

**ENZYME PRETREATMENT OF FATS, OIL AND GREASE FROM RESTAURANT
WASTE TO PROLONG DRAIN FIELD EFFECTIVENESS**

By

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ABSTRACT

ENZYME PRETREATMENT OF FATS, OIL AND GREASE FROM RESTAURANT WASTE TO PROLONG DRAIN FIELD EFFECTIVENESS

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When a fast-food restaurant wastewater containing high strength of FOG is discharged into sewers, it builds up over time and clogs the sewer pipes. Similarly, when a fast-food restaurant wastewater flows into a drain field, it adheres to the surface of inlet pipes, gravel and soil, which will restrict the flow and eventually clog the drain field either physically or biologically. In this study, the Advanced Grease Interceptor System (AGIS) was used for enzymatic pretreatment of wastewater from a fast-food restaurant. This system uses aeration equipment, baffles, and a one-time inoculum with an in-situ condition in order to microbiologically breakdown FOG. The effect of the decomposed FOG in a drain field has not been previously studied, the focus of this research. The objectives of this study were to examine the impact of enzyme treatment on changes of the characteristic of triglyceride, design a bench-scale drain field and assess performance, and determine the impact of wastewater with/without enzyme pretreatment on clogging. LC/MS results showed that enzyme treatment impacted the characteristics of the triglycerides by breaking down the long chain fatty acid and reducing the number of double bonds. Bench-scale trenches with embedded soil moisture sensors for monitoring clogging proved to be a unique, and effective method to investigate the impact of diverse feedstocks. All bench-scale trenches removed a significant amount of COD, BOD₅ and FOG, regardless of their initial concentrations. The results from the laboratory trenches showed that pretreatment of restaurant wastewater using an in-situ enzyme producing bacteria will delay clogging and premature drain field aging.

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KEY TO ABBREVIATIONS

Abbreviation	Full Word
FOG	Fats, Oil and Grease
AGIS	Advanced Grease Interceptor System

Chapter 1: Introduction

Fats, oil and grease (FOG) come from animal meat, cooking oil and dairy products. The chemical structure of FOG is a triglyceride. Each triglyceride is composed of 3 fatty acids connected with glycerol, which is an alcohol with three carbons, each bearing a hydroxyl group. A fatty acid has a long structure with usually 16 or 18 carbon atoms and potentially several double bonds. FOG containing fatty acids with a greater number of double bonds are less biodegradable and more cohesive (sticky). Cohesiveness of FOG results in producing non-biodegradable grease balls.

Restaurants use animal meats, cooking oil and dairy products and produce FOG in their wastewater. Annual grease produced from a single restaurant ranges from 363 to 7,711 kg (800 to 17,000 lbs) (U.S. Environmental Protection Agency 2007). In the United States, the total amount of restaurant grease generated and collected and recycled by renderers in 2010 were 2,061 billion kg and 1,092 kg (4,543 billion lbs and 2,408 billion lbs), respectively. Consequently, only 53 % of greases was recycled (U.S. Environmental Protection Agency 2014).

There are two types of FOG. Yellow grease originates from fryer oil and other such concentrated sources. This FOG is not contaminated with water, wastewater and solid waste and is usually recycled for animal feed and biofuels. Brown grease results from rinsing and washing dishes, sanitizing, mopping floors and washing rags. Unlike yellow grease, brown grease is contaminated and is typically discharged into the wastewater system, which results in sewer blockages as it builds up over time. When sewer pipes are clogged (Figure 1), raw sewage overflows into homes, streets, rivers and parks and people who are exposed to wastewater have a higher chance of contracting diseases.



Figure 1. FOG build up in a sewer (Town of Tyngsborough, 2015)

FOG plays a major role in sewer clogging and is expensive to remove. In 2004, the U.S. EPA reported that there were 20,000 sanitary sewer overflows in the United States. Of these, 49 % were related to FOG clogging coming from restaurants, homes and industrial sources (U.S. Environmental Protection Agency 2004). The United States spends \$25 billion of tax dollars annually to manage FOG build up in sewers (Washtenaw County 2014). The New York Times stated that 62 % of the 15,000 sewer backup complaints in New Your City were related to FOG accumulation (Gregory 2014). FOG issues in sewers cost \$3.5 million and \$4.65 million, in San Francisco and New York City, respectively (San Francisco Water Power Sewer 2012; Gregory 2014). In London, 10 tons of congealed fat and house hold waste blocked and broke a sewer and cost approximately \$450,000 US dollars to repair (Ratcliffe 2015). From these cases, it is clear that the FOG in a sewer is great concern all over the world and proper and sufficient management is needed.

A drain field associated with on-site wastewater disposal is a complete treatment system using microbial, physical and chemical mechanisms. When fast food restaurant discharge wastewater containing FOG into a drain field, FOG adheres to the surface of inlet pipes, gravels

and soil, restricts the flow and eventually clogs the drain field. FOG will not coagulate when the temperature is below 60°C (140 °F) and typical temperature of drain field, at 61 cm (2 ft) below from the ground, is from 3°C (37°F) to 20°C (68°F) (Naylor & Gustin 2015). Therefore, FOG in a drain field will be coagulated and clog the porosity of pipe, gravel, and/or soil. In general, the average of typical life of a drain field is 25 – 30 years (Mckenzie 2010). However, the high strength of FOG in fast-food restaurant wastewater will premature the life of drain field. Wodalski (2012) reported that drain field in a large and quick-serve restaurant was replaced three times in first five years of business, because of FOG (Wodalski 2012).

In order to prevent sewer and drain field clogging, FOG collection and/or treatment systems are required so that the discharge limit of 100 mg/L should not be exceeded (U.S. Environmental Protection Agency 2007). These systems can use physical or biological treatment techniques. The common physical treatment technology is a grease trap. A grease trap intercepts most of the FOG and solids by using baffles in a tank to trap floating FOG and separates it from the wastewater. A grease trap must be pumped when 25 % of it is full (Leverenz et al. 2002) otherwise the grease trap loses efficiency. The efficient grease trap decreases during peak wastewater discharges.

An alternative to the grease trap is to pretreat wastewater containing FOG with enzymes. Enzymes, excreted from certain microbial populations, cleave long chain fatty acids and double bonds. The greater the number of double bonds in a triglyceride, the more difficult it is to biodegrade (de Waard et al. 1993). Once FOG is broken down, it will not be restored back to its original form and it is not cohesive. The use of enzyme treatment at restaurants minimizes the cost of pumping, hauling and disposing of FOG.

1.1. Problem statement

Enzyme treatment of FOG prevents clogging in sewer pipes by converting triglycerides into fragments that do not adhere and cause wastewater constituents to conglomerate (Gajeski 2010). Pretreatment of brown FOG using enzymes in a drain field has not been widely studied. The hypothesis of this research is that enzyme treatment breaks down long fatty acid chains, associated with the triglycerides, reducing clogging potential and extending the life of a drain field.

Drain field clogging from fast-food restaurant wastewater can result from several scenarios, as listed below.

- Kitchen wastewater containing a high concentration of FOG will clog the inlet pipe, even before the wastewater flows into the drain field.
- Kitchen wastewater containing a high concentration of FOG flows into a drain field, but the FOG adheres to the soil surface and clogs the drain field.
- Kitchen wastewater pretreated with enzyme still clogs the drain field because of the high biodegradable organic loading consisting of FOG with low molecular weights and fewer double bonds.

1.2. Objective

The above problem statement and hypothesis lead to the project objectives entailing assessing the effectiveness of enzymatic wastewater pretreatment in preventing the premature aging of a drain field. Sub-objectives follow.

1. Measure changes in the characteristics of triglycerides, resulting from enzymatic pretreatment.
2. Design a bench-scale drain field that represents a field-scale system. Observe the performance of drain field from different characteristics of wastewater.
3. Operate bench-scale drain fields using different wastewater types, with and without enzymatic pretreatment. Assess the differences in performance to estimate if enzymatic pretreatment can potentially prevent premature aging of a drain field. Include soil water moisture sensors to enable in situ clogging monitoring.

Chapter 2: Literature review

This chapter discusses the composition of FOG, FOG treatment technologies, including the grease trap and enzymatic treatment, commercially available enzyme systems and the general operation of a drain field.

2.1. Composition of FOG

FOG is composed of a triglyceride which consists of three fatty acids and a glycerol. Typically, there are five long chain fatty acids that produce FOG, including Myristic acid (C14:0, C₁₄H₂₈O₂), Palmitic acid (C16:0, C₁₆H₃₂O₂), Stearic acid (C16:0, C₁₈H₃₆O₂), Oleic acid (C18:1, C₁₈H₃₄O₂) and Linoleic acid (C18:2, C₁₈H₃₂O₂). The proportion of the five fatty acids that classified as saturated (Myristic acid, Palmitic acid, Stearic acid), monounsaturated (Oleic acid) and polyunsaturated (Linoleic acid) determines the effectiveness of hydrolysis. Monounsaturated and polyunsaturated fatty acids, which consist of double bonds, are more difficult to be degraded than saturated fatty acids (de Waard et al. 1993).

2.2. FOG treatment technologies

Several technologies have been used to treat a FOG. The following reviews each technology in detail.

2.2.1. Grease trap

To prevent sewer blockages, a grease trap is commonly used at restaurants. A typical grease trap collects FOG by cooling and flotation (Metcalf & Eddy 2003). The trap requires maintenance including cleaning every 3 – 6 months (Leverenz et al. 2002). The efficiency of grease traps is relatively low resulting in a typical a FOG effluent concentration of 100 mg/L (Chan 2010; Chu & Ng 2000). Aziz et al. (2011) shows that changing the shape and form of conventional grease abatement devices can improve the FOG capture efficiency. This study found that the 3 most important factors for efficient operation is the inlet configuration, baffle wall arrangement and the HRT. The study was conducted with bench-scale equipment and a computational fluid dynamics model (Aziz et al. 2011).

2.2.2. Enzyme treatment

Many bacteria are able to produce extracellular enzyme, including lipase. Lipase is one of the enzymes that break down FOG, also known as triacylglycerol ester hydrolases. Tang et al. (2012) found that enzyme treatment converts unsaturated fats (containing double bond) to saturated fats (containing single bond), resulting in increased biodegradability. Many researchers (Cavaleiro et al. 2013; Cammarota & Freire 2006; Jeganathan et al. 2006; Kunst et al. 1997; Daverey & Pakshirajan 2015; Wakelin & Forster 1997; Roheim 2003; Tang et al. 2012; Brooksbank et al. 2007; Mazlin 2012; Markossian et al. 2000; Tano-Debrah et al. 1999; Leal et al. 2006; Valladão et al. 2007; Moon & Song 2011) have investigated the effectiveness of enzyme treatment on FOG. These studies showed mixed results due to different enzymes and variables used in the studies, including the type of bacteria, temperature, retention time and dose.

Some processes hydrolyzed FOG by adding enzymes directly. Others hydrolyzed FOG by adding bacteria that excreted the enzymes (in situ enzyme production).

2.2.2.1. Direct enzyme hydrolysis

Previous studies show that optimal temperature, hydraulic retention time (HRT) and dose are the major factors for maximizing efficiency of hydrolysis. Table 1 shows results from studies on the effectiveness of FOG hydrolysis by enzyme.

2.2.2.2. In-situ enzyme production

Previous studies showed that the addition of some microorganisms will excrete enzymes. Table 2 shows the effectiveness of several that were documented in the literature.

Table 1. FOG hydrolysis studies using enzymes

Name	Temperature	HRT	Dose	Results	Reference
<i>Candida rugosa</i>	37°C	24 hours	0.02% (w/w)	Hydrolysis : 52 – 54% of FOG	(Cavaleiro et al. 2013)
<i>Candida rugosa</i>	35°C	3 days	0.5 and 1.5 g of bead/L (0.4 g-lipase/ g -bead)	Hydrolysis : 45 – 54% of FOG	(Jeganathan et al. 2006)
<i>Candida rugosa</i>	35°C	5 hours	0.25 - 1.00%	Hydrolysis : 88% of soybean oil	(Ting et al. 2006)
<i>Candida rugosa</i>	40°C	3 hours	1.5% (w/v)	Hydrolysis : 97% on used frying oil	(Rashid et al. 2014)
<i>Penicillium restrictum</i>	35°C	14 hours	0.1% (w/v)	Hydrolysis : 70% of FOG (initial FOG : 1000 mg/L)	(Leal et al. 2006)
<i>Penicillium restrictum</i>	35°C	22 hours	0.1, 0.5, 1.0% (w/v)	Hydrolysis : 35% of FOG	(Valladão et al. 2007)
<i>Penicillium restrictum</i>	35°C	12 hours	0.1, 0.2 , 0.5% (w/v)	FOG removal : 79 – 90%	(Cammarota et al. 2001)
Pancreatic lipase 250 (PL-250)	25°C	4 hours	25 - 1500 mg/L	Hydrolysis : 35% of neutral fat	(Masse et al. 2001)

Table 2. In-situ enzyme production for FOG hydrolysis

Name	Temperature	HRT	Dose	Results	Reference
<i>Acinetobacter sp.</i>	30 – 35 °C	24 hours	5% (v/v)	FOG removal : 60 – 65%	(Wakelin & Forster 1997)
Bio-Amp (Commercial product) Combination of <i>Pseudomonas fluorescens</i> , <i>Pseudomonas putida</i> , <i>Bacillus subtilis</i> , <i>Bacillus licheniformis</i> , <i>Bacillus thuringiensis</i>	22 ± 2°C	24 hours	32 g of Bio-Amp Pellets	FOG removal : 40%	(Tang et al. 2012)
Unidentified consortium of Gram negative bacteria which was isolated from grease trap, Activated sludge	30 – 35°C	4 days	10% (v/v)	FOG removal : 84 – 96%	(Wakelin & Forster 1998)
<i>F69 (Commercial Product)</i> Various microbes	30°C	21 - 28 days	0.6% (v/v)	FOG removal : 37 – 62%	(Brooksbank et al. 2007)
Bacteria cultured from used cooking oil	30 – 35°C	7 days	10% (v/v)	FOG removal : 88%	(Mazlin 2012)
<i>Bacillus thermoleovorans IHI-91</i>	65°C	7 hours	5 – 10% (v/v)	93 % of triolein (olive oil) was degraded	(Markossian et al. 2000)
<i>JAT (Commerical Product)</i> 15 types of bacteria that isolated from various fatty wastewater samples	20 – 25°C	4 weeks	2% (v/v)	FOG removal : 79%	(Tano-Debrah et al. 1999)
<i>Candida bombicola</i>	30 – 35°C	72 hours	5% (v/v)	FOG removal : over 90%	(Daverey & Pakshirajan 2015)
<i>Pseudomonas aeruginosa LP 602</i>	55°C	24 hours	2% (v/v)	Lipid removal : 70%	(Dharmsthiti & Kuhasuntisuk 1998)

2.2.2.3. Enzyme activity

Enzyme activity is quantity of active enzyme present under a defined condition. Enzyme activity is impacted by type of enzyme and its concentration, temperature, HRT, pH and substrate concentration (Scopes 2002). Evaluation of optimal condition for enzyme activity is critical in order to increase the efficiency. There are several methods to observe enzyme activity such as titrimetric determination, colorimetric assay, spectrophotometric determination, fluorometry, and chromatography (Stoytcheva et al. 2015). The most common method is the titrimetric determination which quantifies a yield of fatty acids produced by hydrolysis of enzyme activity.

2.3. Commercial system

Previous studies showed the feasibility of enzyme hydrolysis on FOG. In this section, full-scale systems using an enzyme for pretreatment before discharge into a sewer or drain field are discussed.

Bio-amp system is developed by NCH Waste Water (West Midlands, United Kingdoms), is used as a pretreatment before a grease trap. Each day, 32 grams of tablets are added into the growth vessel. The vessel is then filled with water and spun for 24 hours at room temperature to excrete the enzymes. Thereafter, the entire content of the vessel is discharged into the sewer system. The system has been used to pretreatment before a grease trap at restaurants (NCH Waste Water 2011). The FOG concentration of grease trap effluent was 281 mg/L. With Bio-amp, the grease trap effluent decreased to 168 mg/L (40 % reduction). The chemical oxygen

demand, total nitrogen, and total phosphorus were reduced by 39 %, 33 % and 56 %, respectively (Tang et al. 2012).

The Advanced Grease Interceptor System (AGIS) was developed by Sustainable Environmental Technologies (Mt. Morris, Michigan). The system is a reactor to generate enzymes and is installed in a 5,678 L (1,500 gal) underground modified septic tank (Figure 2) adjacent to the restaurant and uses a low energy aeration system, baffles and a one-time inoculum of a bacteria population that excretes enzymes to microbiologically breakdown FOG (Sustainable Environmental Technologies 2012). The microbial culture, BIO-FLO, is commercially produced by Bio-Systems International, in Beloit, WI. BIO-FLO is a liquid and contains multiple bacterial cultures and is commonly used for liquid odor control, in a grease trap, drain and for septic tank maintenance (Bio-systems International n.d.). The microbial population excretes extra cellular lipases that cleave long chain fatty acids.



Figure 2. AGIS installed in the septic tank (Sustainable Environmental Technologies 2012)

Figure 3 shows the interior of a pipe before AGIS was installed and 17 months after installation. The use of AGIS at restaurant helps to reduce the cost of pumping, hauling and disposing of FOG.

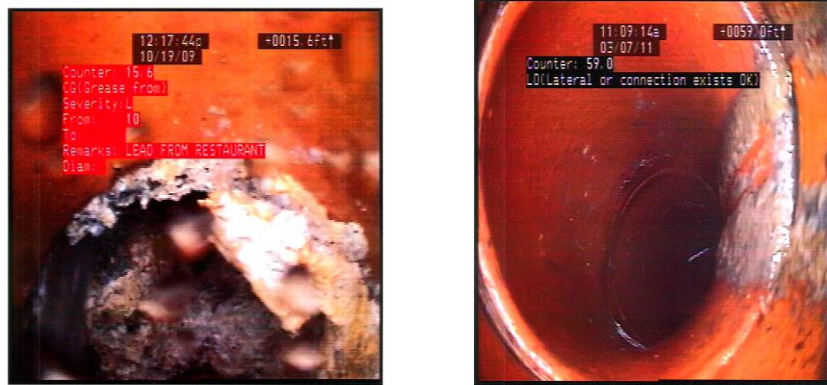


Figure 3. Pipe interior before AGIS treatment and 17 months after implementation
(Sustainable Environmental Technologies 2009)

Aerobic Bacterial Generators (ABG) was developed by SludgeHammer (Petoskey, Michigan). The system is installed in an aerobic treatment tanks and uses 0.07-kW blowers with diffuser a 130.9 m² fixed film media and a bacterial pack. ABG stimulates the growth of bacteria and removes COD, FOG, TP and TKN by 70 %, 89 %, 28 % and 30 %, respectively (Heger et al. 2010). Application of ABG was not only used for sewer but also for drain field (Sludgehammar 2015).

2.4. Operation of drain field

A typical drain field can fail due to either physical or biological clogging. Suspended solids, sludge and FOG plays a major role on physical clogging (Matejcek et al. 2000) whereas biomat plays a role in biological clogging. Physical clogging occurs when the solids and FOG adhere to the inlet pipe, distribution gravel and/or soil. Biological clogging occurs form excessive microorganism growth in the drain field due to large organic, biodegradable loading.

The life of a typical drain field is between 25 and 30 years (Mckenzie 2010). A typical type of soil in a drain field is sandy loam and the total available water capacity is between 20 – 35 % (Peacock & Christensen 2000; Northeast Region Certified Crop Advisor 2010). Once the soil reaches saturation, the porosity in the soil becomes fully filled with water (Figure 4).

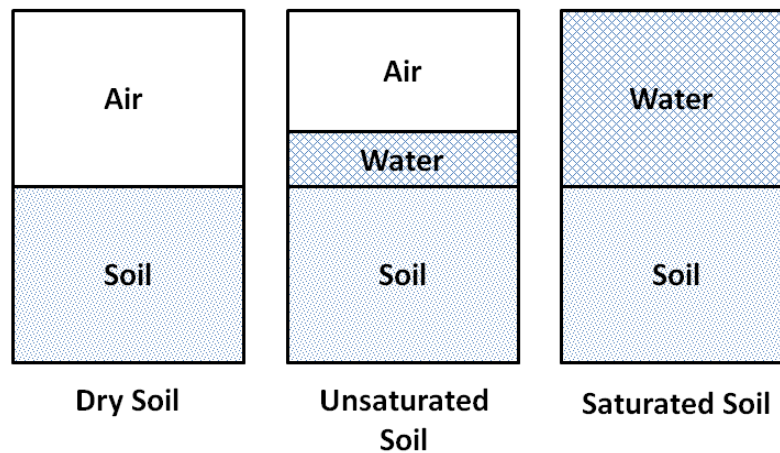


Figure 4. Soil phases from dry to saturation

Biomat formation in the soil, caused by anaerobic microorganisms, restricts the hydraulic flow and will eventually cause the soil to become saturated and the influent to overflow the drain field (NESC 2005). A typical thickness of biomat ranges from 0.7 to 2.5 cm in the soil and gravel below the perforated inlet pipe (May 1996). The biomat decreases filtration rates, reducing the removal of pathogens. High strength organic, biodegradable wastewater loading causes rapid biomat growth, resulting in clogging the soil's porosity (Heger 2013).

Typically, drain fields are placed 61 – 122 cm (2 – 4 ft) under the surface. The most abundant bacteria in the soil down to a depth of 120 cm (47 inch), including within the drain

field (Pettersson & Erland 2003), are *Archaea*, *Acidobacteria*, *Actinobacteria*, *Alphaproteobacteria*, *Bacteroidetes*, *Verrucomicrobia*, *Alphaproteobacteria*, *Etaproteobacteira*, *Gammaproteobactiera*, *Deltaproteobacteria* and *Gemmatimonadetes* (Eilers et al. 2012). Lauber st. al (2013) conducted a study for 7 months and found that the community of bacteria remained same in the temperature range of -5 °C to 28 °C, although the quantity of bacteria may increase or decrease with higher and lower temperatures, respectively.

Chapter 3: Methods

In this chapter, methods to achieve each objective are provided. Included is assessing the impact of an enzymatic fast food restaurant wastewater pretreatment on the type of FOG, the design of a bench-scale drain field and the impact of enzymatic pretreatment on the drain field.

3.1. Enzyme impact on the characteristics of triglycerides

Liquid Chromatography/Mass Spectrometry (LC/MS) combines physical separation and mass analysis and was conducted in order to observe changes in the characteristics of triglycerides impacted by enzymatic pretreatment.

Observation of the changes in the quantity of double bond in a triglyceride determines the impact of the enzyme. The greater number of double bonds, the more cohesive the FOG, resulting in greater negative impacts on the treatment system and less biodegradability.

3.1.1. Enzyme pretreatment system

Fast-food restaurant wastewater containing a high level of FOG, treated with a commercially available bacteria pretreatment system was used for this study. Specifically, the AGIS, which is a reactor to generate enzymes that was installed at fast-food restaurant in Grand Blanc, Michigan was selected for this study, because the system has been demonstrated for over 12 years and was requested to be used by sponsor of the research.

3.1.2. Sample collection and storage

Fast-food restaurant wastewaters with/without AGIS treatment were collected from the restaurant in Grand Blanc, Michigan. Samples were stored in clean glass bottles. If the analysis was delayed more than 4 hours, the samples were adjusted to a pH below 2 with 1:1 hydrochloric acid (HCl) (HACH 2015b). The sample was stored at 0 – 4 °C and analyzed within 28 days of collection.

3.1.3. Sample preparation

FOG was first extracted from the wastewater. To begin the extraction, a 1 L sample of wastewater at room temperature was required. The Xenosep Solid-Phase Extraction method was used, which is EPA approved (1664A). All aluminum dishes were washed three times with approximately 3 mL of Hexane ($\geq 95\%$), Acetone ($\geq 99.5\%$) and Methanol ($\geq 99\%$), in sequence, and pre-heated in 103°C oven.

Following multiple Xenosep Solid-Phase Extraction system procedures, the FOG became solid and was separated from the liquid fraction. Figure 5 - 7 show Xenosep Solid-Phase Extraction system setups and residues.

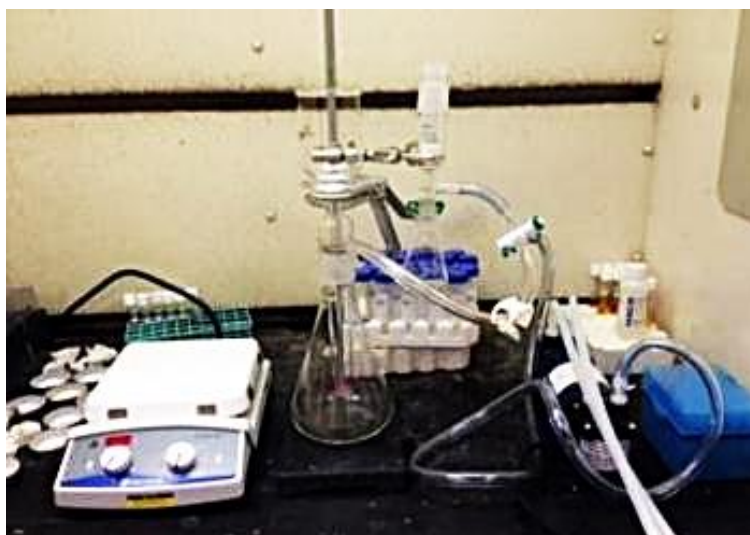


Figure 5. Xenosep Solid-Phase Extraction system setup



Figure 6. FOG extraction of kitchen wastewater treated with AGIS



Figure 7. Solid FOG extracted from samples

3.1.4. Analysis

The extracted FOG was dissolved with isobutyl alcohol ($\geq 99.9\%$) and diluted for the analysis using LC/MS. LC/MS was conducted at the Michigan State University Mass Spectrometry Facility using the Xevo G2-S QToF machine (Figure 8) with an Acquity UPLC BEH C18 1. $1.7\mu\text{m}$ - 2.1×50 mm column. MassLynx V4.1 software was used to set up a method and to analyze the samples. The method was developed with Dr. Daniel Jones from the Department of Chemistry at Michigan State University. Xevo G2-S QToF has autosampler that automatically injected the samples.



Figure 8. Xevo G2-S TOF, Mass Spectrometry Facility, Michigan State University

3.1.5. Data interpretation

Results from the LC/MS were processed using a MarkerLynx XS. Statistical analysis was conducted using an one-way ANOVA in SAS 9.4 with significance level of 0.05. The ANOVA test assumptions were that the samples are independent, normally distributed and the variances are homogenous. The assumptions were tested by normal probability plot and side-by-side box plot. The analysis allowed comparing interaction between treatments using the difference of least squares means.

3.2. Bench-scale drain field design and performance

In order to observe the impact of enzyme pretreated fast-food restaurant wastewater on a drain field, bench-scale drain fields (trenches), including soil moisture sensors embedded within the soil, were designed.

3.2.1. Trench design

All dimensions of the trenches were based on the Michigan criteria for subsurface sewage disposal (Michigan Department of Public Health 1994). Figure 9 shows the schematic of the trench. The feedstock flowed by gravity into the trench. At the bottom of the trench, the treated water exited through a water trap that did not allow air flow into the trench. A typical drain field has multiple inlet pipes, and the maximum width of each is 91 cm (36 inch). Figure 10 shows the dimension of the trenches used for this research.

