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Study of the Bioaugmentation of Grease Separators Using the GOR BioSystem™

Master of Science Thesis, Biotechnology

ALINE DE SANTA IZABEL ALVES

Department of Chemical and Biological Engineering
CHALMERS UNIVERSITY OF TECHNOLOGY
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Examiner: Professor Christer Larsson, Chalmers University of Technology
Supervisor: Niklas Axelsson, Bioteria Technologies

Department of Chemical and Biological Engineering
Chalmers University of Technology
SE-412 96 Göteborg
Sweden
Telephone + 46 (0)31-772 1000

This thesis work was conducted at Bioteria Technologies in Täby, Sweden.

Bioteria Technologies
Tumstocksvägen 13
187 66 Täby
Sweden
Telephone: +46 (0) 8540 889 00
http:// www.bioteria.com

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Dedication

To my beloved grandma **Vanda** and to my parents **Alzidea** and **Jorge Luiz**.

For making me believe that everything is possible, encouraging me to follow my dreams whatever and wherever they were. Your love and support have carried me through.

Abstract

Grease separators (GS) are the first preventive strategy to reduce the entry of fats, oils and grease (FOG) into the sewage system. Since its patent in 1883, very little has been developed in order to improve the function and maintenance of these devices. One of the strategies to upgrade the GS performance is through the use of bioaugmentative systems. However, limited scientific-based knowledge of the performance and bioaugmentation of GS is available to promote sound regulations regarding the use, maintenance and improvement of GSs in Sweden. Thus, an important consideration of this study was to evaluate the function of accepted GSs and the effects of bioaugmentative systems in its performance. Grease separators from three different food-producing establishments (FSEs) (one restaurant from an elementary school and two full service restaurants) located in Danderyd and Stockholm municipalities were evaluated. The GS of one of the full service restaurants was treated with a bioaugmentative system (GOR BioSystem™) and monitored for changes in the effluent quality and FOG accumulation compared to an untreated control period. This evaluation consisted of two cycles, lasting approximately one month (five weeks) each, which was in accordance to the regulation for the intervals between the pump outs in the Stockholm region. The GS from an elementary school in Danderyd municipality was treated and monitored for ten weeks with the same bioaugmentative system (GOR BioSystem™) while one untreated standard GS from a full service restaurant in Stockholm was monitored for five weeks as a control. Regular analysis including FOG (either ether soluble, emulsified and separable fat), biochemical oxygen demand, chemical oxygen demand, total suspended solids, pH, temperature, conductivity, grease cap thickness and bottom solids accumulation and odor was performed. In general, the chemical and physical parameters were similar in both treated and untreated cycles in the full service restaurant analyzed with and without the treatment with GOR BioSystem™. However, lower levels of grease cap development and solids accumulation and odor were achieved in the treated cycle. No grease accumulation was detected in the GS bioaugmented for ten weeks while a considerable amount of both grease and solids accumulated in the control untreated GS after 25 activity days. There were no indications that the bioaugmentation promoted by GOR BioSystem™ led to a deterioration of the effluent quality or released additional amounts of oil and greases in the collection system. Furthermore, it is expected that the use of GOR BioSystem™ can improve the rationalization of the handling of organic waste with the reduction of waste transports.

Keywords: bioaugmentation, *Bacillus* sp., grease separators, wastewater treatment.

Abbreviations

BOD – Biochemical oxygen demand

BOD 7 - Biochemical oxygen demand analyzed after 7 days

COD - Chemical oxygen demand

d – Days

EN- European norm

FOG – Fats, oils and grease

FSE – Food service establishments

GS – Grease separator

WW – Wastewater

ppm – Particles per milion

SS – Swedish standard

SSOs - Sanitary sewer overflows

TN – Total nitrogen

TP – Total phosphorus

TSS – Total suspended solids

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1. Introduction

1.1. FOG-rich residues management

Fats, oils and greases (FOG) management is considered as one of the biggest contemporary challenges of our society. FOG-rich residues present low degradability and the increase of the industrial, commercial and domestic activities, which result in the production of considerable amounts of FOG-rich residues, is of concern. Different FOG management strategies have been continuously developed in order to avoid an environmental catastrophe in the future (Stoll & Gupta 1997, Wakelin & Foster 1997, El-Marsy *et al.* 2004, Montefrio *et al.* 2010, Razaviarani *et al.* 2013).

Fat-rich solid residues and wastewater are produced by the industrial, commercial and residential use of food, hygiene and oil based products such as lubricants, for example. They are generally a problem due to the accumulation in our environment and the odor that they can produce. In kitchen ventilation systems, fat build up prevents heat recovery, increases the risk of fire and require the use of strong chemicals for cleaning. In wastewater, it can build up in the drains causing stoppings, overflows, corrosion, destruction of pumping stations and odor disturbances (Baig and Grenning, 1976). It's also considered a problem due to the creation of anoxic conditions in water systems (Adam *et al.* 2000, Wiyada & Saovanee, 2002).

Figure 1: Problems related to residential and commercial fat processing procedures (Bioteria Technologies 2012).

Increasing fat-rich waste amounts are annually produced, demanding the development of appropriate treatment strategies. Different methods have been developed aiming the treatment of these residues (Wakelin & Foster 1997, Cammarota & Freire 2006, Valladão *et al*., 2007). Also, alternative methods to bio-valorize these materials have been proposed as the use as substrate to produce value added compounds (e.g. microbial lipases, bio lubricants, biogas, cosmetics, etc.) (Montefrio *et al.* 2010, Treichel *et al*., 2010). However, so far, there is no economical, stable, and efficient, easy-to-use and operate process available to stabilize, deodorize or recover energy from commercial and residential fat-rich wastewater and grease separators (GS) solid residues.

1.2. Bioteria Technologies

This project was undertaken at Bioteria Technologies AB in Täby/ Stockholm region, Sweden. Bioteria Technologies AB is a clean-tech Swedish company that develops and market industrial biotechnology based products and services for sustainable buildings, wastewater treatment and waste management.

Bioteria has an intensive research and development of specific products and services for grease removal from wastewater and ventilation systems and also odor control of organic wastes from food producing establishments (FSE).

Figure 2: Bioteria's bio-solutions for kitchen ventilation systems, grease separators and garbage rooms (Adapted from Bioteria Technologies 2013).

1.2.1. GOR BioSystem™

GOR BioSystem™ is a patented technique developed by Bioteria Technologies AB, which aims the reduction of FOG and odor in GSs. This technique is based on a biological process, which is similar to the one used in wastewater treatment plants. This system is based on the inoculation of selective and adapted fat degrading bacteria that stimulate growth of the indigenous aerobic microorganisms present in GSs.

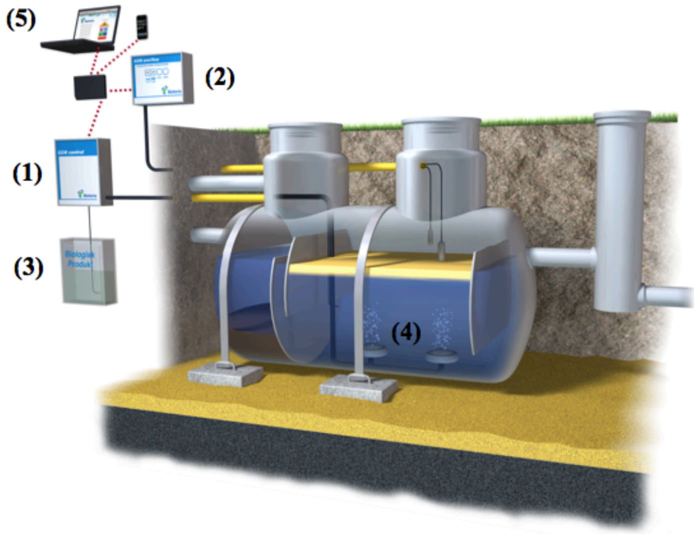


Figure 3: GOR BioSystem™ and its main components. (1) GOR Control, (2) GOR overflow, (3) Bio G, (4) aeration system and (5) service/ online control (Bioteria Technologies 2012).

1.2.1.1. Components

GOR BioSystem™ is composed of a biological product (Bio G) and a patented technique. This system can be installed to a pre-existent GS or in a new GS developed by Bioteria. A description of each of the main components (GOR control, Bio G, aeration system and service) and its main characteristics is given bellow.

- GOR Control

GOR Control is a command control cabinet, which regulates the delivers of air and bioproduct doses in each daily cycle inside of the GS. This dosage is performed in a prescheduled fashion. Its also coupled to the a sensor (GOR overflow) which monitor the water and grease cap levels. The air is introduced continuously every night during the treatment period, while the bioproduct is dosed in the beginning of each treatment cycle only.

- Bio-G

Bio-G contains four special bacterial (*Bacillus* sp.) strains with special fat degrading capabilities. The product is presented in a liquid form, with a certain viscosity that slow down the bacterial sedimentation. The pH level is between 7,0 and 7,5 and bacterial concentration is $1,8 \times 10^8$ spores/ml. The daily dosage is of approximately 100 ml.

- Aeration System

Oxygen (O_2) is a key factor for the complete oxidation of organic compounds. The aeration system is composed of air plates positioned in the bottom of the GS. The number of air plates varies depending on the GS dimensions. Biological aerated systems such as GOR BioSystem™ require a constant addition of air in order to maintain biomass activity. The aerobic environment in the GS is achieved through continuous diffused aeration during the process time of GOR BioSystem™. The air bubbles generated by the air plates increase the oxygen transfer potential.

1.2.1.2. Function

Basically, the GS that composes the GOR BioSystem™ works as a regular GS during the period of regular kitchen activity and as a bioreactor when the kitchen activity ceases and the flow is interrupted. The function of a regular GS will be further explained in the section 2.2. (Grease separators).

The main concern regarding the application of the GOR Biosystem™ is about the factors which can impair the system's function (bioactivity). Among those factors, its possible to mention: physical (temperature, pH) and chemical factors (detergents, chemical agents such as chlorine). The impact that such factors can display in the bioprocess will be further explained in the chapter 2.3.4. (Main concerns regarding bioaugmentative systems and products).

In order to have a better understanding regarding the factors necessary for the evaluation of the effect of this bioaugmentative technique in GSs of large-scale kitchens, key concepts will be further explored in the next chapter.

2. Background

2.1. FOG-rich wastewater

As aforementioned, FOG is a proeminent environmental problem of our society (Stoll & Gupta 1997, Alade *et al.* 2011). FOG- rich wastewater can cause a series of problems in both sewers and wastewater treatment plants worldwide. It can significantly increase operational costs of a sewer system as well as affect the ability of a sewage treatment plant to maintain compliance with their wastewater treatment permit. In wastewater, when released into collection systems, FOG has a tendency to adhere to pipe walls, reducing their capacities. Frequently, these pipe occlusions lead to sanitary sewer overflows (SSOs), which in turn results in the release of contaminated wastewater into the environment (Baig & Grenning 1976, He 2012).

FOG can also cause serious problems in pumping stations (Cox 2011). In such environments FOG-rich wastewater is collected and hold for an interval of time, which will lead to the separation and accumulation of FOG components. The accumulated FOG tends to cover the pumps, cause overheating and effecting the operation of the floats. All this can lead to odor related disturbances, fire hazard and ultimately the failure of the pumping station (Franke 2011).

Furthermore, the presence of FOG in wastewater also results in serious problems to biological treatment systems. High amounts of FOG in the water tends to reduce the cell-aqueous phase transfer rates of substrates, products and oxygen due to the formation of a liquid coat around the microbial flock (Becker *et al.* 1999, Dueholm *et al.* 2000), and can also result in the development of floating sludge with undesirable physical characteristics as well filamentous microorganisms blooms (bulking) (Cammarota & Freire 2006). Additionally, the accumulation of untreated wastewater leads to the decomposition of the organic materials that it contains, which will produce large quantities of malodorous and hazardous gases (Franke 2011).

The typical amount of effluent produced by a regular restaurant ranges between 2-4 gallons/ unity area/ day. Thus it is essential to understand the characteristics and load of wastewater prior further characterizations of the function and performance of a grease separation device (Gross 2004).

2.1.1. General composition of FOG-rich wastewater

FOG-rich wastewater from commercial and residential kitchens activities has a complex composition with high levels of fats and proteins. Considering its high amount of fat, it can more or less be described as an oil-in-water emulsion. The composition varies considerably depending on the activity that produces it and different methods are used to characterize these wastewaters. For instance, different authors have characterized the composition of different FSEs effluents wastewaters by evaluating the nature of the food particles (quantification of carbohydrates, proteins and lipids) and the types of oils (e.g. animal fat, vegetal oil) in order to investigate the correlation of it with FOG deposits in sewage systems (Stoll & Gupta 1997, Wakelin & Forster 1997, Cammarota *et al*. 2001, He *et al.* 2012).

FSE's wastewater can contain large amounts of fats, vegetable oils, lards, shortenings, margarine, butter, grease and other FOG-based products (Davis 2011). The characterization of the wastewater composition is important because of the effect that it may have in the sewage system maintenance and FOG deposits formation.

2.1.1.1. Fats, oils and grease

The acronym FOG commonly includes the fats, oils, waxes and other related constituents that can be found in the wastewater. The compounds, which are liquid at normal temperatures, are called oils, and the ones, which are solids in normal temperature, are called fats. Both are chemically related, composed by carbon and oxygen in different proportions (Metcalf & Eddy 1991).

FOG compounds consist primarily of triglycerides, esters of glycerol and fatty acids (Figure 4). Oil and grease can also contain lower amounts (2-3%) of phospholipids (mainly phosphoglycerides) and trace amounts of steroids and carotenoids (responsible for the color) (Mohn 1997). In waste, lipids are mainly presented as neutral fats and long-chain fatty acids (LCFAs). The profiles of fatty acids are very variable, since they can differ in chain length and be saturated or unsaturated (Valladão *et al*. 2011).

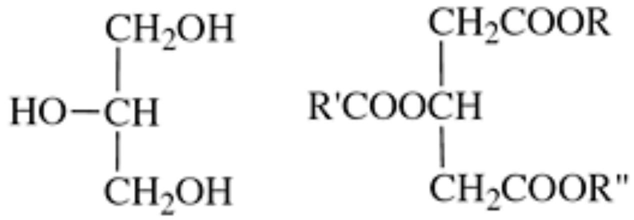


Figure 4: Chemical structure of triglycerides. Glycerol (left), the backbone of triacyl glycerol, and a triglyceride (right) (Kuchel *et al.* 2009).

FOGs are important compounds because of its difficulty of handling and treatment. It separates from water because of its low solubility, which may causes problems such as the adhesion of those compounds to the interior of pipes and tank walls, the reduction of the bio treatability of the wastewater and the production of greasy sludge solids which are difficult to process (Hammer 1996 *apud* Harty 2001).

2.1.2. Wastewater management in Sweden

Water is one of Sweden's foremost natural resources and each municipality is responsible for providing the population with water and for the management of the wastewater that is produced. The wastewater normally goes to a treatment plant where it is treated and then discharged to the receiving water that may be a lake, creek or sea. The management of the wastewater is primarily regulated by the ”Environmental Code” (Miljöbalken - 1998:808), but also by the ”Waste Ordinance” (avfallsförordiningen 2001:1063).

FOG (in special separable fat) is considered as an important wastewater pollutant, and has become an increasing problem. Table 1 shows parameters, which are considered to be primarily harmful to the sewage system. The values given in Table 1 are the values at the connection points between the property line and the sewage system (usually 0.5 meters from the property boundary). Consideration of dilution from other businesses in the same building can be taken. Damage (corrosion and blockage) can occur if one or a combination of these levels is exceeded. Thus, it’s not recommendable that these values are exceeded, even briefly. Each municipality has responsibility to regulate the emission limits and the risk of damage to their own sewage system.

The sewage treatment process is based on the settlement of solids and biological oxidation of the pollutant organic matter. Different methodologies are used depending on the wastewater characteristics. Food service establishments (FSE) affect the environment through the discharge of FOG-rich wastewater to surface watercourses and sewers. Changes in the legal and social obligations regionally and nationally in Sweden are making necessary for these food processing establishments to adopt a more specialized approach to wastewater management.

Table 1: Guideline values for substances that affect pipelines (City Council of Sala Municipality, 2003)

Parameter	Limit Value	Risk for:
pH min.	6.5	Corrosion
pH max.	11.0	Corrosion
Temperature max.	50 °C	-
Conductivity	500 mS/ m	-
Sulfate (summa sulfat SO_4^{2-}, sulfite SO_3^{2-} and thiosulfate $S_2O_3^{2-}$)	400 mg/ L	corrosion in concrete
Magnesium, Mg^{2+}	300 mg/ L	corrosion in concrete
Ammonium, NH_4^+	60 mg/ L	corrosion in concrete
Fat (separable)	50 mg/ L	Clogging

2.1.3. Parameters used to evaluate the effluent quality

The quality of the wastewater has been traditionally controlled by the use of chemical and physical parameters. The primary responsibility for the control of the water pollution lays with the local authorities. Different parameters are used to qualify the effluent wastewater and evaluate the potential of a further treatment strategy. The main parameters for wastewater characterization are the dissolved oxygen (DO), pH, temperature, conductivity, biochemical oxygen demand (BOD), chemical oxygen demand (COD), total organic carbon (TOC), total suspended solids (TSS) and FOG levels.

- Dissolved Oxygen (DO)

Analysis of the DO levels is a key test in water pollution control, wastewater treatments and bioprocesses. It’s also the basis of BOD test, which is an important parameter to evaluate the organic load of residual waters (Rice *et al.* 2012). Aerobic processes require free oxygen to maintain the metabolic processes that produce energy for growth and reproduction of the microorganisms, which participate of such processes. DO is also important in precipitation and dissolution of inorganic substances in water (Rice *et al.* 2012).

DO levels in natural and wastewaters depend on physical, chemical and biological activities in the water body. Oxygen is a poorly soluble gas, and its solubility varies directly with the atmospheric pressure at any given temperature. The solubility of atmospheric oxygen in fresh water ranges between 14.6 mg/L at 0 ^{0}C to about 7.0 mg/L at 35 ^{0}C under normal atmospheric pressure. The amount of DO in WW in movment can be between 1-2 mg/l (Gray 2012).

