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I dedicate this work to my parents Maria and Goce for their sincere love.

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Investigation of co-digestion of food waste and primary sludge at SNJ-wastewater treatment plant

Simjanoski Zlatko

Abstract

Anaerobic co-digestion of different organic waste streams, has proven to be a viable solution for sustainable management of organic fraction of waste, with increased everyday application. Besides the environmentally sound management of organic waste, it enables increased economical performances of employed anaerobic digesters worldwide by increasing the energy recovery from the process itself. A full scale experiments were conducted at SNJ wastewater treatment plant to evaluate the effect of co-digestion of food waste and sewage sludge. The pulse feed resulted, in temporary overload of the system, with sharp increase in the concentrations of acetic acid and consequently 27% increased methane production rate from. During the overload period system remain its stability. Monitoring parameters used during the test were pH, VFA , Alkalinity, COD, TS and TVS.

Laboratory scale, batch test experiments were undertaken, to determine the specific methane yield of different substrates and mixtures of substrates currently used in the co-digestion process at SNJ wastewater treatment plant, as well as to determine maximum food to biomass ratio (gVS substrate/gVS biomass) that can be used for enhanced methane production without causing process perturbations. The blends of sewage sludge and food waste in different ratio showed enhanced cumulative methane production for 36 and 57 % respectively. Organic loading experiment showed that the optimal organic load (food to biomass) is in the range from 1.73~2.1gVS substrate per gVS biomass.

Table of contents

Abstract

List of figures ____ I

List of tables ____ II

List of graphs ____ IV

Introduction ____ 1

Abstract ____ 3

1. Theory ____ 4

1.1 Anaerobic digestion-Historical background ____ 4

1.2 Microbiology of anaerobic digestion ____ 5

1.3 Anaerobic digestion process ____ 9

1.3.1 Hydrolysis ____ 10

1.3.2 Acidogenesis ____ 10

1.3.3 Acetogenesis ____ 11

1.3.4 Methanogenesis ____ 12

1.3.5 Physico-chemical reactions ____ 12

1.4 Process technology ____ 13

1.5 Process parameters ____ 18

1.6 Operational parameters ____ 25

1.7 Monitoring parameters ____ 27

2. Anaerobic co-digestion (AcoD) ____ 29

2.1 Wastewater treatment plant – SNJ ____ 30

3. Materials and methods ____ 34

3.1 Experimental aspects ____ 34

3.2 Experimental procedures and analytical methods ____ 36

4. Results 40
4.1Full scale test experiment 40
4.2 Biomethane potential tests (BMP) 54
4.3 Organic loading test (OL) 57
5. Discussion 62
4. Conclusions 67
4. References 68
APPENDIX I
APPENDIX II

List of figures

Figure 1. The sustainable cycle of anaerobic digestion

Figure 2. Microbial groups and metabolic pathways in AD process

Figure 3. Biochemical pathways of anaerobic digestion process with different process steps duration

Figure 4. Methane production pathways

Figure 5. One stage suspended growth anaerobic reactor

Figure 6 . Schematics of fixed film system

Figure 7. Types of fixed film anaerobic systems

Figure 8. Single-stage digester system

Figure 9. Two stage anaerobic system

Figure 10. Relative growth of methanogens within different temperature ranges

Figure 11. Digestion process time as a function of process temperature

Figure 12. Sludge treatment unit at SNJ –wastewater treatment plant

Figure 13. Schematics of the anaerobic digestion compartments and process flow

List of tables

Table 1. Oxidation-reduction potential of environment and microbial processes

Table 2. Exoenzymatic activity on hydrolytic bacteria on different substrates

Table 3. Substrate affinity of methanogenic microorganisms

Table 4. Classification of methanogenic microorganisms

Table 5. Optimal growth temperature range of some methanogens

Table 6. Exo-enzymes and substrates

Table 7. Major acids produced in acidogenesis stage

Table 8 . Acids, alcohols and nitrogenous compounds suitable for direct utilization by methanogens

Table 9. Fermentation products used indirectly as substrate by methanogens

Table 10. Advantages and disadvantages of suspended growth anaerobic systems

Table 11. Examples of waste streams treated with fixed film reactors

Table 12. Comparison between the mesophilic and thermophilic reactors

Table 13. Growth yield of anaerobic microorganisms

Table 14. Significant nutrients need for the anaerobic microorganisms

Table 15. Effect of free ammonia on anaerobic process

Table 16. Long chain fatty acids that inhibit methane production from acetate

Table17. Average composition of biogas

Table 18. Advantages of Mixing Digester Content

Table 19. Operational conditions for optimal activity of methane forming bacteria

Table 20. Indicators of process instability

Table 21. Conditions causing process upsets in the anaerobic digesters

Table 22. Types and amount of substrates used in the co digestion process at SNJ

Table 24 . Amount of substrates and inoculums used, gas production and methane yield-(BMP)1

Table 25. Amount of substrates and inoculums used, gas production and methane yield-(BMP)2

Table 26. Amount of substrates and inoculums used, gas production and methane yield- (OL)

List of graphs

(20/01/2012)

Graph 1. pH , VFA and alkalinity dynamics in the Buffer Tank 1

Graph 2. COD filtered and VFA dynamics in Buffer Tank 1

Graph 3. Totals solids concentration in Buffer Tank 1 and Digester 2

Graph 4. pH, VFA and Alkalinity dynamics in Digester 2

Graph 5. VFA, COD and methane production rate in Digester 2

Graph 6. Volatile Fatty acids concentrations in Buffer Tank 1

Graph 7. Volatile Fatty acids concentrations in Digester 2

Graph 8. Ion chromatography and titration VFA concentrations

(27/01/2012)

Graph 9. pH , VFA and alkalinity dynamics in the Buffer Tank 1.

Graph 10. COD filtered and VFA dynamics at Buffer Tank 1

Graph 11. pH, VFA and alkalinity concentrations in the Digester 2.

Graph 12. VFA, COD and Methane production rate in Digester 2

Graph 13. Total solids and Total Volatile solids concentrations

Graph 14. Volatile Fatty acids concentrations in Buffer Tank 1

Graph 15. Volatile fatty acids concentrations in Digester 2

Graph 16. Ion chromatography and titration VFA concentrations

(02/03/12)

Graph 17. pH , VFA and alkalinity dynamics in the Buffer Tank 1

Graph 18. COD filtered and VFA dynamics at Buffer Tank 1 over the period of 24 hours

Graph 19. pH, VFA and alkalinity concentrations in the Digester 2

Graph 20. VFA, COD and methane production rate at Digester 2 over 24h period

Graph 21. Total solids and Total Volatile solids concentrations over 24h period

(17/04/2012)

Graph 22. pH , VFA and alkalinity dynamics in the Buffer Tank 1 over 24 hour period

Graph 23. pH, VFA and alkalinity concentrations in the Digester 2 over 24 hour period

Graph 24. Volatile Fatty acids concentrations in Buffer Tank 1

Graph 25. Volatile fatty acids concentrations in Digester 2

Graph 26. Ion chromatography and titration VFA concentrations over 24 hours period

Graph 27. BMP test No. 1, cumulative methane production

Graph 28. BMP test No. 1, daily methane production rate

Graph 29. BMP test No. 2, Cumulative methane production

Graph 30. BMP test No. 2,Daily methane production rate

Graph 31. Methane production ml/day, OL test

Graph 32. Cumulative Methane production (ml), OL test

Graph 33. Specific methane yield and I/S ratios , OL test

Graph 34. Specific methane yield for different S/F ratios, OL test

Graph 35. Total methane production for different I/S ratios, OL test

Graph 36. Total methane production for different S/F ratios, OL test

Graph 37. pH, Alkalinity, VFA, and ammonium concentrations, OL test

INTRODUCTION

The economical and population growth inevitable results in increased natural resources usage, energy consumption and waste generation. The current world's energy supply is dependent on fossil sources (crude oil, lignite, hard coal, natural gas) which exhibits uneven geographical distribution. Utilization of this type of energy carriers has both socio-economic effect on modern societies making them highly dependable of external fuel supply and imply detrimental effect to the environment in terms of emission of green house gases (GHG) leading to global increase in the earth temperature or global warming. On the other hand increasing organic waste stream as part of the municipal or industrial waste streams exhibits detrimental effect to environment when treated in inappropriate manner or not treated at all. For example landfilling of organic waste as a consequence has GHG gases emissions as result for anaerobic decomposition of the waste as well as potential for the pollution of the underground water resources by the landfill leachate.

The term "waste" is defined as any substance or object which the holder discards or intends or is required to be discarded and the term "organic" refer to the biodegradable part of waste(EU directive 75/442/EEC).

At the end of last century EU imposed environmental legislation which aim in reducing the amount of waste generated and it's deposition in the landfills. The documents addressing this issues are EU Directive 75/442/EEC known as waste framework directive and Directive *99/31/EC*. Framework directive established waste management principles and operations lead to reduction, recycling , reuse and disposal of waste. The landfill Directive sets goals for the reduction of organic waste fraction disposed to landfills. One of the Directive's strategies that may lead to these targets is recycling of source separated organic waste by aerobic (composting) or anaerobic (digestion in biogas plants) treatment.

The composting process of organic waste streams results in recycling the nutrients into the soil and the Anaerobic digestion (AD) process leads to methane production which is an energy carrier besides the compost production(Mata Alvarez ,2003).

Mata Alvarez in his book *Biomethanization of organic fraction of municipal solid waste* (OFMSW) reports that that when composted 100kg of Organic fraction of municipal solid waste (OFMSW) 65 kg of compost are produced and 6kWh energy is consumed in the process. On the other hand when employing AD process, from the same amount of (OFMSW) 35kg of compostable matter is produced along side with 22kWh electricity and 44 kWh of heat energy generated .

The other benefits of employing AD process are listed bellow:

- Potential for mitigation of external energy dependence in society,
- Electricity and heat generation
- Reduction in waste volume that should be land filled,
- Use of digestate as a fertilizer (higher homogeneity and better C/N ratio than manure, no carbon footprint compared to chemically produced fertilizers),
- Closed nutrient and carbon cycle,
- Potential for use of diverse types of waste streams,
- Lower water consumption footprint,
- Mitigation of global warming,
- Potential for creation of new jobs(Al Seadi et al. 2008).

The prospects of the process are huge, when considered the fact that the AD process can be employed with various types of organic waste streams which is a viable solution to sustainable waste management practices and considering the fact that approximately 400000 tons of OFMSW per day are produced in the European union countries(Mata Alvarez at al. 2000).The process of biological degradation of different organic waste streams for production of biogas is point of interest and will be elaborated further in the text. Figure 1 Graphically presents the sustainable life-cycle approach of anaerobic digestion.

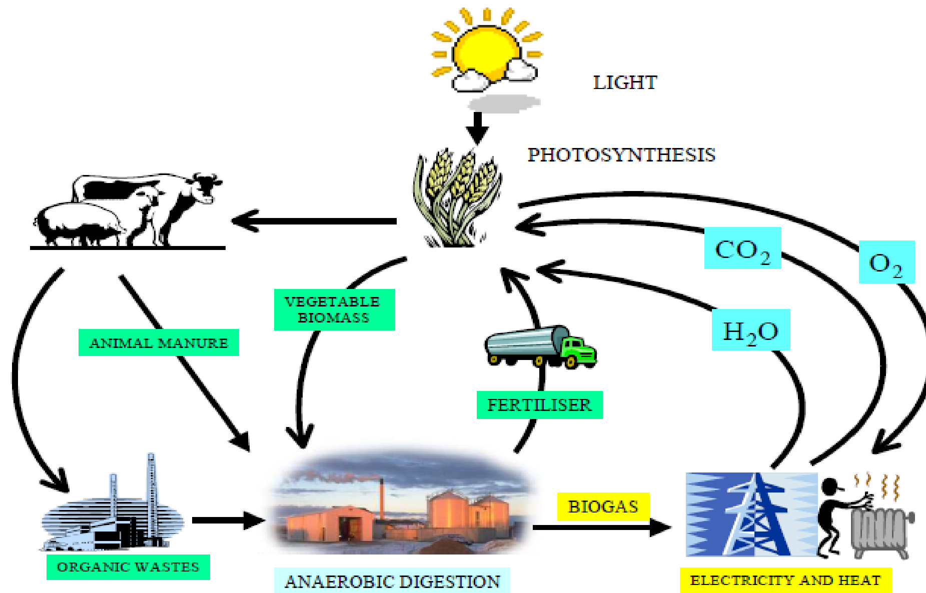


Figure 1. The sustainable cycle of anaerobic digestion (Al Seadi ,2001)

OBJECTIVES

The main purpose of this study is to investigate the effect of anaerobic co-digestion process at SNJ wastewater treatment plant and. Full scale experiment was undertaken to investigate the impact of co-digestion on the gas production rate due to the additional substrate feed. In addition, two laboratory experiments were conducted aiming for determination of methane potential of some of the substrates (primary sludge, food waste) used in process, optimal sludge/food waste ratio for enhanced methane production, as well as to determine the maximum substrate to biomass ratio that can be applied in the process for enhanced production of methane.

1. THEORY

1.1 Anaerobic digestion -historical background

Historical evidence indicates that the AD process is one of the oldest technologies. Biogas was used for heating bath water in Assyria during the 10th century BC and in Persia during the 16th century (Verma,2002). AD advanced with scientific research and, in the 17th century, Jan Baptista Van Helmont established that flammable gases evolved from decaying organic matter. Also, Count Alessandro Volta in 1776 showed that there was a relationship between the amount of decaying organic matter and the amount of flammable gas produced. In 1808, Sir Humphry Davy demonstrated the production of methane production by the anaerobic digestion of cattle manure (Lusk, 1997).

The industrialization of AD began in 1859 with the first digestion plant in Bombay, India. By 1895, AD had made inroads into England where biogas was recovered from a well-designed sewage treatment facility and fueled street lamps in Exeter. Further AD advances were due to the development of microbiology. Research led by Buswell and others in the 1930s identified anaerobic bacteria and the conditions that promote methane production(Lusk, 1997).

In recent times, Europe came under pressure to explore AD market because of two significant reasons: High energy prices and stringent environmental regulations, especially controls on organic matter going to landfills. As a direct consequence at presence more than 600 farm based digesters operate in Europe, where the key factor is their design simplicity. Around 250 of these systems have been installed in Germany alone in the past five years. In addition to farm digesters, Europe leads in large centralized AD systems. Between 1987-95, there were more than 150 new AD plants constructed in Europe (Verma,2002). In Europe, there are 30 large centralized digesters of which 15 are in Denmark alone and 30 more are under construction(Verma,2002).

1.2 Microbiology of anaerobic digestion

Anaerobic digestion is a process of degradation of organic matter by microorganisms in absence of free molecular oxygen, having final product a methane, carbon dioxide, hydrogen sulfide, water and newly synthesized microbial biomass. Process is done by a well balanced microbial ecosystem of several groups of microorganisms that works interactively in the process of complex organic matter breakdown (Chernicharo,2007).The anaerobic microbial system is a diverse one in terms of species, but the work is basically performed by two groups of microorganisms : facultative anaerobes (fermentative) and strict anaerobes with respect to free oxygen presence tolerance. Thus the first group can tolerate oxygen presence but the cell activity is reduced and the second group has zero tolerance to oxygen. Both groups perform best when oxidation reduction potential of the environment range from -100 to -400 mV (Gerardy,2003).Table 1 presents the relationship between the oxidation-reduction potential of the environment and the types of ongoing processes.

Table 1. Oxidation-reduction potential of environment and microbial processes(Gerardy,2003)

Approximate ORP, mV	Carrier Molecule for Degradation of Organic Compounds	Condition	Respiration
>+50	O_2	Oxic	Aerobic
+50 to –50	NO_3^- or NO_2^-	Anaerobic	Anoxic
<–50	SO_4^{2-}	Anaerobic	Fermentation, sulfate reduction
<–100	Organic Compound	Anaerobic	Fermentation, mixed acid production
<–300	CO_2	Anaerobic	Fermentation, methane production

The two groups are differentiated as well of their ability to produce exoenzymes which enables destruction of complex organic matter into simpler soluble products. The hydrolytic fermentative microorganisms produce such enzymes and the strict anaerobes are not capable of doing that. Both groups are capable of producing endoenzymes. The endoenzymes are responsible for soluble substrate utilization inside the microbial cell. Each of the exo and endoenzymes are highly specific regarding the substrate they are degrading so one can imagine that the abundance of different microbial species is necessity for complex organic matter degradation(see Table2.).

Table 2. Exo-enzymatic activity on hydrolytic bacteria on different substrates (Gerardy,2003)

Substrate to be Degraded	Exoenzyme Needed	Example	Bacterium	Product
Polysaccharides	Saccharolytic	Cellulase	*Cellulomonas*	Simple sugar
Proteins	Proteolytic	Protease	*Bacillus*	Amino acids
Lipids	Lipolytic	Lipase	*Mycobacterium*	Fatty acids

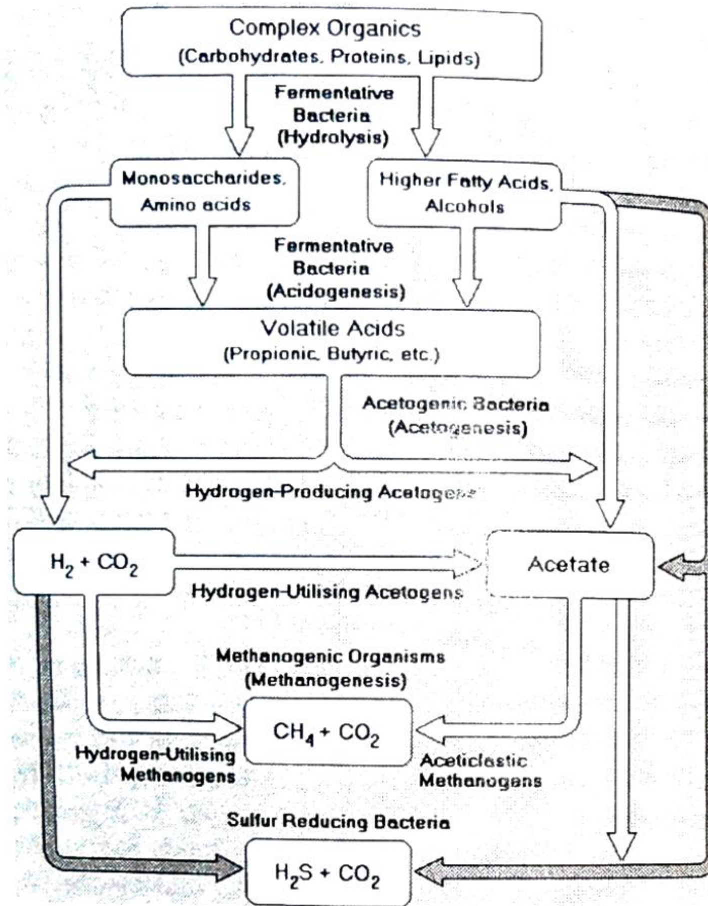


Figure 2. Microbial groups and metabolic pathways in AD process (including sulphure reducing bacteria) (adapted from Lettinga et al. 1996)

The microorganisms are not able to utilize particulate organic matter and this is where the first microbial group appears in the metabolic pathway(see Figure 2.). These group is represented by ***hydrolytic*** group of fermentative bacteria excreting enzymes that attack the organic polymers converting them into soluble monomers which penetrate the cell wall of fermentative bacteria. Fermentative group degrade the soluble products into volatile fatty acids, alcohols, lactic acid , CO_2, ammonia and H_2S as well as new biomass. This consortium of bacterial species also is named as Acidogens are represented by the specie belonging to the *Bacteroidaceae* family and *Clostridia* group. Acidogenic species are anaerobes and form spores enabling them to survive in harsh environments. Next group of bacteria named Acetogens, are intermediate group of bacteria, that oxidizes the product of acidogens and covert them into acetate , hydrogen and CO_2. Acetogenic group of microorganisms lives in syntrophic relationship with the methanogenic group of microorganisms which are strict anaerobes . When acetogens produce acetate also hydrogen is produced which is normally consumed by the sub group of methanogenic groups of bacteria capable producing methane by utilizing hydrogen and carbon dioxide. If by any reason

this relationship is disturbed the hydrogen concentration will start to build up, causing increase in the hydrogen partial pressure which as a consequence has inhibition of the growth of acetogens.

To summarize the above mentioned : *the acetogens are obligate hydrogen producers but can survive only if their methabolic waste-product hydrogen is being constantly removed by the hydrogenotrophic methanogens*.. The methanogens are represented by two sub groups depending of the substrate utilized in the methane production process. One group consumes acetate for methane production and the second group consumes hydrogen in methane build up. Therefore they are named as ***aceticlastic*** *(Methanosaeta,Methanosarcina)* and ***hydrogenotrophic*** (*Methanobacterium, Methanospitillum, Methanobrevibacter*).Table 3 presents the substrate affinity for different methanogenic species.

Table 3. Substrate affinity of methanogenic microorganisms(Gerardy,2003)

Species	Substrate
Methanobacterium formicium	Carbon dioxide, formate, hydrogen
Methanobacterium thermoantotrophicum	Hydrogen, carbon dioxide, carbon monoxide
Methanococcus frisius	Hydrogen, methanol, methylamine
Methanococcus mazei	Acetate, methanol, methylamine
Methanosarcina bakerii	Acetate, carbon dioxide, hydrogen, methanol, methylamine

Methanogens are one of the oldest species of microorganisms belonging to the Domain *Archeobacteria* . The bacterial species can exhibit different shapes and can be find as rods, spirals, cocci like and can be groped as irregular cluster of cells, chains of cells and filaments or sarcina. They are the only species on the planet known for producing methane. This group is classified according the structure, substrate utilized , types of enzymes produced and temperature range of growth into three orders and 4 families (Gerardy,2003). Classification of methanogenic population is presented at Table 4.

Table 4. Classification of methanogenic microorganisms (Gerardy,2003)

Order	Family
Methanobacteriales	Methanobacteriaceases
Methanococcales	Methanococcaceae
Methanomicrobials	Methanomicrobiaceas
	Methanosarcinaceae

Methanogenic population give special feature to AD process due to the capability of some species to degrade biorecalcitrant compounds followed by methane production. Methanogens thrives in heat (some species are found in hydrothermal vents) thus can operate at high temperatures and can sustain increased salinity but are sensitive to pH change in the environment. Table 5. Presents the optimal temperature range for different groups of methogenic microorganisms.

Some species are reported to be sensitive to specific volatile fatty acids from toxicity point of view. They have slowest doubling time of all microbial species involved in the process usually around 3 days (Gerardy,2003).

Table 5. Optimal growth temperature range of some methanogens (Gerardy,2003)

Genus	Temperature Range, °C
Methanobacterium	37–45
Methanobrevibacter	37–40
Methanosphaera	35–40
Methanothermus	83–88
Methanococcus	35–40
	65–91
Methanocorpusculum	30–40
Methanoculleus	35–40
Methanogenium	20–40
Methanoplanus	30–40
Methanospirillum	35–40
Methanococcoides	30–35
Methanohalobium	50–55
Methanohalophilus	35–45
Methanolobus	35–40
Methanosarcina	30–40
	50–55
Methanothrix	35–50

The Microbial consortia of anaerobic digestion process consist of one more group of organisms known as sulphur reducing bacteria. When higher amounts of sulphate ion (SO_4^{2-}) is present in the waste stream this group of microorganisms act on its reduction to hydrogen sulphide (H_2S) which is known to be very toxic for methanogenic population in its unionized form. To avoid the problem in the AD process with H_2S production, COD/SO_4^{2-} in the incoming waste stream should be higher than 10 (Chernicaro,2007). More information about this group of microorganisms can be find in the *Microbiology of anaerobic digesters* by Michael H. Gerardy.

Microbial populations within the anaerobic digestion consortium are changing continuously, depending on different factors such as changes in substrate, presence of toxic substances, environmental parameters such as pH and temperature.

1.3 ANAEROBIC DIGESTION PROCESS

The process represents a complex system of transformations of organic matter in which a (mixture of gasses) biogas is being produced. The composition of biogas produced can fluctuate depending of the substrate (organic matter),digestion system, temperature and other operating parameters discussed in detail further in the text. It is important to stress that the energy carrier in biogas is the methane gas.

The processes of transformation of organic matter can be dived in two main groups depending of microbial community involvement in these reactions as:

- Biochemical or processes mediated by the microbial community or
- Physico-chemical without involvement of microbial community.

Figure 3. presents the duration of each stage in AD process as well as the products of biochemical transformations that take place within each different process step.

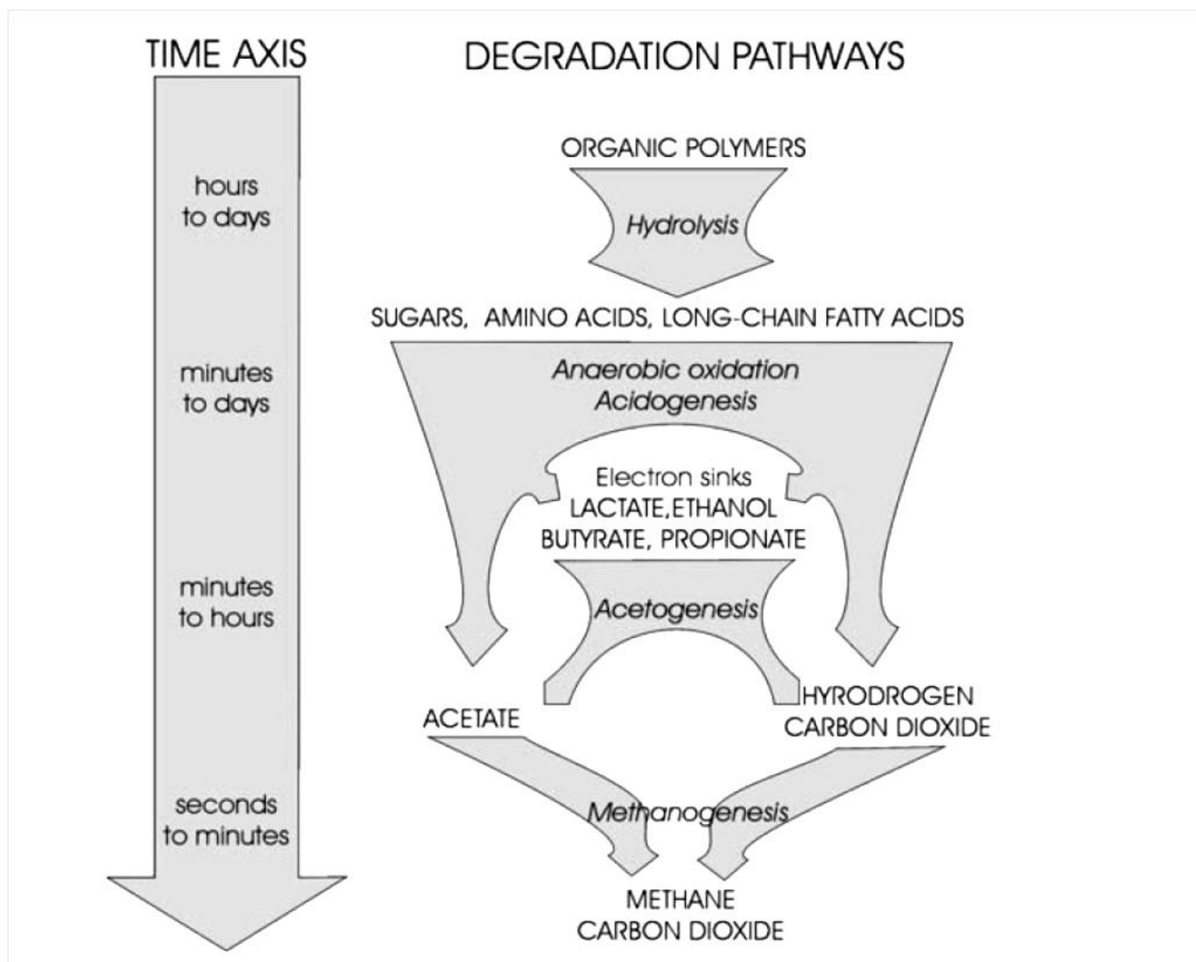


Figure 3. Biochemical pathways of anaerobic digestion process with different process steps duration (Biomethanation 2, 2003)

1.3.1 HYDROLYSIS

As mention above, the organic substrate rarely comes in form of soluble substrates for microbial utilization or if they come they represent only a small fraction of the whole substrate biomass. The complex organic molecules represents nothing more but large numbers of simple organic molecules tied together with the chemical bonds. So the hydrolysis process is all about cutting down these chemical bonds and making the substrate soluble. This process of solubilization is mediated by the microbial activity with excretion of highly specific extracellular enzymes that break down these chemical bonds.. The dominant mechanism of hydrolysis is conducted by the attachment of the microorganisms to the particle producing enzymes in the vicinity of the particle itself, making the solubilization process(ADM1,2001). Table 6, summarizes the hydrolysis mediators ,substrates and consequently hydrolysis products.

Table 6. Exo-enzymes and substrate (Gerardy,2003)

Substrate to be Degraded	Exoenzyme Needed	Example	Bacterium	Product
Polysaccharides	Saccharolytic	Cellulase	*Cellulomonas*	Simple sugar
Proteins	Proteolytic	Protease	*Bacillus*	Amino acids
Lipids	Lipolytic	Lipase	*Mycobacterium*	Fatty acids

To summarize the above mentioned, the hydrolysis process is microbially mediated process of conversion of main organic matter constituents like proteins, fats and polysaccharides into simpler monomers of amino acids, volatile fatty acids (VFA) and monosaccharides.