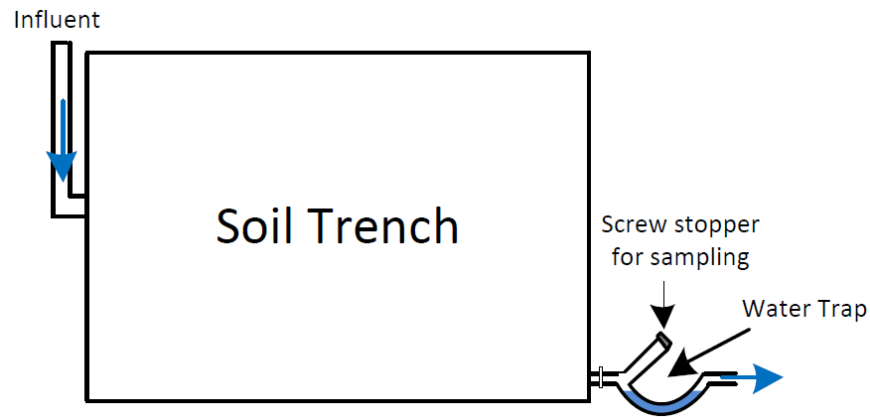


Figure 9. Schematic of trench

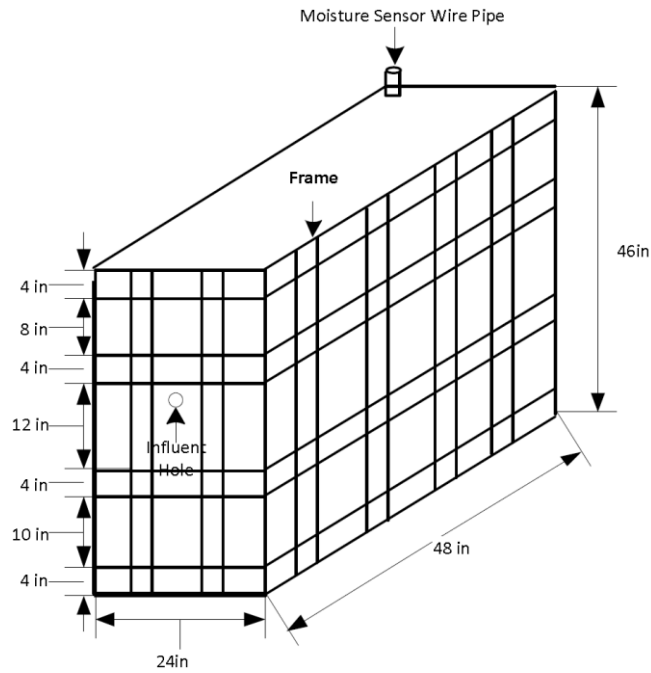


Figure 10. Dimensions of trench

The design of typical drain field is based on hydraulic loading. Equation 1 shows the total surface area required using the typical application rate for sandy loam of 10.2 L/day/m^2 ($0.25 \text{ gal/day/ft}^2$). The hydraulic loading of the bench-scale trench used for this research with a 0.74 m^2

(8 ft²) surface area was 7.57 L/day (2 gal/day). The empty bed contact time (EBCT) of each trench was 60 days. This indicates that a complete exchange of water would take approximately 40 minutes due to the soil porosity. Consequently, the trenches were operated many times this EBCT indicating that equilibrium conditions existed.

$$\text{Total Bottom Area Required} = \frac{\text{Maximum Daily Flow}}{\text{Application Rate}} \quad (\text{Equation 1})$$

The walls of each trench were made of plexiglass and corners were primed and glued. Each trench was supported with wooden frames constructed from 38 x 89 mm (1.5 x 3.5 inch) and 19 x 140 mm (0.75 x 5.5 inch) lumber. All seams were caulked and the inside of the trenches was lined with heavy-duty plastic sheeting by Blue Hawk Inc. (Mooresville, North Carolina).

Figure 11 shows the cross sectional area. The bottom was sloped to allow treated water to exit through the outlet. At the outlet, a 5.08 cm (2 inch) diameter bulkhead were mounted and connected to a water trap.

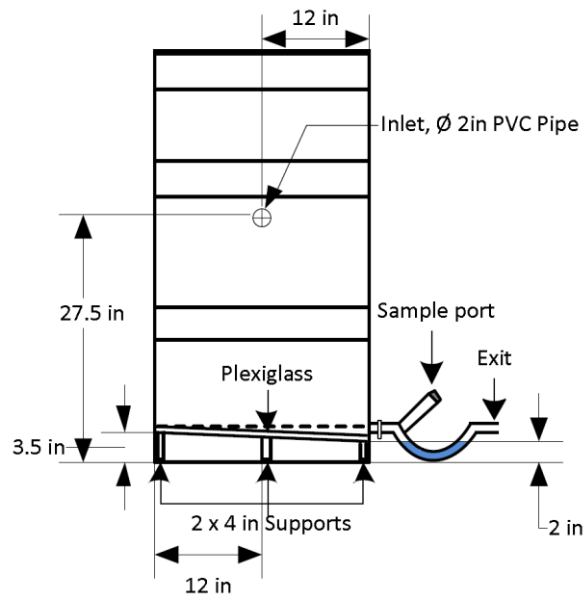


Figure 11. Front view of trench

Figure 12 shows the soil's profile, moisture sensor locations, inlet and outlet. Two barriers with a height, width, and length of 5.1 cm, 10.1 cm and 61 cm (2 x 4 x 24 inch), respectively were mounted on the bottom of the trench in order to capture the exiting water along the distance from the inlet. The depth of the sandy loam that served as the treatment media was 60.9 cm (2 ft). On the top of sandy loam, 15.2 cm (6 inch) depth of gravel was placed then installed inlet pipe with covering 7.6 cm (3 inch) depth of gravel. The top of the trench was covered with 22.9 cm (9 inch) depth of top soil, before wastewater entered, simulating a typical drain field. Sandy loams, top soils, gravels and 6A stones were ordered from Carl Schlegel, Inc. in Lansing, Michigan.

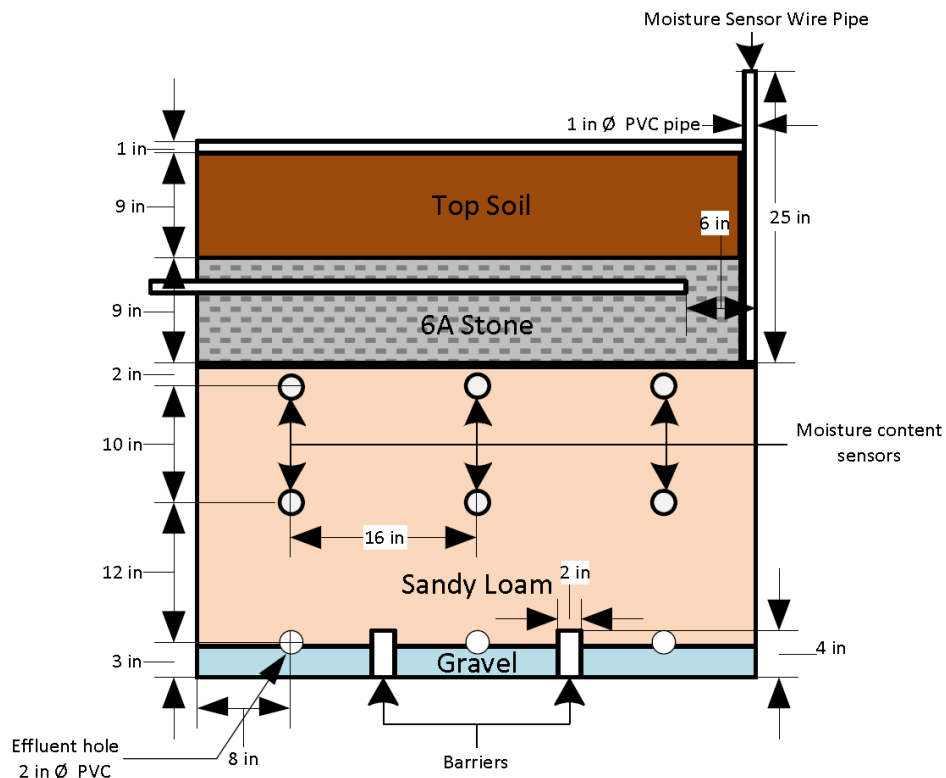


Figure 12. Profile view of trench

Six CS616 soil moisture sensors manufactured by Campbell Scientific were placed at two depths at 3 locations along the length of the trench. All soil moisture sensor wires exited through a 2.5 cm (1 inch) PVC pipe were inserted at the top of the trench and were connected to a CR1000 data logger, manufactured by Campbell Scientific. Three temperature sensors were placed in each between two different depths of sensors. The data was downloaded to a dedicated computer. Figure 13 shows the top view of the soil trench including the CS616 soil moisture sensors, barriers and water traps.

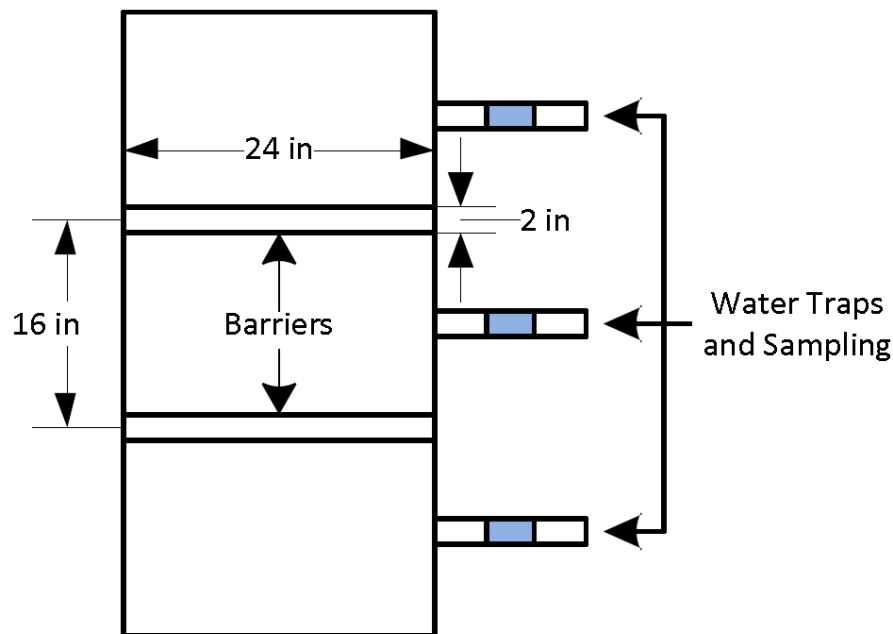


Figure 13. Top view of trench

Figure 14 show the pictures of constructing a bench-scale drain field (trenches). Additional pictures of constructed bench-scale trenches are presented in Appendix A.



Figure 14. Overview of trench construction

3.2.2. Instrumentation

In order to monitor in situ clogging, a CR 1000, AM 16/32 and CS 616 Water Content Reflectometers, manufactured by Campbell Scientific, were used.

The CS 616 water content reflectometer (Figure 15) measures the volumetric water content from 0 % to saturation in a soil using two 30 cm stainless steel rods. The variability between probes is ± 0.5 % volumetric moisture content in dry soil and ± 1.5 % volumetric moisture content in typical saturated soil (Campbell Scientific 2014).

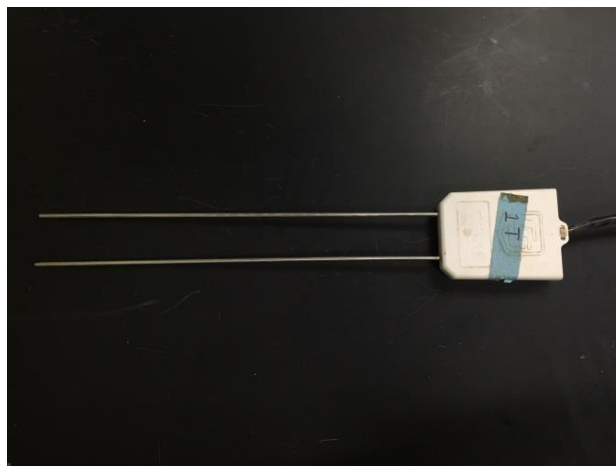


Figure 15. CS 616 Water Content Reflectometer

Because more than 8 of CS 616 sensors were required, a AM 16/32 multiplexer was needed in order to connect CS 616 sensors to datalogger. Figure 16 shows installation of 30 of CS 616 sensors connected to AM 16/32 multiplexer and CR 1000 datalogger.

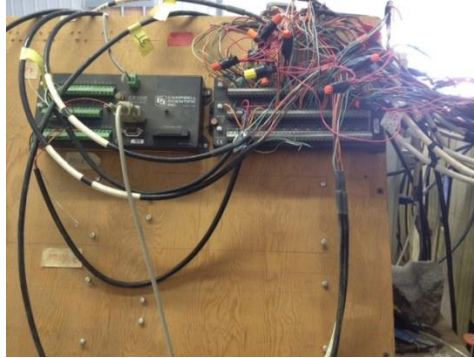


Figure 16. Installation of 30 of CS 616 Water content reflectometer connected with AM 16/32 multiplexer and CR 1000 datalogger

The Temperatures of trenches were monitored using type K thermocouples with PVC-insulated probes and epoxy coated tip by Digi-Sense (Figure 17). The type K thermocouples measure from -250 °F to 221 °F with accuracy of ± 3.0 °F (Cole-Parmer 2015).



Figure 17. Type K thermocouple with PVC-insulated probe and epoxy-coated tip

3.2.3. Analytical

The analysis of trench influent and effluent wastewater parameters (Table 3) shows the validity of simulation and the impact of different characteristics of wastewaters on the drain field. All analyses were conducted with HACH analytical method and following proper Quality Assurance Quality Control (QA/QC) protocols, which is presented in Appendix C.

Table 3. Wastewater analytical parameters

Parameter	HACH Method	U.S. EPA Method	Reference
Chemical oxygen demand (COD)	8000	40 CFR 136.3.	(HACH 2015a)
Biological oxygen demand (BOD ₅)	8043	SM 5210 B	
Fats, Oil and Grease (FOG)	10300	EPA 1664A	
Total phosphate (TP)	8190	EPA 365.1, 365.3	
Total nitrogen (TN)	10208	N/A	
Ammonia	10205	EPA 350.1, 351.1, 351.2	
Nitrate	10206	40 FR 141	

3.2.4. Data interpretation

Nutrient removal caused by the bench-scale trench was compared to the performance of a typical drain field using one-way ANOVA in SAS 9.4 with significance level of 0.05.

Assumptions of the statistical analysis are samples are independent, normally distributed and variances are homogeneous. The assumption was tested by normal probability plot and side-by-side box plot. This demonstrated that the trenches represented a field-scale installation. Direct comparisons of each parameter between two trenches were also compared to determine if enzyme pretreatment was impacting results. The comparison was conducted between Sanitary

trench and rest trenches, Sanitary/kitchen and Sanitary/treated kitchen and Kitchen and Treated kitchen.

3.3. Impact of wastewaters with/without enzyme pretreatment on clogging

Feedstocks were prepared to simulate different conditions, as listed below. Each trench received only one of the following feedstocks.

- *Sanitary wastewater (Sanitary)*

Served as a control that does not cause premature aging of the drain field.

- *Sanitary wastewater mixed with kitchen wastewater without AGIS pretreatment (Sanitary/kitchen)*

Simulates condition for a typical restaurant. Assumed to clog a drain field causing premature of aging.

- *Sanitary wastewater mixed with kitchen wastewater treated with AGIS pretreatment (Sanitary/treated kitchen)*

Test condition.

- *Kitchen wastewater without AGIS pretreatment (Kitchen)*

Extreme condition that is expected to rapidly, physically clog the drain field.

- *Kitchen wastewater treated with AGIS pretreatment (Treated kitchen)*

Extreme condition that is expected to rapidly, biologically clog the drain field.

The sanitary wastewater collection was from a small cluster wastewater treatment system serving 25 homes in Dimondale, Michigan. Kitchen wastewater treated with AGIS pretreatment was collected from a restaurant in Grand Blanc, Michigan with an AGIS that has been operating for over 12 years and discharging to a municipal sewer. To collect kitchen wastewater without AGIS pretreatment, the wastewater had to be mixed from the same restaurant as where the AGIS pretreated kitchen wastewater was collected. This was necessary because there was no direct sample location plus a representative sample over a 24 hour period would have been difficult to obtain. Figure 18 shows the flow diagram from kitchen to AGIS at the sampling site.



Figure 18. Flow diagram of brown grease at a fast-food restaurant

Table 4 shows the composition of the Kitchen wastewater.

Table 4. Feedstock constituents for Kitchen wastewater

Samples	Discharge	Frequency	Total discharge
Washing dishes	37.9 L/discharge (10 gal/discharge)	10 (discharge/day)	378.5 L/discharge (100 gal/discharge)
Rinsing	37.9 L/discharge (10 gal/discharge)	10 (discharge/day)	378.5 L/discharge (100 gal/discharge)
Sanitizing	37.9 L/discharge (10 gal/discharge)	10 (discharge/day)	378.5 L/discharge (100 gal/discharge)
Mopping floor	22.7 L/discharge (10 gal/discharge)	3 (discharge/day)	68.1 L/discharge (18 gal/discharge)
Grill grease	37.9 L/discharge (10 gal/discharge)	1 (discharge/day)	37.9 L/discharge (10 gal/discharge)
Washing rags	151.4 L/discharge (40 gal/discharge)	1 (discharge/day)	151.4 L/discharge (40 gal/discharge)
Total			1,393.0 L/discharge (368 gal/discharge)

The discharge was calculated by measuring the volume of the sinks or tub of units at the restaurant. The frequency of discharge per day was based on a conversation with the manager at

the restaurant. By multiplying these two values, the total amount of discharge was calculated.

Table 5 shows the total wastewater from kitchen and sanitary discharges per day at the restaurant.

Table 5. Total kitchen and sanitary wastewater discharges per day

Samples	Total discharge per day	Comment
Kitchen wastewater	1393 L/day (368 gal/day)	Refer to Table 4
Sanitary wastewater	5564 L/day (1470 gal/day)	35 gpd/seat* 42 seats = 1470 gpd (Metcalf & Eddy 2003)

Table 6 shows the mixture for feedstock in 18.9L (5 gal) carboy.

Table 6. Mixture for feedstock in 18.9L (5 gal) carboy

Samples	Sanitary	Kitchen
Sanitary	18.9 L (5 gal)	0 L (0 gal)
Sanitary/kitchen	3.78 L (1 gal)	15.1 L (4 gal)
Sanitary/treated kitchen	3.78 L (1 gal)	15.1 L (4 gal)
Kitchen	0 L (0 gal)	18.9 L (5 gal)
Treated kitchen	0 L (0 gal)	18.9 L (5 gal)

3.3.1. Sample collection and storage

To save time and cost, enough restaurant samples were collected to operate trenches for two weeks. Treated kitchen wastewater was collected from the AGIS tank using a sump pump. Kitchen wastewater was required the collection of samples from each source in the kitchen as shown in Figure 18. Sanitary wastewater was collected from the centralized septic tank where

the wastewaters from all of the homes were combined before being pumped to the advanced treatment unit. Because this wastewater was biologically active, it was stored at 0 – 4 °C. Before each trip to the restaurant and sanitary system, all carboys were rinsed with water.

3.3.2. Operation

Influent was fed three times every day at approximately 9:30 AM, 1:30 PM, and 8:30 PM to simulate the cleaning schedule at a typical fast -food restaurant. Figure 19 shows the details of the influent line. Note that the holes were opened downward as is in a typical drain field. Feedstocks were pumped into the inlet pipe using a Masterflex pump. Temperature was measured three times a day.

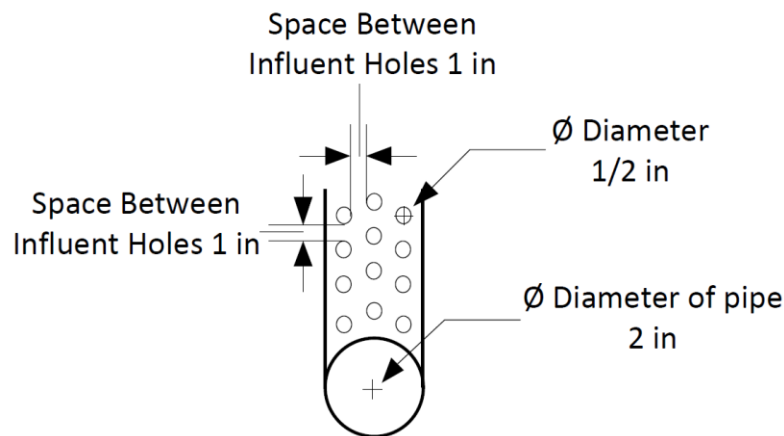


Figure 19. Detail view of influent pipe

The length of the operation was 505 days for all trenches except Kitchen (362 days). The trench receiving Kitchen operation was less, because of a change in experimental plan, due to taking one modified AGIS system offline. The research operated with 4 different phases, as described in Table 7.

Table 7. Operations by phases

Phase	Operation	Log
Phase 1	11/03/13 - 11/19/13 (17 days)	<ul style="list-style-type: none"> • Mixture ratio for Sanitary/kitchen and Sanitary/treated kitchen: <ul style="list-style-type: none"> - Sanitary: 1.5 L/day (0.4 gal/day) - Kitchen: 6.1 L/day (1.6 gal/day) • Influent and effluent parameters tested weekly: COD, FOG, TP and Nitrate
Phase 2	11/20/13 – 02/17/14 (90 days)	<ul style="list-style-type: none"> • Mixture ratio for Sanitary/kitchen and Sanitary/treated kitchen (based on Matcalf and eddy): <ul style="list-style-type: none"> - Sanitary: 6.1 L/day (1.6 gal/day) - Kitchen: 1.5 L/day (0.4 gal/day) • Operation stopped due to heavy ice storm, holidays and maintenance. <ul style="list-style-type: none"> - 12/20/14 - 12/27/14 - 01/26/14 - 01/29/14 - 03/29/14 - 03/30/14 • Influent and effluent parameters tested weekly: COD, FOG, TP and Nitrate • Influent and effluent parameters were tested once: TN
Phase 3	02/18/14 – 06/22/14 (125 day)	<ul style="list-style-type: none"> • Mixture ratio for Sanitary/kitchen and Sanitary/treated kitchen (based on COD result in phase 2): <ul style="list-style-type: none"> - Sanitize: 227.1 L/day (60 gal/day) - Washing Machine: 302.8 L/day (80 gal/day) • The trench receiving Kitchen wastewater began on 03/25/14. • Influent and effluent parameters tested weekly: COD, FOG, TP, TN, Ammonia and Nitrate • Influent and effluent parameters were tested once: BOD₅
Phase 4	06/23/14 – 03/22/15 (273 days)	<ul style="list-style-type: none"> • Mixture ratio for Sanitary/kitchen and Sanitary/treated kitchen (based on COD result in phase 3): <ul style="list-style-type: none"> - Mop Water: 147.6 L/day (39 gal/day) - Washing Machine: 283.9 L/day (75 gal/day) • Influent and effluent parameters tested bi-weekly: COD, BOD₅, TP, TN, Ammonia and Nitrate • Influent and effluent parameters were tested discontinued on 07/14/14: FOG

3.3.3. Analysis

To provide an indication if clogging was occurring, readings from the soil, moisture sensors were monitored. Observation of moisture content in each trench was conducted with CR 1000 datalogger, AM16/32 and CS 616 sensors. Measurements were recorded automatically by CR 1000 datalogger. Downloading data from CR 1000 datalogger was required monthly because the datalogger has a limit of memory.

Soil analysis was conducted on two different days of operation. First soil samplings were collected on the 207th day of operation by using a 1.9 cm (3/4 inch) diameter soil sampler (Figure 18). This small diameter soil sampler was used to minimize disruption to the system because the operation was still on-going.

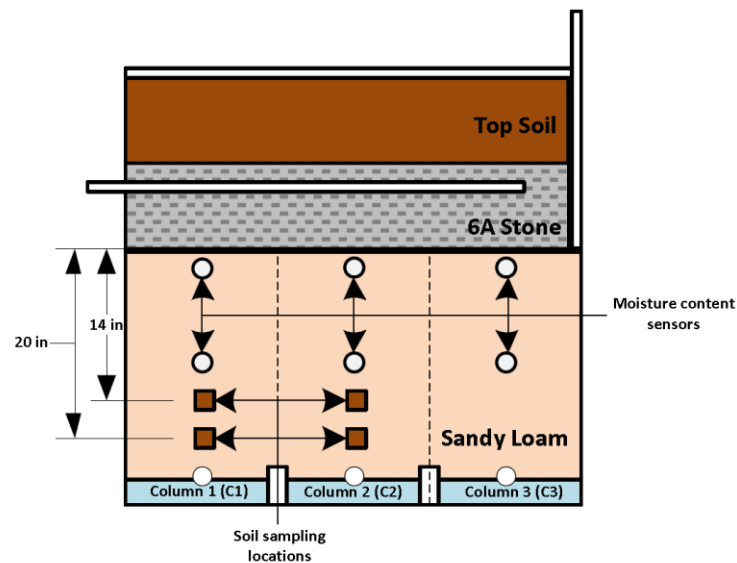


Figure 20. Diagram of soil sampling locations for 1st VS analysis, brown boxes indicate soil sample collection locations.

Collected soil samples were transported in plastic sealed bottles with proper label. Volatile Solids (VS) analysis was conducted following EPA 1684 method. Samples were stored at 4 °C to minimize microbiological decomposition of solids and were not to be held for more than 24 hours. Before the analysis, soil was set at room temperature. VS analysis is an economical method to compare the microorganism populations between different soil treatments.

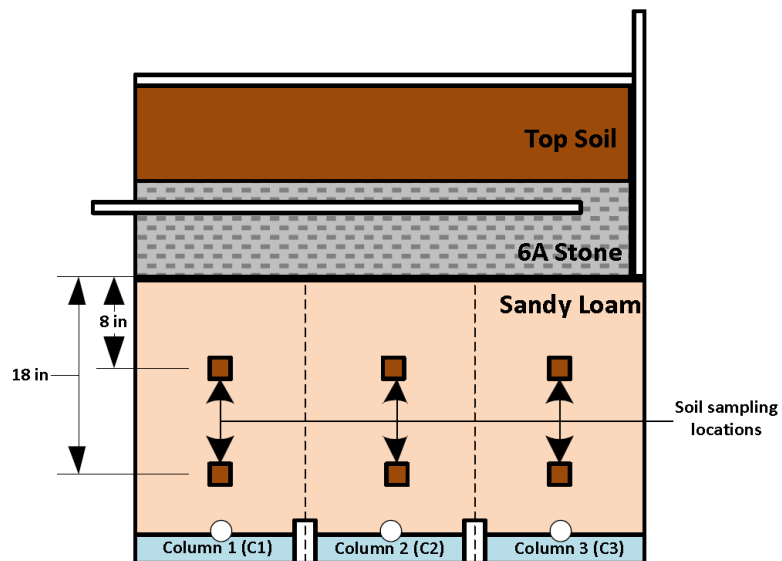


Figure 21. Diagram of soil sampling locations for 2nd VS analysis, brown boxes indicate soil sample collection locations.

