EFFECT OF BIO-AUGMENTATION PRODUCT BiOWiSH® SEPTIC RESCUE ON THE
WASTEWATER TREATMENT PERFORMANCE OF RESIDENTIAL SEPTIC TANKS

A Thesis
presented to
the Faculty of California Polytechnic State University,
San Luis Obispo

In Partial Fulfillment
of the Requirements for the Degree
Master of Science in Civil and Environmental Engineering

by
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March 2019

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 Septic Rescue on the Wastewater Treatment
 Performance of Residential Septic Tanks

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ABSTRACT

Effect of Bio-Augmentation Product BiOWiSH® Septic Rescue on the Wastewater Treatment

Performance of Residential Septic Tanks

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Residential septic systems provide reliable wastewater treatment for over 26 million homes and facilities in the United States, and many more worldwide. When properly maintained, these systems are reliable, low-cost, and long-term treatments for residential wastewater. When neglected, septic systems can fail leading to health and ecological harm through soil and groundwater contamination through the improperly treated wastewater effluent.

This study tested the effect of BiOWiSH® Septic Rescue of BiOWish® Technologies International, Inc. (hereafter referred to as BiOWiSH) on the biological treatment of residential septic tanks. BiOWiSH is meant to act as a bioaugmentation product through the addition of a proprietary blend of *Bacillus* and Lactic Acid producing bacteria to act as a biocatalyst to enhance and encourage a range of hydrolytic, oxidative, and reductive biochemical reaction and promote digestion of bio solids and ammonification within the septic tanks.

To test the effect of BiOWiSH on the treatment of residential septic tanks, four 32-gallon tanks were constructed and filled with water and primary sludge from the primary clarifier at the San Luis Obispo Water Resource Recovery Facility. Two tanks were dosed with the recommended amount of BiOWiSH; one tank had no additive biological treatment and served as the control; one tank was dosed with RID-X® Septic Maintenance, a competitive product (hereafter referred to as RID-X).

Each tank functioned as a plug-flow reactor. Primary sludge and tap water was added daily and effluent was sampled on a daily or weekly basis, based on the parameters being tested. Effluent water samples were tested for removal of ammonia, nitrates, total suspended solids, and biological oxygen demand. Temperature and pH were also recorded.

These analyses indicated no significant advantage from the addition of BiOWiSH in the reduction of ammonia, total suspended solids, or biological oxygen demand over the control tank or the tank dosed with the RID-X competitive product. Nitrates (in the form of nitrate and nitrite) did not form in any of the tanks.

Keywords: Septic Tank, Septic System, Biological Nutrient Removal, BiOWiSH

ACKNOWLEDGMENTS

This thesis truly could not have been completed without the overwhelming love and support from so many in my community. To my thesis advisors, Nirupam Pal, Rebekah Oulton, and Tryg Lundquist, thank you for your long hours and dedication to guiding me through the inception, research, and finally analysis of this study. In addition, thank you for your countless hours of instruction and education throughout my undergraduate career, which I extend to all CE/ENVE faculty. To the entire BiOWiSH family, thank you for your faith in this project and the financial and educational help that you continuously provided.

Special thanks are sent to Joelle Arakaki, Elizabeth Villanueva, Patrick Kalvass, and Emily Holland who individually and as a group helped this project reach completion, through technical help, hours spent sampling together in lab, and their abundant friendship. To Don Heye, your educational and financial help will forever be an outstanding gift to my family.

Most importantly, thank you to my incredible family for the love and support that you have each given through my lifetime, and throughout this study. To my parents, you were the motivation that drove me to finish, and create a life and thesis that I could be proud of. To my sisters, you are my everyday joy and I appreciate the constant love and support you give. And to my husband and best friend, none of this would have been possible without you.

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Chapter 1

INTRODUCTION

Though septic systems are acknowledged as long-term and effective methods to facilitate the treatment of wastewater, the systems are prone to failing and creating groundwater and surface water when not properly maintained (Environmental Protection Agency, 2002). This section reviews the biological, chemical, and mechanical principles to achieve effective treatment of wastewater through residential septic systems, as well as common reasons to why the systems can fail.

To address the contamination from failed and failing septic systems, BiOWiSH® Technologies International, Inc. has created two bio-augmentation products, Septic Rescue and Septic Maintenance (hereafter referred to as BiOWiSH). Composed of BiOWiSH's proprietary blend of "metabolically cooperative" microorganisms, these cultures are claimed to accelerate the biological removal of nutrients from wastewater (BiOWiSH, n.d.). Septic Rescue and Septic Maintenance are designed to rapidly digest organic solid waste, eliminate odor causing compounds, and reduce biological oxygen demand (BOD), total suspended solids (TSS), FOG (Fats, Oil, and Grease), phosphorus, ammonia (NH_3), and nitrates ($\text{NO}_3^-/\text{NO}_2^-$).

Chapter 2

BACKGROUND

The following sections review the important role septic systems play in the treatment of residential wastewater throughout the United States, as well as the traditional methods of contaminant removal within the systems. This section also includes an overview of past BiOWiSH studies testing the effectiveness of BiOWiSH products.

2.1 The Importance of Septic Systems

Residential septic systems service over 26 million homes and facilities throughout the United States alone (Environmental Protection Agency, 2002). Once considered simply temporary installations for rural homes, it was thought that most would eventually be connected to a centralized treatment facility (Environmental Protection Agency, 2002). However, due to their ability to operate in rural communities and their recognized treatment potentials, public health and environmental protection officials now acknowledge that onsite septic systems provide effective and permanent wastewater treatment (Environmental Protection Agency, 2002).

Figure 1: Data from 1990 and 2013 Showing Distribution of Homes Still Utilizing Septic Systems (Walton, 2015).

These septic systems are recognized worldwide as reliable, low-cost, long-term and permanent approaches to wastewater treatment, but only if they are planned, designed, installed, operated, and maintained properly (Environmental Protection Agency, 2002). Unfortunately, in many cases, these systems are neglected or simply designed incorrectly, and the result is

unprecedented contamination of the local soils and groundwater aquifers (Environmental Protection Agency, 2002; Walton, 2015).

In fact, these septic systems may currently constitute the third most common source of groundwater contamination, are a principal or contributing source of degradation in 32% of all harvest-limited shellfish estuaries, and a known contributor to the overabundance of nutrients in ponds, lakes, and coastal estuaries that lead to eutrophication (Environmental Protection Agency, 2002; Walton, 2015). The United States Environmental Protection Agency (USEPA) also estimates that over 168,000 viral illnesses and 34,000 bacterial illnesses occur annually as a result of consumption of water contaminated by improperly treated wastewater from onsite systems reaching local groundwater sources (Environmental Protection Agency, 2002). Over 28,821 miles of streams are designated as “threatened or impaired” by the United States Environmental Protection Agency due to failing septic and sewage pits (Walton, 2015). Septic tanks can be beneficial but left unchecked can create tremendous environmental effects.

Though these numbers alone show that septic systems can be harmful and detrimental to the health of communities and ecosystems, the true effects may still be unknown. While there is ample research into septic system design, significantly less research analyzes the environmental and health effects from the widespread failure of septic systems. Few organizations provide reliable numbers on the amount of septic systems in operation throughout the nation, and there is little data on how many of those systems may be failing (Walton, 2015). The United States Census Bureau stopped collecting county-level data on septic systems in 1990 because no federal program regulated septic systems (Walton, 2015). National data is gathered every 2 years by the American Housing Survey, but with a much smaller sample size (Walton, 2015). Additional research and regulatory oversight will be needed in the future to accurately characterize these impacts (Environmental Protection Agency, 2002).

2.2 Traditional Residential Wastewater Treatment Using Septic Systems

The modern residential septic system consists of an enclosed septic tank and a soil absorption field, also known as a subsurface wastewater infiltration system, or more commonly, a leach field (Environmental Protection Agency, 2002).

Figure 2: Overview of Residential Septic System (Environmental Protection Agency, 2002).

The tank itself is an anaerobic environment meant to collect wastewater, segregate any settleable solids, accumulate, consolidate, and store unsettlable solids, and function as an anaerobic digester that promotes partial digestion of all retained organic matter (Environmental Protection Agency, 2002; Bounds, 1997). The tank is designed to be a pretreatment, similar to primary clarifiers, that then sets the stage for further biological treatment, adsorption, filtration, and infiltration in the following leach fields (Figure 2).

Figure 3: Residential Septic Tank Diagram (Flowhawks)

Vital to this study is the understanding of the stratification that develops in properly functioning septic tanks. The tanks themselves, when not overloaded by excessive amounts of wastewater at one time, function as plug-flow reactors. This severely limits any mixing or heating and allows particles to naturally ascend or descend causing stratification, or septic layers (Bounds, 1997). The floating scum and settled sludge is generally completely anaerobic. The tanks “clear zone,” its effluent, can range be either anoxic or anaerobic (Bounds, 1997).

If this stratification does not occur, septic tanks often fail. Outflowing solids can plug the infiltrative soils and prevent the vital biological, physical, and chemical treatment that those soils provide. This study specifically designed model septic tanks to encourage this stratification and prevent any mixing of the scum and sludge layer. It also looked at the impact of adding the biological aid in reducing total suspended solids (TSS), which is often a cause of leach field clogging and failure.

Following the septic tank, effluent enters the soil through a leach field or similar methods. Though this process was not modeled in this study, it is important to understand the complete treatment process that residential wastewater undergoes before discharge. These technologies

can include aerobic to anaerobic biological treatment in suspended or fixed-film reactors, physical and chemical treatments, soil infiltration, fixed media filtration, and even disinfection (Environmental Protection Agency, 2002). The most common is through aerated infiltration systems, more commonly referred to as leach fields.

Figure 4: Cross Section View of Standard Leach Field Infiltration (R&R Landworks)

In these aerated infiltrative systems, oxygen is naturally supplied through the movement of air between soil particles. The oxygen supplied in the infiltrative system forms a biomat within the first few centimeters of the unsaturated soil and is an aerobic zone, providing more oxygen than the anaerobic septic tanks. In this context, the USEPA defines a biomat as the soil component in which residual particulate matter accumulates and is trapped in the pores of the soil matrix, providing a source of carbon and nutrients for the active biomass (Environmental Protection Agency, 2002).

In the unsaturated vadose zone below the surface, the septic effluent is under negative pressure due to capillary and adsorptive forces in the soil matrix, which allows an additional flow of air through open soil pores and gives oxygen to microbes growing on the surface of soil particles (Environmental Protection Agency, 2002). Water then continues to percolate and can join the natural groundwater aquifer (Environmental Protection Agency, 2002). If pollutants remain in the wastewater, the groundwater table can become contaminated, causing health

problems if the water is used as a drinking source and environmental concerns if water reaches surface waters.

2.2.1 Factors Causing Failing Systems

Consequences of a failed or failing septic system include toilets, drains, and showers draining slowly or backing up, foul odor emitted in the home, or a pooling of water, muddy soil, or sewage above the leach field (BiOWiSH, n.d.). Failed and failing systems can cost thousands of dollars in repair, usually consisting of emptying non-digested solids from the tank. Removing the built-up solids from the tank encourages native septic tank microbes to continue solids digestion and prevents excess solids in the tank effluent from overflowing into the clogged leach field (Environmental Protection Agency, 2002).

As described in the following sections, septic systems fail due to a disturbance in the biological and chemical environment of the septic tank, often leading to septic tanks allowing solids to enter and clog the leach field. A common cause of this failure is from the overuse and flushing of disinfectants, bleach, chlorine, or other chemicals that upset the natural microorganisms within the tank (BiOWiSH, n.d.).

Contamination of non-degradable materials, such as latex products, rubber gloves, plastics, metals, sand, or soil can cause buildup and blockage preventing microorganisms from effectively digesting biosolids in the tank. Excessive water use, such as emptying spas, running multiple loads of laundry, or excessively long showers, can also cause failures as the large quantities of water flush the septic tank of their native microbes, stir up settled solids, and shortens the overall retention and treatment times within the tank (BiOWiSH, n.d.).

2.3 Average Residential Septic Tank Contaminant Concentrations

Contaminant concentrations of typical residential septic tanks differ significantly due to the size of tank, the number of people using the tank, and the loading applied to the system as a whole. A general description of typical wastewater contaminants and their concentrations is supplied in Table 1 below (Environmental Protection Agency, 2002).

