Biodegradation of Fat, Oil, and Grease (FOG) in Wet Wells

by

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Abstract

Fat, oil, and grease (FOG) in wastewater can cause foul odor, sewer line blockage, and may interfere with sewage treatment. FOG control is approached with physical, chemical, and biological methods Many cities, including Edmonton, Alberta, Canada, have effectively applied commercial biological products to control FOG. Analysis of samples collected from wet wells in Edmonton was undertaken to examine the factors that influence the FOG control performance of commercial biological products. Field sampling showed a seasonal variation of FOG and COD concentrations indicating that the higher temperature in the summer-autumn term compared to the winter-spring term benefited FOG removal. The lowest FOG concentration (49.3 mg/L) was observed when the products were applied with a mixer on in summer-autumn term, which suggests the importance of oxygen and thorough mixing. Based on the results of wet well sample analyses, bench-scale experiments investigated the impacts on FOG removal of product dosage, initial COD, and temperature. Addition of 1000 times the recommended dosage of the commercial products increased FOG removal from 35.5% (achieved at the recommended dosage) to 41.1% in 14 days with an initial COD of 600 mg/L in 14 days. FOG removal increased from 29.8% to 48.0% with an increase in temperature from 15 °C to 32 °C. Suggestions to improve FOG control with commercial biological product application are proposed.

Key words: FOG, biodegradation, commercial product, influencing factors.

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List of Abbreviations

FOG ……..…..Fat, oil, grease

SSOs ……..….Sanitary Sewer Overflows

PS ……..…..Pump Station

COD ……..…….Chemical Oxygen Demand

BOD …….……Biological Oxygen Demand

FFAs ……...Free Fatty Acids

LCFA ……..…….Long Chain Fatty Acid

ICP-MS ……..…..Inductively Coupled Plasma- Mass Spectrometry

IC ……..…Ion Chromatography

GADs ……...…. Grease Abatement Devices

1. Introduction

1.1 Background

1.1.1 Fat, oil, grease (FOG), leading concerns in sewer systems

Wastewaters that are rich in fat, oil, and grease (FOG) are attracting increasing attention as populations grow and industrial activities expand. Effective approaches to FOG control are imperative because problems in sewer systems can affect public health and damage the environment. Fat, oil, and grease are components of layer of lipid-rich material deposited on pipes and wells that carry and store wastewater generated during cooking and food processing (Long et al., 2012). Fat, oil, and grease are composed of triglycerides, esters of glycerol and three fatty acids. Differences in the physical properties (e.g., consistency) of fat, oil, and grease are due to the type and physical status of the fatty acids that comprise the triglycerides.

In addition to the daily production of wastewater from private residences, FOG pollution is generated by the dairy industry (Brooksbank et al., 2006), slaughterhouses (Batstone et al., 2007), food processing plants (Cammarota &Freire, 2006), and other industries that process fatty substances. FOG deposits are endemic in sewer systems (including pipe lines, pump stations, wet wells) and downstream wastewater treatment plants, often causing sewer overflows (SSOs). The United States Environmental Protection Agency (USEPA) estimates that there are 40,000 SSOs/year in the U.S. alone, 40% of which are caused by sewer main blockages; 47% of those blockages are FOG related; and there are 5,000 to 8,000 FOG related SSOs/year (Sorenson, 2009). FOG deposits adhere to the interior walls of pipes, reducing sewer pipe diameters or even blocking pipes completely

(Ashley et al., 2000). The release of sewage during SSOs contributes to odor and water pollution, and exposes the environment to pathogens that are a threat to public health (Bridges, 2003). Without treatment, FOG builds up in wastewater treatment plants downstream, where a lipid coating can form biological flocs, blocking cell-aqueous phase transfer rates of substrates, products, and oxygen (Chao &Yang, 1981). FOG buildup in wastewater can induce sludge bulking (Reddy et al., 2003) which will impair wastewater treatment, reduce regular sedimentation, and cause biomass losses (Perle et al., 1995).

Environment and public health concerns mandate a comprehensive understanding of FOG properties and effects so that FOG control can be implemented in practical applications.

Approaches in FOG control can be categorized as physical/chemical and biological. Chemical hydrolysis of FOG can introduce long fatty acids into wastewaters which might inhibit microbial activities and impact the diversity of microorganisms in wastewater (Hanaki et al., 1981; I. Angelidaki et al., 1992) reducing wastewater treatment efficiency and often producing unpleasant odors. Physical methods take advantage of FOG's low density compared to that of water which allows FOG deposits to be separated from wastewater with grease traps, tilted plates, and dissolved air flotation devices.

Grease traps are wide-spread physical-based FOG pretreatment devices applied in municipal wastewater systems, food industries, and some residences. When FOG polluted wastewater flows into a grease trap, FOG will float to the surface while the water will continue to flow to the wastewater collecting system. FOG layers can be removed manually or by addition of chemicals.

Tilted plates are a modification of the grease trap. Tilted plates are parallel gravity separators that provide high surface area while occupying less than 10% of the volume of a conventional grease trap (Willey, 2001), lending the FOG trapping device increased mobility. In dissolved air flotation, micro-bubbles attach to FOG particles promoting their rise to the top of the water body where they are easily removed. Dissolved air flotation devices require high energy for FOG layer skimming, a disadvantage of this method. Laboratory experiments and computer simulations are applied to design physical methods that will achieve higher FOG removal efficiency.

Physical methods need human assistance to remove the FOG layers that accumulate. The necessity for human resources increases the cost to municipalities which spend millions of dollars each year to implement FOG cleaning and maintain a related infrastructure (Agency, 1979). Furthermore, these techniques are inefficient in reducing dissolved and emulsified fats which can restrict the oxygen transfer rate and thus impairing biological treatment (Chao &Yang, 1981).

Compared to physical/chemical approaches to FOG control, the lower cost, higher FOG removal efficiency, and easier maintenance of biological approaches have increased their popularity. Biological methods degrade FOG or accelerate its hydrolysis using competent bacteria species, lipases, surfactants, and commercial supplements. Research on biological approaches concentrates on isolating competent bacteria species, finding optimum working conditions, refining carrier structure design for FOG removal improvement, combining biological methods with physical processes, and studying the effect of applications in natural conditions. Commercial biological products and

supplements have been designed to enhance FOG removal. Examples of such products have been applied to reduce wastewater FOG in applied and Edmonton, Canada.

1.1.2 Introduction to Edmonton's sewer services

The City of Edmonton is continuously ameliorating its drainage infrastructure to ensure a better public service. The city's sewer system is subject to FOG pollutants in the wastewater collected from residences and industries'. Approximately 200 blockages per year occur in Edmonton's sewers, costing more than $1.2 million annually. City of Edmonton Bylaw No.9675 stipulates rules for the disposal of fats, oils, and grease from commercial, institutional, and domestic sources. The City encourages residents to store fats and grease in a disposable container and take containers with four or more liters of used cooking oil to an Eco Station.

Sewage pump stations are like nodes in the sewer system, they are distrubited over the city, each station collecting wastewater and runoff from its serving area nearby and pumping them out to a downstream wastewater treatment plant via conveyance pipes. As wastewater is stored in pump station until the water level comes to a designated value, FOG deposits have time to form inside the pump station. FOG deposits adhere to the exterior walls of pipes and to the interior walls of the pump stations as well as to the interior walls of the pipes that hold the wastewater. To reduce the human resource requirement for manual removal of FOG, biological products have been applied in several pump stations in the City of Edmonton. Thesis products are described in Section 1.1.3.

1.1.3 Introduction to FOG control products

The City of Edmonton has applied two types of commercial biological products for FOG control in local pump stations: Bio-Brick™ (Genesis Biosciences) and Bio-Block™ (Regent Biologic Inc.). Both products are bacteria-laden solid blocks containing enzymes, multiple microorganisms, surfactants, and microorganism nutrients. When added to wet wells and lift stations, the blocks gradually dissolve over 30–120 days, continuously degrading waste materials. The naturally occurring *Bacillus* species (Brooksbank et al., 2006) is used in both products. Genesis Biosciences' Bio-Brick contains surfactants, enzymes, colorants, and a *Bacillus* spore blend (including *B. amyloliquefaciens*, *B. pumilis*, *B. licheniformis*, *B. megatarium*). Regent Biologic's Bio-Block contains four *Bacillus* strains—*B. subtilis*, *B. polymyxa*, *B. licheniformis*, and *B. megatarium.* Many *Bacillus* species secrete large quantities of amylase and protease enzymes which catalyse the breakdown of starch and protein.

When Regent Biologic's Bio-Block or Genesis Biosciences' Bio-Brick is added to a sewage system, surfactants in the solid block act to disperse FOG deposits, and enzymes contained in the solid block or produced by microorganisms in the block catalyse the breakdown of large molecules in the deposits to form simpler organic compounds such as fatty acids and amino acids; bacteria further cleave the fatty acids to low molecular weight hydrocarbons and finally to carbon dioxide and water. Apart from the *Bacillus* in the products, the dispersion of the FOG particles and the breakdown of large molecules in FOG enable other bacterial species in the wastewater to degrade the smaller organic molecules into carbon dioxide and water. For example, lipases secreted from competent bacteria will accelerate the hydrolysis of molecules from FOG released by the products.

FOG serves as a nutrient and carbon source for bacteria, which can form biofilms to which FOG particles adhere; the biofilms can then be isolated from wastewater with sieves.

1.2 Objectives

Environmental conditions strongly affect the efficiency of FOG biodegradation because the activities of enzymes and bacteria are highly dependent on pH, temperature, and the availability of oxygen (Mobarak-Qamsari et al., 2012). The presence of ions and heavy metals can strongly inhibit the activity and growth of microorganisms and enzymes (Irfan et al., 2014; Murthy et al., 2014). When Regent Biologic's Bio-Block or Genesis Biosciences' Bio-Brick were applied to sewage systems in the City of Edmonton, FOG removal efficiency was found to be very low in certain wet wells and/or under certain environmental conditions. The reasons for the failure in selective cases are unknown, but could be elucidated with a thorough and controlled investigation of well wastewater characteristics. Bioremediation is a complex but effective technology that may need to be tailored to specific wells to provide efficient sanitary system management. Although biological processes used in FOG control are attracting research interests around the world, little has been done to evaluate factors that can affect commercial biological products' effectiveness and the performance of the products in practical applications. The impact of different environmental factors on commercial product performance could be evaluated more accurately in a laboratory setting. With further research, Regent Biologic's Bio-Block and Genesis Bioscience' Bio-Brick systems could be used more economically and effectively remove FOG from a greater number of Edmonton's wet

wells. The optimal working conditions for biological products have not yet been established. This study can narrow the gaps in our knowledge of the potential of commercial biological products to reduce FOG accumulation in wet wells. The objectives of this project are to:

(1) Evaluate factors that affect the FOG removal efficiency of Bio-Block and Bio-Brick products by field sampling;

(2) Find optimal conditions to improve FOG removal efficiency by bench-scale experiments.

2. Literature review

With the increasing interests in FOG control, studies have been conducted on different aspects of FOG control regarding its formation, characteristics, degradation pathways, physical and chemical methods for FOG removal, and biological degradation.

2.1 FOG deposits formation and characteristics

Chemically, fats, oils, and greases are similar. They are triglycerides, a sort of ester formed by combination of glycerol and three fatty acids (shown in *Figure 2.1*).

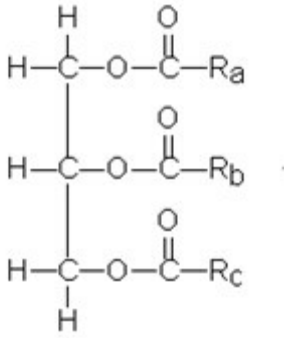


Figure 2.1. Fundamental structure of triglyceride.

Generally, unsaturated fats have relatively lower melting point and are more likely to be liquid while saturated fats have a higher melting point and are more easily to be solidified at room temperature. The glycerides of fatty acids that are liquid at ordinary temperature are called oils; those that are solids are deemed as grease (fats) (McMurry, 1997) .

According to Lissant (1974), FOGs present in wastewater could be categorized based on particle sizes including free, dispersed, emulsified, and dissolved FOGs. For FOG cleaning methods, gravitational separation can be used for free and dispersed FOGs

removal while emulsified and dissolved FOGs require physicochemical and biological treatments for their removal . Despite the fact that FOG deposits is the main reason for sanitary sewer overflows, the mechanisms of FOG deposits formation in sites such as pipe systems and pump stations are not completely clear. To help better understand this issue, researchers have conducted several experiments including FOG formation under laboratory condition, FOG spatial formation and accumulation, influencing factors, and actual FOG deposits analysis.

FOG deposits appear to be adhesive and can be bound to interior pipe walls or internal walls in structures like pump stations. Meanwhile, most of FOG deposits have a grainy, sandstone-like texture and high yield strength when high-pressure jet cleaning is needed for FOG removal (Keener et al., 2008). As FOG deposits is a type of complicated material, they show high variation in physical characteristics such as composition and moisture. Physical characteristics rely hugely on sampling locations, related FOG sources, and even sampling time. For example, in Williams et al. (2012), they found the FOG deposits had a mean moisture content value of 55% with a large range from 15 to 95% though. Such thing can also be found in Keener et al. (2008) with range between 6 and 86%. Sampling locations played an important role in FOG moisture content: generally higher moisture content is easier to be noted in FOG deposits obtained from sewer systems than that from pump stations. A possible reason is the locations differ from each other in environments and sewage characteristics. Also the maturation of the FOG in the network might contribute to the differences. With little impact from sampling locations, the majority (94%) of the FOG solids were found to be volatile among which the extractable oils could make up 15% (Williams et al., 2012). As for metals, the dominant

one is calcium followed by Na, Fe, Al and Mg (Williams et al., 2012). Variations can also be found in characteristics such as yield strength (4 to 34 kPa) and porosity (10 to 24%) (Keener et al., 2008).

In Keener et al. (2008), they proposed three possible categories of FOG deposits based on their formation mechanism. The dominant FOG deposits are classified as metallic salts of fatty acids as observed in 84% of all the FOG deposits samples they collected. Among these samples, layering effects are obvious and distinct indicating an intermittent formation process in practice which can often be seen in restaurants and industries. The second category of FOG deposits is caused by accumulation of lipids from wastes containing highly concentrated lipids. Insignificant metals or minerals can be found among samples of this category which is similar with that of cooking oils. The last and minor category is just mineral deposits without any FOG contents by misidentification.

He et al. (2011) have done a series of experiments regarding FOG deposit formation and its characteristics. In He et al. (2011), they collected grease interceptor effluent from a steakhouse in Cary, NC to provide free fatty acids and used jar test apparatus for a 10 days' run and it was the first documented FOG deposits formation using grease interceptor under laboratory conditions. Compared with FOG deposits samples collected from sewer lines, fatty acids profiles indicated that all of them had similar fatty acids types. The major component of FOG deposits was saturated fat among which palmitic saturated fatty acid was the primary one and Keener et al. (2008)'s work showed a similar analysis results about palmitic being the primary fatty acid in FOG deposits. The observation of palmitic as the primary fatty acids in FOG deposits samples has also been documented in a recent study based on samples collected from different sites in UK

(Williams et al., 2012). According to He et al. (2011)'s comparison among lab-scale FOG deposits product, samples collected from real sites and calcium soap, FOG deposits are likely metallic salts of fatty acid with calcium as the major metal ion and resulted from chemical reaction named saponification. Great property variations in FOG deposits samples including fat content, metals, and saturated fatty acids to unsaturated fatty acids ratios have been reported in recent studies (He et al., 2011; Keener et al., 2008; Williams et al., 2012). He et al. (2011) hypothesized that aggregation between excess calcium or free fatty acids might be another formation mechanism for FOG deposits expect for saponification and they deducted that different FOG sources could have gone through oxidative changes and FOG sources had different concentrations from different samples respectively from the observation that spectral peak intensities for all the samples were quite distinct from each other.

To better analyze impacting factors in FOG deposits formation, researchers took calcium concentration into consideration. Keener et al. (2008) observed higher calcium concentrations in FOG deposits compared with that in wastewater concentration levels. In their study, no correlation between water hardness and high calcium concentrations was noted. From the fact that high concentrations of sulfur and iron (which are usual materials in concrete) were measured in FOG deposits, Keener et al. (2008) and He et al. (2013) proposed that the excess calcium present in FOG deposits might be partly caused by concrete corrosion. He et al. (2013)'s work introduced biogenic concrete corrosion into the formation of FOG deposits through which excess calcium released into water could react with fatty acids and form FOG deposits caused by a charged double layer type compression process. Nevertheless, Williams et al. (2012) observed a correlation between

wastewater hardness and high calcium levels in FOG deposits samples and raised a possible reason: bio calcification. Based on recent literature, He et al. (2013) proposed a relatively complete formation mechanism of FOG deposits which can be seen in *Figure 2.2*.

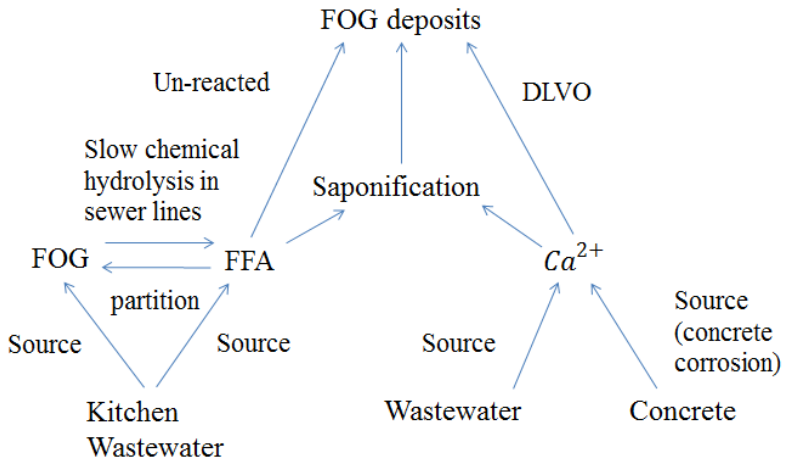


Figure 2.2 Proposed mechanisms of FOG deposits formation in sewer lines adapted from (He et al., 2013)

Generally, there are four contributors in FOG deposits formation: calcium, free fatty acids (FFAs), FOGs, and water. During the formation process, FOGs could be deemed as transporter and a minor source of FFAs in wastewater. Two main sources for FFAs in sewer systems are cooking process and microbial activities in grease interceptors (Canakci, 2007; Monterfrio et al., 2010). Once generated, they would come together with FOGs and stay on wastewater surface. Calcium mainly comes from original wastewater or released by concrete corrosions. Saponification which is the main chemical reaction that FOG deposits be formed could occur at a fast rate at the oil/water or oil/concrete interface with the presence of calcium and FFAs. Other than saponification, the aggregation of excess calcium in wastewater (He et al., 2011; Williams et al., 2012), un-

reacted free fatty acids, and debris in wastewater help the accumulation of FOG deposits on the surface of sewer lines or internal walls in structure (He et al., 2011). Throughout the built up process, the saponified solid act as a core adhered to sewer lines or internal walls with un-reacted FFAs accumulated around it. Due to Van der Waals attraction and electrostatic repulsion, the adhered un-reacted FFAs are able to gather more calcium and other cations towards the solid core matrix. Again, saponification would happen between the un-reacted FFAs and calcium resulting in accumulation of FOG deposits around the solid core matrix. Meanwhile, debris in wastewater could also accumulate and cause the formation of debris layers interspersed with hardened FOG which is consistent with the observation in Keener et al. (2008).

2.2 Methods for FOG removal

In general, physical/chemical and biological methods are most used in FOG control in municipal wastewater. For physical/chemical methods, current researches focus on structure design, technique upgrade for higher FOG loadings, FOG removal improvement and FOG removal estimation. The majority of those studies are laboratory related and computer modeling has been applied sometimes. For biological areas, researchers have been trying to find potential bacteria for FOG degradation, apply combined bacteria species, combine bacteria with enzymes, surfactants, or with physical methods, and optimize operation conditions under lab condition and in practice as well.

2.2.1 Physical/chemical methods

The grease trap method (to achieve floatable FOG separation using gravity), also known as passive and mechanized grease abatement devices (GADs) is the main technique used for separating fat and oil from wastewater (Cammarota &Freire, 2006). Typically, a

grease trap is a rectangular or circular vessel. When FOG containing wastewater passes through the trap under laminar-flow conditions, a proper rate can allow fat, oil and grease inside the water to rise to the surface before they come to the outlet of the trap. After a period of operation, the accumulated FOG layer will be removed manually or mechanically. For its operation, the depth of a typical fat trap is around 1.5m with addition 0.5m added to total liquid depth if accumulation of bottom sludge is considered. *Table 2.1* shows typical surface loading rates applied in practice.

Table 2.1. Typical surface loading rates for different types of water adapted from (Willey, 2001).

Water type	Max surface loading rate ($m^3/m^2/h$)
Margarine wash water	1.5
Acid water	1
Barometric water	3~6

For the aspect of trap design, current design guidelines for grease traps such as Uniform plumbing code (UPC) (IAPMO, 2006) recommend addition of at least one baffle wall configuration to improve separation effects. Contrarily, Aziz et al. (2011) conducted a series of research using experimental results and computational fluid dynamics on alternative inlet, outlet, and baffle wall designs and found that the inclusion of a baffle wall failed to improve oil separation. Moreover, their studies indicated that the high performance of FOG trap might be achieved using shortened inlet pipe, no compartmentalization and flared piping and combination of distributive inlet with a distributive baffle wall (Aziz et al., 2011). Practice suggested FOG removal could fail to meet related regulations easily: high FOG residue within FOG traps get accumulated frequently resulting in manually cleaning up. Furthermore, grease traps are usually

unaesthetic, need more area for construction, and sometimes could cause air pollution around them (Cammarota &Freire, 2006). All these drawbacks require more improvement for grease traps.

As an improvement of traditional grease trap, titled plate separators (TPS) was introduced firstly in petrochemical industry (Willey, 2001). Unlike grease traps, the important factor in the separation process is surface area instead of depth. Tilted plates installed within the vessel can provide many parallel gravity separators resulting lower depth and higher surface area. Consequently, TPS occupy less than 10% of the area of a conventional grease trap (Willey, 2001). Meanwhile, TPS has the advantage of mobility which can bring much more convenience for family and restaurant use (Iggleden, 1978). Several issues thwart the widely application of TPS: readiness to fouling because of the narrow gaps between the plates; long time consumption for plates cleaning; and more strict requirement for pumps and flow control in order to avoid fluctuations and surging. As for FOG layer removal after its formation, directly pouring chemical cleaners has been used in certain practical cases except cleaning manually or mechanically. Nevertheless, it's reported that this process is harmful both for the users and the environment as well (Rashid &Imanaka, 2008).

