Sheikh, Egypt	31.3000	3.94	14.4	475	24	20	3.00	1,425	232.0	6.1
18	Alcalde et al (2003)	Somatic coliphage	Anaerobic	Arad, Israel	31.2560	6.37	24.6	2,300	50	46	5.00	11,500	5,750.0	2.0
19	Alcalde et al (2003)	Somatic coliphage	Anaerobic	Arad, Israel	31.2560	3.52	14.5	2,300	50	46	5.00	11,500	5,750.0	2.0
20	Emparanza-Knorr and Torrella (1995)	Somatic coliphage	Anaerobic	Guardamar del Segura, Spain	38.0897	5.04	18.1	2,100	70	30	2.00	4,200	3,000.0	1.4
21	Soler et al (1995)	Somatic coliphage	Anaerobic	Lorqui-Ceuti, Spain	38.0651	5.08	18.7	2,178	52	42	3.00	6,533	6,050.0	1.1
22	Soler et al (1995)	Somatic coliphage	Anaerobic	San Javier, Murcia, Spain	37.8000	5.05	18.4	1,650	65	25	4.00	3,830	9,200.0	0.4
23	Reinoso et al. (2011)	Somatic coliphage	Anaerobic	Fresno de la Vega, Leon, Spain	42.3363	2.42	5.1	335	15	15	3.75	1,256	3,200.0	0.4
24	Reinoso et al. (2011)	Somatic coliphage	Anaerobic	Fresno de la Vega, Leon, Spain	42.3363	6.05	18.7	335	15	15	3.75	1,256	3,200.0	0.4

Table A2: Continued

Data Point	Authors	Virus Type	Pond Type	K_v CMFM	K_v PFM	K_v DFM	Ci (viruses /L)	Ce (viruses /L)	Log10 Removal	% Removal	d (2005)	SVLR (viruses / ha day)	VVLR (viruses / m3 day
1	El-Deeb Ghazy et al (2008)	Coliphage	Anaerobic	0.09	0.07	0.08	149	98	0.2	34.7%	0.842	729.7	0.02
2	Oragui et al (1987)	Culturable Enteric Virus	Anaerobic	0.67	0.51	0.55	10000	6000	0.2	40.0%	0.183	340,000.0	10.00
3	Bausum et al. (1983)	Culturable Enteric Virus	Anaerobic	2.54	0.35	0.55	180	7.5	1.4	95.8%	0.212	517.5	0.02
4	Bausum et al. (1983)	Culturable Enteric Virus	Anaerobic	0.38	0.16	0.21	148	33.6	0.6	77.3%	0.212	425.5	0.02
5	Bausum et al. (1983)	Culturable Enteric Virus	Anaerobic	0.38	0.16	0.21	52	11.7	0.6	77.5%	0.212	149.5	0.01
6	Iriarte et al. (2013)	F-specific coliphage	Anaerobic	10.60	3.41	6.52	2.24E+04	3.16E+03	0.9	85.9%	0.931	780,618.9	39.03
7	Iriarte et al. (2013)	F-specific coliphage	Anaerobic	17.30	4.22	9.44	1.05E+05	9.77E+03	1.0	90.7%	1.071	3,729,165.8	186.46
8	Alcalde et al (2003)	F-specific coliphage	Anaerobic	3.96	1.09	2.22	2.95E+06	3.31E+05	0.9	88.8%	0.920	73,780,230.7	1475.60
9	Alcalde et al (2003)	F-specific coliphage	Anaerobic	1.27	0.63	0.98	1.41E+06	3.98E+05	0.6	71.8%	0.920	35,313,438.6	706.27
10	Verbyla and Mihelcic (2015)	F-specific coliphage - MS2	Anaerobic	0.14	0.12	0.13	1.45E+02	1.12E+02	0.1	22.4%	0.505	2,127.8	0.07
11	Oragui et al (1987)	Rotavirus	Anaerobic	3.00	1.39	1.68	8.00E+02	2.00E+02	0.6	75.0%	0.183	27,200.0	0.80
12	Oragui et al (1995) andPearson et al. (1995)	Rotavirus	Anaerobic	2.00	1.10	1.39	5.10E+04	1.70E+04	0.5	66.7%	0.337	1,275,000.0	51.00
13	Oragui et al (1995) and Pearson et al. (1995)	Rotavirus	Anaerobic	2.00	1.10	1.39	5.10E+04	1.70E+04	0.5	66.7%	0.337	1,275,000.0	51.00

Table A2: Continued

Data Point	Authors	Virus Type	Pond Type	Kv CMFM	Kv PFM	Kv DFM	Ci (viruses /L)	Ce (viruses /L)	Log10 Removal	% Removal	d (2005)	SVLR (viruses / ha day)	VVLR (viruses / m3 day
14	Oragui et al (1995) and Pearson et al. (1995)	Rotavirus	Anaerobic	2.68	1.30	1.93	1.40E+05	3.80E+04	0.6	72.9%	0.667	2,100,000.0	140.00
15	El-Deeb Ghazy et al (2008)	Rotavirus	Anaerobic	1.47	0.37	0.77	1.00E+04	1.00E+03	1.0	90.0%	0.842	48,842.1	1.63
16	El-Deeb Ghazy et al (2008)	Rotavirus	Anaerobic	16.12	0.75	2.53	1.00E+06	1.00E+04	2.0	99.0%	0.842	4,884,210.5	162.81
17	El-Deeb Ghazy et al (2008)	Rotavirus	Anaerobic	16.12	0.75	2.53	1.00E+04	1.00E+02	2.0	99.0%	0.842	48,842.1	1.63
18	Alcalde et al (2003)	Somatic coliphage	Anaerobic	3.12	0.99	1.90	3.55E+06	4.90E+05	0.9	86.2%	0.920	88,703,347.3	1774.07
19	Alcalde et al (2003)	Somatic coliphage	Anaerobic	1.68	0.74	1.21	1.66E+06	3.80E+05	0.6	77.1%	0.920	41,489,672.7	829.79
20	Emparanza-Knorr and Torrella (1995)	Somatic coliphage	Anaerobic	0.30	0.25	0.27	1.00E+05	7.08E+04	0.2	29.2%	0.429	1,428,571.4	71.43
21	Soler et al (1995)	Somatic coliphage	Anaerobic	0.35	0.30	0.33	5.28E+04	3.82E+04	0.1	27.7%	0.805	1,466,816.3	48.89
22	Soler et al (1995)	Somatic coliphage	Anaerobic	1.61	1.23	1.40	5.05E+04	3.02E+04	0.2	40.2%	0.391	2,815,757.6	121.31
23	Reinoso et al. (2011)	Somatic coliphage	Anaerobic	2.54	1.76	2.26	100	50.118723	0.3	49.9%	1.000	9,552.2	0.25
24	Reinoso et al. (2011)	Somatic coliphage	Anaerobic	0.66	0.59	0.64	199526.23	158489.32	0.1	20.6%	1.000	19,059,222.1	508.25

Table A2: Continued

Data Point	Authors	Virus Type	Pond Type	Location	Latitude	Solar Radiation [kWh/m^2/d]	Air Temp	Surface Area (m^2)	L (m)	W(m)	D (m)	Vol. (m^3)	Q (m^3/d)	HRT (d)
25	Campos et al (2002)	B. fragilis coliphage	Facultative	Choconta, Colombia	5.1500	5.11	18.9	11,025	105	105	2.50	27,000	1,555.0	17.4
26	Campos et al (2002)	B. fragilis coliphage	Facultative	Choconta, Colombia	5.1500	4.84	18.9	16,275	155	105	2.00	33,000	1,555.0	21.2
27	El-Deeb Ghazy et al (2008)	coliphage	Facultative	El-Mofti Kafr El-Sheikh, Egypt	31.3000	5.62	21.3	1,050	48	22	1.50	1,575	232.0	6.8
28	Omura et al (1985)	coliphage	Facultative	Bangkok, Thailand	14.0208	4.83	25.8	1,138	50	23	2.20	2,503	312.8	8.0
29	Botero et al (1997)	coliphage	Facultative	Maracaibo, Venezuela	10.6500	5.11	25.5	1,972	68	29	2.55	5,029	457.1	11.0
30	Botero et al (1997)	coliphage	Facultative	Maracaibo, Venezuela	10.6500	5.11	25.5	2,262	78	29	2.64	5,972	542.9	11.0
31	Botero et al (1997)	coliphage	Facultative	Maracaibo, Venezuela	10.6500	5.11	25.5	2,523	87	29	2.64	6,661	605.5	11.0
32	Herrera and Castillo (2000)	Somatic coliphage	Facultative	La Esmeralda, Melipilla, Chile	-33.6253	6.61	18.2	18,750	250	75	1.80	32,040	2,237.8	5.3
33	Herrera and Castillo (2000)	Somatic coliphage	Facultative	La Esmeralda, Melipilla, Chile	-33.6253	6.61	18.2	20,000	250	80	1.80	34,020	2,237.8	5.5
34	Herrera and Castillo (2000)	Somatic coliphage	Facultative	La Esmeralda, Melipilla, Chile	-33.6253	3.49	12.3	18,750	250	75	1.80	32,040	2,648.2	4.9
35	Herrera and Castillo (2000)	Somatic coliphage	Facultative	La Esmeralda, Melipilla, Chile	-33.6253	3.49	12.3	20,000	250	80	1.80	34,020	2,648.2	4.8
36	Reinoso et al (2008)	coliphage	Facultative	Cubillas de los Oteros, Leon, Spain	42.6056	4.43	11.8	1,073	24	44	1.60	1,717	20.0	75.9
37	Zhenbin et al. (1993)	coliphage	Facultative	Huangzhou City, Hubei Province, China	30.4399	3.67	16.5	390	30	13	1.39	542	125.0	4.3

Table A2: Continued

Data Point	Authors	Virus Type	Pond Type	K_v CMFM	K_v PFM	K_v DFM	Ci (viruses /L)	Ce (viruses /L)	Log10 Removal	% Removal	d (2005)	SVLR (viruses / ha day)	VVLR (viruses / m3 day
25	Campos et al (2002)	B. fragilis coliphage	Facultative	0.86	0.16	0.39	1.58E+03	1.00E+02	1.2	93.7%	1.000	0.86	0.16
26	Campos et al (2002)	B. fragilis coliphage	Facultative	1.01	0.15	0.34	1.78E+02	7.94E+00	1.4	95.5%	0.677	1.01	0.15
27	El-Deeb Ghazy et al (2008)	coliphage	Facultative	0.09	0.07	0.08	97.6	61.6	0.2	36.9%	0.456	0.09	0.07
28	Omura et al (1985)	coliphage	Facultative	10.38	0.55	1.35	2.10E+06	2.50E+04	1.9	98.8%	0.455	10.38	0.55
29	Botero et al (1997)	coliphage	Facultative	0.31	0.13	0.19	70.0	16.0	0.6	77.1%	0.426	0.31	0.13
30	Botero et al (1997)	coliphage	Facultative	0.33	0.14	0.20	70.0	15.0	0.7	78.6%	0.372	0.33	0.14
31	Botero et al (1997)	coliphage	Facultative	0.40	0.15	0.22	70.0	13.0	0.7	81.4%	0.333	0.40	0.15
32	Herrera and Castillo (2000)	Somatic coliphage	Facultative	8.94	0.73	1.39	1.00E+06	2.07E+04	1.7	97.9%	0.300	8.94	0.73
33	Herrera and Castillo (2000)	Somatic coliphage	Facultative	20.37	0.86	1.87	1.00E+06	8.85E+03	2.1	99.1%	0.320	20.37	0.86
34	Herrera and Castillo (2000)	Somatic coliphage	Facultative	1.29	0.41	0.59	1.00E+06	1.36E+05	0.9	86.4%	0.300	1.29	0.41
35	Herrera and Castillo (2000)	Somatic coliphage	Facultative	1.25	0.41	0.59	1.00E+06	1.43E+05	0.8	85.7%	0.320	1.25	0.41
36	Reinoso et al (2008)	coliphage	Facultative	0.01	0.01	0.01	7.24E+04	3.34E+04	0.3	53.9%	1.804	0.01	0.01
37	Zhenbin et al. (1993)	coliphage	Facultative	50.08	1.24	n/a	1.20E+03	5.50E+00	2.3	99.5%	0.433	50.08	1.24

Table A2: Continued

Data Point	Authors	Virus Type	Pond Type	Location	Latitude	Solar Radiation [kWh/m^2/d]	Air Temp	Surface Area (m^2)	L (m)	W(m)	D (m)	Vol. (m^3)	Q (m^3/d)	HRT (d)
38	Zhenbin et al. (1993)	coliphage	Facultative	Huangzhou City, Hubei Province, China	30.4399	3.67	16.5	390	30	13	1.39	542	125.0	4.3
39	Zhenbin et al. (1993)	coliphage	Facultative	Huangzhou City, Hubei Province, China	30.4399	3.67	16.5	390	30	13	1.39	542	250.0	2.2
40	Rao et al (1981)	Culturable Enteric Virus	Facultative	Nagpur, Maharashtra, India	21.1500	4.40	26.3	418	27	15	0.91	382	35.4	10.8
41	Rao et al (1981)	Culturable Enteric Virus	Facultative	Nagpur, Maharashtra, India	21.1500	5.13	25.5	418	27	15	0.91	382	35.4	10.8
42	Rao et al (1981)	Culturable Enteric Virus	Facultative	Nagpur, Maharashtra, India	21.1500	5.13	25.5	418	27	15	0.91	382	35.4	10.8
43	Rao et al (1981)	Culturable Enteric Virus	Facultative	Nagpur, Maharashtra, India	21.1500	5.13	25.5	418	27	15	0.91	382	35.4	10.8
44	Rao et al (1981)	Culturable Enteric Virus	Facultative	Nagpur, Maharashtra, India	21.1500	5.13	25.5	418	27	15	0.91	382	35.4	10.8
45	Rao et al (1981)	Culturable Enteric Virus	Facultative	Nagpur, Maharashtra, India	21.1500	4.80	22.9	418	27	15	0.91	382	35.4	10.8
46	Rao et al (1981)	Culturable Enteric Virus	Facultative	Nagpur, Maharashtra, India	21.1500	4.80	22.9	418	27	15	0.91	382	35.4	10.8
47	Rao et al (1981)	Culturable Enteric Virus	Facultative	Nagpur, Maharashtra, India	21.1500	4.49	20.0	418	27	15	0.91	382	35.4	10.8
48	Rao et al (1981)	Culturable Enteric Virus	Facultative	Nagpur, Maharashtra, India	21.1500	4.49	20.0	418	27	15	0.91	382	35.4	10.8
49	Rao et al (1981)	Culturable Enteric Virus	Facultative	Nagpur, Maharashtra, India	21.1500	4.49	20.0	418	27	15	0.91	382	35.4	10.8
50	Rao et al (1981)	Culturable Enteric Virus	Facultative	Nagpur, Maharashtra, India	21.1500	4.49	20.0	418	27	15	0.91	382	35.4	10.8

Table A2: Continued

Data Point	Authors	Virus Type	Pond Type	K_v CMFM	K_v PFM	K_v DFM	Ci (viruses /L)	Ce (viruses /L)	Log10 Removal	% Removal	d (2005)	SVLR (viruses / ha day)	VVLR (viruses / m3 day
38	Zhenbin et al. (1993)	coliphage	Facultative	0.19	0.14	n/a	5.5	3	0.3	45.5%	0.433	17.6	0.00
39	Zhenbin et al. (1993)	coliphage	Facultative	0.09	0.08	n/a	3	2.5	0.1	16.7%	0.433	19.2	0.00
40	Rao et al (1981)	Culturable Enteric Virus	Facultative	32.94	0.54	1.80	1.21E+03	3.40E+00	2.6	99.7%	0.556	1,027.0	0.11
41	Rao et al (1981)	Culturable Enteric Virus	Facultative	0.29	0.13	0.20	674	162	0.6	76.0%	0.556	570.7	0.06
42	Rao et al (1981)	Culturable Enteric Virus	Facultative	0.58	0.18	0.31	290	40	0.9	86.2%	0.556	245.5	0.03
43	Rao et al (1981)	Culturable Enteric Virus	Facultative	0.23	0.12	0.16	112	32	0.5	71.4%	0.556	94.8	0.01
44	Rao et al (1981)	Culturable Enteric Virus	Facultative	4.07	0.35	0.85	4500	100	1.7	97.8%	0.556	3,810.0	0.42
45	Rao et al (1981)	Culturable Enteric Virus	Facultative	1.96	0.29	0.61	1175	53	1.3	95.5%	0.556	994.8	0.11
46	Rao et al (1981)	Culturable Enteric Virus	Facultative	2.03	0.29	0.62	620	27	1.4	95.6%	0.556	524.9	0.06
47	Rao et al (1981)	Culturable Enteric Virus	Facultative	0.06	0.05	0.05	100	60	0.2	40.0%	0.556	84.7	0.01
48	Rao et al (1981)	Culturable Enteric Virus	Facultative	0.20	0.11	0.14	725	232	0.5	68.0%	0.556	613.8	0.07
49	Rao et al (1981)	Culturable Enteric Virus	Facultative	0.07	0.05	0.06	63	36	0.2	42.9%	0.556	53.3	0.01
50	Rao et al (1981)	Culturable Enteric Virus	Facultative	1.02	0.23	0.43	41	3.4	1.1	91.7%	0.556	34.7	0.00