Table 1: USEPA Onsite Wastewater Treatment Systems Manual Typical Wastewater Concentrations (Environmental Protection Agency, 2002)

Parameter	University of Wis. (1978)	Harkin, et al. (1979)	Ronayne, et al. (1982)	Ayres Associates (1993)	Ayres Associates (1996)
Tanks Sampled	7	33	8	8	1
Location	Wisconsin	Wisconsin	Oregon	Florida	Florida
Number of Samples	150	140-215	56	36	3
BOD5 (mg/L)	138	132	217	141	179
COD (mg/L)	327	445	-	-	-
TSS (mg/L)	49	87	146	161	59
TKN (mgN/L)	45	82	57.1	39	66
TP (mgP/L)	13	21.8	-	11	17

Though the above values are typically accepted as average values for residential septic tank, past studies have shown significant variation in the actual constituent loading levels sampled. A Washington State Department of Health Wastewater Management Program released a 2003 summarizing literature relating to residential septic tank effluent values (Eliasson, 2004). Though their study also looked to differentiate between “typical residential” and “high strength” septic tank effluent, the recorded values are relevant to this research (Table 2).

Table 2: Washington State Department of Health Septic Tank Effluent Values (Eliasson, 2004)

	Capita	Hydraulic Load	BOD5	TSS	pH
		Gpd/ft2	mg/L	mg/L	
Median	4	0.44	178	69	7.46

Average	3.6	0.44	192	87	7.7
Maximum	7	1.07	548	626	8.56
Minimum	1	0.1	32	14	6.6
Standard Deviation	1.4	0.18	91	91	7.82
90th Percentile	5	0.62	300	146	8.02
10th Percentile	2	0.23	89	30	7.02
Total Count	47	42	141	47	140

Another study recorded the variability of septic tank effluent values in 19 separate studies (Table 3).

Table 3: American Society for Testing and Materials “Design and Performance of Septic Tanks” (Bounds, 1997)

	Flow	BOD5	TSS	pH
Source	L/capita/day	mg/L	mg/L	
Kreisli	242	218	114	7.5
Lawrence-Home 1	117	224	130	7.2
Lawrence-Home 2	185	124	70	
Otis et al		125	60	
Otis et al		130	40	
U. Wisconsin		158	51	
Bennet, ASAE		134		
Schmidt-(two)	151	90		7.1
Bounds, 1982 - STEP- (one)	189	118	52	6.9
PHS 2nd Series		178	111	7.4
PHS 3rd Series		92	112	7.5

PHS 4th Series		151	128	7.5
Barshield		223	39	7.1
Ronayne, 1982-(two)	208	217	146	
USEPA 1980 On-Site	167	155	88	
Ziebell, 1974		158	51	
Eastsound, WA, Bounds		214	117	
1996				
Loon Lake, WA, Bounds		90	45	
1996				
Cagle, 1993, Placer, CA -		160	73	
(two)				
Average	180	156	84	

As shown in this section, contaminant concentrations within residential septic tank values differ greatly between studies. The USEPA officially acknowledges that septic tanks in real communities experience these variations, with BOD₅ values ranging from 7 mg/L to 480 mg/L, TSS from 8 mg/L to 695 mg/L, and ammonia levels from less than 1 mg/L to almost 91 mg/L.

For the purposes of this study, the Ohio State University values were used to represent a typical residential septic tank (Peeples & Mancl, 1998). As explained in the following sections, these concentrations were not always matched in the tanks and could affect the overall validity of the study results.

2.4 Biology of Residential Septic Tanks

Septic tanks are passive systems with their own ecosystems in which facultative and anaerobic organisms perform complex biological and chemical processes (Bounds, 1997). Although inflowing wastewater to the septic tank contains high levels of dissolved oxygen (DO), the resident microbes rapidly deplete these oxygen stores as the flow disperses throughout the tank. The remaining tank functions as an anaerobic digester.

The septic tank bacteria are enteric, or those naturally found in the human gut, and are primarily heterotrophic bacteria that work to chemically oxidize and solubilize the organic matter in the tank (Bounds, 1997). The facultative microbes, able to function in both aerobic and anaerobic areas of the tank, solubilize complex organic material to volatile organic acids. Strict anaerobes, generally found in the tank further from the highly oxygenated inlet, ferment these volatile acids into a variety of gases (such as methane, carbon dioxide, and hydrogen sulfide) (Bounds, 1997).

These enteric facultative microbes can take years to grow to a colony size capable of sustaining solids digestion in a residential septic tank (Bounds, 1997). A potential weakness in this study was its relatively short four-week period, which may have not enabled a robust colonization of methane growers to stabilize in the tank and limited the tank's ability to digest inflowing solids.

The most common enteric bacteria in the septic tank include fecal coliforms, fecal streptococcus, lactic acid bacteria, anaerobes. The coccus (spherical), bacillus (rod-shaped), and spirillum (spiral-shaped) bacteria are the predominant digesters (Bounds, 1997). Many of these bacteria are encapsulated by a slime layer of extracellular enzymes that hydrolyze solids by adding water to the organic molecules. The organic molecules are then reduced to a simple fatty acid small enough to be absorbed through the cell wall and be oxidized and metabolized by intracellular enzymes (Bounds, 1997). This process is known as liquefaction and ultimately results in solids becoming water soluble.

Once hydrolyzed, these volatile fatty acids still exert much of their original biochemical oxygen demand but can now transfer to the aerobic infiltrative leach field system for biological removal. BOD removal in the actual septic tank is only 30 to 50% (Environmental Protection Agency, 2002).

Complete digestion, where bacteria convert the volatile fatty acids completely to methane, could reduce the amount of BOD released from the tank. Complete digestion does not usually occur because septic tank temperatures are typically well below the optimum temperature for methane-producing bacteria to flourish. In theory, the BiOWiSH Septic Rescue's active

microbial cultures both enhance and supplement these existing bacteria to spur more complete digestion and treatment.

2.5 Nutrient and Contaminant Removal in Traditional Residential Septic Systems

This section describes specific removal pathways for the various wastewater constituents analyzed in this study. Due to the vastly different environments between the septic tanks and leach fields, these are separated into two distinct sections.

2.5.1 Septic Tanks

Septic “tanks” refer specifically to the anaerobic tank through which influent wastewater passes and solids are allowed to settle. It does not include the infiltration field, leach field, or any secondary treatment following the tank.

2.5.1.1 Environmental and Health Impacts of Nitrates

Nitrate and nitrite are significant groundwater pollutants that affect groundwater and surface water sources throughout the world. Their most notorious effect is causing excessive algal growths in fresh and coastal waters, environments that are often nitrogen limited. These increased algal growths can block sunlight and prevent native plants from growing and create harmful algal blooms that limit the amounts of dissolved oxygen in water sources and alter aquatic ecosystems (Environmental Protection Agency, 2002). The lack of dissolved oxygen in the water systems can cause organism deaths, further degrading habitat conditions. Nitrates in drinking water are also known to cause methemoglobinemia, which reduces the blood’s ability to carry oxygen and can cause health implications in pregnant women, young children, and the elderly (Environmental Protection Agency, 2002).

Although nitrates are common wastewater contaminants following septic systems and leach fields, nitrates are almost non-existent in the septic tank phase of the system. Nitrogen in the wastewater entering the septic tank is almost exclusively in the form of ammonia. Research has shown that in a few instances, nitrates can be found within the tank, though the septic tank effluent is still more than 85% ammonia (Environmental Protection Agency). The remaining nitrogen is present as trace amounts of nitrates or contained in the suspended waste solids.

2.5.1.2 Ammonia Within Septic Tanks

Septic tank influent nitrogen is overwhelmingly in the form of ammonia. Ammonia travels the length of the tank and constitute more than 85% of the nitrogen in septic tank effluent (Environmental Protection Agency). Ammonia is rarely removed within the septic tank as it requires ample dissolved oxygen for transformation through nitrification.

2.5.1.3 Solids Reduction Through Settling

Septic tanks are designed for the settling of solids, similar to primary clarifiers in use at traditional wastewater treatment plants to promote conditions for sludge settlement (Butler & Payne, 2000). The plug-flow design and lack of mixing encourages stratification, the lack of oxygen encourages digestion, and the overall construct allows for effective primary treatment.

The impact of suspended solids reaching the environment and groundwater table include overall changes to aesthetics, hosting microorganisms that can lead to eutrophication in surface waters, or block soil pores in leach fields preventing percolation (Eliasson, 2004). Studies have found that half of the TSS within the tank and one-third of the TSS in the tank effluent are comprised of slowly degrading or inert materials (Eliasson, 2004). Any increase in the amount of slowly degrading solids, or an increase in solids flowing through the tank and reaching the leach field, are common in failing and failed septic tanks.

2.5.1.4 Biological Oxygen Demand Reduction Through Liquefaction

Within a properly functioning septic tank, the settling of solids removes the vast majority of particulate BOD. Untreated colloidal and dissolved BOD passes through the tank and enters the leach fields for further treatment (Environmental Protection Agency, 2002). Studies report that total BOD removed in the septic tank itself can range from only 30-50% to over 65% in some residential systems (Eliasson, 2004; Bounds, 2004).

In residential wastewater, the largest portion of BOD is carbonaceous organic matter, or CBOD (Eliasson, 2004). CBOD is thus the measure of the amount of oxygen that might be used by the microbial and chemical processes that breakdown the carbonaceous material into carbon dioxide, water, and residual organic matter. CBOD is determined by running a standard BOD analysis with a nitrification inhibitor (Eliasson, 2004).

Due to the variability of wastewater, a reliable conversion factor to compare BOD to CBOD is not available for septic systems as is it for traditional wastewater treatment systems. Though CBOD is commonly recorded as the parameter tested in most research experiments, BOD values were requested by the BiOWiSH team in order to compare with previous BiOWiSH studies (Eliasson, 2004).

2.5.2 Leach Fields and Infiltration Systems

Leach fields and infiltration systems refer specifically to the post tank infrastructure in which septic tank effluent drains into native soils.

2.5.2.1 Ammonia Reduction Through Nitrification

The nitrogen cycle describes the natural process in which nitrogen species are transformed. Within the aerated infiltrative surface in leach fields, the ammonia in the septic system effluent can nitrify to nitrate biologically through the process of nitrification. An overview of the nitrogen cycle, with the potential removal pathways, is shown in Figure 5 and described in the sections below.

Figure 5: Nitrogen Cycle with Assimilation, Nitrification, and Denitrification Identified (R&R Landworks, 2016)

In the aerated environment, ammonia oxidizing such as *Nitrosomas* bacteria oxidize ammonia to nitrite (Equation 1). *Nitrosomas* bacteria are most commonly associated with this first oxidation step. *Nitrosococcus*, *Nitrospira*, *Nitrosolobus*, *Nitrosorobrio*, and other autotrophic bacteria are also capable of this oxidation step (Burton, Stensel, & Tchobanoglous, 2014).

Equation 1: Oxidation of Ammonia to Nitrite (Lundquist, 2018)

Nitrification of ammonia ion is completed through nitrite-oxidizing bacteria, such as *Nitrobacter* bacteria, that then oxidize nitrite to nitrate (Lundquist, 2018). *Nitrospina*, *Nitrococcus*, *Nitroeystis*, and *Nitrospira* are other bacteria are also capable of this oxidation step (Burton, Stensel, & Tchobanoglous, 2014).

Equation 2: Oxidation of Nitrite to Nitrate (Environmental Protection Agency, 2002)

If nitrification was occurring in the septic tanks, drops in ammonia concentration would be observed, accompanied by increases in nitrite and nitrate concentrations.

Heterotrophic bacteria and fungi are also capable of facilitating nitrification in infiltration fields, though this process is significantly slower (Environmental Protection Agency, 2002). This nitrification process is observed in much lesser degrees surrounding residential septic systems.

2.5.2.2 Ammonia Reduction Through Assimilation

Though not commonly observed within residential septic tanks, this study did analyze the potential for nitrogen removal through assimilation. During assimilation, bacteria use available ammonia to spur new cell growth, effectively converting ammonia into organic nitrogen. New cell

growth contains approximately 12.5% nitrogen by mass, naturally up-taking soluble ammonia as the reproduce (Lundquist, 2018).

If assimilation was occurring within the septic tanks, steep drops in ammonia concentration would be observed without being accompanied by a rise in nitrite or nitrate concentrations.

2.5.2.3 Nitrate Reduction through Denitrification

Nitrate, under the right conditions, can fully transform to nitrogen gas (N_2) and cease to be a significant environmental concern through the process of denitrification. Facultative aerobes utilize residual oxygen and nitrate as terminal electron acceptors, transforming nitrate to gaseous nitrogen (Burton, Stensel, & Tchobanoglous, 2014).

In the absence of DO or under limited DO concentrations, in facultative microbes the nitrate reductase enzyme in the electron transport respiratory chain is induced, and the nitrate acts as a terminal electron acceptor. Decaying organic matter acts as the electron donor. Denitrifying, heterotrophic microorganisms can then reduce nitrate to nitrogen gas (Burton, Stensel, & Tchobanoglous, 2014).