Dissolved air flotation (DAF) is another important physical process used in FOG control. After the compressed air is introduced into water through nozzles, microbubble clouds can be formed which can attach to the surface of the fat/oil particles resulting in an increase in rise rate (Willey, 2001). To improve the performance of DAF in FOG control, different techniques have applied in the enhancement. Rattanapan et al. (2011) conducted a novel approach using acidification (pH=3) and coagulants (alum, polyaluminum

chloride and ferric chloride) to enhance efficiency of the DAF process. The results turned a notable 80% removal of oil and grease from biodiesel wastewater and a 30% removal in COD (Rattanapan et al., 2011). Le et al. (2012) examined efficiency of microbubble (MB) treatment, microbubble treatment with polyaluminium chloride (PAC) as a coagulant, and MB treatment with cetyltrimethylammonium chloride (CTAC) as a cationic surfactant in the separation of emulsified oil (EO) (1000 mg/L) by flotation. Both the MB treatment with PAC (50 mg/L) and MB treatment with CTAC (0.5 mg/L) showed high EO removal efficiencies of 92% and 89%, respectively (Le et al., 2012). The main concern with DAF process is its operation issues and energy requirement for foam tripping. Although have not been applied in a large scale in practice, some other physical-chemical processes have been evaluated by researchers including microwave irradiation and electrocoagulation (Kuo &Lee, 2009; Tansel &Pascual, 2011; Tir &Moulai-Mostefa, 2008).

In general, physical/chemical processes have been proved to be effective in reducing solidified FOG wastes and FOG layers. Nevertheless, these techniques are prone to fail in reducing dissolved and emulsified fats resulting in reduction of oxygen transfer rates that are important for aerobic biological wastewater treatment downstream (Chao &Yang, 1981). Meanwhile, anaerobic processes can also be affected because of the lipids that can reduce the transport of soluble substrates to the bacterial biomass (Rinzema et al., 1994).

2.2.2 Biological methods

Biological treatment is the process by which targeted wastes are degraded through microbial activities, microbial products like enzymes and so on. With the increasing interests in biological FOG treatment, experiments have been demonstrated on different aspects of biological treatment including FOG degradation pathways, effective strains,

factors that affect treatment efficiency, and operation issues in practice that will be introduced in the following sections.

2.3 Pathway of FOG biodegradation

Pathway of FOG biodegradation is the foundation of biological FOG control processes and provides related theories for further studies and experiments. As a result, the process of how FOG is degraded has been explained by several research groups (Nunn, 1986; Ratledge, 1992). As presented in *Figure 2.3*, once triglycerides are attacked by competent microorganisms using extracellular lipases or phospholipases, free fatty acids will be released and ester bonds within the structures are hydrolyzed (Ratledge, 1992).

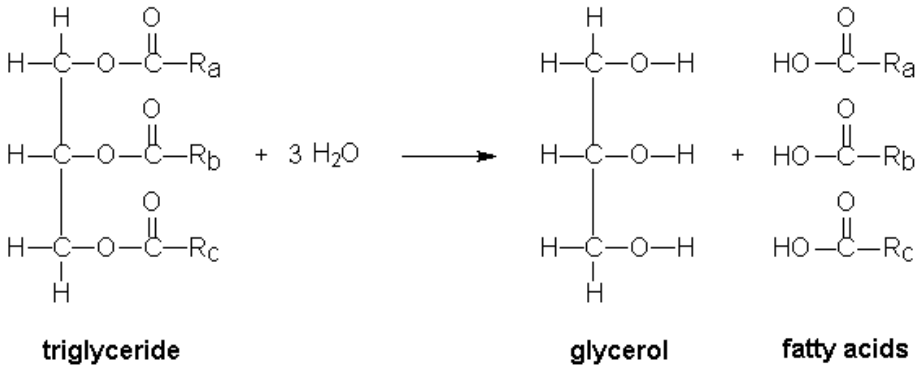


Figure 2.3. Chemical equation for triglyceride hydrolysis (glycerol is formed and fatty acids are released).

Beisson and Tiss (2000) concluded numerous methods for measuring hydrolytic activity and the detection of lipases and suggested that the general triacylglycerol hydrolysis reaction catalyzed by lipases can be expressed in the following format.

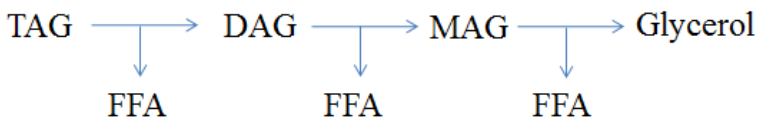


Figure 2.4. General triacylglycerol hydrolysis reaction catalyzed by lipases
Note: (TAG= triacylglycerols, DAG= diacylglycerols, MAG= monoacyglycerols, FFA= free fatty acids)

It's obvious from *Figure 2.4* that for each step in the general triacylglycerol hydrolysis, a free fatty acid will be released and a corresponding type of multi-glycerol will be formed waiting for further hydrolysis which will produce glycerol eventually.

Free fatty acids can be used by a larger group of microorganisms as carbon source. If a microorganism is growing in an environment of fatty acids with the number of carbon atom between C_{14} and C_{18}, including ones with an odd number of carbon atoms, some of the fatty acids can be incorporated into the microorganisms' constituent (Ratledge, 1992). After entering cell body, fatty acids can either be catabolized or directly incorporated into complex lipids for further use. In general, the cyclic β-oxidation is the main process by which fatty acids degradation occurs (Nunn, 1986). The β-oxidation yields a succession of acetyl-CoA units as the fatty acid is progressively shortened by C_2 units. The first step of fatty acid degradation is the activation of the free fatty acid to an acetyl-CoA thioester by acetyl-CoA synthestase (fatty acid: CoA ligase) in which one molecule of adenosine triphosphate (ATP) and CoA per molecule of free fatty acid activated are needed. The next step is called acetyl-CoA dehydrolysis in which acetyl-CoA dehydrogenase is required. Unfortunately, little is known about this sort of enzyme in bacteria (Nunn, 1986). Saturated fatty acids follow the traditional β-oxidation pathway. Nevertheless, the pathway for degradation of unsaturated fatty acids is not determined and two possible

pathways have been proposed: the degradation of unsaturated long chain fatty acids requires complete saturation firstly through which the unsaturated fatty acids could be saturated and ready for further degradation and then followed by the typical β-oxidation pathway (Novak &Carlson, 1970); However, Roy et al. (1986) isolated an anaerobic bligately syntrophic fatty acid degrading acetogenic bacterium (Strain OM) which could ferment all linear saturated fatty acids (C_4 to C_{18}). Meanwhile, they found some mono- and di-unsaturated fatty acids including obleate, elaidate and linolenate could also be oxidized suggesting that β-oxidation of unsaturated fatty acids might occur before saturation. In terms of anaerobic degradation, fatty acids are degraded through β-oxidation pathway to acetate and H_2 and acetate is converted to methane eventually (Long et al., 2012). According to Kim et al. (2004), β-oxidation pathway could be expressed as follow:

$$CH_3(CH_2)_nCOOH + 2H_2O \rightarrow CH_3(CH_2)_{n-2}COOH + CH_3COOH + 2H_2$$

2.4 Analysis of potential species for FOG degradation

As shown in *Table 2.2*, a large number of microorganisms capable of degrading FOG deposits have been identified and may be potential for further application. Markossian et al. (2000) isolated an efficient lipid-degrading thermophilic aerobic bacterium that categorized as *Bacillus* thermoleovorans IHI-91 from an Icelandic hot spring. Being different from regular *Bacillus* species, the optimum temperature for IHI-91 was 65℃. It could secrete high concentration of thermoactive lipases and esterases to degrade a large range of lipids. This isolation have shown the possibility of application of commercial products within a wide temperature range (Markossian et al., 2000).

Mixed microbial cultures have been identified to degrade a variety of oils showing the potential to treat FOG wastewater from different sources (Tano-Debrah et al., 1999; Wakelin &Forster, 1997). Tano-Debrah et al. (1999) developed an inoculum which was a mixed-culture of 15 bacterial isolates from fatty wastewater samples and all of them had demonstrated the ability for FOG (generated from both plant and animal origins) degradation. Despite the fact that the optimum temperature for the inoculum to show FOG removal was 20 to 25℃, they observed the inoculum was active within the temperature range of 8 to 42℃. Wakelin and Forster (1997) compared a range of pure and mixed cultures in degrading vegetable oils, lard and "grease" from a fast-food restaurant grease-trap and found that the removal efficiency depended on FOG materials ranging from 29% for rapeseed oil to 73% for the restaurant grease while activated sludge displayed a relatively more consistent removal in FOG from different sources with the value higher than 90%. Rashid and Imanaka (2008) identified four isolates that belonged to *Bacillus* and found them be able to decrease the suspended solid of the trapped grease from 102 to 40 mg/L and show an extensively removal rate (around 100%) of n-hexane extractable material.

As for application of commercial microbial supplement, the most point is they should not cause a human health hazard or environmental disruption. Additionally, the species should be active in regular conditions, that is to say, the requirement for working condition of these species are reasonable. These criteria limit some potential species for application in commercial products and many of current commercial supplements contain mostly *Bacillus* sp. and closely related bacteria (Brooksbank et al., 2006). Both of the products applied by City of Edmonton, Bio-Brick and Bio-Block contain surfactants,

enzymes, colorants and a *Bacillus* spore blend (including *B. amyloliquefaciens, B. pumilis, B. licheniformis, B. megatarium*). *Figure 2.2* also shows the isolated and identified *Bacillus* that is able to produce lipase and effective in FOG degradation and its fermentation conditions. *Table 2.2* indicates that fermentation of *Bacillus* can occur within a large range of temperature and range of pH value (7.0-9.0) is manageable in practice.

Table 2.2. Potential bacteria for FOG removal
Adapted from (Gupta et al., 2004).

Bacterium/ mixture	pH	Temperature (°C)	Carbon source	Nitrogen source	Reference
Acinetobacter sp.	7	25	Tween-80/ Olive oil	NS	(Barbaro et al., 2001)
Acinetobacter. calcoaceticus	6.8	30	Lactic acid, oleic acid	NS	(Mahler et al., 2000)
Bacillus sp.	7.0	28	Olive oil	Peptone, yeast extract	(Sugihara &Tani, 1991)
*Bacillu*s sp. RSJ 1	9.0	50	Tween-80/ Olive oil	Peptone, yeast extract	(Sharma. R. &Soni, 2002)
Bacillus strain A30-1	9.0	60	Corn oil	Ammonium chloride, yeast extract	(Wang &Srivastava, 1995)
Burkholderia sp.	7.0	45	Glucose, mustard oil	NH_4Cl, $(NH_4)_2HPO_4$	(Rathi et al., 2001)
Pseudomonas sp.	9.0	30	Ground soybean, soluble starch	Corn steep, liquor, $NaNO_3$	(Dong et al., 1999)
Pseudomonas. aeruginosa LP602	7.2	30	Whey, soybean oil, glucose	Ammonium sulfate, yeast extract	(Dharmsthiti &Kuhasuntisuk, 1998)
Pseudomonas. putida 3SK	NS	30	Olive oil	NS	(Lee &Rhee, 1994)
Bacillus sp., *Pseudomonas* sp.	NS	30	Dextrose, triolein	Tryptone, yeast extract	(Lanser &Manthey, 2002)

NS is Not Specified

2.5 Factors that impact FOG removal efficiency

Biodegradation of FOG can be impacted by several factors on which an increasing number of researches have been demonstrated. As one of the main factor, the expression of lipase activity always depends on the presence of carbon and/or a lipid source such as oil or any other inducer. Edible oil in most of countries is based on soybean oil, olive oil, and sunflower oil while mustard oil is more common in countries like India (Chakraborty et al., 2011). Wakelin and Forster (1997) compared the performances of the pure test cultures using different source of FOG substrates including corn, olive, linseed, coconut, rapeseed, FFRG and the biomass yield varied from less than 0.5 g/L to 3.5 g/L. Brooksbank et al. (2006) examined the effect of a microbial supplement on FOG removal using lard, soya, sunflower, rapeseed as FOG sources respectively and found the removal rate varied from 65% to 85% and also suggested that oils must be dispersed for successful microbial growth and biodegradation processes.

Figure 2.4 indicated that the hydrolysis step of FOG would produce glycerol and long chain fatty acids (LCFA) that were either saturate fatty acids with 12 to 14 carbon atoms and unsaturated fatty acids with 18 carbon atoms. Some types of LCFA produced by degradation of glycerol, such as oleic, and linolenic acids, were reported to sustain lipase production from various bacteria and can be toxic to related bacteria (Chakraborty et al., 2011; Ghosh &Saxena, 1996). In natural condition, lipases are generally inducible. During lipases production processes, carbon and nitrogen sources play important roles (Gupta, et al., 2004). According to Gupta et al. (2004), generally organic nitrogen sources were preferred by related bacteria activities.

As for carbon sources, polysaccharides, triacyglycerols, fatty acids, sugars, sugar alcohol and triacylglycerols were beneficial for lipases production. For instance, peptone, yeast extract, and ammonium chloride are suggested for *Bacillus* lipases production (Lanser &Manthey, 2002; Wang &Srivastava, 1995). Mahdi et al. (2012) conducted a series of experiments regarding nitrogen and carbon sources. Mustard oil, coconut oil and maltose were applied as carbon sources and the results suggested that maltose and glucose had an inhibitory effect which was consistent with Eltaweel MA. et al. (2005) results on *Bacillus* sp. strain 42. Shon. et al. (2002) also reported that the addition of organic nitrogen sources such as yeast extract, soytone, and peptone could enhance the removal efficiency of FOGs in their study of FOG degradation using lipase-producing bacterium *Pseudomonas* sp. Strain D2D3. They also conducted a series of experiments regarding oil sources including animal FOG, safflower, fried oil, soybean, and olive oil with the same bacterial strain. Among all the FOG sources, the strain showed the highest removal for olive oil and animal fat (94.5% and 94.4% respectively) and the lowest for safflower oil with the value of 62%. Immanuel G. et al. (2008) indicated the inhibitory and inducible properties of triglycerides on lipase production. Mahdi et al. (2012) examined the effect of inorganic nitrogen sources on lipase production by *Aeromonas* sp. S1 using sodium nitrate, ammonium sulphate and ammonium chloride, respectively. All of them showed inhibitory effect on lipase activity with ammonium chloride as the most inhibiting factor.

Physiological adjustments including pH, temperature, agitation, and incubation period that can affect microorganism activity may impact lipase production consequently. Excessive production of fatty acids can cause drastic decline of pH resulting in inhibition further degradation processes in some cases (Chakraborty et al., 2011). In Mahdi et al.

(2012), the impacts of initial pH and temperature had been examined. The results suggested that lipase production could be affected by pH variation greatly. At pH 8, the maximum enzyme activity was found (195 U mL^{-1}). This was consistent with the results from Immanuel G. et al. (2008). As another important physical factor for bacterial growth and activity, the optimum incubation temperature in this case was 30℃ in which maximum lipase production (195 U mL^{-1}) was observed. Mobarak-Qamsari et al. (2012) studied lipase activity within pH range from 3.0 to 12.0 using *Pseudomonas*. aeruginosa KM110 and found that the maximum enzyme activity was monitored at pH 6.0 and 9.0 and noted that enzyme was not stable at acidic pH condition. In this study, 30 °C and 45 °C were found to help achieve optimum enzyme activity. Jeganathan et al. (2006) observed the optimum pH and temperature for immobilized Candida rugosa lipase were 7.2 and 35 °C that were similar to free lipase. Typically, bacteria favor pH around 7.0 and temperature within 20-45 °C for growth and activity (Mobarak-Qamsari et al., 2012). Lipase production period depends on microbial species from hours to days (Sugihara &Tani, 1991).

2.6 Application of biological treatment processes in FOG control

Generally, biological treatment processes in FOG control can be divided into two categories: aerobic treatment and anaerobic treatment. Brooksbank et al. (2006) investigated the ability of commercial microbial supplements to degrade FOG deposits in bench scale and found that one of the multi-species supplements demonstrated the

capacity of enhancing the degradation of several fats and oils by 37-62% compared with all of the single-species supplements studied.

A drawback of some biological methods was that bacteria could be washed out if not remained properly. To solve this problem and get better FOG removal, Mohamed et al. (2004) tried an immobilization method using a sand biofilm system. The biofilm system was prepared with two species and used to treat vegetable oil and grease from polluted wastewater and it was reported a complete removal of FOG, BOD_5 and COD with 100% when applied two units in sequences. During their study, flow rate through the biofilms and the number of biofilm units applied in treatment sequences were considered as important factors that affected FOG removal percentage and were optimized to achieve the highest FOG removal. Application of a mixture composed of emulsifiers, microorganisms and enzyme for wastewater treatment containing high levels of lipids has also been proved possible (Mendes et al., 2005).

Some commercial available bacterial apparatus for FOG control have been studied as well. Tang et al. (2012) studied the performance of a bio-additive made for the treatment of FOG named Bio-Amp. The main composition of the Bio-Amp unit was pellets loaded by mixed nutrients and five *Pseudomonas* and *Bacillus* strains. A 40% reduction of FOG deposit formation was observed after the treatment indicating less possibility of sewer line blockage. Good nutrients removal in wastewater were also observed: COD, total nitrogen, total phosphorus and total fatty acids were found to be reduced by 39%, 33%, 56%, and 59%, respectively. Considered as a cost-prohibitive method during fat shock loads happening in municipal wastewater, Damasceno et al. (2008) investigated the efficiency of an enzyme pool on an activated sludge system treating dairy wastewater

under fat shock loads and observed a higher COD removal efficiency compared with the control bioreactor. Fat accumulation in the test bioreactor was 3.2 times than the control one. Meanwhile, factors such as turbidity of treated water, recovery time between shock loads all showed positive improvement by the addition of enzyme pool indicating a promising further application of this method.

As for anaerobic treatment, research focuses on pre-hydrolysis and co-digestion. Leal et al. (2006) used an enzyme preparation in two identical upflow anaerobic sludge bed reactors for biological treatment of a synthetic dairy wastewater. Comparison of the two reactors' performance indicated that the hydrolysis step benefited the whole process especially in high oil concentration (1000 mg/L). Jeganathan et al. (2006) evaluated enzyme activity through a 3-day experiment and found that approximately 70% of the enzyme activity remained in the reactor. It reported that the addition of enzyme pool up to 0.5% (w/v) or the rhamnopilid biosurfactant at concentration below 250 mg/L had no inhibitory or toxic effect on the anaerobic microbial consortium (Damasceno et al., 2012). Co-digestion of FOG with municipal biosolids showed a higher removal of FOG (increased by 10-30%), a high COD removal (around 90%) and an increase in gas production (increased by 30-80%) (Long et al., 2012; Rosa et al., 2009).

Although numerous researchers have evaluated effects of different biological treatments for FOG control and have isolated competent bacteria strains that could be used in FOG biodegradation and studied their optimum working conditions, their efficiency in practical application, factors that could impact biological degradation of FOG under real practice conditions have not been fully investigated. Because different sources of limits, most of the experiments conducted by current researchers are under experimental

conditions. Little information regarding real application in municipal wastewater systems could be collected. The application of Bio-Block and Bio-Brick in City of Edmonton is a great opportunity to monitor commercial biological products application in real work. The duration of their application is also useful especially when seasonal factors like temperature, flow rate are considered. The study of their application could provide important analysis and results about biological FOG control in practice. Meanwhile, the combination of field sampling analysis and bench-scale experiments can help make more detailed and complete observations on biological FOG degradation which could be beneficial for further study and practical application. Therefore, the primary objectives can be divided into two parts based on the two-part experiment design. The first part is field sampling and analysis which consists of two seasonal terms based on which influencing factors that could impact the products' performance in FOG removal could be investigated. From the results, some factors that might be of importance could be observed and chosen as focus factors in the second part, bench-scale experiment. In bench-scale experiment, the chosen influencing factors will be studied in order to find optimum conditions for biological FOG removal and make relations with field sampling work to get better FOG removal efficiency.

3. Field sampling and analysis of FOG removal in wet wells

3.1 Introduction

In this chapter, the two products: Bio-Block by Regent Biologic Inc. and Bio-Brick by Genesis Biosciences are tested in practical applications. The City of Edmonton has applied the two products to FOG (fat, oil, grease) deposits in some of the wet wells in pump stations. In Edmonton's case, the problems caused by FOG are mainly unpleasant odors coming from FOG layers and FOG deposits on interior walls and pipe lines inside the wet wells. Like similar biological products, Bio-Block and Bio-Brick are designed to stay inside the wastewater for a period of time (around one month). During the application, the products slowly dissolve and release surfactants, lipase, and functional bacteria. Wastewater keeps entering the wet wells and will be pumped out when it reaches certain water level setting. This dynamic process involves several variables, including flow rate, water temperature, wastewater content, pH, and dissolved oxygen concentration. Some of the factors will exert great impact on biological activities and will thus affect the performance of the FOG removal products. An important function of field sampling work was to monitor these variables to evaluate the impact of Bio-Block and Bio-Brick on the FOG concentration.

The sampling schedule was applied over a long period of time to observe the variation in FOG concentration over the products' active life. Although most parts of the wastewater collecting system in Edmonton are located underground, the water temperature can still be influenced by weather conditions. Field sampling was divided into two sampling terms

--winter-spring sampling term and summer-autumn sampling term according to the weather conditions in Edmonton. Within each sampling term, the products were applied alternately every month, that is, one month Bio-Block was applied and the next month Bio-Brick was applied; each application was done in duplicates to obtain more reliable results. In the summer-autumn sampling term, a mixer was installed inside of one of the sampling sites, PS 155, so that more factors could be involved throughout the sampling work.

The detailed sampling schedule is described in Section 3.2.2. Due to properties of water samples and the requirements of sampling, a patented sampler that could be closed underneath the water was chosen together with two types of sampling bottles made from different materials (plastic and glass). To reduce effect of environment during sampling and during transport to the laboratory, some parameters were tested on site and some pre-treatment were applied to preserve the samples for further tests in the laboratory. From analysis, factors that exerted a significant impact on FOG removal using Bio-Block and Bio-Brick were identified, their impacts were evaluated, and suggestions regarding practical applications of these biological products were made. The second part of this project bench-scale experiments were made possible with the information accumulated by the field sampling and analysis performed in the first part of the project.

3.2 Methodology

3.2.1 Sampling sites

To get a better understanding of the effects of Bio-Block and Bio-Brick applications to wastewater, taking the effects of different locations into consideration, two pump stations

(PS) were chosen as sampling: PS 155 and PS 202. The locations of these two sampling sites can be seen from *Figure 3.1*. Both of the wet wells have good line of sight-straight down access from ground level that allowed samples to be acquired without having to enter the wet wells.