- pH

The pH of a solution is measured as negative logarithm of hydrogen ion concentration at a given temperature. The intensity of the acidic or basic character of a solution is indicated by pH or hydrogen ion concentration in values from 0 (extremely acid) to 14 (extremely basic or alkaline); pH 7 is considered neutral. The pH of mineral waters is between 6 and 9 (Gross 2004). In wastewater it typically tends to fall around 6.5 (Gross 2004). pH is generally constant unless the water quality changes due to anthropogenic and/or natural interferences.

The chemical properties of the organic components and wastewater microorganism's livehood are both strongly influenced by the pH (Davis 2011). Therefore, pH is an important parameter in biological wastewater treatment; very low or high pH values may be deleterious to the resident microbial population.

- Temperature

Temperature is another key parameter, which influences biochemical reactions and also the physical treatment process (Rice *et al.* 2012). Variations in temperature also affect density, which is an important property that helps determine the separation tendencies of the FOG-rich wastewater constituents (Rodis 2004, Gallimore *et al.* 2011). Time will then determinate if substances of higher or lower densities will separate as sediments or floating flocks, for example.

- Conductivity

Conductivity measurement gives an estimative of the variations in the soluble mineral contents of water measuring its ability to pass an electrical current. Conductivity in water is affected by the presence of inorganic dissolved solids such as chloride, nitrate, sulfate, and phosphate anions (ions that carry a negative charge) or sodium, magnesium, calcium, iron, and aluminum cations (ions that carry a positive charge) (Rice *et al.* 2012). Conductivity is also affected by temperature: the warmer the water, the higher the conductivity. For this reason, conductivity is generally reported as conductivity at 25 degrees Celsius (25 ^{0}C). The conductivity of pure water is about 10-6 S/c, or 1 µS/cm (Erickson 1998).

- ***Biochemical Oxygen Demand (BOD)***

Biochemical oxygen demand (BOD) is an empirical standardized laboratory test which quantify the oxygen requirement for the aerobic oxidation of degradable organic matter (e.g. FOG, carbohydrate, proteins – carbonaceous demand) and certain inorganic matter (e.g. sulphides and ferrous ions) in wastewater under controlled conditions of temperature and incubation period (Erickson 1998, Rice *et al.* 2012).

Its one of the most commonly used parameters to define the strength of wastewaters. The methodology of BOD test is to quantify the difference between initial and final dissolved oxygen (DO) of the samples after incubation. BOD is expressed in mg/L or ppm and the test is generally performed after seven days of incubation (BOD 7). Temperature variations can affect the reliability of this test, thus the test is performed at a fixed temperature.

- Chemical Oxygen Demand (COD)

The chemical oxygen demand test allows the measurement of the total quantity of oxygen required for the oxidation of the organic matter present in the wastewater to carbon dioxide and water (Erickson 1998, Rice *et al.* 2012).

It's based upon the fact that most organic compounds can be oxidized by the use of strong chemical oxidizing agents under acid conditions. Thus, it does not depend of the biological assimilability of the substances, resulting in the oxidation of a greater amount of compounds. COD values are greater than BOD values and the difference between both values can be even greater if higher amounts of biologically resistant organic matter (e.g. lignin) are present. The ratio BOD7/COD may give an idea of the ability of a substance to decompose (Erickson 1998).

- Total Organic Carbon (TOC)

In wastewater, the organic carbon is composed of a variety of organic compounds in different oxidation states (Gray 2005). Differently from BOD and COD, TOC is a more convenient and direct expression of the total organic content and it is independent of the oxidation state of the organic matter and does not measure other organically bound elements, such as nitrogen and hydrogen and inorganics which can contribute to the BOD and COD values (Erickson 1998). If a reproducible empirical relationship is established between TOC and COD or BOD, then TOC can be used to estimate the equivalent BOD or COD.

TOC is a rapid test which estimate the amount of organic carbon but which doesn't quantify the difference between the biologically and chemically oxidizable portions of organic compounds. Hence, it cannot replace BOD and COD measurements. Together with the quantification of total nitrogen and total phosphour, it is possible to use TOC values for the estimation of C:N:P ratios. The biodegradability depends largely of this ratio, which in biological treatments should be 100:5:1 (Gray 2005).

- Total Suspended Solids (TSS)

Solids may affect adversely water and effluent quality in a number of ways. Suspended solids are present in sanitary wastewater and many types of industrial wastewater. Suspended solids absorb heat from sunlight, which increases water temperature and subsequently decreases levels of dissolved oxygen (warmer water holds less oxygen than cooler water). Also, suspended particles provide attachment places for other pollutants, such as metals and bacteria. Typically, suspended solids carry a significant portion of organic material, thus significantly contributing to the organic loads of wastewater (solids can contribute up to 60 % of the BOD of a wastewater) (Erickson 1998).

Total suspended solids (TSS) is a water quality measurement of the dry-weight of particles trapped by a filter per volume of water, typically of a specified pore size (0.45 μm). The dry weight is obtained by drying the filter and its content at 103 – 105 ^{0}C of the collected material (Rice *et al.* 2012).

- Fats, Oils and Greases (FOG)

In wastewater, oil and grease can exist in different forms (free, disperse or emulsified) and the differences are based primarily on size. In a oil-water mixture, droplets with sizes greater than 150 micrometers characterize free oil, dispersed oil has a size in a range between 20 and 150 micrometers and emulsified oil has droplets typically smaller than 20 micrometers (Hu 2002).

Tests used to quantify FOG components are not able to determinate an absolute quantity of a specific substance. Rather, it quantifies groups of substances with similar physical characteristics based on their common solubility in an given extracting solvent. Examples of tests to determine the FOG constituents of a wastewater sample are total fat (ether soluble fat), emulsified fat and separable fat (APHA 1998, Rice *et al.* 2012).

Total fat is the determination of the amount of FOG, which can be extracted, in organic solvents such as ether or hexane. Emulsified fat is the amount of the FOG emulsified which also is determinate through an extractive methodology. Separable fat is the relative difference between the values of the total and emulsified fat (APHA 1998). There are different standard methods to determine those parameters. The ones used in this study will be further described in the next chapter, in the section 5.4.1.1. (FOG – ether soluble and separable fat).

2.2. Grease Separators (GS)

The current primary method for the reduction of FOG in wastewater is the use of GSs. Nathaniel Whiting patented the first GS in 1884 (Figure 4). These devices are designed to capture FOG from the wastewater produced by the FSE connected to it. GSs works based on the principle of flotation/ gravitational separation, which is mainly used to remove suspended matter. These matter rise or falls, depending on whether its density is less or greater than of water. The general structure of a GS is presented in the Figure 5.

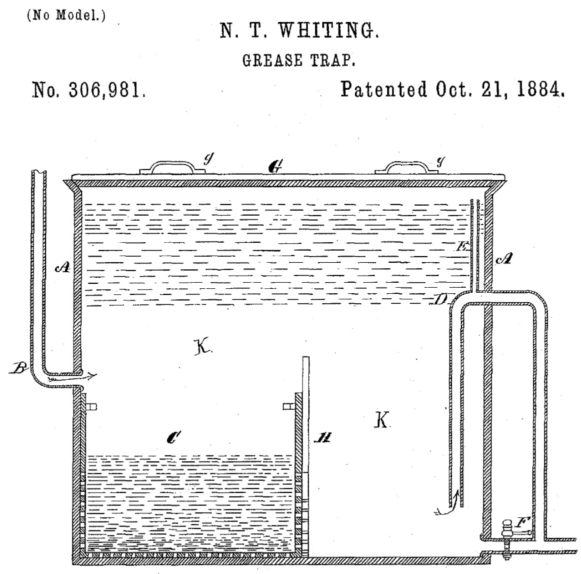


Figure 4: Structure of the first patented grease separator (Whiting, 1884).

GSs allow wastewater to slow down and to provide sufficient time to both solids and FOG to separate from the wastewater stream. When water runs at the right speed through a separator, heavier particles and sludge tends to sink to the bottom of the first compartment. The fat rises and settles to the surface in the next compartment, stays in the separator and can then be sucked away, while the degreased water flows furher out to the sewer system.

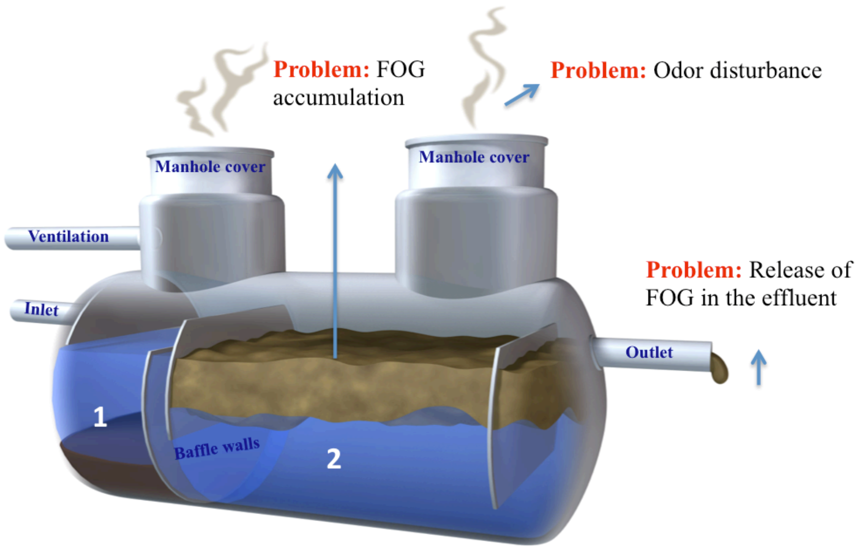


Figure 5: Lateral view of the structure of a regular grease separator and problems related to those devices; (1) slam compartment (2) separation compartment (Adapted from Bioteria Technologies 2011).

There is a problem regarding the classification of grease abatement devices. The terms grease trap and grease interceptor can be found as a synonym to GSs in the literature and no general consensus have been achieved in the correct use of those terms. Most of the studies regarding grease separation devices have been performed in the United States (US). There, grease traps is the term used to classify kitchen grease separation devices smaller than 55 gallons while grease interceptors is the term used to denominate larger outdoor devices with a minimum size of 750 gallons (Engle 2006).

Another example of classification is the one proposed by the International Association of Plumbing and Mechanical Officials (IAPMO), which describes in the plumbing code the smaller devices as hydro mechanical grease interceptors (HGIs) and the larger devices as gravity grease interceptors (GGIs) (IAMPO 2006). In Europe, the regulations regarding the use and maintenance of GSs is described in the European norm EN 1825-2:2002, which in

Sweden is translated in the Swedish standard SS EN 1825-2. The regulations regarding the construction and design of GSs are based on the Standard SS prEN 1825-1.

2.2.1. Factors affecting the performance of GSs

In GSs, the separation processes occur during the time when water is retained into these devices, which should be enough to allow fats to rise. Different environmental (temperature, emulsion properties of the FOG in the wastewater, detergents and other surface active materials used for cleaning) factors may affect the separation time of FOG-rich residues in GSs, and thus the efficiency of such devices (Davis 2011). Thus, those factors should be considered when evaluating the performance of GSs.

The decrease of the temperature into the GS makes FOG components to separate from the stream water, resulting in a degreased effluent. By the contrary, high WW temperature reduces the efficiency of GS to retain FOG compounds. Therefore, the temperatures of water entering in the GS as well the temperature of the separator are of importance (Davis 2011, SS EN 1825-1).

Detergents and other cleaning products are also important variables that need to be considered due to their surfactant properties, which can keep the FOG suspended or emulsified in the wastewater (Davis 2011). This would ultimately allow grease to escape from the separator and cause further problems in the pipelines.

The wastewater characteristics and organic load are also important. Another important (if not the most) factor that affects performance of GSs is the retention time. Retention time is the time that the wastewater remains in the GS and it depended on the area of the GS and the flow rate through the GS. A long retention time may be necessary to allow sufficient separation of the FOG compounds. This is mostly important taking into account that the most FSEs produce very hot wastewater streams during their cleaning operations (Davis 2011).

2.2.2. Regulations regarding the installation and use of grease separators in Sweden

In general, in Sweden it is required that all the FSE (e.g. cafes, bakeries and restaurants, caterers, grocery stores, snack bars, pizzerias, smokehouses and meat industries) have an approved and/ or function tested GS installed in order to prevent major release of fat in the sewage system. The pipes that compose the sewage system are sized to receive wastewater from domestic nature (e.g. with an lower organic strength). According to the ABVA

(*Allmänna vatten- och avloppsanläggningen i Stockholm*/ Public Water and Sewer Facility in Stockholm), FSEs should not emit large quantities of fat in theirs wastewaters.

The Swedish Standard (SS) 1825-2:2002 is the official Swedish version of the European Standard EN 1825-2:2002. It's a national standard that regulate the selection of the nominal size, installation, operation and maintenance of grease GSs. Those GSs should be manufactured according to another standard, the SS EN 1825-1. The standard SS EN 1825-2:2002 does not cover the use of biological additives (i.e. bacteria, enzymes) and does not apply to WW containing light liquids (e.g. grease or oils of mineral origin). It also does not include treating stable emulsions of grease or oil in water.

Stockholm Water Company, which is the regulatory agency responsible for the wastewater management in Stockholm municipality, has reported major problems with blockages in plumbing due to the high levels of emissions of FOG from FSEs. In order to prevent major release of fat, the installation of an approved GS is necessary and required. Even though GS are designed to separate grease from wastewater, they are not capable to receive larger amounts of fat at once. It is therefore not permissible to drain the frying oil or similar directly into the separator and neither in the drain. Large amounts of oil should be poured into special containers for disposal. In Stockholm and Danderyd municipalities there are a number of private contractors who collect grease for recycling. In the following sections the regulations regarding the sizing, construction and maintenance of GSs will be further described.

2.2.2.1. Size and construction

The lack of uniformity of design, sizing and maintenance procedures of GSs may be a result of a lack of scientifically based information regarding GSs performance and effective design. In order to install a GS in a FSE, the property owners must submit an building application to the City Planning Office (*Stadsbyggnadskontoret*). This GS should be approved following specific prescriptions.

Regarding the construction, GSs should be placed in a separate room away from the handling and storage of food in order to avoid any interference in the kitchen activities and food quality. The pump outs must not occur through areas where food is prepared or stored. According to § 47 of the sanitation scheme, individual GSs, septic and closed tanks should be easily accessible for emptying. Lock, manhole or other drainage devices may not be covered. The GS shall be connected to sinks, dishwashers and floor drains.

The rules for dimensioning is based on the nature and quantity of WW to be treated, taking into account parameters such as: the maximum flow rate of WW, the maximum temperature of the WW, the density of grease and oils to be separated and the influence of cleansing and rinsing agents. In cases where more than an usual amount of FOG is expected, the sizing should be performed using a larger separator than calculated or creating a grease storage capacity outside the separation. Another alternative in this case is the increase of the frequency between the pump outs. It is important to remark that the flow on the FSE must not exceed the separator capacity.

Generally, the size of GSs is determined using the following formula:

$$NS = Q_s f_t f_d f_r$$

Where

NS is the GS nominal size that will be calculated

Q*s* is the maximum flow rate entering the separator (l/ s)

f_t is the impedient factor for the temperature of the incoming WW

f_d is the density factor for relevant FOG components

f_r is the hindering factor for the influence of chemical agents which emulsifying properties (e.g. cleansing and rinsing agents).

2.2.3.1. Maintenance

Grease separators shall be inspected, emptied and cleaned routinely. Policy varies significantly from municipality to municipality with regards to the control of GSs, its effluent discharges and maintenance. The property owner is responsible for the GS upkeep and maintenance. According to the standard SS EN 1825-2, all GS should be emptied at least once a month (special frequency agreements can be applied). During the pump outs, the GS should be emptied and cleaned and then filled up with water. The manhole should be properly closed in order to prevent odor related problems. All the FSEs that pursue an GS should have a contract with an approved pump out company.

Different municipalities apply different rules for the maintenance of GS. In Stockholm, for instance, GSs should be pumped out monthly (except for certain activities that have an exception to empty every two months). In Umeå they are less strict regarding the frequency of the pump outs. No monthly empting is required, and the entire GS should be emptied, cleaned and refilled with water at least twice/ year or more often if necessary (UMEVA). In other communities such as Danderyds, Södertäljem Eskilstuna, Västerås, Sigtuna, Bromölla, Strömstad and Katrineholms the use of bio-aerated augmentative systems such as GOR BioSystem^{TM} are already accepted as an alternative to monthly schedule pump outs. It is important to mention that the standard SS EN 1825-2 does not cover the use of bacterial products or enzyme additives, what means that no standard has been made for the use of bioaugmentative systems and products.

2.2.4. Problems

The main problems associated with GSs are the emulsification/ solubilization of fats, the production of hydrogen sulfide (H_2S) and sulfuric acid (H_2SO_4), odor, and the draining practices and disposal of the residues (Angiel 2005, Franke 2011).

Basically oil and water are immiscible and oil can be present as free oil, as an oil sheen and as mixed droplets. At an increased temperature and in turbulent conditions, it can be dissolved or get emulsified. Different chemicals such as solvents and soaps which can also be found in wastewater can improve the solubility and/ or emulsification of oils. As the water temperature slowly drops, the oleaginous emulsified oil starts to separate. In the sequence it thickens, hardens and solidifies turning into fats. Consequently, a layer of fat builds up in the top of the GS or in the pipes of the sewer systems, restricting the WW flow and causing sanitary sewer overflows (SSOs). When such events occur in FSEs it can generate problems such as odors, health risk and operational disturbances.

Hydrogen sulfide (H_2S) is a colorless gas with an offensive strength and characteristic odor. It is considered a toxic waste that is corrosive to metals, flammable and explosive (Franke 2011). The long-term exposition to this gas is also deleterious to humans (Guidotti 1996, Franke 2011). In GSs, it is produced through the anaerobic oxidation of organic compounds by sulphate-reducing bacteria (SRB). Through this process, those bacteria reduce sulfate (SO_4^{2-}) to hydrogen sulfide (S2–, which exists as H2S at lower pH), which is released to the air (Tsuchida *et al*. 2011).

Significant odors are generated by sulfides in the GSs and these odors often are released also in public areas and the kitchen of the restaurant. The rotting processes of solid foods as well

FOG yields gases with disagreeable odor, such as hydrogen sulfide and sulfuric acid, an highly corrosive strong mineral acid (Palmer *et al.* 2000).

Regarding the pump outs and disposal of the residues, the usual procedure for processing the GS sludge and retained grease and solids is to completely remove the contents of the tank and dispose all the liquid, solid and biological material at a municipal wastewater treatment plant or other approved disposal facility. This involves handling of a great volume of water, requiring expensive transportation and disposal and the impact that such transports displays to the environment.