The reaction stoichiometry is dependent on the electron donor, but $C_{10}H_{19}O_3N$ is commonly used to represent the biodegradable organic matter in wastewater (Burton, Stensel, & Tchobanoglous, 2014). Other reaction stoichiometry may be more relevant depending on the actual available nutrient conditions in local soils once anaerobic conditions are met. Equation 3 shows the biological denitrification of nitrate to nitrogen gas assuming standard wastewater nutrients.

Equation 3: Reduction of Nitrate to Nitrogen Gas (Burton, Stensel, & Tchobanoglous, 2014).

If denitrification were occurring in the septic tanks, steep drops in nitrate concentration would be observed.

Nitrate concentrations in septic system effluent reaching local groundwater and surface water sources regularly exceed the 10 mg/L NO₃--N USEPA drinking water standard near infiltration fields and continue to cause groundwater and surface water contamination (Environmental Protection Agency, 2002). Nitrate is a negatively charged ion that is very soluble and moves readily with the percolation soil water and can travel to these fresh water sources for communities.

Denitrification rarely occurs in the anaerobic conditions further below the soil surface. Although denitrifying bacteria use nitrate as an electron acceptor substitute for oxygen, this process only occurs when local soils have high concentrations of carbon or sulfur to act as electron donors (Environmental Protection Agency, 2002; Vaughan, 2013). These nutrient conditions seldom occur below the aerated infiltrative zones of leach fields, and thus it is assumed that all nitrogen applied to the infiltration fields, ultimately leaches into local groundwater (Environmental Protection Agency, 2002).

2.5.2.4 Solids in the Soil Matrix

Solids that make their way through the septic tank and into the infiltrative fields can potentially create significant environmental and health concerns through surface flooding. Large particles can clog the infiltrative surface or soil pores if overloaded (Environmental Protection Agency, 2002). Colloidal solids that pass through can cause cloudiness in surface waters, result in the development of sludge layers that harm aquatic organisms, and lead to excess pathogens reaching communities and aquatic environments (Environmental Protection Agency, 2002).

Solids may be used as nutrients for nitrification and other biological process, and often remain in the biomat and infiltration field. These solids see no significant reduction in systems where excess solids are not effectively settled in the initial tank and can result in costly system failures for homeowners.

2.5.2.5 Biological Oxygen Demand in the Soil Matrix

As described above, BOD is tied to the solids present in residential wastewater. In properly operating septic systems, the particulate BOD is removed by solid collection at the

infiltrative surface and biomat. Any colloidal or dissolved BOD that passes through the biomat is often removed through the aerobic biological processes present in the vadose zone.

High BOD levels can cause low dissolved oxygen concentrations in surface water, create taste and odor problems in well water, and can cause leaching of metals from soil and rock into ground and surface waters (Environmental Protection Agency, 2002).

2.6 BiOWiSH Bacterial Blend

The following section reviews the blend of microbes within BiOWiSH products, it's commercial product application in residential septic systems, and a previous study that tested the applications of the blend on real word systems.

2.6.1 BiOWiSH Bacterial Blend

Biowish Septic Rescue and Septic Maintenance are composed of a proprietary blend of Bacillus and Lactic Acid producing bacteria, including the following (Showell, n.d.);

<i>Bacillus subtilis</i> KLB 34	<i>Bacillus licheniformis</i> (3 different strains)
<i>Bacillus subtilis</i>	<i>Bacillus pumilus</i> (2 different strains)
<i>Bacillus mojavensis</i>	<i>Lactobacillus plantarum</i>
<i>Bacillus amyloliquefaciens</i> (2 different strains)	<i>Pediococcus acidilactici</i>
	<i>Pediococcus pentosaceus</i>

Though these active microbial cultures comprise only 0.1 to 1% of the total product by weight, they provide enough base cultures to inoculate and influence failing septic systems (Product Spec Sheet BiOWiSH Aqua). They act as a biocatalyst to enhance and encourage a range of hydrolytic, oxidative, and reductive biochemical reactions as outlined in previous sections (BiOWiSH Aqua for Freshwater and Surface Water Treatment).

2.6.2 Commercial Product Application

Septic Rescue is marketed as a “revolutionary treatment that restores failing septic systems back to optimal working condition,” saving homeowners the expense of costly pump outs or septic system replacement (BiOWiSH Technologies, n.d.) (Figure 6). Unlike consumer products that are simply preventative, Septic Rescue is claimed to be an effective treatment for septic systems that have already reached a crisis point (BiOWiSH Technologies, n.d.).

Figure 6: BiOWiSH Septic Rescue and Septic Maintenance (BiOWiSH, n.d.)

This product was also specifically designed to be easy for customer use. Residents simply need to completely empty one Septic Rescue packet into their toilet bowl on days 1, 7, and 14 (Figure 7). The product description recommends this incubation occur at night during low water usage.

Figure 7: Commercial Insert for BiOWiSH Septic Rescue Product (BiOWiSH, n.d.)

Following the successful inoculation of Septic Rescue, BiOWiSH Septic Maintenance is recommended for use every three months. This, along with efficient water usage and limiting contamination with toxins, chemicals, or hazardous materials, are the most effective ways to maintain a healthy residential septic system.

2.6.3 Previous BiOWiSH Septic Rescue Research

Many studies have been performed to analyze the effectiveness of the BiOWiSH bacterial blend in the treatment of wastewater, but only one study has specifically analyzed the Septic Rescue and Septic Maintenance blend on residential septic systems. This case study, “The Assessment of BiOWiSH Septic Tank Aid on the Sludge Depth and Effluent Constituents for Several Low Pressure (LPP) Septic Systems in Central North Carolina,” looked at the product’s effects on BOD, TSS, Total Kjeldahl Nitrogen (TKN), FOG, DO, and $\text{NO}_2^-/\text{NO}_3^-$ -N (Vaughan, 2013). Four dosing treatments were studied; 100g/quarter, 50 g/week, 100 g/week, and 200 g/week.

The BiOWiSH blend was added to thirteen real-life septic systems with similar characteristics, flows, and loads in North Carolina. Samples were taken at 0, 7, 14, 30, 60, 90, 120, 150, 180, 210, 240, 270, 300, and 360 days and taken at the septic tank entrance one foot below the tank’s water surface and at a spigot constructed at the end of the septic drain field (Vaughan, 2013).

The overwhelming majority of samples were not detectable for DO and $\text{NO}_2^-/\text{NO}_3^-$ -N, and thus analysis for those parameters were discontinued. There were minimal changes for TKN concentrations for all treatments, including the control (Vaughan, 2013).

For BiOWiSH dosed septic systems, there was generally a decrease in BOD, FOG, and TSS concentrations. The largest decreases occurred during the first four weeks after the initial product additions. All additive treatment concentrations showed a greater and more steady decrease than the control in BOD, FOG, and TSS concentrations (Vaughan, 2013). In addition, the control tanks exhibited more irregular swings in the BOD, FOG, and TSS concentrations between samples than did the BiOWiSH additive tanks (Vaughan, 2013).

The effluent analysis showed that BiOWiSH significantly reduced BOD, TSS, and FOG concentrations over that of control tanks. Additionally, higher BOD, TSS, and FOG concentration reduction percentages were identified in residential tanks with higher initial concentrations of

pollutants (Vaughan, 2013). This suggests that BiOWiSH preforms best in failing and failed septic systems with high constituent concentrations.

Samples were also taken after the effluent had exited the septic system drain field, characterizing the impact of BiOWiSH in the more aerobic infiltrative surface environment. These samples showed no significant reductions to BOD, TSS, TKN, or FOG concentrations compared to the control tanks (Vaughan, 2013). This suggested BiOWiSH had no benefit in the aerated leach fields over control tanks with no additive treatments.

However, the initial leach field influent concentrations of BOD, TSS, TKN, and FOG were low when entering the residential drain fields. The study suggested that it is possible that drain field effluents with higher initial levels of pollutants, characteristic of septic tanks that have failed or are failing, may benefit from the BiOWiSH blend and microbial treatment in the infiltrative systems (Vaughan, 2013).

This study suggested that BiOWiSH was an effective treatment for residential septic tanks experiencing increased BOD, TSS, and FOG loads, but no evidence supported benefits to effluent treated in the infiltrative systems or leach fields (Vaughan, 2013). To validate these claims, further testing and statistical analysis should be performed on the thirteen tanks to understand the variation in real world tanks.

2.6.4 RID-X Septic Maintenance

RID-X Septic Maintenance was chosen as the competitive product to test against the BiOWiSH additive tanks and the control tanks. Although RID-X is marketed as a maintenance product and applied at a maintenance dosage, as compared to the rescue dosage of BiOWiSH, RID-X is widely known as a lead competitor in the treatment of residential septic tanks. Future studies may choose to dose RID-X in a more consistent rescue dosage, or choose an alternate rescue type product to compare against BiOWiSH.

2.7 Alternate Nutrients Considered but Not Tested

BiOWiSH technologies requested the testing of phosphorus in initial design iterations, but this parameter was not pursued. Carbonaceous oxygen demand was also considered for analysis

but was replaced by biological oxygen demand for the reasons described in the following sections.

2.7.1 Phosphorus

Like nitrates, phosphorus is a key plant nutrient that contributes to eutrophication and dissolved oxygen depletion in surface waters. Present in wastewater, conventional residential septic systems are often not adequate in minimizing phosphorus compounds, which leads the nutrient to local groundwater and surface water sources (Environmental Protection Agency, 2002). The amount that leaches through depends on the characteristics of the soil, the thickness of the unsaturated zone that the wastewater percolates through, the applied loading rates, and the age and condition of the overall system (Environmental Protection Agency, 2002).

Phosphorus in onsite treatment wastewater can be removed through two processes. Chemically, it is easily formed into precipitates and sorbs to fixed bed reactors under certain conditions (Burton, Stensel, & Tchobanoglous, 2014). After passing through the septic tank and leach fields, the wastewater enters native soils. If the concentrations are low, $< 5\text{mg/L PO}_4^{3-}\text{-P}$, phosphate will chemisorb onto the surfaces of iron and aluminum minerals in acidic to neutral soils and chemisorb to calcium minerals in neutral to alkaline soils (Environmental Protection Agency, 2002). If these minerals are not available in significant amounts in native soil, or the phosphorus levels too high, effective sorption will not occur. Even with adequate soil capacity to absorb phosphorus, the nutrient can move deeper into the soil profile and affect the overall retention with added loading (Environmental Protection Agency, 2002).

Chemical sorption and precipitation may occur post septic tank and leach field, which was not analyzed in this study.

BiOWiSH Septic Rescue and Septic Maintenance products aimed to reduce the amount of phosphorus through biological process similar to conventional wastewater treatment facilities. Traditionally, biological phosphorus removal involves phosphate uptake by Phosphorus Accumulating Organisms (PAOs) to create phosphorus rich solids, which are then settled out in later steps.

As shown in Figure 8, this biological removal of phosphorus requires the wastewater to first pass through an anaerobic environment, such as a septic tank. Under low DO conditions, the biodegradable solids (bsCOD) ferment and produce volatile fatty acids. PAOs store food under these conditions, assimilating the volatile fatty acids into polyhydroxybutyrate (PHB) storage products. During the creation of PHBs, PAOs internally break phosphorus bonds within themselves for energy and release ortho-phosphates to the surrounding wastewater.

Figure 8: Traditional Biological Phosphorus Removal at Wastewater Treatment Facilities (Lenntech)

When exposed to the aerobic regions, the bacteria undergo rapid metabolism of the stored PHBs. This provides energy for new cells growth, up taking large amounts of orthophosphates present in the wastewater. Phosphorus then accounts for approximately 5-7% of the biomass when formed by PAOs, compared to 1-2% in normal bacteria (Burton, Stensel, & Tchobanoglous, 2014). The bacteria are then generally settled out via a clarifier or other settling tank.

Though this is an effective method of biological phosphorus removal, it only occurs when wastewater enters an anaerobic environment followed by an aerobic environment. This experiment only tested the effectiveness of the BiOWiSH bacterial cultures within the anaerobic septic tank, so phosphorus was not tested for in samples.

The USEPA does not acknowledge substantial biological phosphorus removal in residential septic systems (Environmental Protection Agency, 2002), but the anaerobic tank followed by the aerobic leach fields may provide an environment for removal if POAs are included in the BiOWiSH product. However, the phosphorus rich bacteria may decay in the leach field, decay, and allow phosphorus to release into the environment.

2.7.2 Carbonaceous Oxygen Demand

BiOWiSH Septic Rescue and Septic Maintenance report reductions in BOD from the products in past studies (Vaughan, 2013). The BOD tests, however, require five days of testing and are prone to high degrees of error. The inaccuracies of the test lead to large errors in the BOD data recorded in Experiment 2, and thus switching to testing CBOD was considered. CBOD testing requires significantly less time, yields very clear results, and is prone to less experimental errors.