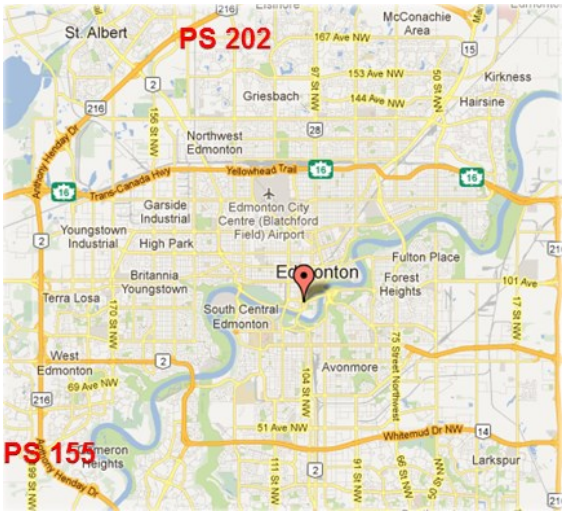


Figure 3.1. Satellite image of locations of two pump stations.

PS 155, named Wedgewood, located at 144 Weaver Drive, Edmonton, services approximately 460 single housing units. Wastewater retention in this wet well (pumps on/off) is approximately 10 min to 1.5 hours depending on the seasons and time of a day. The wet well is non-circular with three pumps submerged under water. PS 202 named Baranow, located at 14550 125 Street, Edmonton, services for approximately 12 services including a school and multi-unit housing. This is a newly developed area that might be further developed in the future. Wastewater retention in this wet well (pumps on/off) is approximately 1 to 3 hours. PS 202 is a noncircular wet well with three pumps submerged under water. Construction data and flow rate information are shown in *Table 3.1*.

Table 3.1. Construction and flow rate information.

Pump station number		PS 155	PS 202
Length (m)		2.115	2.640
Width (m)		2.115	2.640
Height (m)		7.502	8.500
Active volume (L)		4645	4645
Area (m^2)		4.47	6.7
Monthly average flow rate (L/s)	without pump stop time	19.76	113.59
	with pump stop time	9.82	55.38

It's apparent from *Table 3.1* that PS 202 has a much higher flow rate than that of PS 155. A higher flow rate can cause more intensive wastewater turbulence so that the products for which the dissolution rate can be largely affected by water shear can dissolve faster. Nevertheless, a higher flow rate can require the pumps to work more frequently which might reduce the working bacteria concentration inside the wet wells and resulting in a lower FOG removal rate.

The inner conditions of PS 155 and PS 202 are shown in *Figure 3.2*.

(a) (b)

Figure 3.2. Inner condition of PS 155 (a) and PS 202 (b).

A FOG layer can be seen on the wastewater surface inside both of the pump stations shown in *Figure 3.2*. FOG deposits on the interior walls of the wells and the exterior walls of the pipes can also be seen which is more obvious in PS155. PS 202 is deeper underground than PS 155 does, which is the main reason that the water temperature in PS 202 is always higher than the temperature in PS 155. Because of the higher temperature, there is always water vapor in PS 202 during sampling, especially in winter.

3.2.2 Field sampling schedule

The field sampling schedule was drafted based on the weather in Edmonton, rules for product' applications and the working conditions of the target pump stations. Sampling was divided into two sampling terms: winter-spring and summer-autumn. Differences between the two sampling terms included temperature (lower for winter-spring samples than summer-autumn samples) and melt water (higher flow into wastewater collecting system during winter-spring sampling, necessitating more frequent pumping out of the wet wells). A high flow of melt water could dilute the concentrations of functional bacteria, surfactants and lipases as well. The two-term sampling schedule was very necessary to explain FOG and bacteria concentration variations.

Bio-Block and Bio-Brick products are designed to be replaced monthly. During each month, four Bio-Blocks or four Bio-Bricks were applied using webbed pouches submerged in water. Untreated samples without application of Bio-Block and Bio-Brick products were also taken for control measurements. In the winter-spring sampling term, water samples were taken every two weeks and each product was applied for two months to obtain duplicates for each condition.

A mixer was installed in PS 155 before the summer-autumn sampling term began to provide working bacteria with oxygen and help dissolve the products. The working conditions tested and analyzed in PS 155 were (1) no treatment (no products applied), mixer off; (2) no treatment (no products applied), mixer on; (3) product application, mixer off; (4) product application, mixer on. Sampling plan for PS 202 was similar as in winter-spring sampling term.

Table 3.2 and *Table 3.3* depict the field sampling schedule for the two sampling terms.

Table 3.2. Sampling schedule for winter-spring sampling term.

No.	1	2	3	4	5	6	7	8	9
Date	Dec 17	Dec 20	Jan 17	Jan 31	Feb 21	Mar 14	Mar 28	Apr 18	May 2
Condition	Control		Bio-Block	Bio-Block		Control	Bio-Brick	Bio-Brick	

Table 3.3. Sampling schedule for summer-autumn sampling term.

No.		1	2	3	4	5	6	7	8	9	10	11	12	13
Date		Jul 31	Aug 2	Aug 7	Aug 13	Aug 22	Aug 30	Sep 5	Sep 12	Sep 13	Sep 26	Oct 4	Oct 11	Oct 18
Condition	PS 155	Mixer On			Bio-Block				No treatment		Bio-Brick			
					Mixer off		Mixer On				Mixer off		Mixer On	
	PS 202	No Product			Bio-Block						Bio-Brick			

3.2.3 Sampler and sampling method

Sampler

Parameters in a water sample measurements such as dissolved oxygen, COD, nitrite, nitrate, pH, temperature, and FOG concentration can be impacted by the external environment. In this project, the goal was to collect samples that represent in situ water conditions. Therefore, we attempted to minimize changes in water chemistry and other properties. The sampler, the equipment that touches the water sample is a key instrument. Samples were taken just underneath the water surface or between the FOG layer and the water (an area where FOG degradation is prevalent) if there was a thick FOG layer to avoid oxygen contamination from the external environment during the sampling process and to lessen FOG adherence to the interior walls of the sampler after transferring water samples into collectors for further tests.

(a) (b)

Figure 3.3. Snap Sampler by ProHydro. Inc.(a) and sampling bottlers (b): glass bottle (40 mL) on the left; plastic bottle (350 mL) on the right.

The Snap Sampler, a patented (US Pat. 7,178,415) groundwater sampling device (*Figure 3.3*) manufactured by ProHydro, Inc., was used in this project. It applies a double-end-opening bottle. Two lids of each sampling bottle are connected by a spring inside of the sampling bottle that allows the lids to close at the same time so that water samples will not be affected by environmental factors, especially for oxygen. Also, the specially designed lids can seal the samples inside of the sampling bottles with no headspace vapor. To better guarantee water sample quality and avoid FOG adherence, two types of sampling bottles were chosen: a 40 ml VOA glass vial and a 350 ml plastic bottle. The plastic sampling bottles were used to take samples for general water quality tests such as temperature, pH, COD whereas glass sampling bottles were used mainly to do the FOG concentration measurement because FOG is less prone to adhere to glass than plastic.

Sampling method

Before sampling, all sampling bottles were labeled to indicate the location in the water (high position, middle position, and low position) where the sample was to be obtained. During sampling, three linked-in-line Snap Samplers were loaded with one sampling bottle each. The linking mechanism between the two sampler units is shown in *Figure 3.4*.

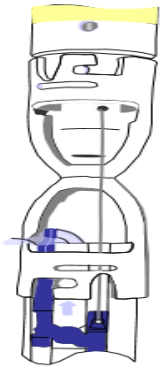


Figure 3.4. Schematic diagram of the connection mechanism of the samplers.

During sampling, the lids remained open until the sampler reached the right position for sampling (the top sampler was placed just underneath water surface or between the wastewater and the FOG layer). All the lids were connected and controlled by a trigger held by the operator. When the sampler reached the designed position, the wastewater was allowed to flow into the sampling bottles for about 0.5 minutes. Then, the operator pulled the control trigger to close the bottles. After ensuring all bottles contained water samples and were sealed, the whole unit was pulled out of the water and the samples was subjected to the pretreatment described in section Water sample pretreatment. To reduce accidental error, three sampling points evenly distributed on the water surface in each pump station were chosen. At each sampling point, two samples were taken: one with three plastic sampling bottles and the other with three glass sampling bottles. All pumps were turned off during sampling to avoid mixing the water and thus destroying FOG layers and introducing oxygen. Furthermore, functioning pumps could possibly impair the plastic samplers.

Water sample pretreatment

Some parameters of interest were measured on site due to their effectiveness for a given period of time and the possibility that they could be impacted after sampling by environmental factors such as temperature. Some parameters could not be measured on site because the equipment required could not be taken to the sampling site or the measurements required time and experimental steps that could not be accommodated at sampling site. Hence, some water sample pretreatments were carried out before the samples were taken to the laboratory.

Temperature and dissolved oxygen measurements were taken on site; care was taken to limit the introduction of oxygen into the bottles when the probe or thermometer was inserted. The shorter the time taken to measure a parameter, the lower the chance of oxygen contamination.

Samples taken with glass sampling bottles were stored directly as they were used for FOG concentration measurement. Temperature and dissolved oxygen were measured for as soon as the samples were taken. Before FOG extraction, all water samples should be mixed together to present the general condition inside the pump station.

Levels of COD, nitrite, nitrate, and ammonium were measured in wastewater samples taken with plastic sampling bottles, and other tests such as IC and ICP-MS were performed. To reduce or restrict the effect of bacterial metabolism inside wastewater samples, the following pretreatment was applied on site. After the sample was mixed, a volume of around 12 mL was filtrated by syringe using a 0.45μm filter to remove bacteria and particles larger than 0.45μm. Filtrated water samples were stored in sealed 15 mL plastic centrifuge tubes, again taking care to reduce contamination with air or other sources, and transported to the laboratory for further testing.

Pretreatment was not applied to water samples that would be used for DNA extraction or pH measurement. To retard biological metabolism, samples were surrounded by ice bags in a cooler until they arrived at the laboratory. In the laboratory samples were stored at 4 °C until testing. All tests were completed within a week after sampling and water chemistry measurements were performed as soon as possible. According to the U.S. Environmental Protection Agency (EPA) Standard methods 4500, nitrite and nitrate

testing should be completed in 48 hours, as nitrite is oxidized to nitrate resulting in variations in their concentrations. Thus, nitrite and nitrate were measured on the day that samples were collected. COD, ammonium, and pH were also measured on the day of collection to reduce the chance of contamination.

3.2.4 Parameters and measurements

All parameters were measured using standard methods, using an EPA approved measurement kit or methods adapted from the literature, as described in sub-sections Temperature to FOG.

Temperature

Sample temperatures were measured on site by inserting a thermometer into the sampling bottles (plastic only) down to the middle position in the water body. After the reading became stable, the data shown on the display was recorded. When reading data, the thermometer was held vertically under water with the graduate lines on the thermometer horizontal. Temperatures at three sampling points were measured at each pump station. The average value of three temperature values was deemed to be the water temperature in that wet well. The temperature was taken right after samples were removed from the water to avoid introducing oxygen into the sampling bottles.

pH

Wastewater pH was measured in the laboratory using a pH meter (B40PCID, SympHony) calibrated weekly with three standard buffers at pH 4, 7, and 10. The wastewater sample was transferred from the sampling bottle to a beaker containing a magnetic stirrer. When the pH probe inserted in the middle of the stirred water sample reached a stable reading

(~0.5 minute) at room temperature the wastewater sample was discarded due to possible contamination from the probe or the open air.

Chemical oxygen demand (COD)

The COD levels of pretreated wastewater samples (filtered using a 0.45 μm filter) were measured with Hach COD Kit (Product # 2125815). Low range (3 to 150 mg/) COD levels were determined with the Reactor Digestion method approved by U.S. EPA for wastewater analysis using Hach Method 8000. After a trial to evaluate COD levels in the wastewater samples, a 20 times dilution rate was chosen. For each measurement, 1.8 mL deionized (DI) water (provided by Hach) was pipetted into the digestion vial before adding 0.2 mL of wastewater sample. A blank measurement was prepared and digested with each sample group by adding 2 mL DI water into a new digestion vial. Digestion vials containing DI water (blank) or samples were mixed well and heated in a COD reactor (Bioscience, Inc., USA) at 150 °C for 2 hours. Each vial was inverted several times while it was still warm and placed in a tube rack to cool to room temperature (~ 1 h). The COD reading was taken with DR 3900 Benchtop Spectrophotometer from Hach. The blank was used to zero the spectrophotometer before sample readings were taken using a preset program in the spectrophotometer (430 COD LR). Vials surfaces were wiped before reading to reduce reading errors.

Ammonium

Ammonium levels in pretreated wastewater samples (filtered using 0.45 μm filter) were measured suing Hach Ammonia Salicylate Method 10205 (approved by the U.S. EPA) with a Hach TNTplus 832 vial with a measurement range of 2 to 47 mg/L NH_3-N. As the wastewater samples were within recommended sample pH range (4 to 8), no further

pretreatment of the samples was needed and no dilutions were made. Sample (0.2 mL) was pipetted into the vial. Flipped the zip with reagent on it over so that the reagent side faced the vial. Vials were capped and shaken 2 to 3 times to dissolve the reagent in the cap. After waiting for 15 minutes, the sample was inverted 2 to 3 times to mix the components completely. A DR 3900 Benchtop Spectrophotometer was used for the ammonium level reading.

Metal ions

Metal ions in the wastewater samples (filtered using .45 μm filters) were measured by inductively coupled plasma mass spectrometry (ICP-MS) using a Perkin Elmer Sciex Elan 9000. Sample pretreatment involved a 50 times dilution using 1% nitric acid (prepared with concentrated nitric acid of trace metal grade). The final dilution was determined by weight (50 g) instead of volume. After preparation, all the samples were transferred to test tubes specially made for the ICP-MS test. A multi-element standard and an internal standard for calibration and a stock solution for sampler rinsing were also transferred to ICP-MS test tubes. Argon was carrier gas. Wastewater samples with lower metal concentration were measured first to reduce metal contamination of the probe. Around 11 minutes was required to calculate the results for each sample.

Anions

Concentrations of wastewater anions—fluoride, chloride, nitrite, sulfate, bromide, nitrate, and phosphate—were measured by ion chromatography (IC). A 100 times dilution for the samples using ultrapure water was required. Diluted samples were filtered using a 0.2 μm filter, transferred to specific IC tubes, and stored at 4 °C until anion measurements were performed. As nitrite and nitrate were included in this test, the samples were not stored

for longer than 48 h. Seven anion solutions (fluoride, chloride, nitrite, sulfate, bromide, nitrate, and phosphate) at different dilution rates (1×, 2×, 5×, 10×, 20×) were used for system calibration. A blank of ultrapure water for sampler rinse was placed after every 7 samples. Each sample required around 4 minutes for anion quantitation and the anion concentration was based on chromatography.

FOG

Pretreatment like filtration can cause some FOG loss, therefore, no pretreatment was applied to the samples that were to be tested for FOG concentration. The samples were mixed before FOG measurement. The partition gravimetric method was used to measure FOG in this project using n-hexane as an extraction liquid in the method described by Greenberg et al. (1992) with some adjustment based on this specific case. A number of labeled flasks were weighed before the extraction for further use. 50 mL of the sample was transferred into a 250 mL separating funnel and acidified to pH 2.0 using hydrochloride acid. Oil and grease content was extracted three times with a 1:1 volume ratio of n-hexane. Each time, the extract was poured into the pre-weighed flasks. When all the extractions were completed, the flasks were placed in the fume hood to evaporate the n-hexane to a constant weight within a reasonable time (usually 2 to 3 days). The final flasks were weighed after evaporation and the weight difference was considered to result from the oil and grease content inside the 50 mL sample. As storage at 4 °C could slow down metabolism to some extent, FOG measurements were performed on the same day as sampling. To aid in quality assurance, all the samples were analyzed in triplicate.

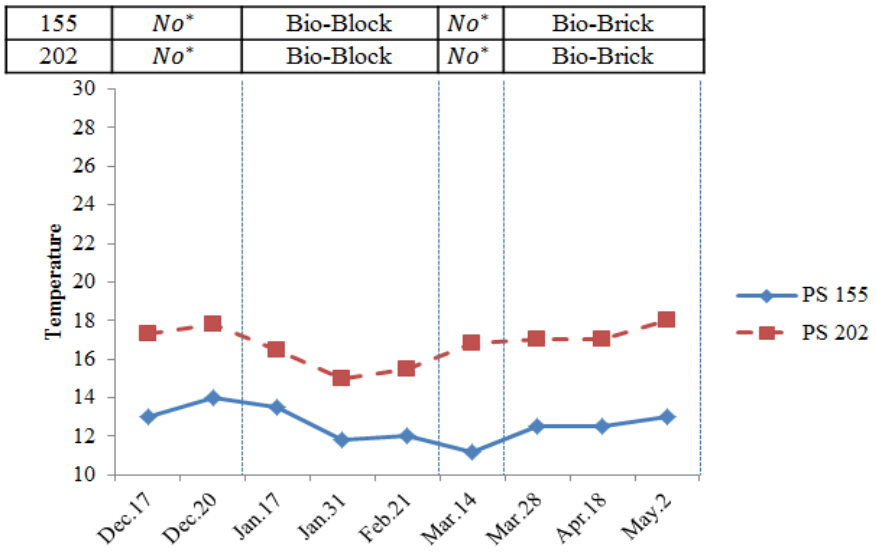
3.2.5 Statistical analysis

To get reliable results and analysis conclusions, triplicated measurements were performed and evaluated. During the statistical analysis process, Student's T-Test was applied when comparing significance in two data groups (differences were deemed as "significant" when $P<0.05$).

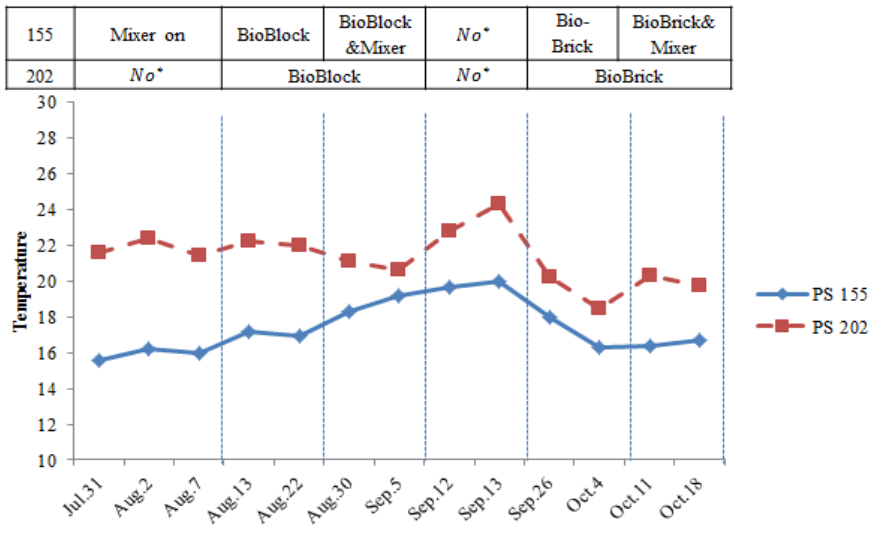
3.3 Results and analysis

3.3.1 Temperature

The temperature data for samples from each pump station throughout the two sampling terms are shown in *Figure 3.5*.



(a)



(b)

Figure 3.5. Sample temperature during the winter-spring sampling term (a) and the summer-autumn sampling term (b).

*Note: No** means no product was applied.

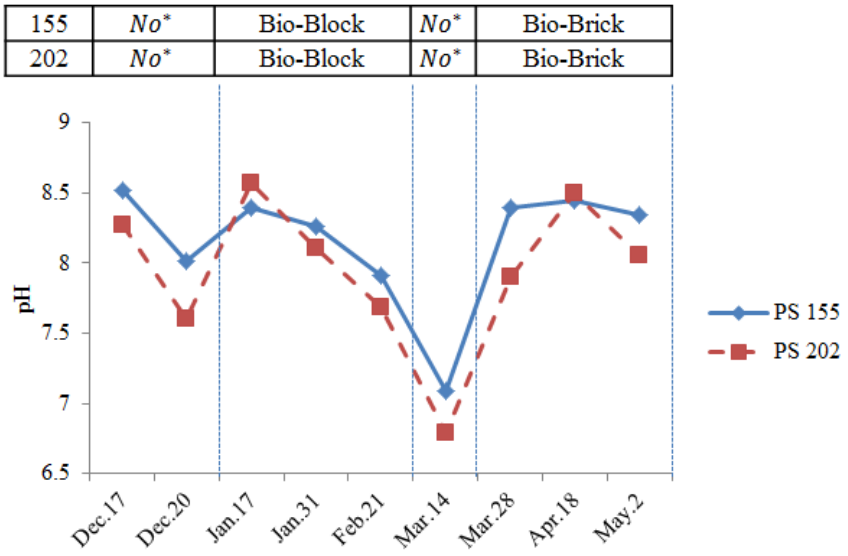
For most part of sewage collecting systems and wet wells are constructed underground, so there was a big difference, particularly during the winter-spring sampling term (Figure 9a) between the water temperature and environmental temperature. According to the Edmonton historical weather report from the Weather Network, the average environmental temperature of the sampling dates in winter-spring sampling term was -5.38 °C which was much lower than what was seen in the figure. On the contrary, a difference between the average environmental temperature (14.47 °C) and the average temperature of the water samples during sampling dates in the spring-summer sampling term was not that obvious. As discussed in section 3.2.1, the depth of the two wet wells played an important role in maintaining a temperature different from environment and a relatively stable temperature. The lower the position of water inside the wet well, the smaller the daily temperature change in the well. That is, the temperature variation in the wet well was not as large as the temperature variation in the air.