The lack of control is another matter of concern. Actually there are no rules regarding the control of GSs and its known that the lack of an appropriate maintenance routine and control can lead not only to problems to the restaurant but also to the whole sewage system. Considering the dimension of the damages that non-functional GSs can have in WW systems, it is crucial to use efficient methodologies to upgrade both the function and maintenance of such devices.

2.3. Bioaugmentation

Bioaugmentation is the method of introducing specialized and actively growing microbial strains into a microbial community aiming to enhance the degradation of certain compounds (such as FOG). Another improvement attributed to bioaugmentative practices is the upgrading of the capacity of microbial communities to adapt to process variations. Thus, it is possible to say that a successful bioaugmentation strategy results in an overall improved treatment.

The inclusion of specific microbes can lead to the enrichment of the overall characteristics of the microbial community (Livingston *et al.* 2007). Although different studies have demonstrated the potential of the use of different microbial strains for the degradation of FOG compounds in lab scale, very few field studies have been reported (Tisinger & Drahos 1997, Wakelin & Forster 1997, Shon *et al.* 2002, EL-Bestaway *et al.* 2005, Livingston *et al.* 2007).

2.3.1. Bioaugmentation as a biotreatment for FOG-rich residues

An environmentally desirable approach for diminishing the entry of high levels of FOG levels in the sewage system is bioaugmentation (He 2011). One attractive strategy is by the introduction of suitable microorganisms in GSs (Rashid & Imanaka 2008). Most microorganisms are capable of degrading FOG compounds, despite the fact that may exists considerable variation in rates of degradation depending on the chemical composition of the waste and on the microbial strain. Certainly, there is a great variation in the ecological and physiological properties of the different microorganisms capable to degrade FOG compounds and also in the range of FOG compounds that they can degrade (Mohn 1997).

The mechanisms of FOG biodegradation has been extensively characterized biochemical- and microbiologically (reviewed by Foster 1992 and Ratledge 1994) (Figure 6). The first step of the biodegradation of fats is the **hydrolysis** of the ester bonds that links the molecule of glycerol to the fatty acids or phosphoric acids that compose the triglycerides. The hydrolysis of FOG is catalyzed by fat degrading enzymes, lipases (triacylglycerol acylhydrolase, EC 3.1.1.3). The reactions of FOG with lipases leads to the hydrolysis of tryacylglycerols to diacylglycerols, monoacylglycerols, fatty acids and glycerol (Sharma *et al.* 2001, Snellman & Colwell 2004). Those enzymes are extracellular, active in free solution or on the surface of the cell wall of microorganisms; thus environmental conditions can affect significantly their activities.

Studies have shown that generally lipases require a pH near to neutral to perform (Mohn 1997). However, its known that lipases with different levels of stabilities have been isolated, increasing the number of processes (with different characteristics) in which they can be applied (Adualema & Gessesse 2012). Since the hydrolytic activity doesn't require oxygen, the lipases can act equally well in the presence or absence of oxygen.

The subsequent step of the FOG biodegradation is the **beta-oxidation**, where the fatty acids liberated from the triglycerides molecules during the hydrolysis will be further decomposed (Wakelin & Forster 1996). The beta-oxidation comprises a stepwise removal of two-carbon units from the fatty acids (acetyl-CoA) (Figure 6). If the fatty acid has an even number of carbons, then the entire chain is degraded to acetyl-CoA. If the fatty acid chain has an odd number of carbons then the last fragment is propionyl-CoA that will be converted to succinyl-CoA (Pepper *et al.* 2014). This is an intracellular step, which will depend on the uptake and transport of the fatty acids molecules to the interior of the microbial cell responsible for its degradation. Acetyl-CoA is a central metabolite (as glycerol), and both can be used in energy metabolism or biosynthesis processes (Rock 2008). The mechanism of beta-oxidation of fatty acids combined with the tricarboxylic acid cycle and the respiratory chain provide more energy per carbon than any other energy source (Zubay 1996).

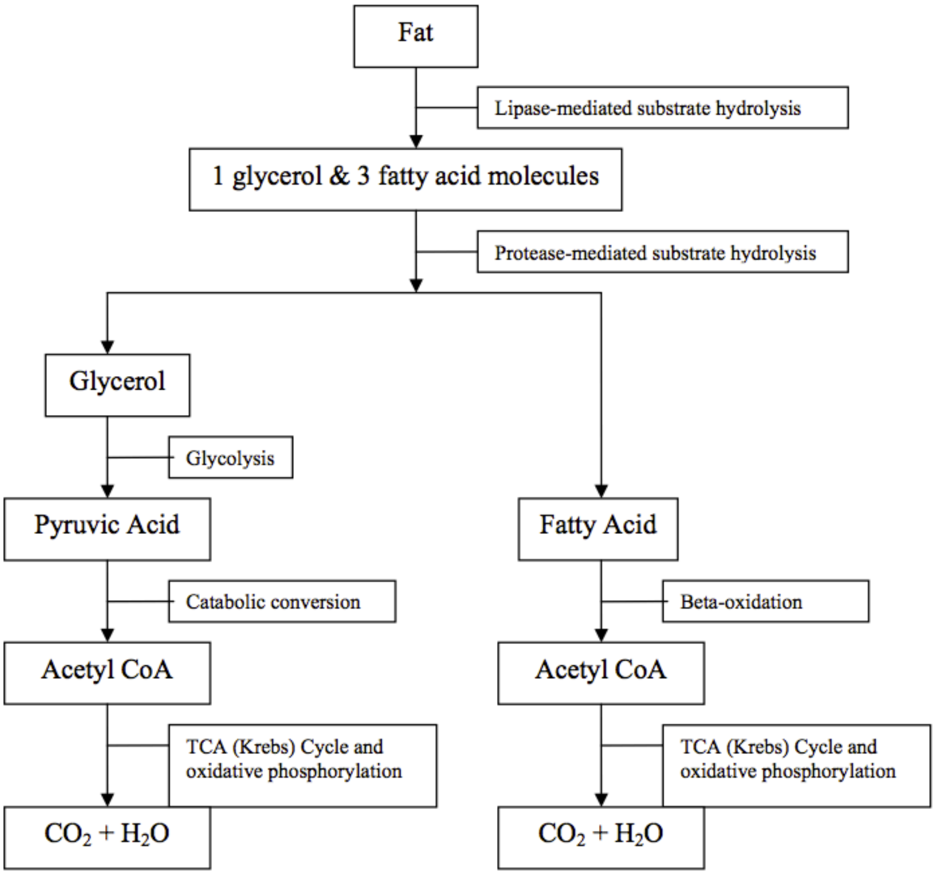


Figure 6: Mechanisms of fat biodegradation

(Adapted from Atkinson *et al.* 1991 and Stainer *et al.* 1976 *apud* Angiel, 2005).

The final products of the complete biodegradation of FOG are CO_2 and biomass. In addition, one or more molecules of water, nitrite, sulphide or methane can be formed, depending on the compound that will serve as the final electron acceptor in the oxidation step (Rock 2008). In aerobic process O_2 is the final electron acceptor.

Most GSs currently in use are unable to efficiently retain dissolved and emulsified fats (Nisola *et al.* 2009). Thus, different methods (physical, chemical and biological) have been developed in order to upgrade the function of the GSs and reduce the frequency (and consequently the costs) of the maintenance (Angiel 2005, Livingston *et al.* 2007, Wong *et al.* 2007, Franke 2011, He *et al.* 2012, Gallimore *et al.* 2011). Different types of bioproducts have been developed for FOG control in wastewater (Brooksbank *et al.* 2007). Some of them are applied directly in the pipelines while others are added to GSs for performance enhancement (Baig & Grenning 1976, Angiel 2005, Livingston *et al.* 2007, Franke 2011, He *et al.* 2012).

Biological treatments of GS include the use of enzymes and/or lipolytic microorganisms (FOG-digesting biomasses). Different strains of microorganisms, with high lipolytic capacities, have been isolated from different types of environments and characterized (Shon *et al.* 2002, Snellman & Colwell 2004, El-Bestawy *et al.* 2005, Cammarota & Freire 2006, Loperena *et al.* 2006, Loperena *et al.* 2009, Treichel *et al.* 2010). Such microorganisms are considered as potential candidates for the use in the formulation of bioaugmentation products as single or mixed cultured or in combination with additives, such as enzymes. Different studies have shown that mixed microbial cultures are able to remove large amounts of grease in contaminated effluents, as free living cultures or when added to support matrices as biofilms (Huban & Plowman 1997, Wakilin & Forster 1997; Tano-Debrah *et al.* 1999, El Masry *et al.* 2004; El-Bestawy *et al.*, 2005; Schade and Lemmer, 2005; Brooksbank *et al.* 2007, Loperena *et al.* 2009).

Even though a considerable amount of bioproducts for the biotreatment of GSs is available in the market, bioaugmentation has shown to be of limited application. Commercial biomasses often demonstrate difficulties to remain established in open systems such as GSs and very little is known regarding long-term assessments of the physical and chemical environmental conditions and microbial ecology of GSs (He 2012).

2.3.2. The use of *Bacillus* in bioaugmentative processes

Bacillus is defined as a group of gram-positive, rod-shaped bacterium that can be aerobic or facultative anaerobic (Ravel & Fraser 2005). An important feature of the bacteria from this genus is the capacity to produce greatly resistant endospores in response to nutritional or environmental stresses (Earl *et al.* 2008). The first studies about *Bacillus* are dated on the late 19^{th} century, and it is considered one of the most characterized bacterial genera. Bacilli research has included classical microbiology, biochemistry, and more modern genomic and proteomic approaches (Alcaraz *et al.* 2010).

The ubiquitous presence of *Bacillus* in different environments reflects the versatile metabolic capabilities of the bacteria present in this widely distributed genus. *Bacillus* strains can be sub-divided in three groups (pathogenic, environmental, and those used for industrial purposes) (Ravel & Fraser 2005). The pathogenic group is represented by *B. anthracis, B. cereus, and B. thuringiensis*. Environmental Bacilli are largely diverse and include *B.*

coahuilensis, B. subtilis, B. pumilus and *B. halodurans*. A good exemplar of an industrial strain is *B. licheniformis* (Ravel & Fraser 2005).

In bioaugmentative processes, this genus is of considerable importance as it includes important lipase producing species and due to its robustness. Different studies have exploited the potential of use different strains of *Bacillus* for the degradation of FOG compounds, in the composition of single species (He *et al*. 2012) or multi-species (Loparena *et al.* 2006, Loparena *et al.* 2009, Mongkolthanaruk & Dharmsthiti 2002) bioproducts.

2.3.3. Necessary environmental conditions for bioaugmentation

As aforementioned, some microorganisms can grow using FOG compounds as primary substrate together with other nutrients such as N, P and trace nutrients. These complementary nutrients can potentially limit the growth on oil and grease and consequently decrease the rate of biodegradation of FOG compounds and prevent the maintenance of that microbial population.

Yet, there are groups of bacteria adapted to live under low nutrient conditions, which are termed as *r* strategists (Andrew & Harris 1986). Examples of an r strategist microorganism are certain species of *Bacillus*. An *r* strategist relies on its high reproductive rates for continued survival in a certain community. Characteristically, *r* strategist microorganisms take over and dominate environments in which resources are temporarily abundant (Andrew & Harris 1986). They can rapidly colonize and degrade large, easily available concentrations or organic matter in short period of time. Also, during low nutrient conditions these bacteria can revert to abundant and resistant spores for dispersion and survival.

Another limiting factor is the insolubility of the FOG compounds. It can limit the availability of the FOG components to the microorganisms and consequently their biodegradation. Generally microorganisms growing on FOG-rich substrates produce surfactants in order to dissolve the FOG and increase its bioavailability to the bioprocess (Cooper & Zajic 1980, Kosaric *et al.* 1983, Maier 2003).

The factors regulating lipase activity are also important. Some lipases are very broad in their substrate activity while others have preferences for specific fatty acids (Thomson 1999). For example, the *B. subtillis* lipase displays substrate-specificity to fatty acids with chain length of eight carbons found in the 1, 3 position of a triglyceride while the *Staphylococcus aureus* lipase has a very broad range of substrate specificity (Thomson 1999). Physico-chemical conditions such as pH, temperature and the presence of inhibitors of enzymatic activity can also affect the activity of lipases (Grupta *et al.* 2004). Thus, it is important to ensure that the metabolic capabilities of the bioproduct are compatible with the substrate that it may degrade and also with the overall environmental conditions.

2.3.4. Main concerns regarding bioaugmentative systems and products

A number of factors potentially present in the GSs environment can prevent the degradation of FOGs by lipolytic microorganisms. Among these factors, it can be mentioned: short retention time, extreme temperatures, pH, presence of toxic compounds (such as chlorine), lack of oxygen and nutrients such as nitrogen and phosphate. Such factors could cause the cessation of the microbial activity.

One of the main problems of most bioaugmentative processes in GSs is the retention time. Generally the high frequency and volume of the wastewater in the FSEs combined with GSs with inappropriate size do not allow sufficiently long retention time. Another problem is an inappropriate process time (i.e. the time for the bioprocess in which the microorganisms will be degrading completely the FOG components). In order to provide a sufficient time for the bioprocess, the focus of the bioaugmentation system has to be in periods with no flow (i.e. when the kitchen of the FSE is not active).

Another challenge in the biotreatment of GSs is the indigenous communities and activated-sludge biomasses. The microorganisms that compose the resident microbial community in GSs generally have a greater diversit y and are better acclimatized to the GS environment and substrates. Different authors have observed that the indigenous microorganisms achieved similar process performances compared to commercial biomasses (Stephenson and Stephenson 1992, Mendoza-Espinoza and Stephenson 1996). However, very little is known about the microbial diversity in GSs and further studies are still necessary (Lucas *et al.* 2012).

Regarding the chemical nature of the FOG degradation products, some fatty acids (long and short fatty acids, unsaturated and branched fatty acids) are recalcitrant (Mohn, 1997). Short fatty acids (with less than eight carbon atoms) tend to be toxic to microorganisms causing membrane disruption. Very long fatty acids (with more than 18 carbon atoms) are difficult to be taken up and metabolized by microorganisms because of their limited solubility and large size. Unsaturated and branched fatty acids are also a problem because the double bonds and tertiary carbon atoms of these fatty acids blocks beta-oxidation. However, despite the recalcitrance of these fatty acids there are some few microorganisms that are capable of degrading them and the lack of such organisms in an environment is the cause of the persistence of those fatty acids (Mohn, 1997).

In Sweden, Stockholm Vatten has demonstrated a concern for the use of biological products in GSs (Axelsson & Stockholm Vatten 1997). According to them, if the FOG degradation occurs in deficiency of oxygen it can result in the production and release of high concentrations of volatile fatty acids in the effluent from the GS. It has been proven that changes in the wastewater composition can result in further problems in the wastewater treatment plants, such as foaming and sludge bulking (Frostell *et al.* 1995). Therefore, a better understanding about the use of bioproducts in GSs is necessary.

3. Aims

FOG management is a significant environmental problem in Sweden, especially regarding the maintenance and management of FOG-rich residues from GSs. The efficiency of GOR Biosystem™ in removing FOG residues and decreasing related problems has been tested by many customers across Sweden (Bioteria Tecnologies 2013). As aforementioned, a great number of municipalities have adopted this technique as an standard methodology to replace the monthly scheduled vacuum pumping, which is known to be costly and detrimental to the environment (Bioteria Technologies 2013).

Despite the potential environmental and economical benefits provided by the implementation of bioaugmentation systems in GSs, there is still a certain objection regarding the efficiency of such systems. In Sweden some governmental and/or municipal authorities still limit or prohibits the use of biological systems including biological addictive in GSs. Thus, the aim of this study was to evaluate the effect of the biological aerated treatment promoted by GOR BioSystem™ on the effluent of restaurants GSs.

3.1. Specific Aims:

- To analyze the effluent quality, the grease and solids removal performance and odor control of a standard untreated GS from an full service restaurant in Stockholm municipality through five weeks using physico-chemical parameters, visual and odor analysis and grease cap and solids accumulation patterns;

- To analyze the effluent quality, the grease and solids removal performance and odor control of a treated GS (with GOR BioSystem™) from an elementary restaurant in Danderyd municipality through ten weeks using physico-chemical parameters, visual and olfactory analysis and grease cap and solids accumulation patterns;

-To compare the effluent quality, the grease and solids removal performance and odor control of one GSs from a full service restaurant at Stockholm municipality with and without Bioteria's bioaugmentation system GOR BioSystem™ through five weeks based on physico-chemical parameters, visual and olfactory analysis and grease cap and solids accumulation patterns.

4. Materials and Methods

Three restaurants in Danderyd and Stockholm municipalities were selected to this field study. One was a restaurant of an elementary school (Restaurant Guld Tuppen/ Vasa Skolan) and two were full service restaurants at the Kungliga Tekniska Högskolan (KTH) campus (Restaurant Brazilia and Restaurant Syster O Bror).

At the Restaurant Brazilia, this study was divided in two different phases: one period when the GS was treated with the GOR BioSystem™ (Phase 1) and one reference period without the use of the GOR BioSystem™ (Phase 2). The dosage of the bioproduct was constant (daily doses) throughout Phase 1. One restaurant GS (Restaurant Syster O Bror) was kept without treatment for 25 activity days as a negative control as well one restaurant GS (Restaurant Guld Tuppen/ Vasa Skolan) was kept under treatment for 44 activity days in order to validate the efficiency of this bioaugmentative system through a longer period of time.

In this study, an activity day was defined as the days in which the restaurants were open for activity during the period of study. The pumping out day was considered as day 0 and the following days in which the restaurants were opened were counted. Weekends and holidays when all the three restaurants evaluated were closed were not included in this counting. This system was adopted for a better basis of comparison and understanding of the results.

Phase 1 lasted 25 activity days in the period between 20th of March to 24th April 2013 and Phase 2 lasted 23 activity days from 13th May to 12th June 2013 at the GS of the Restaurant Brazilia. The GS of the Restaurant Guld Tuppen was evaluated through 44 activity days from 5th March to 6th May 2013 and Restaurant Syster O Bror was evaluated for 25 activity days from the 21th of May to the 19th June 2013. The measurements and samplings for analysis were performed once or twice per week in all the GSs evaluated. The date and the time of each sampling operation was noted.

