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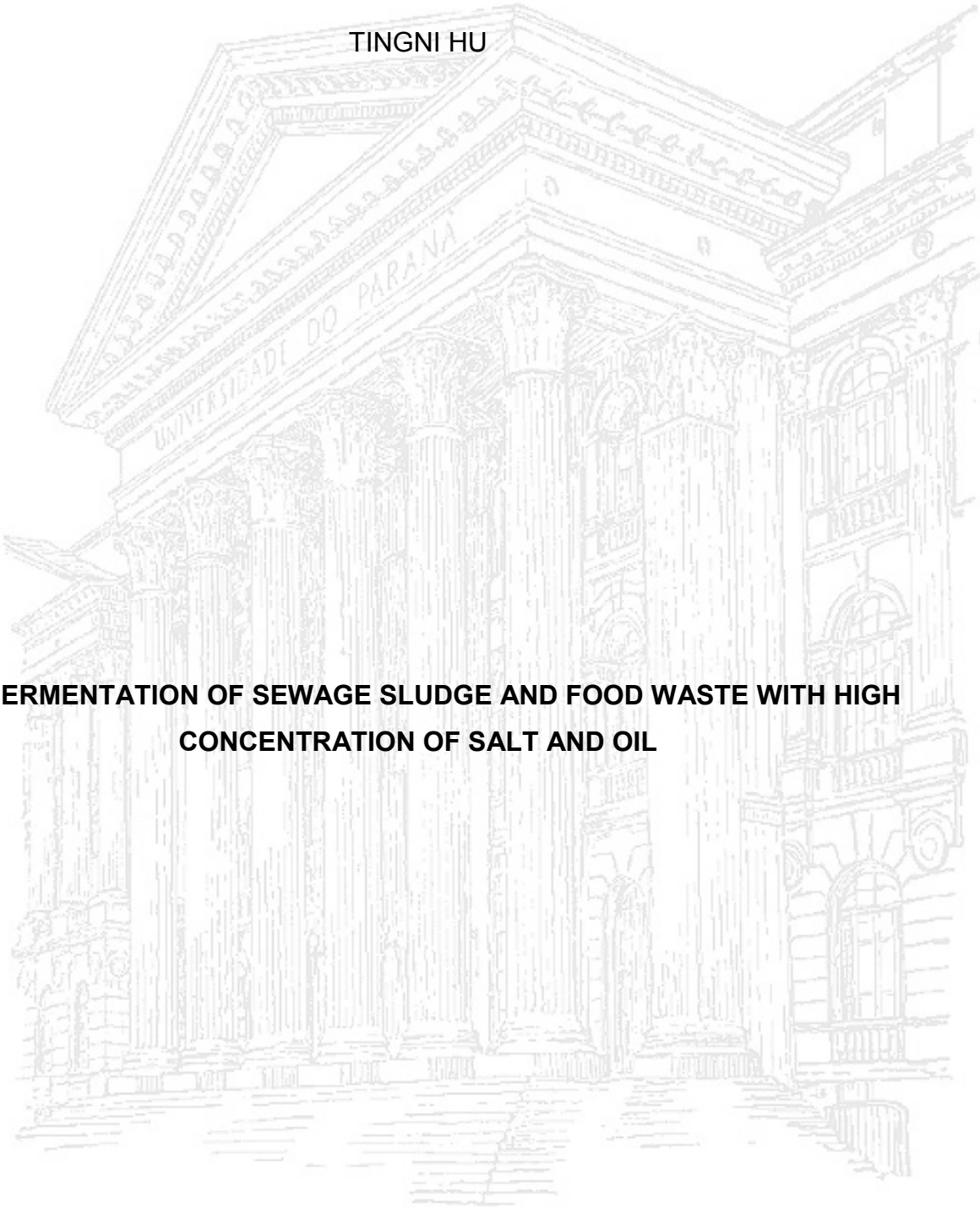

CO-FERMENTATION OF SEWAGE SLUDGE AND FOOD WASTE WITH HIGH CONCENTRATION OF SALT AND OIL

CURITIBA

2017

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Dissertação apresentada como requisito parcial à obtenção do grau de Mestre em Meio Ambiente Urbano e Industrial, no Programa de Mestrado Profissional em Meio Ambiente Urbano e Industrial, Setor de Tecnologia, da Universidade Federal do Paraná em parceria com o Serviço Nacional de Aprendizagem Industrial e a *Universität Stuttgart,* Alemanha.

Orientador: Prof. Dr. Klaus Fischer
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Dipl. –Ing. Jingjing Huang

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Curitiba, 24 de setembro de 2017.

RESUMO

Co-fermentação de lodo de esgoto e resíduo alimentar apresenta muitas vantagens em relação ao tratamento desses resíduos separadamente nos processos tradicionais de mono-fermentação, por isso, torna-se um tópico em desenvolvimento recente na área de tratamento e gerenciamento de resíduos. A composição dos resíduos alimentares se difere pela localidade, por exemplo, na China, têm-se concentrações maiores de sal e de óleo, as quais influenciam na degradação química-biológica do substrato. O presente estudo avaliou os efeitos da alta concentração de sal e de óleo no resto alimentar através da co-digestão com lodo de esgoto. O resíduo alimentar foi preparado em laboratório para simular a fração orgânica de resíduos sólidos urbanos da China, enquanto o lodo de esgoto foi coletado da estação de tratamento de esgoto do Institute for Sanitary Engineering, Water Quality and Solid Waste Management na cidade de Stuttgart (Alemanha). Parâmetros de processo e produção de biogás foram avaliados durante 3 meses de experimento, os quais foram divididos em Experimento Parte I (avaliação variando a concentração de sal), Período de Reabilitação (estabilização do biorreator) e Experimento Parte II (avaliação variando a concentração de óleo). O experimento foi conduzido em um reator tanque agitado contínuo em semi-escala de 210L com 21 dias de tempo de retenção hidráulica e faixa mesofílica (35ºC). Uma composição especificada de proteínas, carboidratos e lipídios foi estabelecida para manter uma taxa de carga orgânica constante (2,3 g SV/L.d) na alimentação manual do digestor semi-contínuo diariamente. A produção de biogás foi determinada diariamente assim como os testes em laboratório para analisar o processo através de parâmetros como, por exemplo, medidas do pH para amostras de entrada e saída do reator, concentração de amônia, condutividade para determinar a concentração de sal, sólidos totais, sólidos voláteis do efluente do reator e valores de FOS/TAC. Os resultados mostraram que a co-digestão de lodo de esgoto e resíduo alimentar produziu maior volume de biogás e rendimento de metano em comparação com a mono-digestão de lodo de esgoto somente. Além disso, o processo de co-fermentação pode ser inibido com maior concentração de sal, medido no digestor (1,2 g/L), enquanto maior teor de gordura no resíduo alimentar (42%) aumenta a produção volumétrica de biogás e metano. As análises dos parâmetros de processo mostraram que a estabilidade é maior degradando substrato de alto teor de óleo (Experimento Parte II) do que material com alta concentração de sal (Experimento Parte I). Contudo, a degradação de substrato com alto teor de óleo gerou um período de atraso na produção de biogás, enquanto que com alta concentração de sal não houve período de retardo, embora sua taxa tenha sido menor. Para concluir, o processo de co-digestão representa uma possibilidade para o tratamento da fração orgânica de resíduos sólidos urbanos, uma vez que a biodegradabilidade do processo alcançou 97% e portanto, poderia tornar-se uma alternativa para aumentar a eficiência da produção de energia renovável na estação de tratamento de efluente.

Palavras-chave: Digestão anaeróbica; Fermentação; Tratamento de resíduo; Resíduo alimentar; Lodo de esgoto; Fração orgânica de resíduo sólido urbano; Produção de biogás; Gordura.

ABSTRACT

Co-fermentation of sewage sludge and food waste presents many advantages over the treatment of these wastes separately in the traditional mono-fermentation process, which makes it a recent and developing topic regarding waste management and treatment. Food waste composition differs depending on the location, for example, in China it has a higher salt and oil content, which influences the chemical-biological degradation of the substrate. The present study assesses the effects of high salt concentration and oil content in the food waste through the co-digestion with sewage sludge. The fed food waste was prepared in the lab to simulate the organic fraction of municipal solid waste from China whereas the sewage sludge was collected from the wastewater treatment plant from the Institute for Sanitary Engineering, Water Quality and Solid Waste Management in Stuttgart city (Germany). Process parameters and biogas production has been evaluated over 3 months of experiments, which were divided as Part I Experiment (assessment with changing salt concentration), Rehabilitation period (stabilization of the bioreactor) and Part II Experiment (assessment with changing oil concentration). The experiment was conducted in a semi-scale continuous-stirred-tank-reactor (CSTR) of 210 L with 21 days of hydraulic retention time and mesophilic range (35ºC). An established composition of protein, carbohydrate and lipids were determined to set a constant organic load rate (2,3 g VS/L.d) to feed manually the semi-continuous digester daily. Biogas production was determined daily as well as lab tests to assess the process through parameters such as pH measurements with the input and output samples from the reactor, ammonium concentration, conductivity to determine the salt concentration, total solids, volatile solids of the reactor's effluent and the FOS/TAC values. The results showed that the co-digestion of sewage sludge and food waste produced higher biogas volume and methane yield in comparison with digestion of only sewage sludge. Furthermore, the co-fermentation process can be inhibited with a higher concentration of salt, measured in the digester (1,2 g/L), while higher fat content in the food (42%) enhances volumetric biogas and methane production. Analysis of the process parameters showed a more stable degradation using a high oil content substrate (Part II Experiment) than with high salt concentration material (Part I Experiment). However, the degradation of substrates with high oil content caused the appearance of a lag period in the biogas production while with high salt concentration no lag period could be observed although its rate was lower. To conclude, the co-digestion process represents a possibility for the treatment of organic fraction of municipal solid waste since biodegradability of the process achieved 97%, thus it would be an alternative to enhance the efficiency of renewable energy production in the wastewater treatment plant.

Keywords: Anaerobic digestion; Fermentation; Waste treatment; Food waste; Sewage sludge; Organic fraction of municipal solid waste; Biogas production; Fat.

ZUSAMMENFASSUNG

Co-Fermentation von Klärschlamm und Lebensmittelabfällen bietet viele Vorteile gegenüber der Behandlung dieser Abfälle in dem traditionellen Monovergärungsverfahren, was es zu einem neueren und sich entwickelnden Thema hinsichtlich der Abfallbehandlung und -verwaltung macht. Die Zusammensetzung der Lebensmittelabfälle ist ortsabhängig, zum Beispiel hat sie in China einen höheren Salz- und Ölgehalt, was den chemisch-biologischen Abbau des Substrats beeinflusst. In der vorliegenden Studie werden die Auswirkungen von hoher Salzkonzentration und Ölgehalt in den Lebensmittelabfällen durch die Co-Vergärung mit Klärschlamm bewertet. Der Nahrungsmittelabfall wurde im Labor vorbereitet, um den organischen Anteil der städtischen, festen Abfälle aus China zu simulieren, wärend der Klärschlamm von der Kläranlage des Institut für Siedlungswasserbau, Wassergüte- und Abfallwirtschaft in der Stadt Stuttgart (Deutschland) gesammelt wurde. Die Prozessparameter und die Biogasproduktion wurden über 3 Monate in drei unterschiedlichen Experimenten evaluiert, bestehend aus dem Teil I-Experiment (Bewertung mit wechselnder Salzkonzentration), einer Rehabilitationsperiode (Stabilisierung des Bioreaktors) und dem Teil II-Experiment (Bewertung mit wechselnder Ölkonzentration). Das Experiment wurde in einem halb-kontinuierlichen Rührkesselreaktor (CSTR) von 210L mit 21 Tagen hydraulischer Retentionszeit und mesophilen Bereich (35°C) durchgeführt. Eine etablierte Zusammensetzung von Protein, Kohlenhydrat und Lipiden wurde bestimmt, um eine konstante organische Belastungsrate (2,3 g oTS/L.d) einzustellen, um den halbkontinuierlichen Kocher täglich zuzuführen. Sowohl die Biogasproduktion als auch die Laborversuche wurden täglich durchgeführt, um den Prozess durch Parameter wie pH-Messwerte bei den Eingangs- und Ausgangsproben aus dem Reaktor, die Ammoniumkonzentration, die Leitfähigkeit zur Bestimmung der Salzkonzentration, Gesamtfeststoffe, flüchtige Feststoffe des Reaktorabwassers und die FOS / TAC-Werte zu bestimmen. Die Ergebnisse zeigten, dass die Co-Fermentation von Klärschlamm und Lebensmittelabfall erzeugte im Vergleich zur Fermentation von nur Klärschlamm ein höheres Biogasvolumen und eine Methanausbeute. Außerdem kann das Co-Fermentationsverfahren mit einer höheren Salzkonzentration (1,2 g/L) gehemmt werden, während ein höherer Fettgehalt (42%) die volumetrische Biogas- und Methanproduktion erhöht. Die Analyse der Prozessparameter zeigt, dass das Verfahren zum Abbau von Substrat mit hohem Ölgehalt (Teil II-Experiment) stabiler ist, als das Verfahren mit hoher Salzkonzentration (Teil I-Experiment). Zusätzlich, bewirkte der Abbau von Substraten mit hohem Ölgehalt das Auftreten einer Verzögerungszeit in der Biogasproduktion. Bei hoher Salzkonzentration hingegen verlief der Abbau ohne Verzögerungszeit, unabhängig von einer niedrigeren Produktionsrate. Abschließend stellt der Co-Fermentationsprozess eine Möglichkeit für die Behandlung von OFMSW dar, da die biologische Abbaubarkeit des Prozesses 97% erreicht hat und ist damit eine Alternative zur Steigerung der Effizienz der erneuerbaren Energieerzeugung in der Kläranlage.

Stichwort: Anaerobe Fermentation; Abfallbehandlung; Lebensmittelabfall; Klärschlamm; Organischen Anteil der städtischen festen Abfälle; Biogasproduktion; Fett.

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LIST OF ABBREVIATIONS

AD	-	Anaerobic digestion
GPR	-	Biogas production rate
°C	-	Celsius
cm	-	Centimeter
C	-	Conductivity
CSTR	-	Continuous stirred tank reactor
m^3	-	Cubic meter
d	-	Day
Ft	-	Factor for the calculation of the salt concentration
FW	-	Food waste
i	-	Fresh material
g	-	Gram
h		hour
HRT	-	Hydraulic retention time
ISWA	-	Institut für Siedlungswasserbau, Wassergüte- und Abfallwirtschaft
kg	-	Kilogram
LFKW	-	Lehr- und Forschungsklärwerk
L	-	Liter
LCFA	-	Long chain fatty acid
M	-	Mass
MPR	-	Methane production rate
mg	-	Miligram
MSW	-	Municipal solid waste
N	-	Normal
OF	-	Organic fraction
S	-	Siemens
Sc	-	Salt content
SS		Sewage sludge
SBP	-	Specific biogas production

SMY	-	Specific methane yield
TAC	-	Total inorganic carbonate
TS	-	Total solids
VFA	-	Volatile fatty acid
FOS	-	Volatile organic substances
VS	-	Volatile solids
V	-	Volume
w		weight
WWTP	-	Wastewater treatment plant

SUMMARY

1 INTRODUCTION

Co-fermentation, as known as co-digestion also, is the process of biodegradation of organic matter from different origins and compositions simultaneously in the absence of air by the action of microorganisms. Usually, substrates with a different organic content are commonly mixed, such as agricultural and industrial residue like silage and animal manure, the organic fraction (OF) of municipal solid waste (MSW), sewage sludge from water waste treatment plants (WWTP), etc.

In this process, besides the degradation of organic matter leading to the substrate biochemical stabilization, the main product of interest is the biogas production, which is a gas mixture composed mainly of methane (50-60%) and carbon dioxide (30-40%) in general, depending on the process performance, this values can change and also there is possible to have formation of trace elements, such as volatile organic compounds and hydrogen sulphide (CARREAS, 2013). The energetic value of biogas is determined by the concentration of methane, which is around 20 and 25 MJ/m^3 compared with 33 and 38 MJ/m^3 for natural gas (WERNER ET AL., 1989).

Many studies are being conducted in the area of co-fermentation of two or more substrates to improve the processes of mono-fermentation in the production of biogas. It is a recent technological reality employed on an industrial scale because co-digestion confers a number of technical, economic and environmental advantages to the process.

Regarding economic viability, sharing the same treatment facility for different kinds of waste can be one benefit of co-digestion, through the unified management methodologies, the reduction of investments and operational costs. In addition, the use of substrates with different physicochemical characteristics may allow greater process efficiency (CARREAS, 2013).

The energy production from the traditional anaerobic mono-fermentation of sewage sludge in WWTP is limited by the low efficiency of the biogas production. In the co-fermentation, according to Poggi-Varaldo and Oleszkiewicz (1992), food waste with high organic content and high biodegradable material content can be considered suitable co-substrates in sewage treatment plants providing a better yield of biogas production and high load of degradable organic matter.

The same result was concluded by Sosnowski, P. et al (2003) in the comparison of mono-fermentation of sewage sludge from a WWTP (experiment 1) and co-fermentation of the sludge with the organic fraction of MSW (experiment 2). After 35 days of experiments, the average cumulative biogas production showed that the volume of biogas generated in the co-fermentation process is almost double than with mono-fermentation of sludge only, with respectively 460 $dm^3/g\ VS_{added}$ and 240 $dm^3/g\ VS_{added}$ approximately.

In addition, co-fermentation makes the compensation of essential nutrients for the balance of the system. An important parameter to be controlled in bacterial metabolism in methane synthesis is the ratio carbon/nitrogen chemical balance. In this case, a substrate, for example, sewage sludge with high nitrogen content, can serve as a chemical complement to another co-substrate, e.g. food waste, to obtain a better anaerobic biodegradation (CARREAS, 2013).

Furthermore, energy recovery of waste can be achieved through biogas generation and reuse of material through the formation of digestate, a byproduct that can be used for soil conditioning as fertilizer. In this way, the reuse of waste can be in accordance with the German legislation Closed-loop Waste Management Act (Kreislaufwirtschaftsgesetz - KrWG) and the European Union legislation, Waste Framework Directive.

Additionally, through the digestion treatment process, the disposal of organic wastes to the landfills can be avoided, which is common in countries where the environmental legislation allows or the waste management is not well structured, like in countries of emerging economies. This way, it would improve the environment, since it avoids the proliferation of animals and insects that transmit diseases, the release of greenhouse gases into the atmosphere, as well as the generation of leachate, which can cause the contamination of water bodies and soil.

Co-digestion is the most relevant topic in recent years in the research area on organic matter fermentation, according to Mata-Alvarez, J. et al. (2014), In the study carried out by the author showed that the organic matter degradation involving sewage sludge and the organic fraction of MSW is the most commonly mix studied in articles published between the years 2010 and 2013. However, further researches in this area still need to be improved and developed, since there is a great variation of the physical-chemical characteristics of the substrates, especially regarding the organic fraction of MSW, which characteristics and material compositions are

different from place to place. The composition of the food waste is consisted by a mixture of complex organic matter (proteins, carbohydrates, and fats), minerals and among other compounds, such as vitamins and water.

The food waste composition may vary regarding the social-cultural and geographic aspects. Regarding the salt content, a survey conducted by the Harvard School of Public Health and the University of Cambridge in 2010 shows that the daily sodium intake varies from country to country, e.g. in Germany, for the adult population, the daily intake is approximately 3700 mg per capita, while in China the presence of salt is major for an adult daily consumption: 5200 mg. This high concentration of salt content (NaCl mainly) in food waste may impact in the microbial activity in the anaerobic process due to the rise in the osmotic pressure leading to the dehydration in the bacterial cell wall (YERKES, 1997). Therefore, the digestion process would be affected, and consequently decrease in biogas production and failure of the process. In this case, the dissolved ion in aqueous mean Na+ was considered the potential factor that influences in the toxicity caused by salt.

In China, the organic fraction of municipal solid waste is account to 40-50% of the weight and the food waste (FW) generation is increasing 10% per year, it means that a high organic content can be biodegraded and energy recovery can be obtained by an anaerobic process as stated by Dai, et al. (2013). In the study carried out by the author, the addition of FW in dewatered sludge improved the system stability as well as increased greatly volumetric biogas production.

Still in relation to the FW composition in China, the high concentration of oil can be verified in a study conducted by BMJ in 2010, which showed that Chinese people is one of the biggest fat consumers in the world with more than 2000 mg per day of omega 3, a polyunsaturated long chain fatty acid (MICHA, 2014).

In this case, long chain fatty acids (LCFA) can also be toxic to the bacteria. Conforming to Demeyer and Henderickx (1967) and Galbraith and Miller (1973), LCFAs are adsorbed to a cell membrane, thereby causing its interferences in the transport and protection processes, resulting in the inhibition of the digestion performance.

By the importance of co-fermentation of food waste in sewage sludge and regarding the inhibitory substances in the high concentration of salt and oil in Chinese food waste, the present thesis will assess the influence of salt (mineral) and oil (fat) content in the anaerobic digestion (AD) process.

2 OBJECTIVES

The general objective of this study is to evaluate the effect of salt and oil supplement in the anaerobic co-digestion of food waste and sewage sludge through the behavior of gas production and process stability.

The specific objectives of this study are:

- To determine experimentally the volumetric biogas (GPR and SBP) and methane production (MPR and SMY) according to different salt concentration load;
- To determine experimentally the volumetric biogas (GPR and SBP) and methane production (MPR and SMY) according to different oil concentration load;
- To determine and to evaluate the optimal and critical concentrations of salt;
- To determine and to evaluate biogas production with high-fat content on FW;
- To assess process parameters;
- To calculate the biodegradation rate.

3 LITERATURE REVIEW

3.1 SUBSTRATES

As long as the food composition vary from place to place, the characterization of the food waste in the specific region has to be done for the biogas production assessment.

Anaerobic digestion (AD) is suitable for the treatment of organic waste due to its high biodegradability (CARREAS, 2013). They are mainly comprised by three groups: carbohydrates, proteins and lipids, each one of these substrates is detailed in the following text.

Carbohydrates have chemical constitution $C_x(H2O)_y$, which contains C, H, and O, the latter in the same proportion as in water. Carbohydrates has one or more alcoholic groups (-OH) and an aldehyde (-CHO) or ketone (-CO-) group. They are composed by monosaccharides, disaccharides and polysaccharides (VICENZI, 2017).

Proteins contain carbon (50 - 55%), hydrogen (6 - 8%), oxygen (20 - 24%), nitrogen (15 - 18%) and sulfur (0,2 - 0,3%). They are high molecular weight polymers whose basic units are amino acids connected by peptide bonds forming long chains in various geometric structures and chemical combinations (VICENZI, 2017).

Lipids are formed by carbon, hydrogen, oxygen and may also have phosphor, nitrogen and Sulphur. They are generally insoluble in water and soluble in organic solvents such as ethyl ether, chloroform, benzene and alcohols (VICENZI, 2017). Among lipids are fat and oil, which are triglycerides of several types of fatty acids. Here, free fatty acids or volatile fatty acids (VFA) are light molecules of carboxylic acid with aliphatic chain until 4 carbons, such as formic, acetic, propionic and valeric acids. While the fatty acids that are bound to other organic components, as glycerol, forming a long aliphatic chain (4 - 28 C) are called long chain fatty acids (LCFA).

Biogas production depends primarily on the amount of substrate consumed or degraded by the bacteria. Usually it can be expressed by the chemical oxygen demand (COD) and volatile solids (VS), or sometimes based in total solids (TS). The Table 1 describes the potential biogas production for different substrates, such as

food waste and WWTP sludge (CARREAS, 2013).

TABLE 1 - POTENTIAL BIOGAS PRODUCTION BASED ON TS FOR FOOD WASTE AND WWTP SLUDGE

Waste	Potential biogas production (Nm^3/t TS)	Methane content (%)
Organic fraction of MSW	400 - 700	60 - 65
WWTP sludge	380 - 400	65 - 75

SOURCE: ADAPTED FROM CARREAS (2013).

The Table 2 states the type of organic content in each fresh material, as well as content of volatile solids and potential biogas production per amount of digested waste (ANGELIDAKI and AHRING, 2002).

TABLE 2 - ORGANIC CONTENT OF SUBSTRATES AND POTENTIAL BIOGAS PRODUCTION BASED ON THE AMOUNT OF FRESH MATERIAL

Type	Organic content	Volatile solids (%)	Biogas production (m^3/t)
Soybean oil/ margarine	90% vegetal oils	90	800 - 1000
Sewage sludge	Carbohydrates, proteins, lipids	3 - 4	17 - 22
Concentrated sludge	Carbohydrates, proteins, lipids	15 - 20	85 - 110
Organic fraction of MSW	Carbohydrates, proteins, lipids	20 - 30	150 - 240

SOURCE: ANGELIDAKI AND AHRING (2002).

Anaerobic digestion of fat has a greater theoretical biogas yield and produces more methane content comparing to degradation of protein and carbohydrate, which values are shown in Table 3.

