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Comparison of Bacterial and Viral Reduction Across Different Wastewater Treatment Processes

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Comparison of Bacterial and Viral Reduction Across Different Wastewater Treatment Processes

by

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A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science in Environmental Engineering
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DEDICATION

To Mom and Dad, Shrey and my professors, you are such an inspiration.
ACKNOWLEDGEMENTS

I would like to acknowledge and thank god who has given me the patience and strength to complete this work. I would also like to thank my parents for all their love, support, prayers, and belief in me. I would like to thank Shrey for all the encouragement, for always being there by me and patiently supporting me through the course of my studies. I would also like to thank my friends for being a support system and for being my family miles away from home. I would also like to thank Amulya Miriyala and Siesta Williams for helping me through the formatting part; I would like to thank Bhagyashree Soni and her friends for helping me with the statistical analysis.

To my advisors, James Mihelcic and Sarina Ergas and my committee member Stewart Oakley, thank you for believing in me and for all the support and efforts to help me grow as an engineer.
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ABSTRACT

Today billions of people live without access to basic sanitation facilities, and thousands die every week due to diseases caused by fecal contamination associated with improper sanitation. It has thus become crucial for decision makers to have access to relevant and sufficient data to implement appropriate solutions to these problems. The Global Water Pathogen Project [http://www.waterpathogens.org/] is dedicated to providing an up-to-date source of data on pathogen reduction associated with different sanitation technologies that are important if the world is to achieve the Sustainable Development Goals (SDGs) related to health and sanitation provision. In this research, a subset of the Global Water Pathogen Project (GWPP) data is used to access the reduction of bacteria and viruses across different mechanical and natural sanitation technologies. The order of expected removal for bacteria during wastewater treatment was reported as highest for a membrane bioreactor (4.4 log10), waste stabilization pond (2.3 log10), conventional activated sludge (1.43 log10), anaerobic anoxic oxic activated sludge (1.9 log10), trickling filter (1.16 log10), and upflow anaerobic sludge blanket reactor (1.2 log10).

Furthermore, the order of expected removal for viruses was reported as highest for a membrane bioreactor (3.3 log10), conventional activated sludge (1.84 log10), anaerobic anoxic oxic activated sludge (1.67 log10), waste stabilization pond (1 log10), upflow anaerobic sludge blanket reactor (0.3 log10) and trickling filter (0.29 log10). It was found that hydraulic retention time (HRT) had a statistically significant relation to the reduction of bacteria in an anaerobic, anoxic oxic treatment system. Similarly, a significant
relation was found between the number of waste stabilization ponds in series and the expected reduction of bacteria. HRT was also found to be a significant factor in virus reduction in waste stabilization ponds. Additionally, it was observed that waste stabilization ponds, trickling filters, and UASB reactors could obtain a greater reduction in bacteria (5-7 log10) when combined with additional treatment (e.g., chemical disinfection or use of maturation ponds). Also, mechanized systems, such as activated sludge systems and membrane bioreactors, obtained a greater reduction (2-3 log10) of viruses when compared to a natural system. It was concluded that the selection of the best suitable technology for pathogen reduction depends on environmental, design, and operational factors as well as considering the performance of specific wastewater treatment systems individually as well as when combined with other treatment technologies that may provide added removal of microbial constituents.
CHAPTER 1 INTRODUCTION

1.1 Background

Human existence is highly dependent on access to clean water and sanitation. Worldwide, clean water and adequate sanitation are two elementary factors required to live a healthy and sustainable life. Accordingly, in resolution 64/292, the United Nations (UN) General Assembly recognized access to sanitation and safe water as a human right. Also, because water is a finite resource and readily-available freshwater is limited, improper water management may lead to water scarcity. This is very evident as, today, around 40% of the world's population is affected by water scarcity, which is projected to increase with increasing global population (UN, 2016).

Furthermore, even with modernization and advancement in technology, around 2.4 billion people around the world still lack access to basic sanitation services, 946 million people practice open defecation (UNICEF & WHO, 2015), nearly 1.8 billion people use fecally contaminated sources of drinking water (WHO, 2016), and almost 800 children die every day due to waterborne diarrheal diseases. Recent analysis also shows that lack of sanitation compels more girls to drop out of school or make them vulnerable to sexual assault (UN, 2016). Finally, surface water in many parts of the world is exposed to extensive pollution, as more than 80% of untreated wastewater and domestic sludge is discharged without any treatment (WWAP, 2017). The upstream discharge could end up being used as an untreated drinking water or irrigation source located downstream, which is indirect reuse of wastewater, which would transpose health risks to the downstream
user. With the world’s urban population also increasing at a rapid rate, the stress of providing adequate sanitation in cities is great. Due to high infrastructure management gaps and the shortage of a highly skilled workforce, community-based models employed to manage sanitation in rural areas and the centralized utility models used to manage sanitation in urban areas may not be effective in reducing exposure to pathogens in collected and partially treated wastewater (Verbyla, Oakley, & Mihelcic, 2013). All this is reported to hold back the economic growth and social development of impacted populations (UNICEF & WHO, 2015). In fact, inadequate sanitation is also known to cost billions of dollars in terms of lost economic potential which eventually can adversely impact a country’s economic growth. (UNICEF & WHO, 2015)

Accordingly, amongst the many sustainable development needs, access to clean water and sanitation has become a pressing issue. To ensure a better functioning public health system, the global community has to take initiatives in providing adequate sanitation (Mara & Evans, 2017). The UN has thus made access to sanitation a major component of the Sustainable Development Goals (SDGs) where Target #2 of the SDG #6 is to “by 2030, achieve access to adequate and equitable sanitation and hygiene for all and end open defecation, paying special attention to the needs of women and girls and those in vulnerable situations” (UN, 2015).

Since all seventeen SDGs are interconnected (Zhang et al., 2016) if the sanitation target is met, that should result in lowered disease burdens, improved nutritional levels to reduce stunting in children, increased female education and better job opportunities. Also, if the wastewater is safely managed, (i.e., treated to safe levels that do not increase
exposure to pathogens), it can act as a source of scarce resources that includes water, nutrients, and energy and also provide future economic opportunities. (WorldBank, 2017).

According to the Joint Monitoring Program (JMP), “basic” access to water includes a source which is protected from external contamination, particularly human excreta. When the global development community talks about providing sanitation and hygiene, proper management of human excreta is crucial. This is because inadequate hygiene, exposure to human excreta, and improper methods of disposal of excreta contribute to the spread of waterborne diseases such as cholera, diarrhea, and hookworm infections (Feachem et al., 1983). Many authors (Prüss-Ustün et al., 2014) (Mihelcic et al., 2009) have historically recognized this relationship between improper sanitation and diseases. Even with a basic sanitation facility, if wastewater does not undergo proper treatment, or there is a fault in the design, or there is improper fecal sludge management, the spread of excreta related disease and environmental degradation is inevitable (Naughton & Mihelcic, 2017). The primary cause of these waterborne diseases is exposure to ‘pathogens’1 present in the excreta. Microorganisms that originate in the gastrointestinal tracts of humans (enteric pathogens) are extremely dangerous as they play a critical role in disease transmission. Because many of these pathogens are infectious as soon as they are excreted, they pose a potential environmental threat of making other humans and animals susceptible to health risks (Naughton & Mihelcic, 2017). Therefore, of all the human excreta (feces and urine), feces is reported as the most dangerous because it is

---

1 Pathogens are microorganisms that invade, infect and damage human body and the ability of these microorganisms to cause diseases is referred to as ‘pathogenicity’ (Madigan, Martinko, Bender, Buckley, & Stahl, Microbial Interactions with Humans, 2015). There are many different types of pathogens that are capable of causing disease in humans. They can be broken down into viruses, bacteria, protozoa and helminths (Alberts, 2002).
more highly concentrated with pathogens than urine. Appendix A provides several figures that show the reported concentration of viruses, bacteria, protozoa, and helminths, respectively, that are found in feces (reported as counts per wet gram). These values show the extent in the number and concentration of pathogens reported in feces; however, note that the data may vary with regions and season because of rates of infectivity. The data in Appendix A also show that the concentrations of harmful pathogens that are excreted in human feces can range from 1 to 1011 units per wet gram of feces (Mcjunkin, 1982). Each pathogen listed in these four figures also has a health hazard associated with it. Because many of these pathogens are known to be highly stable in aqueous environments, they can potentially affect a large segment of the population if transmitted through water. A priority of water and sanitation provision should thus be the maintenance of these pathogens at a level to reduce or eliminate associated health risks (Aw, 2018).

The F-Diagram proposed by Wagner and J.N. Lanoix in 1958 links provision of sanitation and elimination of waterborne disease as it clearly depicts routes through which the human body (mouth) can be infected by pathogens (found in fecal matter) via contaminated hands. Soil, food, and other surfaces contaminated by human feces are other routes through which excreta-related infections are transmitted. Furthermore, the majority of these pathogens enter the water by its contamination with human excreta, and when this water (or a contaminated irrigated crop) is consumed in sufficient amounts, it may lead to health implications making water an indirect medium for transmission of excreta-related pathogens (Naughton & Mihelcic, 2017).
Table 1.1 provides examples of health hazards associated with a list of common waterborne pathogens. There have also been reports which suggest that 1.4 million diarrheal children deaths could be prevented by the provision of clean water, food and hygiene facilities (Prüss-Ustün et al., 2014). It has also been reported that poor water quality, sanitation, and hygiene contributes to 5.7% of the total disease burden worldwide caused by diarrhea, schistosomiasis, trachoma, ascariasis, trichuriasis and hookworm diseases (WHO & OECD, 2003). Improper management of human excreta can also provide a breeding ground for flies, mosquitoes, and cockroaches which will provide additional routes of disease transmission (Feachem et al., 1983). For example, the World Health Organization (WHO) (2014) reports that for water related infections, there are 600,000 deaths by malaria annually, 10,000 deaths due to Japanese encephalitis, and 12,500 deaths due to dengue that is caused by mosquitoes. Reports also demonstrate that improving water quality, sanitation and hygiene has the ability to avoid 9.1% of the total disease burden and 6.3% of all deaths in the world (Prüss-Üstün et al., 2014).

Therefore, systematic management and disposal of human waste, provision of wastewater treatment, water source protection that leads to improvement of microbial water quality, elimination of infectious hosts and proper sanitation awareness and hygiene education can minimize transmission of pathogens from person to person, through human interaction, water or food chain and therefore protect the public health (Carr & Strauss, 2001). There are several technological options to appropriately treat human excreta. Even though there is no single sanitation technology that would fit for all circumstances, extensive research and experience show that a well-designed, operated and maintained system can lead to better health outcomes (Carr & Strauss, 2001).
Table 1.1 Examples of Diseases Caused by Pathogens Found in Feces (Ashbolt, 2004); (WHO, 2014); (Ramees et al., 2017); (CDC, 2018)

<table>
<thead>
<tr>
<th>Pathogen type</th>
<th>Microorganism</th>
<th>Disease Caused</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacteria</td>
<td><em>Escherichia coli</em></td>
<td>Diarrhea, gastroenteritis</td>
</tr>
<tr>
<td></td>
<td><em>Salmonella</em></td>
<td>Diarrhea, fever, and gastroenteritis.</td>
</tr>
<tr>
<td></td>
<td><em>Shigella</em></td>
<td>Shigellosis</td>
</tr>
<tr>
<td></td>
<td><em>Arcobacter</em></td>
<td>Gastroenteritis</td>
</tr>
<tr>
<td>Viruses</td>
<td><em>Rotavirus</em></td>
<td>Gastroenteritis</td>
</tr>
<tr>
<td></td>
<td><em>Norovirus GI</em></td>
<td>Gastroenteritis</td>
</tr>
<tr>
<td></td>
<td>Hepatitis A</td>
<td>Highly contagious liver infection</td>
</tr>
<tr>
<td></td>
<td>Hepatitis E</td>
<td>Acute jaundice and in some cases fulminant liver failure</td>
</tr>
<tr>
<td>Protozoa</td>
<td><em>Giardia</em></td>
<td>Diarrheal illness called Giardiasis</td>
</tr>
<tr>
<td></td>
<td><em>Cryptosporidium</em></td>
<td>Diarrheal disease called Cryptosporidiosis</td>
</tr>
<tr>
<td>Helminth</td>
<td><em>Ascaris lumbricoides</em></td>
<td>Ascariasis; no symptoms. Heavy infections cause intestinal blockage and impaired growth in children.</td>
</tr>
</tbody>
</table>

The important factors that need to be considered when selecting any sanitation technology is its ability to protect the environment and convey human health benefits. The system should separate the user from the excreta, impede any vectors from contacting the excreta and most importantly should be able to inactivate the pathogens and other environmental pollutants (Carr & Strauss, 2001).