The second soil sampling was conducted on the day after the last day of operation. Soil sampling locations were as shown in Figure 19. Soil sampling was completed by scooping soils out of trench and stored in sealed zip-lock bags with proper label and the VS was measured.

3.3.4. Data interpretation

The CR1000 datalogger automatically downloaded readings from the moisture sensors as a comma separated variable file. This data was transferred to a spreadsheet bi-weekly, due to the memory limits in the datalogger and to prevent loss of data in case of a malfunction. The file was opened in Microsoft Excel and plotted using a scatter graph in order to compare different locations of sensors for each trench. A statistical analysis of this data was not possible because there were not replicate trenches for each condition because of budget constraints.

A statistical analysis of the VS level in the soil result was conducted using an one-way ANOVA in SAS 9.4 with significance level of 0.05. Assumptions of the statistical analysis are samples are independent, normally distributed and variances are homogeneous. The assumption was tested by normal probability plot and side-by-side box plot. Difference of least squares means allowed comparing the interactions. The comparison was conducted between the Sanitary trench and rest trenches, Sanitary/kitchen and Sanitary/treated kitchen and Kitchen and Treated kitchen.

Chapter 4: Result and Discussion

Result and discussion for each objective are presented in this chapter. Included are sections on the enzyme impact on the characteristics of triglycerides, bench-scale drain field design and the impact of wastewaters with and without enzyme pretreatment on potential clogging.

4.1. Enzyme impact the characteristics of triglycerides

The LC/MS analysis was compared between Kitchen wastewater and Treated kitchen wastewater in order to observe the impact on the triglyceride. Three replications were conducted for each sample. Figure 22 and Figure 23 show the relative proportions of double bonds in triglycerides of Kitchen wastewater and Treated kitchen wastewater.

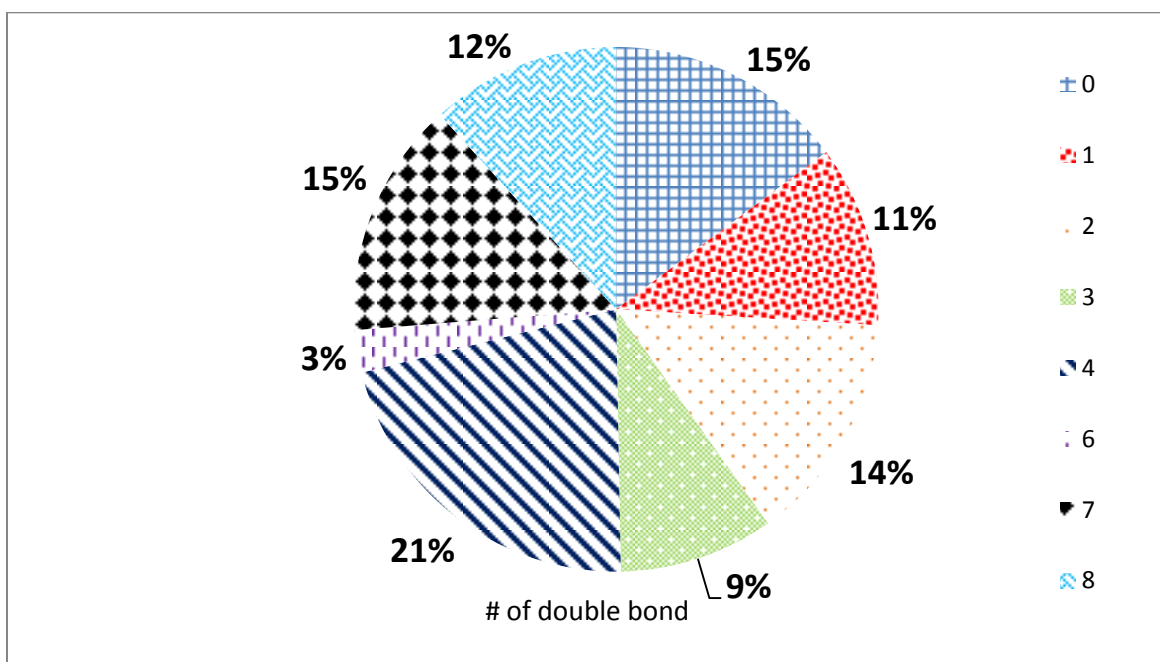


Figure 22. Relative proportion of double bonds in triglycerides of Kitchen wastewater. The values are the total number of double bonds that were found in the triglycerides.

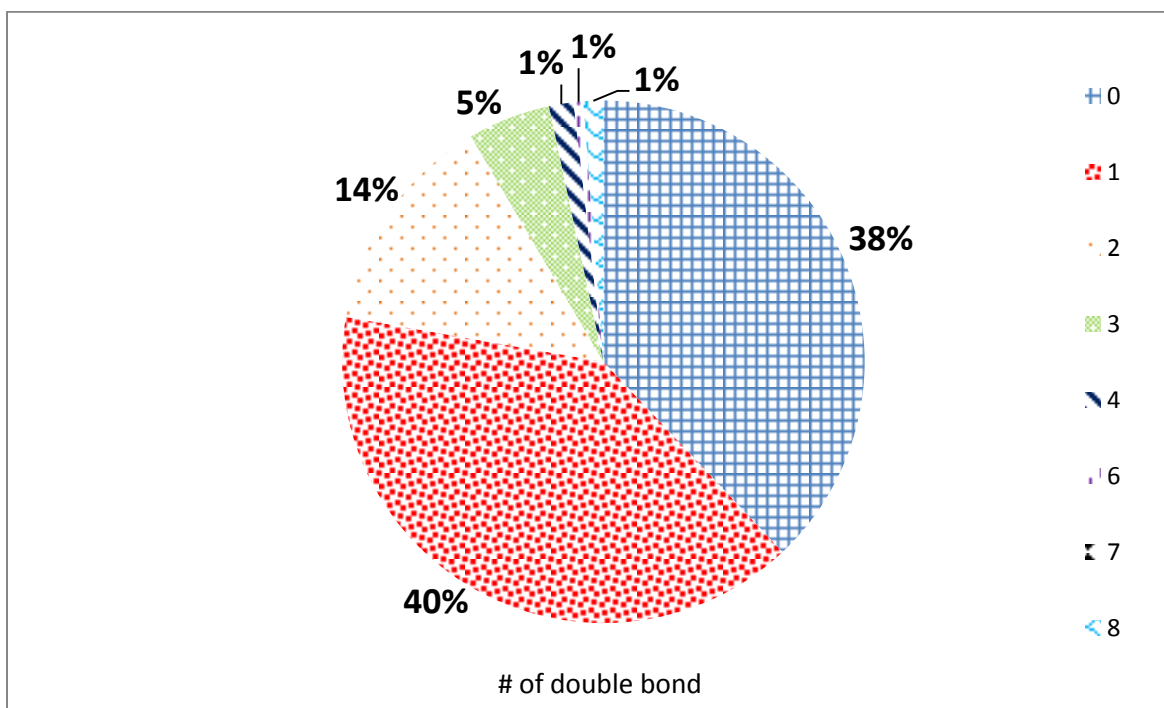


Figure 23. Relative proportion of double bonds in triglycerides of Treated kitchen wastewater, The value are the total number of double bonds that were found in the triglycerides.

Results show that the relative proportion of single bond and one double bond in triglycerides of Treated kitchen wastewater were significantly increased from 15 to 38 % and from 11 to 40 %, respectively (ANOVA, $P\text{-value} < 0.001$, $\alpha = 0.05$). Result displayed that greater than four double bonds in triglycerides of Kitchen wastewater were significantly reduced from 60 % to 8 % by the enzyme treatment (ANOVA, $P\text{-value} < 0.001$, $\alpha = 0.05$). Therefore, result show that enzyme treatment impacted on characteristics of triglyceride in fast-food restaurant wastewater.

4.2. Bench-scale drain field design and performance

Bench-scale trenches were operated for 505 days and influent and effluent concentrations of COD, BOD₅, FOG, TP, TN, ammonia and nitrate were monitored. Removal of these constituents in the trench receiving Sanitary wastewater was compared to a typical field drain field (Hossain 2010). Additionally, removals of these constituents for the other feedstocks are also presented in this Chapter to gauge the effectiveness of the trenches for the variety of wastewaters. Results are discussed in sequential phases as each had different conditions, as described in Chapter 3.

4.2.1. Phase 1

In Phase 1, influent concentrations of COD, TP, FOG and nitrate for each trench were analyzed (Table 8). Effluent concentration of the parameters were not tested as this phase was designed simply to determine if the sanitary wastewater matched the characteristics reported in the literature and to compare the non-treated and treated kitchen wastewater. Also during this Phase, there was not a trench being operating with Kitchen waste only (kitchen waste not treated with AGIS). This resulted because resources only allowed for the construction of 5 trenches and one was being used for a variation of the AGIS process, as requested by the sponsor that proved to be ineffective (data not presented). Consequently, a comparison with the Treated kitchen wastewater was not possible.

Table 8. Parameter analysis of influent concentration of each trench in Phase 1

Analysis	Sanitary	Sanitary/ kitchen	Sanitary/ treated kitchen	Kitchen	Treated kitchen
	Influent	Influent	Influent	Influent	Influent
COD (mg/L)	85.0	577.0	833.0	2315.0	2659.0
FOG (mg/L)	19.7	165.3	109.2	431.2	391.6
TP (mg/L)	53.2	20.1	19.4	17.6	19.0
Nitrate (mg/L-N)	4.3	2.7	2.5	2.2	1.2

The influent concentrations of COD from Sanitary/kitchen and Sanitary/treated kitchen were approximately 7 and 10 times higher than Sanitary, respectively. The influent concentration of COD in the Treated kitchen trench was approximately 31 times higher than Sanitary. The influent concentration of TP and nitrates for all trenches were consistent. The COD, TP, FOG and nitrate influent concentrations of Sanitary wastewater were found to be similar to the drain field influent for a typical sanitary wastewater. These concentrations are 120 – 150 mg/L, 15 mg/L, 10 – 30 mg/L, and < 1 mg/L for COD, FOG, TP and nitrate, respectively (Metcalf & Eddy 2003; Hammerlund & Glotfelty 2008; Cui et al. 2003).

4.2.2. Phase 2

Based on Phase 1 results, the formulations of the feedstocks with kitchen wastewater were changed to better match field conditions. These changes included altering the mixture ratio for Sanitary/kitchen and Sanitary/treated kitchen to 6.1 L/day (1.6 gal/day) of sanitary

wastewater and 1.5 L/day (0.4 gal/day) of kitchen wastewater, based on Metcalf and Eddy (Metcalf & Eddy 2003). Figures 24 – 28 show the influent and effluent values of COD, FOG, TP, TN and nitrate for each trench during this Phase. As in Phase 1, there was not a trench being operating with Kitchen waste only. The error bars represent the standard deviation.

Figure 24 shows the average of influent and effluent concentration of COD for each trench in Phase 2.

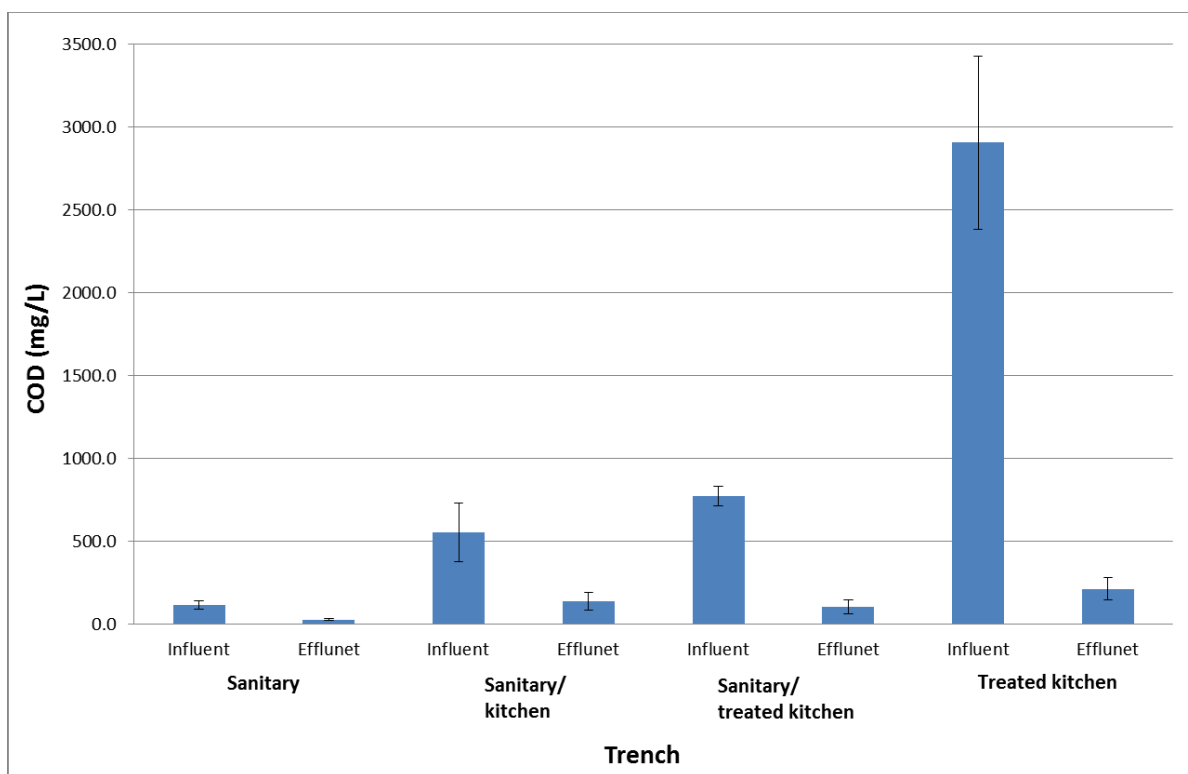


Figure 24. Average influent and effluent concentrations of COD in Phase 2

Even though there was a wide range of influent COD concentration, from 100 to 3,000 mg/L, all bench-scale trenches removed significant amounts of COD. More than 80 % of the mass was removed by the trenches, even though the effluent levels for the each feedstock

containing kitchen wastewater were found to be statistically different. The COD Influent concentration of the trench receiving Sanitary wastewater was 118 mg/L, which is close to typical drain field COD influent concentration of 120 – 150 mg/L (Cui et al. 2003). However, data on the typical COD in the effluent was not found in the literature.

Figure 25 shows the average of influent and effluent concentration of FOG for each trench in Phase 2.

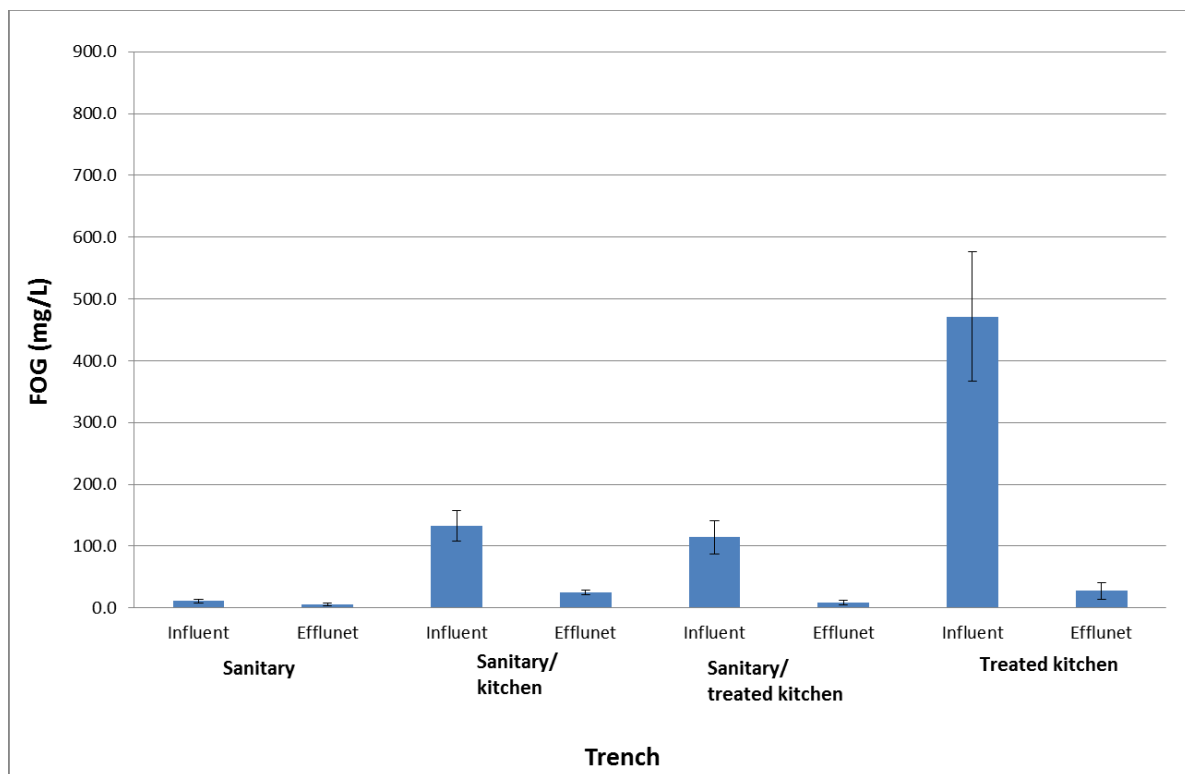


Figure 25. Average influent and effluent concentrations of FOG in Phase 2

Similar to COD result, even though there was a wide range of influent FOG concentrations, from 10 to 471 mg/L, bench-scale trenches reduced FOG concentration significantly. The FOG influent concentration of the trench receiving Sanitary wastewater is 10.9

mg/L, which is close to typical drain field FOG influent concentration of 15 mg/L (Hammerlund & Glotfelty 2008). Removal rate of the trench receiving Sanitary/treated kitchen wastewater statistically higher than the trench receiving Sanitary/kitchen (ANOVA, P-value=0.0007, $\alpha=0.05$). This is hypothesized based on Sanitary/treated kitchen wastewater becoming more biodegradable after enzyme treatment, resulting in better FOG removal by a drain field.

Figure 26 shows average of influent and effluent concentrations of TP for each trench in Phase 2.

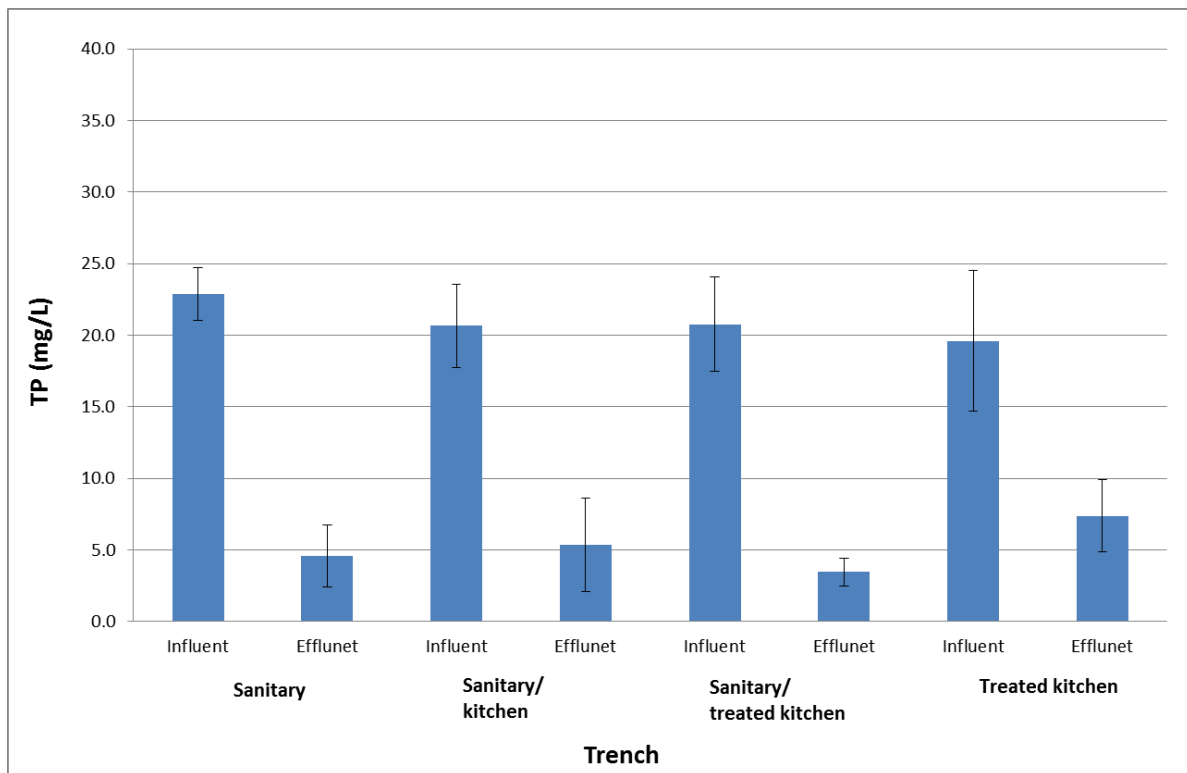


Figure 26. Average influent and effluent concentrations of TP in Phase 2

Unlike COD and FOG results, all TP influent concentrations were statistically consistent (ANOVA, P-value=0.1379, $\alpha=0.05$) and all bench-scale trenches reduced TP concentration significantly. However, the Treated kitchen trench did not remove the phosphorus down to the

same levels as the others (ANOVA, P-value=0.0007, $\alpha=0.05$) for unknown reasons. The TP influent and effluent concentration of the trench receiving Sanitary wastewater are 22.9 mg/L and 4.6 mg/L, which is close to typical drain field TP influent and effluent concentration of 10 – 30 mg/L and 4.9 mg/L, respectively (Hossain 2010; Metcalf & Eddy 2003).

Figure 27 shows influent and effluent concentrations of TN for each trench in Phase 2.

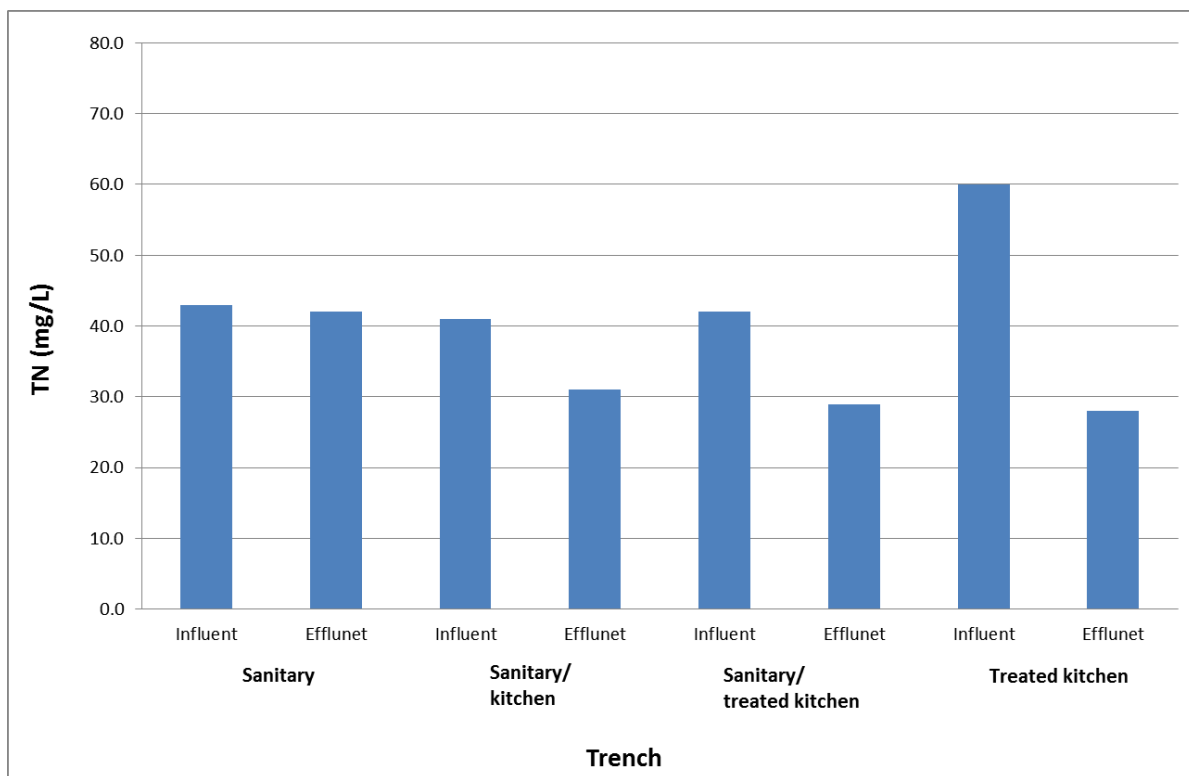


Figure 27. Average influent and effluent concentrations of TN in Phase 2

Result shows all TN concentration of influent were consistent, except treated kitchen. All trenches except Treated kitchen did not removed TN significantly. Statistical analysis including average and standard deviation could not be calculated because TN analysis was conducted only once at the end of Phase 2. The TN influent and effluent concentration of the trench receiving

Sanitary wastewater were 43 mg/L and 42 mg/L, respectively, which is close to typical drain field TN influent and effluent concentrations of 25 - 60 mg/L and 48 mg/L, respectively (Hossain 2010; Metcalf & Eddy 2003).

Figure 28 shows average of influent and effluent concentration of nitrate for each trench in Phase 2.