1.3.2 ACIDOGENESIS

Acidogenesis is better known as fermentation process whereby electrons released from substrate are ultimately transferred to molecules obtained from the breakdown of those same substrate. Fermentation is anaerobic respiration that means no external electron acceptor is present. In this stage the products from the hydrolysis stage, monosaccharides, amino acids and volatile fatty acids are transformed into acetate, hydrogen and CO_2 with pool of around 70% and the rest are organic acids and alcohols with pool of 30 % (Chernicaro,2007). Some of the substrate is being used for microbial biomass production. The dominant product in this stage are acids therefore it is called acidogenesis and of main importance is the acetate that can be directly used by the methanogenic microorganisms in methane production. The most important intermediate VFA produced in this stage are propionic, butyric , lactic and formic acid and small amount of valeric acid(see Table 7.). When degrading complex organic matter acetic acid and propionic acid add with around of 85% of organic matter converted into methane gas and the rest 15 % results from degradation of formic and butyric acid(Chernicharo,2007).

Table 7. Major acids produced in acidogenesis stage(Gerardy,2003)

Name	Formula
Acetate	CH_3COOH
Butanol	$CH_3(CH_2)_2CH_2OH$
Butyrate	$CH_3(CH_2)_2CH_2COOH$
Caproic acid	$CH_3(CH_2)_4COOH$
Formate	$HCOOH$
Ethanol	CH_3CH_2OH
Lactate	$CH_3CHOHCOOH$
Methanol	CH_3OH
Propanol	$CH_3CH_2CH_2OH$
Propionate	CH_3CH_2COOH
Succinate	$HOOCCH_2CH_2COOH$

Table 8 . Acids, alcohols and nitrogenous compounds suitable for direct utilization by methanogens (Gerardy,2003)

Substrate	Chemical Formula
Acetate	CH_3COOH
Formate	$HCOOH$
Methanol	CH_3OH
Methylamine	CH_3NH_2

1.3.3 ACETOGENESIS

Some of the fermentation products can be directly utilized by the methanogenic microorganisms as it is acetate, alcohols and methylamine (Table 8) but products like propionic and butyric acids must be converted first into acetate which is then consumed by the methanogens. Part of the fermentative substrate used by acetogens is converted into new acetogenic biomass. This process of anaerobic oxidation of volatile fatty acids with carbon chains longer than two units and ethanol (alcohols with carbon chain longer than one unit) into acetate, hydrogen and CO_2 is known as acetogenesis.Table 9. summarizes the main intermediates derived in fermentation process for acetate production in this stage.

Table 9. Fermentation products used indirectly as substrate by methanogens(Gerardy,2003)

Substrate	Chemical Formula
Ethanol	CH_3CH_2OH
Butyrate	$CH_3CH_2CH_2COOH$
Propionate	CH_3CH_2COOH

1.3.4 METHANOGENESIS

This is the stage where the biochemical transformation of organic substrate ends. During this stage the methanogens both aceticlastic (acetate consuming) and hydrogenotrophic (hydrogen consuming) convert the substrate into methane gas and newly synthesized biomass . The former group contribute with around 65 % of methane produced and the later one with the rest 26 % and the rest amount from methanol(Figure 4.), (Gerardy,2003). Two genera of methanogens comprise the aceticlastic group: *Methanosaeta* and *Methanosarcina*. Methanosarcina prevails at acetate concentration above 10^{-3} M and Methanosarcina when concentration is bellow this value Methanosarcina has higher growth yield and is less pH sensitive than Methanosaeta(ADM1,2001). Hydrogenotrophs are very diverse group with very important function of regulating the hydrogen partial pressure in the environment enabling the optimal activity of acidogens and acetogens(Chernicharo,2007).

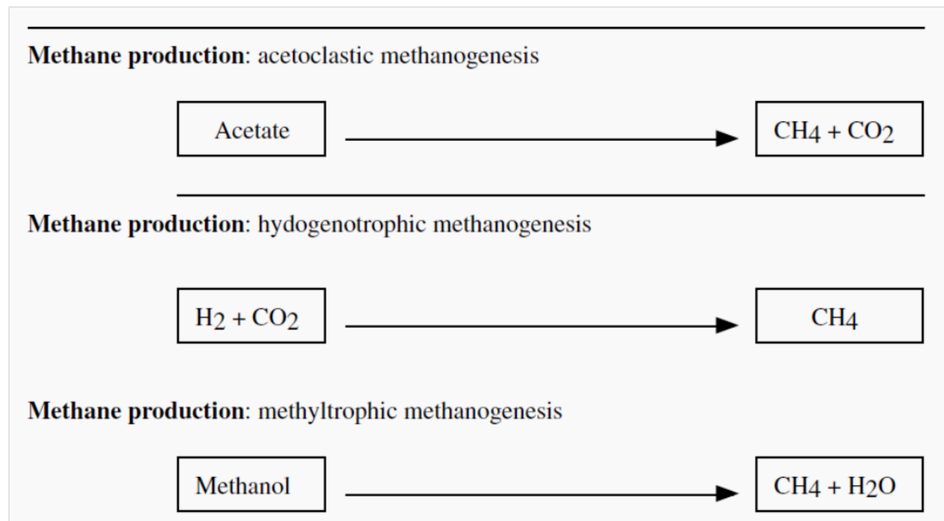


Figure 4. Methane production pathways (Gerardy,2003)

1.3.5 PHYSICO-CHEMICAL REACTIONS

Physico-chemical processes AD process are represented with ion (dissociation/association)-equilibrium reactions and liquid- gas transfer. Equilibrium reactions are strongly influenced by the temperature through the change in equilibrium coefficients. Precipitation and solubilization of ions are i.e liquid-solid transformations and these reactions are of great importance especially for those systems with high levels of cations that readily form carbonate precipitates Mg^{2+} and Ca^{2+} that influences the buffering capacity. The three main components of liquid-gas transfer are CO_2,CH_4, and H_2(Batstone et al.2005, Mata Alvarez et al.2011). The gas transfer is strongly influenced by the solubility of the gasses, mixing regime and temperature.

1.4 PROCESS TECHNOLOGY

The anaerobic digestion technology is well established at presence, and numerous organic waste streams can be utilized in the biogas production. The process takes place in anaerobic reactors that can have different configuration and operational mode depending of the type of substrate used, way of growth of bacteria, temperature and operation.

Based on the "strength" of substrate used, two main types of systems can be differentiated as "dry" or "wet" systems depending of the organic matter in the waste stream represented as solids concentration or Total solids (%). Thus systems treating waste streams with solids concentration TS lower than 15% solids are considered to be wet systems. Systems treating waste streams for values higher than 15 % in the range of 20-40% the systems are considered to be dry ones (Mata Alvarez, 2003).

Depending on the way the biomass is retained in the reactor two types of anaerobic digesters can be differentiated:

- **Suspended growth**, where microbial biomass is homogenously distributed in the reactor,
- **Fixed film reactors**, where microbial biomass is attached to a specific carrier like rocks, or other types of carriers.

Suspended growth reactors are most commonly used for treatment of insoluble high strength organic waste i.e waste streams where the substrate should undergo hydrolysis(Figure 5). They might have intermittent or continuous mixing regime. This is the most common type of reactors used at presence for wastewater sludge digestion as well as organic waste streams originating from other sources.

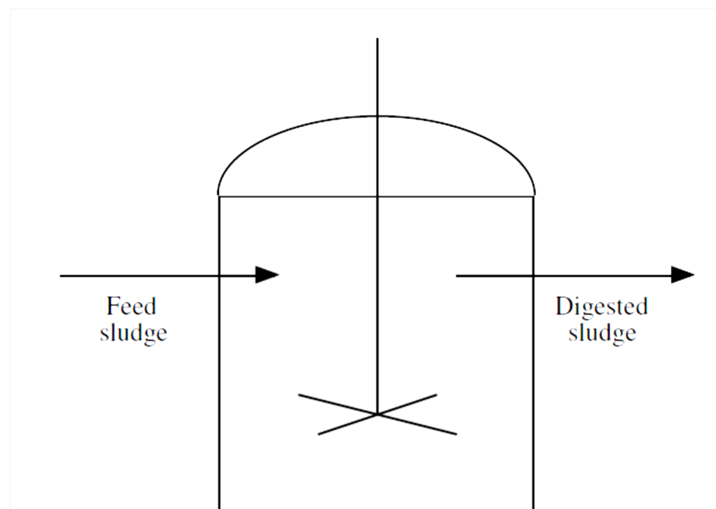


Figure 5. One stage suspended growth anaerobic reactor(Gerardy,2003)

Suspended growth reactors has averaged Solids retention time (SRT) ,time that particulate matter spends in the reactor or Hydraulic retention time (HRT), time that liquid spends in the reactor from 10 to 30 days .When the biomass is not recycled in the system SRT=HRT. HRT in the fixed-film reactors is usually shorter than HRT in suspended growth systems.. Pros and cons of suspended growth reactors are presented at Table 10.

Table 10. Advantages and disadvantages of suspended growth anaerobic systems(Gerardy,2003)

Advantages	Suitable for the treatment of particulate, colloidal, and soluble wastes
	Toxic wastes may be diluted
	Uniform distribution of nutrients, pH, substrate, and temperature
Disadvantages	Large digester volume required to provide necessary SRT
	Treatment efficiency may be reduced due to loss of particulate and colloidal wastes and bacteria in digester effluent

Fixed film reactors are based on the microbial biomass retention on media for sufficient long time for growth thus enabling long SRT's and short HRT's. The most common type of media used for anaerobic fixed film reactors includes rocks, gravel or plastic based carrier(see Figure 6.) The principle of work is based on the flow-through of waste stream and the soluble organic component is readily utilized by the bacteria .The particulate matter attaches to biomass and undergo solubilization and biochemical transformation. The system can be configured as "up-flow" or "down-flow" depending on the waste stream feed direction. These types of systems has proven their good performances treating industrial wastewaters and sludge containing toxic substances due to the adaptation period given by SRT for the methanogens (Gerardy,2003).

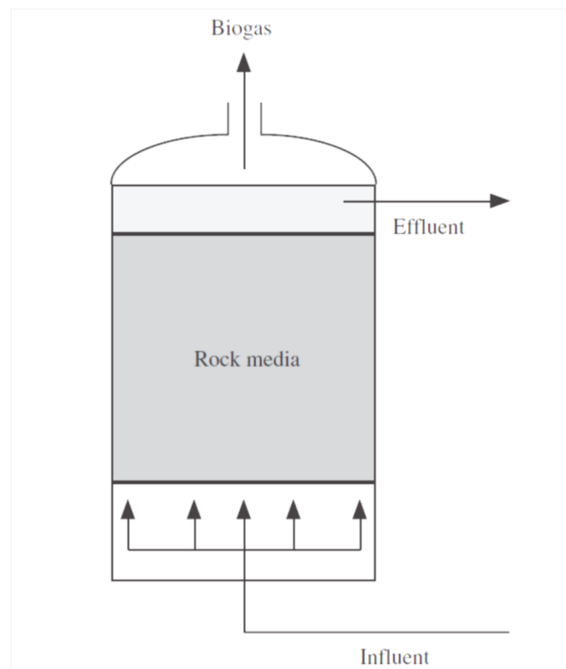


Figure 6. Schematics of fixed film system (Gerardy,2003)

At presence various types of fixed film reactor are used in order to increase the process efficiency. These are presented at Figure 7.In addition Table 11 summarizes the most common waste streams treated with the fixed film anaerobic reactors.

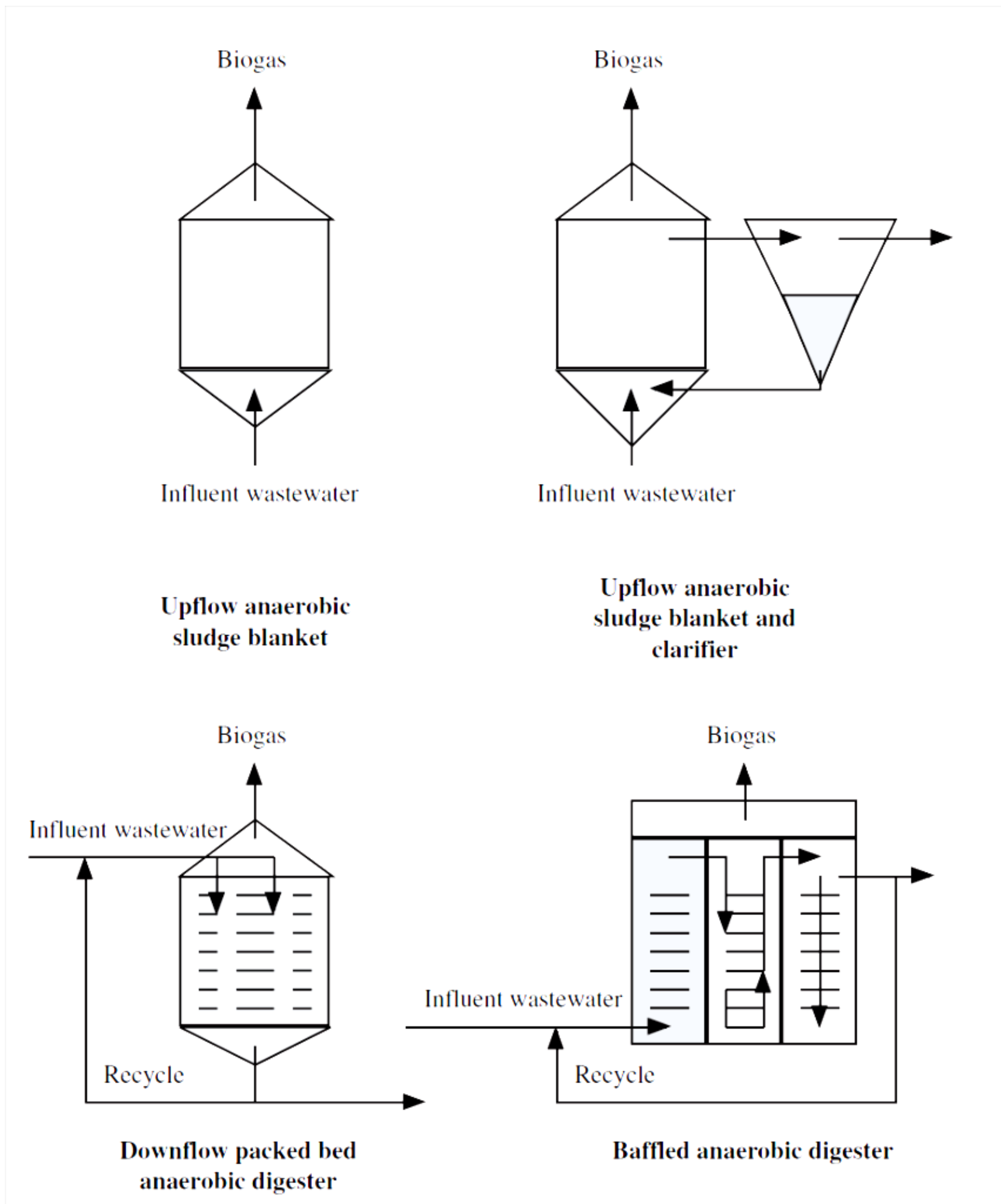


Figure 7. Types of fixed film anaerobic systems(Gerardy,2003)

Table 11. Examples of waste streams treated with fixed film reactors (Gerardy,2003)

Airport deicing fluids
Contaminated groundwater
Industrial wastewaters containing high concentrations of carbohydrates
Industrial wastewaters containing high concentrations of nitrogenous compounds
Low-strength wastewaters (<600 mg/l COD) at relatively short HRTs (<6 hours)

Depending on the configuration two types of anaerobic digestion systems can be differentiated: “single stage” and “two stage” systems (Figure 8 and Figure 9) .

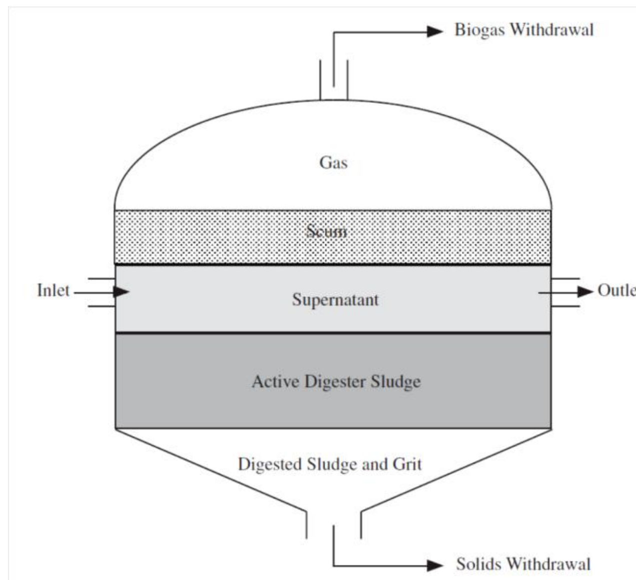


Figure 8. Single-stage digester system(Gerardy,2003)

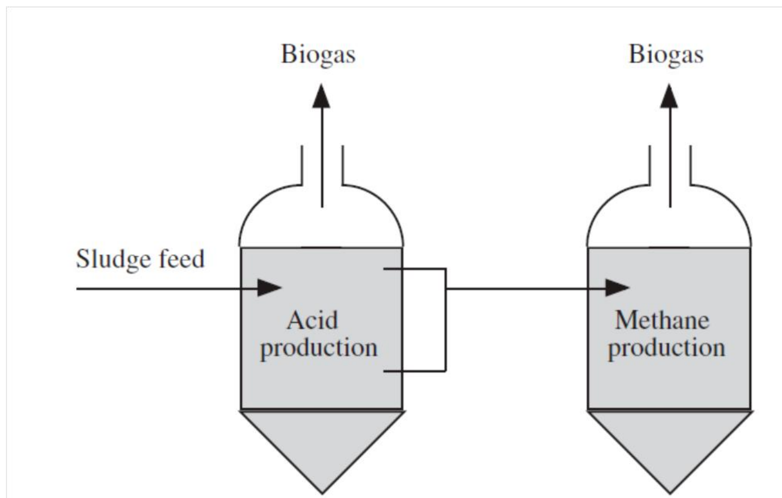


Figure 9. Two stage anaerobic system(Gerardy,2003)

The advantage of the two stage system over the one stage system lies in the fact that the process is more stable. In the one stage system the acid production and methane generation process simultaneously takes place in the same digester. The acidogens growth rate is higher and they are more resistant to process perturbations than methanogens thus when changes appears the system more easily gets upset because the rate of acids production is much higher than the rate of consumption. The two stage system is resolving this issue by splitting the acid production and methane formation processes in two different vessels and the acid generation and consumption can be more easily controlled.

Depending on the operating temperature systems can be differentiated as:

- Mesophilic – with working temperature within the range of 30-35°C
- Thermophilic- with working temperature within the range of 50-60°C

The mesophilic type of operation is most common because of two main reason: the cost for temperature heating are smaller and that the dominant part of microbial anaerobic population is mesophilic. The thermophilic operation range exhibits advantages in terms of higher rate of biogas production and pathogens destruction while they require higher cost for temperature adjustment and are more sensitive to temperature variations. This type of system is suitable for industrial waste streams where heat can be used to maintain the optimal temperature (Gerardy,2003). Advantages and disadvantages of meso and thermophilic temperature operated systems are summarized at Table 12.

Table 12. Comparison between the mesophilic and thermophilic reactors (Gerardy,2003)

Feature	Mesophilic Digester	Thermophilic Digester
Loading rates	Lower	Higher
Destruction of pathogens	Lower	Higher
Sensitivity to toxicants	Lower	Higher
Operational costs	Lower	Higher
Temperature control	Less difficult	More difficult

At presence high solids systems are employed as well for treatment of different organic waste streams. Further information about these technologies can be found in (Biomethanation II, 2003).

1.5 PROCESS PARAMETERS

The anaerobic digestion process is a complex one exhibiting close syntrophic relations between different microbial groups with different physiological and environmental conditions requirements. Therefore the process id strongly influenced by the environmental conditions , thus , the efficiency of conversion of organic substrate into biogas is highly dependent on conditions for growth and activity of anaerobic microorganisms. The following parameters are considered to be of crucial importance for the optimal working conditions: ***temperature, pH, alkalinity, VFA, nutrient supply, presence of toxic and inhibitory substances***(Al Saidi, 2008)**.**

TEMPERATURE

Temperature is one of the key parameters influencing the effectiveness of anaerobic digestion process. Usually, the two most common operated ranges are mesophilic (30-35°C) and thermofilic (50-60°C) due to the fact that most of methanogens spicies activity is highest in these ranges. At themperature between 40 and 50°C the methanogens are inhibited and the temperature of 42°C is considered to be point of transition from meso to thermophilic range(Gerardy,2003).

Temperature directly affect the growth of the methanogenic population and the rate of biochemical reactions in the process. The effect of temperature over the reaction rates can be determined using Arhenius equation:

$$K=K_0\ e\left(\frac{-E}{R\ Tabs}\right) \qquad (1)$$

K- reaction rate
K_0- constant
E- activation energy (cal/mole)
R- gas constant (1.98 cal/mole K)
T_{abs}- absolute temperature (K)

With the temperature rise the maximum growth yield of bacteria (μ_{max}) rise until it reaches the maximum, and then the sharp decrease in the growth appears(Figure 10). The decrease in the yield happened due to two competiting processes microbial growth and microbial decay (bacterial biomass loss). The net growth rate can be calculated using the Arhenius equation as :

$$K_{net}= K_1\ e\left(\frac{-E1}{R\ Tabs}\right)- K_2\ e\left(\frac{-E2}{R\ Tabs}\right) \qquad (2)$$

K_{net}- net growth rate
K_1 - bacterial synthesis rate
K_2 - bacterial decay rate

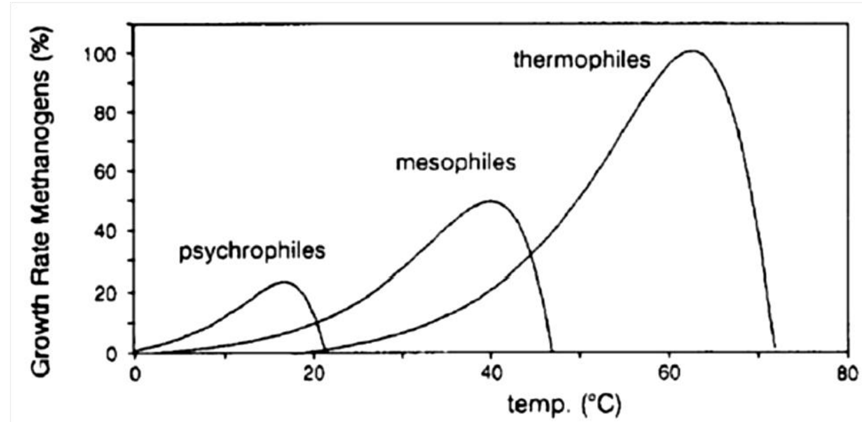


Figure 10. Relative growth of methanogens within different temperature ranges (Angelidaki. et al. 2004).

Although the methanogenic activity is increased for 25-50% resulting in cumulative higher methane production within the thermophilic range there are certain risks of higher process instability in terms of ammonia inhibition due to the fact that the fraction of unionized ammonia (NH_3) increases with temperature, which is reported more toxic than the ionized (NH_4^+)one, because of its capability to penetrate through the cell membrane, as well as temperature fluctuation sensitivity (Chernicaro,2007). In addition some authors reports higher level of residual volatile acids in output of the process , within the thermophilic range(Gerardy,2003).The methanogenic activity is highly sensitive to temperature fluctuations compared to the constant working temperature. In that way authors reports that the fluctuation within the termophilic reactors should be lower than 1°C and 2-3°C in mesophilic system per day(Gerardy,2003). The increase in temperature directly influence the rise in enzymatic activity within the process and the conversion of organic matter into the final biogas product. The higher the temperature, the lower will be the time that organic solids should spend in reactor in order to be converted in biogas and residues. Thus the overall digestion process time is being shorten (see Figure 11.) .

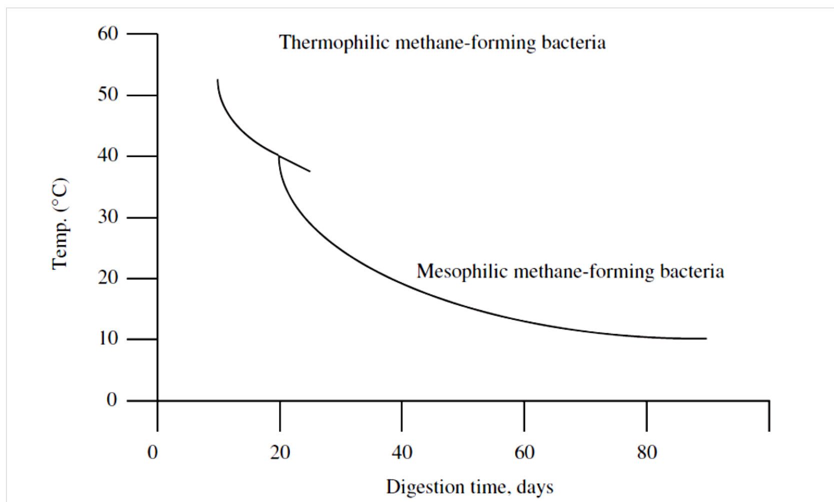


Figure 11. Digestion process time as a function of process temperature(Gerardy,2003)

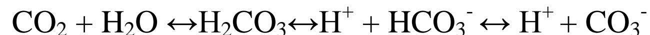
pH and ALKALINITY

pH is a measure of the acidity or basicity of an aqueous solution. As such this parameter represent the environmental conditions in the anaerobic systems and influence the process in two main ways: ***directly*** ,influencing the enzymes activity by changing their structure or ***indirectly***,influencing the toxicity of certain compounds present in the anaerobic system environment(Chernicharo,2007). In this context enzymatic activity of the acidogens is not intact when the pH levels are above 5.0 but the enzymatic of methanogenic population is severely affected below pH levels of 6.2. The optimum pH range for growth of acidogenic microbial population range from 5.0 to 6.0 and for the methanogenic population ranges from 6.6 to 7.4. Different authors presents different optimal values of pH for both groups of bacteria but the optimal operational range for the anaerobic digestion process is reported to be between 6.8 and 7.2 with most stable operation at pH from 7.0 to 7.2(Gerardy,2003).

Nevertheless, the acidogenic bacteria are reported to be much more resistant to pH change and the acids production can proceed even in pH values of 4.5(Chernicharo,2007). In opposite the methanogenic population is much more sensitive to pH change and therefore the pH values should be kept within the above mentioned range that suit optimal growth of methanogenic population. The inhibition of methanogenic activity appears when pH drops below 6.0 and above 8.0(Chernicharo,2007).

The pH values are directly influenced by alkalinity concentration in the system or buffering capacity in the system. Buffer capacity represent the ability of the system to maintain constant values of the pH through grouping of $[H^+]$ and $[OH^-]$ ions in the solution. The presence of alkalinity is of utmost importance in balancing the anaerobic digestion process as it prevents sharp changes in the pH adversely affecting the process itself.

Alkalinity in the anaerobic digestion process is primarily presented in the form of bicarbonate and alkalinity of volatile fatty acids. When organic compound is degraded CO_2 is released and production of carbonic acid, bicarbonate and carbonate alkalinity is created .



$$CO_2 + H_2O \leftrightarrow H_2CO_3 \leftrightarrow H^+ + HCO_3^- \leftrightarrow H^+ + CO_3^-$$

The amount of carbonic acid is directly proportional to the concentration on CO_2 in the gaseous phase when the concentrations in the liquid phase and gas phase of the reactor are balanced. The concentration of CO_2 in the liquid can be estimated using Henry's law equation:

$$[CO_2] = K_H P_{CO2} \qquad (3)$$

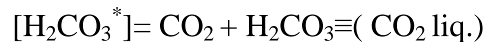
$[CO_2]$- saturation concentration of CO_2 in the liquid phase (mole)
K_H - Henry's law constant for CO_2 water balance (mole/atm L)
P_{CO2} - partial pressure of CO_2 (atm)

The relationship between alkalinity and pH is given with the following formula (Chernicharo,2007):

$$pH= pK_1 + \frac{[HCO\bar{3}]}{[H2CO3*]} \tag{4}$$

pK_1- log ($1/K_1$)

K_1 - constant of apparent ionization ($4.45 * 10^{-7}$ at 25°C)



$$[H_2CO_3^*]= CO_2 + H_2CO_3 \equiv (CO_2 \text{ liq.})$$

Thus the amount of carbonic acid can be estimated using the equation (4) by calculating the partial pressure of CO_2.

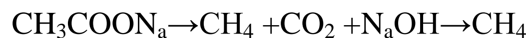
In addition, alkalinity can be created when organic-nitrogen compounds are being degraded and ammonium bicarbonate is created .

$$NH_3 + H_2O + CO_2 \rightarrow NH_4HCO_3$$

Thus, the alkalinity in the system is directly affected by the type of organic matter being degraded. Nitrogen rich organic substrates boost the alkalinity concentration up to proportional value of nitrogen concentration present in the substrate. When these concentrations are high the present ammonia concentrations in the system becomes toxic for the methanogenic population.

