HE, XIA. Characterization of Grease Interceptors for Removing Fat, Oil and Grease (FOG) and Mechanisms of FOG Deposit Formation in Sewer Systems. (Under the direction of Dr. Francis L. de los Reyes III).

Significant amounts of fat, oil and grease (FOG) in wastewater are discharged from Food Service Establishments (FSEs), multifamily housing, and single family homes. FOG must be separated from wastewater before it enters the sewage system, primarily due to its propensity to cause blockages in sanitary sewer collection lines. These FOG blockages, in the property owner’s sewer lateral or the town/city sewerage system lead to sanitary sewer overflows (SSOs) that cause untreated sewage to flow onto streets and travel to storm drains, creeks, and other surface waters. Of the estimated tens of thousands of sanitary sewer overflows (SSOs) that occur each year in the United States, approximately 48% are due to line blockages, of which 47% are related to FOG deposits that constrict the cross-sectional access of pipe. The presence of FOG in wastewater also results to significant problems in conventional biological treatment systems.

Reduction in the levels of FOG is thus highly desirable. Hence, grease interceptors (GIs) are installed between wastewater effluent points and the sewer system to allow FOG to be trapped. A potential approach for reducing the levels of FOG in the GI effluent is bioaugmentation. However, there have been very few long-term assessments of the physico-chemical and microbial characteristics of full-scale grease interceptors (GIs). In the first phase of this study, Full-scale GIs were monitored over a year with and without bioaugmentation (treated and untreated cycles). Statistically significant differences between treated and untreated cycles were detected for several chemical and physical parameters. The
treated cycles had lower BOD and COD at the grease interceptor outlet. While the combined treated cycle data did not show lower FOG concentrations in the GI outlet compared to the combined untreated cycle data, comparison of individual treated and untreated cycles show a positive effect due to the addition of product. Differences in the microbial community structure between treated and untreated cycles were detected by Terminal Restriction Fragment Length Polymorphism (T-RFLP) analysis and the clone and sequencing results. The shifts in the microbial composition were revealed. Taken together, the data shows that the addition of biological products results in changes in the GI chemistry and microbial ecology; these changes had no adverse effects, and in some cases positive effects, on COD, BOD, and FOG degradation in grease interceptors.

Despite the central role that FOG deposits play in SSOs, little is known about the mechanisms of FOG deposit formation in sanitary sewers. In the second phase of this study, FOG deposits were first formed under laboratory condition from the reaction between free fatty acids and calcium chloride. The FTIR data revealed that the FOG deposits (both laboratory-produced and natural FOG deposits) are metallic salts of fatty acids by comparisons with FOG deposits and pure calcium soaps. We demonstrated that calcium, the dominant metal in FOG deposits, was found to be released from concrete under different pH conditions. Oil played the role as a carrier of free fatty acids instead of the source of FFAs in surface reaction that is responsible for FOG deposit formation in sewer lines. The effect of different fatty acids on surface reaction was determined. The data indicated that stickier solid was formed on concrete surface and more severe concrete corrosion occurred when using
unsaturated fatty acids than saturated fatty acids. Taken together, a comprehensive understanding of the mechanisms of FOG deposit formation in sewer systems was proposed.

By applying the knowledge of the proposed mechanisms, a pilot-scale pipe loop system was set-up to simulate gravity flow pipelines and pump-station wet wells, and to directly assess FOG deposit formation in sewer lines under controlled conditions.
Characterization of Grease Interceptors for Removing Fat, Oil and Grease (FOG) and Mechanisms of FOG Deposit Formation in Sewer Systems

by
Xia He

A dissertation submitted to the Graduate Faculty of North Carolina State University in partial fulfillment of the requirements for the Degree of Doctor of Philosophy

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APPROVED BY:

Francis L. de los Reyes III
(Chair of Advisory Committee)

Joel J. Ducoste
(Vice-chair of Advisory Committee)

Detlef Knappe
(Advisory Committee)

Lisa O. Dean
(Advisory Committee)
DEDICATION

I dedicate this work to Guorong Yang and Ge He for their unconditional love and patience.
BIOGRAPHY

Xia He received her Bachelor of Science degree in Water Supply & Drainage Engineering from Xi’an University of Technology in 2004. She then joined the Environmental Science & Engineering at Shanghai Jiao Tong University, where she attended a wastewater control group and got involved in a research project funded by the National Nature Science Foundation of China to study nitrogen removal from wastewater by heterotrophic nitrification. She finished her Master of Science degree in 2007 and joined Dr. de los Reyes’ research group at North Carolina State University in August 2007 to work on her doctoral degree; she studied about fat, oil and grease (FOG) removal from wastewater by bioaugmentation and FOG deposit formation mechanisms in sewer lines.

Xia He’s research interests encompass projects for wastewater control. Specific areas of research include the novel biological reactor design, nutrients removal from wastewater, dynamics of microbial populations in biological systems, blockages in sewer systems, and energy generation from wastewater.
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TABLE OF CONTENTS

LIST OF TABLES................................................................................................................viii
LIST OF FIGURES................................................................................................................ix
CHAPTER 1.  INTRODUCTION...........................................................................................1
  Introduction.......................................................................................................................1
  Background......................................................................................................................4
  References.....................................................................................................................8
CHAPTER 2.  PHYSICO-CHEMICAL CHARACTERIZATION OF GREASE
INTERCEPTORS WITH AND WITHOUT BIOLOGICAL PRODUCT ADDITION..............12
  Abstract.........................................................................................................................12
  Introduction.....................................................................................................................13
  Methods & Materials.....................................................................................................14
  Experimental design and sample collection.................................................................14
  Physical and chemical characterization.........................................................................16
  Statistical analysis.........................................................................................................18
  Results and Discussion.................................................................................................19
  Physical characteristics of full-scale GIs.......................................................................19
  Protein, carbohydrate and volatile fatty acids...............................................................21
  BOD and COD removal from full-scale GIs.................................................................23
  Fat, Oil and Grease removal from full-scale GIs...........................................................25
  Conclusions....................................................................................................................27
  References.....................................................................................................................29
CHAPTER 3.  MICROBIAL CHARACTERIZATION OF GREASE INTERCEPTORS FOR
REMOVING FAT, OIL AND GREASE (FOG) IN SEWER LINES........................................31
  Abstract ........................................................................................................................31
  Introduction ....................................................................................................................32
  Methods and materials .................................................................................................33
  Sampling and storage ....................................................................................................33
  DNA extraction method selection .................................................................................34
  PCR amplification .........................................................................................................35
  Terminal restriction fragment length polymorphism (T-RFLP) ....................................35
  Cloning and sequence libraries.....................................................................................36
  Results ..........................................................................................................................37
  Selection of DNA extraction method ..........................................................................37
  Terminal restriction fragment length polymorphism (T-RFLP) ....................................39
LIST OF TABLES

CHAPTER 2. PHYSICO-CHEMICAL CHARACTERIZATION OF GREASE INTERCEPTORS
WITH AND WITHOUT BIOLOGICAL PRODUCT ADDITION
Table 1. Analytical tests performed at various sampling points in each GI………………17
Table 2. Ranges of measured physical characteristics of full-scale GIs …………………..20
Table 3. Mean concentrations of protein and carbohydrate in both GIs .................22
Table 4. Mean concentrations and statistical analysis of VFAs in GI effluent ..........23

CHAPTER 3. MICROBIAL CHARACTERIZATION OF GREASE INTERCEPTORS FOR
REMOVING FAT, OIL AND GREASE (FOG) IN SEWER LINES
Table 1. DNA yield, A260/A280 and A260/A230 of three DNA extraction methods……..38

CHAPTER 4. EVIDENCE FOR FAT, OIL AND GREASE (FOG) DEPOSIT FORMATION
MECHANISMS IN SEWER LINES
Table 1. Fatty acid composition of FOG deposits ...........................................71
Table 2. Calcium and total fat amounts in FOG deposits ...............................72
LIST OF FIGURES

CHAPTER 1. INTRODUCTION

Figure 1. The formation of FOG blockage in the sewer..............................................1
Figure 2. Schematic of grease interceptor..............................................................4
Figure 3. FOG deposits in a pipe before leaning.....................................................7

CHAPTER 2. PHYSICO-CHEMICAL CHARACTERIZATION OF GREASE INTERCEPTORS WITH AND WITHOUT BIOLOGICAL PRODUCT ADDITION

Figure 1. Sampling points within and downstream of two full-scale grease interceptors.......................................................16
Figure 2. Depth of FOG layer in two compartments for GI in Ca........................................20
Figure 3. COD at outlet for GI in Ca..............................................................................24
Figure 4. COD at outlet for GI in JO.............................................................................25
Figure 5. BOD at the outlet for both GIs......................................................................25
Figure 6. Effluent FOG concentration in both GIs.....................................................28
Figure 7. Effluent FOG concentration in two treated cycles for both GIs..................29

CHAPTER 3. MICROBIAL CHARACTERIZATION OF GREASE INTERCEPTORS FOR REMOVING FAT, OIL AND GREASE (FOG) IN SEWER LINES

Figure 1. T-RFLP results for three DNA extraction methods....................................39
Figure 2. T-RFLP result for the biological product fed in the treated cycle...............40
Figure 3. T-RFLP results showing the microbial composition (as T-RF percentages) in the top layer of the GI Ca.................................................................42
Figure 4. T-RFLP results showing the microbial composition (as T-RF percentages) in the top layer of the GI JO.................................................................44
Figure 5. T-RFLP results showing the microbial composition (as T-RF percentages) at bottom of the GI Ca.................................................................46
Figure 6. T-RFLP results showing the microbial composition (as T-RF percentages) at bottom of the GI JO.................................................................47
Figure 7. T-RFLP results showing the microbial composition (as T-RF percentages) in the effluent of the GI Ca.................................................................49
Figure 8. T-RFLP results showing the microbial composition (as T-RF percentages) in the effluent of the GI JO.................................................................50
Figure 9. Distribution of clone sequences for Ca.......................................................52
Figure 10. Phylogenetic tree of sample CT2E............................................................53
Figure 11. Phylogenetic tree of sample CT3E............................................................54
Figure 12. Distribution of clone sequences for Jordan Oaks.....................................56
Figure 13. Community similarity analysis of oil layer in Ca.......................................58
Figure 14. Community similarity analysis of oil layer in JO.......................................59

CHAPTER 4. EVIDENCE FOR FAT, OIL AND GREASE (FOG) DEPOSIT FORMATION MECHANISMS IN SEWER LINES

Figure 1. FOG deposits formed under laboratory conditions....................................69
CHAPTER 5. MECHANISMS OF FAT, OIL AND GREASE (FOG) DEPOSIT FORMATION IN SEWER LINES

Figure 1. Calcium was released in de-ionized water when pH was maintained at 3, 4, 5, 6, 7 and 8 respectively ...............................................................94
Figure 2. Solids formed on concrete surface with the addition of oil in GI effluent .......95
Figure 3. Baseline corrected infrared spectra of white solids formed on concrete, beaker bottom and brown particles in solution .............................................96
Figure 4. Baseline corrected infrared spectra of soybean oil, sample under no pH adjustment, white solid sample at pH=8 and white solid sample at pH=9 .......98
Figure 5. Solids formed on the concrete surface with addition of different fatty acids ....102
Figure 6. (a) The weight of solid scraped off the concrete surface; (b) The total weight of solid both from concrete surface and solution ....................................102
Figure 7. Calcium released from concrete block with different types of fatty acids ....103
Figure 8. Baseline corrected infrared spectra of solid samples formed on concrete surface with addition of different types of fatty acids ..........................103
Figure 9. The pathway of the propagation in free radical process ..........................104
Figure 10. Proposed mechanisms of FOG deposit formation in sewer lines ..........105

CHAPTER 6. THE FORMATION OF FAT, OIL AND GREASE (FOG) DEPOSIT IN PILOT-SCALE PIPE LOOP SYSTEM

Figure 1. The pilot-scale pipe loop system ..........................................................111
Figure 2. Three concrete blocks were placed in the last section of PVC pipeline .......111
Figure 3. Solids formed in influent tank .............................................................112
Figure 4. Solids formed in effluent tank .............................................................113
Figure 5. Baseline corrected infrared spectra of solid samples taken from two tank walls and the concrete blocks suspended in the two tanks .......................113
Figure 6. Solids formed in PVC pipes ..............................................................115
Figure 7. Solids formed on the concrete surface in PVC pipe ..............................116
Figure 8. Baseline corrected infrared spectra of solid samples taken from three PVC sections and three concrete blocks in PVC pipeline ..........................116
CHAPTER 1

INTRODUCTION

Significant amounts of fat, oil and grease (FOG) in wastewater are discharged from Food Service Establishments (FSEs), multifamily housing, and single family homes. FOG must be separated from wastewater before it enters the sewage system, primarily due to its propensity to cause blockages in sanitary sewer collection lines. As shown in Figure 1, FOG first clings to the sewer pipe walls, then builds up one layer at a time making a smaller, narrower path for the water to travel through, leading eventually to system failure.

![Sewer Blockage Formation](https://www.dunn-nc.org/works/downloads/FOG.PDF)

**Figure 1** The formation of FOG blockage in the sewer (cited from a fact sheet for the proper disposal of fats, oil & grease: www.dunn-nc.org/works/downloads/FOG.PDF)

These FOG blockages, in the property owner’s sewer lateral or the town/city sewerage system lead to sanitary sewer overflows (SSOs) that cause untreated sewage to flow onto streets and travel to storm drains, creeks, and other surface waters. The US EPA estimated that there are approximately 23,000 to 75,000 SSOs in the United States each year,
corresponding to a discharge of 3 to 10 billion gallons of untreated wastewater (U.S. EPA, 2004). Of these SSOs, fifty percent occurred as a result of line blockage, and the single largest cause of these blockages (47%) was attributed to FOG accumulation in sewer lines. The raw sewage in SSOs contains pathogenic bacteria, viruses, protozoa, helminths and other organisms. SSOs may impact drinking water sources, affect the public through recreational or direct exposure, affect shellfish harvested from areas contaminated by sewage, lead to fish kills, or lead to outbreaks of toxic algae or dinoflagellates (U.S. EPA, 2004). The presence of FOG in wastewater also results to significant problems in conventional biological treatment systems. These problems are known to reduce the cell-aqueous phase transfer rates of substrates, products and oxygen through the formation of a liquid coat around the biological floc (Becker et al., 1999; Dueholm et al., 2000). In addition, filamentous microorganism blooms (bulking) and floating sludge with undesirable physical characteristics may develop (Cammarota and Freire, 2006).

Reduction in the levels of FOG is thus highly desirable. Hence, grease interceptors (GIs) are installed between wastewater effluent points and the sewer system to allow FOG to be trapped. Conventionally designed GIs can remove free and dispersed FOG by gravitational separation, whereas emulsified and dissolved FOG would pass to downstream sewer lines (Nisola et al., 2009). A potential approach for reducing the levels of FOG in the GI effluent is bioaugmentation. However, there have been no long-term assessments of the physical, chemical characteristics and microbial ecology of GIs.
In the first phase of this research, two 1000 gallon full-scale GIs were monitored over a year. A comprehensive understanding of the physical chemical characteristics of GIs as well as the effects of bioaugmentation on FOG removal is described in chapter 2: Physico-chemical characterization of grease interceptors with and without biological product addition. Chapter 2 has been accepted for publication in Water Environment Research. The microbial ecology of full-scale GIs and the change in microbial communities with the addition of a biological product is described in Chapter 3: Microbial characterization of grease interceptors for removing fat, oil and grease (FOG) in sewer lines. Chapter 3 is formatted for submission to Journal of Applied Microbiology.

Despite the central role that FOG deposits play in SSOs, very little is known about the mechanisms of FOG deposit formation in sanitary sewers. During the second phase of this research, the mechanisms of FOG deposit formation were studied, and the findings from experiments are described in two papers. One paper has been published in Environmental Science & Technology shown and is in Chapter 4 of this dissertation: Evidence for fat, oil and grease (FOG) deposit formation mechanisms in sewer lines. The other paper will be submitted to Environmental Science & Technology and is in Chapter 5 of this dissertation.

By applying the knowledge of the mechanisms of FOG deposit formation described in Chapters 4 and 5, a pilot-scale pipe loop system was set-up to simulate gravity flow pipelines
and pump-station wet wells, and to directly assess FOG deposit formation in sewer lines under controlled conditions. These results are presented in Chapter 6.

Background

Wastewater FOG is typically a mixture of fats, lipids and oil from anthropogenic sources. The current primary method for reducing FOG accumulations in sewer lines (and thus preventing SSOs) is the use of passive and mechanized grease traps or grease interceptors (GIs). GIs (Figure 2) allow wastewater flows to slow down, and with sufficient time, FOG and solids separate from wastewater. FOG and solids are held by the grease interceptor until they can be removed and disposed by rendering or land application.

Figure 2 Schematic of grease interceptor (FOG report, Town of Cary)

Several prevention strategies have been used to limit SSOs. The most common methods are source control, mechanical cleaning and grease control products (Occiano et al. 2008).
Mechanically cleaning sewer lines is an effective but expensive method for treating grease-clogged pipelines. In this method, pipelines are cleaned using high-pressure water or rodding tools, and the dislodged debris and grease are collected by a truck from a downstream manhole.