All the GS were vacuum pumped prior the beginning of each activity cycle by an approved pump out company. After the vacuum pumping, the GS from Restaurant Brazilia was treated with chlorine in the pump out event between phases 1 and 2 in order to eliminate the effect of the bioproduct during the untreated cycle.

4.1. Study sites

In the beginning of the study, an interview was performed with the responsible for the kitchen of each of the restaurants in order to obtain information regarding the kitchen activities.

4.1.1. Restaurant Syster O Bror

Syster O Bror is a full service restaurant located at the KTH campus, in Stockholm. It serves a different range of meals, drinks and a salad buffet daily during the lunch time and also has à la carte menu. This restaurant also offers dinners and catering service.

The restaurant is open from Monday to Thursday from 10:00 am to 03:30 pm and at Fridays from 10:00 am to 06:30 pm. The lunch is served between 10:00 am to 02:00 pm.

4.1.1.1. Restaurant Syster O Bror GS information

Different from the other GS evaluated in this study, this one was composed of only one separation compartment. It is a 7 L/ s tank made of glass fiber and installed outdoor.

4.1.2. Restaurant Brazilia

Restaurant Brazilia is a lunch restaurant located at the KTH campus, in Stockholm. It serves a different range of meals and also drinks, salads and coffee. Between 300 and 350 meals are served daily during the lunch. Regarding the meals, they offer gluten- and lactose-free options as well alternatives with low carbohydrates and vegetarian. The kitchen is specialized in the Swedish home cooking/ cross cooking, and they also produce their own bread, buns and muffins. This restaurant also offers catering services.

The restaurant is open from Monday to Friday from 09:00 am to 03:00 pm and the lunch is served between 10:30 am to 02:00 pm. The kitchen is open between 08:00 am to 04:00 pm.

The kitchen is staffed with 5 professionals per shift and is equipped with two dishwashers. Generally the kitchen personal did not use chlorine in their activity.

4.1.2.1. Restaurant Brazilia GS information

The GS installed at Restaurant Brazilia is made of glass fiber and is located outside of the restaurant. The actual flow capacity of this GS is 4 L/s which is considered undersized according to SS EN 1525. Based on the standard information, this GS should be sized to have a flow capacity of 10 L/s, which would be more than twice of the actual capacity and size.
An effluent filter specially designed by Bioteria AB was installed in the effluent compartment during the treatment cycle at this GS, as a complement to the biotreatment. This effluent is still under test, and it was removed during the untreated cycle.

4.1.3. Restaurant Guld Tuppen

Restaurant Guld Tuppen is a restaurant located at a elementary high school (Vasa Skolan) in Danderyd Municipality. Every day between 550 and 570 meals are served during the lunchtime, and afternoon snacks to about 200 people between students and employees. The kitchen has the goal of cook a balanced nutritious food and it produce most of the food themselves in order to minimize the amount of finished goods.

This restaurant usually offers three to four dishes daily, one vegetarian. In addition to the hot food they also serve a varied salad buffet. As a drink, they offer the choice of to organic low-fat milk and water. For all meals are served hard bread with margarine.

The kitchen is staffed with five professionals per shift and is equipped with one big dishwasher. The higher period of activity of the dishwasher occurs between 11:00 am and 01:00 pm. The kitchen activities start at 07:00 am and ends at 03:30 pm. The lunch is served between 10:45 am and 12:30 am and the snacks are served between 02:00 pm and 03:00 pm. The kitchen staff was instructed to avoid the use of chlorine in their activity.

4.1.3.1. Guld Tuppen GS information

The GS installed at Restaurant Guld Tuppen is made of glass fiber and is located outside of the restaurant. It is a GS with a flow capacity of 15 L/s. No effluent filter was installed during the treated cycle.

4.2. Determination of the variability of the samples

An initial hourly effluent sampling test was performed in the GS of the restaurants Guld Tuppen and Brazilia in order to determinate an appropriate sampling schedule based in the measurement of the variability in the GS effluents characteristics. Samples were collected each hour during the kitchen activity hours (between 08:00 am -05:00 pm). Composite samples were made manually by the collection of 250 mL aliquots each 15 min that were added together to obtain a final 1 L composite sample for each hour. The samples were analyzed for total oil and grease, COD, TSS, temperature and pH.

4.3. Sampling Methodology

Effluent samples were taken from the outlet compartment from the GS with a manual sampler specially developed by Jorma Paloosari (technical developer at Bioteria Technologies AB). This manual sampler was composed by a collecting tube, which connected to a sampling jar and the manual pump device. The tube was inserted inside of the outlet compartment and the samples were pumped out.

Composite samples from the effluent were prepared typically in specific times (Brazilia from 10:00 pm to 10:45 pm, Syster O Bror from 10:25 am -11:10 am Vasa Skolan from 01:00 pm - 01:45 pm). It is important to remark that all the sampling operations were performed when there was a flow in (and consequently out) of the GS. The optimal time for sampling was determined based on the data obtained in a preliminary variability study (*data not shown*).

The effluent samples were characterized using physical (temperature, pH, conductivity) and chemical (BOD, COD, TOC, TN, TP, emulsified fat, separable fat and ether soluble fat) parameters. The temperature, conductivity and pH were measured *in situ* or immediately after removing the sample from the separator, directly in the sampling jar while the chemical parameters were analyzed by a external accredited Swedish laboratory (Eurofins Environment Sweden AB).

The sampling operations started with the preparation of the sampling materials. All the sampling bottles were properly identified with the data such as sample ID, date, company name and reference code of the test to be performed. Then, the conductivity probe was placed in the effluent compartment of the GS, together with the sampler collector tube. Samples (approximately 1 L) were pumped out from the effluent compartment in intervals of 15 minutes. These samples were mixed inside of the sampler jar and then the pH and the temperature were immediately measured after this procedure. After that, an 1 L aliquot was collected using a graduate beaker, which was further separated in four 250 mL aliquots in the four composite samples bottles (C1, C2, C3 and C4). We had an special attention to keep the order when filling the composite bottles with the subsequent aliquots and when using them to fill the test bottles (C1 for the ether soluble analysis, C2 for TSS, C3 for emulsified fat analysis, C4 for BOD, TOC, PSL2Y pack) as shown in Figure 7.

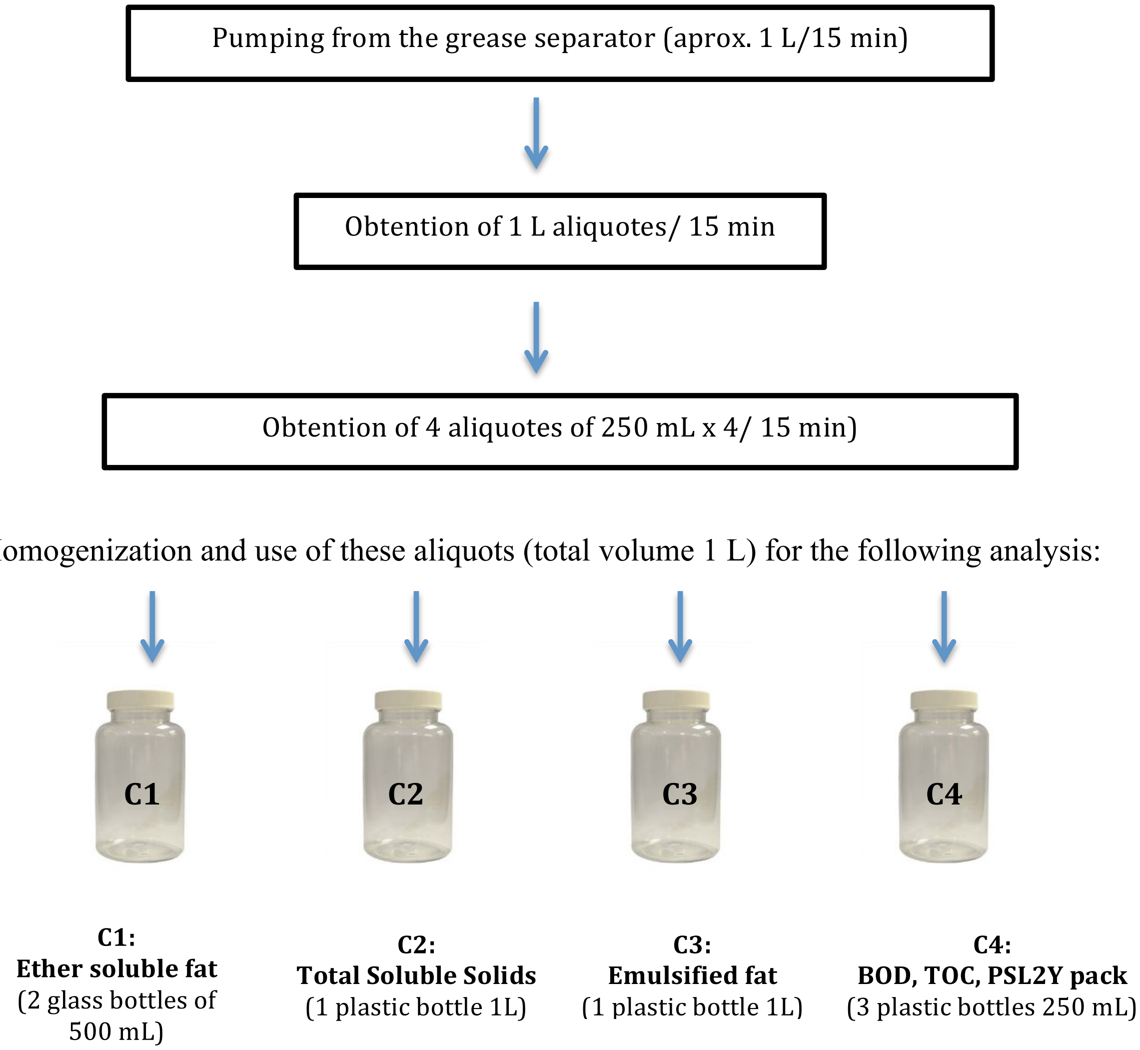


Figure 7: Schematic representation of the sampling methodology.

4.4. Analytical Methods

The effluent samples obtained from the GSs evaluated in this study were analyzed using different chemical and physical characterization methodologies. The selected methodologies will be further described in the following sections.

4.4.1. Chemical Analysis

All the chemical analysis (FOG, TSS, COD, BOD, TOC, TN, TP) was performed by Eurofins Environment Sweden. Eurofins conducts accredited analytical activities in accordance with ISO 17025 and almost all analytical activities covered by the accreditation. Eurofins is also certified according to ISO 9001 and 14001.

4.4.1.1. FOG (ether soluble and separable fat)

The samples for ether soluble fat analysis were prepared by placing two aliquots of 200 mL of the composite samples in two 500 mL glass bottles. The samples for the quantification of the amount of the emulsified fat for the calculation of separable fat were prepared in 1 L plastic bottles, which were filled with 1 L of the samples. All the samples were kept in ice from the collection and preparation until the transport to the laboratory. The samples were analyzed using the standard methods adopted by Eurofins Environmental Sweden (SS 28211 for emulsified fat and SS 28211 for ether soluble fat). The amount of separable fat was calculated based on the difference between the values of the ether soluble fat and the emulsified fat.

4.4.1.2. TSS

The samples for TSS were prepared by the addition of a 1 L aliquot of the composite sample content to a 1 L plastic bottle. After the preparation, this sample was maintained on ice until the arrival to the laboratory. These samples were analyzed using the standard method EN872 by Eurofins Environment Sweden.

4.4.1.3. BOD, COD, TOC, TP and TN

Three aliquots of 250 mL of the composite samples were used for the determination of the BOD 7d, CODcr, TOC, TN, and TP. Two separated aliquots were used for the TOC and BOD 7d tests while a single aliquot was used for the determination of CODcr, N and P. These samples were maintained on ice from the collection and differently from the other samples they were frozen at Bioteria's laboratory prior the shipment to Eurofins's laboratory. The samples were analyzed by the methods EN1484 (TOC), Spectroquant (CODcr), NS EN 1899 1-2 (BOD 7d), EN ISO 11905-1/KONE (N) and SS-EN ISO 15681-2:2005/TrAAcs (P).

4.4.2. Physical Analysis

4.4.2.1. pH, Conductivity and Temperature

PH, conductivity and temperature were measured with an Thermo Scientific Orion Star A324 pH/ISE portable multiparameter meter. This instrument was calibrated at the beginning of each sampling day.

The pH was calibrated at 4.0, 7.0 and 10.0. It was measured in the sampling jar immediately after the removal of the sample from the GS. The conductivity was calibrated with two conductivity standards at 12.9 mS/ cm and 1413µS/cm. It was measured directly in the water phase of the outlet tee of the GS. The temperature was also measured directly in the water phase of the outlet tee of the GS.

4.4.3. Visual Analysis

The grease cap shape and appearance was visually observed at each sampling occasion. It was noted if the fat cake was presented in a hard or soft form.

4.4.4. Odor Evaluation

The odor strength was noted and characterized at each sampling operation. The odor was classified in different levels of strength, in a scale from zero to five as displayed in Table 2. It is important to mention that all the evaluations were made by the same individual, which was trained with an experienced GS specialist from Bioteria Technologies AB.

Table 2: Different levels of strength adopted in the odor classification.

Classification	Odor strength
0	No odor
1	Very weak odor
2	Weak odor
3	Moderate odor
4	Strong odor
5	Very strong odor

4.4.4. Sludge Judge Tests

The fat layer thickness and the level of solids accumulated in the bottom were measured with an sludge judge with an metric scale coupled (Normec Tec AB). The measurements were made in triplicate and the values obtained in each sampling date were noted for comparison.

4.5 Statistical Analysis

In the present study, basic statistic analysis was used to assess the degree of variability of the physico-chemical parameters evaluated. The mean, minimum and maximum and standard deviation values were calculated using as basis the data obtained in the sampling operations (physical parameters) and from the laboratory analysis (chemical parameters). The standard deviation was calculated using the formula presented in the figure 8. The significance of the difference between the treated and untreated cycles at Restaurant Brazilia was verified by the statistical T-test.

$$S = \sqrt{\frac{\Sigma(X - \overline{X})^2}{N}}$$

where S = the standard deviation of a sample,
Σ means "sum of,"
$\underline{X}$ = each value in the data set,
$\overline{X}$ = mean of all values in the data set,
N = number of values in the data set.

Figure 8: Standard deviation formula. (Rieber at University of Georgia website).

5. Results

Based on the results of the samples variability study (*data not shown*), the sampling time was chosen to be in a appropriate period of time for each of the restaurants analyzed, to ensure that the data was collected at relatively stable periods and for convenience. At the time of sampling all the restaurants were staffed and in use. Water was flowing in and out of the GS when the samples were obtained.

5.1. Restaurant Syster O Bror

The Restaurant Syster O Bror was used as a basis for comparison, representing the values of a normal and accepted untreated GS from a full service restaurant in Stockholm area. The length of the data collection corresponded with the maintenance frequency of the separator. It was our intention to collect data for a little more than one maintenance cycle (one month – approximately 20 activity days). Thus, this restaurant was evaluated in a period of time of 25 activity days. It is important to remark that there was a decrease (not quantified) in the number of meals served in the end of this untreated period of observation due to the reduction of the campus activities at KTH.

5.1.1. Physical parameters

Table 3 displays the data of the evaluation of the physical parameters (pH, conductivity and temperature) of this untreated GS during the study period.

Table 3: Physical analysis during the untreated cycle at Restaurant Syster O Bror.

		Min	Max	Average	SD
Syster O Bror (untreated)	**pH**	5,52	6,35	5,82	0,2935
	Conductivity	1302	2013	1695,29	294,49
	Temperature	30,7	33,33	32,03	1,62

SD: standard deviation. The conductivity values are in µS/cm and the temperature values are in Celsius grades (^{O}C).

The analysis of the physical parameters shown that the values of the effluent had an average acid pH (5,82) with temperature and conductivity around 32 °C and 1695,29 µS/ cm respectively. The standard deviation shown a slight variance within the data, which is considered insignificant in the parameters analyzed.

5.1.2. Chemical parameters

The ether soluble and separable fat values obtained during the untreated evaluation period are presented in the Table 4. It presented a high variability through the study cycle, which was confirmed by the standard deviation values. FOG analysis revealed no correlation or pattern in the ratios between the ether soluble (considered as total amount of fat) and separable fat.

Table 4: Chemical analysis during the untreated cycle at Restaurant Syster O Bror.

			Min	Max	Average	SD
Syster O Bror (untreated)	**FOG**	**Total fat**	210	560	388,57	111,87
		Separable fat	110	250	172,86	54,69
	BOD7		920	1500	1202,86	186,34
	COD		1400	2100	1771,43	281,15
	TSS		430	760	558,57	115,82
	TOC		390	620	485,71	91,44
	TN		21	53	33,57	13,25
	TP		12	20	15,33	3,97

SD: standard deviation. All the values presented in this table are in mg/ L.

Oxygen demanding components (BOD, COD) are important elements in the evaluation of the composition of the wastewater. They can also indicate the leve of biodegradability of the effluent. As aforementioned, estimations of the total amount of organic carbons, suspended solids, nitrogen and phosphor are also important parameters for the classification of the wastewater quality. BOD, COD, TSS, TOC, TN and TP values are also presented in the Table 4.

BOD values of the effluent from this restaurant varied between 920 mg/ L (25th day of activity) and 1500 mg/ L (12th and 14th days of activity), while COD values ranged between 1400 mg/ L (20th day of activity) and 2100 mg/ L (5th and 7th days of activity). The BOD/COD ratio calculated based on the average values of the effluent was 0,67, which indicates an easily biodegradable effluent. The amount of suspended solids varied between 430 mg/ L (14th day of activity) and 760 mg/ L (20th day of activity).

Another important indicator for the evaluation of the biodegradability potential is the carbon (C): nitrogen (N): phosphour (P) ratio. It can be estimated by the TOC, TN and TP values. TOC values ranged between 390 mg/ L (20th day of activity) and 620 mg/ L (5th day of activity). Total nitrogen ranged between 21 mg/ L (20th day of activity) and 53 mg/ L (7th day of activity), while total phosphor values were between 7,9 mg/ L (20th day of activity) and 20 mg/ L (7th day of activity). The effluent of the restaurant Syster O Bror resented an average C:N:P ratio of 100: 5,38: 3,07 (the recommended ratio for the biotreatment is 100: 5: 1). Due to the high variability of the samples it wasn't possible to detect any clear trend over time for any of the parameters aforementioned.