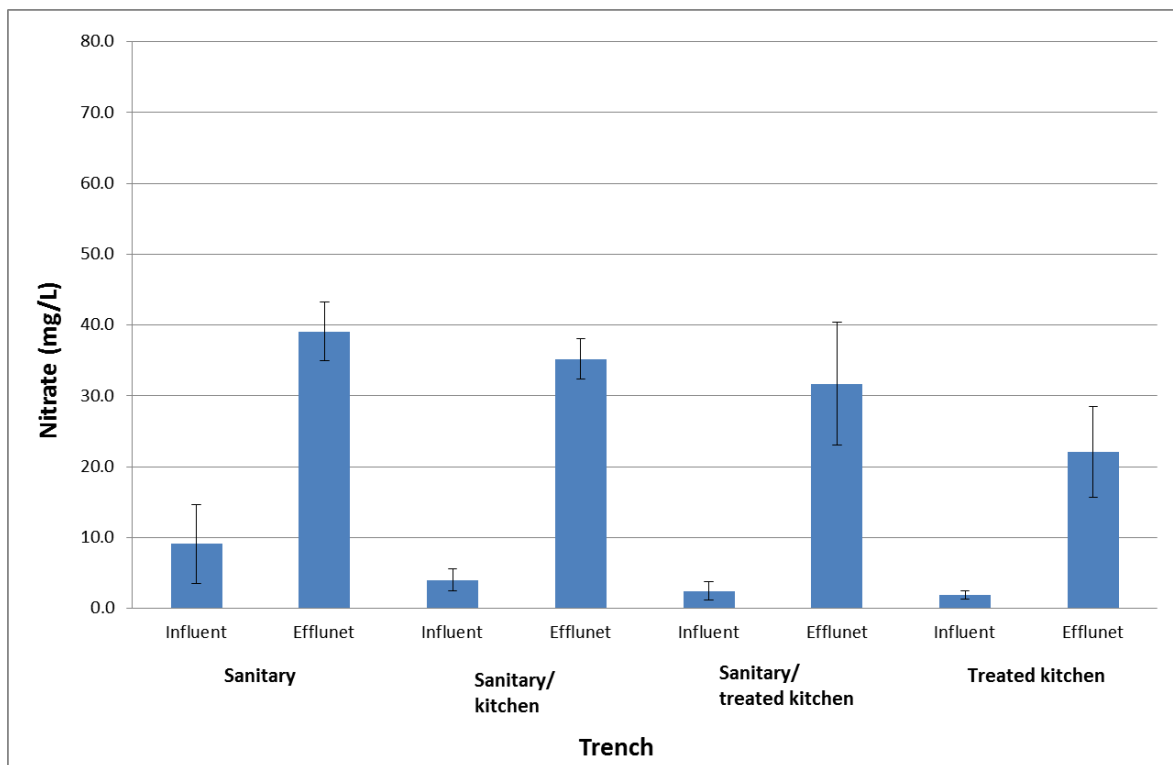


Figure 28. Average influent and effluent concentrations of nitrate in Phase 2

Result shows that all nitrate concentrations increased after treated with the bench-scale trenches. Combined with the relatively consistent influent and effluent TN values for each trench, this indicates that denitrification did not occur. The Nitrate influent and effluent concentrations of the trench receiving Sanitary wastewater were 9.1 mg/L and 39.1 mg/L,

respectively, which is close to typical drain field influent and effluent concentrations of < 1 mg/L and 42 mg/L, respectively (Hossain 2010; Metcalf & Eddy 2003).

4.2.3. Phase 3

In Phase 3, the composition of the non-AGIS treated kitchen wastewater was changed so that it better correspond to AGIS treated kitchen wastewater. As a result, 227.1 L/day (60 gal/day) for Sanitary and 302.8 L/day (80 gal/day) for washing machine wastewater, based on COD result in Phase 2. Also, the previously discussed fifth trench that was receiving feedstock from the modified AGIS system was switched to a feedstock consisting of only not-treated kitchen wastewater (Kitchen). This was hypothesized to serve as the most extreme condition that would likely quickly physically clog the trench. The error bars represent the standard deviation.

Figures 29 – 35 show influent and effluent concentrations of COD, BOD₅, FOG, TP, TN, ammonia and nitrate for each trench in Phase 3.

Figure 29 shows average of influent and effluent concentration of COD for each trench in Phase 3.

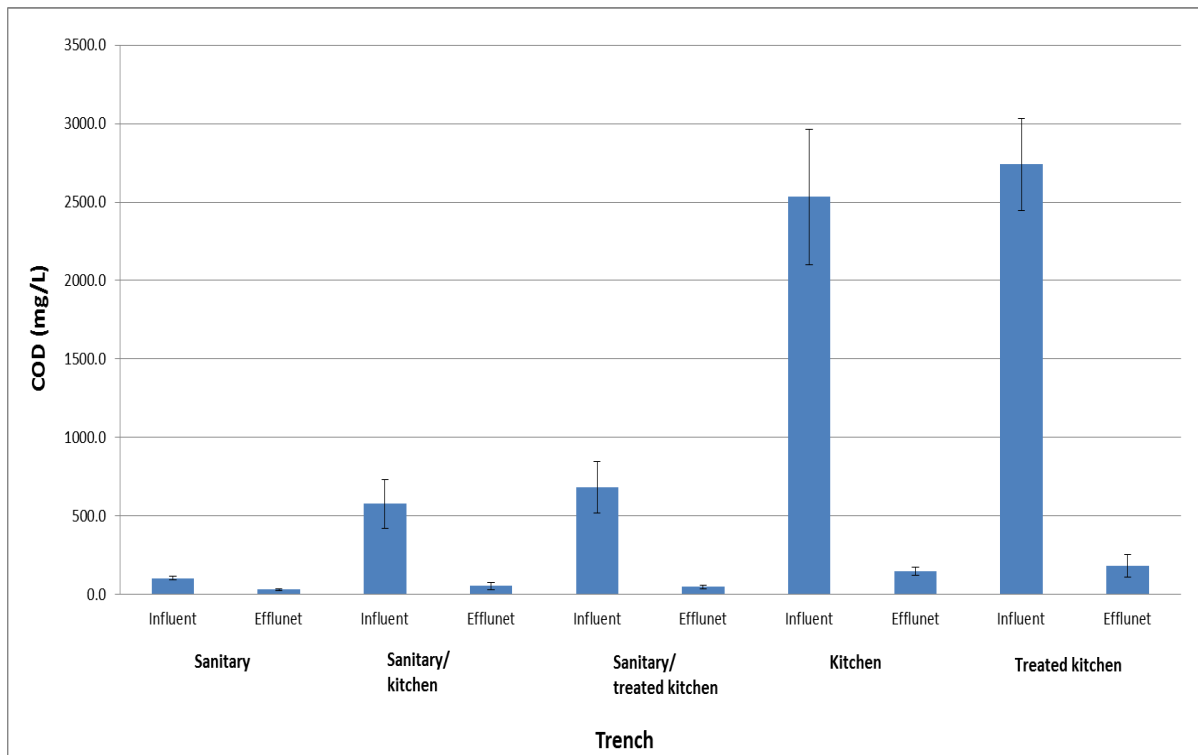


Figure 29. Average influent and effluent concentrations of COD in Phase 3

The influent COD for the Sanitary/kitchen and the Sanitary/treated kitchen were statistically the same with the new formulation (ANOVA, P-value=0.1178, $\alpha=0.05$). Similar to Phase 2, all bench-scale trenches removed significant amounts of COD. The Sanitary trench continued to perform as expected.

Figure 30 shows average of influent and effluent concentration of BOD₅ for each trench in Phase 3.

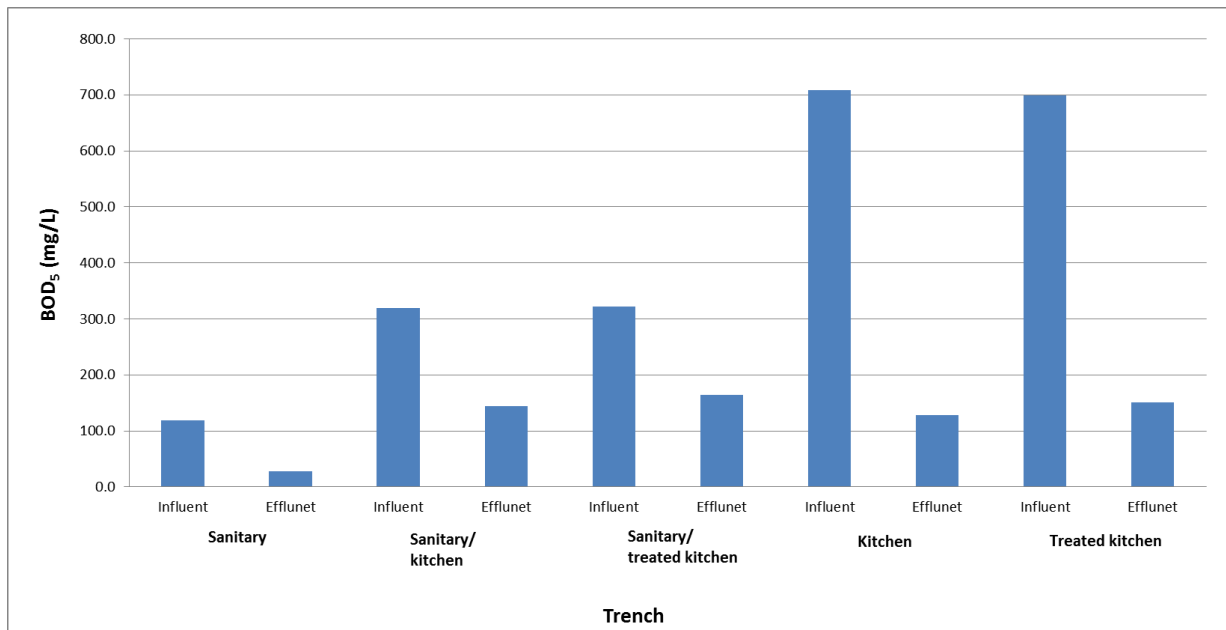


Figure 30. Average of influent and effluent concentration of BOD₅ in Phase 3

Even though there was a wide range of influent BOD₅ concentrations from 100 to 700 mg/L, all bench-scale trenches removed significant amount of as was also found for the COD. Statistical analysis, including average and standard deviation, could not be calculated because the BOD₅ analysis was conducted only once at the end of Phase 3. The BOD₅ influent and effluent concentration of the trench receiving Sanitary wastewater were 119 mg/L and 28 mg/L, respectively, which is fairly close to typical drain field BOD₅ influent and effluent concentration of 140 – 200 mg/L and 1.2 mg/L, respectively (Hossain 2010; Metcalf & Eddy 2003).

Figure 31 shows average of influent and effluent concentration of FOG for each trench in Phase 3.

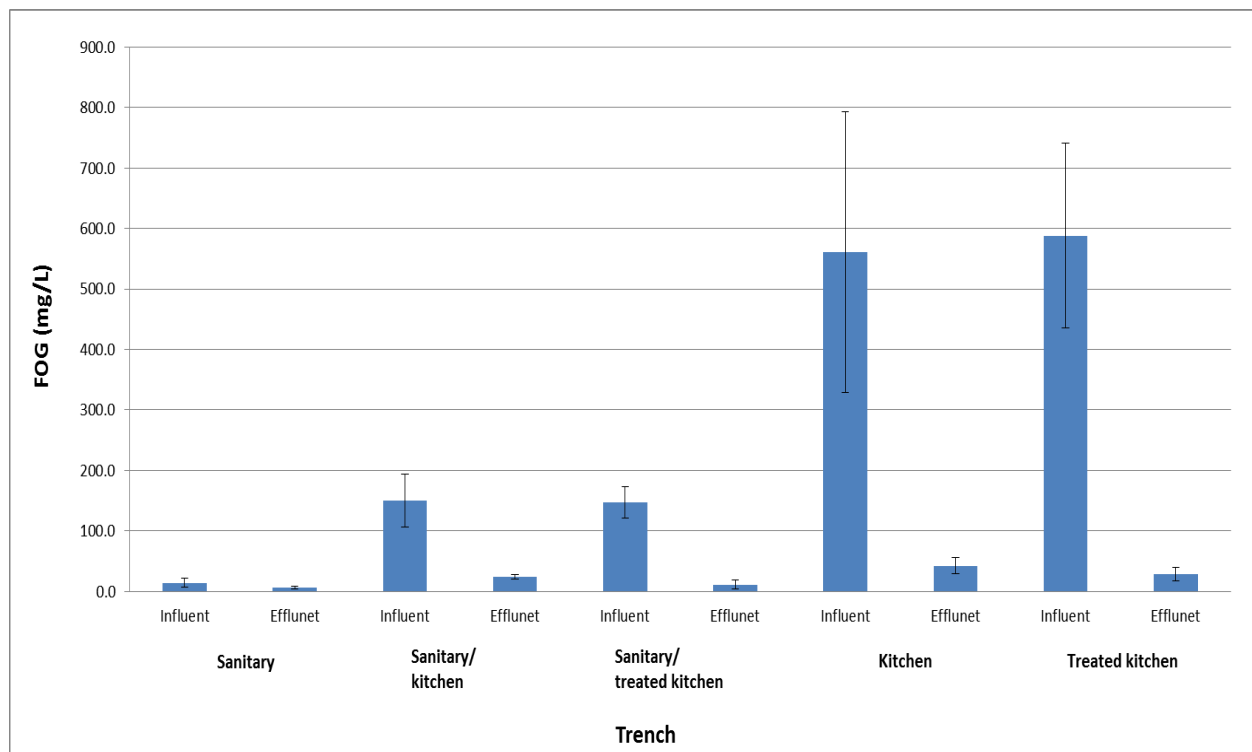


Figure 31. Average influent and effluent concentrations of FOG in Phase 3

Similar results to that in Phase 2 were found in that all bench-scale trenches reduced FOG concentration significantly. The Sanitary trench continued to perform as expected. The removal rate of the trench receiving Sanitary/treated kitchen wastewater was statistically lower than the trench receiving Sanitary/kitchen wastewater (ANOVA, $P\text{-value} < 0.0001$, $\alpha = 0.05$).

Figure 32 shows average of influent and effluent concentrations of TP for each trench in Phase 3.

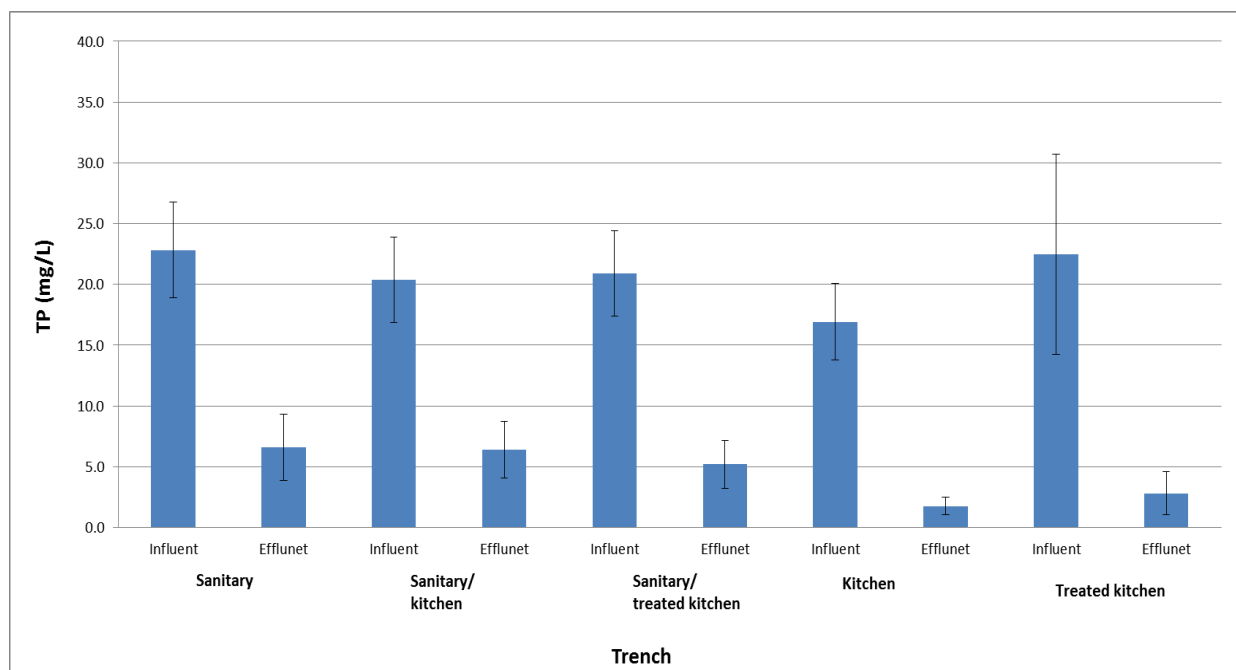


Figure 32. Average influent and effluent concentrations of TP in Phase 3

Similar result to that found in Phase 2 was found in that all bench-scale trenches reduced TP concentration significantly. The Sanitary trench continued to perform as expected.

Figure 33 shows average of influent and effluent concentrations of TN for each trench in Phase 3.

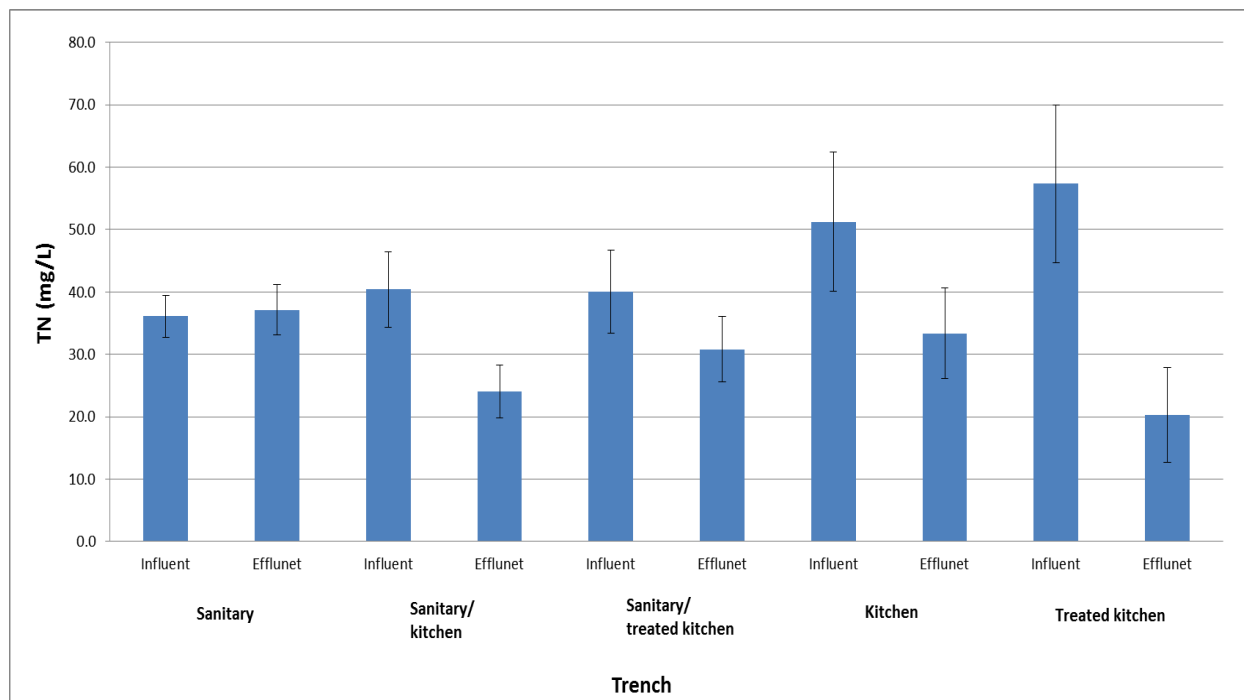


Figure 33. Average influent and effluent concentrations of TN in Phase 3

Similar results to that in Phase 2 were found in that TN in all trenches except Treated kitchen did not removed TN significantly. The Sanitary trench continued to perform as expected. Sanitary/kitchen had better removal of TN than Sanitary/treated kitchen (ANOVA, P-value=0.0025, $\alpha=0.05$). The trench receiving Treated kitchen had a higher removal rate of TN than the trench with AGIS (ANOVA, P-value=0.01252, $\alpha=0.05$). This indicates that the soil in these trenches was saturated and consequently, anaerobic conditions persisted, enhancing the denitrification process. There may have also been a higher level of carbon throughout the soil because of the very high organic loading. This is also essential for denitrification.

Figure 34 shows average of influent and effluent concentrations of ammonia for each trench in Phase 3.

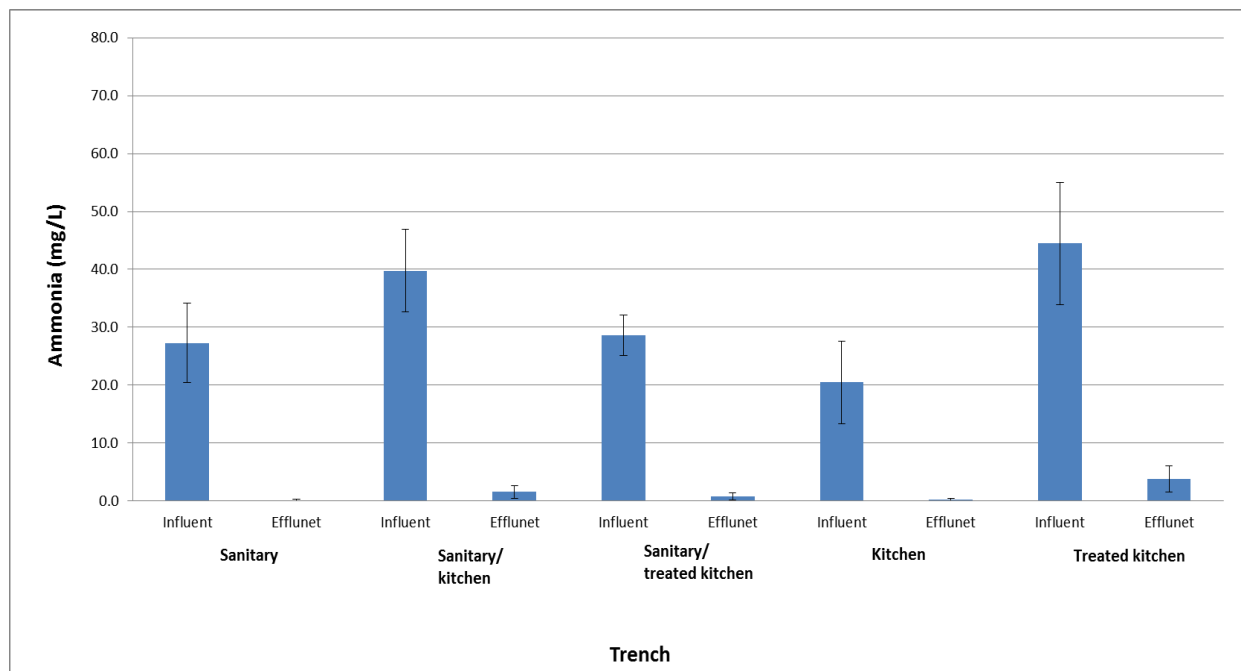


Figure 34. Average influent and effluent concentrations of ammonia in Phase 3

Result show that all bench-scale trenches removed most of the ammonia. Low concentrations of ammonia in effluent indicate that the nitrogen in influent was nitrified to nitrate, resulting in increased nitrate concentration in effluent. The ammonia influent and effluent concentration of the trench receiving Sanitary wastewater are 27.3 mg/L and 0.1 mg/L, respectively, which is close to typical influent and effluent to an actual 20 - 60 mg/L and 0.03 mg/L, respectively (Hossain 2010; Metcalf & Eddy 2003).

Figure 35 shows average of influent and effluent concentrations of nitrate for each trench in Phase 3.

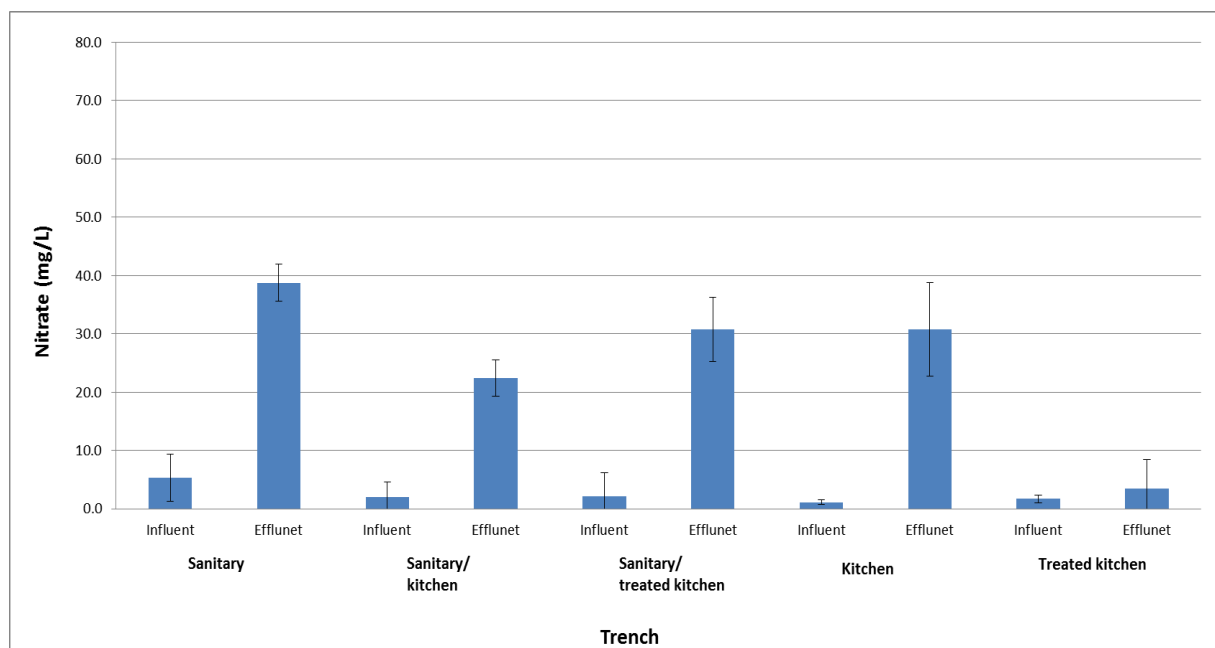


Figure 35. Average influent and effluent concentrations of nitrate in Phase 3

Similar results to that in Phase 2 were observed as the effluent nitrate concentrations in all trenches except Treated kitchen increased after treated with the bench-scale trenches, as expected from ammonia result. The effluent level of nitrate for Sanitary/kitchen was statistically lower (ANOVA, $P\text{-value} < 0.001$, $\alpha = 0.05$) than Sanitary/treated kitchen wastewater. As mentioned in Phase 3, the soil in the trenches receiving Treated kitchen and Sanitary/kitchen wastewater were saturated and, consequently, anaerobic conditioned enhanced the denitrification process. The Sanitary trench continued to perform as expected.

4.2.4. Phase 4

In Phase 4, the composition of the non-AGIS treated kitchen wastewater was changed in order to more closely correlate with AGIS treated kitchen wastewater. This resulted in using

147.6 L/day (39 gal/day) of mop water and 283.9 L/day (75 gal/day) of washing machine wastewater, based on COD results in Phase 3.

Figures 36 – 42 show influent and effluent concentrations of COD, BOD₅, FOG, TP, TN, ammonia and nitrate for each trench in Phase 4. The error bars represent the standard deviation.

Figure 36 shows the average influent and effluent concentrations of COD for each trench in Phase 4.

