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Enhanced degradation of fats, oils and greases in domestic wastewater sewer networks and grease interception systems using peat humic substances

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ENHANCED DEGRADATION OF FATS, OILS AND GREASES IN DOMESTIC WASTEWATER SEWER NETWORKS AND GREASE INTERCEPTION SYSTEMS USING PEAT HUMIC SUBSTANCES

by
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Abstract

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The efficacy of peat humic substances in enhancing the degradation of fats, oils and greases (FOG) was investigated under controlled laboratory conditions using bench scale well-mixed bioreactors. An experimental design was used to evaluate the effects of temperature and peat humic substance (PHS) concentration on FOG degradation in domestic wastewater for a temperature range from 10°C to 30°C and a PHS concentration range from 0 to 20 ppm(v). Factors and interactions significantly affecting the rate of FOG degradation were identified, and models to predict FOG degradation rates as a function of PHS concentration and temperature were developed. The models were used to develop a PHS dosage calculation technique for field operations. Results indicate that PHS can enhance FOG degradation rates by up to a factor of 2, and microbial cell growth rates by up to a factor of 3. Atmospheric hydrogen sulfide generation increased with high PHS concentration at high temperature. The rate of FOG degradation using grease interceptor material was studied at 25°C and a PHS concentration of 500 ppm(v). In these systems, PHS was observed to increase the rate of FOG degradation by up to a factor of 2, and microbial colony growth rates by up to a factor of 5. This work indicates that PHS can enhance FOG degradation rates and increase microbial growth rates in wastewater treatment systems. These results have significant implications for wastewater treatment applications.

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Chapter 1: Introduction

Human population growth has led to increases in liquid and solid wastes as well as air emissions. Wastewater, a combination of liquid or water-carried wastes is removed from all varieties of establishments including residences, institutions, commercial and industrial operations. Untreated wastewater contains nutrients and numerous pathogenic microorganisms which may stimulate the growth of aquatic flora. Where untreated wastewater collects and accumulates, the decomposition of the organic matter it contains can lead to nuisance conditions including the production of malodorous gases. The removal of wastewater from its sources of generation and its subsequent treatment or reuse is necessary to sustain public health and the environment [1], [2].

Increases in legislated regulations regarding wastewater effluents have been a result of high concentrations of biodegradable organic pollutants [3]. Due to the severe consequences associated with the release of pollutants, particularly fats, oils and grease (FOG), the U.S. Environmental Protection Agency, states, and cities regulate the discharge of oil and grease into sanitary sewer collection and treatment systems [4]. FOG is generated everyday by residential sewer usage and commercial establishments. Federal regulations require municipal utility and sewer collection entities to properly manage, operate, and maintain the collection system. The primary means of controlling FOG blockages is to capture and retain FOG materials before discharge into sewer systems through the use of passive interception devices [4].

1.1 Purpose of Experiment

This study examines the potential of peat humic substances as an additive for aiding the remediation of the wastewater pollutants; fats, oils and greases (FOG) and

hydrogen sulfide in sewer networks and grease interceptor systems. Humic substances are a naturally occurring material and their use in wastewater treatment is especially attractive due to their low pollution potential and cost-efficiency [5]. The purpose of this work is to test the efficacy of these substances under controlled laboratory conditions and determine their effect on FOG degradation.

Chapter 2: Literature Review

Water science involving wastewater differs significantly from other water sciences in terms of particulate matter and degree of microbial activity. Wastewater consists of polluted water and solid matter that may clog sewers or settle and lead to undesirable consequences such as sanitary system overflow (SSO), odor nuisance, increased maintenance and wastewater treatment performance problems [6]. The consequences may be severe for local governments and may include responsibility for clearing clogged pipes, regulatory fines, and pollution of local environments [7]. Federal regulations require municipal utilities and sewer collection entities to properly manage, operate, and maintain the wastewater collection network. The primary focus of many capacity management operations maintenance (CMOM) regulations is the prevention of fats, oils and grease (FOG) (including waxes and paraffins) discharge to the collection system [4]. FOG has been found to be a source of capacity reduction and reduced treatment system efficiency.

2.1 Wastewater Components

Constituents in wastewater may come from a variety of sources. Many wastewater sources are rich in organic matter and biomass, often quantified by the chemical oxygen demand (COD). The chemical oxygen demand is a measure of water quality that indicates the mass of oxygen consumed per liter of solution and is the result of the microbial activity in a combination of phases, particularly the biofilm and sediment. In these phases, FOG and sulfur containing pollutants account for a substantial part of oxygen consumption and are associated with many of the common problems of sewer networks and treatment facilities [8]. The general problems associated with FOG include

reduction in cell-aqueous phase transfer rates, sedimentation, development and flotation of low-activity sludge, and system clogging. General problems associated with sulfur-containing pollutants, particularly hydrogen sulfide, include corrosive and toxic properties, unpleasant odors and the ability to contaminate atmospheres [9].

The sulfur-containing chemical species of importance found in wastewater include hydrogen sulfide, sulfates, and the sulfur contained in organic material. Domestic wastewater normally contains these components in varying concentrations depending on the source and hardness of the wastewater [10]. Domestic sewage normally contains 3 to 6 mg L^{-1} of sulfur-containing organic material, present mainly as proteinaceous matter, and also in the form of sulfonates derived from household detergents. Hydrogen sulfide and other malodorous sulfur compounds are formed from the reduction of these sulfur-containing organic materials [11]. Atmospheric hydrogen sulfide can be considered the principal source of many of the problems associated with these species in wastewater. Hydrogen sulfide, H_2S, is a weak diprotic acid that chemically dissociates to the species HS^- and S^{2-}. The proportion of these species is primarily a function of pH and to a lesser extent, temperature and ionic strength. Hydrogen sulfide crosses the aqueous-atmospheric phase boundary as a function of process conditions [10]. This results in maintenance hazards and environmental nuisances for municipalities. Hydrogen sulfide may also be oxidized from the sewer atmosphere by microbial activity to form sulfuric acid (H_2SO_4) which subsequently attacks surfaces and other parts of wastewater facilities (pumping stations, manholes, reservoirs, etc.) [11]. Microbial activity involving sulfide generation (sulfate reduction) also has a direct influence to the content of organic matter measured as COD [10].

A significant fraction of the COD from municipal wastewaters is composed of fats, oils and grease. The FOG fraction of COD is composed of a variety of different molecules that represent a degree of biodegradability lower than other wastewater COD fractions such as proteins and carbohydrates [12]. The accumulation of pollutants with low biodegradability leads to several problems impacting wastewater treatment systems including: non-optimal operational performance (clogging, fouling, reduction of separation efficiency), non-optimal microbial activity, and operational nuisance (increased maintenance, cost and foul odors) [9].

High FOG accumulation in treatment systems, particularly pumping and aeration systems, may promote the concentration of filamentous microorganisms. High concentrations of these organisms may cause undesirable pollutant characteristics and treatment difficulties such as the formation of scum and stable viscous foams at pumping stations and sewage treatment works [9], [13]. Scum and foams also hinder biomass flocculation, sedimentation and generate unpleasant odors. FOG is also capable of forming lipid coats on the biological floc in wastewater. Lipid coating resistance reduces biological-aqueous phase transfer rates, inhibiting natural biological degradation. Hydrolysis of FOG produces long-chain fatty acids which are also reported to inhibit the activity of various microorganisms [9], [14].

From wastewater sources such as domestic and commercial kitchens, a significant portion of FOG is made up of fats and oils, also known as lipids. Lipids are a family of naturally occurring organic compounds that are relatively insoluble in water. Lipids consist of fatty acid molecules chemically bonded with glycerol by ester linkages and are biologically assembled by dehydration reactions and disassembled by hydrolytic

reactions [15]. A simple lipid consists of three fatty acid molecules bonded to the three-carbon molecule, glycerol, and is also known as a triglyceride. The accumulation of fatty acids is a source of microbial inhibition [14]. Fatty acids consist of long hydrocarbon chains (attributing hydrophobic properties), with a carboxyl group at one end, varying in chain length and degree of saturation [15]. Generally, carbon chains consisting of 16 to 18 carbon atoms in length are designated as long-chain fatty acids (LCFA), commonly found in edible fats and oils [13]. The component fatty acids in domestic and commercial wastewater source edible fats and oils vary considerably. Most land-animal lipids contain saturated LCFA and most plant and fish lipids contain unsaturated fatty acids [16].

Lipids are essential components of all cells. Biologically, the major functions of lipids are cellular structure and energy storage. The fatty acid composition of cells varies between species and within species due to temperature (growth at low temperatures favors shorter-length fatty acids, higher temperatures longer length fatty acids). Fatty acids are good electron donors for microorganisms [16]. Energy from fatty acids are oxidized by a process called beta oxidation. The products of this process may be acetate, carbon dioxide, and methane depending on the microbial species involved. A significant amount of microorganism families capable of metabolizing lipids in wastewater environments are presently known and many are yet to be discovered.

2.2 Wastewater Treatment Process Conditions

Dissolved oxygen, pH and oxidation-reduction potential (ORP) are characteristics of wastewater which are central to biological wastewater pollutant transformation. They may fluctuate as a function of sewer microbial activity and wastewater source. The pH of many commercial wastewater sources is often basic due to the presence of equipment

cleaning solutions and degreasers [4]. Wastewater pH subsequently decreases upon entering the collection system due to microbial activity including the fermentation of FOG (production of acetic acid), oxidation of hydrogen sulfide and the formation of volatile acids under anaerobic conditions [10]. Decreasing pH is often observed in sewer networks as a function of relatively high wastewater residence time, high COD and temperature in pipes. The oxidation-reduction potential, in which dissolved oxygen (as an electron acceptor) is pivotally important, governs the mode of microbial transformations in sewer wastewater. The ORP is the difference in electric potential measured between a platinum electrode and a hydrogen standard electrode. In an aqueous medium, the ORP is an approximate measure of the equilibrium existing between the reducing and oxidizing substances in water. Generally, positive values of ORP correspond to oxidizing conditions - negative values correspond to reducing conditions [10], [11]. Dissolved oxygen influences both the pH and ORP and is a determining factor for microbial respiration. The dissolved oxygen concentration in wastewater is a function of the initial oxygen concentration, oxygen consumption rate, and bulk water reaeration. Sewer networks are designed to allow for reaeration of wastewater yet dissolved oxygen concentrations are subject to large fluctuations [17].

Temperature is one characteristic of wastewater that has a significant impact on pollutants as applied to wastewater collection systems. Biological activity including the utilization of FOG and reduction of sulfates takes place at a range of temperatures in collection systems which are responsible for the selection of dominant species. There are two main classes of microorganisms considered in biological remediation of FOG in wastewater: mesophilic and thermophilic. Temperatures that provide optimal growth for

mesophilic organisms range from 20°C to 45°C and range from 45°C to 80°C for thermophilic organisms [18]. Thermophilic temperatures are typically seen in digesters treating wastewater with a defined composition and high fat content such as those found in the dairy or meat processing industries [9]. Commercial grease interceptors have also been known to experience temperatures close to the thermophilic range [4]. Mesophilic temperatures are found in most parts of a sewer network. However, psychrophilic organisms also exist in sewer networks, exhibiting optimal growth at temperatures which do not exceed a maximum of 20°C. Some microorganisms found in sewer systems can grow over a wide range of temperatures; for example: *E. coli* has the ability to grow over a temperature range of 8°C to 48°C with optimal growth occurring at 39°C [18]. However, generally only mesophilic and psychrophilic microorganisms play a major role in biological in-sewer processes.

2.3 Microbial Activity

Microbial respiration in sewage systems is a function of the concentration of dissolved oxygen in the bulk liquid and by wastewater component and are referred to as aerobic, anoxic and anaerobic. During aerobic conditions (oxygen > 0.5 mg/L) microorganisms may utilize oxygen as a terminal electron acceptor (TEA) for metabolic respiration. When the oxygen in the bulk liquid has been depleted, anoxic modes of metabolism occur to continue to provide the microorganisms with energy. Anoxic respiration utilizes TEAs which are less thermodynamically favorable for microbial respiration (compared with oxygen) such as nitrate. Addition of nitrate to promote anoxic processes (denitrification) is a well-studied method for avoiding anaerobic conditions [17]. Anaerobic (oxygen < 0.5 mg/L) conditions lead to many of the problems associated

with wastewater collection systems. Microorganisms capable of anaerobic respiration rely on metabolic pathways which produce smaller energy gains when compared with pathways utilizing oxygen as a TEA [18]. Sulfate has been reported as a common TEA for anaerobic microbial activity [10]. For example, acetate may be mineralized through the three aforementioned processes as expressed by the following stoichiometric equations:

Aerobic respiration: $CH_3COOH + 2O_2 \rightarrow H_2O + CO_2 + HCO_3^-$
Anoxic respiration: $5CH3COOH + 8NO_3^- \rightarrow 4N_2 + 10CO_2 + 6H_2O + 8OH^-$
Anaerobic respiration: $CH_3COOH + SO_4^{2-} \rightarrow S^{2-} + H_2O + CO_2 + HCO_3^-$

Aerobic transformations of pollutants are characterized by high heterotrophic biomass activity including excelled growth of the biofilm and suspended phases and corresponding organic substrate hydrolysis, degradation, and consumption. Aerobic wastewater quality changes include reduced biodegradability and increased compatibility with mechanical treatments. The magnitude of aerobic transformation varies significantly depending on initial wastewater quality and sewer system conditions. Using oxygen as a TEA, organic carbon can be completely mineralized to carbon dioxide by a single microorganism [19]. Studies have shown that the rate of uptake of dissolved oxygen by microorganisms present in domestic sewage varies from about 2 mg/L at 15°C and may increase to values about 20 mg/L as the sewage ‘ages’ within the sewerage system under aerobic conditions. The average is approximately 14 mg/L h at 15°C [16].

Aerobic FOG metabolism by microorganisms has been documented by several researchers [13]. The initial attack on triglycerides by microorganisms is extracellular and involves the hydrolysis of the ester bonds by lipolytic, hydrolytic enzymes (lipases) which remove the fatty acids from the glycerol molecules [9]. Once hydrolyzed, fatty

acids may enter a microbial cell and either catabolized directly or incorporated into complex lipids. Glycerol is released into the bulk liquid [20]. Lipases have been found by researchers to be both highly- or non-selective in regards to attacking lipids containing specific fatty acids. One reason for this difference in fatty acid selectivity has been suggested to be due to the substrate dependency of a microbial population. The removal of FOG by different organisms has been investigated in batch-growth studies and results indicate that removal could be significantly affected by the substrate specificity of the induced extra-cellular lipases, the physical and chemical characteristics of the substrate, and the pH of the culture medium [21], [22].

Anaerobic transformations are characterized by hydrolysis and fermentation of organic substrate, methanogenesis, and sulfate reduction. The rate at which anaerobic transformations proceed is relatively lower than aerobic transformations [17]. The consumption of readily biodegradable (fermentable) organic substrate is generally slower than production of fermentable substrate from hydrolysis. Slight net production of fermentable substrate may be expected of this mode of transformation [17]. Anaerobic decomposition (particularly in inundated wetland soils) requires many interdependent microbial processes (known as syntrophy) and can generate both CO_2 and methane as end products of organic substrate mineralization [20].

Anaerobic transformations in sewer networks are generally associated with sulfide generation by sulfate reducing bacteria (SRB) [11]. SRB are widespread in aquatic and terrestrial environments that become anoxic or anaerobic as a result of microbial decomposition processes. SRB may conduct dissimilatory reduction (producing an inorganic compound from an organic compound). The genera group I (non-acetate

oxidizers) of SRB can utilize lactate, pyruvate, ethanol, or certain fatty acids as electron donors reducing sulfate to sulfide. The genera of group II (acetate oxidizers) specialize in the oxidation of fatty acids, particularly acetate, reducing sulfate to sulfide. The sulfide generation rate is a function of several factors including pH, temperature, nutrients, presence of biofilm on sewer surfaces, presence of sulfate reduction inhibitors, and the oxidation-reduction potential (ORP). Sulfate is often not the limiting nutrient in sulfide generation unless its concentration is below 10-15 mg/L [11]. Wastewater components required for SRB activity include electron-donating organic matter (including FOG) and sulfate or sulfur-containing organic substrate (TEAs). Wastewater pH has an influence over sulfide generation, but rates appear to be highest for pH ranges between 6.5 and 8.0, which is typical of domestic wastewater [11]. A small amount of reduced sulfur is assimilated by the bacteria, but most is released into the external environment as sulfide ions [10].

Anaerobic treatment of lipid rich wastewater or solid waste is difficult in practice due to significant accumulation of LCFA derived from hydrolyzed lipids [17]. Long chain fatty acids are toxic to anaerobic bacteria and sulfate reducing bacteria [14], [23]. The unionized form of these acids, namely the long chain free fatty acids may decrease the availability of ATP to many microbial species. However, it has also been demonstrated that improved microbial fatty acid biodegradation and tolerance can be achieved by substrate acclimatization [14].

2.4 Wastewater Facilities

Sewer networks collect source wastewater and discharge at wastewater treatment facilities. Traditional facilities consist of three general types of wastewater treatment

processes: the primary and secondary treatments which remove the bulk of pollutants and tertiary treatment which decreases the concentration of pollutants to meet regulatory standards before discharge or recycle [5]. Primary treatment (generally physical processing and/or chemical addition) removes settable solids and secondary treatment (biological processes) breaks down waste stream pollutants to non-toxic or benign products. Tertiary treatment, including adsorption on activated carbon, ion exchange and chemical oxidation may be expensive and affected greatly by the efficacy of the primary and secondary treatments [5], [24]. In several instances, traditional wastewater treatment has been found to be too expensive or insufficient in reducing the concentration of pollutants in wastewater streams [5]. Many industrial and commercial establishments generate wastewaters for which current treatment technologies remain unacceptable and unvalidated [24]. Wastewater treatment design has traditionally considered treatment to start at the “end-of-pipe” which had limited the improvement of treatment processes to treatment facilities. However, it has been observed that chemical and biological in-sewer processes affect the sewer itself and subsequent treatment facility performance [17]. Thus, the “sewer as a bioreactor” concept has become corollary to “pipe-and-plant treatment” allowing for improved engineering, sustainability and treatment performance. Currently, rather than existing as solely a collector and transporter of wastewater, the sewer itself may be considered a complex processing system that transforms pollutants and characteristics of wastewater [17].

2.5 Sewer Design

Design and investment in the understanding of sewer networks has, in general, mainly focused on the physical aspects of sewer performance (hydrology, hydraulics and

solids transport). More recently, significant research advancements have included more consideration for microbial activity such as reaeration, water velocity and materials of construction [17], [25]. Low cost, naturally occurring, sustainable and non-polluting treatment solutions are ideal for enabling increases in organic matter removal efficiency and promoting high quality effluents [17].

In-sewer wastewater quality transformations generally take place in four phases: suspended water, sewer sediments, biofilm and sewer atmosphere. Chemical and biological processes occur *in situ* in these phases and by the exchange of material across phase boundaries. Influencing factors include wastewater constituents and microbial activity which are affected by the sewer design which determines sewer process conditions. The sewer networks in many municipalities are the result of years of investment and are continually maintained and revised with pollution control strategies [17]. Volumetric flow capacity is a main factor in the design of sewer networks accounting for the daily fluctuation of sewer usage (referred to as time series) and also fluctuation due to precipitation. This design factor is critical to avoiding septic system overflow and has a large impact on microbial activity. High water velocity causes increase in dissolved oxygen concentration and high rates of microbial activity associated with aerobic respiration. However, high water velocity causes a loss of system biomass in both aqueous and sediment phases decreasing the overall microbial activity. Conversely, periods of low water velocity may cause high sedimentation of suspended solids and microbial activity leading to a high oxygen uptake rate and anaerobic activity. During these different flow periods chemical components of wastewater may undergo significant chemical and microbial transformation, especially in warm temperature regions or sewer

sections. Due to the high and uncontrolled fluctuation of the time series, both pollutant concentrations and process conditions may fluctuate heavily [17]. Therefore, biological wastewater transformation processes proceed under significantly variable system conditions. In addition, models for the degradation of well-defined chemical compositions of FOG which allow for more deterministic modeling of biological processes become less accurate when applied to sewer network systems [25].

In addition to fluctuation in water velocity, sewer networks may be subject to seasonal temperature variation and stratification depending on the region of interest. It is reported that in cold weather, the efficiency of the biological processes may decrease and many of the pollutants of concern, particularly FOG, which solidifies at low temperature, become resistant to biological degradation [12]. Thermal energy is a significant factor driving microbial growth and activity. Sewer temperatures may fluctuate by as much as 19°C between the extremes of seasons depending on the geographical location of the sewer network [26]. Temperature stratification may be a significant factor in pretreatment systems and in wet wells. Plumes of high temperature water have been hypothesized to displace pollutants and facilitate their transport through the collection system [4]. Water networks may also experience temperature stratification also influencing biological transformations [26].