However, the conversion of organic substrate leads to fatty acids production in the process that consumes the alkalinity and as a consequence the pH starts to decrease, but when these acids are being consumed by the methanogens the CO_2 is produced and the bicarbonate alkalinity is restored and the pH change is balanced . This is normal trend when operational conditions are stable. In case, some imbalance is present in the system, and by any cause methanogenic activity is decreased the alkalinity production will also decrease (due to the continuous CO_2 stripping in gas phase) leading to acids accumulation in the reactor further lowering the pH down. This trend of lowering pH value further inevitably leads to complete inhibition of methanogenic activity and biogas production. The negative trend can be detected immediately by measuring the alkalinity concentration. Therefore the alkalinity should be considered as one of the key process parameters for evaluation of stability of the process.

As a result from the interactions between the volatile fatty acids and alkalinity the alkalinity of fatty acids is created.



$$CH_3COON_a \rightarrow CH_4 + CO_2 + N_aOH \rightarrow CH_4$$

VFA alkalinity has buffering range between 3.75 and 5.75 and thus it is not of substantial importance for the anaerobic digestion process. In practice the bicarbonate alkalinity is

calculated when volatile fatty acids alkalinity is subtracted from the total alkalinity, using the following equation (Chernicharo,2007).

$$BA = TA - 0.85 \times 0.83 \times VFA = TA - 0.71 \times VFA \tag{5}$$

BA- bicarbonate alaklinity(mg $CaCO_3$/L)
TA- total alkalinity (mg $CaCO_3$/L)
VFA- concentration of volatile fatty acids (as mg acetic acid/L)
0.85- correction factor that considers 85% ionization of acids to the titration end point,
0.83- conversion factor from acetic acid into alkalinity.

NUTRIENTS

The nutritional needs of anaerobic population are determined by the chemical composition of the cell of the microorganisms. Microbial biomass composition is very versatile. Thus for practical reason of determination of nutrient needs empirical composition is being used as $C_5H_7NO_2$. Anaerobic population has significantly lower growth yield compared to aerobic/anoxic microorganisms (0.4-0.6 gVSS/gCOD), thus the amount of nutrient demand is much lower (Table 13).

Table 13. Growth yield of anaerobic microorganisms (Gerardy,2003)

Bacterial Group	Yield (kg VSS/kg COD)
Volatile acid-forming bacteria	0.15
Methane-forming bacteria	0.03

According to Lettinga *et al*. (1996) the following expression can be used for determination of nutritional needs :

$$N_r = S_0 \times Y \times N_{bac} \times \frac{TSS}{VSS} \tag{6}$$

N_r- nutrient requirement (g/L)
S_0- Influent COD concentration (g/L)
Y - bacterial growth yield (gVSS/gCOD)
N_{bac}- concentration of the nutrient in the bacterial cell (g/gVSS)
TSS/VSS – Total solids/volatile solids of the bacterial cells (usually 1.14)

Regarding the amount needed for different type of nutrients they are classified into two main groups : ***macro*** and ***micro*** nutrients. First group is represented by nitrogen and phosphorus and later one includes cobalt, nickel, sulfide ,iron and molybdenum (Table 14). The two macronutrients nitrogen and phosphorus comes from ammonia nitrogen (NH_4^+ -N) and orthophosphate (HPO_4^{-}P) and anaerobes are able to utilize them only in soluble form. The micro nutrients are of special importance for the enzymatic activity of the anaerobic microorganism (Gerardy,2003).

Lettinga *et al*. (1996) reports some averaged optimal values in the organic mater composition in the form of COD:N:P ratio for nutrients supply to anaerobic population : He proposes as optimal values COD:N:P ratio of 1000:5:1 for the low growth anaerobes (Y=0.05) and COD:N:P ratio of 350:5:1 for the anaerobes with higher growth yield (0.15).

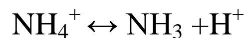
Table 14. Significant nutrients need for the anaerobic microorganisms (Gerardy,2003)

Nutrient	Micronutrient	Macronutrient	Minimum Recommended (% of COD)
Cobalt	X		0.01
Iron	X		0.2
Nickel	X		0.001
Nitrogen		X	3–4
Phosphorus		X	0.5–1
Sulfur	X		0.2

TOXYCITY

Group of compounds of interest causing toxicity in anaerobic digestion process are ammonia, heavy metals ,sulfide and Long chained fatty acids (LCFA) . At the present text more attention will be placed on toxic and inhibitory effect of ammonia ,heavy metals and LCFA. More information about sulfide toxicity can be found at Chernicharo (2007).

Ammonia is an important compound for the anaerobic digestion process for several reasons. Primarily it represents a source of nutrients for the anaerobic population. In addition it represents supplement to buffer capacity of the system balancing the adverse effect of pH change. Ammonia in the anaerobic digestion system is present as free NH_3 and as saline (ionic form) NH_4^+. The saline form is used as a nutrient and the free form of ammonia is considered as toxic, but at large concentrations both are considered to have inhibitory effect over the methanogenic population activity (Gerardy,2003) Which form of ammonia will dominate in the system is directly dependent of the pH value. When the pH values are lower or equal to 7.2 the dominating form is ammonium ion NH_4^+ in opposite when concentration of H^+ ion is lower i.e pH is higher than the free ammonia dominates (Chernicharo,2007). Ammonia concentration ranges and its toxicity effect of microbial population are summarized at Table15.



$$NH_4^+ \leftrightarrow NH_3 + H^+$$

Table 15. Effect of free ammonia on anaerobic process (McCarty, 1964)

Concentration (as N mg/L)	Effect on the process
50-200	Beneficial
200-1000	No adverse effect
1500-3000	Inhibitor for pH > 7.4 to 7.6
> 3000	Toxic

A group of metals and toxic compounds such chromium, cobalt, copper, zinc, cyanides and arsenics are considered to be highly toxic for the anaerobic population in the process. Because of the adverse effect that the group of metals has on the process they are called as “heavy”. Principally the toxic effect of these elements and compounds is reflected as inactivation on the enzymatic system. However when present in trace concentrations they might have beneficial effect on the population (Gerardy,2003).

The concentrations that can be tolerated in the process are directly connected with the sulfide concentration. Although the sulfide by itself has toxic effect, when reacting with the above mentioned group of metals it precipitates as insoluble metal sulfide that has no adverse effect over anaerobic population. Approximately 1.8 mg/l metals are precipitated by 1 mg/l of sulfide (S^{2-}) (Chernicharo, 2007).

Long chain fatty acids have been found to be toxic for the anaerobic microbial population (Table 16). Because the chemical composition and structure of several long-chain fatty acids are similar to those of the lipid components in the cell wall of acetoclastic bacteria and methane-forming bacteria, the fatty acids dissolve in the cell wall. Once dissolved in the cell wall, the acids inhibit the activity of the bacteria at very low concentrations. Long-chain fatty acids concentrations >500 g/l may cause toxicity in anaerobic digesters (Gerardy,2003).

Table 16. Long chain fatty acids that inhibit methane production from acetate (Gerardy,2003)

Fatty Acid	Carbon Units	Saturated/ Unsaturated	Formula
Caprylic (octanoic)	8	Saturated	$CH_3(CH_2)_6COOH$
Capric (decanoic)	10	Saturated	$CH_3(CH_2)_8COOH$
Lauric (dodecanoic)	12	Saturated	$CH_3(CH_2)_{10}COOH$
Myristic (tetradecanoic)	14	Saturated	$CH_3(CH_2)_{12}COOH$
Oleic (*cis*-9-octadecanoic)	18	Unsaturated	$CH_3(CH_2)_7CH{=}CH(CH_2)_7COOH$

BIOGAS

The end product of anaerobic digestion process is production of biogas and the amount of biogas represents the ”health” status of the process itself. Stable anaerobic digestion results in higher amount of biogas produced compared to unstable one. Nevertheless, the biogas represents a mixture of different gasses produced in the process. Two most dominant gases produced are CO_2 and methane. Methane is the only gas that has economic value and is being used for production of heat and electricity. Table 17, list the most common gases produced in the process and their average content in the biogas.

Table17. Average composition of biogas (Al Seadi et al. 2008)

Compound	Chemical symbol	Content (Vol.-%)
Methane	CH_4	50-75
Carbon dioxide	CO_2	25-45
Water vapour	H_2O	2 (20°C) -7 (40°C)
Oxygen	O_2	<2
Nitrogen	N_2	<2
Ammonia	NH_3	<1
Hydrogen	H_2	<1
Hydrogen sulphide	H_2S	<1

The higher the volumetric percentage of the methane in the biogas more the energy can be extracted from the process. With increasing quantities of carbon dioxide in biogas, decreasing heat values of biogas occur and If the carbon dioxide fraction in the biogas increases above 30%, the acid concentration in the sludge increases and the pH drops below 7.0 in case no sufficient alkalinity is present . At pH values below 7.0, significant acid fermentation occurs (Gerardy,2003). Of all the gases produced in the process H_2S-hydrogen sulfide is the most undesirable. If there is substantial amount of this gas the AD equipment may be damaged due to the adverse effect of sulphuric acid created. This gas is scrubbed from the biogas in so called upgrading gas units that are inseparable part of bigger AD plants.

1.6 OPERATIONAL PARAMETERS

A number of parameters are important and have substantial influence over the operation of anaerobic digestion plants. These include: Hydraulic retention time (HRT), Solids retention time (SRT), Organic loading rate (OLR), gas production rate (m^3CH_4/day).

Two significant detention times are of great importance for operation of the digesters.

Hydraulic retention time (HRT): Hydraulic retention time , HRT (days), represents the time that the liquid substrate stays in the anaerobic rector.

$$HRT = \frac{V}{Q} \text{ [d]} \qquad (7)$$

HRT- Hydraulic retention time (days)
V- Volume of digester tank(m^3)
Q- Hydraulic loading rate (m^3/day)

Solids retention time (SRT) : Solids retention time, SRT (days), represents the time that solids (microorganisms) stays in the reactor. This is more important parameter then HRT for several reasons. First of all it is directly connected to the time needed for bacteria to multiply in the

digester. If the time that microorganism spend in the reactor is shorter than the time they need to multiply (generation time) a situation of "washout" occurs i.e the bacteria is been washed out of the system. The second important point is that SRT directly influence the efficiency or competition of the digestion process. The longer the SRT the more complete the digestion process will be and the higher will be the amount of biogas produced and the less residual sludge is left for further handling. According to Rittmann & McCarty *(2001),* the minimum SRT for an anaerobic CSTR at 35ºC is 10 days.

$$\text{SRT} = \frac{Mass\ of\ solids\ in\ the\ reactor}{Mass\ of\ solids\ wasted\ per\ day} = \frac{V*X}{Qw*Xw}\ \text{(days)} \qquad (8)$$

SRT – Solids retention time (days)
V – volume of the reactor (m^3)
X – concentration of solids in the reactor (kg/m^3)
Q_w- flow out of the reactor
X_w – concentration of solids in the effluent stream (kg/m^3).

Organic loading rate (OLR): the organic loading rate can defined as amount (mass) of substrate applied on a daily basis to the reactor's volume. The mass loading can be expressed in terms of Volatile solids- VS (representing the organic part of the solids applied) or in terms of COD applied on a daily basis to the reactor.

$$\text{OLR} = \frac{Q*S}{V}\ (\text{kgVS/m}^3\ \text{d}) \qquad (9)$$

OLR- organic loading rate (kg VS/m^3*d)
Q- flow rate (m^3/d)
S- concentration of VS or COD of the influent stream (kg/m^3)
V- volume of the reactor (m^3).

The organic loading rate must represents the daily load that the reactor is capable to sustain. Increasing the loading rate may lead to digester instability and result in a failure. Different authors recommend different organic loading rate to be optimal for the high rate systems (systems where heat and mixing is applied). Thus, According to Rittmann & McCarty (2001), the recommended organic loading rate for suspended growth anaerobic systems without biomass retention is 1.6- 4.8 kg VSS/(m3*d). Vesilind (1998) reports that the peak organic loading rate for high- rate anaerobic digestion should be 1.9- 2.5 kg VS/(m3*d). Henze et *al.2008,* reports values 5-35 kgCOD/(m^{3*}d) for high rate anaerobic wastewater treatment systems as fixed film or granule with biomass retention.

Gas production rate: it is the amount of m^3 biogas produced per day. This parameter is direct function from other operational parameters like % destruction of organic matter, OLR, SRT as well as temperature, pH and type of the characteristics of the substrate feed into the systems.

Mixing regime: Mixing of the digester content has significant impact over the process efficiency in several ways. Mixing provides better biomass distribution in the reactor making it homogenous, and equalize the temperature inside the reactor. The metabolic activities of acetate-forming bacteria and methane-forming bacteria requires that they should be in close spatial contact. Slow, gentle mixing ensures that contact. Also, mixing provides for efficient hydrolysis of wastes and production of organic acids and alcohols by acid-forming bacteria(Gerardy, 2003).Mixing regime can have intermittent or continuous character and can be done by mechanical applications or gas recirculation. Below in Table 18, are listed some of the advantages of implying mixing in the anaerobic reactors.

Table 18. Advantages of Mixing Digester Content (Gerardy, 2003)

Eliminating or reducing scum buildup
Eliminating thermal stratification or localized pockets of depressed temperature
Maintaining digester sludge chemical and physical uniformity throughout the tank
Rapid dispersion of metabolic wastes (products) produced during substrate digestion
Rapid dispersion of any toxic materials entering the tank (minimizing toxicity)
Prevent deposition of grit

1.7 MONITORING PARAMETERS

Monitoring of the anaerobic digestion process is a key to successful operation of the digester and optimal production of biogas. Following parameters are suggested have been proposed as monitoring parameters for evaluation of process stability: **total solids, volatile solids, organic loading rate, conductivity, pH, alkalinity, temperature, ammonia, total nitrogen, total phosphorus, COD, HRT, SRT, gas production, and gas composition** (Aklaku et al., 2006; Lang & Smith, 2008,Angelidaki et al.2010). (Gerardy,2003) reported operational conditions best suitable for methanogenic population (Table 19.), indicators for process instability and operational conditions responsible for the process upsets (Table 20. and 21.)

Table 19. Operational conditions for optimal activity of methane forming bacteria(Gerardy,2003)

Condition	Optimum	Marginal
Alkalinity, mg/l as $CaCO_3$	1500–3000	1000–1500 3000–5000
Gas composition		
Methane, % volume	65–70	60–65 & 70–75
Carbon dioxide, % volume	30–35	25–30 & 35–40
Hydraulic retention time, days	10–15	7–10 & 15–30
pH	6.8–7.2	6.6–6.8 & 7.2–7.6
Temperature, mesophilic	30–35°C	20–30° & 35–40°C
Temperature, thermophilic	50–56°C	45–50° & 57–60°C
Volatile acids, mg/l as acetic acid	50–500	500–2000

Proper manipulation of some of the above mentioned parameters like SRT,OLR,HRT, or temperature by the process operator prevent adverse changes in monitoring parameters like pH,alkalinity,TS,VS,NH_3 or gas production rate thus creating favorable conditions for anaerobic microbial consortia

Table 20. Indicators of process instability (Gerardy,2003)

Indicator	Decrease	Increase
Biogas production	X	
Methane production	X	
Alkalinity	X	
pH	X	
Volatile solids destruction	X	
Volatile acid concentration		X
Percent CO_2 in biogas		X

Table 21. Conditions causing process upsets in the anaerobic digesters (Gerardy,2003)

Condition	Example
Hydraulic overload	Overpumping of dilute feed sludge
Organic overload	Overpumping of concentrated feed sludge
pH changes	Drop in pH (<6.8) and loss of alkalinity
Temperature fluctuations	Overpumping of feed sludge
Toxicity	Specific inorganic and organic wastes
Large withdrawal of sludge	Excess withdrawal of sludge and reduced retention time
Sudden changes	Rapid increase in nitrate ion concentration

2. ANAEROBIC CO-DIGESTION (AcoD)

Anaerobic digestion have had a long history as a single substrate process. However, at presence the process is being used for treatment of multiple organic waste streams. The process of simultaneous treatment of homogenous mixture of different types of organic substrates for enhanced methane production is called **anaerobic co-digestion (AcoD)** . The concept of co-digestion dates back from the seventies when the first research on digestion of sewage sludge and organic fraction of municipal solid waste (OFMSW) were undertaken (Miller et al., 1978). Later on, this trend continued with behavioral investigation of different substrates over the stability and enhancement of the biogas production process. At presence this technology is well established.

Potential of codigestion is high due to numerous different blends and substrates might be used in the co digestion process including:

· Animal manure and slurry
· Agricultural residues and by-products
· Digestible organic wastes from food and agro industries (vegetable and animal origin)
· Organic fraction of municipal waste and from catering (vegetable and animal origin)
· Sewage sludge
· Dedicated energy crops (e.g. maize, miscanthus, sorghum, clover)(Al Saidi, 2008).

The benefits, of employment this practice, can be seen in more stable anaerobic digestion process and optimized biogas production. Consequently, economically more feasible the process become. Process benefits, such as positive microorganisms synergisms and nutrient and moisture balance, enhanced inhibition prevention (ammonia, lipid degradation products), toxic compound dilution (Mata Alvarez et al. 2011). When treating organic wastes with high nitrogen content the problem of ammonia inhibition is exerted. Angelidaki and Ahring, (1993), Chen et al.(2008) Hansen et al.(1998) , reported decrease in methanogenic activity with increasing level of ammonia present. Therefore, the main issue for the co-digestion process lies in balancing the C/N ratio, but the right combination of several other parameters in the co-substrate mixture, such as macro- and micronutrients, pH/alkalinity, inhibitors/ toxic compounds, biodegradable organic matter, and dry matter, are also relevant (Hartmann et al., 2003). For instance, it has been shown that optimum values for the carbon-to-nitrogen (C/N) ratio fall within the range of 20 to 70 for the AD process (Burton and Turner, 2003) but even lower values (12 to 16) have also been reported (Mshandete et al., 2004).

The Process of AcoD is of considerable interest from technical point of view because it is feasible for use at existing wastewater treatment plants for treatment of sewage sludge with various organic wastes.

Cecchi et al. (1988) published a pilot scale study, comparing the performance of different types of OFMSW codigested with sewage sludge and demonstrations that the codigestion process

could be successfully implemented in existing wastewater treatment plants (WWTP), to improve digester performance and thus energy balance. Zupancic et al. (2008) conducted a real scale test at a WWTP of with the average organic loading rate of digesters was 0.8 kg VSS/(m3 d) and they were supplemented with OFMSW to increase the organic loading rate by 25%, reaching 1 kg VSS/(m3d). As a result, biogas production increased by 80% and the specific biogas production increased from 0.39 m3/kg VSS to over 0.6 m3/kg VSS. Bolzonella et al.(2006) from the test conducted at WWTP at Treviso, Italy, reported that addition of around 10 t/d of OFMSW – with a sludge/OFMSW ratio of 60/40 on a VS basis – increased biogas production from 3 500 to 17 500 m3/month, which corresponded to an increase in specific biogas production from approximately 0.13 m3/kg VS, when only waste activated sludge was digested, to 0.43 m3/kg VS in the case of codigestion, with an applied OLR of 0.78 kg VS/(m3 d).

The process of codigestion , depending of the substrate used can give rise to COD/N ratio by supplementing concentrations of carbon source that can be utilized in biological nutrient removal WWTP by enhancing the process of denitrification. Further information on this aspect might be found in (Cecchi et al. 1994; Pavan et al, 1998 and 2000).

Of special interest, for the author of this text, is the co-digestion process of sewage sludge and food waste employed the Regional Wastewater Treatment Plant of Nord-Jæren (SNJ), located at Mekjarvik, Norway.

2.1 Wastewater Treatment Plant of Nord-Jæren (SNJ)

The regional wastewater treatment plant SNJ, is one of the largest wastewater treatment plant in Norway, treating wastewater from five municipalities. The plant was designed with the wastewater treatment capacity of 240 000 PE (person equivalents) or approx. 130 000m^3/day with maximum incoming flow rate of 4.0 m^3/s.

Wastewater treatment facility can be divided in three main units: transportation unit, wastewater treatment unit and sludge treatment unit. Transportation unit is represented by 8 km long tunnel with diameter of 3.5 m, and has a total volume of 77.000 m3. The tunnel serves a role of equalization unit to buffer hydraulic load peaks during wet weather conditions.

The wastewater unit is a chemical treatment one, based on coagulation-flocculation-sedimentation principle. It consist several sub units. The pumping station unit is represented by four pumps with total pumping capacity of 4000 L/s of wastewater from the tunnel . Next unit in the line is the pretreatment unit represented by 4 continuous belt screens, 1m wide with 8 mm openings, with overall capacity of 4000 L/s followed by a sand trap for coarse particles (sand and grit) removal. The preliminary treated wastewater than enters the main treatment unit represented by 8 rectangular sedimentation basins, with overall volume of app. 18200 m^{3}and total surface area of 3750m^2. Each sedimentation basin is equipped with flocculation chamber. Ferric chloride is added to grit chamber and is used for enhancement of the suspended solids removal process. The flocculated wastewater enters the basins where the settling of the solids in the wastewater takes place. The particles sediments on the bottom forming a layer of sludge with average height of 20 cm ,under normal operational conditions. The average content of solids in

the sludge hooper is around 5% solids and no further thickening of the sludge is applied. Sludge from the bottom is further conveyed to sludge treatment unit using pump system. Sludge treatment unit serves several functions : anaerobic digestion, dewatering, drying and pelletation of sludge. The unit consist following compartments:

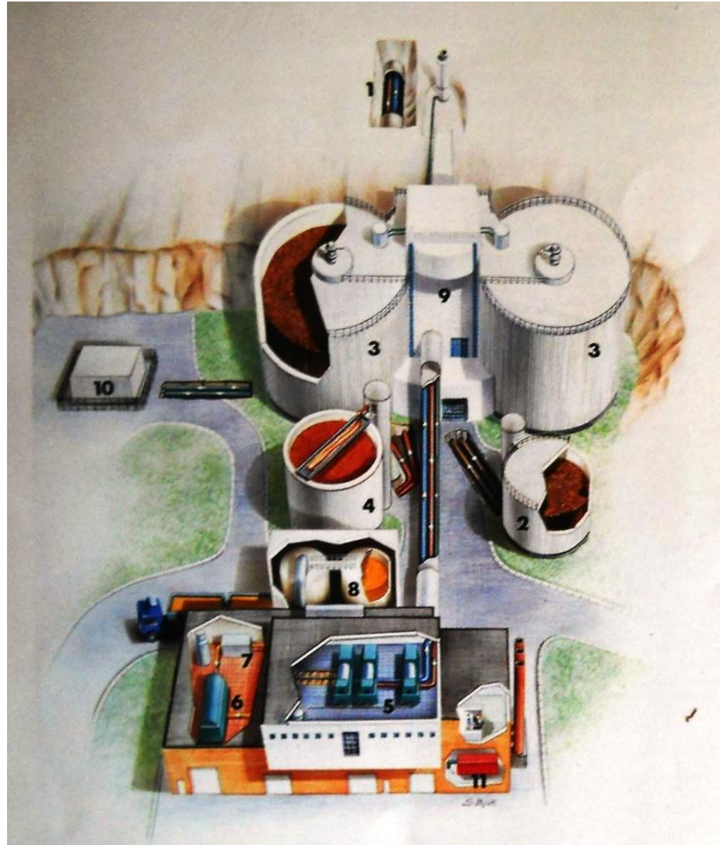


Figure 12. Sludge treatment unit at SNJ –wastewater treatment plant (IVAR,2000)

1. Sludge pumping unit (400) long conveying the sludge from the sedimentation tanks
2. Buffer tank (1) with capacity of 600 m^3 with role of digester load optimization ,
3. Digester tanks (two in number) each has capacity of 3500m^3, with temporary gas storage compartments of around 230m^3,
4. Buffer tank (2) with capacity of 1100m^3 for storage of digested sludge with de-gassing conduit,
5. Dewatering unit represented by centrifuges and polymer addition compartment,
6. Sludge drying unit
7. Pelletation unit
8. Silo for storage and loading of stabilized sludge
9. Central building for machinery , pumps, heat exchangers, and gas handling equipment
10. Gas storage, membrane tank with volume of 74 m^3
11. Central heating unit with gas burner and steam generator.
12. Gas upgrading unit (not shown on the figure).

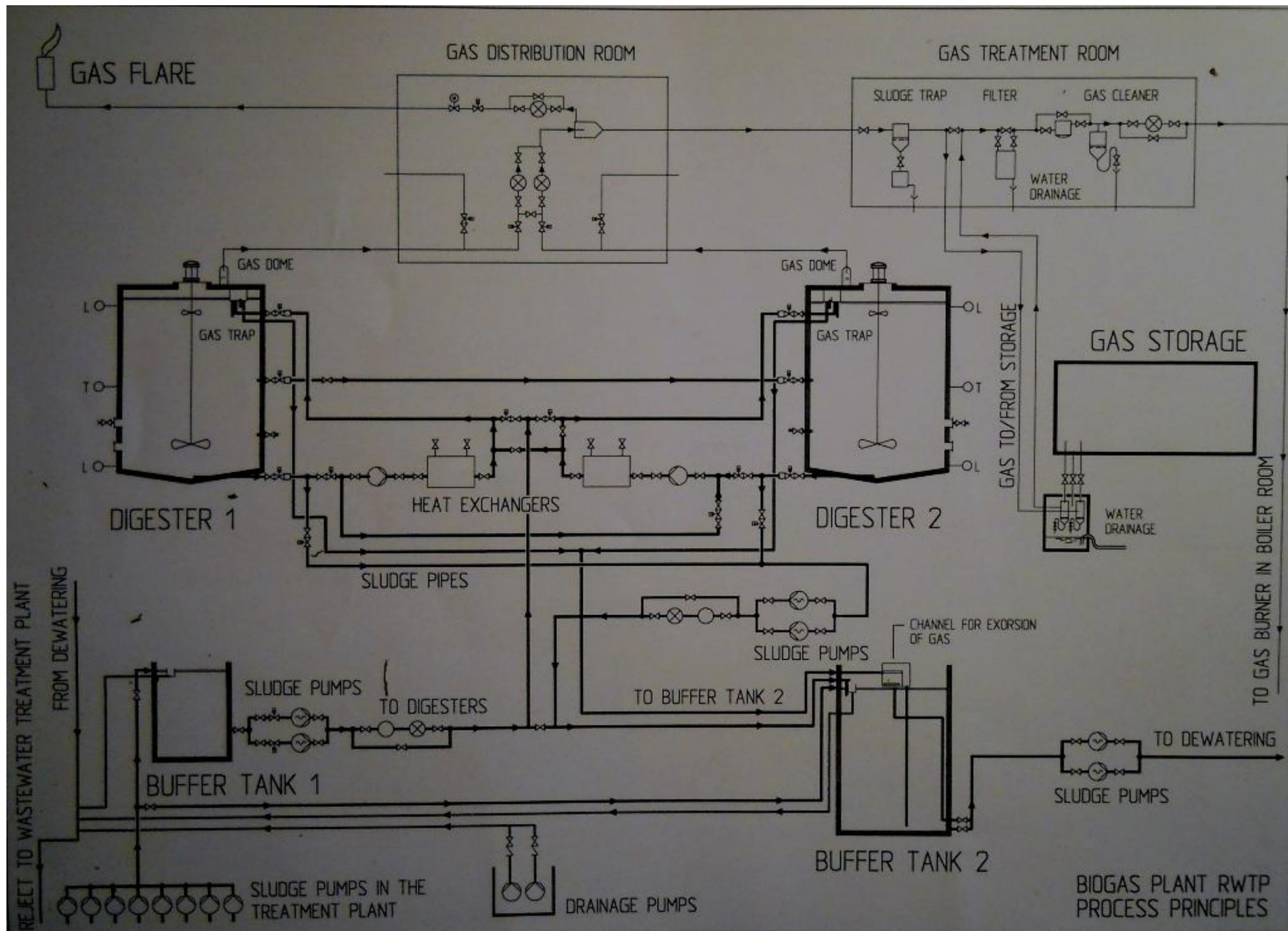


Figure 13. Schematics of the anaerobic digestion compartments and process flow (IVAR,2000)

The process of anaerobic digestion at SNJ starts with the sludge transportation from the sedimentation unit. The sludge with average solids content of 5 % solids is then pumped at Buffer tank 1. This is where the anaerobic digestion leading steps take place. The tank is operated in such a manner to enable balanced load in the digester. Usual daily load of sludge pumped from the tank to the digesters is variable according to the operational process needs and vary within the range of 15-25 m^3 per hour. The Buffer tank 1, is the place where different substrates is being feed for co-digestion with the sludge originating from the wastewater treatment unit. Pumped sludge(mixture of wastes) enters circulation system and is being distributed into digesters. The digesters can be operated in serial or parallel mode. At presence the pumping is operated on an hourly basis switching between Digester 1 and Digester 2. Digesters are mixed pneumatically and the mixing has intermittent character. The temperature inside the digesters is regulated by heat exchangers and kept within the operational range from 37 -39°C. The average daily inflow into digesters range from 300- 450m^3 per day and HRT is kept within the range from 15 to 20 days.

As an end products, from the overall transformation process of organic matter into digesters the biogas is produced which is then distributed to the upgrading unit for CO_2 and H_2S striping and is directed to the gas burner in boiler room. Some of the gas is being stored in gas storage department and the excess gas is directed to the local gas network. The heat produced in the process is mainly used for heating of the digesters and wastewater treatment plant.