TABLE 3 - BIOGAS YIELD AND METHANE CONTENT FOR CARBOHYDRATE, PROTEIN AND FAT

Substrate	Biogas yield (mL/g) [1]	Methane content (%) [2]
Carbohydrates	840	50 - 58%
Proteins	930	50 - 58%
Fat	1430	66 - 73%

SOURCE: [1]: ALVES ET AL. (2009); [2]: GUJER AND ZEHNDER (1983)

3.2 ANAEROBIC PROCESS STAGES

The fermentation process is a biological degradation of organic matter is a process that occurs in the natural environment due to the action of different microorganisms in the absence of air, more specifically, without oxygen, as can be seen in Equation 1 that summarize the general process (CARREAS, 2013):

$$C_6H_{12}O_6 \rightarrow 3\,CO_2 + 3CH_4 + 34{,}4\ \text{calorie} \qquad \text{(Equation 1)}$$

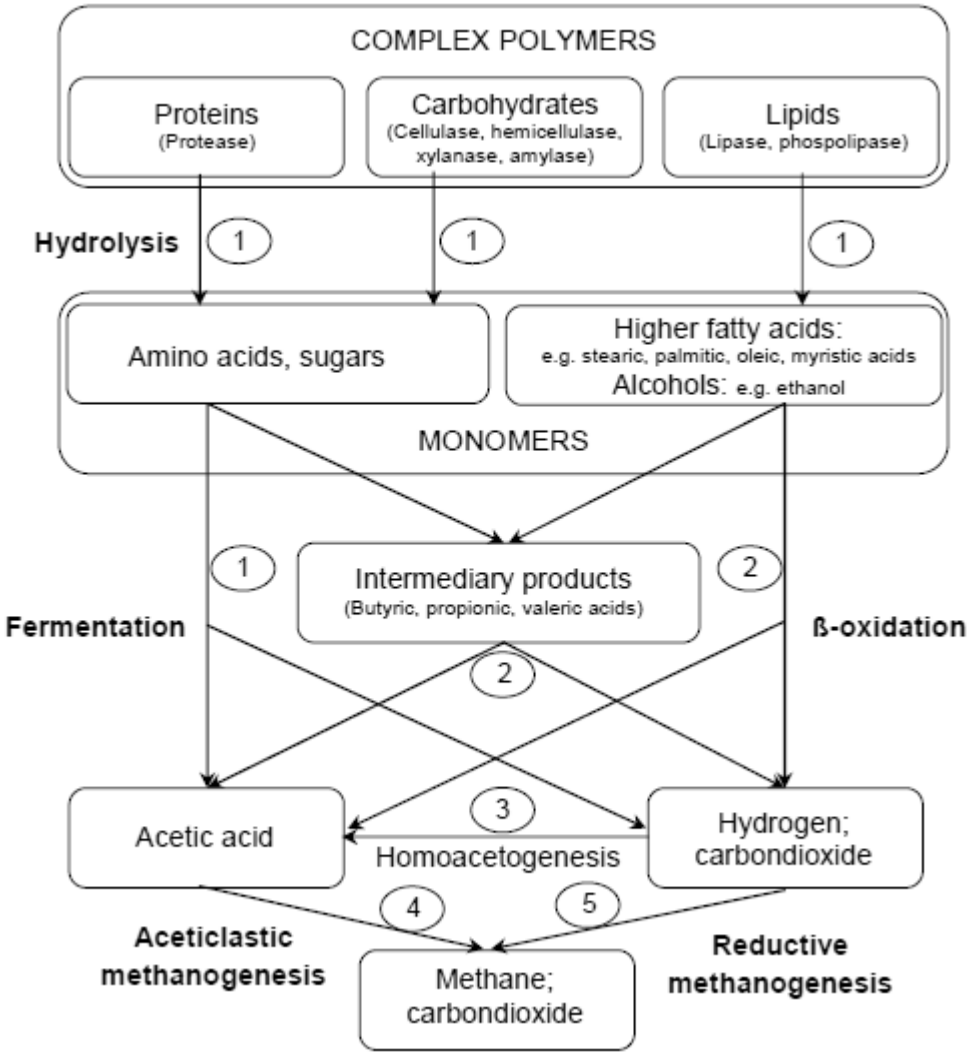
This degradation process is performed by different and specific families of microorganism, most of them, bacteria that consume the substrates by a series of numerous and complex biochemical reactions which usually occur simultaneously.

According to Hill (1977), if a process involves a series of reactions, the overall rate of the reaction is determined by the slowest one, as known as the rate-limiting step.

Generally, the anaerobic decomposition is divided into four stages: hydrolysis, acidogenesis, acetogenesis and methanogenesis, where each one of them can be the limiting phase for the overall reaction rate (LEMA and MÉNDEZ, 2013).

The summary of digestion process can be seen in Figure 1, where the numbers are indicating the family of bacteria that are involved in each phase: 1. Fermentative bacteria; 2. Hydrogen-producing acetogenic bacteria; 3. Hydrogen-consuming acetogenic bacteria; 4. Aceticlastic methanogenic bacteria; 5. Carbon dioxide-reducing methanogenic bacteria (NAYONO, 2010; STRONACH, 1983).

FIGURE 1 - DEGRADATION STEPS OF ANAEROBIC DIGESTION PROCESS



SOURCE: NAYONA (2010).

3.2.1 Hydrolysis

Hydrolysis is the initial stage in the digestion process of complex organic substrates such as proteins, polysaccharides (carbohydrates) and lipids (fat and grease) into soluble polymers and monomers, respectively, amino acids, monosaccharides and fatty acids, since microorganisms can only degrade soluble organic matter that can be transported into the cell passing through the cell wall. Consequently, the hydrolysis step provides organic substrates for subsequent anaerobic stages. This process to degrade complex molecules is performed through the action of extracellular enzymes produced by hydrolytic microorganisms and so far can be the limiting step of the overall process, principally when the residue has a high

solid content or hardly degradable material as lignocellulose materials (CARREAS, 2013)

Hydrolysis rate usually increases with temperature and additionally depends on the size of the particles, due to the availability of surface area for cell adsorption of hydrolytic enzymes. The increase in the rate of reaction can be achieved with physico-chemical pretreatment, whose main impact is the reduction of particle size. Thus, if this step is limiting the digestion process, pretreatment can benefit the overall process by reducing the retention time and furthermore, diminishing reactor sizes (CARREAS, 2013).

The general kinetic term regarding to the disintegration, solubilization and enzymatic hydrolysis of substrates is related to the hydrolysis step in anaerobic fermentation (BATSTONE et al., 2002).

The degradation speed of a substrate is proportional to the rate coefficient of the reaction *K*. In other words, the higher the kinetic coefficient, the faster is the reaction rate, and the more rapid is the reagent consumption and product formation. The Table 4 compares the values of rate coefficients *K* (day^{-1}) of hydrolysis at T = 55ºC for carbohydrates, proteins and lipids. In such way, a carbohydrate has a higher kinetic coefficient than protein and lipid successively. Thus, for the hydrolysis step, degradation of lipids is longer than other organic materials.

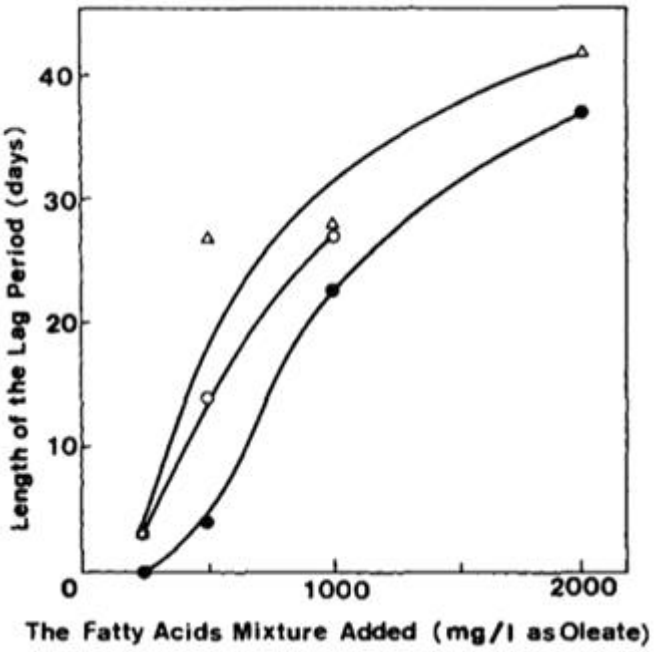
TABLE 4 - KINETIC COEFFICIENT FOR HYDROLYSIS AT 55 ºC

Substrate	**k (day^{-1})**
Carbohydrates	0,025 - 0,2
Proteins	0,015 - 0,075
Lipids	0,005 - 0,010

SOURCE: CHRIST ET AL. (2000).

The Figure 2 shows the increase of the lag period with the rise of fatty acids concentration in anaerobic digestion (HANAKI ET AL., 1981).

FIGURE 2 - LENGTH OF THE LAG PERIOD ACCORDING TO THE CONCENTRATION OF THE FATTY ACIDS ADDED



●: fatty acids mixture alone; ○: acetate in the fatty acids mixture; Δ: n-butyrate in the fatty acids mixture.

SOURCE: HANAKI ET AL. (1981).

3.2.2 Acidogenesis

Also known as fermentation, some molecules produced in the previous hydrolysis step is oxidized to CO_2, H_2 by the anaerobic oxidation pathway, while other monomers are transformed to organic acids (propionic acid, butyric acid, valeric acid) that can be used by acetogenic bacteria in the next step of the process. During this step, alcohols are generated as well (NAYONA, 2010). Thus, if the concentration of ethanol is too high, it can inhibit the process.

The kinetics of this phase is relatively fast, the bacteria that produce acid are growing fat, which has a minimum dubbing time of 30 minutes (CARREAS, 2013).

3.2.3 Acetogenesis

The organic acids and alcohols from the acidogenic phase are converted by acetogenic bacteria in hydrogen, carbon dioxide and acetic acid, here, volatile fatty acids (VFA), such as formic, acetic, propionic, butyric and valeric are also converted

into acetic acid.

The metabolism of acetogenic bacteria is inhibited by high concentrations of hydrogen. Thus, they have a symbiosis relation with bacteria that consume hydrogen, specifically, methanogenic bacteria (CARREAS, 2013).

The kinetic rate oh this step is slower comparing with acidogenesis, because acetogenic bacteria growth is longer than that of acidogenic bacteria. They have a minimum dubbing time from 1,5 to 4 days (CARREAS, 2013).

3.2.4 Methanogenesis

This is the final stage of anaerobic digestion, where acetic acid, hydrogen and carbon dioxide are transformed into methane and carbon dioxide. Here, the reaction has two objectives in the process: one is to produce methane and the other is the removal of gaseous hydrogen, which is toxic to acetogenic bacteria (CARREAS, 2013).

The methanogenic bacteria are strictly anaerobic which means that they live in a total absence of oxygen. The main pathway for methane formation (70%) is done by acetoclastic methanogenic bacteria, which degrade acetic acid, while the rest comes from hydrogenotrophic methanogens, the bacteria that consume hydrogen. Acetoclastic methanogenic bacteria have a slow growth, with minimum doubling time around 2-3 days, and their metabolism not inhibited by the concentration of hydrogen in the biogas (CARREAS, 2013).

3.3 AFFECTING PARAMETERS

The microorganisms' activity depends mostly on the operational parameters and configuration of the digester. To a maximum efficiency of the process, the requirements have to be accomplished: maximum activity of microorganisms, minimum concentration of intermediates and increase the reaction rate of limiting step in the process (CARREAS, 2013).

3.3.1 pH

This parameter determines the biogas production and also its composition.

For example, pH value below 6 cause very low methane content in biogas, therefore, it has less energy qualities (CARREAS, 2013).

The different groups of bacteria present in the digestion process have their own optimum activity levels around pH neutral. For fermentative bacteria, the pH is between 7,2 and 7,4, while for acetogenic bacteria is better in acid medium from 6,0 to 6,2 and for methanogenic bacteria the range is wider between 6,5 and 7,5 (CARREAS, 2013).

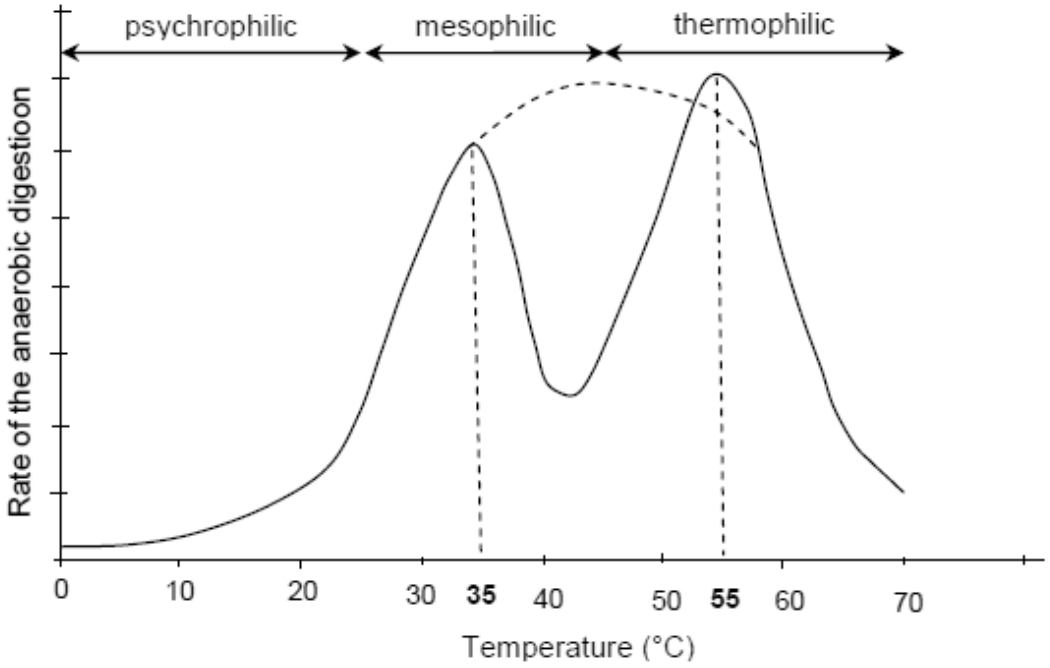
In general, a successful process is developed between 6,0 and 8,3 (BARAZA et al., 2003). When the pH is under 6,5, the activity of methanogenic acetoclastic bacteria decreases, more under than that pH, below 5,5, the activity stops completely. While pH below 4,5, the activity ceases for all kind of microorganisms (LEMA and MENDEZ, 1997).

High concentration of organic acids generated during the fermentation can decrease the pH, in a way that reduce the methane production, furthermore, can cause reactor souring leading to failure of anaerobic process (RITTMANN and MCCARTY, 2012).

3.3.2 Temperature

The temperature affects directly in the bacterial activity. In general, the rate of bacterial growth double for each 10°C increased in temperature, which can varies depending on the bacteria specie, such as methanogenic bacteria that are very sensitive to temperature. The classification of the groups are: psychrophilic that bear temperature from 5 to 20°C, mesophilic that work from 25 to 45°C, where the optimum is around 35 °C, and above 40°C can cause denaturation of the enzymes, finally, the thermophilic can bear high temperature of 45-65°C, which optimum is around 55 °C. The Figure 3 shows the influence of temperature in the rate of degradation in anaerobic process (CARREAS, 2013; NAYONA, 2010).

FIGURE 3 - INFLUENCE OF THE TEMPERATURE IN THE RATE OF DEGRADATION IN ANAEROBIC PROCESS



SOURCE: NAYONA (2010).

Regarding to the anaerobic digestion of waste, the most used temperature range is the mesophilic, although the use of thermophilic condition is increasing currently due to higher processing speed and a better elimination of pathogens. However, thermophilic range is more unstable to changes in operational conditions, and it has more problems of process inhibition by the greater toxicity of certain compounds at high temperatures, for example, ammonia nitrogen and long chain fatty acids (CARREAS, 2013).

3.3.3 Alkalinity

Alkalinity is defined as the capacity of water to neutralize the acid (RITTMANN and MCCARTY, 2012).

It is important to maintain an optimum value of alkalinity in the digestion process, since it buffers sudden changes in pH, such as that produced by the generation of volatile fatty acids. This is a parameter that is given by the concentration of calcium carbonate.

Some studies suggest bicarbonate alkalinity values between 1500 and 5000 mg/l $CaCO_3$, while others show that values above 2500 mg/L of $CaCO_3$ ensure good control of pH and adequate stability. The buffering capacity of the digester is ensured

by keeping a constant ratio of volatile acid/alkalinity <0,25 (WATER ENVIRONMENTAL FEDERATION, 1998).

When carbon dioxide is dissolved, it can also increase the alkalinity of influent. This way, the recirculation of portion of the effluent makes possible the neutralization without adding extra reagents (CARREAS, 2013).

3.3.4 Volatile Fatty Acids (VFA)

Volatile fatty acids (VFA) in anaerobic process are intermediate compounds, such as formic, acetic, propionic and valeric acids, where the two most abundant are acetic and propionic acids. VFA is considered a specific parameter of anaerobic reactor control. This way, the accumulation of VFA in the digester leads to the destabilization of the process causing variation in temperature, pH, organic overloading and consequently inhibition, especially toxic to methanogens. In such case, the methanogens cannot consume hydrogen as fast as VFA are produced, leading to the decrease of the pH, that could cease hydrolysis and acetogenesis. In a mature and stable reactor, VFA content is under 500 mg/L, while inhibition is not achieved for values until 5000 mg/L (CARREAS, 2013; APPELS et al., 2008).

3.3.5 Hydraulic retention time (HRT)

Hydraulic retention time (HRT) defines the extension of time that a substrate or a specific compound targeted for removal will be in contact with the biomass within the digester (CARREAS, 2013). It is defined as the average time that one reactor volume of actively digesting sludge stays within the reactor, calculated by the Equation 2 (RITTMANN and MCCARTY, 2012):

$$\mathrm{HRT} = \frac{\mathrm{V}}{Q} \qquad \text{(Equation 2)}$$

Where:

HRT= hydraulic retention time (d)

V = volume of the reactor (m^3)

Q = influent flow rate (m^3/d)

HRT allows the control of treated effluent flow. If substrate feeding is greater than its degradation, so, the residence time will decrease and in the reactor will be accumulation of intermediate products. Higher HRT means a longer time is available to degrade organic matter. However, if it extends too much, passing the optimum point, the methane yield will decrease, because no more additional substrates will be available to be degraded. Thus, it is important to determine the optimal time (RITTMANN and MCCARTY, 2012).

HRT depends on the type of substrate and the operational temperature. In such way, a higher temperature would decrease the retention time required. Consequently, the digester volume will be lower for degrading a certain material (CARREAS, 2013).

3.3.6 Organic Loading Rate (OLR)

Organic loading rate (OLR) is defined as the quantity of biochemical oxygen demand (BOD) or chemical oxygen demand (COD) fed into the digester volume per day (Tchobanoglous et al., 2003). Or also determined by the mass of volatile solids (VS) added each day per digester volume, calculated by the Equation 3 (VESILIND, 2003)

$$OLR = \frac{Q * C_{VS}}{V_{digester}} = \frac{C_{VS}}{HRT} \qquad \text{(Equation 3)}$$

Where,

OLR = Organic loading rate

Q = volumetric flow rate (m^3/d)

C_{VS} = concentration volatile solids (kg VS/m_3)

$V_{digester}$= digester volume (m^3)

HRT = hydraulic retention time

As can see in the Equation 3, the OLR cannot be considered in absolute terms, but relative to the influent organic load (kg BOD/ m^3.d) or (kg COD/ m^3.d) or (kg VS/ m^3.d).

Therefore, OLR depends on the waste composition and residence time. It is

considered one of the parameters more used to characterize the treating ability of anaerobic reactors.

As mentioned by Rittmann and McCarty (2012), for high rate digestion, the recommended OLR is 1,6 – 4,8 kg VS/(m^3.d), while for low rate process (digestion with no heat, nor mixing) is 0,5 – 1,6 kg VS/(m^3.d). Which is in accordance with Vesilind (2003), that suggested peak OLR for high rate process should be 1,9 – 2,5 kg VS/(m^3.d). In the same way, according to Lemmer (2012), a value from 2,0 – 4,0 kg VS/(m^3.d) is considered as medium organic load rate to be fed in an anaerobic reactor.

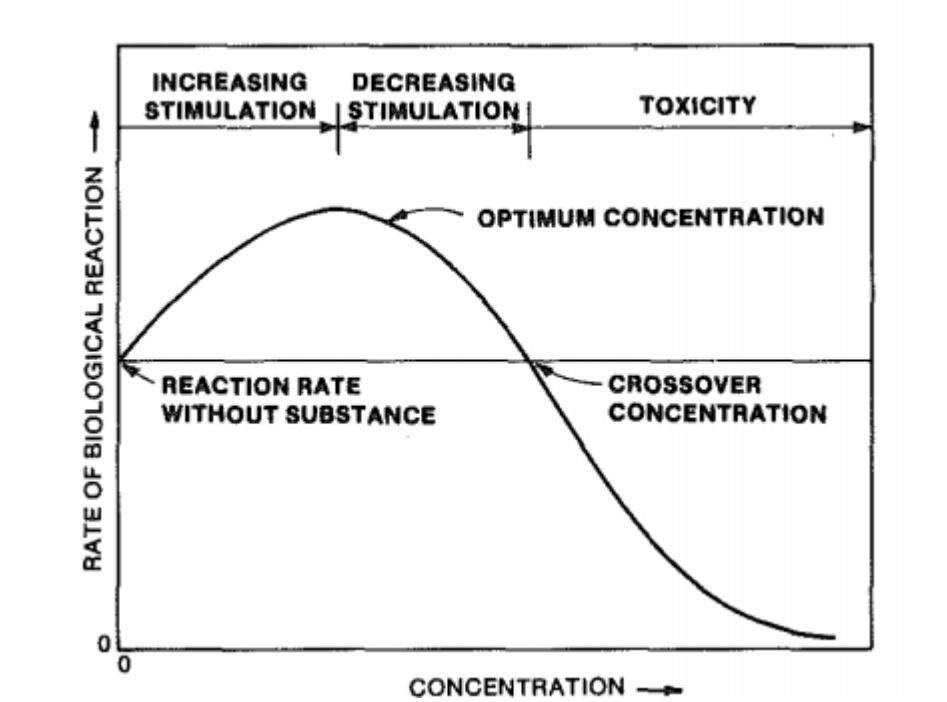
OLR is an important parameter because it can determine the size of the reactor. If OLR is higher than the specified one for operational condition, it can lead to process instabilities and decrease in performance, once methanogenesis can be inhibited, which can result in the accumulation of VFA in the digester. The excessive accumulation of VFA decreases the pH in the digester and consequently can lead to reactor souring and eventually, the failure. Therefore, it is essential that the specified organic loading rate is conservative (CARREAS, 2013).

3.4 TOXICITY AND INHIBITION

As mentioned before, the main indicators to state inhibition in the process are decrease in production of biogas and methane, increase in VFA concentration in the medium leading to decrease in pH values.

Additionally, the anaerobic digestion can also be inhibited by the presence of toxics in the digester and influence the development of bacterial activity. In such way, a compound can be considered toxic in a certain concentration limit, while in lower content can be beneficial for the growth of bacteria, as shown in Figure 4. It is important to consider that temperature change can also favor the formation of toxic substances, such as sulfide, ammonium, VFA, etc.

FIGURE 4 - EFFECT ON THE AEROBIC DIGESTION AS FUNCTION OF THE CONCENTRATION OF A SUBSTANCE



SOURCE: PARKIN AND OWEN (1986).

It should be taken that the limit concentration values for the toxicity of a substance depends on many parameters, such as the environmental conditions (temperature, pH), acclimation or adaptation factors of microorganisms, type of substrates, as well as the influence of other compounds or ions in the system, which can act with synergistic or antagonistically effect.

3.4.1 Salt

Many studies have been conducted regarding the inhibition of biological process due to high salt content. However, some of them present different range for concentration of inhibition due to different operational parameters.

According to Chen et al. (2008), the results from previous investigation on inhibition of the process can vary significantly depending on the waste composition, experimental method and conditions, the type of anaerobic microorganisms, etc. In such way, further studies have to be done in co-fermentation, an environment with so many different chemical compounds, where some of them can act with antagonistic effect, while others have synergistic influence.

According to McCarty and McKinney (1961), the toxicity caused by salts is

principally related to the cation, which due to the osmotic pressure causes dehydration of bacterial cells, as investigated by de Baere *et al.* (1984) and Yerkes *et al.* (1997).

At low concentration, the presence of sodium is indispensable for methanogens, due to the formation of adenosine triphosphate (ATP) or in the oxidation of nicotinamide adenine dinucleotide (NADH), which has an important role in the production of energy for the cell (DIMROTH and THOMER, 1989). In such a way, a sodium concentration around 0,1 – 0,2 g/L is beneficial for the bacterial growth in a mesophilic condition (MCCARTY, 1964; CHEN, 2008).

While the optimal sodium content for mesophilic bacteria vary from 0,23 to 0,35 g/L, depending on the type of inoculum (KUGELMAN and CHIN, 1971; PATEL and ROTH, 1977; CHEN, 2008).

However, higher concentrations of sodium affect the metabolism and consequently on bacterial activity, which a range from 3,5 to 5,5 g/L is considered a moderate inhibition and 8,0 g/L is strongly inhibitory for mesophilic methanogens (MCCARTY, 1964; CHEN, 2008).

The major information found in the literature regarding the salt inhibition is related to the influence of sodium ion (Na^+). While according to Carreas (2003), the limit concentration of sodium chlorite (NaCl) is 40 g/L. So, the NaCl concentrations using limit values from the literature can be calculated with the relation between the molar mass of ion Na^+ (23,0 g/mol) and NaCl (58,4 g/mol). Here, 1 mol of NaCl is dissociated in 1 mol of Na^+ and 1 mol of Cl^-. In this case, no synergistic or antagonistic effect is considered by the ion chloride (Cl^-). The Table 5 summarizes the salt concentration influence in the anaerobic digestion.

TABLE 5 - SALT CONCENTRATION AND ITS INFLUENCE IN ANAEROBIC DIGESTION

Component	**Stimulants**		**Moderate inhibitor**		**Strong inhibitor**
Na+ (g/L) [1]	0,1	0,2	3,5	5,5	8,0
NaCl (g/L) [2]	0,25	0,51	8,89	13,97	20,31
NaCl (g/L) [3]	-		-		40

SOURCE: 1: CHEN (2008); 2: CALCULATED BY THE AUTHOR (2017); 3: CARREAS (2003)

3.4.2 Long chain fatty acids (LCFAs)

As well demonstrated by the literature, the degradation of fatty material is

difficult due to the formation of long chain fatty acids (LCFAs), which shows inhibition limits at low concentrations for gram-positive bacteria, as the methanogens, but not for gram-negative ones (KABARA et al., 1977; ZEIKUS, 1977).

Methanogenic bacteria are inhibited by LCFA when the limit concentration is achieved. According to Rinzema et al. (1994), no adaptation of microorganisms was possible after repeated exposure to toxic concentrations, neither prolonged introduction to non-toxic concentrations. The same results were found by Angelidaki and Ahring (1992), which stated that bacterial growth was not recovered after reached the limit concentration of oleic acid and stearic acid at 1,0 g/L working in a thermophilic condition, similar value of 1,2 g/L for oleic acid and lauric acid was found by Koster and Cramer using mesophilic temperature (1987).

This permanent damage is caused by an interference of LCFA in the membrane cell of microorganisms, which affects the transport and protection functions of the cell wall (DEMEYER AND HENDERICKX, 1967; GALBRAITH AND MILLER, 1973).

Another negative effect influenced by LCFA is related to the sludge washout, when biomass incorporate layers of LCFA creating the flotation of sludge, removal of biomass occurs with the effluent outlet (RINZEMA ET AL., 1994).