2.6 Wastewater Treatment

Many industries including food processing and service have experienced an extensive range of problems related to the treatment of oil- and grease-containing wastewater prior to sewer discharge. Treatment of this wastewater prior to sewer discharge and main wastewater treatment processing is required by Federal and local

regulations and plays an important role in maintaining wastewater processing efficiency and pollution control. Unrestrained FOG from industrial pretreatment systems can be a nuisance to biological treatment systems, especially in conventional mesophilic processes [2], [27]. A variety of pretreatment systems are employed to remove FOG and prevent associated problems, however commercially available pretreatments have, in some cases, been considered to deliver inadequate performance [3], [6]. Many commercially available systems act primarily as solid separators and operate marginally as biological treatment processes [27]. The technology of biodegradation as a pretreatment has not yet been fully exploited in the processing of organic material present in wastewater streams. Operators of conventional pretreatment systems and biological nutrient supplement systems would benefit significantly from any commercial development of a product or process that would improve grease control [4].

A variety of commercial FOG-restraining pretreatment systems are commercially available such as grease interceptors, tilted plate separators, dissolved air flotation systems and physical-chemical treatments. The main technique for separating oils and fats from commercial restaurant and fast-food wastewater is by grease interceptors or grease collection methods. A grease interceptor is primarily a physical separation unit; a vessel through which wastewater passes a series of baffles in laminar-flow conditions at a rate that allows fat/oil particles to rise to the surface before reaching the trap outlet [9]. The separation principle is based on Stoke's Law relating the rising velocity of a lipid particle to its radius, and on the theory that the separation efficiency is independent of depth [9]. In practice, grease interceptors are designed to allow sludge to accumulate at the bottom of the device and fat to accumulate at the top. However, non-optimized design

and sizing of these systems may lead to discharge of FOG pollutants beyond regulatory standards. Also, grease interceptors tend to become unaesthetic in terms of maintenance and may cause localized air pollution [9].

Other commercially acceptable techniques take advantages of different physical properties for improving separation efficiency of FOG. Tilted plate separators provide a high surface area for separation and are also available as pre-packaged units. However, the high surface area may be more susceptible to fouling and cleaning of the system is time-consuming. Dissolved-air flotation systems increase the rate of rise of FOG by forming micro-bubbles which attach to FOG particles. However, high air-loading rates, raw wastewater recycling and water temperature are critical parameters to maintain efficient operation. Chemical-physical treatments reduce organic COD loads in wastewater by protein and fat precipitation or flotation using chemical compounds such as aluminum sulfate, ferric chloride, or more commonly, lyme. These compounds are intended to break fat emulsions and coagulate fat particles, which can then be readily separated by physical means such as flotation or sedimentation. Use of these pretreatments is not expected to be widespread due to high cost of chemical reagents and formation of problematic sludge during flocculation [9].

Significant research advancements have been made toward treatment of defined-composition industrial wastewater [9]. Food processing industries generating high FOG content wastewaters may operate bioreactors for biodegradation of waste streams. These bioreactors may operate in single units or staged in series under a variety of conditions (anaerobic, aerobic, mesophilic or thermophilic). Many industrially operated bioreactors include a chemical or enzymatic hydrolysis pretreatment step for the reduction of FOG

particle size. FOG particle size regulates the biodegradability and bioavailability of substrate materials. Alkaline hydrolysis pretreatments have been mostly tested in waste activated sludge or municipal waste. Treatment with NaOH has been shown to increase the ratio of soluble substrates and reduce the volatile solid content during anaerobic digestion. However, increased pH from alkaline pretreatments may have deleterious effects for subsequent operations and research for alkaline treatments regarding complex waste from wastewater collection systems is insufficient [9].

Biological enzymes, particularly lipases, have been used in the treatment of domestic wastewaters and in the cleaning of sewer systems, cesspools, sinkholes, and in the effluents of restaurants [9]. Several patents exist for the application of hydrolytic enzymes, including hydrolases and lipases, in wastewater treatment. Pretreatment with pancreatic lipases has been reported to reduce FOG particle size by 60% although high doses were required to obtain a substantial reduction in laboratory reactors [27]. Multiple inoculations of the reactors were found to be necessary for establishing proper conditions and microbial activity. Hydrolases, biological enzymes produced by fungi, are capable of degrading the most complex organic compounds. This wide-spectrum degradation of organic compounds enables a considerable increase in organic matter removal efficiency in laboratory bioreactors [9].

2.7 Humic Substance

Addition of humic substance is an attractive means for wastewater remediation due to its natural origin and low pollution potential. It is found among other dissolved organic matter in the natural environment such as compost heaps, marine and lake sediments and peat bogs and constitutes about 60-70% of soil organic matter and 30-50%

of surface water organic matter on earth [5]. Humic substance is widely reported to stimulate plants through a mechanism associated with soil microbial activity and binding of readily absorbed soil contaminants [28]. The concept has been extended to both aerobic and anaerobic digestion of municipal sewage showing substantially increased process activity. In a two-year study of anaerobic digestion of municipal waste with peat humic substance addition, positive observations included an increase conversion of organics to combustible gas, changes in sludge character, reduction of sludge volume, and elimination of odors [29]. Other laboratory tests using activated sludge found aqueous humic substance to have a high affinity for organic material, increasing non-polar organic compound solubility [30]. Other field experiments have demonstrated the successful detoxification of a plant-operated activated sludge aerator contaminated with inhibitory metals [29].

Humic substance is a high-molecular weight polymeric mixture of partially decayed organic materials derived partly from the constituents of microorganisms that have resisted decomposition and partly from refractory plant materials [5]. It exists in both solid and aqueous phases as sub-micron colloids and dissolved anionic macromolecules, respectively; each phase exhibiting unique chemical properties. Extracted humic substance from natural environments is a heterogeneous mixture of molecules containing multiple chemical functional groups. Common chemical (free and bound) functional groups to both aqueous and solid phases include phenolic groups, quinone structures, nitrogen and oxygen as bridge units and COOH groups variously placed on aromatic rings [5]. Aqueous humic substances molecular structure has been described as containing glassy and rubbery domains: small quantities of high surface area

carbonaceous material admixed in a larger amount of solvent-like matter and rigid structures due to intra-molecular forces and site-specific surface interaction [28].

Observed properties of aqueous-phase humic substance (AHS) are the ability to create complexes, increase solubility, and facilitate transport of hydrophobic organic solutes and metals [5], [30]. AHS has been found to have much greater capacity for creating complexes with organic solutes than the capacity for solid phase humic substance to absorb organic solutes. It has been reported that the oxygen-containing functional groups may be largely responsible for regulating properties such as water solubility, acidity, surface activity, and metal complexing capacity [5]. Humic substance has been reported to have the ability to accept terminal electrons from some microorganisms capable of anaerobic oxidation of organic compounds and hydrogen. This electron transport yields energy to support growth and further enhances the capacity for microorganisms to reduce other, less accessible electron acceptors due to an electron shuttling mechanism. These properties suggest that AHS may play a larger role than is currently understood about the oxidation of organic matter and also about the impact on the fate of other environmental contaminants [31].

Chapter 3: Materials and Methods

The materials, methods and equipment used for all experiments are described below. All bioreactor experiments were conducted under controlled laboratory conditions and operated inside a fume hood. An approved job safety analysis was performed before starting experimentation. All experiments followed standard operating procedures including a protocol for the safe-disposal of hazardous materials.

3.1 Peat Humic Substance

Concentrated peat humic substance (PHS) was provided by JSH International in 16 ounce HDPE bottles. The same batch of PHS product was used for all experiments. The PHS is 10% extracted peat humic substance dissolved in water.

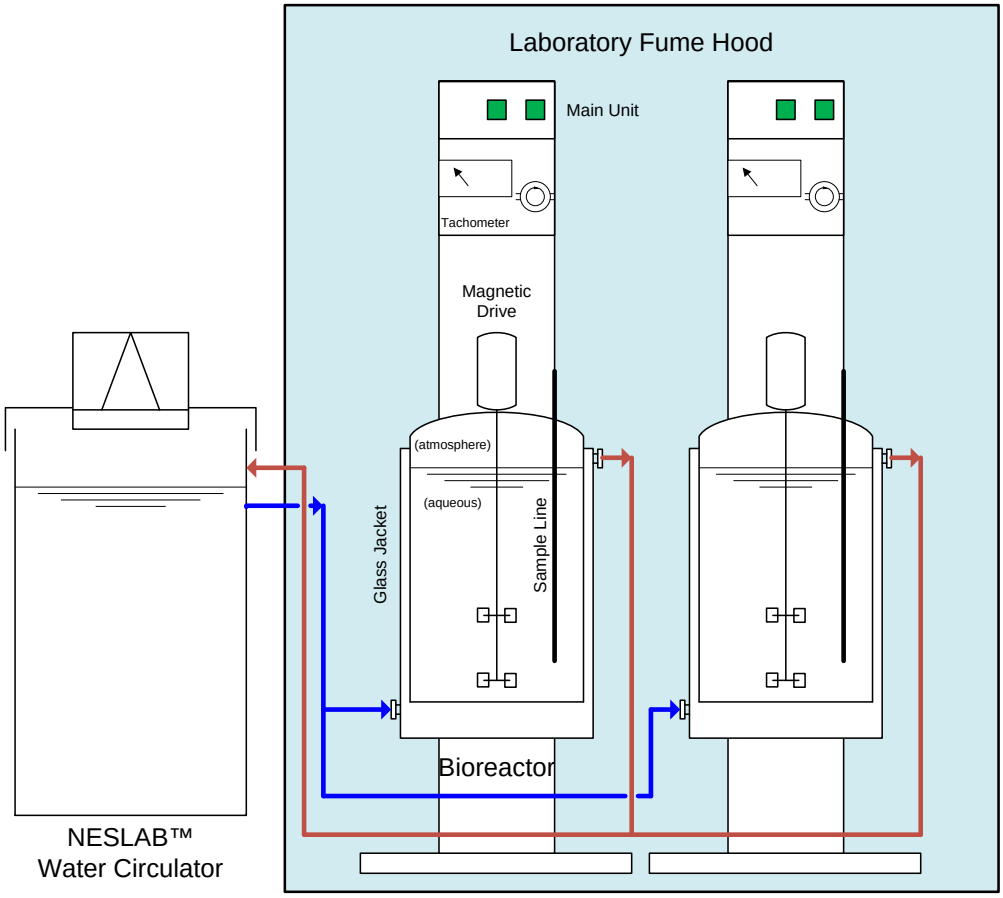
3.2 Bioreactor Experiments

Experimental System

Two Eyela® Heavy Duty Benchtop Fermentors (bioreactors), model MBF250 were used for this work. Each bioreactor consisted of a main unit and a fermentation vessel. The fermentation vessel construction consisted of a 2.5 L borosilicate glass cylinder with a secondary glass wall and a stainless steel lid. The vessel lid was constructed with a magnetic drive system for turning an impeller shaft with two impellers spaced 2.5 in. apart, a four-baffle configuration, and multiple configurable ports. The secondary glass wall was used as a temperature regulating jacket. A NESLAB™ water circulator with an operating range of -10°C to 70°C provided uniform temperature control to both vessels in parallel. Each fermentation vessel docked to a main unit which controlled agitation speed in a range of 100 RPM to 1200 RPM (for runtimes greater than 24 h) and up to a speed of 1500 RPM (for runtimes less than 24 h). A photograph and

schematic of the reactor system with docked bioreactors and temperature control configuration are shown in Figures 1 a and b. Prior to each experiment, bioreactors were hand-washed and sterilized in a BetaStar® autoclave at 123°C at 16 psig for 16min. Total autoclave cycle time was 40 min. Maintenance of rotating parts was performed as needed.

a) Experimental System



b) Schematic of Experimental System

Figures 1 a-b. Bench-top Bioreactor System

Fats, Oils and Greases (FOG) Quantification

The method of FOG quantification used in this work is analogous to United States EPA Method 1664 [32], [33]. EPA method 1664 Revision A quantifies n-hexane extractable material (HEM)) from an aqueous matrix. It is a performance-based method that gravimetrically determines the concentration of hexane extractable material using laboratory grade n-hexane with 99% purity. The limits of detection for Method 1664 are 5 to 1000 mg/L; extendable by sample dilution [34]. The method used in this work determines concentration based on infrared (IR) absorption of HEM thin-films extracted with technical grade n-hexane (98.5% purity). The detection limits of this work are from

10 mg/L with accuracy within 10% to an upper detection dependent upon sample dilution [33].

Sample Extraction & Preparation

Samples were extracted from agitated bioreactors via a liquid sample port. The liquid sample port line was constructed of a 0.25 in. stainless steel tube inserted through a liquid sampling port into the fermentation vessel. The line penetrated to a depth between the two impellers at a radius from the impeller shaft close to the baffles to ensure well-mixed sampling. The tube-end inside the bioreactor had a 45° edge. The opposite end of the sample line was connected by silicon rubber tubing to a syringe (5 – 10 ml) outside of the reactor. The sample line was held in place by a silicon rubber stopper.

The line was cleared of air and debris by passing a minimum of six sample volumes through the syringe to minimize sample contamination and optimize reproducibility. A volume of 5 ml was drawn for each sample and transferred into a new 15 ml polypropylene centrifuge tube. Hexane (OmniSolv® Hexanes 98.5%) was added to each sample for solute extraction and mixed vigorously with a Fisher™ vortexer at 2800 RPM for 45 s. The samples were subsequently centrifuged using a Forma™ centrifuge at 3200 RPM for 3.25 min to ensure well-defined polar (aqueous), non-polar (organic), and solid layers. Figure 2 shows an example of a sample prepared for quantification.

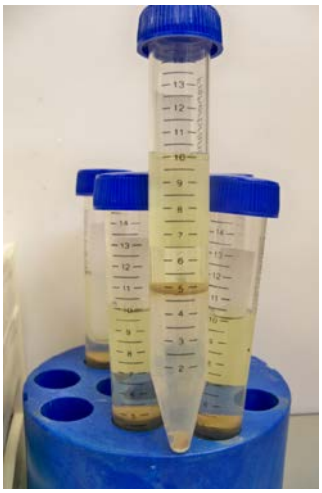


Figure 2. Sample Prepared for FOG Quantification
(Grease interceptor material experiment with clearly defined aqueous, organic and solid phases)

Infrared Absorption (IR)

The Wilks's InfraCal™ Total Organic Carbon / Total Petroleum Hydrocarbon analyzer, model HATR-T2, was used to measure the IR absorption of FOG constituents. The instrument is a compact, fixed-filter, mid-band IR detector with an insignificant optical air path. The detector uses an attenuated total reflectance (ATR) sample plate where a prescribed volume of hexane containing extracted material is deposited on a zirconia crystal window. The hexane and lighter volatiles evaporate, leaving behind a film that is measured by infrared [32].

The InfraCal™ instrument was operated under a fume hood for safety consideration and to expedite the evaporation of volatile materials. Prior to making measurements, the analyzer was switched on and idled for 1 h. HPLC-grade methanol, hexane and Kimwipes™ were used to clean the zirconia detection window before the instrument was zeroed. To check for detector malfunction, the absorbance of pure hexane was tested and compared with known values. From the non-polar extract layer of each

sample, 50 µl were deposited on the detector window using a micropipette. Evaporation of volatile material was allowed for 5 min before measurement of the film. The absorbance of the HEM film was recorded and converted to FOG concentration using the calibration curve in Appendix B.

Rate of FOG Degradation

The FOG degradation rate was determined by linear regression of the concentration measurements as a function of time. The concentration data were readied for analysis using standard statistical techniques. The first regression point was selected after the large molecule breakdown phase observed in the majority of experiments. The last regression point was selected at the naturally occurring end of ingestion period, also observed in the majority of experiments. The end of the FOG ingestion cycle is identified by a second large molecule breakdown phase characterized by an increase in FOG concentration after a general concentration decrease. Each regression contained at least three data points and allowed for the determination of an average degradation rate represented by the slope expressed in mg FOG L^{-1} day^{-1}. This rate is an averaged rate representative of the complex processes occurring in the bioreactor.

Microbial Quantification

For quantification of viable, colony-forming, microbiological units (CFU) present at the start and end of the bioreactor trials, a serial dilution method was used. Liquid assays for colony-forming cell counts from agitated bioreactors were drawn in triplicate and aseptically preserved in centrifuge tubes with Fisher® BioReagents™ glycerol in a sample-cryoprotectant ratio of 10:1. The samples were mixed for 10 seconds at 2800 RPM using a Fisher™ vortex mixer and stored in a laboratory freezer at -70°C.

Preserved biological assays were removed from storage and thawed for quantification. Ten Fisher™ micro-centrifuge tubes were prepared for aseptic dilutions. Each micro-centrifuge tube was filled with 1 mL of nutritionally rich media (Difco™ Lysogeny Broth (LB), Lennox). Dilutions, in a ratio of 1:10, were made by inoculating the first filled micro-centrifuge tube with 100 µL of the thawed sample and vortex mixing for 2 s. Dilutions continued in series by transferring 100 µL of inoculated broth from each previous microfuge tube to the subsequent with mixing until each tube was used.

Each microfuge tube was aseptically plated onto nutritionally rich agar media. Stackable Fisherbrand™ 100 O.D. x 15 mm petri dishes were prepared with LB and agar, To each plate, 100 µL of each dilution sample was deposited and distributed over the plate using a glass rod technique. After an incubation period of 72 h, the plates were photographed. Plates containing between 30 and 300 individual colonies were selected, counted and recorded. The sample cell culture density was calculated using Equation 1:

$$\rho_{brxtr} = \frac{CFU}{V * df} \tag{1}$$

where ρ_{brxtr} is the cell culture density of the liquid sample, *CFU* is the number of counted colonies, *V* is the sample volume of dilution (100 µL), and *df* is the dilution factor. The cell culture density of the liquid sample calculated as CFU ml^{-1} were averaged from triplicate liquid assays to increase the likelihood of accurate representation of the biological population density inside the bioreactors.

Domestic Wastewater Study

The effects of peat humic substance on the rate of FOG degradation were investigated in a replicated experimental design. The dissolved oxygen concentration and system pH were measured for wastewater samples obtained in January 2010; aqueous and

atmospheric hydrogen sulfide concentrations were measured for wastewater samples obtained in July 2010.

Sample Collection

Wastewater grab samples were obtained in the months of January and July from the Monroe Township Utility Department's (MTUD) pumping stations #917 (The Ridings) and #916 (Deschler Farms), respectively. Figure 3 shows pumping station #917 during site research. FOG is visible in an upper left crescent of the well surface. Samples were obtained on-site. The absence of commercial biological stimulants or oxidizers in the pumping stations was verified by the MTUD. A bucket-and-chain device with interchangeable buckets was lowered into the pumping station to obtain samples from the water surface. A 0.5 gal iron bucket fitted with holes in the bottom and another plastic 5 gal bucket were used for collecting surface FOG and wastewater. The pH and dissolved oxygen content of the liquid samples were measured on-site using a Hach™ HQ40d digital meter in conjunction with IntelliCal™ field probes. The liquid and solid samples were stored together in clean 1 gal. glass containers and transported to the laboratory. Samples that were not immediately used were stored in laboratory refrigeration at 4°C.

Sample wastewater obtained for preliminary investigation was collected in October. Sample wastewater obtained for experimental design use was obtained in January and July. January wastewater samples were used within 18 days and the July samples were used within 23 days.

Figure 3. MTUD Pumping Station #917 in October 2009
(FOG visible in upper-left crescent of well surface, bucket device chain visible extending from top right to water surface)

Sample Preparation

Approximately 4 L of the sample wastewater was prepared by suction filtration using a ceramic Coors™ Büchner funnel in conjunction with a 10 L Schott™ glass vacuum collection vessel. Particles with diameter greater than 1 mm were removed. The collected wastewater was divided into two 2 L volumetric flasks, transferred to the fermentation vessels and sealed with the vessel lid. Each fermentation vessel was subsequently docked on a main unit and connected to the water circulator. To simulate the field environment, the vessels were covered with aluminum foil keeping the contents dark. To ensure samples with representative FOG concentrations, the agitation speed was set to 1100 ± 50 RPM and the vessel's temperature was set to the experimental design condition for the duration of the experiment.

Experimental Design

An experimental design was developed to investigate the effect of two factors, temperature and PHS concentration, on the rate of FOG degradation. To minimize the

number of required experiments, a 2^2 factorial design was developed using a PHS concentration range of 1 to 20 parts per million by volume, ppm(v), and seasonal wastewater temperatures with a range of 10°C to 30°C (50°F - 86°F) [35]. Factor ranges were chosen representative of field operating conditions. The design included two center points at 20°C and 10 ppm(v) PHS to test for system nonlinearity. Figure 4 is a graphic representation of the design matrix. Tests were performed two experimental points at a time; each using a similar bacterial sample and the same temperature.