Grease control products also can reduce or eliminate grease blockages in pipelines. Enzymes such as lipases can hydrolyze triglycerides to glycerol and long-chain fatty acids and later to volatile fatty acids, making FOG available to the wastewater’s native microbes. *Candida rugosa* lipases were used in the treatment of domestic wastewaters and in the cleaning of sewer systems, cesspools and sinkholes (Jaeger and Reetz, 1998). The lipases from *Pseudomonas aeruginosa* were used to hydrolyze FOG in the effluents of restaurants (Dharmsthiti and Kuhasuntisuk, 1998). Commercial lipases from other sources (animal and vegetable) were applied in the pretreatment of slaughterhouses effluents (Masse et al., 2001). Lipases have also been used to accelerate the bioaugmentation of polymers (Marten et al., 2003; Sivalingam et al., 2003; Takamoto et al., 2001) and of slurries from oil-well perforations containing synthetic esters emulsified in water (Aliphat et al., 1998). A small amount of enzyme (lipase SEP) was also shown to be promising for increasing biodegradation of FOG wastewater (Rigo et al., 2007).

Microbial products are another option for grease control in the sewer lines. Numerous microorganisms capable of degrading FOGs have been identified and may be potential candidates as components of bioaugmentation products (Brooksbank et al., 2007). Thermophilic vegetable-oil-degrading bacteria have been isolated, raising the possibility that
commercial products could be designed to operate over a wide temperature range (Markossian et al., 2000). Mixed microbial cultures have also been shown to degrade a variety of oils indicating the potential to treat wastewater containing mixed FOGs from the catering industry waste stream (Wakelin & Forster 1997; Tano-Debra et al., 1999). More recent studies have shown that mixed microbial inocula can degrade significant amounts of a variety of fats and oils in lab scale and may thus help keep sewer lines free of grease deposits (Brooksbank et al., 2007). However, most FOG reducing methods are evaluated in lab scale but have not applied in the field.

No long-term assessments of the physical, chemical characteristics and microbial ecology of full-scale GIs have been performed. In addition, no study has investigated the efficacy of commercially available bioaugmentation products under environmental conditions. There is a need to determine the chemical and microbiological effects of biological drain products on grease interceptor characteristics and performance and to address relevant regulatory ordinances.

Very limited knowledge is available on FOG deposits in sewer lines. One of the first studies (Keener et al., 2008) that looked into the properties influencing FOG deposit formation demonstrated that the FOG deposits display an adhesive character and can become securely bound to interior pipe walls (Figure 3). In addition, a majority of FOG deposits also have a grainy, sandstone-like texture and high yield strength, at times requiring high-pressure jet cleaning for removal. The study of the physical properties and chemistry of FOG deposits (Keener et al., 2008) indicated that there are three categories of FOG deposits: the primary
category and majority (84%) of FOG deposits appear to be metallic salts of fatty acids. They exhibit high sample strength and very different fatty acid profiles than common cooking oils (Keener et al. 2008). These samples showed concentrations of saturated fatty acids and calcium well above background levels and distinct layering effects, suggesting an intermittent formation process. A second category of FOG deposits results from the accumulation of lipids from waste discharges of highly concentrated lipids. A third and minor category would be misidentified mineral deposits. In addition, 85% of FOG deposit samples contained calcium as the primary metal or mineral present, with average concentrations of 4255 ppm. There was no correlation found between calcium concentration in FOG deposit samples and water hardness. The FOG deposits preferentially accumulate saturated fats and calcium suggesting that a chemical process is responsible for their formation.

Figure 3 FOG deposits in a pipe before cleaning (Keener et al., 2008)
REFERENCES


Report to Congress: Impacts and Control of CSOs and SSOs; U.S. Environmental Protection Agency: 2004


CHAPTER 2

Physico-chemical Characterization of Grease Interceptors with and without Biological Product Addition

Xia He¹, Jason Osborne², and Francis L. de los Reyes III¹*

¹*Department of Civil, Construction and Environmental Engineering, North Carolina State University, Raleigh, North Carolina. Email: fdelosr@ncsu.edu

²Department of Statistics, North Carolina State University, Raleigh, North Carolina
**ABSTRACT:** Hardened and insoluble fat, oil, and grease (FOG) deposits are the primary cause of sewer line blockages leading to sanitary sewer overflows (SSOs). However, there have been very few long-term assessments of the physico-chemical characteristics of full-scale grease interceptors (GIs), the first “line of defense” against FOG buildup in sewer lines. In this study, we assessed the physico-chemical characteristics of two full-scale GIs (at a restaurant and a retirement community kitchen) over a one year period. Statistically significant differences between bioaugmented and untreated cycles were detected for several chemical and physical properties. The treated cycles had lower BOD and COD at the grease interceptor outlet. While the combined treated cycle data did not show lower FOG concentrations in the GI outlet compared to the combined untreated cycle data, comparison of specific individual treated and untreated cycles show a positive effect due to the addition of product.

**KEYWORDS:** grease interceptor, fats, oils and grease (FOG), bioaugmentation, sanitary sewer overflow (SSO)
INTRODUCTION

The formation of hardened and insoluble FOG deposits is the primary cause of sewer line blockages leading to sanitary sewer overflows (SSOs). Of the estimated tens of thousands of SSOs that occur each year in the United States, approximately 48% are due to line blockages, of which 47% are related to FOG (EPA, 2004). SSOs are not only unlawful releases of untreated wastewater into the waters of the United States; they also introduce significant amounts of environmentally detrimental nutrients into river segments already plagued with algal blooms. Grease-related SSOs resulted in the discharge of about 114,000 m$^3$ (30 million gal) of wastewater, which not only introduced pollutants to the environment, but also exposed the public to pathogens (EPA, 2004). Removal of FOG from wastewater is thus important to ensure that wastewater is disposed of efficiently and economically. However, there have been no long-term assessments of the chemical characteristics and microbial ecology of full-scale grease interceptors (GIs), the first “line of defense” against FOG buildup in sewer lines. Properly designed grease interceptors should allow the retention of FOG until it can be removed physically by manual pumping.

A potential approach for reducing the levels of FOG in the grease interceptor effluent is bioaugmentation. Numerous microorganisms capable of degrading FOG have been identified and are potential candidates as components of bioaugmentation products. Thermophilic vegetable-oil-degrading bacteria have been isolated, which can be designed to operate over a wide temperature range (Markossian et al. 2000). Mixed microbial cultures have demonstrated the potential versatility of bioaugmentation when used to remove FOG from industrial effluents (Wakilin & Forster 1997; Tano-Debra et al. 1999; El-Masry et al. 2004;
El-Bestawy et al. 2005). Multi-species bioaugmentation products have successfully reduced FOG in wastewater in lab scale and demonstrated their potential to minimize FOG accumulation and blockages in sewer lines (Brooksbank et al. 2007). However, there are a number of potential problems in using multi-species products as FOG degraders in the field, such as the variability of environmental conditions and the competition between microorganisms. The aim of the current study is to provide a comprehensive understanding of the chemical characteristics of GIs, which have been largely ignored by the research community. In addition, the effects of biological product addition on FOG removal were investigated in the full-scale GIs.

METHODS & MATERIALS

Experimental design and sample collection

Two 1000 gallon full-scale GIs, from the food service of a retirement home (JO) and a full-service restaurant (Ca.), were monitored for over a year, beginning in December 2007. The GIs were chosen from several candidate sites in Cary, NC based on several criteria, which included: similarity in sizes, the presence of two compartments that can be accessed for sampling, and the ability to obtain a downstream sample. The study was designed to consist of four cycles lasting 60 days each. Each of the GIs was subjected to alternating periods of treatment and non-treatment. The cycles were: Day 1-60 (untreated, no addition of biological product), Day 61-120 (treated, with addition of biological product), Day 121-180 (untreated), Day 181-240 (treated). However, because of onsite issues, it was decided to perform another cycle- Day 241-300 (treated) for both GIs. At the end of each cycle, the GIs were completely pumped clean with a vacuum truck by a commercial GI cleaning service.
During the study, the restaurant staff and workers were blind to whether they were in a treated or untreated cycle and made no efforts to change practices as a result of the trial. The blind nature of the study was ensured by replacing the commercial product with tap water that was dyed the same color as the product.

One of two different bioaugmentation products was added to each treated cycle in both full-scale GIs. Each product was a combination of spore forming beneficial microorganisms (*Bacillus* strain) with a minimum microbial count of $1.49 \times 10^8$ and may or may not have contained other formulation ingredients such as opacifiers (to provide uniform appearance), less than 0.16% dye (for color), less than 0.16% fragrance (for odor control), and a surfactant content of no more than 2.50% total actives by weight that does not emulsify fats, oils and greases. The products used both have a minimum shelf life of 2 years. Bioaugmentation consisted of dosing 21 oz of randomly chosen biological product (from the two products) daily to a drain line in the kitchen, metered by a Power Systems Drain Mate 2000 D-Cell Battery Unit Pump (Power Systems, Irving, TX). Biological products were arranged with a third party to be shipped to the NCSU Laboratory without any markings indicating the source company, or any other product information.

Prior to beginning the study, experiments were conducted at the two food service sites to determine the best time to sample the GIs during the day via a 24-hour sampling experiment and to develop appropriate sampling procedures. These initial experiments showed that a sampling time between 2 and 3 PM resulted in effluent COD and FOG concentrations that were close to the 24 h-average value (data not shown). Approximately 2L samples were
taken between 2 and 3 PM every 2-3 days, from 7 points within and downstream of the GIs, as shown in Figure 1. The influent of GI was collected from point 1 named as inlet1; inside the GI, samples taken from point 2 to point 5 are named as top2, middle 3, bottom4 and top5; the effluent of GI was collected from point 6 named as outlet6; the downstream sample was taken from point 7 named as downstream7. Several characteristics were measured in situ, and samples were stored on ice and immediately transported back to NCSU Environmental Engineering lab for further analysis.

![Sampling points diagram](image)

Figure 1- Sampling points within and downstream of two full-scale grease interceptors

**Physical and chemical characterization**

Physical parameters (pH, DO, ORP, temperature) were measured for all sampling points in situ using a Hanna portable pH/mV meter and a YSI Model 55 Dissolved Oxygen Meter. The depths of FOG in two compartments were measured using a sludge judge (NASCO, Fort Atkinson, WI). Chemical analytical tests were performed in the lab (Table 1) in duplicate,
and measurement techniques were verified in triplicate several times during the course of the study to ensure replicate measurements were within acceptable standard deviations according to standard methods (APHA, 1998).

Table 1. Analytical tests performed at various sampling points in each GI

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Inlet1</th>
<th>Top2</th>
<th>Middle3</th>
<th>Bottom4</th>
<th>Top5</th>
<th>Outlet6</th>
<th>Downstream7</th>
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<td></td>
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<td></td>
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<td>+</td>
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<tr>
<td>COD (total, soluble)</td>
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<td>+</td>
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<td>VFAs</td>
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<tr>
<td>Fat, Oil &amp; Grease</td>
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</tbody>
</table>

BOD<sub>5</sub> was measured according to standard methods (APHA, 1998). COD was measured using a Hach Kit (Hach Co., Loveland, CO). Protein was analyzed using a Modified Lowry Protein Assay Kit (PIERCE, Rockford, IL). Carbohydrate analysis was conducted according to Dubois’ method (Dubois et al, 1956). Fat, Oil & Grease was analyzed by a commercial lab (TRITEST, Raleigh, NC) using EPA Method 1664A (APHA, 1998).
The volatile fatty acids (VFAs) in the wastewater were analyzed using a modified headspace method using a Teledyne Tekmar 7000 headspace autosampler and HP 5890 Gas Chromatography –Flame Ionization Detector. Sample vials were equilibrated at 65°C for 10 minutes, mixed for 5 minutes, then stabilized for 5 minutes. Column DB-FFAP (30m X 0.45 mm X 0.85µm, Agilent, USA) was used to separate the VFAs. The temperature of injector and detector was 250°C. The initial temperature was 50°C for 0.5 min, then ramped 20°C/min to 100°C, maintained at 100°C for 5 min, ramped 8°C/min to 156°C, maintained at 156°C for 3 min, ramped 60°C/min to 240°C, then maintained at 240°C for 5 min. The carrier gas was helium. With this technique, eight fatty acids (acetic, propionic, isobutyric, n-butyric, isovaleric, valeric, isocaproic, hexanoic) were analyzed. The lower quantitation limits for the eight fatty acids were: acetic 21.0mg/L, propionic 20.0mg/L, isobutyric 2.0mg/L, n-butyric 19.1mg/L, isovaleric 2.0mg/L, valeric 5.0mg/L, isocaproic 9.6mg/L, and hexanoic 10.0mg/L.

**Statistical analysis**

Linear models were considered for each of the various chemical characteristics measured (denoted generally by $Y_{ij}$). These models all take the form $Y_{ij} = \mu + \tau_i + E_{ij}$, where $\mu$ is the overall mean value of chemical characteristics, $\tau_i$ denote the effects of the $i^{th}$ biological treatment, $j$ is an index for the days in which the measurements were made, and $E_{ij}$ denotes experimental error or variability not explained by the treatment effect. These errors were assumed to be normally distributed with constant variance. Residual diagnostics did not reveal any obvious violation of this assumption. Separate models were fit for each location. Models were fit using the GLM procedure of the SAS statistical package (SAS, Cary, NC).
RESULTS and DISCUSSION

Physical characteristics of full-scale GIs

The physical properties in both GIs (Table 2) varied widely across all 7 sampling sites due to the variability of influent flow. Within the GIs, the pH was less than 7. For downstream samples, wastewater from other sources (e.g., bathrooms, showers) mixed with GI effluent, resulting in higher pH. The GIs were generally anaerobic. The average ORP in the GIs was in the -100 to +100mV range, which is in the range of sulfate and ferric iron as major electron acceptors (Charpentier et al. 1998). Statistically significant differences were measured in pH and ORP between treated and untreated cycles for the GI in Ca, with a higher pH in the treated cycles that may be due to the addition of biological product. The higher pH may also be an indication of possible enhanced biological activity, possibly reactions aside from fermentation of volatile fatty acids. For the JO site, a higher pH was observed at the beginning in the oil layer, and the pH decreased toward the end of the treated cycle. Compared to the untreated group, lower ORPs were found at most sampling sites in the treated group in both GIs. This is further indication of biological activity in the treated group, which tends to lower ORP as organisms respire. High concentrations of total solids (TS) and volatile solids (VS) were observed at the inlet, top2, and bottom (>10000 mg/L) in both GIs. Even at the outlet, the average TS and VS concentrations were around 2500 mg/L and 1500 mg/L, respectively. Significantly lower TS and VS were observed in the treated group at the outlet in the Ca GI but no significant difference was found in the JO site. The depths of the FOG layer in the two compartments for GI in Ca were significantly lower in the treated cycles (Figure 2), especially in the second compartment, and during the latter stages
of a cycle. This indicates that the effect of the biological product was more pronounced as FOG accumulated toward the end of a cycle.

Table 2. Ranges of measured physical characteristics of full-scale GIs

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Inlet1</th>
<th>Top2</th>
<th>Middle3</th>
<th>Bottom4</th>
<th>Top5</th>
<th>Outlet6</th>
<th>Downstream7</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>JO</td>
<td>5.2-10.5</td>
<td>4.2-6.2</td>
<td>4.2-6.1</td>
<td>3.8-6.0</td>
<td>4.3-6.1</td>
<td>4.2-6.7</td>
</tr>
<tr>
<td></td>
<td>Ca</td>
<td>4.9-11.7</td>
<td>3.9-6.1</td>
<td>4.1-5.9</td>
<td>4.0-5.5</td>
<td>3.9-7.0</td>
<td>4.5-7.0</td>
</tr>
<tr>
<td>DO (mg/l)</td>
<td>JO</td>
<td>1.3-7.1</td>
<td>0.1-1.2</td>
<td>0.1-1.7</td>
<td>0.1-1.7</td>
<td>0.1-1.4</td>
<td>0.2-1.8</td>
</tr>
<tr>
<td></td>
<td>Ca</td>
<td>3.0-8.2</td>
<td>0.2-1.3</td>
<td>0.3-1.9</td>
<td>0.1-1.8</td>
<td>0.2-1.6</td>
<td>0.2-2.2</td>
</tr>
<tr>
<td>Temperature (°C)</td>
<td>JO</td>
<td>20-60</td>
<td>22-40</td>
<td>19-40</td>
<td>21-41</td>
<td>21-40</td>
<td>22-39</td>
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<tr>
<td></td>
<td>Ca</td>
<td>20-52</td>
<td>21-45</td>
<td>22-41</td>
<td>21-38</td>
<td>24-42</td>
<td>26-42</td>
</tr>
<tr>
<td></td>
<td>Ca</td>
<td>-248-277</td>
<td>-48-177</td>
<td>-7-127</td>
<td>70-162</td>
<td>-105-151</td>
<td>-138-111</td>
</tr>
</tbody>
</table>

Figure 2- Depth of FOG layer in two compartments for GI in Ca: (a) depth of FOG layer from the first compartment; (b) depth of FOG layer from the second compartment
Protein, carbohydrate and volatile fatty acids

Protein, carbohydrate and fatty acid levels were measured to assess possible breakdown of influent wastewater (Table 3). A large amount of protein and carbohydrate stayed in the oil layer and bottom of both GIs. The concentrations of protein and carbohydrate fluctuated dramatically in the GIs. Thus, the comparison between untreated and treated cycles using only the average concentrations is misleading, and statistical analysis becomes crucial. For the Ca treated cycle, although the concentration of protein and carbohydrate changed significantly in top2 (p-value<0.0001 for protein, and p-value=0.0006 for carbohydrate) and bottom (p-value<0.0001 for protein, and p-value=0.0127 for carbohydrate) samples, the effluent (p-value=0.6825 for protein, and p-value=0.6406 for carbohydrate) concentration was the same as in the untreated condition. Thus, other effects within the grease interceptor balanced the measured positive effects of bioaugmentation on the top2 and bottom sampling points, and the overall concentration in the effluent remained the same. For the GI in JO, the same pattern was observed with respect to carbohydrate. However the decreased protein concentration in the effluent demonstrated that the bioaugmentation effect varies from one GI to another.
Table 3. Mean concentrations of protein and carbohydrate in both GIs

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Inlet1</th>
<th>Top2</th>
<th>Bottom4</th>
<th>Outlet6</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>untreated</td>
<td>treated</td>
<td>untreated</td>
<td>treated</td>
</tr>
<tr>
<td><strong>Protein</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(mg/l)</td>
<td>505</td>
<td>652.7</td>
<td>915.4</td>
<td>877.1</td>
</tr>
<tr>
<td><strong>Carbohydrate</strong></td>
<td>792.5</td>
<td>826.7</td>
<td>2523.5</td>
<td>473.3</td>
</tr>
<tr>
<td>(mg/l)</td>
<td></td>
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<td></td>
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</table>

When protein and carbohydrate are catabolized under anaerobic conditions, fatty acids are formed. Eight types of fatty acids were tested in this study, including hexanoic, i-caproic, valeric, n-butyric, i-butyric, propionic and acetic. No significant differences in the influent between untreated and treated cycles were observed in both GIs. The scenario was different with respect to the GI effluent (Table 4). Statistically, significant differences in many types of fatty acids were observed in both GIs. These differences in the fatty acid profile are evidence that bioaugmentation changed the metabolic processes in the GIs. At the Ca site, the concentrations of most fatty acids in the treated cycles, compared to the untreated cycles, were higher in the beginning and lower at the end. However, in JO, higher concentrations of valeric, n-butyric, i-butyric, and propionic were observed in the treated cycles throughout the duration of the entire cycle. Only acetic acid showed a lower concentration toward the end of the treated cycles. Again, these site-specific effects indicate that the effect of biological product addition is different, consistent with the physical characteristics and protein.
carbohydrate results. For downstream samples, the effects of bioaugmentation were counteracted by the mixing of wastewater from sinks and restrooms, resulting in no significant differences between treated and untreated cycles for most of the fatty acids measured downstream of the GIs.