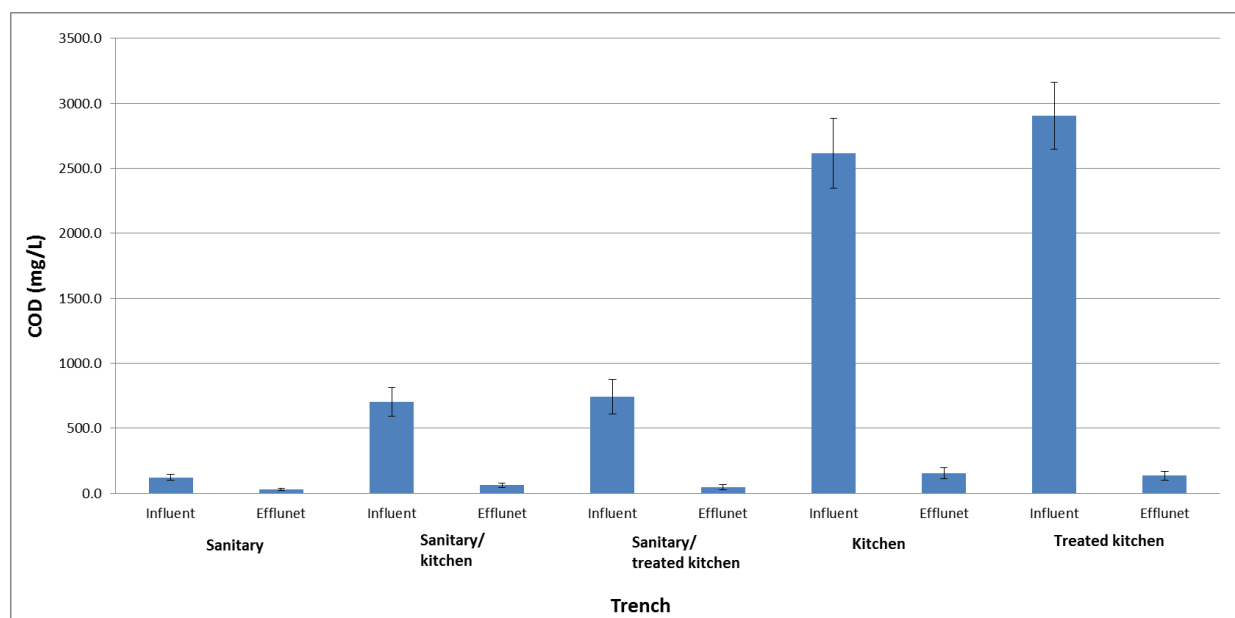


Figure 36. Average influent and effluent concentrations of COD in Phase 4

The influent COD for the Sanitary/kitchen and the Sanitary/treated kitchen were statistically the same with the new formulation (ANOVA, P-value=0.3468, $\alpha=0.05$). Similar result to that in Phase 2 were found as all bench-scale trenches removed significant amounts of COD. The Sanitary trench continued to perform as expected.

Figure 37 shows the average influent and effluent concentrations of BOD₅ for each trench in Phase 4.

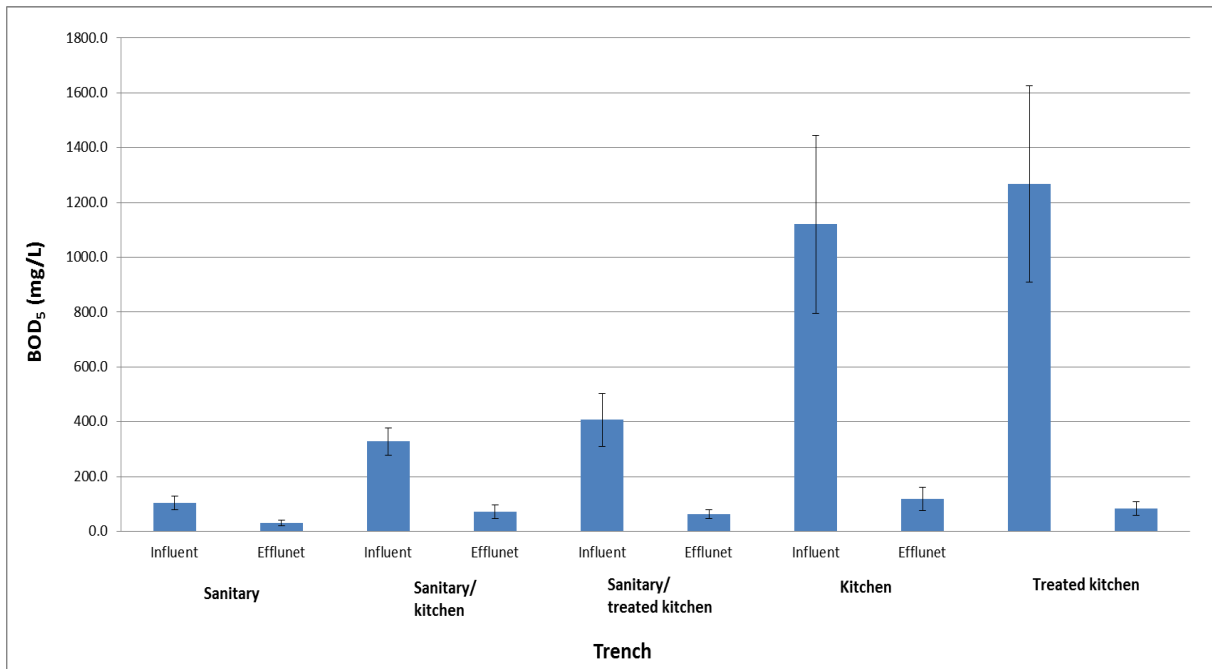


Figure 37. Average influent and effluent concentrations of BOD₅ in Phase 4

Similar results to that in Phase 3 were found as all bench-scale trenches removed significant amount of BOD₅. The Sanitary trench continued to perform as expected.

Figure 38 shows the average influent and effluent concentrations of FOG for each trench in Phase 4.

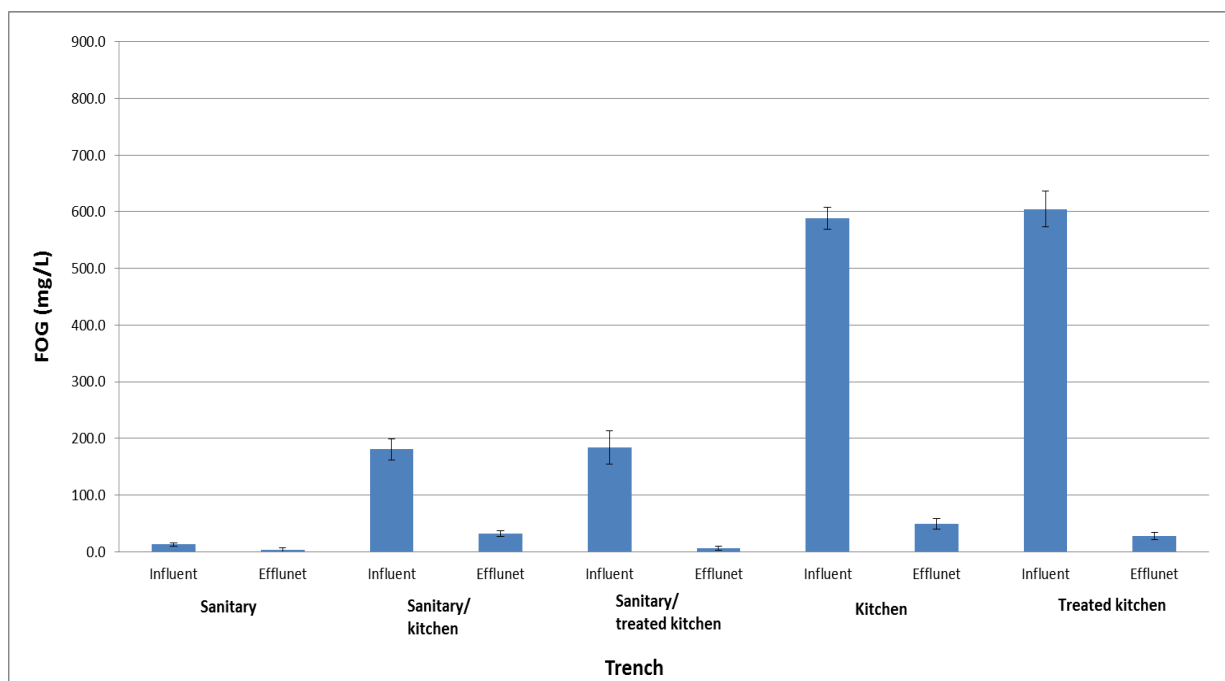


Figure 38. Average of influent and effluent concentrations of FOG in Phase 4

Similar results to that in Phase 2 were found as all bench-scale trenches reduced FOG concentration significantly. The Sanitary trench continued to perform as expected.. The removal rate of the trench receiving Sanitary/treated kitchen was statistically lower (ANOVA, P-value<0.0001, $\alpha=0.05$) than the trench receiving Sanitary/kitchen wastewater, as expected.

Figure 39 shows average influent and effluent concentrations of TP for each trench in Phase 4.

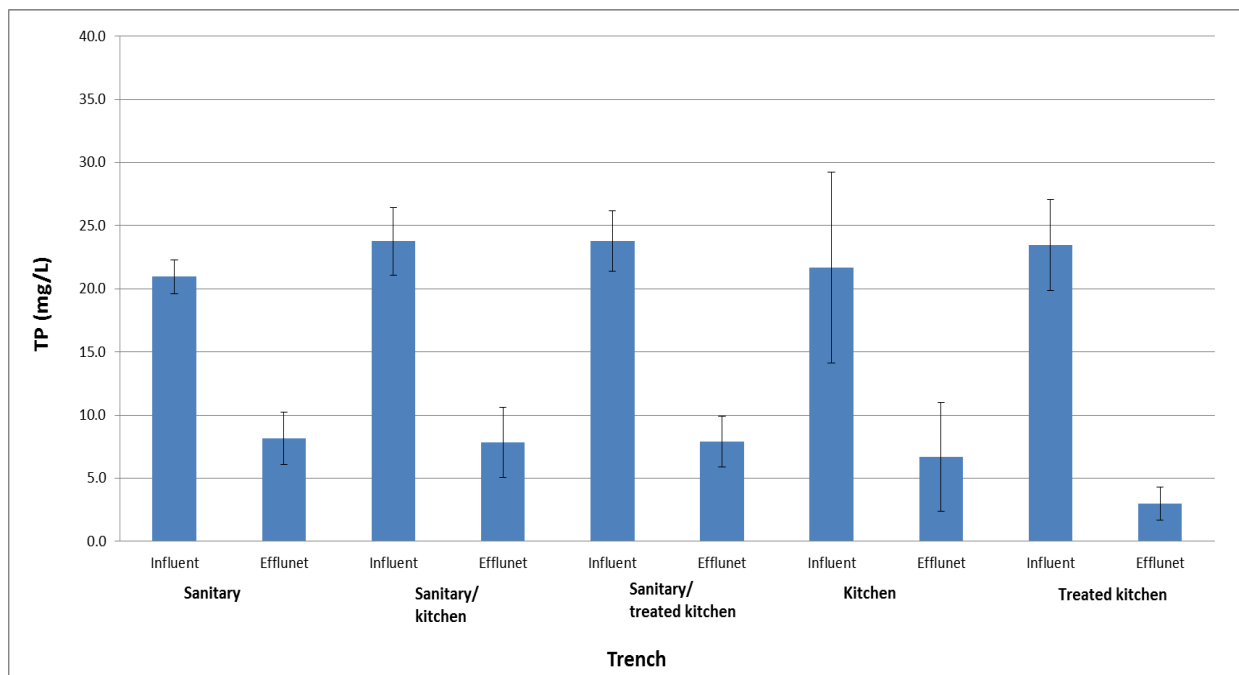


Figure 39. Average influent and effluent concentrations of TP in Phase 4

Similar results to that in Phase 2 were found as all bench-scale trenches reduced TP concentration significantly as expected. The Sanitary trench continued to perform as expected.

Figure 40 shows average influent and effluent concentrations of TN for each trench in Phase 4.

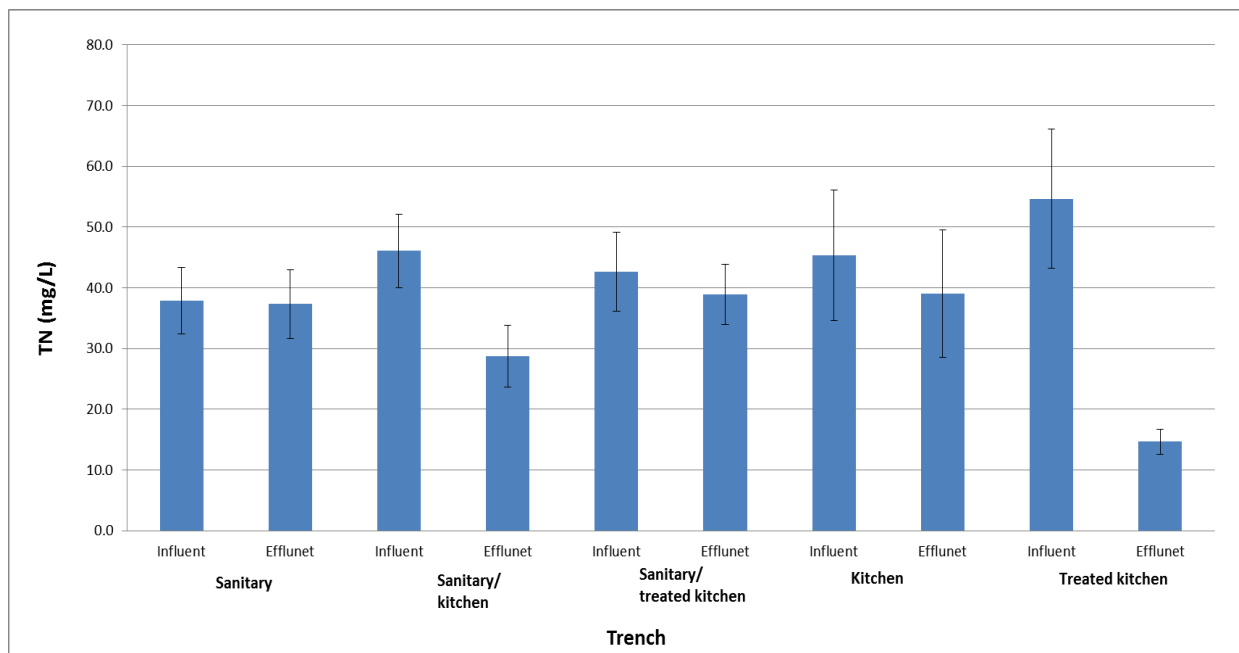


Figure 40. Average of influent and effluent concentrations of TN in Phase 4

Similar results to that in Phase 2 were found as all trenches except the Treated kitchen trench did not removed TN significantly. The Sanitary trench continued to perform as expected. Sanitary/kitchen had a statistical (ANOVA, $P\text{-value} < 0.0001$, $\alpha = 0.05$) better removal rate than Sanitary/treated kitchen.

Figure 41 shows the average influent and effluent concentrations of ammonia for each trench in Phase 4.

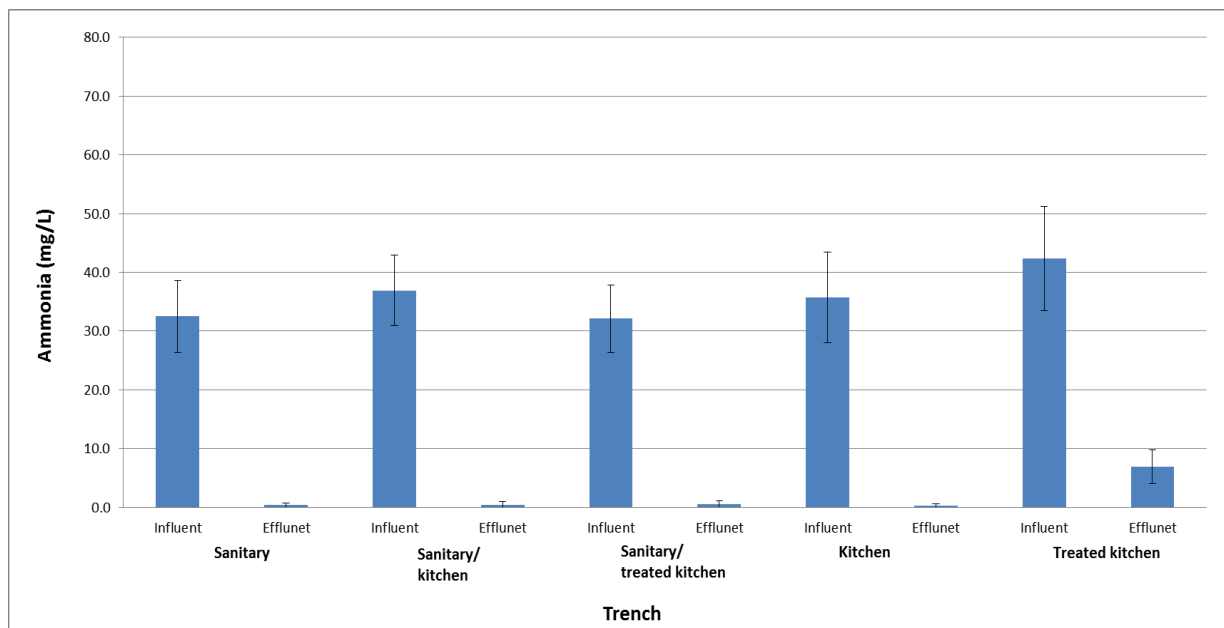


Figure 41. Average influent and effluent concentrations of ammonia in Phase 4

Similar results to that in Phase 2 were found as all bench-scale trenches removed most of ammonia significantly. The Sanitary trench continued to perform as expected.

Figure 42 shows the average influent and effluent concentrations of nitrate for each trench in Phase 4.

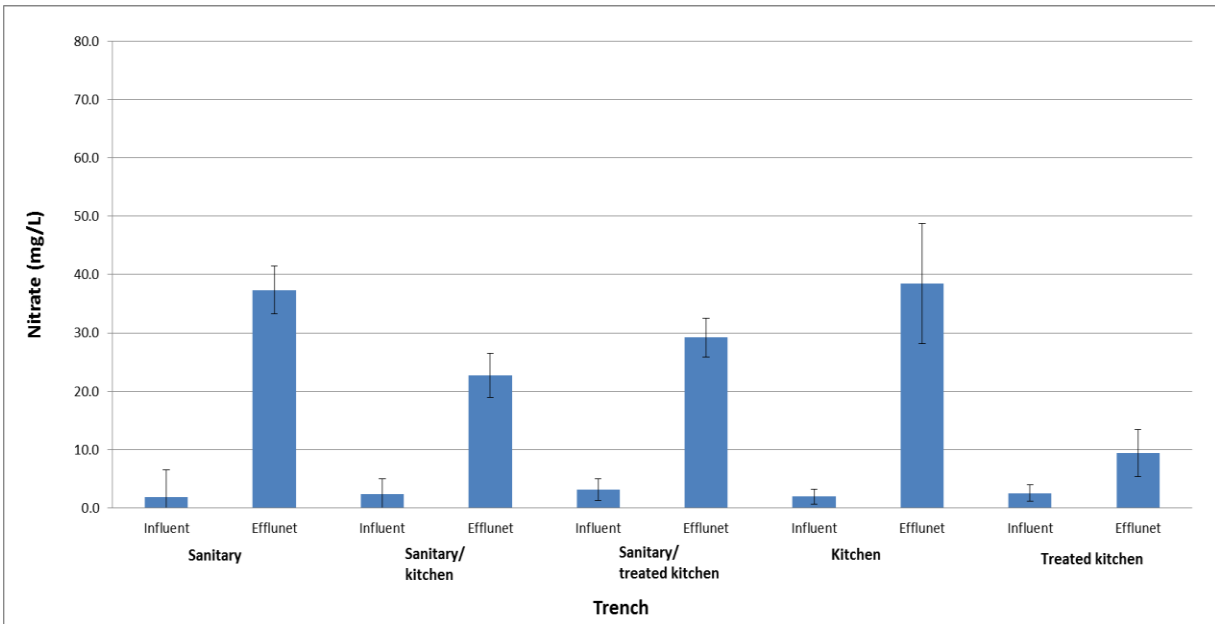


Figure 42. Average influent and effluent concentrations of nitrate in Phase 4

Similar results to that in Phase 2 were found as the nitrate in all trenches increased after treatment except in the Treated kitchen trench. The effluent level of nitrate for Sanitary/kitchen was statically lower (ANOVA, $P\text{-value} < 0.001$, $\alpha = 0.05$) than Sanitary/treated kitchen wastewater, as previously discussed.

Overall, result show that the performance of the bench-scale trench receiving Sanitary wastewater was similar to an actual drain field receiving sanitary wastewater. All trenches achieved significant removals of COD, BOD₅, FOG and TP. Enzyme pretreatment did not reduce the influent COD and FOG. However, the FOG removal rate in the trench with Sanitary/kitchen and Kitchen were substantially lower than the trenches with Sanitary/treated kitchen and Treated kitchen wastewater. After enzymatic treatment, the FOG apparently became more biodegradable because of the lower molecular weight of the triglycerides. All bench-scale trenches nitrified most of the ammonia to nitrate. The trench receiving Kitchen and Sanitary/kitchen wastewater

reduced TN concentrations, indicating that the soil in these trenches was saturated, and consequently, anaerobic, enhancing the denitrification process.

4.3. Impact of wastewater with/without enzyme pretreatment on clogging

In order to study this objective, observation of soil moisture content, soil volatile solids and visual conditions of the inlet pipes were conducted.

4.3.1. Moisture Contents in trenches

All trenches had 6 moisture sensors embedded in the soil at different locations (Figure 43).

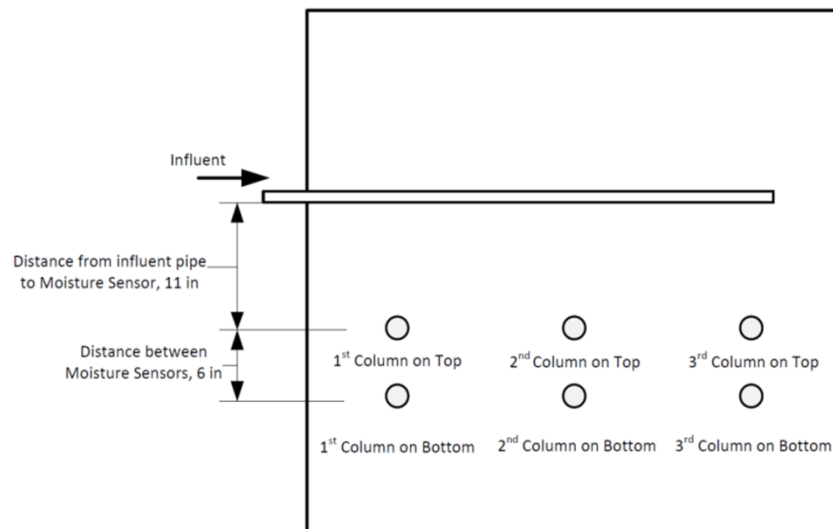


Figure 43. Schematic diagram of moisture sensor locations on a trench. Each circle represents a moisture sensor (not to scale).

Each of the six moisture content sensors was labeled by location as shown in Figure 41.

Figure 44 - 47 show the average daily moisture content for each sensor. The averaged was calculated from measurements made every 10 minutes.

Figure 44 shows daily moisture levels in the trench that received Sanitary wastewater.

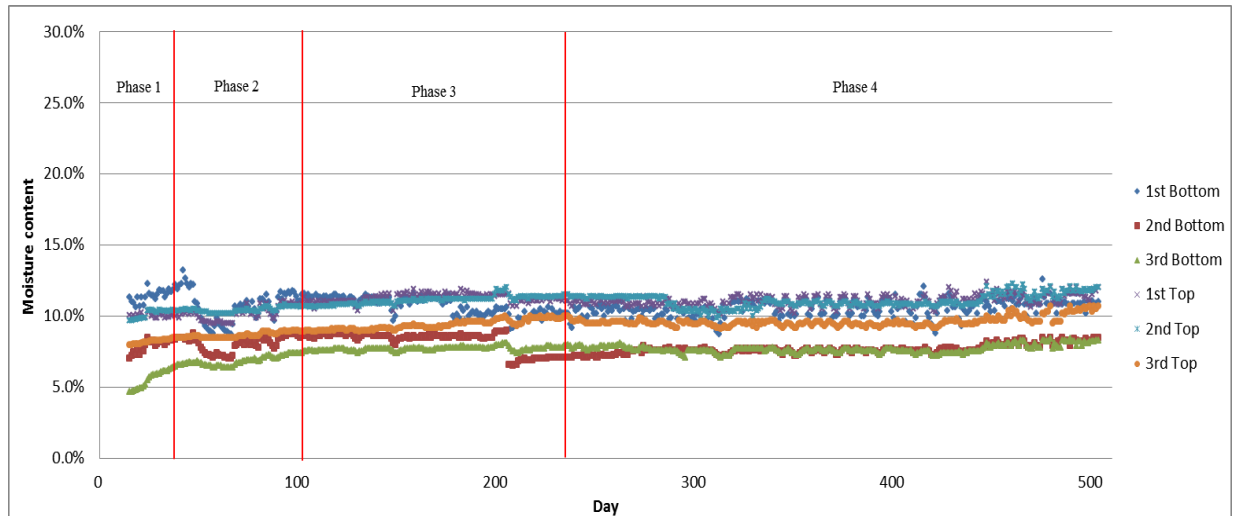


Figure 44. Daily moisture levels in the trench receiving Sanitary wastewater

Results show consistent moisture contents throughout the 505 days of operation. There is no to very low increasing slopes at all locations. The highest moisture content was found at 1st Top location and the lowest was at the 3rd Bottom location. This indicates very little water flowed across the length of the trench.

Figure 45 shows daily moisture levels in trenches that received Sanitary/kitchen (top) and Sanitary/treated kitchen (bottom).

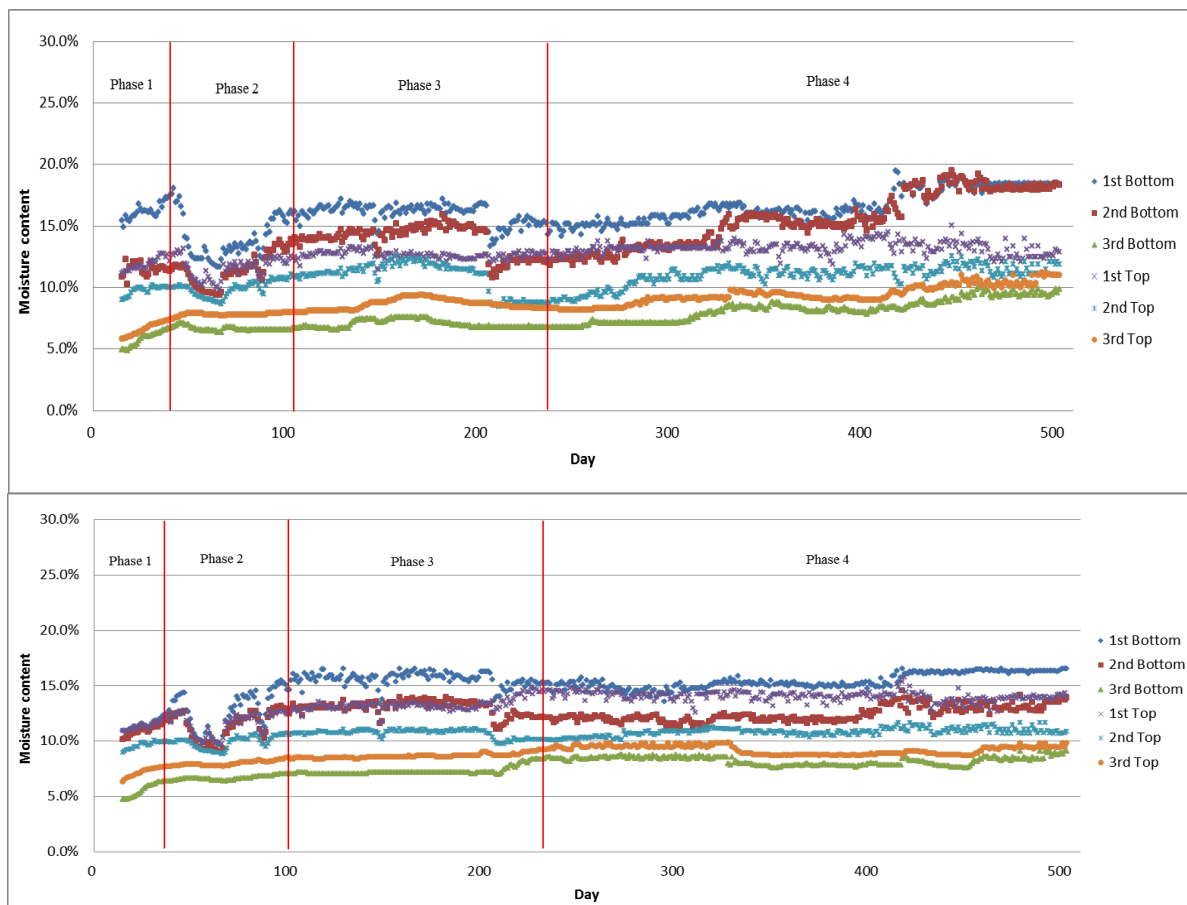


Figure 45. Daily moisture levels in trenches receiving Sanitary/kitchen wastewater (top) and Sanitary/treated kitchen wastewater (bottom)

Results show that the moisture levels in the Sanitary/kitchen trench was not constant. Moisture levels at the 1st bottom and 2nd bottom locations almost reached the saturation (20 – 35%), indicating premature aging of the rain field by clogging of soil porosity. Moisture levels of the Sanitary/treated kitchen trench (bottom graph in Figure 45) were relatively constant during the 505 days of operation, except at the 1st bottom location. Both graphs showed a higher moisture levels than the Sanitary wastewater trench, however, the trench receiving Sanitary wastewater (Figure 44) was substantially lower.