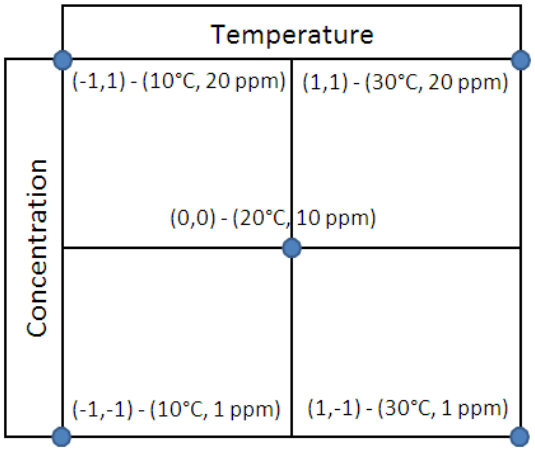


Figure 4. Experimental Design Matrix (Graphical representation)

The experimental design was performed using wastewater obtained from pumping station site #917 in January. The bioreactors were configured for PHS inoculation, liquid sampling, venting, pH, ORP measurement, and wastewater sample capacity of 2 L. Once the bioreactors had reached their specified operating temperature, initial FOG samples were obtained and the vessels were subsequently inoculated with PHS. For each set of experiments, FOG concentration, pH and ORP were measured in 24 h intervals from each bioreactor. For each FOG sample, 1 ml of hexane was used for FOG quantification.

The experimental design was replicated using wastewater obtained from pumping station site #916 in July due to difficulties in obtaining samples at pumping station #917.

The replicate design included the addition of control experiments operated at the design center point temperature of 20°C. Control reactors used the same microbially active wastewater samples as the corresponding PHS dosed reactors. PHS concentrations of 0 and 10 ppm(v) were used for the control and design center point, respectively. The bioreactors were configured for PHS inoculation, liquid sampling, gas sampling, venting and a sample capacity of 2 L. Initial FOG concentration measurement, PHS inoculation, and the hexane extraction quantity were replicated identically. For each set of experiments, FOG concentration, Aqueous and atmospheric hydrogen sulfide concentration (H_2S_{aq} and H_2S_{atm}) were measured in 12 h intervals from each bioreactor. Biological samples were taken before and after each trial for the assessment of colony forming microorganisms.

Dissolved Oxygen and pH

For continuous measurements of pH, two-channel Thermo™ Orion 720A analog meters coupled with pH (channel-1) probes were configured for the bioreactors. The pH probes were calibrated using an internal three point calibration with standards from Fisher™. Probes were held rigidly in their respective ports using modified rubber stoppers to maintain them at the correct depth. Dissolved oxygen (DO) and pH were also measured directly in the unsealed fermentation vessel before and after each experiment using a Hach™ HQ40d digital meter in conjunction with IntelliCal™ field probes.

Atmospheric and Aqueous Hydrogen Sulfide

To measure the atmospheric hydrogen sulfide concentration (H_2S_{atm}), a RKI Instruments™ GD-K71D H_2S_{atm} environmental sample-draw detector was mounted between the two main units. The sample draw detector had a range of 0-30 ppm(v) and

was calibrated using a two point internal calibration with a gas standard from GTS-Welco®. Atmospheric sampling ports from both fermentation vessels were connected to a three-way switching valve with rigid laboratory tubing. The switching valve allowed atmospheric samples to be drawn to the detector, one bioreactor at a time. A dilution junction was incorporated to further dilute concentrated samples with ambient air. An in-line moisture and particulate filter was located immediately upstream from the electrolytic chemical detector. The H_2S_{atm} concentration was read directly from the detection unit. A schematic of the H_2S_{atm} detector sample line is shown in Figure 5.

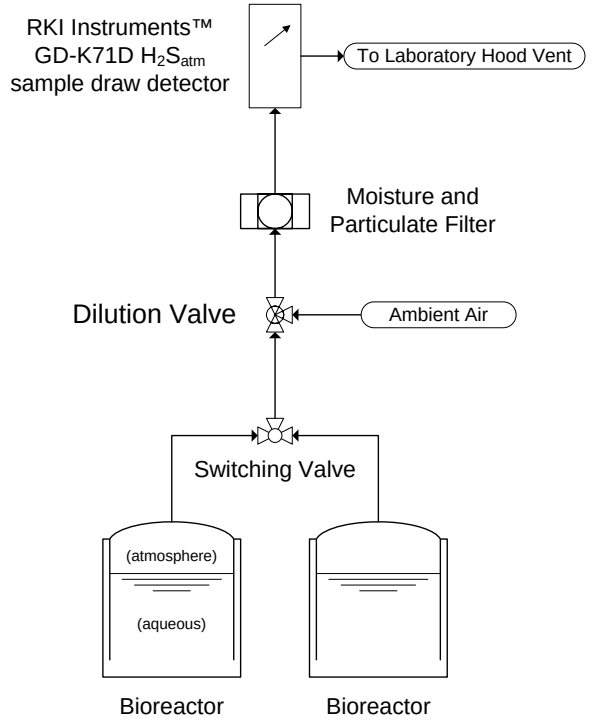


Figure 5. H_2S_{atm} Sample Draw Detection Scheme

The aqueous hydrogen sulfide concentration (H_2S_{aq}) measurements were performed using the extracted wastewater samples for FOG measurement. H_2S_{aq} measurements were performed using a modified method of Cline used for measurement of sulfide in environmental samples [36]. Prepared diamine reagent was contained in a

graduated 100 ml buret. To each bioreactor sample, 1 ml of prepared diamine reagent was dispensed into the centrifuge tube. The sample was prepared for FOG analysis. After centrifugation, 3 ml of the aqueous phase was transferred into a cuvette with a micropipette and stored for 20 minutes to allow color development. The absorbance at a wavelength of 670 nm was measured using a Milton Roy™ Spectronic 21D and recorded. Liquid sulfide calibration data are listed in Appendix B.

Grease Interceptor Material Experiment

Grease interceptor material experiments evaluated the rate of FOG degradation with grease collector material as the main substrate. Experiments were performed at 25°C (77°F), with 5% (volume) grease in tap water. Typically, 16 ounces of PHS are poured in the grease interceptor every 24 hours. Based on an industrial grease interceptor with a liquid holding capacity of 35 gal, the average PHS concentration in the grease interceptor was approximately 500 ppm(v) h^{-1}. Side-by-side experiments were performed with a control and a PHS-dosed bioreactor. Two separate batches of grease samples were used over the duration of these trials. Bioreactors were configured for PHS inoculation, liquid sampling, and venting. FOG concentration was measured in 12 h intervals and biological samples were taken before and after each trial for each bioreactor. Due to the high quantity of extractable content contained in each bioreactor liquid sample, a dilution step was required before IR measurement. To each bioreactor FOG sample, 5 ml of hexane were added, and prepared for quantification. From the prepared sample, 1 mL of the organic layer was diluted in 10 ml of pure hexane and vortexed 5 s to ensure well mixed samples.

Sample Grease Material

Grease collector sample material was provided by JSH International. Samples contained FOG removed from a restaurant grease interceptor. Samples were collected from the grease interceptor before business hours of operation and stored in mason jars. Samples were transported to the Rowan University laboratory and stored in a 4°C refrigerator if not used immediately.

Sample Preparation

Grease samples were heterogeneous in color and consistency when received. Figure 6 shows typical grease interceptor material from a restaurant.

Figure 6. Heterogeneous Restaurant Grease Interceptor Material (Collected 6:00am, April 20, 2010)

Samples were warmed in a scientific oven in a temperature range of 30°C and 37°C for at least 3 h to obtain uniformity and increase material flow-ability. A single main unit was fit with a custom mixing device for mason jar attachment. To prevent overflow during the mixing process, the volume of material in the sample containers was

checked and adjusted. After warming, samples were subsequently docked to the mixing unit, sealed, and blended to a satisfactory uniform consistency.

The grease sample was prepared in a 5% (volume) system by first preheating to 30°C to improve flowing characteristics. Fermentation vessels were loaded with 100 ml of grease measured with graduated cylinders. Tap water (1900 ml) was measured with volumetric flasks and added to the bioreactor. The bioreactors were subsequently sealed and docked on a main unit. Water circulation lines were connected and the bioreactors covered with aluminum foil. Agitation speeds were set to a nominal 1100 RPM.

3.3 Statistical Methods and Analysis

Statistical methods were employed depending on the quantity of data. FOG concentration data were analyzed for the rate of FOG degradation.

Data Preparation

For each FOG concentration measurement, a set of three samples was drawn and the absorbance of the HEM film for each sample was recorded. An average absorbance from each set of data was computed for conversion to FOG concentration. A modified nearest-neighbor method of outlier detection was used in data analysis. The average and standard deviation of the data set were calculated from the data selected. The two-tailed student's t distribution for 95% confidence was compared to the standard calculated t for the selected value [35]. The average absorbance was then converted to FOG concentration using the calibration curve in Appendix B. The calibration used was based on a mixture of olive and rapeseed oil and modeled by a linear, forced-zero trend with an R^2 value of 0.976.

Experimental Design

The experimental design and the replicate used biological samples from January and July. Due to the variation in sewer network conditions and the geographical variation between biological samples, it was hypothesized that the biological consortium from each biological sample could be different. Therefore, the design was analyzed for each season and also with all data combined. The January design was completed without control and center-point side-by-side replicates; therefore, calculated data were used to complete the analysis. A ratio of design center points from the January data and July data was calculated. Two July design control points and one center point were multiplied by this ratio to calculate three January wastewater sample rates 263, 243, and 196 mg FOG L^{-1} day^{-1} found in Table 2.

The response surface method of analysis was selected for application to the 2^2 factorial designs with center points and controls. The response surface method is used for modeling and optimization of systems, in which, a response of interest is influenced by several variables. The form of the relationship between the rate of FOG degradation and the variables, temperature and PHS concentration, is unknown. However, the response surface method serves to find a suitable approximation for the true relationship.[33] Strong variable interaction and nonlinearity were hypothesized for this experiment; therefore, the analysis used second-order polynomial models. Statgraphics Centurion™ v16.1 was used in the construction and analysis of the experimental design. The design was coded for the experimental conditions as shown in Table 1 for the response surface analysis.

Table 1. Experimental Conditions Coded for Design

Temperature, T (°C)	coded	Peat Humic Substance, PHS (µL/L)	coded
30	1	20	1
30	1	1	-0.9
10	-1	20	1
10	-1	1	-0.9
20	0	10	0
20	0	10	0
20	0	0	-1
20	0	0	-1

Chapter 4: Results

The full composition of experiments and raw data are presented in Appendix A. FOG degradation rates and cell counts were obtained using the methods described in the Materials and Methods: Rate of FOG Degradation and Cell Counts sections.

4.1 Domestic Wastewater Study

A replicated 2^2 experimental design was completed for wastewater studies with wastewater samples from January and July. The effects of temperature and PHS concentration on the rate of FOG degradation were studied. Viable colony-forming cells, pH, DO, and atmospheric and aqueous phase hydrogen sulfide concentration were also measured in some experiments. January wastewater sample experiments were conducted over a period of 18 days with each experiment's duration ranging from 112 to 122 hours. July wastewater sample experiments were conducted over a period of 23 days with each experiment's duration ranging from 82 to 84 hours.

FOG Degradation Analysis

Figure 7 is a plot of FOG concentration as a function of time for a typical wastewater experiment. As the figure indicates, there is an increase in FOG concentration at the start of the experiment. The FOG concentration subsequently decreases and, at approximately 3.5 days, ceases to significantly decrease. This characteristic was observed in the majority of the FOG degradation results.

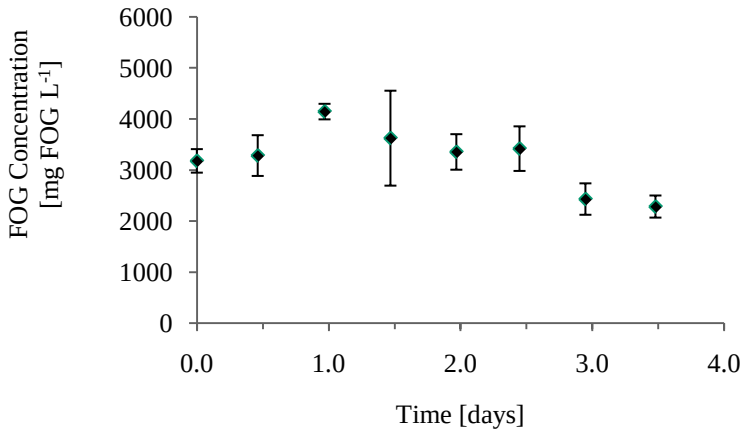


Figure 7. July Wastewater Sample FOG Degradation (T = 30°C, PHS = 20 ppm(v))

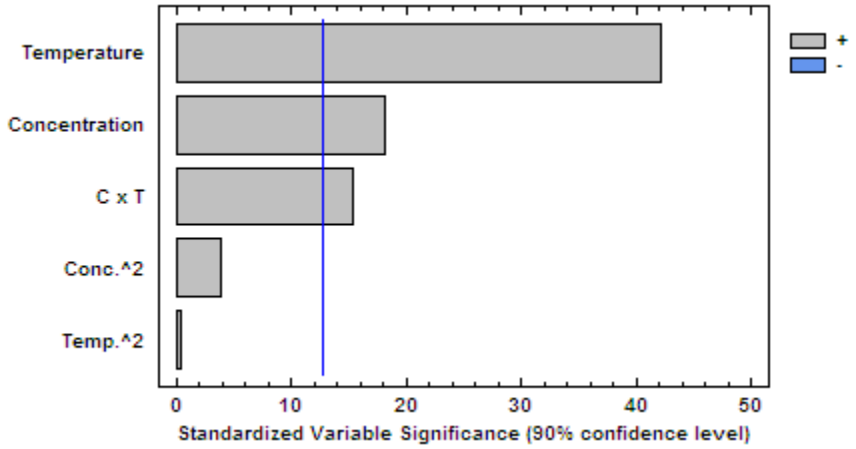
A summary of the experimental design results is presented in Table 2. The rates obtained for the July and January wastewater samples ranged from 113 to 721 and 25 to 590 mg FOG L^{-1} day^{-1}, respectively. The highest observed rate of FOG degradation was 721 mg FOG L^{-1} day^{-1}, which corresponds to a PHS concentration of 20 ppm(v) at a temperature of 30°C from the July wastewater sample.

Table 2. Experimental Design Results

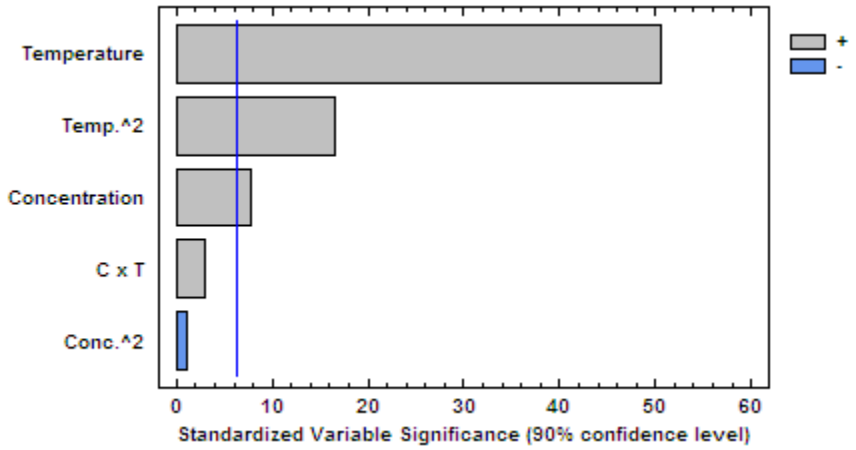
Experimental Condition,		FOG degradation rate,	
Temperature	PHS	January	July
T (°C)	ppm(v) (µL/L)	mg FOG L^{-1} d^{-1}	mg FOG L^{-1} d^{-1}
30	20	590	721
30	1	327	636
10	20	42	148
10	1	25	113
20	10	294	311
20	10	243	257
20	0	263	278
20	0	196	207

Standard analysis of variance and experimental design analyses were carried out. The data were analyzed independently for each (January and July) wastewater sample and as a combined data set. January and July data analyses were performed at the 90%

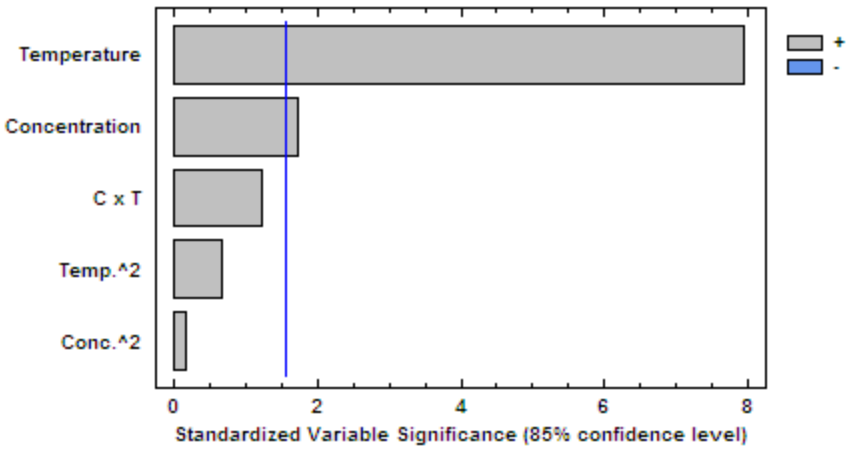
confidence level to be consistent with the 11% deviation observed in FOG concentration measurements and the combined data were analyzed at the 85% confidence level (α = 0.15). Figures 2 a-c are Pareto charts for the January, July and combined wastewater data showing the factors that significantly affect the rate of FOG degradation.



a) January Wastewater Sample



b) July Wastewater Sample



c) Combined Data

Figures 8 a-c. Pareto Variable Significance Charts

The pareto charts identify the significance of the variables studied. Bars which surpass the minimum significant value (vertical line) indicate significance at the specified confidence level. Both water temperature and PHS concentration have a significant effect on the rate of FOG degradation in each analysis, with water temperature being more significant. It is noteworthy that the interaction between water temperature and PHS concentration (C x T) is significant in the January sample and the second order temperature effect is significant in the July sample.

Empirical models were developed for FOG degradation rate as a function of PHS concentration and water temperature. The form of the model is displayed in Equation 2:

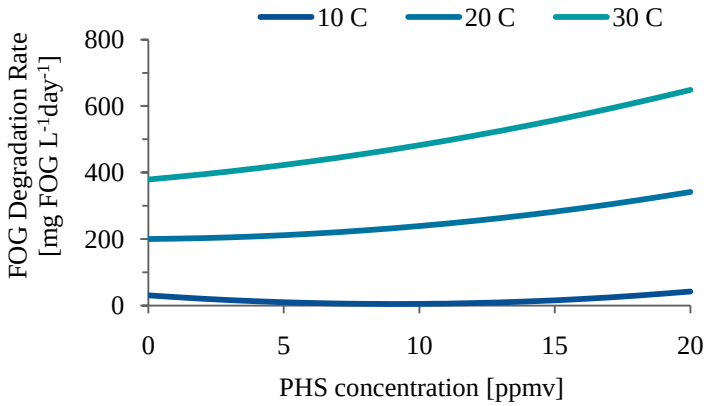
$$R_{FOG} = A + B*[T] + C*[PHS] + D*[T]^2 + E*[T]*[PHS] + F*[PHS]^2 \qquad (2)$$

where R_{FOG} = FOG degradation rate, T = temperature (°C), [PHS] = PHS concentration (ppm(v)), and A-F are model coefficients. The model coefficients and R^2 values for each analysis are summarized in Table 3.

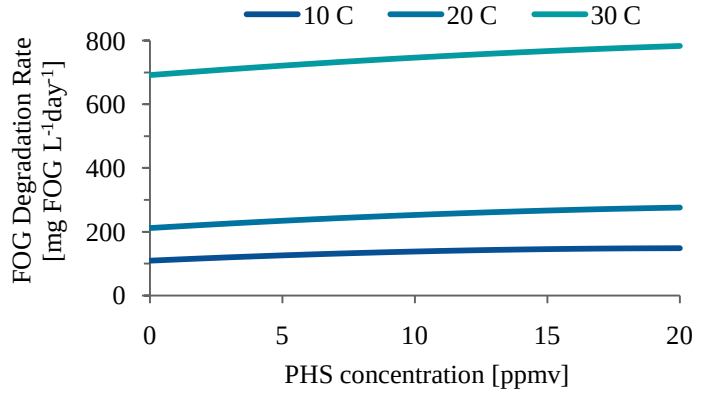
Table 3. Degradation Model Regression Coefficients

		Coefficient					
Data	R^2	A	B	C	D	E	F
January	0.92	-130.30	15.63	-12.20	0.04	0.65	0.32
July	0.92	386.54	-46.67	2.42	1.90	0.13	-0.09
Combined	0.86	-23.76	5.74	-4.89	0.36	0.39	0.11

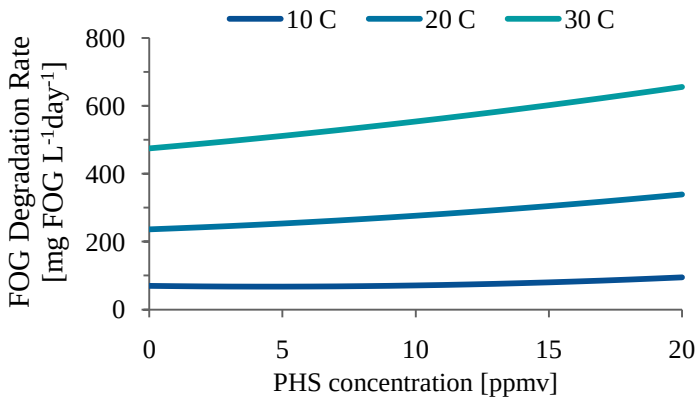
Figures 9 a-c display the model-predicted FOG degradation rate as a function of PHS concentration for the temperatures investigated for each data set.



a) January Wastewater Sample



b) July Wastewater Sample



c) Combined Data

Figures 9 a-c. Empirical Response Surface Analysis Models (FOG degradation rate as a function of time)

Dissolved Oxygen and pH

The results of the dissolved oxygen measurements are presented in Table 4. Dissolved oxygen (DO) concentration ranged from 3.40 to 4.99 mg/L at the start of the experiment and from 0.2 to 0.63 mg/L at the end of the experiment.