The digested sludge is pumped to the Buffer tank 2 through the gas extracting channel. From Buffer tank 2 the sludge is directed to dewatering (centrifugation) and drying . Finally the pellets are being produced. Pellets are further used as fertilizer and undergo strict monitoring control for pathogen and heavy metals presence.

At presence different organic substrates are used in AD process at SNJ wastewater treatment plant.Table 22 summarizes the types and amounts of different organic substrates utilized in the process.

Table 22. Types and amount of substrates used in the co digestion process at SNJ (Ydestebø,2012).

SUBSTRATES	SOURCE	AMOUNT FEEDED AT SNJ (Tons/year)	Average solids content (TS)
Primary sludge	WWTP-SNJ	125000	5.5
Food waste	Catering services	6400	25
Waste activated sludge	WWTP- VIk	2200	7
Waste activated sludge	WWTP- Grødaland	2000	20
Other organic waste	Industry	1400	10-20

3. MATERIALS AND METHODS

Four full scale test were conducted to evaluate the effect of co-digestion of food waste and sewage sludge at SNJ. The co-digestion process is conducted in a pulse mode with feeding of high solids content food waste into the buffer tank. This pulse mode addition of food waste, represents a temporary overload for the process and the system response was evaluated using some of the most relevant parameters for control of the anaerobic digestion process like: pH, Alkalinity, VFA concentration (Angelidaki et al. 2010). In addition COD and solids concentrations were evaluated. Production of biogas was recorded through the on-line instrumentation system at the wastewater treatment plant.
In order to investigate the process response to the pulse load grab samples from buffer tank 1 and digester 2 were taken "before" and "after" the food waste pumping into the system.

3.1 EXPERIMENTAL ASPECTS

Full scale experiments: the tests were carried out at SNJ wastewater treatment plant. Objective of the full scale test experiment was to evaluate the effect of pulse feed of additional substrate in the system on gas production. For the purpose of the test, samples were taken for further analysis of several different parameters from the feed unit (Buffer Tank 1) and digesters. Sampling was done within an interval of 24 hours and samples were taken prior the food waste addition in the process and afterward. The number of samples taken, was organized in such manner that most of them were taken in approximate time interval for a period of 2 hours after the food waste addition , with approximate time distance between each sampling of 30 min. This was done with intention of evaluation of the spike response in gas production. During this time interval of 2 hours the operation of digesters was set in such manner that only one of the digesters was fed during this interval in order to get better insight of the codigestion effect over the gas production. After this period of 2 hours , samples were taken in reduced time intervals, finishing next day approximately 24 hours after the test starting time. The samples taken prior the addition of waste serves role of reference values for the evaluation of the effect of co-digestion. Total number of full scale test experiments is four and they were conducted within the period January-April 2012.

Methane potential test (BMP) & Organic loading (OL) determination test

These tests were conducted under controlled laboratory conditions using the Automatic methane potential test system (AMPTS II). The system consist three different units A,B and C. Unit A or ***sample incubation unit*** ,consist 15 vials-rectors (500 mL) at which the inoculum (digester sludge) is mixed with the substrate . The reactors consist slow rotation agitator for media mixing. The reactors are settled into a water bath with constant temperature of 37°C. Produced biogas in the reactors is then transported to Unit B and finally to Unit C. Unit B is ***CO_2-fixing unit***. It consist 15 small vials (100 mL) with alkaline solution (NaOH solution) for acid fraction gasses

(CO_2 and H_2S) removal, only allowing passage of CH_4 produced in Unit A. Unit C is a ***gas volume measuring device*** and works on a liquid displacement & buoyancy with a multi-flow cell arrangement (15 cells). This unit measures the volume of CH_4 passed produced in Unit A, and digital pulse is generated when certain amount of methane flows through. This unit is connected to a computer and data are generated using online network serving software. More information about setup, operation and maintenance of the automated system can be found at the "AMPTS II" , operation and maintenance manual (Bioprocess control, 2011).

The purpose of the methane potential test was to evaluate the ***methane yield*** NmL CH4 produced per gVS added, from different substrates and blend of substrates with different ratios. Two methane potential tests were carried out in a batch mode.

The first methane potential test started on 13.02.2012. In this experiment undigested sludge from SNJ wastewater treatment plant and yeast extract were evaluated for methane production. The experiment consisted two blanks (digester sludge), and six reactors (triplicates) for the undigested sludge and yeast extract.

The second methane potential test started on 05.03.2012. Following substrates and blends of substrates were evaluated: primary sludge, food waste, mixture of sludge and food waste in a 75-25% ratio and mixture of primary sludge and food waste in a 50-50% ratio on a mass weight basis. In these experiment three blanks were used and three reactors for each of four above mentioned substrates and blend of substrates.

Both experiments were terminated when the production rate of methane fell below 50 mL per day.

The organic loading test was conducted with final aim to determine the maximum organic loading or ***substrate to biomass*** ratio that the SNJ anaerobic treatment unit can handle without causing process instability. The amount of mixture of substrates used in the experiment was 100g and inoculum amount was 200g. The food waste used for experiment had 12 % VS, and sludge had a concentration of 5.1% VS, and biomass was represented with 1.85% VS. For the test purpose several different mixtures with increasing food to undigested sludge ratio were prepared as 0:100, 20:80 ,40:60, 60:40, 80:20 and 100:0 on a mass weight base. By increasing the food waste to sludge ratio the organic substrate loading based on volatile solids content increase gradualy from 5.1% to 12% VS and the following actual substrate to biomass ratios were evaluated 2.75:1 ,3.45:1, 4.21:1 ,4.97:1, 5.67:1, 6.46:1 (%VS substrate/% VS biomass).

The complete data for the amount of inoculum and substrates used in the laboratory test are presented in Appendix II.

3.2 EXPERIMENTAL PROCEDURES AND ANALYTICAL METHODS

The full scale experiment was based on taking grab samples at previously determined time intervals and samples analysis for the following parameters: **pH, VFA , Alkalinity ,TS,TVS, and COD**. Special attention was put on pH, VFA and alkalinity concentration as most representative parameters for the evaluation of the effect of codigestion at SNJ wastewater treatment plant(Anngelidaki et al. 2010). Basically most of the analysis were done at SNJ laboratory except the Ion Chromatography test for the evaluation of presence of different fatty acids that was undertaken at the laboratory at University of Stavanger.

Sampling procedure: Samples were taken from Buffer Tank 1 , Digesters 2 and food waste delivery trucks. For that purpose polyethylene plastic bottles of 500mL were used.

Further, samples were evaluated for the pH and conductivity values. Both parameters were measured using instrument type "WTW Multi 340i". The instrument was calibrated before measurement start up , with two different solutions at pH values of 4.00 and 7.00 respectively.

For the analysis of VFA and alkalinity, 5 point titration procedure was applied (R.E Moosbrugger et al. 1992). For the titration purpose the samples were subjected to centrifugation followed by filtration. 50 mL from each sample was added to conical flasks and put for centrifugation at 8000 RPM's for period of 15 min. Centrifugation unit type was Heraeus, Biofuge Primo- Thermo scientific. Centrifuged samples,were further filtered. Filtration was done using 2 pieces of 0.45 µm paper filters (GF/C) for better liquid-solid separation(Ydestebø,2012). Part of the supernatant was further put to titration and part was frozen for Ion Chromatography test and COD analysis. Titration sample was 50 mL both for the Buffer tank and digester sludge with 5 and 10 times dilutions respectively. Acid used for titration was 0.02 M HCl and for the pH adjustment was used solution of NaOH 0.045 M.

Data obtained from the titration procedure were incorporated into TITRA 5 software application for determination of the VFA and alkalinity concentrations as mg/L acetic acid and mg/L of H_2CO_3 alkalinity respectively.

Totals Solids (% mass weight) content of the samples was determined using the instrument Sartorius-Thermo control oven. Clean aluminum plate was first put for measurement on an analytical balance ,type Sartorius-basic. Approximately 2.5 g of sample sludge was poured onto the plate and put for dying at 105° C for approximate time period of 30 minutes. After the drying has finished the plate sample was measured on the balance an value was recorded for further determination of TVS i.e organic fractions of solids. TS content was read directly from the instrument. Further the sample was put for burning at 550°C in the Carbolite Furnaces oven for 2 hours. The sample was cooled down to room temperature and measured on the analytical balance.

TVS (% mass weight) concentration of sample was determined using following equation:

$$\text{TVS, as \% TS} = \frac{A - B}{A - C} \times 100\ (\%)$$

where:

A = weight of residue plus dish before burning,(g)
B = weight of residue plus dish after burning,(g) and
C = weight of dish, (g).

Chemical oxygen demand (COD) values were determined using closed reflux method (APHA et al., 2006). Total and Soluble (filtered) COD values were determined during the test.

For the Total COD determination 2g of sludge was diluted into 200 mL of distilled water. The solution was further homogenized using mechanical mixer. For the soluble COD samples were conserved with H_2SO_4 stored at the 4°C temperature until the actual measurement was done. Digestion solution was prepared by dilution of 10.216 g $K_2Cr_2O_7$, previously dried at 150°C for 2 hours into 500 ml distilled water, 167 ml concentrated H_2SO_4 and 33.3g H_2SO_4. These ingredients were dissolved, cooled to room temperature and diluted to 1000 ml. Sulphuric acid solution was prepared by adding 5.5 g Ag_2SO_4 per kg concentrated H_2SO_4 (ρ=1.84 kg/l), mixed and left to dissolve for 2 days.

Before the COD test start up the samples was diluted by 50 times for the buffer tank sludge and 20 times for the digester sludge. 2.5 ml of sample , 1.5 mL of digestion solution and 3.5 mL of sulphuric acid solution were poured into the clean vials. Further samples were put for digestion into heat reactor type HACH for a 2h period. The vials should be fasten tight before putted for digestion. When digestion process was finished the samples were left to cool down for about 30 min and than were submitted for the reading part. COD values reading was done using Spectrophotometer type (Spectroquant® Pharo 300, MERCK). Before the reading start up the instrument was calibrated standard solution. Standard was prepared by dissolving 0.425 g potassium hydrogen phtalate ($C_8H_5O_4K$) in 1000 ml distilled water. Standard strength equals COD value of 500mg/L. Blank sample was prepared using distilled water and clean vial free from scratches and impurities.

Ion chromatography tests were conducted for the determination of concentration of different volatile acids in the samples both from buffer tank and digester. The test was conducted using instrumentation type Dionex ICS-3000. Frozen samples were put to unfreeze and dilutions were prepared 20 and 10 times for the buffer tank and digester sample respectively. Each sample was filtered further, using 5 ml syringe and 0.2μm syringe filter. Approximately 2 ml of each sample was poured into small vials specialized for ion chromatography test. For the calibration purpose two different standards of 50 mg/l containing Lactic, Propionic and Formic acid and Acetic , Butyric and Valeric acid were prepared.

Methane potential tests and organic loading test:

Before the tests start up 3M NaOH solution was prepared for the CO_2 striping unit. Solution was prepared using 120g NaOH in 750 mL water. In addition Thymolphthalein solution was prepared using 40mg in 9 ml 99.5% ethanol and 1ml water added at the end. This solution was further mixed with the NaOH solution 5 ml/ 1L of NaOH, and serves role of pH indicator i.e when the NaOH solution becomes impaired the color will change from blue to colorless and the liquid is replaced.

For the test purpose freshly taken digester sludge,primary sludge and food waste, from SNJ were used and the TS and TVS content were determined in advance. Based on the percentage of solids in the substrates and inoculum , AMPTS II system automatically estimated the amount both for inoculum and substrate per reactor needed.
The inoculums to substrate ratio was chosen to be 2:1. The total volume of the reactors was 650mL. Each of the reactors was stripped with nitrogen gas to create anaerobic conditions. The striping gas contained 0 percent CO_2.

The average methane content for the test purpose in BMP system was set to average value of 60 and 65 % vol. in biogas produced. For both experiments the total amount of sample per reactor used was 300g. Temperature in the water bath was set to constant 37°C. The volume of the water inside the water bath was controlled in order to keep it constant. Mixing regime has intermittent character and was set to 43% from maximum speed with active mixing intervals of 180 sec. Finally ,the substrates were inoculated with digester sludge and the experiment was started.

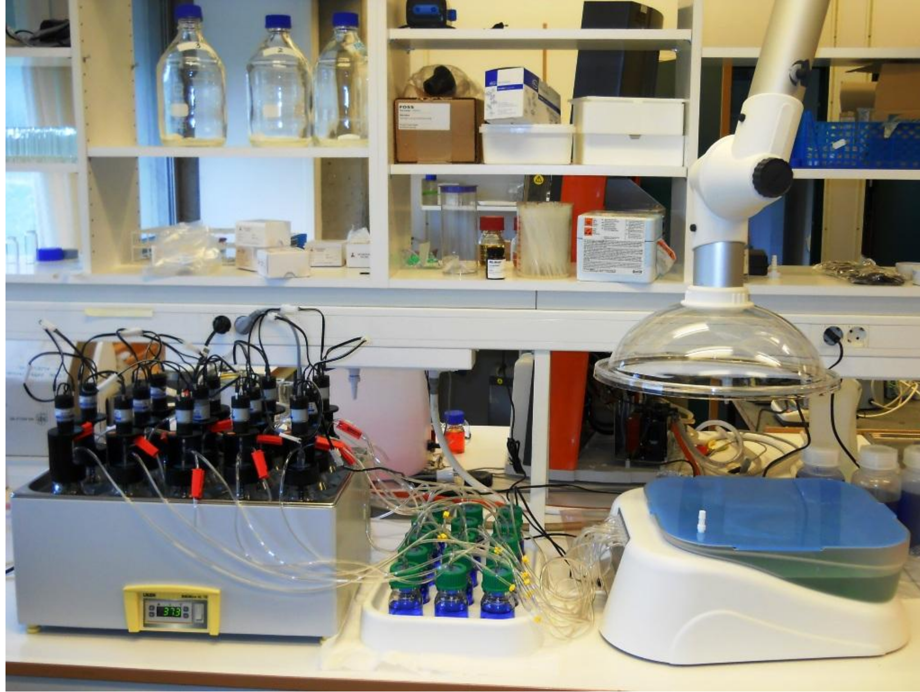


Figure13. Experimental setup for the methane potential and organic loading test.

The methane potential or specific methane production yield was determined using the following formula:

$$MP = (V_{substrate\&inoculum} - V_{inoculum} (\frac{Mincolum,sample}{Minocolum,blank})) / Mvs,_{substrate} \qquad (10)$$

where:

MP – is the normalized volume of gas produced per gram VS of substrate added (Nl/gVS)

$V_{substrate\&inoculum}$ – is the accumulated volume of gas produced from the reactor with both inoculum and substrate.

Vinoculum - is the mean value of the accumulated volume of gas produced by the blanks.

$M_{incolum,sample}$ – is the mass of inoculum in the sample (g VS)

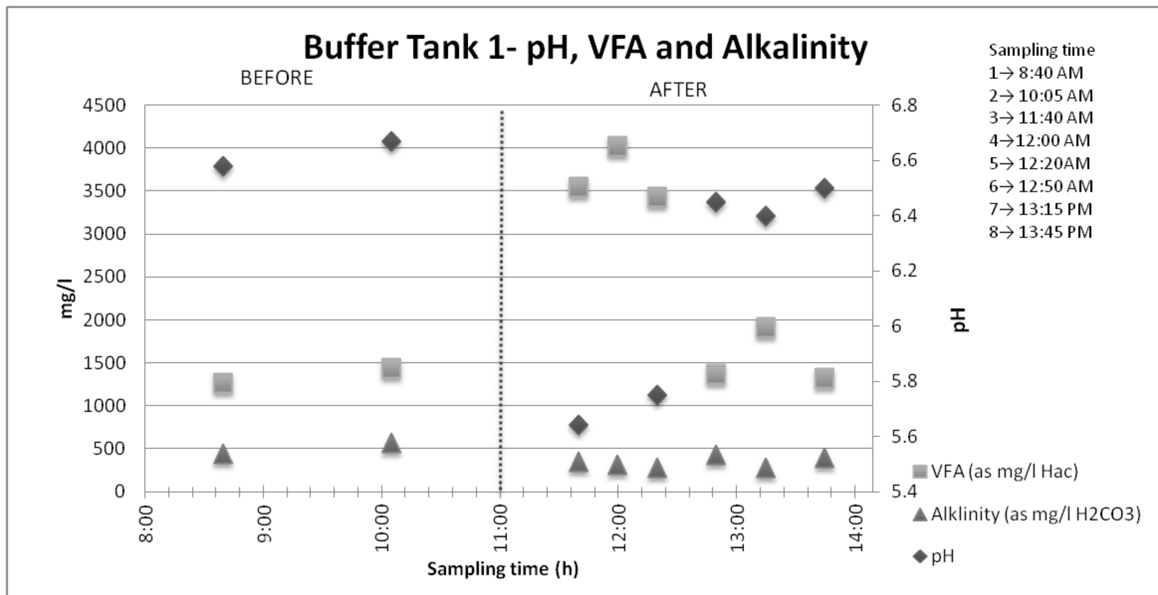
$M_{incololum, blank}$ – is the mass of inoculm in the blank (g VS)

$Mvs,_{substrate}$ – mass of substrate(mixture of substrates) in the sample.

4. RESULTS

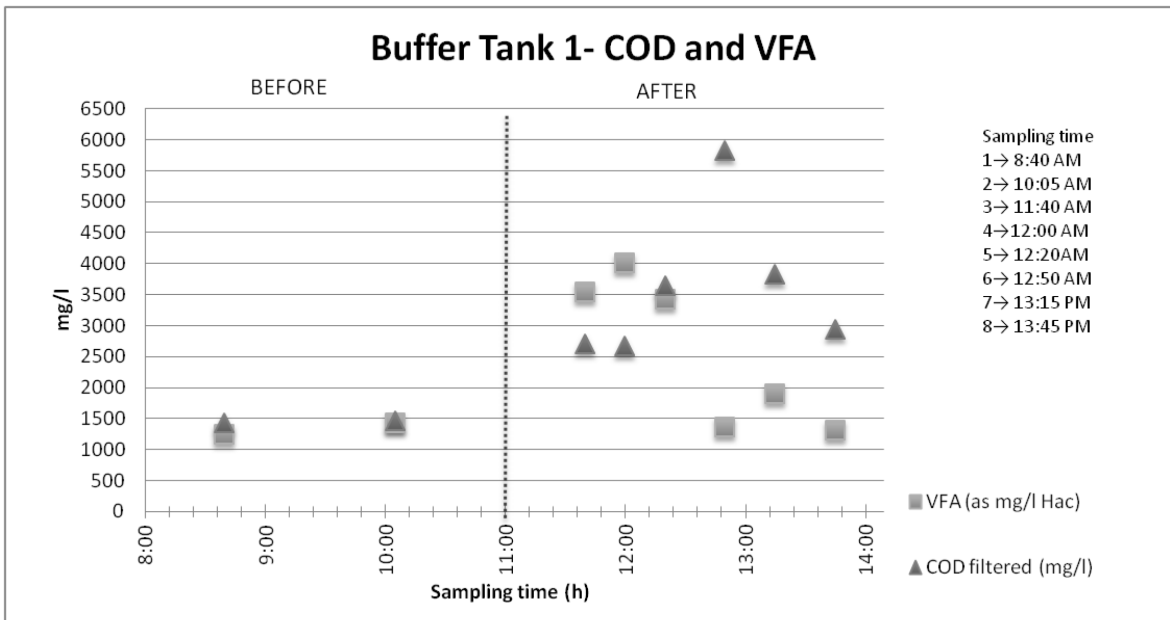
Full scale test No. 1 (20.01.2012)

pH was monitored right after the samples were taken and Graph 1 shows the behavior of Buffer Tank before and after the food waste is being feed.



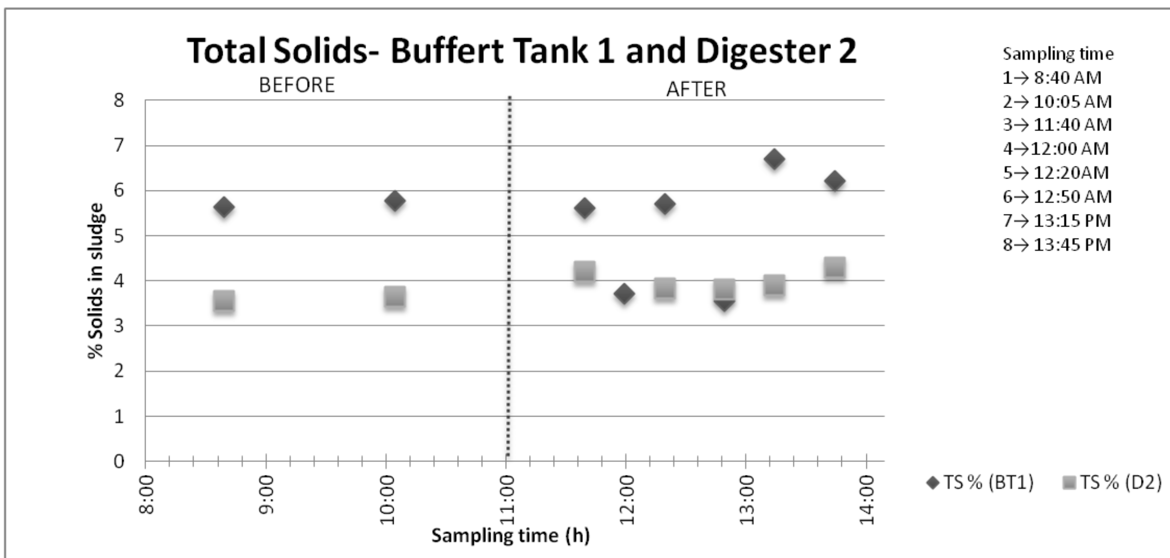
Graph 1. pH , VFA and alkalinity dynamics in the Buffer Tank 1.

The Buffer Tank is place where the initial steps of anaerobic digestion i.e hydrolysis and acidification take place and we see high dynamic in presented parameters. Right after the pulse we see increase in the VFA concentrations from around 1300 mg/l HAc (as acetic acid) to around 3500 mg/l. Simultaneously the pH drop from 6.6 to around 5.6 with trend to recover to previous values over the time. The alkalinity dynamics shows drop within the range from 500 mg/l to around 300 mg/l.



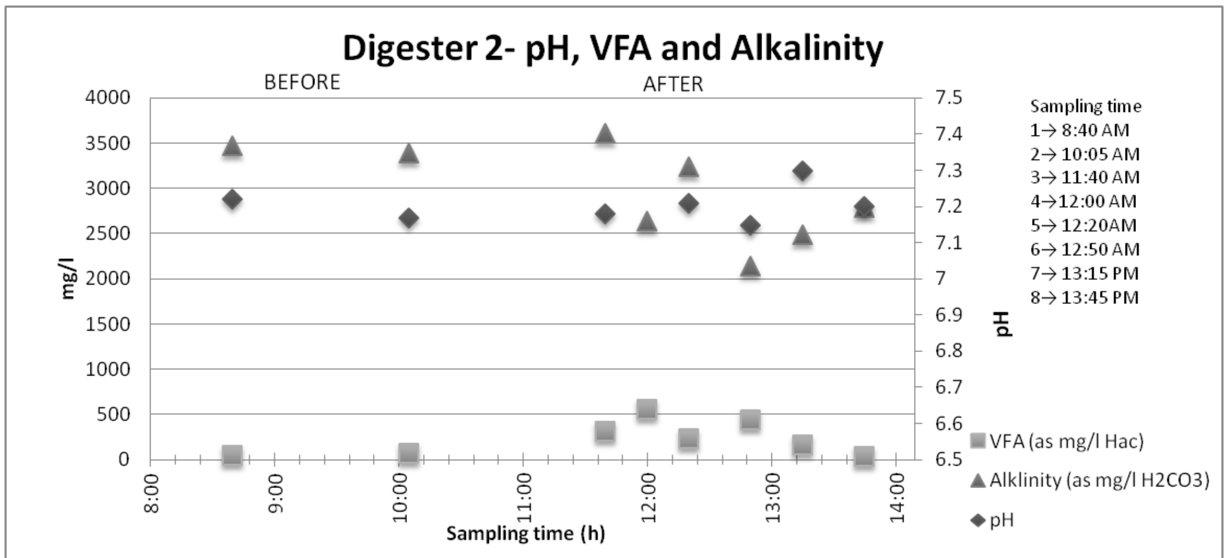
Graph 2. COD filtered and VFA dynamics in Buffer Tank 1

Graph 2 shows the dynamics between the VFA concentrations in buffer tank and soluble COD values. The soluble COD dynamics shows increase in the concentrations after the addition of food waste from background value of around 1500 mg/l up to 6000 mg/l. Bellow in the Graph 3 the dynamics in solids concentrations in the Buffer Tank 1 and Digester 2 are presented.



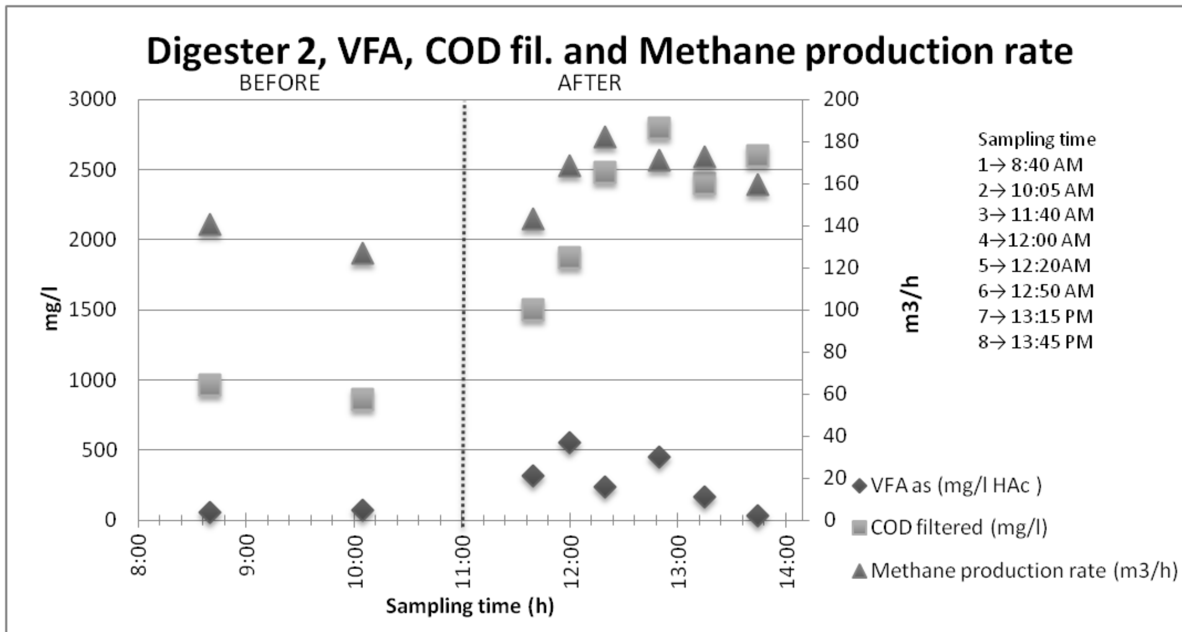
Graph 3. Totals solids concentration in Buffer Tank 1 and Digester 2

The TS values for the sample taken at 12.00 showed extraordinary high value of 13% which the author of this text suspect is due to analytical error with the instrumentation used for solids determination.



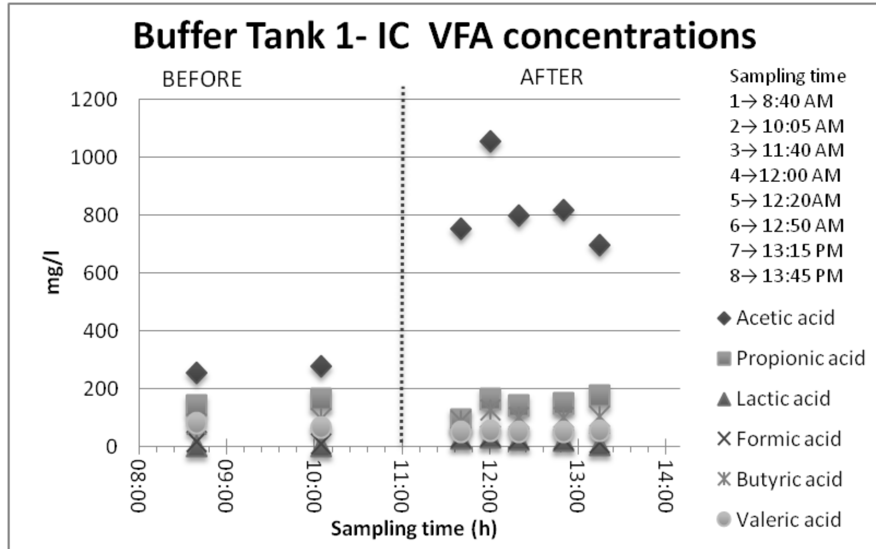
Graph 4. pH, VFA and Alkalinity dynamics in Digester 2

For the digester 2, the values of pH , before and after the addition of food waste is fairly constant and within the range of 7.1 to 7.3 (+/- 0.2) units. However we see rise in the concentration of VFA within the range from around 70mg/l before the feed pulse up to 550 mg/l after the food waste was pumped in the system. Alkalinity dynamics show decrease in the alkalinity concentration after the feed pulse within the range from 3500 mg/l down to 2200 mg/l as bicarbonate alkalinity. Tchobanogolous reported optimum range for alkalinity in the digester from 3000-5000 mg/l (Tchobanogolous et al.,2004).