Table A2: Continued

Data Point	Authors	Virus Type	Pond Type	Location	Latitude	Solar Radiation [kWh/m^2/d]	Air Temp	Surface Area (m^2)	L (m)	W(m)	D (m)	Vol. (m^3)	Q (m^3/d)	HRT (d)
51	Rao et al (1981)	Culturable Enteric Virus	Facultative	Nagpur, Maharashtra, India	21.1500	5.50	23.7	418	27	15	0.91	382	35.4	10.8
52	Rao et al (1981)	Culturable Enteric Virus	Facultative	Nagpur, Maharashtra, India	21.1500	5.50	23.7	418	27	15	0.91	382	35.4	10.8
53	Rao et al (1981)	Culturable Enteric Virus	Facultative	Nagpur, Maharashtra, India	21.1500	5.50	23.7	418	27	15	0.91	382	35.4	10.8
54	Rao et al (1981)	Culturable Enteric Virus	Facultative	Nagpur, Maharashtra, India	21.1500	6.22	28.7	418	27	15	0.91	382	35.4	10.8
55	Rao et al (1981)	Culturable Enteric Virus	Facultative	Nagpur, Maharashtra, India	21.1500	6.77	32.7	418	27	15	0.91	382	35.4	10.8
56	Rao et al (1981)	Culturable Enteric Virus	Facultative	Nagpur, Maharashtra, India	21.1500	4.95	30.3	418	27	15	0.91	382	35.4	10.8
57	Rao et al (1981)	Culturable Enteric Virus	Facultative	Nagpur, Maharashtra, India	21.1500	4.95	30.3	418	27	15	0.91	382	35.4	10.8
58	Rao et al (1981)	Culturable Enteric Virus	Facultative	Nagpur, Maharashtra, India	21.1500	3.89	26.7	418	27	15	0.91	382	35.4	10.8
59	Rao et al (1981)	Culturable Enteric Virus	Facultative	Nagpur, Maharashtra, India	21.1500	3.72	25.8	418	27	15	0.91	382	35.4	10.8
60	Rao et al (1981)	Culturable Enteric Virus	Facultative	Nagpur, Maharashtra, India	21.1500	5.13	25.5	418	27	15	1.22	510	42.5	12.0
61	Rao et al (1981)	Culturable Enteric Virus	Facultative	Nagpur, Maharashtra, India	21.1500	5.13	25.5	418	27	15	1.22	510	42.5	12.0
62	Rao et al (1981)	Culturable Enteric Virus	Facultative	Nagpur, Maharashtra, India	21.1500	5.13	25.5	418	27	15	1.22	510	42.5	12.0
63	Rao et al (1981)	Culturable Enteric Virus	Facultative	Nagpur, Maharashtra, India	21.1500	4.80	22.9	418	27	15	1.22	510	42.5	12.0

Table A2: Continued

Data Point	Authors	Virus Type	Pond Type	K_v CMFM	K_v PFM	K_v DFM	Ci (viruses /L)	Ce (viruses /L)	Log10 Removal	% Removal	d (2005)	SVLR (viruses / ha day)	VVLR (viruses / m3 day
51	Rao et al (1981)	Culturable Enteric Virus	Facultative	0.14	0.08	0.11	100	40	0.4	60.0%	0.556	84.7	0.01
52	Rao et al (1981)	Culturable Enteric Virus	Facultative	0.12	0.08	0.10	100	43	0.4	57.0%	0.556	84.7	0.01
53	Rao et al (1981)	Culturable Enteric Virus	Facultative	0.52	0.17	0.29	263	40	0.8	84.8%	0.556	222.7	0.02
54	Rao et al (1981)	Culturable Enteric Virus	Facultative	0.38	0.15	0.24	375	73	0.7	80.5%	0.556	317.5	0.03
55	Rao et al (1981)	Culturable Enteric Virus	Facultative	2.18	0.30	0.64	1475	60	1.4	95.9%	0.556	1,248.8	0.14
56	Rao et al (1981)	Culturable Enteric Virus	Facultative	2.33	0.30	0.66	157	6	1.4	96.2%	0.556	132.9	0.01
57	Rao et al (1981)	Culturable Enteric Virus	Facultative	3.38	0.34	0.79	750	20	1.6	97.3%	0.556	635.0	0.07
58	Rao et al (1981)	Culturable Enteric Virus	Facultative	3.70	0.34	0.82	2825	69	1.6	97.6%	0.556	2,391.8	0.26
59	Rao et al (1981)	Culturable Enteric Virus	Facultative	3.27	0.33	0.77	4100	113	1.6	97.2%	0.556	3,471.3	0.38
60	Rao et al (1981)	Culturable Enteric Virus	Facultative	2.26	0.28	0.62	674	24	1.4	96.4%	0.556	684.8	0.06
61	Rao et al (1981)	Culturable Enteric Virus	Facultative	0.75	0.19	0.35	290	29	1.0	90.0%	0.556	294.6	0.02
62	Rao et al (1981)	Culturable Enteric Virus	Facultative	6.61	0.37	0.97	4500	56	1.9	98.8%	0.556	4,572.0	0.38
63	Rao et al (1981)	Culturable Enteric Virus	Facultative	2.64	0.29	0.66	1175	36	1.5	96.9%	0.556	1,193.8	0.10

Table A2: Continued

Data Point	Authors	Virus Type	Pond Type	Location	Latitude	Solar Radiation [kWh/m^2/d]	Air Temp	Surface Area (m^2)	L (m)	W(m)	D (m)	Vol. (m^3)	Q (m^3/d)	HRT (d)
64	Rao et al (1981)	Culturable Enteric Virus	Facultative	Nagpur, Maharashtra, India	21.1500	4.80	22.9	418	27	15	1.22	510	42.5	12.0
65	Rao et al (1981)	Culturable Enteric Virus	Facultative	Nagpur, Maharashtra, India	21.1500	4.49	20.0	418	27	15	1.22	510	42.5	12.0
66	Rao et al (1981)	Culturable Enteric Virus	Facultative	Nagpur, Maharashtra, India	21.1500	4.49	20.0	418	27	15	1.22	510	42.5	12.0
67	Rao et al (1981)	Culturable Enteric Virus	Facultative	Nagpur, Maharashtra, India	21.1500	4.49	20.0	418	27	15	1.22	510	42.5	12.0
68	Rao et al (1981)	Culturable Enteric Virus	Facultative	Nagpur, Maharashtra, India	21.1500	4.49	20.0	418	27	15	1.22	510	42.5	12.0
69	Rao et al (1981)	Culturable Enteric Virus	Facultative	Nagpur, Maharashtra, India	21.1500	4.62	20.5	418	27	15	1.22	510	42.5	12.0
70	Rao et al (1981)	Culturable Enteric Virus	Facultative	Nagpur, Maharashtra, India	21.1500	5.50	23.7	418	27	15	1.22	510	42.5	12.0
71	Rao et al (1981)	Culturable Enteric Virus	Facultative	Nagpur, Maharashtra, India	21.1500	6.22	28.7	418	27	15	1.22	510	42.5	12.0
72	Rao et al (1981)	Culturable Enteric Virus	Facultative	Nagpur, Maharashtra, India	21.1500	6.77	32.7	418	27	15	1.22	510	42.5	12.0
73	Rao et al (1981)	Culturable Enteric Virus	Facultative	Nagpur, Maharashtra, India	21.1500	4.95	30.3	418	27	15	1.22	510	42.5	12.0
74	Rao et al (1981)	Culturable Enteric Virus	Facultative	Nagpur, Maharashtra, India	21.1500	4.95	30.3	418	27	15	1.22	510	42.5	12.0
75	Rao et al (1981)	Culturable Enteric Virus	Facultative	Nagpur, Maharashtra, India	21.1500	3.89	26.7	418	27	15	1.22	510	42.5	12.0
76	Rao et al (1981)	Culturable Enteric Virus	Facultative	Nagpur, Maharashtra, India	21.1500	3.72	25.8	418	27	15	1.22	510	42.5	12.0

Table A2: Continued

Data Point	Authors	Virus Type	Pond Type	K_v CMFM	K_v PFM	K_v DFM	Ci (viruses /L)	Ce (viruses /L)	Log10 Removal	% Removal	d (2005)	SVLR (viruses / ha day)	VVLR (viruses / m3 day
64	Rao et al (1981)	Culturable Enteric Virus	Facultative	3.36	0.31	0.74	620	15	1.6	97.6%	0.556	629.9	0.05
65	Rao et al (1981)	Culturable Enteric Virus	Facultative	0.19	0.10	0.14	100	30	0.5	70.0%	0.556	101.6	0.01
66	Rao et al (1981)	Culturable Enteric Virus	Facultative	0.67	0.18	0.33	725	80	1.0	89.0%	0.556	736.6	0.06
67	Rao et al (1981)	Culturable Enteric Virus	Facultative	1.23	0.23	0.46	63	4	1.2	93.7%	0.556	64.0	0.01
68	Rao et al (1981)	Culturable Enteric Virus	Facultative	0.92	0.21	0.39	41	3.4	1.1	91.7%	0.556	41.7	0.00
69	Rao et al (1981)	Culturable Enteric Virus	Facultative	19.67	0.46	1.43	237	1	2.4	99.6%	0.556	240.8	0.02
70	Rao et al (1981)	Culturable Enteric Virus	Facultative	0.36	0.14	0.22	263	50	0.7	81.0%	0.556	267.2	0.02
71	Rao et al (1981)	Culturable Enteric Virus	Facultative	0.78	0.20	0.36	375	36	1.0	90.4%	0.556	381.0	0.03
72	Rao et al (1981)	Culturable Enteric Virus	Facultative	2.48	0.29	0.65	1475	48	1.5	96.7%	0.556	1,498.6	0.12
73	Rao et al (1981)	Culturable Enteric Virus	Facultative	1.55	0.25	0.52	157	8	1.3	94.9%	0.556	159.5	0.01
74	Rao et al (1981)	Culturable Enteric Virus	Facultative	10.33	0.40	1.15	750	6	2.1	99.2%	0.556	762.0	0.06
75	Rao et al (1981)	Culturable Enteric Virus	Facultative	8.64	0.39	1.08	2825	27	2.0	99.0%	0.556	2,870.2	0.24
76	Rao et al (1981)	Culturable Enteric Virus	Facultative	5.09	0.34	0.88	4100	66	1.8	98.4%	0.556	4,165.6	0.34

Table A2: Continued

Data Point	Authors	Virus Type	Pond Type	Location	Latitude	Solar Radiation [kWh/m^2/d]	Air Temp	Surface Area (m^2)	L (m)	W(m)	D (m)	Vol. (m^3)	Q (m^3/d)	HRT (d)
77	Rao et al (1981)	Culturable Enteric Virus	Facultative	Nagpur, Maharashtra, India	21.1500	3.72	25.8	418	27	15	1.22	510	42.5	12.0
78	Rao et al (1981)	Culturable Enteric Virus	Facultative	Nagpur, Maharashtra, India	21.1500	4.40	26.3	418	27	15	1.52	637	47.2	13.5
79	Rao et al (1981)	Culturable Enteric Virus	Facultative	Nagpur, Maharashtra, India	21.1500	5.13	25.5	418	27	15	1.52	637	47.2	13.5
80	Rao et al (1981)	Culturable Enteric Virus	Facultative	Nagpur, Maharashtra, India	21.1500	5.13	25.5	418	27	15	1.52	637	47.2	13.5
81	Rao et al (1981)	Culturable Enteric Virus	Facultative	Nagpur, Maharashtra, India	21.1500	5.13	25.5	418	27	15	1.52	637	47.2	13.5
82	Rao et al (1981)	Culturable Enteric Virus	Facultative	Nagpur, Maharashtra, India	21.1500	4.80	22.9	418	27	15	1.52	637	47.2	13.5
83	Rao et al (1981)	Culturable Enteric Virus	Facultative	Nagpur, Maharashtra, India	21.1500	4.49	20.0	418	27	15	1.52	637	47.2	13.5
84	Rao et al (1981)	Culturable Enteric Virus	Facultative	Nagpur, Maharashtra, India	21.1500	4.49	20.0	418	27	15	1.52	637	47.2	13.5
85	Rao et al (1981)	Culturable Enteric Virus	Facultative	Nagpur, Maharashtra, India	21.1500	4.49	20.0	418	27	15	1.52	637	47.2	13.5
86	Rao et al (1981)	Culturable Enteric Virus	Facultative	Nagpur, Maharashtra, India	21.1500	4.49	20.0	418	27	15	1.52	637	47.2	13.5
87	Rao et al (1981)	Culturable Enteric Virus	Facultative	Nagpur, Maharashtra, India	21.1500	4.62	20.5	418	27	15	1.52	637	47.2	13.5
88	Rao et al (1981)	Culturable Enteric Virus	Facultative	Nagpur, Maharashtra, India	21.1500	4.62	20.5	418	27	15	1.52	637	47.2	13.5
89	Rao et al (1981)	Culturable Enteric Virus	Facultative	Nagpur, Maharashtra, India	21.1500	5.50	23.7	418	27	15	1.52	637	47.2	13.5

Table A2: Continued

Data Point	Authors	Virus Type	Pond Type	K_v CMFM	K_v PFM	K_v DFM	Ci (viruses /L)	Ce (viruses /L)	Log10 Removal	% Removal	d (2005)	SVLR (viruses / ha day)	VVLR (viruses / m3 day
77	Rao et al (1981)	Culturable Enteric Virus	Facultative	5.48	0.35	0.91	1002	15	1.8	98.5%	0.556	1,018.0	0.08
78	Rao et al (1981)	Culturable Enteric Virus	Facultative	7.41	0.34	0.94	1.21E+03	1.20E+01	2.0	99.0%	0.556	1,369.3	0.09
79	Rao et al (1981)	Culturable Enteric Virus	Facultative	1.24	0.21	0.43	674	38	1.2	94.4%	0.556	760.9	0.05
80	Rao et al (1981)	Culturable Enteric Virus	Facultative	1.19	0.21	0.42	290	17	1.2	94.1%	0.556	327.4	0.02
81	Rao et al (1981)	Culturable Enteric Virus	Facultative	13.81	0.39	1.17	4500	24	2.3	99.5%	0.556	5,080.0	0.33
82	Rao et al (1981)	Culturable Enteric Virus	Facultative	2.16	0.25	0.57	1175	39	1.5	96.7%	0.556	1,326.4	0.09
83	Rao et al (1981)	Culturable Enteric Virus	Facultative	0.07	0.05	0.06	100	50	0.3	50.0%	0.556	112.9	0.01
84	Rao et al (1981)	Culturable Enteric Virus	Facultative	0.37	0.13	0.22	725	120	0.8	83.4%	0.556	818.4	0.05
85	Rao et al (1981)	Culturable Enteric Virus	Facultative	0.31	0.12	0.19	63	12	0.7	81.0%	0.556	71.1	0.00
86	Rao et al (1981)	Culturable Enteric Virus	Facultative	0.53	0.16	0.27	41	5	0.9	87.8%	0.556	46.3	0.00
87	Rao et al (1981)	Culturable Enteric Virus	Facultative	1.31	0.22	0.45	150	8	1.3	94.7%	0.556	169.3	0.01
88	Rao et al (1981)	Culturable Enteric Virus	Facultative	17.48	0.41	1.27	237	1	2.4	99.6%	0.556	267.5	0.02
89	Rao et al (1981)	Culturable Enteric Virus	Facultative	0.39	0.14	0.22	100	16	0.8	84.0%	0.556	112.9	0.01

Table A2: Continued

Data Point	Authors	Virus Type	Pond Type	Location	Latitude	Solar Radiation [kWh/m^2/d]	Air Temp	Surface Area (m^2)	L (m)	W(m)	D (m)	Vol. (m^3)	Q (m^3/d)	HRT (d)
90	Rao et al (1981)	Culturable Enteric Virus	Facultative	Nagpur, Maharashtra, India	21.1500	5.50	23.7	418	27	15	1.52	637	47.2	13.5
91	Rao et al (1981)	Culturable Enteric Virus	Facultative	Nagpur, Maharashtra, India	21.1500	5.50	23.7	418	27	15	1.52	637	47.2	13.5
92	Rao et al (1981)	Culturable Enteric Virus	Facultative	Nagpur, Maharashtra, India	21.1500	6.22	28.7	418	27	15	1.52	637	47.2	13.5
93	Rao et al (1981)	Culturable Enteric Virus	Facultative	Nagpur, Maharashtra, India	21.1500	6.77	32.7	418	27	15	1.52	637	47.2	13.5
94	Rao et al (1981)	Culturable Enteric Virus	Facultative	Nagpur, Maharashtra, India	21.1500	4.95	30.3	418	27	15	1.52	637	47.2	13.5
95	Rao et al (1981)	Culturable Enteric Virus	Facultative	Nagpur, Maharashtra, India	21.1500	4.95	30.3	418	27	15	1.52	637	47.2	13.5
96	Rao et al (1981)	Culturable Enteric Virus	Facultative	Nagpur, Maharashtra, India	21.1500	3.89	26.7	418	27	15	1.52	637	47.2	13.5
97	Rao et al (1981)	Culturable Enteric Virus	Facultative	Nagpur, Maharashtra, India	21.1500	3.72	25.8	418	27	15	1.52	637	47.2	13.5
98	Rao et al (1981)	Culturable Enteric Virus	Facultative	Nagpur, Maharashtra, India	21.1500	3.72	25.8	418	27	15	1.52	637	47.2	13.5
99	Rao et al (1981)	Culturable Enteric Virus	Facultative	Bhilai, Chhattisgarh, India	21.2100	5.44	23.0	51,213	320	160	1.22	62,439	34,070.0	1.8
100	Rao et al (1981)	Culturable Enteric Virus	Facultative	Bhilai, Chhattisgarh, India	21.2100	6.19	27.5	51,213	320	160	1.22	62,439	28,390.0	2.2
101	Rao et al (1981)	Culturable Enteric Virus	Facultative	Bhilai, Chhattisgarh, India	21.2100	6.71	30.9	51,213	320	160	1.22	62,439	28,390.0	2.2
102	Rao et al (1981)	Culturable Enteric Virus	Facultative	Bhilai, Chhattisgarh, India	21.2100	6.58	32.8	51,213	320	160	1.22	62,439	28,390.0	2.2