Table 4. Dissolved Oxygen Results

Temperature, T (°C)	Peat Humic Substance, PHS (µL/L)	Starting Dissolved Oxygen, DO (mg/L)	Ending Dissolved Oxygen, DO (mg/L)
30	20	3.40	0.63
30	1	3.40	0.34
10	20	4.99	0.40
10	1	4.99	0.25
20	10	4.39	0.30
20	10	4.39	0.20

The pH of the January wastewater samples is shown in Figure 10. The pH of the experimental trials ranged from 5.3 to 6.9. The pH range within each trial varied between 0.3 to 1.1 pH units. The average pH was 5.77 ± 0.13 at the 95% confidence level.

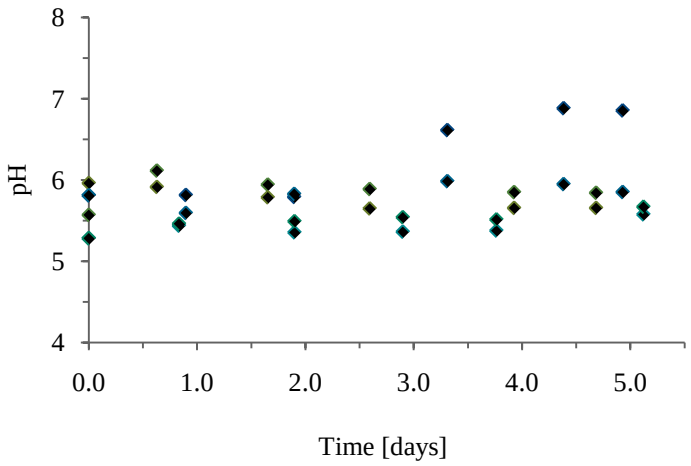


Figure 10. January Wastewater Sample Bioreactor pH (Compilation of all experiments)

Aqueous and Atmospheric Hydrogen Sulfide

The concentration of aqueous hydrogen sulfide, H_2S_{aq}, measurements ranged from 4.6 to 12.5 mg/L. Data indicated that the fluctuations in aqueous sulfide concentration in both control and PHS-supplemented bioreactors were statistically identical. Figure 11 is an example of the aqueous sulfide concentration between a control and PHS-supplemented bioreactor. For temperatures of 20-30°C, each set of side-by-side experiments exhibited the highest H_2S_{aq} concentration at the beginning of the experiment followed by a sharp decrease and subsequent smaller fluctuations in concentration.

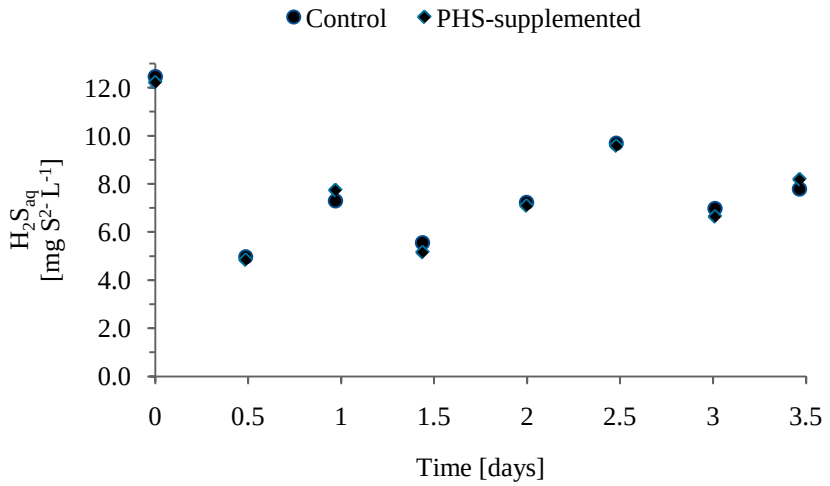


Figure 11. July Wastewater Sample H_2S_{aq} Concentration (T = 20°C, PHS = 10 ppm(v), 0 (Control))

The atmospheric hydrogen sulfide concentration measurements ranged from 2 to 60 ppm(v). Similar to the aqueous hydrogen sulfide data, the atmospheric hydrogen sulfide concentration was highest at the beginning of the experiment and was followed by an initial sharp decrease. Smaller fluctuations and a gradual increase in atmospheric sulfide concentration were observed for temperatures of 20°C and 30°C. At an operating temperature of 10°C, atmospheric sulfide was below the detection limits of the equipment. At temperatures of 20°C and 30°C, the PHS-supplemented bioreactors produced more atmospheric hydrogen sulfide in a range of 1.8 to 3.2 ppm(v) greater than the control as shown in Figure 12.

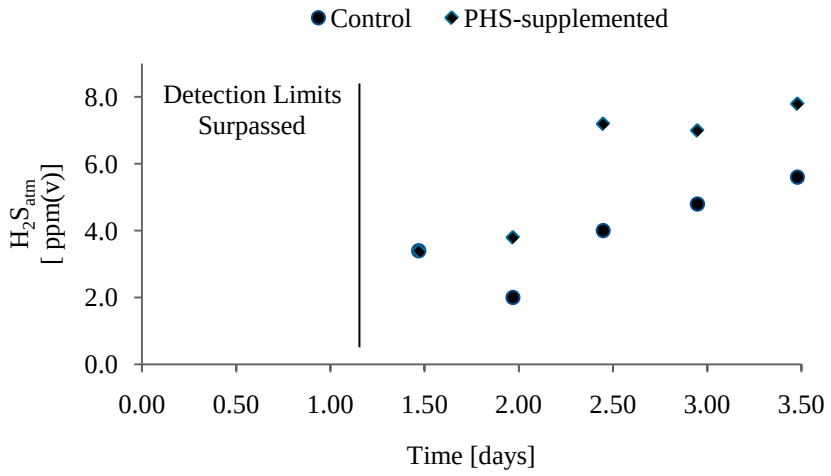


Figure 12. July Wastewater Sample H_2S_{atm} Concentration (T = 20°C, PHS = 10 ppm(v), 0 (Control))

Cell Counts

Table 5 lists the results for the viable CFU concentration (CFU/mL) for experiments from the July wastewater samples. The concentrations are reported for a temperature range of 20°C to 30°C and a PHS concentration range of 0 to 20 ppm(v). The increase in viable cell concentration in the bioreactors ranges from 12 to 102 times more than the original starting concentration. The data indicate increasing PHS concentration and temperature both increase the viable colony forming units in the bioreactor.

Table 5. July Wastewater Sample Microorganism Counts

Temperature, (°C)	PHS, (µL/L)	Viable CFU Concentration, start CFU mL^{-1}	end CFU mL^{-1}	Concentration Increase, per Trial	Ratio
30	1	2.7E+04	1.6E+06	58	1.8
	20	3.5E+04	3.6E+06	102	
20	0 (Control)	4.1E+04	5.3E+05	12	3.1
	10	2.9E+04	1.1E+06	37	

4.2 Grease Interceptor Material Experiment

Grease interceptor material experiments were conducted at 25°C and 5% grease in tap water by volume.

Fog Degradation Analysis

Bioreactors were tested to ensure their equivalency by running two control reactors side by side. The results of the equivalency test are shown in Figure 13.

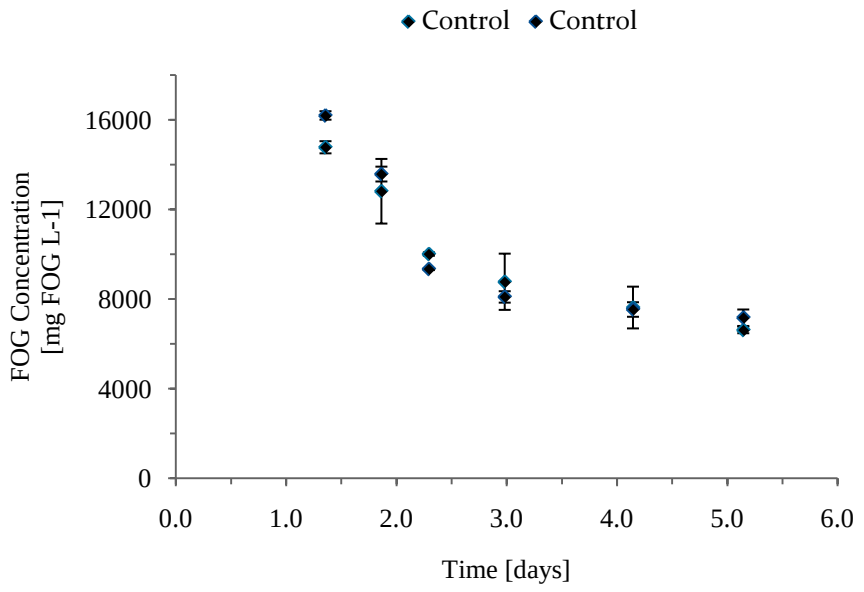


Figure 13. Bioreactor Equivalency Experiment (5% (vol.) Grease, T = 25°C, PHS = 0 (Control))

The figure indicates no significant difference (95% confidence level) exists between bioreactors. Bioreactor experiments were performed side by side with bioreactor controls. Therefore all experimental results are reported as ratios of FOG degradation rates affected by PHS relative to FOG degradation rates from control bioreactors. Figure 14 is an example of experimental results from a side-by-side experiment and control.

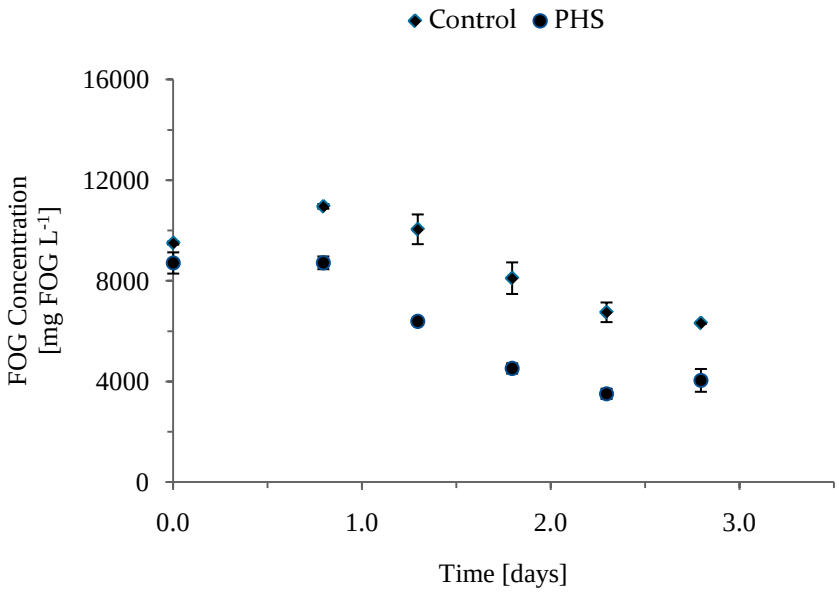


Figure 14. Grease Interceptor Experiments 9 & 10 (5% (vol.) Grease, T = 25°C, PHS = 500 ppm(v), 0(Control))

A significant difference (95% confidence level) exists in the FOG concentration as a function of time in the control bioreactor and the PHS dosed bioreactor. Figure 14 shows an initial increase in FOG concentration which is also observed in the majority of FOG degradation experiments. The results of the replicate experiments are presented in Table 6. The measured ratios of FOG degradation rate varied from 0.9 to 2.2.

Table 6. Grease Interceptor FOG Degradation Results

Reactor Trial, Designation	Peat Humic Substance, PHS (μL L^{-1})	FOG Degradation Rate, mg FOG L^{-1} d^{-1}	FOG Degradation Rate, Ratio
G8	0	756	1.7
G7	500	1322	
G9	0	2320	1.5
G10	500	3471	
G11	0	2167	1.1
G12	500	2367	
G13	0	1429	0.9
G14	500	1303	
G16	0	1814	2.2
G17	500	4011	

Cell Counts

Cell count samples were acquired at the start and end of the experiments. Table 7 is a typical set of cell count data for an experiment.

Table 7. Grease Interceptor Microorganism Counts

Reactor	Experiment, Sample	Colony Forming Unit Concentration, CFU mL^{-1}	Concentration Increase, per trial
Control	Start	7.87E+04	18.7
	End	1.55E+06	
PHS	Start	4.25E+04	82.5
	End	3.55E+06	

The increase in CFU concentration ranges from 18.7 to 82.5 times more than the original starting concentration. The data indicate the rate of cell growth in the PHS-dosed bioreactor is approximately 4.5 times greater relative to the control.

Chapter 5: Discussion

This work is an investigation of the degradation of fats, oils and grease and reduction of hydrogen sulfide in domestic wastewater collection systems and commercial grease interception systems. The results are discussed here with reference to available literature related to FOG degradation and microbial activity in wastewater.

5.1 Domestic Wastewater Study

FOG Degradation

The rate of FOG degradation was selected as a characteristic for the evaluation of peat humic substance additive performance. Degradation, in this work, is defined as a breakdown of molecules into smaller molecules or decomposition of a compound by stages; exhibiting well-defined intermediate products. An increase in FOG concentration from the initial FOG concentration as measured by the InfraCal™ infrared detector was observed in most experiments as shown in Figure 7. The results suggest that this is a beginning stage of FOG degradation and may be the result of both the reduction of FOG particle size and increase in FOG bioavailability. One possible explanation for the reduction in particle size may be the mixing intensity (applying shearing force) coupled with the release of extracellular enzymes such as lipases or hydrolases, initially reduced FOG particle size and increased the concentration of free lipids and fatty acids. Smaller particles have a high surface area to volume ratio and offer a larger surface area for bacterial colonization [27]. Smaller FOG particles are also more soluble in hexane due to the larger surface area were found to be more easily detected by the method of quantification used in this work. Literature also suggests that under anaerobic conditions, it is also likely for microbial activity to produce more fermentable material than is

consumed [17]. This increase in concentration is also consistent with an observed lag phase approximately 24 hours in length before a reduction in FOG concentration. This is consistent with literature reporting a lag phase of approximately 24 hours before significant degradation of FOG material [13]. However, some experiments exhibited no lag phase. This might suggest that microbial flora present in the bioreactors had been acclimatized to their environment. Microbes that have acclimatized may not exhibit evidence of a developmental lag phase [16].

Dissolved Oxygen and pH

The dissolved oxygen concentration was initially observed to be under aerobic conditions and proceed to or approach anaerobic conditions for bioreactor trials in which DO was measured listed in Table 4. The high content of FOG may contribute to high chemical and biological oxygen demand. Reported rates for oxygen uptake rates in domestic sewage have been reported in a range from 2 to 20 mg L^{-1} h^{-1} with an average rate of 14 L^{-1} h^{-1}. For the 2 L experimental system it may be reasonable to assume that the time taken to transition between aerobic and anaerobic microbial respiration was less than 24 hours [10]. The role of PHS in the stimulation of microorganisms may change during the aerobic-anaerobic transition however it is reported to have positive effects in each regime [29]. The pH was observed to range between 5.3 and 6.9 for bioreactor trials in which pH was measured as shown in Figure 10. An average pH of 5.77 indicated the biodegradation experiments took place in a slightly acidic environment. This pH range suggests that the experimental medium has been subject to microbial activity, particularly organic matter fermentation [4], [12]. In this range of pH the production and quantity of hydrogen sulfide are near maximum [11].

Aqueous and Atmospheric Hydrogen Sulfide

The measured aqueous and atmospheric hydrogen sulfide concentrations were difficult to analyze. Aqueous phase H_2S concentrations were measureable, however their degree of fluctuation as shown in Figures 11 and 12 did not allow for meaningful interpretation. One possible explanation for the high initial H_2S_{aq} concentrations detected in most experiments may be due to the activity of sulfate reducing bacteria during sample storage. The subsequent sudden decrease and fluctuation of H_2S_{aq} may be caused by equalization with H_2S_{atm} upon vigorous agitation. Sulfide concentration fuctuation may also be caused by microbial activity involving microbial sulfate reduction and sulfide re-oxidation cycles [19], [37].

The generation of atmospheric H_2S was observed over the full range of the experimental detection system; however, no H_2S_{atm} was detected at a temperature of 10°C. It is hypothesized that H_2S_{aq} did not evolve from the aqueous phase in detectable quantities due to high solubility at low temperature, or increased gaseous density prevented detectable quantities of H_2S_{atm} from entering the analysis stream. In agreement with H_2S_{aq} measurements, a high initial concentration was detected for experimental temperatures 20°C and 30°C followed by a sudden decrease. The initial trend in H_2S_{atm} concentration may correspond to the initial non-equilibrium of aqueous hydrogen sulfide and intense agitation may have facilitated degassing of the saturated aqueous medium. Continuing after the initial concentration trend, a gradual increase in H_2S_{atm} concentration was observed for experimental temperatures of 20°C and 30°C which is attributed to the activity of sulfate reducing bacteria. SRB inhabit many aquatic and terrestrial environments that transition between aerobic and anaerobic conditions [10],

[11]. High PHS dosed reactors produced more H_2S_{atm} than low PHS dosed reactors and controls. Literature studies indicate humic substances are capable of both providing both electron accepting and donating capability for organisms for highly oxidized and reduced humic substances, respectively [37]. The capability of humic substances to bind, create complexes with, and increase the organic partitioning coefficient is also well-documented [30]. It is hypothesized that due to the possible high chemical oxygen demand exerted on the medium, the addition of PHS provided a degree of stimulation by three mechanisms. Initially, PHS functioned as an electron acceptor, replacing sulfate as a terminal electron acceptor for microbial respiration. In the second mechanism, PHS increased the solubility of sulfur-containing organic compounds, and created PHS-sulfur organic compound complexes. Once reduced, PHS functioned as a thermodynamically favorable electron donor, increasing the short term availability of an easily fermentable sulfur-containing substrate.

Cell Counts

Colony-forming microorganism concentration measurements were performed for the July wastewater sample for organisms limited to aerobic conditions capable of growing on a nutrient-rich media. It was observed that the rate of cell growth increased as a function of PHS concentration and temperature. These results shown in Table 5 were expected and consistent with available literature.

Experimental Design Analysis

The experimental design analysis of FOG degradation rate as a function of PHS and temperature found that both factors significantly impacted the degradation rate in wastewater samples. Temperature and PHS were both expected to contribute to FOG

degradation. As shown in Table 2, the highest observed rates of FOG degradation for each wastewater sample occurred at high temperature and high doses of PHS. Therefore, it is inferred that the optimal conditions for FOG degradation were outside of the parameter ranges tested. The highest FOG degradation rate, 721 mg FOG L^{-1} day^{-1} or 0.03 g FOG L^{-1} h^{-1}, compares well with the pilot and industrial scale study of Grulois *et al*. (1993). For semi-continuous processes treating grease residue, a special consortium of biomass was found to degrade lipids between 0.03 and 0.04 g FOG L^{-1} h^{-1} in a mesophilic temperature range of 25 to 30°C [38]. A first order kinetic model was applied to calculate the apparent degradation rate constant, k, as a measure of the rate of biochemical degradation [39]. The k values ranged from 0.02 day^{-1} at a temperature of 10°C to 0.25 day^{-1} at 30°C. These values are close to the accepted typical values for municipal wastewater (0.17 day^{-1}) and petroleum oils (0.11 day^{-1}) [40]. Wastewater samples collected during January were found to have lower FOG degradation rates compared to wastewater samples collected during July for the same experimental conditions. These results indicate that the samples may have contained different microbial communities. For wastewater samples collected in January, it was additionally found that the interaction of temperature and PHS concentration is significant for FOG degradation. This would suggest the increased microbial activity associated with high temperatures caused a larger response toward PHS as a degradation stimulant. It has been observed in both wastewater samples that the temperature is a more significant parameter in enhancing biological FOG degradation. Warmer temperatures allow for the increased microbial activity due to increased solubility and subsequent bioavailability of FOG and also reduction in the amount of energy required for microorganism activity [41]. For

wastewater samples collected in July, it was additionally observed that the second order effect of temperature significantly impacted FOG degradation. One possible explanation for this is that the microbial community contained in the July wastewater sample was more sensitive to temperature changes in the range tested. Microbial communities will perform optimally in environments similar to those in which they have been acclimatized [12]. Tolerance for temperature shock and performance of biodegradation after adjustment varies among mixed microbial communities [18]. Empirical models were created for predicting FOG degradation rates in wastewater as a function of temperature and PHS concentration for application to field trials. The models suggest that PHS has a greater effect on microbial activity in the January wastewater sample and also has a greater effect at high temperature. This may also be explained by the interaction between temperature and PHS on biodegradation.