According to the Washington State Department of Health report Septic Tank Effluent Values, CBOD should not be used to characterized septic tank effluent (Eliasson, 2004). There is a lack of reliable CBOD data on residential septic systems available through the USEPA and independent sources. Also, unlike domestic wastewater collected and treated at wastewater treatment plants, there is no conversion factor that can easily predict BOD5 values from CBOD5 levels. BOD was still utilized for Experiment 2, and the errors noted as part of the analysis.

2.8 Research Objectives

Although septic systems have the potential to provide safe and reliable treatment for residential wastewater treatment, the environmental and health risks from failing systems pose significant concerns for communities that rely on septic systems. The lack of low cost and readily available commercial products to restore these failing systems results in unchecked groundwater contamination and high costs for residents when replacement or physical maintenance becomes required.

The purpose of this study is to test the effect of the BiOWiSH commercial product on the biological treatment of wastewater in residential septic tanks. Specifically, this study compares effluent from tanks treated with BiOWiSH, a leading competitive product RID-X® Septic

Maintenance (hereafter referred to as RID-X), and a control tank with no additive treatments.

Specific goals of this study included:

1. Construct model-scale septic tanks to accurately represent the properties of residential septic tanks
2. Develop a Standard Operation Procedure for sampling from the model-scale tanks
 - a. Compare sampling methods and frequencies
3. Measure effluent concentrations of the following parameters between the BiOWiSH additive tanks, control tank, and tank dosed with a main competitive product;
 - a. Nitrates
 - b. Ammonia
 - c. Biological oxygen demand
 - d. Total suspended solids
4. Analyze and compare effluent concentrations to determine potential benefits of BiOWiSH on the biological treatment of residential septic systems over competitive products or no product addition.

Chapter 3

METHODS AND MATERIALS

Prior to testing BiOWiSH's effect on the treatment of residential septic tank wastewater, model-scale septic tanks were constructed to represent septic tanks and enable efficient sampling. Pre-experiments were also performed to test the variation in sludge to be added to the septic tanks.

To simplify the sampling and analysis process, this study was performed as two distinct experiments. Experiment 1 tested the effect of BiOWiSH on the reduction of ammonia and nitrates within the septic tank, with samples taken on a daily basis. Experiment 2 tested the effect of BiOWiSH on the reduction of TSS and BOD, with samples taken every 5 to 7 days.

The model-scale septic tanks were constructed to represent failed or failing septic tanks. To do this, the ammonia, TSS, and BOD levels chosen were at the higher end of the typical residential tank concentrations. Tanks were also dosed with predigested sludge sourced from the primary clarifier from the San Luis Obispo Water Resource Recovery Facility, which is more indicative of slowly digesting sludge that would be found persisting in failing or failed septic tanks.

Septic tanks can fail when an influx of chemicals, or a lack of influent nutrients, starves the native enteric bacteria populations. The model-scale tanks portray these decreased bacterial populations as the tanks were initially dosed with the predigested sludge and tap water, but not given time to grow bacteria populations before the start of the experiments.

An analysis of the pH in the model-scale tanks showed slightly lower than expected pH values. This may also suggest a failing septic tank environment and is further explained in the following sections.

3.1 Fabrication of Model Scale Septic Tanks

Four model-scale septic tanks were constructed according to the specifications outlined in the Ohio State University article "Laboratory Scale Septic Tanks." Their purpose, like those in that study, was to produce a daily supply of effluent that closely matched the values of residential septic tanks, shown in Table 4 below (Peebles & Mancl, 1998). Though initial test runs were

performed to reach these values, inconsistencies in the collected primary sludge resulted in these values being variable.

Table 4: Values Used for This Study (Peeples & Mancl, 1998)

Nutrient	Concentration
BOD₅	140 mg/L
TSS	75 mg/L
NH₃-N	30 mg/L
pH	9-Jun

Initial experiments were performed to estimate a sludge dilution to yield the appropriate TSS, thus mimicking residential septic tank influent. Sludge was collected following the primary clarifier at the San Luis Obispo Water Resource Recovery Facility. Each experiment's sludge was collected at a single time. Sludge following the primary clarifier was chosen over solids present at the treatment center's headworks due to its lesser potential for contamination and ability to be safely collected onsite. Although the primary clarifier sludge was predigested, the sludge still retained ample BOD concentrations and many of the nutrients present in raw sludge.

The results of these tests are outlined in the Results and Conclusions section and show the variation within the same sludge sample. A dilution of 1:50 was ultimately chosen due to its ease of measurement. NH₃-N solution was added daily to represent the daily influx of residential septic tanks and bring the effluent to the correct ammonia concentration.

Four laboratory septic tanks were constructed from 32-gallon cylindrical polyethylene containers with an inner diameter of 22 inches. To prevent scum disturbance during experimental filling, loose floating baffles were constructed from Styrofoam wall insulation with a thickness of one inch. The baffle essentially created two compartments within the tanks; the top section that could be accessed daily for sludge and solution addition, and the lower portion that could remain relatively undisturbed and maintain an anaerobic condition. Figure 9 shows the construction of these tanks, and Figure 10 shows the tanks after construction.

Figure 9: Septic Tank Construction (Peeples & Mancl, 1998)

Figure 10: Construction of Septic Tanks

Small bowls were glued to the top center of each baffle to hold the daily addition of primary sludge and chemical solutions. The bowls, which had two small holes drilled on the sides to facilitate slow draining, doubled as mixing bowls while also allowing the sludge dilution, chemical solutions, and added tap water to smoothly flow through those small holes and over the top of the bowls onto the floating baffle. Both limited the disturbance to the scum accumulating at the top of the tanks.

A series of PVC pipes were installed in each tank to further minimize any scum disturbance, as well as prevent any disturbance to the settling sludge layer at the bottom. Approximately 2 inches from the bottom of the tanks, a 1-inch hole was drilled and fitted with 1-inch female threaded joints. These joints were initially sealed with epoxy but leaked when tanks were filled to capacity in tap run trials. The joints were then sealed completely through a combination of rubber gaskets and Super Glue®.

Inside the tanks, a 20 cm length of 1-inch PVC pipe extended to the center before elbowing upward, further extended by a 25-inch PVC pipe. Effluent entered this vertical pipe through 4 cm long slats cut radially on the top of the pipes. Half of a plastic petri dish was then glued to the top of the pipe to prevent scum and any abnormally large sediment clusters from entering the effluent.

To enable efficient effluent collection on the exterior of the tank, a 10-cm pipe extended from the bottom thread and elbowed up to a 15-cm pipe section. Moving upward, a 1-inch ball valve, 25 cm long pipe section, and a T-joint with an open pipe extended up to the height of the septic tank. This pipe was meant to remain open to the environment to prevent suction forming within the line, as well as be a point of entry in the case of a clogged line. Neither of these proved to be an issue in the two experiments.

Unlike the Ohio State University study, these scale septic tanks did not have a bottom valve and drain. Although this would have made disposal much more efficient, they were omitted due to initial concern with leaks. In addition, due to space limitations at the sampling location, there were no tap water inlets equipped with spray nozzles. Instead, tap water was manually added via a garden hose. Care was taken to ensure that tap water was added in gentle streams to facilitate mixing of added ingredients, without disturbing the tank interior.

Initial concerns with temperature fluctuations were taken into consideration as the tanks were stored outdoors. Each tank was wrapped with a 1-inch egg carton foam mattress topper, a fleece heated blanket, and secured using Bungee Cords. The heated blankets malfunctioned but remained as an added layer of insulation. Temperature data and graphs are shown in the Results and Analysis section.

3.2 Additions and Sampling from Model Scale Septic Tanks

A complete Standard Operating Procedure was created specifically for this study and can be found in Appendix A.

Each tank received 500 mL of 1:50 sludge dilution, which was made daily from refrigerated sludge. To ensure the most homogenous sludge sample possible, all sludge was collected from the primary sludge collection facility at the San Luis Obispo Water Resource Recovery Facility from the same day. To store, 10 mL of sludge was placed in 15 mL Falcon tubes and frozen, below -18 °C, and thawed the day before use. In initial runs, sludge was added every day, though in later runs the appropriate amount of diluted sludge was added every 2 to 3 days.

Figure 11: Sludge Collected from the San Luis Obispo Water Resource Recovery Facility

In Experiment 1, when ammonium was being analyzed, 687 mg of 90 mg/L $\text{NH}_4\text{Cl-N}$ was aqueously added to each tank.

Early analysis of effluent samples showed a lower than expected pH for the tanks. This might be due to the creation of acidic H_2S , though some acidity may have been caused by the addition of the NH_4Cl solution. For this reason, an initial titration determined that 1 mL of 10N NaOH neutralized the 687 mL of NH_4Cl solution, and thus should be added daily as well.

Five-gallon buckets were placed below each discharge ports, the ports were opened, and water was collected. At least 1 L was emptied into the bucket to remove water sitting in the internal piping system, and another 2 to 3 liters was removed based on suggestion from the Ohio State University study. After these 3 to 4 L were removed, a sample of the effluent was taken and placed in a sealable vial. For sample runs measuring nitrates and ammonia, 40 mL glass VOA vials were utilized for sample collection. For sample runs measuring TSS and BOD, samples were collected in 1 L plastic containers.

Solutions were then added to each tank, the holes in the plastic bowl delivery system unplugged from sludge particles, and each tank was filled to its original full level with tap water per the suggestions of the Ohio State University Study. The excess 3 to 4 L of wastewater, collected in buckets but not used for sampling, was disposed in a sanitary sewer. Each collected sample was immediately tested for pH and temperature and refrigerated at 4 °C for up to 14 days before testing. Experiment 1 samples testing for ammonia and nitrate concentrations were acidified using 3 drops of 96% H₂SO₄ before storage.

Initial experimental discussions considered the use of actual residential septic tank effluent and laboratory made growth media. Effluent was not sourced from residential septic systems due to a health and safety concern from sampling. Additional insurance would have been required to access these tanks, and safety protocols would be needed to protect against hazards from sampling from the tanks. Likewise, there would have been additional error due to the inherent differences in individual septic tank design and household water usage and influent conditions, which could not be accurately captured in this study.

A nutrient broth was considered in place of septic sludge but was not used due to concerns that the lack of enteric pathogens native to the sludge would be absent. These bacteria are meant to work in conjunction with the microbes added through BiOWiSH, and their absence could disrupt the growth of BiOWiSH microbes. There was also discussion into potential competition from other bacteria and microbes inherent in the sludge that would not be accurately characterized if a growth or nutrient media was used. These analyses are also the reason that the

primary sludge collected from the San Luis Obispo Wastewater Resource Recovery facility was not autoclaved.

3.3 Product Preparation and Addition

BiOWiSH Septic Rescue was added according to the instructions outlined in the consumer instructional packet. The tanks were incubated with BiOWiSH on days 0, 7, and 14 during the initial run, and on days 0, 8 and 23. The variability of these times was due to scheduling conflicts and equipment availability. The competitor product was incubated on day 0, according to its product description and not re-incubated.

Each BiOWiSH packet contains 100 grams of product. Assuming an average single family residential septic tank size of 1250 gallons and a 32-gallon laboratory tank capacity, 2.56 grams of BiOWiSH was needed to be added to each BiOWiSH tank (Van Delden, n.d.). However, past studies suggested that adding small amounts of BiOWiSH may not give a representative amount of bacterial cultures, and thus a 100g/L aqueous solution was prepared.

This 100 g/L solution was made by adding 10 grams of BiOWiSH to 100 mL of water and stirring for 30 minutes using a magnetic stir bar on a stir plate until fully dissolved. After the solution was dissolved, it was placed at room temperature for approximately two hours before being added to the tanks. This process was meant to mimic the consumer packet instructions of emptying the entire packet into the toilet, letting sit, and then flushing. Using this saturated solution, 25.6 mL of the BiOWiSH solution was added each incubation period.

Using the same preparation method, the competitor blend was made by adding 10 mg of product to 100 mL of water and stirred. This solution included a denser base, and did not fully suspend, so the solution was continually stirred until incubation. Using this hyper-saturated solution, 70.8 mL of the competitor solution was added at Day 0.

3.4 Sampling Periods

A four-week sampling period was chosen due to the results supplied by the case study “The Assessment of BiOWiSH Septic Tank Aid on the Sludge Depth and Effluent Constituents for Several Low-Pressure Pipe (LPP) Septic Systems in Central North Carolina” (Vaughan, 2013). This study showed that the most significant results of the BiOWiSH addition, regardless of

dosage, occurred within the first 2-4 weeks after the initial incubation period. A four-week sampling period was chosen to allow ample time for chemical and biological processes to occur, based on the data presented in the Vaughan study.