Table A2: Continued

Data Point	Authors	Virus Type	Pond Type	K_v CMFM	K_v PFM	K_v DFM	Ci (viruses /L)	Ce (viruses /L)	Log10 Removal	% Removal	d (2005)	SVLR (viruses / ha day)	VVLR (viruses / m3 day
90	Rao et al (1981)	Culturable Enteric Virus	Facultative	0.16	0.09	0.12	100	31	0.5	69.0%	0.556	112.9	0.01
91	Rao et al (1981)	Culturable Enteric Virus	Facultative	0.40	0.14	0.23	263	41	0.8	84.4%	0.556	296.9	0.02
92	Rao et al (1981)	Culturable Enteric Virus	Facultative	0.85	0.19	0.35	375	30	1.1	92.0%	0.556	423.3	0.03
93	Rao et al (1981)	Culturable Enteric Virus	Facultative	2.25	0.26	0.58	1475	47	1.5	96.8%	0.556	1,665.1	0.11
94	Rao et al (1981)	Culturable Enteric Virus	Facultative	1.38	0.22	0.46	157	8	1.3	94.9%	0.556	177.2	0.01
95	Rao et al (1981)	Culturable Enteric Virus	Facultative	1.61	0.23	0.49	750	33	1.4	95.6%	0.556	846.7	0.06
96	Rao et al (1981)	Culturable Enteric Virus	Facultative	2.61	0.27	0.62	2825	78	1.6	97.2%	0.556	3,189.1	0.21
97	Rao et al (1981)	Culturable Enteric Virus	Facultative	37.89	0.46	1.61	4100	8	2.7	99.8%	0.556	4,628.4	0.30
98	Rao et al (1981)	Culturable Enteric Virus	Facultative	1.38	0.22	0.46	1002	51	1.3	94.9%	0.556	1,131.1	0.07
99	Rao et al (1981)	Culturable Enteric Virus	Facultative	19.64	1.97	4.41	3.33E+02	9.00E+00	1.6	97.3%	0.500	2,215.3	0.18
100	Rao et al (1981)	Culturable Enteric Virus	Facultative	12.81	1.53	3.30	350	12	1.5	96.6%	0.500	1,940.2	0.16
101	Rao et al (1981)	Culturable Enteric Virus	Facultative	8.41	1.35	2.70	117	6	1.3	94.9%	0.500	648.6	0.05
102	Rao et al (1981)	Culturable Enteric Virus	Facultative	8.26	1.34	2.67	1150	60	1.3	94.8%	0.500	6,375.1	0.52

Table A2: Continued

Data Point	Authors	Virus Type	Pond Type	Location	Latitude	Solar Radiation [kWh/m^2/d]	Air Temp	Surface Area (m^2)	L (m)	W(m)	D (m)	Vol. (m^3)	Q (m^3/d)	HRT (d)
103	Oragui et al (1987)	Culturable Enteric Virus	Facultative	EXTRABES, Campina Grande, Brazil	-7.2306	5.58	25.1	4.5	3.0	1.5	3.35	15	3.0	5.0
104	Bausum et al. (1983)	Culturable Enteric Virus	Facultative	Jonestown, MS, USA	34.3208	5.09	23.5	16,900	130	130	1.95	32,955	450.0	73.2
105	Bausum et al. (1983)	Culturable Enteric Virus	Facultative	Jonestown, MS, USA	34.3208	5.09	23.5	14,884	122	122	1.95	29,024	450.0	64.5
106	Bausum et al. (1983)	Culturable Enteric Virus	Facultative	Jonestown, MS, USA	34.3208	3.18	7.6	16,900	130	130	1.95	32,955	450.0	73.2
107	Bausum et al. (1983)	Culturable Enteric Virus	Facultative	Jonestown, MS, USA	34.3208	3.18	7.6	14,884	122	122	1.95	29,024	450.0	64.5
108	Bausum et al. (1983)	Culturable Enteric Virus	Facultative	Jonestown, MS, USA	34.3208	4.95	16.6	16,900	130	130	1.95	32,955	450.0	73.2
109	Bausum et al. (1983)	Culturable Enteric Virus	Facultative	Jonestown, MS, USA	34.3208	4.95	16.6	14,884	122	122	1.95	29,024	450.0	64.5
110	Bausum et al. (1983)	Culturable Enteric Virus	Facultative	Shelby, MS, USA	33.9431	5.10	23.8	45,796	214	214	1.20	54,955	1,140.0	48.2
111	Bausum et al. (1983)	Culturable Enteric Virus	Facultative	Shelby, MS, USA	33.9431	3.25	8.9	45,796	214	214	1.20	54,955	1,140.0	48.2
112	Bausum et al. (1983)	Culturable Enteric Virus	Facultative	Shelby, MS, USA	33.9431	4.96	17.4	45,796	214	214	1.20	54,955	1,140.0	48.2
113	Bausum et al. (1983)	Culturable Enteric Virus	Facultative	El Paso, TX, USA	31.9487	5.64	21.4	409,944	744	551	1.50	614,916	23,000.0	26.7
114	Bausum et al. (1983)	Culturable Enteric Virus	Facultative	El Paso, TX, USA	31.9487	4.43	8.2	409,944	744	551	1.50	614,916	23,000.0	26.7
115	Bausum et al. (1983)	Culturable Enteric Virus	Facultative	El Paso, TX, USA	31.9487	6.52	16.8	409,944	744	551	1.50	614,916	23,000.0	26.7

Table A2: Continued

Data Point	Authors	Virus Type	Pond Type	K_v CMFM	K_v PFM	K_v DFM	Ci (viruses /L)	Ce (viruses /L)	Log10 Removal	% Removal	d (2005)	SVLR (viruses / ha day)	VVLR (viruses / m3 day
103	Oragui et al (1987)	Culturable Enteric Virus	Facultative	1.00	0.36	0.57	6.00E+03	1.00E+03	0.8	83.3%	0.500	40,320.0	1.20
104	Bausum et al. (1983)	Culturable Enteric Virus	Facultative	1.45	0.06	0.24	514	4.8	2.0	99.1%	1.000	136.9	0.01
105	Bausum et al. (1983)	Culturable Enteric Virus	Facultative	0.36	0.05	0.13	4.8	0.2	1.4	95.8%	1.000	1.5	0.00
106	Bausum et al. (1983)	Culturable Enteric Virus	Facultative	0.38	0.05	0.13	32	1.1	1.5	96.6%	1.000	8.5	0.00
107	Bausum et al. (1983)	Culturable Enteric Virus	Facultative	0.04	0.02	0.03	1.1	0.3	0.6	72.7%	1.000	0.3	0.00
108	Bausum et al. (1983)	Culturable Enteric Virus	Facultative	2.25	0.07	0.28	83	0.5	2.2	99.4%	1.000	22.1	0.00
109	Bausum et al. (1983)	Culturable Enteric Virus	Facultative	0.01	0.01	0.01	0.5	0.3	0.2	40.0%	1.000	0.2	0.00
110	Bausum et al. (1983)	Culturable Enteric Virus	Facultative	1.27	0.09	0.29	791	12.7	1.8	98.4%	1.000	196.9	0.02
111	Bausum et al. (1983)	Culturable Enteric Virus	Facultative	0.39	0.06	0.16	52	2.6	1.3	95.0%	1.000	12.9	0.00
112	Bausum et al. (1983)	Culturable Enteric Virus	Facultative	3.64	0.11	0.44	53	0.3	2.2	99.4%	1.000	13.2	0.00
113	Bausum et al. (1983)	Culturable Enteric Virus	Facultative	2.79	0.16	0.49	348	4.6	1.9	98.7%	0.741	195.2	0.01
114	Bausum et al. (1983)	Culturable Enteric Virus	Facultative	1.21	0.13	0.34	87	2.6	1.5	97.0%	0.741	48.8	0.00
115	Bausum et al. (1983)	Culturable Enteric Virus	Facultative	0.16	0.06	0.10	74	14.3	0.7	80.7%	0.741	41.5	0.00

Table A2: Continued

Data Point	Authors	Virus Type	Pond Type	Location	Latitude	Solar Radiation [kWh/m^2/d]	Air Temp	Surface Area (m^2)	L (m)	W(m)	D (m)	Vol. (m^3)	Q (m^3/d)	HRT (d)
116	Bausum et al. (1983)	Culturable Enteric Virus	Facultative	Kermit, TX, USA	31.8185	5.56	21.5	17,272	127	136	2.60	44,907	3,000.0	15.0
117	Bausum et al. (1983)	Culturable Enteric Virus	Facultative	Kermit, TX, USA	31.8185	4.23	9.0	17,272	127	136	2.60	44,907	3,000.0	15.0
118	Bausum et al. (1983)	Culturable Enteric Virus	Facultative	Kermit, TX, USA	31.8185	6.23	17.4	17,272	127	136	2.60	44,907	3,000.0	15.0
119	Bausum et al. (1983)	Culturable Enteric Virus	Facultative	Beresford, SD, USA	43.0951	4.75	16.8	33,150	255	130	1.70	56,355	1,140.0	49.4
120	Bausum et al. (1983)	Culturable Enteric Virus	Facultative	Beresford, SD, USA	43.0951	4.75	16.8	30,000	250	120	1.70	51,000	1,140.0	44.7
121	Bausum et al. (1983)	Culturable Enteric Virus	Facultative	Beresford, SD, USA	43.0951	2.61	-5.6	33,150	255	130	1.70	56,355	1,140.0	49.4
122	Bausum et al. (1983)	Culturable Enteric Virus	Facultative	Beresford, SD, USA	43.0951	2.61	-5.6	30,000	250	120	1.70	51,000	1,140.0	44.7
123	Bausum et al. (1983)	Culturable Enteric Virus	Facultative	Beresford, SD, USA	43.0951	4.58	7.5	33,150	255	130	1.70	56,355	1,140.0	49.4
124	Bausum et al. (1983)	Culturable Enteric Virus	Facultative	Beresford, SD, USA	43.0951	4.58	7.5	30,000	250	120	1.70	51,000	1,140.0	44.7
125	Bausum et al. (1983)	Culturable Enteric Virus	Facultative	Lennox, SD, USA	43.3466	4.75	16.8	21,000	140	150	1.50	31,500	760.0	41.4
126	Bausum et al. (1983)	Culturable Enteric Virus	Facultative	Lennox, SD, USA	43.3466	2.61	-5.6	21,000	140	150	1.50	31,500	760.0	41.4
127	Bausum et al. (1983)	Culturable Enteric Virus	Facultative	Lennox, SD, USA	43.3466	4.58	7.5	21,000	140	150	1.50	31,500	760.0	41.4
128	Zhenbin et al. (1993)	Culturable Enteric Virus	Facultative	Huangzhou City, Hubei Province, China	30.4399	3.67	16.5	390	30	13	1.39	542	125.0	4.3

Table A2: Continued

Data Point	Authors	Virus Type	Pond Type	K_v CMFM	K_v PFM	K_v DFM	Ci (viruses /L)	Ce (viruses /L)	Log10 Removal	% Removal	d (2005)	SVLR (viruses / ha day)	VVLR (viruses / m3 day
116	Bausum et al. (1983)	Culturable Enteric Virus	Facultative	0.32	0.12	0.22	7.5	1.3	0.8	82.7%	1.071	13.0	0.00
117	Bausum et al. (1983)	Culturable Enteric Virus	Facultative	0.18	0.09	0.14	33.6	9.1	0.6	72.9%	1.071	58.4	0.00
118	Bausum et al. (1983)	Culturable Enteric Virus	Facultative	0.07	0.05	0.06	11.7	5.6	0.3	52.1%	1.071	20.3	0.00
119	Bausum et al. (1983)	Culturable Enteric Virus	Facultative	3.15	0.10	0.29	94	0.6	2.2	99.4%	0.510	32.3	0.00
120	Bausum et al. (1983)	Culturable Enteric Virus	Facultative	0.00	0.00	0.00	0.6	0.5	0.1	16.7%	0.480	0.2	0.00
121	Bausum et al. (1983)	Culturable Enteric Virus	Facultative	0.04	0.02	0.03	44	15.2	0.5	65.5%	0.510	15.1	0.00
122	Bausum et al. (1983)	Culturable Enteric Virus	Facultative	0.13	0.04	0.07	15.2	2.2	0.8	85.5%	0.480	5.8	0.00
123	Bausum et al. (1983)	Culturable Enteric Virus	Facultative	0.49	0.07	0.14	50	2	1.4	96.0%	0.510	17.2	0.00
124	Bausum et al. (1983)	Culturable Enteric Virus	Facultative	0.09	0.04	0.05	2	0.4	0.7	80.0%	0.480	0.8	0.00
125	Bausum et al. (1983)	Culturable Enteric Virus	Facultative	7.79	0.14	0.66	162	0.5	2.5	99.7%	1.071	58.6	0.00
126	Bausum et al. (1983)	Culturable Enteric Virus	Facultative	0.44	0.07	0.19	216	11.3	1.3	94.8%	1.071	78.2	0.01
127	Bausum et al. (1983)	Culturable Enteric Virus	Facultative	0.32	0.06	0.15	17	1.2	1.2	92.9%	1.071	6.2	0.00
128	Zhenbin et al. (1993)	Culturable Enteric Virus	Facultative	0.13	0.10	n/a	1.32	0.84	0.2	36.4%	0.433	4.2	0.00

Table A2: Continued

Data Point	Authors	Virus Type	Pond Type	Location	Latitude	Solar Radiation [kWh/m^2/d]	Air Temp	Surface Area (m^2)	L (m)	W(m)	D (m)	Vol. (m^3)	Q (m^3/d)	HRT (d)
129	Zhenbin et al. (1993)	Culturable Enteric Virus	Facultative	Outskirts of Huangzhou City, Hubei Province, China	30.4399	3.67	16.5	390	30	13	1.39	542	125.0	4.3
130	Symonds et al. (2014)	Culturable Enteric Virus	Facultative	Yungas, Bolivia	-15.6517	3.92	16.9	1,375	50	28	1.80	2,475	150.0	16.5
131	Malherbe and Coetzee (1965); Malherbe and Strickland-Cholmley (1967b)	Culturable Enteric Virus	Facultative	Olifantsvlei, Johannesburg, South Africa	-26.3331	5.60	17.9	1,012	41	21	1.22	1,050	54.5	19.3
132	Malherbe and Coetzee (1965); Malherbe and Strickland-Cholmley (1967b)	Culturable Enteric Virus	Facultative	Olifantsvlei, Johannesburg, South Africa	-26.3331	5.60	17.9	506	21	20	1.22	512	54.5	9.4
133	Malherbe and Coetzee (1965); Malherbe and Strickland-Cholmley (1967b)	Culturable Enteric Virus	Facultative	Olifantsvlei, Johannesburg, South Africa	-26.3331	5.60	17.9	253	21	10	1.22	256	54.5	4.7
134	Lewis et al. (1986)	Culturable Enteric Virus	Facultative	Near Christchurch, New Zealand	-43.497	3.67	11.2	10,000	160	63	1.30	13,000	228.6	38.0
135	Pedahzur et al. (1993)	F-specific coliphage	Facultative	Sha'alvim, Israel	31.872	5.57	19.8	1,500	50	30	1.00	1,500	300.0	5.0
136	Pedahzur et al. (1993)	F-specific coliphage	Facultative	Sha'alvim, Israel	31.872	5.57	19.8	1,500	50	30	1.00	1,500	300.0	5.0
137	Pedahzur et al. (1993)	F-specific coliphage	Facultative	Sha'alvim, Israel	31.872	5.57	19.8	1,500	50	30	1.00	1,500	300.0	5.0
138	Pedahzur et al. (1993)	F-specific coliphage	Facultative	Sha'alvim, Israel	31.872	5.57	19.8	1,500	50	30	1.00	1,500	300.0	5.0
139	Campos et al (2002)	F-specific coliphage - RNA	Facultative	Choconta, Colombia	5.1500	5.11	18.9	11,025	105	105	2.50	27,000	1,555.0	17.4
140	Campos et al (2002)	F-specific coliphage - RNA	Facultative	Choconta, Colombia	5.1500	5.11	18.9	16,275	155	105	2.00	33,000	1,555.0	21.2
141	Campos et al (2002)	F-specific coliphage - RNA	Facultative	Choconta, Colombia	5.1500	4.84	18.9	11,025	105	105	2.50	27,000	1,555.0	17.4