5.1.3. Visual analysis

Figure 9 displays an schematic representation of the GS installed at restaurant Syster O Bror. The results of the visual observation of the GS of Restaurant Syster O Bror during the course of the untreated control cycle are presented at the Figure 9.

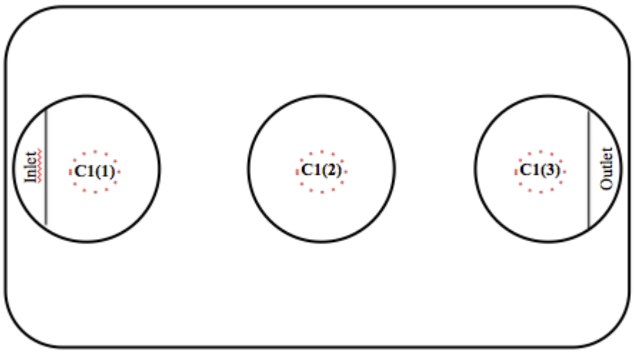


Figure 9: Schematic representation of the grease separator installed at Restaurant Syster O Bror. C1(1), C1(2) and C1(3) represent the 3 different areas of separation compartment.

Visually, the grease cap was presented as a dark, very thick, compact and hard layer. In this untreated GS, we could observe fat accumulation already after five days of activity (Figure 10). At that date, apparently grease started to solidify. In the course of the study period, we could observe a progressive liquefaction of the grease cap on the top, which started to be presented as an oil layer. During the study period it was also possible to observe the build-up of a grease layer in the walls of the effluent compartment. The thickness of this grease layer wall was very irregular and of approximately 2 mm (Figure 11).

Figure 10: Visual analysis of the GS at the Restaurant Syster O Bror during the course of the study (without treatment with GOR BioSystem^{TM})

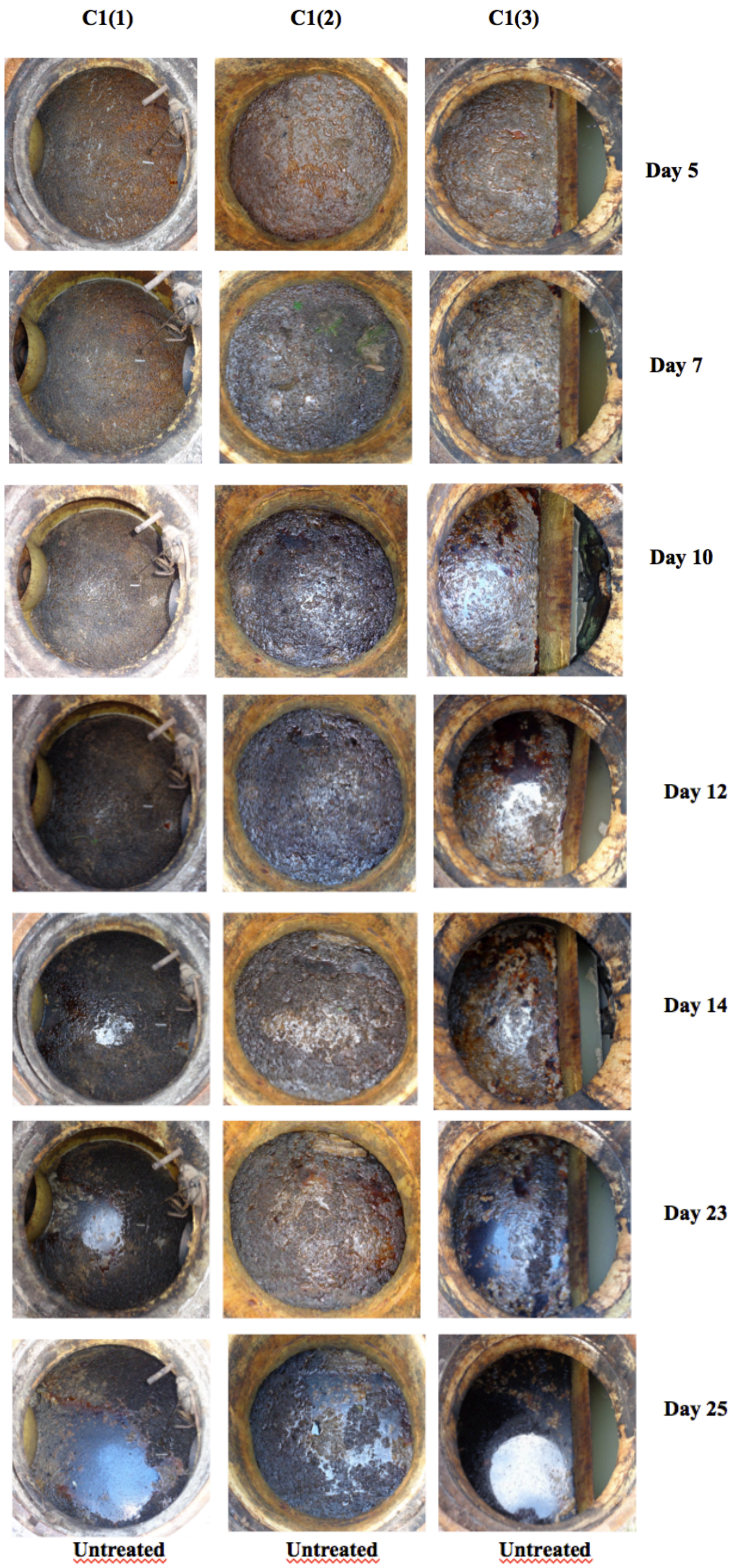


Figure 11: Fat builds up in the effluent compartment of the GS at Restaurant Syster O Bror.

5.1.4. Odor evaluation

Is was possible to detect a very strong and markedly unpleasant odor already in the first week of observation at this GS. The progressive accumulation of FOG and other organic residues lead to an enhancement of the odor strength, which at the end of the study period (25 days) was classified in the highest level (5 - very strong).

5.1.5. Sludge judge test

The results of the tests with the sludge judge are presented in the Table 5. A solid fat layer of approximately 4 cm thickness, was observed already at approximately seven days of activity. We could observe a general increase of the thickness of this layer and in the amount of solids in the most of the compartments observed (C1(1), C1(2) and C1(3)) throughout the study period. It was not possible to perform the sludge judge test in the compartment C1(3) in the last day of observation (23th day of activity) due to problems with the sludge judge in the field. At the end of the untreated control cycle (25 days) the grease cap thickness reached 19 cm and the solids accumulation 15 cm at the intermediate part of the GS (compartment C1(2)).

It was observed in several sampling operations that the grease cap was presented as a triphasic layer composed by an oil layer in the top, an intermediate layer with bigger particles and an third layer with fine suspended particles (Figure 12).

Table 5: Profile of accumulation of settable solids and grease cap (GC) thickness at Restaurant Syster O Bror.

	7 days		12 days		14 days		23 days	
	Grease cap	**Sludge**	**Grease cap**	**Sludge**	**Grease cap**	**Sludge**	**Grease cap**	**Sludge**
C1(1)	4,0	17	16,5	17	17	22	15	22
C1(2)	4,5	7	10	11	7,5	13	19	15
C1(3)	8,5	5	2	10	5,5	15	n/a	n/a

All the measurements presented in this table are in cm.

Figure 12: Sludge judge test showing the triphasic disposition on the grease cap at C1(2) at Restaurant Syster O Bror after 14 days of activity.

5.2. Restaurant Brazilia

The GS installed at Restaurant Brazilia was evaluated as an apparently undersized (according to the SS EN 1825-2:2002) but accepted GS at Stockholm area. Both treated and untreated cycles were performed during the period of time that corresponded to the maintenance frequency of the separator (one month/ 23- 25 activity days). It is important to remark that there was a decrease in the number of meals served during the untreated cycle due to the reduction of the campus activities at KTH (Figure 13). We suspect that the reduction of the number of customers lead to changes in the kitchen activities and consequently in the organic load discharged in the effluent. This decrease was of approximately minus 90 customers (approximately 27 %) in the 1st week, minus 120 customers (approximately 37 %) in the 2nd week and – 160 customers (49 %) in the 3rd week of study during the treated cycle. That was not possible to determinate the approximate decrease in the 4th week of the study.

Figure 13: Average number of meals served at restaurant Brazilia during the treated cycle with GOR BioSystem™ and untreated cycle. The data referent to the number of meals served during the 4th week of activity during the untreated cycle was not available.

5.2.1 Physical parameters

The data of the evaluation of the physical parameters (pH, conductivity and temperature) is displayed in Table 6.

Table 6: Physical analysis during the treated and untreated cycles at Restaurant Brazilia.

		Min	Max	Average	SD
Brazilia (treated)	**pH**	4,83	5,91	5,41	0,37
	Conductivity	600	975	817,1	146,4
	Temperature	14,78	23,83	21	3,22
Brazilia (untreated)	**pH**	4,88	5,73	5,25	0,28
	Conductivity	651	1344,25	955,21	239,83
	Temperature	19,25	31,3	25,32	3,91

SD: standard deviation. The treated cycle refers to the study period in which the GS was under treatment with GOR BioSystem™. The conductivity values are in µS/cm and the temperature values are in Celsius grades (^{O}C)

No remarkable difference was detected between the values of the physical parameters from the treated cycle with GOR BioSystem™ and the untreated cycle. The analysis of the physical parameters shown that the effluent pH displayed a variation between 4,82 (11th day of activity) and 5,58 (20th day of activity) in the treated cycle and between 4,87 (18th day of activity) and 5,72 (10th day of activity) in the untreated cycle. No significant difference was observed in the pH between the cycles ($t= 0,567$, $df= 11$, $p > 0,05$). The conductivity ranged between 600 µS/cm (11th day of activity) and 975 µS/cm (17th day of activity) in the treated cycle and between 651 µS/cm (15th day of activity) and 1344,25 µS/cm (10th day of activity) in the untreated cycle. No significant difference was observed in the conductivity between the cycles ($t= 1,29$, $df= 10$, $p > 0,05$). The temperature ranged between 14,77°C (11th day of activity) and 23,82 °C (17th day of activity) in the treated cycle and between 19,25 °C (3rd day of activity) and 31,30 °C (24th day of activity) in the untreated cycle. No significant difference was observed in the temperature between the cycles ($t= 2,033$, $df= 11$, $p > 0,05$). The variation of the physical parameters evaluated through the study period is illustrated in more details the graphs presented in the Figures 14, 15 and 16.

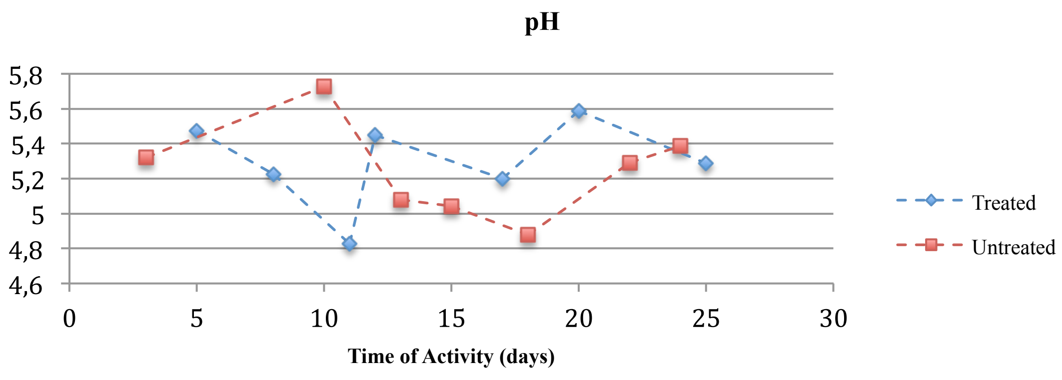


Figure 14: pH variation in the effluent of Restaurant Brazilia during the study period (treated with GOR BioSystem™ and untreated cycles).

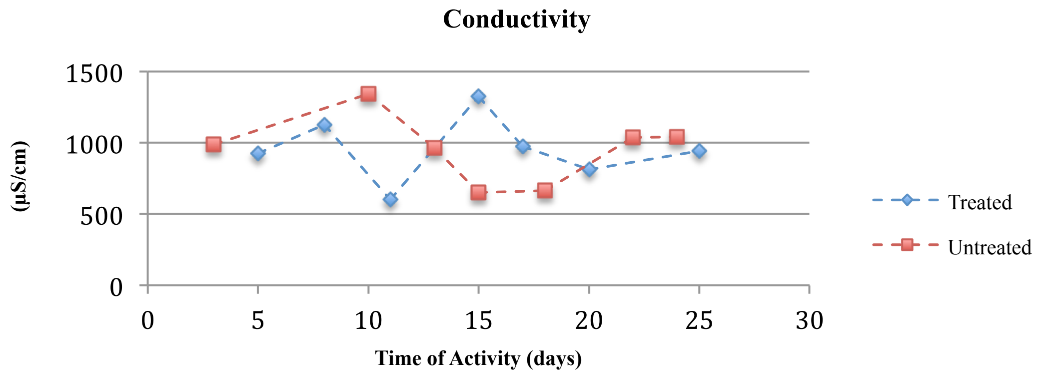


Figure 15: Conductivity variation in the effluent of Restaurant Brazilia during the study period (treated with GOR BioSystem™ and untreated cycles).

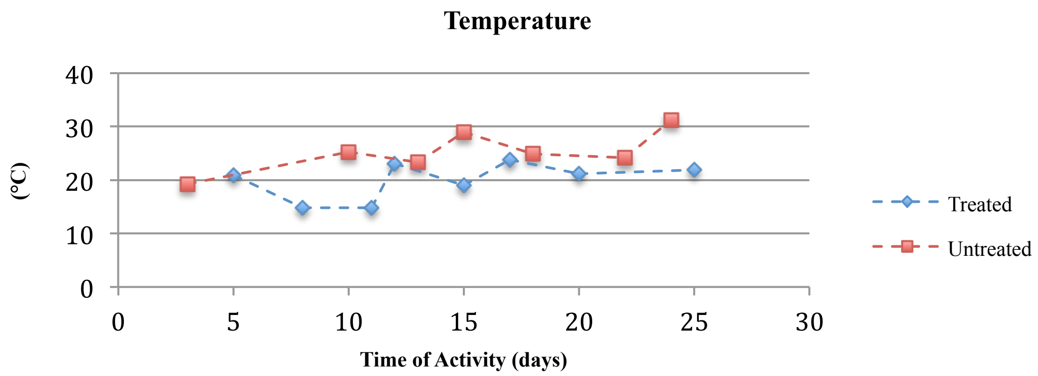


Figure 16: Temperature variation in the effluent of Restaurant Brazilia during the study period (treated with GOR BioSystem™ and untreated cycles).

5.2.2 Chemical parameters

The values of the chemical characterization (FOG, BOD7, COD, TOC, TSS, TN and TP) of the effluent during the treated cycle with GOR BioSystem™ and untreated cycle are listed in the Table 7. It is important to remind the decrease in the number of meals served during the untreated cycle (Figure 13), which probably affected the organic load discharged in this GS. Therefore when comparing the results of both cycles it is important co consider this decrease during the period without the treatment with GOR BioSystem™.

Table 7: Chemical analysis during the untreated cycle at Restaurant Brazilia.

			Min	Max	Average	SD
Brazilia **(treated)**	**FOG**	**Total fat**	220	480	327,14	95,17
		Separable fat	80	210	121,43	48,11
	BOD7		840	1600	1185,71	246,9
	COD		1400	3100	2014,29	539,84
	TSS		190	580	361,43	129,15
	TOC		430	890	590	168,33
	TN		19	40	28,43	8,12
	TP		5,7	12	8,49	2,13
Brazilia **(untreated)**	**FOG**	**Total fat**	140	520	252,86	129,96
		Separable fat	67	300	129	83,2
	BOD7		720	1200	1000	178,61
	COD		1300	2200	1657,14	287,85
	TSS		160	620	366,67	75,96
	TOC		400	590	485,71	74,8
	TN		9,9	45	22,84	11,4
	TP		3,4	10	6,24	2,11

SD: standard deviation. The treated cycle refers to the study period in which the GS was under treatment with GOR BioSystem^{TM}. All the values presented in this table are in mg/ L.

A high variability was observed in the values of both types of fat evaluated in both treated and untreated cycles and it was not possible to detect a trend in relation to the amount of FOG versus time. Regarding the total amount of fat (ether soluble fat), the minimum value detected in the treated cycle with GOR BioSystem^{TM} was 220 mg/ L (8th day of activity) while the maximum was 480 mg/ L (11th day of activity). In the untreated cycle, the total amount of fat varied between 140 mg/L (18th day of activity) and 520 mg/L (24th day of activity). However, no significant difference was observed in the total amount of fat between the cycles ($t= 1,129$, $df= 12$, $p > 0,05$). Separable fat values varied between 80 mg/ L (8 th and 17th day of activity) and 210 mg/ L (12th day of activity) in the treated cycle and between 67 mg/ L (18th day of activity) and 300 mg/ L (24th day of activity) in the untreated cycle. No significant difference was observed in the amount of separable fat between the cycles ($t= 0,192$, $df= 12$, $p > 0,05$). The variation of the FOG levels at the effluent of Restaurant Brazilia during the periods with and without the treatment with GOR BioSystem^{TM} can be observed in the graphs presented in the Figures 17 and 18.

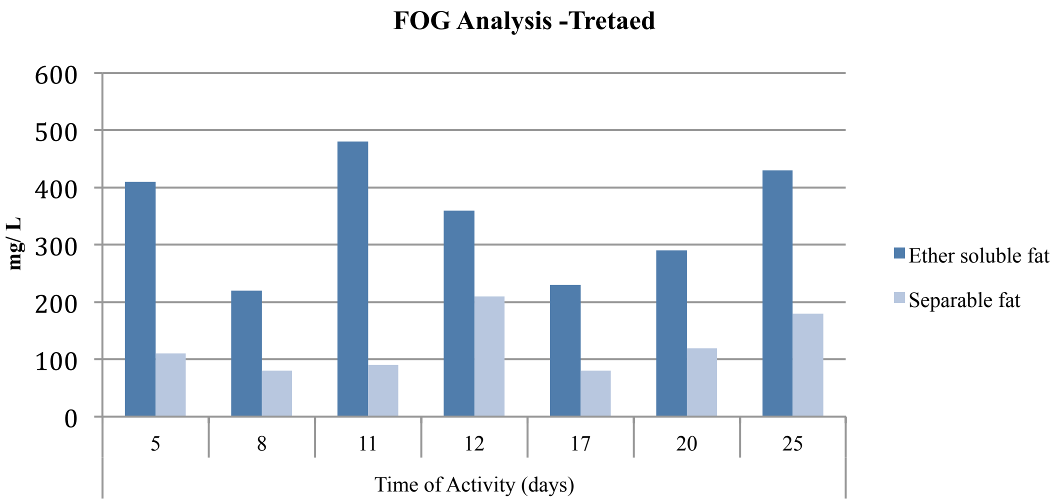


Figure 17: FOG analysis on the effluent of the grease separator of Restaurant Brazilia during the treated cycle with GOR BioSystem^{TM}.

