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Abstract

Biodegradation of organic matter by anaerobic digestion is an old sludge treatment technique. The process offers many advantages with effective conversion of a broad range of organic wastes. The major application of anaerobic digestion is the stabilization of concentrated sludges produced from waste water treatment plants (WWTP) and industrial concentrated wastes. A advantages with anaerobic digestion (AD) processes are reduced disposal costs through reduction in waste volumes of waste at the same time converting the organic carbon into biogas, an environmentally sound energy source. The key parameter is determining the biodegradable COD ($_{Bio}$COD) of influent sludges and characterisation of the organic materials into fractions of polysaccharides, protein and lipids. Investigation and analysis of the dynamic behaviour of digesters with models is useful tool in optimizing, modification and design.

The system investigated in this study is the AD reactor at WWTP SNJ in Stavanger, Norway. The AD reactor was monitored in two periods, normal conditions or "steady state" and one injected pulse load for "dynamic state". The measured and monitored parameters in the two periods were added together and by the model ADM1, systemised and implemented into the software Aquasim for parameter estimations. The extended period of modelling was 150 days.

Solids analysis was conducted for process characterization and potential for biogas production. During steady state conditions influent sludge TS was 7 [%] and the VS fraction 74.7 [%], digested sludge TS of 3 [%] of which 48.4 [% was volatile], totally removed COD of 20440 [kgCOD/d] with average biogas production 13076 [Nm^3/d]. Organic loading rate (OLR) was fluxing from 1.1- 4.2 [kgVS/m^3d]. The stability of reactor pH, volatile fatty acids (VFA`s) and alkalinity (ALK) was analysed. The alkalinity in reactors was measured to be 4500[mg/l] (as $CaCO_3$). Mineralization and measurement of cations NH_4^+, Ca^{2+}, Mg^{2+}, Na^+ and K^+ was conducted by Ion Chromatography (IC) for evaluation of ammonium concentrations and alkalinity developments. Ammonium concentration was 690 [mg/l NH_4-N] in steady state analysis.

For dynamic state analysis the reactor where tested by injecting a high COD content waste directly into one reactor while using the parallel reactor as a reference. Investigation of reactor behavior and intermediates interactions was conducted under this stress test. Intensive measurement at of pH, VFA`s and ALK was processed and analyzed. Dynamics in VFA`s, pH and ALK was significantly during the pulse load. pH level decreased from 7.2 to 6.95 and VFA`s dynamics from 209[mg/l] to 1170[mg/l] (as HAc). Lactate (HLa) and propionic acid (HPr) showed high dynamics in the first phase, especially HLa from zero to 286[mg/l]. The ALK was decreasing from 4900-3940[mg/l] (as $CaCO_3$). The interactions and

mineralization processes showed increasing values at ammonium concentration from normal state at 690 to 907[mg/l NH_4-N] for the pulse load. Toxic levels of free ammonia were calculated to 47.5[mg/l as NH_3-N].

The AD process is stable and capable of handling the investigated acting high level pulse loading of strong COD waste. Disintegration and hydrolytic process in the system buffer tank is underestimated and further strategy for this compartment has to be reconsidered to enhance the overall anaerobic process. Significant fermentation in the buffer tank is evident from data, and could be exploited in future process upgrades.

Modelling co-digestion of alternative organic wastes using the anaerobic digestion model no 1 and implementation into the process simulation tool Aquasim for system analysis and parameter estimations is complex. Preliminary modelling results show high intermediates dynamics even with “correction actions” on the model. Simulated process state variables of the ADM deviates significantly from measured values during the pulse load test, and analysis based on this model has to be further investigated for modelling evaluation. The model result for buffer tank is interesting and should be paid further attention. The result show that significantly enhanced fermentation (including hydrolysis and disintegration of particulates) can be achieved by increasing the buffer tank temperature, resulting in higher overall biogas potential and higher loading rate capacity of downstream methanogenic processes. Further investigations by non-steady state pulse loading experiments should be performed for model calibration and validation, and optimization of co-digestion operational strategies.