Table A2: Continued

Data Point	Authors	Virus Type	Pond Type	K_v CMFM	K_v PFM	K_v DFM	Ci (viruses /L)	Ce (viruses /L)	Log10 Removal	% Removal	d (2005)	SVLR (viruses / ha day)	VVLR (viruses / m3 day
129	Zhenbin et al. (1993)	Culturable Enteric Virus	Facultative	0.37	0.22	n/a	0.84	0.32	0.4	61.9%	0.433	2.7	0.00
130	Symonds et al. (2014)	Culturable Enteric Virus	Facultative	2.25	0.22	0.52	42	1.1	1.6	97.4%	0.550	45.8	0.00
131	Malherbe and Coetzee (1965); Malherbe and Strickland-Cholmley (1967b)	Culturable Enteric Virus	Facultative	1.14	0.16	0.34	3455	150	1.4	95.7%	0.512	1,861.7	0.18
132	Malherbe and Coetzee (1965); Malherbe and Strickland-Cholmley (1967b)	Culturable Enteric Virus	Facultative	0.46	0.18	0.32	150	28	0.7	81.3%	0.952	161.6	0.02
133	Malherbe and Coetzee (1965); Malherbe and Strickland-Cholmley (1967b)	Culturable Enteric Virus	Facultative	0.24	0.16	0.20	32	15	0.3	53.1%	0.476	69.0	0.01
134	Lewis et al. (1986)	Culturable Enteric Virus	Facultative	0.02	0.02	0.02	190.54607	102.3293	0.3	46.3%	0.391	43.6	0.00
135	Pedahzur et al. (1993)	F-specific coliphage	Facultative	1.12	0.38	0.63	630.00	95.35	0.8	84.9%	0.600	1,260.0	0.13
136	Pedahzur et al. (1993)	F-specific coliphage	Facultative	1.01	0.36	0.59	630.00	104.55	0.8	83.4%	0.600	1,260.0	0.13
137	Pedahzur et al. (1993)	F-specific coliphage	Facultative	1.39	0.41	0.73	630.00	79.31	0.9	87.4%	0.600	1,260.0	0.13
138	Pedahzur et al. (1993)	F-specific coliphage	Facultative	1.06	0.37	0.61	630.00	99.85	0.8	84.2%	0.600	1,260.0	0.13
139	Campos et al (2002)	F-specific coliphage - RNA	Facultative	2.24	0.21	0.64	100000	2511.8864	1.6	97.5%	1.000	141,043.1	5.76
140	Campos et al (2002)	F-specific coliphage - RNA	Facultative	0.10	0.05	0.08	2.51E+03	7.94E+02	0.5	68.4%	0.677	2,400.0	0.12
141	Campos et al (2002)	F-specific coliphage - RNA	Facultative	0.76	0.15	0.36	6.31E+04	4.47E+03	1.2	92.9%	1.000	88,992.2	3.63

Table A2: Continued

Data Point	Authors	Virus Type	Pond Type	Location	Latitude	Solar Radiation [kWh/m^2/d]	Air Temp	Surface Area (m^2)	L (m)	W(m)	D (m)	Vol. (m^3)	Q (m^3/d)	HRT (d)
142	Campos et al (2002)	F-specific coliphage - RNA	Facultative	Choconta, Colombia	5.1500	4.84	18.9	16,275	155	105	2.00	33,000	1,555.0	21.2
143	Alcalde et al (2003)	F-specific coliphage	Facultative	Arad, Israel	31.2560	6.37	24.6	29,000	200	145	2.50	72,500	8,529.4	8.5
144	Alcalde et al (2003)	F-specific coliphage	Facultative	Arad, Israel	31.2560	3.52	14.5	29,000	200	145	2.50	72,500	8,529.4	8.5
145	Verbyla and Mihelcic (2015)	F-specific coliphage - MS2	Facultative	Belding, Michigan, USA	43.0804	5.32	18.4	68,798	443	155	2.00	137,596	6,434.5	21.4
146	Verbyla and Mihelcic (2015)	F-specific coliphage - MS2	Facultative	Belding, Michigan, USA	43.0804	5.32	18.4	60,704	372	163	2.00	121,272	6,434.5	18.8
147	Da Silva et al. (2008)	Norovirus GI	Facultative	Daoulas, Northwest France	48.3585	3.23	10.6	10,000	120	83	0.80	8,000	300.0	13.3
148	Oragui et al (1987)	Rotavirus	Facultative	EXTRABES, Campina Grande, Brazil	-7.2306	5.58	25.1	5	3	2	3.35	15	3.0	5.0
149	Oragui et al (1995) and Pearson et al. (1995)	Rotavirus	Facultative	Catingueira, Campina Grande, Brazil	-7.2306	5.34	25.0	26	13	2	1.00	26	8.6	3.0
150	Oragui et al (1995) and Pearson et al. (1995)	Rotavirus	Facultative	Catingueira, Brazil	-7.2306	5.34	25.0	26	12.90	2.00	1.33	34.3	8.6	4.0
151	Oragui et al (1995) and Pearson et al. (1995)	Rotavirus	Facultative	Catingueira, Brazil	-7.2306	5.34	25.0	26	12.90	2.00	1.67	43.1	8.6	5.0
152	Oragui et al (1995) and Pearson et al. (1995)	Rotavirus	Facultative	Catingueira, Brazil	-7.2306	5.34	25.0	26	12.90	2.00	2.00	51.6	8.6	6.0
153	Oragui et al (1995) and Pearson et al. (1995)	Rotavirus	Facultative	Catingueira, Brazil	-7.2306	5.34	25.0	24	4.90	4.90	2.00	48.0	8.0	6.0
154	Oragui et al (1995) and Pearson et al. (1995)	Rotavirus	Facultative	Catingueira, Brazil	-7.2306	5.34	25.0	4	4	1	1.50	6	3.2	2.0

Table A2: Continued

Data Point	Authors	Virus Type	Pond Type	K_v CMFM	K_v PFM	K_v DFM	Ci (viruses /L)	Ce (viruses /L)	Log10 Removal	% Removal	d (2005)	SVLR (viruses / ha day)	VVLR (viruses / m3 day
142	Campos et al (2002)	F-specific coliphage - RNA	Facultative	10.50	0.25	0.88	4.47E+03	2.00E+01	2.4	99.6%	0.677	4,267.9	0.21
143	Alcalde et al (2003)	F-specific coliphage	Facultative	0.88	0.25	0.47	331131.12	38904.514	0.9	88.3%	0.725	973,915.1	38.96
144	Alcalde et al (2003)	F-specific coliphage	Facultative	0.45	0.18	0.30	3.98E+05	8.32E+04	0.7	79.1%	0.725	1,170,903.4	46.84
145	Verbyla and Mihelcic (2015)	F-specific coliphage - MS2	Facultative	0.73	0.13	0.23	112.20185	6.7608298	1.2	94.0%	0.351	104.9	0.01
146	Verbyla and Mihelcic (2015)	F-specific coliphage - MS2	Facultative	0.66	0.14	0.25	6.76E+00	5.01E-01	1.1	92.6%	0.438	7.2	0.00
147	Da Silva et al. (2008)	Norovirus GI	Facultative	0.11	0.05	0.08	39810717	10000000	0.6	74.9%	0.694	11,943,215.1	1492.90
148	Oragui et al (1987)	Rotavirus	Facultative	0.37	0.21	0.28	200	70	0.5	65.0%	0.500	1,344.0	0.04
149	Oragui et al (1995) and Pearson et al. (1995)	Rotavirus	Facultative	0.37	0.25	0.27	17000	8100	0.3	52.4%	0.155	56,666.7	5.67
150	Oragui et al (1995) and Pearson et al. (1995)	Rotavirus	Facultative	0.18	0.13	0.14	1.70E+04	1.00E+04	0.2	41.2%	0.155	56,525.0	4.25
151	Oragui et al (1995) and Pearson et al. (1995)	Rotavirus	Facultative	0.29	0.18	0.20	1.70E+04	6.90E+03	0.4	59.4%	0.155	56,780.0	3.40
152	Oragui et al (1995) and Pearson et al. (1995)	Rotavirus	Facultative	0.35	0.19	0.22	1.70E+04	5.50E+03	0.5	67.6%	0.155	56,666.7	2.83
153	Oragui et al (1995) and Pearson et al. (1995)	Rotavirus	Facultative	0.44	0.21	0.34	1.70E+04	4.70E+03	0.6	72.4%	1.000	56,666.7	2.83
154	Oragui et al (1995) and Pearson et al. (1995)	Rotavirus	Facultative	0.36	0.27	0.31	38,000	22,000	0.2	42.1%	0.333	285,000.0	19.00

Table A2: Continued

Data Point	Authors	Virus Type	Pond Type	Location	Latitude	Solar Radiation [kWh/m^2/d]	Air Temp	Surface Area (m^2)	L (m)	W(m)	D (m)	Vol. (m^3)	Q (m^3/d)	HRT (d)
155	El-Deeb Ghazy et al (2008)	Rotavirus	Facultative	El-Mofti Kafr El-Sheikh, Egypt	31.3000	2.80	16.4	1,056	48	22	1.50	1,584	232.0	6.8
156	El-Deeb Ghazy et al (2008)	Rotavirus	Facultative	El-Mofti Kafr El-Sheikh, Egypt	31.3000	3.04	14.8	1,050	48	22	1.50	1,575	232.0	6.8
157	El-Deeb Ghazy et al (2008)	Rotavirus	Facultative	El-Mofti Kafr El-Sheikh, Egypt	31.3000	3.94	14.4	1,050	48	22	1.50	1,575	232.0	6.8
158	Alcalde et al (2003)	Somatic coliphage	Facultative	Arad, Israel	31.2560	6.37	24.6	29,000	200	145	2.50	72,500	8,529.4	8.5
159	Alcalde et al (2003)	Somatic coliphage	Facultative	Arad, Israel	31.2560	3.52	14.5	29,000	200	145	2.50	72,500	8,529.4	8.5
160	Emparanza-Knorr and Torrella (1995)	Somatic coliphage	Facultative	Guardamar del Segura, Spain	38.0897	5.04	18.1	16,875	225	75	2.00	33,750	3,000.0	11.3
161	Campos et al (2002)	Somatic coliphage	Facultative	Choconta, Colombia	5.1500	5.11	18.9	11,025	105	105	2.50	27,000	1,555.0	17.4
162	Campos et al (2002)	Somatic coliphage	Facultative	Choconta, Colombia	5.1500	5.11	18.9	16,275	155	105	2.00	33,000	1,555.0	21.2
163	Campos et al (2002)	Somatic coliphage	Facultative	Choconta, Colombia	5.1500	4.84	18.9	11,025	105	105	2.50	27,000	1,555.0	17.4
164	Campos et al (2002)	Somatic coliphage	Facultative	Choconta, Colombia	5.1500	4.84	18.9	16,275	155	105	2.00	33,000	1,555.0	21.2
165	Soler et al (1995)	Somatic coliphage	Facultative	Lorqui-Ceuti, Spain	38.0651	5.08	18.7	13,825	248	56	2.00	27,650	6,050.0	4.6
166	Soler et al (1995)	Somatic coliphage	Facultative	Lorqui-Ceuti, Spain	38.0651	5.08	18.7	29,950	360	83	2.00	59,900	6,050.0	9.9
167	Soler et al (1995)	Somatic coliphage	Facultative	San Javier, Murcia, Spain	37.8000	5.05	18.4	29,000	395	73	2.00	47,000	9,200.0	5.1

Table A2: Continued

Data Point	Authors	Virus Type	Pond Type	K_v CMFM	K_v PFM	K_v DFM	Ci (viruses /L)	Ce (viruses /L)	Log10 Removal	% Removal	d (2005)	SVLR (viruses / ha day)	VVLR (viruses / m3 day
155	El-Deeb Ghazy et al (2008)	Rotavirus	Facultative	1.32	0.34	0.58	1000	100	1.0	90.0%	0.458	2,197.0	0.15
156	El-Deeb Ghazy et al (2008)	Rotavirus	Facultative	1.33	0.34	0.58	1.00E+04	1.00E+03	1.0	90.0%	0.456	22,095.2	1.47
157	El-Deeb Ghazy et al (2008)	Rotavirus	Facultative	1.33	0.34	0.58	1.00E+02	10	1.0	90.0%	0.456	221.0	0.01
158	Alcalde et al (2003)	Somatic coliphage	Facultative	0.43	0.18	0.29	489778.82	104712.85	0.7	78.6%	0.725	1,440,525.9	57.62
159	Alcalde et al (2003)	Somatic coliphage	Facultative	0.06	0.05	0.05	3.80E+05	2.57E+05	0.2	32.4%	0.725	1,118,204.1	44.73
160	Emparanza-Knorr and Torrella (1995)	Somatic coliphage	Facultative	0.14	0.08	0.10	70766.77	27976.39	0.4	60.5%	0.333	125,807.6	6.29
161	Campos et al (2002)	Somatic coliphage	Facultative	0.59	0.14	0.31	6.31E+04	5.62E+03	1.1	91.1%	1.000	88,992.2	3.63
162	Campos et al (2002)	Somatic coliphage	Facultative	0.02	0.02	0.02	5.62E+03	3.98E+03	0.2	29.2%	0.677	5,372.9	0.26
163	Campos et al (2002)	Somatic coliphage	Facultative	1.39	0.19	0.51	3.98E+05	1.58E+04	1.4	96.0%	1.000	561,502.6	22.93
164	Campos et al (2002)	Somatic coliphage	Facultative	74.63	0.35	1.56	1.58E+04	1.00E+01	3.2	99.9%	0.677	15,142.9	0.75
165	Soler et al (1995)	Somatic coliphage	Facultative	0.23	0.16	0.18	38200	18450	0.3	51.7%	0.225	167,168.2	8.36
166	Soler et al (1995)	Somatic coliphage	Facultative	0.11	0.07	0.08	1.85E+04	8.90E+03	0.3	51.8%	0.231	37,269.6	1.86
167	Soler et al (1995)	Somatic coliphage	Facultative	0.19	0.13	0.14	30200	15500	0.3	48.7%	0.186	95,806.9	5.91

Table A2: Continued

Data Point	Authors	Virus Type	Pond Type	Location	Latitude	Solar Radiation [kWh/m^2/d]	Air Temp	Surface Area (m^2)	L (m)	W(m)	D (m)	Vol. (m^3)	Q (m^3/d)	HRT (d)
168	Soler et al (1995)	Somatic coliphage	Facultative	San Javier, Murcia, Spain	37.8000	5.05	18.4	29,000	395	73	2.00	47,000	9,200.0	5.1
169	Ceballos et al (1995)	Somatic coliphage	Facultative	Sape, Paraiba, Brazil	-7.0948	5.86	25.7	26,000	200	130	2.20	57,200	950.4	60.2
170	Reinoso et al. (2011)	Somatic coliphage	Facultative	Fresno de la Vega, Leon, Spain	42.3363	2.42	5.1	8,481	142	70	2.00	16,962	3,200.0	4.1
171	Reinoso et al. (2011)	Somatic coliphage	Facultative	Fresno de la Vega, Leon, Spain	42.3363	6.05	18.7	8,481	142	70	2.00	16,962	3,200.0	4.1
172	El-Deeb Ghazy et al (2008)	coliphage	Maturation	El-Mofti Kafr El-Sheikh, Egypt	31.3000	5.62	21.3	635	32	20	1.40	889	232.0	3.8
173	Omura et al (1985)	coliphage	Maturation	Bangkok, Thailand	14.0208	4.83	25.8	4,800	120	40	1.30	6,240	312.0	20.0
174	Ohgaki et al. (1986)	coliphage	Maturation	Bangkok, Thailand	14.0208	5.56	27.0	4,800	120	40	1.30	6,240	312.0	20.0
175	Botero et al (1997)	coliphage	Maturation	Maracaibo, Venezuela	10.6500	5.11	25.5	1,500	60	25	1.40	2,100	636.4	3.3
176	Botero et al (1997)	coliphage	Maturation	Maracaibo, Venezuela	10.6500	5.11	25.5	1,500	60	25	1.40	2,100	420.0	5.0
177	Botero et al (1997)	coliphage	Maturation	Maracaibo, Venezuela	10.6500	5.11	25.5	1,500	60	25	1.40	2,100	636.4	3.3
178	Botero et al (1997)	coliphage	Maturation	Maracaibo, Venezuela	10.6500	5.11	25.5	1,500	60	25	1.40	2,100	420.0	5.0
179	Botero et al (1997)	coliphage	Maturation	Maracaibo, Venezuela	10.6500	5.11	25.5	1,500	60	25	1.40	2,100	636.4	3.3
180	Botero et al (1997)	coliphage	Maturation	Maracaibo, Venezuela	10.6500	5.11	25.5	1,500	60	25	1.40	2,100	420.0	5.0

Table A2: Continued

Data Point	Authors	Virus Type	Pond Type	K_v CMFM	K_v PFM	K_v DFM	Ci (viruses /L)	Ce (viruses /L)	Log10 Removal	% Removal	d (2005)	SVLR (viruses / ha day)	VVLR (viruses / m3 day
168	Soler et al (1995)	Somatic coliphage	Facultative	0.13	0.10	0.11	1.55E+04	9.29E+03	0.2	40.1%	0.186	49,172.4	3.03
169	Ceballos et al (1995)	Somatic coliphage	Facultative	2.41	0.08	0.26	1.90E+06	1.30E+04	2.2	99.3%	0.650	694,523.1	31.57
170	Reinoso et al. (2011)	Somatic coliphage	Facultative	0.37	0.22	0.29	50.118723	19.952623	0.4	60.2%	0.493	189.1	0.01
171	Reinoso et al. (2011)	Somatic coliphage	Facultative	4.62	0.73	1.46	158489.32	7943.2823	1.3	95.0%	0.493	598,002.4	29.90
172	El-Deeb Ghazy et al (2008)	coliphage	Maturation	0.25	0.18	0.22	61.6	31.4	0.3	49.0%	0.620	225.1	0.02
173	Omura et al (1985)	coliphage	Maturation	0.20	0.08	0.11	25000	5000	0.7	80.0%	0.333	16,250.0	1.25
174	Ohgaki et al. (1986)	coliphage	Maturation	0.35	0.10	0.16	2.00E+03	2.50E+02	0.9	87.5%	0.333	1,300.0	0.10
175	Botero et al (1997)	coliphage	Maturation	0.33	0.23	0.27	16	7.6	0.3	52.5%	0.417	67.9	0.00
176	Botero et al (1997)	coliphage	Maturation	0.12	0.10	0.11	7.6	4.7	0.2	38.2%	0.417	21.3	0.00
177	Botero et al (1997)	coliphage	Maturation	1.03	0.45	0.64	15	3.4	0.6	77.3%	0.417	63.6	0.00
178	Botero et al (1997)	coliphage	Maturation	0.23	0.15	0.18	3.4	1.6	0.3	52.9%	0.417	9.5	0.00
179	Botero et al (1997)	coliphage	Maturation	0.22	0.16	0.19	13	7.6	0.2	41.5%	0.417	55.2	0.00
180	Botero et al (1997)	coliphage	Maturation	0.29	0.18	0.22	7.6	3.1	0.4	59.2%	0.417	21.3	0.00