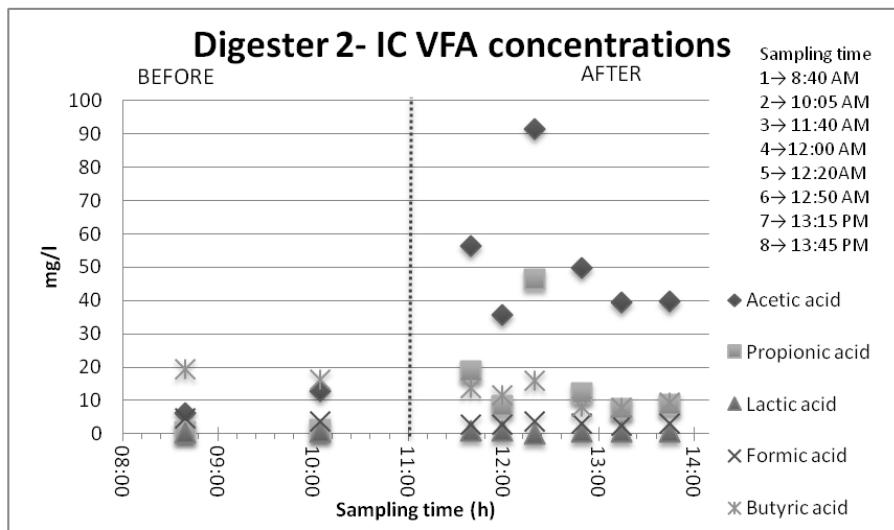
Graph 5. VFA, COD and methane production rate in Digester 2

Graph 5 shows the dynamics of the VFA, soluble COD and methane production rate. The graph above shows increase of the three observed parameters after the feed pulse. Concentrations of the soluble COD increase from a background value of about 850 mg/l up to 2800 mg/l and the methane production rate increase from background value of 127 up 182 m3/h.



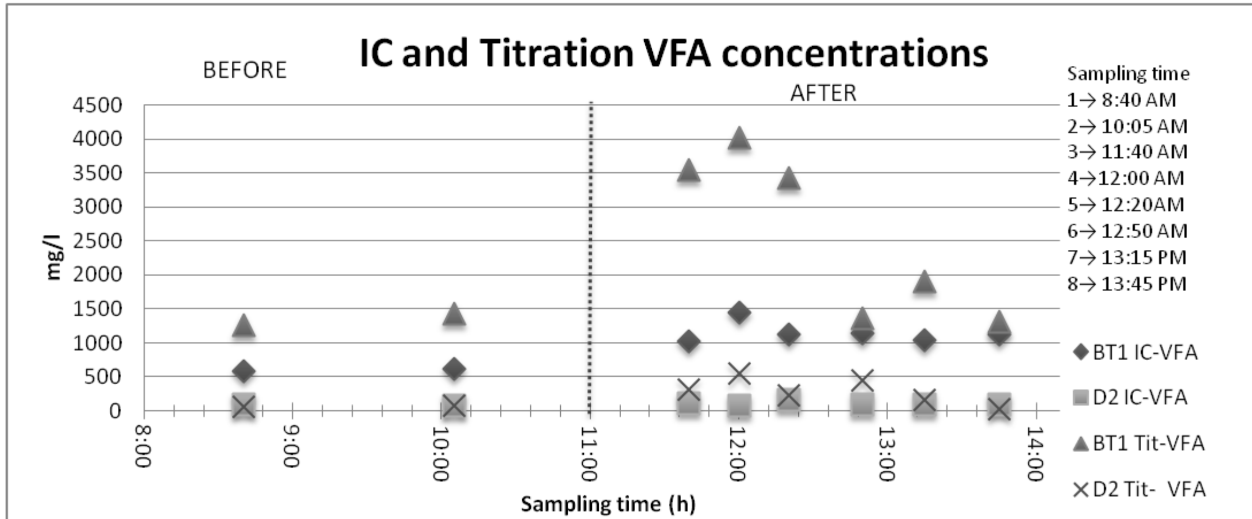
Graph 6. Volatile Fatty acids concentrations in Buffer Tank 1

The Ion chromatography test revealed the pool of concentrations for different fatty acids in the buffer tank. As can be seen from Graph . the two dominant acids are Acetic and Propionic. The pool of acetic acid dramatically increase after the addition of food waste. The average pool of acetic acid and propionic acid before the pulse feed is around 71% of total fatty acids concentration and this pool increase to 83 % after the pulse feed. The third most dominant acid is butyric acid and the rest of the acids are at trace levels.



Graph 7. Volatile Fatty acids concentrations in Digester 2

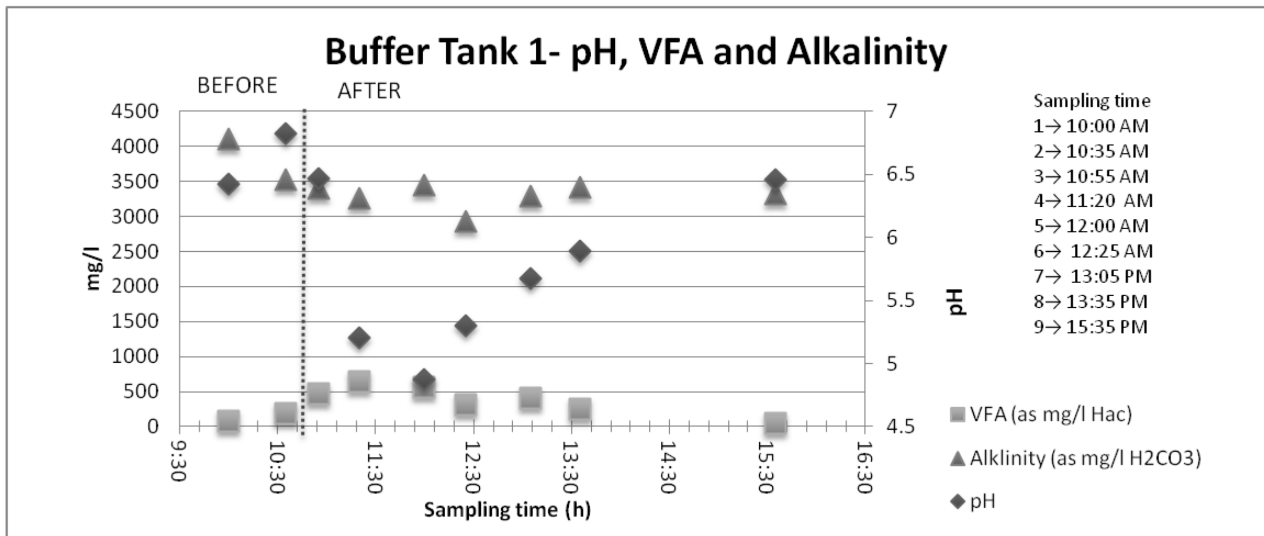
Picture in the Digester is somewhat similar. The concentrations of Acetic acid and Propionic sharply rise after the pulse feed. Unusual thing at this case is the extraordinary high value of Valeric acid that naturally comes from solubilization and fermentation of proteinaceous matter. Bellow in text a comparison is presented in Graph 8, regarding the total VFA concentrations obtained during the Ion chromatography test and 5 point titration for determination of VFA concentrations.



Graph 8. Ion chromatography and titration VFA concentrations

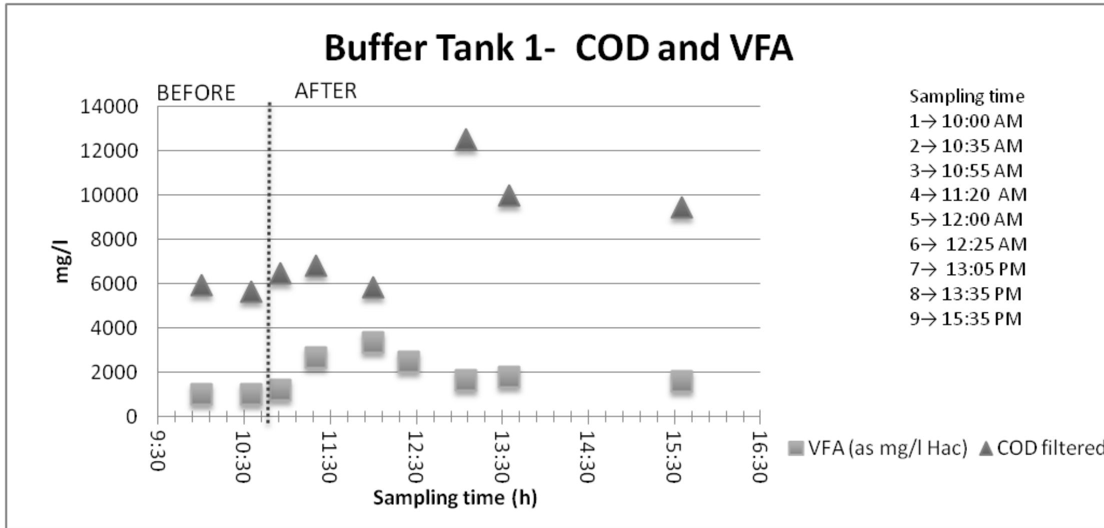
<u>Full scale test No. 2 (27.01.2012)</u>

The test conducted on 27.02.2012 revealed following dynamics of the process parameters.



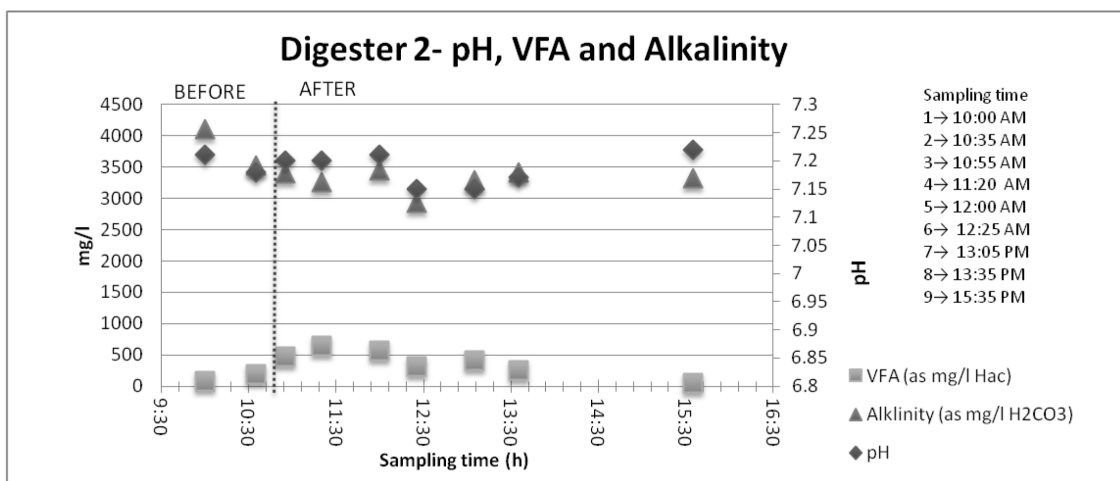
Graph 9. pH , VFA and alkalinity dynamics in the Buffer Tank 1.

Graph 9 reveals that concentration of VFA in the Buffer tank before the addition of food waste was around 180 and after rose up to around 650 mg/l HAc. pH values shows sharp decline from background value of around 6.8 to 4.87 after the feed pulse with trend to recover to background values over the time. Alkalinity concentrations shows decline of background values from around 400mg/l bicarbonate alkalinity down to 130 mg/l followed with trend of recovering to background values over the time.



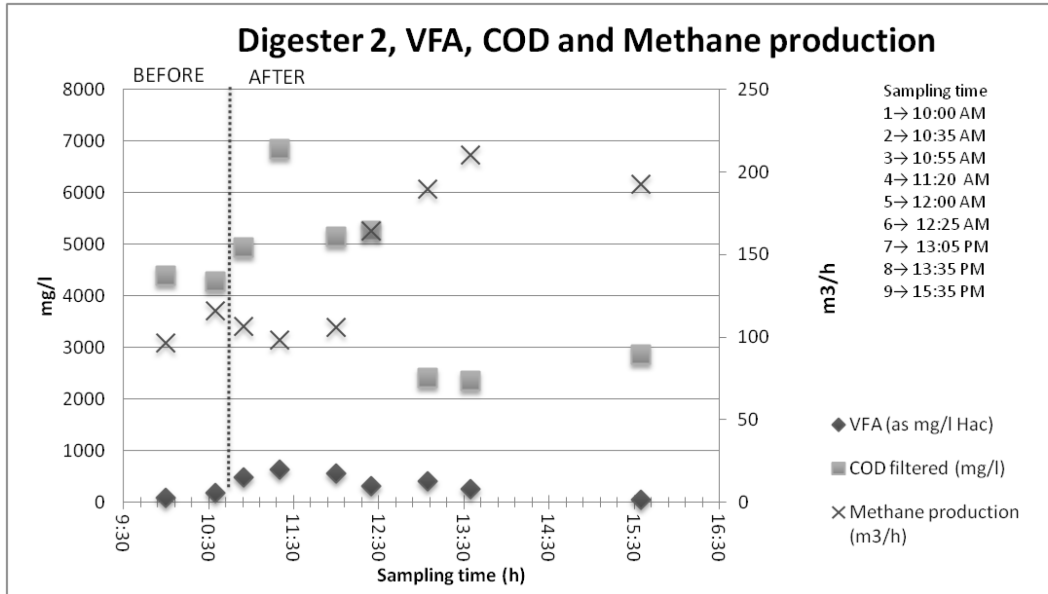
Graph 10. COD filtered and VFA dynamics at Buffer Tank 1

Soluble chemical oxygen demand (COD) values vary significantly before and after the food waste was feed to BT1. During the observed period of time the concentrations of COD rise from background values of about 5600 mg/l up to 12500mg/l which is more than double than the initial concentration.



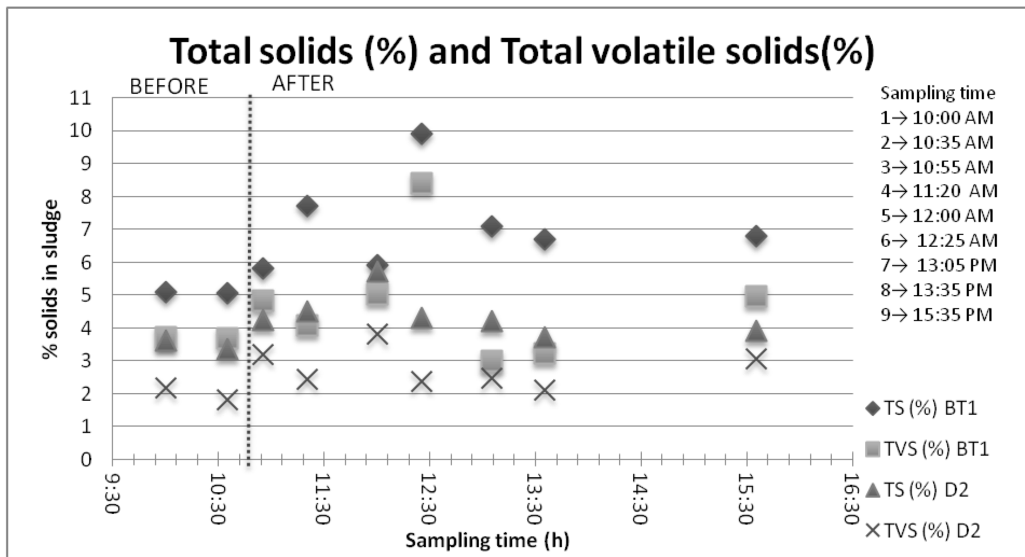
Graph 11. pH, VFA and alkalinity concentrations in the Digester 2.

Dynamics in digester 2 (Graph 11) reveal an increase in the VFA concentrations within range 100-640 mg/l HAc with quite insignificant variations in the pH within the range from 7.15 to.7.25. As result from the temporary overload the alkalinity decrease from background value around 4000 mg/l down to 2900mg/l. This trend is temporary under normal operation, and over the time the alkalinity concentrations restore to background values.



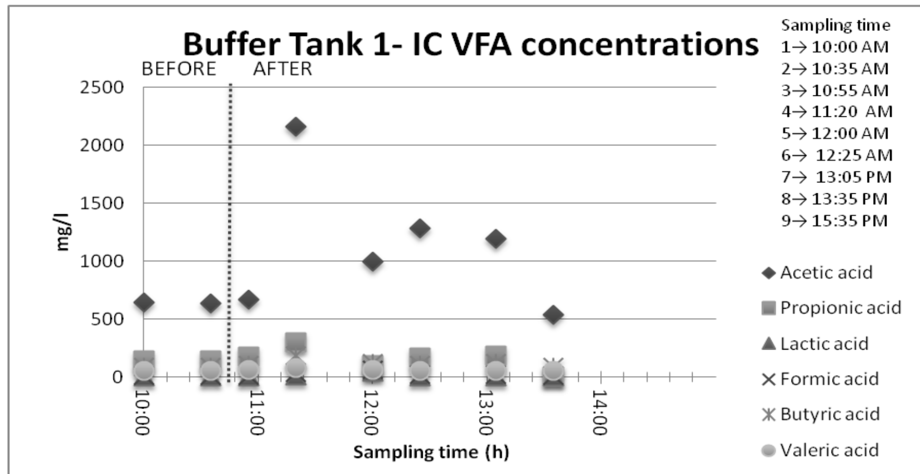
Graph 12. VFA, COD and Methane production rate in Digester 2

As a result of the addition of food waste the value of soluble COD rise for approximately 50% of the background values from around 4300 up to 6850 mg/l. Consequently the methane production rate increase from background value of around 115 m3/h up to pick value of 210 m3/h.



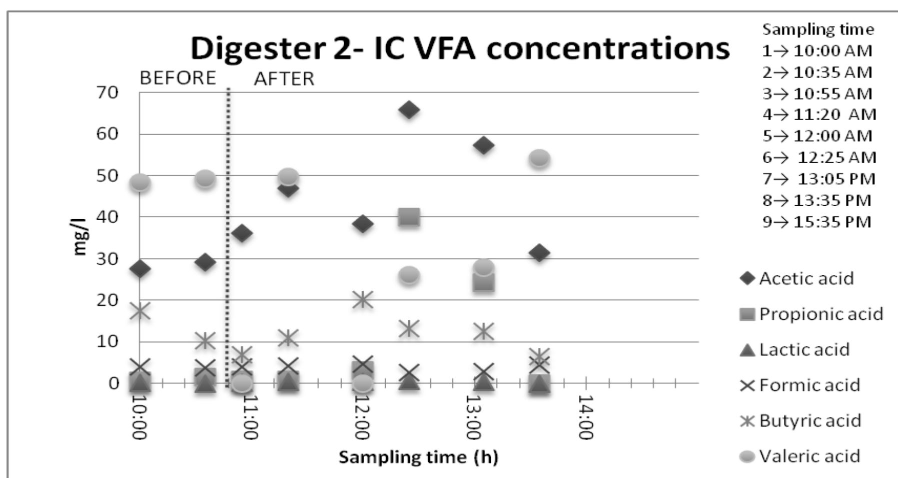
Graph 13. Total solids and Total Volatile solids concentrations

Graph 13 shows the dynamics in solids content in both buffer tank and digester sludge. From the graph one can observe slight increase in solids concentration in Buffer tank after the high solids food waste is being added. We see rise in the values from 5.1 up to 9.9 % of TS in the buffer tank. This trend in solids concentration rise is not so dramatic in the digester due to high volume and the values for TS vary within the range of 3.5 to 4.5 % solids. The TVS solids concentration show good correlation with the TS values and for buffer tank sludge average concentration of TVS vary from 70-80 and 50-60 % for the Digester sludge respectively. Full data for the solids concentrations from the full scale experiments are presented in the Appendix 1.



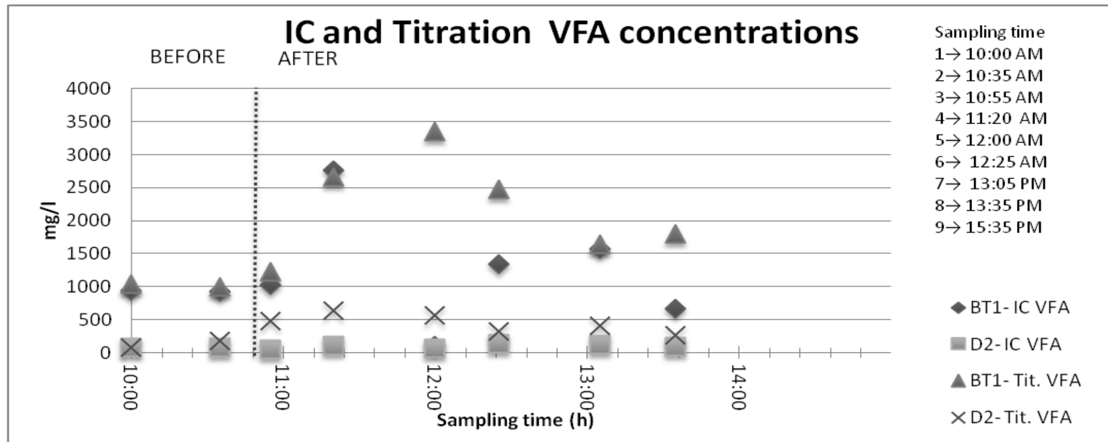
Graph 14. . Volatile Fatty acids concentrations in Buffer Tank 1

The data analysis on different VFA concentrations from the Ion chromatography test revealed similar behavior in the buffer tank as those from the first full scale test. The dominant pool, forms acetic and propionic acid. It is noticeable that the acetic acid concentration rise sharply after the impulse feed. The average pool of acetic and propionic acid is 84% from the total VFA concentrations.



Graph 15. Volatile fatty acids concentrations in Digester 2

The trend of sharp rise in the amount of acetic acid due to pulse feed of food waste can be clearly distinguished. Similar fashion is present for the propionic and butyric acids.

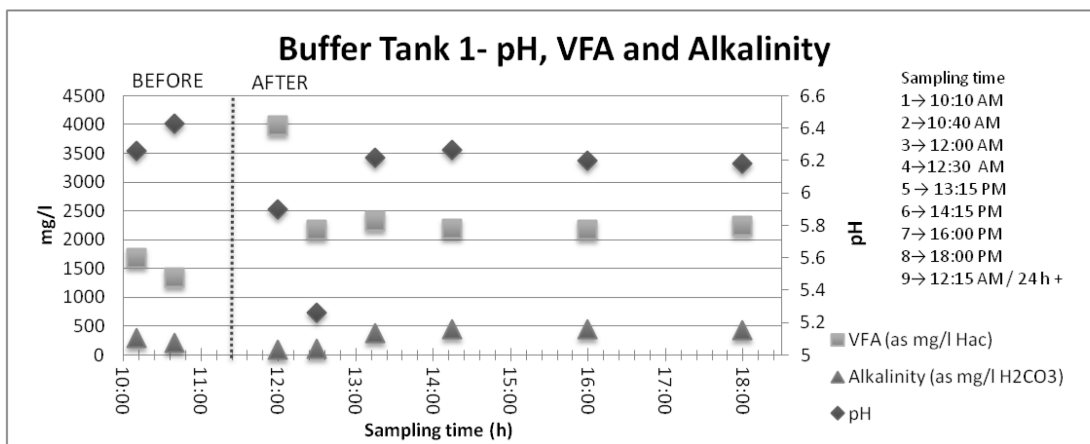


Graph 16. Ion chromatography and titration VFA concentrations

Graph 16 show some deviation between the VFA concentrations from IC test and titration method. These deviations might occur because the analytical method for preparation of IC test is far more complex than titrations method and possible errors during sample preparations should not be excluded.

<u>Full scale test No. 3 (02.03.2012)</u>

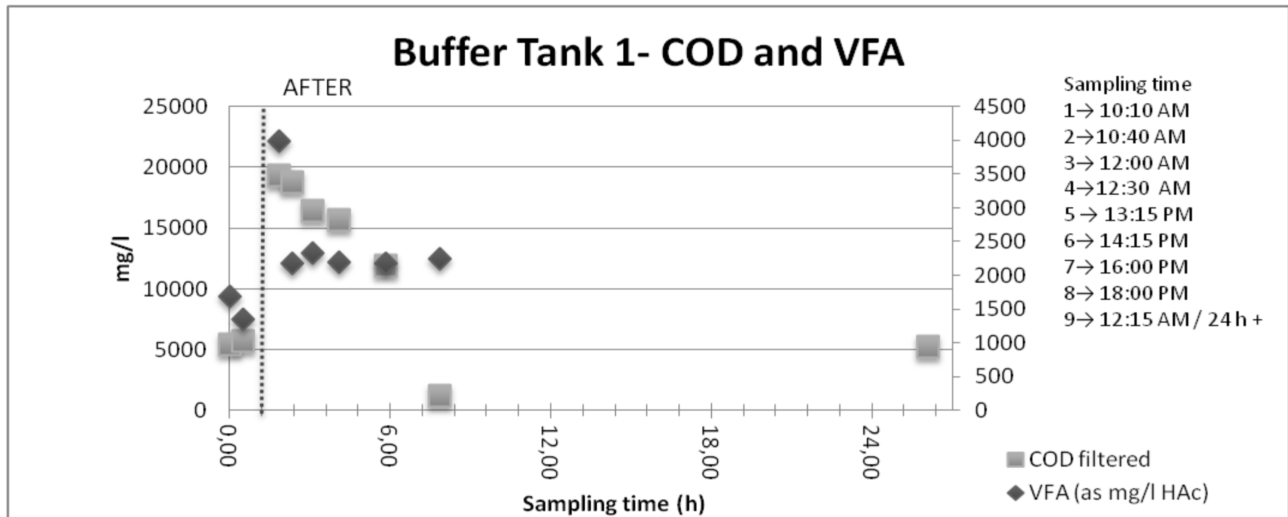
Full scale test number 3 revealed interesting findings regarding the anaerobic digestion process.



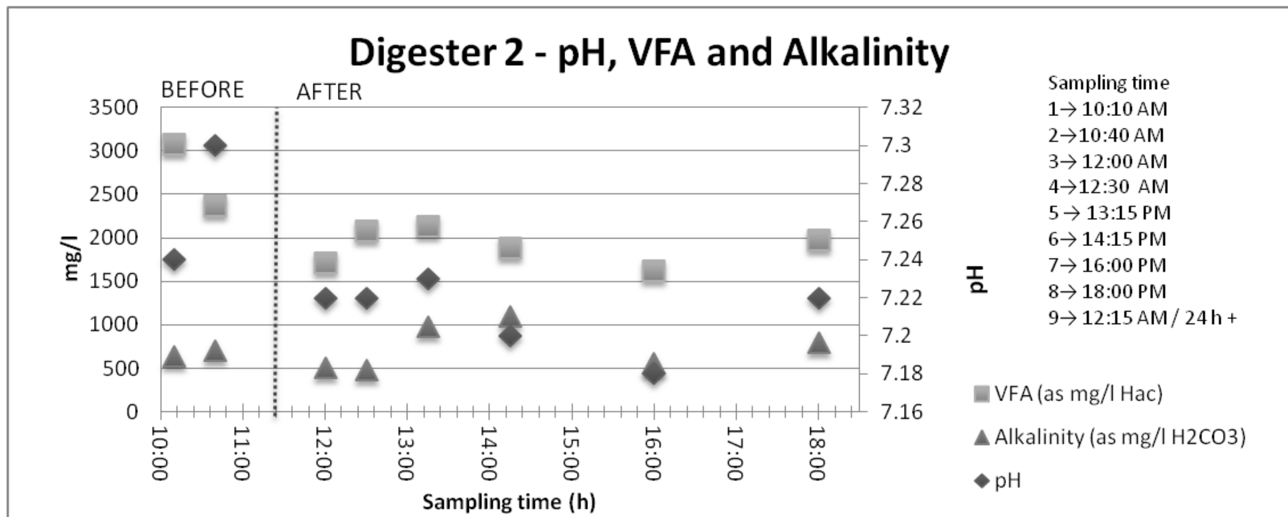
Graph 17. pH , VFA and alkalinity dynamics in the Buffer Tank 1

Dynamics of the observed parameters from the buffer tank sludge reveal increase in the VFA concentrations from the background value of around 1400 up to 4000 mg/l HAc right after the pulse feed. Consequently sharp drop can be seen in pH value from 6.3 down to 5.3 as well as drop in alkalinity concentrations from around 300 down to 100 mg/l for the same period of time. The trend of recovery in pH and alkalinity one can observe in similar fashion as within previous full scale tests. In similar fashion one can observe sharp rise in the concentration of the soluble COD(Graph18).

This increase is almost 400% if compared background values before and after the food waste addition. However, as can be seen from the Graph 18 COD concentration over the time start to decrease reaching the background value after the period of 24 hours.