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INVESTIGATION AND ANALYSIS OF ANAEROBIC DIGESTION SNJ IVAR

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Table of contents

List of Figures

List of Tables

List of Graphs

1 Introduction

The fermentation process is among the oldest biological technologies utilised by mankind, initially for food and beverage production. The process is also called anaerobic digestion (AD) and defined as processes where organic material is available and degraded in absence of oxygen (O_2). The organic material is being decomposed and mineralized into compounds like NH_4^+, PO_4^{3-}, S^{2-} and water (H_2O) in to the solution. The gasses carbon dioxide (CO_2) and methane (CH_4) is the end product of the fermentation and anaerobic degradation process. The whole process is also called biogas process when organic matter is degraded by microorganisms under anaerobic conditions. The biogas process generates in general 30-40 % carbon dioxide (CO_2) and 60-70% methane (CH_4). Some minor quantities of nitrogen, hydrogen, ammonia and hydrogen sulphide (usually less than 1% of the total gas volume) are also generated (Angelidaki et al.).

Anaerobic digestion consists of a complex series of reactions with a wide range of microorganisms involved. The sum of these organisms and reactions are able to degrade a wide range of substrate materials. The process occurs naturally in places where organic material is available and the redox potential is low or absent, zero oxygen condtions.

The organic matter is serving the microorganisms with substrate for bioenergetics. The organisms obtain energy via coupled reduction and oxidation reactions involving electron transfer from an electron donor to an electron acceptor (Ekama G.A). In this case electron acceptor is not available outside the organism and has to be generated intracellurlarly. The redox bioprocess needs these alternative electron acceptors internally generated for fermentation and oxidation processes. From the organic compound, microorganisms are intracellurlarly generating electron acceptors and the substrate carbon who is degraded is acting as electron donor (Tchobanoglous et al.,2003).

The organic matter is degraded into carbon atoms at various oxidation/reduction states. When biodegradation and fermentation occurs, the substrate carbon is found in either in its most oxidized form, CO_2, or in the most reduced form, CH_4 (Angelidaki et al.).

The organics, the wasted sludge from wastewater treatment plant (WWTP) and industry is the source and substrate for the bioprocess. The sludge provides substrate for the microorganism and traditionally divided into three particulate substrate groups. The groups are specified based on the chemical composition into polysaccharides, lipids and proteins. These groups are often called polymeric substances or polymers for polysaccharides (sugars), lipids (fats) and proteins. The size characterisation with dead end filtration of organic substrate is divided into different physical size in µm. The particulate organics is referred to the suspended solids (SS) at ca.0.65 µm and larger course particulates. The small organics or polymers is in range of 0,01-1 µm, and these polymeric organics is almost

exclusively related to the colloidal and macromolecular fraction. Very large polymers like starch, cellulose and deoxyribonucleic acid (DNA) is large enough to be regarded as colloidal. Therefore the fractionation of the organics in sludges are mainly consisting of colloidal and particulate aggregates, with polymers creating cellular fragments into coagulated conglomerates (Kommedal,2003). Characterisation of the sludge loading into this polymeric and particulate carbon is important design, process and controlling parameter for biogas production.

Anaerobic fermentation and oxidation are used primary for the treatment of waste sludge and high-strength organic waste, and interest in biogas plants to produce the renewable energy source “biogas” is increasing for energy production. The technology has positive net energy production and can replace the fossil fuel sources. Biogas production has a commercial potential for energy production and a direct positive effect on the carbon dioxide gas production (Batstone et al., 2002). The number of plants is increasing as well as the average plant size. A trend of the last years is the growing interest in substituting natural gas by converting biogas into natural gas networks. The option to convert biogas to (bio) natural gas quality is primarily relevant for large-scale biogas plants. Due to increasing investment and operating costs, the need for a fully developed design and optimized operation increases for profitable operation of large-scale plants. The development of an appropriate model for the complete fermentation process is an important step in this direction (Gerber et al.,2008).

There are existing many models vary with respect to their objectives and complexity. Some are comparatively simple models developed only for calculate the maximum biogas rate, which will be theoretical produced from organic substances. Another similar type of models for calculating a biogas rate and digestion rates, because not every substrate component is degradable at the same conversion rate. Lignin, for instance, is degradable difficultly or only very slowly; in contrast fats is degradable very well. The application of these models does not allow for dynamic test and calibration. Therefore, more complex models were established due to the kinetics for growth of microorganisms. When kinetics is taken into account, the activity of microorganisms “the growth rate” and the biogas production rate can be investigated. Appropriate models for a variety of substrates, different loading rates and bioenergetics were achieved for process management.