Figure 46 shows the daily moisture levels for the trench that received Kitchen wastewater.

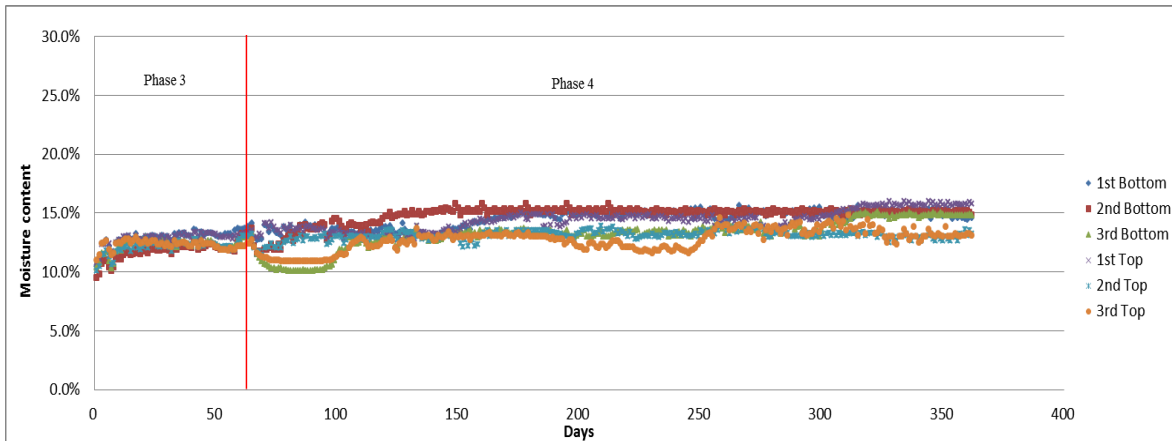


Figure 46. Daily moisture level of the trench receiving Kitchen wastewater

Result show that moisture levels did not changed significantly during the 362 days of operation. Note that this condition was not operated for as long as the others as the trench originally received a feedstock from a modification to the AGIS system consisting of an effluent filter that was proven to be detrimental. The relatively low, constant moisture levels seem unusual but results from the inspection of the soil and inlet lines demonstrated clogging that only allowed water to very slowly flow into the soil. This was also directly demonstrated after 178 days of operation when the feedstock started backing up in the inlet pipe, making it difficult to add the wastewater to the trench.

Figure 47 shows the daily moisture levels of the trench that received Treated kitchen wastewater.

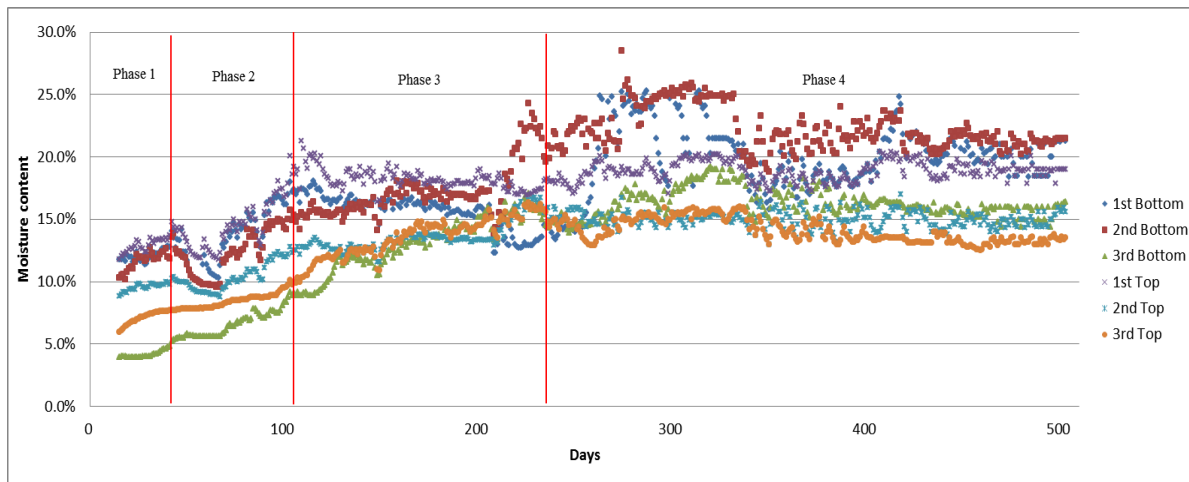


Figure 47. Daily moisture levels in the trench receiving Treated kitchen wastewater

The trench receiving Treated kitchen wastewater had the highest moisture level of all the conditions. This trench also had the highest biodegradable organic loading, and consequently was demonstrated to have the highest level of microorganisms (discussed in VS section). The graph showed a higher moisture levels than the Sanitary wastewater trench.

To gain a better perspective on the change in moisture content, monthly plots of the 10 minute moisture contents values were produced over a 24 hour period beginning on 254th day of operation and 88th day for the Kitchen trench. These plots could not be routinely produced due to memory limitations of the CR 1000 datalogger.

Figure 48 - 50 show the moisture level for the 10 minutes segment. The peaks in the figures represent a hydraulic flow of feedstock.

Figure 48 shows the moisture level of the trench that received Sanitary wastewater.

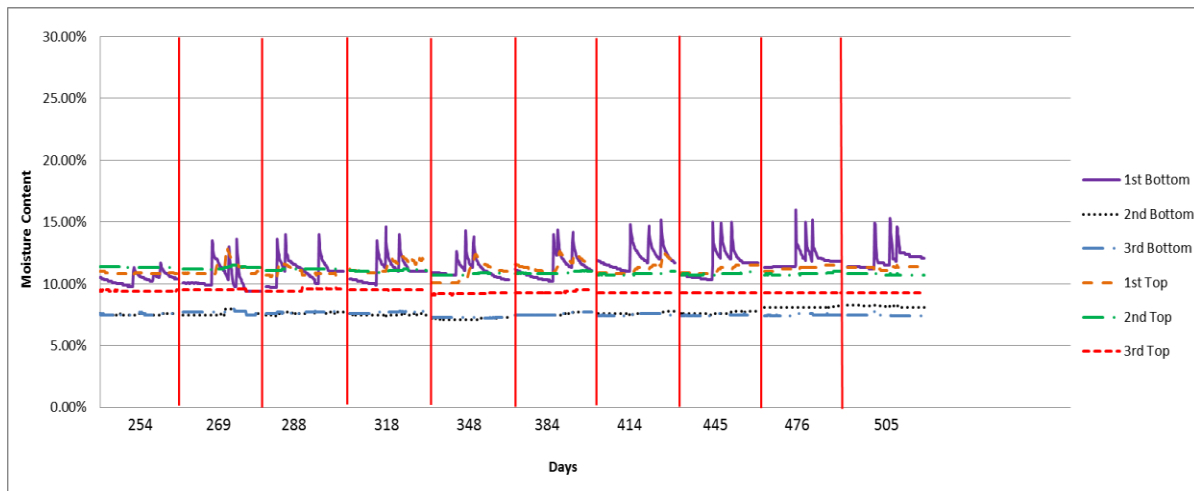


Figure 48. Moisture content of trench receiving Sanitary wastewater (10 minutes segments)

Only the 1st bottom location was impacted during 505 days of operation. The other locations showed consistent moisture contents.

Figure 49 shows the 10 minute moisture level of the trenches that received Sanitary/kitchen and Sanitary/treated kitchen trenches.

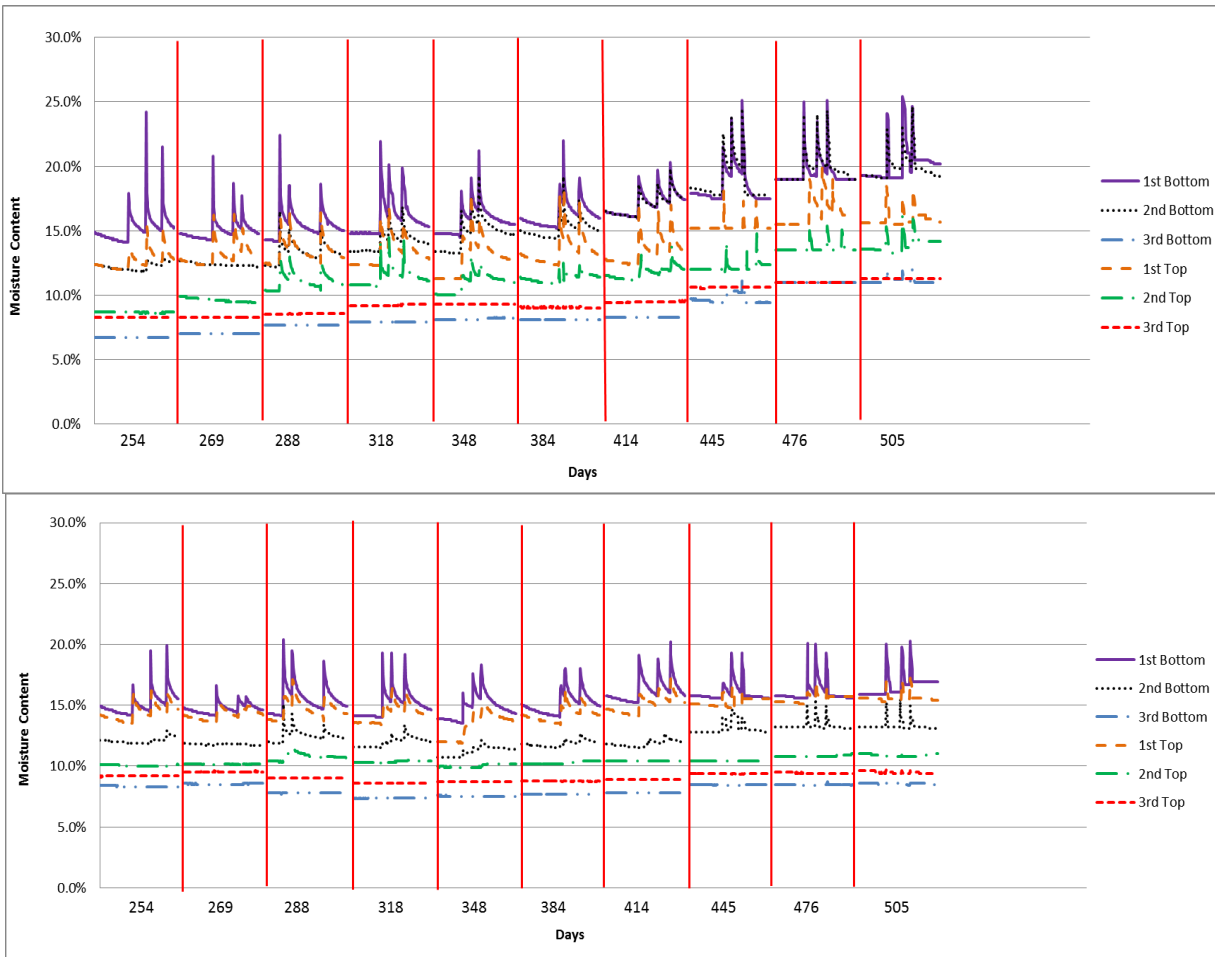


Figure 49. Moisture levels of trenches receiving Sanitary/kitchen (top) and Sanitary/treated kitchen (bottom) (10 minutes segments)

The soil moisture levels were greater for the Sanitary/kitchen trench, although both received close to the same COD and FOG concentrations. More impacted locations and higher peaks of moisture levels are hypothesized to cause the premature of aging of the drain field. Both graphs showed higher moisture levels than the Sanitary wastewater trench.

Figure 50 shows the moisture levels of the trenches that received Kitchen (top) and Treated kitchen (bottom).

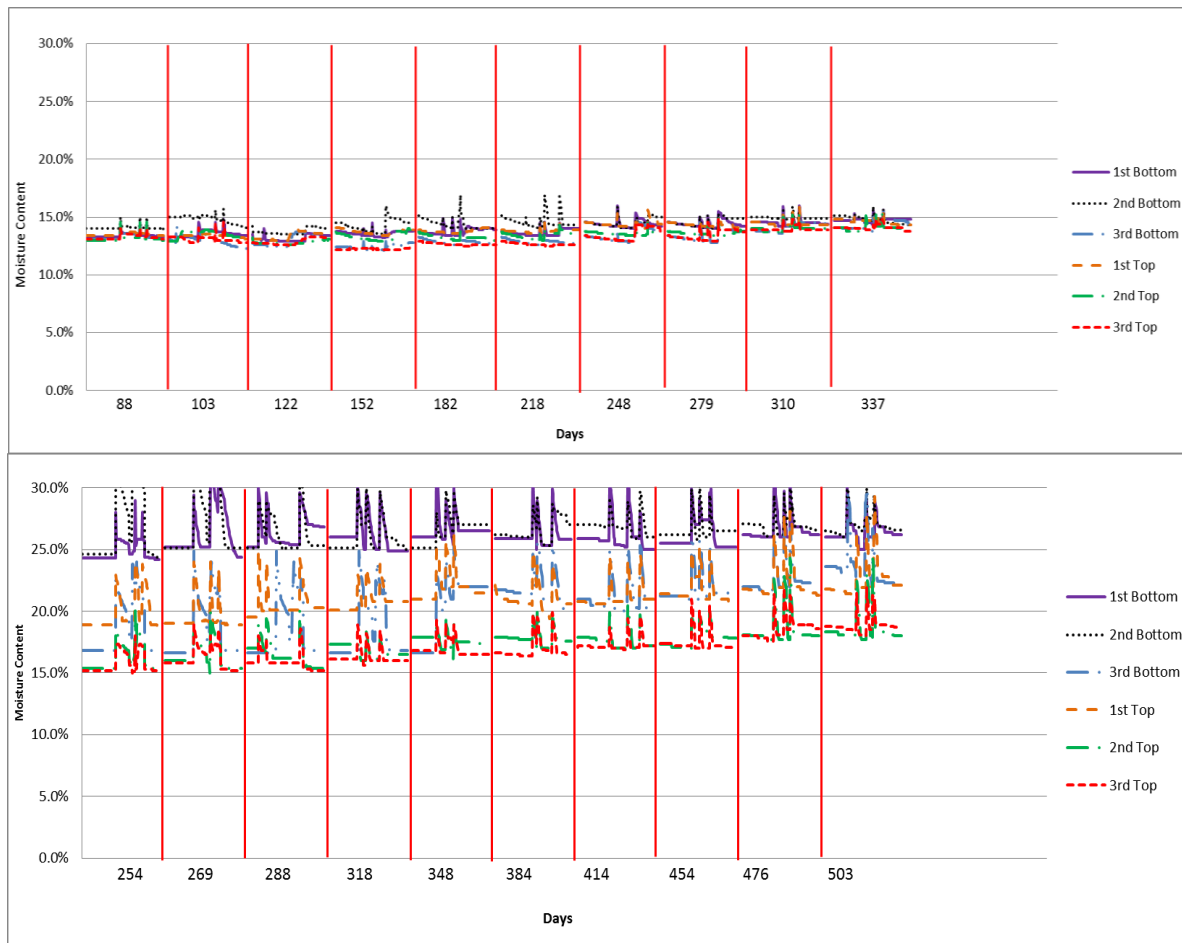


Figure 50. Moisture levels of trenches receiving Kitchen (top) and Treated kitchen (bottom) (10 minutes segments)

Result shows a clear difference between the trenches. Water started to surface in the Treated kitchen trench on day 242 of operation. This was at first surprising but data from the soil VS and visual examination of the inlet pipe and soil after the systems were shut down, discussed in the next subsections, and provides the explanation. The trench receiving kitchen wastewater showed a higher moisture levels than the Sanitary wastewater trench.

4.3.2. Volatile solids

The VS concentration is an indicator of the microorganism population within the soil (Ellis 2004). Figure 51 and 52 show the VS (mg/g) concentration collected on the 207th day of operation for each trench at depths of 35.6 cm (14 inch) and 50.8 cm (20 inch) and each soil column within the trench. The diagram of the soil sampling locations is shown in Figure 20.

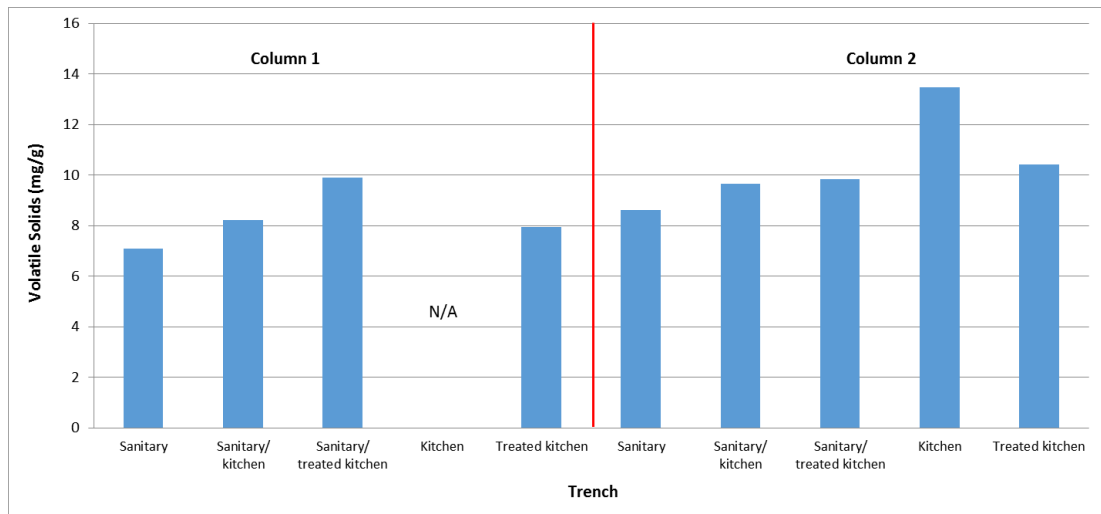


Figure 51. VS at 35.6 cm (14 inch) depth for columns 1 and 2

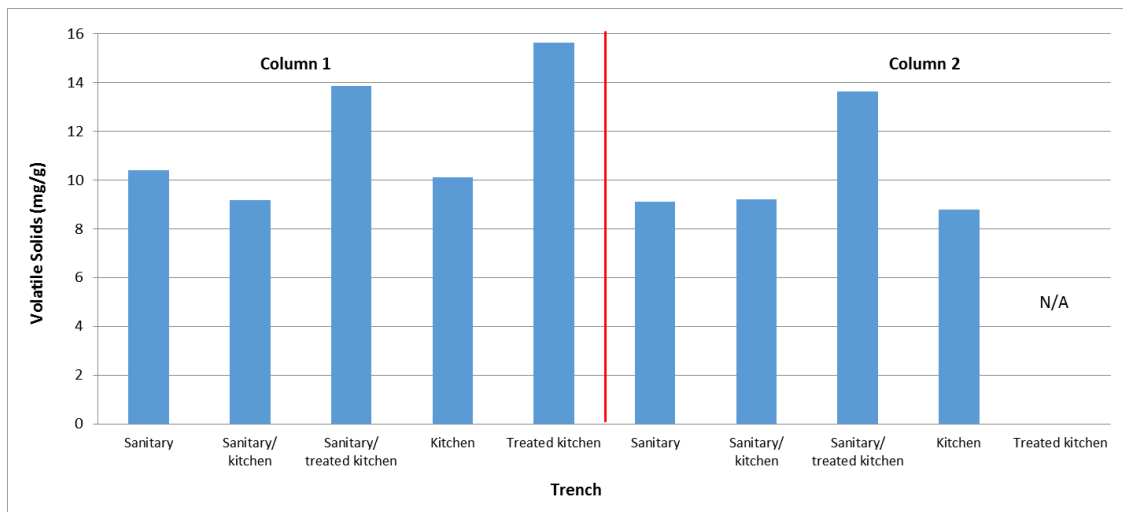


Figure 52. VS at 50.8 cm (20 inch) depth for columns 1 and 2

The values of column 1 (Kitchen) at 35.6 cm (14 inch) depth and column 2 (Treated kitchen) at 50.8 cm (20 inch) are not available because of laboratory errors. Only one sample was analyzed for this VS analysis without any replications because the operation was on-going and collecting more samples would have increased the risk of disrupting the system.

The results show that Sanitary/treated kitchen of column 1 at 35.6 cm (14 inch) and 50.8 cm (20 inch) depths had slightly higher VS levels than the Sanitary/kitchen trench soil. In the column 2 at 50.8 cm (20 inch) depth, higher VS level was found in the Sanitary/treated kitchen than Sanitary, Sanitary/kitchen and Kitchen. This indicates that enzyme pretreatment increased the biodegradable organic matter (microorganism population) in the soil because the wastewater that was treated with enzyme became more biodegradable.

The second soil analysis was conducted on the day after operation. Three replicates for each column at 20.3 cm (8 inch) and 45.7 cm (18 inch) depth from the top of sandy loam were collected (Figure 21). Figure 53 - 57 show the VS concentrations of each trench, in each column and at depths of 20.3 cm (8 inch) and 45.7 cm (18 inch) from the top of sandy loam layer. The error bars represent the standard deviation.

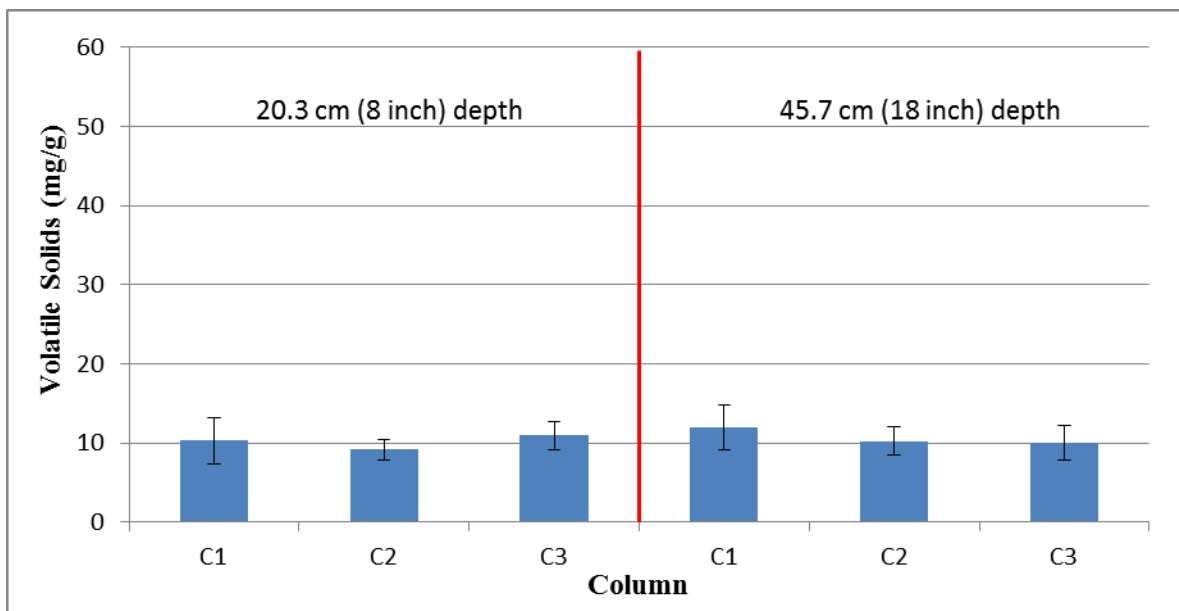


Figure 53. VS of Sanitary trench at depths of 20.3 cm (8 inch) and 45.7 cm (18 inch) from the top of the sandy loam

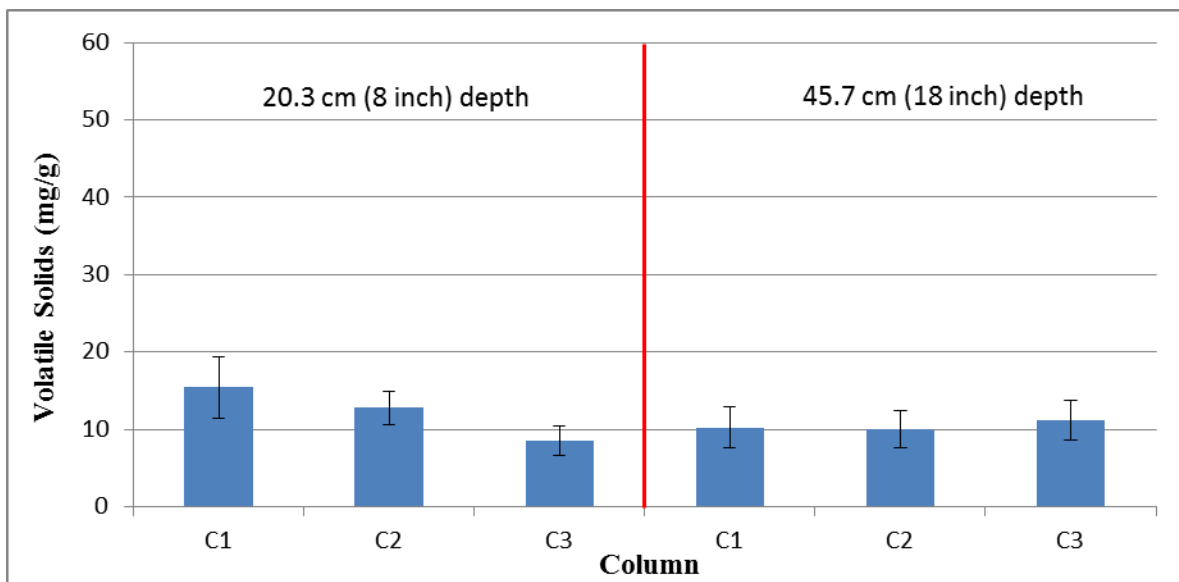


Figure 54. VS of Sanitary/kitchen trench at a depths of 20.3 cm (8 inch) and 45.7 cm (18 inch) from the top of sandy loam