The effect of the depth of the wet wells could be observed by comparing data from two pump stations on the same day. The water temperature in PS 202 with depth of 8.5 m was always higher than the water temperature in PS 155 with a depth of 7.5 m in both sampling terms. The average temperature difference between PS 155 and PS 202 was around 4 °C. Considering that all the samples were taken at the same time each day, the impact of the water source on the water temperature could be ignored. However, there was still a temperature variation in each sampling term. Furthermore, the temperature trends in the pump stations were similar and were consistent with the historical temperature record. Apart from this, the temperature showed a seasonal change if data from the two sampling terms were compared. The water temperature in both wet wells in

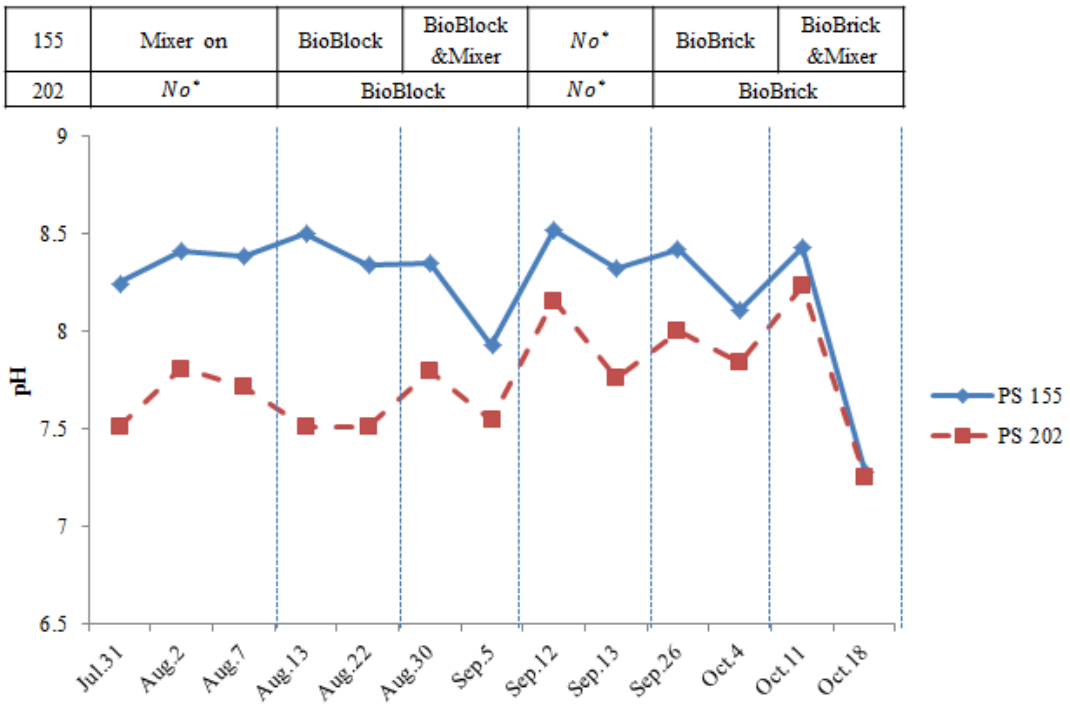
the summer-autumn sampling term were on average 5 °C higher than that of the winter-spring sampling term. As temperature is one of the major factors that can influence metabolism, bacterial activity, and related lipases activity, FOG biological removal efficiency would be expected to be higher during the summer-autumn sampling term with higher water temperature and that was the observed result. According to the reports in the literature, the temperature was still lower than the optimum temperature range for *Bacillus*. Another thing to note is that the temperature did not show change significantly when a mixer was applied inside PS 155, possibly because (1) water came in and was pumped out periodically; this could reduce the heat generated when the mixer was on and (2) because of the large water body surrounding the mixer, the heat generated by mixing was not sufficient to increase the water temperature.

3.3.2 pH

The pH data for each pump station throughout the two sampling terms are shown in *Figure 3.6*.



(a)



(b)

Figure 3.6. pH of each pump station during the winter-spring sampling term (a) and the summer-autumn sampling term (b).

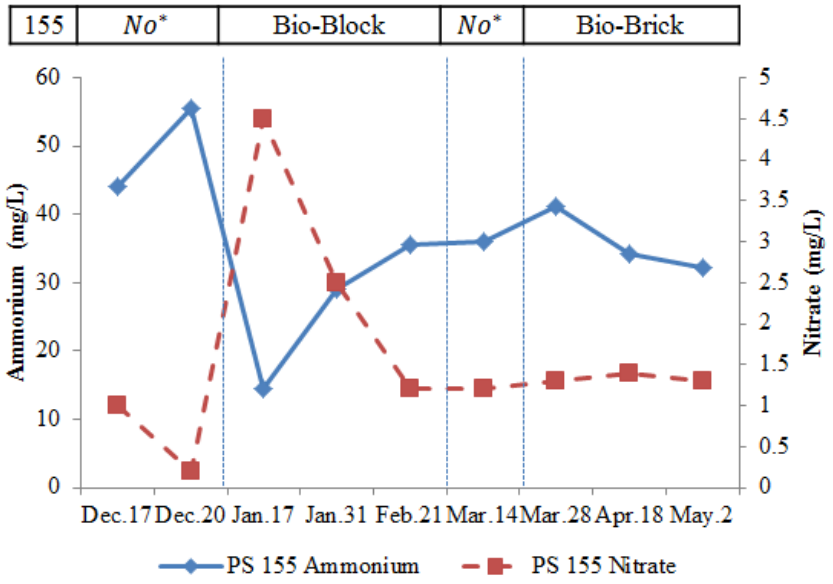
*Note: No** means no product was applied.

The pH of PS 155 was a little bit higher than that of PS 202 in the winter-spring sampling term. Change trends in both pump stations were similar, especially in the highest and lowest pH values. Two obvious pH drops could be identified in *Figure 3.6*: December 17 to December 20; January 17 to March 14. A tiny pH drop can be observed when Bio-Brick was applied. In the summer-autumn sampling term, the pH of PS 155 was higher than the pH of PS 202 and the difference in each sampling point is relatively higher compared to that in the winter-spring sampling term. Furthermore, pH values during the summer-autumn sampling term for each pump station were much more stable, fluctuating in a narrow range, and finally came close to each other. An obvious pH drop at the end of this sampling term was observed when Bio-Brick was applied. No evident change in average pH between the two sampling terms for each pump station was observed: the average pH values for PS 155 are 8.15 and 8.25 for winter-spring and summer-autumn sampling term respectively and the average values for PS 202 are 7.94 and 7.14 for winter-spring and summer-autumn sampling term respectively.

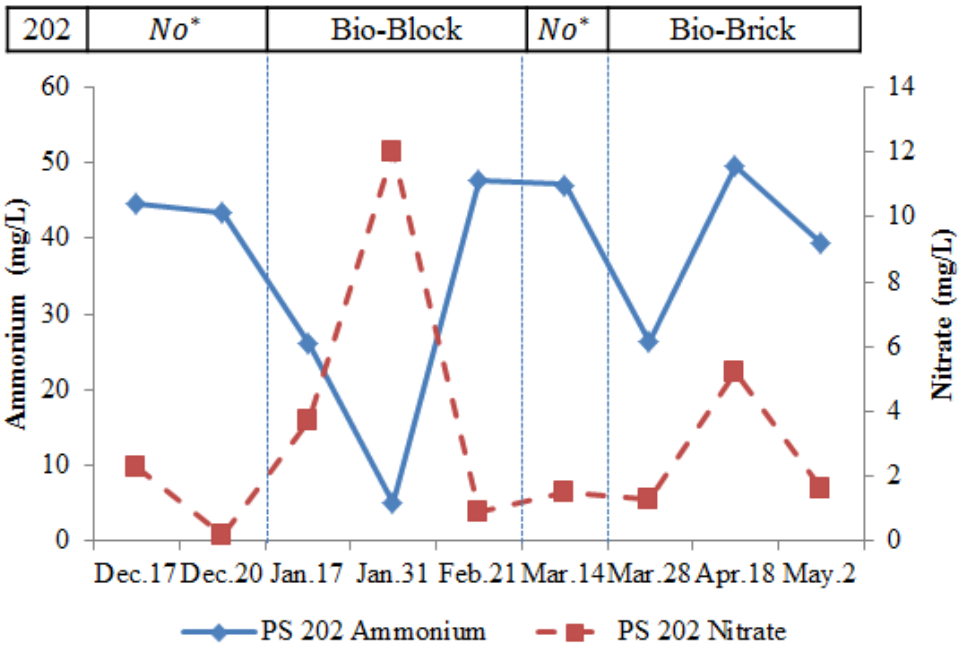
pH value can be related to many factors inside the water, including water source and biological activity. Hydrolysis of FOG components might release fatty acids into the wastewater resulting in a pH drop. This might contribute to the pH drops observed in this study. Overall, pH values indicate that the wastewater was alkalescent during the majority of this study.

3.3.3 Ammonium and nitrate

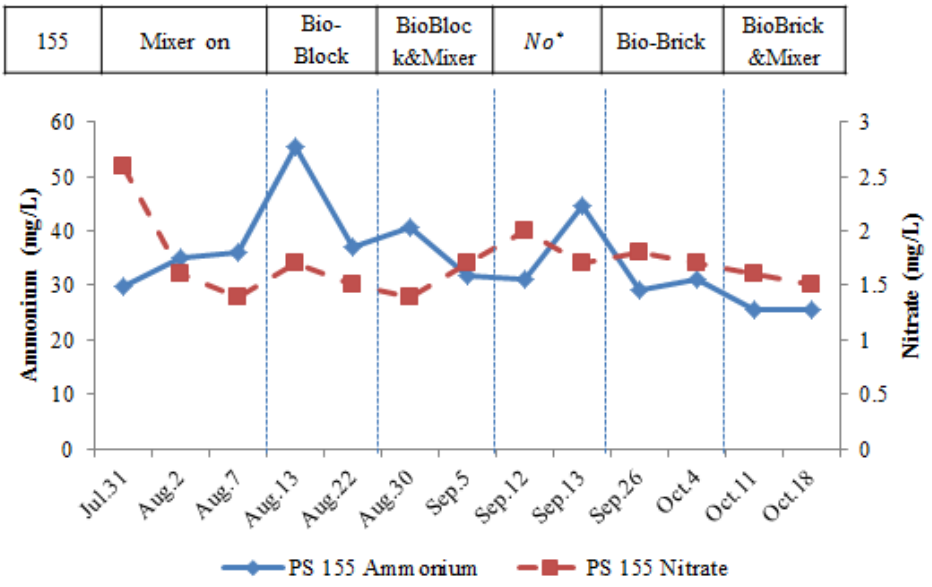
Ammonium and nitrate level in each pump station during the two sampling terms are shown in *Figure 3.7*.



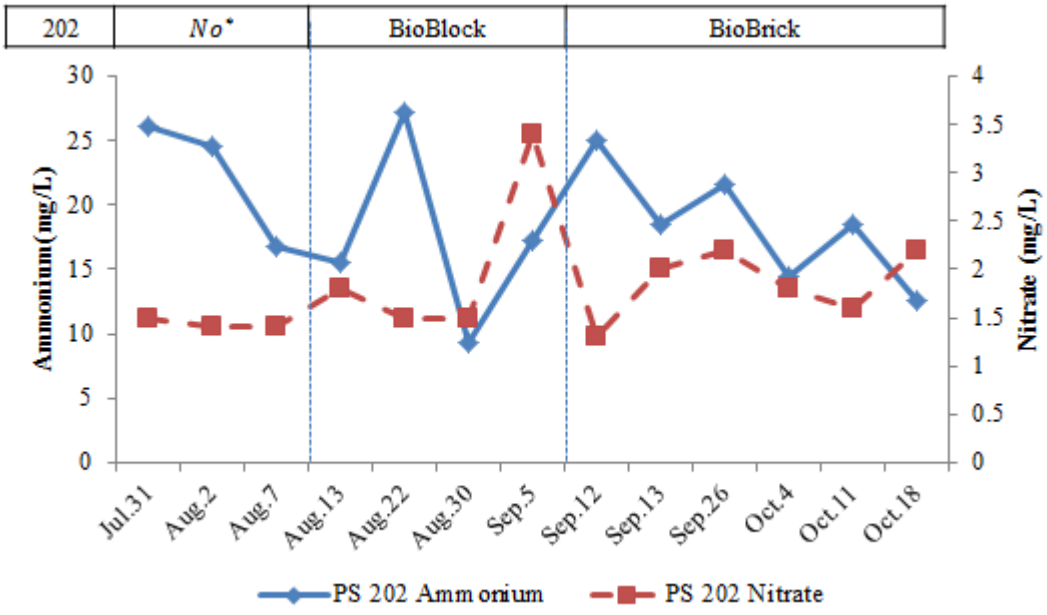
(a)



(b)



(c)



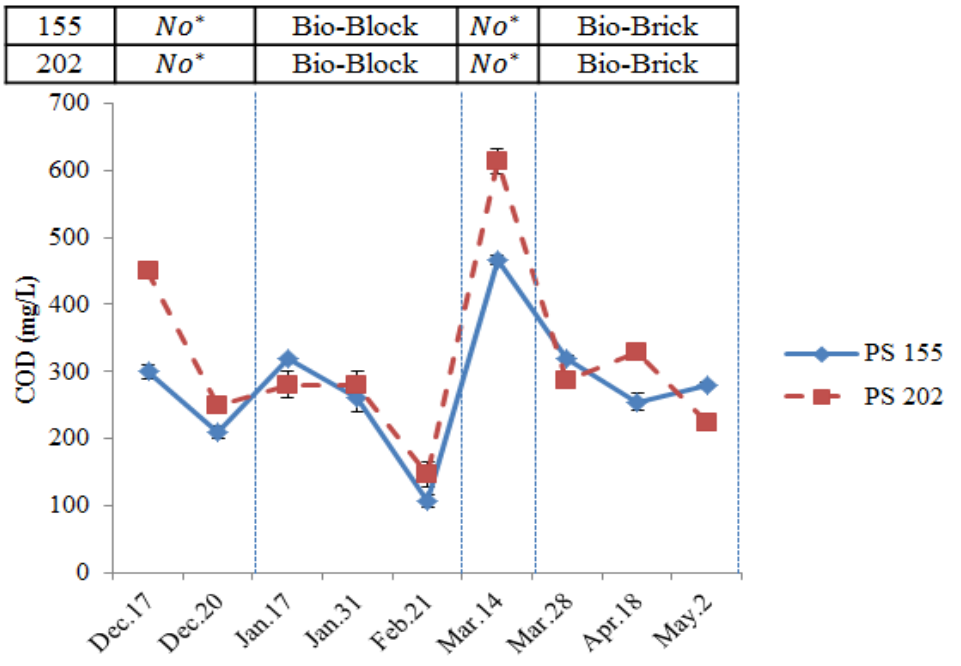
(d)

Figure 3.7. Ammonium and nitrate of each station during each sampling term.
Note: (a) and (b): ammonium and nitrate in winter-spring sampling term for PS 155 and PS 202, respectively; (c) and (d): ammonium and nitrate in summer-autumn sampling term for PS 155 and PS 202. *No** means no product was applied.

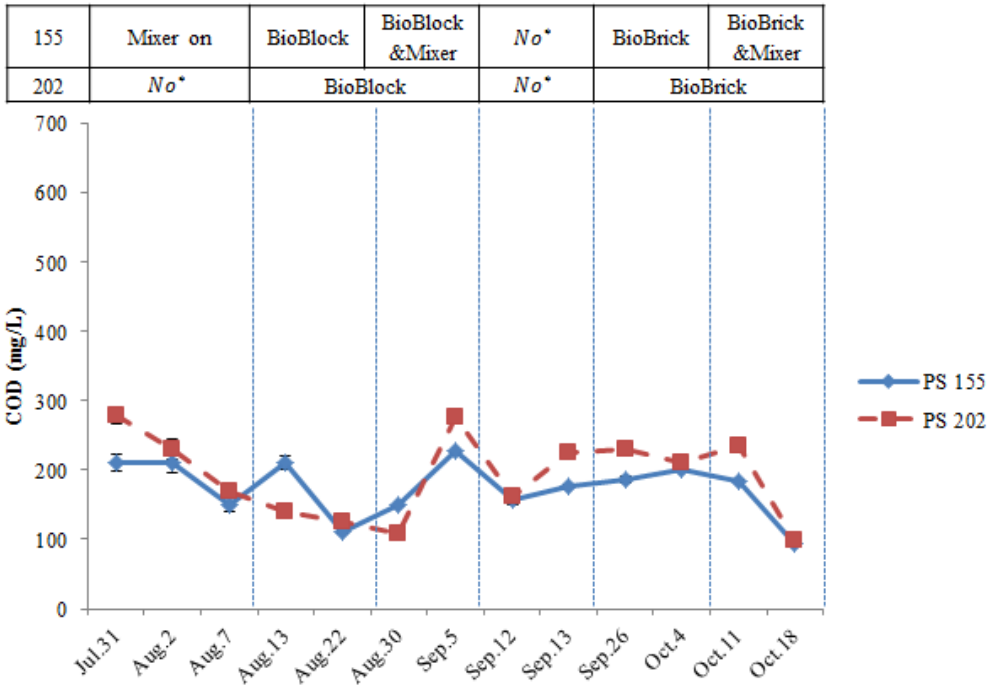
Inorganic nitrogen exists in wastewater mainly in three forms: ammonium, nitrite, and nitrate; nitrite being easily oxidized to nitrate is thus less stable than ammonium and nitrate. Both sampling terms showed fluctuations in ammonium and nitrate concentrations, with more vigorous ammonium and nitrate fluctuations occurring during the winter-spring sampling term than the summer-autumn sampling term. The ammonium and nitrate concentrations in both wet wells were in the range of their reported concentrations in the municipal systems. Nitrate could serve as an electron acceptor which would help the FOG degradation process. Nevertheless, the results showed that nitrate concentrations in the wastewater samples of both pump stations were very low (less than 3 mg/L). This could contribute to the low FOG removal efficiency observed in some of product applications.

3.3.4 COD

As a major wastewater parameter, COD levels are indicative of water quality and biological processes. The COD in each pump station during both sampling terms is shown in *Figure 3.8*.



(a)



(b)

Figure 3.8. COD in each pump station during the winter-spring sampling term (a) and the summer-autumn sampling term (b).

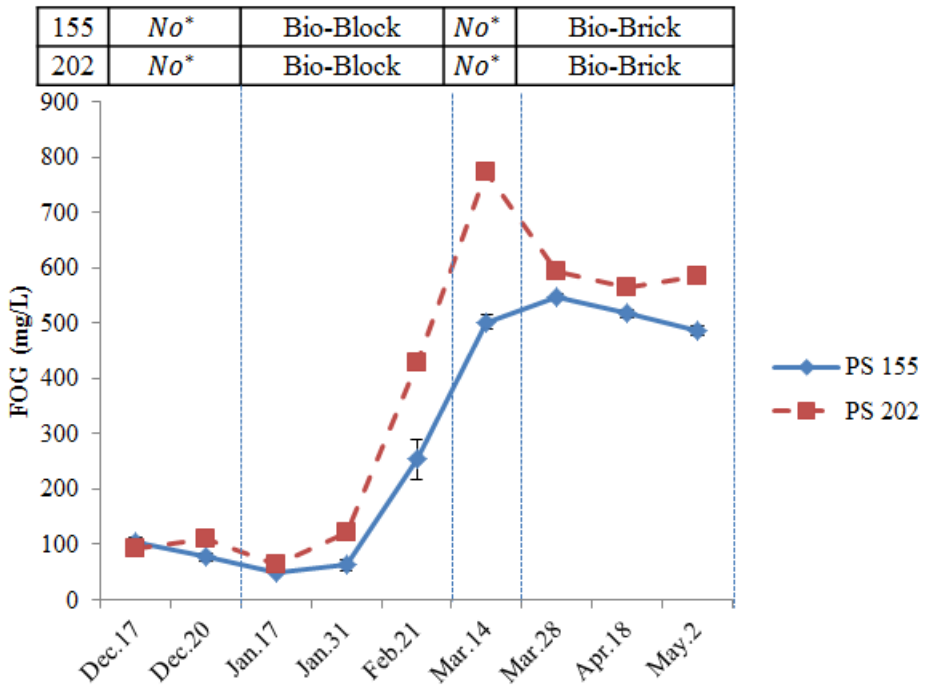
*Note: No** means no product was applied.

It is notable that COD change trends of PS 155 and PS 202 were generally similar in both sampling terms. At all times the COD was higher in PS 202 than in PS 155 (279.7 mg/L and 317.63 mg/L for PS 155 and PS 202 in winter-spring sampling term, respectively; 177.7 mg/L and 190.89 mg/L for PS 155 and PS 202 in summer-autumn sampling term, respectively). The difference in PS 155 and PS 202 average COD values was more obvious in the winter-spring sampling term which was similar to other analyzed parameters. Meanwhile, COD showed huge fluctuations during each sampling term in both pump stations. Although both sampling terms displayed COD fluctuations, the COD varied more significantly in the winter-spring sampling term than in the summer-autumn sampling term. Possible influencing factors included an unstable wastewater flow rate, biological activities in the wastewater, quality of the wastewater source. In the winter-spring sampling term, two obvious COD drops could be identified: January 17 to February 21 and March 14 to May 2 during which Bio-Block and Bio-Brick were applied. Surfactants in the products dissolved in the wastewater helping to break up FOG into little drops that could be more easily biodegraded. Meanwhile, functional bacteria contained in the products could degrade hydrolyzed FOG (in the forms of fatty acids). Fatty acids could also be hydrolyzed by extant bacteria in the wastewater. FOG degradation was assisted by Bio-Block and Bio-Brick resulting in a drop in COD. When Bio-Block was applied in the winter-spring sampling term, the COD dropped more significantly than when Bio-Brick was applied during that term. It can therefore be deduced that Bio-Block decreases COD level more efficiently than Bio-Brick in cooler weather.

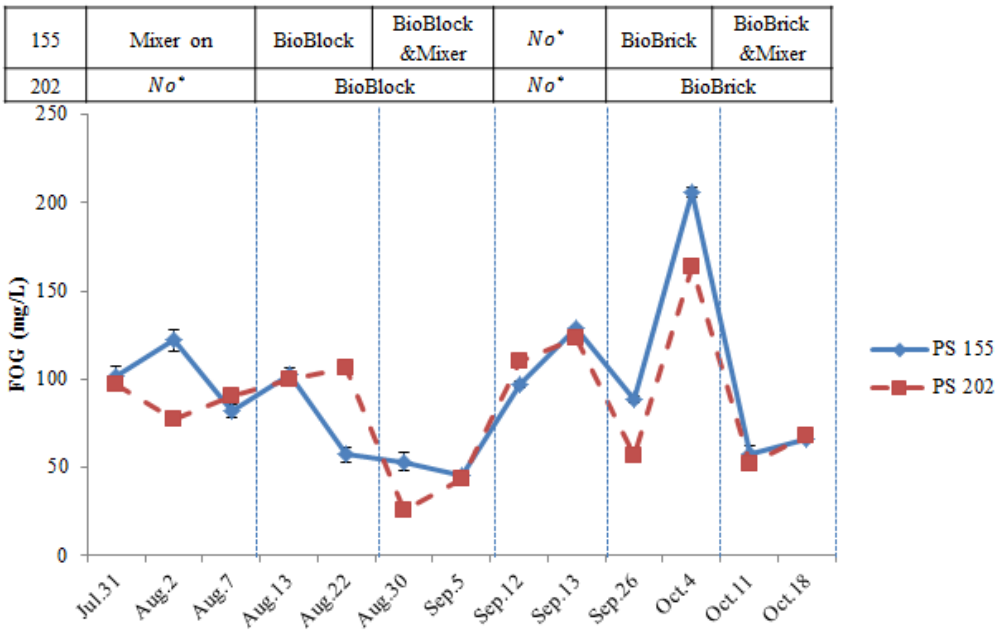
Furthermore, it seems that the effect of Bio-Block could last longer than that of Bio-Brick. From April 18 to May 2, the COD stabilized in the Bio-Brick-treated wet well instead of decreasing, indicating that Bio-Brick was not as effective as when it was initially applied. During the summer-autumn sampling term, COD drop trends can also be found when two products were applied. Although COD dropped generally during product application, it continued to fluctuate. When the mixer was turned on there was an increase in COD when Bio-Block was applied. The winter-spring sampling term generally has a higher COD level than the summer-autumn sampling term. Temperature might have played an important role in the COD level. The average temperature in the winter-spring sampling term is around 5 °C lower than that in the summer-autumn sampling term (section 3.3.1), thus, bacterial growth and metabolism would be expected to be slower in the winter-spring sampling term. Meanwhile, low temperature might limit the activity of fat hydrolyzing enzymes which are helpful in COD reduction. Temperature will impact bacteria both contained in wastewater and the Bio-Block and Bio-Brick products.