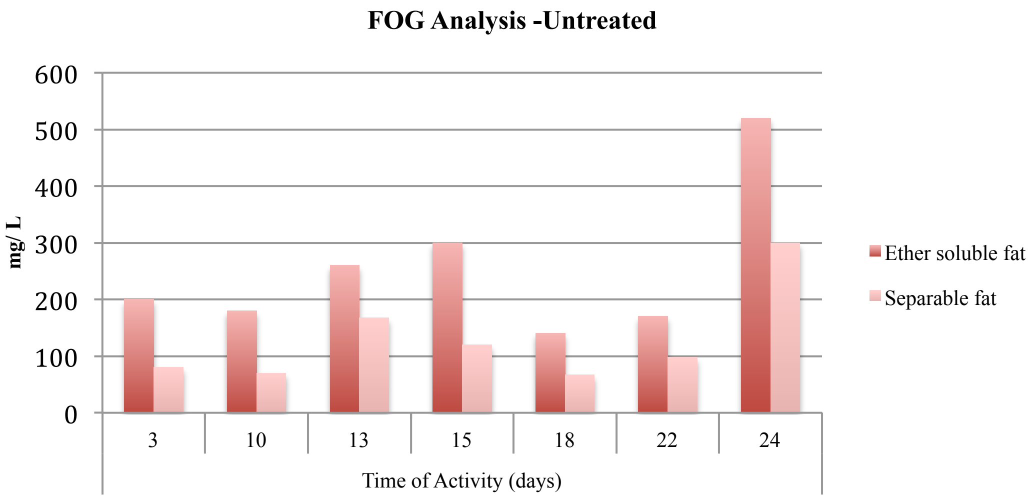


Figure 18: FOG analysis on the effluent of the grease separator of Restaurant Brazilia during the untreated cycle (without the treatment with GOR BioSystem^{TM}).

In relation to BOD, the values detected at the effluent from this restaurant were in a range between 840 mg/ L (17th day of activity) and 1600 mg/ L (15th day of activity) in the treated cycle with GOR BioSystem^{TM} and between 720 mg/ L (24th day of activity) and 1200 mg/ L (8th and 25th day of activity) in the untreated cycle. No significant difference was observed in BOD between the cycles ($t= 1,49$, $df= 12$, $p > 0,05$). COD values ranged between 1400 mg/ L (20th day of activity) and 3100 mg/ L (15th day of activity) in the treated cycle and between 1300 mg/ L (3rd day of activity) and 2200 mg/ L (24th day of activity) in the untreated cycle.

No significant difference was observed in the amount of COD between the cycles (t= 1,38, df= 12, p > 0,05). The BOD/COD ratio calculated based on the average values of the effluent was 0,58 in the treated cycle and 0,60 in the untreated cycle, which indicates an easily biodegradable effluent from both effluents.

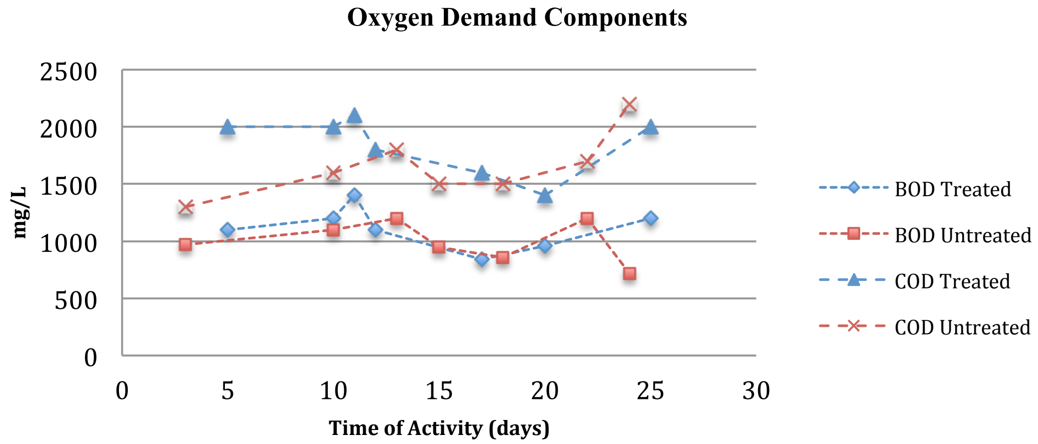


Figure 19: BOD and COD analysis on the effluent of the grease separator of Restaurant Brazilia during the treated cycle with GOR BioSystem™ and untreated cycle.

The load of suspended solids varied between 190 mg/ L (8th day of activity) and 720 mg/ L (25th day of activity) in the treated cycle and between 140 mg/ L (18th day of activity) and 620 mg/ L (24th day of activity) in the untreated cycle. No significant difference was observed in the levels of suspended solids between the cycles (t= 0,085, df= 12, p > 0,05). TOC values ranged between 430 mg/ L (20th day of activity) and 890 mg/ L (15th day of activity) in the treated cycle and between 400 mg/ L (15th day of activity) and 590 mg/ L (22th day of activity) in the untreated cycle. No significant difference was observed the amount of TOC between the cycles (t= 1,38, df= 12, p > 0,05).

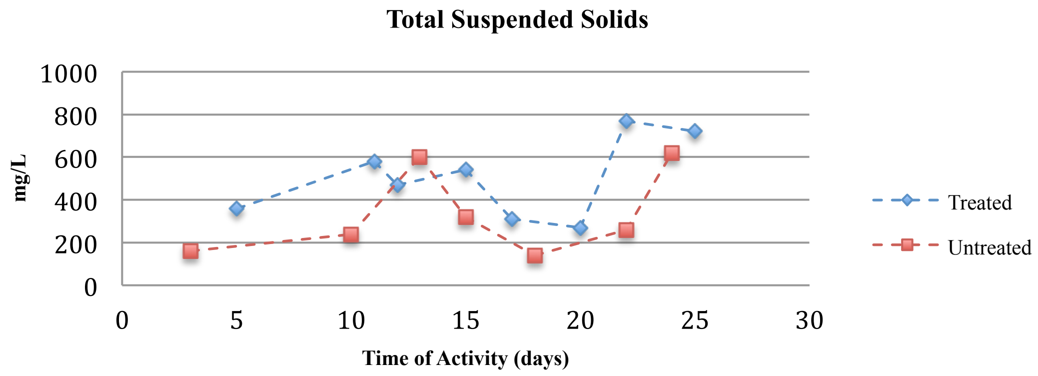


Figure 20: TSS analysis on the effluent of the grease separator of Restaurant Brazilia during the treated cycle with GOR BioSystem^{TM} and untreated cycle.

Total nitrogen ranged between 19 mg/L (11th day of activity) and 40 mg/L (15th day of activity) in the treated cycle and between 9,9 mg/L (18th day of activity) and 45 mg/L (22th day of activity) in the untreated cycle. No significant difference was observed the amount of nitrogen between the cycles (t= 0,977, df= 12, $p > 0,05$). Total phosphor values were detected in a range between 5,7 mg/ L (11th day of activity) and 12 mg/ L (15th day of activity) in the treated cycle and between 3,3 mg/L (18th day of activity) and 10 mg/L (10th day of activity) in the untreated cycle. No significant difference was observed the amount of total phosphor between the cycles (t= 1,38, df= 12, $p > 0,05$). The effluent of the restaurant Brazilia presented an average C:N:P ratio of 100: 4,81: 1,43 in the treated cycle and of 100: 4,70: 1,28.

5.2.3. Visual analysis

Figure 21 displays an schematic representation of the GS installed at restaurant Brazilia. We could observe the formation of a more developed grease cap layer during the untreated cycle, already in the third day of activity. The grease cap was then compact and hard. In the treated cycle, the grease cap developed much less and with a different consistence, visually much softer. Those visual differences are presented in the Figure 22 and partially quantified in the sludge judge analysis (section 5.2.5.).

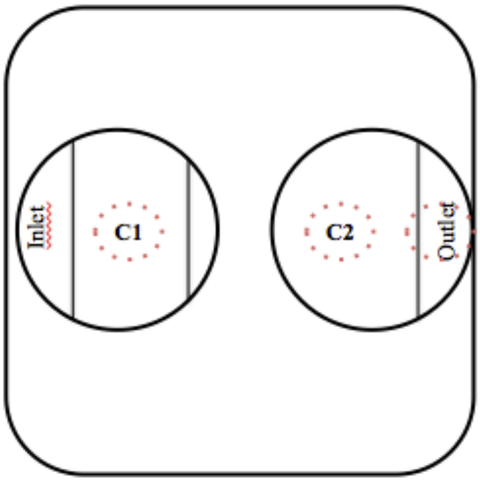
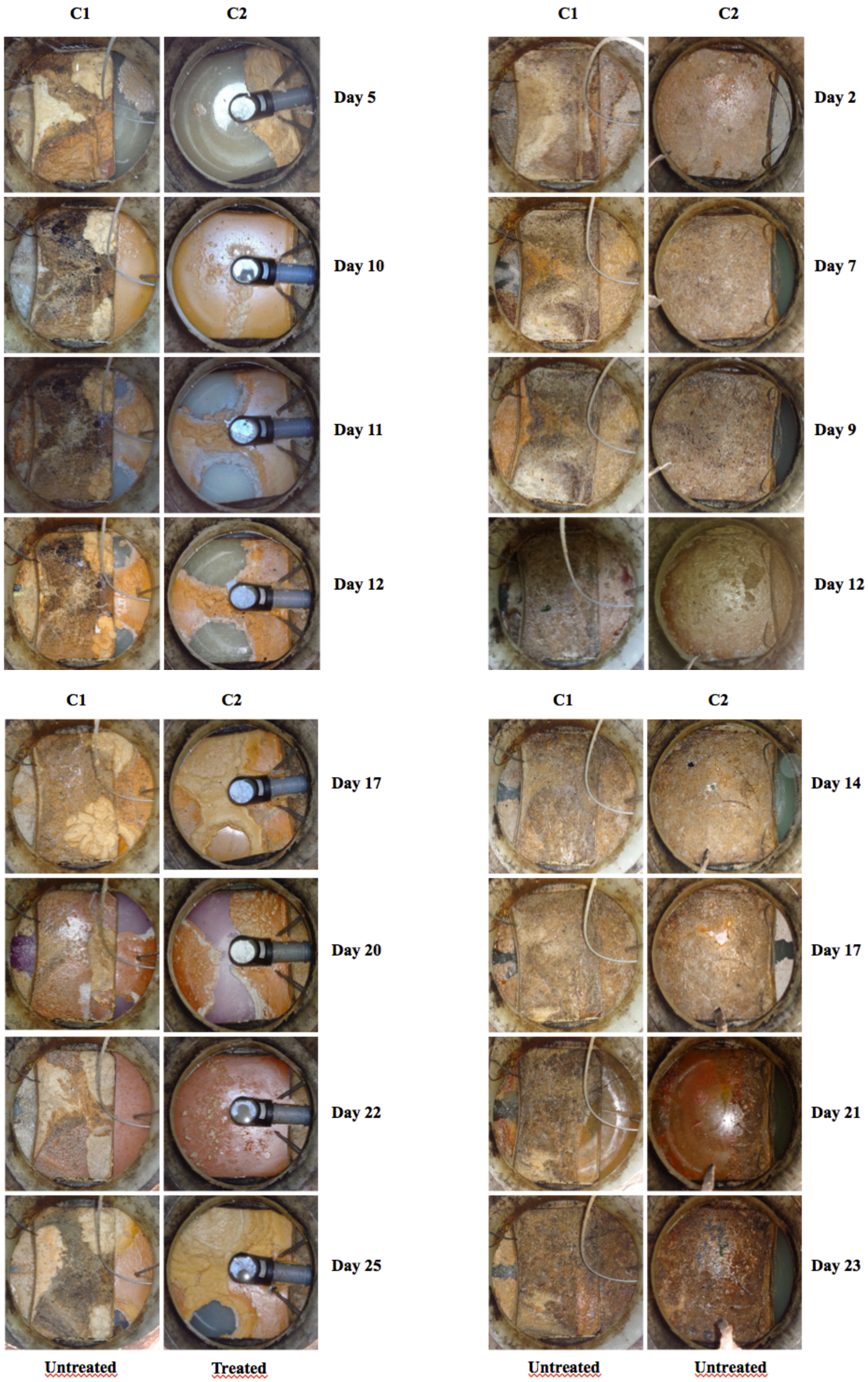


Figure 21: Schematic representation of the grease separator installed at Restaurant Brazilia. C1 (slam compartment) and C2 (separation compartment) represent the 2 different functional areas of this grease separator.

Figure 22: Visual analysis of the GS at Restaurant Brazilia during the course of the study (with and without the treatment with GOR BioSystem™).



5.2.4. Odor evaluation

It was possible to detect a remarkable difference between the odor detected during the treated cycle with GOR BioSystem^{TM} and in the untreated cycle. The odor was clearly reduced during the treated cycle compared with the untreated control period of observation.

A weak odor was detected from the second week of evaluation (12th day of activity) during the treatment with GOR BioSystem^{TM}. At the end of this evaluation period, the odor was still classified in the second level of strength (weak odor). During the untreated cycle, it was possible to detect a pungent odor already in the first week of the study (2nd and 7th days of activity). The strength of odor increased through the study cycle and at the end of the observation period (25th day of activity) it was classified in the fifth level of strength (very strong odor).

5.2.5. Sludge judge test

The sludge judge test was performed in five dates during the treated cycle with GOR BioSystem^{TM} and in three days during the untreated cycle. During the untreated cycle, it was possible to observe a progressive development of the grease cap, which had 4 cm after nine days of activity and 9,5 cm after 23 days of activity. An interesting observation regarding the appearance of the grease cap layer was that it was presented as a triphasic layer of oil and grease in the untreated cycle at the compartment 2, which wasn't detected during the treated cycle (Figure 23).

Treated **Unreated**

Figure 23: Sludge judge test at Restaurant Brazilia. The sample of the treated cycle was taken at the 22th day of activity under the treatment with GOR BioSystem^{TM} while the sample of the untreated cycle was taken at the 23th day of activity without the treatment with GOR BioSystem^{TM}.

The results of the tests with the sludge judge are presented in the Tables 8 and 9. During the period under treatment with GOR BioSystem^{TM}, the grease cap layer did not present a progressive increase in thickness. It had 2 cm of thickness after eight days of activity, presenting a further decrease (such as the one observed after 12 days of activity) when it was detected as a 0,5 cm layer. However, we can say that the grease cap maintained an average of 2 cm of thickness during the 25 days of activity observed during the treatment with GOR BioSystem^{TM}, which is almost 80 % inferior to the thickness of the grease cap formed during the study period without the treatment with GOR BioSystem^{TM} (which reached 8,5 cm after 23 days of activity). It was also possible to observe an increase in the thickness of the grease cap at the slam compartment (C1) during the treated cycle (approximately 54 %). During the untreated cycle it was observed a grease cap layer of 5,5 after 23 days of activity while in the treated cycle it was detected a layer of 8,5 cm.

In relation to the profile of solids accumulation, we could observe a discrete accumulation of sludge in the bottom of the compartment 2 during the untreated cycle, differently from the cycle with the treatment with GOR BioSystem^{TM}, when no solids was detected in any of the samplings occasions. It was possible to observe a high amount of sludge in C1 during the untreated cycle (26 cm) which was almost the triple of the amount detected in the treated cycle (9 cm).

Table 8: Profile of accumulation of settable solids and grease cap thickness during the study period under the treatment with GOR BioSystem^{TM} at Restaurant Brazilia.

	Day 0		10 days		12 days		17 days		22 days		25 days	
	Grease cap	Sludge	Grease cap	Sludge	Grease cap	Sludge	Grease cap	Sludge	Grease cap	Sludge	Grease cap	Sludge
C1	0	0	3,5	11	6	27,5	7,5	17	2,5	14	8,5	9
C2	0	0	2	n/d	0,5	n/d	2	n/d	1	n/d	2	n/d
E	0	0	n/d	n/d	n/d	0,3	n/d	n/d	n/d	n/d	n/d	n/d

All the measurements presented in this table are in cm. C1 (slam compartment), C2 (separation compartment) and E (effluent compartment) represent the different sampling areas of this grease separator

Table 9: Profile of accumulation of settable solids and grease cap thickness during the study period without the treatment with GOR BioSystem™ at Restaurant Brazilia.

	Day 0		9 days		14 days		23 days	
	Grease cap	Sludge	Grease cap	Sludge	Grease cap	Sludge	Grease cap	Sludge
C1	0	0	5,5	11	5	21,5	5,5	26
C2	0	0	4	5	4,5	5	9,5	3
E	0	0	n/d	5	n/d	2	n/d	5

All the measurements presented in this table are in cm. C1 (slam compartment), C2 (separation compartment) and E (effluent compartment) represent the different sampling areas of this grease separator

5.3. Restaurant Guld Tuppen

The GS of the Restaurant Guld Tuppen was evaluated as an accepted GS from an elementary school at Danderyd municipality. The control treated cycle with GOR BioSystem™ was performed during the period of time that corresponded to more than the double of the maintenance frequency of the separator (two and a half months), for approximately 10 weeks consisting of 44 activity days. No substantial difference in the number of meals (i.e. organic load) was observed during this control treated cycle.

5.3.1. Physical parameters

It was observed a overall increase in the values of the physical parameters evaluated at the effluent of the grease separator of Restaurant Guld Tuppen (Table 10) over time. The analysis of the physical parameters revealed a pH variation between 6,04 (11th day of activity) and 6,94 (26th day of activity). The conductivity ranged between 875 µS/cm (22th day of activity) and 1337,25 µS/cm (38th day of activity) while the temperature ranged between 14,9 °C (26th day of activity) and 24,35 °C (33th day of activity). The standard deviation shown a slight variance within the data, which is considered insignificant in relation to the parameters analyzed.