5.2 Grease Interceptor Material Experiment

FOG Degradation

Bioreactors were tested to ensure their equivalency by running control (no PHS) experiments. No significant difference between FOG degradation in the bioreactors exists at the 95% confidence level. For most FOG degradation experiments the trends observed for FOG concentration as a function of time included a characteristic initial increase in concentration followed by a period of decreasing concentration as shown in Figure 14. The mechanism as described for FOG degradation in wastewater experiments previously discussed is likely to also apply to these experiments. FOG degradation rates for PHS-dosed reactors ranged from 0.9 to 2.2 times greater than control reactors. The average apparent degradation rate constant was found to be 0.24 ± 0.10 day-1 at the 95%

confidence interval which is between rate constants reported for municipal wastewater (0.17) and edible oils (0.35) [40]. The large differences observed between comparisons of FOG degradation rates are suggested to be a function of differences in sample preparation and storage. Different storage periods and numbers of warming cycles are hypothesized to have changed the properties of the microbial consortium and FOG substrate present in each sample [9], [41].

Cell Counts

The results of the grease material study cell counts listed in Table 7 have indicated that PHS may stimulate the rate of cellular growth on grease material substrate by up to a factor of 4 to 5, relative to a control. Large variations were observed between trials; most are considered to be a function of sample preparation.

5.3 Experimental Limitations

Extensive care was taken in preparation of the collected data yet high variability was observed in some measurements. This is suggested to be due to the limitations of the experimental system and procedures. It is important to note that this work did not include chemical analysis and characterization of the FOG samples or peat humic substance. Thus, the changes in chemical composition of the FOG material is not known, nor the concentration of PHS during experimentation. Preliminary analyses have identified the mixed microbial cultures acquired from wet well and grease interceptor grab samples as another source of performance variability. The peat humic substance product was also found to contain an average microorganism concentration in a range of 1.85×10^4 CFU mL^{-1}. Given that wastewater experiments used PHS in a range of 2 to 40 μL, it is unlikely that any colony forming units could compete with established

microorganisms. Grease interceptor material experiments used a PHS dosage of 1 mL which may have contributed to the FOG degradation performance.

Chapter 6: Conclusions & Recommendations

The effects of peat humic substance on the rate of FOG degradation in wastewater and grease interceptor materials were studied. The effects of PHS on wastewater pH, dissolved oxygen and hydrogen sulfide concentrations were also investigated in a well-mixed, controlled, batch laboratory bioreactor. Samples used for all experimentation were provided by JSH International including peat humic substance (PHS), grease interceptor and domestic wastewater materials.

For experiments using domestic wastewater, an experimental design was used to investigate the effects of PHS concentration and temperature on FOG degradation. It was found that the FOG degradation rate increased as a function of temperature and PHS concentration and ranged from 10% to 110% relative to control experiments. The data suggest that FOG degradation in these systems proceeds by a two-part biological mechanism involving increasing FOG bioavailability and FOG consumption. The mechanism suggests activity of microorganisms breaks down large FOG molecules and particles initially which is consistent with an observed increase in FOG concentration. This is followed by consumption of smaller FOG molecules and subsequent decrease in FOG concentration. In the experiments using grease interceptor material, the results again supported this two-part mechanism. For grease interceptor material experiments at constant temperature, peat humic substance enhanced FOG degradation rates by up to 120% relative to control experiments. The addition of PHS also increased the growth rate of microorganisms during both wastewater and grease interceptor experiments by up to a factor of 3 and 4, respectively. Peat humic substance was observed to have no effect on the concentration of aqueous hydrogen sulfide, however, increases in hydrogen sulfide

concentration were observed in correspondence with high PHS dosing. Thus, it appears that the PHS products enhance microbial metabolic activity, leading to enhanced FOG degradation rates and H_2S_{atm} generation.

The results of this work have led to several recommendations for further research. Larger scale experimentation, particularly in commercial grease interception systems and domestic wastewater collection systems is needed before peat humic substance additives can be optimally applied in industrial applications. It is recommended that a similar scaled-up study be performed with several method revisions in order to more confidently quantify and describe the effects of peat humic substances in wastewater systems. Futher experiments to verify hypotheses concerning biological degradation of FOG may include add-back studies in which non-sterile and sterile experiments are compared side-by-side. Other recommendations include the use of a standardized and robust microbial culture, use of real-time investigation of microbial activity based on gene and protein expression, use of well-defined aqueous media, improvement of water-quality parameter measurement and improvement of characterization and quantification techniques for both FOG and PHS.

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Appendices

Appendix A: Experimental Data

Table 8. January Sample Wastewater FOG Data

Run #		Sample	Time	Days	a1	a2	a3	d1	d2	T	n1	n2	n3	tav	stdev	t stat	A_{IR_Av}	F_{conav}	Sdev	%SD	% Dec.	Dif.	Slope [ppm/D]	Cycle Regrssn.
E1	Design Code 1,1	1	1/6/10 1:05 PM	0.00	141	134	207	66	7	207	141	134	FALSE	137.5	4.9	19.86	137.5	1301	381	29.3%	0%			
	Vessel T [C] 30	2	1/7/10 10:35 AM	0.90	222	159	134	63	25	222	FALSE	159	134	146.5	17.7	6.04	171.7	1624	429	26.4%	-25%	-25%	360.8	-590
	Agitation [rpm] 1100	3	1/8/10 10:35 AM	1.90	72	77	98	21	5	98	72	77	FALSE	74.5	3.5	9.40	82.3	779	131	16.8%	40%	65%	-845.2	
	PHS [ppmv] 20	4	1/9/10 8:30 PM	3.31	19	19	17	0	2	17	19	19	FALSE	19	0.0	#DIV/0!	18.3	173	11	6.3%	87%	47%	-428.5	
		5	1/10/10 10:15 PM	4.38	16	12	12	4	0	16	FALSE	12	12	12	0.0	#DIV/0!	13.3	126	22	17.3%	90%	4%	-44.1	
		6	1/11/10 11:20 AM	4.93	19	16	12	3	4	12	19	16	FALSE	17.5	2.1	3.67	15.7	148	33	22.4%	89%	-2%	40.5	
E2	Design Code 1,-1	1	1/6/10 1:05 PM	0.00	100	109	124	15	9	124	100	109	FALSE	104.5	6.4	4.33	111.0	1050	115	10.9%	0%			
	Vessel T [C] 30	2	1/7/10 10:35 AM	0.90	93	114	48	21	45	48	93	114	FALSE	103.5	14.8	5.29	85.0	804	319	39.7%	23%	23%	-274.6	-327
	Agitation [rpm] 1100	3	1/8/10 10:35 AM	1.90	88	99	87	11	1	99	88	FALSE	87	87.5	0.7	23.00	87.5	828	63	7.6%	21%	-2%	23.7	
	PHS [ppmv] 1	4	1/9/10 8:30 PM	3.31	42	37	37	5	0	42	FALSE	37	37	37	0.0	#DIV/0!	38.7	366	27	7.5%	65%	44%	-326.9	
		5	1/10/10 10:15 PM	4.38	13	19	15	4	2	19	13	FALSE	15	14	1.4	5.00	15.7	148	29	19.5%	86%	21%	-202.8	
		6	1/11/10 11:20 AM	4.93	9	12	9	3	0	12	9	FALSE	9	9	0.0	#DIV/0!	10.0	95	16	17.3%	91%	5%	-98.3	
E3	Design Code -1,-1	1	1/11/10 5:45 PM	0.00	57	98	118	20	41	57	FALSE	98	118	108	14.1	5.10	91.0	861	294	34.2%	0%			
	Vessel T [C] 10	2	1/12/10 1:45 PM	0.83	106	84	105	1	21	84	106	FALSE	105	105.5	0.7	43.00	105.5	998	118	11.8%	-16%	-16%	164.6	-42
	Agitation [rpm] 1100	3	1/13/10 3:20 PM	1.90	109	20	76	33	56	20	109	FALSE	76	92.5	23.3	4.39	68.3	646	426	65.8%	25%	41%	-329.9	
	PHS [ppmv] 1	4	1/14/10 3:20 PM	2.90	112	92	85	20	7	112	FALSE	92	85	88.5	4.9	6.71	96.3	911	133	14.5%	-6%	-31%	264.9	
		5	1/15/10 12:05 PM	3.76	117	99	76	18	23	76	117	99	FALSE	108	12.7	3.56	97.3	921	194	21.1%	-7%	-1%	10.9	
		6	1/16/10 8:40 PM	5.12	84	88	59	4	25	59	84	88	FALSE	86	2.8	13.50	86.0	814	149	18.3%	5%	12%	-79.0	
		7	1/17/10 9:05 AM	5.64	50	53	50	3	0	53	50	FALSE	50	50	0.0	#DIV/0!	51.0	482	16	3.4%	44%	38%	-640.0	
E4	Design Code -1,1	1	1/11/10 5:45 PM	0.00	54	19	76	22	35	19	54	FALSE	76	65	15.6	4.18	49.7	470	272	57.9%	0%			
	Vessel T [C] 10	2	1/12/10 1:45 PM	0.83	85	53	61	24	8	85	FALSE	53	61	57	5.7	7.00	66.3	628	158	25.1%	-34%	-34%	189.2	-25
	Agitation [rpm] 1100	3	1/13/10 3:20 PM	1.90	68	64	56	4	8	56	68	64	FALSE	66	2.8	5.00	62.7	593	58	9.8%	-26%	7%	-32.5	
	PHS [ppmv] 20	4	1/14/10 3:20 PM	2.90	56	66	61	5	5	FALSE	56	66	61	61	5.0	#VALUE!	61.0	577	47	8.2%	-23%	3%	-15.8	
		5	1/15/10 12:05 PM	3.76	79	65	58	14	7	79	FALSE	65	58	61.5	4.9	5.00	67.3	637	101	15.9%	-36%	-13%	69.3	
		6	1/16/10 8:40 PM	5.12	51	56	41	5	10	41	51	56	FALSE	53.5	3.5	5.00	49.3	467	72	15.5%	1%	36%	-125.4	
		7	1/17/10 9:05 AM	5.64	39	33	36	3	3	FALSE	39	33	36	36	3.0	#VALUE!	36.0	341	28	8.3%	28%	27%	-243.8	
E5	Design Code 0,0	1	1/19/10 7:15 PM	0.00	423	433	479	46	10	479	423	433	FALSE	428	7.1	10.20	445.0	4210	283	6.7%	0%			
	Vessel T [C] 20	2	1/20/10 10:20 AM	0.63	507	495	640	133	12	640	507	495	FALSE	501	8.5	23.17	501.0	4740	761	16.1%	-13%	-13%	843.0	
	Agitation [rpm] 1150	3	1/21/10 10:55 AM	1.65	636	580	572	56	8	636	FALSE	580	572	576	5.7	15.00	576.0	5449	330	6.1%	-29%	-17%	692.7	
	PHS [ppmv] 10	4	1/22/10 9:30 AM	2.59	578	464	700	122	114	700	578	464	FALSE	521	80.6	3.14	580.7	5494	1117	20.3%	-30%	-1%	46.9	
		5	1/23/10 5:30 PM	3.93	715	666	770	55	49	770	715	666	FALSE	690.5	34.6	3.24	717.0	6783	492	7.3%	-61%	-31%	967.4	
		6	1/24/10 11:40 AM	4.68	756	652	732	24	80	652	756	FALSE	732	744	17.0	7.67	713.3	6749	515	7.6%	-60%	1%	-45.8	
E6	Design Code 0,0	1	1/19/10 7:15 PM	0.00	414	385	382	29	3	414	FALSE	385	382	383.5	2.1	20.33	383.5	3628	167	4.6%	0%			
	Vessel T [C] 20	2	1/20/10 10:20 AM	0.63	700	500	508	192	8	700	FALSE	500	508	504	5.7	49.00	504.0	4768	1071	22.5%	-31%	-31%	1814.0	
	Agitation [rpm] 1150	3	1/21/10 10:55 AM	1.65	650	708	640	58	10	708	650	FALSE	640	645	7.1	12.60	666.0	6301	347	5.5%	-74%	-42%	1496.3	-294
	PHS [ppmv] 10	4	1/22/10 9:30 AM	2.59	614	588	636	22	26	588	614	FALSE	636	625	15.6	3.36	612.7	5796	227	3.9%	-60%	14%	-536.2	
		5	1/23/10 5:30 PM	3.93	600	566	612	12	34	566	600	FALSE	612	606	8.5	6.67	592.7	5607	226	4.0%	-55%	5%	-141.9	
		6	1/24/10 11:40 AM	4.68	540	532	570	30	8	570	540	532	FALSE	536	5.7	8.50	547.3	5178	190	3.7%	-43%	12%	-566.6	

Table 9. July Sample Wastewater FOG Data

Run #			Sample	Time	Days	a1	a2	a3	d1	d2	T	n1	n2	n3	tav	stdev	t stat	A_{IR_Av}	F_{conav}	Sdev	%SD	% Dec.	Dif.	Slopes [ppm/D]	Cycle Regrssn.
WW6			1	7/15/10 9:45 PM	0.56	337	320	368	31	17	368	337	320	FALSE	328.5	12.0	4.65	341.7	3232	230	7%	0%			
	Vessel T [C]	30	2	7/16/10 11:15 AM	0.00	319	305	384	65	14	384	319	305	FALSE	312	9.9	10.29	336.0	3179	399	13%	2%	2%	95.3	
	Agitation [rpm]	1000	3	7/16/10 10:20 PM	0.46	345	364	332	19	13	364	345	FALSE	332	338.5	9.2	3.92	347.0	3283	152	5%	-2%	-3%	225.4	
	PHS [ppmv]	20	4	7/17/10 10:30 AM	0.97	546	414	354	132	60	546	FALSE	414	354	384	42.4	5.40	438.0	4144	929	22%	-28%	-27%	1698.3	-721
			5	7/17/10 10:30 PM	1.47	353	372	424	52	19	424	353	372	FALSE	362.5	13.4	6.47	383.0	3623	348	10%	-12%	16%	-1040.7	
			6	7/18/10 10:30 AM	1.97	434	359	350	75	9	434	FALSE	359	350	354.5	6.4	17.67	354.5	3354	436	13%	-4%	8%	-539.3	
			7	7/18/10 10:00 PM	2.45	398	336	350	48	14	398	FALSE	336	350	343	9.9	7.86	361.3	3418	308	9%	-6%	-2%	134.9	
			8	7/19/10 10:00 AM	2.95	283	240	248	35	8	283	FALSE	240	248	244	5.7	9.75	257.0	2431	216	9%	25%	31%	-1974.1	
			9	7/19/10 10:45 PM	3.48	261		222	39	39	FALSE	261		222	241.5	27.6	#VALUE!	241.5	2285	261	11%	29%	5%	-276.0	
WW5			1	7/15/10 9:45 PM	0.56	362	328	286	34	42	286	362	328	FALSE	345	24.0	3.47	325.3	3078	360	12%	0%			
	Vessel T [C]	30	2	7/16/10 11:15 AM	0.00	419	274	320	99	46	419	FALSE	274	320	297	32.5	5.30	337.7	3195	701	22%	-4%	-4%	-207.4	
	Agitation [rpm]	1050	3	7/16/10 10:20 PM	0.46	279	310	283	27	4	310	279	FALSE	283	281	2.8	14.50	281.0	2658	160	6%	14%	17%	-1160.9	
	PHS [ppmv]	1	4	7/17/10 10:30 AM	0.97	431	344	326	87	18	431	FALSE	344	326	335	12.7	10.67	367.0	3472	531	15%	-13%	-26%	1605.0	-636
			5	7/17/10 10:30 PM	1.47	486	360	324	126	36	486	FALSE	360	324	342	25.5	8.00	390.0	3690	805	22%	-20%	-7%	435.2	
			6	7/18/10 10:30 AM	1.97	342	367	346	21	4	367	342	FALSE	346	344	2.8	11.50	351.7	3327	127	4%	-8%	12%	-725.3	
			7	7/18/10 10:00 PM	2.45	516	304	255	212	49	516	FALSE	304	255	279.5	34.6	9.65	358.3	3390	1312	39%	-10%	-2%	131.6	
			8	7/19/10 10:00 AM	2.95	215	210	213	2	3	210	215	FALSE	213	214	1.4	4.00	212.7	2012	24	1%	35%	45%	-2756.2	
			9	7/19/10 10:45 PM	3.48	235	259	253	6	18	235	FALSE	259	253	256	4.2	7.00	249.0	2356	118	5%	23%	-11%	647.0	
WW4			1	7/9/10 12:30 PM	0.00	551	572	559	13	8	572	551	FALSE	559	555	5.7	4.25	560.7	5304	100	2%	0%			-148
	Vessel T [C]	10	2	7/9/10 11:00 PM	0.44	560	553	541	7	12	541	560	553	FALSE	556.5	4.9	4.43	551.3	5216	91	2%	2%	2%	-201.8	
	Agitation [rpm]	1050	3	7/10/10 1:55 PM	1.06	573	544	511	29	33	511	573	544	FALSE	558.5	20.5	3.28	542.7	5134	293	6%	3%	2%	-131.9	
	PHS [ppmv]	20	4	7/10/10 11:45 PM	1.47	538	536	612	74	2	612	538	536	FALSE	537	1.4	75.00	537.0	5080	410	8%	4%	1%	-130.8	
			5	7/11/10 1:00 PM	2.02	546	532	506	14	26	506	546	532	FALSE	539	9.9	4.71	528.0	4995	192	4%	6%	2%	-154.2	
			6	7/11/10 11:55 PM	2.48	605	549	538	56	11	605	FALSE	549	538	543.5	7.8	11.18	564.0	5336	340	6%	-1%	-6%	748.8	
			7	7/12/10 11:00 AM	2.94	638	530	514	108	16	638	FALSE	530	514	522	11.3	14.50	522.0	4939	638	13%	7%	7%	-860.4	
			8	7/12/10 11:30 PM	3.46	536	543	493	7	43	493	536	543	FALSE	539.5	4.9	13.29	539.5	5104	256	5%	4%	-3%	317.9	
WW3			1	7/9/10 12:30 PM	0.00	536	546	580	34	10	580	536	546	FALSE	541	7.1	7.80	554.0	5241	218	4%	0%			-113
	Vessel T [C]	10	2	7/9/10 11:00 PM	0.44	612	567	531	45	36	612	FALSE	567	531	549	25.5	3.50	570.0	5393	384	7%	-3%	-3%	346.0	
	Agitation [rpm]	1050	3	7/10/10 1:55 PM	1.06	570	520	523	47	3	570	FALSE	520	523	521.5	2.1	32.33	521.5	4934	265	5%	6%	9%	-738.3	
	PHS [ppmv]	1	4	7/10/10 11:45 PM	1.47	566	506	535	31	29	566	FALSE	506	535	520.5	20.5	3.14	535.7	5068	284	6%	3%	-3%	327.1	
			5	7/11/10 1:00 PM	2.02	542	489	544	2	53	489	542	FALSE	544	543	1.4	54.00	543.0	5137	295	6%	2%	-1%	125.7	
			6	7/11/10 11:55 PM	2.48	594	532	555	39	23	594	FALSE	532	555	543.5	16.3	4.39	560.3	5301	297	6%	-1%	-3%	360.5	
			7	7/12/10 11:00 AM	2.94	595	535	490	60	45	595	FALSE	535	490	512.5	31.8	3.67	540.0	5109	498	10%	3%	4%	-416.6	
			8	7/12/10 11:30 PM	3.46	549	532	539	10	7	549	FALSE	532	539	535.5	4.9	3.86	540.0	5109	81	2%	3%	0%	0.0	
WW2			1	7/1/10 12:00 PM	0.00	453	456	400	3	53	400	453	456	FALSE	454.5	2.1	36.33	454.5	4300	298	7%	0%			-311
	Vessel T [C]	20	2	7/1/10 9:45 PM	0.41	413	394	440	27	19	440	413	394	FALSE	403.5	13.4	3.84	415.7	3933	219	6%	9%	9%	-904.3	
	Agitation [rpm]	1000	3	7/2/10 12:00 PM	1.00	524	407	367	117	40	524	FALSE	407	367	387	28.3	6.85	432.7	4093	772	19%	5%	-4%	270.9	
	PHS [ppmv]	10	4	7/2/10 10:25 PM	1.43	486	373	384	102	11	486	FALSE	373	384	378.5	7.8	19.55	378.5	3581	589	16%	17%	12%	-1180.7	
			5	7/3/10 12:00 PM	2.00	375	398	399	1	23	375	FALSE	398	399	398.5	0.7	47.00	398.5	3770	128	3%	12%	-4%	334.3	
			6	7/3/10 10:30 PM	2.44	378	394	516	122	16	516	378	394	FALSE	386	11.3	16.25	386.0	3652	714	20%	15%	3%	-270.3	
			7	7/4/10 10:50 AM	2.95	546	432	414	114	18	546	FALSE	432	414	423	12.7	13.67	423.0	4002	677	17%	7%	-8%	681.2	
			8	7/4/10 10:40 PM	3.44	520	439	403	81	36	520	FALSE	439	403	421	25.5	5.50	454.0	4295	567	13%	0%	-7%	594.8	
WW8			1	7/21/10 10:45 AM	0.00	374	396	437	41	22	437	374	396	FALSE	385	15.6	4.73	402.3	3806	302	8%	0%			-257
	Vessel T [C]	20	2	7/21/10 10:25 PM	0.49	347	349	348	1	1	FALSE	347	349	348	348	1.0	#VALUE!	348.0	3292	9	0%	14%	14%	-1057.4	
	Agitation [rpm]	1050	3	7/22/10 10:00 AM	0.97	366	352	341	14	11	366	FALSE	352	341	346.5	7.8	3.55	353.0	3340	119	4%	12%	-1%	98.0	
	PHS [ppmv]	10	4	7/22/10 9:15 PM	1.44	529	419	393	110	26	529	FALSE	419	393	406	18.4	9.46	447.0	4229	683	16%	-11%	-23%	1897.2	
			5	7/23/10 10:40 AM	2.00	503	389	411	92	22	503	FALSE	389	411	400	15.6	9.36	434.3	4109	572	14%	-8%	3%	-214.4	
			6	7/23/10 10:15 PM	2.48	338	322	303	16	19	303	338	322	FALSE	330	11.3	3.38	321.0	3037	166	5%	20%	28%	-2221.6	
			7	7/24/10 11:00 AM	3.01	329	310	331	2	19	310	329	FALSE	331	330	1.4	20.00	330.0	3122	110	4%	18%	-2%	160.3	
			8	7/24/10 9:55 PM	3.47	269	307	310	3	38	269	FALSE	307	310	308.5	2.1	26.33	308.5	2919	216	7%	23%	5%	-447.2	
WW1			1	7/1/10 12:00 PM	0.00	386	342	326	44	16	386	FALSE	342	326	334	11.3	6.50	351.3	3324	294	9%	0%			-278
	Vessel T [C]	20	2	7/1/10 9:45 PM	0.41	383	363	308	20	55	308	383	363	FALSE	373	14.1	6.50	351.3	3324	367	11%	0%	0%	0.0	
	Agitation [rpm]	1000	3	7/2/10 12:00 PM	1.00	457	307	314	143	7	457	FALSE	307	314	310.5	4.9	41.86	310.5	2938	801	27%	12%	12%	-650.6	
	PHS [ppmv]	0	4	7/2/10 10:25 PM	1.43	327	297	298	29	1	327	FALSE	297	298	297.5	0.7	59.00	297.5	2815	161	6%	15%	4%	-283.4	
			5	7/3/10 12:00 PM	2.00	326	263	267	59	4	326	FALSE	263	267	265	2.8	30.50	265.0	2507	334	13%	25%	9%	-543.3	
			6	7/3/10 10:30 PM	2.44	268	315	300	15	32	268	FALSE	315	300	307.5	10.6	5.27	294.3	2785	227	8%	16%	-8%	634.3	
			7	7/4/10 10:50 AM	2.95	341	262	304	37	42	262	341	FALSE	304	322.5	26.2	3.27	302.3	2860	374	13%	14%	-2%	147.3	
			8	7/4/10 10:40 PM	3.44	307	311	320	9	4	320	307	311	FALSE	309	2.8	5.50	312.7	2958	63	2%	11%	-3%	198.3	
WW7			1	7/21/10 10:45 AM	0.00	378	409	385	24	7	409	378	FALSE	385	381.5	4.9	7.86	390.7	3696	154	4%	0%			-207
	Vessel T [C]	20	2	7/21/10 10:25 PM	0.49	367	349	358	9	9	FALSE	367	349	358	358	9.0	#VALUE!	358.0	3387	85	3%	8%	8%	-635.8	
	Agitation [rpm]	1050	3	7/22/10 10:00 AM	0.97	364	346	332	18	14	364	FALSE	346	332	339	9.9	3.57	347.3	3286	152	5%	11%	3%	-209.1	
	PHS [ppmv]	0	4	7/22/10 9:15 PM	1.44	430	379	376	51	3	430	FALSE	379	376	377.5	2.1	35.00	377.5	3571	287	8%	3%	-8%	608.9	
			5	7/23/10 10:40 AM	2.00	367	381	352	14	15	352	367	381	FALSE	374	9.9	3.14	366.7	3469	137	4%	6%	3%	-183.3	
			6	7/23/10 10:15 PM	2.48	334	282	295	39	13	334	FALSE	282	295	288.5	9.2	7.00	303.7	2873	256	9%	22%	16%	-1234.9	
			7	7/24/10 11:00 AM	3.01	314	319	351	32	5	351	314	319	FALSE	316.5	3.5	13.80	316.5	2994	190	6%	19%	-3%	228.5	
			8	7/24/10 9:55 PM	3.47	294	334	349	15	40	294	FALSE	334	349	341.5	10.6	6.33	325.7	3081	269	9%	17%	-2%	190.7	