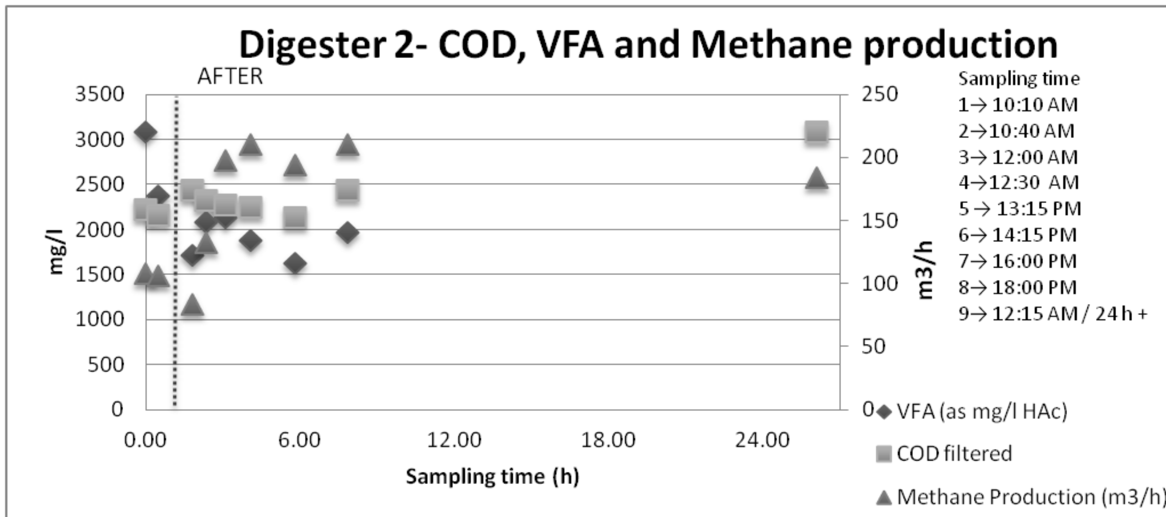
Graph 18. COD filtered and VFA dynamics at Buffer Tank 1 over the period of 24 hours



Graph 19. pH, VFA and alkalinity concentrations in the Digester 2

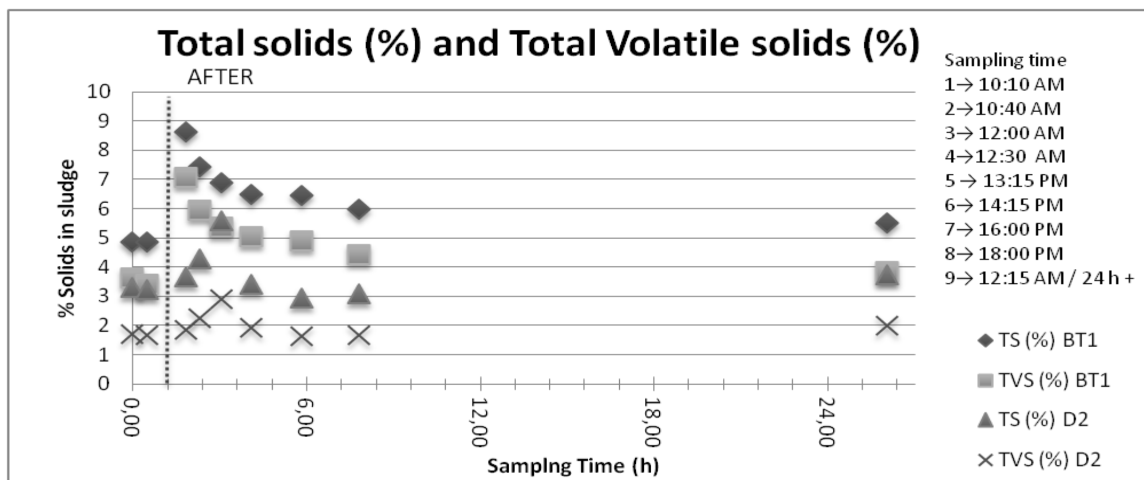
Graph 19 describes the negative trend that was ongoing during the test. From the figure it can be seen that the alkalinity concentration at the digester range from 600 up to 1100 mg/l which is almost 75% less compared with the average concentration from previous full scale test with minimum value of 484 mg/l recorded after the pulse feed. At the same time VFA concentration vary within the range 1700 mg/l up to 3100 mg/l. Actual concentrations recorded during the test are approximately 500% higher than the peak values measured during the previous test. The pH level vary within the range from 7.15 up to 7.3. What actually one can see at this graph is the acidification of the digester that resulted in gas production inhibition over the next couple of

days. The reason for such adverse trend is the failure in the digester heating control . This failure resulted in uncontrolled continuous temperature rise over 50°C. Considering the fact that the methanogenic population in the digester at the plant is adapted to mesophilic conditions could not cope with this temperature rise and becomes inhibited. As a consequence we see accumulation of VFA and consumption of alkalinity. More about the effect of temperature on methanogenic activity can be found at (M.H. ICA et al. 1993).



Graph 20. VFA, COD and methane production rate at Digester 2 over 24h period

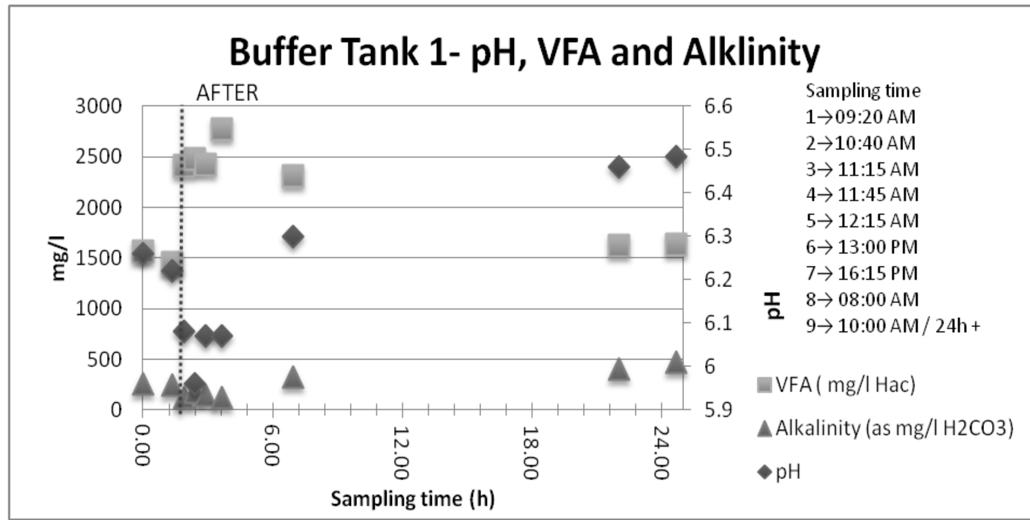
As can be seen from the Graph 20 , the concentration in the VFA is increasing over the 24 hours period and there is a slight decrease in methane production rate. However, although the negative trend is ongoing a clear distinction can be made on the rise of the methane production right after the pulse feed in the digester. The feeding rate to the digester over the 24 hour period was 19 m3/h averaged.



Graph 21. Total solids and Total Volatile solids concentrations over 24h period

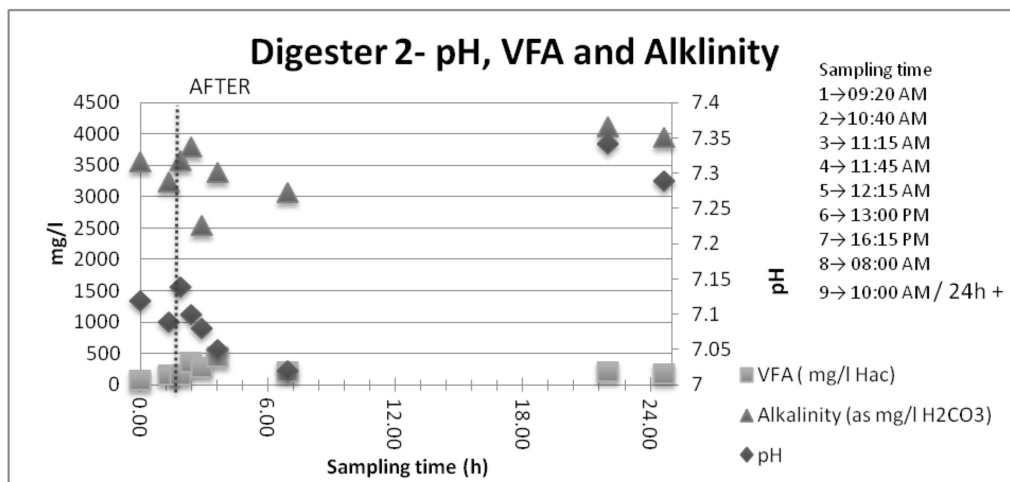
Pulse feed of food waste with high solid content (around 30%) into buffer tank results in increased solids concentration that returns to approximately same background solids concentration after period of time over 24 hours.

Full scale experiment No. 4 (16.04.2012)



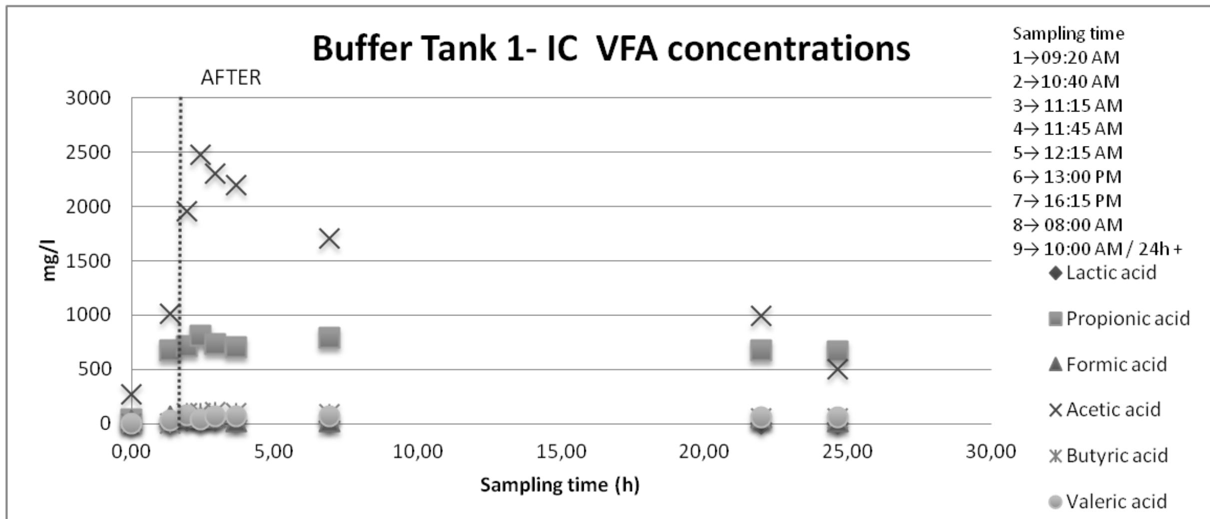
Graph 22. pH , VFA and alkalinity dynamics in the Buffer Tank 1 over 24 hour period

Data analysis from the test revealed similar trend as in previous tests did. After the pulse feed one can observe increase in VFA concentration from background value of around 1500 mg/l up to 2770 mg/l. Consequently the alkalinity concentration start to drop from background value of 260 mg/l down to 120 mg/l. Simultaneously after the pulse feed pH start to drop reaching the lowest value of 5.96 followed by recovery trend over the 24 hour period reaching somewhat higher values than the background values before the pulse feed.



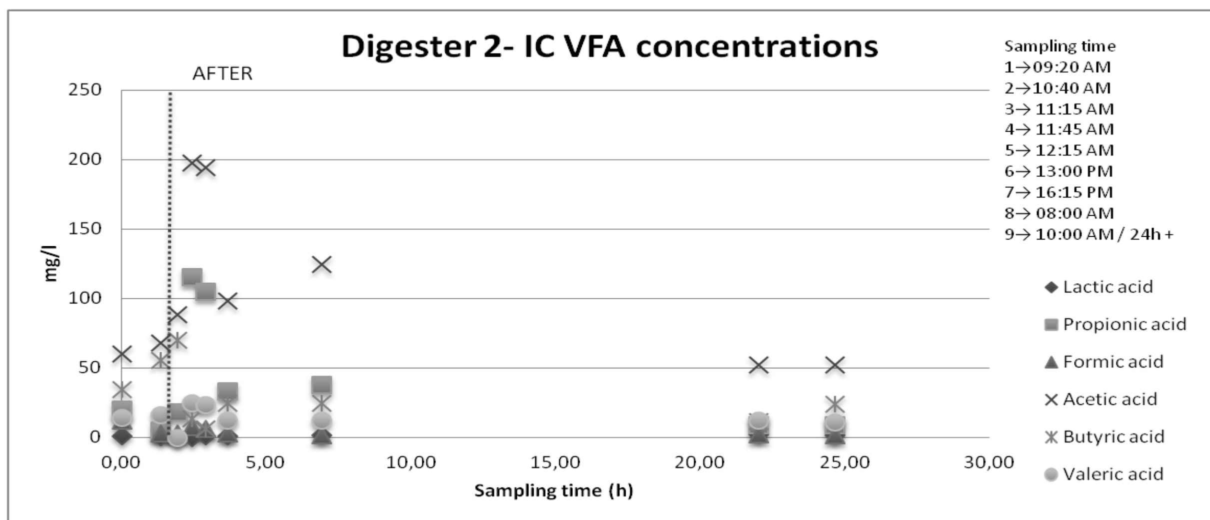
Graph 23. pH, VFA and alkalinity concentrations in the Digester 2 over 24 hour period

Graph 23. shows the dynamics of the observed parameters in digester over the period of 24hours. One can notice that after the pulse feed VFA concentration rise from background values of around 80mg/l up to 450 mg/l HAc reaching the background values after the period of 24 hours. Same trend can be observed for the pH and Alkalinity. Thus , after the pulse pH drop from values around 7.1 to around 7 with trend to recover over the time. The alkalinity concentration decrease from background value of around 3500 down to 2500 mg/l followed by the same recovery trend over the 24 hour period.



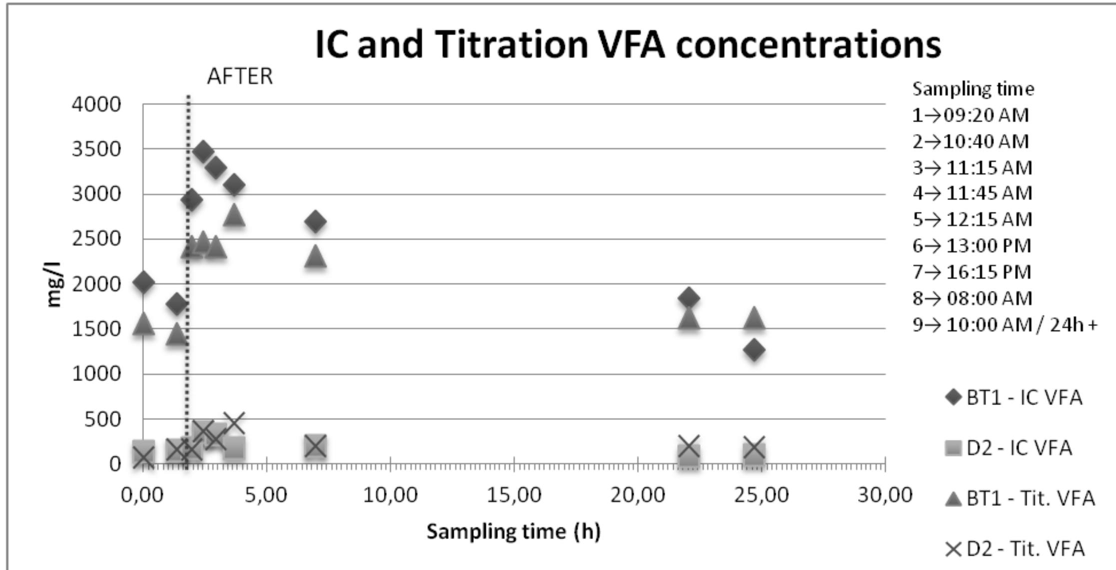
Graph 24. Volatile Fatty acids concentrations in Buffer Tank 1

The ion chromatography test revealed dramatic increase in acetic acid concentration right after the pulse feed of food waste. The pool of propionic and acetic acid is 93% and the pool of acetic acid is 60% on average from total VFA concentrations. The pool of all different fatty acid is at trace level.



Graph 25. Volatile fatty acids concentrations in Digester 2

Similar situation with the VFA concentrations revealed the ion chromatograph test from the digester samples. After the food waste pulse feed the pool of acetic acid and propionic acid rise from around 55% up to maximum of 89% from the total fatty acid concentrations. Third most dominant concentration is the butyric acid with average pool of 18%.



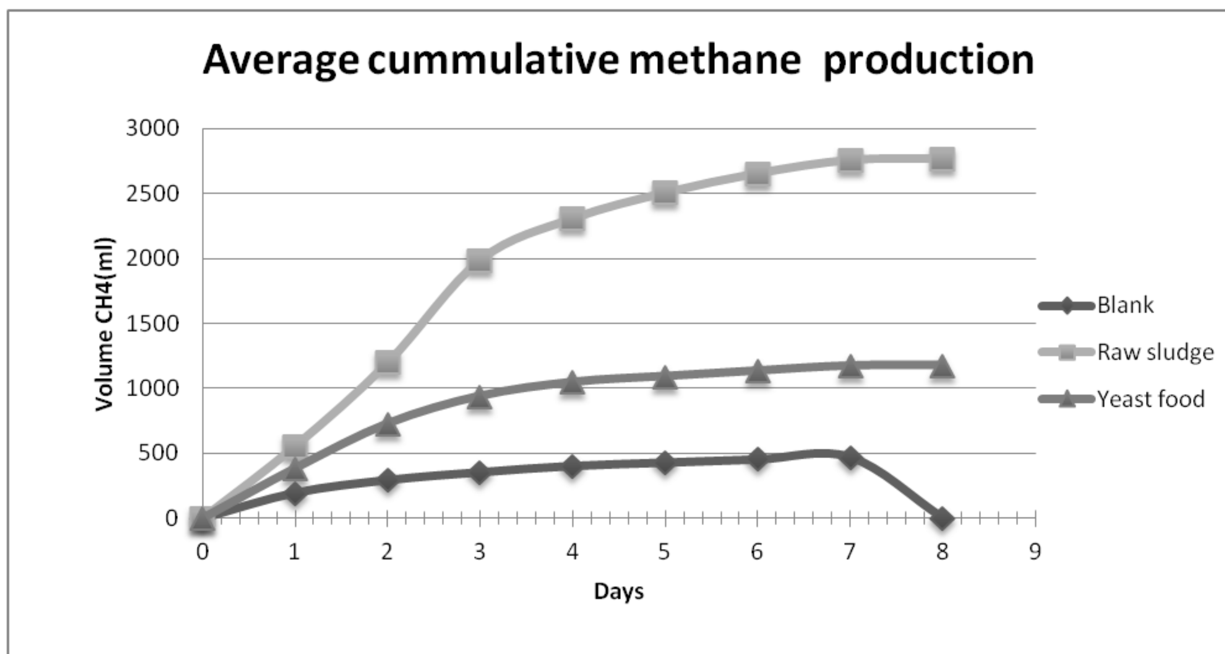
Graph 26. Ion chromatography and titration VFA concentrations over 24 hours period

Biomethane potential tests (BMP)

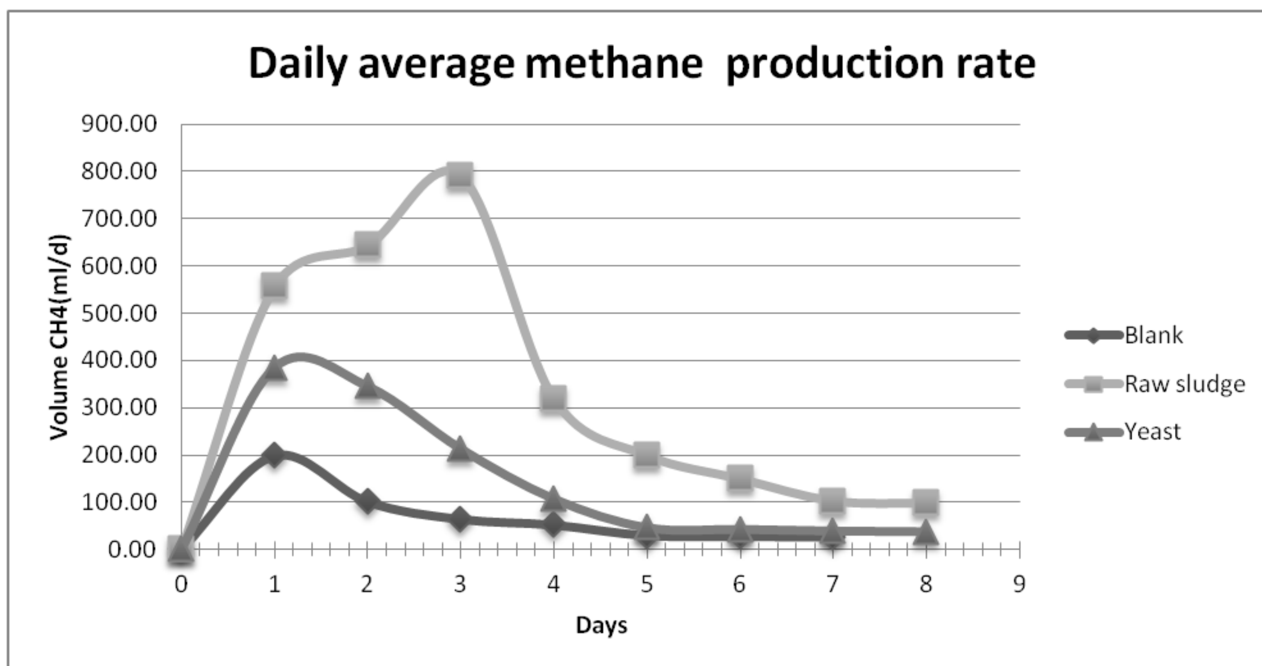
Biomethane potential test №. 1 was conducted with purpose of determination volume of methane production yield per gram VS of organic substrates added. The test was conducted over 8 days period. Substrates used for the test was sewage sludge and yeast extract that serves the role of positive control. Blanks were used for determination of background gas production from the digester sludge that serves as inoculum in the test. Triplicates were used for the blank, sewage sludge and yeast with purpose of achieving higher reliability of the results. Results are summarized in Table 24. Graphs 27 and 28 give inside in the cumulative and daily methane production rate respectively.

Table 24 . Amount of substrates and inoculums used, gas production and methane yield- (BMP) 1.

	Blank (digester sludge)	Sewage sludge	Yeast extract
Percents solids (%) VS	2.15	4.5	3
Substrate VS amount [g]	0	2.6	2.37
Inoculum VS amount [g]	6.45	5.21	4.75
Inocolum/substrate ratio	2	2	2
Average daily methane production (ml/day)	62	317	135
Average cumulative methane production (ml)	496	2771	1178
Methane yield (ml CH_4/g VS added)	123	911	343



Graph 27. BMP test No. 1, cumulative methane production

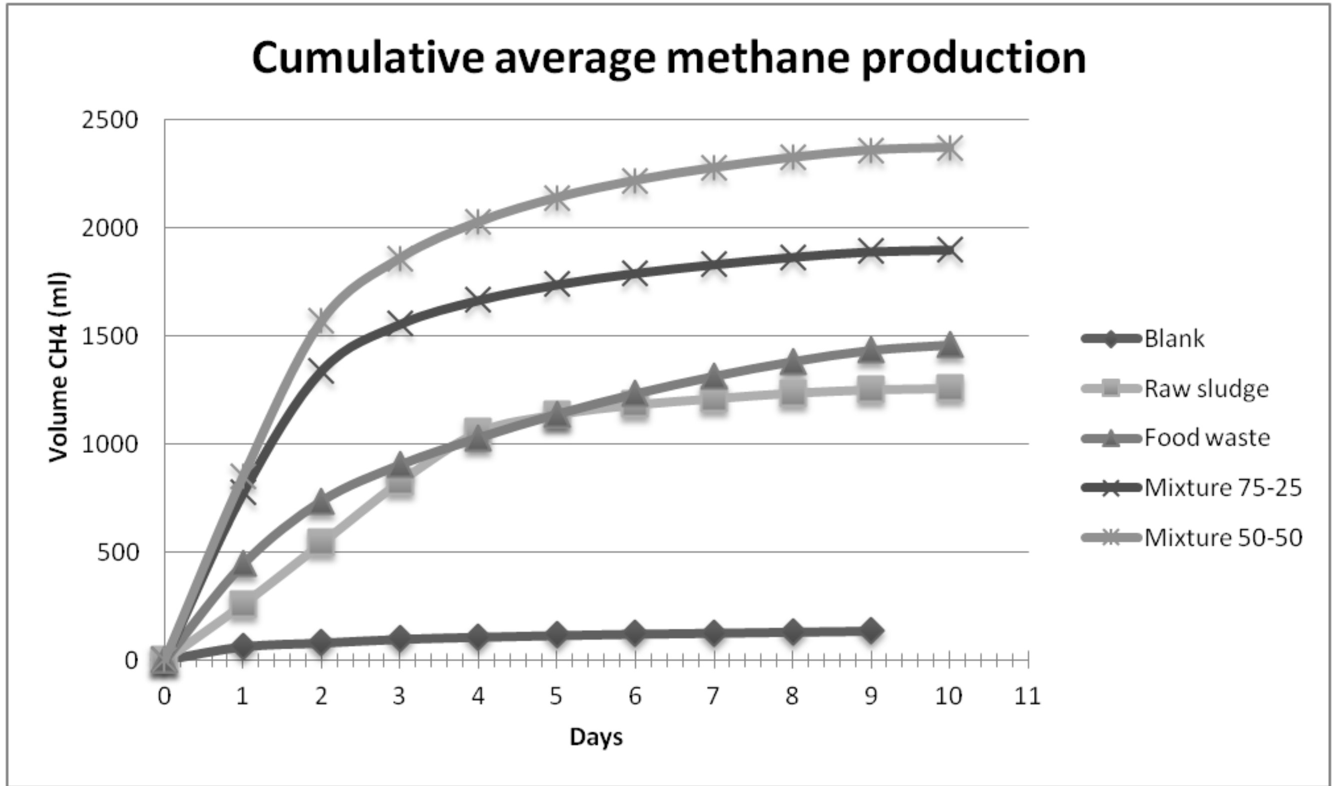


Graph 28. BMP test No. 1, Daily methane production rate

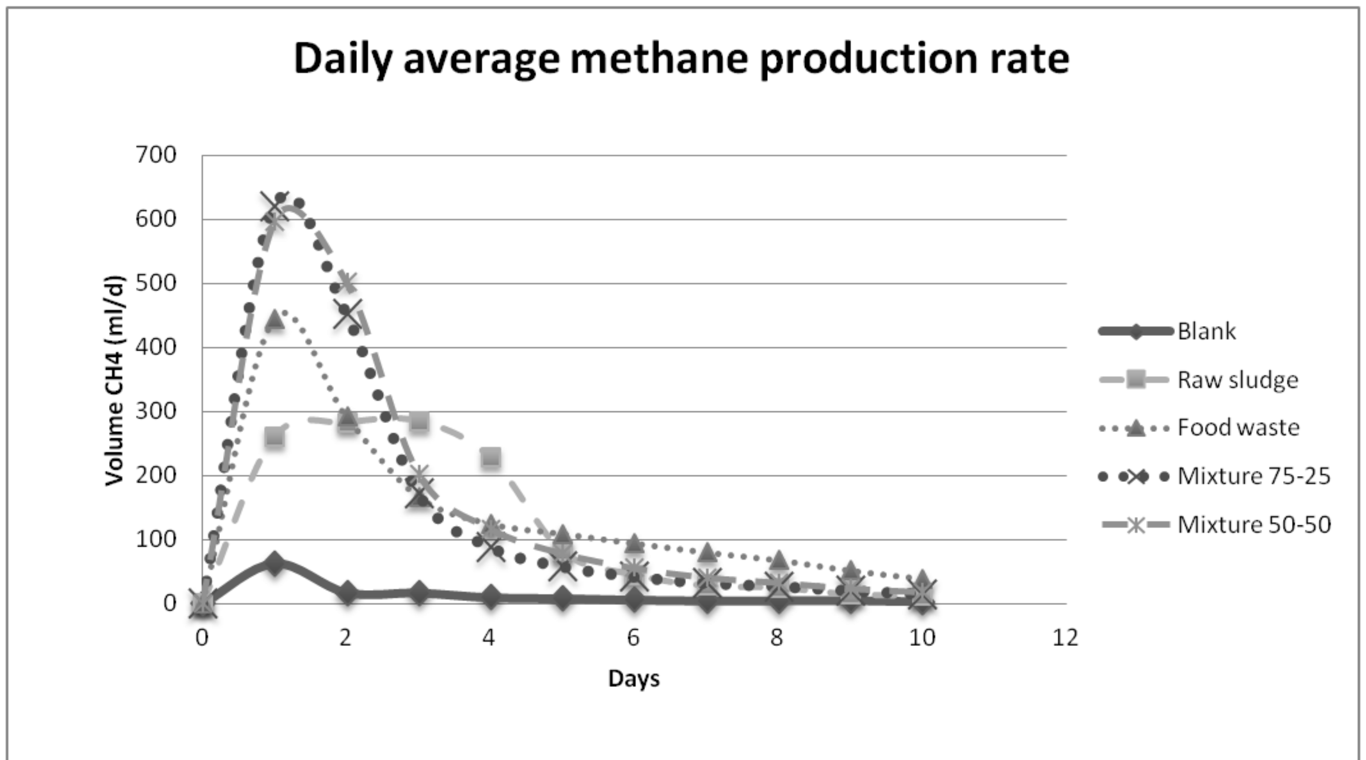
Biomethane potential test №.2 was carried throughout ten days period. Substrates used for the test purpose were: sewage sludge, food waste, mixture of sewage sludge and food waste 75-25% and 50-50% on a mass base weight . Triplicates were used for each of the substrates including inocolum and digester sludge. The experiment ended when daily methane production rate fell below 30 ml. Graphs 29 and 30 give inside in the cumulative and daily methane production rate from the BMP test No. 2 ,respectively.