Table A2: Continued

Data Point	Authors	Virus Type	Pond Type	Location	Latitude	Solar Radiation [kWh/m^2/d]	Air Temp	Surface Area (m^2)	L (m)	W(m)	D (m)	Vol. (m^3)	Q (m^3/d)	HRT (d)
181	Herrera and Castillo (2000)	Somatic coliphage	Maturation	La Esmeralda, Melipilla, Chile	-33.6253	6.61	18.2	23,100	210	110	1.80	40,680	4,475.5	2.2
182	Herrera and Castillo (2000)	Somatic coliphage	Maturation	La Esmeralda,, Chile	-33.6253	3.49	12.3	23,100	210	110	1.80	40,680	5,296.3	1.4
183	Zhenbin et al. (1993)	coliphage	Maturation	Huangzhou City, Hubei Province, China	30.4399	3.67	16.5	450	30	15	1.35	608	250.0	2.4
184	Macdonald & Ernst (1986)	Culturable Enteric Virus	Polishing	West Camden, Australia	-33.6150	4.55	16.0	12,210	407	30	1.00	12,210	900.0	7.0
185	Macdonald & Ernst (1986)	Culturable Enteric Virus	Polishing	West Camden, Australia	-33.6150	4.55	16.0	17,200	430	40	1.00	17,200	900.0	16.0
186	Oragui et al (1987)	Culturable Enteric Virus	Maturation	EXTRABES, Campina Grande, Brazil	-7.2306	5.58	25.1	5	3	2	3.30	15	3.0	4.9
187	Oragui et al (1987)	Culturable Enteric Virus	Maturation	EXTRABES, Brazil	-7.2306	5.58	25.1	5	3.00	1.80	2.80	15.1	3.0	5.0
188	Oragui et al (1987)	Culturable Enteric Virus	Maturation	EXTRABES, Brazil	-7.2306	5.58	25.1	5	3.00	1.80	2.80	15.1	3.0	5.0
189	Morris (1984)	Culturable Enteric Virus	Polishing	Coventry, U.K.	52.3701	2.73	11.4	67,200	368	183	1	67,200	2,400	28.0
190	Salter et al (1999)	Culturable Enteric Virus	Polishing	Holmwood, U.K.	51.1921	2.72	10.7	1,092	28	39	1.00	1,092	500.0	2.2
191	Bausum et al. (1983)	Culturable Enteric Virus	Maturation	Shelby, MS, USA	33.9431	5.10	23.8	22,940	155	148	1.20	27,528	1,140.0	24.1
192	Bausum et al. (1983)	Culturable Enteric Virus	Maturation	Shelby, MS, USA	33.9431	3.25	8.9	22,940	155	148	1.20	27,528	1,140.0	24.1
193	Bausum et al. (1983)	Culturable Enteric Virus	Maturation	Shelby, MS, USA	33.9431	4.96	17.4	22,940	155	148	1.20	27,528	1,140.0	24.1

Table A2: Continued

Data Point	Authors	Virus Type	Pond Type	K_v CMFM	K_v PFM	K_v DFM	Ci (viruses /L)	Ce (viruses /L)	Log10 Removal	% Removal	d (2005)	SVLR (viruses / ha day)	VVLR (viruses / m3 day
181	Herrera and Castillo (2000)	Somatic coliphage	Maturation	4.87	1.12	2.05	8847.0001	755.74339	1.1	91.5%	0.524	17,140.7	0.97
182	Herrera and Castillo (2000)	Somatic coliphage	Maturation	1.23	0.72	0.94	1.43E+05	5.25E+04	0.4	63.3%	0.524	327,552.4	18.60
183	Zhenbin et al. (1993)	coliphage	Maturation	0.62	0.38		2.5	1	0.4	60.0%	0.500	13.9	0.00
184	Macdonald & Ernst (1986)	Culturable Enteric Virus	Polishing	0.17	0.11	0.12	693	318	0.3	54.1%	0.074	510.9	0.05
185	Macdonald & Ernst (1986)	Culturable Enteric Virus	Polishing	0.37	0.12	0.14	318	47	0.8	85.4%	0.093	166.6	0.02
186	Oragui et al (1987)	Culturable Enteric Virus	Maturation	0.30	0.19	0.24	1000	400	0.4	60.0%	0.500	6,720.0	0.20
187	Oragui et al (1987)	Culturable Enteric Virus	Maturation	1.40	0.42	0.73	400	50	0.9	87.5%	0.600	2,240.0	0.08
188	Oragui et al (1987)	Culturable Enteric Virus	Maturation	0.91	0.34	0.55	50	9	0.7	82.0%	0.600	280.0	0.01
189	Morris (1984)	Culturable Enteric Virus	Polishing	2.82	0.16	n/a	4000	50	1.9	98.8%	0.420	1,428.6	0.14
190	Salter et al (1999)	Culturable Enteric Virus	Polishing	3.40	0.98	2.18	2.95E+01	3.50E+00	0.9	88.1%	1.393	135.1	0.01
191	Bausum et al. (1983)	Culturable Enteric Virus	Maturation	0.62	0.11	0.27	12.7	0.8	1.2	93.7%	0.955	6.3	0.00
192	Bausum et al. (1983)	Culturable Enteric Virus	Maturation	0.11	0.05	0.09	2.6	0.7	0.6	73.1%	0.955	1.3	0.00
193	Bausum et al. (1983)	Culturable Enteric Virus	Maturation	0.02	0.02	0.02	0.3	0.2	0.2	33.3%	0.955	0.1	0.00

Table A2: Continued

Data Point	Authors	Virus Type	Pond Type	Location	Latitude	Solar Radiation [kWh/m^2/d]	Air Temp	Surface Area (m^2)	L (m)	W(m)	D (m)	Vol. (m^3)	Q (m^3/d)	HRT (d)
194	Bausum et al. (1983)	Culturable Enteric Virus	Maturation	El Paso, TX, USA	31.9487	5.64	21.4	130,368	448	291	1.50	195,552	23,000.0	8.5
195	Bausum et al. (1983)	Culturable Enteric Virus	Maturation	El Paso, TX, USA	31.9487	4.43	8.2	130,368	448	291	1.50	195,552	23,000.0	8.5
196	Bausum et al. (1983)	Culturable Enteric Virus	Maturation	El Paso, TX, USA	31.9487	6.52	16.8	130,368	448	291	1.50	195,552	23,000.0	8.5
197	Bausum et al. (1983)	Culturable Enteric Virus	Maturation	Lennox, SD, USA	43.3466	4.75	16.8	20,250	135	150	1.50	30,375	1,140.0	26.6
198	Bausum et al. (1983)	Culturable Enteric Virus	Maturation	Lennox, SD, USA	43.3466	2.61	-5.6	20,250	135	150	1.50	30,375	1,140.0	26.6
199	Bausum et al. (1983)	Culturable Enteric Virus	Maturation	Lennox, SD, USA	43.3466	4.58	7.5	20,250	135	150	1.50	30,375	1,140.0	26.6
200	Malherbe and Strickland-Cholmley (1967b)	Culturable Enteric Virus	Maturation	Olifantsvlei, Johannesburg, South Africa	-26.3331	5.60	17.9	26,100	228	114	1.52	39,776	5,678.0	7.0
201	England et al. (1967)	Culturable Enteric Virus	Polishing	Santee, California, USA	32.831	5.23	18.3	64,750	326	190	1.00	61,940	2,064.7	30.0
202	England et al. (1967)	Culturable Enteric Virus	Polishing	Santee, California, USA	32.831	5.23	18.3	64,750	326	190	1.00	61,940	2,065	30.0
203	Lewis et al. (1986)	Culturable Enteric Virus	Maturation	Near Christchurch, New Zealand	-43.497	3.67	11.2	9,000	100	90	1.40	12,600	228.6	25.0
204	Alcalde et al (2003)	F-specific coliphage	Maturation	Arad, Israel	31.2560	6.37	24.6	25,375	175	145	1.50	38,063	5,075.0	7.5
205	Alcalde et al (2003)	F-specific coliphage	Maturation	Arad, Israel	31.2560	3.52	14.5	25,375	175	145	1.50	38,063	5,075.0	7.5
206	Turner and Lewis (1995)	F-specific coliphage	Polishing	Rosedale, Auckland, New Zealand	-36.8404	4.23	16.1	360,000	900	400	2.78	1,000,000	43,000.0	23.3

Table A2: Continued

Data Point	Authors	Virus Type	Pond Type	K_v CMFM	K_v PFM	K_v DFM	Ci (viruses /L)	Ce (viruses /L)	Log10 Removal	% Removal	d (2005)	SVLR (viruses / ha day)	VVLR (viruses / m3 day
194	Bausum et al. (1983)	Culturable Enteric Virus	Maturation	0.78	0.24	0.43	4.6	0.6	0.9	87.0%	0.650	8.1	0.00
195	Bausum et al. (1983)	Culturable Enteric Virus	Maturation	0.19	0.11	0.15	2.6	1	0.4	61.5%	0.650	4.6	0.00
196	Bausum et al. (1983)	Culturable Enteric Virus	Maturation	1.41	0.30	0.61	14.3	1.1	1.1	92.3%	0.650	25.2	0.00
197	Bausum et al. (1983)	Culturable Enteric Virus	Maturation	0.03	0.02	0.02	0.5	0.3	0.2	40.0%	1.111	0.3	0.00
198	Bausum et al. (1983)	Culturable Enteric Virus	Maturation	0.00	0.00	0.00	11.3	10.5	0.0	7.1%	1.111	6.4	0.00
199	Bausum et al. (1983)	Culturable Enteric Virus	Maturation	0.11	0.05	0.09	1.2	0.3	0.6	75.0%	1.111	0.7	0.00
200	Malherbe and Strickland-Cholmley (1967b)	Culturable Enteric Virus	Maturation	0.61	0.24	n/a	15.31	2.90	0.7	81.1%	0.539	33.3	0.00
201	England et al. (1967)	Culturable Enteric Virus	Polishing	0.53	0.09	0.19	15.08	0.89	1.2	94.1%	0.583	4.8	0.00
202	England et al. (1967)	Culturable Enteric Virus	Polishing	0.49	0.09	0.19	13.74	0.87	0.0	93.7%	0.583	4.4	0.00
203	Lewis et al. (1986)	Culturable Enteric Virus	Maturation	0.12	0.06	0.09	102.33	25.70	0.6	74.9%	0.900	26.0	0.00
204	Alcalde et al (2003)	F-specific coliphage	Maturation	6.25	0.52	1.50	3.89E+04	8.13E+02	1.7	97.9%	0.829	77,809.0	5.19
205	Alcalde et al (2003)	F-specific coliphage	Maturation	0.35	0.17	0.26	8.32E+04	2.29E+04	0.6	72.5%	0.829	166,352.8	11.09
206	Turner and Lewis (1995)	F-specific coliphage	Polishing	5.46	0.21	0.54	5.50E+05	4.30E+03	2.1	99.2%	0.444	656,944.4	23.65

Table A2: Continued

Data Point	Authors	Virus Type	Pond Type	Location	Latitude	Solar Radiation [kWh/m^2/d]	Air Temp	Surface Area (m^2)	L (m)	W(m)	D (m)	Vol. (m^3)	Q (m^3/d)	HRT (d)
207	Turner and Lewis (1995)	F-specific coliphage	Polishing	Rosedale, Auckland, New Zealand	-36.8404	4.23	16.1	53,950	830	65	2.78	149,981	43,000.0	3.5
208	Verbyla and Mihelcic (2015)	F-specific coliphage - MS2	Maturation	Belding, Michigan, USA	43.0804	5.32	18.4	30,352	180	169	1.50	45,528	6,434.5	7.1
209	Da Silva et al. (2008)	Norovirus GI	Maturation	Daoulas, Northwest France	48.3585	3.23	10.6	4,500	130	35	1.20	5,400	300.0	9.0
210	Da Silva et al. (2008)	Norovirus GI	Maturation	Daoulas, Northwest France	48.3585	3.23	10.6	4,800	135	36	1.20	5,760	300.0	9.6
211	Symonds et al. (2014)	Norovirus GI	Maturation	Yungas, Bolivia	-15.6517	3.92	16.9	507	39	13	1.50	761	167.1	4.6
212	Symonds et al. (2014)	Norovirus GI	Maturation	Yungas, Bolivia	-15.6517	3.92	16.9	507	39	13	1.50	761	167.1	4.6
213	Da Silva et al. (2008)	Norovirus GII	Maturation	Daoulas, Northwest France	48.3585	3.23	10.6	4,500	130	35	1.20	5,400	300.0	9.0
214	Da Silva et al. (2008)	Norovirus GII	Maturation	Daoulas, Northwest France	48.3585	3.23	10.6	4,800	135	36	1.20	5,760	300.0	9.6
215	Oragui et al (1987)	Rotavirus	Maturation	EXTRABES, Campina Grande, Brazil	-7.2306	5.58	25.1	5	3	2	3.30	15	3.0	4.9
216	Oragui et al (1987)	Rotavirus	Maturation	EXTRABES, Brazil	-7.2306	5.58	25.1	5	3.00	1.80	2.80	15.1	3.0	5.0
217	Oragui et al (1987)	Rotavirus	Maturation	EXTRABES, Brazil	-7.2306	5.58	25.1	5	3.00	1.80	2.80	15.1	3.0	5.0
218	Oragui et al (1995) and Pearson et al. (1995)	Rotavirus	Maturation	Catingueira, Brazil	-7.2306	5.34	25.0	153	17	9	1.00	153	40.2	3.8
219	Oragui et al (1995) and Pearson et al. (1995)	Rotavirus	Maturation	Catingueira, Brazil	-7.2306	5.34	25.0	39	10.40	3.75	0.90	35.1	5.0	7.0

Table A2: Continued

Data Point	Authors	Virus Type	Pond Type	K_v CMFM	K_v PFM	K_v DFM	Ci (viruses /L)	Ce (viruses /L)	Log10 Removal	% Removal	d (2005)	SVLR (viruses / ha day)	VVLR (viruses / m3 day
207	Turner and Lewis (1995)	F-specific coliphage	Polishing	4.64	0.82	0.98	4.30E+03	2.50E+02	1.2	94.2%	0.078	34,272.5	1.23
208	Verbyla and Mihelcic (2015)	F-specific coliphage - MS2	Maturation	0.57	0.23	0.39	5.01E-01	1.00E-01	0.7	80.0%	0.937	1.1	0.00
209	Da Silva et al. (2008)	Norovirus GI	Maturation	0.03	0.03	0.03	1.00E+07	6.31E+06	0.2	36.9%	0.266	6,666,666.7	555.56
210	Da Silva et al. (2008)	Norovirus GI	Maturation	0.47	0.12	0.18	6309573.4	630957.34	1.0	90.0%	0.263	3,943,483.4	328.62
211	Symonds et al. (2014)	Norovirus GI	Maturation	360.37	1.63	4.82	2.06E+06	1.26E+03	3.2	99.9%	0.333	6,804,395.6	453.63
212	Symonds et al. (2014)	Norovirus GI	Maturation	0.26	0.17	0.20	1258	579	0.3	54.0%	0.333	4,147.3	0.28
213	Da Silva et al. (2008)	Norovirus GII	Maturation	0.17	0.08	0.10	6.31E+06	1.58E+06	0.6	74.9%	0.266	4,206,382.3	350.53
214	Da Silva et al. (2008)	Norovirus GII	Maturation	0.77	0.14	0.23	1584893.2	100000	1.2	93.7%	0.263	990,558.2	82.55
215	Oragui et al (1987)	Rotavirus	Maturation	0.27	0.17	0.22	7.00E+01	3.00E+01	0.4	57.1%	0.500	470.4	0.01
216	Oragui et al (1987)	Rotavirus	Maturation	0.40	0.22	0.30	30	10	0.5	66.7%	0.600	168.0	0.01
217	Oragui et al (1987)	Rotavirus	Maturation	0.47	0.24	0.34	10	3	0.5	70.0%	0.600	56.0	0.00
218	Oragui et al (1995) and Pearson et al. (1995)	Rotavirus	Maturation	0.14	0.11	0.13	7.04E+03	4.60E+03	0.2	34.7%	0.507	18,526.3	1.85
219	Oragui et al (1995) and Pearson et al. (1995)	Rotavirus	Maturation	0.45	0.20	0.28	4.60E+03	1.10E+03	0.6	76.1%	0.361	5,914.3	0.66