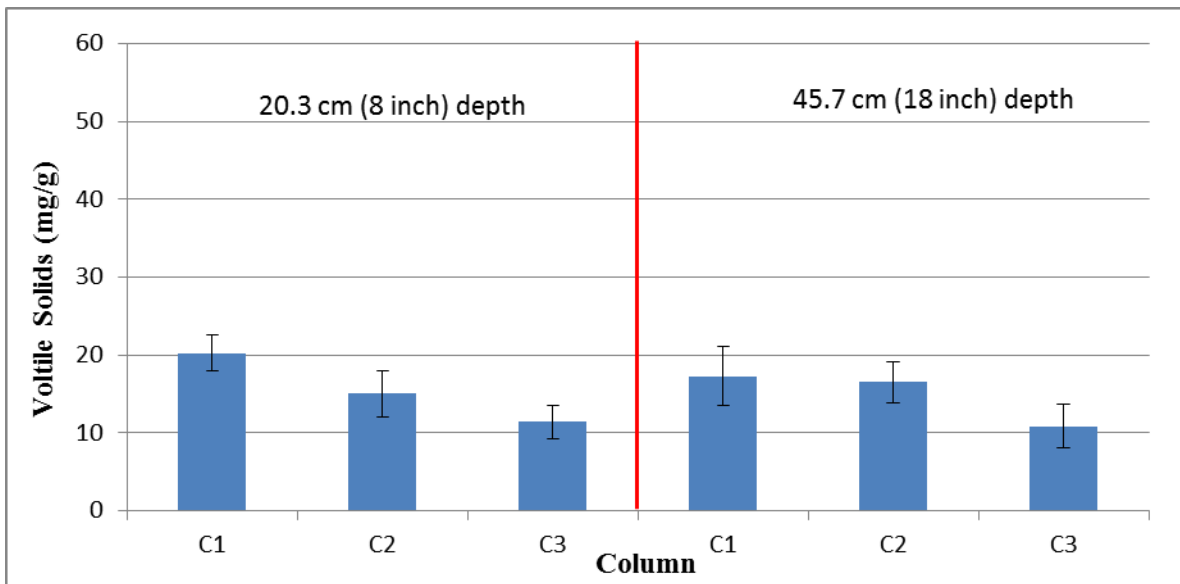


Figure 55. VS of Sanitary/treated kitchen trench at depths of 20.3 cm (8 inch) and 45.7 cm (18 inch) from the top of sandy loam

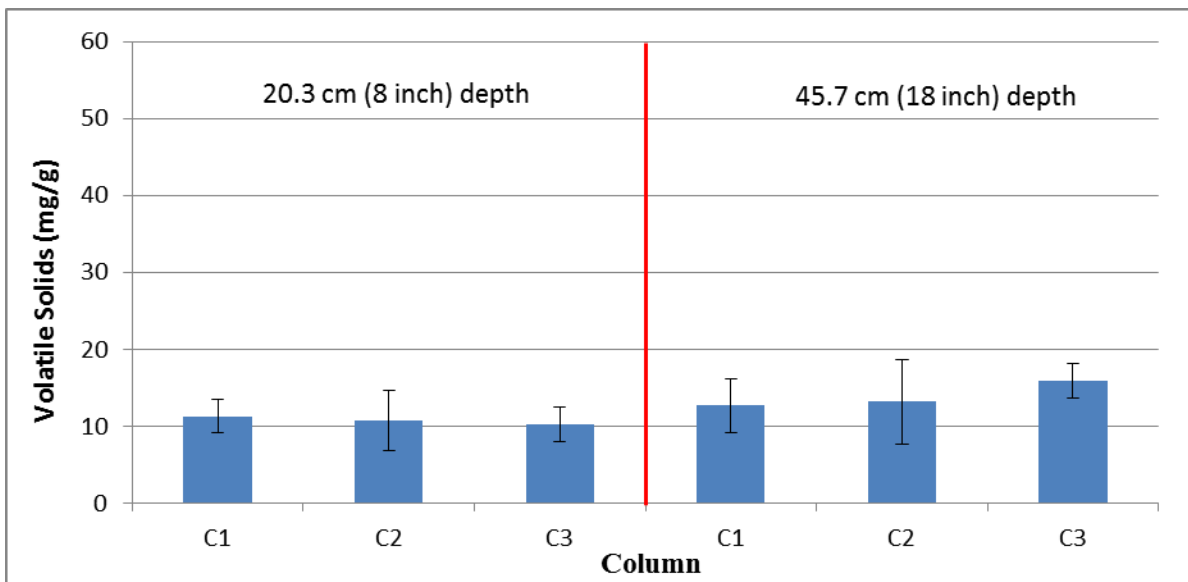


Figure 56. VS of Kitchen trench at depths of 20.3 cm (8 inch) and 45.7 cm (18 inch) from the top of sandy loam

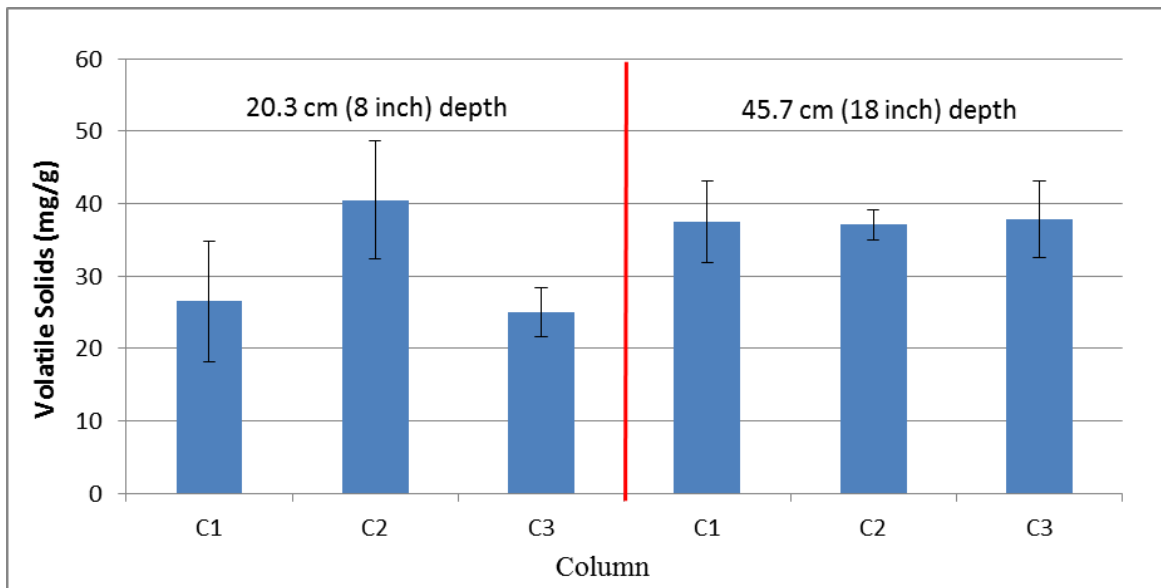


Figure 57. VS Treated kitchen trench at depths of 20.3 cm (8 inch) and 45.7 cm (18 inch) from the top of sandy loam

The VS in the trench that received Treated kitchen is statistically higher than the trench receiving Kitchen wastewater (ANOVA, $P\text{-value} < 0.001$, $\alpha = 0.05$). The VS in the trench receiving Sanitary/treated kitchen wastewater is statistically higher than Sanitary and Sanitary/kitchen wastewater (ANOVA, $P\text{-value} = 0.0025$, $\alpha = 0.05$). VS levels for the trenches that received Sanitary wastewater and Sanitary/kitchen were not statistically different except C1 at a depth of 20.3 cm (8 inch). Results indicate that there is a greater microorganism population in enzyme pretreated wastewater.

4.3.3. Visual Observation

Visual observation of gravel in the trenches, influent pipes and treatment soils are an indication were made when the system was dissassembled. A typical drain field failes due to physical pipe, gravel and/or soil blockages or extensive biomat growth (NESC 2005; Bio-Safe One 2011; Owens & E. Moye Rutledge 2011; Mckenzie 2010; Matejcek et al. 2000) and is often realized by water surfacing. Figure 58 shows the top view of the Sanitary trench. The soil is dry as water from the inlet pipe always progressed downward and across the trench.



Figure 58. Top view of Sanitary trench

The trenches that received Sanitary/kitchen, Sanitary/treated kitchen and Kitchen feedstocks had similar appearances. Figure 59 shows the Treated kitchen trench. Water started surfacing on the 242th days of operation, indicating that the underlying soil was becoming clogged. This was confirmed from the moisture sensor data.



Figure 59. Top view of Treated kitchen trench

The clogging was found to be caused by the extensive growth of biomat (Figure 59). The biomat restricts the flow and eventually clogged the trench.



Figure 60. Biomat was found in the Treated kitchen trench

The COD concentrations of both Kitchen and Treated kitchen wastewater were approximately 30 times higher than sanitary wastewater. However, AGIS treatment transformed the FOG so that it was biodegradable, causing the extensive biomat growth. To manage this large

biodegradable, organic load the sizing of the drain field should be designed based on organic loading, not the standardly used hydraulic loading.

Figure 61 shows the clean condition of the inlet pipe from the Sanitary trench after 505 days of operation.

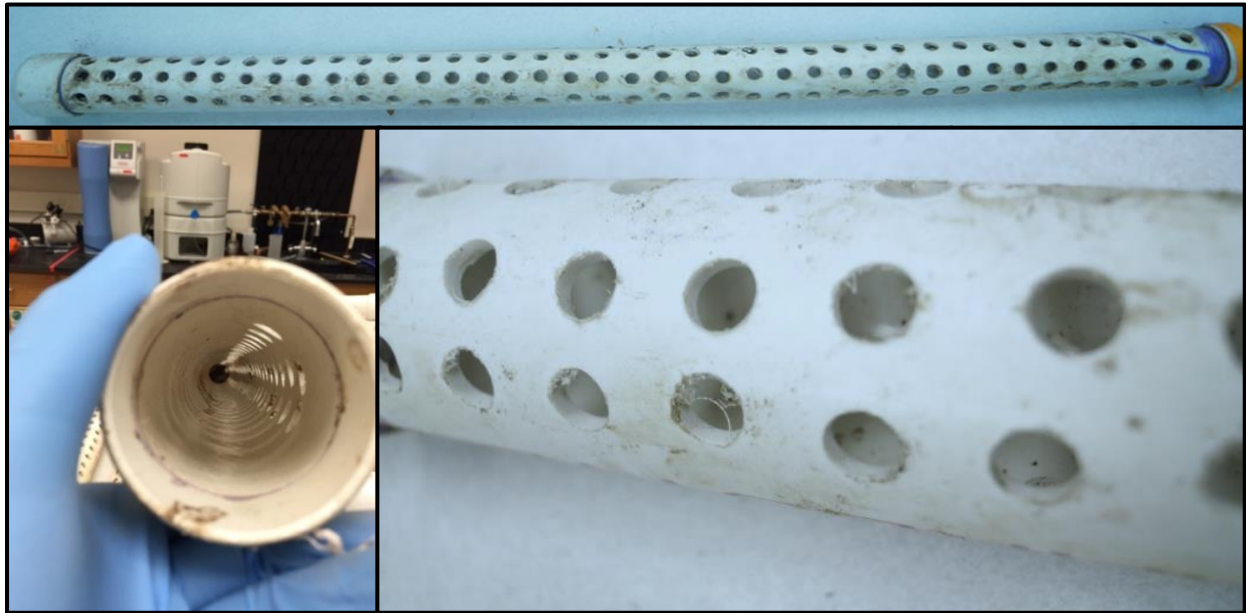


Figure 61. Inlet pipe condition in the Sanitary trench after 505 days of operation

Since sanitary wastewater contains relatively low concentrations of FOG, when compared to fast-food restaurant wastewater, it did not cause physical clogging.

Figure 62 shows the condition of the inlet pipe in the Sanitary/kitchen trench after 505 days of operation.

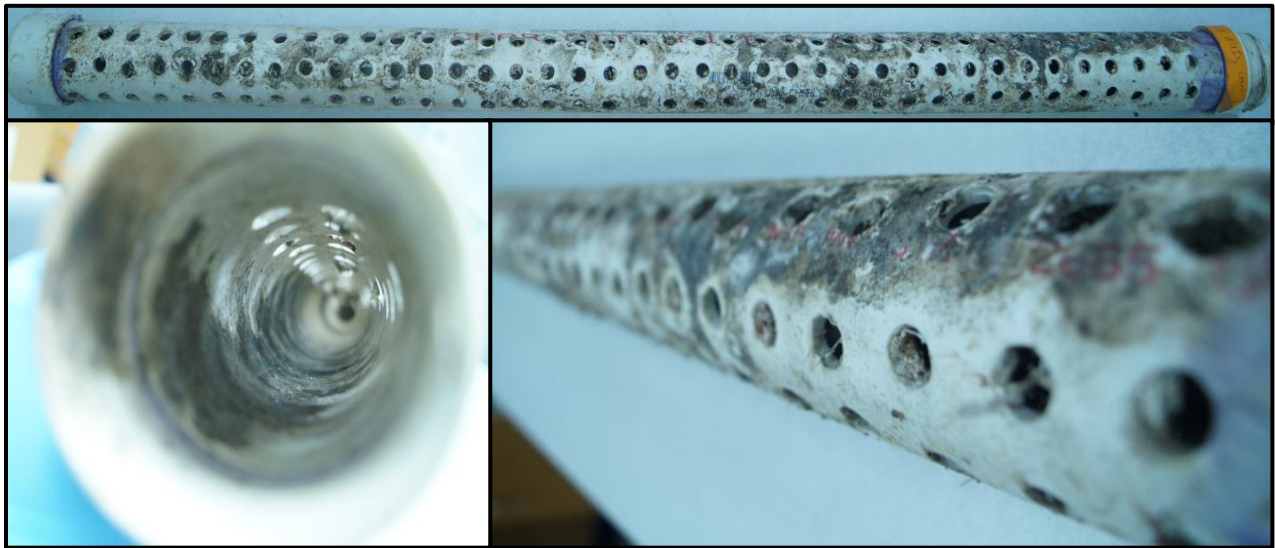


Figure 62. Inlet pipe condition in the Sanitary/kitchen trench after 505 days of operation

Some FOG residues were adhered on both the inner and outer sides of the pipe after 505 days of operation. This solids accumulation has the potential to ultimately clog the pipe.

Figure 63 shows the clean inlet pipe from the Sanitary/treated kitchen trench.

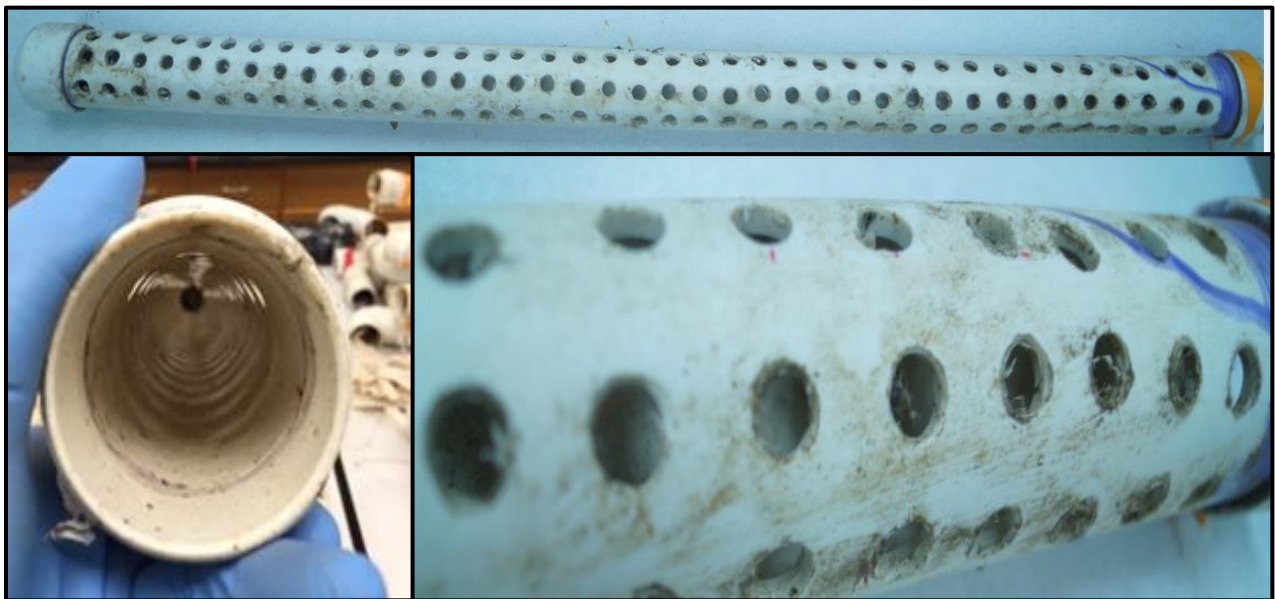


Figure 63. Inlet pipe condition in the Sanitary/treated kitchen trench after 505 days of operation

There is no evidence of FOG accumulation on both the inner and outer sides of the pipe. Result shows that enzyme treatment degraded the cohesiveness of the triglycerides so that FOG in will not physically clog the pipe.

Figure 64 shows the condition of the inlet pipe in the Kitchen trench.



Figure 64. Inlet pipe condition in the Kitchen trench after 337 days of operation

The pipe is plugged with FOG resulting in wastewater back-up within the pipe and in the soil. The feedstock was only able to slowly trickle down into the soil, hence the reason for the low soil moisture levels previously discussed (Figure 46 and Figure 50).

Figure 65 shows the inlet pipe from the Treated kitchen wastewater trench.



Figure 65. Inlet pipe from the Treated kitchen trench after 505 days of operation

Approximately half of the effluent holes were clogged with a layer of biomat.

Chapter 5: Conclusion and recommendations

Three objectives were formulated and studied. The results are first summarized. Thereafter, recommendations are provided.

5.1. Summary

The objectives of this research were to observe changes in the characteristic of triglycerides resulting from enzyme treatment, the development of a bench-scale drain field that simulated actual installations and its performance for various feedstocks and the evaluation of drain field clogging by feedstocks with/without enzyme pretreatment. A summary of the results follows.

- LC/MS analysis showed that enzyme treatment impacted the characteristics of the triglycerides composing the FOG. Specifically, the number of double bonds and the molecular weight of the individual components of the FOG were reduced.
- Bench-scale trenches with embedded soil moisture sensors for monitoring clogging proved to be a unique, effective method to research the impact of diverse feedstocks. Their performance with typical sanitary feedstocks closely correlated to that found in the literature.
- Bench-scale trenches designed based on hydraulic loading effectively removed a significant amount of COD, BOD₅ and FOG, regardless of their initial concentrations.
- Daily graphs of readings from the moisture sensors were not sensitive enough to detect water flow in a drain field. Ten minute segment graphs showed substantial changes in moisture level, including an increase at each feeding and then a slow decrease thereafter.

- Moisture levels in the trenches receiving sanitary wastewater (serving as a control) and the sanitary wastewater mixed with kitchen wastewater that was enzymatically pretreated did not have substantially elevated moisture levels. The trench receiving the sanitary wastewater and untreated kitchen wastewater showed signs of premature aging based on the elevated moisture levels and increasing slopes. The trench receiving only untreated kitchen wastewater had a consistent level of moisture due to the clogged inlet pipe. However, the trench receiving only enzymatic treated kitchen wastewater had the highest level of moisture due to the formation of a biomat.
- VS levels in the trench receiving Sanitary wastewater were consistently low. The trench receiving Sanitary/treated kitchen wastewater showed higher VS levels than the trench receiving Sanitary/kitchen wastewater. The VS levels of trench receiving Kitchen wastewater showed no significant difference in different trench location, whereas significant VS levels in different trench location were found in the trench receiving Treated kitchen wastewater. These results indicate that there is a greater microorganism population in enzyme pretreated wastewater.
- Inlet pipes from the trenches receiving Sanitary wastewater and Sanitary/treated kitchen wastewater were found to be relatively clean. The inlet pipe receiving the Sanitary/kitchen showed signs of clogging because of FOG accumulation. The inlet pipe receiving Kitchen wastewater was clogged. However, the inlet pipe receiving Treated kitchen wastewater was also partially clogged but this was caused by the extensive biomat growth.
- Since the bench-scale drain field meet Michigan Department of Public Health criteria, scaling up will likely perform as the bench-scale drain field. Scaling up may result in a

different quantity of bacteria because of the lower temperature in an actual system, however, the the bacteria community would not be different. Further, FOG does not coagulate at higher temperatures but the ambient temperature in which the trench was operated is not high enough to significantly impact the FOG. However, the bench-scale trench has walls with a width of 61 cm (24 inch), therefore, the spreading of influent water and oxygen may be different with field demonstration drain field which has no walls.

Overall, results from the laboratory trenches show pretreatment of restaurant wastewater using an in-situ enzyme producing bacteria (enzyme generator) will delay clogging and, consequently, premature drain field aging. This research verified that the mechanism was that enzyme treatment helps the FOG to become more biodegradable which reduces the risk of physical clogging. The resulting increase in the biodegradable organic load may increase the growth of the biomat but if the drain field is clogged by a biomat, it can be rested and will recover after several months. However, if it is clogged by the accumulation of FOG, portions of the drain field must be replaced. Designing the drain field based on organic loading, instead of the standard hydraulic loading, is recommended when pretreating with enzymes. Alternatively, constructing two separated drain fields; one for operation and another one at rest, is also an option.

The cost for installing AGIS is approximately \$10,000 and annual operation (aeration) is approximately \$204 (300 W, 24 hours per day, always on) (Duke Energy 2015). This cost is substantially less than replacing a commercial drain field which will cost tens or hundreds of thousands dollar.

5.2. Recommendation

Bench-scale trenches demonstrated the effectiveness of enzymatic pretreatment. Further research is justified to examine additional variables.

- This study used sandy loam for the soil type, which resulted in the significant removal of COD, BOD₅, TP and FOG. The Michigan Criteria for Subsurface Sewage Disposal provides multiple options for soil type, such as coarse sand, medium sand, sandy loam and loamy sand. Depending on the soil type, the hydraulic loading of a drain field can be determined. Repeating this research using these different soil types is suggested.
- Precipitation and snow can impact the performance of a drain field because the efficiency of drain field decreases when there is an increase in the hydraulic loading. Simulation of precipitation is suggested.
- Bench-scale drain field performed identically to a typical drain field during the operation period (505 days). A field demonstration for a longer period of operation is recommended. Field demonstrations are more realistic as seasonal and precipitation influences are included. Installation of moisture content sensors in the field demonstration is recommended.
- Biomat is formed by an excessive anaerobic microorganism, resulting from high organic loadings. A treatment system using enzymatic pretreatment and then an advanced treatment unit may prevent any premature aging due to the increase formation of a biomat which is caused by higher biodegradable organic load. Demonstrating a combination of both systems is recommended.

For future research, the following changes to the experimental design are recommended.

- Formulating kitchen wastewater from its components (rinsing sink, washing dish, mop water, sanitization, washing machine and grill grease) was challenging. In future studies, a septic tank for kitchen wastewater prior to enzyme treatment is suggested so that typical and consistent samples can be obtained.
- Constructing bench-scale trenches was a challenge, because there were no previous studies or instructions. Plexiglass sheets were used for the walls of the bench-scale trenches and rounded wood frames to support the weight of the soil. To avoid leakages, plastic sheeting was embedded in the trench. A major reason for custom building the trenches was that the required container size of 61 x 122 x 122 cm (24 x 48 x 48 inch) was not available. In future studies, a 330 gal intermediate bulk container (IBC) with welded galvanized tubular steel frame is recommended because it is more economical, less time consuming to build and has a lower risk of leaking. The typical size of an IBC container is 122 x 102 x 135 cm (48 x 40 x 53 inch) which is not a correct for the trench. However, a partition wall inside the container can be constructed.
- As moisture content sensors showed the potential clogging of a trench, however, installing oxygen sensors in addition to moisture content sensors is suggested in order to show the potential clogging by a biomat. Since biomat grow occurs in an anaerobic condition, oxygen sensor can display potential biomat formation by determining whether the soil environment is aerobic or anaerobic.
- Average of moisture contents were shown as daily values. This measurement was not sensitive enough to detect water flow. Representing values as average of 10 minute

segments was sensitive enough to detect water flow, however 10 minute segments will overload the datalogger memory without frequent data downloading. Investigating the optimal segment for measuring moisture content is suggested so that the datalogger memory is not overloaded but also to be sensitive enough for water flow detection.

- Cost and performance comparisons of commercial enzyme applications are suggested, including capital cost, operation cost, enzyme activity, FOG removal and nutrient removal.
- Evaluation of enzyme activity is suggested. In order to measure enzyme activity, there are titrimetric determination, colorimetric assay, spectrophotometric determination, fluorometry and chromatography (Stoytcheva et al. 2015). Enzyme activity shows the effectiveness of the enzyme pretreatment and the influence on performance and/or premature of aging the drain field.
- Microbial community analysis is suggested in order to understand the impact of different characteristics of wastewater with/without enzyme pretreatment on the microbial community in a drain field. Bacteria play a significant role in treatment, because each bacterium has a potential function for treatment. Table 9 shows the common phylum, genus and species of microorganisms in a typical drain field soil and species that have a potential function of oil degradation. These are the target genus groups and species that are suggested to be identified and compared during the analysis.

Table 9. Microbial Community in a drain field (Atoyan et al. 2013)

Phylum	Genus and Species	Potential function	Phylum	Genus and Species	Potential function
Acidobacteria	Terriglobus roseus	Extracellular polysaccharide production	α -Proteobacteria	Methylocystis sp.	CH ₄ oxidation
Actinobacteria	Leucobacter komagatae	Biosurfactant production		Labrys sp.	Unknown
	Mycobacterium arupense	Pathogen		Erythrobacter sp.	Aerobic phototrophic bacteria
	Mycobacterium sp.	Pathogen; PAH degradation		Sphingobium sp.	Degradation of phenolic compounds
	Rhodococcus coprophilus	Phenol degradation		Sphingopyxis sp.	Degradation of polyvinyl alcohols
	Acinetobacter	Oil degrader			
	Arthrobacter	Oil degrader			
Bacteroidetes	Flavobacterium succinicans	Cellulose & polysaccharide degradation	β -Proteobacteria	Acidovorax defluvii	Denitrification
	Flavobacterium	Oil degrader		Acidovorax facilis	Degradation of polyhydroxyalkanoates
Firmicutes	Bacillus sp.	Pathogen; various Oil degrader		Thiobacillus sp.	Fe, S & S ²⁻ oxidation
	Clostridium sp.	Pathogen; various		Dechloromonas sp.	Perchlorate reduction
Nitrospirae	Nitrospira sp.	NO ₂ ⁻ oxidation		Rhodocyclus tenuis	Purple, non-S photosynthetic bacteria;
					methanol & formate oxidation
				Zoogloea ramigera	Extracellular polysaccharide production
α -Proteobacteria	Caulobacter sp.	Unknown	δ -Proteobacteria	Desulfovibrio desulfuricans	SO ₄ ²⁻ & NO ₃ ⁻ reduction
	Phenylobacterium sp.	Degradation of chlorinated N-heterocyclics	γ -Proteobacteria	Legionella pneumophila	Pathogen
		& linear alkylbenzenesulfonates		Methylosarcina sp.	Methane oxidation
	Beijerinckia sp.	Non-symbiotic N fixation;		Pseudomonas stutzeri	Pathogen; denitrification; degradation of CCl ₄
		degradation of aromatic compound		Pseudomonas umsongensis	Various
	Afipia sp.	Pathogen		Pseudomonas sp.	Various
	Bradyrhizobium elkanii	Symbiotic N fixation		Luteibacter rhizovicius	Chitin degradation
	Nitrobacter vulgaris	NO ₂ ⁻ oxidation		Lysobacter sp.	Glucan & chitin degradation
	Methylocystis parvus	CH ₄ oxidation		Thermomonas sp.	Fe ²⁺ oxidation; NO ₃ ⁻ reduction
			Proteobacteria	Achromobacter	Oil degrader
				Alcaligenes	Oil degrader
				Pseudomonas spp.	Oil degrader

Transforming FOG to become more biodegradable is not only beneficial to sewer clogging but also increasing biogas production in anaerobic digestion as shown in

- Table 10. Further studies to investigate the cost benefit are warranted.