Table 10: Physical analysis during the treated cycle at Restaurant Guld Tuppen.

		Min	Max	Average	SD
Guld Tuppen (treated)	**pH**	6,04	6,94	6,47	0,26
	conductivity	875	1337,25	1157,92	147,77
	temperature	14,9	24,35	20,2	3,0

SD: standard deviation. The conductivity values are in µS/cm and the temperature values are in Celsius grades (^{O}C).

5.3.2 Chemical parameters

The values of the chemical analysis of the effluent from the GS installed at Restaurant Guld Tuppen under treatment with GOR BioSystem^{TM} are listed in the Table 10. Regarding the total amount of fat (ether soluble fat), the minimum value detected was 110 mg/L (23th day of activity) while the maximum was 240 mg/L (38th day of activity). The separable fat values varied between 0 mg/L (23th and 41th days of activity) and 70 mg/L (14th day of activity). The mean value is under the standard limit value of 50 mg of separable grease per liter, which is a positive value from a sewage network perspective.

BOD values of the effluent from this restaurant varied between 490 mg/L (5th day of activity) and 740 mg/L (16th day of activity), while COD values ranged between 820 mg/L (41th day of activity) and 1400 mg/ L (28th day of activity). The BOD/COD ratio calculated based on the average values of the effluent was 0,54, which indicates an easily biodegradable effluent.

Table 11: Chemical analysis during the treated cycle at Restaurant Guld Tuppen.

			Min	Max	Average	SD
Guld Tuppen (treated)	**FOG**	**Total fat**	110	240	195,71	36,52
		Separable fat	0	70	30	21,12
		BOD7	490	740	613,64	85
		COD	820	1400	1140	191
		TSS	69	330	204,93	63,86
		TOC	250	410	333,64	53,44
		TN	11	36	23,55	6,25
		TP	6,5	49	20,14	10,86

SD: standard deviation. All the values presented in this table are in mg/L.

The amount of suspended solids varied between 69 mg/ L (5th day of activity) and 330 mg/ L (14th day of activity). TOC values ranged between 250 mg/ L (5th day of activity) and 410 mg/ L (11th day of activity). Total nitrogen ranged between 11 mg/ L (41th day of activity) and 36 mg/ L (28th day of activity), while total phosphor values were between 6,5 mg/ L (41th day of activity) and 49 mg/ L (5th day of activity). The effluent of the restaurant Guld Tuppen presented an average C:N:P ratio of 100: 7,05: 6,03.

5.3.3. Visual analysis

Figure 24 displays an schematic representation of the GS installed at Restaurant Guld Tuppen. We could not observe the formation of a grease cap layer over the compartment 2 neither compartment 1 during the control treated cycle after ten weeks of observation. Even over C1 the grease cap was relatively thin and discontinuous (Figure 25).

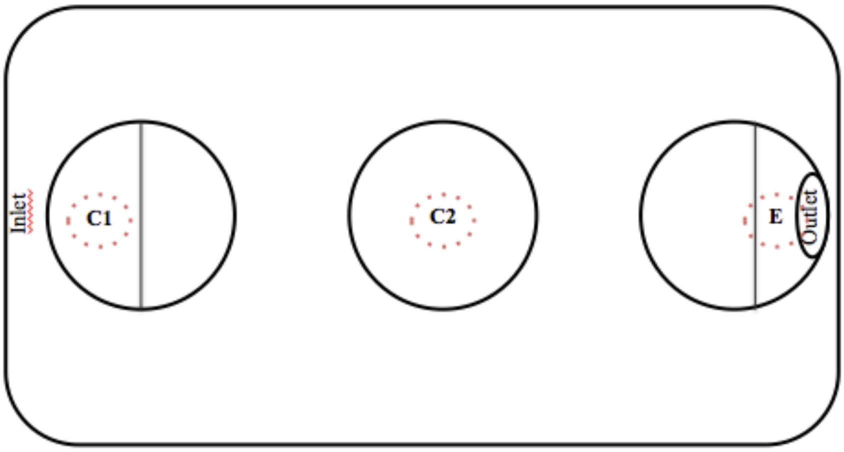


Figure 24: Schematic representation of the grease separator installed at Restaurant Guld Tuppen. C1 (slam compartment), C2 (separation compartment) and E (effluent compartment) represent the 3 different functional areas of this grease separator.

Figure 25: Visual analysis of the GS at Restaurant Guld Tuppen during the course of the study with the treatment with GOR BioSystem™(part 1).

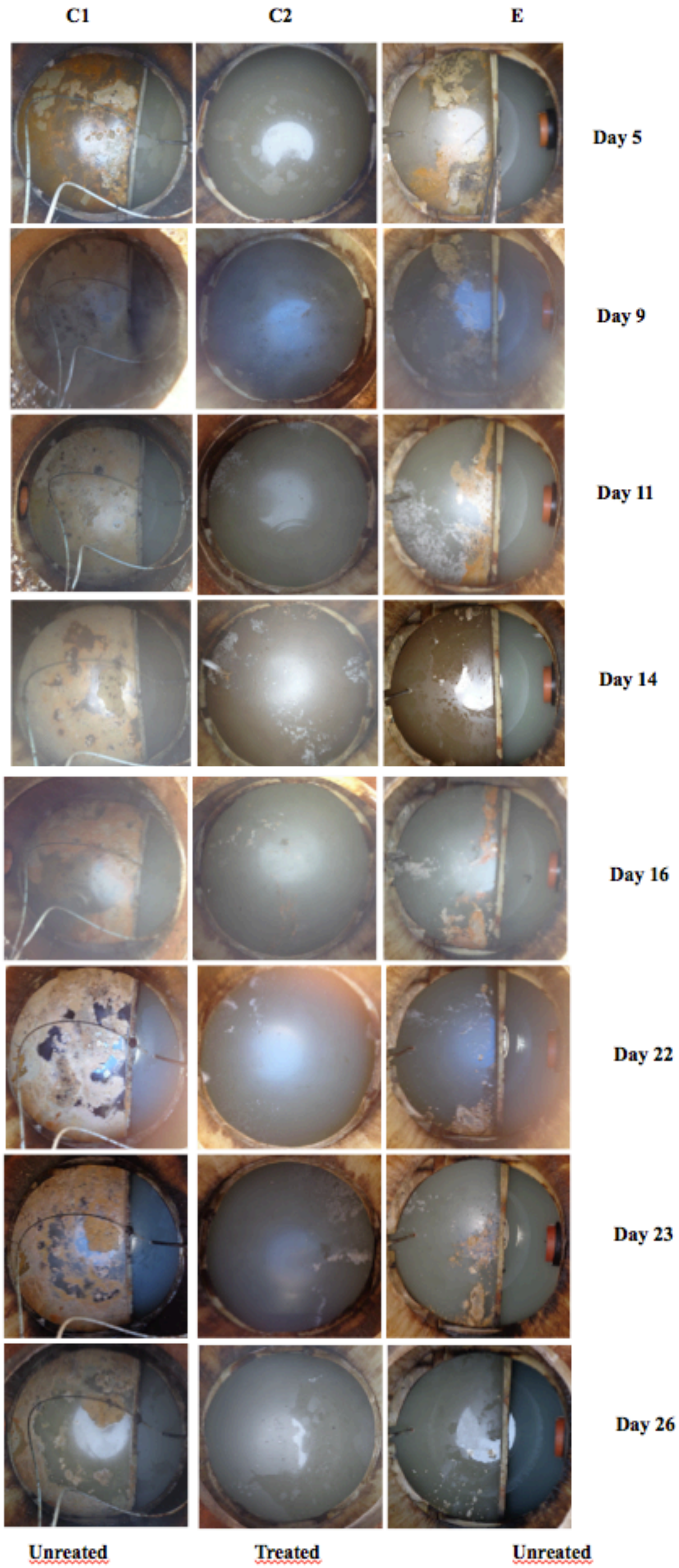
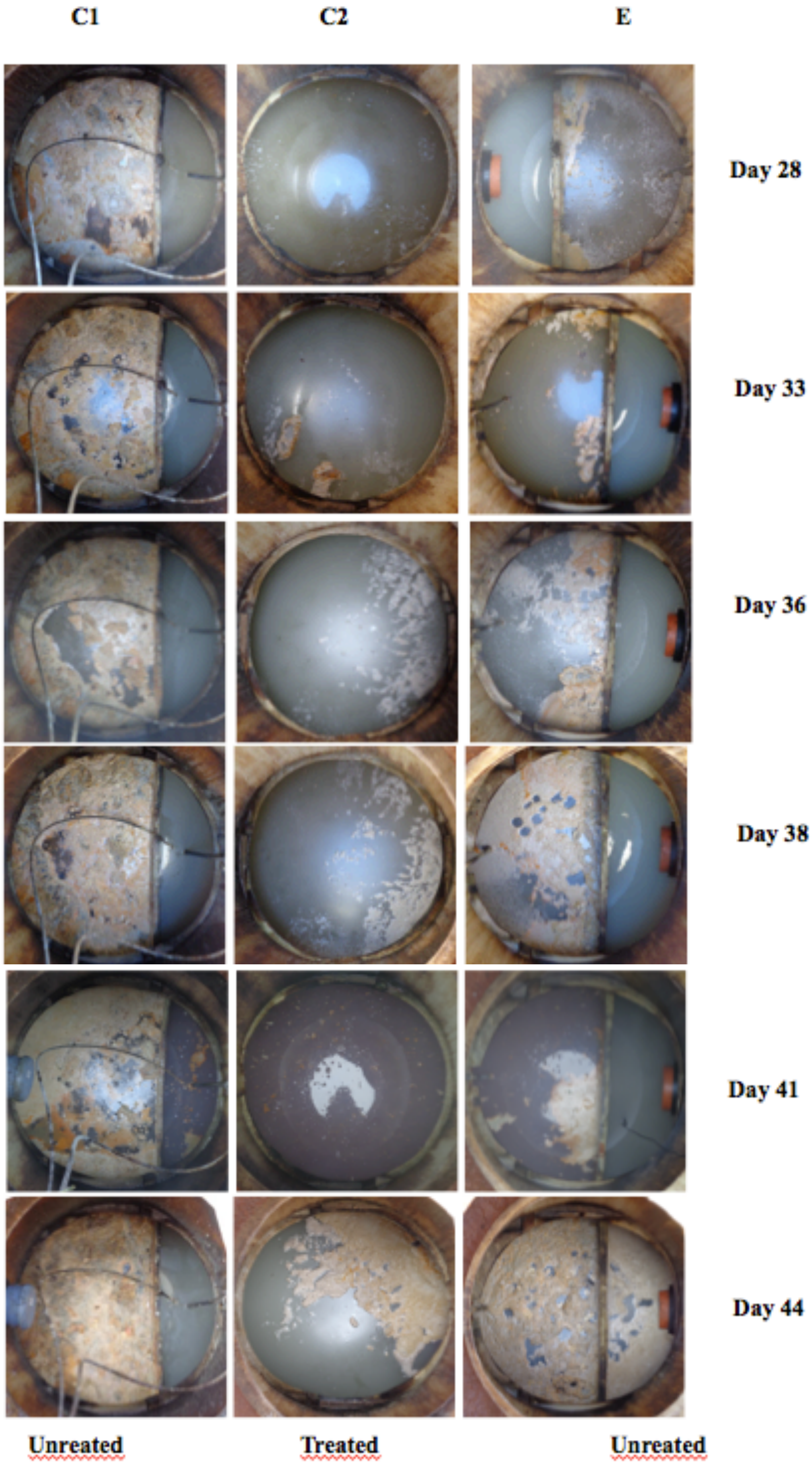


Figure 25 (continuation): Visual analysis of the GS at Restaurant Guld Tuppen during the course of the study with the treatment with GOR BioSystem^{TM}(part 2).



5.3.4. Odor evaluation

No disturbing odor was detected at the GS installed at Restaurant Guld Tuppen during the study period under the treatment with GOR BioSystem™. It was noticed a very characteristic (but not disturbing) odor in a very low strength through the observation period, which was classified in the lowest level of strength (very weak odor - level 1). The quality of this odor (smell characteristics) was also very different from the ones detected in the other GSs evaluated, which presented in general a pungent and unpleasant odor.

5.3.5. Sludge judge test

The sludge judge test was performed in eight dates during the study period at Restaurant Guld Tuppen. The results of the tests with the sludge judge are presented in the Table 12. No grease cap was detected over the bioaugmented zone of the GS (C2) until the 36th day of activity. After that period, only a discrete layer of 0,5 cm was observed at the 41th day of observation over C2. No solids were accumulated in the bottom of this compartment during the study period.

No grease cap was developed over the effluent compartment and we observed a very inconstant development of the GC over C1. The maximum value detected for the GC thickness over C1 was 7 cm at the 36th day of observation. Regarding the solids, we could follow a gradual accumulation in the bottom of C1 (which reached 55 cm after 41 days of activity). At the effluent compartment, we detected a more discrete accumulation (which reached 25 cm after 36 days of activity). The solids accumulated in the effluent displayed visual differences from the ones detected at C1: it seems clear/ greyish and it’s suspected to be biomass.

Table 12: Profile of accumulation of sludge and grease cap thickness at the grease separator treated with GOR BioSystem™ at Restaurant Guld Tuppen.

	Day 0		5 days		9 days		11 days		14 days		22 days	
	Grease cap	Sludge	Grease cap	Sludge	Grease cap	Sludge	Grease cap	Sludge	Grease cap	Sludge	Grease cap	Sludge
C1	0	0	n/d	0,5	n/d	n/d	3	15	n/d	15	0,7	30
C2	0	0	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d
E	0	0	n/d	n/d	n/d	18	n/d	18	n/d	18	n/d	20

All the measurements presented in this table are in cm. Day 0 corresponds to the day in which the grease separator was pumped out.

(continuation)

	Day 26		36 days		41 days	
	Grease cap	Sludge	Grease cap	Sludge	Grease cap	Sludge
C1	n/d	32	7	40	0,5	55
C2	n/d	n/d	n/d	n/d	0,5	n/d
E	n/d	23,5	n/d	25	n/d	19

5.4. Summary

In this study three different GSs were evaluated in relation to the impact of its performance in the effluent quality, FOG and settable solids removal and odor control. It is important to highlight that comparisons between the results obtained from these three GSs are not appropriated, thus the performance of each of those GSs have to be analyzed individually.

Different analytical strategies were adopted in this study. One GS was kept without bioaugmentative treatment during a interval of 25 activity days (at Restaurant Syster O Bror), while a second one was treated for 25 activity days and after a pump out and sterilization procedures was kept without treatment for a similar period of time (at Restaurant Brazilia) and a third separator was treated for almost the double length of time (44 activity days) under continuous bioaugmentation with GOR BioSystem™.

In relation to the effect of the treatment in the effluent quality, it was possible to observe that only the GS maintained under continuous treatment with GOR BioSystem™ over ten weeks (GS at Restaurant Guld Tuppen) presented pH and separable fat values which were in accordance to the recommended limit values (Table 13). All the GSs evaluated failed in relation to the temperature of the effluent and all of those presented acceptable conductivity values.

Table 13: Effluent Quality Analysis

	Recommended Values	Syster O Bror (untreated)	Brazilia (treated)	Brazilia (untreated)	Vasa Skolan (treated)
pH	6,5 – 11	5,82	5,41	5,25	6,47
Temperature	0-11	32,03	21	25,32	20,2
Conductivity	500000	1695,29	817,1	955,21	1157,92
Separable fat	50	172,86	121,43	129	30

The values presented at this table are based on the pH, temperature, conductivity and separable fat mean values obtained at restaurants Syster O Bror (untreated), Brazilia (untreated and treaded with GOR BioSystem™) and Guld Tuppen (treaded with GOR BioSystem™). The recommended values are based on the City council of Sala municipality recommendations (Sala Municipality 2003).

The analysis of the grease cap thickness shown that the untreated cycles (at Restaurant Syster O Bror and Restaurant Brazilia) presented significant higher levels of grease accumulation in relation to the cycles with treatment with GOR BioSystem™ (at Restaurant Brazilia and Restaurant Guld Tuppen) (Figure 26).

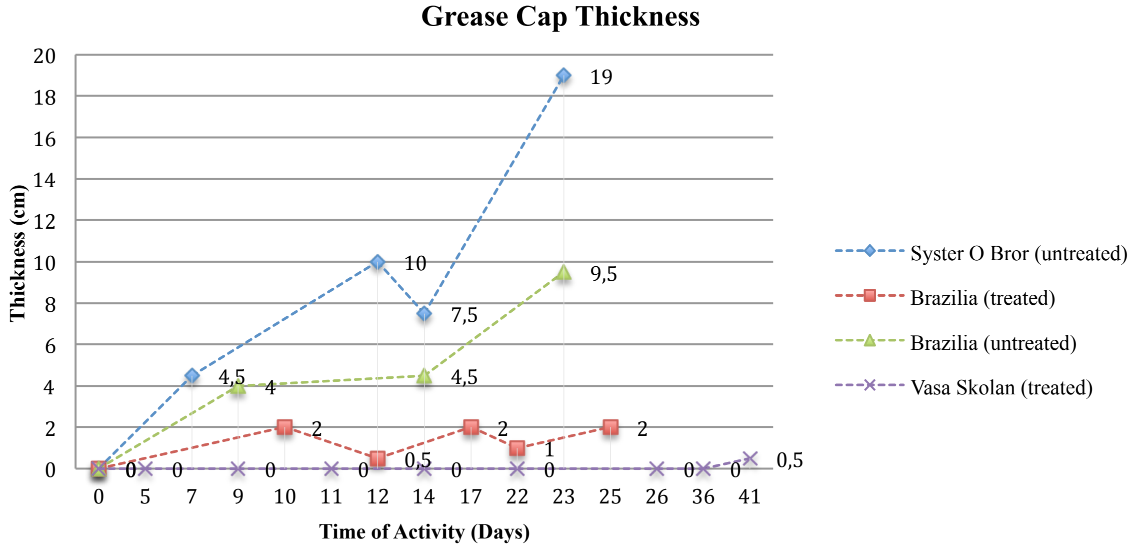


Figure 26: The values presented at this graph are based on the analysis of the grease cap thickness at restaurants Syster O Bror (untreated), Brazilia (untreated and treaded with GOR BioSystem™) and Guld Tuppen (treaded with GOR BioSystem™). The time of activity was determined as the period posterior to the complete pump out of the grease separator, being the pump out day marked as day 0.