Table 4. Mean concentrations and statistical analysis of VFAs in GI effluent

<table>
<thead>
<tr>
<th></th>
<th>Ca</th>
<th>JO</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>untreated</td>
<td>treated</td>
</tr>
<tr>
<td>Hexanoic</td>
<td>3.6±12</td>
<td>0.4±4.2</td>
</tr>
<tr>
<td>i-caproic</td>
<td>1.0±7.9</td>
<td>0</td>
</tr>
<tr>
<td>Valeric</td>
<td>34.8±58.2</td>
<td>18.5±21.9</td>
</tr>
<tr>
<td>i-valeric</td>
<td>3.2±6.6</td>
<td>2.2±4.1</td>
</tr>
<tr>
<td>n-butyric</td>
<td>45.2±44.7</td>
<td>29.5±38.4</td>
</tr>
<tr>
<td>i-butyric</td>
<td>7.6±31</td>
<td>1.4±3.6</td>
</tr>
<tr>
<td>Propionic</td>
<td>194.7±207.5</td>
<td>149.5±166.9</td>
</tr>
<tr>
<td>Acetic</td>
<td>142.5±285</td>
<td>75.9±84.8</td>
</tr>
</tbody>
</table>

BOD and COD removal from full-scale GIs

High concentrations of COD (tCOD of 3000 - 4000mg/L; sCOD of 1000 - 2000mg/L) and BOD were found in the inlet and outlet of both GIs. The average effluent tCOD was 1500 - 2500mg/L; the average effluent sCOD was around 1000mg/L (Figures 3 and 4). Statistical analysis demonstrated that there were no significant differences in influent COD and influent BOD between untreated and treated cycles in both GIs. Although no significant difference
was found in tCOD of effluent, lower sCOD was found in the treated cycles for GI in Ca (Figure 3). Since there were no significant differences in effluent protein and carbohydrate, the lower sCOD may be due to lower levels of fatty acids. A lower GI effluent BOD was also observed in Ca (Figure 5), indicating that bioaugmentation helped decrease the BOD concentration in the grease interceptor. The downstream wastewater represented not only the effluent from the grease interceptor, but also wastewater from sinks and restrooms (and showers in JO). Thus, it is difficult to ascertain the effect of bioaugmentation on downstream samples. For the GI in JO, a lower effluent COD and BOD were observed in treated cycles (Figures 4 and 5), which might be attributed to the lower protein and the change in concentration of fatty acids. It is interesting to note that bioaugmentation resulted in either a positive effect (lower BOD in the downstream toward the end of the cycle) or no effect on downstream BOD. Ideally, a mass balance of COD and BOD should allow the delineation of the additional organic matter degradation due to biological product addition. However, the high variability in the influent precludes a mass balance analysis of the data.

Figure 3- COD at outlet for GI in Ca: (a) total COD; (b) soluble COD
Fat, Oil and Grease removal from full-scale GIs

Fat, oil and grease (FOG) analyses were performed on samples taken from the inlet, outlet and downstream. The influent FOG concentration fluctuated from 6 mg/L to 13620 mg/L. This high variability in influent FOG is expected, given the variability inherent in kitchen operations, type of food, and the episodic nature of the flows. Because of these variations,
there was no significant influent FOG concentration difference between untreated and treated cycles in both GIs. The effluent FOG concentrations did not fluctuate as much as the influent; the average concentration was between 150 to 200mg/L (Figure 6). The comparison between untreated cycles and treated cycles (p-value=0.6812 in Ca, p-value=0.2008 in JO) demonstrated that taking into account all treated and untreated cycles, bioaugmentation did not have a significant effect on FOG degradation in both GIs. However, there were differences in the effects of the biological products added in two different treated cycles in Ca and JO. In Ca, treated cycle3 had a lower FOG concentration than treated cycle4 at the end of cycle (different biological products were used) (Figure 7). Untreated cycle2 was chosen as the cycle to compare to treated cycle3, since untreated cycle1 was carried out in the winter and untreated cycle2 and treated cycle3 occurred in summer/fall. Comparison of effluent FOG for untreated cycle2 and treated cycle3 showed a significant difference (p-value=0.0036). The statistical analysis also showed that effluent FOG concentration in cycle3 was lower than in cycle2. Since there was no significant inlet FOG concentration difference between cycle2 and cycle3, the lower effluent FOG concentration in cycle3 indicated that addition of biological product resulted in FOG degradation in Ca. In JO, a different scenario is evident. The comparison between treated cycle3 and cycle4 (p-value=0.1839) showed that the two biological products have almost the same effect on FOG degradation (Figure 7). Untreated cycle2 was chosen for comparison with treated cycle3 for the same reason cited above. Analysis showed a significant difference between untreated cycle2 and treated cycle3 (p-value = 0.04). Thus, addition of biological product had a positive effect on FOG degradation in both JO and Ca, with perhaps a greater positive effect in Ca. It thus becomes evident that the effectiveness of the biological products is affected by the GI type, and may
be due to differences in kitchen wastewater characteristics. With respect to downstream FOG, the statistical analysis revealed that the difference between untreated cycle2 and either treated cycle3 in Ca or treated cycle3 in JO can be ignored. Though bioaugmentation demonstrated its capability to degrade FOG in both grease interceptors as discussed above, this effect is dampened by the inclusion of other wastewater flows (from bathrooms, showers, and other sources).

CONCLUSIONS
Statistically significant differences between treated and untreated cycles were detected for several physical and chemical characteristics. The changes in these parameters (e.g., pH, ORP) provide evidence for increased microbial activity during the treated cycles. The treated cycles had lower BOD and COD at the grease interceptor outlet. The shifts in volatile fatty acid profiles also show that the addition of biological product resulted in changes within the grease interceptors. While the combined treated cycle data (cycles 3 and 4) did not show lower FOG concentrations in the GI outlet compared to the combined untreated cycle data (cycle 1 and cycle 2), comparison of individual treated and untreated cycles shows a positive effect due to the addition of product. The effects of biological product addition were also different for the two GIs. In general, the Ca GI showed a higher, positive effect on effluent characteristics due to product addition. For example, the depth of the FOG layer in the Ca GI was significantly lower for the treated cycle than in the untreated cycle. Thus, the effect of biological product addition depends on the type of product used, the characteristics of the specific GI and food waste, and additional factors that may be present during the cycle. However, in all cases the addition of biological product did not result in any adverse effect-
GI effluent characteristics were similar or better in treated cycles compared to those in untreated cycles. There is also no evidence of passing grease downstream due to the addition of biological products. The positive effect of bioaugmentation provides evidence to help keep sewer lines free of FOG deposits, and then contribute to prevent sanitary sewer overflows. Future studies should include a longer duration between GI cleanout. It is possible that greater differences between treated and untreated cycles become more pronounced toward the end of longer cycles and less FOG in treated cycle will enter the downstream sewer lines.

Figure 6- Effluent FOG concentration in both GIs: (a) FOG for GI in Ca; (b) FOG for GI in JO
Figure 7- Effluent FOG concentration in two treated cycles for both GIs: (a) FOG for GI in Ca; (b) FOG for GI in JO

REFERENCES


CHAPTER 3

Microbial characterization of grease interceptors for removing fat, oil and grease (FOG) in sewer lines

Xia He, Jason So, Francis de los Reyes III
Department of Civil, Construction and Environmental Engineering, North Carolina State University

Abstract: Fat, oil and grease (FOG) in wastewater create problems including the production of foul odors, the blockage of sewer lines leading to overflows, and interference with proper operation of wastewater treatment works. While many microbial species have been found to degrade FOG, the microbial communities in full-scale grease interceptors (GIs), the first “line of defense” preventing FOG from entering the sewer lines, have not been studied. In this study, the microbial ecology in GIs was evaluated and the effects of bioaugmentation on grease interceptor performance were determined using Terminal Restriction Fragment Length Polymorphism (T-RFLP) and 16S rRNA gene clone library construction. Differences in the microbial community structure between bioaugmented and control cycles were detected by T-RFLP and clone library analysis. Even though the dominant TRFs in the biological product were not detected, the addition of product resulted in shifts in the microbial composition.

Key words: grease interceptors, microbial community, bioaugmentation, fat, oil and grease (FOG), T-RFLP, 16S rRNA
**Introduction**

Fat, oil and grease (FOG) in wastewater create problems including the production of foul odors, interference with proper operation of wastewater treatment works and the blockage of sewer lines leading to sanitary sewer overflows (SSOs). Fifty percent of SSOs occur as a result of line blockings with the largest source of these blockages (47%) attributed to FOG deposits that accumulate in sewer lines (U.S. EPA 2004). SSOs can potentially release high pathogens, nutrients, and solids loadings that result in harm to public health and the environment. Many microbial species have been found to have abilities to degrade oil (Quek et al., 2006; Sadouk et al., 2009; Nisola et al., 2009; Zhou and Shen, 2010). Bioaugmentation has also demonstrated its advantage in oil degradation in contaminated soil (Hua et al., 2010). Multi-species bioaugmentation products have successfully reduced FOG in wastewater in lab scale and demonstrated their potential to minimize FOG accumulation and blockages in sewer lines (Brooksbank et al., 2007). However, there is no previous research on the microbial communities in full-scale grease interceptors (GIs), the first “line of defense” preventing the FOG from entering the sewer lines. In addition, the effect of bioaugmentation on the microbial communities in full-scale GIs has not been reported.

In this study, two 1000 gallon full-scale GIs, from the food service of a retirement home (JO) and a full-service restaurant (Ca), were monitored for over a year. The study was designed to consist of four cycles lasting 60 days each. Each of the GIs was subjected to alternating periods of treatment (addition of generic biological product) and non-treatment. Our previous research (He et al., in press) showed significant differences between treated and untreated cycles for several physical and chemical parameters, such as oxidation-reduction
potential (ORP), volatile fatty acids (VFAs), biochemical oxygen demand (BOD), chemical oxygen demand (COD) and FOG. The changes in these parameters provided evidence for increased microbial activity with bioaugmentation. However, the effects of biological product addition were different for the two GIs, which may be due to the different dominant bacteria present. Therefore, to obtain a more comprehensive understanding of full-scale GIs and the bioaugmentation effect on GI performance, this study aimed to investigate the microbial ecology of full-scale GIs and the change in microbial communities when biological product is added. Samples from both GIs for one untreated cycle and one treated cycle were used to assess the microbial communities using terminal restriction fragment length polymorphism (T-RFLP) coupled with cloning and sequencing of the 16S rRNA gene.

Methods and materials

Sampling and storage

Samples were taken twice a week from the oil layer (top layer), solids layer (bottom layer) and effluent of grease interceptor in an untreated cycle and a treated cycle for both GIs. All samples were centrifuged at 4000 xg for 15 min in 15 ml tubes. The pellets were transferred to 2 ml tubes and frozen at -80 °C. Each cycle lasted for 60 days, and samples at approximately day 30 and day 60 were obtained for DNA extraction, T-RFLP and generation of clone libraries. The biological product that was added in the treated cycle was also analyzed using T-RFLP.
DNA extraction method selection

The DNA extraction method from a complex matrix may affect the results of microbial community analysis (Carrigg et al., 2007; LaMontagne et al., 2001). Since the effect of DNA extraction method on grease interceptor samples has not yet been reported, it is important to ensure that the most appropriate method is used. Two commercial DNA extraction kits, PowerSoil™ DNA isolation kit (MoBio Laboratories, Inc., Carlsbad, CA) and FastDNA® SPIN Kit for Soil (BIO 101, Inc., Vista, CA), and a method developed in our lab were compared for their efficiency and effectiveness in extracting DNA from the grease interceptor samples. For the laboratory method, a low pH aluminum sulfate solution was added to 0.25 g GI sample. The pH in the solution was measured, adjusted to pH 6.5 by adding HCl, then raised to 9.0~9.5 with NaOH. The sample was vortexed briefly, then 0.25 g acid washed glass beads and 400 ul lysis solution were added. The sample was bead beat at max speed for 1 min and the tubes were centrifuged for 5 min at 13,200 xg. The supernatant was transferred, after which 0.5 volume of 7.5 mol l^{-1} ammonium acetate was added. The tube was incubated on ice for 10 min, centrifuged at 13,200 xg for 5 min, then 1 volume of 100% isopropanol was added to the supernatant, and the tube mixed and incubated at room temperature for 5 min. The samples were centrifuged again at 13,200 xg for 5 min, then 1 ml of 70% ethanol was added to the supernatant to precipitate DNA. The DNA pellet was washed with ethanol, and resuspended in TE buffer for further use. One sample from the top layer was divided into nine equal weight samples respectively. Triplicate DNA extractions were performed for each DNA extraction method. Nucleic acids concentrations were measured spectrophotometrically using a Nanodrop spectrophotometer.
**PCR amplification**

PCR amplification of the 16S rRNA gene region was carried out using universal bacterial primers 8F (5’-AGAGTTTGATCCTTGGCTCAG-3’) and 1492R (5’-GCATACCTTGTACGACTT-3’) (Liu et al., 1997; Hongoh et al., 2003) with the fluorescent tag 6-FAM labeled at the end of 5’ of the 8F primer. The Epicentre FailSafe™ PCR enzyme mixture and 2X reaction buffer F was used to provide the most reliable amplification for these samples. The PCR program included an initial denaturation at 94°C for 5 minutes followed by 28 cycles of denaturation at 94°C for 1 minute, annealing at 56°C for 1 minute, and elongation at 72°C for 60 s. A final elongation step at 72°C for 30 minutes was added to avoid incomplete products. The products were pooled and verified visually using 1.5% agarose gel electrophoresis in 1X TBE and SYBR Green I staining (Molecular Probes).

**Terminal restriction fragment length polymorphism (T-RFLP)**

Prior to enzymatic digestion, the PCR amplicons were purified using Wizard PCR Preps DNA purification system (Promega, Madison, Wis.). Purified PCR products (approximately 200 ng) were digested separately with 5 U of tetrameric restriction endonucleases, HhaI, MspI and RsaI (Promega, Madison, Wis), and the fluorescently-labeled terminal restriction fragment was precisely measured using an automated DNA sequencer (Liu et al., 1997). Three replicate PCRs were performed for each sample using Bacterial primers (Liu et al., 1997; Hongoh et al., 2003) labeled fluorescently at the 5’ end. Restriction digests were incubated at 37°C for 4 h. To analyze the unique terminal restriction fragments (T-RFs) or operational taxonomic units (OTUs), 2μl of digested samples were denatured at 94°C for 5 minutes and quick-cooled on ice. Then, 2 μl of each denatured sample containing the size
standards (for determination of fragment sizes) were injected onto a capillary system (ABI 3100 Genetic Analyzer, Applied Biosystems) for electrophoretic separation of fragments. T-RFLP profiles were then analyzed using GeneScanTM software (version 3.7, Applied Biosystems). Analysis of the electropherograms allowed calculation of diversity. Analysis allowed identification of the ribotypes and calculation of species/group levels. T-RFLP was performed twice for a treated or untreated cycle for each GI.