Table 25. Amount of substrates and inoculums used, gas production and methane yield - (BMP) 2.

	Blank (digester sludge)	Sewage sludge	Food waste	Mixture 75-25 %	Mixture 50-50 %
Percent solids (%) VS	1.9	5.25	22.5	6.7	7.4
Substrate VS amount [g]	0	2.41	2.73	3.11	3.43
Inoculum VS amount [g]	5.7	4.83	5.46	4.82	4.82
Inocolum/substrate ratio	2	2	2	1.55	1.4
Average daily methane production (ml/day)	13	115	134	174	217
Average cumulative methane production (ml)	136	1259	1459	1900	2370
Methane yield (ml CH_4/g VS added)	30	475	486	572	657



Graph 29. BMP test No. 2,Cumulative methane production



Graph 30. BMP test No. 2, Daily methane production rate

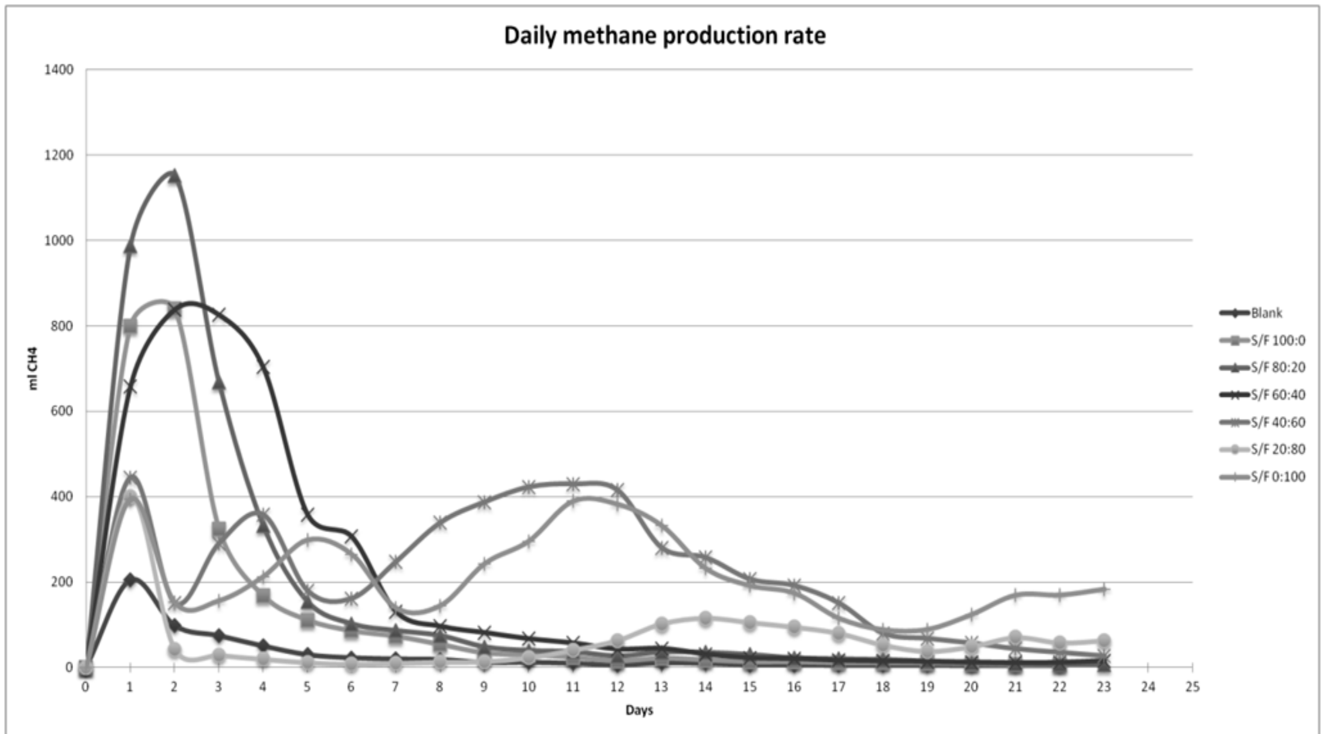
Organic loading test

The test was conducted for a period of 23 days. Different loadings were used use to determine maximum loading ratio substrate/inoculum for enhanced methane production. The evaluated ratios used in experiment (gVS substrate/g VS inoculum) were increased in the range from (1.37:1,1.73:1,2.1:1,2.48:1,2.83:1,3.23:1), while the mass of inoculum and substrates used was kept constant at 200g and 100 g respectively. Results from the test are summarized in Table 26.

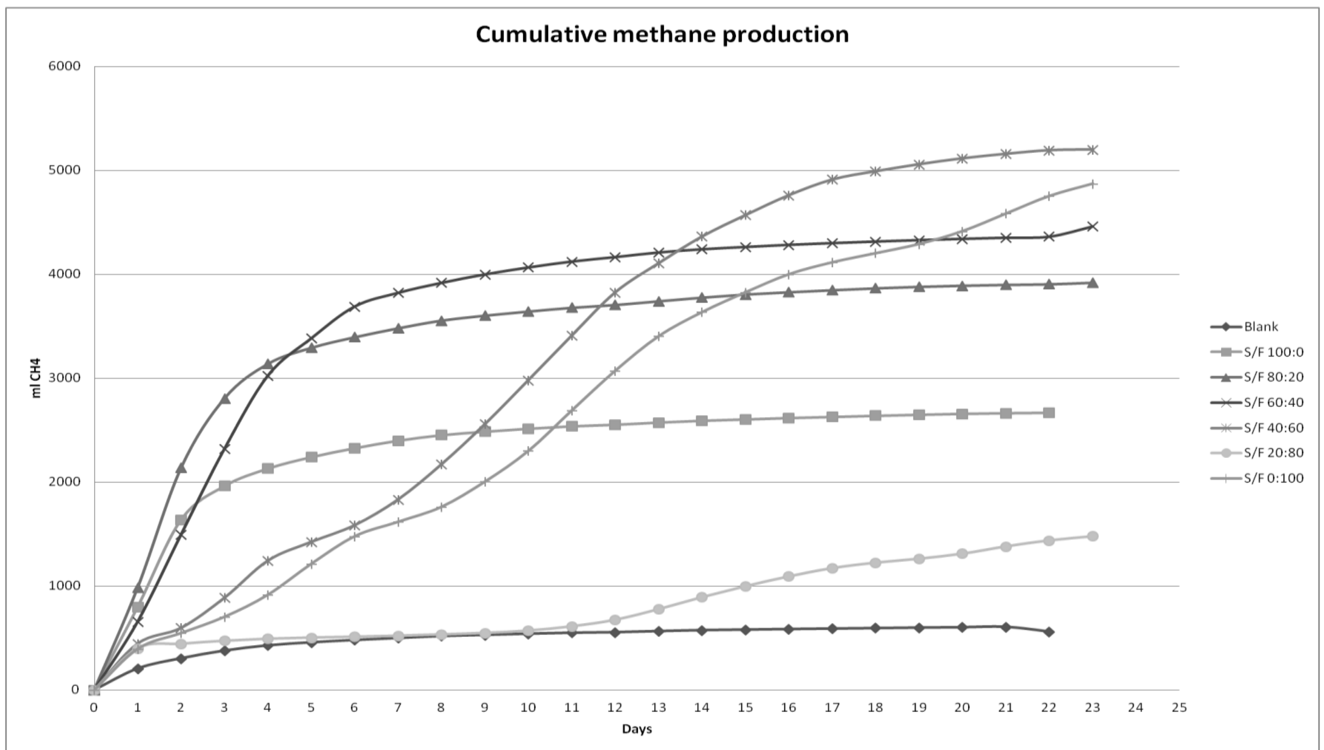
Table 26. Amount of substrates and inoculums used, gas production and methane yield- (OL)

	Blank (inoculum)	Sludge/Food 100:0	Sludge/Food 80:20	Sludge/Food 60:40	Sludge/Food 40:60	Sludge/Food 20:80	Sludge/Food 0:100
Percent solids (%) VS	1.85	5.1	6.4	7.8	9.2	10.5	11.96
Substrate VS amount [g]	0	5.1	6.4	7.8	9.2	10.5	11.96
Inoculum VS amount [g]	5.55	3.7	3.7	3.7	3.7	3.7	3.7
Inocolum/substrate ratio	0	0.72	0.57	0.47	0.4	0.35	0.3
Average daily methane production (ml/day)	27	116	163	182	217	63	206
Average cumulative methane production (ml)	607	2669	3905	4362	5201	1478	4871
Methane yield (ml CH_4/g VS added)	109	404	509	481	499	83	356

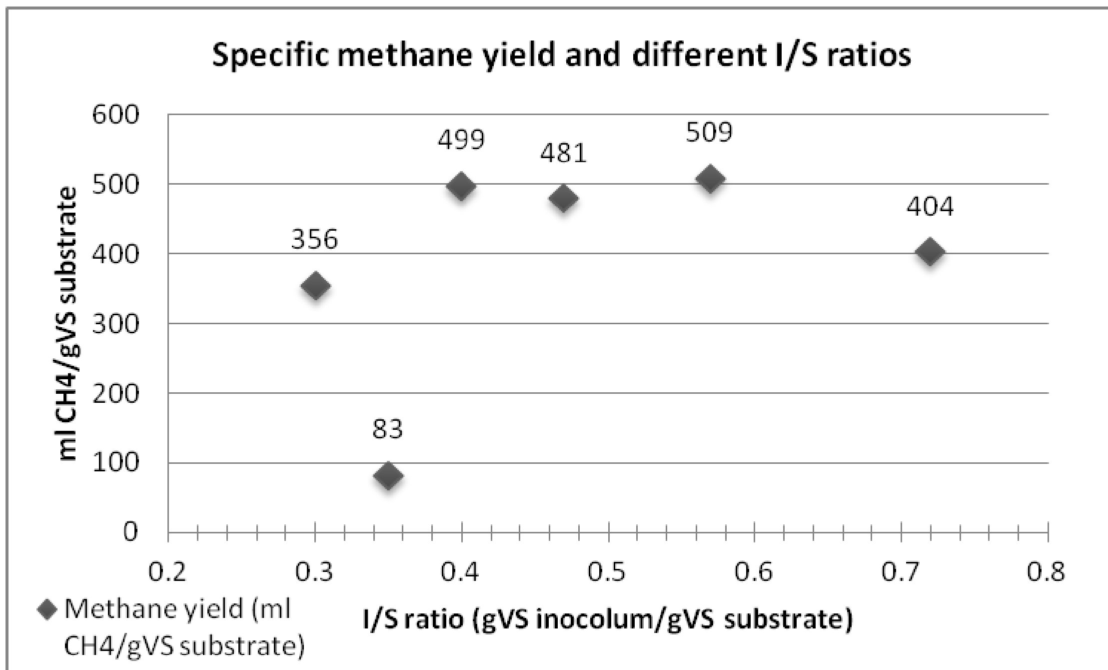
Graph 31 gives and insight in the cumulative methane production during the 23 days test period for the different organic loading ratios and Graph 32 gives an insight in the daily rate of methane production. Further Graph 33 and 34 desrcibe the relationship between the specific methane yield (ml CH_4/gVS substrate) and I/S ratios as well as dependance from the food content in the samples. Graphs 35 and 36 give insight between the cumulative methane production ,I/S ratios and different S/F ratios used in experiment.



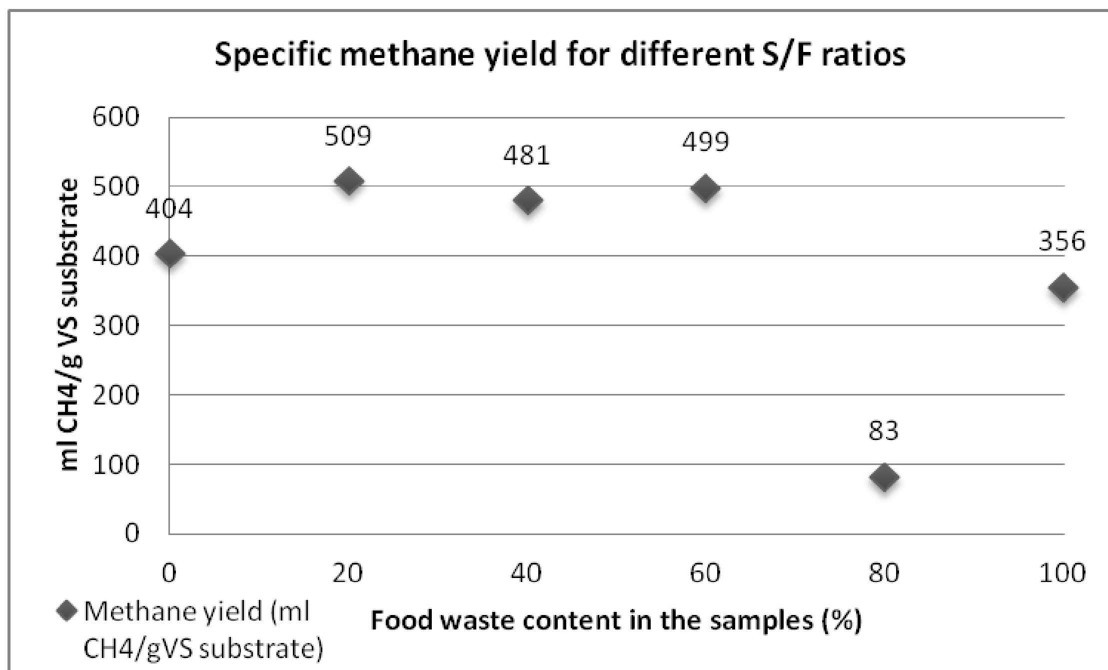
Graph 31. Methane production ml/day, OL test



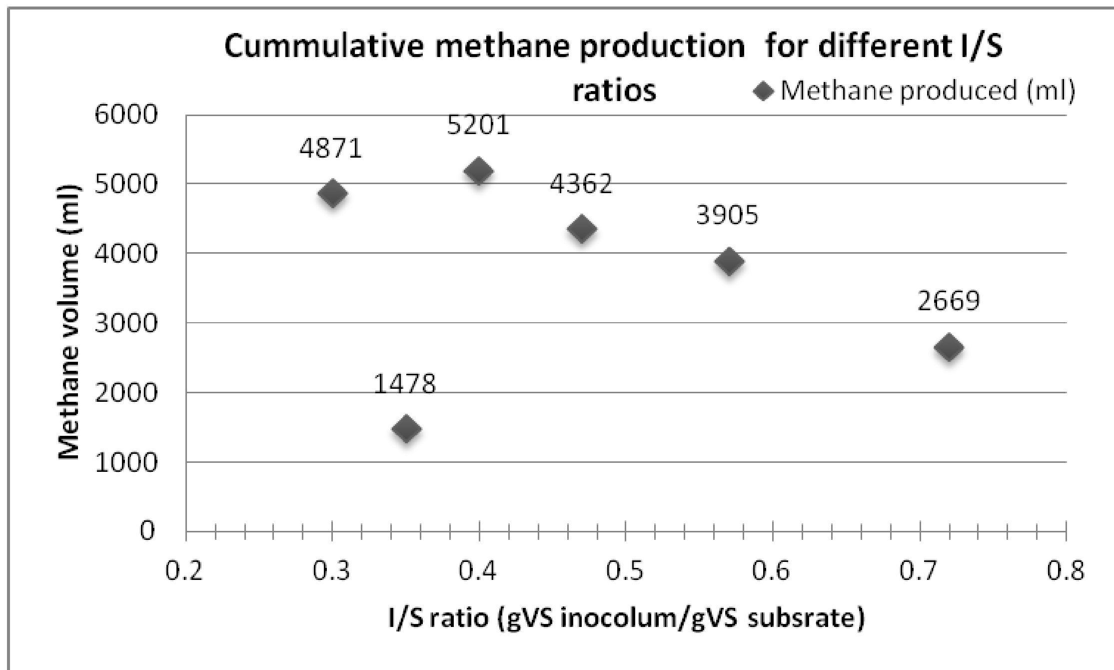
Graph 32. Cumulative Methane production (ml), OL test



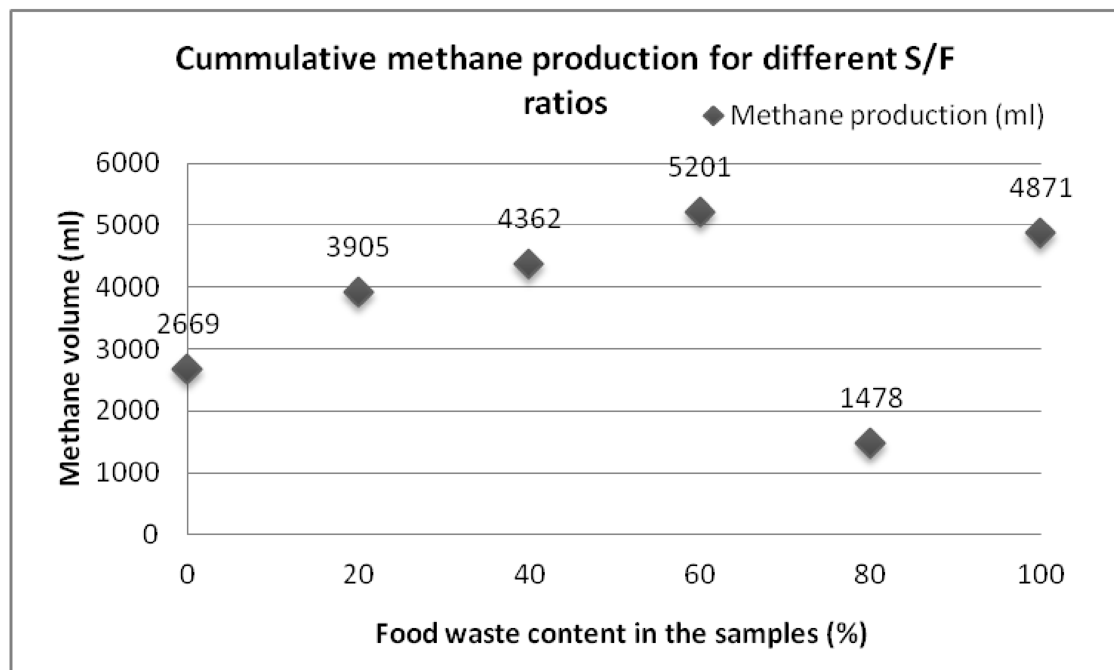
Graph 33. Specific methane yield and I/S ratios , OL test



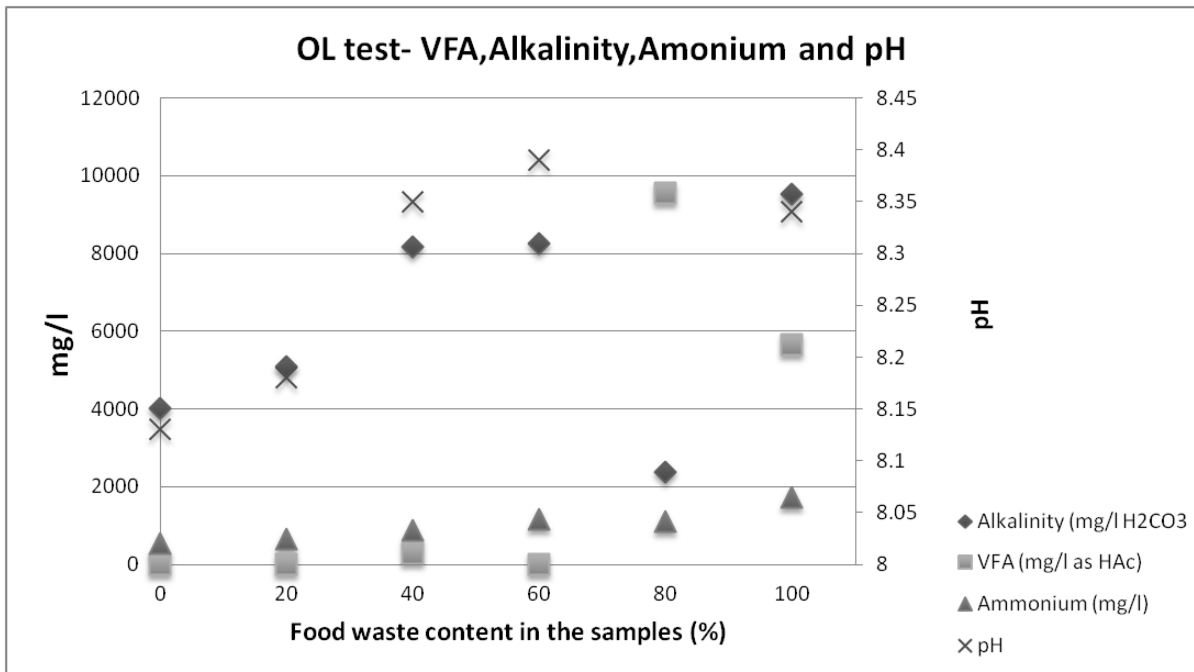
Graph 34. Specific methane yield for different S/F ratios, OL test



Graph 35. Total methane production for different I/S ratios, OL test



Graph 36. Total methane production for different S/F ratios, OL test



Graph 37. pH,Alklinity, VFA, and ammonium concentrations, OL test

5. DISCUSSION

The behavior and dynamics of the observed parameters described well the effect of temporary organic overload and the system (Buffer Tank and Digester) response. A distinctive and clear picture about the process status can be reported through the interrelationship between the monitored parameters.

The pulse feed of high solid organic content food waste in the buffer tank 1 resulted in increase in the VFA concentrations within the range from 1000 up to 4000mg/l HAc. As a consequence of the increase in the acids concentrations decrease in the alkalinity can be observed within the range from 500 down to 100 mg/l as H_2CO_3, resulting in sharp pH drop from background value (before the pulse feed) of 6.5 down to 5.0 and recovers in the following couple of hours.

The Ion chromatography test gave in insight into the dynamics of different fatty acids concentrations. After the pulse we see a sharp increase in the Acetic acid that is typical for system overload (Biomethanation II,2003). This can be explained through the fact that the Buffer tank itself is the place where hydrolysis and acidogenesis takes place. However, the dramatic and sharp rise in the acids concentrations can be partially connected with the fact that the food waste undergo thermal sterilization process treatment and partial hydrolysis and solubilization is to be expected before it is feed in the system.

Different pools of acids describes the process and microbial system health status(B. K. Ahring, 1995). Analysis of the IC test showed and average pool of 85 % for acetic and propionic acid and the rest pool of 15 % is represented by butyric (third most dominant), valeric, formic and lactic acid (Graph .6,14,24). For instance before the pulse the average concentration of HAc is around 515 mg/l and 1300mg/l for the rest of the observed period. Similar trend can not be observed for propionic acid and butyric acid because concentrations of these two main intermediates are quite stable. Aarrestad,(2011) reports high level of lactic acid in the buffer tank. However test conducted in this study failed to confirm reported findings.

Measured values for the soluble COD in (BT1) shows a significant correlation with the VFA concentrations. Thus, before the pulse we observe background values of 4300 mg/ on average, with increase up to 8500 mg/l for the rest of the observed period. Question may be raised why the total COD is not observed and the answer to this question lie in the fact that due to the high dilution it is more efficient to observe the soluble COD response to the pulse feed.

Dynamics in the Digester are somewhat similar as the behavior in the buffer tank with respect to the VFA and alkalinity concentrations. However this is not the case with the pH. Observed values for the pH in the digester exhibits quite stable trend with variations within values from 7.1 to 7.3. This is considered to be and optimal operating range for the digesters (Gerardy,2003). However it is very interesting to appoint one fact regarding the pH behavior in the Digester. Due to the acidification of the digestion that was observed when conducting the third full scale test

one may expect that due to dramatically increased VFA concentration and alkalinity drop, pH response should consequently follow. However, this did not happen, and during that test the pH ranged within 7.2 and 7.3. This appoints the fact that the pH should not be used as "standalone" or the only parameter for the process monitoring because it is directly dependant on the buffering capacity of the system (Murto,2000).

Under normal operating regime, an average increase in the VFA concentration from background value of 100 mg/l up to 320 mg/l , with maximum recorded value of 640 mg/l for the rest of the observed period. In contrast when the acidification of the digester took place the average VFA concentration was averaged on 2100 mg/l which is almost 700% higher than the averaged value after the pulse feed, and almost 20 times fold increase from the background values. Values for the alkalinity under normal operating regime vary from 3550 mg/l down to 3200 mg/l. Literature reports optimal value for the alkalinity from 3500 to 5000mg/l (Gerardy,2003) The averaged value for the alkalinity, in digester, during the acidification event was 720mg/l.

The Ion chromatography test on digester samples revealed similar dynamics as in the Buffer tank. After the pulse feed increase in the acetic acid was recorded. Noticeable is the increase in the propionic and butyric acid. This fact can be explained with the temporally overload of the digested by the pulse feed. Literature reports that butyric acid is one of key parameters for process monitoring under overload conditions in the digesters (Murto,2000).

One of the primary goals of the co-digestion of food waste with the sewage sludge at SNJ wastewater treatment plant is the increase of the methane production. As a consequence of addition of daily averaged 25 m3 of food waste with VS contents of around 27% the rate of methane production was raised from background value of 127 m3/h up to 154 m3/h during the observed period of time.

The solids analyses reveled that on average, buffer tank sludge concentration has total solids content of 6.3% with volatile part of 72% and the digester sludge comprise 3.8% of total solids with 57% volatile content. Aarested (2011), reported values of TS at 7 % and VS 74.7 % for the Buffer tank sludge and TS at 3 % and VS at 48.4 % for the digester sludge.

Biomethane potential tests (BMP)

Biomethane potential tests were carried on, with purpose to determine the specific methane production yield (ml CH_4/gVS added) of separate substrates and mixtures of different ratios of substrates used in co-digestion process at SNJ wastewater treatment plant. For the purpose of the test, an inocolum to substrate ratio (I/S) was chosen to be 2 as an optimal one reported in the literature (Chynoweth, 1993). The mixtures used in test were sludge to food waste 75-25% and 50-50% on a mass weight base.

The digester sludge i.e inoculum showed different methane potential yields. Within the first BMP test the methane yield was reported to be 123 ml/gVS and for the second test 30ml/gVS.

The BMP test revealed two different values for the specific methane yield of sewage sludge with values of 911 Nml and 475 Nml per gram of VS added. The difference in the values is considerable and the author of this study considers that this due to different organic loadings used in the test. However the value of 475ml/g VS added is more realistic compared to the theoretical value of 490ml.. For instance Sosnowski reported value of 293 ml/g VS added for mixture of thickened waste activated sludge and primary sludge in ratio 1:1(Sosnowski et al. 2003).The difference in values can be partially explained by the difference in the constituents of the sludge it self.

The methane yield for the food waste was found to be 486 Nml/g VS added which is to be expected due to the higher VS fraction (around 90%) compared to the sewage sludge.The cumulative methane production of the food waste was found to be slightly higher compared to the sewage sludge. Also the average daily rate of methane production was 134ml/day and 15% higher than the sludge's 115 ml/day.

Methane yield from the mixtures was found to be higher than those from the sewage sludge and food waste individually. For the ratio of 75-25 % the methane yield was found to be 572Nml/gVS with total cumulative methane production of 1900 ml of CH_4. For the mixture of 50% sludge and 50% food waste the methane yield was found to be 657 Nml/gVS with cumulative gas production of 2370ml CH_4. Similar findings was reported by (Sosnowski et al. 2003). However the I/S ratio in the cases of mixtures was 1.55 and 1.4 respectively.

Organic loading test

The test revealed and increase in the cumulative methane production as the amount of organic content vas raised. However this was not case with all the different mixtures ratios of sewage sludge and food waste. From the Graph 31 It can be seen that the volume of gas production is not proportional to ratio of food waste added. For instance the sample with sludge to food waste ratio S/F 100:0 produced 2669 ml CH_4 and the sample with Sludge to food ratio 80:20 produced 3905 ml or 32% more methane compared with sample where no food waste was added. As one should expect this increase ratio should be expected for the remaining samples but this is not the case. For example the sample with S/F ratio of 60:40 produced 4362 ml CH_4 which is only 10.5% increase compared with the sample with S/F ratio of 80:20. Sample with S/F ratio of 40:60 produced only 17 % more gas compared to sample S/F 80:20 and this increase in gas production is only 10.5% for the sample with S/F ratio of 0:100. Sample with S/F ratio 20:80 produced only 1457 mlCH_4(Graph 32 and 36).

This tendency in disproportion between the increased organic loading and methane produced is probably result of overload of the samples. Graph. 30 give a clear and distinctive picture about the behavior of different samples. From the graph one can notice the decrease in the daily methane when the food ratio content in substrate was raised from 40 up to 100%. The averaged daily gas production per gram VS added shows the following order as the fraction of food waste was increased from 0 to 100: 79.2, 79.5, 61.6, 54.2, 7.9 and 29.8ml/gVS respectively. The decrease in the gas produced may be explained by the fact that in samples with food waste content higher than 20% we see inhibition of the methanogenic population either by VFA or ammonia concentration.