Table 10. Literature reviews of enzyme activity on biogas production

Sludge	Enzymes	Results	Reference
Biological Sludge	Mixture of lipase, glycosidic enzymes	Improved solubilisation of organic matter (20 - 40 %) Reduced vitality Increased methane production(60 %)	(Wawrzynczyk et al. 2003)
Primary & waste activated sludge mixture(WWTP)	Mixtures of enzymes	Increased methane yield(60 %)	(Davidsson & Jansen 2006)
Mixed waste water sludge	Two glycosidic enzymes	Increase methane production (20%) Improved dewatering properties	(Wawrzynczyk et al. 2008)
Anaerobically digested sludge	Protease, lipase, & other	Improved dewatering properties	(Ayol et al. 2008)
Primary sewage sludge (WWTP)	Mixture of enzymes	Reduction of total solids (80 %)	(Roman et al. 2006)
Effluents form slaughterhouse	Lipase (<i>Penicillium restrictum</i>)	High COD removal (85 %) Increased bio gas production (37 ml to 175 ml)	(Valladão et al. 2007)
Nile perch processing waste water	Lipase (<i>Penicillium restrictum</i>)	Methane yield increment (76 %)	(Gumisiriza et al. 2009)
Synthetic waste water (COD 10,000-12,000 & BOD ₅ 7,000-9000 mg/L and oil & grease 200-1200 mg/L)	Lipase (<i>Penicillium restrictum</i>)	High COD removal (91 %) Oil & grease removal (82 %) Similar Biogas production (before and after) Cost effectiveness (1 % w/v) for oil & grease 700-800 mg/L	(Cammarota et al. 2001)
Synthetic waste water (powder milk and fat)	Lipase (<i>Penicillium restrictum</i>)	High COD removal (90%) Same methane percentage in biogas (~65%)	(Leal et al. 2006)
Food waste (vegetable: grain: meat =49:31:17(W/W))	Mixture of enzymes (Carbohydraz :protease :Lipase) (Note: Lipase from <i>Candida rugosa</i>)	At 1:2:1 of C:P:L, Achieved High COD removal (>95 %), VSS reduction (61 %), and Methane content(67-75 %)	(Moon & Song 2011)
Pet food waste water	Lipase (<i>Candida rugosa</i>)	Achieved 90% of Overall COD and oil&grease removal Methane to COD yield of 80 %	(Jeganathan et al. 2006)
Meat-processing waste	Lipase (<i>Candida rugosa</i>)	Achieved high COD removal (60 %) Fat hydrolysis (28 %) Methane production was same	(Cavaleiro et al. 2013)

APPENDICES

APPENDIX A: Construction pictures



Figure 66. Effluent pipes



Figure 67. Trench construction



Figure 68. Three effluent outlets



Figure 69. Moisture sensors locations



Figure 70. Inside of inlet distribution pipe

APPENDIX B: Analytical result

Table 11. Phase 2 performance result

		Sanitary		Sanitary/kitchen		Sanitary/treated kitchen		Treated kitchen	
		Influent	Effluent	Influent	Effluent	Influent	Effluent	Influent	Effluent
COD (mg/L)	n	12	12	12	12	12	12	12	12
	Avg.	118.3	28.1	554.4	137.4	772.8	105.9	2907.8	213.5
	Std.	25.3	4.7	178.0	54.4	58.8	41.6	523.1	68.2
FOG (mg/L)	n	12	6	12	6	12	6	12	6
	Avg.	10.9	5.4	132.5	25.7	114.3	8.8	471.3	27.3
	Std.	2.6	1.7	24.8	3.8	27.0	4.2	104.7	13.5
TP (mg/L)	n	12	12	12	12	12	12	12	12
	Avg.	22.9	4.6	20.7	5.4	20.8	3.5	19.6	7.4
	Std.	1.9	2.2	2.9	3.3	3.3	1.0	4.9	2.5
TN (mg/L)	n	1	1	1	1	1	1	1	1
	Avg.	43.0	42.0	41.0	31.0	42.0	29.0	60.0	28.0
	Std.	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Nitrate (mg/L)	n	12	12	12	12	12	12	12	12
	Avg.	9.1	39.1	4.0	35.2	2.5	31.7	1.8	22.1
	Std.	5.5	4.1	1.6	2.9	1.3	8.7	0.6	6.4

Table 12. Phase 3 performance result

		Sanitary		Sanitary/kitchen		Sanitary/treated kitchen		Kitchen		Treated kitchen	
		Influent	Effluent	Influent	Effluent	Influent	Effluent	Influent	Effluent	Influent	Effluent
COD (mg/L)	n	12	12	12	12	12	12	12	12	12	12
	Avg.	101.8	30.3	576.4	52.9	683.3	46.8	2533.6	145.5	2740.0	179.8
	Std.	11.8	6.3	156.8	21.3	165.0	11.2	432.0	26.4	291.7	72.7
BOD (mg/L)	n	1	1	1	1	1	1	1	1	1	1
	Avg.	119.0	28.0	318.8	143.9	321.4	164.9	707.8	128.4	698.8	150.4
	Std.	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
FOG (mg/L)	n	12	3	12	3	12	3	12	3	12	3
	Avg.	14.6	6.5	150.3	25.0	147.4	11.1	560.5	42.7	587.8	29.2
	Std.	8.0	2.7	44.3	3.7	25.5	7.4	231.5	13.5	152.8	10.9
TP (mg/L)	n	12	12	12	12	12	12	12	12	12	12
	Avg.	22.8	6.6	20.4	6.4	20.9	5.2	16.9	1.8	22.5	2.8
	Std.	4.0	2.7	3.5	2.3	3.5	2.0	3.1	0.7	8.2	1.8
TN (mg/L)	n	12	12	12	12	12	12	12	12	12	12
	Avg.	36.1	37.1	40.4	24.0	40.1	30.8	51.3	33.4	57.3	20.3
	Std.	3.4	4.0	6.0	4.2	6.7	5.2	11.2	7.2	12.7	7.6
Ammonia (mg/L)	n	12	12	12	12	12	12	12	12	12	12
	Avg.	27.3	0.1	39.8	1.5	28.6	0.8	20.5	0.2	44.5	3.8
	Std.	6.8	0.2	7.2	1.1	3.5	0.6	7.1	0.3	10.6	2.3
Nitrate (mg/L)	n	12	12	12	12	12	12	12	12	12	12
	Avg.	5.3	38.7	2.0	22.4	2.1	30.7	1.1	30.8	1.7	3.5
	Std.	4.0	3.2	2.6	3.1	4.1	5.5	0.4	8.0	0.7	4.9

Table 13. Phase 4 performance result

		Sanitary		Sanitary/kitchen		Sanitary/treated kitchen		Kitchen		Treated kitchen	
		Influent	Effluent	Influent	Effluent	Influent	Effluent	Influent	Effluent	Influent	Effluent
COD (mg/L)	n	20	20	20	20	20	20	20	20	20	20
	Avg.	119.8	29.3	703.9	59.9	740.6	47.5	2615.4	152.9	2903.8	133.4
	Std.	22.4	7.0	110.7	19.0	132.3	19.5	268.2	40.9	256.3	35.6
BOD (mg/L)	n	19	15	18	18	18	19	18	18	19	17
	Avg.	102.6	31.4	327.9	72.4	406.6	63.3	1120.3	118.5	1267.9	84.3
	Std.	24.8	9.9	50.1	24.9	95.6	16.7	325.4	43.0	359.1	25.1
FOG (mg/L)	n	3	3	3	3	3	3	3	3	3	3
	Avg.	12.8	2.6	180.3	32.0	184.3	6.5	588.7	48.7	605.0	27.3
	Std.	3.0	3.3	19.2	4.6	29.3	3.5	19.6	9.5	31.6	6.5
TP (mg/L)	n	20	20	20	20	20	20	20	20	20	20
	Avg.	21.0	8.2	23.8	7.8	23.8	7.9	21.7	6.7	23.5	3.0
	Std.	1.3	2.1	2.7	2.8	2.4	2.0	7.6	4.3	3.6	1.3
TN (mg/L)	n	20	20	20	20	20	20	20	20	20	20
	Avg.	37.9	37.3	46.1	28.8	42.7	38.8	45.4	39.0	54.7	14.6
	Std.	5.5	5.7	6.0	5.1	6.5	5.0	10.8	10.5	11.4	2.1
Ammonia (mg/L)	n	20	20	20	20	20	20	20	20	20	20
	Avg.	32.5	0.3	36.9	0.4	32.1	0.5	35.7	0.3	42.4	6.9
	Std.	6.1	0.4	6.0	0.6	5.8	0.7	7.7	0.4	8.9	2.9
Nitrate (mg/L)	n	20	20	20	20	20	20	20	20	20	20
	Avg.	1.9	37.4	2.4	22.7	3.1	29.2	1.9	38.4	2.6	9.4
	Std.	4.7	4.1	2.6	3.8	1.9	3.3	1.3	10.3	1.4	4.0

APPENDIX C: QAQC

Table 14. Relative percentage difference between standards

	Phase 1	Phase 2	Phase 3	Phase 4	Average
COD (%)	9.6	8.4	10.2	12.1	10.1
BOD (%)	N/A	N/A	15.4	11.4	13.4
FOG (%)	12.1	10.4	11.2	8.9	10.7
TP (%)	6.9	7.2	12.1	4.5	7.7
TN (%)	N/A	4.2	5.2	4.9	4.8
Ammonia (%)	N/A	N/A	3.1	3.6	3.4
Nitrate (%)	5.6	3.4	4.2	2.4	3.9
Average	8.6	6.7	8.8	6.8	7.7

Table 15. Relative percentage difference between replicates

	Phase 1	Phase 2	Phase 3	Phase 4	Average
COD (%)	9.1	6.8	7.7	9.4	8.3
BOD (%)	N/A	N/A	13.2	10.1	11.7
FOG (%)	5.9	4.6	6.4	5.8	5.7
TP (%)	3.5	4.4	3.1	4.9	4.0
TN (%)	N/A	4.5	5.8	6.2	5.5
Ammonia (%)	N/A	N/A	11.5	10.4	11.0
Nitrate (%)	4.2	4.9	5.8	6.1	5.3
Average	5.7	5.0	7.6	7.6	7.3

REFERENCES

REFERENCES

- Atoyan, J. et al., 2013. Microbial Community Structure of a Leachfield Soil: Response to Intermittent Aeration and Tetracycline Addition. *Water*, 5(2), pp.505–524. Available at: <http://www.mdpi.com/2073-4441/5/2/505/> [Accessed December 2, 2014].
- Ayol, A. et al., 2008. Aerobic and anaerobic bioprocessing of activated sludge: floc disintegration by enzymes. *Journal of environmental science and health. Part A, Toxic/hazardous substances & environmental engineering*, 43(13), pp.1528–1535.
- Aziz, T.N. et al., 2011. Performance of Grease Abatement Devices for Removal of Fat, Oil, and Grease. *Journal of Environmental Engineering*, 137(1), pp.84–92.
- Bio-Safe One, 2011. The truth about drain field replacement. , p.1. Available at: <http://www.biosafeone.com/bio112.html> [Accessed August 12, 2013].
- Bio-systems International, BIO-FLO. *Bio-systems International M.S.D.S.* Available at: <http://www.biobugs.com/Products.html>.
- Brooksbank, a. M., Latchford, J.W. & Mudge, S.M., 2007. Degradation and modification of fats, oils and grease by commercial microbial supplements. *World Journal of Microbiology and Biotechnology*, 23(7), pp.977–985.
- Cammarota, M.C. & Freire, D.M.G., 2006. A review on hydrolytic enzymes in the treatment of wastewater with high oil and grease content. *Bioresource Technology*, 97(17), pp.2195–2210.
- Cammarota, M.C., Teixeira, G. a. & Freire, D.M.G., 2001. Enzymatic pre-hydrolysis and anaerobic degradation of wastewaters with high fat contents. *Biotechnology Letters*, 23(19), pp.1591–1595.
- Campbell Scientific, 2014. CS616-L Water Content Reflectometer. Available at: <https://www.campbellsci.com/cs616-l>.
- Cavaleiro, a. J. et al., 2013. Biochemical methane potential of raw and pre-treated meat-processing wastes. *Bioresource Technology*, 129, pp.519–525. Available at: <http://dx.doi.org/10.1016/j.biortech.2012.11.083>.
- Chan, H., 2010. Removal and recycling of pollutants from Hong Kong restaurant wastewaters. *Bioresource Technology*, 101(17), pp.6859–6867. Available at: <http://dx.doi.org/10.1016/j.biortech.2010.03.104>.
- Chu, W. & Ng, F.-L., 2000. Upgrading the conventional grease trap using a tube settler. *Environment International*, 26(1), pp.17–22.

- Cole-Parmer, 2015. Digi-Sense Type-K PVC-Ins Probe. Available at:
http://www.coleparmer.com/Product/Digi_Sense_Type_K_PVC_Ins_Probe_Epoxy_Coated_Tip_Mini_Con_Undgrd_10Ft_20_Gauge/EW-08466-04#Specs.
- Cui, L.-H. et al., 2003. TREATMENT AND UTILIZATION OF SEPTIC TANK EFFLUENT USING VERTICAL FLOW CONSTRUCTED WETLANDS AND HYDROPONIC CULTIVATION OF VEGETABLES. *Journal of environmental sciences (China)*, 15(1), pp.75–82.
- Daverey, A. & Pakshirajan, K., 2015. Treatment of dairy wastewater containing high amount of fats and oils using a yeast-bioreactor system under batch, fed-batch and continuous operation. *Desalination and Water Treatment*, (June), pp.1–7. Available at:
<http://www.tandfonline.com/doi/abs/10.1080/19443994.2014.1003609>.
- Davidsson, A. & Jansen, 2006. Pre-treatment of wastewater sludge before anaerobic digestion – Hygienisation, Ultrasonic treatment and Enzyme dosing. *Vatten*, 64(4), pp.335–340.
- Dharmsthiti, S. & Kuhasuntisuk, B., 1998. Lipase from *Pseudomonas aeruginosa* LP602: biochemical properties and application for wastewater treatment. *Journal of Industrial Microbiology and Biotechnology*, 21(1-2), pp.75–80.
- Duke Energy, 2015. Electric Appliance Operating Cost List. *Duke Energy*, p.8. Available at:
http://www.duke-energy.com/pdfs/Appliance_OpCost_List_Duke_v8.06.pdf.
- Eilers, K.G. et al., 2012. Digging deeper to find unique microbial communities: The strong effect of depth on the structure of bacterial and archaeal communities in soil. *Soil Biology and Biochemistry*, 50, pp.58–65. Available at:
<http://linkinghub.elsevier.com/retrieve/pii/S0038071712001137> [Accessed October 25, 2014].
- Ellis, T., 2004. Chemistry of wastewater. *Eolss Publishers*, p.1. Available at:
<http://www.eolss.net/eolssamplechapters/c06/e6-13-04-05/e6-13-04-05-txt-03.aspx>
 [Accessed January 1, 2015].
- Gajeski, G., 2010. Enzymes help cut through fats, oils and greases in septic tank, drain line and sewer applications. *Lenzyme*, p.1. Available at:
http://www.lenzyme.com/Grease_Education_Clearing_the_F.html [Accessed January 7, 2015].
- Gregory, K., 2014. New York Tries to Rid Its Sewers of FOG (Fat, Oil and Grease). *The New York Times*. Available at: http://www.nytimes.com/2014/02/15/nyregion/new-york-tries-to-clear-its-sewers-of-fog-fat-oil-and-grease.html?_r=0.

- Gumisiriza, R. et al., 2009. Enhancement of anaerobic digestion of Nile perch fish processing wastewater. *African Journal of Biotechnology*, 8(2), pp.328–333. Available at: <http://www.ajol.info/index.php/ajb/article/view/59806>.
- HACH, 2015a. EPA Compliant Methods. *HACH Company*. Available at: <http://www.hach.com/epa> [Accessed January 1, 2015].
- HACH, 2015b. Oil and Grease. , Edition 9(Method 10300).
- Hammerlund, D. & Glotfelty, B., 2008. Onsite Sewage Disposal Of High Strength Wastewater High Strength Waste. *Source Water Assessment Program*, p.1.
- Heger, S., 2013. *Loading rates How much can the soil take?*,
- Heger, S.F., Schmidt, D.R. & Janni, K.A., 2010. AEROBIC AND MEDIA FILTER TREATMENT SYSTEMS FOR MILK HOUSE WASTEWATER ON SMALL DAIRY OPERATIONS. *American Society of Agricultural and Biological Engineers*, 26(2), pp.319–328.
- Hossain, F., 2010. *Comparison of Traditional Standard Drainfield with Innovative B&G Treatment Bed for Nutrient Removal from Septic Tank Wastewater*. University of Central Florida. Available at: http://etd.fcla.edu/CF/CFE0003271/Hossain_Fahim_201008_Ph.D.pdf.
- Jeganathan, J., Bassi, A. & Nakhla, G., 2006. Pre-treatment of high oil and grease pet food industrial wastewaters using immobilized lipase hydrolyzation. *Journal of Hazardous Materials*, 137(1), pp.121–128.
- Kunst, F. et al., 1997. The complete genome sequence of the gram-positive bacterium *Bacillus subtilis*. *Nature*, 390(6657), pp.249–256.
- Leal, M.C.M.R. et al., 2006. Effect of enzymatic hydrolysis on anaerobic treatment of dairy wastewater. *Process Biochemistry*, 41(5), pp.1173–1178.
- Leverenz, H., Tchobanoglous, G. & Darby, J., 2002. *REVIEW OF TECHNOLOGIES FOR THE ONSITE TREATMENT OF WASTEWATER IN CALIFORNIA*, California.
- Markossian, S. et al., 2000. Isolation and characterization of lipid-degrading *Bacillus thermoleovorans* IHI-91 from an icelandic hot spring. *Extremophiles : life under extreme conditions*, 4(6), pp.365–371.
- Masse, L., Kennedy, K.J. & Chou, S., 2001. Testing of alkaline and enzymatic hydrolysis pretreatments for fat particles in slaughterhouse wastewater. *Bioresource Technology*, 77(2), pp.145–155.

- Matejcek, B.C., Erlsten, S. & Bloomquist, D., 2000. *Determination of Properties and the Long Term Acceptance Rate of Effluents from Food Service Establishments that Employ Onsite Sewage Treatment*, Gainesville, Florida.
- May, R., 1996. Problems Associated with the Use of Gravel in Septic Tank Leachfield Systems. *Journal of Environmental Health* 1, 59(2).
- Mazlin, M.A., 2012. *BIODEGRADATION OF USED COOKING OIL BY INDIGENOUS BACTERIA*. UNIVERSITI TEKNOLOGI MALAYSIA.
- Mckenzie, C., 2010. How to Maintain your Drainfield. *AquaKlear Engineering*. Available at: <http://www.aquaklear.com/blog/sewage-treatment-systems/how-to-maintain-your-drainfield/>.
- Metcalf & Eddy, 2003. *Wastewater Engineering Treatment, Disposal, and Reuse* Third Edit., McGraw-Hill.
- Michigan Department of Public Health, 1994. Michigan Criteria for Subsurface Sewage Disposal.
- Moon, H.C. & Song, I.S., 2011. Enzymatic Hydrolysis of FoodWaste and Methane Production Using UASB Bioreactor. *International Journal of Green Energy*, 8(3), pp.361–371.
- Naylor, S. & Gustin, A., 2015. Geothermal Ground Source Heat Pumps and Geology in Indiana. *Indiana Geological Survey*. Available at: <http://igs.indiana.edu/Geothermal/HeatPumps.cfm>.
- NCH Waste Water, 2011. Overview of BioAmp system. *NCH Europe*, p.1. Available at: <http://www.bioamp.co.uk/overview> [Accessed January 1, 2015].
- NESC, 2005. Drainfield Rehabilitation. , 16(1), pp.1–8.
- Northeast Region Certified Crop Advisor, 2010. Competency Area 2: Soil hydrology AEM. *Cornell University*. Available at: <http://nrcca.cals.cornell.edu/soil/CA2/CA0212.1-3.php> [Accessed January 1, 2015].
- Owens, P.R. & E. Moye Rutledge, 2011. Septic Drain Field Design and Maintenance. *Phosphorus Best Management Practices*.
- Peacock, W. & Christensen, L., 2000. *Interpretation of soil and water analysis*, Oakland, Calibronia: UCANR. Available at: <http://books.google.com/books?hl=en&lr=&id=rMoAET18u9YC&oi=fnd&pg=PA115&dq=Interpretation+of+Soil+and+Water+Analysis&ots=0gLv7wMDO2&sig=ySYQik0IYLyDgQPt->

4P8NWdeVT0\http://books.google.com/books?hl=en&lr=&id=rMoAET18u9YC&oi=fnd
&pg=PA115&dq=Interpretation+.

- Pettersson, M. & Erland, B., 2003. Temperature-dependent changes in the soil bacterial community in limed and unlimed soil. *FEMS Microbiology Ecology*, 45(1).
- Rashid, R., Zaharudin, N.A. & Idris, A., 2014. Enzymatic hydrolysis of used-frying oil using *Candida rugosa* lipase. *Jurnal Teknologi (Sciences and Engineering)*, 67(3), pp.101–107.
- Ratcliffe, R., 2015. 10-tonne fatberg removed from west London sewer. *theguardian*. Available at: <http://www.theguardian.com/uk-news/2015/apr/21/huge-10-ton-fatberg-removed-chelsea-sewer-london>.
- Roheim, J., 2003. Low cost automated on site system for growing and dispensing vegetative bacteria. *Proceedings of the Water Environment Federation, Industrial Wastes*, pp.634–647.
- Roman, H.J., Burgess, J.E. & Pletschke, B.I., 2006. Enzyme treatment to decrease solids and improve digestion of primary sewage sludge. *African Journal of Biotechnology*, 5(May), pp.963–967.
- San Francisco Water Power Sewer, 2012. Commercial Fats, oil and grease(FOG) control. Available at: <http://sfwater.org/index.aspx?page=480>.
- Scopes, R.K., 2002. Enzyme Activity and Assays. *Encyclopedia of Life Sciences*, pp.1–6. Available at: [http://www.life.illinois.edu/biochem/455/Lab exercises/B-gal/enzymology.pdf](http://www.life.illinois.edu/biochem/455/Lab%20exercises/B-gal/enzymology.pdf).
- Sludgehammar, 2015. HOW IT WORKS. *Sludgehammar*, p.1. Available at: <http://www.sludgehammer.net/>.
- Stoytcheva, M. et al., 2015. Analytical methods for lipases activity determination : A review Analytical Methods for Lipases Activity Determination : A Review. , (July).
- Sustainable Environmental Technologies, 2012. AGIS Technology. Available at: <http://set-agis.com/agis-technology/>.
- Sustainable Environmental Technologies, 2009. *Sagebrush Cantina Fenton Township*,
- Tang, H.L., Xie, Y.F. & Chen, Y.C., 2012. Use of Bio-Amp, a commercial bio-additive for the treatment of grease trap wastewater containing fat, oil, and grease. *Bioresource Technology*, 124, pp.52–58. Available at: <http://dx.doi.org/10.1016/j.biortech.2012.08.012>.
- Tano-Debrah, K. et al., 1999. An inoculum for the aerobic treatment of wastewaters with high concentrations of fats and oils. *Bioresource Technology*, 69(2), pp.133–139.

- Ting, W.J. et al., 2006. Application of binary immobilized *Candida rugosa* lipase for hydrolysis of soybean oil. *Journal of Molecular Catalysis B: Enzymatic*, 42(1-2), pp.32–38.
- Town of Tyngsborough, 2015. *Fats, Oils And Grease (FOG)*, Massachusetts. Available at: <http://www.tyngsboroughma.gov/government/departments/sewer/what-not-to-flush-online-guide/>.
- U.S. Environmental Protection Agency, 2014. *Food Waste Scoping Analysis*,
- U.S. Environmental Protection Agency, 2007. National Pretreatment Program. , 30 CFR 403.
- U.S. Environmental Protection Agency, 2004. Report to Congress on Impacts and Control of Combined Sewer Overflows and Sanitary Sewer Overflows. Available at: <http://water.epa.gov/polwaste/npdes/cso/2004-Report-to-Congress.cfm>.
- Valladão, A.B.G., Freire, D.M.G. & Cammarota, M.C., 2007. Enzymatic pre-hydrolysis applied to the anaerobic treatment of effluents from poultry slaughterhouses. *International Biodeterioration and Biodegradation*, 60, pp.219–225.
- De Waard, P. et al., 1993. Heteronuclear NMR analysis of unsaturated fatty acids in poly(3-hydroxyalkanoates). Study of beta-oxidation in *Pseudomonas putida*. *The Journal of biological chemistry*, 268(1), pp.315–319.
- Wakelin, N.G. & Forster, C.F., 1997. An investigation into microbial removal of fats, oils and greases. *Bioresource Technology*, 59(1), pp.37–43. Available at: <http://linkinghub.elsevier.com/retrieve/pii/S0960852496001344>.
- Wakelin, N.G. & Forster, F., 1998. THE AEROBIC TREATMENT OF GREASE-CONTAINING FAST FOOD RESTAURANT WASTEWATERS. *Trans IChemE*, 76(February).
- Washtenaw County, 2014. FOG-Fats, Oil and Grease. Available at: http://www.ewashtenaw.org/government/departments/environmental_health/food_safety/eh_fatsoilsgrease.html.
- Wawrzynczyk, J. et al., 2003. Alternative method for sludge reduction using commercial enzymes. In *Aqua Enviro Technology Transfer: Eighth CLWEM/Aqua Enviro European Biosolids and Organic Residuals Conference*. Wakefield West Yorkshire, UK, pp. 1–5.
- Wawrzynczyk, J. et al., 2008. The function of cation-binding agents in the enzymatic treatment of municipal sludge. *Water Research*, 42(6-7), pp.1555–1562.
- Wodalski, E., 2012. Up To the Challenge. *Pumper*, p.1. Available at: http://www.pumper.com/editorial/2012/03/up_to_the_challenge.