However, just because a sanitation technology is classified as improved, does not mean it will improve public health because today only 34% of rural and 26% of urban sanitation prevent human contact with excreta (WWAP, 2017). Wastewater treatment is a crucial part of a water management cycle and disposal of untreated wastewater is known to cause disease outbreaks caused by contamination of drinking water sources, degradation of aquatic ecosystems, and other social and environmental problems.
Furthermore, because of the lack of infrastructure, financing, and technical incapability, 80% of global wastewater is discharged without treatment especially in developing countries (WWAP, 2017).

Wastewater treatment is a combination of processes that inactivate, remove, or kill pathogens and manage other environmental pollutants (e.g., BOD, TSS) by a combination of physical, chemical and biological processes. Both mechanized and natural treatment techniques are also available to wastewater planners and designers. The treatment process typically starts with the removal of large particles, which include various unit processes such as grit removal and screening. Primary treatment employs unit processes like sedimentation and removes suspended solids. Secondary treatment uses biology-based processes such as activated sludge, waste stabilization ponds, trickling filters, and packed bed reactors for the removal of dissolved organics (e.g., BOD). The next treatment step is tertiary or advanced treatment which aims to remove nutrients and/or toxins from water by employing filtration, screening, air stripping, ion exchange, precipitation, and oxidation. Disinfection is considered as a type of tertiary treatment in much of the world and is carried out by either chemical processes like ozonation, chlorination or physical process like UV irradiation. The sludge produced during treatment may be processed by thickening, dewatering, or drying and made biologically stable before disposal (EPA, 2004). The sludge contains elevated concentrations of pathogens and also must be treated to inactivate them (Mihelcic, 2018).

The Global Water Pathogen Project (GWPP) is a resource that allows a wastewater planner to determine the fate of different types of pathogens during wastewater processing by different sanitation technologies. The GWPP was initiated by
the International Hydrological Program of United Nations Educational, Scientific, and Cultural Organization (UNESCO) and Michigan State University. This project aims to develop a knowledge reserve on disease risks caused by water and establish intervention measure to reduce the mortality associated with water pathogens, unsafe drinking water, and lack of basic sanitation. It also aims to update the current benchmark reference (Feachem et al., 1983) which since its publication in 1983, has been used as a key guide in sanitation practices and also aims to gather information on new and emerging pathogens. The main motive behind the GWPP project is to serve as a guide for waterborne pathogens and to provide significant data which can be used as an information sharing network to carry out a risk assessment and ensure water safety around the world. The primary focus of Section IV of the GWPP is to report pathogen removal expected for the use of individual sanitation technologies. It thus provides access to up to date information on pathogen reduction across individual sanitation unit processes. There is an additional chapter in Section IV of the GWPP that reports pathogen reduction across an entire treatment plant of multiple unit processes that employs natural and mechanized treatment. The data reported for entire treatment plant is further classified to provide information about pathogen removal, with and without disinfection. The GWPP thus provides an up-to-date source of data on pathogen reduction with the development of sanitation technologies that is important if the SDG target related to sanitation provision is to be met.

1.2 Research Objectives and Tasks

The overall goal of this thesis is to use a subset of the GWPP data to assess the reduction of pathogens (i.e., bacteria and viruses) across wastewater treatment (i.e.,
sanitation) technologies that employ mechanical and natural treatment processes. This research will also include in the discussion how pathogen reduction across these different treatment technologies can be improved with the deployment of disinfection. The overall goal of this research will be met by the following objectives:

a. Assess the key factors in which mechanized and natural systems achieve greater reduction since developing countries are not likely to implement mechanized systems, etc.

b. Assess how hydraulic retention time (HRT) affects reduction in bacteria and virus concentrations for some mechanical and natural wastewater treatment technologies.

c. Assess the extent of improvement in bacteria and virus reduction when a specific mechanical or natural treatment technology is followed by an additional unit process that provides disinfection.

It is hoped that this research will support decisions that are currently being made on the types of sanitation technologies that will best lead to improved public health and potential to safely reuse treated wastewater for the water and nutrients resources it contains. Though the results and discussion of this thesis research are focused on analyzing data obtained for bacteria and viruses only, a reader should note that Chapter 2 provides discussion on the fate of bacteria, viruses, protozoa, and helminths during wastewater treatment. The reason for adding discussion on two additional pathogen types is to provide readers with useful information on the fate of all pathogen classes during wastewater treatment because the occurrence of specific classes of pathogens may differ based on one’s geographical location.
CHAPTER 2 LITERATURE REVIEW

2.1 Review of Global Water Pathogen Project (GWPP)

The Global Water Pathogen Project aims to be a resource for a worldwide audience to provide significant data on health, sanitation, disinfection and, risks related to pathogens in excreta and water. It aims to build a knowledge source that highlights the removal, resistance, and persistence of pathogen in wastewater treatment along with the aim to contribute towards the achievement and implementation of multiple SDG’s. Table 2.1 summarizes the information available in various sections of this resource.

Table 2.1 Summary of Chapters in Global Water Pathogen Project

<table>
<thead>
<tr>
<th>Section</th>
<th>Resource Summary</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Health hazards of excreta (theory and control)</td>
<td>Provides an overview on the importance of sanitation through statistical information on the global sanitation challenge, highlights relationship between sanitation and diseases, quantifies the health impacts of these diseases, provides a detailed understanding of various critical aspects of waterborne pathogens, discusses the economic value of improved sanitation and addresses issues related to gender and sanitation.</td>
</tr>
<tr>
<td>2. Indicators and microbial source tracking markers</td>
<td>Provides information regarding the classification, importance, determination methods, occurrence, persistence, resistance and application of indicator organisms and the density of these indicators in feces, sewage, sludge for target-oriented water quality investigations and sustainable management of water safety. Information on the current use of Bacteriophages, Fecal Indicator Bacteria (FIB) and the viral MST markers in developed, developing and emerging regions is available. It also provides a brief insight on the application and stability of indicators in treatment and disinfection processes which can eventually be used to select the most suitable and efficient method for treatment and disinfection investigation in various natural and engineered systems.</td>
</tr>
</tbody>
</table>
Table 2.1 (Continued)

<table>
<thead>
<tr>
<th>Section</th>
<th>Resource Summary</th>
</tr>
</thead>
<tbody>
<tr>
<td>3. <strong>Specific excreted pathogens (Environmental and Epidemiology aspects)</strong></td>
<td>Provides a detailed description of pathogens of public health concern that are found in wastewater and waste contaminated water by elaborating the epidemiology, classification, transmission, vaccination, occurrence, and persistence in the environment. It also provides information on the reduction of these pathogens by different sanitation systems as well as the effect of disinfection on these pathogens.</td>
</tr>
<tr>
<td>4. <strong>Management of risks from excreta and wastewater</strong></td>
<td>This section provides insight into how various physical, environmental and biological factors affect pathogen persistence. It explains how knowing persistence time for various pathogens acts as a helpful tool in estimating public health impact, designing transport models and selection of various sanitation technologies. The section summarizes pathogen reduction by considering various reduction mechanisms adopted by various sanitation technologies. It provides data on specific pathogen removal in specific unit sanitation processes and reviews the effect of various disinfection agents on individual pathogen class followed by advantages and disadvantages posed by implementing these practices and discusses the need to improve emergency response to prevent disease outbreaks and also to promote the development of new, easy to use disinfection methods.</td>
</tr>
<tr>
<td>5. <strong>Case Studies</strong></td>
<td>Provides case study information on the framework of safe sanitation system and its application, regulations for safe system design, system planning through evaluation of alternative scenario and managing risks by targeting pathogen sources.</td>
</tr>
</tbody>
</table>

2.2 Pathogens in Wastewater

Enteric pathogens excreted in human feces are known to be present in high concentrations in domestic wastewater. Upon excretion, many pathogens are known to be highly infectious and stable in water and pose a risk of environmental transmission which can eventually adversely impact public health. Because feces are a source of pathogens, there is a direct relation between the number of pathogens excreted in feces and the resulting risk of pathogen transmission. Apart from this, their persistence and infectivity play a vital role in the transmission of health risks. The type of pathogens
associated with fecal matter includes bacteria, viruses, protozoa and helminths (Aw, 2018). The presence of fecal-based pathogens in untreated wastewater has been a cause of several health risks and illnesses (Lam et al., 2015) from as mild as Gastroenteritis to as severe as diarrhea, typhoid, hepatitis A and sometimes chronic diseases (Aw, 2018). To understand the health hazard and removal efficiency of pathogens from wastewater, it is important to consider qualities like size, surface charge, and resistance forms. Therefore, it is vital to understand each pathogen class in greater detail as addressed in the following subsections.

2.2.1 Bacteria

Bacteria are prokaryotic (single cell) organisms that are the simplest and lowest form of life on earth (Madigan et al., 2015). The majority of them have a double-stranded DNA packed in a single chromosome (Aw, 2018). The size of each cell can be as small as 1-2 µm, and the volume occupied by 1,000 bacterial cells is approximately $10^{-12}$ ml (Mara & Horan, 2003). The classification is based on the different existing species which fall under various genera which are a subdivision of the 14 kingdoms where each kingdom is classified by the 16S ribosomal RNA (Madigan et al., 2015).

Out of these few thousand bacteria that have been identified, only a few are pathogenic. The pathogenic bacteria are classified into two types: Autochthonous (native to the place where found) and Allochthonous (imported to the ecosystem). Pathogenic bacteria are known to cause plant, animal and human diseases; as an example, *E. coli* and *Salmonella* infect both humans and animals, making them significant environmental reservoirs (Aw, 2018). Under favorable conditions, bacteria multiply by binary fission or budding, and some bacteria can reproduce in 30 minutes and thus this rapid growth factor
is responsible for the fast progression of diseases (Mara & Horan, 2003). The major routes of fecal-oral transmission of these infectious bacteria are through direct host contact and consumption of infected food or water (Mihelcic et al., 2009). As one example, vibrio cholera is transmitted via fecally contaminated food, drinking water, wastewater and additionally have marine reservoirs (Aw, 2018). Certain bacterial species are known to form endospores, a complex structure containing all reproductive information of the cell and coated with multiple protein layers (Metcalf & Eddy, 2014). Additionally, these structures are known to be tremendously resistant to heat, disinfection, and dehydration which helps them to remain inactive in the environment for a decade.

2.2.2 Viruses

Viruses are simple structured organisms that consist of nucleic acid center (DNA or RNA) and are covered by an outer shell protein called a capsid (Madigan et al., 2015). The size of a virus is approximately 10^{-2} to 10^{-1} \mu m, and they are spherical (Crittenden et al., 2012). They have no metabolism and are completely dependent on their hosts for survival and reproduction, making them obligatory intercellular parasites (Aw, 2018) but they can remain infectious outside the host cell and infect another host cell (Mara & Horan, 2003). Once a cell is infected, the virus has the potential to subvert the host cell metabolism to produce new viruses and result in complete lysis of host cell (Madigan et al., 2015). These organisms can rapidly adapt to changing conditions making them genetically diverse (Aw, 2018). The extracellular state in which a virus is resistant from its surrounding environment is called the Virion (Crittenden et al., 2012). When attacking a host cell, all viruses follow a pattern of attachment, penetration, replication, and release (Mara & Horan, 2003) as well as demonstrate high host species specificity (Aw, 2018).
Because of this negligible size when compared to bacteria, they cannot be detected under a normal microscope. Not until 1931, when microscopes were invented, were these microorganisms considered as filterable agents and even today, it is difficult to identify them under the electronic microscope unless they are present in wastewater (1-100 units per liter) (Crittenden et al., 2012). The widely known pathogenic viruses include adenovirus, norovirus, hepatitis A and E, rotavirus, etc. These viruses are enteric are known to cause Gastroenteritis, Hepatitis, meningitis, etc. (Aw, 2018). Although many infectious diseases are caused by viruses, these pathogens are not prominently known for their disease-causing capacities when compared to bacteria (Crittenden et al., 2012).