3.5 Sampling Frequency

In accordance with the Ohio State University study, sludge was added, and samples were taken from the tank daily for the initial experiments. Although this was the recommended sampling procedure, a Washington State Department of Health – Wastewater Management Program report in 2003 suggested that these samples may not be a representative sample for real life sampling of septic tanks. The study, titled “Septic Tank Effluent Values,” states that “the collection of frequent samples even over short periods of time to provide results representing average operating conditions, such as a 30-day average, is not practical for the purpose of monitoring septic tank performance on an on-going basis in the field (Eliasson, 2004).” Although daily samples may not accurately capture the slow changes in a septic tank biology, taking samples over the four-week period should give a representative view of the tank environment.

The Washington study does continue by recommending single samples from residential tanks, creating a snapshot of septic tank performance. These however still do not necessarily represent the effluent characteristics of the tank at any other time. The inconsistent sampling methods and the disagreement between the most effective method may be another reason for the extremely high variability present in septic tank effluent literature.

The potential inaccuracy of daily samples, the potential that daily samples and the water removed was upsetting the biology of the tanks, and the time commitment required in Experiment 1 was adjusted for the second experiment. In Experiment 2, which tested BiOWiSH Septic Rescue’s effectiveness on TSS and BOD, samples were taken on a roughly weekly basis. Though this is potentially a more accurate representation of the internal biology of each tank, it did provide less overall sample points for the study.

3.6 Ammonia Analysis

Prior to ammonia analysis, samples were filtered through 0.22 um cellulose esters membranes (GSWP04700) to remove any particulate matter. Samples were then placed in 15 mL

Falcon™ tubes, and ammonia levels were tested using a Timberline Instruments TL-2800 Ammonia Analyzer. It used an eight-roller peristaltic pump to direct each sample, a caustic solution, and a buffer absorbing solution in a single tubular membrane assembly. Each sample was then internally mixed with the caustic solution, resulting in a mixture with a pH off 11 or higher. (Timberline Instrument, n.d.)

The samples were stored at a pH of less than 2 to enable all ammonia species to remain in the soluble ammonium form, which prolonged its storage life and prevented any loss of ammonia gas during storage and transport. When mixed with the caustic solution, that rise to a pH of 11 would result in almost all ammonium ions being converted to ammonia gas, as represented in Figure 12.

Figure 12: Ammonia Speciation Graph (Kunz & Mukhtar, 2016)

As the sample and caustic mixture flowed over the tubular membrane, dissolved ammonia gas diffused through a membrane wall and was further dissolved by a buffered solution, flowing on the inside of the tube, with a pH of 6.

Ammonia concentrations were measured in the parts-per-billion range by measuring the change in electrical conductance of the absorbing solution to the concentration of ammonium ion

present in the sample (Timberline Instrument, n.d.). It was crucial to first make standards of known ammonia concentrations in order to create a five-point calibration curve. The use of this curve yields the concentration of ammonia in the unknown samples.

3.7 Nitrate and Nitrite Analysis

Prior to nitrate and nitrite analysis, samples were filtered through 0.22 μm cellulose esters membranes (GSWP04700) to remove any particulate matter. Nitrate and nitrite were both analyzed via ion chromatography. Ion chromatographs measure concentrations of major anions and cations in the parts-per-billion range by separating the ions based on their interaction with an internal resin (Dionex, 2018). Samples pass through this resin column followed by a specialized eluent. Ions then begin separating from the column and can be measured and identified by their respective retention times and conductivity readings (Dionex, 2018).

All samples were pre-filtered through 0.22- μm Millipore Membrane filters. Extremely turbid samples were pre-filtered through 5- μm Millipore Membrane filters and then through the 0.22- μm filters. Nitrocellulose membranes were used to prevent nitrate losses that had previously been identified when glass fiber filters were utilized. Each sample was then transferred to 6-mL Dionex Polyvials and sealed with Dionex caps (filter caps were not used) and stored at 4°C for up to two weeks.

Samples were then loaded individually into the Dionex: DX-120 Ion Chromatograph (IC). Before each sampling period, the eluent reservoir was refilled with a 9 mM Na_2CO_3 solution and the IC primed with the new eluent. Within the IC, samples were mixed with the eluent and pass through the ion exchange resin contained in the separator column. This efficiently separated the ions based on their affinities. The mixture then passed through the suppressor that eliminates background conductivity for each sample. The mixture reached a conductivity cell where the Dionex Chromeleon software measured each sample's electrical conductivity. (Dionex, 2018)

The Dionex Chromeleon software presented each sample with graphs comparing electrical conductivity versus retention time. Most samples showed three distinct peaks: nitrate, nitrite, and sulfate (from acidification to preserve samples). By analyzing the calibration samples included in each run, retention times were matched with the appropriate ions, and a calibration

curve was created to translate the electrical conductivity areas to ion concentrations. (Dionex, 2018)

Standard calibration samples were run with both acidified and non-acidified to ensure that the sulfur concentrations in the samples were not affecting the nitrate and nitrite concentrations. In addition, differing conductivity areas for identical standard samples, tested in different runs, proved that calibration runs were required for each individual run.

3.8 Total Suspended Solids Analysis

Total suspended solids were measured according to the ASTM D5907-18 method of Non-Filterable Matter. Fisherbrand G4 Glass Fiber Filters (5.5 cm, cat. No 09-804-55c) were prepared by applying a vacuum and rinsed with no less than 100 mL reverse-osmosis water. They were then placed in pre-ashed aluminum weigh boats, in stacks of 15 to 20, and placed in the 1000 °C muffle furnace for fifteen minutes. The top and bottom filters were discarded to avoid contamination, and the remaining “de-ashed” filters placed in a desiccator to prevent moisture from bonding to the filters.

Filters were then weighed on a Fisher Science scale (item ALF104) and weights recorded. Volumes of samples were then filtered to yield at least 2.5 mL of dried residue. Because no large flocs were identified, samples were vigorously shaken for two minutes instead of blended. The resulting filters showed no large flocs or specs. Filters were then dried for no less than one hour at 105°C, and then weighed. Each sample was run in duplicate, and some trials were run in triplicate for added redundancy.

3.9 Biological Oxygen Demand Analysis

The Carbonaceous Oxygen Demand (CBOD₅) of each sample was found following the APHA Standard Methods 1995 Edition, Sections 5210 A and B. Though the procedures were followed precisely, some error was identified and acknowledged in the Results and Discussion section of this study.

Before actual CBOD₅ tests were run, an assortment of pre-lab measurements was required. Each 300 mL sample bottle was adequately rinsed with deionized water and autoclaved according to factory instructions. Approximately 8L of dilution water was prepared by adding 2

HACH BOD nutrient buffer pillows (6 mL concentrate, Product # 148266) to reverse osmosis water. The solution is then shaken vigorously to saturate with oxygen.

The ProODO Optical Dissolved Oxygen Probe (YSI Item #626279) was calibrated before each test run, with calibration temperature and DO recorded. Blanks were made by adding dilution water, shaken vigorously to saturate, and then filled with dilution water without sample or seed before testing for initial DO. This was to determine if there was any unforeseen error from residual bacterial growth.

Standards determined the appropriate amount of seed added to standards. To create standards, bottles were filled halfway with dilution water, shaken, and 6.27 mL of Glutamic Acid (alternately named glutamate standard) was added. Unfiltered primary influent from the San Luis Obispo Water Resource Recovery Facility was added as “seed bacteria,” a 1.25 mL dose determined prior by another graduate research team at California Polytechnic State University (Rodrigues, 2013). One dose of nitrification inhibitor was added before the bottle was shaken, filled with dilution water, and tested for initial DO.

To determine the CBOD₅ of septic tank samples, the bottles were filled halfway with dilution water before the shaken and undiluted sample was added. Volumes of samples were added based on estimates that hoped to achieve a final DO of no less than 2 mg/L and achieve at least a 1 mg/L change from the initial DO. One dose of nitrification inhibitor was then added, the bottle was shaken, and then filled with dilution water.

All blanks, standards, and samples were prepared within a two-hour period. Initial DO readings were taken, and all bottles were placed in a dark incubator at 20 °C. After five days, final DO readings were taken.

3.10 Quality Assurance / Quality Control (QA/QC)

QA/QC methods are critical to determine the validity of all laboratory tests performed. Sample runs were tested with spikes to determine the accuracy of the standard solutions and needed to fall within $\pm 15\%$ of the expected value for the run to be accepted. Splits, or sample duplicates, test the precision of the sampling methods and instrumentation, and needed to fall within $\pm 10\%$ difference in values to be accepted. Continued calibration verification was also

implemented in each run to ensure instrumentation accuracy and needed to fall within 10% of the expected value. Samples that did not meet these requirements were not included in final analysis.

When analyzing ammonia concentrations via the Timberline instrument, calibration standards and a calibration curve was prepared prior to each run. R^2 values needed to be >0.99 for each of these runs.

When analyzing BOD concentrations, samples with final DO readings less than 2 mg/L or that underwent a change less than 1 mg/L from the initial DO were not included in the analysis. The APHA Standard Methods recommends not including data from runs in which the test blanks experienced DO variations in excess of 0.1 mg/L DO. In order to account for the change in DO and have an appropriate representation of data, error propagation was applied to all samples during those runs.

Chapter 4

RESULTS AND ANALYSIS

The following sections outline the results of the pre-experiment sludge characteristic tests, Experiment 1 testing ammonia and nitrates, and Experiment 2 testing BOD and TSS levels.

4.1 Initial Sludge Characteristic Tests

An initial series of tests was performed to summarize the characteristics of the primary sludge collected from the San Luis Obispo Water Resource Recovery Facility. These tests were completed in accordance with all QA/QC and methodology as prescribed in previous sections.

4.1.1 Total Suspended Solids

Primary tests looked to determine the continuity of suspended solids of the initially collected sludge. Although the sludge was collected from the San Luis Obispo facility on the same day and vigorously mixed, the semi-solid nature of the waste suggested that consistency was not an appropriate assumption. This initial series of tests sought to quantify the suspended soil variability within the sludge.

To determine the average TSS of the primary sludge, dilutions were made of 1:30 to 1:80 sludge to DI water. The samples were then vigorously mixed via a magnetic stir rod and stir plate for ten minutes each and stored for 12 hours at 4°C. The samples were then tested according to Section 3, Methods and Materials. The laboratory results for TSS, adjusted for dilution factors, is shown in Figure 13.

Figure 13: TSS of Diluted Samples, Adjusted

A dilution of 1:50 was ultimately chosen due to its ease of measurement and daily application to the tanks. The 1:50 dilution was also the closest to the 75 mg/L TSS value trying to be achieved.

The level of “noise,” or variability namely due to variations within the sludge is important to consider when analyzing the sample results. Significant changes observed due to the addition of BiOWiSH would be needed to suggest any impacts to its addition.

4.1.2 Ammonia

Ammonia levels were tested in three different dilutions over six hours.. Three identical 5-gallon buckets were filled with 2 liters of primary sludge, collected the previous day and stored at 4 °C. One bucket was dosed with 5 mg/L aqueous BiOWiSH, one with 10 mg/L phosphorous, and one bucket had no additions to act as a Control. The phosphorous addition was a parameter not pursued in later studies.

Figure 14 shows the variability of ammonia levels in these three buckets. Samples of sludge were taken from each bucket and diluted to three different Falcon™ tubes for ammonia analysis. Figure 14 represents the average ammonia concentration of the three samples taken, with an error bar showing the standard deviation to represent the variability.

Figure 14: Initial Ammonia Concentrations to Show Variability in Sludge Concentrations

Initial sludge characteristic tests show the range of variability within a single sludge sample. This variation has the potential to skew data in future studies and is mentioned in the following Results and Conclusions sections.

4.2 Experiment 1 – Ammonia and Nitrate Analysis

Experiment 1 tested the effect of BiOWiSH on the reduction of ammonia and nitrate concentrations within septic tank effluent. Sludge and ammonia solution was added on a daily basis, and daily samples were collected.

4.2.1 Temperature and pH

Temperature and pH did not remain within expected levels throughout the duration of the test period. Temperatures ranged between 14.6 °C (Day 15) and 30.7 °C (Day 23). No temperature values were taken in the night hours, and all tanks showed similar trends in temperature. The pH of the tanks ranged between 5.1 (Day 23) and 6.22 (Day 0). Average residential septic tanks range between 6 and 9, above most of the recorded values.

The lower pH values further promote that these model-scale tanks were representing failed or failing septic tanks, where lower pH values are more common, and where BiOWiSH microbes would need to flourish to facilitate enhanced biological treatment of the influent wastewater.