In order to get insight in the samples behavior pH, VFA and alkalinity analyses were conducted using 5 point titration method and Ion chromatograph test after the experiment was terminated. The pH values varied in the range from 8.05 up to 8.35, with exception on sample with S/F ratio of 20:80 with pH of 7.8. The alkalinity in samples increased as the content of food was increased within the range of 3870 mg/l for the inocolum up to 9500 mg/l in the sample with 100% food waste as substrate. This can be explained by the increasing food content in the samples which is known to be protein rich and increasing the nitrogenous compounds content contributes the buffering capacity in the process. The same trend was observed for the ammonium concentration rising from 612mg/l up to 1726 mg/l for the sample with 100% food waste substrate. Titration analysis showed VFA concentrations for the samples with S/F ratio of 100:0 and 80: 20 were negligible with increasing values from 274, 9500 and 5670 mg/l as acetic acid for the samples with S/F ratios of 40:60,20:80 and 0:100%(Graph 37).

The decrease in the methane production in the reactor with the S/F ratio higher than 80:20 in terms of food waste can be explained by the acidification process and rapid increase in the VFA concentrations after the experiment startup. Graph 30. clearly shows this effect. After the experiment was started due to the high organic load the increase in the VFA is dramatic resulting

in pH drop than consequently ends up with inhibition of the methanogenic population. This is especially noticeable with the sample with S/F ratio 20:80 where after initial rise in of gas production during the first two days nearly complete inhibition can be detected starting to recover at the final days of the experiment.

However the samples with S/F ratio 60:40, 40:60 and 0:100 were not completely inhibited although the inhibition can be very well noticed from the Graph 30. Behavior of these systems can be explained with initial acidification that cause pH drop but after some time methanogenic population start to cope with the increased VFA concentrations and restore the gas production. This recovery however is not complete. As they continue degrading the protein reach food waste ammonia concentration start to increase resulting in pH of over 8.3. High levels of pH leads to higher presence of the free form NH_3 which is reported to be more toxic than the ionized form NH_4 resulting in methanogenic population inhibition.. At the same high VFA concentrations were maintained due to the degradation of complex organic matter. Possible explanation for the behavior of these systems can be described with inhibited state of gas production. Free ammonia inhibition result in VFA accumulation, which in turn lower pH and decrease the ratio of free ammonia, with the result that free ammonia inhibition is relieved. Due to this self-stabilizing mechanism, processes can be maintained in a stable ammonia inhibited state, where a balance between VFA concentration and ammonia loading exist (Biomethanation II,2003). Angelidaki and Ahring (1995), reported values of free ammonia concentration of 1.1g-N/litre or more to cause inhibition in batch cultures at pH 8.0 (reactor pH).

Overloading process is characterized by accumulation of acetic acid and accumulation of propionic and butyric acid is a sign of process imbalance between the acidogenesis and acetogenesis process. The results from the ion chromatography test reports this situation in the samples with food waste content higher form 20%. For instance the propionic acid concentrations in the samples with,60,80 and 100% food content were found to be 183, 4523 and 5327 mg/l. At the same time acetic acid concentrations were found to be 193mg/l, 3715 and 920 mg/l. Thus the averaged ratios HAc/HPr from the observed reactors were 1 ,0.82 and 0.17 respectively. Literature reports and optimal ratio of 1.4 between acetic and propionic acid for stable anaerobic digestion systems (Biomethanation II,2003).

6. CONCLUSIONS

The anerobic codigestion is a feasible way to increase the methane production at SNJ wastewater treatment plant. The temporary overload of the system caused by the pulse feed of food waste resulted in 27% averaged increase in the rate of methane production during the observation period. The full scale tests confirmed the theory behind the phenomenon of temporary organic overload on system.

Blending of sewage sludge and food waste (co-digestion) in mixture ratios 75 :25 and 50:50 showed and increase in cumulative methane production yield from 36% up to 57% compared to the sewage sludge sample due to the higher organic loading.

The organic loading (food to biomass) test revealed that the optimal organic loading ratios lies within the range 1.73~2.1:1 (gVS substrate/gVS biomass) that corresponds to mixing ratio between sludge and food waste the range 80:20 up to 60:40. The ratio of 60:40 showed some inhibition but the system retain overall stability. Higher organic loadings ratios resulted in methanogenic biomass inhibition and consequently inhibited methane production.

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APPENDIX I

Full scale test data (20.01.2012)

	Sample 1		Sample 2		Sample 3		Sample 4		Sample 5		Sample 6		Sample 7		Sample 8	
	BT1	D2	BT1	D2	BT1	D2	BT1	D2	BT1	D2	BT1	D2	BT1	D2	BT1	D2
pH	6.58	7.22	6.67	7.17	5.64	7.18			5.75	7.21	6.45	7.15	6.4	7.3	6.5	7.2
Conductivity	5.15	9.24	5.18	9.1	7.9	9.05			7.4	9.18	7.2	8.66	5.75	9.02	5.72	8.76
TS (%)	5.62	3.55	5.78	3.7	5.6	4.2	3.7	13.7	5.7	3.82	3.54	3.81	6.7	3.9	6.21	4.3
VFA(mg/lHAc)	1259	53	1435	95	3544	320	4017	555	3431	238	1370	448	1914	163	1315	33
Alkalinity (mg/l) H_2CO_3	444	3473	567	3460	351	3609	317	2640	274	3242	434	2143	279	2497	395	2796
COD fil. (mg/l)	1440	960	1480	880	2715	1500	2680	1870	3650	2480	5840	2800	3840	2400	2950	2600
COD.Tot (mg/l)*100	570	310	450	450	280	480	440	550	280	780	550	740	400	400	660	1250
Gas (m3/h) CH_4 68% $_{aver.}$		207		187		211		248		268		252		254		235

Full scale test data (27.01.2012)

	Sample 1		Sample 2		Sample 3		Sample 4		Sample 5		Sample 6		Sample 7		Sample 8		Sample 9	
	BT1	D2	BT1	D2	BT1	D2	BT1	D2	BT1	D2	BT1	D2	BT1	D2	BT1	D2	BT1	D2
pH	6.42	7.21	6.82	7.18	6.47	7.2	5.2	7.2	4.87	7.21	5.3	7.15	5.67	7.15	5.89	7.17	6.46	7.22
Conductivity	3.93	9.86	3.98	9.88	4.05	9.92	6.14	10	9.47	9.72	6.96	9.71	6.38	10.18	5.88	9.74	5.38	9.83
TS (%)	5.1	3.6	5.06	3.35	5.81	4.24	7.71	4.5	5.9	5.7	9.9	4.29	7.1	4.2	6.7	3.7	6.79	3.9
TVS (%)	73	60	73	54	83	75	53	54	85	67	85	55	42	59	48	57	73	78
COD.Tot (mg/l)*100	760	710	913	505	931	675	1177	613	1100	594	1600	530	2340	594	897	612	970	607
COD fil. (mg/l)	5950	4380	5650	4275	6500	4930	6800	6850	5840	5150	0	5250	12500	2400	10000	2350	9450	2850
Alkalinity (mg/l) H_2CO_3	418	4103	390	3527	325	3397	253	3261	138	3439	283	2924	280	3290	321	3412	397	3320
VFA(mg/lHAc),	1041	80	1000	180	1218	475	2660	639	3350	565	2467	315	1643	408	1798	256	1597	44
Gas (m3/h) CH_4 69% $_{aver.}$		140		168		154		142		153		238		275		305		279

Full Scale test data (02.03.2012)

	Sample 1		Sample 2		Sample 3		Sample 4		Sample 5		Sample 6		Sample 7		Sample 8		Sample 9	
	BT1	D2	BT1	D2	BT1	D2	BT1	D2	BT1	D2	BT1	D2	BT1	D2	BT1	D2	BT1	D2
pH	6.26	7.24	6.43	7.3	5.9	7.22	5.26	7.22	6.22	7.23	6.27	7.2	6.2	7.18	6.18	7.22		
Conductivity	3.42	8.75	4.02	8.78	5.97	8.86	5.1	8.74	4.49	8.41	4.48	8.44	4.24	8.65	3.98	8.74		
TS (%)	4.88	3.33	4.88	3.23	8.63	3.68	7.43	4.29	6.89	5.59	6.51	3.42	6.46	2.96	5.98	3.11	5.51	3.75
TVS (%)	75	52	70	52.7	82.2	51	80	53	78	52	77.4	56.4	76.3	56.4	74.4	54.6	70	53.6
COD fil. (mg/l)	5422	2221	5656	2160	19340	2443	18760	2330	16420	2280	15700	2250	11920	2132	1192	2438	5246	3091
Alkalinity (mg/l) H_2CO_3	301	639	216	710	103	510	104	484	384	987	448	1100	444	562	431	792		
VFA(mg/lHAc),	1688	3082	1345	2374	3980	1715	2173	2089	2331	2132	2186	1886	2168	1628	2237	1975		
Gas (m3/h) CH_4 70% $_{aver.}$		155		152		120		190		283		301		277		301		263

Full Scale test data (17.04.2012)

	Sample 1		Sample 2		Sample 3		Sample 4		Sample 5		Sample 6		Sample 7		Sample 8		Sample 9	
	BT1	D2	BT1	D2	BT1	D2	BT1	D2	BT1	D2	BT1	D2	BT1	D2	BT1	D2	BT1	D2
pH	6.26	7.12	6.22	7.09	6.08	7.14	5.96	7.1	6.07	7.08	6.07	7.05	6.3	7.02	6.46	7.343	6.4	7.29
TS (%)	5.81	3.39	6.44	3.2	6.83	3.82	8.41	2.75	8.47	3.93	8.84	3.73	6.98	3.91	5.46	3.48	5.34	3.52
TVS (%)	79.9	57	78.1	52	76.4	54.7	83	56.3	84.5	54.5	76.8	51.3	78	49.2	70.9	55.3	72.8	56.5
Alkalinity (mg/l) H_2CO_3	260	3561	240	3249	121	3588	171	3800	146	2544	118	3399	325	3069	402	4124	469	3945
VFA(mg/lHAc),	1568	82	1455	163	2412	169	2475	372	2423	275	2772	454	2313	210	1625	207	1627.6	185.4
Gas (m3/h) CH_4 68% $_{aver.}$		235		227		253		274		305		288		292		186		185

Ion chromatography data (20.01.2012/27.01.2012)

	(mg/l)	Acetic acid	Propionic acid	Lactic acid	Formic acid	Butyric acid	Valeric acid
Sample 1	BT1	253.82	141.77	0.79	17.05	76.97	80.67
	D2	6.32	0.50	0.33	4.48	19.24	84.81
Sample 2	BT1	274.73	164.87	0.88	8.96	96.42	64.30
	D2	12.71	1.06	0.41	3.75	16.11	62.19
Sample 3	BT1	754.37	92.80	26.93	31.35	87.11	50.04
	D2	56.22	18.67	0.88	2.66	13.74	27.68
Sample 4	BT1	1053.68	162.38	30.56	34.85	123.57	55.06
	D2	35.56	8.36	0.96	2.69	11.42	28.59
Sample 5	BT1	796.67	141.71	23.75	27.47	99.89	50.77
	D2	91.37	46.28	0.00	3.63	15.82	25.60
Sample 6	BT1	817.08	149.51	18.36	23.69	99.46	49.75
	D2	49.83	12.08	0.47	3.02	8.20	41.02
Sample 7	BT1	695.04	174.61	4.67	11.75	103.61	52.88
	D2	39.44	7.47	0.55	2.57	7.82	51.53
Sample 8	BT1	732.13	203.79	3.72	13.15	115.96	56.95
	D2	39.78	8.83	0.57	3.13	9.26	56.99
Sample 1	BT1	643.52	142.12	0.00	8.88	86.61	56.23
	D2	27.76	0.65	0.09	4.11	17.63	48.44
Sample 2	BT1	637.61	141.59	0.00	8.94	84.15	53.75
	D2	29.24	1.51	0.00	3.78	10.36	49.28
Sample 3	BT1	670.11	179.16	2.55	11.91	98.79	57.54
	D2	36.30	0.89	0.11	4.07	7.02	0.00
Sample 4	BT1	2159.92	297.36	13.15	36.96	182.35	76.30
	D2	46.95	0.89	0.32	4.16	11.08	49.83
Sample 5	BT1	995.66	104.32	47.15	60.57	115.53	62.41
	D2	38.51	3.04	0.52	4.71	20.21	0.00
Sample 6	BT1	1283.00	166.40	5.65	16.15	101.07	52.30
	D2	65.94	39.99	0.57	2.75	13.15	26.00
Sample 7	BT1	1195.71	183.35	5.42	16.40	115.45	52.59
	D2	57.41	24.22	0.43	2.88	12.52	27.82
Sample 8	BT1	536.40	0.00	4.92	12.54	87.20	49.46
	D2	31.49	0.00	0.00	4.57	6.43	54.30

Ion chromatography test (17.04.2012)

	(mg/l)	Lactic acid	Propionic acid	Formic acid	Acetic acid	Butyric acid	Valeric acid
Sample 1	BT1	1.34	619.00	26.67	687.79	0.00	0.00
	D2	0.99	20.07	12.49	60.38	34.60	14.02
Sample 2	BT1	0.83	673.68	63.49	1008.16	3.15	31.67
	D2	0.77	4.98	3.34	68.43	56.03	15.99
Sample 3	BT1	24.23	717.02	62.33	1960.63	95.70	76.18
	D2	0.00	18.01	3.17	88.29	70.36	0.00
Sample 4	BT1	15.01	806.44	54.13	2473.20	93.73	35.49
	D2	0.00	115.39	7.37	197.95	13.39	24.77
Sample 5	BT1	31.58	728.08	59.69	2304.88	106.58	69.94
	D2	1.05	105.24	6.05	194.23	6.29	23.44
Sample 6	BT1	29.09	703.94	18.18	2192.54	99.48	65.82
	D2	1.04	33.56	3.23	98.75	24.89	11.99
Sample 7	BT1	18.39	791.26	20.92	1707.79	83.30	71.64
	D2	1.61	38.08	1.81	124.87	24.53	12.05
Sample 8	BT1	2.94	678.61	53.27	995.57	51.94	59.65
	D2	2.30	8.87	2.71	52.28	11.55	12.29
Sample 9	BT1	0.00	663.89	16.09	497.27	42.95	55.59
	D2	1.64	8.87	2.04	52.69	23.86	11.20

APPENDIX II

Biomethane potential test No. 1

Cumulative methane production

Day	Volume [Nml]	Volume [Nml]	Volume [Nml]	Volume [Nml]Raw sludge	Volume [Nml]	Volume [Nml]	Yeast food Volume [Nml]	Yeast food 2 Volume [Nml]
0	0	0	0	0	0	0	0	0
1	204.1	196.6	196.5	544.9	562	560.6	402.1	367.2
2	310	294.4	295.5	1156.8	1225.2	1214.7	751.3	706.7
3	377.5	356.6	356.4	1957.8	1969.9	2043.5	931.4	950.8
4	431.4	409	405.1	2278.9	2262.5	2385.1	1007.6	1087.6
5	463.2	438.7	430.8	2474.1	2446.8	2602	1041.6	1144.7
6	492.8	466.3	455.4	2624.2	2578	2768.5	1076.2	1194.3
7	523.7	489.4	473.4	2725.6	2668	2884.1	1108.3	1240
8				2739.7	2677.7	2896.7	1114.1	1242.5

Daily methane flow rate

Day	Flow [Nml/day]	Flow [Nml/day]	Flow [Nml/day]	Flow [Nml/day]	Flow [Nml/day]	Flow [Nml/day]	Yeast food Flow [Nml/day]	Yeast food 2 Flow [Nml/day]
0	0	0	0	0	0	0	0	0
1	204.1	196.6	196.5	544.9	562	560.6	402.1	367.2
2	105.9	97.7	99	612	663.2	654.1	349.1	339.5
3	67.5	62.2	60.9	801	744.7	828.8	180.2	244.1
4	53.9	52.4	48.7	321	292.6	341.6	76.1	136.7
5	31.8	29.7	25.8	195.2	184.3	216.9	34	57.2
6	29.6	27.5	24.6	150.2	131.2	166.5	34.7	49.6
7	31.7	25.6	20.1	101.4	90	115.5	32	45.6
8				97.2	84.4	110.2	35.3	39

Biomethane potential test No. 2

Cumulative methane production

Day	Blank Volume [Nml]	Blank Volume [Nml]	Blank Volume [Nml]	Raw sludge Volume [Nml]	Raw sludge Volume [Nml]	raw sludge Volume [Nml]	Food waste Volume [Nml]	Food waste Volume [Nml]	Food waste Volume [Nml]	Mixture 75-25 Volume [Nml]	Mixture 75-25 Volume [Nml]	Mixture 75-25 Volume [Nml]	Mixture 50-50 Volume [Nml]	Mixture 50-50 Volume [Nml]	Mixture 50-50 Volume [Nml]
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
1	65	58.1	66	233	261.8	285.1	372.7	435	527.9	677.7	810.3	837.3	856.3	816	882.1
2	82.4	74.7	84.9	471.7	552.2	603.3	660.7	746.3	805.4	1287.9	1343.7	1391.2	1582.6	1550.7	1572.7
3	100.2	91.9	101.5	699	845.5	937.3	823.7	905.4	984.8	1509.3	1546.4	1612.1	1871.1	1847.9	1858.6
4	110.4	102.2	110.3	954.4	1054.7	1158.5	939.3	1020.1	1129.8	1619.8	1652.8	1726.8	2036.4	2015.5	2028.2
5	119.6	111.2	116.9	1054.1	1124.6	1235.8	1042	1119.9	1255.7	1691.2	1722.9	1802.9	2144.9	2126.1	2145.9
6	124.7	118.3	122.6	1099.4	1166.2	1285.9	1129.7	1207.8	1363	1742.3	1775.6	1857.5	2222.4	2205.3	2228.4
7	130.5	122.5	126.5	1127.9	1192.1	1317	1204.6	1284.9	1451.9	1782.3	1815	1899	2279.9	2264.1	2287.9
8	137.1	127.5	130.1	1160.6	1212	1340.9	1267.1	1353.8	1525	1815.8	1849	1934.3	2326	2313.2	2334.2
9	142.1	132.6	133.4	1176.1	1225.9	1358	1314	1410.4	1577.2	1840.7	1874.3	1960.8	2359.5	2349.7	2366.7
10			135.7	1183.5	1232	1361.6	1337.8	1435.2	1603	1848.4	1883.8	1966.6	2368.4	2363	2378.5

Daily methane flow

Day	Blank Flow [Nml/day]	Blank Flow [Nml/day]	Blank Flow [Nml/day]	Raw sludge Flow [Nml/day]	Raw sludge Flow [Nml/day]	raw sludge Flow [Nml/day]	Food waste Flow [Nml/day]	Food waste Flow [Nml/day]	Food waste Flow [Nml/day]	Mixture 75-25 Flow [Nml/day]	Mixture 75-25 Flow [Nml/day]	Mixture 75-25 Flow [Nml/day]	Mixture 50-50 Flow [Nml/day]	Mixture 50-50 Flow [Nml/day]	Mixture 50-50 Flow [Nml/day]
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
1	65	58.1	66	233	261.8	285.1	372.7	435	527.9	677.7	810.3	837.3	856.3	816	882.1
2	17.4	16.6	18.9	238.6	290.4	318.2	288	311.3	277.5	610.2	533.4	553.8	726.3	734.6	690.7
3	17.8	17.2	16.6	227.3	293.3	334	163.1	159.1	179.4	221.4	202.7	220.9	288.5	297.2	285.9
4	10.2	10.3	8.8	255.3	209.2	221.2	115.6	114.7	144.9	110.6	106.5	114.6	165.3	167.6	169.6
5	9.2	9	6.6	99.7	69.9	77.3	102.7	99.8	125.9	71.4	70.1	76.2	108.6	110.6	117.7
6	5.1	7.1	5.7	45.3	41.7	50.1	87.7	87.9	107.3	51.1	52.7	54.6	77.4	79.2	82.4
7	5.8	4.2	3.9	28.5	25.8	31.1	74.9	77.1	88.9	40	39.4	41.4	57.5	58.8	59.6
8	6.6	5	3.6	32.7	19.9	23.9	62.4	68.8	73.1	33.5	34	35.3	46.1	49.1	46.3
9	7.2	6.3	3.3	15.4	13.9	17.1	46.9	56.6	52.2	24.9	25.3	26.6	33.5	36.5	32.5
10			3.3	10.9	11.9	15.3	33.9	46.4	37.3	19.2	17.4	22.2	25.6	27.9	24.8

Organic loading test (OL)

Cumulative methane production

Day	Blank Volume [Nml]	Blank Volume [Nml]	Sludge-Food 100:0 Volume [Nml]	Sludge-Food 100:0 Volume [Nml]	Sludge-Food 80:20 Volume [Nml]	Sludge-Food 80:20 Volume [Nml]	Sludge-Food 60:40 Volume [Nml]	Sludge-Food 60:40 Volume [Nml]	Sludge-Food 40:60 Volume [Nml]	Sludge-Food 40:60 Volume [Nml]	Sludge-Food 20:80 Volume [Nml]	Sludge-Food 20:80 Volume [Nml]	Sludge-Food 0:100 Volume [Nml]	Sludge-Food 0:100 Volume [Nml]
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
1	177.7	233.7	749.6	848.3	1016.9	956.3	695.4	620.2	458.4	431.5	403.9	396.8	339.2	449
2	266.4	341.3	1530.4	1747.4	2183.5	2094.6	1577.9	1414.6	637.4	554.4	450.3	436	478.9	612.9
3	334.9	421.6	1844	2082.1	2820.3	2793.2	2407.5	2237.9	1018.7	753.6	482.6	461.3	640.2	763.8
4	382.8	473.8	2007	2254.9	3146	3131.5	3104.8	2947.3	1347.8	1139.3	505.3	477.7	851.9	977.2
5	410.1	505.6	2116.4	2366.7	3300.1	3285.6	3476.2	3292.7	1515.3	1331.1	518.8	487.1	1124.1	1301.5
6	430.6	529.5	2200.8	2453.1	3402.1	3387.3	3782.5	3599	1740.8	1428.6	528.4	493.2	1414.8	1540.1
7	448.6	550.2	2272.7	2526.6	3490.2	3471.3	3911.5	3732	2090.8	1574	540.5	500.9	1573.7	1658.7
8	466.3	569.2	2322.3	2583.7	3563.8	3546.5	4009.7	3826.3	2502.4	1840.5	555.5	511.2	1729.3	1792
9	476.5	581.4	2354.1	2619.2	3609.6	3596.1	4091.6	3907	2958.9	2158.5	573.1	521.2	1973.7	2033.2
10	488.7	592.6	2381.8	2649	3648.6	3635.7	4158.4	3974.1	3461.1	2500.9	601.5	538.8	2249.3	2349.1
11	498.4	601.4	2404	2673.6	3686.1	3671.2	4219.1	4027.3	3936.8	2883.7	650.7	571.4	2584.5	2792.5
12	503.8	606.4	2418.1	2689.2	3714.6	3695.9	4266.1	4066	4400.7	3250.2	724.3	624.3	2916.9	3224.3
13	514.5	616.7	2438.7	2711.1	3753.3	3729.1	4306.8	4113.9	4623.7	3587.8	833.6	719.4	3250.1	3555.1
14	524.3	624.9	2456.4	2728.2	3792.4	3760	4335.9	4146.9	4748.8	3976.5	947.9	836.2	3539.9	3728
15	529.4	630.9	2468.5	2741.8	3825.3	3786.9	4357.6	4169.9	4846.9	4291	1048.2	946.2	3760.9	3889.4
16	534.5	636.3	2481.8	2754.4	3840.4	3815.9	4377.7	4190.8	4928.7	4591.9	1139.6	1043.9	3920.5	4076.8
17	539.5	640.2	2492.8	2765.2	3851.5	3844	4394	4207.5	4995.9	4826.2	1215.5	1128	4031.8	4195.1
18	544.6	644.1	2503.1	2776.4	3863	3868.8	4408.9	4222.6	5059.8	4922	1261	1187.7	4122.8	4279.9
19	549.7	647.9	2513.6	2786.3	3874.6	3885.7	4423.2	4235.5	5117.3	4999.4	1299.8	1224.4	4209.4	4370.6
20	554.3	651.7	2522.3	2795.2	3883.6	3897.9	4435.4	4247.9	5164.7	5065.5	1361.1	1260.7	4312.5	4514.7
21	558.6	654.9	2529.7	2800.4	3889.9	3908.3	4446.4	4258.5	5198	5119	1442.1	1320.1	4439.7	4725.5
22	561		2534.6	2803.4	3893.5	3915.9	4459.6	4265.1	5223.1	5163.5	1513	1364.6	4602.6	4901
23						3919.8	4460.7		5225.6	5175.6	1563.6	1393.3	4741.9	4999.5

Daily methane flow

Day	Blank Flow [Nml/day]	Blank Flow [Nml/day]	Sludge-Food 100:0 Flow [Nml/day]	Sludge-Food 100:0 Flow [Nml/day]	Sludge-Food 80:20 Flow [Nml/day]	Sludge-Food 80:20 Flow [Nml/day]	Sludge-Food 60:40 Flow [Nml/day]	Sludge-Food 60:40 Flow [Nml/day]	Sludge-Food 40:60 Flow [Nml/day]	Sludge-Food 40:60 Flow [Nml/day]	Sludge-Food 20:80 Flow [Nml/day]	Sludge-Food 20:80 Flow [Nml/day]	Sludge-Food 0:100 Flow [Nml/day]	Sludge-Food 0:100 Flow [Nml/day]
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
1	177.7	233.7	749.6	848.3	1016.9	956.3	695.4	620.2	458.4	431.5	403.9	396.8	339.2	449
2	88.7	107.5	780.8	899	1166.6	1138.2	882.6	794.4	179	122.9	46.5	39.2	139.7	163.9
3	68.5	80.3	313.7	334.7	636.7	698.6	829.6	823.3	381.3	199.2	32.3	25.2	161.3	150.8
4	47.9	52.2	163	172.8	325.7	338.2	697.2	709.4	329.2	385.7	22.7	16.5	211.7	213.4
5	27.3	31.8	109.4	111.8	154.1	154.1	371.5	345.4	167.4	191.8	13.5	9.4	272.2	324.3
6	20.5	23.9	84.4	86.4	102	101.7	306.3	306.3	225.5	97.5	9.6	6.1	290.7	238.7
7	18	20.8	71.9	73.4	88.1	84	128.9	132.9	350.1	145.4	12.1	7.7	158.9	118.6
8	17.7	19	49.6	57.2	73.6	75.1	98.2	94.3	411.5	266.5	15	10.3	155.6	133.3
9	10.2	12.2	31.8	35.5	45.8	49.7	81.9	80.8	456.5	318	17.5	10	244.4	241.2
10	12.1	11.3	27.7	29.8	39	39.6	66.8	67.1	502.2	342.4	28.4	17.6	275.6	315.9
11	9.7	8.7	22.2	24.6	37.4	35.4	60.8	53.2	475.7	382.9	49.2	32.6	335.2	443.5
12	5.4	5.1	14.2	15.6	28.6	24.7	47	38.6	463.9	366.5	73.6	52.9	332.4	431.8
13	10.6	10.3	20.6	21.9	38.7	33.2	40.7	47.9	223	337.5	109.3	95.1	333.3	330.8
14	9.9	8.2	17.7	17.1	39.1	30.9	29.1	33	125.1	388.8	114.3	116.8	289.7	172.9
15	5.1	6	12.2	13.6	32.9	26.9	21.8	23.1	98	314.5	100.3	110	221	161.3
16	5.1	5.3	13.3	12.6	15.1	28.9	20	20.9	81.8	300.9	91.3	97.7	159.6	187.5
17	5.1	3.9	11	10.8	11	28.2	16.3	16.7	67.2	234.3	75.9	84.1	111.3	118.3
18	5.1	3.9	10.3	11.2	11.5	24.8	14.9	15.2	63.8	95.8	45.6	59.7	91	84.8
19	5.1	3.8	10.5	9.9	11.7	16.9	14.3	12.9	57.6	77.4	38.8	36.7	86.6	90.6
20	4.6	3.8	8.7	8.9	9	12.2	12.2	12.4	47.4	66.1	61.3	36.3	103.1	144.1
21	4.3	3.8	7.4	5.2	6.3	10.3	11	10.6	33.2	53.5	80.9	59.4	127.3	210.8
22	4.3		6.9	5.2	6.1	7.6	13.2	9.9	25.2	44.5	71	44.5	162.9	175.5
23						7	15.5		22.3	30.8	77.9	47.9	212.9	152.6

5 point titrations and Ion Chromatography results

	Alkalinity (mg/lH2CO3)	VFA mg/l Hac	pH	Ammonium (mg/l)	Lactic acid	Propionic acid	Formic acid	Acetic acid	Butyric acid	Valeric acid
Blank	3827	23	8.05	613	0	41	74	69	61	0
S/F 100:0	4023	5	8.13	533	0	188	50	51	49	0
S/F 80:20	5084	0	8.18	653	0	270	73	51	179	0
S/F 60:40	8178	274	8.35	880	0	45	102	96	50	0
S/F 40:60	8248	0	8.39	1161	0	183	49	193	55	0
S/F 20:80	2361	9548	7.79	1102	7	4524	345	3715	2626	1946
S/F 0:100	9516	5658	8.34	1727	0	5373	116	919	261	581