2.2.3 Protozoa

Protozoa are single-celled eukaryotic organisms with 35,000 varieties of species identified. They display a large physiological and morphological diversity (Madigan et al., 2015). These unicellular organisms have special ‘organelles’ that are used for feeding, excretion, and motion. Their size is approximately 1-10 µm (Crittenden et al., 2012). Depending upon the mode of nutrition for obtaining energy, protozoa are divided into four categories: photoautotrophic (primary producers), chemoheterotrophic, heterotrophic (helpful in BOD removal) and predation (feeding on algae, bacteria and other protozoa (Madigan et al., 2015). Depending on their ability to move, they can further be classified as motile or non-motile. Motile protozoa are flagellates, ciliates, and amoeba which carry out movement by flagella, ciliates, and amoeboid respectively whereas the non-motile protozoa are classified as coccidian’s and microsporidia (Crittenden et al., 2012).

Protozoa multiply in two different ways, the simpler organisms by binary fission and cyst formation (e.g., Giardia). Under stressful conditions, higher order organisms like
Cryptosporidium multiply through complex sexual and asexual methods (Crittenden et al., 2012), (Madigan et al., 2015). Sexual diversity is stated to lead to environmental adaption as this helps in maintaining genetic diversity in these organisms (Crittenden et al., 2012). When protozoa come in contact with a host cell, it usually excystate the hosts (Crittenden et al., 2012). For example, Giardia release trophozoites and Cryptosporidium release sporozoites inside the host cells. It is difficult for pathogenic protozoa to survive outside host cells and therefore to survive in outside conditions for a longer time, they tend to form environmental resistant spores, cysts, and oocysts (Crittenden et al., 2012). Protozoa are also known to be parasitic in nature and therefore cause diseases when transmitted via fecal-oral routes. They are known to be especially harmful to immunosuppressed individuals and young children and can cause severe diarrhea, vomiting, nausea, and cramps (Metcalf & Eddy, 2014). Due to their large size and predation on bacteria, they act as an effluent polisher in biological wastewater treatment processes (Crittenden et al., 2012). They are found in wastewater all around the world and due to the formation of environmental resistant forms of Cysts or (oo)cysts, are highly resistant to conventional disinfection (chlorination). It has however been found that UV disinfection is highly effective in removing Cryptosporidium oocysts and Giardia cysts (Metcalf & Eddy, 2014).

2.2.4 Helminths

Helminths are parasitic worms (Metcalf & Eddy, 2014). They come in various shapes (elongated, round, flat) and size (Crittenden et al., 2012). They are classified into three major groups: phyla - roundworms called Nematoda, flatworms called Platyhelminthes, and segment worms referred to as Annelida (Metcalf & Eddy, 2014).
Helminths reproduce through eggs (20 µm -80 µm) (Cisneros & Rendon, 2007). A helminth egg is not infective by itself after excretion and has a latency period of 18 days to a number of weeks depending on environmental factors (Keas, 1999). Once it is consumed by the host, it goes on to produce larva which then grows inside the host body and cause various complications. Once matured, it produces more eggs, which are then released into the environment via excreta and the cycle repeats itself (Cisneros & Rendon, 2007). Helminth eggs are known to be one of the most resistant biological particles because it is made up of multiple layers which act as a protective barrier from various acids, bases, organic solvents, and salts (Cisneros & Rendon, 2007). Nematodes (an abundant animal group on earth) and platyhelminths are the major cause of infection in humans. *Ascaris lumbricoides* (Nematoda phylum) infects nearly half a billion-people making it the most predominant pathogenic infection (Metcalf & Eddy, 2014). As previously mentioned, because of the high resistive nature of helminth eggs, chlorination and anaerobic digestion are ineffective in their reduction (Metcalf & Eddy, 2014)

### 2.3 Pathogen Transmission in the Environment

There are numerous factors that affect pathogen transmission in the environment. As is known, human feces are the major source, as well as an entry point, of pathogens into the environment; therefore, concentration and loading of pathogens in feces play a major role in their transmission. Out in the environment, the capability of the organism to survive and infect a host cell also plays a vital role in their fate and ultimate transmission to a human host. Figure 2.1 visually reviews the important factors that contribute to the transmission of these pathogens in the environment (Aw, 2018).
Figure 2.1 Pathogen and Environmental Characteristics that Affect Pathogen Transmission (image generated by the author of this thesis using information from Aw, 2018.)

2.4 Microbial Indicators

Indicators are the set of organisms that are used as a key tool to examine the potential presence of pathogens in water and wastewater and monitor water quality (Ashbolt, Grabow, & Snozzi, 2001). These organisms are nonpathogenic, common in the human gut, easy to measure and have similar survival and death conditions as some pathogens such as bacteria. It is important to note that even though these organisms are used for Quantitative Microbial Risk Assessment (QMRA), their application is very specific and depends on the type of problem being addressed (Farnleitner & Blanch, 2017). Table 2.2 summarizes how indicators are classified based on a specific application.
Table 2.2 Different Types of Indicator Organisms (Ashbolt et al., 2001); (Farnleitner & Blanch, 2017)

<table>
<thead>
<tr>
<th>Type of Indicators</th>
<th>Application</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fecal Indicator</td>
<td>Detect the presence of fecal contamination from human and animal sources and allows one to infer the presence of pathogens. Example: <em>E. coli</em></td>
</tr>
<tr>
<td>Treatment Indicators</td>
<td>Used to recognize the efficiency of the treatment process in pathogen reduction. Example: Total coliforms from chlorine disinfection.</td>
</tr>
<tr>
<td>Indicators for mobility and fate</td>
<td>Used to access microbial transport in soil and groundwater systems. These can be fecal indicators or treatment indicators depending on the type of study being conducted.</td>
</tr>
<tr>
<td>Host-associated fecal indicators</td>
<td>Also referred to as a microbial source to as Microbial Source Tracking (MST) markers are used to detect specific types of pathogens. Example: F-RNA coliphage used to detect viruses.</td>
</tr>
</tbody>
</table>

2.5 Different Unit Processes in Wastewater Treatment

Wastewater treatment plants use a combination of physical, chemical and biological processes to treat wastewater to meet the effluent standards. The common unit processes used for wastewater treatment are discussed in the following subsections.

2.5.1 Physical Unit Process

This type of unit process employs physical techniques to remove coarse materials, fine solids, small particles, and suspended solids (Metcalf & Eddy, 2014). Examples of basic physical unit processes are screening, grit removal, mixing, flocculation, gravity settling, flotation, and sedimentation. Other processes such as membrane filtration, depth filtration, and surface filtration are employed during advanced treatment for the removal of suspended solids and dissolved organic compounds (Metcalf & Eddy, 2014).
2.5.2 Chemical Unit Process

Chemicals and chemical reactions can be employed to bring about changes in the wastewater constituents (Metcalf & Eddy, 2014) which can be later removed with physical processes. The most basic chemical unit processes involve chemical precipitation, adsorption, gas transfer, and disinfection (Metcalf & Eddy, 2014). Chemical precipitation involves the addition of chemicals to wastewater, causing certain constituents to precipitate (Metcalf & Eddy, 2014). The addition of oxygen in the activated sludge process is an example of gas transfer (Metcalf & Eddy, 2014). Disinfection involves the use of chlorine, ozone or which helps reduce pathogens. Discussed in more detail in section 2.7.

2.5.3 Biological Unit Processes

Biological unit processes employ microbial metabolism to bring about the reduction of biodegradable organic constituents found in wastewater (Metcalf & Eddy, 2014). The microorganisms consume the organic matter present in wastewater and either convert it to biological cell tissues, which are later eliminated via settlement or another physical process or convert it to gases (e.g., CO2) which are diffused in the atmosphere (Metcalf & Eddy, 2014). Biological processes can also be employed to remove nitrogen and phosphorous from the aqueous phase.

2.6 Different Wastewater Treatment Processes

The assembly of different unit processes placed together to achieve a set degree of removal of constituents from wastewater results in pretreatment, primary, secondary, tertiary and advanced treatment (Metcalf & Eddy, 2014). Each treatment process is selected on the basis of the need for removal of selected constituents and the degree to which this removal is desired. Pretreatment and primary treatment usually employ
physical unit processes, secondary treatment involves the use of biological and chemical unit processes, and the tertiary treatment general employs all types of unit processes (Metcalf & Eddy, 2014). This section provides information on each treatment process, discusses the primary mechanisms involved and the pathogen removal efficiency in each of them.

2.6.1 Pretreatment

A soon as the wastewater enters the treatment plant, it goes through pretreatment. Pretreatment can involve screening of large debris, cans, and bottles (EPA, 2004). The main function of pretreatment is screening and removal of coarse solids like stones, grits and sand (EPA, 2004). Several sources (e.g., Feachem et al., 1983; Marin et al., 2015) have reported that pathogen reduction does not take place during pretreatment which aligns with the information summarized by (Oakley, 2018b).

Table 2.3 Pretreatment Unit Processes
(LeChevallier & Keung Au, 2004)

<table>
<thead>
<tr>
<th>Unit Process</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Screening</td>
<td>Screening provides a physical barrier which has uniform size openings that are used to remove coarse materials. Depending on the size of the openings, screens can be classified as coarse (&gt; 6 mm), fine (0.5 – 6 mm) and micro (&lt; 0.5 mm) (Metcalf &amp; Eddy, 2014).</td>
</tr>
<tr>
<td>Grit Removal</td>
<td>Grit removal is the process that uses gravity settling to remove grit, sand, gravel, cinders, and other heavy solids that enter a treatment plant with stormwater. It is important to remove these particles as they may damage the equipment’s and cause other operational problems downstream (EPA, 2004).</td>
</tr>
</tbody>
</table>

Table 2.3 provides a brief description of two different unit processes used for pretreatment. It can be seen from previous information on pathogen size as well as the table above that the size of each pathogen class is much smaller than the available
screens size, as a result promoting easy passage of pathogens through them. Therefore, it is expected there will be no pathogen removal during pretreatment.

2.6.2 Primary Sedimentation

The process of primary sedimentation involves the removal of suspended organic solids by gravity sedimentation (Metcalf & Eddy, 2014). The sedimentation tanks come in various shapes (rectangular or circular), and size depends on the hydraulic loading. This process should be designed to achieve a removal efficiency of 50% to 70% suspended solids and 25% to 40% BOD removal with an average retention time of 1.5 to 2.5 hours (Metcalf & Eddy, 2014). Sludge that settles to the bottom of the tank is further treated and dewatered before further use or disposal. (EPA, 2004).

The reduction of pathogens is reported to be extremely low during primary settling and occurs due to entrapment, adsorption or retention of pathogens which tend to be associated with settling floc particles. The reduction of helminths is reported to be 0 to \(< 1 \log_{10}\) and that of bacteria, virus, and protozoa as \(0 – 1 \log_{10}\). When advanced treatments such as chemically enhanced primary treatment (CEPT) which employs coagulation-flocculation process in addition to sedimentation (Metcalf & Eddy, 2014) and advanced primary treatment (APT) which employs coagulation-flocculation in addition to high rate lamellar settlement (high rate clarification with very short HRT) (Metcalf & Eddy, 2014) are employed, the reduction may increase to \(1 – 3 \log_{10}\) for helminths and \(1 – 2 \log_{10}\) for bacteria, virus and protozoa. Therefore, more suspended solids are formed and removed due to chemical floc formation, leading to increased reduction efficiency of pathogens in the CEPT and APT process (Oakley, 2018b).
Sedimentation of pathogens by retention in flocs has been suggested as a major factor for pathogen reduction in this process by Oakley (2018b). Due to limited availability of literature, Oakley inferred that the mechanism of sedimentation of pathogens (retention in flocs) is similar to the mechanism of floc formation (i.e., coalescence) and the rate of floc formation. Oakley also reported that removal of pathogens by sedimentation is highly dependent on the terminal settling velocity of the particular pathogens. He showed that with existing design overflow rates, the settling velocity of individual pathogens is too small and therefore no independent settlement will occur. Finally, Oakley also suggested that by lowering the overflow rate with an adequate range of HRT and with proper basin hydraulic design, enhanced pathogen removal could be achieved.

Other physical factors, such as wind and water temperature, can also affect pathogen removal through the sedimentation (Oakley, 2018b). Lower water temperature is known to increase the water viscosity which directly reduces the rate of sedimentation. In addition, a slight change in water temperature (1 degree Celcius) is known to generate density currents which may lead to hydraulic short-circuiting in the sedimentation tanks (Metcalf & Eddy, 2014). Both these temperature related effects influence the sedimentation process. Oakley (2018b) infers that circular cells would be formed in sedimentation tanks due to the wind which will eventually reduce the volumetric capacity of the tank. Additional pathogen removal in this process could be enhanced if the proper and correct dose of chemicals are added since this would escalate the coagulation and flocculation process.
2.6.3 Secondary Treatment

The main function of the secondary treatment is the removal of dissolved organic matter (biochemical oxygen demand (BOD)) and ammonia nitrogen conversion, if nitrification is required for discharge. This process can remove up to 90% of these wastewater constituents (EPA, 2004). There are two major classifications of processes that can be employed to carry out this removal, attached growth processes, and suspended growth processes.