**Cloning and sequence libraries**

Bacterial clone libraries were constructed using DNA extracted from the GI samples. Clone libraries were generated by inserting PCR products into the pGEM-T Easy vector (Promega, Madison WI), in accordance with the manufacturer’s protocol. Colony PCR was performed by picking transformants using a sterile plastic pipette tip and placing them into a 96-well plate containing a PCR cocktail of 25 μl of FailSafe PCR system reaction mix F (Epicentre; Madison, WI), 0.6 μL FailSafe enzyme mix (Epicentre), 0.25 μmol l⁻¹ of each primer and sterile pure water added to a total volume of 50 μl. The forward primer used for colony PCR was the T7 primer (5’-TAATACGACTCACTATAGGG-3’) and the reverse primer used was SP6 (5’-TATTTAGGGTACACTATAG-3’). PCR conditions for colony PCR were: a lysis step at 95°C for 10 min, an initial denaturing step at 95°C for 5 min, followed by 40 cycles of denaturation at 95°C for 1 min, annealing at 55°C for 1 min, extension at 72°C for 1.5 min, and final extension at 72°C for 10 min. Plates containing amplified inserts were submitted to the NC State University Genome Sciences Laboratory (Raleigh, NC) for purification and sequencing. Sequences were analyzed for presence of chimeras using RDP (Ribosomal Database Project, www.rdp.cme.msu.edu), then analyzed by BLAST (Basic Local Alignment
Search Tool, [www.ncbi.nlm.nih.gov](http://www.ncbi.nlm.nih.gov) for closest matches in the NCBI (National Center for Biotechnology Information) nr (unaligned) 16S rRNA database. The sequences were also subjected to restriction fragment analysis in silico to determine matches with the T-RFLP results. In this way, several T-RFs from the T-RFLP analysis were matched to nearly full-length sequences determined by cloning-sequencing. To construct the phylogenetic tree, all sequences were trimmed with the universal primer 8F (5’-AGAGTTTGATCCTTGGCTCAG-3’). The trimmed sequences were aligned (greengenes.lbl.gov), and the aligned sequences were analyzed by chimera check (greengenes.lbl.gov). Non-chimeric sequences were used to input to clustalX and MEGA5 to generate phylogenetic trees.

**Results**

**Selection of DNA extraction method**

The DNA yield, A260/A280 and A260/A230 ratios of each DNA extraction method are shown in Table 1. In terms of DNA yield, the lab method produced the highest DNA concentration, and the MOBIO kit produced the lowest DNA. There were no significant differences among the three DNA extraction methods with respect to the ratio of A260/A280. However, the lab method resulted in the lowest amount of contaminants that absorb at 230 nm. The BIO101 extract had the highest amount of contaminants that absorb at 230 nm. The extracted top layer DNAs were further analyzed using Terminal Restriction Fragment Length Polymorphism (T-RFLP). The result is shown in Fig. 1. Each unique terminal restriction fragment (T-RF) represents a specific “ribotype” in the microbial community that occurs at a 1% or higher level in the community. The MOBIO kit resulted in the highest number of
fragments. The three methods showed similar T-RF profiles. The BIO101 kit was slightly different from the other two methods with respect to the dominant fragments 222 and 593. Although the lab method gave the highest DNA yield, ratios of A260/A280 and A260/A230, MOBIO detected more T-RFs (more “phylotypes”), and thus a higher richness. Thus, MOBIO PowerSoil DNA Isolation Kit was chosen as the method for extracting DNA from all grease interceptor samples.

Table 1. DNA yield, A260/A280 and A260/A230 of three DNA extraction methods

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</thead>
<tbody>
<tr>
<td>Top1</td>
<td>Bio101  kit</td>
<td>58.4</td>
<td>1.68</td>
<td>0.13</td>
<td>3.62</td>
<td>1.69</td>
<td>0.73</td>
<td>338.13</td>
<td>1.99</td>
<td>2.46</td>
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<tr>
<td>Top2</td>
<td>MOBIO kit</td>
<td>66.1</td>
<td>1.7</td>
<td>0.13</td>
<td>5.96</td>
<td>1.89</td>
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Figure 1 T-RFLP results for three DNA extraction methods

Terminal restriction fragment length polymorphism (T-RFLP)

PCR was used to amplify genes encoding 16S rRNA from total community DNA. The PCR product was digested with restriction enzymes HhaI, MspI, RsaI, and the fluorescently labeled terminal restriction fragment was measured by using an automated DNA sequencer. The T-RFLP results (Figure 1) were all based on restriction enzyme MspI because MspI digestion resulted in a higher richness (number of terminal restriction fragments, or T-RFs) compared to HhaI and RsaI.

Biological product

The biological product added in the treated cycle to both GIs was analyzed using T-RFLP. The relative abundance of different ribotypes, or operational taxonomic units (OTUs)
(represented by terminal restriction fragments) is shown as percentages (level of specific T-RF as a percentage of the total levels of all T-RFs). There are six terminal restriction fragments (T-RFs) with relative abundance above 1% in the pie chart (Fig. 2). Bacteria with relative abundance less than 1% are classified as “others”. In the biological product, 59.8% bacteria were from a phylotype with T-RF length equal to 164. The second dominant phylotype had relative abundance of 15.8% in the biological product with T-RF length equal to 556. The third dominant phylotype covered 5.4% in the biological product with T-RF length equal to 159.

**Figure 2** T-RFLP result for the biological product fed in the treated cycle

*Fat, oil and grease layer of full-scale GI*

The T-RFLP results for the oil layer (top layer) in Ca are shown in Fig. 3. In sample CT2M, T-RFs 298, 90, 566 and 177 were the dominant groups in the microbial community. None of the T-RFs (relative abundance above 1%) was present in the biological product. T-RF 556 and 159 (which were dominant in the biological product) had relative abundance of 0.85%
and 0.70% in CT2M, respectively. The relative abundance of T-RF 164 was less than 0.5% in replicates of CT2M. This indicates that the organisms in the biological product were not present in high quantities in the untreated cycle samples taken at Day 30 of the cycle. At the end of the untreated cycle (CT2E), T-RFs 298, 90, 566 and 177 were still the dominant groups in the microbial community. The relative abundance of T-RF 90 decreased and the relative abundances of the other three groups increased. The relative abundance of T-RF 159 increased to 1.32% but no T-RF 164 and 556 were found. Although T-RF 556 and an increasing abundance of T-RF 159 were observed in the untreated cycle, the total amount of these three fragments (that are present in the biological product) was low, representing only about 1% of the microbial community in the oil layer. In treated cycle, T-RFs 298, 566, 177 and 579 were the four dominant groups in CT3M. There were clear shifts in the microbial community in CT3M as compared to CT2M. Compared to CT2M, T-RF 90 decreased, while T-RF 579 increased. The most dominant group or species was T-RF 177 in CT3M, as opposed to T-RF 298 in CT2M. There were 0.21% T-RF 556 and 4.04% T-RF 159 in CT3M. Although the relative abundance of T-RF 164 and T-RF 556 were almost the same as in CT2M, the higher abundance of T-RF 159 in CT3M was significant. The difference between CT2M and CT3M indicated the impact of bioaugmentation on microbial community in the oil layer using TRF 159 as a marker. It is also reasonable to state that the phylotype associated with T-RF 159 in the biological product was favored over the other dominant species in the biological product in Ca. For CT3E, the dominant T-RFs switched from 177, 298, 566, 579 to 298, 566, 159, and 287. The relative abundances of T-RF 556 and T-RF 159 changed to 0.52% and 11.85%, respectively, and still no T-RF 164 was found. Again the species or group associated with T-RF 159, which was one of the dominant species in the
biological product, was favored, and the abundance of T-RF 159 increased significantly in the treated cycle.

**Figure 3** T-RFLP results showing the microbial composition (as T-RF percentages) in the top layer of the GI Ca. The label “2” (x-axis) refers to untreated cycle 2; and “3” is for treated cycle 3. “M” refers to the middle (approx. Day 30) of a 60-day cycle; “E” refers to the end (approx. Day 60). Thus the figures show the changes over time (middle vs. end of a cycle) and the differences between treated and untreated cycles.
The same comparison was done in the GI named JO. Samples from untreated cycle 2 and treated cycle 3 were chosen for T-RFLP analysis. The T-RFLP results are shown in Fig. 4. In the untreated cycle 2 in JO, the dominant species were T-RFs 298, 566, 90 and 869. T-RF 869 was not dominant in Ca, and is thus unique to JO. From the middle period (Day 30) to the end, the level of T-RF 298 decreased but the relative abundance of other dominant T-RFs did not change. The levels of T-RF 380 increased and new T-RFs appeared toward the end of the cycle. The relative abundances of T-RF 164 and T-RF 556 (found in the biological product) in the top layer samples were both below 0.5%. Of the T-RFs in the biological product, only T-RF 159 was found in JT2E with a relative abundance of 1.36%. In the treated cycle 3 in JO, the dominant T-RFs in both JT3M and JT3E were still 298, 566, 90 and 869. The level of T-RF 298 decreased from the middle period to the end of treated cycle 3. T-RF 566 decreased and the relative abundance of T-RF 90 and T-RF 869 increased over time. T-RFs 164, 556 and 159, the dominant species in the biological product, were not detected.
Figure 4 T-RFLP results showing the microbial composition (as T-RF percentages) in the top layer of the GI JO. The label “2” (x-axis) refers to untreated cycle 2; and “3” is for treated cycle 3. “M” refers to the middle (approx. Day 30) of a 60-day cycle; “E” refers to the end (approx. Day 60). Thus the figures show the changes over time (middle vs. end of a cycle) and the differences between treated and untreated cycles.

Solid layer of full-scale GI

In the solid layer in Ca, the dominant phylotypes were T-RF 90, 298 and 566 (Fig. 5). No T-RF 164, 556 and 159 were found in the bottom, which indicates that the biological product did not migrate to the GI bottom layer but tended to go up to the top layer as expected. After
comparing T-RFs between top layer and bottom layer, the unique species in top are T-RFs 579, 159, 281, 57, 127, 207, 222, 236, and 355. The unique species at the bottom are T-RFs 549, 124, 304, 161, 340, 810 and 869. Most of these unique bottom species tended to decrease in abundance with bioaugmentation, except for T-RF 549 and 869, which showed a slight increase. The changes in unique species in top and bottom samples indicate the ability of bioaugmentation to affect the structure of microbial communities. In JO, the scenario is similar. The dominant phylotypes were T-RF 298, 566 and 90 (Fig. 6). The dominant T-RFs in the biological product, T-RF 164, 556 and 159 were not detected in the bottom sample, indicating that the added biological product did not migrate to the bottom layer. By comparing the T-RFs in the top layer and the bottom layer, we can determine that the unique phylotypes at the top were T-RFs 159, 185, 274, 342, 364 and 380. Only T-RF 159 was also found in top layer in Ca. The unique top phylotypes tended to decrease in relative abundance from greater than 1% to less than 1% in treated cycle 3, which is not what happened in Ca. The unique phylotypes in the bottom layer were T-RFs 810, 387, 124, 654, 64, 177, 806, 180, 240, 281, 340, and 394. T-RFs 124, 340 and 810 were also unique phylotypes in the bottom layer in JO. Most of the unique bottom phylotypes tended to increase in abundance toward the end of untreated cycle 2. Most of these phylotypes also decreased in abundance toward the end of treated cycle3, except for T-RF 240 and T-RP 340, which were detected only in treated cycle3. The difference between untreated cycle and treated cycle indicated that while the dominant phylotypes in the biological product did not migrate to the bottom layer, changes in the microbial community at the bottom correlated with addition of product.
**Figure 5** T-RFLP results showing the microbial composition (as T-RF percentages) at bottom of the GI Ca. The label “2” (x-axis) refers to untreated cycle 2; and “3” is for treated cycle 3. “M” refers to the middle (approx. Day 30) of a 60-day cycle; “E” refers to the end (approx. Day 60). Thus the figures show the changes over time (middle vs. end of a cycle) and the differences between treated and untreated cycles.
Figure 6  T-RFLP results showing the microbial composition (as T-RF percentages) at bottom of the GI JO. The label “2” (x-axis) refers to untreated cycle 2; and “3” is for treated cycle 3. “M” refers to the middle (approx. Day 30) of a 60-day cycle; “E” refers to the end (approx. Day 60). Thus the figures show the changes over time (middle vs. end of a cycle) and the differences between treated and untreated cycles.

**Effluent of full-scale GIs**

In Ca, the dominant phylotype in the effluent from the untreated cycle 2 are T-RF 90, 298 and 93 (Fig. 7). No unique phylotype was found in the effluent based on the T-RF
comparison with top and bottom layers, the dominant species in top and bottom affected the microbial community structure in the effluent. One exception is that the relative abundances of T-RF 159 and 556 were 2.4% and 1.2% respectively at the end of untreated cycle. Since the amounts of these two phylotypes were quite low at the top (around 1% in total) and bottom (none was found) in untreated cycle 2, these functional bacteria may exist in the second compartment of the GI. However, T-RF 164 was not detected. In treated cycle 3, the effluent was affected mostly by the top but not the bottom layer because the dominant T-RFs in the effluent were the dominant T-RFs in top layer. These were T-RFs 177, 566 and 298 in the middle period and T-RF 298, 566 and 159 at the end. The relative abundances of T-RF 159 and T-RF 556 were 6.7% and 1.5%, respectively, at the end of treated cycle. The increased amount of T-RF 159 can be attributed to the addition of the biological product. The dominant T-RFs in effluent, for GI in JO, were 90, 298, 566, 93, 549 and 869 (Fig. 8). All these T-RFs can be found in both top and bottom layers. The unique T-RFs not present in top and bottom but in effluent were T-RF 300, 590, 168, 97 and 295. Compared to untreated cycle 2, the relative abundances of T-RFs 90 and 298 were higher and a lower number of T-RFs were obtained in treated cycle 3.
Figure 7 T-RFLP results showing the microbial composition (as T-RF percentages) in the effluent of the GI Ca. The label “2” (x-axis) refers to untreated cycle 2; and “3” is for treated cycle 3. “M” refers to the middle (approx. Day 30) of a 60-day cycle; “E” refers to the end (approx. Day 60). Thus the figures show the changes over time (middle vs. end of a cycle) and the differences between treated and untreated cycles.
Figure 8 T-RFLP results showing the microbial composition (as T-RF percentages) in the effluent of the GI JO. The label “2” (x-axis) refers to untreated cycle 2; and “3” is for treated cycle 3. “M” refers to the middle (approx. Day 30) of a 60-day cycle; “E” refers to the end (approx. Day 60). Thus the figures show the changes over time (middle vs. end of a cycle) and the differences between treated and untreated cycles.

Cloning and sequence libraries
To accomplish identification based on nearly full-length 16S rRNA sequences, cloning-sequencing was performed on 4 samples: untreated and treated Ca top samples at the end of
the cycles (CT2E and CT3E), and untreated and treated JO top samples at the end of the cycles (JT2E and JT3E). In CT2E, 77 clones (80% of the total clones) were identified as their closely related 16S rRNA sequences by BLAST. CT2E is dominated by 8 species (Fig. 9): *Mitsuokella* sp. DJF RR21, *Selenomonas* sp. WG, *Lactobacillus* sp., *Prevotella multisaccharivorax*, *Thermoanaerobacterium thermosaccharolyticum*, uncultured *Veillonella* sp., *Megasphaera elsdenii*, and uncultured *Prevotella* sp.. After alignment and chimeric check, 14 sequences were available for phylogenetic analysis (Fig. 10). Of 14 sequences, phylogenetic analysis distinctly grouped the bacterial clonal sequences into the *Lactobacillus* sp., the *Thermoanaerobacterium thermosaccharolyticum*, the *Prevotella* sp., the *Selenomonas* sp. WG and the *Mitsuokella* sp. DJF RR21. Comparison of in silico digestion of the sequence with the T-RFLP analysis allowed the identification of three T-RFs: T-RF 298 was identified as *Mitsuokella*, *Selenomonas*, or *Megasphaera*; T-RF 177 was identified as *Lactobacillus* sp.; T-RF 159 was identified as *Thermoanaerobacterium thermosaccharolyticum*, which is a thermophilic organism consistent with the observation of higher temperatures at the Ca. In CT3E, 87 clones (91% of the total clones) were identified by BLAST. Compared with CT2E, the common dominant species found in CT3E are *Mitsuokella* sp. DJF RR21, *Selenomonas* sp. WG, *Prevotella multisaccharivorax*, *Thermoanaerobacterium thermosaccharolyticum*, and *Megasphaera elsdenii*. New dominant species in CT3E were *Lactobacillus delbrueckii*, *Caloramotro uzoniensis* and *Chlorobibacterium* (Fig. 9). After alignment and checking for chimeras, 38 sequences were available for phylogenetic analysis. The phylogenetic tree of CT3E was constructed with additional reference sequences from the GenBank database providing classification of the sequences to the family taxonomic level as shown in Fig. 11. In addition to the 3 T-RFs identified in
CT2E, three more T-RFs were identified from the T-RFLP analysis in CT3E, which are T-RF 287 identified as *Acidaminococcaceae bacterium*; T-RF 522 identified as *Caloramator viterbiensis* or *Caloramator sp.* 45B; and T-RF 207 identified as *Chlorobi bacterium* or *Bacterpodetes*. Also, the percentage of T-RF 159 (biological product) is increased from 6% in CT2E to 17% in CT3E. The difference between CT2E and CT3E is consistent with the observation from T-RFLP that demonstrates the bioaugmentation effect on the microbial communities.