The kinetics and the growth rate of microorganisms are depending on the quantity and the composition of the substrate. Many models are precisely adapted to a special substrate or a few numbers of substrates. Therefore, a transfer of the model to processes using different substrates is very complex without experimental results. For investigations of the substrate carbon, only models can be used, which consider the major organic fraction of used substrate, divided into carbohydrates, fats and proteins (Gerber et al.,2008).

Advanced simulations by an adequate mathematical model are a novel tool for calibration of the fermentation process. Development of models where achieved to better understand and utilisation the knowledge of AD. More kinetics where incorporated into the models, with use of substrate level uptake by Monod-type kinetics. Allowing variable yields with basic kinetics by Michaelis-Menten expressions used for depolymerisation kinetics (Batstone et al., 2002).

Anaerobic Digestion Model no. 1 (ADM1), by the IWA Task Group for Mathematical Modelling of anaerobic digestion process (Batstone et al., 2002), has develop a model as a useful tool for analyzing anaerobic degradation processes. The model is regarded as common platform, which simulation applications for a wide range of specific processes should be developed.

ADM1 was used to assess and present operations of the digesters at the WWTP, SNJ in Stavanger. The model is one of the most comprehensive and complex one taking into account that the model has a number of shortcomings and compromises. For evaluation and calibration of the anaerobic reactors, ADM1 where implemented into AQUASIM. The software AQUASIM is designed computer program for modelling and evaluating of aquatic systems (Reichert, 1998). The digesters were investigated for steady-state and dynamic conditions by the model ADM1 and simulated by the system analysis tool AQUASIM.

For modeling the process the substrate carbon has to be measured for the different input sludge load. Therefore, processing and using different substrates is very complex without experimental data. With steady-state and dynamic conditions, different loading rates and combination of different types waste, experimental analysis and standard measurement is required. Operational measurements and additional off line analysis, such as chemical oxygen demand analysis (COD) for investigating the COD flux through system, solids analysis with total solids (TS), volatile- (VS), and fixed solids(FS). Measurement of pH, experimental analysis of alkalinity (ALK) and volatile fatty acids (VFA), was performed especial for the reactors and the behaviour of intermediate formation and transport. The model was validated and calibrated by comparing the simulations with the measured values at SNJ plant survey.

1.2 The master thesis project.

The objective of this study is an examination of the AD reactors at SNJ under two different organic loading regimes;

1. Ordinary loading conditions referred to as the “Steady state”.

2. Dynamic differential loading conditions denoted the “Dynamic state”.

Based on these two test conditions, analysis of AD behaviour by modelling the digesters using the acclaimed model ADM1 and the software AQUASIM computer program will be performed. The following tasks were undertaken as part of this master thesis project:

- Theory study of AD process in general and research of studies of similar investigations.
- Investigation of operational conditions for the reactors and buffer compartments.
- Implementation of ADM1 to the reactor system with use of AQUASIM, and evaluation of steady state and dynamic simulation.
- Evaluating of AD process in reactors with Ion Chromatograph (IC), where volatile fatty acids (VFA`s) were analysed for the behaviour of intermediate formation and transport.
- Calculated biogas productions based on analytical and measured data with biogas obtained from the monitoring and controlling computers at the plant.
- Determination of COD flux and solids analysis of TS, VS solids and calculation of reduction during the digestion process.
- Estimation of solids retention time (SRT), volumetric loadings and organic reduction.
- Calculating Organic loading rate (OLR).
- Calculating the mass balance for sludge throughout the system.
- Characterization of alternative organic wastes.
- Evaluation of biogas potential by co-digestion of alternative organic wastes
- Identification and evaluation of possible inhibitors and toxic fractions during high organic loading linked to co-digestion.

  Primarily this project has a learning perspective, and the main objective is description and analysis of the anaerobic digestion process, and its' operation. Furthermore, evaluation of the possibility of enhancing the biogas production and increase organic loading by co-digestion of alternative organic wastes will be evaluated.

2 Anaerobic digestion and fermentation

For anaerobic process analysis and use of ADM1 by Batstone et al.(2002) and implementing it to the numeric mathematical software AQUASIM model by Reichert. (1998), the fundamental of fermentation process and kinetics has to be fully investigated and understood. AD and fermentation processes are advanced process; it is demanding knowledge in wide range of biochemical and physic-chemical processes based on fundamentals in microbiology. Knowledge in microbiology is well known from studies and conferences like this from 1984; summary of the "Working party No.1" in Athens, The National Technical University, in May 1984. Session 2:

"Microbiology of anaerobic digestion.