Oleic acid ($C_{18}H_{34}O_2$) is the most abundant LCFA contained in wastewater (HWU ET AL., 1998). There are many studies in the literature regarding the toxicity caused by oleate as a LCFA molecule. Hwu et al. (1997) concluded that thermophilic bacteria are more sensible to LCFAs than mesophilic. The author showed in another study that oleic acid inhibition is more correlated to the physical conditions of sludge than to its biological characteristics (for example, the methanogenic bacteria activity, the acclimation of sludge), where higher specific area sludge, as suspended and flocculent sludge, are greater inhibited than granular ones (HWU ET AL., 1996).

3.4.3 Ammonia

Ammonia in the digestion process is formed after the degradation of proteins, nucleic acids and urea (GONZÁLEZ-FERNÁNDEZ, AND GARCÍA-ENCINA, 2009). A low level of ammonia is essential to bacteria growth, while an excess concentration is toxic to the process, leading to failure.

Instability in the anaerobic process can be also related to the high

concentration of ammonia produced in the digester. According to Hejnfelt and Angelidaki (2009) and McCarty (1964), the inhibited level can start from 1500 mg/L to 7000 mg/L, which as stated by Chen et al. (2008), it depends on the condition of the environment (temperature, pH), the type of substrates and acclimation periods. Nevertheless, above 3000 mg NH4–N/L the process is inhibited at any pH values (MCCARTY, 1964). Even for an already adapted environment, the toxicity starts from 3000–4000 mg NH4–N/L (ANGELIDAKI AND AHRING, 1993; RAJAGOPAL, 2013).

On the other hand, with lower concentration of ammonia nitrogen, below 500 mg/L, the negative aspect is related to the decrease in buffer capacity of the system, and also, it means that less nitrogen in biomass as nutrient, which can cause lower methane yield (PROCHÁZKA ET AL., 2012; RAJAGOPAL, 2013).

3.5 CONTINUOUSLY STIRRED TANK REACTOR (CSTR)

In a Constant or Continuous Stirred Tank Reactor (CSTR), variables such as temperature and concentration do not vary with the position inside the reactor. Thus, these characteristics of the effluent stream are identical to that within the reactor. However, they vary over the time (LEVENSPIEL, 1999).

In WWTP the predominant fermenter or digester used is CSTR model. In this case, the reactor ensures that the digestate is continuously and completely mixed. Generally, the retention time used is between 14 – 28 days, depending on the feeding material and the temperature of operation (VERMA, 2002).

It can be operated in semi-continuous or continuous regime, which means that residue is fed either periodically (semi-continuously) or continuously to the reactor. In some cases, depending on the waste to be treated, the digester is purged periodically to avoid concentration of toxic compounds.

According Rittmann and McCarty (2012), for CSTR anaerobic digester working at 35 °C the minimum HRT is 10 days, while the starting time is from 30 to 90 days.

4 METHODS

4.1 BIOGAS REACTOR

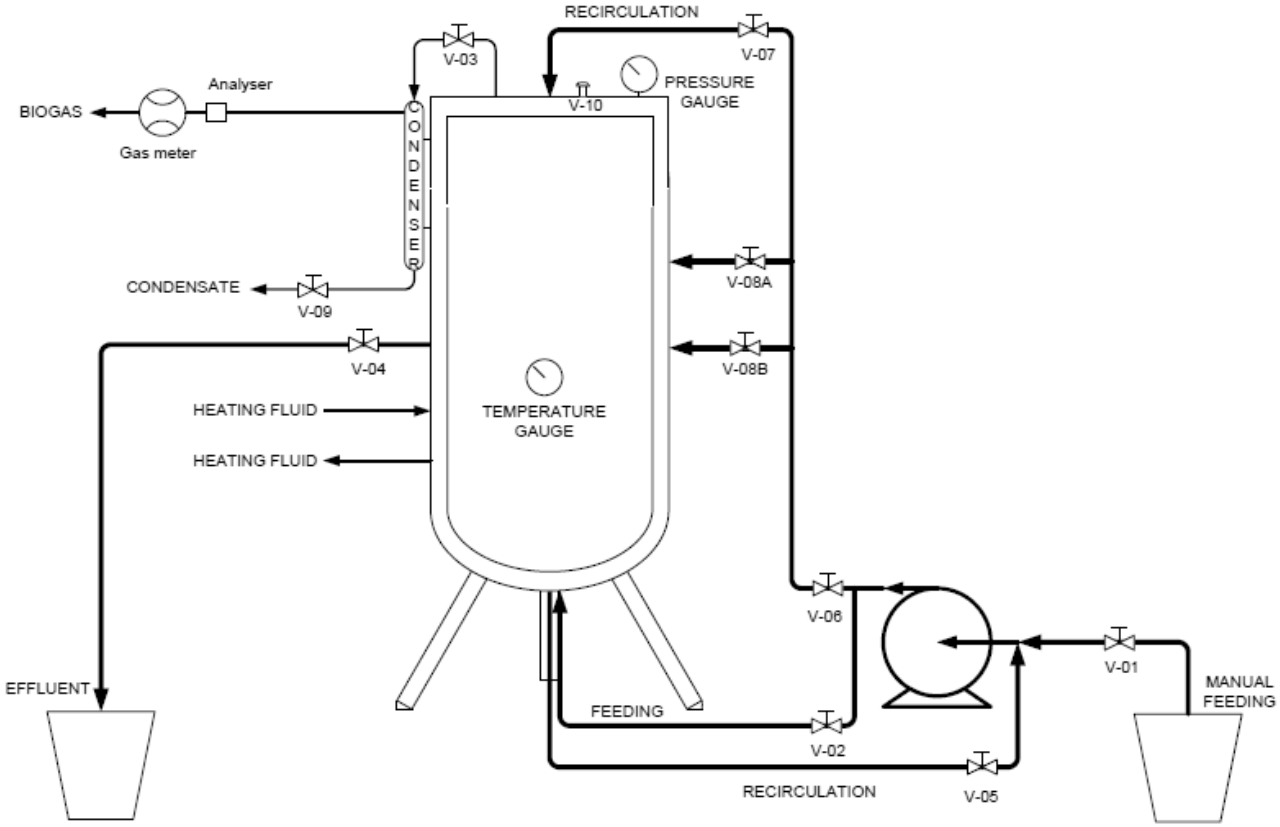
The experiment with the reactor was conducted in the Treatment Plant for Education and Research Lehr- und Forschungsklärwerk (LFKW) from the Institute for Sanitary Engineering, Water Quality and Solid Waste Management or *Institut für Siedlungswasserbau, Wassergüte- und Abfallwirtschaft* (ISWA) of the University of Stuttgart.

The model of the reactor can be considered as a Continuous Stirred Tank Reactor (CSTR), which operates at a mesophilic temperature around 35 ºC and slightly pressurized, manometric pressure around 0,01 bar. The total volume of the reactor is 250 L, however, the liquid working volume inside the reactor is approximately 210 L and the hydraulic retention time is around 21 days.

The Figure 5 represents the operation of the reactor in a semi-continuous system, which the recirculation of the material occurs continuously from the bottom of the reactor controlled by the valve V-05 pumped to the top passing through the valves V-06 and V-07. Currently, the auxiliary valves V-08A and V-08B are closed. During weekdays, the feedstock is manually fed using the valve V-01, where the material is pumped into the anaerobic reactor by the valve V-02. The gas produced by the process is collected in the top of the reactor controlled by the valve V-03 where goes to the gas composition analyzer measuring CO2 and CH4 and after to the gas meter to measure the gas volume in a full-time operation. In case of a condensate forming in the biogas pipe, it is collected in the condenser and removed by the valve V-09. The effluent of the process formed by a thick sludge material is removed from the reactor every day after the feeding time by the valve V-04. The effluent is removed from the reactor at a rate proportional to the feeding introduced.

There is a safety valve in the reactor, in case of high pressure generated into the reactor, V-10 opens to release the gas accumulated in the process.

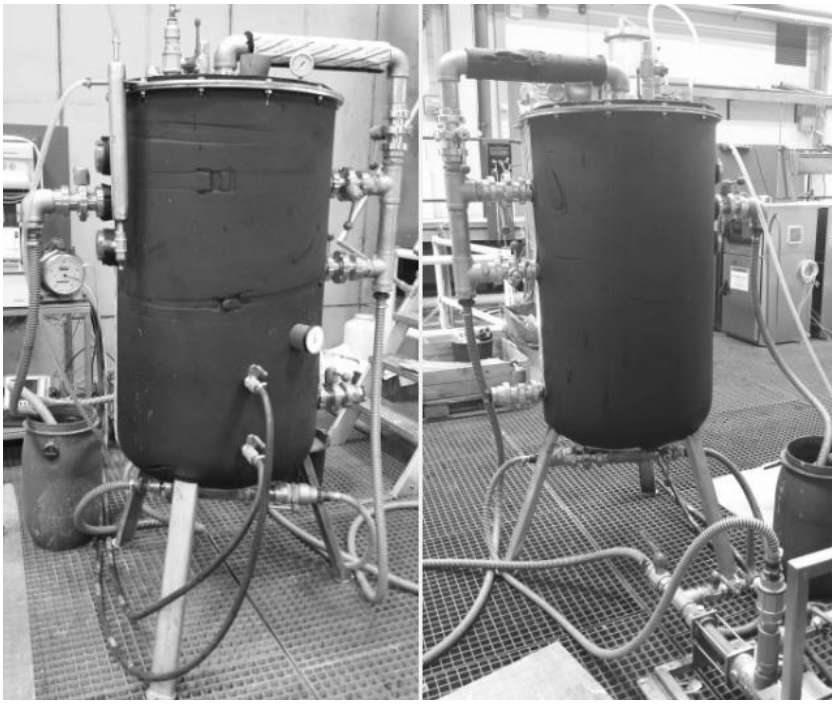
FIGURE 5 - SKETCH OF THE ANAEROBIC REACTOR



SOURCE: Author (2017).

The Figure 6 shows the front and back views of the anaerobic reactor previously described.

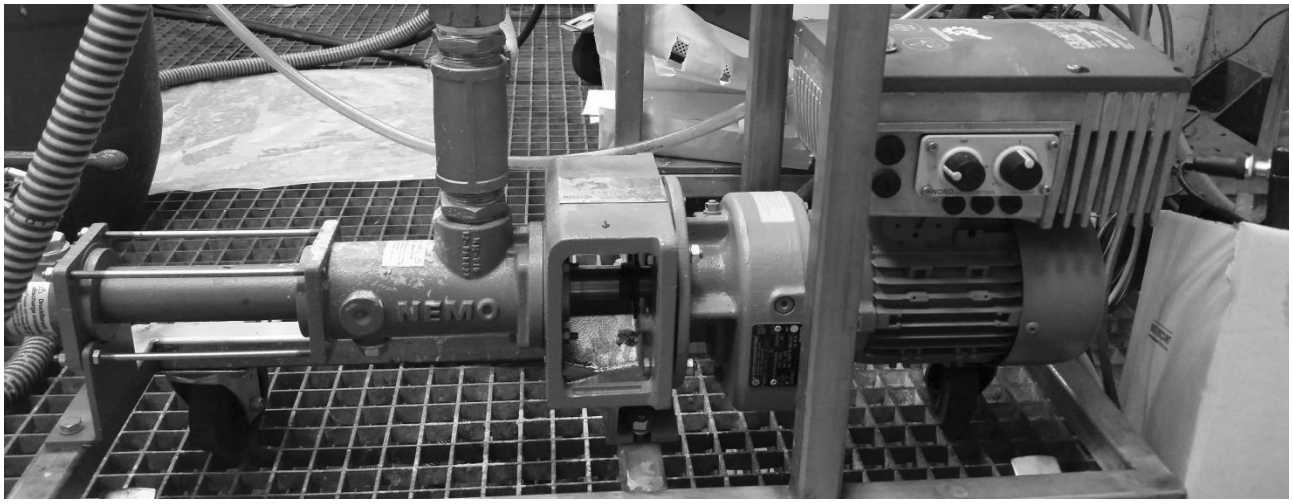
FIGURE 6 - FRONT AND BACK VIEWS OF THE ANAEROBIC REACTOR IN LFKW, ISWA



SOURCE: Author (2017).

The pump used in the operation to feed and continuously circulate the material from the bottom to the top of the anaerobic reactor is provided by the company Netzsch, model NEMO® BY Progressing Cavity Pumps. It can be used for different physical-chemical conditions of the materials, such as a liquid mixture with solid content as the one used in this study. The Figure 7 shows the pump used in the reactor arrangement.

FIGURE 7 - PROGRESSIVE CAVITY PUMP FROM NETZSCH COMPANY USED FOR THE FEEDING AND CIRCULATION IN THE REACTOR



SOURCE: Author (2017).

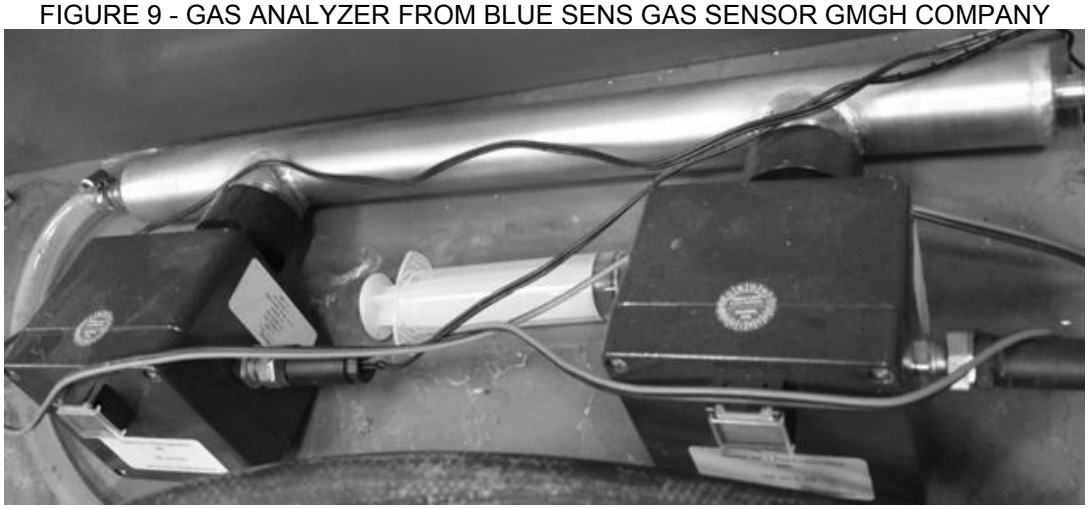
The reactor is thermally isolated by a jacket to keep the temperature constantly suitable to the bacteria to degrade the substrates by their metabolic activities. In this case, the temperature inside the reactor is around 35 ºC, working in a mesophilic condition. To heat the reactor, there is a heating device with distilled water bath, see in Figure 8 which exchanges heat with a thermal fluid that passes through a spiral pipe around the outside of the reactor vessel keeping the required constant temperature to the system.

FIGURE 8 - HEATING DEVICE FROM THE COMPANY JULABO

SOURCE: Author (2017).

The biogas generated in the process is collected in the top of the reactor, where subsequently is located a gas composition analyzer to characterize methane and carbon dioxide from the gaseous outflow. Afterwards, the volume of gas can be obtained by a gas meter from the company Ritter Apparatebau GmbH. All these data can be recorded in a data logger installed in the system, which can store information about biogas volume and composition every 10 minutes. The Figure 9 presents the gas sensor to characterization of CH_4 and CO_2 and the Figure 10 states the gas meter and data logger respectively from left to right side.

FIGURE 9 - GAS ANALYZER FROM BLUE SENS GAS SENSOR GMGH COMPANY



SOURCE: Author (2017).

FIGURE 10 - GAS METER FROM RITTER APPARATEBAU GMBH COMPANY AND DATA LOGGER FROM ENDRESS+HAUSER COMPANY

SOURCE: Author (2017).

As the results in biogas volume are generally presented in the literature as normal volume of gas (Nm3 or NL for normal liter), calculated at standard conditions (0°C and 1atm), the values of measured temperature and pressure need correction regarding need temperature, pressure and vapor pressure correction.

In this study, the pressure was measured around 1atm, so, do not need to be corrected, neither the vapor pressure, once the gas stream was measured without water vapor, which was condensed and removed in a previous step in the reactor arrangement. Thus, the temperature is the only parameter to be adjusted. The correction is based on the ideal gas equation: $PV = nRT$, which provides a correction factor regarding the temperature stated in the Table 6 (FAUSTZAHLEN BIOGAS, 2013).

TABLE 6 – TEMPERATURE CORRECTION FACTOR FOR THE NORMAL VOLUME OF BIOGAS

Temperature (°C)	Correction factor
25	0,971
30	0,962
35	0,951
40	0,936

SOURCE: Author (2017).

In this case, as the temperature in the reactor is 35°C, the measured volume of biogas has to be multiplied by 0,951, stating the as normal volume of gas.

4.2 FEEDSTOCK

This study is focused on the determination of biogas production behavior through co-fermentation of organic fraction of municipal solid waste and sewage sludge from wastewater treatment plant (WWTP).

As a first experiment set, food waste from the University of Stuttgart's canteen was collected and mixed with primary sludge from ISWA's WWTP. However some problems arose, such as the non-homogeneity and the variable amount of salt in the food waste, which makes unfeasible the study by the influence of fixed variation of the salt content, and another problem was regarding the high organic load of the input material leading to the overload and toxicity of the process.

For these reasons, the input material was changed to a controlled sample that can be manipulated and that is viable for the well-functioning of the process since they have similar characteristics of the input material described.

The primary sludge was changed to secondary sludge from the same WWTP from ISWA to lower the organic load, while the food waste was simulated by a mixture of materials required for its same characterization through protein, carbohydrate and fat content. In this case, kitchen oil, cooked noodle, soy powder and kitchen salt were used, as can be seen in Figure 11.

FIGURE 11 - FROM LEFT TO RIGHT: KITCHEN OIL, COOKED NOODLE, SOY POWDER AND KITCHEN SALT USED TO SIMULATE FOOD WASTE

SOURCE: Author (2017).

The Table 7 presents the summary information for the TS, the VS in dry

basis (%) and wet basis (g/kg fresh material), the COD as well as the composition of protein, fat and carbohydrate of the substrate materials: rapeseed oil, cooked noodle, soy powder and secondary sludge.

TABLE 7 - PARAMETERS OF THE SUBSTRATES IN ANAEROBIC CO-DIGESTION

Parameters	**Substrates**			
	Noodle	Soy powder	Rapeseed oil	Secondary sludge
TS (%)	88,5	88	-	0,84
VS (%)	99	93,3	99,9	69,1
VS (g/kg fresh material)	876,2	821,04	999	5,8
COD (g/L)*	283,5	1053,7	2384,2	8,77
Protein (%)	12,0	58,6	-	-
Fat (%)	1,2	2,1	100	-
Carbohydrate (%)	71,0	21,3	-	-

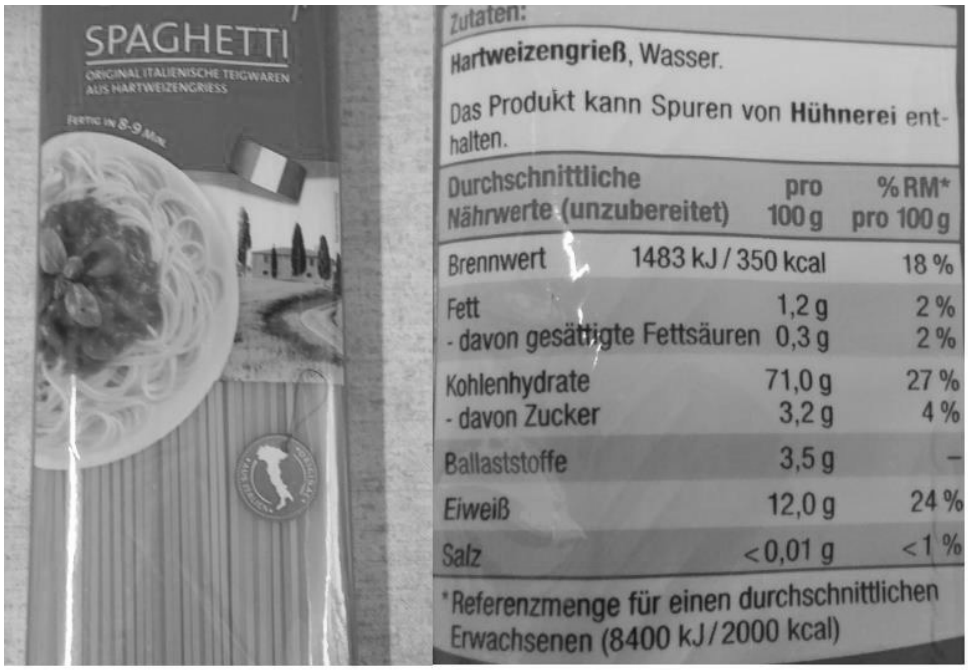
* COD values were defined by YAN, 2017.

SOURCE: Author (2017).

4.2.1 Noodle

As noodle has high carbohydrate content, 71% of the mass according to the information available in the package, as seen in Figure 12, it was prepared to simulate part of the carbohydrates present in the food waste composition in this study.

FIGURE 12 - NOODLE USED AS PART OF THE SUBSTRATES



SOURCE: Author (2017).

4.2.2 Soy powder

The soy powder was used as the main part of protein content presented in the food waste. According to the lab analysis, the protein content represents 71,4% of the volatile solids for the soy powder, which means that a great part of degradable organic matter in soy powder is related to the protein content.

4.2.3 Salt

This is the first part of the study conducted in LFKW. The salt used in the experiments was kitchen salt, considering that the composition is mainly made by sodium chloride (NaCl), more than 99%. Thus, the present study will focus on the influence of sodium (Na) as light metal ion for the biogas production in the co-fermentation process.

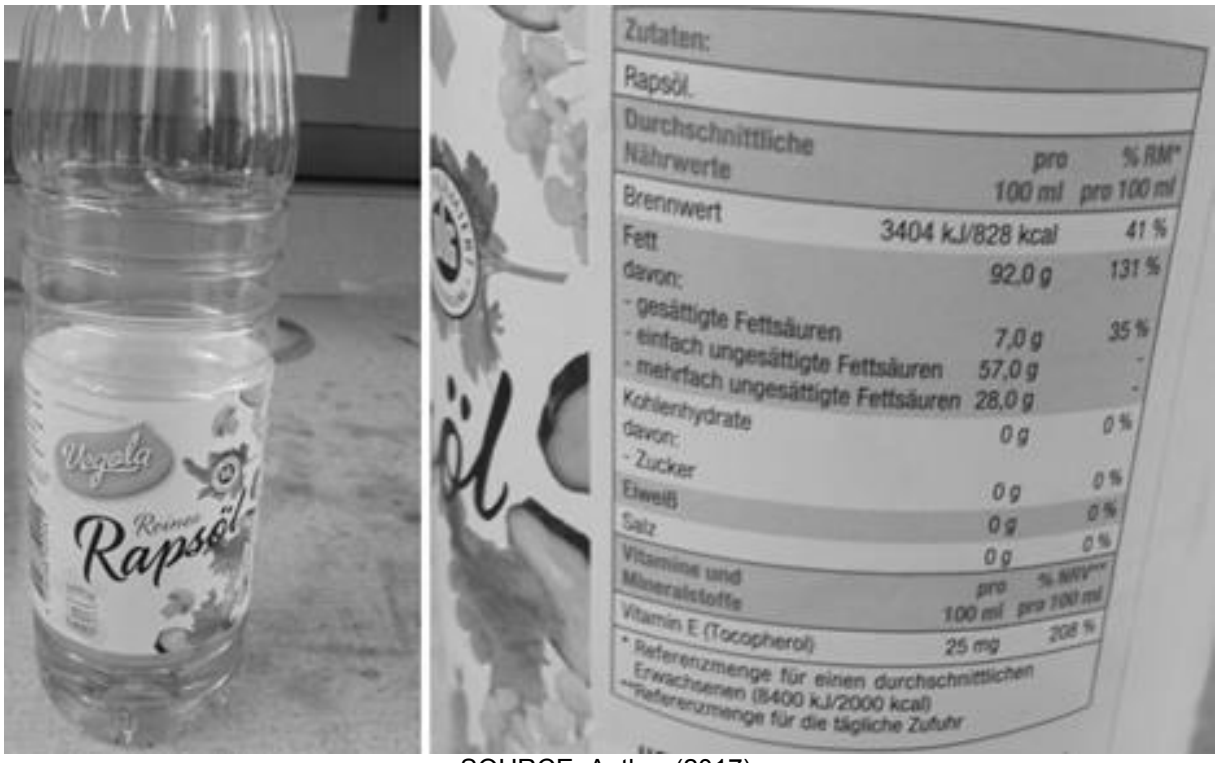
4.2.4 Rapeseed oil

This is the second part of the study conducted in LFKW. However, the initial composition of food waste in the salt study also requires a determined amount of oil. So, the rapeseed oil was used in both parts of the investigation.

As kitchen oil has a high concentration of fat, it was used to determine the influence of fatty acids of food waste in the anaerobic process. The concentration of oil was increased week by week to study the biogas production behavior according to the specified level of fat.

The composition of fat in the kitchen oil can be seen in Figure 13. In which, the main fatty acid presented is monounsaturated molecules (62%), mainly oleic acid. Following by polyunsaturated fatty acids with 30,4%, such as linoleic acid and linolenic acid. And finally, low concentration of saturated fatty acids, 7,6%.

FIGURE 13 - RAPESEED OIL USED IN THE REACTOR FEEDING



SOURCE: Author (2017).

4.2.5 Secondary sludge

The secondary sludge, also known as excess activated sludge, was taken from the sedimentation tank outflow in LFKW treatment plant. It means that this flow

contains a very low concentration of easily degradable carbon. According to Yan (2017), which has made the characterization of this secondary sludge in a previous study, the mean value for the total solid (TS) content is 0,84%, while the volatile solid (VS) is 69,1%. The chemical oxygen demand (COD) is very low compared with the other substrates in the mixture, 8,77 g/L.

The secondary sludge provides essential nutrients and minerals for the digestion process and it works as a mean to help in the homogenization of the mixture to feed the reactor. Every day, a sample of secondary sludge was taken to fill 10 L of material together with the prepared mix food, as can be seen in Figure 14.