The result of the grease cap accumulation was in accordance to the results obtained in the odor evaluation. It was possible to detect markedly stronger odors with disturbing characteristics during the untreated cycles in relation to the cycles under the treatment with GOR BioSystem™ (Figure 27).

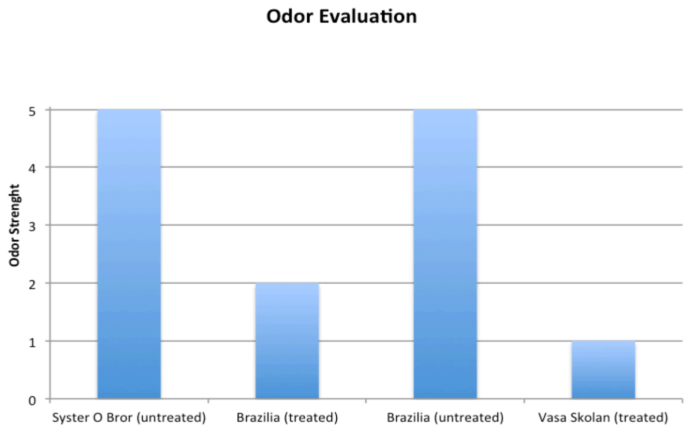


Figure 27: The values presented at this graph are based on the olfactory analysis of the odor from the grease separators at restaurants Syster O Bror (untreated), Brazilia (untreated and treaded with GOR BioSystem™) and Guld Tuppen (treaded with GOR BioSystem™). The odor strength was classified in a scale from 5 (very strong) to 0 (not detectable).

6. Discussion

This project was set out to examine the impact of the biotreatment promoted by GOR BioSystem™ on the quality of the effluent and grease removal of different GSs from different FSEs in Sweden. During the course of this study, three different GSs with different characteristics were evaluated. They were different in size (the GS from the Restaurant Brazilia was considered under-dimensioned based on the SS EN 1525, for example), design (the GS from the Restaurant Syster O Bror lacks the sludge section) and kitchen routine practices/ menus.

It is known that different factors can affect the performance of both GSs and possibly of the bioaugmentative strategy. For instance, the GS design, sizes as well the composition of the WW, which was introduced in the GS can interfere with the dynamics of the FOG separation and consequently the bioprocess (Rodis 2004, Korsha 2011) Thus, it is important to take into consideration that comparisons of the performances between the three different GS evaluated are inappropriate. However, the comparison between treated and untreated cycles in the same GS, such as the evaluation performed at Restaurant Brazilia, can provide valuable indicators of the impact of the GOR BioSystem™ on the effluent quality, grease accumulation profile and odor control.

One of the major challenges to GS field research comprises the adequacy and accuracy of the sampling methodologies. There are many factors that can affect the sample quality such as the nature of the sample matrix (highly hydrophobic and heterogeneous), the high variability of the organic load during the operation times of the restaurants and the difficulty in accessing the sampling points (He 2012). Another initial issue was the availability of GSs for study. GSs are mostly located outdoor and underground, thus conventional installations do not permit an identical side-by-side comparison of untreated and treated waste streams. Additionally, adverse environmental aspects such as external temperature and weather conditions (such as very low temperatures and snow in the winter) can also impact the sampling operation strategy and impair the quality of the analysis.

Very few GS incorporate sample ports. What we mainly found in the field was the access to the effluent compartment through an effluent sanitary tee where FOG and other floating solids tended to accumulate. An example of this was presented in the results from Restaurant Syster O Bror (Figure 11) during the study period without treatment with GOR BioSystem™. This accumulation could have influenced the quality of the results, since occasional release of this accumulated material can lead to false results.

Another aspect that we faced in the field during this study was the challenge of obtaining reliable and representative samples due to the lack of a standardized methodology and protocols for the sampling operations in GSs. Furthermore, GS presents a high variability of influent flow and content (Lesikar *et al*. 2006. Livingston *et al*. 2007, He *et al.* 2012). Consequently, flow and effluent characteristics may also change on an hourly, daily and/ or seasonal basis due to variations in the restaurant operation and routines (Livingston *et al*. 2007, He *et al.* 2012). It is known that the organic load of the wastewater is generally directly related to the food production activity in the kitchens (Lowry 1994). The choice of performing one-hour composite samples and the preliminary variability study were the strategies adopted in this study as an attempt to minimize the effects of this high variability. However, we believe that further studies are necessary in order to determine a more accurate and reliable strategy for sampling GSs.

Concerning the results obtained in the analysis of the effluent's physical parameters, it is important to remark that temperature, conductivity and pH are important environmental factors. When measured in the effluent, they reflect the bioactivity that occurred inside the GSs, therefore it can be seen as indicators of changes in the effluent quality due to bioprocessing. Comparing the results from the treated cycle with GOR BioSystem^{TM} and the untreated cycle at Restaurant Brazilia, no impact was observed in neither the pH, temperature nor conductivity values. The values obtained at restaurants Syster O Bror and Guld Tuppen were also within the range reported in the literature. Also, it is possible to affirm that all those parameters remained within an expectable range for bacterial activity based on the effluent quality recommendations (vide Table 13).

In a study comparing the treatment of GS for three months with a bacterial product with a three months baseline period, Angiel (2005) observed pH between 4,98 and 6,2 (baseline) and 4,61 and 6,65 (treated cycle) and and temperature between 25,6 and 42,7 °C (baseline) and 27,7 and 40,1 °C (treated cycle). He (2012) monitored two different 1000 gallons GSs or a one-year period which was divided in four cycles (two treated and two untreated) lasting 60 days each. This author found pH values between 4,2 and 7,0 and temperatures between 22 and 42 °C in the effluent of both GSs evaluated in both types of cycles. Livingston *et al.* (2007) analyzed the effect of bioaugmentation in four different restaurants GSs. In general, these authors observed average ph values ranging between 5,4 and 6,1 in all four GS and temperature values between 20,9 and 29,9 °C.

In relation to the chemical analysis, one of the most important parameters is the level of separable fat. This parameter is used as a standard in the recommended values for the control of effluent quality due to the deleterious effects of FOG in WW systems and treatment plants. Mostly the levels of total and separable fat varied greatly throughout the weeks of the study in all of the restaurants analyzed. The only GS that released an average value of separable fat in accordance to the standard limits was the one that was submitted to a longer treatment with GOR BioSystem^{TM} (at Restaurant Guld Tuppen). This result can corroborate the positive effects that GOR BioSystem^{TM} can play in the maintenance of the GSs, keeping an improved function and overall performance.

Livingston and collaborators (2007) observed a gradual increase in the oil and grease levels in the effluent of GSs with and without bioaugmentation, which was not observed in the present study. However, it is important to note that the results observed in the present study were obtained over a shorter period of observation (approximately five weeks cycles at Restaurant Brazilia and ten weeks at Restaurant Guld Tuppen). It is possible that more expressive results would be obtained over a longer period of observation.

The analysis of BOD and COD give insights about the composition of the wastewater, indicating the amount of organic and inorganic compounds that can be oxidized. The correlation between BOD and COD can also indicate the biodegradability level of the effluent. Both BOD and COD presented distinct values in all the GSs analyzed being the average values of the effluent from the GS that was treated for a longer period (Restaurant Guld Tuppen) considerably lower. However, according to the BOD/COD ratios its possible to observe that all the effluents analyzed are potentially biodegradable. Most of the studies found in the literature report the results of BOD 5, which are not comparable with the results obtained in the BOD 7 tests performed in the present study.

Regarding the amount of TSS (another standard parameter), it was possible to observe an overall variation through the different cycles in the different restaurants evaluated. Stockholm Water Company (the agency regulating the WW in Stockholm region) limit the discharge of TSS to 300 mg/ L. This information is relevant, considering the fact that the GSs of the two of the restaurants analyzed are located in the Stockholm city (Restaurant Brazilia and Restaurant Syster O Bror). Based on that limit, only the effluent from the GS treated for a longer period of time (Restaurant Guld Tuppen) could match the Stockholm Water requirements, with an average discharge of 204,93 mg/L.

The values obtained in the standard untreated GS (Restaurant Syster O Bror) was the highest, exceeding in more than 80 % the limit, while the average values obtained with and without the treatment with GOR BioSystem™ at Restaurant Brazilia exceeded aproximatelly 20 % (20,47 % duting the treated cycle and 22,22 % during the untreated cycle). Its important to remark that no statistical difference could be observed between the treated and untreated cycles at the Restaurant Brazilia, therefore the addition of the microbial product didn't affect the amount of sediments released in the effluent.

Barden & Burgess (1999) reported a significant reduction in the TSS levels in 10 restaurants over a 13-week period compared to a 7 weeks baseline period. This can indicate that a longer period of observation can be necessary for the detection of significant reduction of TSS levels. On the contrary, another authors (Livingston *et al.* 2007) observed an increase in the effluent of 3 of 4 different restaurants analyzed for 4 cycles of 8 to 11 weeks due to the bioaugmentation using a bioproduct composed by bacterial spores and surfactants.

TOC, TN and TP values are important parameters that give insights about the nutritional content of the effluent of the GS and potentially refect the nutritional conditions within those tanks. These parametes can be used to estimate the C:N:P ratios; the optimal ratio for the biodegradation of organic residues in wastewater is 100:5:1. It was possible to observe similar C:N:P ratios between the treated and untreated cycles at Restaurant Brazilia, which were close to the optimal ratio. The effluent from the GS from Restaurant Guld Tuppen (treated with GOR BioSystem™ for 10 weeks) presented an excess of both nitrogen (41 %) and phosphor (503 %) while the effluent of GS from the Restaurant Syster O Bror (untreated for 5 weeks) presented an lower excess in the nitrogen (7,6 %) and phosphor (207 %) levels. Different authors have discussed the variations in the composition of the WW from different FSE, and such differences in the C:N:P ratios can be directly related to the type of food prepared in the restaurants (Lesikar *et al*. 2006. Livingston *et al*. 2007, He *et al.* 2012).

In general, it is possible to say that no statistical difference was observed in all the chemical parameters analyzed between the treated and untreated cycles. At Restaurant Brazilia this fact can reinforce the hypothesis that longer periods of time with a higher amount of samples values can be necessary to observe significative differences. The authors He and collaborators (2012) observed statistically significant differences between bioaugmented and untreated cycles for several chemical and physical parameters. These authors analyzed two full-scale

GSs (from a restaurant and a retirement community kitchen) over a one-year period, and they observed lower BOD and COD values at the GS outlet during the treated cycles than in the untreated cycles. However, the bioaugmentation strategy did not include an aeration system and they observed that most of the GSs were anaerobic. Thus, metabolic differences are expected and it was not possible to make a comparison with the data of this study.

Samplings for the different cycles of this study occurred during different seasons (cycle 1 in Restaurant Brazilia occurred in late winter/ early spring and cycle 2 late spring/ early summer). It is possible that the external environmental temperature could have affected the temperature dispersion inside the GSs. Moreover, in some cases such as in Restaurants Syster O Bror and Brazilia, differences in the organic load were observed as a result of the gradual decrease of the number of meals served at the end of the academic activities in the campus. It is an very important information in relation to Restaurant Brazilia, because this decrease occurred during the course of the untreated cycle. Continuing this study by having a better accuracy with the seasonal conditions in both treated and untreated cycles would be beneficial to account for the variations in sales (i.e. organic load) and environmental factors that would affect the physico-chemical conditions inside of the GS.

Regarding the analysis of the grease cap development, bottom solids accumulation and the visual analysis, interesting results were obtained as well good considerations for further studies. One interesting result of this study was the detection of phase separation between the grease and oil content in the grease cap, especially during the untreated cycles (Figures 11 and 23). Such separation was not observed during the treated cycles (e.g. Figure 23). It is suspected that it may occur as a result of the bioaugmentative process promoted by GOR-BioSystem™, since oil is more accessible to the microbial/ enzymatic attack than the bigger and more complex grease particles (Lowry 1994).

It was observed that this liquid/oil layer had the consistence of emulsified grease or oil and it is possible that it is a product of the first stages of FOG catabolism. Bacteria begin their metabolism outside of the cell by producing surfactants and excrete enzymes that break larger molecules into smaller ones. These smaller molecules (such as fatty acids and glycerol) can be assimilated by the cells and be further metabolized (Wakelin & Forster 1996, Rock 2008). Another possibility is due to the changes in the menu characteristics. However, no significant differences were detected in the menus during the period of this study corresponding to both treated and untreated cycles (*data not shown*).

Aerated bio systems, such as the GOR BioSystem™, are proven to soften and greatly reduce the grease cap, eliminating the odor and clarifying the water (Lowry 1994). Aeration increases oxygen and the ubiquitous aerobic bacterial populations creating a mini-waste water treatment plant environment. The aeration also leads to changes in the consistency of the grease layer. This can be mainly attributed by the increase of the metabolic activity of the aerobic bacteria over the grease cap components. In this study, the analysis of the effects of the biotreatment over the grease cap formation (Figure 26) shown that GOR BioSystem^{TM} (an aerated biotreatment system) contributed to the maintenance of lower levels of FOG accumulating inside the GS compared to the levels found in untreated GSs.

It was also possible to observe a remarkable difference between the odor strength released from the GSs treated with GOR BioSystem^{TM} and the untreated GSs (Figure 27). According to Lowry (1994), the reduction or elimination of anaerobic bacteria will restrict or eliminate the production of H_2S and CH_4, which will lead to an odor control effect. Odor is considered as an issue in FSEs due to the disturbances that it can cause in the restaurants' operations. Thus, an effective treatment against odor is highly desired and in this study, the GOR BioSystem^{TM} presented satisfactory results in relation to odor control in GSs.

About the sludge judge tests, it is important to discuss the effects that aeration can have in the grease cap thickness during the treated cycles. It is possible for the air bubbles to get trapped in big organic matter molecules that will float and compose the grease cap. Thus, it would be necessary to have a more detailed analysis of the grease cap composition in order to determine the amount and quality of the components of the grease cap and have more substantial data regarding the effects of the bioaugmentation on the grease cap.

In consideration of the effects of bioaugmentation on the accumulation of solids in the GS, especially in the separation compartment, it was not possible to reach a conclusion taking into account the results obtained in this study. In the restaurant Guld Tuppen, for example, it was possible to detect solids in the effluent compartment during the treated cycle. This material is suspected to be biomass due to its the visual characteristics and the retention in the effluent compartment could have happened due to the GS design. Aerobic systems powered by the addition of mechanical aeration devices are expected to slightly promote the increase of biomass in GSs. However, further analysis is necessary in order to confirm this hypothesis.

Also, in relation to the solids accumulation profile, the results obtained at Restaurant Guld Tuppen (treated with Gor BioSystem^{TM} for 10 weeks) were different from the results obtained at Restaurant Brazilia. In the untreated cycle at Restaurant Brazilia, we observed a discrete accumulation (which ranged between 3 and 5 cm of settable solids), which was different from the period under treatment with GOR BioSystem™ in which no accumulated solids were detected even after 25 days of observation. No solids were detected in the effluent compartment. One hypothesis about what could had lead to this result was due to an enhancement of the biodegradation of the solids promoted by the bioaugmentative system (GOR BioSystem™), which could then be flushed away in the water flow together with the biomass.

It is also important to mention that during the sludge judge test the degree of compactibility of the grease cap or solids layer was not evaluated. Then, even though the values of the accumulation of solids seemed high (such as the solids accumulated in the effluent compartment at the GS from the Restaurant Guld Tuppen), quantitative differences in the weight and composition could occur. One hypothesis about the accumulation of the solids in this compartment is that it was due to the design of the effluent compartment and positioning of the effluent outlet, which enables the trapping of the biomass and other particles from the sludge and separation sections. Hence, more accurate studies are necessary in order to validate those results.

This study provided interesting data about the function and optimization of GSs through the use of bioaugmentative systems. It is possible to affirm that none of the physical or chemical data collected in this study indicated that the environment at the GSs was unsuitable for the bioactivity of the GOR BioSystem™, which would contradict the disclaimed bio-function. However, further studies are necessary for a greater understanding of the bioaugmentative processes promoted by GOR Biosystem™ in GSs and improvement of this new technology.

7. Conclusion

The present study investigated the effects of the bioaugmentation with GOR-BioSystem™ in the effluent, solid residues accumulated and odor in different restaurant GSs. Based on the results obtained during this study, it can be concluded that the use of the GOR BioSystem™ does not evidentially contribute to changes in the composition of the effluent in any noticeable way. No significant changes in the concentration of oils and greases and other organic components (t.ex. BOD, COD, TSS, TOC, TN and TP) in the effluent of the restaurants analyzed during the bioaugmentation with the aforementioned bio system was detected. The bioactivity displayed in the GSs subjected to this biotreatment confirmed that the physical environment provided by the GOR BioSystem™ was within the boundaries of a suitable environment for aerobic metabolism.

The results also supported the efficiency of GOR BioSystem™ in odor control and FOG removal inside of the GS. Foul odors were greatly reduced in the treated GSs (at Restaurant Guld Tuppen and at Restaurant Brazilia) and the layer of grease accumulated without the treatment at Restaurant Brazilia was considerably biodegraded during the treatment period with the use of GOR BioSystem™.

8. Future Work

The research community has largely neglected grease separators. Aspects about the physico-chemical characteristics of GS and the potential for biotreatment are still not fully understood. Furthermore, studies aiming to provide comprehensive understanding of the environmental conditions of GS are essential. As suggested by He and collaborators (2012), future studies should include cycles with longer duration between the pump outs. This would possible allow greater differences between treated and untreated cycles, which may become more preeminent toward the end of longer cycles.

GSs are very dynamic systems and the evaluation of its performance is a challenge due to the high numbers of variables in the system. One strategy to reduce the number of variables during the sampling procedures in a further study would be to sample in the same day of the week (or same activity day) in order to have a better comparison basis for treated and untreated cycles. And also to take consideration of the seasonal effects in both sampling operation and GS performance.

Finally, FOG samplings and analysis is a very complex due to the nature of the sample and the lack of homogeneity of FOG-water mixtures. It's important to find ways to improve the sampling methodology in order to increase the reliability of the samples from GSs. Also, in order to have a better understanding regarding the bioprocesses involved in the bioaugmentation of GSs it is essential, among other factors, to look at the chemistry and physical characteristics of wastewater from different FSE activities, to study theirs potential of separation and biodegradability patterns.

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