2.6.3.1 Attached Growth Processes

An attached growth process has microorganisms that grow on a supporting surface. The microbial growth on the fixed films is used to remove dissolved organic matter from wastewater by passing it through the microbial layer (biofilms). Biofilms are a biologically active matrix of cells and extracellular substances that are attached to a solid surface (Garrett, Bhakoo, & Zhang, 2008). Examples of specific unit processes are trickling filters, fluidized bed reactors, integrated fixed-film activated sludge, moving bed bioreactors, packed bed filters, and rotating biological contactors (Metcalf & Eddy, 2014).

A trickling filter “is a non-submerged, aerobic fixed film biological reactor that uses rocks or plastic packaging over which the wastewater is distributed for treatment” (Metcalf & Eddy, 2014). There have been advancements in the material used to support the biological film and bed materials now sometimes consist of plastic balls, interlocking sheets of corrugated plastic, and various other types of synthetic media (EPA, 2004). Different types of microorganisms like bacteria, algae, and fungi grow on the surface of these media forming a biomass layer (Metcalf & Eddy, 2014). The mechanism of formation of this biomass layer involves three steps: 1) adsorption (accumulation) of
organisms on the bed material, 2) attachment and consolidation of organism on the surface by formation of polymer bridges and, 3) colonization as well as division of organisms on the surface (Garrett et al., 2008). The wastewater flows vertically over the slime layer where heterotrophic organisms found in the slime consume the organic matter in the wastewater and also grow (EPA, 2004). As the microorganisms are heterotrophic in nature, they need periodic oxygen supply which is maintained by the unsaturated nature of the media. This means the voids in the media gets refilled with air as water moves downwards through the filter (Oakley & von Sperling, 2017). The slime layer keeps growing with time and after a certain point gets detached creating a need for secondary sedimentation for its removal (EPA, 2004). To avoid clogging of the media, primary treatment is required prior to the use of trickling filters (Metcalf & Eddy, 2014).

When a trickling filter is combined with secondary sedimentation the expected reduction is reported as $1 - 2 \log_{10}$ for bacteria, $0 - 1 \log_{10}$ for protozoa, $0 - 2 \log_{10}$ for viruses, and $1 - 2 \log_{10}$ for helminths. Pathogens are removed by retention in the biofilm through adsorption, sedimentation of the slogged biofilm and by predation by other microorganisms in the biofilm. Various other factors like hydraulic and organic loading rate, peak wastewater flows, etc. also contribute to pathogen reduction which is discussed in detail in the later section (Oakley & von Sperling, 2017).

An anaerobic media filter is a submerged fixed film biological reactor that uses a submerged media for biofilm growth. An anaerobic biofilm grows on this submerged media, and when wastewater is passed through it, the soluble organic matter is removed through anaerobic digestion (Metcalf & Eddy, 2014). The media always remains submerged making sure that an anaerobic environment is maintained in the biofilm as
well as the media. Wastewater in a media filters can flow in either an upward or downward direction. Also, this system is quite effective in removal of low concentrations of suspended solids. Anaerobic media filters are known to produce very little biofilm sloughing and therefore does not require an additional secondary sedimentation process.

Similar to the trickling filter, pathogen removal in an anaerobic media filter is caused by either retention in biofilm through adsorption or by predation by other microorganisms in the biofilm. Some other factors that influence pathogen removal include hydraulic retention time, hydraulic and organic loading rate, media clogging, and the peak wastewater flow which are discussed in detail in the following paragraph. The removal of helminths was recorded as 0.32 to 1.02 log10 reduction, and there are not many numerical data identified to show pathogen reduction of bacteria, viruses, and protozoa through anaerobic media filter (Oakley & von Sperling, 2017).

One of the major mechanisms involved in pathogen removal in the attached growth process is retention in the biofilms (Oakley & von Sperling, 2017) The retention occurs as a result of attachment and adsorption. Weaver & Sinton (2009) suggest that in water, bacteria and viruses have a net negative charge leading to mutual repulsion with other bacteria and viruses but in higher ionic conditions like wastewater and marine water the electric double layer is compressed, and the resulting repulsion is overcome and allows bacterial parasites to attach to particles. The report of Weaver & Sinton (2009) also suggests that pathogens can be held together via weak linkage (attraction forces) (hydrophobic interactions and Van der Waals force or by physical attachment) or by cellular appendages or extracellular polymers excreted from cells.
The surface charge of a virus plays an important role in its sorption; this surface charge is pH dependent and the pH at which the surface charge switches its sign is called the isoelectric point (Michen & Graule, 2010). Xagoraraki, Yin, & Svambayev (2014) suggest that viruses have a positive charge when the pH of the solution is below its isoelectric point. Therefore they will be adsorbed to the negatively charged surface. In contrast, they have a negative charge when the solution pH is above its isoelectric point resulting in their adsorption to the positive surface.

One additional factor that influences pathogen reduction in an attached growth process is pathogen predation. Literature also shows that with well-designed and operated hydraulic distribution, lower hydraulic and organic loading rate and proper recirculation also enhance pathogen removal could be achieved (Oakley & von Sperling, 2017).

2.6.3.2 Suspended Growth Processes

Unlike the attached growth process, the microorganisms in this process remain in suspension. The suspended microorganisms tend to perform well leading to efficient BOD removal. With the elimination of organic compounds, the microbial biomass tends to grow, and the excessive biomass later settles down. There are a number of different processes that employ this mechanism like activated sludge process, sequencing batch reactors, and oxidation ditches (EPA, 2004). The activated sludge process is the most traditional and widely employed suspended growth process (Metcalf & Eddy, 2014). This unit process is located after primary treatment and is followed by secondary clarifiers where gravity separation is used to separate the biomass from the effluent (Metcalf & Eddy, 2014). They can also be followed by advanced treatment (disinfection) or anaerobic
digestion. These systems are designed based on the applied loading rate, i.e. the BOD mass to volumetric loading rate of the reactor (Naughton & Rousselot, 2017). To increase the efficiency of this process, the settled biomass is fed as return activated (because of the presence of millions of active microorganisms) sludge to the beginning of the process. As aeration is a crucial step for the steady performance of this system, a number of different aeration techniques are employed to maintain the oxygen level, for example, mechanical aeration and forced aeration techniques (Naughton & Rousselot, 2017). Some different types of activated sludge process can be created by making a slight variation in the reactor shapes, method of aeration and settlement processes. Table 2.4 classifies different activated sludge systems based on variations in designs.

Table 2.4 Classification of Activated Sludge Processes. (Mihelcic, Fry, Myre, Phillips, & Barkdoll, 2009)

<table>
<thead>
<tr>
<th>Variation in Reactors</th>
<th>Variation in Aeration</th>
<th>Variation in Settlement Processes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oxidation ditches</td>
<td>Step feed aeration process</td>
<td>Contact stabilization</td>
</tr>
<tr>
<td>Sequencing batch reactors</td>
<td>Extended aeration</td>
<td></td>
</tr>
<tr>
<td>Fixed film activated sludge (IFAS)</td>
<td>Anaerobic, anoxic and oxic (A2O) system</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Moving bed biofilm reactor (MBBR)</td>
<td></td>
</tr>
</tbody>
</table>

Table 2.5 shows there are a number of factors that influence the removal of pathogens in activated sludge systems including environmental factors, operational factors, microbiological factors, physical-chemical factors, and adsorption factors. Although adsorption on sludge and predation are cited as the major factors for pathogen reduction in the activated sludge process, it should be noted that in a full-scale system all the factors are interconnected; therefore, isolating one or two major factors can be
difficult. The average pathogen reduction by activated sludge process was observed to be 1.5 log10 reduction for bacteria, 1.8 log10 reduction for viruses and viral indicators, 1.3 log10 reduction for protozoa and 0.65 log10 reduction for helminths (Naughton & Rousselot, 2017).

Table 2.5 Factors Affecting Pathogen Removal in Activated Sludge Process
Adapted from (Naughton & Rousselot, 2017)

<table>
<thead>
<tr>
<th>Factors</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Environmental</td>
<td>• Higher the water Temperature, higher the pathogen removal e.g. some viruses may be sensitive to heat and UV while bacteria perform greater adsorption at a higher temperature as it leads to higher viscosity.</td>
</tr>
<tr>
<td></td>
<td>• The presence of certain viral species varies with seasons.</td>
</tr>
<tr>
<td></td>
<td>• Rain leads to pathogen dilution causing lower reduction.</td>
</tr>
<tr>
<td></td>
<td>• Wind reduces bioaerosol.</td>
</tr>
<tr>
<td>Operational</td>
<td>• Higher hydraulic retention time provides a longer time for pathogen predation, natural decay, and inactivation.</td>
</tr>
<tr>
<td></td>
<td>• Longer solids retention time means longer adsorption time.</td>
</tr>
<tr>
<td></td>
<td>• Flow rate, high flow rate means lower HRT meaning reduced pathogen reduction.</td>
</tr>
<tr>
<td></td>
<td>• Type of process, A2O systems remove bacteria more than conventional activated sludge due to the presence of numerous different species and Oxidation ditch remove protozoa better than A2O and conventional system due to longer HRT.</td>
</tr>
<tr>
<td>Microbiological</td>
<td>• Predation by other organisms due to the presence of a diverse group of organisms. Also reduced by autotrophic organisms (nitrifying and phosphorous removing organisms).</td>
</tr>
<tr>
<td>Physical and Chemical</td>
<td>• Removal of CBOD is correlated with removal of coliphages.</td>
</tr>
<tr>
<td></td>
<td>• Lower pH leads to better adsorption, and higher pH reduces adsorption but leads to better inactivation as pH affects the charge on viruses.</td>
</tr>
<tr>
<td></td>
<td>• Chemical can increase reduction of coliforms and coliphages.</td>
</tr>
<tr>
<td></td>
<td>• Pathogens are adsorbed on suspended solids, therefore; higher removal of suspended solids leads to higher pathogen reduction.</td>
</tr>
<tr>
<td>Adsorption</td>
<td>• Adsorption onto sludge particles and removal is caused in secondary clarifiers or membrane filtration.</td>
</tr>
</tbody>
</table>
An anaerobic sludge blanket reactor employs an anaerobic biological process for the removal of soluble organic compounds. The wastewater in this process is passed through a flocculated or granulated sludge blanket and the production of sludge is quite low as most of the sludge gets retained in the reactor itself. This process is temperature sensitive as the growth of anaerobic bacteria reduces with decreasing temperature. Therefore, this sanitation technology is not employed in colder regions.

The upflow anaerobic sludge blanket reactor (UASB), expanded granular sludge bed (EGSB) and anaerobic baffled reactors (ABR) are all examples of anaerobic sludge blanket reactors. In a UASB reactor, the influent enters through the bottom of the reactor and passes through a floating sludge blanket where anaerobic microbes attached to the flocs helps in reduction of organic compounds. These reactors are also divided into three sections, one to collect the methane produced by microbial activity, the treated wastewater, and the sludge. The only difference between a UASB and EGSB reactor is that in EGHB process, some of the wastewater is recirculated to obtain an increased contact time (Oakley, von Sperling, & Verbyla, 2017).

This system is not expected to have a lot of pathogen reduction efficiency as the primary design function is BOD removal and methane production. The reported reduction in pathogen concentration is between 0-1.5 log10. The major factors that contribute to the pathogen inactivation include pathogen retention in sludge, physical-chemical processes like temperature, upflow velocity, hydraulic overloading, sludge accumulation, and gas production (Oakley & von Sperling, 2017).
2.6.4 Natural Systems

Natural systems consist of a number of different types of artificially developed pond treatment systems that employ different physical and biological unit processes as discussed in the remainder of this section.

A waste stabilization pond (WSP) is an open basin that is surrounded by earth embankments and employs natural biological and physical processes for removal of organic matter and pathogens. Table 2.6 describes different types of ponds with information provided on typical pond depth requirements, location in the overall treatment train, and a focus on removed constituents.