Table 10. July Sample Wastewater H_2S_{aq} and H_2S_{atm} Data

Temp. [C]		20		10		30		20	
PHS conc. [ppmv]		0	10	1	20	1	20	0	10
H_2S_{aq} [mg L^{-1}] Sample	1	11.1	9.8	4.9	5.4	12.0	11.1	12.2	12.5
	2	6.4	5.3	5.3	5.2	8.2	8.7	4.9	5.0
	3	4.6	4.9	7.4	7.6	7.4	6.8	7.7	7.3
	4	7.1	7.2	8.0	8.3	7.3	7.5	5.2	5.6
	5	8.7	8.1	8.6	8.0	6.4	6.8	7.1	7.2
	6	9.3	9.0	7.4	7.8	9.6	9.6	9.6	9.7
	7	7.5	6.8	5.8	6.4	10.3	9.7	6.6	7.0
	8	7.3	7.5	5.8	6.1	9.0	9.2	8.2	7.8
H_2S_{atm} [ppmv] Sample	1	21.0	15.8	0.0	0.0	19.4	29.4	20.6	20.6
	2	19.4	-	0.0	0.0	28.4	p/d	0.0	0.0
	3	1.8	0.0	0.0	0.0	21.8	p/d	0.0	0.0
	4	6.4	2.0	0.0	0.0	3.4	3.4	0.0	0.0
	5	23.2	4.0	0.0	0.0	2.0	3.8	1.8	3.4
	6	26.8	3.2	0.0	0.0	4.0	7.2	2.6	2.8
	7	15.0	2.2	0.0	0.0	4.8	7.0	0.0	0.0
	8	1.8	0.0	0.0	0.0	5.6	7.8	0.0	0.0