FIGURE 14 - LEFT: SAMPLE OF SECONDARY SLUDGE; RIGHT: PREPARED FOOD MIXTURE TO FEED THE REACTOR

SOURCE: Author (2017).

4.3 FEEDING METHOD AND PARAMETERS

The preparation of input materials was done in the laboratory of BVK in ISWA from Monday to Friday in order to feed the reactor daily.

The noodle was cooked in boiled water for 10 minutes, after, the water was removed using a sieve and then the noodle was mixed in a blender for about 10 minutes with some secondary sludge to auxiliary the homogenization. A specified amount of oil, noodle, and soy was also added to the blender to mix all these material up until getting a homogeneous mixture to feed the reactor, as shown in Figure 15.

FIGURE 15 - PREPARED FOOD MIXTURE TO FEED THE REACTOR.

SOURCE: Author (2017).

The mixture of food added to secondary sludge was prepared with specific organic loading rate (OLR), protein, carbohydrate, fat and salt contents according to the weekly plan.

The organic loading rate (OLR) was calculated and established as OLR=2,3gVS/L.day to be used with the same value keeping constant feed for the organic materials daily. According to Lemmer (2012), this value is considered as a medium organic load to be fed in an anaerobic reactor for biogas production.

The calculation was done using the TS (%) and VS (%) values from Table 7 to obtain the VS (g/kg fresh material) by multiplying both variables. So, the OLR can be obtained by the Equation 4:

$$OLR = \frac{\sum \frac{VS_i * M_i}{1000}}{V} \qquad \text{(Equation 4)}$$

Where,

i is each one of the fresh material: noodle, soy, oil and sludge;

VS_i is the volatile solids in g/kg of the fresh material i;

M_i is the weight of the fresh material i (g);

V is the volume of the digester, in this case, 210L.

The summary of the calculation and results is shown in Table 8.

TABLE 8 - AMOUNT OF ORGANIC SUBSTRATES AND OLR

Experiments	**Parameter**	**Fresh materials, i**			
		Noodle	Soy powder	Rapeseed oil	Secondary sludge
	VS (g/kg fresh material)	876,2	821,04	999	5,8
Part I	Weight of fresh material (g)	220	200	63	10000
	OLR (g VS/L.d)	2,3			
Part II					
Week 1	Weight of fresh material (g)	233	212	42	10000
Week 2		208	188	84	10000
Week 3		181	165	126	10000
Week 4		155	141	168	10000
Week 1	OLR (g VS/L.d)	2,3			
Week 2		2,3			
Week 3		2,3			
Week 4		2,3			

SOURCE: Author (2017).

The Figure 16 shows experiment schedule and the quantity of each material added to feed the reactor during this whole study. Week by week, there is a variation in the salt input for 5 weeks (5 different concentrations) of test during the experiments in the Part I. For the oil assessment (Part II), the experiments ran for 4 weeks (4 different concentrations) of test. There were 4 weeks between these 2 parts of experiments for metabolic rehabilitation and stabilization of the microorganisms present in the reactor due to further change in the feeding conditions and previous toxicity of the process with high salt concentration in the mean.

FIGURE 16 - SCHEDULE FOR THE EXPERIMENTS PART I AND PART II

	Monday	Tuesday	Wednesday	Thursday	Friday
Jun 2017	5	6	7	8	9
	Holiday	Part I: 63 g oil + 80g salt + 220 g noodle + 200 g soy bean powder + 2nd sludge			
	12	13	14	15 Holiday	16
	Part I: 63 g oil + 160g salt + 220 g noodle + 200 g soy bean powder + 2nd sludge				
	19	20	21	22	23
	Part I: 63 g oil + 240g salt + 220 g noodle + 200 g soy bean powder + 2nd sludge				
	26	27	28	29	30
	Part I: 63 g oil + 390g salt + 220 g noodle + 200 g soy bean powder + 2nd sludge				
Jul 2017	3	4	5	6	7
	Part I: 63 g oil + 520g salt + 220 g noodle + 200 g soy bean powder + 2nd sludge				
	10	11	12	13	14
	Reactor stabilization: 2nd sludge				
	17	18	19	20	21
	Reactor stabilization: 63 g oil + 220 g noodle + 200 g soy bean powder + 2nd sludge				
	24	25	26	27	28
	Reactor stabilization: 63 g oil + 220 g noodle + 200 g soy bean powder + 2nd sludge				
	31	1	2	3	4
	Reactor stabilization: 63 g oil + 220 g noodle + 200 g soy bean powder + 2nd sludge				
Aug 2017	7	8	9	10	11
	Part II: 42 g oil + 233 g noodle + 212 g soy bean powder + 2nd sludge				
	14	15	16	17	18
	Part II: 84 g oil + 207 g noodle + 188 g soy bean powder + 2nd sludge				
	21	22	23	24	25
	Part II: 126 g oil + 181 g noodle + 165 g soy bean powder + 2nd sludge				
	28	29	30	31	1
	Part II: 168 g oil + 155 g noodle + 141 g soy bean powder + 2nd sludge				

SOURCE: Author (2017).

The same information is summarized in Table 9, where the concentration of salt (Part I) and oil (Part II) is based in the weight of food (noodle plus soy).

TABLE 9 - SUMMARY OF THE AMOUNT OF FRESH MATERIAL USED TO SIMULATE FOOD WASTE AND ITS CONCENTRATION

		Weight of fresh material (g)				
Experiment	**Week**	**Oil**	**Noodle**	**Soy**	**Salt**	**Concentration of salt or oil in the food (%)**
Part I	1st	63	220	200	80	14
	2nd				160	25
	3rd				240	33
	4th				390	45
	5th				520	52
Part II	1st	42	233	212	-	9
	2nd	84	208	188		18
	3rd	126	181	165		27
	4th	168	155	141		36

SOURCE: Author (2017).

In this study, the inoculum used is sludge from the LFKW WWTP. During the time between experiments Part I and Part II, reactor rehabilitation time, the microorganisms present in the inoculum was set to acclimate to the new medium and conditions of operation. The experiments in this period were conducted in the digester working with moderate organic loading rate and constantly monitoring operating parameters. According to the literature, digesters that have been started slowly eventually offer greater stability.

Daily, the feeding of the 250L capacity CSTR anaerobic reactor is manually made, by closing the process valves (V-03, V05 and V-06 according to the Figure 5) and opening the feeding valves (V-01 and V-02) to pump the liquid mixture from the bucket into the reactor. After the feeding, the opposite process has to be done to close the feeding valves and open the process' one. After the feeding, the effluent from the reactor has to be discarded, so approximately 10L of effluent is removed daily by opening the valve V-04.

4.3.1 Experiment Part I: salt influence

To simulate similar characteristics of the food waste from China, a study about its characterization was previously determined through the literature review. In this case, the study is conducted based in Chengdu city, which according to Yang (2012), the organic fraction content corresponds to and 48% of carbohydrate, 35% of protein and 17% of fat.

Thus, the calculation of mass quantity for each one of the feeding materials has to be done to simulate that composition in food waste.

For the first part of the study regarding to the salt influence evaluation (Part I), a mass balance was done for each one of the substances: protein content ($Total\ Protein$), carbohydrate content ($Total\ Carbohydrate$) and fat content ($Total\ Fat$) according to the following Equations 5.1, 5.2, and 5.3 respectively:

$$Total\ Protein = M\ protein, oil + M\ protein, noodle + M\ protein, soy$$

(Equation 5.1)

$$Total\ Carbohydrate = M\ carbohydrate, oil + M\ carbohydrate, noodle + M\ carbohydrate, soy$$

(Equation 5.2)

$$Total\ Fat = M\ fat, oil + M\ fat, noodle + M\ fat, soy$$

(Equation 5.3)

From now on, we can call "i" as a symbol to represent the fresh material: oil, noodle or soy.

So, returning to the Equation 5.1:

$M\ protein, i$ is the weight (g) of protein in the fresh material i and can be calculated by Equation 6:

$$M\ protein, i = \frac{\%\ protein, i}{VS_{\ wet\ basis}, i} * VS_{ABS}, i \qquad \text{(Equation 6)}$$

Where, $\%\ protein, i$ is the composition of protein in the fresh material i and can be found in the information on the product package.

$VS_{\ wet\ basis}, i$ is the amount of volatile solids (g) in the fresh material i (g) and calculated according to Equation 7:

$$VS_{\ wet\ basis} = \frac{TS * VS}{10000} \qquad \text{(Equation 7)}$$

Where, TS is the total solids (%) and VS is the volatile solids (%). They were determined experimentally in the lab.

Returning to Equation 6, VS_{ABS}, i is the absolute volatile content in (g), which can be calculated by the Equation 7:

$$VS_{ABS}, i = VS_{\ wet\ basis}, i * Mi \qquad \text{(Equation 7)}$$

In which, Mi is the weight of fresh material i (g) that is needed to fulfill the required composition of protein, carbohydrate and oil in the mixture, which can be obtained by Equations 8.1, 8.2, and 8.3 respectively:

$$Protein\ in\ the\ mixture\ (\%) = \frac{Total\ Protein}{Total\ Protein + Total\ Carbohydrate + Total\ Fat}$$

(Equation 8.1)

$$Carbohydrate\ in\ the\ mixture\ (\%) = \frac{Total\ Carbohydrate}{Total\ Protein + Total\ Carbohydrate + Total\ Fat}$$

(Equation 8.2)

$$Fat\ in\ the\ mixture\ (\%) = \frac{Total\ Fat}{Total\ Protein + Total\ Carbohydrate + Total\ Fat}$$

(Equation 8.3)

As mentioned before, the established composition of protein, carbohydrate and fat in the mixture is 35%, 48% and 17% respectively.

All the values obtained by the lab experiments for TS and VS, as well as the calculated values for the mentioned parameters were done on the program Microsoft Excel, as can be seen in Table 10.

TABLE 10 – PARAMETERS FOR SIMULATED MIXTURE DETERMINED ACCORDING TO THE FOOD WASTE COMPOSITION

Parameters	**Fresh material**			**Prepared food mixture**
	Oil	Noodle	Soy	
TS (%)	-	88,50	88,00	
VS (%)	99,9	99,00	93,30	
VS wet basis (g/g fresh material)	-	0,88	0,82	
Weight of fresh material (g)	63,0	220,0	200,0	
VS absolute (g)	63,0	192,8	164,2	
Protein (%)	-	12,0	58,6	
Fat (%)	-	1,2	2,1	-
Carbohydrate (%)	-	71,0	21,3	
Protein (%) / VS wet basis	-	13,7	71,4	
Fat (%) / VS wet basis	-	1,4	2,6	
Carbohydrate (%) / VS wet basis	-	81,0	26,0	
M protein (g)	-	26,4	117,2	
M fat (g)	63,0	2,6	4,3	
M carbohydrate (g)	-	156,2	42,7	
Total Protein (g)	-	-	-	143,6
Total Fat (g)				69,9
Total Carbohydrate (g)				198,9
Protein in mixture (%)				35%
Fat in mixture (%)				17%
Carbohydrate in mixture (%)				48%

SOURCE: Author (2017).

As result, the amount of fresh material determined to be used in the prepared mixture for the oil is 63 g, the noodle is 220 g and soy is 200g, which corresponds to the required composition of food waste in Chengdu: 48% of carbohydrate, 35% of protein and 17% of fat. And it means that the morphological content of the simulated food mixture was: 13% oil, 46% noodle and 41% soy weight.

The proportion of volatile solids in the whole mixture of secondary sludge added with prepared food is 12%, while the VS content of food mixture in this set represents 88%, as shown in Table 11.

TABLE 11 - WEIGHT PROPORTION OF PREPARED FOOD AND SECONDARY SLUDGE

Parameters	**Fresh material**			
	Oil	Noodle	Soy	Secondary Sludge
VS wet basis (g/g fresh material)	-	0,88	0,82	0,0058
Weight of fresh material (g)	63	220	200	10000
VS absolute (g)	63	192,8	164,2	58
VS TOTAL (g)	478			
VS Food Mixture (%)	88			
VS Secondary Sludge (%)	12			

SOURCE: Author (2017).

As kitchen salt (NaCl) is an inorganic compound, its use does not affect the change in this organic composition of food waste. So, these calculated quantities for each one of the fresh materials can remain the same while the salt increment can be done weekly. It has to be complied with the established OLR=2,3g VS/L.d.

Table 12 describes the quantities and salt concentration added in the prepared food containing 63g of oil, 220g of noodle and 200g of soy (total weight 483g), as well as in the mixture of 10L added with sludge to feed the reactor during the first part of the experiments.

TABLE 12 - SALT CONCENTRATION DURING THE EXPERIMENTS PART I

Week	**Amount of salt added (g)**	**Volume of mixture (L)**	**Salt concentration in the mixture (g/L)**	**Weight of food – regardless the salt (g)**	**Salt content in the food (g salt added/g food)**
1st	80	10	8	483	0,17
2nd	160		16		0,33
3rd	240		24		0,5
4th	390		39		0,81
5th	520		52		1,08

SOURCE: Author (2017).

4.3.2 Experiment Part II: oil influence

The second part of the study involves the evaluation of oil influence in biogas production.

To comply with the OLR established for this study, $^{2,3\ \text{g of VS}}/_{\text{L. day}}$, the amount of each fresh material were calculated according to the mentioned method previously explained, with increase in the oil concentration in the prepared food starting with 9%, 18%, 27% and finishing in 36% after 4 weeks. The amount of oil added and its concentration in the input mixture with sludge (10L), as well as in the prepared food is shown in Table 13.

TABLE 13 - OIL CONCENTRATION DURING THE EXPERIMENTS PART II

Week	Oil (g)	Weight of prepared food (g)	Oil concentration in the food (%)	Volume of mixture (L)	Oil concentration in the mixture (g/L)
1st	42	487	9	10	4,2
2nd	84	480	18		8,4
3rd	126	472	27		12,6
4th	168	464	36		16,8

SOURCE: Author (2017).

Using this concentration of oil in the food, it means that the composition of the food also changes weekly. Thus, it was evaluated in this second part of the study, a smaller oil concentration and others greater than the one determined for the food waste in China, where it had 35% protein, 48% carbohydrate and 17% fat. As shown in Table 14, the protein and carbohydrate compositions also change in order to keep the organic load constant, these contents had to be changed while the oil concentration increases. In such way, the concentration of fat in the food was calculated using the same method described in section 4.3.1, which increased weekly from 12% to 22%, passing through 32%, ending in 42%.

TABLE 14 - COMPOSITION OF SIMULATED FOOD WASTE IN THE PART II EXPERIMENT

Parameters	Week 1	Week 2	Week 3	Week 4
Protein in mixture (%)	37	33	29	24
Carbohydrate in mixture (%)	51	48	40	34
Fat in mixture (%)	12	22	32	42

SOURCE: Author (2017).

Regarding the concentration of oleic acid, the main LCFA present in the rapeseed oil (62%) and the most notable one in the literature, the amount added in the food waste or its concentration added in the reactor is shown in Table 15.

It is important to notice that this concentration of oleic acid does not mean the real concentration in the reactor, which due to the retention time can accumulate the compound for more than a week.

TABLE 15 - AMOUNT AND CONCENTRATION OF OLEIC ACID ADDED TO THE REACTOR

Week	Oil (g)	Oleic acid concentration in oil (%)	Oleic acid (g)	Oleic acid added in the reactor (g/L)
1st	42	62	26,04	0,12
2nd	84		52,08	0,25
3rd	126		78,12	0,37
4th	168		104,16	0,50

SOURCE: Author (2017).

4.4 LABORATORY TESTS

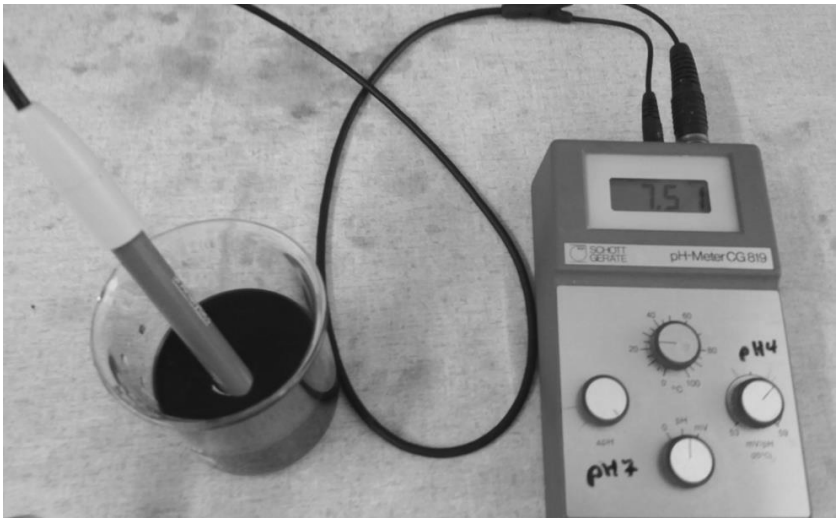
Daily, experimental tests performed in the BVK laboratory were done to assess and to control the fermentation process by determining the physical and chemical characterization of the incoming and outgoing substances of the anaerobic reactor. Since it is a CSTR model reactor, a perfect mixture of the materials can be considered, so the parameters such as concentration, pH and temperature collected in the reactor output represents the conditions within the reactor in the anaerobic process.

The parameters assessed are pH measurements for reactor's input and output materials, ammonium content in the process, conductivity to determine the salt concentration, total solids (TS), volatile solids of the effluent, as well as the assessment of the fermentation process by FOS/TAC value. The measured values are presented in the Appendix 2.

4.4.1 pH measurement

The pH measurements were carried out daily using the pH meter of the company SCHOTT GERATE, from the series CG819. It consists of two electrodes, one of reference and one of measurement, and a galvanometer connected to a scale of pH units. This scale is generally between pH 1 and 14, as showed in the Figure 17.

FIGURE 17 - MEASUREMENT OF SLUDGE SAMPLE WITH PH METER



SOURCE: Author (2017).

First, the calibration of the pH meter with buffers 7 and 4 (for acid solutions) was done. Distilled water was then used to wash the electrode before making any measurement and after, dry the electrode. Finally, the pH of the sample was determined by reading the value in the pH meter.

The pH values measured for this study were done for the secondary sludge (input material) to control the material that was fed into the reactor, as well as the mixture for the input substrate and the effluent from the reactor to assess the process.

4.4.2 Total Solids (TS) and Volatile Solids (VS)

The values of Total Solids (TS) and Volatile Solids (VS) were determined using an empirical method, following the standard of DIN EN 15934 and DIN 15935 respectively.

The samples were collected daily from the output material from the reactor,

which was weighed in a porcelain crucible with a predetermined weight, and then the liquid sample is taken for drying in an oven for 24 hours at a temperature of 105 ºC. The Figure 18 presents lab oven with its operational conditions.

FIGURE 18 – OPERATIONAL CONDITIONS OF THE OVEN TO DRY THE EFFLUENT SAMPLE AT 105 ºC

SOURCE: Author (2017).

The determination of the TS value in weight % was done through the Equation 9, where the mass of the crucible has to be discounted in both cases:

$$TS\ (\%) = \frac{Md}{Mw} * 100 \qquad \text{(Equation 9)}$$

Where Mw is the weight (g) of the initial (wet) sample;
Md the weight (g) following drying.

For the determination of Volatile Solids, the samples from the 105°C drying process were heated to 550°C in a muffle oven, Figure 19. When the ash was ready, i.e. no black residues of organic matter were left, the samples were removed from the muffle, placed in a desiccator to cool and the weights were measured when it reaches room temperature.

FIGURE 19 - MUFFLE OVEN USED TO BURN THE EFFLUENT SAMPLE AT 550°C

SOURCE: Author (2017).

The determination of the VS value in weight % was done through the Equation 10, where the mass of the crucible has to be discounted in both cases:

$$VS\ (\%) = \frac{Md - Mc}{Md} * 100 \qquad \text{(Equation 10)}$$

Where Md is the sample weight (g) of the previous drying process;

Mc is the mass (g) of the sample after combustion in the muffle.

The Figure 20 shows the sample from the reactor since the initial condition, after heated at 105°C and burned at 550°C.

FIGURE 20 – FROM THE LEFT TO THE RIGHT: SAMPLE OF EFFLUENT IN INITIAL CONDITION; AFTER DRIED AT 105 ºC; AND AFTER HEATED AT 550 ºC

SOURCE: Author (2017).

4.4.3 Biodegradability

According to Nielfa et al. (2015), the experimental biodegradability of an anaerobic process can be calculated by the Equation 11:

$$BD_{VS}(\%) = \frac{VS_i - VS_f}{VS_i} * 100 \qquad \text{(Equation 11)}$$

Where,

BD_{VS} is the experimental biodegradability (%);

VS_i is the initial volatile solid content in absolute basis in grams (g);

VS_f is the final volatile solid content, after the anaerobic process in absolute basis in grams (g)

4.4.4 $\mathrm{NH_4^+}$

The equilibrium concentration is established between unionized ammonia ($\mathrm{NH_3}$) and ionized ammonia ($\mathrm{NH_4^+}$) in aqueous solutions according to the Equation 12 (RAJAGOPAL *et al.*, 2013):

$$NH_4^+ + OH^- \leftrightarrow NH_3 + H_2O \qquad \text{(Equation 12)}$$

Due to the equilibrium, in the fermentation process unionized ammonia exists with other forms of nitrogen compound, such as ionized ammonia ($\mathrm{NH_4^+}$), carbonate

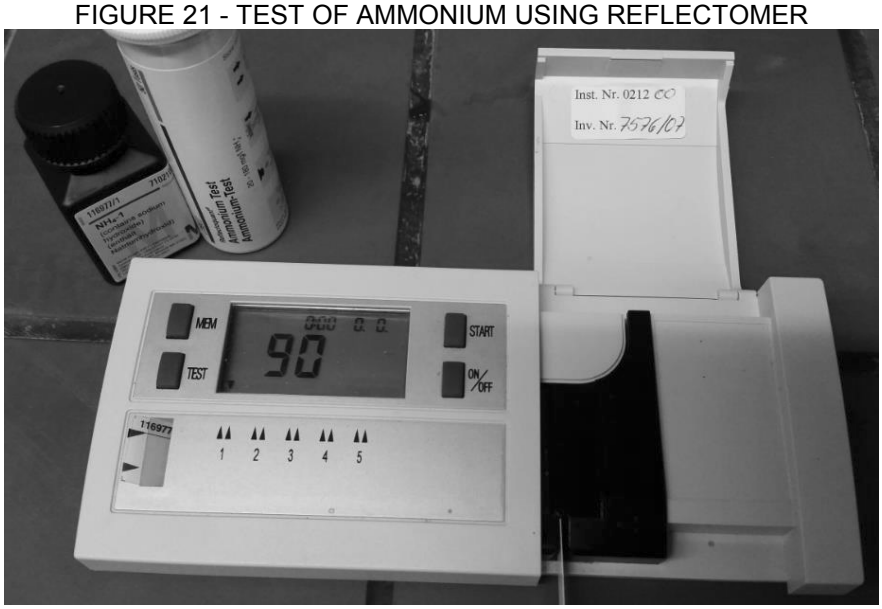
(NH_2COOH) and struvite ($NH_4MgPO_4 \cdot 6H_2O$) (HAFNER AND BISOGNI, 2009).

Failure of the fermentation process due to high ammonia content is related to the inhibition of microbial activity, while an optimum ammonia concentration ensures sufficient buffer capacity for the methanogenic process (RAJAGOPAL *et al.*, 2013).

Thus, the measurement of ammonium in the fermentation process was done daily to monitor its performance and stability. A sample of the effluent from the reactor was taken to measure the concentration of ammonium.

Firstly, the sample was diluted with distilled water in a ratio 1:10 and then, the solution was set into a lab shaker to be homogenized and to extract the solid sample material. Following this, the sample was filtered through a filter paper from Macherey-Nagel, type MN 615 ¼. Then, 5 mL of pretreated sample was filled into the test vessel and 10 drops of reagent NH4-1 were added and swirled. After that, one test strip was immersed in the sample for 2 seconds and immediately inserted into a reflectometer to read the value of ammonium in mg/L. The Figure 21 shows the materials and reflectometer device used to the ammonium test.

FIGURE 21 - TEST OF AMMONIUM USING REFLECTOMER



SOURCE: Author (2017).

A ratio (1:10) for dilution was used, so the result from the display in the reflectomer needs to be multiplied by 11 to have the concentration of NH_4^+ in mg/L. However, in the literature is very common to find the references values for ammonium expressed based on the mass of nitrogen, which means $NH_4 - N$ in mg/L. For this reason, there is a conversion factor of 0.776.

4.4.5 FOS and TAC

According to Hach Company© (2015), the determination of FOS/TAC ratio can serve as an indicator for the assessment of fermentation processes. It is a method calculated empirically according to the Nordmann standard.

In fact, FOS is related to the Volatile Organic Substances, which can correspond to the volatile fatty acids content, the unit is mg/L acetic acid equivalents. While, TAC is regarded to the Total Inorganic Carbonate, which serves as an estimation of the alkaline buffering capacity of the sample, as unit: mg CaCO3/L (Lossie, 2008).

The assessment of the biogas production process can be done by using the ratio of FOS/TAC values. According to Lossie (2008), a ratio between 0,3 and 0,4 is considered normal in practice. However, it can differ from plant to plant, which historical evaluation of this value need to be verified regularly by each single case because it can differ depends on the process, as well as the substrate input characterization. In general, the indication for the process' assessment can be considered according to the values described in Table 16, which corresponds to the experience values of DEULA-Nienburg.

TABLE 16 - ASSESSMENT OF FERMENTATION PROCESS ACCORDING TO THE FOS/TAC RATIO

FOS / TAC value	Background	Measure
> 0,6	Plant heavily overloaded	Suspend feeding
0,5-0,6	Plant overloaded	Reduce feeding
0,4-0,5	Plant heavily loaded	Increase monitoring
0,3-0,4	Plant loaded	Maintain feeding
0,2-0,3	Plant under loaded	Increase feeding slowly
<0,2	Plant very under loaded	Increase feeding rapidly

SOURCE: ADAPTED FROM LOSSIE (2008).