**Figure 9** Distribution of clone sequences for Ca. CT2E is the sample for top layer, untreated cycle 2, end of cycle. CT3E is the sample for top layer, treated cycle 3, end of cycle.
Figure 10 Phylogenetic tree of sample CT2E
Figure 11 Phylogenetic tree of sample CT3E
The clone library at JO was quite different from the clone library at Ca. First, in silico digestion of the sequence with the T-RFLP analysis allowed the identification of only one T-RF in both JT2E and JT3E: T-RF 298 was identified as *Mitsuokella*, *Selenomonas*, or *Megasphaera*. It should be noted that T-RF 298 is the most dominant fragment in the T-RFLP analysis, is also found as dominant fragment in Ca, and the clone library shows that these organisms are the most dominant in these samples. The clone library for the JO untreated sample was very similar to that of the treated sample (Fig. 12), although the treated sample had a higher diversity and included a few more less dominant species. JT2E was dominated by about 6 species: *Mitsuokella* sp. DJF RR21, *Mitsuokella jalaludinii* M9, *Megasphaera elsdenii*, *Selenomonas sp.* WG, *Prevotella multisaccharivorax*, and *Dialister succinatiphilus*. In many of the clone sequences, the similarity to the database sequences were less than 97%, suggesting that many of the clone sequences represented closely related, but not the same species. In addition to the above species, JT3E included *Prevotella ruminicola* and *Firmicutes*. The similarity between treated and untreated cycles for JO was consistent with the T-RFLP results, and show the possibility that the biological product was not detected by the analysis. Because 96 clones were randomly chosen and sequenced, the detection limit was higher than 1%, and it is likely that organisms in the biological product were present at less than 1% of the GI population.
Discussion

The microbial community analysis using T-RFLP showed that the dominant T-RFs in the biological product were not always detectable in the treated cycles. The most dominant “phylotype” in one product (T-RF 164) was not detected in the GI samples. This may be due to difficulties in extracting the cells or the DNA from the oily GI sample matrix. While additional DNA extraction techniques in oily GI samples can be further explored in the future, the comparison of DNA extraction methods shows that the most commonly used methods result in similar communities. It is likely that the product addition resulted in specific organisms reaching levels of less than 1% of the total microbial community. In some instances, the increase in several dominant T-RFs can be attributed to product addition, such as the increase of T-RF 159 from 1% in CT2E to 12% in CT3E. This increase in T-RF 159 corresponded to a lower FOG concentration observed in treated cycle 3, compared to untreated cycle 2 (He et al. 2011). Thus it can be hypothesized that T-RF 159, from the biological product, was contributing to FOG degradation during the treated cycle.
overall microbial communities can be described using a calculated Bray-Curtis similarity index. This index takes into account both presence and levels of specific T-RFs. The similarity comparison in microbial communities CT2M, CT2E, CT3M and CT3E are shown in Fig. 13, which is a multidimensional scaled (MDS) plot. The microbial communities of CT2M and CT2E are overlapping, showing very similar communities. However, the microbial communities in the treated cycles are very different, and the microbial communities shifted over time (CT3M vs. CT3E). It is clear that the addition of biological product changed the structure of microbial community in Ca. In particular, the organism/group corresponding to T-RF 159 in the biological product became dominant and most likely contributed to increased FOG degradation in the Ca. However, the dominant species of biological product were not detected in JO, and could be the reason why the addition of biological product in Ca resulted in better FOG degradation in Ca (p-value=0.0036) compared to JO (p-value=0.0402) discussed previously (He et al. in press). Although not as pronounced, the similarity MDS plot shows the qualitative shift in microbial communities due to addition of product in JO (Fig. 14). It is not likely that the dominant species of bioaugmentation are the direct cause of the lower FOG concentration during the treated cycle. The lower FOG concentration in treated cycle 3 (He et al. 2011) may be due to the change in microbial community structure. With respect to the effluent of Ca, the difference between untreated cycle 2 and treated cycle 3 revealed that bioaugmentation not only affected the microbial community at the top and bottom locations, but also influenced the microbial community structure in the effluent. It would also affect the downstream FOG degradation because the dominant species in the effluent were from the top layer in the grease interceptor. In all cases, differences in the microbial community structure between
treated and untreated cycles were detected. Thus, the addition of biological product resulted in shifts in the microbial composition. Taken together, the data shows that the addition of products results in changes in the GI chemistry and microbial ecology. These changes were not negative but, in some cases, positive as lower COD and FOG concentration were observed in treated cycles in both GIs (He et al. 2011).

**Figure 13** Community similarity analysis of oil layer in Ca. There are 11 squares in the figure. Two blue squares represent replicates of communities in CT2M. Red, light blue, and green squares represent triplicates of communities in CT2E, CT3M, and CT3E respectively.
Figure 14 Community similarity analysis of oil layer in JO. Two blue squares represent replicates of communities in JT2M. Red, light blue, and green squares represent communities in JT2E, JT3M, and JT3E respectively.

Though many of the sequences from both GIs are from novel (hitherto unreported) bacteria, given the less than 97% similarity with sequences in the 16S rRNA databases, the cloning and sequencing results are consistent with the T-RFLP analysis. Several organisms that can ferment a wide variety of substrates, such as sugars, were detected. Many were organisms that are known to be rumen or human feces isolates. Several organisms could degrade glycerol. One organism (*Selemonas*) had a particular species that demonstrated lipolytic activity (*Selemonas lipolytica*). These organisms were favored in the anaerobic environments of GIs, and took advantage of the food waste (a wide variety of substrates such as sugars) to dominate the microbial community. Though sequence analysis shows slight differences in the microbial community structures of JO treated and untreated cycles,
significant difference is found in Ca as the additional organisms that thermophilic were detected.

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**References**


CHAPTER 4

Evidence for fat, oil and grease (FOG) deposit formation mechanisms in sewer lines

Xia He¹, Mahbuba Iasmin¹, Lisa O. Dean², Simon E. Lappi³, Joel J. Ducoste¹ and Francis L. de los Reyes III¹*

¹Department of Civil, Construction and Environmental Engineering, North Carolina State University, Raleigh, North Carolina 27695

²Department of Food, Bioprocessing, and Nutrition Sciences, North Carolina State University

³Department of Chemistry, North Carolina State University
Abstract:
The presence of hardened and insoluble fats, oil, and grease (FOG) deposits in sewer lines is a major cause of line blockages leading to sanitary sewer overflows (SSOs). Despite the central role that FOG deposits play in SSOs, little is known about the mechanisms of FOG deposit formation in sanitary sewers. In this study, FOG deposits were formed under laboratory conditions from the reaction between free fatty acids and calcium chloride. The calcium and fatty acid profile analysis showed that the laboratory-produced FOG deposit displayed similar characteristics to FOG deposits collected from sanitary sewer lines. Results of FTIR analysis showed that the FOG deposits are metallic salts of fatty acid as revealed by comparisons with FOG deposits collected from sewer lines and pure calcium soaps. Based on the data, we propose that the formation of FOG deposits occurs from the aggregation of excess calcium compressing the double layer of free fatty acid micelles, and a saponification reaction between aggregated calcium and free fatty acids.

Key words: fats, oils and grease (FOG) deposit, sanitary sewer overflows, metallic salt of fatty acid, ATR-FTIR
Introduction

As the numbers and density of commercial food preparation and serving facilities increase, so do the amounts of fats, oils and grease (FOG) that are routinely discharged into sewer collection systems. Of the estimated tens of thousands of sanitary sewer overflows (SSOs) that occur each year in the United States, approximately 48% are due to line blockages, of which 47% are related to FOG deposits that constrict the cross-sectional access of pipe (1). Despite the central role that FOG deposits play in SSOs, very little is known about the mechanisms of FOG deposit formation in sanitary sewers. Examination of the physical properties and chemistry of FOG deposit samples from 23 cities around the United States (2), showed that FOG deposits display an adhesive character, have a grainy, sandstone-like texture and high yield strength. In addition, 16 of 19 FOG deposit samples (84%) contained greater than 50% lipid content, with the primary lipid being palmitic, a saturated fat and 85% of FOG deposit samples contained calcium as the primary metal, with average concentrations of 4255 mg/L (2). The preferential accumulation of fats and calcium further suggests that FOG deposits may be metallic salts of fatty acids and chemical saponification may be responsible for their formation (2). Calcium ions are naturally present in domestic and industrial wastewater and high levels of free fatty acids have been found in wastewater due to processes such as food frying (3). Additionally, calcium may be released from biologically induced concrete corrosion (4-6). While the saponification process may be a plausible explanation for the formation of these deposits due to their chemical constituents and physical structure, proof for this mechanism requires additional data, including the actual formation of FOG deposits under saponification condition. The objective of this study is to
verify the hypothesis that FOG deposit formation is the result of a saponification reaction between free fatty acids and metal ions such as calcium.

**Materials and Methods**

**Formation of FOG deposits under laboratory conditions.** Grease interceptor (GI) effluent from a steakhouse in Cary, NC was collected and used as the source of free fatty acids. The reaction was performed using a jar-test apparatus (Phipps & Bird JarTester\textsuperscript{TM}). In each beaker, 1L GI effluent was added and mixed with calcium chloride salt (CaCl\textsubscript{2}.2H\textsubscript{2}O) at varying concentrations. The mixing speed was set at 20 rpm and operated continuously at 20 °C for 10 days. On day 10 of the reaction process, the solution in each beaker was filtered through a wet-strengthened qualitative filter paper (>\(25 \mu\text{m}\)) using a vacuum pump to collect formed FOG deposits. The filter paper with the FOG deposits was then dried at 105 °C overnight, and the concentration of FOG deposit was determined as total suspended solids (7). Vegetable oil (canola) mixed with the same amount of calcium chloride and exposed to the same conditions as the GI effluent samples was used as control. FOG deposits were not expected to form in the control samples since no free fatty acids would be produced by hydrolysis to react with calcium salt under laboratory conditions.

**Sampling of FOG deposits in full scale field sewer lines.** Three FOG deposit samples from sanitary sewer lines in Cary, NC were obtained to compare their chemical makeup with the FOG deposit formed in the lab. One FOG deposit sample was from an apartment area (apartment), one sample from a food service establishment (shopping center 1), and one sample was from a commercial, food service and retail area of a shopping center (shopping center 2). Sites were chosen with input from utility personnel, based on past experience with
sewer lines that form FOG deposits. The wastewater at all the sampling sites represented a mixture of wastewater from food service and bathroom activities. Samples were placed on ice and stored in the lab at 4 °C.

**Fatty acid profile.** Samples of the deposits were directly saponified and converted to fatty acid methyl esters according to AOCS Ce 2-66 (8) and analyzed using gas chromatography (GC). In brief, 0.5 to 1.0 grams of sample were weighed in triplicate into glass screw topped tubes. Each tube was spiked with 0.5 mg tridecanoin (C13:0) in ethanol to serve as an internal standard. One mL of 0.5 N NaOH in methanol was added to each and the tubes were heated for 10 min at 85°C in a water bath. After cooling, 1 mL of 14% boron trifluoride in methanol was added to each tube. The tubes were recapped, vortexed, and returned to the water bath for 10 min. After cooling, 1 mL of water, followed by 1 mL of hexane was added to each tube. The tubes were vortexed at top speed for 30 sec and then allowed to stand to form layers. The top (organic) layer containing the fatty acid methyl esters was removed and dried over sodium sulfate. The fatty acid methyl esters were analyzed using a Perkin Elmer Autosystem XL GC (Sheldon, CT) fitted with a capillary BPX-070 column (SGE Inc., Austin, TX) using a flame ionization detector (FID). The column length was 30 m with an internal diameter of 0.25 mm and a film thickness of 0.25 μm. The temperature gradient was 60 °C with a 2 min hold time, increased at 4°C per min to 180 °C and then increased at 10 °C to a final temperature of 235 °C. The run time was 27.7 min. The carrier gas used was helium at a flow rate of 40 psi. The injection was split at 150 mL/min. The results were reported as percent of the total fatty acids based on peak areas as per the official method (AOCS Ce 1f-96) (8) and the total fatty acids were calculated based on the ratio of internal standard to the fatty acid peaks present when compared to a standard mixture (Kel Fir Fame 5 Standard Mix,
Matreya, Pleasant Gap, PA). The standard mixture of fatty acid methyl esters was run with each sample set to determine retention times and recoveries.

**Calcium analysis.** Calcium concentration was determined using a Perkin Elmer 2000 inductively-coupled plasma optical emission spectrometer (ICP-OES). A solid sample was placed in acid-washed porcelain crucible, and then put into muffle furnace, ramping up the temperature 100 °C every hour until 500 °C was reached. The sample was maintained at 500 °C for 16 hours. After cooling the sample, 2 mL deionized water was used to rinse residue toward the center of the crucible. 4 mL of 6N HCl was then added, and the sample was heated on a hot plate at 95 °C for 45 minutes until the sample was completely dry. The sample was then cooled and another 4 mL of 6N HCl was added to the sample with subsequent warming on the hot plate for 15 minutes. After cooling, the acid solution was filtered through a Whatman filter paper into a 25 mL glass volumetric flask and brought to the volume with deionized water. The sample was then analyzed by ICP-OES for calcium. Since the FOG deposit formed in the lab was attached to the filter paper, both filter paper and FOG deposit were simultaneously digested. The calcium concentration in the FOG deposit was determined by subtracting the calcium concentration of filter paper (0.034 mg). The liquid sample was diluted 10-fold with 1% HCl and 1% HNO₃. After dilution, the liquid sample was analyzed by ICP-OES.

**Formation of calcium soap.** An alkali hydrolysis of the vegetable (or animal) fats similar to the method used by Poulenat et al. (9) was performed to produce calcium soap at room temperature since the average temperature in the sanitary sewer collection system was observed to be 5 to 25 °C (10). Calcium chloride (9.8%wt) was added to a solution of sodium hydroxide (0.6%wt) and de-ionized water (14.9%wt). The solution was allowed to
cool to the room temperature (22 °C). The oil fat (Pure Wesson Canola Oil, ConAgra Foods, Omaha, 74.7%wt) at room temperature was gradually added and mixed to the solution. The mixture was stirred at 450 rpm using a Stir-Pak Laboratory Mix Impeller (Cole Parmer, 23-2300 rpm). The calcium soap sample for FTIR analysis from the batch reactor was collected after four hours of mixing.

**Fourier transform infrared (FTIR) spectrometer analysis.** FTIR analysis was performed for the FOG deposit sample created in the lab, a calcium soap developed from calcium chloride and canola oil, three FOG deposit samples from the sewer collection systems, pure lard, and three pure fatty acids (palmitic acid, oleic acid and linoleic acid). Infrared absorption spectra of these samples were determined with a Digilab FTS-6000 Fourier Transform Infrared (FTIR) spectrometer using a mounted crystalline Zinc Selenide attenuated total internal reflection (ATR) sampling attachment (Pike Technologies inc., MIRacle™ Single Reflection ATR). The infrared light is focused onto the photodiode of a liquid nitrogen-cooled, wide band mercury-cadmium-telluride (MCT) detector with a linearized normal spectral response of 450 to 7000 cm⁻¹. The spectra were converted into absorbance units by taking the negative of the log ratio of a sample spectrum to that of an air spectrum. The data were then computed with a data processing programs (Microcal Origin, v7.0, Microcal Software Inc., Northampton, MA.).
Results and Discussion

**FOG deposit formation under laboratory conditions.** FOG deposits formed under laboratory conditions is shown in Figure 1. The white pinpoint particles started to form at day 2, with the size of the particles increasing until its maximum observed size was achieved at day 7. To our knowledge, this is the first reported formation of FOG deposits from GI effluent under laboratory conditions. No solids were formed in the control beaker, which contained calcium, and the vegetable oil. The vegetable oil was observed on the surface at all times within the control beaker. These results are consistent with the hypothesis that in the absence of free fatty acid, calcium salt will not react to form a FOG deposit.

![Figure 1](image1.png)

**FIGURE 1.** FOG deposits formed under laboratory conditions. (a) Photo was taken at day 10 when free fatty acids reacted with calcium salt in 1L beaker; (b) Close-up of FOG deposit particles.

Similar fatty acid profiles (Table 1) were found in FOG deposit samples (R1, R2, R3) formed in the lab and FOG deposit samples taken from sewer lines (apartment, shopping center 1 and
shopping center 2). Saturated fat was the major component and palmitic was the primary saturated fatty acid in all FOG deposit samples, consistent with the results of Keener et al. (2). Monounsaturated fat was the second major component in all FOG deposit samples. Although the percentages of monounsaturated fat in FOG deposit samples from sewer lines were higher than those of the deposits formed in the lab, low percentages (around 10%) of monounsaturated fat in FOG deposits were observed in 12 FOG deposits from sewer lines (2). Oleic was the primary monounsaturated fat in the FOG deposits formed in the lab and in those collected in the apartment area and shopping center 1. In addition, linoleic was the primary polyunsaturated fat in all FOG deposit samples, similar to the results of Keener et al. (2).
**TABLE 1. Fatty acid composition of FOG deposits**

<table>
<thead>
<tr>
<th>Sample</th>
<th>Total fat $^a$ (g/g)</th>
<th>Saturated fat $^b$ (%)</th>
<th>Primary saturated fat</th>
<th>Monounsaturated fat $^c$ (%)</th>
<th>Polyunsaturated fat $^d$ (%)</th>
<th>Primary polyunsaturated fat</th>
</tr>
</thead>
<tbody>
<tr>
<td>R1</td>
<td>0.344</td>
<td>78.8</td>
<td>Palmitic</td>
<td>9.8</td>
<td>Oleic</td>
<td>0.8</td>
</tr>
<tr>
<td>R2</td>
<td>0.255</td>
<td>57.5</td>
<td>Palmitic</td>
<td>9.0</td>
<td>Oleic</td>
<td>0.6</td>
</tr>
<tr>
<td>R3</td>
<td>0.18</td>
<td>70.6</td>
<td>Palmitic</td>
<td>14.0</td>
<td>Oleic</td>
<td>0.7</td>
</tr>
<tr>
<td>Apartment</td>
<td>0.261</td>
<td>56.5</td>
<td>Palmitic</td>
<td>38.3</td>
<td>Oleic &amp; palmitoleic</td>
<td>1.0</td>
</tr>
<tr>
<td>Shopping center 1</td>
<td>0.393</td>
<td>38.7</td>
<td>Palmitic</td>
<td>37.2</td>
<td>Oleic</td>
<td>15.3</td>
</tr>
<tr>
<td>Shopping center 2</td>
<td>0.489</td>
<td>64.7</td>
<td>Palmitic</td>
<td>31.7</td>
<td>Palmitoleic</td>
<td>0.6</td>
</tr>
</tbody>
</table>

$^a$ Total fat content was calculated from 1 g FOG deposit sample

$^b$ Saturated fat is shown in percentage of the total fat

$^c$ Monounsaturated fat is shown in percentage of total fat

$^d$ Polyunsaturated fat is shown in percentage of total fat

Three reactions (R1, R2 and R3) of GI effluent and calcium chloride were assessed at calcium concentrations of 50, 400 and 750 mg/L respectively. Increasing concentrations of calcium were explored to determine any impact on the amount of FOG deposit formed. As mentioned earlier, biological reactions that induce corrosion of concrete pipes (4-6) may release excess calcium beyond that found in typical wastewaters. As additional calcium concentration was increased from 50 mg/L to 750 mg/L, the resulting FOG deposit weight
also increased (Table 2). From R1 to R3, increasing levels of calcium led to higher calcium levels measured in the FOG deposits.