Figure 15: Experiment 1, Temperature Analysis

Figure 16: Experiment 1, pH

The decreasing and acidic pH may be from sulfur compounds forming within the anoxic tanks. Although no tests were performed to quantify the sulfur contents of the sampling tanks, all

effluents smelled strongly of H₂S, or rotten eggs. Equation 4 shows a generic anaerobic wastewater reaction in which sulfate (SO₄²⁻), plentiful in wastewater and primary sludge, is biologically reduced to form sulfide (S₂⁻).

Equation 4: Sulfate Reduction to Sulfur

Sulfide can then combine with hydrogen to form hydrogen sulfide (H₂S, which can then oxidize biologically to sulfuric acid in the headspace of the tank and the low DO regions at the top water layer (Equation 5). Presence of H₂S was not tested due to a lack of proper available equipment. However, the presence of H₂S may explain the lower pH than is generally found in residential tanks, as it is highly soluble in water and acts as a weak acid (PubChem, 2019).

Equation 5: Hydrogen Sulfide Production

Laboratory safety measures and precautions were followed to prevent placing students in dangerous positions from sulfur related fumes. Adequate air circulation and decontamination measures were followed during each sampling event.

4.2.2 Visual Analysis of Samples

Daily sample effluent underwent several visual changes throughout Experiment 1. At the beginning of the run, all tank effluents were light in color. By Day 9, Tanks A and B with the BiOWiSH additions were slightly darker in color (Figure 17). By Day 13, Control Tank C began to show a color change (Figure 18). By Day 19, all sample tanks were similar in color (Figure 19).

Figure 17: Experiment 1, Day 9 Color Variation

Figure 18: Experiment 1, Day 13 Color Variation

Figure 19: Experiment 1, Day 19 Color Variation

The change in color was observed before analytical test results were available. The color change was originally considered a sign that the BiOWiSH additive tanks were actively affecting the tank biology. Samples lost their dark color after being acidified and stored at 4 °C for about 24 hours. The color loss did not appear to affect the turbidity of samples.

The visual color analysis was a completely subjective examination, with no quantitative data collected using a colorimeter or spectrometer. These experimental methods were not utilized as the samples quickly lost their color once acidified and stored. This may be due to residual biological or chemical processes, or caused by the acidification of samples before storage.

4.2.3 Nitrate and Nitrite

Nitrate (NO_3^-) and nitrite (NO_2^-) were analyzed in the daily wastewater effluent samples. The maximum nitrate level detected was 0.4 mg/L NO_3^- -N, well below the USEPA Drinking Water Standard of 10 mg NO_3^- -N. Most samples were non-detects, with levels below the Ion Chromatographer detection limits of approximately 0.5 mg NO_3^- -N.

The maximum nitrite level detected was 0.5 mg/L NO_2^- -N. Most samples were non-detects, with levels below the Ion Chromatographer detection limits.

No increase of nitrate or nitrite was observed, which may have suggested nitrification. The absence of nitrification is due to the low levels of oxygen within the tanks. If nitrification had occurred, and nitrate was present in significant quantities, denitrification may have occurred.

4.2.4 Ammonia

Ammonia (NH_3) was analyzed in the daily wastewater effluent samples, shown in Figure 20.

Figure 19: Experiment 2, Ammonia Analysis

Ammonia levels at the start of the run varied between 7.4 mg/L and 8.5 mg/L NH_3 -N. Throughout the duration of the run, ammonia levels varied, peaking at 12.8 mg/L NH_3 -N.

In the first four days, there was a general increase in ammonia in all four tanks. This suggests a stabilization period in the tank where the initial ammonia load was lower than expected, and the ammonia added each day upset the ammonia balance.

By Day 9, Tanks A (BiOWiSH addition), C (Control) and D (RID-X) experienced slight drops in ammonia. At Day 20, drops in ammonia concentrations were observed in all four tanks. This drop did not correspond with a rise in nitrate or nitrite levels, as expressed in the previous section, so it is unlikely that any nitrification or denitrification occurred. Ammonia levels also did not steadily decrease over time. The slight drops at Day 9 and 20 may have been due to an excess of water being removed for sampling, increased evaporation of the tank, or the previous day's sludge sample containing uncharacteristically low levels of ammonia.

The general increasing trend observed in ammonia levels was not anticipated and is likely due to a tank stabilization period and should be considered in future iterations of this study. Ammonia as NH_4Cl was initially added to the residential septic tanks with the intent to bring all tanks to the 30 mg/L $\text{NH}_3\text{-N}$ outlined in the experimental setup, but starting ammonia levels were significantly less, starting between 7.4 mg/L and 8.5 mg/L $\text{NH}_3\text{-N}$. This discrepancy may be due to a laboratory error, where less NH_4Cl was added than needed, or due to the added ammonia solution settling within the tank initially. Future studies should be vigilant to ensure that initial ammonia levels more closely match the 30 mg/L $\text{NH}_3\text{-N}$ level at the initial sampling.

Final ammonia levels show that the tanks with BiOWiSH additions, Tanks A and B, performed better than the competition Tank D with RID-X. Tank D began with the lowest ammonia levels and finished the run with the highest ammonia levels. The Control Tank C performed better than all other tanks at the end of the experiment, with the lowest ammonia levels, though levels were similar to Tank B with BiOWiSH.

No evidence of nitrification or ammonia assimilation were observed. The ammonia analysis shows no significant benefit in ammonia reduction from the addition of BiOWiSH. Adding the BiOWiSH product does suggest advantages over RID-X in ammonia reduction but shows no clear advantage over a control tank with no added products.

4.3 Experiment 2 – TSS and BOD Analysis

Experiment 2 tested the effect of BiOWiSH on the reduction of BOD and TSS concentrations within septic tank effluent.

4.3.1 Temperature and pH

Temperature and pH did not remain within expected levels throughout the duration of the test period. Temperature ranged between 15.6 °C (Day 3) and 23.3 °C (Day 8). Ambient and night temperature values were not taken, but all tanks showed similar trends in temperature. The pH of the tanks ranged between 5.78 (Day 12) and 6.49 (Day 37). Average residential septic tanks range between 6 and 9, above the recorded values.

Figure 20: Experiment 2, Temperature Analysis

Figure 21: Experiment 2, pH Analysis

The acidic pH may be from sulfur compounds forming within the anoxic tanks, as described in the Experiment 1 analysis. The general increasing trend of the pH in all tanks suggests that the tank biologies were gradually matching those of residential septic tanks. This further supports the ideal that, although not ideal due to time restraints, longer sampling periods may be more beneficial in future studies.

4.3.2 Visual Analysis of Samples

Similar to Experiment 1, visual changes in the septic effluent were observed throughout Experiment 2. The change in color was observed before analytical test results were available.

By Day 4, visual analysis of the samples showed that Tank A (BiOWiSH addition) was noticeably darker in color than other tanks. Tank C (Control) and Tank D (RID-X) also showed lesser color changes, with Tank B (BiOWiSH) remaining light in color (Figure 23).

Initially, the darker coloration in the BiOWiSH additive tanks in Experiment 1 and in one of the BiOWiSH tanks in Experiment 2 suggested increased microbial growth in these tanks. The following sections compare this visual analysis to actual BOD and TSS data.

A: BiOWiSH Addition B: BiOWiSH Addition C: Control D: RID-X

Figure 22: Experiment 2, Day 4 Color Variation

By Day 8, all sample tanks were similar in color. As in Experiment 1, sample lost their dark color after being acidified and stored at 4 °C for about 24 hours. However, the larger water samples did show that the black coloring did seem to precipitate and drop to the bottom of the sampling container (Figure 24). For TSS sampling of the stored samples where any precipitation was observed, samples were vigorously mixed using a hand mixer to ensure all flocs were broken. No flocs were later observed in any TSS sampling filters.

Figure 23: Experiment 2, Precipitation of Coloring After Sample Preservation

The visual color analysis was a completely subjective examination, with no quantitative data collected through the use of a colorimeter or spectrometer. These experimental methods were not utilized as the samples quickly lost their color once acidified and stored.

4.3.3 Total Suspended Solids

Total suspended solids (TSS) was analyzed roughly on a weekly basis in the wastewater effluent samples, shown in Figure 25. Throughout the run, the TSS values were consistently lower than the 75 mg/L tank average of residential septic tanks.

Figure 24: Experiment 2, Total Suspended Solids Analysis

TSS levels at the start of the run varied between 16.7 mg/L and 31.7 mg/L. Throughout the duration of the run, TSS levels varied, peaking at 71 mg/L. The tight distribution of TSS levels in the first 8 days suggests that the four tanks started the run at comparable levels. Clear distinctions between the TSS levels in the tanks became clear around day 12.

The increase in TSS of all four tanks between Days 12 and 23 suggests bacterial growth was insufficient to decrease the daily addition of septic sludge.

There was a steep drop in TSS of all four tanks between Days 23 and 29. This drop suggests that the microbial population within the tanks had reached significant enough numbers to offset the daily amount of added sludge and facilitate ample digestion of solids within the septic

effluent. There was a decrease in overall tank temperature recorded between Day 23 and Day 29. It is possible that the temperature decrease resulted in lower digestion rates of settled sludge and less gasses being emitted from the sludge digestion, reducing solids from suspending into the tank from the settled sludge.

Alternately, this drop could indicate a systematic failure due to microbial death or removal. TSS values may have dropped if the biology within the tank reached a plateau of growth around Day 23. The first stages may have represented an inoculation period, where the tanks flourished under high nutrient conditions. When the readily available food was used, and bacterial competition began, cell growth may have occurred. If this is the case, then the addition of any additive treatment would not benefit tanks past this 23 Day inoculation period.

TSS values may have dropped due to large quantities of sample water being removed via sampling and evaporation and replaced with clean tap water. This system failure was not observed during the testing period.

Tank B with BiOWiSH recorded the highest TSS concentrations although, as mentioned in the previous section, it was the effluent with the lightest color variation. This suggests that the observed darker color is caused by colloidal particles instead of suspended solids, and that the addition of BiOWiSH microbes increased the total amount of suspended solids without facilitating higher levels of solids digestion.

The Control performed better than all additive treatments. Tank B with BiOWiSH and Tank D with RID-X reported the highest TSS values. The TSS analysis shows no significant benefit in TSS reduction from the addition of BiOWiSH over RID-X or a control tank with no added products.

4.3.4 Pre- Versus Post- Inoculation Total Suspended Solids Test Results

An additional test was performed to determine if there were measurable differences between samples taken immediately pre- and post- inoculation of BiOWiSH and RID-X, and to test the possible effect of water being removed during the sampling process in the Control. In the TSS sampling period on Day 23, samples were taken prior to and approximately 1 hour after inoculation, in an attempt for the added materials to disperse laminarly through the tank.

Sample BOD values for runs in all other sampling periods were taken 1 hour after inoculation, as determined in the original Standard Operating Procedure for sampling. Percent differences ranged from below 3% to over 22%, as outlined in Table 5 below.

Table 5: Percent Differences from Pre- and Post- Inoculation Sampling for Total Suspended Solids

	Pre-Inoculation	Post Inoculation	Percent Difference
A BiOWiSH Additive	48	47	2.08%
B BiOWiSH Additive	71	57.5	19.01%
C Control	39.5	48.5	22.78%
D RID-X Additive (Competition)	58	54.5	6.03%

The 19.01% increase in TSS in the BiOWiSH Tank B suggests that the inoculation of the bacterial product may have a direct impact on the tank's effluent. The increase in TSS might suggest the added BiOWiSH product was beneficial in providing bacteria to decrease TSS. The inconclusive results of the TSS data for the remainder of the study, however, does not suggest conclusive impacts of the BiOWiSH biological product.

The 22.78% difference in the Control Tank suggest that the largest cause of discrepancy in TSS is due to sampling methods. The Control Tank was not dosed with any additional biological product and was dependent only on the native bacteria present and sustained by the added biomass. The substantial rise in TSS suggests that the sampling methodology, which requires several liters of water to be removed for sampling, may decrease the microbial population substantially enough to disrupt the tank's biology and function.

4.3.5 Biological Oxygen Demand

Biological oxygen demand (BOD) was analyzed roughly on a weekly basis in the wastewater effluent samples, shown in Figure 26. The target BOD of 140 mg/L was approached by the BiOWiSH B Tank near the conclusion of the experiment but was not reached by the other tanks.

Three sampling periods included control BOD vials that surpassed the 0.1 mg/L DO change from initial to final DO. Due to time limits and an inability to retest these samples, these samples were still considered for analysis, but error bars were created for each point by propagating through the error inherent from the change in DO concentrations within the test blanks (Figure 26).

BOD levels at the start of the run varied between 17.7 mg/L DO and 70.4 mg/L DO. The general increase in BOD suggests the beneficial microbial populations within the tank were not sufficient to break down the contaminants within the tanks.