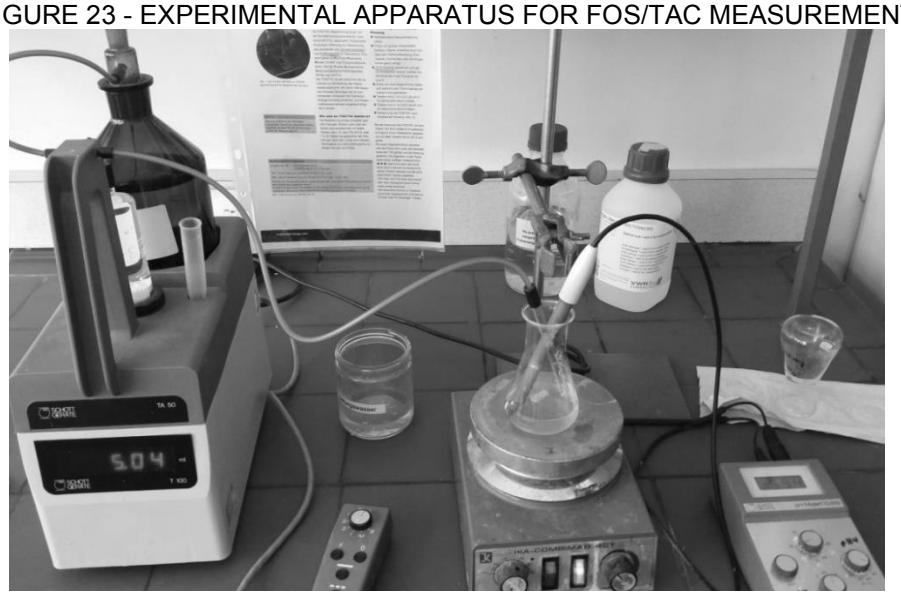
A sample of reactor's effluent is firstly filtrated by a filter paper from Macherey-Nagel, type MN 615 ¼, Figure 22. This separation step is necessary to avoid the influence of the suspended solids in the tests with the filtrate.

FIGURE 22 - PREPARATION OF EFFLUENT SAMPLE IN THE FILTER

SOURCE: Author (2017).

The experimental apparatus was set using 20 mL of sample in an Erlenmeyer. The substrate was homogenized by a magnetic stir bar and a pH-meter was used to read the pH value for the titration using 0,1 N of sulfuric acid solution (H_2SO_4), as shows the Figure 23.

FIGURE 23 - EXPERIMENTAL APPARATUS FOR FOS/TAC MEASUREMENT



SOURCE: Author (2017).

When the pH reaches at 5,0, the volume in mL of sulfuric acid solution was read in the titrator, after which is possible to calculate the TAC value in mg/L of $CaCO_3$. The following titration can measure the FOS value when pH reaches at 4,4, which express mg/L of acetic acid (CH_3COOH). The calculation of the TAC and FOS values can be done by the Equations 13 and 14 respectively (Lossie, 2008):

$$TAC = Volume\ (mL)\ of\ H2SO4\ consumed\ from\ begging\ to\ pH\ 5{,}0\ \ x\ 250$$

(Equation 13)

$$FOS = (Volume\ (mL)\ of\ H2SO4\ consumed\ from\ pH\ 5{,}0\ to\ pH\ 4{,}4\ x\ 1{,}66 - 0{,}15)\ x\ 500$$

(Equation 14)

4.4.6 Conductivity and salt content

Conductivity is a measure of the ability of water to transmit electrical current and it is proportional to the ions dissolved in a solution. The conductivity meter is a device that contains electrodes in which a charged current flows between them. The more ions are dissolved in a solution, higher is the value displayed by the conductivity meter.

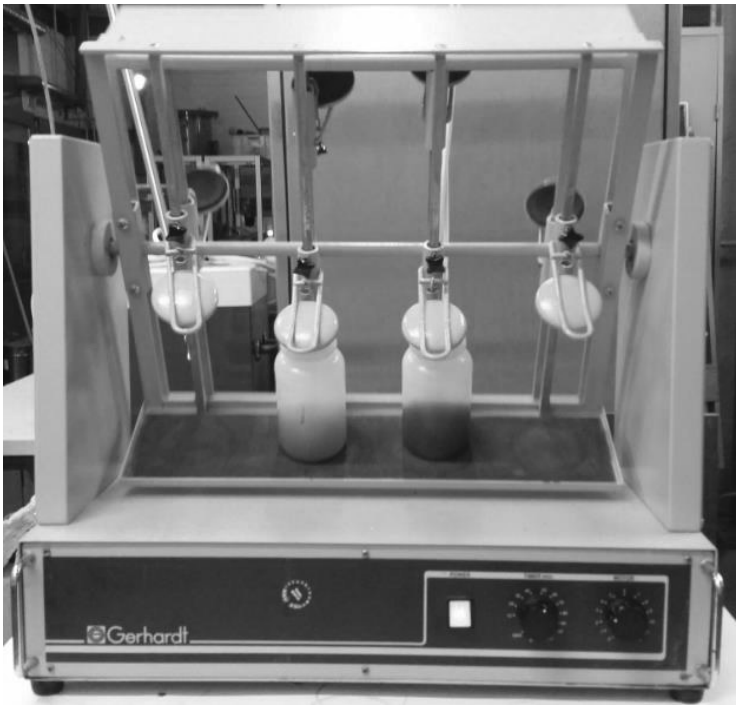
The conductivity of the effluent from the reactor was measured to calculate later the salt concentration within the CSTR. Assuming that is a perfect mixing reactor, the concentration of salt in the output material has the same concentration at any point within the reactor.

The conductivity of the sample was measured in order to calculate the salt content according to the method from VDLUFA Methods book (FCQAO, 1994).

The sample of effluent was collected daily and prepared with distillate water in a ratio 1:10. It means that 20g of effluent was diluted with 200 mL of water. After that, the bottle containing the prepared sample was placed on a lab shaker for 2h, Figure 24. It was necessary to the extraction of salt in the digestate material and consequent dissolution of sodium ion to the water.

FIGURE 24 - SAMPLE PLACED IN THE SHAKER TO EXTRACT THE DISSOLVED SOLIDS IN THE

EFFLUENT



SOURCE: Author (2017).

Following this, the prepared sample was filtered and the filtrate was placed to the conductivity measuring device where the sensors were completely submerged into the sample and the value of conductivity was recorded when the readout was equilibrated, as shown in Figure 25.

FIGURE 25 - MEASUREMENT OF CONDUCTIVITY

SOURCE: Author (2017).

The salt content calculation can be obtained by Equation 15:

$$Sc = C * Ft \quad \text{(Equation 15)}$$

Where,

Sc = salt content in the fresh material, with an extraction ratio of 1 + 10, the value corresponds to the KCl concentration of the solution in (mg/L)

C = Conductivity of the sample extract in (10^{-4} S/cm)

Ft = Factor for the calculation of the salt concentration from the conductivity, taking the temperature into consideration.

Here, the adaption for the sodium chloride concentration was done in order to replace the calcium chloride through their molar mass stated in the Method from FCQAO (1994).

So, at 25°C: Ft = 584,4 (mg/L)/14,12 (10^{-4} S/cm) = 52.80 (mg/L)/(10^{-4} S/cm).

5 RESULTS

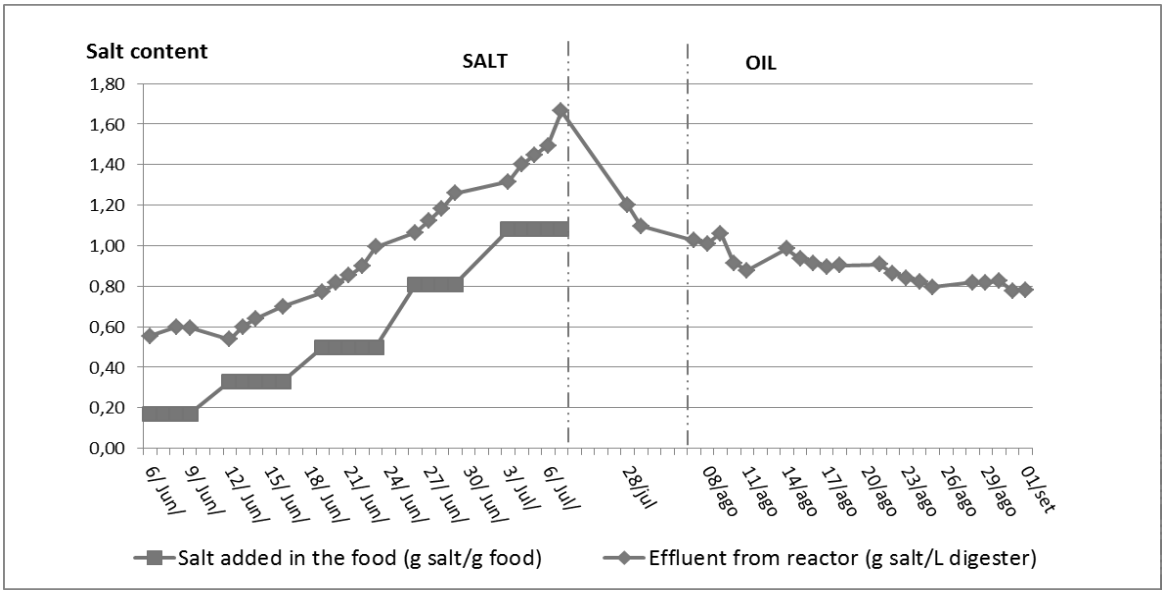
The anaerobic digestion process was monitored and assessed through the system parameters, such as pH, volatile solids (VS) reduction, concentration of toxic substances such as salt, fat and ammonia, volatile fatty acids (VFA) and buffer capacity, as well as operational conditions, like temperature and pressure of the reactor. The biogas volume produced and methane yield were also determined in order to evaluate the success of the co-fermentation process.

The graphics below are showing the results for each parameter analyzed during the whole period of study, including experiment Part I (salt content), followed by the rehabilitation and stabilization period, and finally, experiment Part II (assessment of oil content). The results values are presented in the Appendix 3.

5.1 SALT CONTENT

The salt content can be determined through the conductivity measured with the reactor effluent, which ranged from 0,54 to 1,66 g/L for the Part I period and remained more constant between 0,78 and 1,06 g/L in the experiment Part II. This is because there was no feeding of additional kitchen salt in the reactor for the second period. Whereas in the first part was fed additionally 0,17; 0,33; 0,50; 0,81; 1,08 g salt/g food during the weeks 1, 2, 3, 4 and 5 respectively, as shown by the Figure 26.

FIGURE 26 - SALT CONTENT IN THE CO-FERMENTATION PROCESS



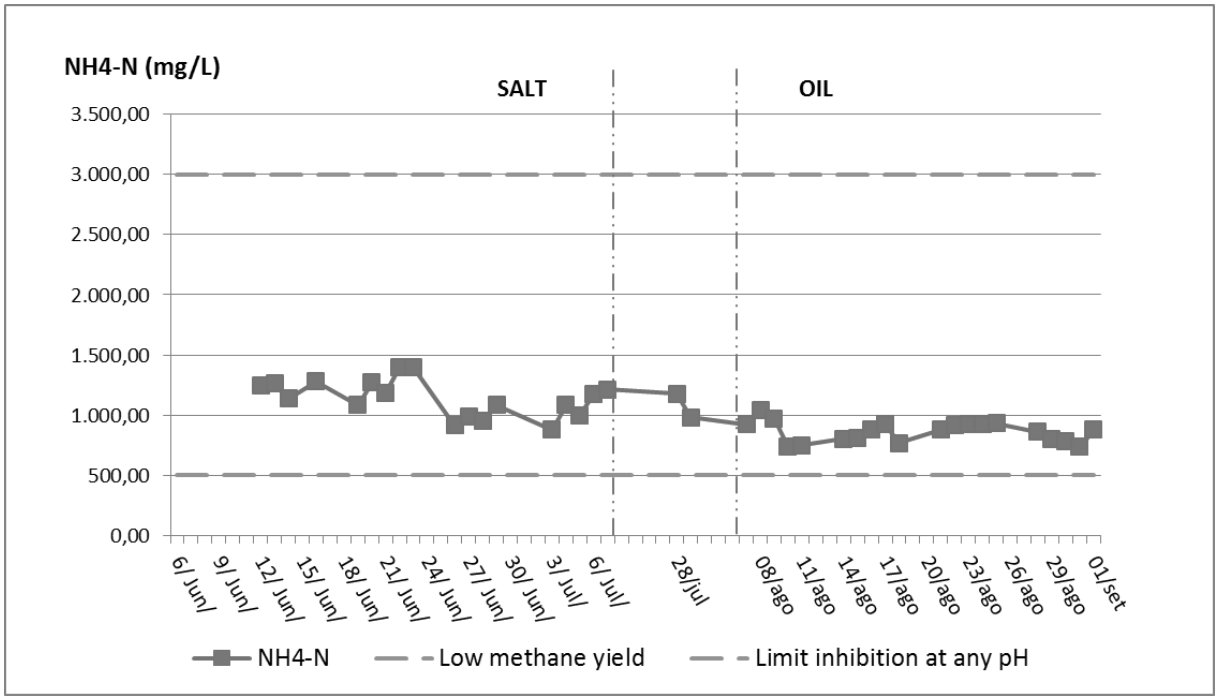
SOURCE: Author (2017).

5.2 AMMONIUM CONCENTRATION

The ammonium concentration varied modestly between 734 and 1400 mg NH4-N/L throughout the study period, thus, it was within the limits established according to the literature exposed in the section 3.4.3 of this thesis, where 3000 mg/L is the maximum limit of ammonia nitrogen for which the process does not begin inhibition and 500 mg/L is the minimum concentration to maintain the buffer capacity and nitrogen as nutrient in the digester.

Comparing the experiment Part I with Part II, a slight decline is noticed in Part II, which has an average of 862 mg NH4-N/L, while Part I is 1142 mg NH4-N/L. As the ammonia concentration is stable in the process, the calculated average the whole process is approximately 1000 mg NH4-N/L or 1.0 g NH4-N/L with the conditions of the current process. The variation in the concentration is ranged from 734 to 1400 mg NH4-N/L. Figure 27 shows the results obtained for the concentration of ammonium in the AD.

FIGURE 27 - CONCENTRATION OF AMMONIUM IN THE CO-FERMENTATION PROCESS



SOURCE: Author (2017).

5.3 FOS AND TAC

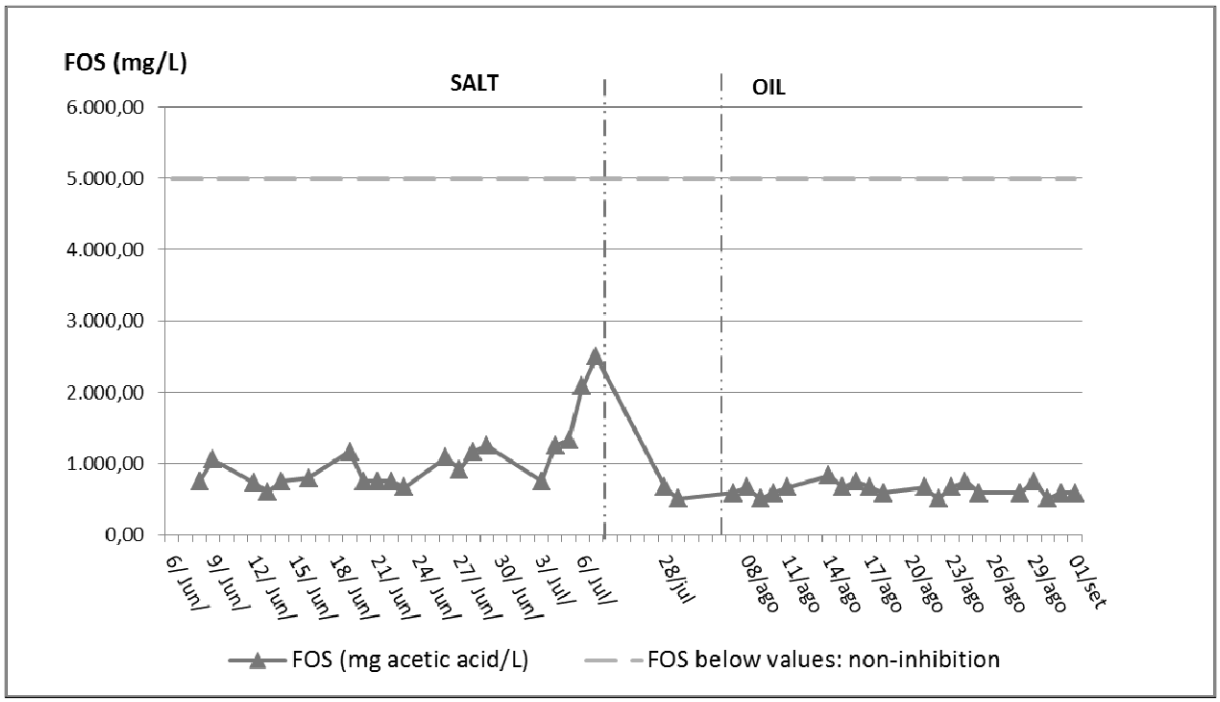
The results generated for the FOS, which is indicating the volatile fatty acids

content of the process, show that the whole process worked below the limit specified as inhibitory according to the literature, when VFA content is under 500 mg/L, see section 3.3. 4.

However, in the Part I experiment, for a higher salt content, a substantial increase in the FOS value in the last week could be observed. In other words, more VFA was formed. The VFA variation in Part I varied from 606 to 2498 mg/L of acetic acid, and an average of 1058 mg/L.

After this period, from the reactor rehabilitation period until the end of experiment Part II, it is observed that the VFA level remained constant and stable even when the reactor received a higher oil load or fat concentration. Thus, the calculated average for Part II is 639 mg/L. The Figure 28 presents the FOS results in the whole period of study.

Figure 28 - FOS (Volatile Organic Substances) concentration in the CO-FERMENTATION PROCESS



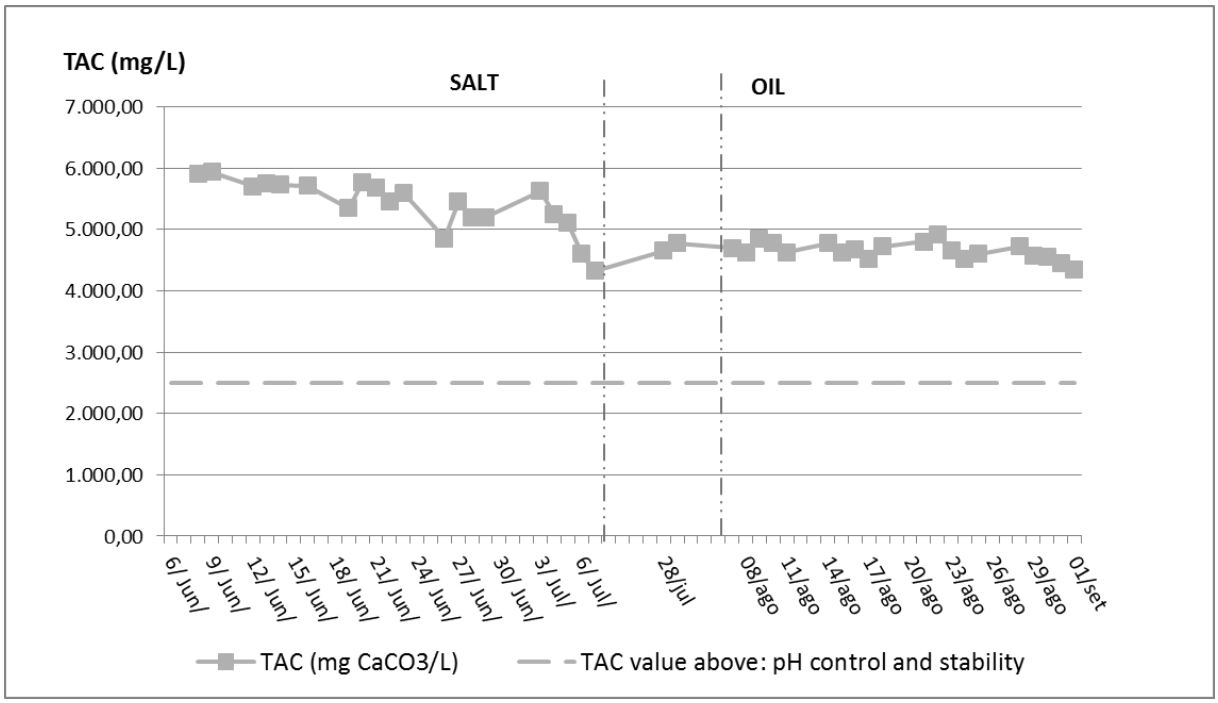
SOURCE: Author (2017).

The values obtained through the TAC measurement are above the minimum limit established according to the literature: 2500 mg L of $CaCO_3$, see section 3.3.3. Thus, the process can maintain a relative stability regarding the alkalinity, as shown in Figure 29.

However, in the last week of experiments with salt, there was a slight

tendency to decrease the alkalinity of the process. In this period, the TAC variation was from 5935 to 4325 mg/L of $CaCO_3$, with an average of 5409 mg/L. In Part II, the alkalinity of the system remained more stable, with an average of 4653 mg/L of $CaCO_3$.

FIGURE 29 - TAC (VOLATILE ORGANIC SUBSTANCES) CONCENTRATION IN THE CO-FERMENTATION PROCESS



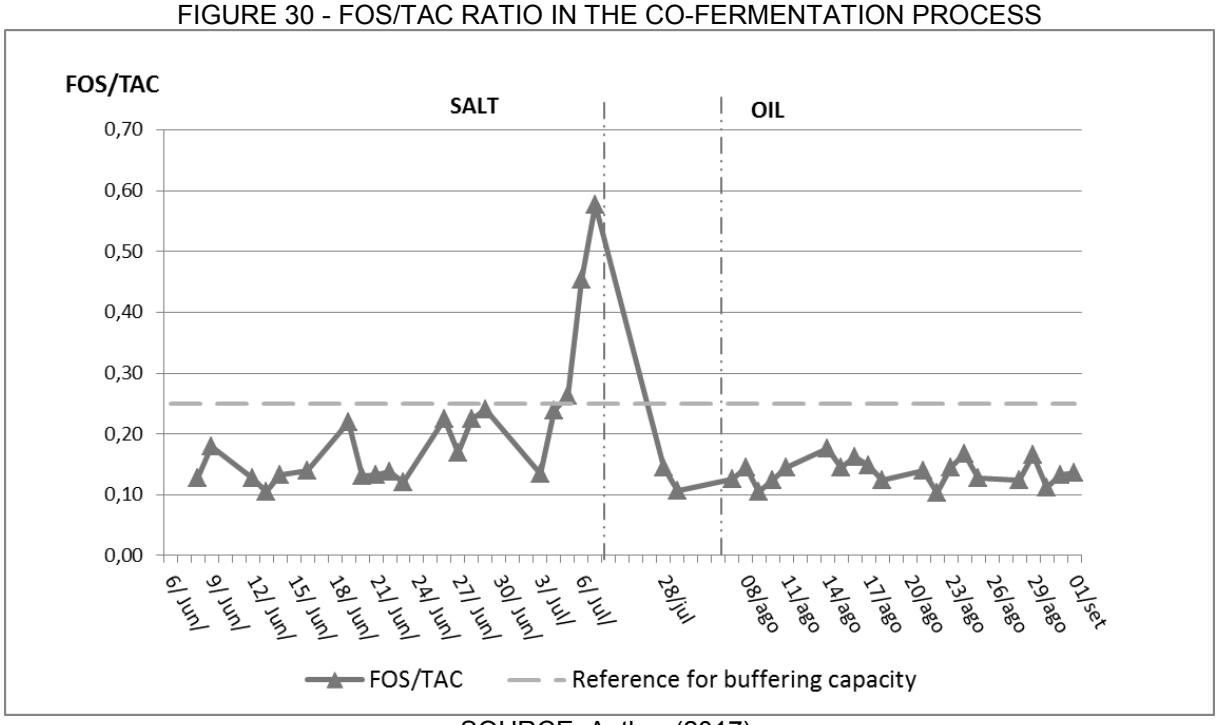
SOURCE: Author (2017).

To better understand the process buffer capacity, it is necessary to combine the FOS and TAC values by the ratio: FOS/TAC.

Just in the last week of salt feeding that the values passed over the limit established according to the literature, which states that FOS/TAC ratio should be under 0,25, see section 3.3.3.

Besides that, the variation of FOS/TAC is greater in Part I than in Part II, as can be seen in Figure 30.

FIGURE 30 - FOS/TAC RATIO IN THE CO-FERMENTATION PROCESS



SOURCE: Author (2017).

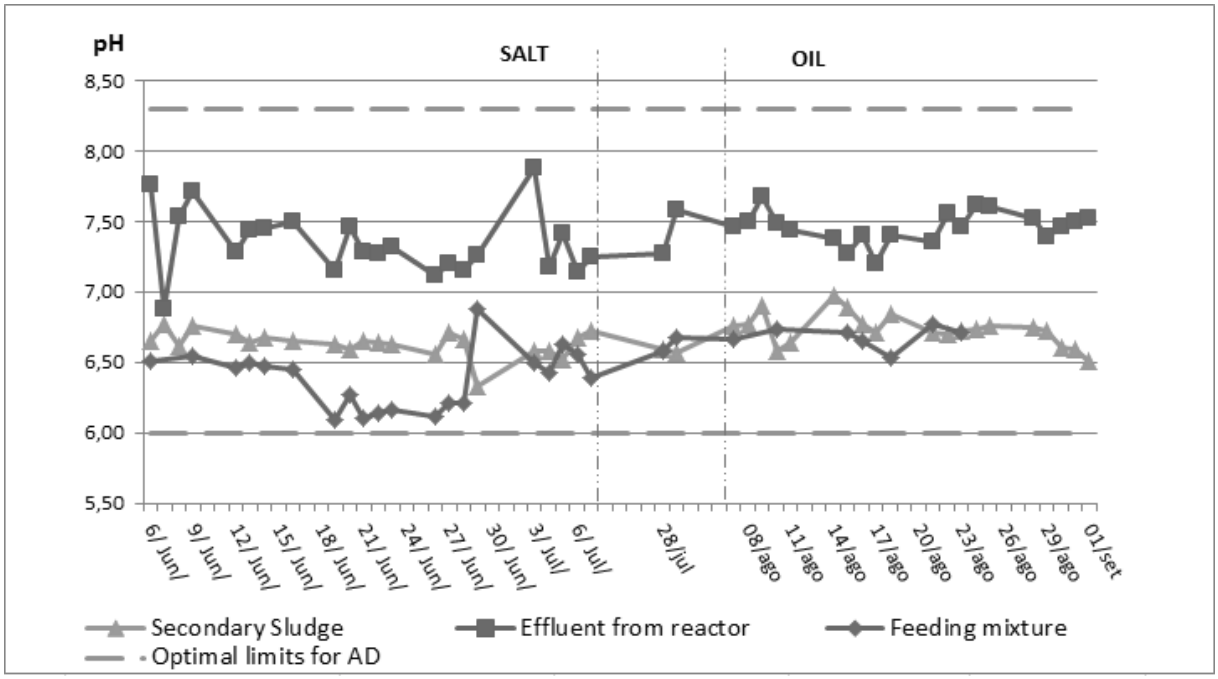
5.4 PH VALUES

The pH measurement gives an idea of the process acidity or alkalinity. In such a way, the result shown in Figure 31 presents for the whole study period, the pH of the influent (both the feeding mixture and its sludge) and the reactor effluent were controlled within the limits established according to the reference value for the optimal result in anaerobic digestion process according to the literature from Baraza et al., (2003), between 6,0 and 8,3.