3.3.5 FOG concentration

Bio-Block and Bio-Brick performance was mainly evaluated by the FOG levels shown in *Figure 3.9*.



(a)



(b)

Figure 3.9. FOG levels in each pump station during the winter-spring sampling term (a) and the summer-autumn sampling term (b).

Note: No^* means no product was applied.

Fluctuations were observed in both sampling terms and in the winter-spring sampling term, changes in FOG levels in the two pump stations were similar. There was an identical low FOG level from December 17 to January 31 after which the FOG concentration increased quickly reaching summits on March 14 (PS 155) and March 28 (PS 202) in the winter-spring sampling term. During the time Bio-Brick was applied, FOG concentration decreased slowly. The low FOG concentration at the beginning reflected a manual clean-up of the FOG layer before the sampling term began. Manual cleaning might cut a main FOG source and stop solidified FOG from dissolving and hydrolyzing. After cleaning, the FOG concentration increased in both pump stations even though Bio-Block was applied. When Bio-Brick was applied, the FOG began to decrease slowly. It is possible that the rate of FOG increase in the wastewater was faster than the rate of FOG biodegradation by the products. Thus, a higher dosage of product might help in the winter-spring sampling term. More fluctuation was observed during the summer-autumn sampling term than in the winter-spring term. The trends were similar but differences exist in some of the points. In PS 202, although some fluctuations existed, the general trend could be described as follow: FOG was at a relatively stable level and then dropped when Bio-Brick was applied; once Bio-Brick (what left in the net pouches) was removed, FOG began to accumulate and stabilized at a low concentration till Bio-Brick was applied.

In PS 155, when a mixer was installed, the working condition became more complicated. When no products were applied and there was only a mixer working, the FOG stayed at a relatively high concentration till Bio-Block was introduced in the wet well. After turning on the mixer, the FOG concentration decreased to its lowest level. During the time no

treatment was performed (September 12 - September 13: no products and no mixer), the FOG concentration began to increase again even during the first two weeks when Bio-Brick was applied. Similar to the FOG level behavior when Bio-Block was applied, the FOG level decreased to a low level as Bio-Brick and the mixer worked together. Each product application appeared to be facilitated by the mixer.

3.3.6 Metal ion results

The ICP-MS analysis presented in *Table 3.4* provides information about metal ions present in the water samples.

Table 3.4. ICP-MS results.

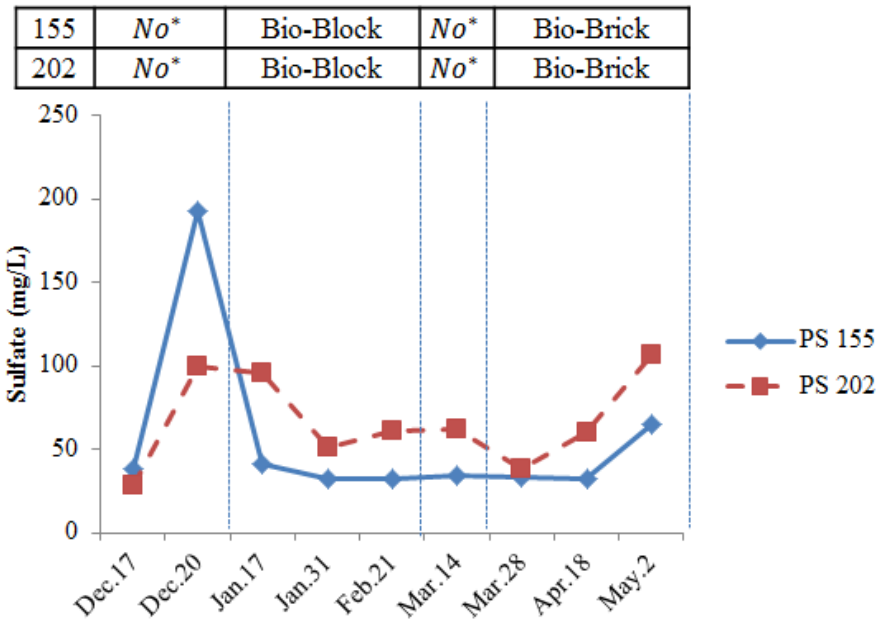
PS number	PS 155		PS 202	
Term	Winter-spring	Summer-autumn	Winter-spring	Summer-autumn
B (mg/L)	0.0774	0.2174	0.0908	0.0876
Na (mg/L)	54.5338	56.8747	71.0379	59.0971
Mg (mg/L)	14.1464	16.8064	21.1332	25.8188
Si (mg/L)	2.8467	3.8281	3.3853	4.0132
P (mg/L)	3.0664	3.4242	3.3853	2.5032
K (mg/L)	14.5173	16.9485	22.1693	14.6146
Ca (mg/L)	80.1027	114.0559	120.2700	153.1525
Fe (mg/L)	0.0091	0.9323	0.7725	0.8850
Zn (mg/L)	0.0981	0.3916	0.2367	0.3409
Sr (mg/L)	0.7192	0.8073	0.8912	1.0326

For most of metal ions, the two pump stations had a similar metal profile and there was no obvious seasonal change in metal concentrations. The calcium concentration deserves more attention than that of other metals because it is reported by several studies that calcium can help the build-up of FOG layers (He et al., 2011; Keener et al., 2008) although there is no specific concentration level that is supposed to be beneficial for FOG layer formation. He et al. (2011) and Keener et al. (2008) proposed that the excess calcium present in FOG deposits might be partly caused by concrete corrosion. He et al.

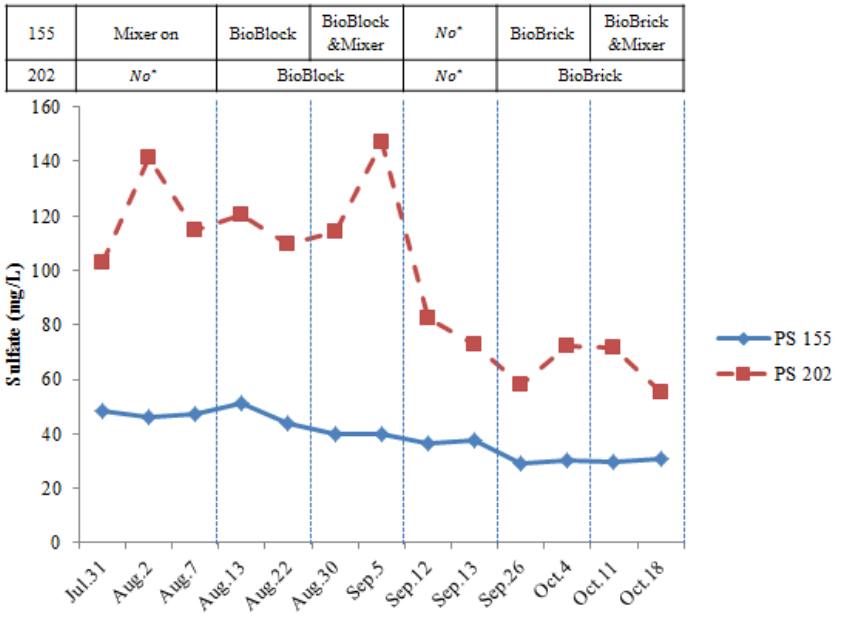
(2011) tried to form FOG deposit with calcium concentration of 50 mg/L to 750 mg/L and found that the resulting FOG deposit weight also increased. They also found that increasing levels of calcium led to higher calcium levels in FOG deposits which could support the important role of calcium in FOG formation. Thus, methods to reduce calcium concentration or restrict corrosion of concrete might mitigate FOG build-up in pump stations.

3.3.7 Anion results

IC analysis can provide information about anions in water samples including fluoride, chloride, sulfate, phosphate, bromide. Sulfate concentrations in each pump stations during both sampling terms are shown in *Figure 3.10*.



(a)



(b)

Figure 3.10. Sulfate concentration in each pump station in (a) winter-spring sampling term, (b) summer-autumn sampling term.

*Note: No** means no product was applied.

During the winter-spring sampling term, changes in sulfate concentration in both pump stations were similar with PS 202 having a relatively higher sulfate concentration. On December 20, both pump stations showed a sudden crest after which the sulfate level decreased and stayed at a stable level increasing slightly at the last sampling point. The difference between PS 155 and PS 202 during the summer-autumn sampling term is much more significant than during the winter-spring sampling term. For each sampling date, the sulfate concentration in PS 202 is around twice that of PS 155. In general, the sulfate concentrations in both wet wells were much higher than the typical sulfate concentration in untreated domestic wastewater as shown in *Table 3*.5.

Table 3.5. Typical composition of untreated domestic wastewater adapted from George et al. (1991).

Contaminants	Unit	Weak	Medium	Strong
COD	mg/L	250	500	1000
Nitrogen	mg/L	20	40	85
Nitrite	mg/L	0	0	0
Nitrate	mg/L	0	0	0
Phosphorus	mg/L	4	8	15
Sulfate	mg/L	20	30	50
Grease	mg/L	50	100	150

Table 3.5 indicates a maximum concentration of 50 mg/L in typical untreated domestic wastewater whereas maximum sulfate concentrations above 100mg/L were observed in the two pump stations and the average sulfate concentration in PS 202 during the summer-autumn sampling term was 97.1 mg/L.

3.4 Discussion of field sampling results

Comparisons of the sampling term results in the two pump stations, two pump stations, the working conditions, and evaluation of Bio-Block and Bio-Brick products are discussed in section 3.4.1-3.4.2.

3.4.1 Comparison between sampling terms

Table 3.6 contains selected parameters for the winter-spring and summer-autumn sampling terms.

Table 3.6. Main parameters of two sampling terms.

Parameter	PS No.	Winter-Spring Term		Summer-Autumn Term	
		Value	St.d	Value	St.d
pH	155	8.15	0.42	8.33	0.16
	202	7.94	0.51	7.70	0.20
COD (mg/L)	155	279.70	90.93	177.69	36.78
	202	317.63	129.71	190.89	60.31
Temperature	155	12.61	0.82	17.68	1.58
	202	16.77	0.93	22.04	1.02
FOG (mg/L)	155	267.56	208.63	87.88	28.53
	202	369.78	258.93	85.77	30.35
NH_4(mg/L)	155	35.81	10.54	37.96	7.64
	202	36.59	13.88	20.04	5.69

As indicated in *Table 3.6*, minor differences in pH values can be found between the two sampling terms. pH values are related to factors like water source, chemical reactions, and biological activities. The pH range in both wet wells was not restrictive to *Bacillus* metabolism throughout product application.

An average difference of 5 °C between the two sampling terms in both pump stations is noted in *Table 3.6* As wastewater collecting systems are underground for the most part,

temperatures in each sampling term were stable (standard deviation ≤ 1.58). Ammonium concentrations in PS 155 did not change significantly between the two sampling terms but underwent a decrease of approximately 62% in PS 202 from the winter-spring sampling term to the summer-autumn sampling term, which may indicate that nitrification was more limited in winter-spring sampling term in PS 202.

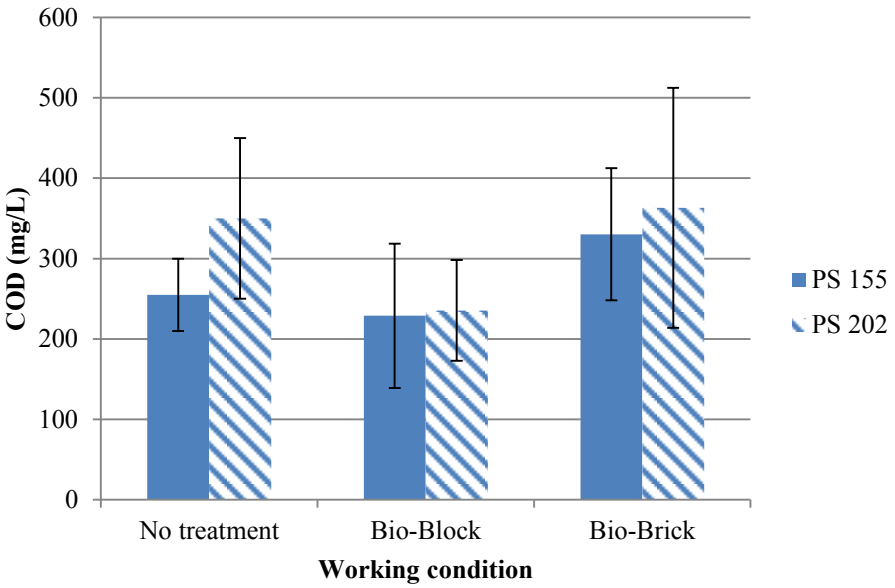
Both COD and FOG concentrations dropped sharply from the winter-spring sampling term to the summer-autumn sampling term. The high standard deviations suggest that their values are not stable in each sampling point which is consistent with *Figure 3.8* and *Figure 3.9*. From the winter-spring sampling term to the summer-autumn sampling term, there is an average decrease in FOG of 67.2% and 76.8% in PS 155 and PS 202, respectively and an average decrease in COD of 36.5% and 39.9% in PS 155 and PS 202, respectively. Besides wastewater sources, warmer temperature is a main cause for the drops of FOG and COD concentrations. Warmer temperature can affect FOG and COD removal by (1) helping to create an environment that is beneficial for bacterial growth, metabolism, and other biological activities and thus assisting *Bacillus* and other bacterial species in FOG and COD degradation, (2) increasing the activity of enzymes that degrade FOG and COD, (3) softening FOG to make it more accessible to degradation by *Bacillu*s and other bacteria.

3.4.2 Evaluation of different products and working conditions

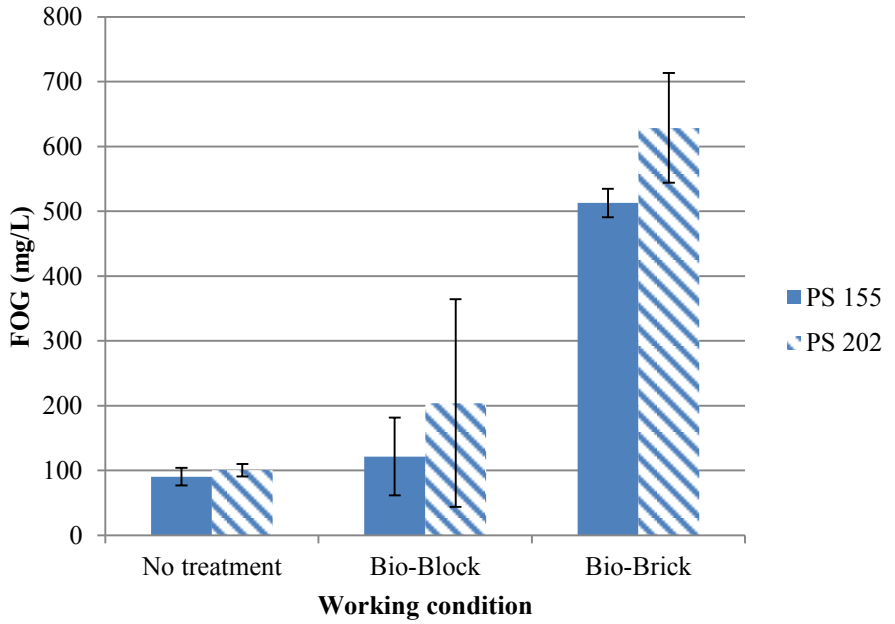
COD and FOG are the main parameters used in this project to evaluate the performance of Bio-Block and Bio-Brick in degrading FOG (fat, oil, grease) deposits in wet wells under different working conditions.

3.4.2.1 Winter-spring sampling term

Concentrations of COD and FOG in pump stations 155 and 202 using different products in the winter-spring sampling term are shown in *Figure 3.11*.



(a)



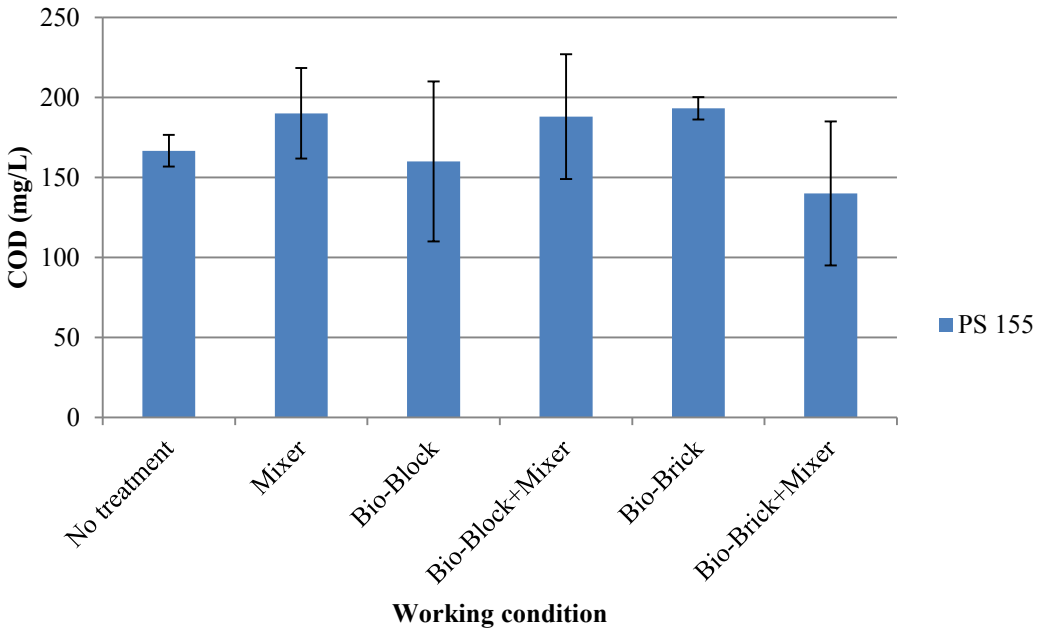
(b)

Figure 3.11. Concentrations COD (a) and FOG (b) using different products in the winter-spring sampling term.

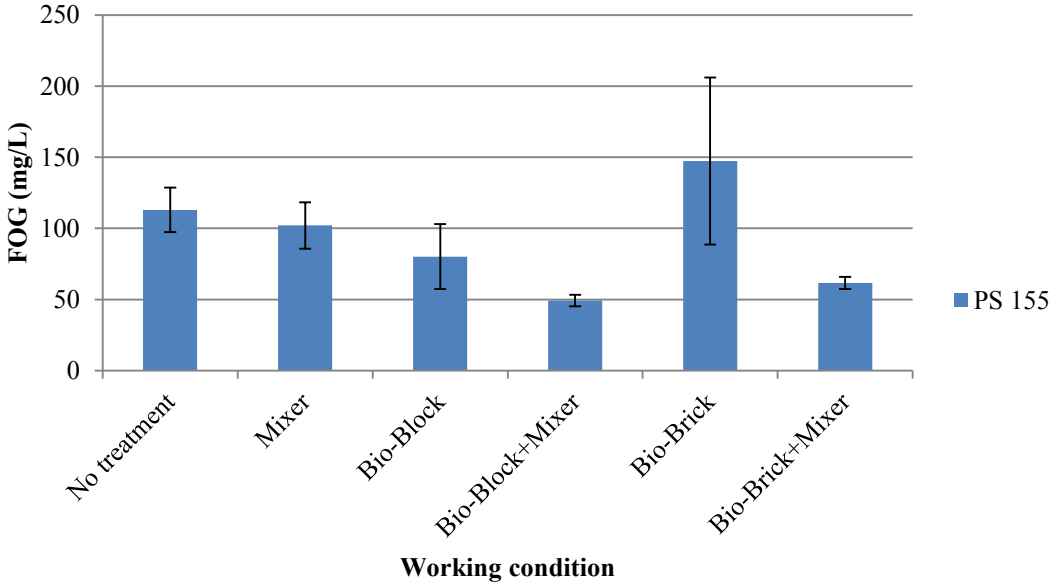
In *Figure 3.11*, no significant improvement of COD removal could be observed in the winter-spring sampling term compared with the no treatment working condition ($P>0.05$). A FOG concentration increase could be observed after solidified FOG was cleaned manually while the products were applied, indicating the products have failed to meet FOG removal expectation. The work pattern of the pumps inside the wet wells dictates that wastewater will be pumped out to downstream and go to wastewater treatment finally. Hydraulic residence time is of great importance for biological activity inside wet wells. Thus, the hydraulic residence time might be too short for both product and extant bacteria to multiply and degrade COD and FOG. Low temperature in the wet wells might limit bacterial activity and enzymatic activity causing the rate of FOG reduction to be slower than the rate of FOG accumulation and resulting in an increase in FOG concentration. Bio-Block and Bio-Brick products cannot reduce FOG within the winter-spring sampling term.

3.4.2.2 Summer-autumn sampling term

A mixer installed in PS 155 created more working conditions in that pump stations. Therefore, PS 155 and PS 202 will be discussed separately. COD and FOG concentrations in PS 155 under different working conditions in the summer-autumn sampling term are shown in *Figure 3.12*.



(a)



(b)

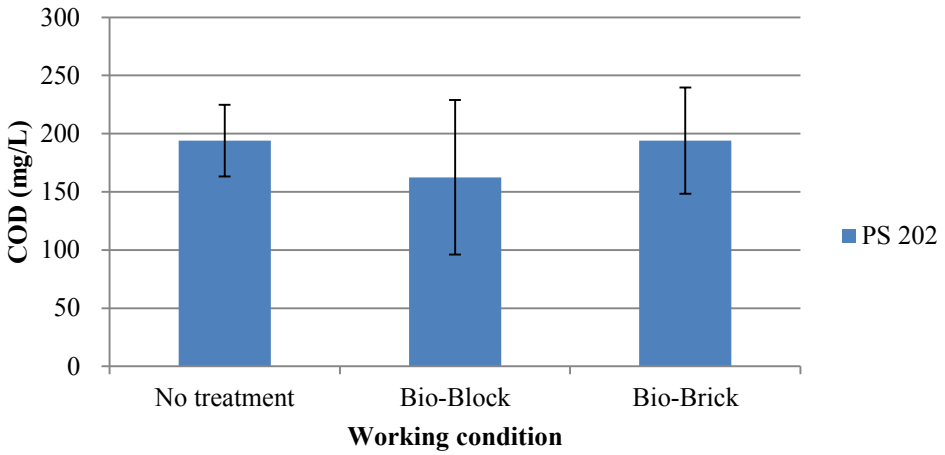
Figure 3.12. Concentrations of COD (a) and FOG (b) in PS 155 under different working conditions during the summer-autumn sampling term.