Although data is not available for BOD on Day 8, Tank B with the BiOWiSH addition reported the highest BOD values. By the end of the sample run, both BiOWiSH additive tanks reported higher BOD values than the Control and RID-X tanks.

Figure 25: Experiment 2, Biological Oxygen Demand Analysis

Samples with final DO readings less than 2 mg/L or that underwent a change less than 1 mg/L from the initial DO were not included in the analysis. Due to multiple dilutions failing to reach these requirements, no values are available for the RID-X competition after 23 days or for the

BiOWiSH B Tank after 8 days. Due to these gaps in data, general trends and end-point analyses were used to compare tanks.

Tank B with BiOWiSH recorded the highest BOD concentrations although it was the effluent with the lightest color variation. This suggests that the observed darker color coincides with increased BOD and bacteria growth, and that the addition of BiOWiSH microbes increased the overall amount of BOD and bacteria concentrations without facilitating higher levels of solids digestion.

The BOD analysis shows no significant benefit in BOD reduction from the addition of BiOWiSH over RID-X or a control tank with no added products.

4.3.6 Pre- Versus Post- Inoculation BOD Test Results

An additional test was performed to determine if there were measurable differences between samples taken immediately pre- and post- inoculation of BiOWiSH and RID-X, and to test the possible effect of water being removed during the sampling process in the Control. In the BOD sampling periods beginning on Days 8 and 23, samples were taken prior to and approximately 1 hour after inoculation, in an attempt for the added materials to disperse laminae through the tank.

Sample BOD values for runs beginning on Days 1 and 29 were taken 1 hour after inoculation, as determined in the original Standard Operating Procedure for sampling.

Percent differences ranged from below 3% to over 46%, as outlined in Table 6 below. Not all samples were observed for this potential change.

Table 6: Percent Differences from Pre- and Post- Inoculation Sampling for Biological Oxygen Demand

	Day 8			Day 23		
	Pre-Inoculation	Post Inoculation	Percent Difference	Pre-Inoculation	Post Inoculation	Percent Difference
A BiOWiSH Additive	37.85	31.80	15.99%	82.80	98.10	18.48%
B BiOWiSH Additive	-	-	-	142.20	-	-
C Control	49.90	51.30	2.81%	60.10	87.75	46.01%
D RID-X Additive (Competition)	70.90	68.90	2.82%	-	-	-

The considerable changes in BOD observed in Day 8's BiOWiSH Tank A, and Day 23's BiOWiSH Tanks B's suggests that the inoculation of the bacterial product may have a direct impact on the BOD of the tank's effluent. BOD decreased in Day 8's BiOWiSH Tank A and increased in Day 23's BiOWiSH Tank B. The inconsistency of the effect of the biological product, and the inconclusive result of the BOD data for the remainder of the study, does not suggest conclusive impacts of the BiOWiSH biological product.

The substantial 46% difference in Day 23's Control Tank suggests the largest cause of discrepancy is due to sampling methods. The Control Tank was not dosed with any additional biological product and was dependent only on the native bacteria present and sustained by the added biomass. The substantial rise in BOD suggests that the sampling methodology, which requires several liters of water to be removed for sampling, may decrease the microbial population substantially enough to disrupt the tank's biology and function. No significant change was observed in pre- or post- inoculation samples for the Control Tank when sampled on Day 8.

4.4 Key Findings

The following sections condense the key findings of Experiment 1 and Experiment 2.

4.4.1 Experiment 1 – Ammonia and Nitrate Analysis

The absence of DO in the tanks prevented nitrification of ammonia to nitrates. Although Tanks A and B with BiOWiSH added performed better than the RID-X competition, they did not show significant advantages over the control tank with no additives. The ammonia analysis shows no significant benefit in ammonia reduction from the addition of BiOWiSH.

Denitrification did not occur due to the lack of nitrates present or created through nitrification.

Although no significant benefits were observed during this experiment, the BiOWiSH products may provide some benefit in an overall residential septic system when introduced to the aerobic environment in the leach field. These adjustments are further outlined in the Conclusions section.

4.4.2 Experiment 2 – Total Suspended Solids and Biological Oxygen Demand Analysis

The Control Tank performed better than all additive treatments, resulting in the lowest TSS values. The TSS analysis shows no significant benefit in TSS reduction from the addition of BiOWiSH over RID-X or a control tank with no added products.

The steep drop in TSS values over all four tanks suggested that, after Day 23, each biology of the tanks may have reached sufficient levels to offset the daily addition of sludge and facilitate ample digestion of solids. Because there was no drop in BOD levels over the same period, it is unlikely that there was significant digestion. Other potential reasons for this TSS drop were discussed in Section 4.3.3 Total Suspended Solids.

Tests comparing pre- and post- inoculation TSS values showed that TSS both increased and decreased after sampling and inoculation. Results were inconclusive but suggest that the sampling methodology may decrease the microbial population substantially enough to disrupt the tank's biology and function.

The BOD analysis shows no significant benefit in BOD reduction from the addition of BiOWiSH over RID-X or a control tank with no added products.

The BiOWiSH treated Tank B had the highest values for BOD by the end of the experiment, and the highest levels of TSS for the first 23 days. The visual sample analysis

showed that Tank B was the last of the four tanks to present significant color change, as discussed in Section 4.3.2 Visual Analysis of Samples

This suggests that the color change, where effluent gained a darker hue, indicates additional microbial growth within the tank. The additional bacteria growth resulted in higher levels of TSS and BOD, where the bacteria remains suspended in the tank effluent. The increased level of bacteria did not suggest reductions in BOD and TSS from increased digestion of solids, as was anticipated.

Future studies should visually analyze the septic tank effluent and watch for color changes, which would suggest the tanks are increasing in bacterial growth but not adequately digesting solids. This analysis applies only to Tank B, not to the other BiOWiSH additive Tank A.

Tests comparing pre- and post- inoculation BOD values showed that BOD both increased and decreased after sampling and inoculation. Results were inconclusive, but like TSS suggest that the sampling methodology may decrease the microbial population substantially enough to disrupt the tank's biology and function.

Chapter 5

CONCLUSIONS AND FUTURE IMPROVEMENTS

An analysis of the goals of this study and the results are shown in Table 7 below;

Table 7: Analysis of Study Goals

Study Goal	Result and Conclusion
Construct model-scale septic tanks to accurately represent the properties of residential septic tanks	The Ohio State University study was utilized as an outline for the construction of model-scale septic tanks. Edits were made to reduce potential for leaks.
Develop a Standard Operation Procedure for sampling from the model-scale tanks	A Standard Operating Procedure was developed (Appendix A) to facilitate sample collection from the tank supernatant while limiting tank disturbance and mixing. Sample analysis did show significant differences in pollutant concentrations when samples pre- and post-product addition. Future studies are needed to determine the best sampling frequencies and methodology for testing effluent using these model-scale tanks.
Measure effluent concentrations of nitrates, ammonia, biological oxygen demand, and total suspended solids between the BiOWiSH additive tanks, control tanks, and RID-X tank	Nitrates were not recorded in significant concentrations in any of the model-scale septic tanks. Ammonia, biological oxygen demand, and total suspended solids were measured following standard methods as described in the Methods and Materials section.

Analyze and compare effluent

concentrations to determine

potential benefits of BiOWiSH on

the biological treatment of

residential septic systems

No significant reductions in nitrates, ammonia, biological

oxygen demand, or total suspended solids was measured

as the result of the addition of BiOWiSH

The variability in sample results, potentially due to the variations within the sampled sludge, would require significant observed changes to determine if BiOWiSH effectively improved the condition of the tanks. These significant differences were not observed.

Although the addition of BiOWiSH did not suggest any significant benefits to the performance of septic tanks, additional studies are needed to fully analyze its potential contributions to septic systems as a whole. As described in Section 2.2, Traditional Residential Wastewater Treatment Using Septic Systems, much of the biological and chemi-physical treatment of septic effluent occurs in the aerobic infiltrative zone. And as suggested in Experiment 2, the addition of BiOWiSH in Tank B may augment and increase the bacteria and microbes within the tank. These added microbes may prove beneficial to ammonia, nitrate, BOD, and TSS removal later in the septic system when exposed to aerobic environments.

5.1 Proposed Design Improvements

The model scale septic tanks were efficient and easy to sample from, but several improvements can be made.

Near the end of the second experimental run, the PVC piping structure that contained the ball valve and discharge ports on two tanks became dislodged. This did not cause any leaking from the tanks but did require additional support to hold the sampling port upright in the correct position. Reducing the distance that the PVC piping is built away from the tank or increasing the support would eliminate this problem.

Each tank was meant to have a bottom discharge point for easier effluent discharge at the end of the experiment, as outlined in the original Ohio State University report. These were

omitted due to initial leakage concerns. Adding a valve on the side of the tank, lower than the discharge point, is an option for future studies if leakage concerns are pacified.

The plastic bowls attached to the floating baffles, meant to facilitate slow transfer of influent to the tank, was often clogged upon sludge addition. Increasing the holes from 2 – 1/8 inch holes to 4 – 1/4 inch holes enabled the daily sludge additions to flow more smoothly to the tank with less clogging, while still limiting the floating scum disturbance.

5.2 Proposed Experimental Methods Improvements

Although the sampling procedures outlined in the Ohio State University study were effective at providing a baseline for sampling procedure, some adjustments could be made to ensure a more accurate analysis of the tanks' performance.

As described in the Results and Analysis section, significant changes in TSS and BOD were observed when samples were taken pre- and post- inoculation of biological additives. This is most likely due to the several liters of water needed to be removed to gather a representative sample of the tank biology. To limit the impact that this water has on the sampled levels, TSS and BOD should only be sampled post-inoculation of product addition. Pre-inoculation data should be replaced with data taken the previous day.

To further limit the impact of water removal, samples could be taken on an every-other day basis with sludge still added daily. This would decrease the water removed and allow more time for the tanks' bacterial cultures to grow.

In future studies, more robust pre-experiments should be performed to determine a more appropriate sludge dilution. This would enable starting values of BOD, TSS, and NH_3 to be closer to the expected average septic tank values and yield more appropriate results.

Finally, if possible, longer sampling times may be better representative of an actual residential septic tank. To offset this longer sampling period, more septic tanks could easily be added. Early samples should still be taken to determine the length needed for tank pollutant concentrations to fully stabilize.

5.3 Future Projects and Parameters

Though many of the outcomes of this study proved inconclusive, there are several follow-up studies that would yield informative data.

The most important follow-up study would incorporate an aerobic zone to further replicate a complete residential septic system, not solely the septic tank. Residential septic tanks are followed by a complex soil matrix, which allows air and oxygen the opportunity to enter the system. It is generally this section that allows further treatment through biological processes, adsorption, filtration, and infiltration into the underlying soils. The past BiOWiSH study did not show significant pollutant concentration reductions in these leach fields.

An additional aeration step could easily be added to the existing experimental set-up. Effluent sampled from the model scale septic tanks should be placed in an incubator and aerated via bubble aerators. Placing them in the incubator will control the temperature while limiting outward contamination. If possible, the physical adsorption could also be measured by allowing effluent from the aeration section, or effluent straight from tanks, to simply percolate through a soil column.

Due to time and labor constraints, the only parameters tested in this study were BOD₅, NH₄⁺, NO₃⁻/NO₂⁻-N, TSS, and temperature/pH. For a more robust and complete understanding of this product's capabilities, as well as understand what parameters in residential septic tanks could be improved, a further study should consider the impacts on phosphorus and FOG. These would be most influential when studied after aeration and absorption environments.

Several iterations of this study could be repeated to further test the effect of sampling effluent pre- or post- product inoculation. The effect of sampling frequency could also be analyzed to determine if daily or weekly samples were most appropriate. Statistical analysis on minimum detectable difference should also be performed to determine the number of total tanks and duplicates needed to provide more robust data analysis on the effect of BiOWiSH.

Another potential study could look at optimizing the dosage of the BiOWiSH products in residential septic tanks. For this study, the factory recommended dosage was utilized, though

further studies may show that an increase in product corresponds with advanced treatment, as was shown in the only BiOWiSH case study available.

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APPENDICES

Methods for Sampling Effluent from Model Scale Septic Tanks
Created: January 2018 by Kimberly LaMar

For Help: Kimberly LaMar 209-275-5324

Background:

Septic tanks provide safe and reliable wastewater treatment for many rural communities, though their final effluent has the potential to cause environmental and human health concerns. These tanks were specially designed according to an Ohio State University to mimic the characteristics of residential septic tanks, and enable collection and sampling of effluent and scum (for FOG testing). Samples collected can be then frozen or acidified for preservation for future testing.

The following are instructions for sampling of effluent only. Please see additional methods for proper collection of scum and sludge from tanks.