Natural wastewater treatment systems have the capability of pathogen reduction through hostile environmental conditions or natural death. Wetlands are known to reduce pathogens with varying but notable effectiveness (Kadlec & Wallace, 2009). The different mechanisms that contribute to pathogen removal involve sunlight, sedimentation, and other physical-chemical and microbiological factors.

As seen in Table 2.7, it should be noted that these factors affect different types of pathogens in diverse ways and this table gives a brief idea about it. As reported by (Verbyla, von Sperling, & Maiga, 2017), the average removal efficiency of different pathogens in waste stabilization ponds is expected to be 4 log10 for virus, protozoa and helminths and 6 log10 for bacteria.

Table 2.6 Types of Waste Stabilization Pond Systems
Created using data from (Verbyla, von Sperling, & Maiga, 2017)

<table>
<thead>
<tr>
<th>Type of pond</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anaerobic ponds</td>
<td>• The main aim is the removal of carbon-containing organic matter.</td>
</tr>
<tr>
<td></td>
<td>• 3-5m deep.</td>
</tr>
<tr>
<td></td>
<td>• Places first in a series of ponds</td>
</tr>
</tbody>
</table>
Table 2.6 (Continued)

<table>
<thead>
<tr>
<th>Type of pond</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Facultative ponds</td>
<td>• The main aim is the removal of carbon-containing organic matter.</td>
</tr>
<tr>
<td></td>
<td>• Shallow ponds with a depth of 1.5m to 3m.</td>
</tr>
<tr>
<td></td>
<td>• Places first or second in a series of ponds</td>
</tr>
<tr>
<td></td>
<td>• Helminth egg removal</td>
</tr>
<tr>
<td>Maturation ponds</td>
<td>• Pathogen removal.</td>
</tr>
<tr>
<td></td>
<td>• Shallow ponds, &lt; 1.5m in depth.</td>
</tr>
<tr>
<td></td>
<td>• Placed last in a series of ponds</td>
</tr>
<tr>
<td>Aerated ponds</td>
<td>• The main aim is the removal of carbon-containing organic matter.</td>
</tr>
<tr>
<td></td>
<td>• Usually placed first in a series of ponds</td>
</tr>
<tr>
<td>High rate algal ponds</td>
<td>• Removal of organic matter and recovery of algae or energy.</td>
</tr>
<tr>
<td></td>
<td>• Used by themselves or placed between anaerobic and maturation ponds.</td>
</tr>
</tbody>
</table>

Sunlight is the main source of UV radiation in a waste stabilization pond and wetland systems. Kadlec & Wallace (2009) suggest that the UV wavelength range that is most effective in pathogen reduction is 240nm – 280nm. Kadlec & Wallace (2009) also mention that only 1% of the total solar radiation that falls on the natural system is in this range and inferred that the principle mechanism causing pathogen reduction by sunlight was photooxidation and direct hit radiation damage. Mathematically the solar inactivation rate is expressed as

\[ k_s = \frac{k'_s}{K_L h} \]

where,

\[ k_s = \text{overall solar inactivation rate coefficient, (m}^2/\text{J}) \]

\[ k'_s = \text{intrinsic solar inactivation rate coefficient, (m}^2/\text{J}) \]

\[ K_L = \text{light attenuation coefficient, (m}^{-1}) \], and,

\[ h = \text{water depth, (m)} \]
Table 2.7 Mechanisms Involved in Pathogen Removal in a Waste Stabilization Pond
Adapted from (Verbyla, von Sperling, & Maiga, 2017) with permission under the creative commons attribution 4.0 international license

<table>
<thead>
<tr>
<th>Type of pathogen</th>
<th>Removal mechanism</th>
</tr>
</thead>
</table>
| Bacteria         | • More sensitive to sunlight exposure than viruses.  
                    • High water temperature leads to a higher reduction rate.  
                    • Other factors involve hydraulic retention time, pH, dissolved oxygen, pond depth, number of ponds in series, turbidity, etc. |
| Viruses          | • Sunlight exposure is an important removal mechanism.  
                    • High water temperature leads to a higher reduction rate.  
                    • Other factors involve hydraulic retention time, pH, dissolved oxygen, pond depth, number of ponds in series, and turbidity. |
| Protozoa         | • The most important factor is hydraulic retention time and sedimentation.  
                    • Other factors include sunlight exposure, temperature, and pH. |
| Helminths        | • Primary removal mechanism is hydraulic retention time, and sedimentation and other factors do not contribute much towards reduction. |

In this equation it is seen that the pond depth is inversely proportional to the solar inactivation rate, suggesting that the intensity of radiation on the top of a section of the pond will be higher than the deeper sections; therefore, light penetration is reduced which eventually reduces the pathogen reduction. Because solar disinfection depends on water depth, the presence of dense vegetation (emergent, submerged or floating) will reduce pathogen inactivation (Kadlec & Wallace, 2009).

Another important factor that affects pathogen reduction is the association of organisms with other particles present in wastewater. The association provides shielding which lowers the inactivation rate. Therefore, the number of suspended solids and optical absorbance play a vital role in the effectiveness of the radiations (Kadlec & Wallace, 2009). The inactivation rate of pathogens is calculated by the following equation.
\[ N = N_0 e^{(-k_i t)} \]

where,

\( k_i \) = inactivation rate coefficient, \((m^2/J)\)

\( N_0 \) = initial number of dispersed organisms,

\( N \) = survival number of dispersed organisms,

\( I \) = intensity of UV light in solution, \((J/m^2.d)\), and

\( t \) = time, \((d)\)

Kadlec & Wallace (2009) infer that studies show the UV susceptibility of bacterial pathogens and state that inactivation of viruses would require six to eight times stronger dosage of UV radiations than that required for coliform inactivation. In a wetland system, Kadlec & Wallace (2009) inferred that nematodes, rotifers, and protozoa (flagellated and ciliated) are the main predators of bacteria. The predation of viruses by these organisms is to a limited extent in comparison to bacteria (Verbyla, von Sperling, & Maiga, 2017). Kadlec & Wallace (2009) summarize various studies the predation rate of E. coli and other bacteria and states that 90% reduction of fecal bacteria is caused by protozooplankton gazing. They also state that a higher temperature leads to higher gazing.

The removal of pathogens in WSP systems through filtration and settling is associated with submerged plants and biofilms linked with them. These components trap the pathogens on their ‘sticky traps,’ and the result is the considerable removal of microorganisms of all size (Kadlec & Wallace, 2009). Kadlec & Wallace (2009) also infer how light exposure and a higher number of submerged surfaces enhance pathogen removal. Also, Verbyla, von Sperling, & Maiga (2017) infer that low turbulence (quiescent condition approaching laminar flow) can lead to better sedimentation.
Apart from the above-mentioned factors, Verbyla, von Sperling, & Maiga (2017) infer others factors like high pH, high temperature, and dissolved oxygen (DO) can enhance pathogen removal. They state that in WSP systems, at high pH and high temperature the availability of ammonia increases which has a sanitizing effect on bacteria. Other design features like the employment of a greater number of ponds in series as well as ensuring a long hydraulic retention time also play a major role in pathogen removal.

2.7 Disinfection

After passing through the entire treatment process, disinfection is a crucial step in the reduction of pathogens from wastewater for protection of public health. Depending on the desired effluent quality, design parameters, and other environmental and economic factors, the wastewater is disinfected by either using physical agents like UV irradiation, sunlight exposure, and by applying chemical disinfection through the addition of chlorine, ammonia, and lime. Heat is not used for wastewater disinfection but can be used for disinfection of sludge.

Pathogen inactivation through addition of a chemical disinfection is caused by chemical degradation; i.e., oxidation of a cell's metabolic functions (proteins, lipids, nucleic acid, DNA, RNA). The exposure/contact time and concentration of the disinfectant plays a significant role in determining the efficiency of the disinfectant. Apart from this, the chemical used should be applicable to different pathogens, cost-effective, and not produce harmful disinfection byproducts (Kohn, Decrey, & Vinnerås, 2017).

Pathogen inactivation through physical disinfection is caused by physically damaging the cell structure and disrupting cell functions using heat, sunlight, radiation,
sonication, UV irradiation, and hydrodynamic pressure. These agents have several different mechanisms through which the cells are damaged. Application of heat denatures the proteins and enzymes in a cell. The nucleic acid in a microbial cell absorbs the photons from UV irradiation, causing the formation of photoproducts that disrupts cell replication and transcription. Organic matters present in wastewater absorbs sunlight to produce different oxides which damage protein, cell membrane, nucleic acid, and amino acid causing pathogen inactivation. Sonication causes mechanical failure of the cell membrane and cell wall via pressure when the voids generated by acoustic cavitation collapses. Radiation can also cause direct damage to cell DNA and RNA.

2.8 Expressing Pathogen Reduction in Sanitation System

The change in pathogen concentration in wastewater along the sanitation chain can be expressed using the terminology described in Table 2.8. As the concentration of pathogens highly varies, it is appropriate to describe these pathogen concentrations with respect to its order of magnitude and not focus on its digital accuracy. Therefore, log reduction values can be used to appropriately describe the reduction efficiency (difference in the influent and effluent pathogen concentration). Methods to calculate the log reduction values are described in detail in the next Chapter.

Table 2.8 Terminology Describing Fate of Pathogens (von Sperling, Verbyla, & Mihelcic, 2018)

<table>
<thead>
<tr>
<th>Term</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pathogen Removal</td>
<td>The physical eradication of pathogens from contaminated water and wastewater.</td>
</tr>
<tr>
<td>Pathogen Inactivation</td>
<td>The loss of capability of pathogens due to their physical destruction of pathogens in wastewater and sludge.</td>
</tr>
<tr>
<td>Pathogen Reduction</td>
<td>The collective effect of pathogen removal and inactivation in a sanitation system.</td>
</tr>
<tr>
<td>Term</td>
<td>Definition</td>
</tr>
<tr>
<td>---------------------</td>
<td>-------------------------------------------------------------------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>Pathogen Growth</td>
<td>The reproduction of pathogens by replication in a sanitation system. It should be noted that, due to the absence of a host, the pathogens do not regrow in the sanitation system.</td>
</tr>
</tbody>
</table>
CHAPTER 3 METHODS

3.1 Data Considerations

The data considered for this research were collected from individual chapters located on the GWPP website (http://www.waterpathogens.org/) and were used to generate tables and figures in this study. The data on fate and inactivation of viruses and bacteria for the sanitation technologies of activated sludge, trickling filter and waste stabilization ponds was made available to the author of this thesis by Dr. Colleen Naughton and Dr. Stewart Oakley, the lead writers of the respective GWPP chapters. Also, the following GWPP chapters were referenced to obtain data for log_{10} reduction of bacteria, viruses, and indicators for membrane bioreactors (Verbyla & Rousselot, 2018) and UASB reactors (Oakley, von Sperling, & Verbyla, 2017). Because limited research has been done on the fate and transport of pathogens, no additional information was found by the author, and therefore no additional data was added to the existing data provided by the GWPP. At some places, the data made available by the authors considered limited studies which were from old resources, but no change was made to this data to keep this study consistent. The existing data were statistically analyzed to validate the confidence of the data as well as to assess the influence of HRT and some other factors on the log_{10} reduction efficiency of different treatment technologies.