**TABLE 2. Calcium and total fat amounts in FOG deposits**

<table>
<thead>
<tr>
<th>Sample</th>
<th>FOG deposit weight (mg)</th>
<th>Calcium in FOG deposit (mg)</th>
<th>Total fat in FOG deposit (mg)</th>
<th>Total fat/calcium (mg/mg)</th>
<th>Calcium/FOG deposit (mg/mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>R1</td>
<td>52.6</td>
<td>2.15</td>
<td>18.07</td>
<td>8.40</td>
<td>0.0409</td>
</tr>
<tr>
<td>R2</td>
<td>93.5</td>
<td>4.61</td>
<td>23.81</td>
<td>5.16</td>
<td>0.0493</td>
</tr>
<tr>
<td>R3</td>
<td>123.5</td>
<td>6.06</td>
<td>22.23</td>
<td>3.67</td>
<td>0.0491</td>
</tr>
<tr>
<td>Apartment</td>
<td>415.2</td>
<td>21.35</td>
<td>108.16</td>
<td>5.07</td>
<td>0.0514</td>
</tr>
<tr>
<td>Shopping center 1</td>
<td>145.5</td>
<td>0.13</td>
<td>57.22</td>
<td>438.42</td>
<td>0.0009</td>
</tr>
<tr>
<td>Shopping center 2</td>
<td>143.5</td>
<td>1.78</td>
<td>70.10</td>
<td>39.41</td>
<td>0.0124</td>
</tr>
</tbody>
</table>

Total fat in the FOG deposit increased from R1 to R2, indicating that additional calcium reacted with surplus free fatty acids. Total fat, however, remained constant at 23 mg from R2 to R3, suggesting that although more calcium was added, no more free fatty acids were available to react with calcium.

The total fat to calcium ratio is higher in FOG deposit samples collected from shopping centers than those formed under laboratory conditions, which may have been caused by different reaction conditions such as the finite amount of available free fatty acids to react with excess calcium under lab batch conditions. The FOG deposits from the shopping centers
were likely the result of long-term reactions with higher concentrations of available free fatty acids that were continuously discharged from food service establishments. However, with the same fatty acid substrate (GI effluent), in R2 and R3, the total fat concentration remained at 23 mg, but the total fat to calcium ratio decreased from 5.16 in R2 to 3.67 in R3. The decreased ratio suggests that there may be other processes aside from saponification that led to accumulation of calcium within these deposits.

The results in Table 2 suggest that there may be two processes involved in FOG deposit formation. In the first process, calcium tends to accumulate around fatty acid micelles due to a DLVO type process (11) (i.e., compression of charged double layer) due to the slightly negative carboxylic end of the free fatty acids. In the second process, free fatty acids react with calcium to form calcium based fatty acid salts through a saponification reaction. The slightly negative carboxylic ends of unreacted free fatty acids continue to attract positive calcium ions, since the saponification reaction may be slow compared to the transport of calcium ions towards the solid deposit (i.e., a reaction limited process) (9). Due to the slower saponification reaction, it is hypothesized that more calcium than the stoichiometric amount needed for saponification would accumulate in the deposit. Research is needed, however, to confirm the involvement of a double layer compression process along with a saponification reaction to create solid FOG deposits in sewer lines.

**FTIR analysis.** FTIR is a simple and powerful technique that is widely applied to determine oil and grease in water (12-15), oily materials in different chemical processes (16-19), trans fat in food (20, 21), and fatty acids and fatty acid salts (9, 22-24). If the saponification hypothesis is correct, then the calcium soap should be detected in the FOG deposit. In the infrared spectra, when free fatty acids react with calcium salt and the “hard” metallic salts of
fatty acids (soaps) is formed, the carbonyl group stretching vibration at 1745 cm\(^{-1}\) of triacylglycerols (TAG) disappears and three characteristic calcium soap bands appear: (i) the carboxylate ion symmetric stretching vibration, \(v_1\) at 1422 cm\(^{-1}\); (ii) the carboxylate ion asymmetric stretching vibration, \(v_2\) at 1577 cm\(^{-1}\); and (iii) the metal-oxygen bond vibration at 665 cm\(^{-1}\) (9, 24). Poulenat et al. (9) identified four regions that can be attributed to the formation of calcium soaps: region 1: 4000-3000 cm\(^{-1}\); region 2: 1800-1350 cm\(^{-1}\); region 3: 1350-1180 cm\(^{-1}\) and additional side band near 720 cm\(^{-1}\); region 4: near 670 cm\(^{-1}\).

**Infrared spectrum of FOG deposit formed in the lab.** The infrared spectra of the FOG deposit formed in the lab, and calcium soap made from canola oil and calcium chloride are shown in Figure 2. The lab FOG deposit infrared spectra appear quite similar to the pure calcium soap infrared spectra. Comparisons were made between these two samples based on the four characteristic regions of calcium soap discussed in Poulenat et al. (9).
FIGURE 2. Baseline corrected infrared spectra of FOG deposit formed in the lab and calcium soap

In region 1 between 4000-3000 cm\(^{-1}\), the band has been associated with O-H stretching vibration of hydrated water (9). Soaps have a broad absorption around 3400 cm\(^{-1}\), which is characteristic of hydrogen bonding and the polar head groups of the soap molecule (24,25). This strong absorption band at 3400 cm\(^{-1}\) is observed in both the FOG deposit and calcium soap as shown in Figure 2. Both the lab-scale FOG deposit and the pure calcium soap spectra display the presence of water (liquid) (located within the broad peak and between 3500 and 3700 cm\(^{-1}\) (26). The presence of water is not surprising since both fatty acid salts
contained water during the saponification process. Four bands, located at 3004, 2955, 2922 and 2851 cm\(^{-1}\), represent the frequencies of the aliphatic chains of the soap (9). These bands display no significant modifications as a result of saponification.

In region 2, the total disappearance of the stretching vibration at 1745 cm\(^{-1}\), attributed to the frequency of the ester bond in triglycerides (TAG), should be observed for calcium soap formation (9). Two modes of vibration, attributed to the carboxylate group of the fatty acid metallic salt, were expected: the symmetric stretching vibration, \(\nu_1\); and the asymmetric vibration, \(\nu_2\). The appearance of two absorption bands \(\nu_1\) and \(\nu_2\), of the carboxylate group instead of a single band at 1745 cm\(^{-1}\), shows that calcium soaps possess an ionized structure and that calcium-oxygen bonds in the soaps have an ionic character (26). In calcium soaps, these two stretching vibrations were split into two or three bands: \(\nu_2\) was split into 1577 and 1541 cm\(^{-1}\), and \(\nu_1\) was split into 1468, 1435 and 1422 cm\(^{-1}\) (9). All five bands were observed in the infrared spectra of the FOG deposit and calcium soap made in the lab as shown in Figure 2. The disappearance of the band at 1745 cm\(^{-1}\) was also observed in the FOG deposit. The existence of the band at 1745 cm\(^{-1}\) in the calcium soap sample (Figure 2), however, may be due to excessive canola oil that was used to react with calcium. Un-reacted canola oil was visually present when the calcium soap was analyzed.

In region 3, the spectral region of the aliphatic chains is sensitive to the crystallization of soap (9). Many absorption bands in this region are approximately equally spaced and with weak intensities. Poulenat et al. (2003) observed a 722 cm\(^{-1}\) singlet for their calcium soap spectrum. This band near 720 cm\(^{-1}\) is likely due to the rocking vibration of successive methylene groups, \((\text{CH}_2)\) found in calcium (9) and other metallic soaps (28). The two characteristic bands (1350-1180 cm\(^{-1}\) and an additional side band near 720 cm\(^{-1}\) are
found in both the lab-scale FOG deposit and calcium soap as shown in Figure 2. In region 4, the calcium-oxygen bond absorption band is at 665 cm$^{-1}$ (9). This band was present in the infrared spectra of both samples. An unknown cluster of spectral peaks was found around 1000-1300 cm$^{-1}$ in both the lab-scale FOG deposit and the pure calcium soap spectra that was not present in any other FTIR spectra analyzed in this study. These additional peaks may be due to organic constituents within the FOG source (unknown for the lab-scale FOG deposit and canola oil for the pure calcium soap) containing possible unsaturated trans double bonds that displays peaks near this region (26).

Overall, the strong similarity between FOG deposit and calcium soap infrared spectra, particularly the four absorption bands of Poulenat et al. (9) suggest that FOG deposit is likely a metallic salt of fatty acid made of calcium and formed as a result of saponification. The question then is whether FOG deposits from actual sewer lines have similar spectral profiles as with the pure calcium soap and the lab-scale FOG deposits, and in addition, if these spectral signatures differ in pure fatty acids or lipid samples.

**Infrared spectra of FOG deposits from sewer lines.** The infrared spectra of three FOG deposit samples are shown in Figure 3. The FOG deposit sample from the apartment area has a strong infrared spectral similarity to the FOG deposits formed in the lab. The absorption bands in four characteristic parts of the calcium soap were present in the FOG deposit sample from the apartment area, including the broad band at around 3400 cm$^{-1}$ (region 1), the disappearance of absorption band at 1745 cm$^{-1}$ and appearance of two absorption bands of $\nu_1$ and three absorption bands of $\nu_2$ (region 2), the singlet at 722 cm$^{-1}$ (region 3) and the calcium-oxygen band at 665 cm$^{-1}$ (region 4). The FOG deposits from shopping centers 1 and 2 display some similarity but differ from the lab FOG deposit and
apartment area samples. All samples displayed the absorption bands from characteristic regions 1, 3 and 4 in their infrared spectra. Significant differences, however, were noted in region 2 (1800-1350 cm$^{-1}$). In this region, although the absorption band at 1745 cm$^{-1}$ was not present, two strong intensity absorption bands appeared at around 1700 cm$^{-1}$. Absorption bands at 1577 cm$^{-1}$ and 1541 cm$^{-1}$ were observed in $\nu_2$ from shopping center 2 but only 1541 cm$^{-1}$ was observed in $\nu_2$ from shopping center 1. Three other bands at 1462, 1430 and 1411 cm$^{-1}$ appeared in both shopping center samples.

FIGURE 3. Baseline corrected infrared spectra of three FOG deposit samples from sewer lines
The differences noted in region 2 could be attributed to other materials accumulating in the FOG deposit, which led to the overlap of other absorption bands. In particular, the calcium-oxygen bond was present in region 4, but unlike the lab-scale and apartment area FOG deposit samples, the peak of the broad band was not at 665 cm\(^{-1}\) but at 680 cm\(^{-1}\). It is likely that other materials accumulating in the FOG deposit affected the appearance of absorption bands. As discussed earlier, a large amount of fatty acids accumulated in shopping center FOG deposits. It is likely that the accumulation of fatty acids or unsaturated oil such as canola affected the appearance of absorption bands since a number of different food service establishments that potentially discharge a wide variety of lipid contents are present in the shopping center.

**Infrared spectra of pure fatty acids and fat.** Three dominant fatty acids measured in the FOG deposits (palmitic, oleic and linoleic) were subjected to FTIR analysis (Figure 4). In the region of 1800-1350 cm\(^{-1}\) (region 2), for palmitic acid, a single strong intensity band was found at 1690 cm\(^{-1}\). Three other bands also appeared in the palmitic acid spectrum at 1470, 1430 and 1411 cm\(^{-1}\). For oleic acid, a single strong intensity band was located at 1704 cm\(^{-1}\) with three other bands located at 1464, 1430 and 1411 cm\(^{-1}\). The linoleic acid spectrum also displayed a single strong intensity band at 1704 cm\(^{-1}\) with three other bands observed at 1460, 1430 and 1411 cm\(^{-1}\).
FIGURE 4. Baseline corrected infrared spectra of palmitic, oleic and linoleic acids

The three strong intensity bands at 1690 cm\(^{-1}\), 1704 cm\(^{-1}\), 1704 cm\(^{-1}\), from palmitic, oleic and linoleic respectively, may account for the two strong intensity bands observed near 1700 cm\(^{-1}\) in shopping center FOG deposit samples. The three bands located at 1464, 1430 and 1411 cm\(^{-1}\) in the shopping center FOG deposits are very similar in position to the three bands observed in the oil standards. There were no bands located at 1577 and 1541 cm\(^{-1}\). However, the weak intensity bands at 1577 and 1541 cm\(^{-1}\) were observed in the FOG deposits. The
results of the fatty acid spectra appear to support the hypothesis that five characteristic bands of calcium soap are present but with weak intensities and are masked by the bands attributed to the accumulation of fatty acids. In the region at around 670 cm\(^{-1}\), palmitic had an absorption band at 680 cm\(^{-1}\), while oleic and linoleic had shoulders at 680 cm\(^{-1}\). These differences in peaks for the fatty acids investigated could explain why the spectral peak of FOG deposits from shopping centers was at 680 cm\(^{-1}\) instead of 665 cm\(^{-1}\) as suggested by Poulenat et al. (9).

Lard or an equivalent saturated fat is another possible candidate accumulating in the FOG deposit as it solidifies at room temperature. The infrared spectrum of lard is displayed in Figure 5. In the region of 1800-1350 cm\(^{-1}\), a strong intensity absorption band was located at 1731 cm\(^{-1}\) instead of near 1700 cm\(^{-1}\). No absorption band was observed at or near 670 cm\(^{-1}\). Consequently, lard was not likely one of the materials that accumulated in the shopping center FOG deposits.

FIGURE 5. Baseline corrected infrared spectrum of lard
Therefore, the FOG deposit from the apartment area is likely the product of saponification reaction as is the lab-scale FOG deposit. However, the FOG deposits from the shopping centers are not only the products of the reaction between free fatty acids and calcium but also contained un-reacted fatty acids such as palmitic, oleic and linoleic acids. These un-reacted free fatty acids may likely draw calcium and other cations towards the solid FOG deposit matrix based on the effects of van der Waals attraction and electrostatic repulsion (DLVO theory) (11). However, more research needs to be performed to prove this additional mechanism of drawing calcium towards the FOG deposit.

FTIR analysis demonstrated a strong similarity between the lab-scale FOG deposit and calcium soap as shown in their infrared spectra, particularly in the absorption bands of four characteristic regions previously identified for calcium soap. Analysis of these four infrared spectral band regions indicated that FOG deposits are likely metallic salts of fatty acid made of calcium and formed as a result of saponification. However, the difference among FOG deposit samples, such as different total fat to calcium ratios and appearance of additional bands due to the un-reacted fatty acids (e.g., palmitic, oleic and linoleic), indicated that some FOG deposits are not only formed by the reaction between free fatty acids and a metal but are also aggregates made of excess calcium or fatty acids based on DLVO theory. Although the spectral peak positions of the different samples analyzed in this study are not significantly shifted, peak intensities were different. It is possible that the different FOG sources may have gone through oxidative changes (i.e., become more oxygenated), causing the fatty acid salt to contain more polar that lead to band shifts as well as changes in intensity. In addition, changes in intensity could be the result of different FOG source concentrations. Nonetheless,
the results of this study shed light on the formation mechanisms of FOG deposits, and will
ultimately lead to an improved understanding of possible measures to prevent FOG deposit
from forming and blocking sewer lines.

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CHAPTER 5
Mechanisms of Fat, Oil and Grease (FOG) Deposit Formation in Sewer Lines
Xia He, Lisa O. Dean, Simon E. Lappi, Francis L. de los Reyes III, Joel J. Ducoste

Abstract:
FOG deposits that form in sewer lines have been shown to be metallic salts of fatty acids in previous research. In this study, we demonstrated that calcium, the dominant metal in FOG deposits, was released from concrete under different pH conditions. In the presence of a concrete block, FOG deposits were first formed on the concrete surface when a small amount of oil was added to grease interceptor effluent. The added oil played a role as a carrier instead of the acting as the source of free fatty acids (FFAs) in the saponification reaction. The effect of different fatty acids on the reaction at the concrete surface was determined. The results show that unsaturated fatty acids formed a more viscous, “stickier” solid on concrete surface and resulted in more severe concrete corrosion compared to saturated fatty acids. The results were integrated into a more comprehensive model of the mechanisms of FOG deposit formation.

Introduction
Sanitary sewer overflows (SSOs) occur when sewer lines are blocked by debris, roots, and deposits that are typically thought of as arising form or composed of fat, oil and grease (FOG). The FOG originated from wastewater discharged from Food Service Establishments (FSEs), multifamily housing, and single family homes, and FOG deposits are estimated to be responsible for 47% of the line blockages that lead to sanitary sewer overflows (SSOs) (1).
In previous research, high concentrations of fatty acids and calcium were found in FOG deposits, collected from 23 cities around the United States (2). In subsequent research, we were able to form FOG deposits by mixing the two major components (i.e., free fatty acids and calcium) (3). FTIR analysis and comparisons with FOG deposits and pure calcium soaps provided evidence that both laboratory-produced and natural FOG deposits are calcium salts of fatty acid and formed as a result of saponification (3). Since FOG deposits display an adhesive character and strongly adhere to interior pipe walls above the water mark (2), the reaction between free fatty acids and calcium may occur as a surface reaction.