The pH of the secondary sludge coming from the WWTP remained practically constant, throughout the study period, around 6,5 and 6,7. While its mixture with the prepared food presented a slight variation for the Part I experiment period, nevertheless, it still remains in the range of 6,0 – 6,7.

Also, reactor effluent kept relatively constant throughout the evaluated process, ranging from 7,2 to 7,8 regardless of the point on June 7th, which gave pH 6,9, probably caused by measurement error in the sample.

FIGURE 31 - RESULTS FROM THE PH MEASUREMENT IN THE CO-FERMENTATION PROCESS



SOURCE: Author (2017).

5.5 GAS PRODUCTION

The experiments for evaluation of biogas production behavior through the co-fermentation of sewage sludge influenced by an increased salt concentration, as well as high oil concentration in the food were carried out from Monday to Friday.

Figure 33 shows the general result from the daily feeding in the CSTR. It represents the concentration profile for the 2 main gaseous flows produced by the anaerobic reactor: methane content (CH_4) and carbon dioxide (CO_2) in % vol. In addition, there is the biogas volume (L) produced in this reactor for the whole period of this study: Part I, rehabilitation and stabilization period and Part II. Each experiment part will be further discussed. The volume of biogas produced was recorded by the data logger from each 10 minutes, so that was possible to draw a profile curve in Microsoft Excel.

Regarding the biogas production, a peak in the volume of biogas can be observed every day after the feeding. It can be noticed that every Monday, the biogas production is lower comparing with other weekdays due to the fact that there were not substrate easy available to be degraded because there were weekends, which has no additional feeding in the reactor, which can be seen in Figure 33, the decline in biogas volume in normal liter (NL) produced for this period. This break was

necessary to start a new experimental condition to the reactor weekly. Also, by the reason of the retention time in the reactor, during the week, the biogas production rises every day slightly more, so, the volume of gas generated in Friday is modestly greater than Monday.

The results from the experiments of Part I is related to the salt influence in the biogas production from 06/06 until 07/07. While for the oil assessment, Part II, occurred from 07/08 until 01/09. In this mean time, there was a break for rehabilitation of the reactor and stabilization (08/07 until 06/08) due to changes in feeding conditions and a hydraulic retention time of approximately 21 days.

Generally, the graph shows that when methane content decreases, the concentration of CO_2 increases and the opposite also occurs. It is normal because they are the 2 principal gases in the whole mixture, accounting for more than 95%.

Additionally, regarding the operational behavior of the reactor, it can be considered well controlled. The changes in the results are related to the variations in the operational conditions, in this case, change in the input substrates fed into the process. Thus, these are expected results and not unexpected deviations, which can be confirmed with the results from the effluent sample analysis.

The only abnormality observed in the graph occurred in 14th of August when the concentration of methane and CO_2 dropped abruptly at the same time. It happened when the pressure within the reactor was slightly higher (P≈0,03 bar) than the normal operation (P≈0,01 bar) and approximately double of the effluent volume was collected from the reactor, as shown in Figure 32.

FIGURE 32 - DOUBLE VOLUME OF SLUDGE COLLECTED FROM THE REACTOR IN 14/08

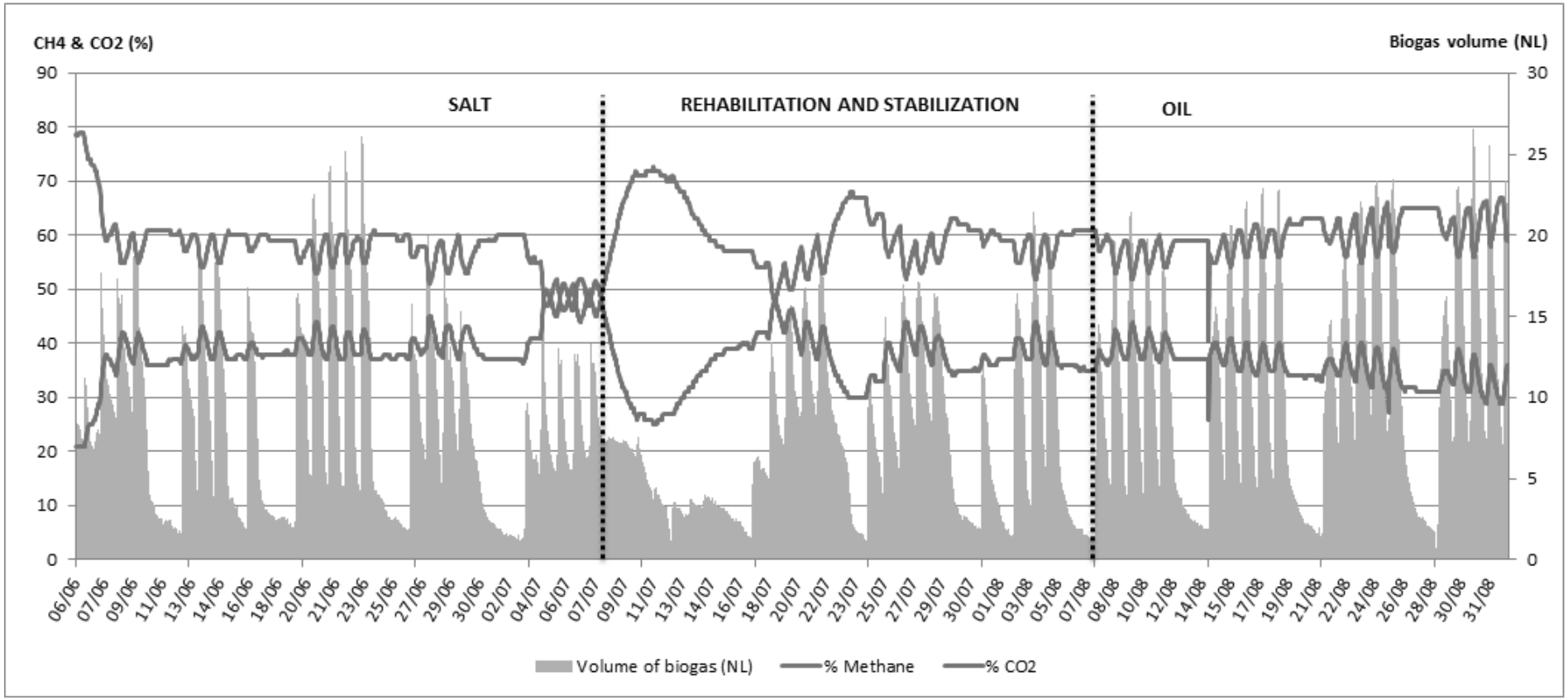
SOURCE: Author (2017).

The experiment with salt content (Part I) shows that the biogas production reaches a maximum volume in the third week, after which, a tendency of decline is observed for the next two weeks. Methane yield reduced as well in the 5th week.

During the rehabilitation and stabilization period, in the 1st week, the digester was fed with sludge from the WWTP with the aim to remove the high accumulation of salt content due to the previous experiment. The following 3 weeks, the co-fermentation process was set again with prepared food mixed with sludge. It is possible to see that, for the 1st week (10th – 14th of July), the mono-fermentation of sludge presents the lowest biogas volume production compared with other periods of study, which the sludge was co-fermented with simulated food waste. For that period, the methane concentration is the highest. However, in terms of methane yield measured by the volume of methane produced this is not as relevant as it seems in Figure 33.

Additionally, for the Part II experiment, the biogas production and methane concentration tend to increase stably week by week with higher concentration of oil (fat) input in a co-digestion process, as shown in Figure 33. The daily calculated biogas production values are presented in the Appendix 4.

FIGURE 33 – BIOGAS PRODUCTION VOLUME AND CONCENTRATION IN METHANE AND CO2 FOR THE WHOLE PERIOD OF EXPERIMENTS



SOURCE: Author (2017).

Additionally, from the data recorded by the data logger is possible to observe, comparing the experiments Part I and II, a time delay to reach the peaks (maximum values for gas production) after every day feeding at 12h. The data show that in Part I, the biogas production peaks are reached 1h approximately later 13h or 14h, while in Part II, the time lag to achieve the peaks increases week after week. In which the first occurred between 16h and 18h, while in the second week between 19h and 23h; third week between 19h and 1h of the next day, and fourth week 17h and 1h of the next day, as shown in Table 17.

TABLE 17 – HOURS OF THE DAY IN WHICH BIOGAS ACHIEVES THE PEAK OF PRODUCTION

Experiment	Week	Monday	Tuesday	Wednesday	Thursday	Friday
Part I	1st	Between 13h and 14h				
	2nd					
	3rd					
	4th					
	5th					
Part II	1st	17h	16h	17h	17h	18h
	2nd	23h	20h	19h	19h	19h
	3rd	1h of the next day	20h	20h	20h	19h
	4th	1h of the next day	19h	18h	17h	18h

SOURCE: Author (2017).

The Figure 34 shows the results calculated for the Gas Production Rate (GPR) in NL/L digester.d and the Specific Biogas Production (SBP) in NL gas/g VS. GPR shows the amount of gas generated per volume of reactor per day, where the volume of digester is 210L and the feeding was done one a day. While SBP is related to the volume of biogas produced per amount of VS added every day, an established amount of 478 g/d. Both have similar graphics curve behavior because they have constant basis for volume biogas production, which means that the volume of the reactor and the organic load considered is the same every day.

They are important parameters because usually in the literature, the biogas volume is calculated in normal conditions and regarding the volume of digester or the organic load material added, so, the results can be compared.

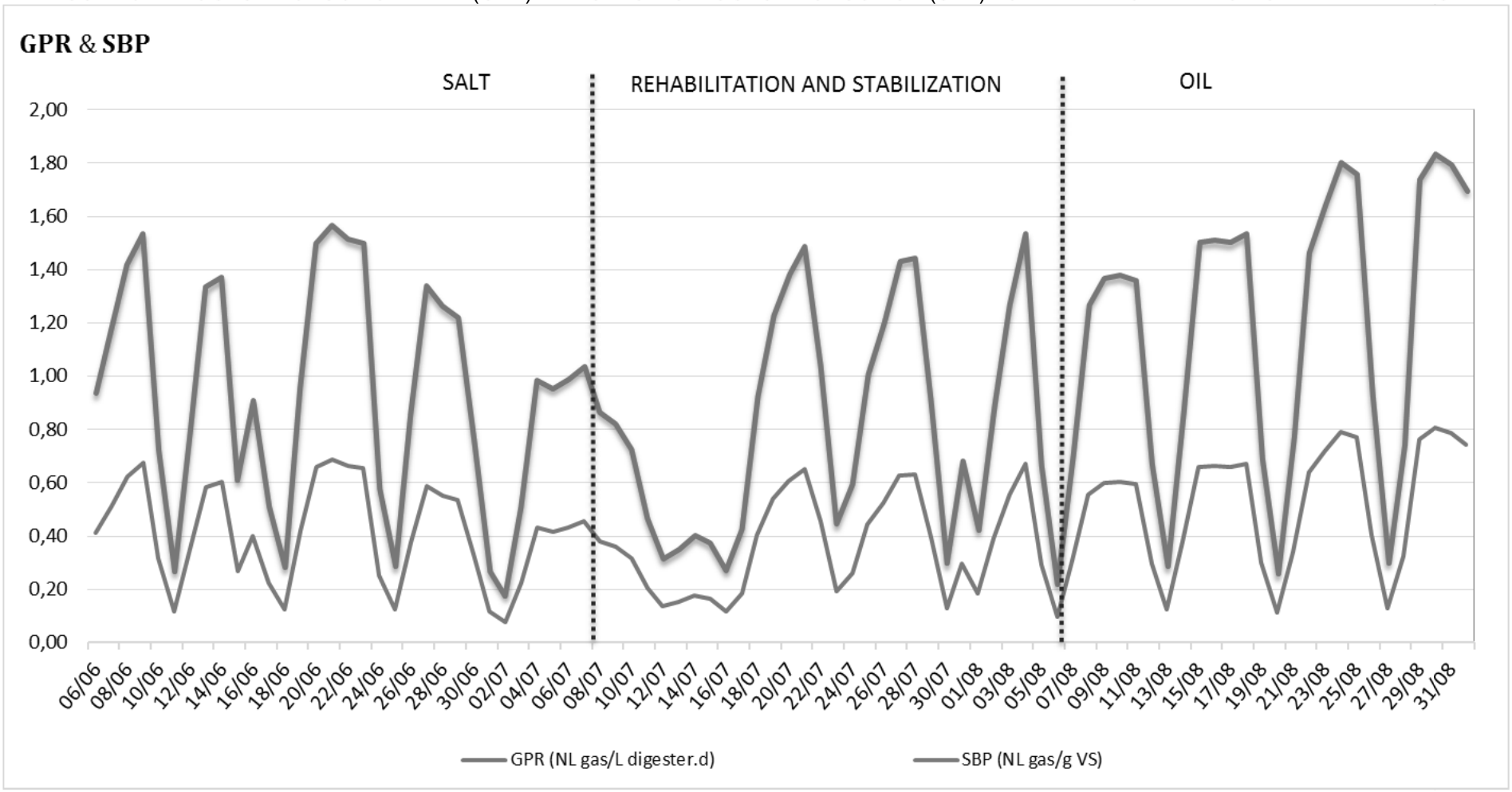
The behavior of GPR and SBP is the same as previously stated, which for co-fermentation with a high concentration of salt, an inhibition process can be noticed

since the 4th week. Then, the digestion of sludge alone has a lower generation of biogas, in the 1st week of rehabilitation of the reactor. Finally, the co-fermentation with increased concentration of oil leads to raising biogas formation.

The maximum GPR for the evaluation with salt is achieved with 1,57 NL/L digester.d. While, in the rehabilitation time, using the same OLR as the Part I experiment but regardless the salt in the mixture, the maximum GPR is 1,53 NL/L digester.d. Whereas the maximum GPR for oil assessment (Part II) is the same for the whole process, which is 1,83 NL/L digester.d.

For the maximum SBP in Part I, the value achieved is 0,69 NL gas/g VS, when for the rehabilitation period, it is 0,67 NL gas/g VS, and for Part II is 0,81 NL gas/g VS, which is maximum also for the whole process. All these results can be seen in Figure 34.

FIGURE 34 - BIOGAS PRODUCTION RATE (GPR) AND SPECIFIC BIOGAS PRODUCTION (SBP) FOR THE WHOLE PERIOD OF EXPERIMENTS



SOURCE: Author (2017).

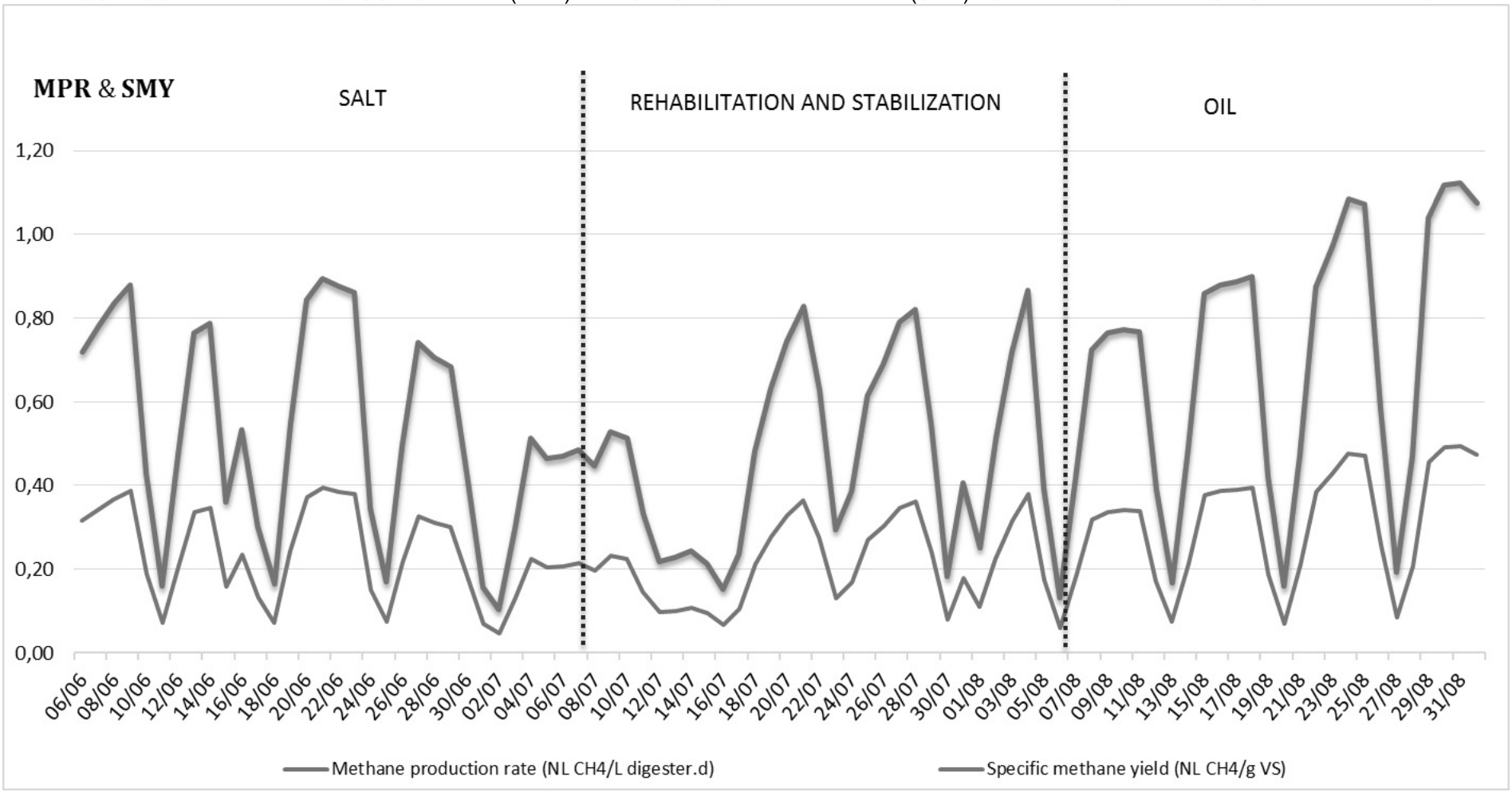
Similar to the GPR, the Methane Production Rate (MPR) states the normal volume of methane produced per liter of digester per day (NL CH_4/L digester.d), while the Specific Methane Yield (SMY) is a parameter analogous to the SBP, expressing the volume of methane produced per weight of VS (NL CH_4/g VS).

Figure 35 shows the results from the calculation of methane production. It is an important parameter because this is the product of interest. Methane is the fuel product that has aggregate energy value, when it enters into combustion, releases energy in the form of heat that can be used later. They were calculated as well to be available for comparison with the results from the literature.

In this case, the maximum MPR is 0,9 NL CH_4/L digester.d for the Part I, while the followed rehabilitation time has 0,87 NL CH_4/L digester.d and the process maximum MPR is achieved in the Part II experiment with 1,13 NL CH_4/L digester.d.

The maximum SMY in Part I experiment is 0,39 NL CH_4/g VS, while in the rehabilitation period is 0,38 NL CH_4/g VS, and the maximum SMY for the whole process is determined in Part II with 0,49 NL CH_4/g VS. The theses results are shown in Figure 35.

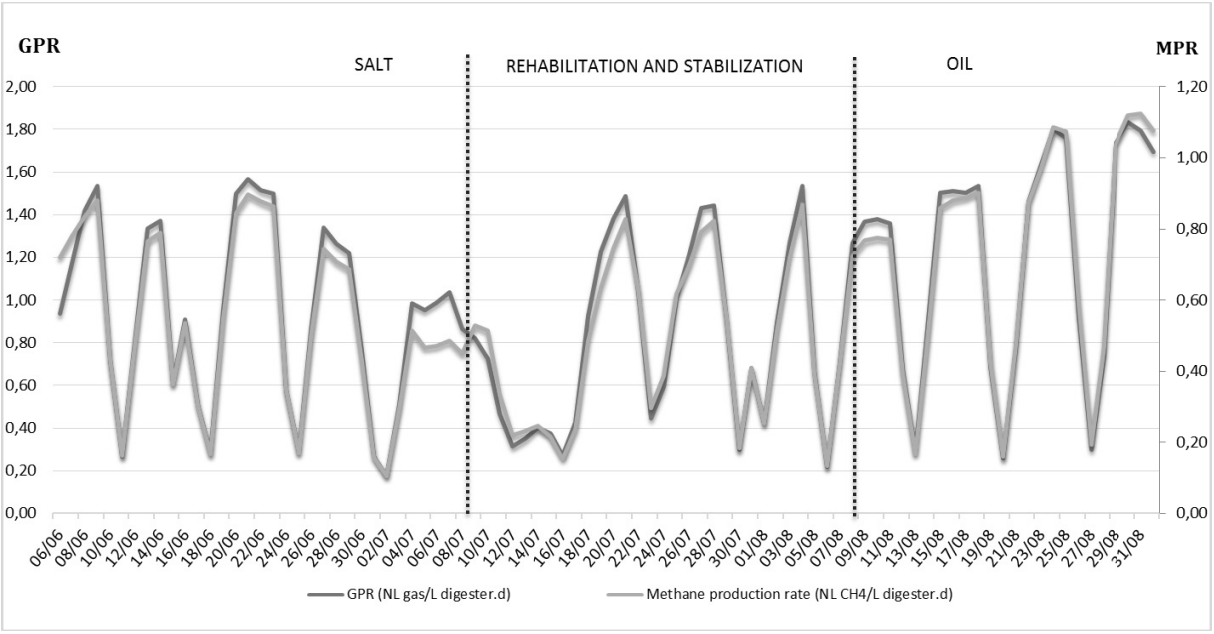
FIGURE 35 - METHANE PRODUCTION RATE (MPR) AND SPECIFIC METHANE YIELD (SMY) FOR THE WHOLE PERIOD OF EXPERIMENTS



SOURCE: Author (2017).

Comparing the biogas (GPR) and methane (MPR) production curves, it can be seen in Figure 36 similar behaviors, regardless of the period from 04/07 to 11/07. During this time, the digestion process was affected by the influence of high salt concentration. In such a way, methane volume has decreased more than the biogas volume, thus proving that co-fermentation increases biogas production also increases methane yield.

FIGURE 36 - COMPARISON OF BIOGAS AND METHANE PRODUCTION



SOURCE: Author (2017).

Figure 37 states the same results previously shown for the GPR and MPR, now only for the experiments Part I. Thus, it is included the salt concentration within the reactor calculated through the effluent conductivity as well as the salt content added in the food mixture. It is possible to see that increasing the salt concentration, the anaerobic process reaches in a maximum biogas around 1,6 L/L digester.d in the third week of experiments, when the maximum salt concentration was 0,8 g/L in the reactor. And then, starting from the 4th week, there is a decrease of gas production with higher salt content around 1,2 g/L. This decline proceeds for the following week as well. The same behavior is observed to the methane production.

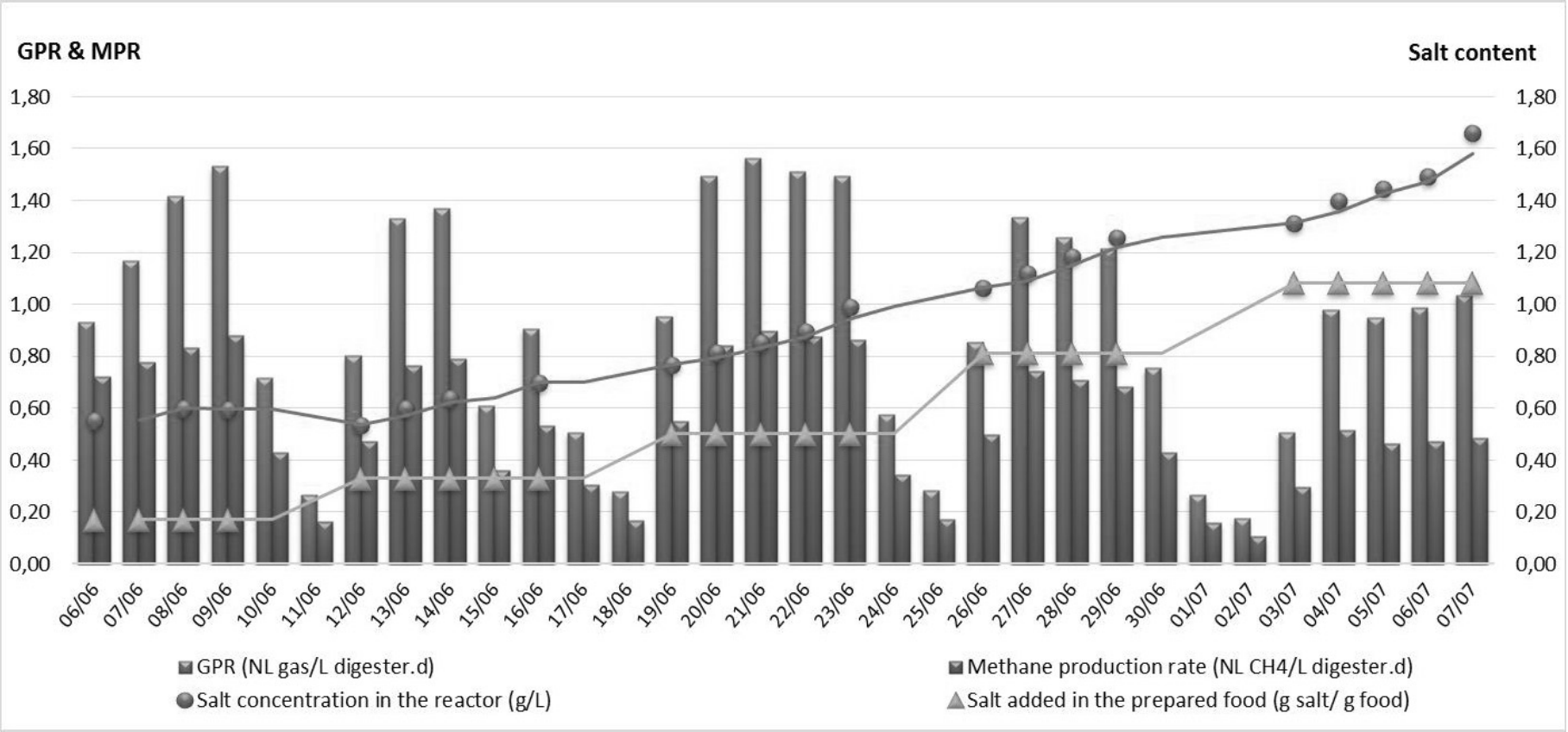
A study previously conducted in the same reactor from Yan (2017) showed that the method of quantification of oil content through the measurement of COD parameter is not suitable due to its hydrophobic property, and it was necessary to use an emulsifier that may has interfered in the results. In the same study, the author

also proved that COD and VS have a similarity, where the measurement of VS seems to be more reliable. Thus, for the present study, VS of effluent was determined to assess the biodegradability of the process, since the input VS was set to 478 g/d.