Table 11. Grease Interceptor Material FOG Data

			Subsample			1					2					3																		
PHS [ppmv]	Run #	Sample	Time	Days	vD [mL]	vDt [mL]	vsti [mL]	vTti [mL]	aIR	C	vDt [mL]	vsti [mL]	vTti [mL]	aIR	C	vDt [mL]	vsti [mL]	vTti [mL]	aIR	C	ld1	ld2	T	n1	n2	n3	tav	stdev	t stat	µg/mL BRxtr	SD [ppm]	%SD	% Dec.	Rate
		1	3/4/10 3:00 PM	0.00																														
		2	3/5/10 11:35 PM	1.36	1.0	10.6	4.5	10.0	173	**19084**	10.5	4.5	10	137	**14970**	10.3	4.66	10.33	132	**14586**	4114	384	19084	FALSE	14970	14586	14778	271	22.44	14778	271	1.8%	0%	
		3	3/6/10 11:45 AM	1.86	1.0	10.2	4.7	9.8	130	**12670**	10.3	4.8	10	140	**14325**	10.2	4.75	10	113	**11450**	1654	1221	14325	12670	FALSE	11450	12060	863	3.71	12815	1443	11.3%	13%	
0	G5	4	3/6/10 10:05 PM	2.30	1.0	10.4	4.5	10.0	93	**10065**	10.4	4.5	10	92	**9957**	10.3	4.5	10	83	**8897**	108	1060	8897	10065	9957	FALSE	10011	77	20.60	10011	77	0.8%	32%	
		5	3/7/10 2:35 PM	2.98	1.0	10.3	4.5	10.0	95	**10183**	10.3	4.5	10	78	**8361**	10.3	4.5	9.75	76	**7776**	1822	585	10183	FALSE	8361	7776	8068	413	7.23	8773	1255	14.3%	41%	
		6	3/8/10 6:30 PM	4.15	1.0	10.4	4.5	9.9	81	**8607**	10.4	4.7	10	72	**7509**	10.3	4.75	10	66	**6753**	1098	756	8607	FALSE	7509	6753	7131	535	3.90	7623	932	12.2%	48%	
		7	3/9/10 6:30 PM	5.15	1.0	10.3	4.7	9.9	67	**6790**	10.4	4.7	10	63	**6571**	10.3	4.7	10	63	**6507**	219	63	6790	FALSE	6571	6507	6539	45	7.95	6623	148	2.2%	55%	
		1	3/4/10 3:00 PM	0.00																														
		2	3/5/10 11:35 PM	1.36	1.0	10.4	4.6	9.8	157	**16065**	10.3	4.7	10	134	**13841**	10.3	4.7	10.25	151	**16333**	267	2224	13841	16065	FALSE	16333	16199	189	17.63	16199	189	1.2%	0%	
		3	3/6/10 11:45 AM	1.86	1.0	10.3	4.8	10.0	136	**13915**	10.3	4.5	9.5	136	**13253**	10.2	4.75	10	134	**13577**	338	325	13915	FALSE	13253	13577	13415	230	3.08	13582	331	2.4%	16%	
0	G6	4	3/6/10 10:05 PM	2.30	1.0	10.5	4.0	9.8	90	**10281**	10.4	4.5	10	86	**9308**	10.35	4.4	9.9	87	**9371**	911	63	10281	FALSE	9308	9371	9339	45	29.93	9339	45	0.5%	42%	
		5	3/7/10 2:35 PM	2.98	1.0	10.3	4.5	10.0	78	**8361**	10	4.8	9.8	83	**7852**	10.4	4.6	10	76	**8076**	285	224	8361	FALSE	7852	8076	7964	158	3.55	8096	255	3.1%	50%	
		6	3/8/10 6:30 PM	4.15	1.0	10.4	4.5	9.8	72	**7403**	10.3	4.6	9.8	72	**7297**	10.3	4.7	9.9	78	**7905**	502	106	7905	7403	7297	FALSE	7350	75	10.48	7535	325	4.3%	53%	
		7	3/9/10 6:30 PM	5.15	1.0	10.2	4.5	9.8	72	**7295**	10.3	4.5	9.6	75	**7455**	10.4	4.5	9.7	66	**6754**	159	542	6754	7295	7455	FALSE	7375	113	7.80	7168	367	5.1%	56%	
		1	3/15/10 3:00 PM	0.00																														
		2	3/16/10 9:20 AM	0.76	1.0	10.1	5.8	11.0	91	**9130**	10	4.75	9.9	71	**6919**	10.1	4.75	9.9	80	**7874**	1257	955	9130	FALSE	6919	7874	7396	675	3.63	7974	1109	13.9%	0%	1322.3
		3	3/16/10 8:40 PM	1.24	0.7	10.4	4.9	10.1	63	**9209**	10.3	4.8	10	62	**8976**	10.4	4.75	10	56	**8265**	233	711	8265	9209	8976	FALSE	9093	165	7.10	8817	492	5.6%	-11%	
500	G7	4	3/17/10 8:40 AM	1.74	1.0	10.1	4.8	9.9	76	**7407**	10	4.5	9.55	67	**6402**	10	5	10	69	**6528**	879	126	7407	FALSE	6402	6528	6465	89	14.98	6465	89	1.4%	19%	
		5	3/17/10 10:00 PM	2.29	1.0	10.1	4.8	10.0	63	**6321**	10	4.75	10	55	**5464**	10.1	4.8	10.1	60	**6077**	244	614	5464	6321	FALSE	6077	6199	172	6.04	5954	442	7.4%	25%	
		6	3/18/10 11:00 AM	2.83	1.0	10.3	4.8	10.0	66	**6753**	10.1	4.5	9.6	68	**6628**	10.1	4.5	9.6	61	**5945**	125	682	5945	6753	6628	FALSE	6690	89	11.88	6442	435	6.7%	19%	
		7	3/19/10 11:00 AM	3.83	1.0																													
		1	3/15/10 3:00 PM	0.00																														
		2	3/16/10 9:20 AM	0.76	1.0	10.0	4.6	9.7	64	**6176**	10.15	4.7	9.8	63	**6171**	10	4.75	9.9	68	**6626**	450	5	6626	6176	6171	FALSE	6173	4	170.70	6173	4	0.1%	0%	756.1
		3	3/16/10 8:40 PM	1.24	0.7	10.4	5.0	10.2	63	**9209**	10.4	4.8	10	67	**9888**	10.2	4.75	10	84	**12159**	2271	679	12159	9209	9888	FALSE	9549	480	7.69	10419	1545	14.8%	-69%	
0	G8	4	3/17/10 8:40 AM	1.74	1.0	10.0	4.6	9.6	50	**4730**	10	4.75	9.75	57	**5393**	10	4.9	9.9	59	**5582**	189	662	4730	FALSE	5393	5582	5487	134	8.00	5235	447	8.5%	15%	
		5	3/17/10 10:00 PM	2.29	1.0	10.1	4.5	9.9	51	**5263**	10.1	4.75	9.9	47	**4626**	10.2	4.8	9.9	50	**4921**	342	296	5263	FALSE	4626	4921	4774	209	3.31	4937	319	6.5%	20%	
		6	3/18/10 11:00 AM	2.83	1.0	10.0	4.8	9.8	56	**5351**	10.2	4.5	9.5	45	**4342**	10	4.8	10	42	**4132**	1009	210	5351	FALSE	4342	4132	4237	149	10.60	4609	651	14.1%	25%	
		7	3/19/10 11:00 AM	3.83	1.0																													
		1	3/18/10 3:30 PM	0.00	1.0	10.1	4.6	9.75	119	**11712**	10	4.7	9.8	98	**9457**	10.1	4.75	9.9	97	**9547**	2165	90	11712	FALSE	9457	9547	9502	63	49.23	9502	63	0.7%	0%	
		2	3/19/10 10:35 AM	0.80	1.0	10.1	4.8	9.9	112	**11023**	9.95	4.75	10	133	**13146**	10.1	4.8	9.8	114	**10893**	2123	130	13146	11023	FALSE	10893	10958	92	33.67	10958	92	0.8%	-15%	2319.8
		3	3/19/10 10:35 PM	1.30	0.7	10.2	4.8	9.8	68	**9468**	10.2	4.8	9.9	75	**10649**	10.1	4.5	9.6	72	**10025**	624	557	10649	9468	FALSE	10025	9746	394	3.24	10047	591	5.9%	-6%	
0	G9	4	3/20/10 10:35 AM	1.80	1.0	10.2	4.6	9.7	90	**8815**	10.2	4.8	9.9	80	**7874**	10.2	4.8	9.8	79	**7623**	941	251	8815	FALSE	7874	7623	7749	177	8.50	8104	628	7.8%	15%	
		5	3/20/10 10:35 PM	2.30	1.0	10.1	4.5	9.6	68	**6628**	10.1	4.75	9.9	73	**7185**	10.1	4.6	9.7	66	**6433**	557	195	7185	6628	FALSE	6433	6530	138	6.72	6748	390	5.8%	29%	
		6	3/21/10 10:35 AM	2.80	1.0	10.1	4.6	9.8	64	**6299**	10.1	4.9	10	58	**5653**	9.9	4.75	9.8	67	**6338**	39	646	5653	6299	FALSE	6338	6318	28	33.98	6318	28	0.4%	34%	
		7	3/21/10 10:35 PM	3.30	1.0																													
		1	3/18/10 3:30 PM	0.00	1.0	10.1	4.75	9.9	93	**9153**	10.1	4.75	9.9	88	**8661**	10	4.75	9.8	87	**8313**	492	348	9153	FALSE	8661	8313	8487	246	3.83	8709	422	4.8%	0%	
		2	3/19/10 10:35 AM	0.80	1.0	10.1	4.8	9.8	90	**8686**	10	4.75	9.9	87	**8478**	10	4.75	9.8	94	**8982**	296	208	8982	8686	8478	FALSE	8582	147	3.85	8715	253	2.9%	0%	3471.4
		3	3/19/10 10:35 PM	1.30	0.7	9.6	4.6	9.8	48	**6415**	10.2	4.6	9.7	46	**6468**	10.1	4.75	9.75	46	**6279**	54	135	6279	6415	6468	FALSE	6441	38	6.06	6387	97	1.5%	27%	
500	G10	4	3/20/10 10:35 AM	1.80	1.0	10.1	4.9	10.0	47	**4581**	10.2	4.75	9.8	44	**4288**	10.2	4.75	9.8	48	**4678**	97	292	4288	4581	FALSE	4678	4630	69	7.00	4516	203	4.5%	48%	
		5	3/20/10 10:35 PM	2.30	1.0	10.0	4.5	9.6	38	**3667**	10.1	4.8	10	36	**3578**	10.2	4.75	9.9	33	**3280**	89	298	3280	3667	3578	FALSE	3622	63	7.65	3508	203	5.8%	60%	
		6	3/21/10 10:35 AM	2.80	1.0	10.2	4.8	9.9	42	**4154**	10.1	4.75	9.9	36	**3543**	10.1	4.85	10	45	**4429**	275	611	3543	4154	FALSE	4429	4292	194	5.45	4042	453	11.2%	54%	
		7	3/21/10 10:35 PM	3.30	1.0																													
		1	3/24/10 11:25 AM	0.00	1.0	10.1	4.75	9.8	89	**8589**	10.1	4.5	9.7	94	**9341**	10.2	4.7	9.8	88	**8662**	680	72	9341	8589	FALSE	8662	8626	51	19.75	8626	51	0.6%	0%	
		2	3/24/10 11:05 PM	0.49	1.0	10.3	4.7	10.0	125	**12849**	10.3	4.75	10	120	**12278**	10.35	4.8	10.2	107	**11315**	571	963	11315	12849	12278	FALSE	12564	404	4.37	12147	775	6.4%	-41%	2167.0
		3	3/25/10 1:00 PM	1.07	1.0	10.1	4.8	9.8	118	**11275**	10.1	4.6	9.6	120	**11466**	10.1	4.75	9.8	106	**10230**	191	1045	10230	11275	11466	FALSE	11371	135	11.94	10991	666	6.1%	-27%	
0	G11	4	3/25/10 9:05 PM	1.40	1.0	10.2	4.5	9.6	136	**13386**	10.2	4.8	10	136	**13649**	10.1	4.75	9.9	119	**11712**	262	1674	11712	13386	13649	FALSE	13518	186	13.76	13518	186	1.4%	-57%	
		5	3/26/10 11:10 AM	1.99	1.0	10.0	4.5	9.7	92	**9052**	10	4.75	9.8	92	**8791**	9.9	4.7	9.75	96	**9081**	29	261	8791	9052	FALSE	9081	9067	21	18.78	9067	21	0.2%	-5%	
		6	3/26/10 10:40 PM	2.47	1.0	10.2	4.5	9.6	83	**8170**	10.2	4.5	9.6	80	**7874**	10.2	4.8	10	78	**7828**	295	46	8170	FALSE	7874	7828	7851	33	13.75	7851	33	0.4%	9%	
		7	3/27/10 12:00 PM	3.02	1.0	10.0	4.7	9.8	80	**7644**	9.9	4.7	9.8	81	**7738**	9.95	4.7	9.75	77	**7321**	94	323	7321	7644	7738	FALSE	7691	66	7.88	7568	219	2.9%	12%	
		1	3/24/10 11:25 AM	0.00	1.0	10	4.6	9.75	101	**9842**	10.1	4.8	10	93	**9242**	10.1	4.75	10	92	**9230**	600	11	9842	FALSE	9242	9230	9236	8	105.67	9236	8	0.1%	0%	
		2	3/24/10 11:05 PM	0.49	1.0	10.3	4.8	10.2	137	**14418**	10.3	4.8	10.1	106	**10949**	10.3	4.9	10.25	133	**13867**	551	2918	10949	14418	FALSE	13867	14143	389	11.60	13078	1864	14.3%	-42%	2366.9
		3	3/25/10 1:00 PM	1.07	1.0	10.2	4.8	9.8	115	**10933**	10.1	4.6	9.7	112	**10916**	10.1	4.9	10	114	**11111**	178	17	11111	10933	10916	FALSE	10924	12	22.50	10924	12	0.1%	-18%	
500	G12	4	3/25/10 9:05 PM	1.40	1.0	10.1	4.9	10.0	120	**11696**	10.2	4.7	9.8	114	**11221**	10.2	5	10.1	115	**11319**	376	98	11696	FALSE	11221	11319	11270	70	8.65	11412	251	2.2%	-24%	
		5	3/26/10 11:10 AM	1.99	1.0	9.9	4.8	9.8	111	**10500**	10	4.9	10	104	**10036**	10.1	4.75	9.8	99	**9554**	464	482	9554	10500	10036	FALSE	10268	328	3.07	10030	473	4.7%	-9%	
		6	3/26/10 10:40 PM	2.47	1.0	10.2	4.8	9.8	86	**8382**	10.2	4.5	9.7	85	**8531**	10.15	4.5	9.7	84	**8389**	142	7	8531	8382	FALSE	8389	8385	5	41.92	8385	5	0.1%	9%	
		7	3/27/10 12:00 PM	3.02	1.0	10.1	4.8	9.8	107	**10275**	10.2	4.7	9.8	91	**8957**	10	4.7	9.75	88	**8409**	1318	548	10275	FALSE	8957	8409	8683	388	5.81	9214	959	10.4%	0%	
		1	4/21/10 2:30 PM	0.00	1.0	9.9	4.7	9.75	104	**9838**	9.9	4.6	9.6	90	**8430**	10	4.75	9.75	84	**7947**	1409	482	9838	FALSE	8430	7947	8188	341	6.84	8738	983	11.2%	0%	
		2	4/22/10 1:55 AM	0.48	1.0	10.0	4.8	9.8	101	**9555**	9.9	4.7	9.8	97	**9267**	9.8	4.6	9.7	92	**8700**	288	566	8700	9555	9267	FALSE	9411	204	4.93	9174	435	4.7%	-5%	
		3	4/22/10 2:15 PM	0.99	1.0	10.1	4.5	9.5	180	**17200**	9.7	4.6	9.6	122	**11196**	10	4.75	9.75	103	**9745**	6004	1451	17200	FALSE	11196	9745	10470	1026	9.27	12713	3952	31.1%	-45%	
0	G13	4	4/22/10 11:15 PM	1.36	1.0	10.1	4.8	9.8	97	**9269**	10.1	4.75	9.75	92	**8791**	10	4.5	9.5	89	**8420**	478	371	9269	FALSE	8791	8420	8605	262	3.58	8827	425	4.8%	-1%	
		5	4/23/10 9:50 AM	1.81	1.0	10.0	4.5	9.6	112	**10808**	10.1	4.6	9.75	120	**11810**	10.1	4.5	9.6	100	**9746**	1002	1061	9746	10808	11810	FALSE	11309	709	3.12	10788	1032	9.6%	-23%	1429.6
		6	4/23/10 11:45 PM	2.39	1.0	10.2	4.5	9.8	92	**9322**	10.2	4.5	9.7	88	**8832**	10.1	4.6	9.8	85	**8447**	490	385	9322	FALSE	8832	8447	8639	272	3.55	8867	439	4.9%	-1%	
		7	4/24/10 11:30 AM	2.88	1.0	10.2	4.8	10.0	100	**10132**	10.2	4.5	9.5	101	**9746**	10.1	4.5	9.75	89	**8929**	386	817	8929	10132	9746	FALSE	9939	273	5.23	9603	614	6.4%	-10%	
		8	4/24/10 11:30 PM	3.38	1.0	10.1	4.7	9.8	84	**8107**	10	4.5	9.7	87	**8560**	10.1	4.5	9.6	92	**8967**	407	453	8107	FALSE	8560	8967	8763	288	3.23	8545	430	5.0%	2%	
		1	4/21/10 2:30 PM	0.00	1.0	10	4.75	9.75	86	**8136**	10	4.75	9.75	95	**8988**	10	4.6	9.7	102	**9843**	855	851	9843	8136	8988	FALSE	8562	602	3.01	8989	853	9.5%	0%	
		2	4/22/10 1:55 AM	0.48	1.0	9.7	4.6	9.7	89	**8331**	9.9	4.6	9.7	86	**8216**	9.9	4.6	9.7	85	**8120**	115	96	8331	FALSE	8216	8120	8168	68	3.40	8222	105	1.3%	9%	
		3	4/22/10 2:15 PM	0.99	1.0	10.1	4.8	9.8	177	**16913**	10.1	4.6	9.6	114	**10893**	10.1	4.6	9.6	91	**8695**	6020	2198	16913	FALSE	10893	8695	9794	1554	6.48	12167	4254	35.0%	-35%	
500	G14	4	4/22/10 11:15 PM	1.36	1.0	10.0	4.6	9.7	93	**8974**	10.1	4.7	9.8	107	**10429**	10.1	4.5	9.55	102	**9844**	585	869	8974	FALSE	10429	9844	10136	414	3.97	9749	732	7.5%	-8%	
		5	4/23/10 9:50 AM	1.81	1.0	10.1	4.7	9.8	127	**12317**	10.1	4.9	10	96	**9357**	10.1	4.7	9.85	110	**10826**	1491	1470	12317	FALSE	9357	10826	10091	1039	3.03	10833	1480	13.7%	-21%	1302.5
		6	4/23/10 11:45 PM	2.39	1.0	10.1	4.8	9.9	88	**8661**	10.1	4.75	9.9	99	**9744**	10.2	4.8	10	99	**9936**	192	1083	8661	FALSE	9744	9936	9840	136	12.28	9447	687	7.3%	-5%	
		7	4/24/10 11:30 AM	2.88	1.0	10.2	4.6	9.8	138	**13850**	10.2	4.75	10	122	**12362**	10.1	4.5	9.7	120	**11925**	1488	437	13850	FALSE	12362	11925	12143	309	7.82	12712	1009	7.9%	-41%	
		8	4/24/10 11:30 PM	3.38	1.0	10.2	4.7	9.8	99	**9649**	10.1	4.6	9.8	78	**7751**	10.1	4.7	9.8	92	**8967**	682	1215	7751	9649	FALSE	8967	9308	482	4.56	8789	961	10.9%	2%	
		1	4/21/10 2:30 PM	0.00	1.0	10	4.6	9.7	104	**10036**	10	4.5	9.5	95	**8988**	10	4.5	9.5	82	**7758**	1048	1230	7758	10036	8988	FALSE	9512	741	3.35	8927	1140	12.8%	0%	
		2	4/22/10 1:55 AM	0.48	1.0	9.8	4.6	9.7	94	**8890**	9.8	4.7	9.8	88	**8322**	9.9	4.6	9.7	78	**7452**	567	870	7452	8890	8322	FALSE	8606	401	4.07	8221	724	8.8%	8%	
		3	4/22/10 2:15 PM	0.99	1.0	10.0	4.7	9.7	94	**8893**	9.9	4.6	9.7	140	**13375**	10	4.6	9.7	86	**8299**	4482	594	13375	8893	FALSE	8299	8596	420	16.09	8596	420	4.9%	4%	
500	G15	4	4/22/10 11:15 PM	1.36	1.0	10.0	4.6	9.6	105	**9934**	10	4.75	9.7	94	**8804**	10.1	4.5	9.6	88	**8577**	1130	227	9934	FALSE	8804	8577	8691	161	10.94	9105	727	8.0%	-2%	
		5	4/23/10 9:50 AM	1.81	1.0	10.1	4.8	9.8	82	**7797**	9.95	4.55	9.65	77	**7393**	10.15	4.75	9.8	101	**9796**	1999	403	9796	7797	7393	FALSE	7595	285	10.91	8329	1286	15.4%	7%	
		6	4/23/10 11:45 PM	2.39	1.0	10.1	4.8	9.8	100	**9555**	10	4.75	9.9	96	**9355**	10.2	4.6	9.8	85	**8531**	201	824	8531	9555	9355	FALSE	9455	142	9.22	9147	543	5.9%	-2%	
		7	4/24/10 11:30 AM	2.88	1.0	10.1	4.5	9.7	126	**12521**	10.1	4.7	9.75	118	**11388**	10.1	4.75	9.9	106	**10433**	1133	956	12521	FALSE	11388	10433	10910	676	3.37	11447	1046	9.1%	-28%	
		8	4/24/10 11:30 PM	3.38	1.0	10.1	4.7	9.8	88	**8577**	10.2	5.4	10.5	106	**10434**	10.2	4.6	9.7	86	**8465**	1857	112	10434	8577	FALSE	8465	8521	79	34.17	8521	79	0.9%	5%	
		1	5/6/10 12:35 PM	0.00	1.0	10.2	4.7	9.9	179	**17964**	10.1	4.6	9.75	182	**17912**	10.2	4.75	9.8	176	**17154**	52	759	17154	17964	17912	FALSE	17938	37	30.24	17938	37	0.2%	0%	1814.9
		2	5/7/10 12:20 AM	0.49	1.0	10.3	4.8	10.0	157	**15911**	10.1	4.7	9.8	172	**16764**	10.2	4.75	9.75	175	**16887**	124	853	15911	FALSE	16764	16887	16826	87	14.81	16826	87	0.5%	6%	
		3	5/7/10 1:00 PM	1.02	1.0	10.3	4.8	10.0	165	**16722**	10.2	4.8	9.9	161	**16003**	10.2	4.7	9.8	158	**15552**	719	451	16722	FALSE	16003	15552	15777	319	4.19	16092	590	3.7%	10%	
0	G16	4	5/7/10 9:15 PM	1.36	1.0	10.1	4.7	9.9	165	**16397**	10.1	4.7	9.9	163	**16198**	10.1	4.7	9.8	160	**15594**	199	604	15594	16397	16198	FALSE	16298	141	7.08	16063	418	2.6%	10%	
		5	5/8/10 10:00 AM	1.89	1.0	10.3	4.6	9.8	154	**15457**	10.2	4.6	9.8	156	**15656**	10.2	4.8	10	142	**14251**	199	1206	14251	15457	15656	FALSE	15556	141	13.10	15556	141	0.9%	13%	
		6	5/8/10 8:45 PM	2.34	1.0	10.1	4.7	9.8	150	**14620**	10	4.7	9.8	139	**13413**	10	4.7	9.8	139	**13413**	1206	0	14620	FALSE	13413	13413	13413	0	#DIV/0!	13413	696	5.2%	25%	
		7	5/9/10 9:00 AM	2.85	1.0	10.2	4.7	9.7	144	**13896**	10.1	4.6	9.6	137	**13091**	10.1	4.5	9.5	112	**10702**	805	2389	10702	13896	13091	FALSE	13493	569	6.93	12563	1661	13.2%	30%	
		8	5/9/10 10:50 PM	3.43	1.0	10.2	4.6	9.8	130	**12921**	10	4.7	9.8	101	**9746**	10.1	4.7	9.75	97	**9361**	3175	385	12921	FALSE	9746	9361	9554	272	17.49	9554	272	2.9%	47%	
		1	5/6/10 12:35 PM	0.00	1.0	10.2	4.75	9.8	181	**17641**	10	4.7	9.8	185	**17852**	10.2	4.9	10			211	211	FALSE	17641	17852		17747	149	#VALUE!	17747	149	0.8%	0%	
		2	5/7/10 12:20 AM	0.49	1.0	10.2	4.5	9.7	191	**19169**	10.1	4.7	9.8	178	**17349**	10.2	4.8	10	183	**18366**	803	1017	17349	19169	FALSE	18366	18767	568	3.53	18294	912	5.0%	-3%	4011.0
		3	5/7/10 1:00 PM	1.02	1.0	10.2	4.7	9.8	174	**17127**	10.1	4.9	10	165	**16082**	10.2	4.7	9.75	154	**15010**	1045	1072	15010	17127	16082	FALSE	16604	739	3.05	16073	1059	6.6%	9%	
500	G17	4	5/7/10 9:15 PM	1.36	1.0	10.1	4.7	9.9	158	**15701**	10	4.7	9.9	134	**13184**	10.1	4.5	9.7	156	**15503**	199	2318	13184	15701	FALSE	15503	15602	141	24.33	15602	141	0.9%	12%	
		5	5/8/10 10:00 AM	1.89	1.0	10.2	4.8	9.9	142	**14114**	10	4.75	9.9	154	**15007**	10.2	4.7	9.8	152	**14961**	45	847	14114	FALSE	15007	14961	14984	32	38.38	14984	32	0.2%	16%	
		6	5/8/10 8:45 PM	2.34	1.0	10.1	4.6	9.7	140	**13645**	9.9	4.6	9.7	130	**12419**	10.1	4.7	9.8	154	**15010**	1365	1226	15010	13645	12419	FALSE	13032	867	3.23	13691	1296	9.5%	23%	
		7	5/9/10 9:00 AM	2.85	1.0	10.1	4.6	9.7	100	**9746**	10	4.9	9.9	86	**8136**	10.1	4.75	9.8	89	**8589**	1157	453	9746	FALSE	8136	8589	8363	320	6.11	8824	830	9.4%	50%	
		8	5/9/10 10:50 PM	3.43	1.0	10.1	4.6	9.6	91	**8695**	10	4.75	9.8	84	**8026**	8.5	4.6	9.6	146	**11741**	3045	669	11741	8695	8026	FALSE	8361	473	10.11	9488	1980	20.9%	47%	
		1	5/6/10 12:35 PM	0.00	1.0	10.2	4.8	9.9	153	**15060**	10.2	4.75	9.9	158	**15704**	10	4.75	9.75	170	**16083**	379	645	15060	FALSE	15704	16083	15894	268	4.40	15616	517	3.3%	0%	
		2	5/7/10 12:20 AM	0.49	1.0	10.2	4.7	9.8			10.2	4.75	9.9	166	**16499**	10.1	4.8	10	152	**15105**	1394	1394	FALSE		16499	15105	15802	986	#VALUE!	15802	986	6.2%	-1%	
		3	5/7/10 1:00 PM	1.02	1.0	10.2	4.8	9.9	158	**15552**	10.2	4.8	9.9	144	**14313**	10.1	4.8	10	143	**14211**	1239	102	15552	FALSE	14313	14211	14262	72	25.27	14262	72	0.5%	9%	
500	G18	4	5/7/10 9:15 PM	1.36	1.0	10.2	4.9	10.0	136	**13386**	10.1	4.8	9.9	139	**13548**	10	4.6	9.75	125	**12181**	161	1206	12181	13386	13548	FALSE	13467	114	15.96	13467	114	0.8%	14%	
		5	5/8/10 10:00 AM	1.89	1.0	10.2	4.8	9.9	143	**14144**	10.2	4.6	9.75	122	**12126**	10.1	4.8	10	140	**13913**	231	1786	12126	14144	FALSE	13913	14028	163	16.46	14028	163	1.2%	10%	
		6	5/8/10 8:45 PM	2.34	1.0	10.0	4.9	10.0	153	**14764**	10.1	4.9	10	137	**13353**	10	4.75	9.9	131	**12765**	1412	587	14764	FALSE	13353	12765	13059	415	5.81	13627	1027	7.5%	13%	
		7	5/9/10 9:00 AM	2.85	1.0	10.1	4.8	9.8	100	**9555**	10	4.8	9.75	98	**9179**	10.2	4.8	9.8	91	**8781**	377	397	8781	9555	9179	FALSE	9367	266	3.11	9172	387	4.2%	41%	
		8	5/9/10 10:50 PM	3.43	1.0	8.1	4.6	9.6	178	**13640**	8.2	4.75	9.8	154	**12066**	8.2	4.8	9.9	148	**11711**	1574	355	13640	FALSE	12066	11711	11889	251	9.86	12473	1027	8.2%	20%	

Table 12. January Sample Wastewater pH Data

Sample	Experiment								
	E1 & E2	E3 & E4	E5 & E6	E1	E2	E3	E4	E5	E6
	Trial Length [days]			pH					
1	0.00	0.00	0.00	5.81	5.81	5.28	5.28	5.57	5.96
2	0.90	0.83	0.63	5.81	5.59	5.44	5.46	6.11	5.91
3	1.90	1.90	1.65	5.79	5.83	5.35	5.49	5.94	5.79
4	3.31	2.90	2.59	6.61	5.98	5.36	5.54	5.89	5.65
5	4.38	3.76	3.93	6.88	5.95	5.38	5.51	5.85	5.65
6	4.93	5.12	4.68	6.85	5.85	5.58	5.67	5.84	5.65
7	5.00	5.64	4.68	7.14	6.32	5.87	5.93	6.00	6.11
8		5.68				6.04	5.82		

Table 13. July Sample Wastewater Microbial Quantification Data

Trial	Set	Count Dilution 1	2	3	4	5	6
WW1C1	1	48					
	2	28					
	3	37					
WW1E1	1						
	2						
	3						
WW2C1	1	23					
	2	13					
	3	21					
WW2E1	1						
	2						
	3						
WW3C1	1		270	46	57		
	2			75			
	3			87			
WW3E1	1				131		
	2				133		
	3				115	58	
WW4C1	1		299	59			
	2			53			
	3			56			
WW4E1	1			219	39	60	
	2			209			
	3			115			
WW5C1	1		26	4			
	2		26	3			
	3		28	4			
WW5E1	1			173	22	1	
	2			184	25	1	
	3			129	13	1	
WW6C1	1		31	4			
	2		40	4			
	3		35	3			
WW6E1	1				46	3	
	2				48	4	
	3				15	1	
WW7C1	1				100	3	
	2				58	10	
	3				52	0	
WW7E1	1					22	
	2					170	
	3					79	
WW8C1	1				29	3	
	2				15	13	
	3				29	0	
WW8E1	1					180	2
	2					21	3
	3					87	0

Table 14. Wastewater Study Microbial Quantification Data Summary

Trial Sample	*Count avg.*	*Population [CFU/mL]*	*Temperature [C]*	*PHS [ppmv]*	*Increase per trial*
WW1C1	38	3.8E+03	20	0 (Control)	
WW1E1					
WW2C1	19	1.9E+03		10	
WW2E1					
WW3C1	69	6.9E+05	10	1	18
WW3E1	126	1.3E+07			
WW4C1	56	5.6E+05		20	3
WW4E1	181	1.8E+06			
WW5C1	27	2.7E+04	30	1	58
WW5E1	162	1.6E+06			
WW6C1	35	3.5E+04		20	102
WW6E1	36	3.6E+06			
WW7C1	70	7.0E+06	20	0 (Control)	12
WW7E1	90	9.0E+07			
WW8C1	24	2.4E+06		10	38
WW8E1	96	9.6E+07			

Table 15. Grease Interceptor Material Study Microbial Quantification Data

Trial	Set	Count Dilution 1	2	3	4	5	6
R16B	1		76				
	2		91				
	3		71				
R16E	1			145	25		
	2			163			
	3			157			
R17B	1		47				
	2		38				
	3		60				
R17E	1			135			
	2			256	28		
	3			225	43		
R18B	1		35				
	2		46				
	3		32				
R18E	1			174	16		
	2			148	13		
	3			146			

Table 16. Grease Interceptor Material Microbial Quantification Data Summary

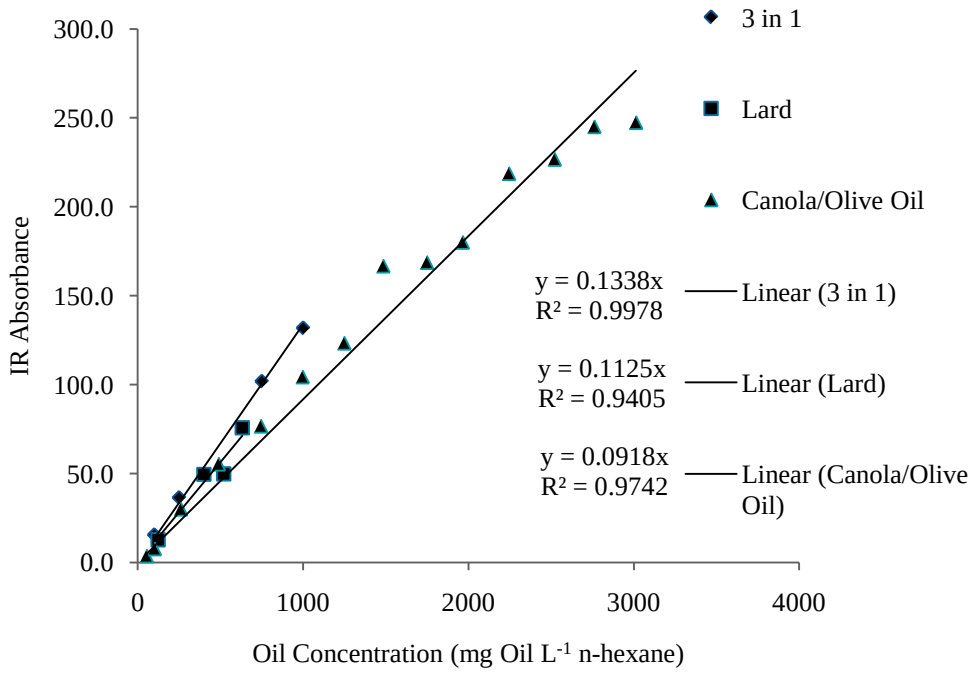
PHS ppm(v)	*Reactor Sample*	*Population [CFU/mL]*	*Conc. Increase per trial*
500	7B	3.24E+04	121.1
	7E	3.93E+06	
0 (Control)	8B	2.47E+04	126.1
	8E	3.11E+06	
0 (Control)	9E	7.60E+06	
500	10E	3.80E+07	
0 (Control)	13B	3.30E+07	1.1
	13E	3.53E+07	
500	14B	3.10E+07	1.2
	14E	3.73E+07	
500	15B	5.30E+07	1.4
	15E	7.50E+07	
0 (Control)	16B	7.87E+04	19.7
	16E	1.55E+06	
PHS	17B	4.25E+04	83.5
	17E	3.55E+06	
500	18B	3.35E+04	43.9
	18E	1.47E+06	