Similar to the winter-spring sampling term, no significant difference in COD values among all the working conditions could be detected in the summer-autumn sampling term. Although the COD concentration was stable throughout the whole sampling term, the values were much lower than those in the winter-autumn sampling term. Other working conditions had similar COD levels compared to the no treatment condition. Possibly, the product was not very effective in COD removal. Or the concentration of the product was not high enough for it to show a distinct COD removal effect. It is also known that the hydrolysis of FOG may lead to the reduction of FOG concentration and an increase in the COD concentration. However, a higher temperature during this sampling term might have improved the activity of all competent bacteria, thus lowering the COD level. Differences among all working conditions can be clearly identified with respect to the FOG concentration.

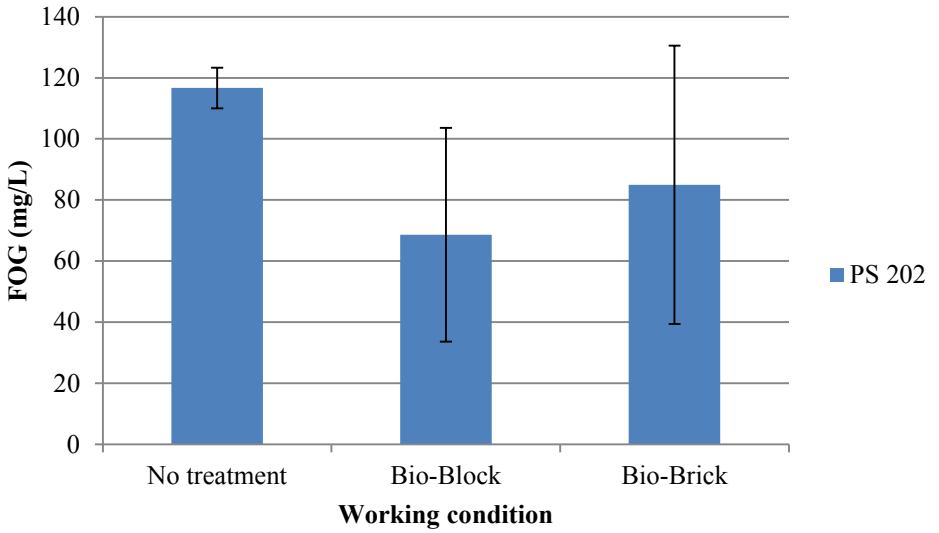
With only mixer working, FOG decreased slightly (9.73%). When Bio-Block was applied, 29.07% FOG removal was achieved compared with the no treatment working condition. The most significant improvement in FOG removal was observed when both the product and the mixer were applied (56.37% and 45.44% for Bio-Block and Bio-Brick, respectively). In this case, the application of a mixer inside of the wet well considerably improved the performance of both products. The use of a mixer can improve FOG clearance in several ways: (1) a mixer introduces oxygen to wastewater which might provide more available electron acceptors for bacterial degradation of FOG and other organics, (2) Secondly, a mixer aids bacterial release from the product and blends bacteria product, and wastewater efficiently to advance FOG degradation, (3) the water turbines caused by mixer can break up solid FOG deposits making them accessible to

hydrolysis. Data in *Figure 3.9* indicates that when only Bio-Brick was applied the FOG concentration was higher than that in the no treatment working condition. A longer experimental time might improve Bio-Brick performance.

Concentrations of COD and FOG in PS 202 under different working conditions in the summer-autumn sampling term are shown in *Figure 3.13*.



(a)



(b)

Figure 3.13. Concentrations of COD (a) and FOG (b) in PS 202 under different working conditions in the summer-autumn sampling term.

Similar to the experimental results for PS 155, no distinct differences in COD concentrations were observed in PS 202 in all three working conditions during the summer-autumn sampling term. However, in the summer-autumn sampling term the COD concentrations are much lower than those in the winter-spring sampling term (see

section 3.4.1). Furthermore, the results are consistent with those of PS 155 in that the products did not perform well with respect to COD removal. 41.16% and 27.15% FOG removal was achieved with Bio-Block and Bio-Brick, respectively. The results of Bio-Block and Bio-Brick application to remove FOG in PS 202 can be analyzed in a similar manner to those in PSS 155.

3.5 Conclusion for field sampling

The performance of Bio-Block and Bio-Brick in FOG degradation under different environment and working conditions was monitored by collecting wet well wastewater samples over two seasons. In the winter-spring sampling term, the products failed to meet the expectation of FOG removal as FOG concentration increased throughout the term. FOG removal improved significantly in the summer-autumn sampling term compared to the winter-spring sampling term indicating the importance of temperature in biological degradation. The low winter temperatures prevalent in Edmonton reduced bacterial activity from the Bio-Block and Bio-Brick products and from bacteria contained in wastewater in the wet wells. Growth, metabolism, multiplication, and enzymatic (e.g., lipase) activities increased during the summer-autumn sampling term, improving FOG degradation. It is notable that COD did not change much with different working conditions, including no treatment, during the summer-autumn sampling term. This observation suggests that Bio-Block and Bio-Brick products might not be of great help in COD removal. COD degradation might rely on the whole competent bacterial species inside the wet wells. Throughout the two sampling terms, the numbers of blocks for each product was four. The dosage of the two products can deemed to be same considering the same weight of each block (10 pounds). In the summer-autumn sampling term which

showed a FOG removal improvement, Bio-Block performed better than Bio-Brick in FOG control. Furthermore, Bio-Block takes less time to dissolve in water and this allows the product to degrade FOG in a shorter period of time. Based on these analyses presented here, Bio-Block is preferable to Bio-Brick for FOG removal under conditions tested in this study. The best results were observed in PS 155 with the mixer on and the application of Bio-Block or Bio-Brick. The mixer can increase oxygen concentration in the wet wells, provide more chances for the product to get in touch with FOG, and increase the dissolution rate of the products. Consequently, a mixer is recommended to improve the performance of Bio-Block and Bio-Brick products.

4. Bench-scale experiments

4.1 Introduction

Field sampling work revealed several important factors that might impact the application of Bio-Block and Bio-Brick to FOG removal in wet well wastewaters, including temperature, product dosage, effect of mixing, and COD concentration in the wet well. For example, the products demonstrate better performance in FOG removal at relatively higher temperatures because bacteria are more prolific and active under these conditions. Also, FOG removal efficiency is higher when the product is mixed thoroughly with the wastewater. Other factors that need to be considered are pumping frequency and flow rate of wastewater. Only a few of these factors were tested during field sampling work because of practical limitations. Bench-scale experiments can provide additional evidence to support hypotheses and findings drawn from the field sampling work. In bench-scale experiments, a batch reactor running that imitates the conditions in a wet well can be controlled more easily than a wet well to test factors that influencing Bio-Brick performance with respect to FOG control under laboratory conditions.

4.2 Methodology

4.2.1 Oily synthetic wastewater

The composition of the synthetic wastewater used in these experiments, based on Yang et al. (2012) was as follow: D-glucose, urea 30 mg/L, NaCl 150mg/L, $NaHCO_3$ 30 mg/L, KH_2PO_4 12.75mg/L, $Na_2HPO_4 \cdot 12H_2O$ 0.030 mg/L, NH_4Cl 0.117mg/L, $MgSO_4 \cdot 7H_2O$ 12 mg/L, $CaCl_2$ 6 mg/L, $FeCl_3$ 0.25 mg/L, $MnSO_4 \cdot H_2O$ 6 mg/L. The dosage of D-glucose was based on the COD value required for each experiment run. The final pH was

controlled by HCl and NaOH to make it a neutral condition. Ultrapure water was used to eliminate impacts from unknown trace metals. As some of the ingredients for synthetic wastewater are at very low concentrations, a 10× concentrated stock synthetic wastewater was prepared. Because ingredients can mixdegrade or change in an autoclave, the prepared synthetic wastewater stock was autoclave separately, D-glucose, NH_4Cl, and $NaHCO_3$ were filtrated using 0.2 μm pore filters; other ingredients were used to make the 10-× stock solution and then autoclaved. Stock solutions were stored at 4 °C until use. When needed, the 10-× stock was diluted and added the other three filtered ingredients. Stock solutions were not stored for more than one month. All preparations were carried out in a biological safety cabinet. To prepare an oily synthetic wastewater, a commercial canola (Compliments, Canada) was chosen as the oil source. According to Statistic Canada (2011), canola oil took about 50% of all oil consumed by Canadians which makes canola oil as one of the main source in FOG in Canada. Only one oil source was used in these experiments. Oil was added to the bottles filled with 50 mL synthetic wastewater and well mixed.

4.2.2 Bench-scale experiment design and set up

Based on analyses of the field sampling work, bench scale experiments focused on the effects of product dosage, COD concentration, and temperature on FOG removal in wastewater. Product dosage is directly related to the bacterial concentration in the wastewater. A higher product dosage would be expected to increase the bacterial activity that degrades FOG. The COD concentration is an important condition in bacterial metabolism, as it is likely that bacteria can degrade simple organic substances more easily than long chain cyclic compounds. Therefore, FOG biodegradation might be

influenced by the presence of organics content and thus the COD concentration in the wastewater. In addition, bacterial and enzyme activities increase as the temperature rises from cold to moderate, thus temperature is a main factor in the bench-scale study. The three factors—product dosage, COD concentration, and temperature were evaluated separately in each run of the bench-scale experiments.

To control water quality, COD, and FOG concentration, synthetic wastewater was used in the laboratory experiments to eliminate effects from bacterial species that might exist in real wastewater without destroying natural wastewater contents (see section 4.2.1). A glucose dosage based on the required COD concentration was used to supply the COD. Tests were performed on 50 mL of synthetic oily wastewater contained in 250 mL glass bottles that were cleaned and autoclaved before each experiment. Because Bio-Brick showed better FOG removal performance and dissolved more easily in water than Bio-Block, Bio-Brick was used in the bench-scale experiments.

To accelerate the interaction with the wastewater and to get a more accurate amount of product needed to perform FOG degradation, a calculated dose of Bio-Brick was pre-dissolved in freshly prepared synthetic wastewater using a centrifuge tube; the mixture was vortexed until the product completely dissolved and the volume of solution needed based on the product dosage requirement was calculated. The product solution was pipetted into a 250 mL bottle loaded with synthetic wastewater. Generally, a 50 mL reaction mixture (synthetic water + product solution) was used in each 250 mL bottle. To improve the FOG removal rate and imitate the mixer used in PS 155, reaction mixtures were placed on a shaker during the experiment. Bottles were covered with autoclaved aluminum foil so that oxygen could get in and contaminants from the outer environment

could be avoided, additions of nutrients and bacteria were performed quickly to avoid contact between the reaction mixture and the outer environment.

Bacteria inoculation and addition of water were performed in a biological safety cabinet that supplied a continuous aseptic flow. Experimental conditions were controlled as follows: temperature was controlled using an incubator shaker (Innova 44, New Brunswick Scientific Co. Inc., USA) with a temperature range of 4 °C to 80 °C; COD concentration was controlled by the amount of D-glucose added to the synthetic wastewater; a two-week experiment run time was performed because preliminary experiments failed to demonstrate ideal FOG removal and bacterial growth in one-week trials. Based on factors of interests in this study, experimental runs and one blank were designed. Triplicates were tested for each working condition; COD and FOG concentrations were tested in each run. The detailed experimental design is shown in *Table 4.1* to *Table 4.3*.

The impact of product dosage on FOG removal

An experimental design to test the dosage of Bo-Brick impact on FOG reduction in wastewater at two bacteria conditions is shown in *Table 4.1*.

Table 4.1. Bench-scale experiment design (dosage).

Bacteria Concentration (CFU/mL)	COD (mg/L)	Temperature (°C)
10^9 10^6 0	600	32

Genzyme recommended a 0.12 mg/L dosage of Bio-Brick to combat FOG in wastewater. Plate counting evaluated the bacterial concentration at the recommended dosage to be 10^6 CFU/mL. Consequently, 10^6 CFU/mL (0.12 mg/L dosage of Bio-Brick) and 10^9 CFU/mL (0.12 g/L dosage of Bio-Brick) bacterial concentrations were tested. An experiment at each bacterial concentration was run for 14 days at 32 °C and a COD of 600 mg/L in an incubator shaker (120rpm). FOG extracted from each bottle after the two weeks experiment was compared with initial FOG concentration to calculate the FOG removal percentage. Similarly, initial and final CODs were compared.

The impact of COD concentration on FOG removal

An experimental design to test the Bio-Brick impact on FOG reduction in wastewater at three COD concentrations is shown in *Table 4.2*.

Table 4.2. Bench-scale experiment design (COD).

Initial COD (mg/L)	Bacteria Concentration (CFU/mL)	Temperature (°C)
50 300 500 0	10^9	32

The influence of COD concentration (50, 300, and 500 mg/L) on FOG removal under laboratory conditions were tested at a bacterial concentration of 10^9 CFU/mL (0.12 g/L of

Bio-Brick product) and a temperature of 32 °C. COD concentrations were controlled by changing the dosage of D-glucose for each group. COD and FOG concentrations were measured after a 14 day treatment in an incubator shaker (120 rpm) and compared with initial COD and FOG concentrations.

From the observation of COD variation and FOG removal calculated, the impact of COD concentration on the Bio-Brick product's performance was evaluated.

The impact of temperature on FOG removal

An experimental design to test the Bio-Brick impact on FOG reduction in wastewater at three temperatures with bacterial concentration of 10^9 CFU/mL is shown in *Table 4.3*.

Table 4.3. Bench-scale experiment design (temperature).

Temperature (°C)	Bacteria Concentration (CFU/mL)	Initial COD (mg/L)
15	10^9	250
	0	
22	10^9	
	0	
32	10^9	
	0	

Three temperature levels, 15, 22, and 32 °C were tested in the experiments shown in Table 10. At each temperature, blanks were run to compare results with those of the experimental group at the same temperature. The bacteria concentration for all experimental groups was 10^9 CFU/mL (0.12 mg/L of Bio-Brick product) and the initial COD concentration in all groups was 250 mg/L, which was close to the average COD value in the field sampling work. 15 °C was similar to the temperature measured in wet wells, especially in winter. 32 °C was tested to create a relatively more beneficial environment for bacterial metabolism. Experiments at 15 and 32 °C were carried out in

an incubator shaker (120 rpm) with a preset temperature, while experiments at 22 °C were run in a similar shaker under room temperature as an intermediate working condition. After a 14-day treatment, FOG removal was calculated for each temperature condition based on initial and final FOG concentrations. Similarly, initial and final COD concentrations were measured.

4.2.3 Parameters

The two most important parameters in oily wastewater control are COD and FOG concentrations. Their measurements and pretreatments are introduced in the following sections of COD and FOG.

COD

Initial and final COD concentrations were measured using the method mentioned in section 3.3.4. As the measuring range of Hach vial is 5~150 mg COD/L, dilutions were performed based on initial and final COD values. The samples were filtrated using 0.45 μm filters before COD measurements. Experimental results indicated that more than 99.99% FOG was removed after this filtration.

FOG

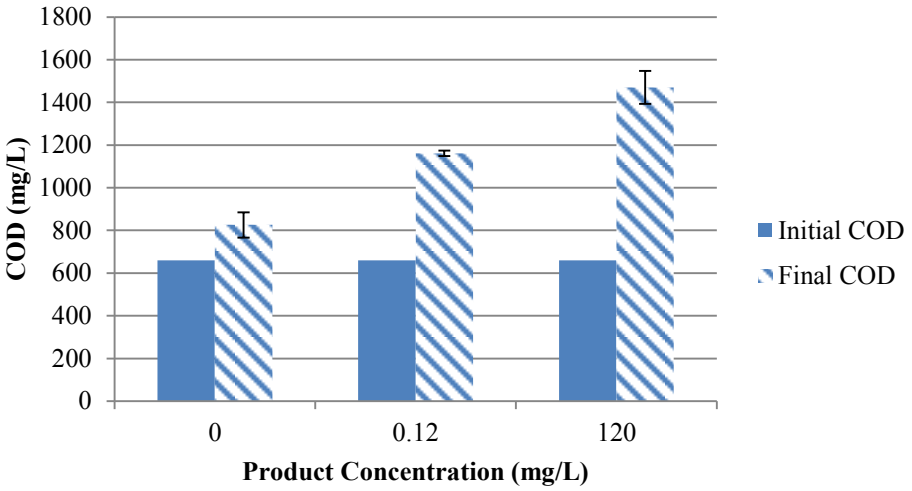
FOG extraction process in the field sampling work was applied for the laboratory testing; n-hexane was used for FOG extraction (see section 3.3.5).

4.3 Results and analysis

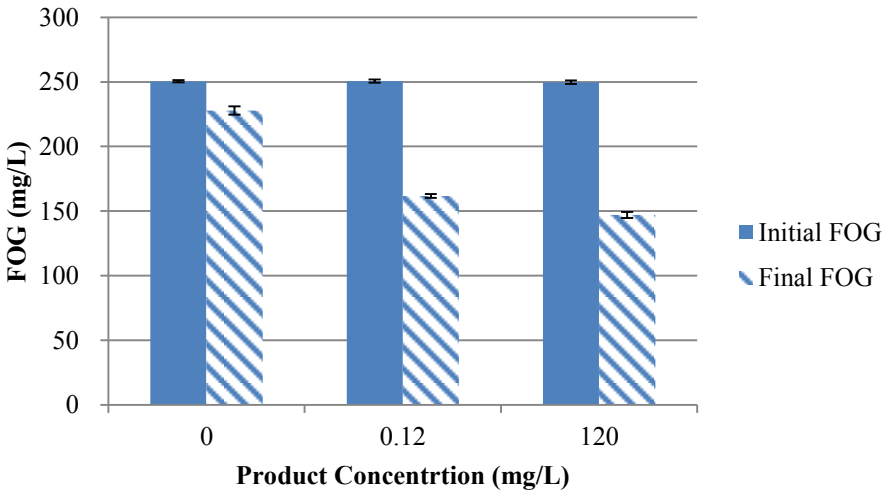
4.3.1 Impact of product dosage

Product dosage controls the initial bacteria concentration in the wastewater and thus affects the FOG removal performance during the experiment. To evaluate the impact of

product dosage on FOG removal, two Bio-Brick doses were applied which resulted in wastewater bacteria concentrations of 10^6 CFU/mL and 10^9 CFU/mL. COD and FOG were measured throughout the 14-day experiment and the results are shown in *Figure 4.1*



(a)



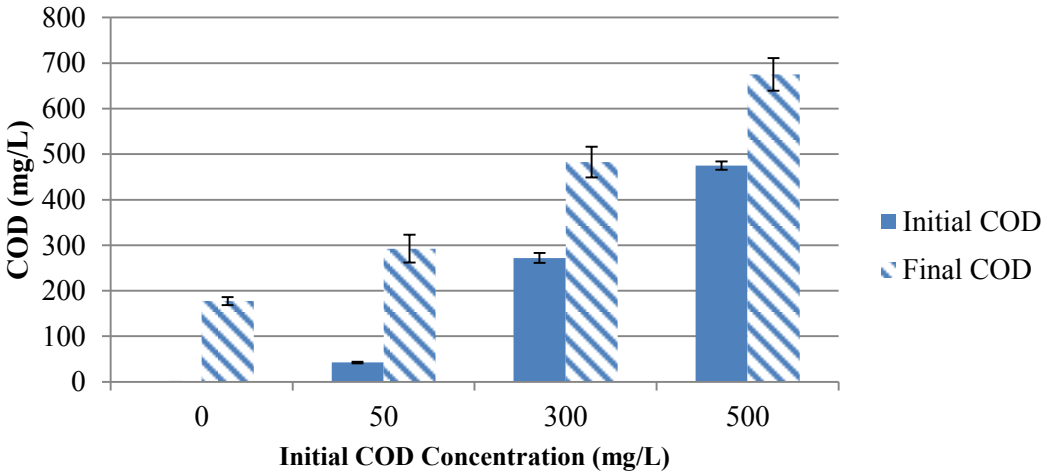
(b)

Figure 4.1. Initial and final COD (a) and FOG removal (b) under different product dosage.

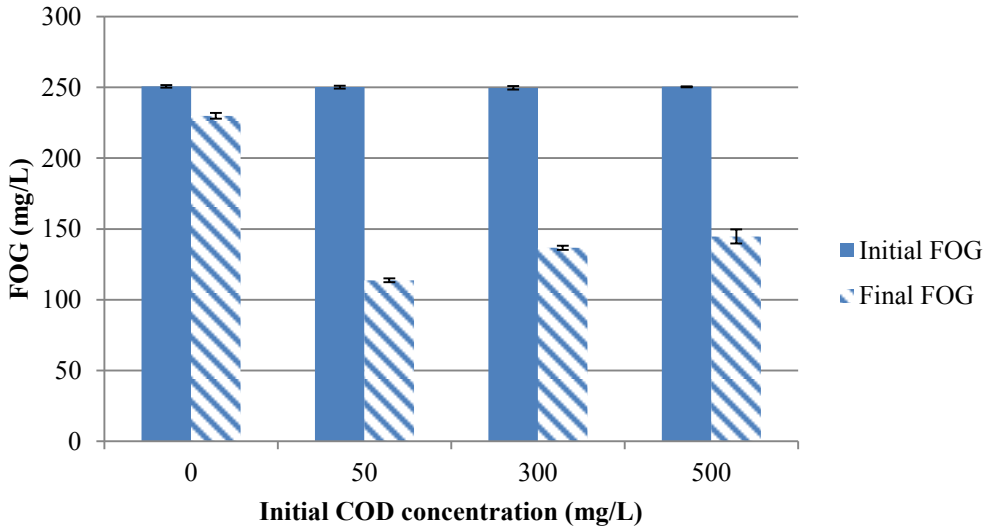
COD increased significantly in all three experimental groups: The COD in the product concentration of 0 mg/L increased by 37.5%, COD in product concentration of 0.12 g/L increased by 122.8%, and COD in product concentration of 0.12 mg/L increased by 75.9%. Hydrolysis of oil could generate fatty acids and soluble glycerol that could be counted as COD sources. As all bottles were shaking continuously for 14 days, the process would help accelerate the dissolution and hydrolysis of the oil inside the water. This might be the main reason COD changed so much after treatment. Meanwhile, bacterial, enzyme, and chemical activity could also increase the COD concentration: *Bacillus*, lipase, and surfactants contained in the products could enhance the process of oil hydrolysis. Moreover, bacterial activity might generate more small carbon units from oil resulting in an increase in COD. Both showed high FOG removal 41.1% and 35.5% (P=0.01), respectively. FOG removal in control group could result from oil hydrolysis and/or oil loss during the extraction process. COD concentration of 50 mg/L with a higher bacteria concentration performed better in FOG removal. An improvement in FOG removal might result from an increasing in Bio-Brick product.