The surface reaction responsible for FOG deposit build up on pipe walls is still unclear. Although calcium ions are naturally present in domestic and industrial wastewater, there was no correlation found between calcium concentration in FOG deposit samples and water hardness (2). Another possible source of calcium is from biologically induced concrete corrosion (4-6) since calcium hydroxide is one of the major components in concrete. The objectives of this study were to examine the calcium leaching from concrete and to investigate the surface reaction to further understand the mechanisms of FOG deposits formation in sewer lines.

Materials and Methods

**Calcium leaching from concrete.** The concrete sample used in this study was obtained from the concrete lab in the Department of Civil, Construction and Environmental Engineering, NCSU. The major components of the concrete sample were cement (19%), sand (31%), coarse aggregate (42%) and water (8%). The concrete sample was cut into
rectangular shapes with dimensions of 5x2x2 cm. Calcium leaching tests were performed using a jar-test apparatus (Phipps & Bird Jar Tester) at different pH levels. A single concrete block (5x2x2 cm) was suspended vertically so that half of the concrete block was submerged in 1 L de-ionized water or GI effluent collected from a steakhouse in Cary, NC. The mixing speed was set at 20 rpm and operated continuously at 20 °C for 24 days. The pH was maintained at 3, 4, 5, 6, 7 and 8 in six beakers each with either de-ionized water or GI effluent. Daily additions of sodium hydroxide or sulfuric acid were used to maintain the target pH in each beaker. Liquid samples were collected every three days from each beaker with de-ionized water for calcium analysis (3). For beakers with GI effluent, liquid samples were collected at day 1 and day 24 for calcium analysis.

**Formation of FOG deposits on concrete surface.** Concrete, a commonly used sewer pipe material, was selected to investigate the formation of FOG deposits on sewer pipe walls. 1 g soybean oil was added to a mixed system that included 1 L GI effluent, calcium chloride (400 mg/L Ca\(^{2+}\)), and a single rectangular shaped concrete block (5x2x2 cm) that was suspended vertically with half of it submerged. The mixing speed was set to 20 rpm and the system was operated continuously at 20 °C for 14 days. A sample prepared as described above but with no soybean oil was used as a control. Solid samples were scraped off the concrete blocks from both mixing systems at day 14 for Fourier Transform Infrared (FTIR) Spectrometer analysis (3).

**The role of oil in the saponification surface reaction.** These experiments were performed using a jar-test apparatus (Phipps & Bird Jar Tester). In each beaker, 1 g soybean
oil was added to 1 L calcium chloride solution with a calcium concentration of 400 mg/L. The mixing speed was set at 20 rpm and the system was operated continuously at 20 °C for 32 days. Three pH conditions were considered. Two of them were basic (pH=8 and pH=9) to mimic a slightly alkaline environment in the presence of a concrete block. Sodium hydroxide and sulfuric acid were used to maintain pH to the assigned value in each beaker every day. For the third condition, no pH adjustment was performed, and the initial pH was 7. 

Duplicate tests were performed for each pH condition. A solid sample was collected on day 32 in each beaker for FTIR analysis. Another three scenarios were studied in three 300 mL beakers with concrete blocks: 100 mL soybean oil; mixture of 25 mL soybean oil and 75 mL de-ionized water; and 100 mL soybean oil with the addition of 1 g palmitic acid (C16:0). In each beaker, half of the concrete block was submerged. The beakers were placed on a multi-position magnetic stirrer (Fisher Scientific Thermix Stirrer Model 120S) under mild mixing intensity conditions. Observations were performed at 20 °C for 24 days.

**The effect of different types of fatty acids on saponification surface reaction.** Palmitic (C16:0), oleic (C18:1), and linoleic (C18:2) acids (Fisher & Fluka), three major fatty acids present in FOG deposits (2, 3), were selected to study the effect of different types of fatty acids on the saponification surface reaction. Palmitic acid (0.25 g and 1 g), 0.25 g oleic acid and 0.25 g linoleic acid were added, each to 300 mL beakers with 25 mL soybean oil and 75 mL de-ionized water in each beaker. Three replicates were performed. The beakers were placed on a multi-position magnetic stirrer (Fisher Scientific Thermix Stirrer Model 120S) under mild mixing intensity conditions. After overnight mixing, a concrete block (5x2x2 cm) was added and suspended with in the vertical position and half submerged in the solution.
Observations were performed at 20 °C for 32 days. The pH was measured every 2 days. After 32 days, the solid that accumulated on the concrete surface was scraped off. The solids in solution were collected by filtering the solution through a wet-strengthened qualitative filter paper (> 25 µm). Both solid sample were dried overnight at 105 °C and the dry samples were analyzed by FTIR.

**Results and Discussion**

**Calcium leaching from concrete.** Even though a large amount of calcium was found in the FOG deposit samples, no correlation between calcium concentration in FOG deposit samples and water hardness was found (2, 3). A possible source of calcium in sewer systems is concrete since calcium may be released due to Microbially Induced Concrete Corrosion (MICC) (4-6). The production of sulfuric acid by microorganism activities is the key step in MICC (4). The pH thus an important factor that affects the amount of calcium released from concrete. When the concrete blocks were placed in de-ionized water under different pH conditions, the calcium concentration in solution increased at higher rates with decreasing pH (Figure 1). These results suggest that the concrete block experienced higher corrosion rates with decreasing pH. Since calcium oxide is a major component in cement (i.e., 64%) (7), the resulting pH of the moisture on the concrete surface could reach 13 (4). When the concrete sample was hydrated in this study, the calcium hydroxide released can react with sulfuric acid to form CaSO₄, which can then react with Ca aluminate present in the cement mixture to produce ettringite ((CaO)₃·Al₂O₃·(CaSO₄)₃·32H₂O) (4). Ettringite is an expansive product and can result in small cracks in concrete (4). It changes into gypsum (CaSO₄·2H₂O) when the pH is decreasing (8). Gypsum and ettringite may be removed by the flow thereby
accelerating the corrosion process (9). At pH = 3, the calcium release displayed a linear profile and achieved a calcium concentration of 418 mg/L on day 24. At higher pH levels (≥ 4), the calcium leaching trends were similar but not linear. The big gap between pH = 3 and pH = 4 may be due to the lime buffering. At lower pH (pH = 3), the environment was very acidic and aggressive, resulting in a significant release of calcium hydroxide from concrete sample and the inability to buffer the solution. Even the calcium from calcium silicate was released. But at higher pH, calcium hydroxide may not be fully released and still able to buffer in the system. For pH 6, 7 and 8, the released calcium concentrations were 127, 111, and 72 mg/L respectively on day 24. Thus, even for around neutral conditions, considerable amounts of calcium were released from the concrete sample. When concrete blocks were placed into the GI effluent solution, lower concentrations of calcium leaching from concrete were observed for all six pH conditions than in de-ionized water. On day 24, the released calcium concentrations from concrete were 381, 130, 89, 90, 53 and 45 mg/L at pH 3, 4, 5, 6, 7, and 8, respectively. Again, a sharp decrease in calcium concentration between pH 3 and 4 was observed. Calcium was released from concrete near neutral pH in both de-ionized water and GI effluent demonstrating that concrete, as a source, is responsible for a significant amount of calcium present in the wastewater in sewer system.
Figure 1  Calcium was released in de-ionized water when pH was maintained at 3, 4, 5, 6, 7 and 8 respectively.

Formation of FOG deposits on the concrete surface. Imaging of sewer lines has revealed that most FOG deposits are found along the pipe cross-section above the low-flow water mark (2). These photographic depictions of FOG deposits suggested the preferential accumulation of FOG deposit precursor chemicals at the water surface. Such accumulation may occur with the addition of non-polar material such as oil which is also a potential source of FOG deposit chemical precursor. Samples subjected to 1 g oil addition to the system that had half of the concrete block submerged in GI effluent displayed white solid formation within 2 days at the concrete and water interface (Figure 2). These white solids were also found on glass wall and beaker bottom. However, most of the white solids adhered to concrete surface above the water mark. In addition to the white solids, brown particles were observed in solution. Analysis of the white solids collected on concrete and beaker bottom
using FTIR revealed that they were soap according to the four characteristic absorption bands associated with calcium soap (3) (Figure 3). Moreover, two characteristic bands of calcium soap were also identified from the FTIR spectral analysis of the brown particles. The spectral signal of the organic part of the other FTIR Ca soap characteristic regions were weak, which showed that less of free fatty acids were available to react with calcium. These results represent the first time that FOG deposits were formed on concrete surfaces under laboratory conditions. Under control conditions, without the addition of oil, solid did form as free fatty acids from GI effluent reacted with calcium leaching from concrete. However, none of these solids accumulated on the concrete surface above water mark. Though few particles were attached to submerged concrete surface, unlike the natural FOG deposits that display an adhesive character and can become securely bound to interior pipe walls, these submerged solids would quickly detach once the mixing speed was increased to 50 rpm. These results suggest that the presence of oil may be required to facilitate the FOG deposit formation on sewer pipe walls.

![Figure 2 Solids formed on concrete surface with the addition of oil in GI effluent](image)
Based on our knowledge, two hypotheses may responsible for the role of oil in surface reaction to form FOG deposit on sewer pipe walls: (i) when oil was exposed to a base concrete surface, it would undergo hydrolysis from the release of calcium hydroxide to form free fatty acids then react with calcium to form soap; (ii) oil acts as a non-polar partitioner of that transport any free fatty acids to the air-water interface and then react with the calcium available on the concrete surface, but not play a role in the release of free fatty acids. To prove the first hypothesis, 1 g oil was added to 1 L calcium chloride solution under three pH conditions (pH=8, pH=9, without pH adjustment). When the pH was maintained at 9, the oil started to display the formation of white solids at day 28. When the pH was kept at 8, the oil
began to display the formation of white solids at day 32. Finally, with no pH adjustment, there was no solid formed. The samples on the surface were collected and examined by FTIR. The spectra of three samples together with pure oil sample are shown in Figure 4. The FTIR result confirmed that only oil was present since the spectrum was the same as the pure oil for the sample with no pH adjustment. Under basic conditions, the spectrum of sample obtained at pH=8 was the same as the one at pH=9. Compared with pure oil, three significant differences were noted for pH=8 and pH=9 samples. First, a significant peak was displayed at 665 cm⁻¹, which was one of three characteristic calcium soap bands representing calcium-oxygen bond (8). Another difference was the appearance of a significant peak at 3400 cm⁻¹ associated with calcium soap. The third difference was the appearance of the band at 970 cm⁻¹. It falls within the region where absorption bands describe the appearance of glycerol appeared (10), a product of oil hydrolysis. Although the bands for the other two characteristic regions were not observed for samples collected under pH=8 and 9 conditions, the appearance of these other three bands indicate that the saponification was ongoing. These samples were likely the intermediates during the soap formation. However, at pH below 10, the soap formation process due to oil hydrolysis (hypothesis (i)) was so slow that it can’t be the explanation of the soap formation on concrete surface within 2 days as mentioned above. Therefore, the oil acted as a carrier not a source of free fatty acids in surface reaction during the soap formation in the pH=8 and 9 conditions. To get a comprehensive understanding of the role of oil in sewer pipe walls, the concrete blocks were placed in pure oil, oil and water mixture, and the mixture of palmitic acid and pure oil. After 24 days mixing, no solid was observed on concrete surface under all three conditions. Without the presence of water, calcium hydroxide may not be released because the pH was around 7 in both pure oil and
mixture of oil and palmitic acid systems and soap was not formed even though palmitic acid dissolved in oil and was available to react with calcium. With the presence of water, little amount of calcium hydroxide may be released from concrete since the pH was increased from 7 to 8 at day 12 and then stayed at 8. The fact that no solid was formed in 24 days was another evidence to support the conclusion that oil acted as a carrier not a source of free fatty acids in the surface reaction in sewer lines.

Figure 4 Baseline corrected infrared spectra of soybean oil, sample under no pH adjustment, white solid sample at pH=8 and white solid sample at pH=9.

The effect of different type of fatty acids on the saponification surface reaction. The generation of free fatty acids due to cooking process or microbial activities (11, 12) triggers
saponification leading to the formation of FOG deposits in sewer lines. Palmitic acid, oleic acid and linoleic acid were three major free fatty acids by examining the fatty acid characterization of grease interceptor FOG, (13). These three fatty acids were also found to be major fatty acids contributing to FOG blockages (2, 3). When concrete block was exposed to the mixture of oil and water, with the addition of palmitic acid, the solid began to build up on concrete surface within 2 days and kept accumulating. After 32 days mixing, the soft and not sticky solid was surrounding the concrete surface as shown in Figure 5. The solids formed on the concrete surface and in solution were measured and shown in Figure 6. There was average 9.4 g solid scraped off the concrete surface for Palmitic_2 but only 1.2 g for Palmitic_1 (Figure 6a). No solid was found in Palmitic_1 solution but 6.3 g solid was obtained in solution of Palmitic_2 (Figure 6b). The results of Figure 6 indicated that the amount of solid formation is proportional to the addition amount of palmitic acid. The pH was around 8 for Palmitic_1 and reduced to 7 as more palmitic acid was added. Low concentrations of calcium were released from concrete in Palmitic_1 & 2 as shown in Figure 7. Similar amount of calcium was obtained in solutions in Palmitic_1 (17.1 mg/L Ca\(^{2+}\)) and in Palmitic_2 (14.3 mg/L Ca\(^{2+}\)). In terms of the FTIR result, high similarity was also found between Palmitic_1 and Palmitic_2. Their infrared spectra are different from the infrared spectrum of pure oil as they both displayed two additional bands: one was the band at 970 cm\(^{-1}\) representing the existence of glycerol; the other one was a characteristic band of calcium soap at around 1577 cm\(^{-1}\) which represents the carboxylate ion asymmetric stretching vibration (10) as shown in Figure 8a. Though other two characteristic bands of calcium soap were not found, the appearance of bands at 970 cm\(^{-1}\) and 1577 cm\(^{-1}\) indicated the saponification had also begun in these samples but the soap forming process was likely
not complete at day 32. In the Oleic samples, the solid was not formed on the concrete surface as quickly as in the Palmitic_1&2 samples. However, the Oleic samples were stickier and displayed higher aggregation around the concrete surface. When the concrete block was removed, there was 1.1 g solid affixed to the concrete surface (Figure 5a) and 16.9 g solid left in solution. The total amount of solid in the Oleic sample was higher than Palmitic_1 and Palmitic_2. Another difference was that the pH for Oleic did not remain constant as in Palmitic_1&2, but dropped suddenly to 5 at around day 23. The drop of pH likely accelerated the calcium leaching from the concrete sample as the average calcium concentration was equal to 521.3 mg/L, a concentration much higher than the amount in Palmitic_1&2 (Figure 7). The infrared spectrum of the solid in Oleic sample was also different from Palmitic_1&2. Two characteristic bands of calcium soap appeared at 665 cm\(^{-1}\) representing the calcium oxygen bond and at 3400 cm\(^{-1}\) representing O-H stretching vibration of hydrated water in soap, which were not part of the spectral analysis of Palmitic_1&2. Glycerol was also found for the Oleic sample given the existence of absorption band at 970 cm\(^{-1}\). Though the characteristic band at 1577 cm\(^{-1}\) was not found, an absorption band at 1588 cm\(^{-1}\) appeared in that region, which may shift to 1577 cm\(^{-1}\) if more time was allowed for the reaction. The saponification process was not complete in the Oleic samples as the bands were likely in the process of shifting. For the linoleic acid samples, all solids were affixed to the concrete block (Figure 5d). The pH dropped from 8 to 5 at day 15. A possible reason for the pH drop in both the Oleic and Linoleic samples is the oxidative rancidity of unsaturated fatty acids. The oxidative rancidity reaction occurred in three stages. The initiation reactions produced small numbers of free radicals (14). In this case, the free radical may be generated by the reaction between oxygen and elemental ferrous since 1-3% of ferrous oxide is present in slag.
which is one component in concrete aggregate (7). The reaction goes as $2\text{O}_2^{2-} + 2\text{H}^+ + 3\text{Fe}^{2+} \rightarrow \cdot\text{OH} + \text{OH}^- + 3\text{Fe}^{3+}$ (15). A small number of free radicals can trigger the propagation to give lipid peroxide as shown in Figure 9 (16). At the final stage, the ferric, released from cement, would react with lipid peroxide (RCOOH) as $\text{Fe}^{3+} + \text{RCOOH} \rightarrow \text{Fe}^{2+} + \text{H}^+ + \text{RCOO}^-$. The production of $\text{H}^+$ would accelerate the calcium hydroxide being released from concrete and cause the pH to drop in the solution. Since there are two double bonds in linoleic acid and one double bond in oleic acid, twice RCOOH should be produced that leads to twice $\text{H}^+$ produced and twice calcium released in Linoleic than in Oleic. This difference in unsaturation would explain why the pH dropped several days ahead in Linoleic than in Oleic. The calcium analysis revealed that 1001 mg/L calcium was released in Linoleic solution. The Linoleic calcium release amount was almost twice the calcium released in Oleic and further supports the reaction mechanism that lead to the pH drop. The FTIR spectral analysis also revealed similar profile for both Oleic to Linoleic (Figure 8). The only difference of absorption bands was in the region between 1550 cm$^{-1}$ to 1650 cm$^{-1}$. The absorption band at 1630 cm$^{-1}$, instead of 1588 cm$^{-1}$, was observed in Linoleic, which may be additional evidence to support the hypothesis of band shifting from 1630 cm$^{-1}$ to 1580 cm$^{-1}$ and then to 1577 cm$^{-1}$ as the formation of carboxyl group of soap occurs. According to the FTIR results, the similarity between Oleic and Linoleic as well as the difference between Oleic, Linoleic and Palmitic_1&2 revealed that the steps of saponification are different between saturated fatty acids and unsaturated fatty acids. In general, when saturated fatty acids, such as palmitic acid, reacted with calcium, less sticky solid may be formed and less corrosion on concrete surface. However, for unsaturated fatty acids, such as oleic and linoleic acids, with increasing number
of double bonds, stickier tacky solid may be formed and more corrosion can occur and result in significant amounts of calcium released from the concrete sample.