It should be noted that the GWPP pathogen reduction data were collected from a large number of studies and the data are typically reported as an average mean for the log reduction value from all these data. The average mean is calculated by taking an
average of all values divided by the sum total of all values. Some of the literature reports the log reduction as a range of minimum and maximum observed values, a mean value of this range was considered as the mean log10 reduction. Therefore, the data used for this study is also an average mean. The method used to calculate the reduction efficiency of pathogen, concentration was adopted from the following reference (von Sperling, Verbyla, & Mihelcic, 2018). In that case, the % reduction efficiency was calculated as follows

\[
\%E = \frac{N_0 - N}{N_0} \times 100 \quad (3-1)
\]

where,

- \( E \) = reduction efficiency,
- \( N_0 \) = influent pathogen concentration, and
- \( N \) = effluent pathogen concentration

As the concentration of pathogens is expected to be high in the influent and some effluents, representing the percentage reduction efficiency in terms of log reduction value is the most convenient method. The log reduction value (LVR) is the difference in the log-transformed values of pathogen concentration reported in the influent and effluent (von Sperling, Verbyla, & Mihelcic, 2018).

\[
LVR = \log_{10} N_0 - \log_{10} N \quad (3-2)
\]

where,

- \( \log_{10} N_0 \) = log reduced value of influent pathogen concentration, and
- \( N \) = log reduced value of effluent pathogen concentration.
Because both the reduction efficiency and log reduction values are dependent on the influent and effluent pathogen concentration, they are related as shown in the following two equations (von Sperling, Verbyla, & Mihelcic, 2018).

\[
\text{LVR} = \log_{10} \left( \frac{100}{100 - \%E} \right) \ldots (3-3)
\]

\[
\%E = 100 \times \left(1 - 10^{-LVR} \right) \ldots (3-4)
\]

The log reduction efficiency of each unit process in series is also additive (von Sperling, Verbyla, & Mihelcic, 2018). Therefore, this concept was used to compare the estimate of the expected reduction in pathogen concentrations across the different unit processes. It was assumed that the data provided for the waste stabilization ponds, activated sludge systems and trickling filter sanitation technologies were linearly distributed and ranged in the 95% confidence interval (p > 0.05). The standard deviation of this data was calculated by using Equation (3-5) (Al-Saleh & Yousif, 2009).

\[
\sigma = \sqrt{\frac{1}{N} \sum_{i=1}^{N} (x_i - \mu)^2} \ldots (3-5)
\]

where,

\( \sigma \) = standard deviation,

\( \mu \) = mean,

\( x_i \) = individual data point values, and

\( N \) = total number of data points

For data obtained for the sanitation technologies of membrane bioreactor and a UASB, because the data were reported in a range, the standard deviation was calculated using Equation (3-6) where the 99.7% values of the standard curve were considered as the lowest and highest range of the available data.

\[
\mu \pm 3\sigma \ldots (3-6)
\]
where,

\[ \mu = \text{mean, and} \]

\[ \sigma = \text{standard deviation} \]

For example, the reported bacterial log\(_{10}\) reduction for a UASB reactor was reported to range from 0.8 to 1.6 (Oakley et al., 2017). Therefore the mean log\(_{10}\) reduction was calculated as 1.2 for this range and, the standard deviation was calculated using Equation 3-6 to be 0.133. The results of this analysis can be found in Appendix B. Using all the above data, bar graphs were plotted with bars representing mean log\(_{10}\) reduction and error bars representing the standard deviations observed in the data.

Next, the log\(_{10}\) reduction data was statically analyzed by using the software ‘R’ as well as by ANOVA, to check its relative dependency on HRT and a couple of other factors including the number of ponds and temperature comparison (only for natural treatment technologies). The detailed R codes and results generated by both methods are provided in Appendix B.
CHAPTER 4 RESULTS AND DISCUSSION

4.1 Pathogen Removal in Different Unit Process from GWPP Data

Table 4.1 shows the log reduction value reported by the GWPP for bacteria and viruses for the following sanitation technologies: waste stabilization ponds, activated sludge, trickling filters, membrane bioreactors, and UASB reactors.

Table 4.1 $\log_{10}$ Reduction of Bacteria and Viruses in Different Sanitation Technologies Reported in the GWPP

<table>
<thead>
<tr>
<th>Type of Sanitation System</th>
<th>Sanitation Technology</th>
<th>Bacteria</th>
<th>Viruses</th>
<th>Indicators</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mechanized System</td>
<td>Conventional Activated Sludge</td>
<td>1.43 n= 15</td>
<td>1.84 n= 55</td>
<td>1.8</td>
</tr>
<tr>
<td></td>
<td>Anaerobic Anoxic and Oxic Activated Sludge System</td>
<td>1.9 n=6</td>
<td>1.67 n=11</td>
<td>2.27</td>
</tr>
<tr>
<td></td>
<td>Trickling Filter</td>
<td>1.16 n=1</td>
<td>0.29 n=2</td>
<td>1.41</td>
</tr>
<tr>
<td></td>
<td>UASB Reactor</td>
<td>1.2</td>
<td>0.3</td>
<td>1.1</td>
</tr>
<tr>
<td></td>
<td>Membrane Bioreactor</td>
<td>4.4</td>
<td>3.3</td>
<td>4.6</td>
</tr>
<tr>
<td>Natural System</td>
<td>Waste Stabilization Pond</td>
<td>2.3 n=16</td>
<td>1 n=45</td>
<td>1.6</td>
</tr>
</tbody>
</table>
4.2 Reduction of Bacteria

The reduction of bacterial pathogens was observed to be the highest ($4.4 \log_{10}$) across a membrane bioreactor (Table 4.1 and Figure 4.1). Verbyla & Rousselot (2018) infer that high removal through such a system is mainly due to size exclusion (retention of pathogens on the membrane) and this mechanism is highly dependent on the pore size of the membrane which may employ a micro or ultrafiltration membrane. As previously reported the size of a bacteria is as small as 1-2 µm; therefore the ratio of pathogen size to membrane pore size will make retention easy (Verbyla & Rousselot, 2018).

![Figure 4.1 Bacterial Removal in Different Sanitation Technologies](image_url)

Figure 4.1 Bacterial Removal in Different Sanitation Technologies
(Also Shown in Table 4.1)

Figure 4.1 also shows the $\log_{10}$ reduction of bacteria through an anaerobic anoxic oxic (A2O) system is expected to be $1.9 \log_{10}$ and this is comparatively higher than the
1.43 log\textsubscript{10} reduction expected for a conventional activated sludge system. Naughton & Rousselot (2017) suggested that one important mechanism for pathogen reduction in A2O systems is the hydraulic retention time. When an ANOVA p-test was performed on the log reduction and HRT data obtained from Dr. Naughton, HRT was found to not be a significant factor (p = 0.27) in the log reduction of bacteria. Figure 4.2 shows that when the regression was performed on the data set (n=6) to determine the impact on HRT on bacteria reduction in A2O systems, the data were highly scattered, and the model was able to explain only 29% of the variability of the response data around the mean. The figure also visually shows the 95% confidence interval. Looking at the regression line, it can be said that an increase in HRT should result in an increase in the log\textsubscript{10} reduction. The low change in prediction value in comparison to change in response value suggests that HRT is not a significant factor in determining the log reduction of bacteria in an A2O system. Naughton & Rousselot (2017) also suggested that the most important factor of pathogen reduction in A2O systems was adsorption to sludge (which highly depends on pH) and predation. Generally, in A2O systems pathogens that are less sustained in anoxic conditions fall prey to the anoxic organisms present in the water environment of part of the reactor. It would make sense that longer HRT would also result in greater predation and eventually higher log\textsubscript{10} reduction but the variation in results here could be due to shorter HRT used in developing the regression (only 0.33, 0.25 and 0.49 days). Therefore, an analysis of more data is suggested to better understand this relationship. Data on other factors including pH, temperature, and flow can also be studied to understand the relationship of sedimentation in A2O systems.
Figure 4.2 Significance of HRT for Log_{10} Reduction on Bacteria in an Anaerobic Anoxic Oxic Sanitation System

Because no HRT data were available for bacterial reduction in a conventional activated sludge system, an analysis could not be performed but it is expected that the removal of bacteria would be caused due to adsorption and settlement and therefore, a longer HRT should prove to benefit bacteria removal.

The log_{10} reduction of bacteria in a UASB reactor is expected to be 1.2 (Table 4.1 and Figure 4.1). The reduction is most likely due to combination of sedimentation, and retention of bacterial pathogens in the sludge which is affected by the pathogen settling velocity as well as due to the presence of chemicals (NH_{3}, fatty acids, aldehydes) which may prove to be toxic for pathogen metabolism (Oakley et al., 2017).
A 2.3 $\log_{10}$ reduction of bacteria is expected when employing a waste stabilization pond (Table 4.1 and Figure 4.1). The data available had one prediction factor as the number of ponds in series which were classified as either 2 ponds or 3 ponds in series. The data were analyzed for factors including the month of sample collected, the concentration of pathogen, HRT and number of ponds in series. The analysis found that the number of ponds in series is the most important factor in determining the log reduction of bacteria as the ANOVA analysis gave a significant relationship ($p= 0.043$) with 95% confidence. It was also found that 3 ponds in series gave a 0.75 $\log_{10}$ higher reduction of bacteria than 2 ponds in series. Also, HRT was observed to be a significant factor ($p= 0.038$) with 95% confidence in bacterial reduction as predicted by the ANOVA analysis.

Figure 4.3 shows that the $R^2$ value is low (27%) for a highly significant HRT data, this trend indicates that even though the data points are scattered (away from the line), and the model defines a low percentage of data the prediction variable (HRT) can still provide information regarding the response variable ($\log_{10}$ reduction). This can also be because the data may contain many unexplained variables that can have a direct influence on the prediction factor (HRT) that impacts the relation of HRT with the response factor. The regression line shows that log reduction of bacteria would decrease with an increase in HRT which is not intuitive. The regression line in Figure 4.3 suggests the log reduction of bacteria concentration is expected to decrease in a waste stabilization pond with an increase in HRT. This conclusion does not make sense based on the fundamental mechanisms of sedimentation as the primary reduction methods in this particular sanitation technology. Careful examination of the data (obtained through the GWPP) that made up this figure shows some of the problems working with a limited data set. For
example, there are 16 data points that make up the analysis and these data were obtained from three separate studies that measured the bacterial reduction in waste stabilization ponds in three different locations around the world (Morocco, India, Brazil). Only three studies were considered as no additional information was found by the author, and therefore no additional data was added to the existing data provided by the GWPP.

In the Morocco study (Hassani et al., 1992), two waste stabilization ponds in series were studied where the 1st pond was 2.3 m in depth and the second pond had 1.6 m of depth. This study reported the log reduction of Aeromonas bacteria (3 different species). Both ponds had an HRT reported as 22 days and reported a log$_{10}$ reduction of 1.5 for the deeper pond and 1.9 for the shallower pond. Samples were collected twice a month from February to June. The water temperature varied between 9.9 degrees Celsius to 34 degrees Celsius with a mean temperature of 21 degrees Celsius. The study reported that reduction was greater for warm (temperature above 25 degrees Celsius) months ($p<0.01$) than for cold months. Thus, within the same study, there is an expected difference in bacteria reduction with a shallower pond depth and higher temperature that both are expected to result in greater bacteria reduction.

The study in India (Joshi, Parhad, & Rao, 1973) reported 12 data points from 2 different pond systems. These 12 data points were obtained for pond systems that consisted of two cells and 3 cells in series (each system had 6 data points which developed from taking a geometric mean of 4 station points). The reduction of *Salmonella* was studied for both these systems. The reported pond depth was 1.2 m for both systems, and the samples were taken collected between March to August. The total HRT for the two-pond systems was 12.3 days while the total HRT for the three-pond system was 7
days. Furthermore, the two-cell system had submerged interconnections while the three-cell system was connected by a surface overflow arrangement. Even though most of the other parameters were similar, log\textsubscript{10} reduction values ranged from 1log - 2.5 log for the two-cell system while the log\textsubscript{10} reduction for three-cell system ranged from 2.5log – 3.4 log. The authors hypothesized that the two-cell system with the below ground baffle connectedness resulted in the movement of flocs near the bottom of the first cell into the second cell and these flocs contained bacteria which resulted in increased bacteria in the effluent of the second pond (which resulted in a lower overall reduction of bacteria). In contrast, the authors hypothesized that the surface baffle connected system had greater amounts of algal particles overflowing into the second and third cells, which allowed for greater predation of bacteria by the algae and thus great observed reduction of bacteria in that system.

The study in Brazil (Oragui et al., 1995) provided only 2 data points obtained from 2 different facultative ponds with similar HRT (5 days) and pond depth (3.4 m). The only variable that differed between the two ponds was different influent concentration, in one pond the influent concentration was 8/100 ml, and in the second the influent concentration was 20/100 ml. The reported log reduction was almost similar 1.9 and 2 in both systems. Thus, lack of data here and overall makes it difficult to interpret the impact of HRT on bacteria reduction in waste stabilization ponds.

These results are evidence that show the efficiency of pathogen reduction in a natural treatment system depends on many correlated factors and that poorly maintained systems would cause lower pathogen reduction, which was suggested by Verbyla et al. (2017). The analysis did not show any significant relation (p=0.644) between the month
(season) in which the samples were collected and the log reduction of bacteria. Also, because very limited data on the depth of the pond was available from the GWPP data and original references, a future study on the significance of pond depth and water turbidity on pathogen reduction is recommended because the depth of the pond will determine the penetration capability of the sunlight as discussed in Chapter 2.