Job Hazard Analysis

See the Sampling Effluent from Model Scale Septic Tanks Job Hazard Analysis for the specific hazards identified in each work step and the safe work procedures to avoid those hazards.

Test Duration:

Approximately 1 hour

Materials/Reagents:

*Denotes materials that should be brought to deck

- 4 - 500 mL reagent bottles*
- 2 - 250 mL reagent bottles or other like-size glassware*
- 4 - 10 mL Timberline vials of primary sludge (located in the refrigerator)
- Laboratory Scale
- Metal spatula (like ones used to measure chemicals)*

- 4 - 50 mL Timberline vials, labeled, for sample collection*
- 5 gallon bucket*
- 90 mg/L $\text{NH}_4\text{Cl-N}$ (1 L per run)*
- 30 mg/L KH_2PO_4 (1 L per run)*
- Temperature and pH probe
- 0.1 N NaOH with 1 mL pipette*

Instrument/Equipment Location:

Septic tanks are located at the southeast corner of Building 13's second story deck. Access to the deck is through Room 13-201.

Personal Protective Equipment Required:

Safety Glasses (goggles if wearing contacts) and nitrile gloves are required. Lab coats and/or plastic aprons are recommended due to potential for effluent to splash during collection.

Sample Preservation:

Test for temperature and pH within 15 minutes of collection. Acidify samples to <2 pH using H_2SO_4 or other acid to prevent volatilization of ammonia and then refrigerate at 4°C until analyzing. If samples cannot be acidified, freeze at -18°C .

Solution Preparation

50:1 Sludge Dilution *MADE DAILY*

- 1) Rinse a labeled 500 mL reagent bottle with DI water.
- 2) Place reagent bottle on lab scale and tare (zero).
- 3) Fill reagent bottle with ~200 mL tap water.
- 4) Using metal spatula and a DI bottle, empty 10 mL sludge Timberline vial into reagent bottle. Place dirty Timberline vial in appropriate container for later cleaning.
- 5) Fill reagent bottle with tap water until 500 mL by weight.

- 6) Repeat to make 4 reagent bottle sludge dilutions.

90 mg/L Ammonium chloride as Nitrogen

MADE EVERY OTHER DAY

- 1) Rinse the labeled 2 L reagent bottle with DI water.
- 2) Place reagent bottle on lab scale and tare (zero).
- 3) Fill reagent bottle with ~ 1 L DI water.
- 4) Add 687.24 mg NH_4Cl to bottle, rinsing weigh boat with DI water.
- 5) Fill reagent bottle with DI water until 2 L by weight.
- 6) Add magnetic bar and stir with magnetic stir plate for at least 5 minutes.

30 mg/L Potassium phosphate monobasic as Phosphorus

MADE EVERY OTHER DAY

- 1) Rinse the labeled 2 L reagent bottle with DI water.
- 2) Place reagent bottle on lab scale and tare (zero).
- 3) Fill reagent bottle with ~ 1 L DI water.
- 4) Add 263.66 mg KH_2PO_4 to bottle, rinsing weigh boat with DI water.
- 5) Fill reagent bottle with DI water until 2 L by weight.
- 6) Add magnetic bar and stir with magnetic stir plate for at least 5 minutes.

0.1 N NaOH

Make When Needed

- 1) Rinse a 100 mL volumetric flask with DI water
- 2) Fill flask with ~50 mL DI water
- 3) Using a pre-calibrated micropipette, transfer 1 mL of 10 N NaOH stock solution (found in chemical cabinet) to volumetric flask.
- 4) Fill flask with DI water until the meniscus is at the fill line.
- 5) Invert until well mixed. Label and store in chemical cabinet.

Sampling Procedure

1. Visually check all tanks for any leaks. If leak is identified, call contact (on top of methods) IMMEDIATELY for instruction.
2. Remove tank lids. Be aware that water will have condensed on the bottom of the lid and will drip when removed.
3. Using a metal spatula, remove any residual sludge/solids from the bowl and gently place in the tank on the side of the foam baffle.
4. Place an orange 5-gallon bucket below the discharge port on the front side of the tank. DO NOT FORGET THIS STEP. If you do, you will pour wastewater everywhere.
5. Using two hands (one on the valve and one holding the piping system), open the blue valve and allow at least 1 L to flow into the bucket. (This can be roughly estimated using the pre-measured 1 L plastic tub beside the tank). Allow 2-3 L of water to drain, and then fill the 50 mL Timberline vial with the sample. (It is easier to collect the sample first in the small plastic tub, close the valve, and then fill up the vial directly from that small tub).
6. Using two hands, securely close the blue valve. Ensure that there are no leaks.
7. Repeat steps 3-6 for each tank, being sure to label the Timberline vials appropriately.
8. Using pre-measured 250 mL reagent bottles or other glassware, transfer 250 mL of the 90 mg/L $\text{NH}_4\text{Cl-N}$ and 250 mL of the 30 mg/L KH_2PO_4 into each of the plastic bowls. These can drain either through the small hole on its side, or as an overflow over the bowl. If overflowing solutions, do so in a steady and moderate speed to minimize scum disturbance. The solutions will run through the hole or over the side and drain to the side of the floating baffle.
9. Using pre-calibrate pipettes, add 1 mL of 0.1 N NaOH to each bowl.
10. When solutions have finished draining, completely empty the 500 mL sludge dilutions into each of the plastic bowls. Shake before pouring and rinse with DI water if solids remain.
11. Clogging of the bowls' holes are common due to solids in sludge solution. Clear these using the metal spatula, but don't be concerned if clogging persists.

12. Fill tank, via overflow in each bowl, with tap water, until tanks reach original level.

Remember to fill slowly to minimize scum disturbance. This generally further dilutes and overflows much of the sludge solids, making it easier to de-clog.

13. Ensure all bowls are empty. Securely replace lids.

14. Record temperature and pH of all samples. Acidify samples to <2 pH using H_2SO_4 or other acid to prevent volatilization of ammonia and then refrigerate at 4°C until analyzing.

If samples cannot be acidified, freeze at -18°C .

15. Dispose of excess effluent from sampling and rinse 5-gallon bucket.

16. Remake $90\text{ mg/L NH}_4\text{Cl-N}$ and $30\text{ mg/L KH}_2\text{PO}_4$ if needed.

Cleaning Sludge Timberline vials:

- 1) Rinse vials three times with tap water.
- 2) Using a wetted paper towel, wash rim and top of vial (and lid if needed) to clean dried sludge that often accumulates.
- 3) Rinse three times with DI water.
- 4) Soak overnight in labeled container of Alconox and DI water mixture.
- 5) Rinse with DI water, then soak overnight in labeled container of DI water.
- 6) Let dry completely before returning to clean vial tub.

Note: manufacturing supplier of these vials requires soaking DI water before reuse, and it is vital to ensure these are clean as they are used for other lab samples.

Job Hazard Analysis
Methods for Sampling Effluent from Model Septic Ranks
California Polytechnic State University, San Luis Obispo, Building 13 Labs and Deck

Created by:	Kimberly Merilles	January 2019
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Work Steps and Tasks	Hazards Identified	Control/Safe Work Procedures
1. Sludge Collection; includes lifting sludge vault lid and reaching into sump to collect sludge.	Driving hazards	Perform a vehicle inspection before operating. If using Cal Poly vehicles, ensure that vehicles are correctly checked out and Driver Safety lessons are up to date.
	Onsite hazards - varied	Check into Front Office of facility. Alert nearby workers of presence and be aware of alarms. Plan evacuation routes in case of emergencies.
	Back strain from lifting sludge vault lid, bending and leaning to collect sludge, and carrying buckets of sludge	Use proper lifting form, including lifting with the knees instead of the back, keeping loads close to the body and avoiding twisting motions, and using a buddy if necessary. Take multiple loads or use carts/dollies to prevent carrying heavy loads significant distances.
	Pathogen and contaminant contact	Wear safety glasses (goggles if wearing contacts), nitrile gloves, a lab coat, long pants, and closed toe shoes. A splash apron is optional but recommended. Avoid contact with sludge, and decontaminate and wash all affected equipment after sampling.
	Breathing in potential contaminants (such as H ₂ S, pathogens)	If available, bring portable gas meter to measure H ₂ S concentrations. When not available, use best judgement in avoiding contaminated air. Open sludge vault and allow it to air out before collecting sludge. Take frequent breaks. Position yourself upwind.
	Hand safety and pinch points from closing sludge vault lid	Wear cut resistant gloves if possible. If not available, use additional care to avoid pinch points when replacing sludge vault lid. The lid is heavy, and using a second person/buddy is recommended.

2. Solution Preparation	Chemical contact during measuring and stirring	Wear safety glasses (goggles if wearing contacts), nitrile gloves, a lab coat, long pants, and closed toe shoes. A splash apron is optional but recommended. Read SDS sheets to each chemical prior to handling to understand hazards and properly treat if you come into contact with them. Avoid skin contact with chemicals and solutions. Rinse all affected sink and/or use eye wash station if contacted with any chemicals.
	Cut hazards from broken glass	Wear closed toe shoes at all times in the laboratory. If glass is broken, carefully sweep glass into broken glass container near sink. Avoid picking up broken pieces of glass. If you do get cut, clean well and visit the Campus Health Center.
	Hot plate hazard	The stir plate can also be used as a hot plate. When turning on the stir plate, do not immediately walk away; watch to make sure that the stir knob was correctly turned. Accidentally turning on the hot plate can cause burns and can shatter glassware.
3. Sampling Wastewater From Tank	Pathogen and contaminant contact	Wear safety glasses (goggles if wearing contacts), nitrile gloves, a lab coat, long pants, and closed toe shoes. A splash apron is optional but recommended, as water may splash with water flowing out of tanks. Avoid contact with wastewater. Rinse all affected areas in the sink and/or use eye wash station if contacted with any chemicals.
	Back strain from lifting filled buckets of wastewater	Use proper lifting form, including lifting with the knees instead of the back, keeping loads close to the body and avoiding twisting motions, and using a buddy if necessary. Take multiple loads or use carts/dollies to prevent carrying heavy loads significant distances.
	Breathing in potential contaminants (such as H ₂ S, pathogens)	If available, bring portable gas meter to measure H ₂ S concentrations. When not available, use best judgement in avoiding contaminated air. Open tanks and allow to air out. Take frequent breaks. Position yourself upwind.

	Biological Hazards	Bees are often found on the deck and are attracted to any standing water (such as thin films left in buckets or accumulated during rain). Wasps have also made nests nearby and in the foam insulation covers. Be aware of bees, wasps, and other insects and avoid when possible. If any workers are allergic, have them avoid sampling if insects are present and have an Epi-pen on hand.
	Weather	The deck is subject to direct sunlight and can become incredibly warm in summer months. The direct sunlight also reflects bright light off the white pavement. Wear sunscreen, tinted safety goggles or a baseball cap, and proper clothing to prevent strain from the sun. Stay hydrated and watch for signs of heat stroke and heat exhaustion. If heat stroke or heat exhaustion symptoms occur, visit the Campus Health Center or a medical office immediately.
4. Sludge and Solution Addition to Tanks	Pathogen and contaminant contact	Wear safety glasses (goggles if wearing contacts), nitrile gloves, a lab coat, long pants, and closed toe shoes. A splash apron is optional but recommended, as water may splash with solutions flowing into tanks. Avoid contact with wastewater. Rinse all affected areas in the sink and/or use eye wash station if contacted with any chemicals.
	Breathing in potential contaminants (such as H ₂ S, pathogens)	If available, bring portable gas meter to measure H ₂ S concentrations. When not available, use best judgement in avoiding contaminated air. Open tanks and allow to air out. Take frequent breaks. Position yourself upwind.
5. Filling Tanks with Tap Water, Dumping Excess Wastewater	Slip/trip/fall hazard from extended hose and water splashing onto pavement	Be watchful of steps, avoiding wet areas when possible. Clear pathways and identify any potential tripping hazards that cannot be moved.

	Back strain from lifting filled buckets of wastewater	Use proper lifting form, including lifting with the knees instead of the back, keeping loads close to the body and avoiding twisting motions, and using a buddy if necessary. Take multiple loads or use carts/dollies to prevent carrying heavy loads significant distances.
6. Sample Preservation and Cleaning Equipment	Pathogen and contaminant contact	Wear goggles when dealing with concentrated acid, nitrile gloves, a lab coat, long pants, and closed toe shoes. Avoid contact with wastewater samples. If acid comes into contact with skin or clothing, rinse well to prevent chemical burns. If acid comes into contact with water, use the eye wash station and visit the Campus Health center or a medical office if irritation persists.
	Cut hazards from broken glass	Wear closed toe shoes at all times in the laboratory. If glass is broken, carefully sweep glass into broken glass container near sink. Avoid picking up broken pieces of glass. If you do get cut, clean well and visit the Campus Health Center.