As the COD was not determined to correlate with organic matter degradation, in this case, degradation of oil neither an analytical method was not available to quantify the real concentration of oleic acid in the reactor (LCFA), then the results shown in Figure 38 present the variation of biogas and methane production according to the oil concentration, as well as the fat concentration in the food added to the feeding of the reactor. Here, oil is the fresh material rapeseed oil, while fat is the lipid, a general term for fatty acids.

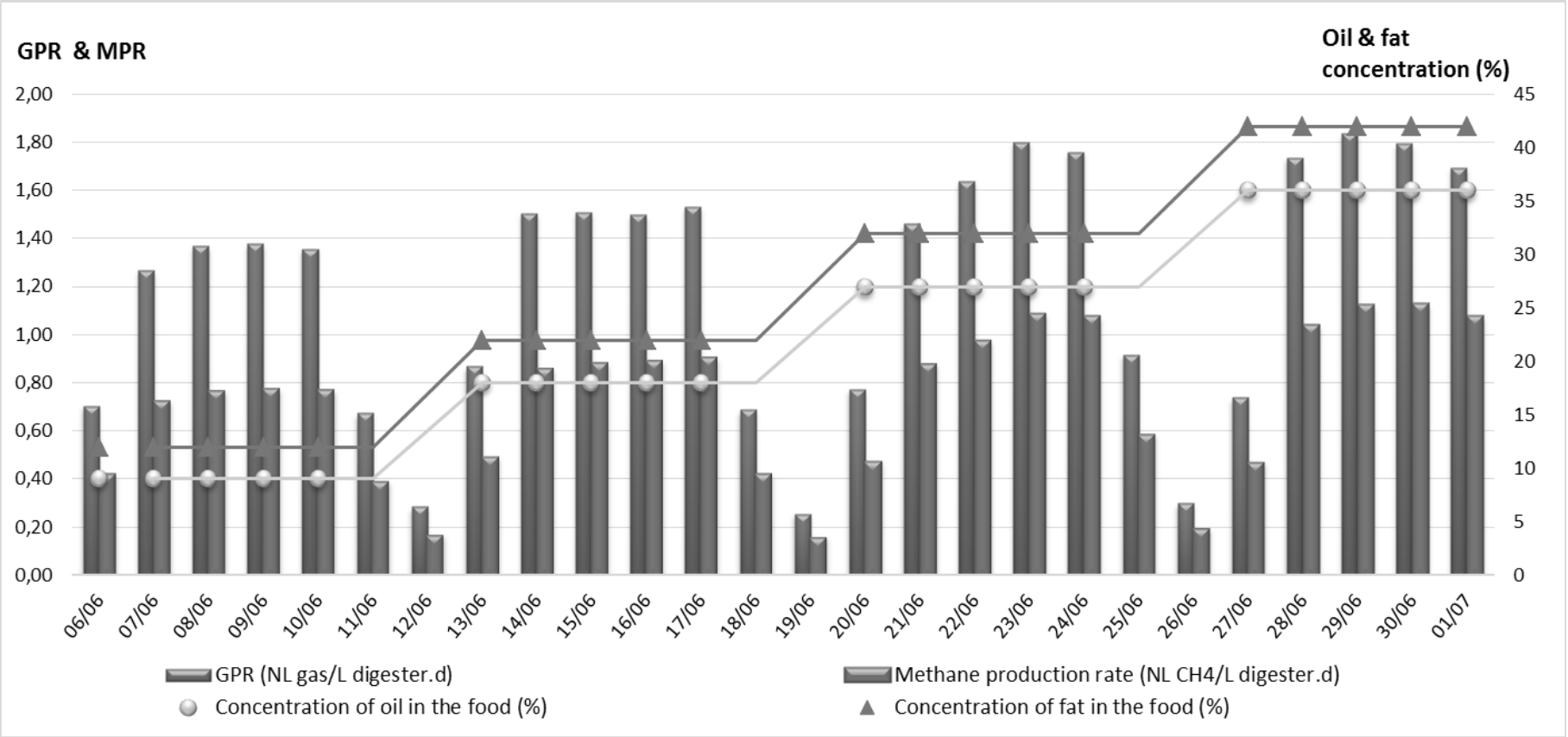
It can be seen from the Figure 38 that when the amount of oil increases, so does the production of biogas and methane. In the last week (4th), a slight gas volume increased comparing with the previous one (3rd), which can also be visualized in the graph of Figure 33. For this amount of gas, a higher concentration of oil is required, which is 36% in the food, or relating to the fat content means 42% of lipid, or in relation to the calculated oleic acid concentration added is 0,50 g/L.

FIGURE 37 - BIOGAS PRODUCTION ACCORDING TO THE CONCENTRATION OF SALT



SOURCE: Author (2017).

FIGURE 38- BIOGAS PRODUCTION ACCORDING TO THE CONCENTRATION OF OIL



SOURCE: Author (2017).

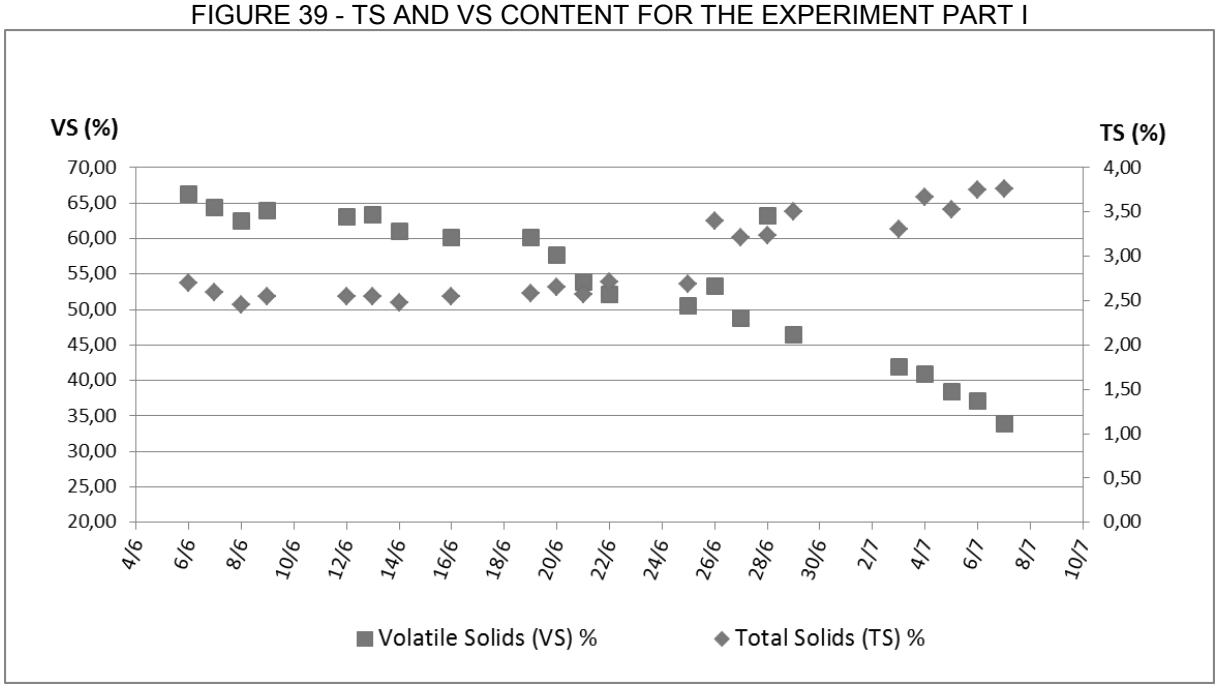
5.6 TOTAL SOLIDS AND VOLATILE SOLIDS

The contents of total solids and volatile solids are shown in Figure 39 and Figure 40 for the Part I and Part II experiment respectively.

As observed in Figure 39, during the experiments with the gradual increase of salt in the reactor feed, the content of total solids in the output is increased weekly as well. Where no change during the first two weeks was observed, with a weekly average of 2,57% for the first week and 2,53% for the second week, in the third week there is a slight increase (2.63%) and in the fourth week there is a more significant increase of total solids, with 3,17%, also in fifth week with 3,64%.

On the other hand, there was a decrease in the volatile solids content observed week by week, which for the first, the average is 64,30%, decreasing to 61,91%, 55,94%, 51,53% and 39,37% in the second, third, fourth and fifth week respectively.

FIGURE 39 - TS AND VS CONTENT FOR THE EXPERIMENT PART I

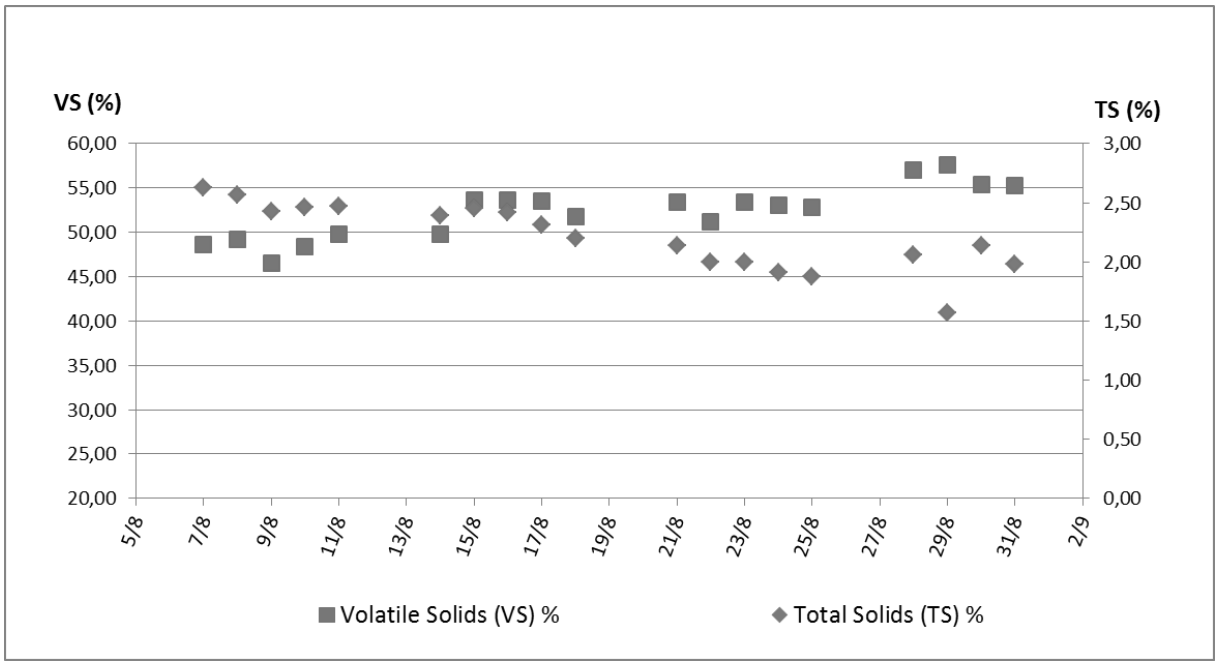


SOURCE: Author (2017).

In relation to the total solids of the experiment art II, there was a slight weekly reduction in the values, decreasing from 2,51% (first week) to 2,35% (second week), 1,98% (third week), and finally 1,94% (fourth week). Meanwhile, the concentration of volatile solids gradually rises week after week, starting with an average of 48,50% at week 1, increasing to 52,46%, 52,77% and 56,33% at weeks 2, 3 and 4 respectively,

as shown in Figure 40.

FIGURE 40 - TS AND VS CONTENT FOR THE EXPERIMENT PART II

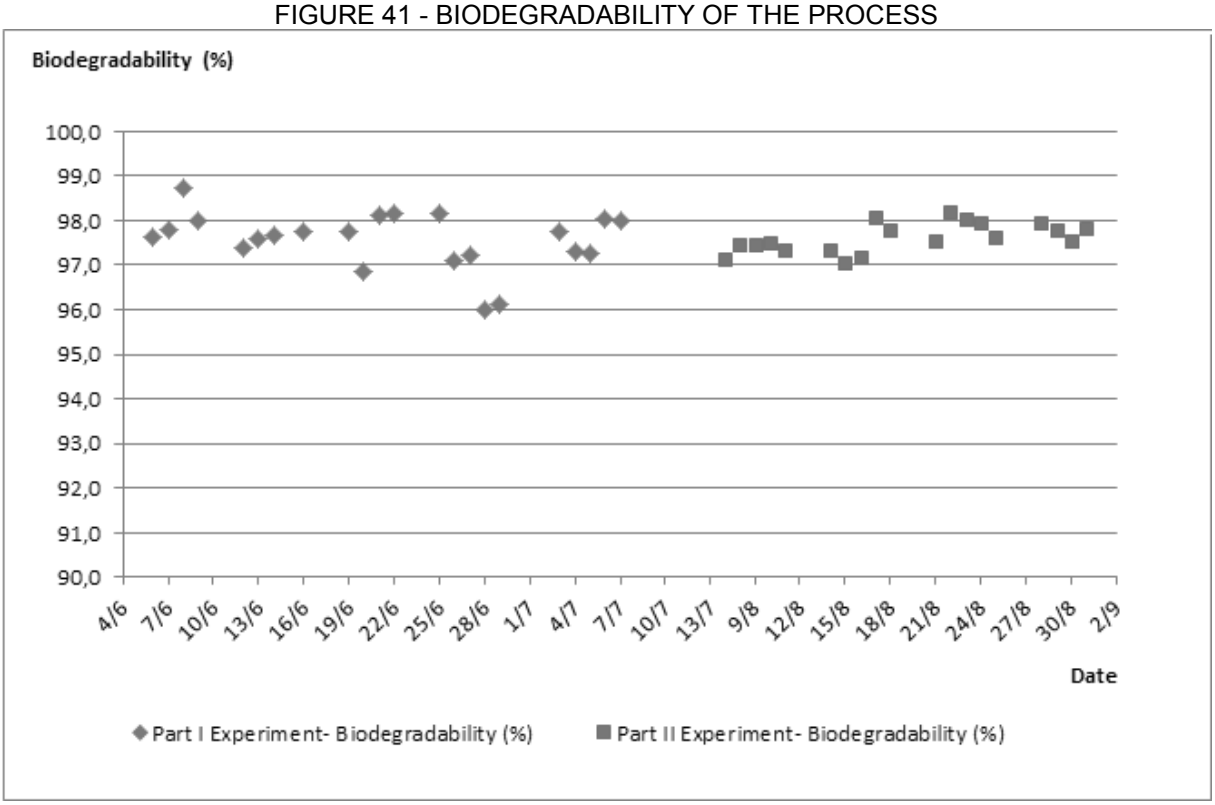


SOURCE: Author (2017).

5.7 BIODEGRADABILITY OF THE PROCESS

The biodegradability of the process calculated for Part I regarding the salt assessment has a similar average result comparing with Part II for oil assessment. In both cases, the biodegradability is more than 96%, as shown in Figure 41.

FIGURE 41 - BIODEGRADABILITY OF THE PROCESS



SOURCE: Author (2017).

An average biodegradability for the overall process is more than 97%. The results of the experiments Part I and Part II are shown in Table 18, the details of calculation are presented in the Appendix 1.

TABLE 18 - EXPERIMENTAL BIODEGRADABILITY OF THE ANAEROBIC PROCESS

Parameter	Part I	Part II
Average input volatile solids, VS_0 (g)	478	478
Average output volatile solids, VS_f (g)	11,6	11,1
Average Biodegradability, BD (%)	97,6	97,7

SOURCE: Author (2017).

6 DISCUSSION

The results obtained with the co-fermentation of sewage sludge and food waste demonstrate that the process worked satisfactorily stable for the established conditions. In this case, by previously setting the same OLR (2,3g VS/L.d) for the whole experiment (Part I, rehabilitation period and Part II) allowed the analysis of the influence of specific substances that are considered in the existing literature as process inhibitors in the biogas production: salt (mainly referring ion sodium), long chain fatty acids (mainly by oleic acid) and ammonia as the result from the degradation process of biomass. The experiments were conducted basing on the composition of food waste in Chengdu/China, which has 35% protein, 17% fat and 48% carbohydrate.

The Part I of the study was performed with salt concentration (NaCl) in the food increasing from 0,17 g/g; 0,33 g/g; 0,50 g/g; 0,81 g/g and 1,08 g/g from week 1 to week 5. Although these concentrations are considered high relative to their usual content in food waste when mixed with sewage sludge, it becomes diluted to feed the reactor, this concentration in relation to the digester volume becomes more realistic, ranging from 0,54 to 1,66 g/L. A low concentration of salt is essential for the production of energy for the bacterial cell. Therefore, biogas production reaches a maximum volume in the third week of feed, with a salt concentration of approximately 0,9 g/L, generating biogas production rate (GPR) of 1,57 NL/L digester.d and methane production rate (MPR) of 0,9 NL/L digester.d. However, it was observed that the inhibition of the process started already from the fourth week of experiments, where with 1,2 g/L of salt concentration, the biogas and methane productions start to decrease, as can be seen in Figure 37.

It means that for the current process of co-fermentation, the maximum concentration of salt tolerable by microorganisms before its metabolic activity turn to be affected is around 1,2 g/L. The hypothesis is that due to the high salt concentration, an increase in osmotic pressure has occurred and consequently, dehydration of the cell membrane, as suggested by the literature (YERKES, 1997).

For the Part II experiment, the concentration of lipid was gradually increased because according to the literature, the inhibition of AD process is irreversible, causing damage to the membrane structure of bacteria cell.

Although the actual concentration of oleic acid in the reactor was not able to

be determined, the added concentration of oleic acid was calculated to get an idea of its value in the reactor once it is known by the literature that above 1,2 g/L (KOSTER AND CRAMER, 1987) the process can be inhibited. No inhibition of LCFA was possible to see in this study with a maximum concentration of oleic acid added in the digester of 0,5 g/L. Therefore, the results in Figure 38 shows a tendency to raise the gas production with the increment on oil concentration. It is in agreement with the statement of the literature, as shown in Table 3, for substrates with higher fat content, the biogas yield as well as methane concentration are larger. In this case, a maximum biogas (GPR=1,83 NL/L digester.d and SBP=0,81NL/g VS) and methane (MPR=1,13 NL CH4/L digester.d and SMY=0,49 NL CH4/g VS) production could be obtained for the whole process by feeding the reactor with 42% of fat in the FW.

From the Table 17 is possible to see that for the whole period of Experiment Part I, the biogas production peak is reached 1 hour later after the feeding. While for the Experiment Part II (with higher fat content), the peaks were reached at different span times after the feeding, in which an average of 5 hours of delay is verified for the first week and 9 hours for the following 3 weeks of experiments.

According to Christ, et al. (2000), the first order kinetic constant values for the hydrolysis step in AD process is lower for lipids (0,005 – 0,010 d^{-1}) in comparison with proteins (0,015 – 0,075 d^{-1}) and carbohydrates (0,025 – 0,200 d^{-1}), as shown in Table 4 (CHRIST ET AL., 2000). Considering that the overall reaction rate is determined by the slower reaction and generally it is determined by the hydrolysis step for systems containing high solid content and suspended solids, as characterized for the present study. In this case, the fat degradation is slower compared with other substances, since it has a lower kinetic coefficient. Therefore, the increase of fat concentration in the food composition (Part II) affects the hydrolysis step, which takes longer, consequently delaying the overall reactions for the biogas production, as seen in the lag period stated in Table 17.

In relation to pH, the mixture of food prepared in the laboratory with secondary sludge resulted in a slightly acidic medium for the feed of the reactor, input pH varied between 6,0-6,7. While the pH of the reactor's effluent was more neutral, ranged slightly from 7,2 to 7,8. It means that the alkalinity increased after the digestion process. This can be explained by the fact that during the degradation of organic matter, the nitrogen content present in the substrate is degraded into smaller molecules, such as ammonia that in contact with aqueous mean is converted into

ammonium, a base solution that raises the pH. It can be seen from Figure 31 that the pH provided a favorable environment to the activity of methanogenic acetoclastic bacteria since it remained around 7,2, within the limits considered optimal, despite having more variation of the pH in the Part I period than in Part II.

The results obtained for the ammonium concentration in the reactor remained relatively constant and within the optimal limits of operation, as shown in Figure 27. It means that the process for microbial activity was not interfered by the formation of ammonia nitrogen, where the average concentration is 1,0 g NH4-N /L. The modest reduction in ammonia concentrations of Part I (1142 mg NH4-N/L) and Part II (862 mg NH4-N/L) may be related to the decrease of protein source (soy) in the mixture for bacteria degradation, as shown in Table 9. Consequently, less biomass to be degraded and converted to nitrogen compounds.

The results obtained for volatile fatty acids (VFA) through the measurement of FOS (Volatile Organic Substances), Figure 28, show abrupt variation only in the last week of experiments using a high concentration of salt (average 1,5 g salt/L digester). During Part I experiment, the average VFA concentration is 1058 mg acetic acid/L, however, in the last week, this value reached 2498 mg acetic acid/L. It means that the increase in salt concentration (1,5 g salt/L digester) resulted in an accumulation of intermediate products The methanogenic bacteria were not able to remove the VFA fast enough, leading to the decrease of methane formation (see Figure 33) and the slight instability in pH values (see Figure 31). It can be considered a moderate inhibition of bacterial activities by the high concentration of salt in the process.

The values generated for TAC (Volatile Organic Substances) are relatively stable and within the limit specified by the literature. Figure 29 shows that the alkalinity had slight tendency to decline in the last week of experiment Part I.

FOS/TAC ratio presents a larger variation in Part I than Part II, as seen in Figure 30. In addition, the limit determined by the literature (0,25) in the last week of experiment with the salt has been exceeded leading to instabilities in the process control by the pH, see Figure 31. Despite this variation of pH, the process is still controlled within the determined limits. For the current process, the stability of the buffer capacity occurs for FOS/TAC ranging from 0,1 to 0,2.

Regarding the result for the total solids (TS) in the Part I experiment, an increase in the values is presented weekly due to the rise of salt quantity and

concentration in the input flow of the reactor. Thus, the amount of solid material (salt) that cannot be degraded or assimilated by the bacteria in the digester increases and consequently a higher elimination of the excess salt from the digester starting from the fourth week is shown in Figure 39. On the other hand, a decrease of volatile solids occurred in parallel as a result of the reduction of solids that can be degraded by the bacteria.

For the TS and VS values in the experiment Part II, no drastic variation is observed in Figure 40 due to the feed with liquid oil, which does not cause changes in the TS and VS values. The slight variation observed is related to the modest weekly difference in feed composition added to the reactor, as shown in Table 9.

The biodegradability of the process resulted in more than 97%. It means that the organic matter was degraded by the process of anaerobic digestion with high efficiency.

7 CONCLUSION

The present study assessed the influence of a higher concentration of salt and oil in the food waste to be co-digested with sewage sludge regarding process stability, production of biogas and methane as well as the efficiency of the degradation.

The experiments were conducted in a semi-scale 210 L reactor, model continuous stirred tank reactor (CSTR) operating in a semi-continuous regime with hydraulic retention time around 21 days. The operational parameters were set at a mesophilic temperature about 35 ºC and slightly pressurized, the manometric pressure of 0,01 bar.

The co-fermentation process was determined based on the characterization of food waste in Chengdu/China, which is composed of 35% (w/w) of protein, 17% of fat and 48% of carbohydrate. Besides that, an established organic load rate (2,3g VS/L.d) was used as a reference to feeding the reactor daily.

In such a way, the process demonstrated to be more stable during the feeding with high oil content (Part II experiment) than the feeding with high salt concentration (Part I experiment), though the analysis of parameters that control the process, such as ammonia concentration (average of 1000 mg NH4-N/L), VFA, alkalinity, pH (ranged from 7,2 to 7,8) and FOS/TAC.

In addition, co-digestion of sewage sludge with food waste produced a larger volume of biogas and also a higher methane yield, in comparison with digestion of only sewage sludge.

Furthermore, the results showed that the process can be inhibited with higher concentration of salt (1,2 g/L), while high content of oil can improve volumetric biogas and methane production (maximum GPR = 1,83 NL/L digester.d and maximum MPR = 1,13 NL CH4/L digester.d) using an oil concentration of 36% in the food, in oher words, it means 42% of the fat or lipid content. In the present study, inhibition limit was not reached regarding the influence of long chain fatty acid (LCFA), which had a maximum of 0,50 g/L of oleic acid added to the reactor.

The addition of long chain fatty acids caused the appearance of the lag period in the biogas production from the degradation of substrates with high oil content (Part II). While biogas production from the feeding of high salt concentration (Part I) proceed without lag period although its rate was lower (maximum GPR = 1,57 NL/L

digester.d).

Regarding the biodegradability of the process, it is possible to note that the degradation of organic matter was performed successfully since the efficiency of the digestion process achieved more than 97% of biodegradability.

In general, the results obtained with the present study allowed knowing better the anaerobic co-fermentation performance of sewage sludge with food waste in a semi-scale reactor. In this way, the co-digestion process represents an additional possibility for the disposal, treatment, and utilization of the organic fraction of municipal solid waste as well as enhances WWTP's renewable energy production efficiency.