Appendix B: Calibration Data

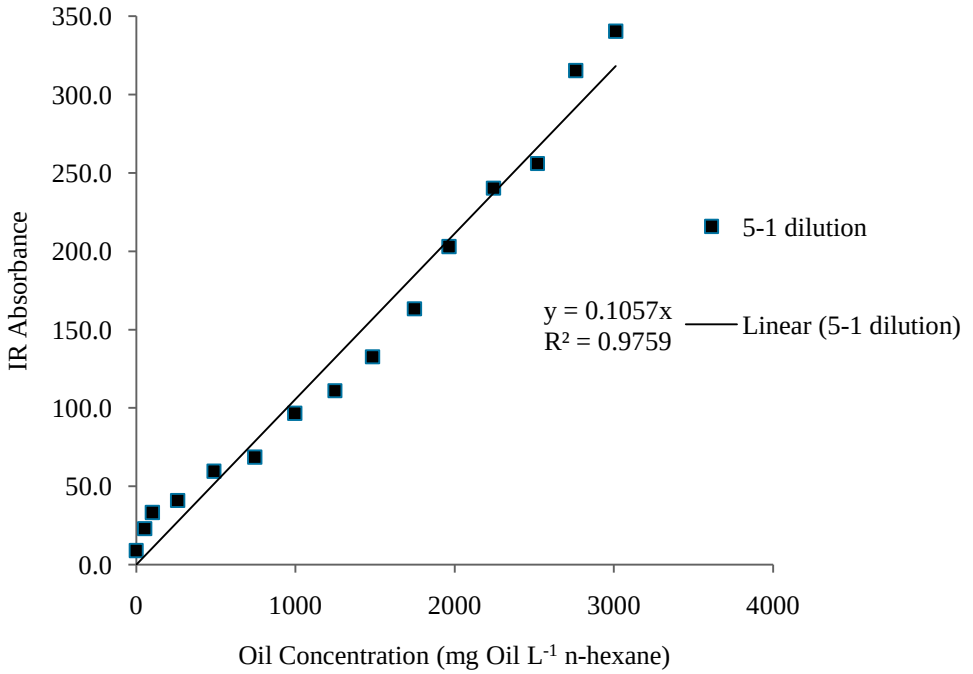
Table 17. FOG Concentration Calibration Data

Organic Carbon Solute	*Concentration [mg Oil L^{-1} n-hexane]*	*IR Absorbance*						
		a1	*a2*	*a3*	*a4*	*a5*	*avg.*	*StDev*
3-in-1 factory standards	100	18	14	14	16		15.5	1.9
	250	35	39	36	37	35	36.4	1.7
	750	95	101	107	103	103	101.8	4.4
	1000	135	131	132	126	135	131.8	3.7
Lard	520	45	46	54	54		49.8	4.9
	124	12	12	15	12		12.8	1.5
	400	52	47	48	51		49.5	2.4
	632	75	78	75	75		75.8	1.5
90% Canola, 10% Olive Oil	53	4	4	3			3.7	0.6
	102	7	8	8			7.7	0.6
	260	34	27	29			30.0	3.6
	488	53	56	57			55.3	2.1
	744	74	81	75			76.7	3.8
	996	102	108	103			104.3	3.2
	1248	133	122	115			123.3	9.1
	1484	179	168	153			166.7	13.1
	1748	171	173	162			168.7	5.9
	1964	166	184	190			180.0	12.5
	2244	216	222	218			218.7	3.1
	2520	229	230	221			226.7	4.9
	2760	256	242	237			245.0	9.8
	3012	246	246	250			247.3	2.3
90% Canola, 10% Olive + Extraction Process	0	9	8	10			9.0	1.0
	53	19	23	27			23.0	4.0
	102	27	36	37			33.3	5.5
	260	39	34	50			41.0	8.2
	488	68	58	53			59.7	7.6
	744	67	71	68			68.7	2.1
	996	93	98	99			96.7	3.2
	1248	106	111	116			111.0	5.0
	1484	140	123	135			132.7	8.7
	1748	155	167	168			163.3	7.2
	1964	196	205	208			203.0	6.2
	2244	246	234	241			240.3	6.0
	2520	232	258	267	267		256.0	16.6
	2760	316	315	315			315.3	0.6
	3012	366	348	322	326		340.5	20.5

Figures 15 a-b. FOG Calibration



a) Organic Carbon Source Comparison



b) 10% Olive, 90% Canola Oil Calibration with Extraction Process

Table 18. Aqueous Hydrogen Sulfide Concentration Calibration Data

H_2S_{aq} Conc.	Absorbance (670nm)
0.7	0.02
2.4	0.13
5.0	0.28
7.2	0.42
9.6	0.60

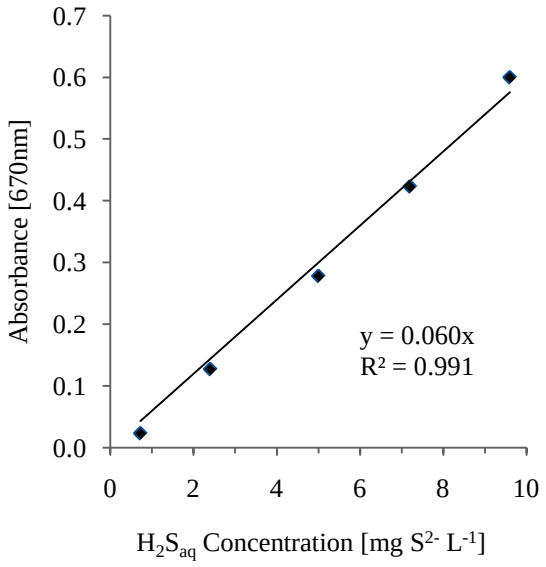


Figure 16. H_2S_{aq} Calibration

Appendix C: Other Experiments

Sterile Oil/Water & Non-sterile PHS Experiment

This test was performed to assess the effect of PHS in an initially sterile environment containing edible cooking oil to simulate FOG. The test was conducted at a temperature of 30°C and a PHS concentration of 10 ppm(v). Experimental methods were in the testing and refining stage during this experiment. The raw data and result of this experiment are shown in Table AB and Figure C, respectively. It was determined from this experiment that PHS does not show a significant impact in the mineralization of FOG without the presence of a biological consortium.

Table 19. Sterile Oil/Water – Non-sterile PHS Experiment Data

			A_{IR}					Conc.	
Sample	***Time***	***day***	***1***	***2***	***3***	***avg.***	***std.***	***avg.***	***std.***
1	11/18/09 6:27 PM	0.00	281	251	323	285	36.2	2192	280
2	11/19/09 11:25 AM	0.71	195	134	77	135	59.0	1042	455
3	11/19/09 6:10 PM	0.99	94	218	143	152	62.5	1167	482
4	11/20/09 8:47 AM	1.60	299	354	393	349	47.2	2682	365
5	11/20/09 9:40 AM	1.63	369	345	365	360	12.9	2766	101
6	11/21/09 5:47 PM	2.97	387	405	410	401	12.1	3081	95
7	11/23/09 10:20 AM	4.66	420	351	365	379	36.5	2912	282
8	11/23/09 6:15 PM	4.99	363	335	345	348	14.2	2674	111

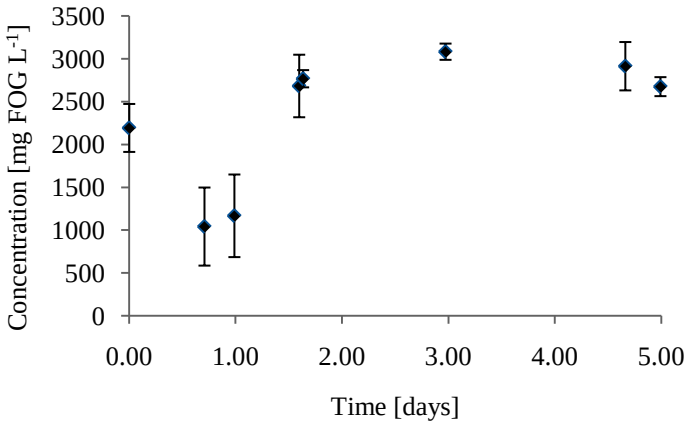


Figure 17. Sterile Oil-Water – Non-sterile PHS Experiment Results

Preliminary Wastewater Experiment

Table 20. Preliminary Wastewater Experiment Data

			A_{IR}												conc.	
Sample	***Time***	***day***	***a1***	***a2***	***a3***	***a4***	***a5***	***a6***	***a7***	***a8***	***a9***	***a10***	***avg.***	***std.***	***avg.***	***std.***
1	11/10/09 4:10 PM	0.00	253	207	213	222							223.8	20.5	1721.5	158.9
2	11/10/09 5:15 PM	0.05	250	235	234	235							238.5	7.7	1834.9	60.7
3	11/10/09 6:15 PM	0.09	238	229	206	242							228.8	16.1	1760.0	125.5
4	11/10/09 8:15 PM	0.17	187	244	215	212							214.5	23.3	1650.4	181.0
5	11/11/09 11:45 AM	0.82	151	215	196	199							190.3	27.5	1464.0	212.8
6	11/11/09 6:15 PM	1.09	227	206	210	223							216.5	10.1	1665.8	79.2
7	11/12/09 10:30 AM	1.76	160	158	174	170							165.5	7.7	1273.8	61.1
8	11/12/09 4:50 PM	2.03	158	124	129								137.0	18.4	1054.7	142.8
9	11/13/09 9:45 AM	2.73	82	83	96.5	81.5							85.8	7.2	660.8	57.0
10	11/14/09 1:10 PM	3.88	47	45	47.5	52	52	45.5					48.2	3.1	371.9	25.6
11	11/16/09 11:45 AM	5.82	58.5	58	59	68.5	92.5	72	65.5	66	61.5	57	65.9	10.6	507.9	83.3

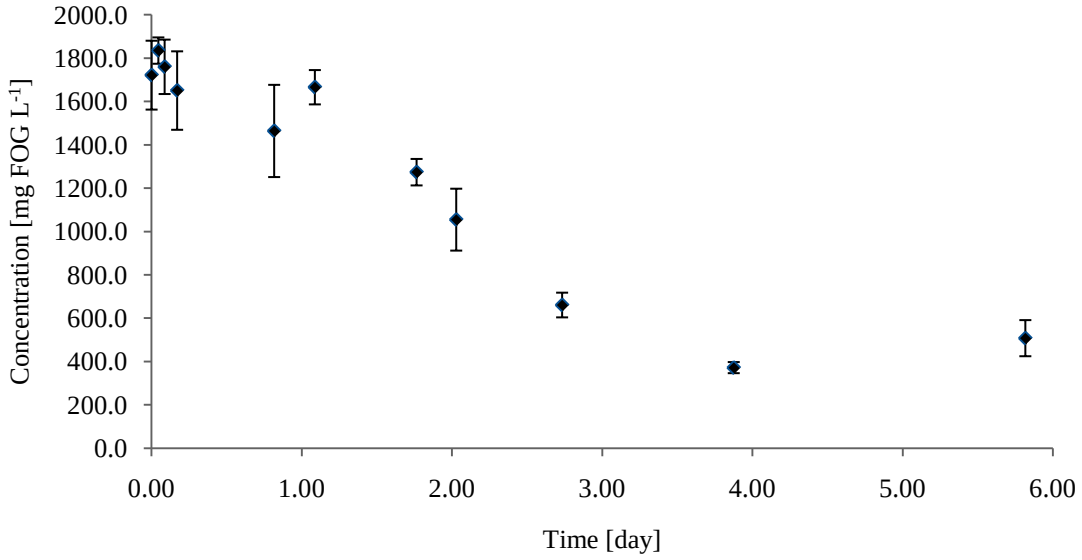


Figure 18. Preliminary Wastewater Experiment Results

Batch Jar Screening Experiment

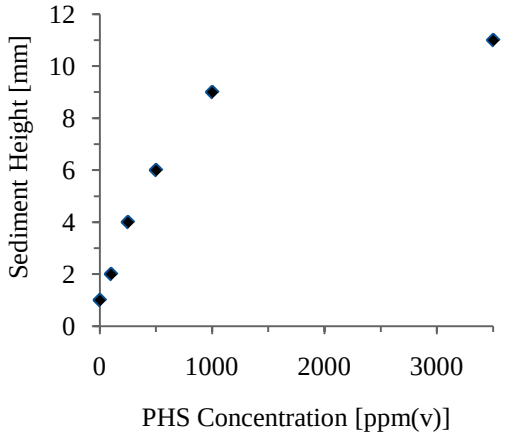
The batch jar screening experiment was designed to monitor long-term effects of PHS in batch, high grease, non-agitated environments. Uniform restaurant grease samples (refer to Sample Grease Material: Sample Preparation) were used in these experiments. Six new, clean, identical 800 mL mason jars were filled with 500 ml of PHS solution (measured using a volumetric flask) and 200 ml of grease (measured using a graduated cylinder. PHS concentrations tested were 0, 100, 250, 500, 1000 and 3500 ppm(v).

Samples were sealed and stored in a laboratory fume hood to minimize agitation or interruption of forming biological structures. For a period of 45 days, the ambient temperature ranged from 18°C to 21°C during which high-resolution photographs were taken at intervals ranging from 1 to 7 days. Using a ruler and white paper background, sample characteristics were identified and measured. The observed liquid turbidity, layer color, and height of interface boundaries: atmosphere/organic, organic/aqueous and aqueous/solid sediment were recorded.

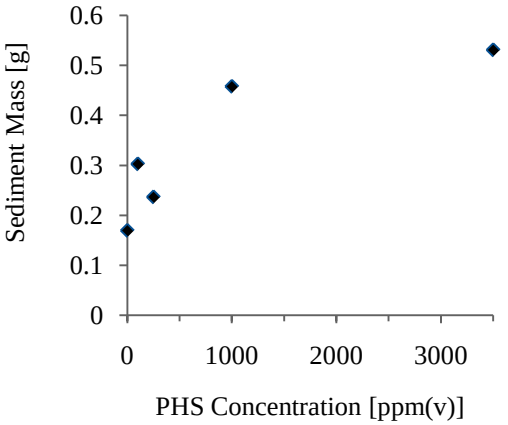
Batch experiments were disassembled for further quantification. Parameters for further quantification included aqueous phase dissolved FOG concentration, solid sediment mass and aqueous phase biological concentration. To access the aqueous phase, the organic sample phase was bypassed to collect sediment and aqueous samples. A double pipette tip-in-tip device was used to access the aqueous phase and a peristaltic pump with hose assembly was used to collect sediment. The sediment was pumped to a suction filtration apparatus using a paper-filtered ceramic Coors™ Büchner funnel in conjunction with a 2 L glass vacuum collection vessel. The dry weight of the wet sediment, deposited on the filter paper, was determined using a Denver Instrument™ IR-

35 moisture analyzer. Liquid assays from each sample were analyzed for FOG content (refer to FOG Quantification) and were preserved for future biological quantification.

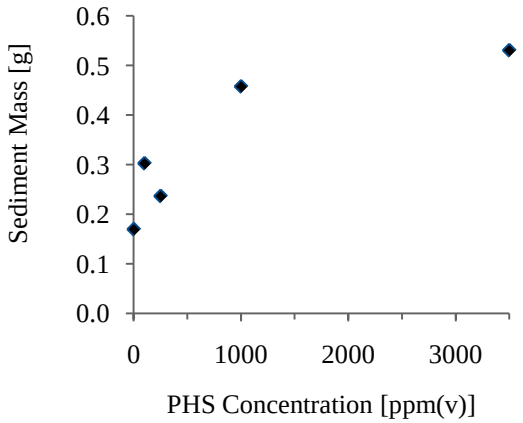
Static batch grease material experiments show the interaction of PHS at varying concentration and grease material at room temperature in a controlled, minimally agitated system. After a period of 40 days of observation the grease material experiments were further quantified for analysis. Figures 15 a-c are plots of the observed height of settled material (sediment) collected, quantified sediment mass and aqueous phase FOG concentration as functions of PHS concentration in the batch experiments.



a) Sediment Height



b) Sediment Mass



c) Aqueous Phase FOG Concentration

Figures 19. a-c. Batch Experiment Results (40 day period).

Sediment height increased with increasing PHS concentration from 1 to 11 mm. Most of the sedimentation occurred at the beginning of the experiment. During the course of the experiment, the aqueous phases of some batches were observed to change in color and opacity over time. Batch experiments containing PHS concentrations equal to and lower than 250 ppm(v) were found to exhibit a cloudy white aqueous phase which did not completely clear over the duration of the experiment. Experiments with PHS concentrations greater than 250 ppm(v) were found to have initially dark opaque aqueous phases that gradually became clear. Upon disassembly, batch experiments were quantified for sediment mass and aqueous phase FOG concentration.

The dry mass of the sediment ranged from 0.170 to 0.531 g. The measurement at a PHS concentration of 500 ppm(v), was removed due to procedural errors in quantification. FOG quantification of the aqueous phase also follows a similar trend as the sediment height and dry mass. The FOG concentration present in the aqueous phase ranges from 280 to 570 mg FOG L^{-1} with the maximum at a PHS concentration of 1000 ppm(v).

Chemical Reduction of H_2S_{aq} Concentration Experiment

Experiments were performed to determine the effect of peat humic substance on aqueous hydrogen sulfide. A 2^2 factorial experimental design was performed in triplicate using aqueous sulfide concentrations in a range of 3 to 7 mg L^{-1}, PHS concentrations of 20 to 380 ppm(v) and ambient laboratory temperature of 18 to 21°C. Sulfide quantification was performed using the method described by Cline.[35] The method utilizes a single crystalline color-forming reagent, *N*,*N*-di-methyl-*p*-phenylenediaminesulfate. The reagent provides applicability to a wide range of aqueous sulfide quantification including stability for quantification of low sulfide concentrations and a simple procedure for standardization. The method was specifically adapted for quanitification of sulfides in concentrations between 0.03 and 32 mg L^{-1} and is free of salt effects and temperature dependence.[16]

Batch experiments were performed in stirred laboratory beakers. A sulfide solution of 50 mg L^{-1} was prepared from solid Na_2S and purified deionized water (pDI). Aqueous sulfide solutions were prepared by dilution of the concentrated sulfide solution with pDI to experimental concentrations at a volume of 50 mL. To each experimental batch of aqueous sulfide solution, a dose of peat humic substance was added via micropipette. Control batches were inoculated with a 0.5 mL of pDI in place of PHS. A dose of acidic diamine reagent was added and the batch was mixed by stir-bar. Allowing 20 minutes for color to develop, 10 mL of each batch was transferred to a centrifuge tube, and centrifuged at 2500 rpm for 3 min. prior to spectrophotometric analysis for minimization of measurement interference due to suspended particles. Samples were subsequently transferred to clean cuvettes and absorbance of light at a wavelength of 670

nm was measured using a Spectronic 21D. Resulting sulfide concentrations were calculated using the calibration curve in Appendix B.

Analysis of the experimental design was completed by combining all data from the triplicate experimental design. All data sets were prepared using the procedure described in the Statistical Methods and Analysis section. The percent sulfide reduction was calculated by comparing the aqueous sulfide concentrations of the control batches to the experimental batches dosed with peat humic substance using Equation 3.

$$\%reduction = \frac{C_{SC} - C_{SE}}{C_{SC}} \quad (3)$$

Where C_{SC} is the concentration of aqueous sulfide in the control experiments and C_{SE} is the concentration of aqueous sulfide in the experimental batches dosed with PHS. A factorial analysis for screening experimental designs was used to determine the significant trends in the data using Statgraphics Centurion.

A 2^2 factorial experimental design was completed in triplicate for aqueous sulfide reduction with peat humic substances. The effects of sulfide concentration and PHS concentration were studied. The reduction of aqueous sulfide ranged from 11% to 47% of the initial concentration. Results indicate that the highest percentage of sulfide reduction occurs at an initial sulfide concentration of 3 mg L^{-1} with a PHS concentration of 380 ppm(v). A pareto analysis of the experimental design indicates that PHS concentration is a significant positive factor for sulfide reduction at the 95% confidence level.

Table 21. Non-biological H_2S_{aq} Concentration Experiment Data

Design Code		$H_2S_{aq\ (nom.)}$	*PHS Conc.*	*Absorbance* $_{670}$				H_2S_{aq}	
		mg L^{-1}	*ppm(v)*	*a1*	*a2*	*a3*	*avg.*	*mg* L^{-1}	*% dec.*
Sulfide	PHS	3	0	0.304	0.319	0.300	0.308	3.11	
		5	0	0.450	0.390	0.398	0.413	4.17	
		7	0	0.744	0.742	0.756	0.747	7.55	
-1	-1	3	20	0.207	0.272	0.257	0.245	2.48	20.3%
-1	1	3	380	0.163	0.193	0.202	0.186	1.88	39.5%
0	0	5	200	0.326	0.335	0.333	0.331	3.35	19.7%
1	-1	7	20	0.622	0.594	0.657	0.624	6.31	16.5%
1	1	7	380	0.583	0.570	0.541	0.565	5.70	24.4%