Table A2: Continued

Data Point	Authors	Virus Type	Pond Type	Location	Latitude	Solar Radiation [kWh/m^2/d]	Air Temp	Surface Area (m^2)	L (m)	W(m)	D (m)	Vol. (m^3)	Q (m^3/d)	HRT (d)
219	Oragui et al (1995) and Pearson et al. (1995)	Rotavirus	Maturation	Catingueira, Campina Grande, Brazil	-7.2306	5.34	25.0	39	10.40	3.75	0.90	35.1	5.0	7.0
220	Oragui et al (1995) and Pearson et al. (1995)	Rotavirus	Maturation	Catingueira, Brazil	-7.2306	5.34	25.0	39	10.40	3.75	0.64	25.0	5.0	5.0
221	Oragui et al (1995) and Pearson et al. (1995)	Rotavirus	Maturation	Catingueira, Brazil	-7.2306	5.34	25.0	39	10.40	3.75	0.39	15.2	5.1	3.0
222	Oragui et al (1995) and Pearson et al. (1995)	Rotavirus	Maturation	Catingueira, Brazil	-7.2306	5.34	25.0	39	10.40	3.75	0.39	15.2	5.1	3.0
223	Oragui et al (1995) and Pearson et al. (1995)	Rotavirus	Maturation	Catingueira, Brazil	-7.2306	5.34	25.0	14	10.40	1.30	0.39	5.3	5.3	1.0
224	Oragui et al (1995) and Pearson et al. (1995)	Rotavirus	Maturation	Catingueira, Brazil	-7.2306	5.34	25.0	31	8.45	3.70	0.60	18.8	3.8	5.0
225	Oragui et al (1995) and Pearson et al. (1995)	Rotavirus	Maturation	Catingueira, Brazil	-7.2306	5.34	25.0	31	8.45	3.70	0.60	18.8	3.8	5.0
226	Oragui et al (1995) and Pearson et al. (1995)	Rotavirus	Maturation	Catingueira, Brazil	-7.2306	5.34	25.0	31	8.45	3.70	0.60	18.8	4.5	4.2
227	Oragui et al (1995) and Pearson et al. (1995)	Rotavirus	Maturation	Catingueira, Brazil	-7.2306	5.34	25.0	4	4	1	1.50	6	3.2	2.0
228	Oragui et al (1995) and Pearson et al. (1995)	Rotavirus	Maturation	Catingueira, Brazil	-7.2306	5.34	25.0	4	3.60	1.20	1.50	6.5	3.2	2.0
229	Oragui et al (1995) and Pearson et al. (1995)	Rotavirus	Maturation	Catingueira, Brazil	-7.2306	5.34	25.0	4	3.60	1.20	1.50	6.5	3.2	2.0
230	Oragui et al (1995) and Pearson et al. (1995)	Rotavirus	Maturation	Catingueira, Brazil	-7.2306	5.34	25.0	4	3.60	1.20	1.50	6.5	3.2	2.0
231	Oragui et al (1995) and Pearson et al. (1995)	Rotavirus	Maturation	Catingueira, Brazil	-7.2306	5.34	25.0	4	3.60	1.20	1.50	6.5	3.2	2.0

Table A2: Continued

Data Point	Authors	Virus Type	Pond Type	K_v CMFM	K_v PFM	K_v DFM	Ci (viruses /L)	Ce (viruses /L)	Log10 Removal	% Removal	d (2005)	SVLR (viruses / ha day)	VVLR (viruses / m3 day
219	Oragui et al (1995) and Pearson et al. (1995)	Rotavirus	Maturation	0.45	0.20	0.28	4.60E+03	1.10E+03	0.6	76.1%	0.361	5,914.3	0.66
220	Oragui et al (1995) and Pearson et al. (1995)	Rotavirus	Maturation	0.41	0.22	0.29	4.60E+03	1.50E+03	0.5	67.4%	0.361	5,888.0	0.92
221	Oragui et al (1995) and Pearson et al. (1995)	Rotavirus	Maturation	0.43	0.28	0.34	4.60E+03	2.00E+03	0.4	56.5%	0.361	5,980.0	1.53
222	Oragui et al (1995) and Pearson et al. (1995)	Rotavirus	Maturation	0.43	0.28	0.34	4.60E+03	2.00E+03	0.4	56.5%	0.361	5,980.0	1.53
223	Oragui et al (1995) and Pearson et al. (1995)	Rotavirus	Maturation	4.68	1.74	2.07	4.60E+03	8.10E+02	0.8	82.4%	0.125	17,940.0	4.60
224	Oragui et al (1995) and Pearson et al. (1995)	Rotavirus	Maturation	306.47	1.47	5.05	1.53E+03	1	3.2	99.9%	0.438	1,840.0	0.31
225	Oragui et al (1995) and Pearson et al. (1995)	Rotavirus	Maturation	76.47	1.19	3.50	1.53E+03	4	2.6	99.7%	0.438	1,840.0	0.31
226	Oragui et al (1995) and Pearson et al. (1995)	Rotavirus	Maturation	182.30	1.58	5.05	1.53E+03	2	2.9	99.9%	0.438	2,190.5	0.37
227	Oragui et al (1995) and Pearson et al. (1995)	Rotavirus	Maturation	0.42	0.30	0.35	2.20E+04	1.20E+04	0.3	45.5%	0.333	165,000.0	11.00
228	Oragui et al (1995) and Pearson et al. (1995)	Rotavirus	Maturation	0.16	0.14	0.15	1.20E+04	9.10E+03	0.1	24.2%	0.333	90,000.0	6.00
229	Oragui et al (1995) and Pearson et al. (1995)	Rotavirus	Maturation	0.23	0.19	0.21	9.10E+03	6.20E+03	0.2	31.9%	0.333	68,250.0	4.55
230	Oragui et al (1995) and Pearson et al. (1995)	Rotavirus	Maturation	0.79	0.47	0.58	6.20E+03	2.40E+03	0.4	61.3%	0.333	46,500.0	3.10
231	Oragui et al (1995) and Pearson et al. (1995)	Rotavirus	Maturation	1.21	0.62	0.80	2.40E+03	7.00E+02	0.5	70.8%	0.333	18,000.0	1.20

Table A2: Continued

Data Point	Authors	Virus Type	Pond Type	Location	Latitude	Solar Radiation [kWh/m^2/d]	Air Temp	Surface Area (m^2)	L (m)	W(m)	D (m)	Vol. (m^3)	Q (m^3/d)	HRT (d)
232	Oragui et al (1995) and Pearson et al. (1995)	Rotavirus	Maturation	Catingueira, Brazil	-7.2306	5.34	25.0	4	3.60	1.20	1.50	6.5	3.2	2.0
233	El-Deeb Ghazy et al (2008)	Rotavirus	Maturation	El-Mofti Kafr El-Sheikh, Egypt	31.3000	3.04	14.8	635	32	20	1.40	889	232.0	3.8
234	El-Deeb Ghazy et al (2008)	Rotavirus	Maturation	El-Mofti Kafr El-Sheikh, Egypt	31.3000	3.94	14.4	635	32	20	1.40	889	232.0	3.8
235	Symonds et al. (2014)	Rotavirus Group A	Maturation	Yungas, Bolivia	-15.6517	3.92	16.9	507	39	13	1.50	761	167.1	4.6
236	Symonds et al. (2014)	Rotavirus Group A	Maturation	Yungas, Bolivia	-15.6517	3.92	16.9	507	39	13	1.50	761	167.1	4.6
237	Symonds et al. (2014)	Rotavirus Group A	Polishing	Yungas, Bolivia	-15.56	3.92	16.9	1,260	60	21	1.50	1,890	96.4	19.6
238	Betancour (2013); Betancour et al. (2013)	Rotavirus Group A	Maturation	Ecilda Paullier, San Jose, Uruguay	-34.352	4.63	17.9	1,980	45	44	1.50	2,970	188.0	15.8
239	Betancour (2013); Betancour et al. (2013)	Rotavirus Group A	Maturation	Ecilda Paullier, San Jose, Uruguay	-34.352	4.63	17.9	1,980	45	44	1.50	2,970	188.0	15.8
240	Betancour (2013); Betancour et al. (2013)	Rotavirus Group A	Maturation	Ecilda Paullier, San Jose, Uruguay	-34.352	4.63	17.9	1,980	45	44	1.50	2,970	188.0	15.8
241	Betancour (2013); Betancour et al. (2013)	Rotavirus Group A	Maturation	Ecilda Paullier, San Jose, Uruguay	-34.352	4.63	17.9	1,980	45	44	1.50	2,970	188.0	15.8
242	Betancour (2013); Betancour et al. (2013)	Rotavirus Group A	Maturation	Ecilda Paullier, San Jose, Uruguay	-34.352	4.63	17.9	1,980	45	44	1.50	2,970	188.0	15.8
243	Betancour (2013); Betancour et al. (2013)	Rotavirus Group A	Maturation	Ecilda Paullier, San Jose, Uruguay	-34.352	4.63	17.9	1,980	45	44	1.50	2,970	188.0	15.8
244	Alcalde et al (2003)	Somatic coliphage	Maturation	Arad, Israel	31.2560	6.37	24.6	25,375	175	145	1.50	38,063	5,075.0	7.5

Table A2: Continued

Data Point	Authors	Virus Type	Pond Type	K_v CMFM	K_v PFM	K_v DFM	Ci (viruses /L)	Ce (viruses /L)	Log10 Removal	% Removal	d (2005)	SVLR (viruses / ha day)	VVLR (viruses / m3 day
232	Oragui et al (1995) and Pearson et al. (1995)	Rotavirus	Maturation	1.09	0.58	0.74	7.00E+02	2.20E+02	0.5	68.6%	0.333	5,250.0	0.35
233	El-Deeb Ghazy et al (2008)	Rotavirus	Maturation	2.35	0.60	1.12	1.00E+03	1.00E+02	1.0	90.0%	0.620	3,653.5	0.26
234	El-Deeb Ghazy et al (2008)	Rotavirus	Maturation	2.35	0.60	1.12	10	1	1.0	90.0%	0.620	36.5	0.00
235	Symonds et al. (2014)	Rotavirus Group A	Maturation	517.55	1.71	5.23	4535000	1925	3.4	100.0%	0.333	14,950,549.5	996.70
236	Symonds et al. (2014)	Rotavirus Group A	Maturation	0.35	0.21	0.26	1925	741	0.4	61.5%	0.333	6,346.2	0.42
237	Symonds et al. (2014)	Rotavirus Group A	Polishing	1.68	0.18	0.35	204	6	1.5	97.1%	0.350	156.1	0.01
238	Betancour (2013); Betancour et al. (2013)	Rotavirus Group A	Maturation	1.23	0.19	0.49	39700	1950	1.3	95.1%	0.978	37,694.9	2.51
239	Betancour (2013); Betancour et al. (2013)	Rotavirus Group A	Maturation	4.75	0.27	0.95	1.14E+04	1.50E+02	1.9	98.7%	0.978	10,824.2	0.72
240	Betancour (2013); Betancour et al. (2013)	Rotavirus Group A	Maturation	0.80	0.17	0.38	2.05E+03	1.50E+02	1.1	92.7%	0.978	1,946.5	0.13
241	Betancour (2013); Betancour et al. (2013)	Rotavirus Group A	Maturation	3.17	0.25	0.79	7.67E+03	1.50E+02	1.7	98.0%	0.978	7,282.6	0.49
242	Betancour (2013); Betancour et al. (2013)	Rotavirus Group A	Maturation	0.98	0.18	0.43	2.10E+04	1.28E+03	1.2	93.9%	0.978	19,939.4	1.33
243	Betancour (2013); Betancour et al. (2013)	Rotavirus Group A	Maturation	0.01	0.01	0.01	3.24E+04	2.94E+04	0.0	9.3%	0.978	30,763.6	2.05
244	Alcalde et al (2003)	Somatic coliphage	Maturation	0.90	0.27	0.52	104712.85	13489.629	0.9	87.1%	0.829	209,425.7	13.96

Table A2: Continued

Data Point	Authors	Virus Type	Pond Type	Location	Latitude	Solar Radiation [kWh/m^2/d]	Air Temp	Surface Area (m^2)	L (m)	W(m)	D (m)	Vol. (m^3)	Q (m^3/d)	HRT (d)
245	Alcalde et al (2003)	Somatic coliphage	Maturation	Arad, Israel	31.2560	3.52	14.5	25,375	175	145	1.50	38,063	5,075.0	7.5
246	Reinoso et al. (2011)	Somatic coliphage	Maturation	Fresno de la Vega, Leon, Spain	42.3363	2.42	5.1	3,169	80	40	1.50	4,754	3,200.0	1.0
247	Reinoso et al. (2011)	Somatic coliphage	Maturation	Fresno de la Vega, Leon, Spain	42.3363	6.05	18.7	3,169	80	40	1.50	4,754	3,200.0	1.0
248	Jurzik et al. (2015)	Human Adenovirus	Polishing	Bochum (North Rhine-Westphalia, Germany)	51.4435	3.38125	10.275	127,000	730	174	2	300,000	75,000	4.0
249	Jurzik et al. (2015)	Somatic coliphage	Polishing	Bochum (North Rhine-Westphalia, Germany)	51.4435	3.38125	10.275	127,000	730	174	2.00	300,000	75,000	4.0

Data Point	Authors	Virus Type	Pond Type	K_v CMFM	K_v PFM	K_v DFM	Ci (viruses /L)	Ce (viruses /L)	Log10 Removal	% Removal	d (2005)	SVLR (viruses / ha day)	VVLR (viruses / m3 day
245	Alcalde et al (2003)	Somatic coliphage	Maturation	0.10	0.08	0.09	2.57E+05	1.45E+05	0.3	43.8%	0.829	514,079.2	34.27
246	Reinoso et al. (2011)	Somatic coliphage	Maturation	4.01	1.61	2.44	19.952623	3.9810717	0.7	80.0%	0.500	201.5	0.01
247	Reinoso et al. (2011)	Somatic coliphage	Maturation	18.95	2.99	6.01	7943.2823	398.10717	1.3	95.0%	0.500	80,209.9	5.35
248	Jurzik et al. (2015)	Human Adenovirus	Polishing	0.02	0.02	0.02	6.20E+03	5.80E+03	0.0	6.5%	0.225	36,614.2	1.55
249	Jurzik et al. (2015)	Somatic coliphage	Polishing	1.97	0.55	0.77	7.10E+04	8.00E+03	0.9	88.7%	0.225	419,291.3	17.75

APPENDIX B: RESULTS

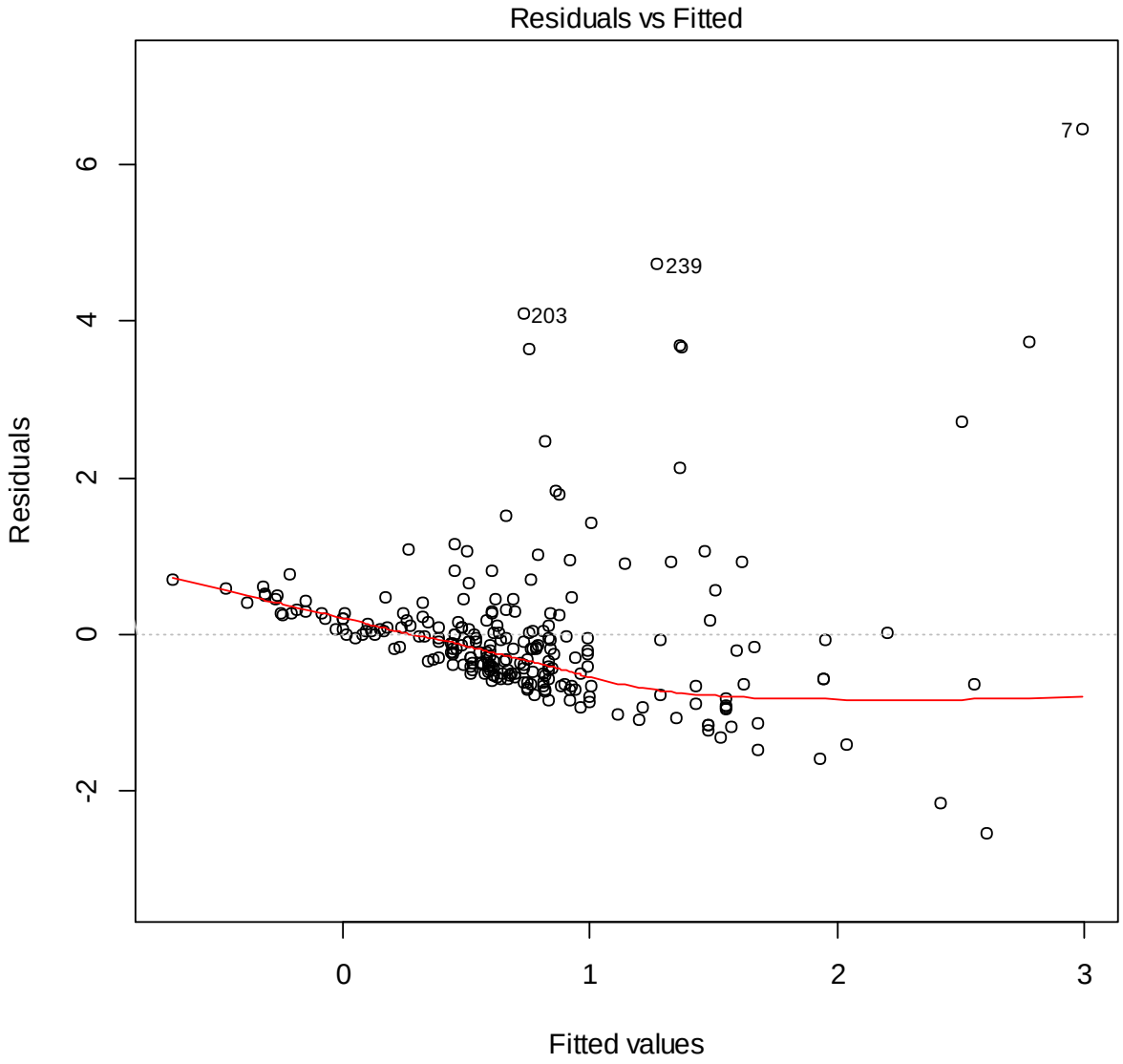

Figure B1: Residuals plotted against the fitted values for the regression equation with original data. $y(K_{v,DFM})$ = x(virus) + x(pond) + x(solar radiation) + x(temperature) + x(HRT) + x(depth) + x(vvlr) + x(svlr); used as a diagnostic test for the homoscedasticity assumption of multiple linear regression.