4.3.2 Impact of COD concentration

D-glucose (50, 300, and 500 mg COD/L, respectively) was the main source of COD. Changes in COD before and after treatment and FOG removal rates are shown in *Figure 4.2*.



(a)



(b)

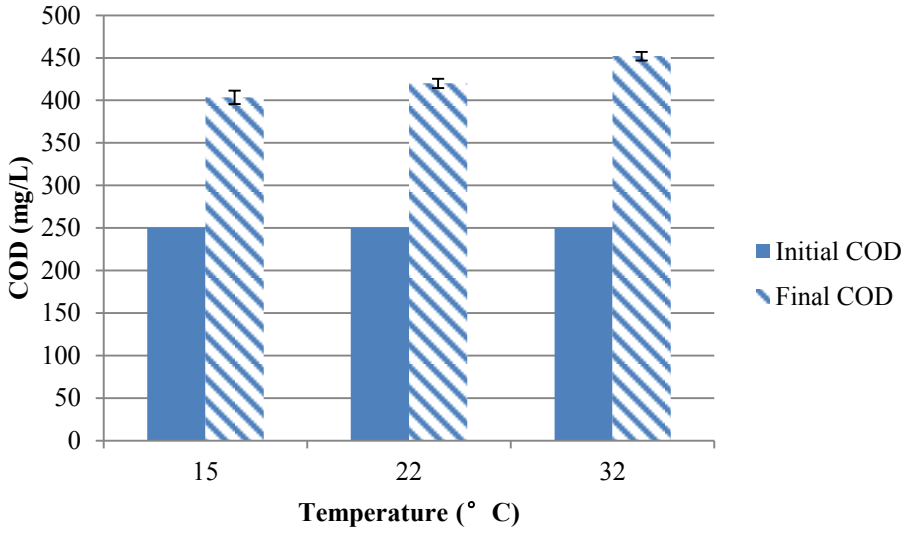
Figure 4.2. Initial and final COD (a) and FOG removal (b) under different initial COD concentration.

As shown in *Figure 4.2* (a), COD increased in varying degrees for all the groups. Initial COD of 50 mg/L with lowest initial COD concentration was observed to have the highest increasing percentage of 590.6% while COD concentration of 300 mg/L increased by 77.5% and COD concentration of 500 mg/L increased by 42.2%. Both hydrolysis and

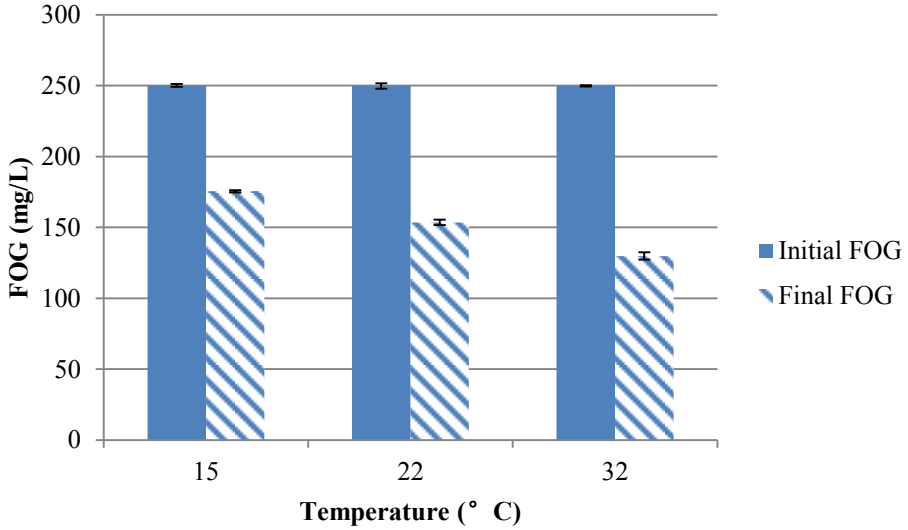
bacterial activity could contribute to an increase in COD. In initial COD concentration of 0 mg/L, the COD might mainly come from oil hydrolysis. Initial COD of 50 mg/L was found to have the highest FOG removal (~54.5%). FOG removal for initial COD concentrations of 300 mg/L and 500 mg/L were 45.2% and 42.2%, respectively. The FOG removal decreased when the COD concentration in the reaction mixtures increased. Presumably *Bacillus* was forced to consume oil when D-glucose concentration was low or had been consumed suggesting that a higher COD concentration could thwart the efficiency of *Bacillus* in FOG degradation.

4.3.3 Impact of temperature

Three temperatures were applied to Bio-Brick enhanced FOG removal experiments using incubator shaker: 15 °C, 22 °C, and 32 °C for each experimental group, respectively. COD changes and FOG removal after 14-day treatments are depicted in *Figure 4.3*.



(a)



(b)

Figure 4.3. Initial and final COD (a) and FOG removal (b) at different temperature.

COD in blanks and experiment groups increased compared to initial COD concentrations of 250 mg/L. COD increased with temperature in each blank were 26% at 15 °C, 33.2% at 22 °C, and 40.1% at 32 °C. This suggested that a higher temperature can accelerate the

hydrolysis of oil. The increase of COD with temperature could also be found in all the experiment groups (with product added) in which bacteria would be expected to hydrolyze oil and degrade FOG resulting in an increase in COD. As expected, the highest FOG removal (48.0%) was observed at the highest temperature in this experiment (32 °C). FOG removal increased with the increasing temperature (29.8% at 15 °C, and 38.5% at 22 °C) as shown in *Figure 4.3* (b) which was consistent with observation in COD changes.

4.4 Discussion of bench-scale experiment

The bench-scale experiments demonstrated impacts on FOG removal from product dosage, initial COD concentration, and temperature. An increase in product dosages increases the initial bacteria concentration within a system and can improve FOG removal significantly. In *Figure 4.2* (b), the difference in FOG removal between product concentration of 0.12 g/L and product concentration of 0.12 mg/L was not as distinctive as expected (P=0.01). A possible reason is that the temperature applied throughout that run was 32 °C, which is beneficial for bacterial growth.

In Tang et al. (2012), a commercial bio-additive named Bio-Amp produced a significant increase in readily biodegradable COD fractions which might be consistent with the observation that COD increased after treatment.

In the experiment using different initial COD concentrations, the group applying 50 mg COD/L showed the highest FOG removal. Thus, it can be assumed that the initial COD concentration can affect the bacterial tendency to degrade oil. To be more specific, the easily degradable fractions in COD might influence FOG removal for the reason that

bacteria could possibly tend to degrade those fractions counted as COD under their existence and degrade oil afterward or in a small scale at the same time. When the initial COD was 0 mg/L, the product showed the lowest FOG removal which might be attributed to the fact that bacteria still need some initial COD in metabolism. Higher temperature is beneficial for bacterial growth and metabolism and enhances enzyme activity. Brooksbank et al. (2006) used commercial microbial supplements to degrade FOG deposits in bench scale and found that FOG removal rates varied from 37-62% depending on species (single or multiple). In Mohamed et al. (2004), a complete removal of FOG, BOD_5, and COD was achieved when two biofilm system units were applied in sequences and immobilized using sand. The highest FOG removal throughout our bench-scale experiment was 54.5% at 32 °C with a COD concentration of 50 mg/L.

Although the batch reactor used in this experiment did not cause bacteria loss from the product, the removal was still lower compared with Brooksbank et al. (2006) and Mohamed et al. (2004). Several reasons could possibly help explain this. First, the construction of the system used in these experiments was simpler than other systems that used bio-film which might have allowed bacteria to contact target organics more completely. Meanwhile, bacteria in bio-film system are more active. Second, water contents (like metals) might make significant changes in biological activity. Some trace metals could enhance the degradation ability of competent bacteria species. Third, bacterial diversity in our bench-scale experiment was too limited and the increase in COD might be attributed to that fact. As the Bio-Brick product accelerated FOG hydrolysis and broke FOG into some smaller units, COD increased. However, the limited species of bacteria failed to consume a large part of the COD. Brooksbank et al. (2006) suggested

that multiple-species bio-supplements were more competitive in FOG and other nutrient degradation compared with single-species supplements. Therefore, diversity of competent bacteria species should be taken into consideration in biological product design and application.

4.5 Conclusions derived from bench-scale experiments

In bench-scale experiments, Bio-Brick dosage, initial COD concentration, and temperature were studied separately to determine their impact they might have on FOG removal in wastewater. Although a higher FOG removal was observed with bacteria concentration 1000 times higher than the recommended value, the difference between the two bacterial concentrations was not significant. In the study of initial COD concentration impact, a lower readily biodegradable COD concentration encouraged the FOG biodegradation, presumably because the low COD concentration drove the bacteria to consume oil. FOG removal increased with the increasing temperature: the highest FOG removal 48.0% occurred at 32 °C. Thus, temperature has a crucial role in biological FOG treatment.

5. Discussion: field sampling work & bench-scale experiment

It is necessary to combine results from field sampling work and bench-scale experiment together to elucidate factors' impact on the products' performance and analyze possible approaches to ameliorate the application of biological products.

5.1 Product dosage

Literatures on FOG biodegradation mainly focus on bacteria species, lipases, working conditions, and treatment structure design. Nevertheless, limited research has investigated the impact of product dosage on FOG removal. The poor performance of Bio-Block and Bio-Brick during practical application especially in winter has caught attention on the issue of product dosage. Apart from the recommended dosage of 0.12 mg/L Bio-Block, product of 120 mg/L was tested in this study. The result showed that the more concentrated product could improve FOG removal by 41.1% FOG compared to 35.5% removal with recommended dosage under the same conditions (P=0.01). The increase in dosage can be meaningful considering the working condition in practical application.

The temperature observed during two sampling terms varies from 12 °C to 22 °C. During winter-spring sampling term, temperature usually varied from 12 °C to 16 °C. Low temperature could restrict growth and reproduction of competent bacteria. Thus, the final concentration might be lower than expected in practice.

Furthermore, wastewater would be pumped out periodically once water level came to the designed one. Hydraulic residence time of the product was probably short for the bacteria

to demonstrate FOG removal ability. Based on the two points, it is necessary to increase product dosage when applied in wet wells.

5.2 Temperature

Both field sampling work and bench-scale experiment results reveal the importance of temperature in biological treatment processes. There is significant drop of FOG concentration from winter-spring to summer-autumn sampling term in both PS 155 (267.6 mg/L to 87.88 mg/L with P=0.003) and PS 202 (369.78 mg/L to 85.77 mg/L with P=0.002). Similar phenomenon has been observed in bench-scale experiment: FOG removal increases from 29.8% to 48.0% with temperature increases from 15 °C to 32 °C.

Research has been carried out regarding optimum temperature for bacterial activity and lipase activity for FOG removal. According to Becker et al. (1999), thermophilic conditions are beneficial for FOG to become more available to bacteria and their enzymes. Mahdi et al. (2012) observed the highest lipase activity produced from *Aeromonas* sp.S1 within a temperature range from 25 °C to 30 °C. In Mobarak-Qamsari et al. (2012)'s study of *Pseudomonas.* aeruginosa KM110, 30 °C and 45 °C were found to help achieve optimum enzyme activity. Jeganathan et al. (2006) observed the optimum temperature for immobilized Candida rugosa lipase was 35 °C. Typical temperature range for competent bacteria in FOG biodegradation is within 20-45 °C (Mobarak-Qamsari et al., 2012). It is apparent that water temperature inside of wet wells is below this range for most of the time. Although located underground, water temperature is inevitably related to environment temperature. Edmonton experiences low temperature throughout the year causing the long-last low temperature in wet wells. Consequently, low temperature might

be one of the main reasons that both products fail to meet expectation on FOG removal during practical application.

5.3 COD concentration

Generally, COD concentrations in both pump stations stay at a high level. For PS 155, average COD is 279.7 mg/L in winter-spring sampling term and 17.69 mg/L in summer-autumn sampling term. For PS 202, average COD is 317.6 mg/L in winter-spring sampling term and 190.9 mg/L in summer-autumn sampling term. It is evident that COD is relatively lower in summer-autumn sampling term in both wet wells which can be possibly attributed to higher bacteria activity at higher temperature in summer-autumn sampling term. All competent bacteria species can benefit from proper temperature in COD removal improvement. All the three runs in bench-scale experiment show increased COD concentrations after treatment instead of drops observed in most of related literatures. It is likely caused by the fact that the simple structure of bacterial species in batch reactors limits the ability of COD reduction. Although *Bacillus* can break oil molecule into smaller units, the ability of further degradation of the smaller units and fatty acids inside of batch reactors is limited. Bacterial species in wet wells could be much more complicated compared to experiment condition which might be beneficial for COD removal.

Bacteria might tend to degrade easily biodegradable organics within a system and turn to consume other organic sources that can be potentially used. In this case, FOG removal could be impacted by initial COD level especially the one has larger easily biodegradable fractions. Bench-scale experiment suggests that the product shows the highest FOG

removal with lowest COD concentration (controlled by dosage of D-glucose) with the value of 54.5%. FOG removal decreases as initial COD concentration increases. This observation can potentially help explain why the products cannot perform well in a condition with high COD concentration.

5.4 Mixing effect

The mixer installed in PS 155 during summer-autumn sampling term has improved FOG removal working together with both Bio-Block and Bio-Brick. Besides effect from mixer, working pumps in wet wells can also evoke turbulence in water and introduce more oxygen. This would increase the chance for bacteria to get in touch with target organics and enhance biodegradation ability. During bench-scale experiment, a shaker is used for treatment to supply force that can cause water turbulence. However, the effect of shaker is not as significant as a mixer. For the reactor incubated using a shaker, the water surface is not often disturbed. Usually, water surface will keep stable and not interact with water underneath. In practice, installing a mixer to provide turbulence and shear force to accelerate product dissolve rate is a potential approach to enhance FOG degradation by biological products.

5.5 Other factors

Except for proper temperature and a high product dosage, some other factors might contribute to the increase in FOG removal during bench-scale experiment compared to field work observation as well. Four bacteria species in batch reactors all belong to *Bacillus* and the species structure is not as complicated as practical working condition. There is no other bacteria present in the batch reactors. For the field samples, competition

for oxygen, some crucial nutrients that are useful for bacterial metabolism might exist during the application of products in wet wells. If this is the case, the ability of FOG biodegradation gained from the products could be weakened. Therefore, in the future, it is necessary to conduct studies on competition between different bacteria species in a water system to reduce the reaction that can impair treatment performance. Another main reason is there is almost no bacteria loss in bench-scale experiment which is a characteristic for batch reactor. Nevertheless, a serious competent bacteria loss exists due to working pumps: bacteria keeps been released from the product and pumped out by the pumps. Consequently, it is hard to maintain a stable high bacteria concentration in wet wells. Thus, a potential method is to try to immobilize bacteria and gain a desirous level.

6. Conclusion and suggestions

FOG that can cause numerous problems especially in sewer systems has drawn increasing number of studies on its formation, properties, and treatment methods. Among all the approaches, biodegradation is a promising one for the low cost and effective FOG removal. The City of Edmonton has applied two products, Bio-Block and Bio-Brick to relieve FOG problems in their wet wells. The FOG removal effectiveness of these two products is not as good as expected. To monitor practical application of the two products and find out parameters that can exert impact on their performance, this project is launched. This product is performed in two different phases including field sampling work and bench-scale experiment.

Field sampling work results indicate that higher temperature might enhance the ability of biodegradation for FOG and COD. Meanwhile, it is observed that the products tend to dissolve more quickly at a relatively higher temperature which is beneficial for them to demonstrate good FOG removal. Mixer installed in PS 155 during summer-autumn sampling term improved FOG removal effectively when working together with the products. Under this condition, the FOG degradation rates were 56.37% and 45.44% for Bio-Block and Bio-Brick, respectively. A mixer is recommended for the reason that it might increase oxygen concentration in the wet wells, provide more chances for the product to get in touch with FOGs, and increase dissolving rate of the products.

At the second stage of the project, bench-scale experiments were performed which evaluated the impact of product dosage, initial COD concentration, and temperature on FOG removal. A dosage 1000 times greater than the recommended value is found to be

effective in FOG removal improvement, which led to 41.1% removal in 14 days. It was also observed that FOG removal decreased from 54.5% to 42.2% when COD concentrations increased from 50 mg/L to 500 mg/L. Further, the result of experiment on temperature impact is consistent with field sampling work that a proper temperature (30 °C) is beneficial for bacterial metabolism and lipase activity.

Several suggestions can be drawn from the results of this project regarding practical application. Firstly, adding the products in the upstream or inlet of the pump stations will allow increased treatment time and enhance FOG removal in pump stations. Secondly, applying the mixer together with the product can help to improve FOG removal efficiency in the pump stations. Thirdly, enhanced product concentration in the pump stations is expected to improve FOG removal. Fourthly, encourage public's awareness about FOG problems and collect FOG in a separated way to cut off FOG source in sewer systems is necessary.

References

Agency, U.S. Environmental Protection. (1979). Process Desgin Manual - Sludge Treatment and Disposal. 625/621-679-011.

Ashley, R.M., Fraser, A., Burrows, R., & Blanksby, J. (2000). The management of sediment in combined sewers. *Urban Water, 2*(4), 263-275.

Aziz, Tarek N., Holt, Leon M., Keener, Kevin M., Groninger, John W., & Ducoste, Joel J. (2011). Performance of Grease Abatement Devices for Removal of Fat, Oil, and Grease. *Journal of Environental Engineering-ASCE, 137*(1), 84-92. doi: 10.1061//asce/ee.1943-7870.0000295

Barbaro, S. E., Trevors, J. T., & al., et. (2001). Effects of low temperature, cold shock, and various carbon sources on esterase and lipase activities and exopolysaccharide production by a psychrotrophic Acinetobacter sp. *Canadian Journal of Microbiology 47*(3), 194-205.

Batstone, D, Keller, J, & Newel, B. (2007). Model development and full scale validation for anaerobic treatment of protein and fat based wastewater. *Water Science and Technology, 36*(6-7), 423-431.

Becker, P., Koster, D., Popov, M.N., Markossian, S., Anlianileian, G., & Markl, H. (1999). The biodegradation of olive oil and the treatment of lipid-rich wool scouring wastewater under aerobic thermophilic conditions. *Water Research, 33*, 653-660.

Beisson, F., & Tiss. (2000). Methods for lipase detection and assay: A critical review. *European Journal of Lipid Science and Technology, 102*(2), 133-153.

Bridges, O. (2003). Double trouble: health risks of accidental sewage release. *Chemosphere, 52*, 1373-1379.

Brooksbank, A.M., Latchford, J.W., & Mudge, S.M. (2006). Degradation and modification of fats, oils and grease by commercial microbial supplements. *World Journal of Microbiology and Biotechnology, 23*(7), 977-985. doi: 10.1007/s11274-006-9323-1

Cammarota, M. C., & Freire, D. M. (2006). A review on hydrolytic enzymes in the treatment of wastewater with high oil and grease content. *Bioresource Technology, 97*(17), 2195-2210. doi: 10.1016/j.biortech.2006.02.030

Canakci, M. (2007). The potential of restaurant waste lipids as biodiesel feedstocks. *Bioresource Technology, 98*(1), 183-190. doi: 10.1016/j.biortech.2005.11.022

Chakraborty, C., Chowdhury, R., & Bhattacharya, P. (2011). Experimental studies and mathematical modeling of an up-flow biofilm reactor treating mustard oil rich wastewater. *Bioresource Technology, 102*(10), 5596-5601. doi: 10.1016/j.biortech.2011.02.010

Chao, AC., & Yang, W. (1981). Biological treatment of wool scouring wastewater. *Journal of the Water Pollution Control Federation, 53*(3), 311-317.

Damasceno, F. R., Cammarota, M. C., & Freire, D. M. (2012). The combined use of a biosurfactant and an enzyme preparation to treat an effluent with a high fat content. *Colloids Surf B Biointerfaces, 95*, 241-246. doi: 10.1016/j.colsurfb.2012.03.003

Damasceno, F. R., Freire, Denise M. G., & Cammarota, Magali C. (2008). Impact of the addition of an enzyme pool on an activated sludge system treating dairy wastewater under fat shock loads. *Journal of Chemical Technology & Biotechnology, 83*(5), 730-738. doi: 10.1002/jctb.1863

Dharmsthiti, S., & Kuhasuntisuk, B. (1998). Lipase from Pseudomonas aeruginosa LP602: Biochemical properties and application for wastewater treatment. *Journal of Industrial Microbiology & Biotechnology, 21*(1-2), 75-80.

Dong, H., Gao, S., Han, S. P., & Cao, S.G. (1999). Purification and characterization of a Pseudomonas sp. lipase and its properties in non-aqueous media. *Biotechnology and Applied Biochemistry, 30*(3), 251-256.

Eltaweel MA., Abd Rahman RNZR., Salleh AB., & Basri, M. (2005). An organic solvent - stavle lipase from Bacillus sp. strain 42. *Ann Microbiol, 55*, 187-192.

George, Tchobanoglous;, Franklin, L., Burton;, & Stensel, H. David. (1991). *Wastewater Engineering. Treatment Disposal Reuse*. New York: McGraw-Hill.

Ghosh, P.K., & Saxena, R.K. (1996). Microbial lipases: production and application. *Science progress, 79*.

Greenberg, A.E., Clesceri, L.S., & Eaton, A.D. (1992). *Standard Methods for the Examination of Water and Wastewater*. New York: American Public Health Association, American Water Works Association, Water Pollution Control Federation.

Gupta, R., Gupta, N., & Rathi, P. (2004). Bacterial lipases: an overview of production, purification and biochemical properties. *Appl Microbiol Biotechnol, 64*(6), 763-781. doi: 10.1007/s00253-004-1568-8

Hanaki, K., Nagase, M., & Matsuo, T. (1981). Mechanism of Inhibition Caused by Long-chain Fatty-acids in Anaerobic-digestion Process. *Biotechnology and Bioengineering, 23*(7), 1591-1610.

He, X., de los Reyes, F. L., 3rd, Leming, M. L., Dean, L. O., Lappi, S. E., & Ducoste, J. J. (2013). Mechanisms of fat, oil and grease (FOG) deposit formation in sewer lines. *Water Research, 47*(13), 4451-4459. doi: 10.1016/j.watres.2013.05.002

He, X., Iasmin, M., Dean, L. O., Lappi, S. E., Ducoste, J. J., & de los Reyes, F. L., 3rd. (2011). Evidence for fat, oil, and grease (FOG) deposit formation mechanisms in sewer lines. *Environ Sci Technol, 45*(10), 4385-4391. doi: 10.1021/es2001997

I. Angelidaki, L. Ellegaard, & Ahring, B.K. (1992). Compact automated displacement gas metering system for measurement of low gas rates from laboratory fermentors. *Biotechnol Bioeng, 39*, 351-353.

IAPMO, International Association of Plumbing and Mechanical Officials. (2006). Uniforma plumbing code (UPC). Ontario, Canada.