To conclude, further research efforts can be improved in this field regarding:

- Continuation of the tests with higher concentrations of oil in the digester in order to determine the inhibition limit of LCFA;
- Analytical tests with the reactor effluent to measure the actual concentration of LCFA, such as oleic acid. Thus, the toxicity of the process relative to specific compounds can be determined and compared with literature review;
- Carry out simulation studies of the process to estimate and to predict the theoretical methane production and comparing with the results of the present experimental study in order to have a better control of the process.

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APPENDIX 1 – DETERMINATION OF TS AND VS VALUES AND BIODEGRADABILITY

	Date	Crucible weight	Crucible weight + Effluent sample	Weight after dried	Weight after burned	Weight of effluent (g)	Weight of dried sample (g)	Weight of burned sample (g)	Total Solids (TS) %	Fixed Solids (FS) %	Volatile Solids (VS) %	VS (g org/g fresh)	VSf, final (g)	VS0, initial (g)	Biodegradability (%)
Part I	6/6	62,31	125,98	64,03	62,89	63,67	1,72	0,58	2,70	33,72	66,28	0,2	11,4	478	97,6
	7/6	65,6	128,49	67,23	66,18	62,89	1,63	0,58	2,59	35,58	64,42	0,2	10,5	478	97,8
	8/6	62,73	101,91	63,69	63,09	39,18	0,96	0,36	2,45	37,50	62,50	0,2	6	478	98,7
	9/6	50,41	109,27	51,91	50,95	58,86	1,5	0,54	2,55	36,00	64,00	0,2	9,6	478	98,0
	12/6	65,9	143,58	67,88	66,63	77,68	1,98	0,73	2,55	36,87	63,13	0,2	12,5	478	97,4
	13/6	64,61	136,5	66,44	65,28	71,89	1,83	0,67	2,55	36,61	63,39	0,2	11,6	478	97,6
	14/6	62,44	135,9	64,26	63,15	73,46	1,82	0,71	2,48	39,01	60,99	0,2	11,1	478	97,7
	16/6	61,41	131,38	63,19	62,12	69,97	1,78	0,71	2,54	39,89	60,11	0,2	10,7	478	97,8
	19/6	65,74	134,61	67,52	66,45	68,87	1,78	0,71	2,58	39,89	60,11	0,2	10,7	478	97,8
	20/6	78,74	177,63	81,36	79,85	98,89	2,62	1,11	2,65	42,37	57,63	0,2	15,1	478	96,8
	21/6	65,91	130,01	67,56	66,67	64,1	1,65	0,76	2,57	46,06	53,94	0,1	8,9	478	98,1
	22/6	64,6	127,03	66,29	65,41	62,43	1,69	0,81	2,71	47,93	52,07	0,1	8,8	478	98,2
	25/6	65,6	129,75	67,32	66,45	64,15	1,72	0,85	2,68	49,42	50,58	0,1	8,7	478	98,2
	3/7	65,73	143,68	68,31	67,23	77,95	2,58	1,5	3,31	58,14	41,86	0,1	10,8	478	97,7
	26/6	61,43	138,2	64,04	62,65	76,77	2,61	1,22	3,40	46,74	53,26	0,2	13,9	478	97,1
	27/6	62,46	147,56	65,19	63,86	85,1	2,73	1,4	3,21	51,28	48,72	0,2	13,3	478	97,2
	28/6	62,32	155,79	65,34	63,43	93,47	3,02	1,11	3,23	36,75	63,25	0,2	19,1	478	96,0
	29/6	78,79	192,9	82,79	80,93	114,11	4	2,14	3,51	53,50	46,50	0,2	18,6	478	96,1
	4/7	97,77	183,03	100,9	99,62	85,26	3,13	1,85	3,67	59,11	40,89	0,2	12,8	478	97,3
	5/7	83,06	179,13	86,45	85,15	96,07	3,39	2,09	3,53	61,65	38,35	0,1	13	478	97,3
	6/7	65,9	133,47	68,43	67,49	67,57	2,53	1,59	3,74	62,85	37,15	0,1	9,4	478	98,0
	7/7	85,85	160,39	88,65	87,7	74,54	2,8	1,85	3,76	66,07	33,93	0,1	9,5	478	98,0
STABILIZATION	28/7	62,69	147,14	65,11	64,09	84,45	2,42	1,4	2,87	57,85	42,15	0,1	10,2	478	97,9
	4/8	62,71	125,49	64,36	63,62	62,78	1,65	0,91	2,63	55,15	44,85	0,1	7,4	478	98,5

	Date	Crucible weight	Crucible weight + Effluent sample	Weight after dried	Weight after burned	Weight of effluent (g)	Weight of dried sample (g)	Weight of burned sample (g)	Total Solids (TS) %	Fixed Solids (FS) %	Volatile Solids (VS) %	VS (g org/g fresh)	VSf, final (g)	VS0, initial (g)	Biodegradability (%)
Partll	7/8	83,07	190,53	85,89	84,52	107,46	2,82	1,45	2,62	51,42	48,58	0,1	13,7	478	97,1
	8/8	106,92	202,91	109,38	108,17	95,99	2,46	1,25	2,56	50,81	49,19	0,1	12,1	478	97,5
	9/8	78,69	186,69	81,31	80,09	108	2,62	1,4	2,43	53,44	46,56	0,1	12,2	478	97,4
	10/8	83,07	182,9	85,53	84,34	99,83	2,46	1,27	2,46	51,63	48,37	0,1	11,9	478	97,5
	11/8	106,84	209,35	109,37	108,11	102,51	2,53	1,27	2,47	50,20	49,80	0,1	12,6	478	97,4
	14/8	83,22	188,91	85,75	84,49	105,69	2,53	1,27	2,39	50,20	49,80	0,1	12,6	478	97,4
	15/8	81,97	188,34	84,58	83,18	106,37	2,61	1,21	2,45	46,36	53,64	0,1	14	478	97,1
	16/8	78,75	182,16	81,25	79,91	103,41	2,5	1,16	2,42	46,40	53,60	0,1	13,4	478	97,2
	17/8	65,6	139,25	67,3	66,39	73,65	1,7	0,79	2,31	46,47	53,53	0,1	9,1	478	98,1
	18/8	97,75	190,2	99,78	98,73	92,45	2,03	0,98	2,20	48,28	51,72	0,1	10,5	478	97,8
	21/8	83,64	186,02	85,83	84,66	102,38	2,19	1,02	2,14	46,58	53,42	0,1	11,7	478	97,6
	22/8	83,23	167,42	84,91	84,05	84,19	1,68	0,82	2,00	48,81	51,19	0,1	8,6	478	98,2
	23/8	83,12	171,28	84,88	83,94	88,16	1,76	0,82	2,00	46,59	53,41	0,1	9,4	478	98,0
	24/8	83,12	178,88	84,95	83,98	95,76	1,83	0,86	1,91	46,99	53,01	0,1	9,7	478	98,0
	25/8	83,23	197,43	85,37	84,24	114,2	2,14	1,01	1,87	47,20	52,80	0,1	11,3	478	97,6
	28/8	97,77	187,95	99,49	98,51	83,48	1,72	0,74	2,06	43,02	56,98	0,1	9,8	478	97,9
	29/8	83,37	181,25	85,21	84,15	117,42	1,84	0,78	1,57	42,39	57,61	0,1	10,6	478	97,8
	30/8	83,13	200,79	85,26	84,08	99,73	2,13	0,95	2,14	44,60	55,40	0,1	11,8	478	97,5
	31/8	78,72	182,86	80,6	79,56	94,98	1,88	0,84	1,98	44,68	55,32	0,1	10,4	478	97,8

APPENDIX 2 – PARAMETERS MEASURED EXPERIMENTALLY

	Date	pH			Conductivity (mS/cm)		NH4-N Measured	TAC - pH 5	FOS - pH 4,4
		Secondary Sludge	Feeding mixture	Effluent from reactor	Feeding mixture	Effluent from reactor			
Part I	06/06/ 2017	6,66	6,51	7,76		1,34		11,99	18,81
	07/06/ 2017	6,77		6,88				12,04	14,42
	08/06/ 2017	6,62		7,54		1,45		23,65	24,65
	09/06/ 2017	6,76	6,55	7,72		1,44		23,74	25,11
	12/06/ 2017	6,7	6,47	7,29	1,19	1,3	146	22,82	23,79
	13/06/ 2017	6,65	6,5	7,44	1,86	1,45	148	22,98	23,8
	14/06/ 2017	6,68	6,48	7,45	2,03	1,55	133	22,9	23,9
	16/06/ 2017	6,66	6,45	7,5	2,38	1,69	150	22,85	23,9
	19/06/ 2017	6,63	6,1	7,16	3,51	1,86	127	21,4	22,9
	20/06/ 2017	6,6	6,27	7,47	3,51	1,97	149	23,1	24,1
	21/06/ 2017	6,66	6,11	7,29	3,5	2,06	139	22,7	23,7
	22/06/ 2017	6,64	6,14	7,28	3,63	2,17	164	21,8	22,8
	23/06/ 2017	6,63	6,17	7,32		2,4	164	22,4	23,3
	26/06/ 2017	6,56	6,12	7,12		2,57	107	19,4	20,8
	27/06/ 2017	6,71	6,22	7,21		2,71	116	21,8	23
	28/06/ 2017	6,67	6,21	7,16		2,86	112	20,8	22,3
	29/06/ 2017	6,33	6,88	7,26	5,01	3,04	127	20,8	22,4
	03/07/ 2017	6,58	6,5	7,88		3,18	103	22,5	23,5
	04/07/ 2017	6,58	6,43	7,18		3,38	127	21	22,6
	05/07/ 2017	6,52	6,63	7,42		3,5	117	20,4	22,1
	06/07/ 2017	6,68	6,56	7,15		3,61	138	18,4	21
	07/07/ 2017	6,73	6,4	7,25	6,4	4,02	142	17,3	20,4
stabiliz ation	28/07/ 2017	6,6	6,58	7,27	0,22	2,9	138	18,6	19,5
	04/08/ 2017	6,57	6,68	7,59	0,21	2,65	115	19,1	19,8

	Date	pH			Conductivity (mS/cm)		NH4-N Measured	TAC - pH 5	FOS - pH 4,4
		Secondary Sludge	Feeding mixture	Effluent from reactor	Feeding mixture	Effluent from reactor			
Part II	07/08/2017	6,76	6,67	7,47	0,21	2,48	108	18,8	19,6
	08/08/2017	6,77		7,5		2,44	122	18,5	19,4
	09/08/2017	6,91		7,68		2,56	114	19,4	20,1
	10/08/2017	6,58	6,74	7,49	0,21	2,21	86	19,1	19,9
	11/08/2017	6,64		7,44		2,12	88	18,5	19,4
	14/08/2017	6,98		7,38	0,21	2,38	94	19,1	20,2
	15/08/2017	6,9	6,72	7,28	0,2	2,26	95	18,5	19,4
	16/08/2017	6,77	6,66	7,41		2,21	103	18,7	19,7
	17/08/2017	6,71		7,21		2,16	108	18,1	19
	18/08/2017	6,85	6,54	7,41		2,18	90	18,9	19,7
	21/08/2017	6,72	6,77	7,36		2,19	103	19,2	20,1
	22/08/2017	6,7		7,56		2,09	107	19,7	20,4
	23/08/2017	6,73	6,72	7,47		2,03	108	18,6	19,5
	24/08/2017	6,74		7,62		1,99	108	18,1	19,1
	25/08/2017	6,76		7,61		1,92	109	18,4	19,2
	28/08/2017	6,75		7,53		1,98	101	18,9	19,7
	29/08/2017	6,73		7,4		1,97	94	18,3	19,3
	30/08/2017	6,61		7,47		2	92	18,2	18,9
	31/08/2017	6,6		7,5		1,88	86	17,8	18,6
	01/09/2017	6,51		7,52		1,89	80	17,4	18,2

APPENDIX 3 – PARAMETERS OF THE PROCESS AFTER CALCULATION

	Date	Salt content					
		Feeding mixture	Effluent from reactor (g salt/L digester)	NH4-N	TAC (mg CaCO3/L)	FOS (mg acetic acid/L)	FOS/TAC
Part I	06/06/2017		0,55				
	07/06/2017						
	08/06/2017		0,60		5912,50	755,00	0,13
	09/06/2017		0,60		5935,00	1062,10	0,18
	12/06/2017	0,63	0,54	1246,26	5705,00	730,10	0,13
	13/06/2017	0,98	0,60	1263,33	5745,00	605,60	0,11
	14/06/2017	1,07	0,64	1135,29	5725,00	755,00	0,13
	16/06/2017	1,26	0,70	1280,40	5712,50	796,50	0,14
	19/06/2017	1,85	0,77	1084,07	5350,00	1170,00	0,22
	20/06/2017	1,85	0,82	1271,86	5775,00	755,00	0,13
	21/06/2017	1,85	0,85	1186,50	5675,00	755,00	0,13
	22/06/2017	1,92	0,90	1399,90	5450,00	755,00	0,14
	23/06/2017		0,99	1399,90	5600,00	672,00	0,12
	26/06/2017		1,06	913,35	4850,00	1087,00	0,22
	27/06/2017		1,12	990,18	5450,00	921,00	0,17
	28/06/2017		1,18	956,03	5200,00	1170,00	0,23
	29/06/2017	2,65	1,26	1084,07	5200,00	1253,00	0,24
	03/07/2017		1,32	879,21	5625,00	755,00	0,13
	04/07/2017		1,40	1084,07	5250,00	1253,00	0,24
	05/07/2017		1,45	998,71	5100,00	1336,00	0,26
	06/07/2017		1,49	1177,97	4600,00	2083,00	0,45
	07/07/2017	3,38	1,66	1212,11	4325,00	2498,00	0,58
stabilization	28/07/2017	0,12	1,20	1177,97	4650,00	672,00	0,14
	04/08/2017	0,11	1,10	981,64	4775,00	506,00	0,11
Part II	07/08/2017	0,11	1,03	921,89	4700,00	589,00	0,13
	08/08/2017		1,01	1041,39	4625,00	672,00	0,15
	09/08/2017		1,06	973,10	4850,00	506,00	0,10
	10/08/2017	0,11	0,91	734,10	4775,00	589,00	0,12
	11/08/2017		0,88	751,17	4625,00	672,00	0,15
	14/08/2017	0,11	0,99	802,38	4775,00	838,00	0,18
	15/08/2017	0,11	0,94	810,92	4625,00	672,00	0,15
	16/08/2017		0,91	879,21	4675,00	755,00	0,16
	17/08/2017		0,89	921,89	4525,00	672,00	0,15
	18/08/2017		0,90	768,24	4725,00	589,00	0,12
	21/08/2017		0,91	879,21	4800,00	672,00	0,14
	22/08/2017		0,87	913,35	4925,00	506,00	0,10
	23/08/2017		0,84	921,89	4650,00	672,00	0,14
	24/08/2017		0,82	921,89	4525,00	755,00	0,17
	25/08/2017		0,79	930,42	4600,00	589,00	0,13
	28/08/2017		0,82	862,14	4725,00	589,00	0,12
	29/08/2017		0,82	802,38	4575,00	755,00	0,17
	30/08/2017		0,83	785,31	4550,00	506,00	0,11
	31/08/2017		0,78	734,10	4450,00	589,00	0,13
	01/09/2017		0,78	880,00	4350,00	589,00	0,14

APPENDIX 4 – DAILY GAS PRODUCTION

Date	BIOGAS PRODUCTION (L)	GPR (L gas/L digester.d)	CO2 (%)	CH4 (%)	CO2 (L)	CH4 (L)	CH4 (L/L digester.d)	GPR (NL gas/L digester.d)	Methane production rate (NL CH4/L digester.d)	SBP (NL gas/g VS)	Specific methane yield (NL CH4/g VS)
17/05	178,2	0,85	42,9	41,6	76,5	74,2	0,35	0,81	0,34	0,35	0,15
06/06	206,3	0,98	22,2	77,1	45,9	159,1	0,76	0,93	0,72	0,41	0,32
07/06	258,2	1,23	31,1	66,7	80,2	172,2	0,82	1,17	0,78	0,51	0,34
08/06	313,1	1,49	37,7	58,9	118,0	184,5	0,88	1,42	0,84	0,62	0,37
09/06	338,6	1,61	39,4	57,3	133,6	194,2	0,92	1,53	0,88	0,67	0,39
10/06	158,9	0,76	37,1	60,0	59,0	95,3	0,45	0,72	0,43	0,32	0,19
11/06	58,8	0,28	36,3	60,9	21,4	35,8	0,17	0,27	0,16	0,12	0,07
12/06	177,3	0,84	37,8	59,0	67,0	104,6	0,50	0,80	0,47	0,35	0,21
13/06	294,1	1,40	39,6	57,5	116,3	169,0	0,80	1,33	0,77	0,59	0,34
14/06	302,8	1,44	39,5	57,5	119,8	174,1	0,83	1,37	0,79	0,60	0,35
15/06	134,5	0,64	37,8	59,6	50,8	80,1	0,38	0,61	0,36	0,27	0,16
16/06	200,4	0,95	38,5	58,9	77,1	118,1	0,56	0,91	0,53	0,40	0,23
17/06	112,4	0,54	38,1	59,5	42,8	66,9	0,32	0,51	0,30	0,22	0,13
18/06	62,1	0,30	38,1	59,0	23,7	36,7	0,17	0,28	0,17	0,12	0,07
19/06	211,0	1,00	39,3	57,5	82,9	121,4	0,58	0,96	0,55	0,42	0,24
20/06	330,5	1,57	40,5	56,4	133,9	186,4	0,89	1,50	0,84	0,66	0,37
21/06	345,8	1,65	39,9	57,2	138,0	197,9	0,94	1,57	0,90	0,69	0,39
22/06	334,1	1,59	39,3	58,0	131,4	193,6	0,92	1,51	0,88	0,66	0,39
23/06	330,4	1,57	39,6	57,6	130,9	190,3	0,91	1,50	0,86	0,66	0,38
24/06	127,2	0,61	37,3	60,0	47,5	76,3	0,36	0,58	0,35	0,25	0,15
25/06	63,0	0,30	37,6	59,7	23,7	37,6	0,18	0,29	0,17	0,13	0,07
26/06	188,5	0,90	38,9	58,1	73,4	109,6	0,52	0,85	0,50	0,38	0,22
27/06	295,1	1,41	41,0	55,6	120,9	164,0	0,78	1,34	0,74	0,59	0,33
28/06	278,4	1,33	40,5	56,1	112,8	156,1	0,74	1,26	0,71	0,55	0,31
29/06	268,7	1,28	40,3	56,3	108,3	151,3	0,72	1,22	0,69	0,53	0,30
30/06	166,6	0,79	39,9	56,7	66,5	94,5	0,45	0,75	0,43	0,33	0,19
01/07	58,9	0,28	37,0	59,2	21,8	34,9	0,17	0,27	0,16	0,12	0,07
02/07	38,9	0,19	37,0	60,0	14,4	23,3	0,11	0,18	0,11	0,08	0,05
03/07	112,2	0,53	38,1	58,4	42,7	65,5	0,31	0,51	0,30	0,22	0,13
04/07	216,8	1,03	44,0	52,4	95,4	113,5	0,54	0,98	0,51	0,43	0,23
05/07	210,2	1,00	47,7	49,0	100,3	102,9	0,49	0,95	0,47	0,42	0,20
06/07	218,3	1,04	49,0	47,7	107,0	104,0	0,50	0,99	0,47	0,43	0,21
07/07	228,8	1,09	49,8	46,9	114,0	107,3	0,51	1,04	0,49	0,46	0,21
08/07	191,0	0,91	44,9	51,9	85,8	99,1	0,47	0,86	0,45	0,38	0,20
09/07	181,1	0,86	33,2	64,5	60,1	116,9	0,56	0,82	0,53	0,36	0,23
10/07	159,9	0,76	27,2	70,9	43,4	113,3	0,54	0,72	0,51	0,32	0,23
11/07	102,4	0,49	25,8	71,9	26,4	73,6	0,35	0,46	0,33	0,20	0,15
12/07	69,1	0,33	27,3	70,3	18,9	48,5	0,23	0,31	0,22	0,14	0,10
13/07	76,7	0,37	31,0	66,3	23,8	50,9	0,24	0,35	0,23	0,15	0,10
14/07	88,7	0,42	34,8	61,2	30,9	54,2	0,26	0,40	0,25	0,18	0,11
15/07	82,1	0,39	38,0	58,0	31,2	47,6	0,23	0,37	0,22	0,16	0,09
16/07	59,6	0,28	39,3	57,0	23,4	34,0	0,16	0,27	0,15	0,12	0,07
17/07	93,6	0,45	40,3	56,0	37,8	52,4	0,25	0,42	0,24	0,19	0,10
18/07	204,2	0,97	43,7	52,4	89,2	107,1	0,51	0,92	0,48	0,41	0,21
19/07	270,7	1,29	44,6	51,6	120,8	139,7	0,67	1,23	0,63	0,54	0,28
20/07	304,6	1,45	42,0	54,1	128,0	164,8	0,78	1,38	0,75	0,61	0,33

Date	BIOGAS PRODUCTION (L)	GPR (L gas/L digester.d)	CO2 (%)	CH4 (%)	CO2 (L)	CH4 (L)	CH4 (L/L digester.d)	GPR (NL gas/L digester.d)	Methane production rate (NL CH4/L digester.d)	SBP (NL gas/g VS)	Specific methane yield (NL CH4/g VS)
21/07	327,5	1,56	40,4	55,8	132,4	182,9	0,87	1,48	0,83	0,65	0,36
22/07	229,1	1,09	36,1	60,6	82,7	138,8	0,66	1,04	0,63	0,46	0,28
23/07	98,0	0,47	30,5	66,8	29,9	65,5	0,31	0,44	0,30	0,19	0,13
24/07	131,0	0,62	31,4	65,1	41,2	85,3	0,41	0,59	0,39	0,26	0,17
25/07	222,4	1,06	35,4	61,1	78,7	136,0	0,65	1,01	0,62	0,44	0,27
26/07	264,2	1,26	38,5	57,9	101,7	153,0	0,73	1,20	0,69	0,53	0,30
27/07	315,6	1,50	41,1	55,3	129,9	174,6	0,83	1,43	0,79	0,63	0,35
28/07	317,9	1,51	39,4	57,1	125,2	181,5	0,86	1,44	0,82	0,63	0,36
29/07	199,7	0,95	36,6	60,4	73,1	120,6	0,57	0,90	0,55	0,40	0,24
30/07	65,3	0,31	35,0	61,9	22,8	40,4	0,19	0,30	0,18	0,13	0,08
31/07	149,7	0,71	36,2	60,1	54,3	90,0	0,43	0,68	0,41	0,30	0,18
01/08	93,0	0,44	36,5	60,0	33,9	55,8	0,27	0,42	0,25	0,19	0,11
02/08	197,1	0,94	38,6	57,5	76,0	113,4	0,54	0,89	0,51	0,39	0,23
03/08	279,5	1,33	39,5	56,9	110,5	159,1	0,76	1,27	0,72	0,56	0,32
04/08	338,4	1,61	39,5	56,6	133,6	191,4	0,91	1,53	0,87	0,67	0,38
05/08	146,2	0,70	36,6	59,5	53,5	87,0	0,41	0,66	0,39	0,29	0,17
06/08	48,2	0,23	35,6	60,8	17,2	29,3	0,14	0,22	0,13	0,10	0,06
07/08	155,4	0,74	36,2	59,8	56,2	92,8	0,44	0,70	0,42	0,31	0,18
08/08	279,7	1,33	38,7	57,2	108,2	159,9	0,76	1,27	0,72	0,56	0,32
09/08	301,6	1,44	39,8	56,1	120,1	169,3	0,81	1,37	0,77	0,60	0,34
10/08	303,9	1,45	39,6	56,3	120,4	171,0	0,81	1,38	0,77	0,60	0,34
11/08	299,4	1,43	39,3	56,7	117,6	169,9	0,81	1,36	0,77	0,60	0,34
12/08	148,4	0,71	37,7	58,3	56,0	86,5	0,41	0,67	0,39	0,30	0,17
13/08	62,9	0,30	37,0	59,0	23,3	37,1	0,18	0,28	0,17	0,13	0,07
14/08	191,7	0,91	37,5	56,5	71,9	108,4	0,52	0,87	0,49	0,38	0,22
15/08	331,8	1,58	38,4	57,1	127,6	189,6	0,90	1,50	0,86	0,66	0,38
16/08	332,9	1,59	37,4	58,4	124,4	194,5	0,93	1,51	0,88	0,66	0,39
17/08	331,0	1,58	36,7	59,2	121,4	196,1	0,93	1,50	0,89	0,66	0,39
18/08	338,1	1,61	37,0	58,9	125,2	199,0	0,95	1,53	0,90	0,67	0,40
19/08	151,4	0,72	34,7	61,5	52,5	93,2	0,44	0,69	0,42	0,30	0,19
20/08	56,4	0,27	34,0	62,8	19,1	35,4	0,17	0,26	0,16	0,11	0,07
21/08	170,4	0,81	34,8	61,5	59,4	104,7	0,50	0,77	0,47	0,34	0,21
22/08	322,4	1,54	36,4	59,9	117,4	193,3	0,92	1,46	0,88	0,64	0,38
23/08	361,5	1,72	36,7	59,4	132,6	214,8	1,02	1,64	0,97	0,72	0,43
24/08	397,7	1,89	35,9	60,3	142,6	239,6	1,14	1,80	1,09	0,79	0,48
25/08	388,1	1,85	34,6	61,0	134,2	236,8	1,13	1,76	1,07	0,77	0,47
26/08	202,5	0,96	32,7	63,9	66,3	129,4	0,62	0,92	0,59	0,40	0,26
27/08	65,8	0,31	31,2	65,0	20,6	42,8	0,20	0,30	0,19	0,13	0,09
28/08	162,9	0,78	32,0	63,7	52,0	103,8	0,49	0,74	0,47	0,32	0,21
29/08	383,2	1,82	35,3	59,9	135,3	229,4	1,09	1,74	1,04	0,76	0,46
30/08	405,0	1,93	34,3	61,0	138,7	247,0	1,18	1,83	1,12	0,81	0,49
31/08	396,1	1,89	32,5	62,7	128,8	248,4	1,18	1,79	1,13	0,79	0,49
01/09	374,1	1,78	32,0	63,5	119,8	237,6	1,13	1,69	1,08	0,74	0,47