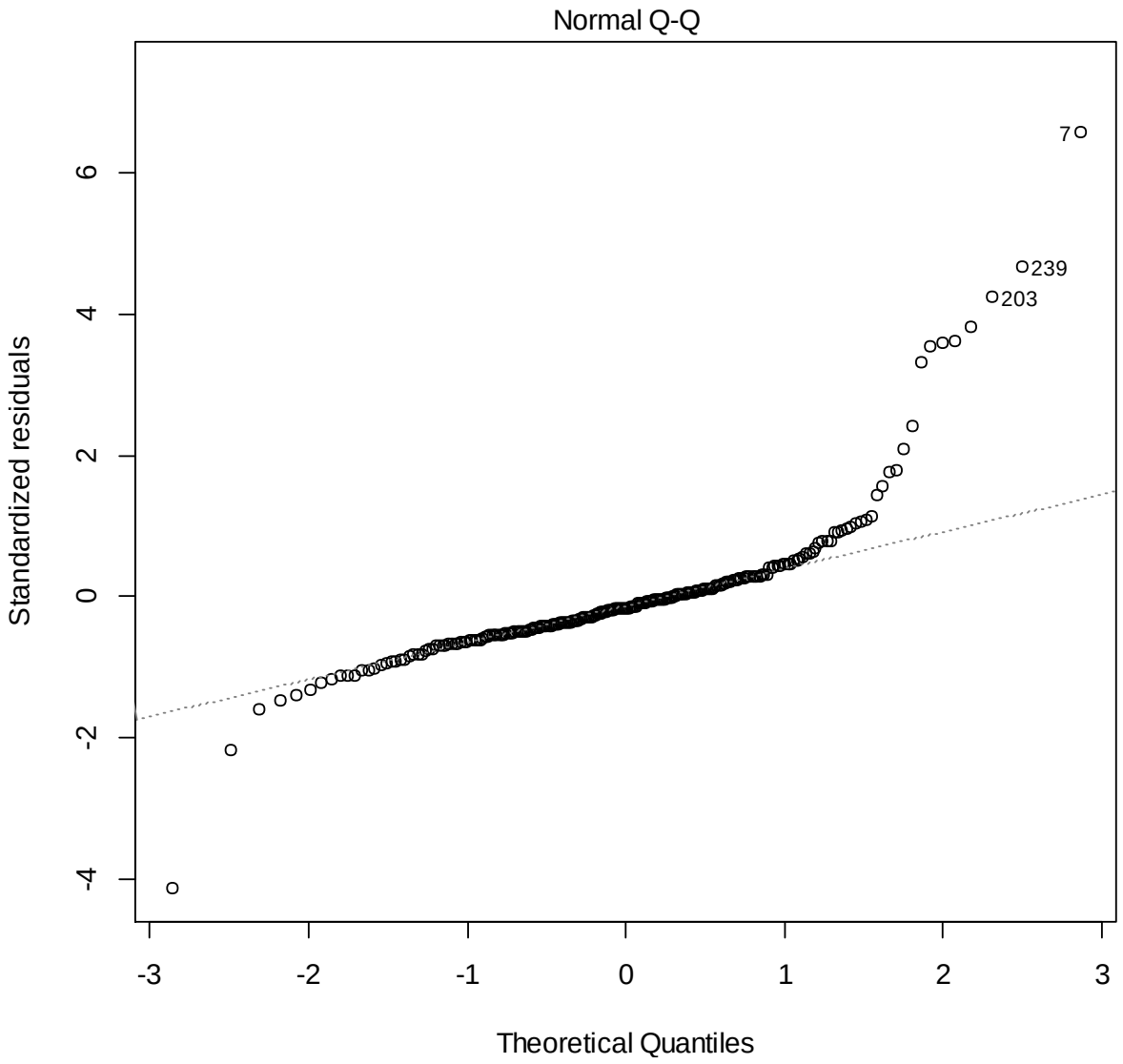

Figure B2: Q-Q plot for the regression equation with original data. $y(K_{v,DFM})$ = x(virus) + x(pond) + x(solar radiation) + x(temperature) + x(HRT) + x(depth) + x(vvlr) + x(svlr); used as a diagnostic test for the normality of the residual error assumption of multiple linear regression.

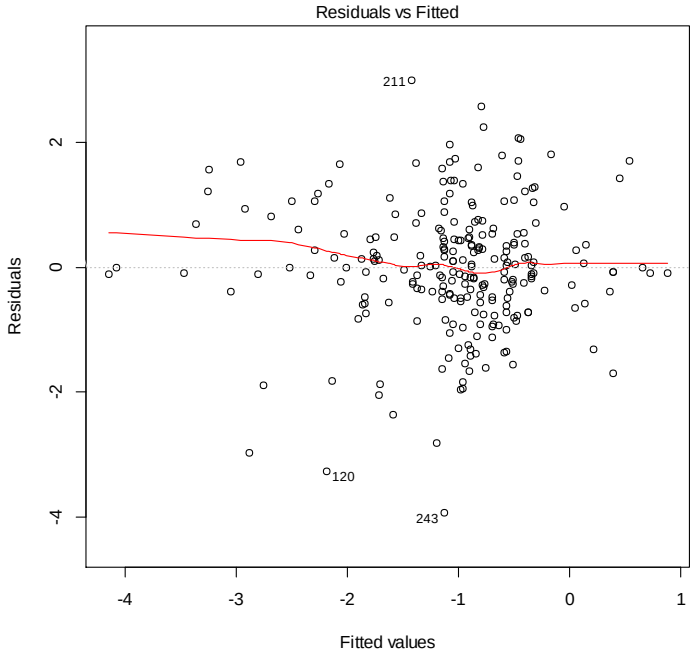

Figure B3: Residuals plotted against the fitted values for the regression equation using logarithmically transformed $K_{v,app}$ values. $y(\ln K_{v,DFM})$ = x(virus) + x(pond) + x(solar radiation) + x(temperature) + x(HRT) + x(depth) + x(vvlr) + x(svlr); used as a diagnostic test for the homoscedasticity assumption of multiple linear regression.

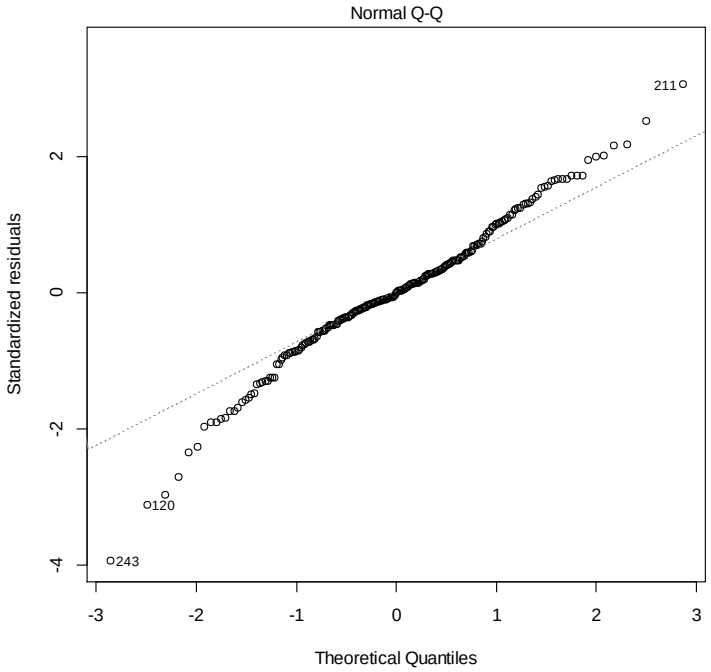

Figure B4: Q-Q plot for regression equation using logarithmically transformed $K_{v,app}$ values. y $(\ln K_{v,DFM})$ = x(virus) + x(pond) + x(solar radiation) + x(temperature) + x(HRT) + x(depth) + x(vvlr) + x(svlr); used as a diagnostic test for the normality of the residual error assumption of multiple linear regression

Table B1: Variance inflation factors for alternative regression equations; used as a multi-collinearity diagnostic

	Temp	**HRT**	**Depth**	**SVLR**
Equation 14	1.154667	1.352585	1.06104	1.230134
Equation 15	1.154523	1.151954	1.016274	
Equation 16	1.147211	1.147211		
Equation 17	1.169056	1.249358	1.073227	1.131268
Equation 18	1.168721	1.16296	1.018117	
Equation 19	1.159057	1.159057		
Equation 20	1.154667	1.352585	1.06104	1.230134

Table B2: Pearson's correlation coefficients between explanatory variables; used as a multi-collinearity diagnostic

	Solar Radiation	Depth	Temp	HRT	SVLR	VVLR
Solar Radiation	1					
Depth	0.04854	1				
Temp	**0.66707**	-0.1103	1			
HRT	-0.1626	-0.086	-0.3266	1		
SVLR	0.04308	0.38421	0.10744	-0.4329	1	
VVLR	0.04385	0.2999	0.12723	-0.4441	**0.99539**	1

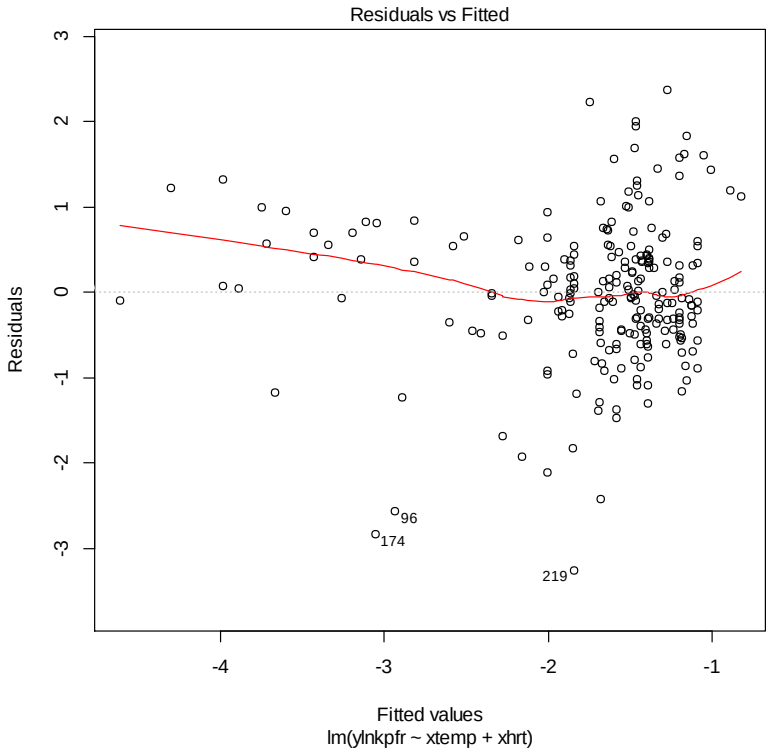

Figure B5: Residuals plotted against the fitted values for the regression Equation 16. These are used as a diagnostic test for the homoscedasticity assumption of multiple linear regression.

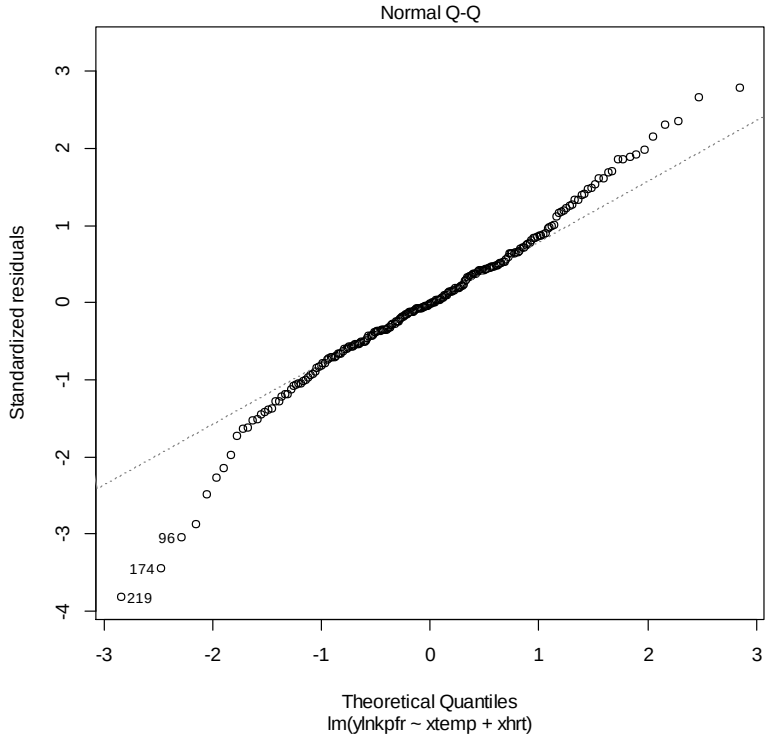

Figure B6: Q-Q plot for regression Equation 16. This is used as a diagnostic test for the normality of the residual error assumption of multiple linear regression

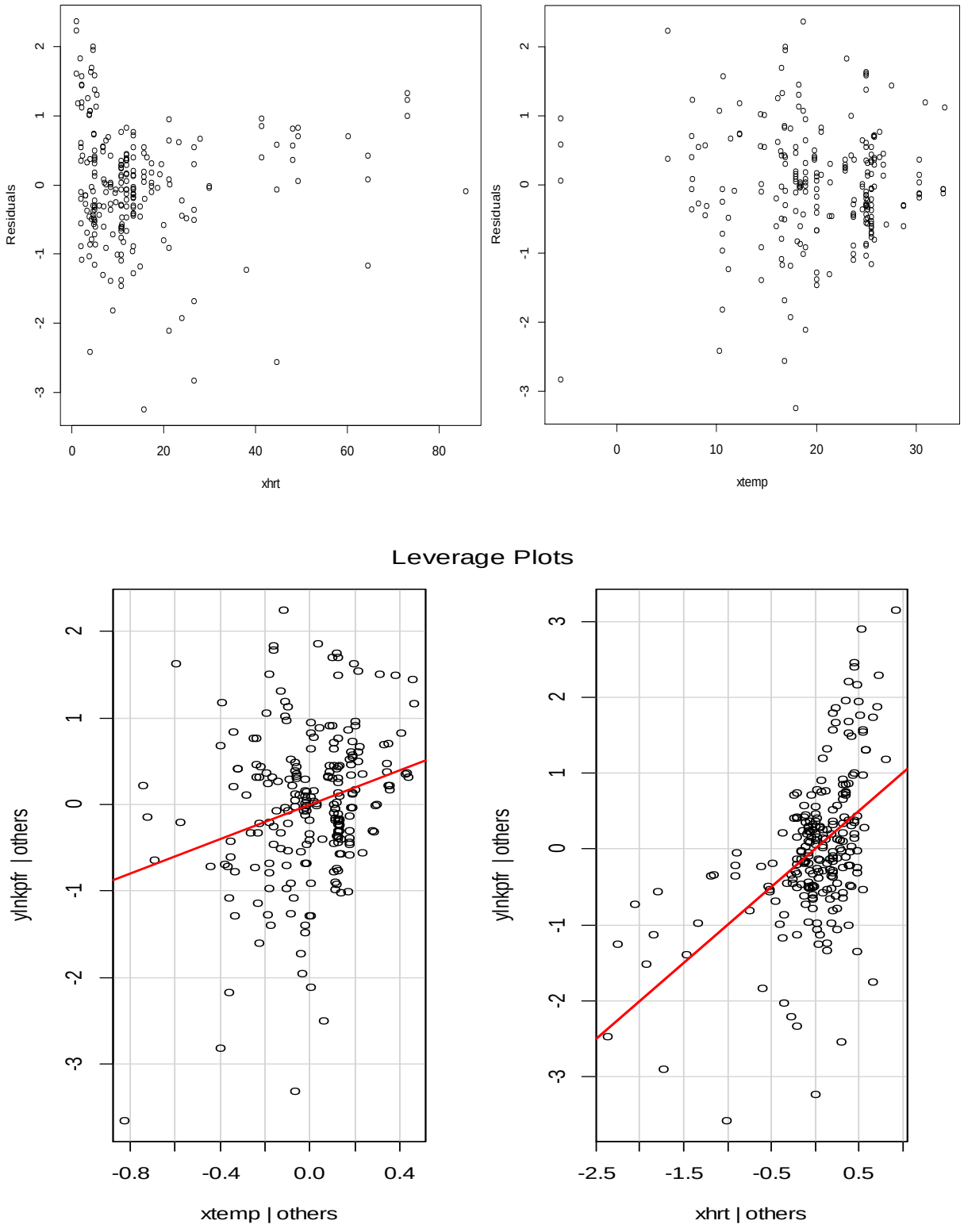

Figure B7: Linearity check for Equation 16 with residuals against residuals plots and with leverage plots

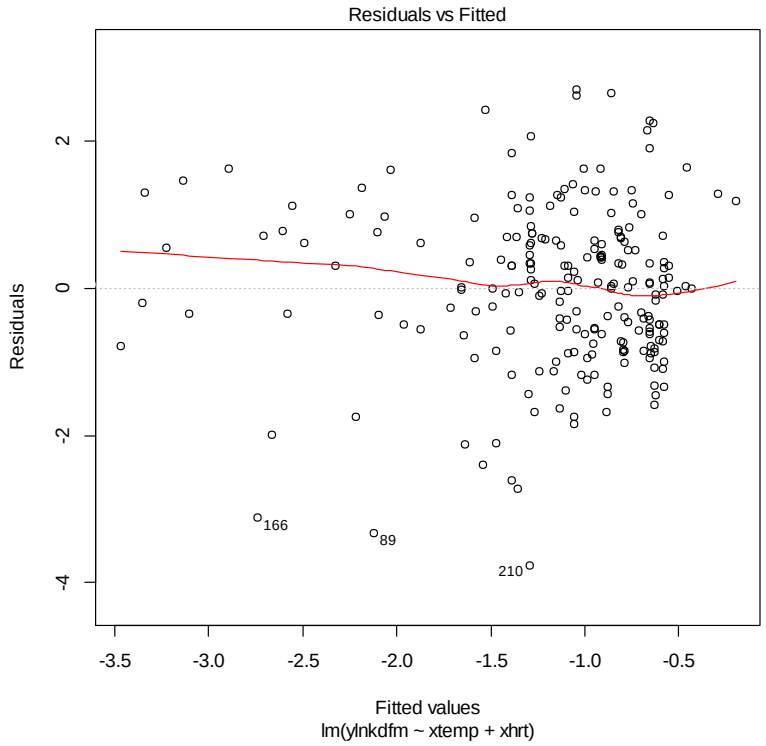

Figure B8: Residuals plotted against the fitted values for the regression Equation 19. These are used as a diagnostic test for the homoscedasticity assumption of multiple linear regression.