Iggleden, G.J. (1978). Design and Application of Tilted Plate Separator Oil Interceptors. *Chemistry & Industry*(21), 826-831.

Immanuel G., Esakkiraj P., Jebadhas A., Iyapparaj P., & A., Palavesam. (2008). Investigation of lipase production by milk isolate Serratia rubidae. *Food Technol Biotechnol, 20*, 60-65.

Irfan, M., Ahmad, A., & Hayat, S. (2014). Effect of cadmium on the growth and antioxidant enzymes in two varieties of Brassica juncea. *Saudi Journal of Biological Sciences, 21*(2), 125-131.

Jeganathan, J., Bassi, A., & Nakhla, G. (2006). Pre-treatment of high oil and grease pet food industrial wastewaters using immobilized lipase hydrolyzation. *J Hazard Mater, 137*(1), 121-128. doi: 10.1016/j.jhazmat.2005.11.106

Keener, Kevin M., Ducoste, Joel J., & Holt, Leon M. (2008). Properties Influencing Fat, Oil, and Grease Deposit Formation. *Water Environment Research, 80*(12), 2241-2246. doi: 10.2175/193864708x267441

Kim, Sang-Hyoun, Han, Sun-Kee, & Shin, Hang-Sik. (2004). Kinetics of LCFA Inhibition on Acetoclastic Methanogenesis, Propionate Degradation and β-Oxidation. *Journal of Environmental Science and Health, Part A, 39*(4), 1025-1037. doi: 10.1081/ese-120028411

Kuo, Chin-Hsing, & Lee, Chon-Lin. (2009). Treatment of a Cutting Oil Emulsion by Microwave Irradiation. *Separation Science and Technology, 44*(8), 1799-1815. doi: 10.1080/01496390902775869

Lanser, A.C., & Manthey, L.K. (2002). Regioselectivity of new bacterial lipases determined by hydrolysis of triolein. *Current Microbiology, 44*(5), 336-340.

Le, TV., Imai, T., Higuchi, T., Doi, R, Teeka, J., & Sun, XF. (2012). Separation of Oil-in-Water Emulsions by Microbubble treatment and the Effect of Adding coagulant or Cationic Surfactant on Removal Efficiency *Water Science and Technology, 66*(5), 1036-1043. doi: 10.2165/wst.2012.276

Leal, Márcia C. M. R., Freire, Denise M. G., Cammarota, Magali C., & Sant'Anna, Geraldo L. (2006). Effect of enzymatic hydrolysis on anaerobic treatment of dairy wastewater. *Process Biochemistry, 41*(5), 1173-1178. doi: 10.1016/j.procbio.2005.12.014

Lee, S. Y., & Rhee, J. S. (1994). Hydrolysis of triglyceride by the whole cell of pseudomonas putida 3SK in two-phase batch and continuous reactor systems. *Biotechnology and Bioengineering, 44*(4), 437-443.

Lissant, K. J. (1974). *Emulsions and emulsion technology Part I* (Vol. 1). New York: Marcel Dekker.

Long, J. Hunter, Aziz, Tarek N., Reyes, Francis L. de los, & Ducoste, Joel J. (2012). Anaerobic co-digestion of fat, oil, and grease (FOG): A review of gas production and process limitations. *Process Safety and Environmental Protection, 90*(3), 231-245. doi: 10.1016/j.psep.2011.10.001

Mahdi, Bayan A., Bhattacharya, Amrik, & Gupta, Anshu. (2012). Enhanced lipase production from Aeromonas sp. S1 using Sal deoiled seed cake as novel natural substrate for potential application in dairy wastewater treatment. *Journal of Chemical Technology & Biotechnology, 87*(3), 418-426. doi: 10.1002/jctb.2740

Mahler, G. F., Kok, R. G., Cordenons, A., Hellingwerf, K. J., & Nudel, B. C. (2000). Effects of carbon sources on extracellular lipase production and lipA transcription in Acinetobacter calcoaceticus. *Journal of Industrial Microbiology & Biotechnology, 24*(1), 25-30.

Markossian, S., Becker, P., Markl, H., & Antranikian, G. (2000). Isolation and Characterization of Lipid-degrading Bacillus Thermoleovorans IHI-91 from an Icelandic Hot Spring. *Extremphiles, 4*(6), 365-371.

McMurry, J. (1997). *Organic Chemistry*

Mendes, AA., de Castro, HF., Pereira, EB., & Furigo, A. (2005). Application of lipases for wastewater treatment containing high levels of lipids. *QUIMICA NOVA, 28*(2), 296-305.

Mobarak-Qamsari, E., Kasra-Kermanshahi, R., Nosrati, M., & Amani, T. (2012). Enzymatic Pre-Hydrlysis of High Fat Content Dairy Wastewater as a Pretreatment for Anaerobic Digestion. *International Journal of Environmental Research, 6*(2), 475-480.

Mohamed, H., El-Masry, El-Bestawy., Ebtesam, & El-Adl., Nawal I. (2004). Bioremediation of Vegetable Oil and Grease from Polluted Wastewater Using a Sand Biofilm System. *Journal of Microbiology & Biotechnology, 20*, 551-557.

Monterfrio, M.J., Tai, X., & Obbard, J.P. (2010). Recovery and pretreatment of fats, oil and grease from grease interceptors for biodiesel production. *Applied Energy, 87*, 3155-3161.

Murthy, S., Bali, G., & Sarangi, SK. (2014). Effect of lead on growth, protein and biosorption capacity of Bacillus cereus isolated from industrial effluent. *Journal of Environmental Biology, 35*(2), 407-411.

Nisola, G.M., Cho., Eul Saeng, Shon., Ho Kyong, & Tian., Dan. (2009). Cell immobilized FOG-Trap system for Fat, Oil, and Grease Removal from Restaurant Wastewater. *Journal of Environmental Engineering, 135*, 876-884.

Novak, J.T., & Carlson, D.A. (1970). The Kinetics of Anaerobic Long Chain Fatty Acid Degradation. *Journal of Water Pollution and Control, 42*(11), 1932-1943.

Nunn, WD. (1986). A Molecular View of Fatty-Acid Catabolism in Escherichia-Coli. *Microbiological Reviews, 50*(2), 179-192.

Perle, M., KImchie, Sh., & Shelef, G. (1995). Some biochemical aspects of anaerobic degradation of dairy wastewater. *Water Research, 29*, 1549-1554.

Rashid, Naeem, & Imanaka, Tadayuki. (2008). Efficient Degradation of Grease Using Microorganisms. *Journal of The Chemical Society of Pakistan, 30*(4), 612-617.

Rathi, P., Saxena, R.K., & Gupta, R. (2001). A novel alkaline lipase from Burkholderia cepacia for detergent formulation. *Process Biochemistry, 37*(2), 187-192.

Ratledge, C. (1992). Microbial Oxidations of Fatty Alcohols and Fatty Acids. *Journal of Chemical Technology and Biotechnology, 55*(4), 399-400.

Rattanapan, Cheerawit, Sawain, Aneak, Suksaroj, Thunwadee, & Suksaroj, Chaisri. (2011). Enhanced efficiency of dissolved air flotation for biodiesel wastewater treatment by acidification and coagulation processes. *Desalination, 280*(1-3), 370-377. doi: 10.1016/j.desal.2011.07.018

Reddy, K., Drysdale, GD., & Bux, F. (2003). Evaluation of activated sludge treatment and settleability in remediation of edible oil effluent. *Water SA, 29*(3), 245-250.

Rinzema, A., Boone, M., Van Knippenberg, K., & Lettinga, G. (1994). Bactericidal Effect of Long Chain Fatty Acids in Anaerobic Digestion. *Water Environmental Research*(66), 40-49.

Rosa, D. R., Duarte, I. C., Saavedra, N. K., Varesche, M. B., Zaiat, M., Cammarota, M. C., & Freire, D. M. (2009). Performance and molecular evaluation of an anaerobic system with suspended biomass for treating wastewater with high fat content after enzymatic hydrolysis. *Bioresour Technol, 100*(24), 6170-6176. doi: 10.1016/j.biortech.2009.06.089

Roy, F., Samain, E., Dubourguier, H.C., & Albagnac, G. (1986). A New Obligately Proton Reducing Anaerobe Oxidizing Saturated and Unsaturated Long CHain Fatty Acids. *Arch. Mircobiology, 11*(145), 142-147.

Sharma. R., & Soni, S.K. (2002). Production of extracellular alkaline lipase from a Bacillus sp. RSJ1 and its application in ester hydroysis. *Indian Journal of Microbiology, 42*(1), 49-54.

Shon., Ho Kyong;, Dan, Tian;, Dae-young, Kwon;, Chang-suk, Jin;, Tae-jong, Lee;, & Wook-Jin, Chung;. (2002). Degradation of fat, oil, and grease (FOGs) by lipase-producing bacterium Pseudomonas sp strain D2D3. *Journal of Microbiology & Biotechnology, 12*(4), 583-591.

Sorenson, Kyle. (2009). *Oil and Grease Removal Technologies*. Paper presented at the EPA Region 8 Conference.

Sugihara, A., & Tani, T. (1991). Purification and characterization of a noval thermostable lipase from Bacillus sp. *Journal of Biochemistry, 109*(2), 211-216.

Tang, H. L., Xie, Y. F., & Chen, Y. C. (2012). Use of Bio-Amp, a commercial bio-additive for the treatment of grease trap wastewater containing fat, oil, and grease. *Bioresource Technology, 124*, 52-58. doi: 10.1016/j.biortech.2012.08.012

Tano-Debrah, K., Fukuyama, S., Otonari, N., Taniguchi, F., & Ogura, M. (1999). An inoculum for the aerobic treatment of wastewater with high concentrations of fats and oils. *Bioresource Technology*(74), 231-239.

Tansel, B., & Pascual, B. (2011). Removal of emulsified fuel oils from brackish and pond water by dissolved air flotation with and without polyelectrolyte use: pilot-scale investigation for estuarine and near shore applications. *Chemosphere, 85*(7), 1182-1186. doi: 10.1016/j.chemosphere.2011.07.006

Tir, M., & Moulai-Mostefa, N. (2008). Optimization of oil removal from oily wastewater by electrocoagulation using response surface method. *J Hazard Mater, 158*(1), 107-115. doi: 10.1016/j.jhazmat.2008.01.051

Wakelin, N.G., & Forster, C.F. (1997). An investigation into microbial removal of fats oils and greases. *Bioresource Technology*(59), 37-43.

Wang, Y., & Srivastava, K.C. (1995). Thermostable alkaline lipase from a newly isolated thermophilic Bacillus, strain A30-1 (ATCC 53841). *Journal of Fermentation and Bioengineering, 79*(5), 433-438.

Willey, R. (2001). Fats, oils, and greases: the minimization and treatment of wastewaters generated from oil refining and margarine production. *Ecotoxicol Environ Saf, 50*(2), 127-133. doi: 10.1006/eesa.2001.2081

Williams, J. B., Clarkson, C., Mant, C., Drinkwater, A., & May, E. (2012). Fat, oil and grease deposits in sewers: characterisation of deposits and formation mechanisms. *Water Research, 46*(19), 6319-6328. doi: 10.1016/j.watres.2012.09.002

Yang, Bin, Chen, Guanghao, & Chen, Guohua. (2012). Submerged membrane bioreactor in treatment of simulated restaurant wastewater. *Separation and Purification Technology, 88*, 184-190. doi: 10.1016/j.seppur.2011.12.026

Appendix A: Raw Data

Field sampling raw data(Winter-Spring Term)

Date	Dec 17	Dec 20	Jan 17	Jan 31	Feb 21	Mar 14	Mar 28	Apr 18	May 2
PS 155									
pH	8.52	8.01	8.39	8.26	7.91	7.09	8.39	8.45	8.34
COD(mg/L)	300.0	210.0	320.0	260.0	106.7	466.7	320.0	255.0	279.0
T(℃)	13.0	14.0	13.5	11.8	12.0	11.2	12.5	12.5	13.0
FOG(mg/L)	104.0	77.0	48.0	63.0	254.0	501.0	546.0	517.0	487.0
Ammonium (mg/L)	44.0	55.5	14.5	29.0	35.5	36.1	41.2	34.3	32.2
Nitrate (mg/L)	1.0	0.2	4.5	2.5	1.2	1.2	1.3	1.4	1.3
PS 202									
pH	8.27	7.60	8.57	8.11	7.68	6.79	7.90	8.50	8.05
COD(mg/L)	450.0	250.0	280.0	280.0	146.7	613.3	286.7	328.0	224.0
T(℃)	17.3	17.8	16.5	15.0	15.5	16.8	17.0	17.0	18.0
FOG(mg/L)	91.0	110.0	62.0	122.0	428.0	774.0	592.0	564.0	585.0
Ammonium (mg/L)	44.6	43.5	26.2	5.0	47.6	47.1	26.3	49.7	39.3
Nitrate (mg/L)	2.3	0.2	3.7	12.0	0.9	1.5	1.3	5.2	1.6

Field sampling raw data(Summer-Autumn Term)

Date	Jul.31	Aug.2	Aug.7	Aug.13	Aug.22	Aug.30	Sep.5	Sep.12	Sep.13	Sep.26	Oct.4	Oct.11	Oct.18
PS 155													
pH	8.25	8.41	8.39	8.5	8.34	8.35	7.93	8.52	8.32	8.42	8.11	8.43	7.28
COD(mg/L)	210.0	210.0	150.0	210.0	110.0	149.0	227.0	156.7	176.5	186.2	200.1	185.0	95.0
T(℃)	15.6	16.2	16.0	17.2	16.9	18.3	19.2	19.7	20.0	18.0	16.3	16.4	16.7
FOG(mg/L)	102.0	122.0	82.0	103.0	57.3	53.3	45.3	97.3	128.7	88.7	206.0	57.3	66.0
Ammonium (mg/L)	29.8	35.1	36.0	55.6	37.1	40.6	31.9	31.0	44.5	29.0	31.1	25.7	25.4
Nitrate (mg/L)	2.6	1.6	1.4	1.7	1.5	1.4	1.7	2.0	1.7	1.8	1.7	1.6	1.5
PS 202													
pH	7.51	7.81	7.72	7.51	7.51	7.80	7.55	8.16	7.76	8.00	7.84	8.23	7.25
COD(mg/L)	280.0	230.0	170.0	140.0	126.0	108.0	276.0	163.2	224.8	230.8	210.7	234.7	99.7
T(℃)	21.6	22.4	21.4	22.2	22.0	21.1	20.6	22.8	24.3	20.2	18.5	20.3	19.7
FOG(mg/L)	97.0	77.0	90.0	100.0	106.0	25.3	43.3	110.0	123.3	56.7	163.3	52.0	68.0
Ammonium (mg/L)	26.2	24.6	16.8	15.6	27.2	9.3	17.2	25.0	18.5	21.6	14.5	18.5	12.6
Nitrate (mg/L)	1.5	1.4	1.4	1.8	1.5	1.5	3.4	1.3	2.0	2.2	1.8	1.6	2.2

Metal ion raw data (Winter-Spring Term)											
Date	PS	B	Na	Mg	Si	P	K	Ca	Fe	Zn	Sr
Dec 17	155	NA	46.5322	13.7736	1.8945	2.8920	14.2711	75.6570	0.0000	0.0514	0.6542
	202	0.1022	74.8804	22.3678	3.5564	3.8402	26.8977	129.6409	2.5203	0.0202	0.8458
Dec 20	155	0.0115	43.0281	14.3396	2.4052	4.5991	16.3975	77.3501	0.2604	0.0464	0.7323
	202	0.1048	61.2310	34.1501	3.8352	4.0048	20.9187	162.8512	1.2949	0.0901	1.1339
Jan 17	155	0.0678	57.8455	13.5465	2.1239	2.5756	12.7011	77.1662	0.2636	0.0596	0.7180
	202	0.0000	85.9913	17.7378	2.7284	3.2459	23.0328	94.0834	0.6593	0.0327	0.7745
Jan 31	155	0.0000	52.4531	13.0303	2.2578	3.2150	16.4016	83.9870	0.0000	0.1511	0.7103
	202	0.0485	60.5773	20.1071	2.1256	3.3810	25.0649	99.9426	0.5981	0.0453	0.8460
Feb 21	155	0.1304	49.8821	16.1809	3.3103	3.3232	17.2509	94.3749	1.4570	0.0926	0.7396
	202	0.0254	50.3966	16.8485	3.9379	3.2137	21.5059	97.9709	0.2892	0.2876	0.8236
Mar 14	155	0.1205	48.3358	11.8515	3.3421	2.5828	12.6493	40.4650	0.0000	0.1871	0.5818
	202	0.0000	68.5210	17.3791	2.6489	2.8116	19.5258	102.7135	0.1594	0.3193	0.8000
Mar 28	155	0.0292	72.4222	13.5992	2.0154	2.6862	13.7677	78.6597	0.0000	0.1185	0.7817
	202	0.1557	62.0758	15.3858	4.6666	2.1492	15.9062	97.2546	0.2186	1.1774	0.7630
Apr 18	155	0.0322	59.3904	15.7694	4.5403	3.6807	15.0785	90.8269	0.2136	0.0632	0.7746
	202	0.1080	104.3356	23.9210	3.8191	6.1478	25.7015	159.8655	0.7485	0.0396	1.0806
May 2	155	0.1500	60.9148	15.2269	3.7304	2.0434	12.1377	102.4377	0.0000	0.1129	0.7803
	202	0.0000	71.3324	22.3019	3.1495	3.3599	20.9706	138.1074	0.4639	0.1177	0.9533

Metal ion raw data (Summer-Autumn Term)											
Date	PS	B	Na	Mg	Si	P	K	Ca	Fe	Zn	Sr
Jul 31	155	0.3745	78.6233	20.1818	4.6071	2.6761	15.3657	158.4258	0.7791	0.2151	1.0164
	202	0.0810	67.0617	27.2851	3.9542	2.0801	15.2953	166.1757	0.8683	0.0478	1.0539
Aug 2	155	0.2181	66.9583	17.6223	4.8079	3.0087	16.7264	118.6079	0.9681	0.8168	0.7958
	202	0.0597	76.2225	37.6657	4.2275	1.8755	1.3356	182.9927	0.8107	0.4993	1.2964
Aug 7	155	0.1522	49.2378	17.3663	4.0487	3.0605	14.7451	120.9161	0.6846	0.4111	0.8073
	202	0.0640	63.7568	30.0089	4.1706	3.0215	17.4702	209.6550	1.3613	0.3844	1.2239
Aug 13	155	0.2650	58.2372	16.2790	5.6578	5.2973	18.9845	112.9075	1.2391	0.3401	0.7742
	202	0.1591	65.9758	32.7555	4.9989	3.7974	15.8255	201.2124	1.4349	0.4525	1.1696
Aug 22	155	0.2792	55.3379	15.7863	3.7138	2.8747	15.9095	104.9223	0.7626	0.4030	0.7431
	202	0.3608	74.5587	27.0775	4.3235	1.9554	15.3905	91.0378	0.7908	0.4223	1.1418
Aug 30	155	0.0985	43.0308	16.6459	4.2721	3.6232	17.6795	111.2327	1.0256	0.5091	0.7791
	202	0.0074	48.1781	29.4626	3.3001	0.9655	11.7762	179.1194	0.7843	0.3052	1.1981
Sep 5	155	0.1812	72.2858	20.5582	6.1350	4.0203	24.1063	140.6451	3.8048	0.4170	0.8314
	202	0.0090	51.6040	25.3967	5.3871	1.5894	2.7759	203.0657	0.7486	0.2904	1.0009
Sep 12	155	0.0864	47.9052	15.9093	3.0417	3.3984	17.2116	98.3434	1.0923	0.3545	0.7237
	202	NA	52.6425	22.2707	3.9910	4.0768	24.8468	153.8126	0.7192	0.6279	0.9524
Sep 13	155	0.0649	39.1737	13.9605	2.2198	4.1712	19.4611	90.1397	0.5256	0.3924	0.6567
	202	0.0303	60.0270	24.9573	3.5904	2.6798	16.5363	136.4247	1.4414	0.6114	0.9758
Sep 26	155	0.4279	62.3462	12.5381	3.4243	2.8823	13.8740	73.0278	0.4122	0.6231	0.6080
	202	0.0187	53.4414	21.5364	3.8853	3.9332	20.3895	107.9862	1.0972	0.4912	0.8953
Oct 4	155	0.2213	50.9963	14.0212	3.2787	2.5695	14.4665	94.3696	0.1378	0.4681	0.7445
	202	NA	47.5331	19.0204	1.9898	1.6113	16.8067	128.4835	NA	0.1438	0.8633
Oct 11	155	0.1711	52.8031	14.4805	2.0036	3.2724	14.9964	90.4910	0.4738	0.0722	0.7550
	202	0.0062	62.8529	20.8873	3.0264	3.1429	17.9981	122.4807	0.1642	0.0602	0.8520
Oct 18	155	0.2855	62.4356	23.1338	2.5549	3.6604	16.8038	168.6979	0.2149	0.0678	1.2603
	202	0.1677	44.4077	17.3200	5.3267	1.8122	13.5435	108.5364	0.3994	0.0956	0.8003

Bench-scale experiment (dosage) raw data

Bacteria (CFU/mL)	Initial FOG (mg/L)	Final FOG (mg/L)	Removal (%)	Initial COD (mg/L)	Final COD (mg/L)
No	250.8	222.5	11.30	645	1294
	249.3	231.3	7.22		1149
	251.2	229.7	8.56		1234
10^9	249.7	148.8	40.43	669	1487
	251.4	148.7	40.86		1468
	248.0	143.7	42.07		1456
10^6	252.4	163.3	35.32	664	1263
	249.6	159.9	35.93		1144
	250.0	162.0	35.19		1076

Bench-scale experiment (COD) raw data

Initial FOG (mg/L)	Final FOG (mg/L)	Removal (%)	Initial COD (mg/L)	Final COD (mg/L)
251.4	229.8	8.60	0	190
249.4	227.4	8.81	0	172
251.3	232.4	7.53	0	170
251.6	115.6	54.07	45	255
249	112.5	54.83	42	330
249.8	113.3	54.63	40	292
248.6	136.4	45.15	276	473
251.3	135.1	46.23	257	447
248.9	138.7	44.26	283	528
250.7	151.8	39.45	485	626
249.9	141.5	43.39	477	690
250.5	140.9	43.76	463	710

Bench-scale experiment (temperature) raw data

Temperature	Initial FOG (mg/L)	Final FOG (mg/L)	Removal (%)	Initial COD (mg/L)	Final COD (mg/L)
15℃	250.4	174.6	30.28	250	406
	248.8	175.6	29.44		412
	251.2	176.4	29.76		393
22℃	251.0	151.8	39.53		426
	247.0	152.7	38.18		413
	251.2	156.1	37.87		421
32℃	250.5	133.6	46.67		453
	249.2	128.3	48.51		462
	249.7	127.6	48.90		441