**Figure 4.3 Significance of HRT for Log\textsubscript{10} Reduction on Bacteria in Waste Stabilization Ponds**

From Figure 4.1 we can conclude that a membrane bioreactor would provide the best result in a log\textsubscript{10} reduction of bacterial pathogens, and the next best technology that could achieve high log reduction would be a waste stabilization pond. As discussed above, a significant relationship (95%) was found between the log reduction of bacteria and the number of ponds in series for a waste stabilization pond system. Figure 4.4 provides supporting evidence to this result as it can be seen that with an increase in number of maturation ponds in series the log reduction of various bacteria can increase.
from 2.3 $\log_{10}$ reduction for one waste stabilization pond to up to 5 to 7 $\log_{10}$ reduction for employing multiple maturation ponds in series. This is because maturation ponds are designed to be shallow to enhance pathogen reduction by UV light. This overall reduction is even higher than the 4.4 $\log$ reduction reported for a membrane bioreactor. A similar log reduction capacity ($\approx 2 \log_{10}$) was observed for both a UASB reactor and trickling filter employed alone as shown in Figure 4.1. This lower removal is expected because the design of these systems emphasizes on the removal of organic pollutants. These technologies, however, can be used to remove greater amounts of pathogens if integrated with other sanitation technologies. For example, Figure 4.4 shows that when a UASB system is coupled with facultative and maturation ponds in series, log reduction as high as 5 $\log_{10}$ can be expected for a bacterial indicator (*E. coli*). The data for figure 4.4 was obtained from Oakley (2018a).

![Figure 4.4 Log Reduction of Bacteria When Additional Treatment is Provided to a Facultative Pond or UASB System](image)

**Figure 4.4 Log Reduction of Bacteria When Additional Treatment is Provided to a Facultative Pond or UASB System**

<table>
<thead>
<tr>
<th>Log Reduction Value</th>
<th>Facultative and Maturation Ponds in Series</th>
<th>Facultative Pond Followed By Two Maturation Ponds in Series</th>
<th>Facultative Pond Followed By Three Maturation Ponds in Series</th>
<th>Anaerobic pond followed by Facultative and Maturation Ponds in Series</th>
<th>UASB Reactor Followed by a Secondary Facultative Pond in series</th>
<th>UASB Reactor Followed by a Secondary Facultative and Two Maturation Ponds in series (UASB/F2/M1/M2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lower range in overall reduction</td>
<td>1.87</td>
<td>4.84</td>
<td>4.89</td>
<td>4.26</td>
<td>3</td>
<td>5.17 1.24 5 2.45 5.74</td>
</tr>
<tr>
<td>Higher range in overall reduction</td>
<td>4.84</td>
<td>4.89</td>
<td>4.26</td>
<td>3</td>
<td>5.17 1.24 5 2.45 5.74</td>
<td></td>
</tr>
</tbody>
</table>
The higher log reduction efficiency observed by employing additional treatment was also found to be true even for the conventional activated sludge process. The reported reduction of bacteria in Figure 4.1 \((1.5 \log_{10})\) for conventional activated sludge system was observed to increase in Figure 4.5 to as high as \(6-7 \log_{10}\) reduction when the activated sludge is combined with filtration and disinfection. Data for figure 4.5 was obtained from Oakley (2018a).

![Graph showing log reduction of bacteria](image)

**Figure 4.5 Log Reduction of Bacteria in an Activated Sludge System When Additional Treatment is Provided**

Muga & Mihelcic (2008) provide an analysis of the suitability of different treatment technologies depending on environmental, societal and economic factors. According to Muga & Mihelcic (2008) highly mechanized systems like activated sludge require greater economic investment as well as higher energy requirements which makes them less
suitable for smaller communities and developing countries. In contrast, mechanized sanitation technologies like a UASB reactor or trickling filter require much less economic investment and energy inputs. Therefore, the use of facultative waste stabilization ponds, trickling filters, and UASB reactors that are integrated with additional treatment provided by maturation ponds, for example, is recommended to wastewater managers to provide for better bacterial reduction when treated wastewater is discharged to the environment or considered for a reuse application.

4.3 Reduction of Viruses

The reduction of viruses is seen to be the highest at $3.3 \log_{10}$ reduction for a membrane bioreactor system as shown in Figure 4.6. This expected removal is lower than reported for bacteria (Figure 4.1). As discussed in Chapter 2, the size of viruses are smaller compared to bacteria and Verbyla & Rousselot (2018) infer that due to this small size ($10^{-2} \mu m$ to $10^{-1} \mu m$), viruses can easily pass through the existing membrane pore sizes, making reduction through size exclusion difficult but the development of cake layers on these membranes reduce the nominal pore sizes and may lead to viral retention. Also, through the mechanism of adsorption, which was also discussed in Chapter 2, virus particles attach to suspended solids that increases the overall size of the particle and eventually helps in retention on the membrane (Verbyla & Rousselot, 2018). As seen in Figure 4.6, the removal of viruses is expected to be $1.84 \log_{10}$ in a conventional activated sludge system and $1.67 \log_{10}$ in an anaerobic, anoxic oxic activated sludge systems. Naughton & Rousselot (2017) state that the main mechanism of viral removal in this system is through adsorption to sludge. The comparatively lower log reduction of virus in A2O systems may be the result of pathogen resuspension in the water during the returned
activated sludge (Naughton & Rousselot, 2017). Another important pathogen reduction mechanism as discussed in Chapter 2 should be hydraulic retention time. The ANOVA analysis performed on the reference data (n=24) suggested no significant relation (p=0.56) between hydraulic retention time and log reduction of viruses.

![Figure 4.6 Virus Removal in Different Sanitation Technologies](image)

As shown in Figure 4.7, when the $R^2$ value was plotted, a very scattered dataset was observed with only 15% of the model capable of explaining the data. The figure also provides a 95% confidence interval. Also, there are a large number of outliers; that is, the data is out of range (highly scattered) which the regression line is not able to define. Additionally, as the slope of the line is close to zero it can be said that the variation in HRT has little impact on the log reduction. Naughton & Rousselot (2017) discuss how HRT is an important design factor to support predation whereas SRT (solids retention time) is an important operation factor that would support the adsorption mechanism. As discussed above because adsorption to sludge is believed to be a major mechanism for
virus reduction in activated sludge systems, the relation between SRT and log reduction of viruses would be able to better explain the reduction. However, because the available data on SRT related to log reduction of viruses was limited, statistical analysis could not be performed to support the observation, but the reference data did show a mean 2.2 log_{10} reduction for an average SRT of 7.5 days. From the understanding of removal mechanisms of viruses involved in activated sludge process stated in Chapter 2, some other important factors that could be tested to analyze the reduction of viruses would be the temperature, pH, and its ionic strength of water.

Figure 4.7 Significance of HRT for Log_{10} Reduction of Viruses in a Conventional Activated Sludge System
The reduction of viral pathogens was reported to be low (0.3 log$_{10}$ reduction) for both a trickling filter and UASB reactor (Figure 4.6). As discussed in Chapter 2, the main mechanism of virus reduction in a trickling filter is expected to be adsorption to biofilms. Oakley et al. (2017) suggest the main reasons for the virus reduction in a UASB reactor could be the solids retention time and contact with settling sludge (adsorption). Even though a statistical analysis was not performed to support these claims, through the knowledge gathered from the literature reviewed in this thesis, it could be concluded that one of the most important factors of viral reduction in mechanized systems is adsorption of viral pathogens to sludge.

Figure 4.6 shows that the reduction for viral pathogens in a waste stabilization pond was 1 log$_{10}$. As mentioned in Chapter 2 the main mechanism of pathogen reduction in natural systems is through solar disinfection and settling. Verbyla et al. (2017) suggest that other factors including HRT, water turbidity, and pond depth also play a crucial role in viral pathogen reduction in natural systems. The reference data had three prediction factors for which data was available including hydraulic retention time, pond depth and temperature. All waste stabilization ponds in the study that make up the results presented here are facultative ponds and no maturation pond was present. When an ANOVA analysis was performed on HRT as the prediction factor, the results showed a highly significant (p= 0.002) relation with 95% confidence between HRT and log$_{10}$ reduction. The $R^2$ plot of this relationship can be seen in Figure 4.8. For this linear regression model, the $R^2$ value measures the proportion of the variation in log reduction which is explained by the independent variable HRT. Here the $R^2$ value of 20% indicates that the model explains 20% of the variability of the response data around its mean. Even though the trend
indicates a significant relation between the prediction value and response value, the low $R^2$ value is a result of a large number of outliers which the regression line is not able to define. Verbyla et al. (2017) state that the performance of the natural system (waste stabilization pond) is dependent on multiple interrelated factors and identification of the contribution of a single factor towards its performance is not possible. Additionally, the slope of the line in Figure 4.8 suggests that according to this model, the log_{10} reduction of viral pathogens is expected to increase with the increase in hydraulic retention time.

![Graph](image)

Figure 4.8 Significance of HRT for Log_{10} Reduction of Viruses in a Waste Stabilization Pond
When statistical analysis was performed on temperature as the prediction variable and \( \log_{10} \) reduction as the response variable, it resulted in 90% confidence \((p=0.07)\) that temperature is a significant factor responsible for the reduction of viruses. The mean temperature from the raw data was 17.7 degrees Celsius. Linden & Murphy (2017) suggest that viral reduction can be caused by solar heating by coagulating proteins and enzymes within the viral cells. From the information presented in Chapter 2, the inactivation of viruses can also be a result of the combination of solar heating and UV light. ANOVA analysis of pond depth and log reduction resulted in an insignificant relationship \((p=0.48)\) between the two variables (due to lack of pond depth data, a similar analysis for bacterial reduction was not performed). Even though Verbyla et al. (2017) suggests that pond depth is an important factor, he also suggests that high water turbidity can lower the inactivation of viral pathogens, therefore, additional variable for water turbidity is required to correctly predict the effect of pond depth on viral reduction.

The log reduction of viral pathogens was reported to be the greatest with employing membrane bioreactor technology and the second best \((1.6 -1.8 \log_{10})\) reduction was observed through an activated sludge system as seen in Figure 4.6. Both these systems are energy intensive and require large economic investment (Muga and Mihelicic, 2008) making them unsuitable for many developing nations and smaller communities. Figure 4.6 reports that the reduction of viral pathogens through a waste stabilization pond is 1 \( \log_{10} \). Even though natural systems require less financial and environmental investment, low virus reduction may make them unsuitable for discharge to the environment or in a water reuse scenario. However, even though the log reduction of viruses was reported to be the lowest through a UASB reactor and a trickling filter \((\approx \)
0.3 log10), when additional treatment like chlorination was applied, log reduction of viruses was doubled as seen in Figure 4.9. This higher removal of pathogens has also been reported for a UASB reactor followed by several maturation ponds (Symonds, et al., 2014). Furthermore, UASB reactors are a less energy intensive option in comparison to other mechanized systems (Cornejo, Zhang, & Mihelcic, 2013). As with bacteria reduction, a UASB reactor and trickling filter can still be used for virus reduction if they are integrated with additional treatment that increases pathogen removal. Figure 4.9 also shows increased log reduction of viruses by an activated sludge system when chlorination was added. The data for figure 4.9 was obtained from Oakley (2018a).

![Figure 4.9 Log Reduction of Virus When Additional Treatment is Provided to an Activated Sludge System and Trickling Filter System](image-url)
CHAPTER 5 CONCLUSIONS AND RECOMMENDATIONS

The reduction of bacteria and viruses across wastewater treatment technologies was studied by: i) determining whether mechanized or natural wastewater treatment technologies achieve greater reduction of these pathogens; ii) assessing how HRT impacts reduction in bacteria and virus concentrations; and iii) assessing the extent of improvement in bacteria and virus reduction when specific treatment technologies are followed by an additional unit process(es) that provides further pathogen removal. The efficiency of mechanized as well as natural wastewater treatment systems to achieve high reduction of pathogens relies on a number of prediction factors that may be interconnected and independent analysis of these prediction factors to determine its significance in $\log_{10}$ reduction of bacteria and viruses can result in predictions that may contradict the literature. Therefore, to correctly predict the results, the knowledge of each of these factors is a prerequisite. The above analysis identified as well as analyzed a few of these variables and their effect on the reduction of bacteria and viruses. Additional studies and data of each of these prediction factors is needed to better understand their influence on pathogen reduction.