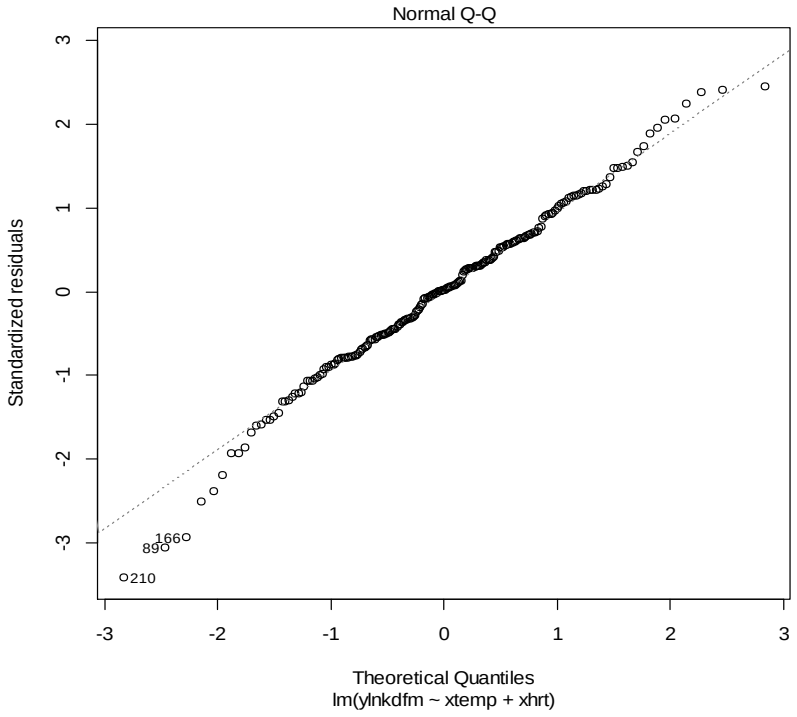

Figure B9: Q-Q plot for regression Equation 19. This is used as a diagnostic test for the normality of the residual error assumption of multiple linear regression

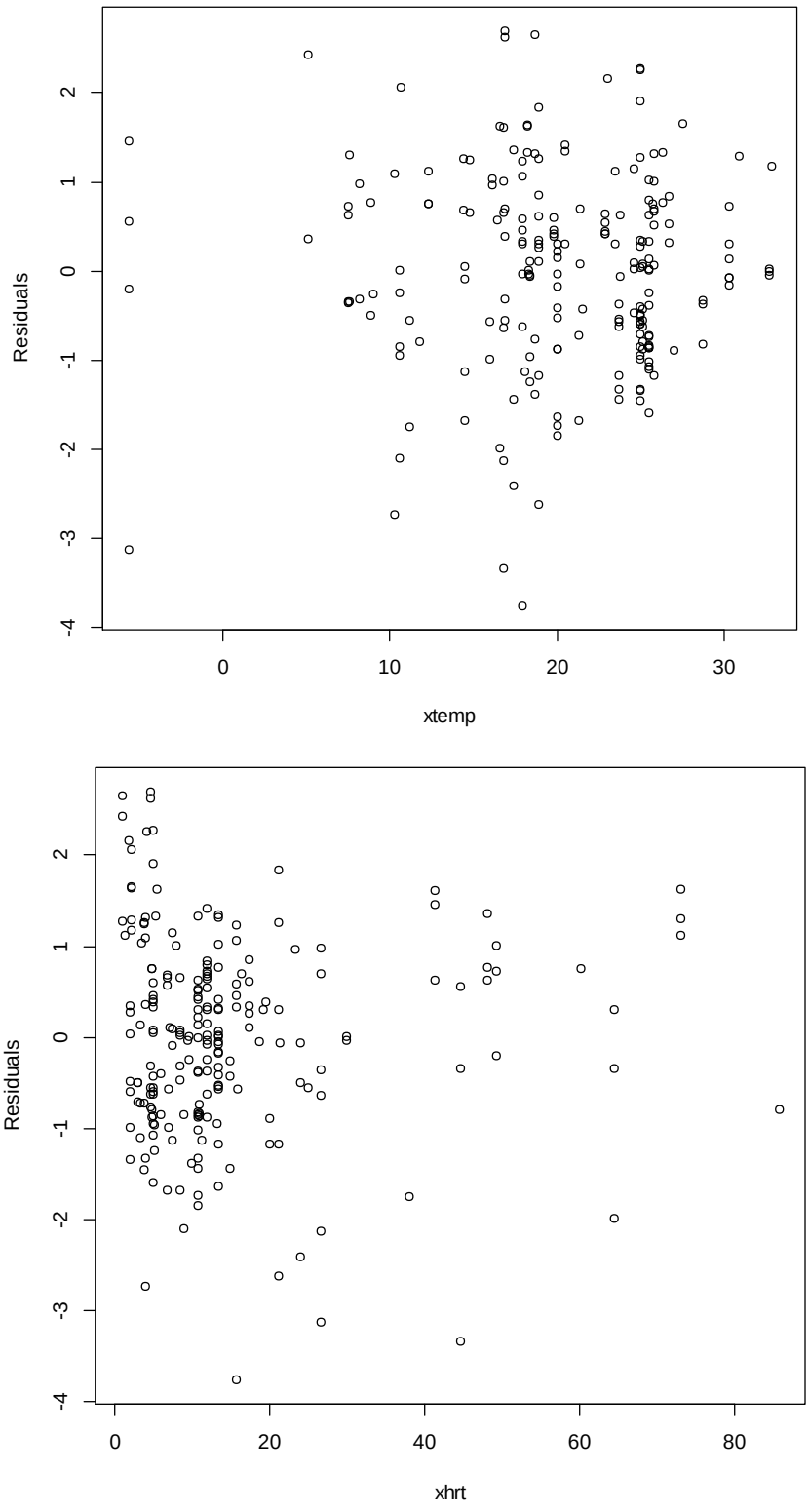

Figure B10: Linearity check for Equation 19 with residuals against residuals plots and with leverage plots

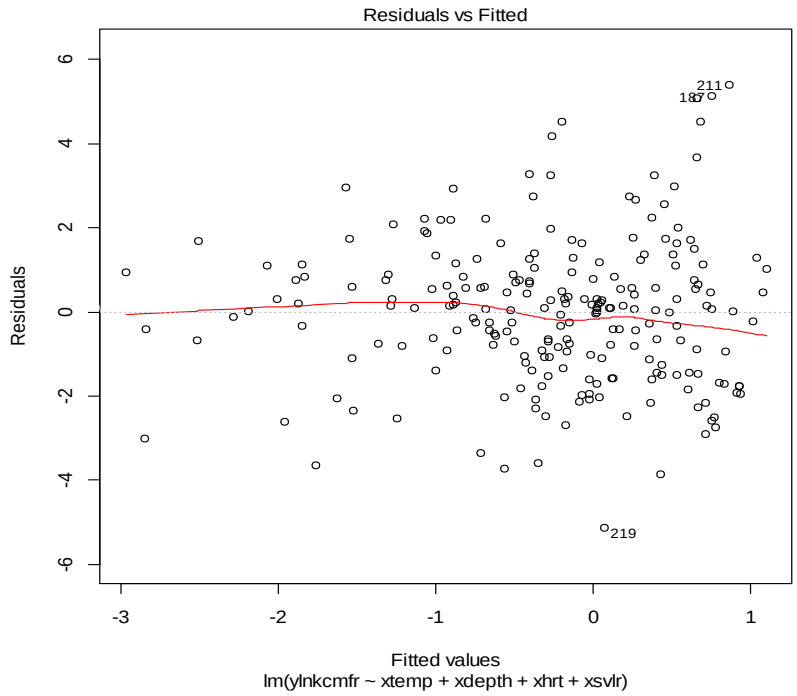

Figure B11: Residuals plotted against the fitted values for the regression Equation 20. These are used as a diagnostic test for the homoscedasticity assumption of multiple linear regression

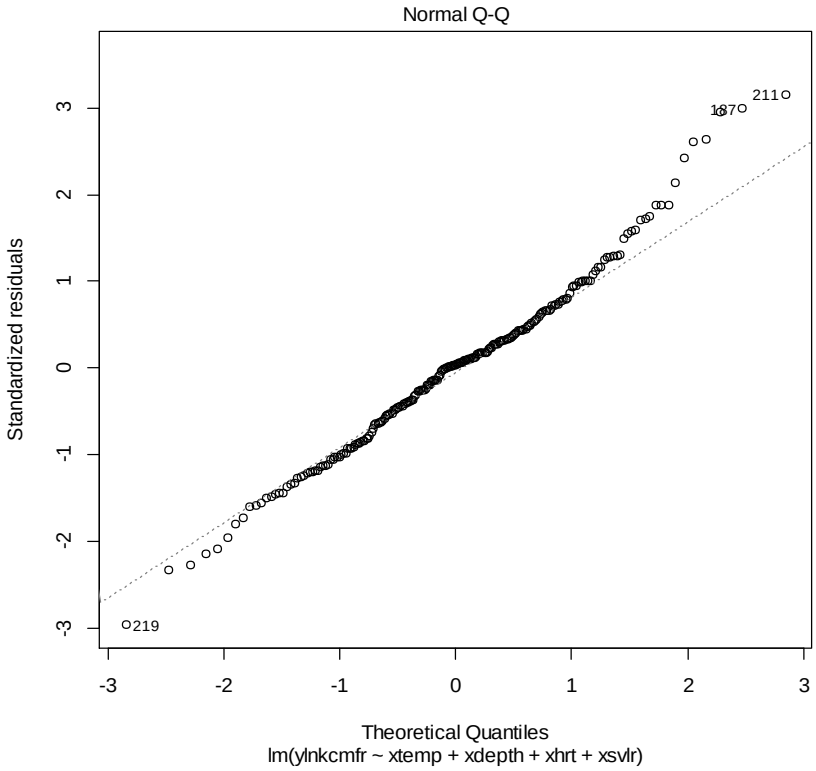

Figure B12: Q-Q plot for regression Equation 20. This is used as a diagnostic test for the normality of the residual error assumption of multiple linear regression

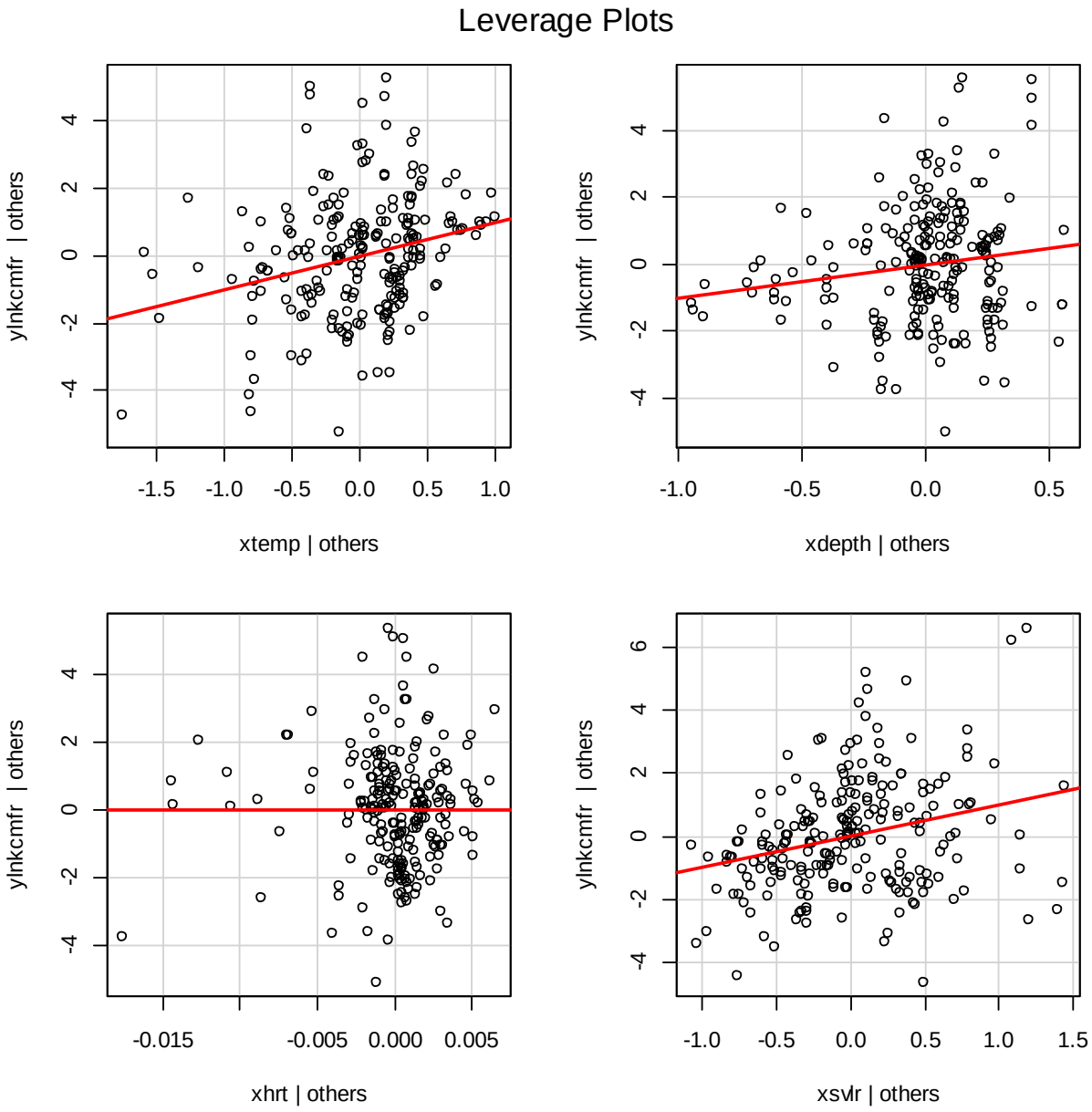

Figure B13: Linearity check for Equation 20 with leverage plots

APPENDIX C: COPYRIGHT PERMISSIONS

The copyright permission from Elsevier for Figure 2.1 is displayed below.

ELSEVIER LICENSE
TERMS AND CONDITIONS

Dec 01, 2015

This is a License Agreement between Kel Vannoy ("You") and Elsevier ("Elsevier") provided by Copyright Clearance Center ("CCC"). The license consists of your order details, the terms and conditions provided by Elsevier, and the payment terms and conditions.

All payments must be made in full to CCC. For payment instructions, please see information listed at the bottom of this form.

Supplier	Elsevier Limited The Boulevard,Langford Lane Kidlington,Oxford,OX5 1GB,UK
Registered Company Number	1982084
Customer name	Kel Vannoy
Customer address	1016 E Clifton St TAMPA, FL 33604
License number	3752271064471
License date	Nov 18, 2015
Licensed content publisher	Elsevier
Licensed content publication	Water Research
Licensed content title	A review of virus removal in wastewater treatment pond systems
Licensed content author	Matthew E. Verbyla,James R. Mihelcic
Licensed content date	15 March 2015
Licensed content volume number	71
Licensed content issue number	n/a
Number of pages	18

Start Page	107
End Page	124
Type of Use	reuse in a thesis/dissertation
Portion	figures/tables/illustrations
Number of figures/tables/illustrations	1
Format	electronic
Are you the author of this Elsevier article?	No
Will you be translating?	No
Original figure numbers	Figure S2
Title of your thesis/dissertation	Modeling the Extent of Virus Removal in Waste Stabilization Ponds to Support Reuse of Wastewater
Expected completion date	Nov 2015
Estimated size (number of pages)	100
Elsevier VAT number	GB 494 6272 12
Permissions price	0.00 USD
VAT/Local Sales Tax	0.00 USD / 0.00 GBP
Total	0.00 USD

Entire order details and publisher terms and conditions are available by clicking the link below.
http://s100.copyright.com/CustomerAdmin/PLF.jsp?ref=28c2eb4a-5acf-431f-9d00-a8726ac8429a

ABOUT THE AUTHOR

Kelly “Kel” James Vannoy grew up in Billings, Montana and graduated from California State University, Chico in 2014 with a Bachelor of Science in Civil Engineering. Some of his engineering and multi-disciplinary interests include: sustainable development engineering, integrated solid waste and wastewater management, resource recovery from waste streams, potable water infrastructure, management of water resources, renewable and appropriate energy systems, aquaponics, and permaculture. As an undergraduate, Kel had the privilege to work on a design feasibility study for the wastewater management master plan for Lake Atitlán, Guatemala. This experience sparked his interest in sustainable development engineering, compelled him to study Spanish abroad in Chile for a semester, and motivated him to further his knowledge by pursuing a Master of Science in Environmental Engineering at the University of South Florida.

As for professional experience, Kel has held engineering positions with ERM, Inc. and CH2M Hill. Kel plans to begin his engineering consulting career in Latin America, with a particular focus on implementing sustainable and economically viable waste management and water infrastructure projects that benefit communities and safeguard public and environmental health. His long term vision is to split time living in mountain ecovillages in the pacific northwest and pacific southwest regions of North and South America, respectively, and contribute to a resilient water-energy-food nexus in these locations. Outside of academic and professional interests, Kel enjoys backpacking, snowboarding, traveling, philosophy, cooking, and all things related to sustainability and self-reliance.