Figure 5 Solids formed on the concrete surface with addition of different fatty acids: (a) Palmitic_1: addition of 0.25 g palmitic acid; (b) Palmitic_2: addition of 1 g palmitic acid; (c) Oleic: addition of 0.25 g oleic acid; (d) Linoleic: addition of 0.25 g linoleic acid

Figure 6 (a) The weight of solid scraped off the concrete surface; (b) The total weight of solid both from concrete surface and solution
Figure 7 Calcium released from concrete block with different types of fatty acids

Figure 8 Baseline corrected infrared spectra of solid samples formed on concrete surface with addition of different types of fatty acids
Proposed mechanisms of FOG deposit formation in sewer lines. The results of this study suggest that there are four major components attributing to FOG deposit formation on sewer pipe walls: calcium, free fatty acids (FFAs), FOG (or oil), and water. Here, the oil acts as a primary carrier and a minor source of FFAs in sewer pipelines. FOG deposits will likely not form in the absence of any of the four components. The general understanding of FOG deposit formation in sewer lines can be demonstrated in Figure 10. When FFAs, produced from cooking process (11) or generated by microbial activities in grease interceptor (13), were discharged into sewer pipelines, they would partition into oil and flow on wastewater surface. With the presence of calcium that is at the interface of oil and water or oil and concrete, saponification occurs at a fast rate. The results suggest that the type of free fatty acids may influence the adhesive quality of the soap produced. The more unsaturated fatty acids present in the wastewater, the more adhesive soap would likely be formed. The built-up process of FOG deposit on sewer lines is not only caused by soap formation, but also due to its adhesive character to capture the oil with un-reacted free fatty acids in wastewater (3).
Soap will act as a core affixed to the sewer pipe walls. The un-reacted FFAs would move toward and stick to the core. These un-reacted free fatty acids may likely draw calcium and other cations toward the solid core matrix based on the effects of van der Waals attraction and electrostatic repulsion (DLVO theory) \(^3\). Saponification will occur between un-reacted FFAs and calcium on the matrix to form more soap cores that result in FOG deposit accumulation on sewer pipe walls. Because of the adhesive character, surface charge, and flow restrictions in sewer lines, the debris in wastewater would also accumulate and result in the formation of debris layers interspersed with hardened FOG.

Figure 10 Proposed mechanisms of FOG deposit formation in sewer lines
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CHAPTER 6

The formation of fat, oil and grease (FOG) deposit in pilot-scale pipe loop system

Abstract:
A study was performed to form FOG deposits in a simulating sewer pipeline system, which can provide deeper understanding into the mechanisms of FOG deposit formation in sanitary sewers and further help to make strategies to maintain a sustainable sewer collection system. In this study, a pilot-scale pipe loop system was set-up to simulate gravity flow pipelines and pump-station wet wells. Significant amount of calcium was consumed, and the FTIR results indicated soap was formed in the pipe loop system. The sticky “hard” soap was found to be built up on the two tank walls above water mark, but the softer and less sticky solid was obtained at the bottom of PVC pipeline. The results of this study revealed that hard FOG deposits were formed at the spot where the oil was accumulated. The free fatty acids that did not partition into oil can react with calcium to form soft and not sticky soap that settle down at downstream pipeline. These results also supported the speculation that oil plays an important role as a carrier of free fatty acids in pipe surface saponification reaction that leads to the formation of FOG deposits above low-flow water mark.

Introduction
Hardened and insoluble fats, oil, and grease (FOG) deposits are the primary cause of sewer line blockages leading to sanitary sewer overflows (SSOs). As the numbers and densities of commercial food preparation and serving facilities increase, so does the amount of FOG that
are routinely discharged into the nation’s sanitary sewer systems. Of the estimated 75,000 SSOs that occur each year in the United States, approximately 48% are due to line blockages, of which 47% are related to FOG (EPA, 2004). SSOs are not only unlawful releases of untreated wastewater into the waters of the United States; they also introduce significant amounts of environmentally detrimental nutrients into river segments already plagued with algal blooms. Grease-related SSOs resulted in the discharge of about 114,000 m$^3$ (30 million gal) of wastewater, which not only introduced pollutants to the environment, but also exposed the public to pathogens (EPA, 2004). In a previous study, FOG deposits were recreated under laboratory conditions by mixing the two major components (i.e., free fatty acids and calcium) (He et al. 2011). The results of He et al. provided evidence that both laboratory-produced and natural FOG deposits are metallic salts of fatty acid made of calcium and formed as a result of saponification. Their further study proposed a hypothesis that oil plays an important role in FOG deposit formation above water mark on sewer pipe walls. However, no FOG deposit has been formed above water mark in a pilot-scale sewer pipeline system. In this study, to provide a deeper understanding into the mechanisms of FOG deposit formation in sanitary sewers and further help to make strategies to maintain a sustainable sewer collection system, a pilot-scale pipe loop system was set-up to simulate the potential for FOG deposit formation in gravity flow pipelines and pump-station wet wells. Experimental tests were performed to directly assess FOG formation in sewer lines under controlled conditions.
Methods and Materials

A pilot scale sewer collection system was set-up as shown in Figure 1. The size of the two tanks in the pipe loop system was (base: 1.5 ft x 1.5 ft, height: 2.5 ft). These tanks were connected by a 10 ft long, 3 inch diameter PVC pipe. A stainless pump (HAYWARD, POWER-FLO MATRIX) was used to recirculate the water in this system. Around 30 gallons of grease interceptor effluent (taken from full service Steakhouse, Cary, NC), 150 ml soybean oil, and 100mg/L Ca^{2+} (calcium chloride) were added to the pilot system. Concrete blocks were added to the pilot system to explore both surface saponification as well as the adhesive properties of FOG deposits on the concrete surface. Two concrete blocks were half submerged in the two tanks. Another three concrete blocks were placed in the PVC section that was close to the effluent tank as shown in Figure 2. The flow rate in the pilot system was 22 gpm to achieve a sewer velocity of 2 ft/sec. The system was operated for 42 days. Pictures of solid formation in this system were taken twice a week. At day 42, solid samples produced during the pilot study were collected from the two tanks, three different sections of the PVC pipe, and scraped from the five concrete blocks. Solids were measured after drying in oven at 105 °C overnight. The dry solid samples were analyzed by Fourier Transform Infrared (FTIR) Spectrometer to assess the bands associated with these samples. The metal analysis, including calcium, iron, magnesium and potassium, was performed on the liquid samples taken from the two tanks at day 42.
Results and Discussion

Solids continued to accumulate in the two tanks, the three PVC pipe sections and the five concrete blocks during 42 days observation. The difference of the adhesive properties among the concrete surface, the PVC pipe surface, and the stainless drum wall surface was not visually significant. As shown in Figure 3, solid buildup was observed on the tank wall and on the concrete block surface above the water mark. The same phenomenon was observed in effluent tank as the solids accumulated above water mark on both effluent tank wall and the suspended concrete block as shown in Figure 4. The results displayed differences in the dry
weight of solids collected from the two tank walls, which were 7.1 g in the influent tank and 1.9 g in the effluent tank. One possible explanation for the difference in solids distribution may be due to the difference in oil distribution. In the presence of oil, the free fatty acids would partition into it and react with calcium to form calcium soap on the surface. Most of the oil was observed to stay on the influent tank surface allowing for more soap to form in influent tank compared to the effluent tank. Using FTIR to examine the solid samples from both tanks, the results revealed that they were all calcium based salts of fatty acid or soap as shown in Figure 5. Additional evidence of saponification that occurred in the pipe loop system was assessed by the change of calcium concentration in the liquid. At the beginning, the additional calcium (calcium chloride) was 100 mg/L and calcium in GI effluent was 11 mg/L (Total calcium: 111 mg/L). Calcium was also released from the five concrete blocks during the operation. Thus, the available concentration of calcium in this pilot system was higher than 111 mg/L. However, at day 42, the concentration of calcium in the liquid was 69 mg/L. In this close system, as the pH was around 7, the calcium would not be precipitated but to react with free fatty acids to form calcium soap.

Figure 3 Solids formed in influent tank: (a) the influent tank with suspended concrete block; (b) the suspended concrete block in influent tank
Figure 4 Solids formed in effluent tank: (a) the effluent tank with the suspended concrete block; (b) the suspended concrete block in effluent tank

Figure 5 Baseline corrected infrared spectra of solid samples taken from two tank walls and the concrete blocks suspended in the two tanks.

In the PVC pipeline, the solids were observed in three pipe sections as shown in Figure 6. In the closet pipe section (section 3) to the influent tank (Figure 6a), solids were found
throughout the pipe wall. However, nearly no solid was adhered to the pipe wall above water mark in section 1 & 2 as shown in Figure 6 b & c. The solid found above water mark in section 3 may be due to the occurrence of forming in the influent tank, and the soap formed in the influent tank wall was lifted up by air bubble and entered into PVC pipeline, which would adhere to the top of pipe wall. No air bubble reached pipe section 1 & 2, which resulted in the fact that no solid was formed on the top pipe wall in section 1 & 2. The oil was another factor to affect the solid formation in the PVC pipeline. However, no oil was visually observed in the PVC pipeline. Consequently, the effect of oil on solid formation in the PVC pipeline was minor in this case. The dry weight of solids collected from the three pipe sections were 0.64 g in section 3, 1.59 g in section 2 and 2.84 g in section 1. The increasing solid weight from upstream to downstream would be a result of saponification taking place and the settling of solids along the flow. The FTIR result provided evidence of saponification in PVC pipeline as the characteristic bands of calcium soap appeared in all spectra of the solid samples collected from the three pipe sections (Figure 8). Compared to the solid sample collected in the influent tank wall, the solid samples in three pipe sections were softer and not sticky as the “hard” soap in the influent tank. Without partitioning into oil in the influent tank, it is hypothesized that less free fatty acids would enter the pipeline and react with calcium. Consequently, less soap would be formed and later settle down that result in the solids becoming softer and less sticky. Concerning with the concrete blocks in downstream pipeline, solids were found to accumulate below water mark shown in Figure 7. Similarly, soap was also found in these three solid samples according to the FTIR result shown in Figure 8. They were not as hard as the solids in the influent tank. The purpose of placing concrete blocks at the downstream pipeline was to simulate the FOG deposits.
accumulating on the top as in the sewer lines. However, since oil was not present, albeit small amounts of soap were formed, significant amounts of solids not buildup on the PVC pipe walls above water mark. A control test was performed in the pipe loop system with the addition of only the GI effluent and calcium chloride. This control pipe loop test displayed no “hard” soap formation similar to the solids shown in Figure 3. The solids obtained in the control pilot test were more like the solids found in the pipeline in this system. These types of solid formation with physical characteristics seems to support the proposed mechanism suggested earlier (Chapter 5) that oil plays an important role as a carrier of free fatty acids in the surface saponification reaction that leads to FOG deposits above low-flow water mark (Keener et al. 2008). Overall, as the pipe loop system was set-up to simulate the gravity flow pipelines and pump-station wet wells, the comparisons of dry weight among solid samples and the evidence of saponification provided by FTIR results indicate that the FOG deposit was more likely formed in wells or manhole than in pipelines.

Figure 6 Solids formed in PVC pipes: (a) PVC pipe section 3; (b) PVC pipe section 2; (c) PVC pipe section 1
Figure 7 Solids formed on the concrete surface in PVC pipe: (a) concrete_3 in PVC pipe; (b) concrete_2 in PVC pipe; (c) concrete_1 in PVC pipe

Figure 8 Baseline corrected infrared spectra of solid samples taken from three PVC sections and three concrete blocks in PVC pipeline

Conclusions

The sticky “hard” soap produced in the pilot sewer system was found to accumulate on two tank walls above the water mark, but the softer and less sticky solid was obtained at the bottom of PVC pipeline. This phenomenon would be attributed to the distribution of oil
since oil as the carrier of free fatty acids stayed in the tanks instead of pipeline that led to the occurrence of the surface saponification reaction to form FOG deposits above the water mark. This observation also supports the proposed mechanisms in Chapter 5 that the process of FOG deposit accumulation on pipe walls requires the presence of oil. The free fatty acids that did not partition into oil can react with calcium in water and settle down at downstream pipeline. However, since it was soft and not sticky, it may be washed away when the flow rate is sufficient high in the sewer lines.

References


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CHAPTER 7
CONCLUSIONS AND RECOMMENDATIONS

Conclusions

Due to the central role that fat, oil and grease (FOG) deposits play in sanitary sewer overflows (SSOs), the objectives of this study focused on two aspects: (i) reduction in the levels of FOG entering the sewer system by bioaugmentation in grease interceptors (GIs); (ii) mechanisms of FOG deposit formation in sewer lines. It is the first time that chemical and microbial characterization of full-scale GIs has been reported and the mechanisms of FOG deposit formation have been proposed based on lab data and field work. A comprehensive understanding of the effect of bioaugmentation in GIs and mechanisms of FOG deposit formation has been achieved, which provided deep insight that contributed to prevent sewer blockages and reduce SSOs.

Full-scale GIs were monitored over a year with and without bioaugmentation (treated and untreated cycles). Statistically significant differences between treated and untreated cycles were detected for several chemical and physical parameters. The treated cycles had lower BOD and COD at the grease interceptor outlet. While the combined treated cycle data did not show lower FOG concentrations in the GI outlet compared to the combined untreated cycle data, comparison of individual treated and untreated cycles show a positive effect due to the addition of product. Differences in the microbial community structure between treated and untreated cycles were detected by Terminal Restriction Fragment Length Polymorphism (T-RFLP) analysis and the clone and sequencing results. The shifts in the microbial composition
were revealed. The effects of biological product addition were also different for the two GIs. In general, the Ca GI showed a higher, positive effect on effluent characteristics due to product addition. Thus, the effect of biological product addition depends on the type of product used, the characteristics of the specific GI and food waste, and additional factors that may be present during the cycle. However, in all cases the addition of biological product did not result in any adverse effect- GI effluent characteristics were similar or better in treated cycles compared to those in untreated cycles. Taken together, the data shows that the addition of products results in changes in the GI chemistry and microbial ecology; these changes had no adverse effects, and in some cases positive effects, on COD, BOD, and FOG degradation in grease interceptors.

FOG deposits were first formed under laboratory condition from the reaction between free fatty acids and calcium chloride. While comparing laboratory-produced FOG deposits to natural FOG deposits collected from sewer lines, the similar characteristic was found based on the calcium and fatty acid profile analysis. The FTIR data further revealed that the FOG deposits (both laboratory-produced and natural FOG deposits) are metallic salts of fatty acids by comparisons with FOG deposits and pure calcium soaps. There are four major components attributing to the FOG deposit formation in sewer lines: calcium, free fatty acids (FFAs), FOG and water. Though calcium is naturally present in water, another source of calcium in sewer lines is concrete. When FFAs, produced from cooking process or generated by microbial activities such as in grease interceptors, were discharged in sewer lines, they would partition into FOG that flows on wastewater surface and then react with the calcium on the interface of oil and water or oil and concrete to form soap. Based on the types of FFAs,
different levels of adhesive soap would be produced, which can become bound to sewer pipe walls and draw un-reacted FFAs and cations towards the solid FOG deposit matrix.

**Recommendations for Future Work**

1) When biological product is applied in full-scale GIs, a longer duration between GI cleanout should be used. In this study, the cycles were limited to 60 days because of town ordinances. It is possible that greater differences between treated and untreated cycles may become more significant toward the end of longer cycles.

2) The most dominant “species” in one product (TRF 164) was not detected in the GI samples. This may be due to difficulties in extracting the cells or the DNA from the oily GI sample matrix. Additional DNA extraction techniques in oily GI samples should be explored in the future. The adjustment of the composition of biological product, such as increasing TRF159 in this study, may lead to more positive effect on FOG removal.

3) The timing to add biological product should be optimized based on the 24 hour flow rate data. A lower flow rate would bring a higher retention time of biological product in GI. Thus, when biological product is added during low flow rate, it will stay in GI longer and biodegrade more FOG.

4) When adding different types of fatty acids to the system with oil, water and concrete, solids affixed to concrete were not exactly calcium soap. In other words, the saponification process was not complete yet. A longer reaction time is needed to fulfill the whole process.
5) Since soap was formed fast with small amount of oil present in the system with GI and concrete but formed slowly when small amount of fatty acid (0.25g) added to the system with 25 ml oil, 75 ml water and concrete, the ratio of oil/fatty acid may determine the rate of FOG deposit formation.

6) Though hypothesis was given to explain the effects of different fatty acids on FOG deposit formation on concrete, more work is required to prove the hypothesis and improve the understanding the role of different fatty acids that play in FOG deposit formation.

7) To develop a method to measure oil and free fatty acids in wastewater. The ratio of oil and fatty acids will be useful to predict the FOG deposit formation. Also, based on the understanding of the effect of different types of fatty acids on FOG deposit formation, to know the major fatty acids in wastewater would be another reference to indicate the potential of FOG deposit formation in sewer lines.