The bacterial reduction is best achieved by deployment of a more expensive wastewater treatment technology like a membrane bioreactor which is estimated to achieve $4.4 \log_{10}$ reduction of bacteria. In comparison a facultative waste stabilization pond is expected to achieve $2.3 \log_{10}$ reduction of bacteria which was higher than reported for the technologies of activated sludge, trickling filter and a UASB reactor. Mechanical
treatment technologies like membrane bioreactors and activated sludge are however more expensive technologies that require more skilled labor and advanced technical knowledge as compared to design and operation of natural systems, such as waste stabilization ponds, or less mechanized sanitation technologies, such as a UASB reactor and trickling filter. When these systems are integrated with chemical disinfection or maturation ponds that employ UV light, increased log reduction of bacteria is expected. In these cases, the log reduction of bacteria in a waste stabilization pond system increased to 5-7 $\log_{10}$ reduction, and in a system that uses a UASB reactor, bacteria reduction can be increased to 5 $\log_{10}$ reduction when multiple maturation ponds in series are placed after the UASB reactor. A similar increase in log reduction was observed for conventional activated sludge systems (6-7 $\log_{10}$) when filtration and disinfection were integrated with this technology. Therefore, these types of systems may be the most cost-effective and reliable treatment systems to achieve bacterial reduction of treated wastewater effluent.

The highest (3.3 $\log_{10}$) viral reduction is expected through the employment of a membrane bioreactor, but it is also seen that activated sludge achieves approximately 2 $\log_{10}$ reduction for viruses, which was lower than that reported for bacteria by similar technologies. The performance of more mechanized systems for viral reduction was better as compared to the natural system (1 $\log_{10}$). As mentioned above the mechanical treatment technologies are more expensive and require more skilled labors and advanced technical knowledge as compared to natural systems or less mechanized treatment systems like UASB reactors and trickling filters. When these systems are integrated with filtration and disinfection, the increase in log reduction was observed for conventional
activated sludge systems (2-4 log\textsubscript{10}) and trickling filters (2.5-2.8 log\textsubscript{10}). Even though, these types of systems are the more costly and yet they are cost-effective treatment systems to achieve a viral reduction in wastewater effluent.

Further study as well as field studies on the performance of these wastewater treatment technologies when coupled with advanced tertiary treatment (disinfection) or use of UV sunlight needs to be carried out to understand the efficiency of different disinfection technology on bacterial and viral pathogens. It is also recommended that additional studies be carried out to identify the predictive factors that would be responsible for these reductions. Better understanding, as well as more data on the predictive factors like SRT, HRT, pond depths, temperature, flowrate, pH, can be further used to statistically analyze the correlation of these factors and log reduction of bacteria and viruses. This will help in understanding the significance of each factor in relation to log reduction of pathogens.
REFERENCES


APPENDIX A SUPPORTING INFORMATION FOR CHAPTER 1

Figure A.1 Concentration of Viruses Reported in Human Feces

Figure A.2 Concentration of Bacterial Pathogens in Human Feces
Figure A.3 Concentration of Protozoan Pathogens in Human Feces

Figure A.4 Concentration of Helminth in Human Feces
APPENDIX B SUPPORTING INFORMATION FOR CHAPTER 4

B.1 Data for Figure 4.1 and 4.4

Table B.1 Mean $\log_{10}$ Reduction and Standard Deviations for Bacteria Data in Different Treatment Technologies

<table>
<thead>
<tr>
<th>Treatment Technology</th>
<th>Mean Log10 Reduction</th>
<th>Standard Deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trickling Filter (n=1)</td>
<td>1.16</td>
<td>NA</td>
</tr>
<tr>
<td>UASB</td>
<td>1.2</td>
<td>0.133</td>
</tr>
<tr>
<td>Membrane Bioreactor</td>
<td>4.4</td>
<td>1</td>
</tr>
<tr>
<td>Conventional Activated Sludge (n=15)</td>
<td>1.43</td>
<td>0.77</td>
</tr>
<tr>
<td>A2O Activated Sludge (n=6)</td>
<td>1.9</td>
<td>0.7</td>
</tr>
<tr>
<td>Waste Stabilization Ponds (n=16)</td>
<td>2.3</td>
<td>0.66</td>
</tr>
</tbody>
</table>

Table B.2 Mean $\log_{10}$ Reduction and Standard Deviations for Virus Data in Different Treatment Technologies

<table>
<thead>
<tr>
<th>Treatment Technology</th>
<th>Mean Log10 Reduction</th>
<th>Standard Deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trickling Filter (n= 2)</td>
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<td>0.08</td>
</tr>
<tr>
<td>UASB</td>
<td>0.3</td>
<td>0.1</td>
</tr>
<tr>
<td>Membrane Bioreactor</td>
<td>3.3</td>
<td>1.2</td>
</tr>
<tr>
<td>Conventional Activated Sludge (n=55)</td>
<td>1.84</td>
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</tr>
<tr>
<td>A2O Activated Sludge (n=11)</td>
<td>1.67</td>
<td>0.92</td>
</tr>
<tr>
<td>Waste Stabilization Ponds (n=45)</td>
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<td>0.7</td>
</tr>
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</table>
B.2 ANOVA Results for Various Parameters of Different Treatment Technologies

### SUMMARY OUTPUT

#### Regression Statistics
- Multiple R: 0.53566465
- R Square: 0.28693661
- Adjusted R Square: 0.10867077
- Standard Error: 0.66272129
- Observations: 6

#### ANOVA

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<th>MS</th>
<th>F</th>
<th>Significance F</th>
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</thead>
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<td>Regression</td>
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<td>0.7069353</td>
<td>1.60959948</td>
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<tr>
<td>Residual</td>
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</table>

#### Coefficients

<table>
<thead>
<tr>
<th>Coefficients</th>
<th>Standard Error</th>
<th>t Stat</th>
<th>P-value</th>
<th>Lower 95%</th>
<th>Upper 95%</th>
<th>Lower 95.0%</th>
<th>Upper 95.0%</th>
</tr>
</thead>
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<tr>
<td>Intercept</td>
<td>0.28697238</td>
<td>1.30244031</td>
<td>0.22033438</td>
<td>-3.3291817</td>
<td>3.9031264</td>
<td>-3.3291817</td>
<td>3.9031264</td>
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<tr>
<td>X Variable 1</td>
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<td>3.67533015</td>
<td>1.26869992</td>
<td>-5.5414613</td>
<td>14.8672434</td>
<td>-5.5414613</td>
<td>14.8672434</td>
</tr>
</tbody>
</table>

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Figure B.1 ANOVA Analysis Results for HRT vs. Log Reduction of Bacterial Pathogens in A2O System

### SUMMARY OUTPUT

#### Regression Statistics
- Multiple R: 0.12287398
- R Square: 0.01509802
- Adjusted R Square: -0.0296703
- Standard Error: 5.33210053
- Observations: 24

#### ANOVA

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<th>Source</th>
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<th>Significance F</th>
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<td>0.33724812</td>
<td>0.56732391</td>
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<td>Residual</td>
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<td>625.488513</td>
<td>28.431296</td>
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<td>Total</td>
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<td>635.076914</td>
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#### Coefficients

<table>
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<th>Standard Error</th>
<th>t Stat</th>
<th>P-value</th>
<th>Lower 95%</th>
<th>Upper 95%</th>
<th>Lower 95.0%</th>
<th>Upper 95.0%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept</td>
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<td>0.17937938</td>
<td>-7.7033667</td>
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<tr>
<td>X Variable 1</td>
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<td>0.58073068</td>
<td>-2.7563586</td>
<td>4.90043013</td>
<td>-2.7563586</td>
<td>4.90043013</td>
</tr>
</tbody>
</table>

---

Figure B.2 ANOVA Analysis Results for HRT vs. Log Reduction of Viral Pathogens in Conventional Activated Sludge System
SUMMARY OUTPUT

HRT

Regression Statistics

<p>| | | | | |</p>
<table>
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<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Multiple R</td>
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<tr>
<td>R Square</td>
<td>0.27103027</td>
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</tr>
<tr>
<td>Adjusted R Square</td>
<td>0.21896101</td>
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</tr>
<tr>
<td>Standard Error</td>
<td>4.6638273</td>
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<td></td>
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</tr>
<tr>
<td>Observations</td>
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</tbody>
</table>

ANOVA

<table>
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</thead>
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<td>Regression</td>
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<td>113.219509</td>
<td>5.20518713</td>
<td>0.03868323</td>
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<tr>
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<td>21.7512851</td>
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<td></td>
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</table>

Coefficients | Standard Error | t Stat  | P-value | Lower 95% | Upper 95% | Lower 95.0% | Upper 95.0% |
-------------|----------------|---------|---------|-----------|-----------|-------------|-------------|
Intercept    | 20.374003      | 4.43459199 | 4.5943354 | 0.00041693 | 10.8627491 | 29.8852569 | 10.8627491 | 29.8852569 |
X Variable 1 | -4.1627616     | 1.82458192 | -2.2814879 | 0.03868323 | -8.0761006 | -0.2494226 | -8.0761006 | -0.2494226 |

Figure B.3 ANOVA Analysis Results for HRT vs. Log Reduction of Bacterial Pathogens in Waste Stabilization Pond System

SUMMARY OUTPUT

Ponds in series

Regression Statistics

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<table>
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<td>Multiple R</td>
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</tr>
<tr>
<td>R Square</td>
<td>0.4882928</td>
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</tr>
<tr>
<td>Adjusted R Square</td>
<td>0.4517428</td>
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<tr>
<td>Standard Error</td>
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</tr>
<tr>
<td>Observations</td>
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ANOVA

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<th>SS</th>
<th>MS</th>
<th>F</th>
<th>Significance F</th>
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<td>Residual</td>
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<td>Total</td>
<td>15</td>
<td>7</td>
<td></td>
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</tr>
</tbody>
</table>

Coefficients | Standard Error | t Stat  | P-value | Lower 95% | Upper 95% | Lower 95.0% | Upper 95.0% |
-------------|----------------|---------|---------|-----------|-----------|-------------|-------------|
Intercept    | 0.55392428     | 0.4809578 | 1.15171077 | -0.477627614 | 1.58547618 | -0.4776276 | 1.58547618 |
X Variable 1 | 0.72328604     | 0.19788673 | 3.6505075  | 0.00259871 | 0.298861218 | 1.14771087 | 0.29886122 | 1.14771087 |

Figure B.4 ANOVA Analysis Results for Number of Ponds in Series vs. Log Reduction of Bacterial Pathogens in Waste Stabilization Pond System
Figure B.5 ANOVA Analysis Results for HRT vs Log Reduction of Viral Pathogens in Waste Stabilization Pond System

B.3 ‘R code’ for Various Parameters to Determine p-Value of Different Treatment Technologies

The code below is an example of R code written to conduct p-test of prediction factors like HRT, Pond depth and temperature with log reduction for viral removal in waste stabilization ponds.

```r
virus<-data[which(data$Pathogen.Type=="Virus"),]
virus_model <- lm(Log10_removal ~ Pathogen + Concentration + Treatment.Factor.1.Value + Treatment.Factor.2.Value + Treatment.Factor.3.Value, data=virus)
summary(virus_model)
```

Call:
```
lm(formula = Log10_removal ~ Pathogen + Concentration + Treatment.Factor.1.Value + Treatment.Factor.2.Value + Treatment.Factor.3.Value, data = virus)
```

Residuals:
```
    Min  1Q Median  3Q  Max
-1.36625 -0.37737  0.02936  0.24855  1.52365
```

Coefficients:
```
                  Estimate Std. Error t value  Pr(>|t|)    Lower 95%    Upper 95%    Lower 95%0    Upper 95%0
Intercept       0.614011559 0.151112657   4.06327021 0.000201655 0.309263842 0.918759277
X Variable 1    0.013572143 0.004191526   3.237995378 0.002321931 0.005119124 0.022025161
```
```
| Estimate | Std. Error | t value | Pr(>|t|) |
|----------|------------|---------|---------|
| (Intercept) | 4.050e-01  | 4.058e-01 | 0.998   | 0.32435 |
| PathogenRotavirus | -3.875e-01 | 5.013e-01 | -0.773  | 0.44419 |
| Concentration | -3.435e-06 | 2.294e-05 | -0.150  | 0.88173 |
| Treatment.Factor.1.Value | 1.580e-02  | 5.307e-03  | 2.977   | 0.00499 ** |
| Treatment.Factor.2.Value | -1.231e-01 | 1.600e-01  | -0.770  | 0.44621 |
| Treatment.Factor.3.Value | 2.341e-02  | 1.281e-02  | 1.827   | 0.07535 . |

---

Signif. codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Residual standard error: 0.6188 on 39 degrees of freedom

Multiple R-squared: 0.3099, Adjusted R-squared: 0.2215

F-statistic: 3.503 on 5 and 39 DF, p-value: 0.01
```