Anaerobic digestion is a process where various of bacterial populations interfere. The role played by three groups of bacteria is presented. First, the fermentative bacteria which are responsible for the hydrolysis and fermentation of biological polymers. Their action results in the production of intermediary metabolites like succinate, lactate, etc. In the second stage, acetogenesis, different type of bacteria are involved : others have been called "Hydrogen producing acetogenic bacteria" (HPAB) or syntrophic bacteria. They produce mainly acetate, hydrogen and carbon dioxide. H_2, CO_2 *and acetate are degraded during the third step: methanogenesis. Methanogens are the best known members of the bacterial consortium responsible of the anaerobic digestion process. Some emphasis is put on the interrelations between different groups of bacteria. Interspecies hydrogen transfer occurring between methanogens and other bacteria is discussed*". (Bruce, A.M., et al.,1984.)

In this chapter the fundamentals and the process will be exploratory due to the intention of increasing the theoretical knowledge of the issue "*conversion processes in anaerobic digestion*".

2.1 Conversion processes in anaerobic digestion

Degrading of organic matter in anaerobic processes can be dividing into two main types, biochemical - and physic-processes;

1. Biochemical processes.

These processes are normally catalysed by intra – or extracellular enzymes. The products from enzyme interactions act as a pool of available organic material. Disintegration of composites material, particulate organics into smaller constituents and their subsequent enzymatic hydrolysis to soluble monomers are extracellular processes. Digestion of soluble monomers mediated by microorganisms is intracellular, this process result in biomass growth and decay.

2. Physico-chemical processes.

During this process anaerobic digestion contains three broad types of chemical and physical processes. There is liquid and gas interactions, transfer processes and solubility of solids. These three broad types of chemical and physical processes are designed into fig 2.1.

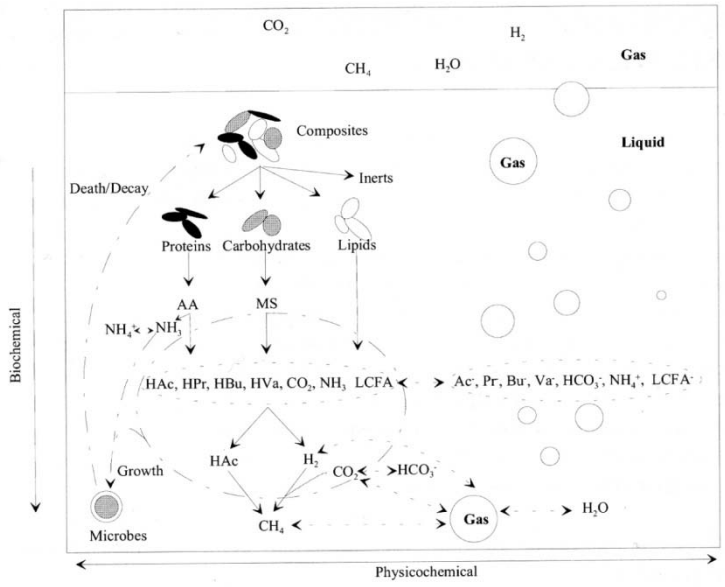


Figure 2.1 Conversion processes in anaerobic digestion used in IWA Model No 1 (ADM1) (Batstone et al,.2002).

1. Liquid – liquid processes such as ion association/dissociation (rapid)
2. Liquid-gas transfer (rapid/moderate depending on mixing)
3. Liquid solids processes such as precipitation and solubilisation (medium to slow)

All of these interactions are very important for effective biochemical processes. Proper mixing rate is significantly with a temperature optimum for the microorganisms (Tchobanogolous et al.,2004).

2.2 COD flux in conversion processes

In general characterization of COD is divided into 1.biodegradable and 2.unbiodegradable with fractionations;

1.Biodegradable COD ($_{Bio}$COD)

-Soluble and particulate.

2.Unbiodegradable COD ($_{UnBio}$COD)

-Soluble and particulate.

The biodegradable COD ($_{Bio}$COD) is undergoing a degradation process by the microorganism. The soluble fraction is considered to be small molecules utilized directly by the organism and termed "readily biodegradable COD" ($_{RB}$COD). The particulate fraction is a complex composite matter that has to undergo converting processes into smaller or simpler compounds. By hydrolysis the particulate is converted into $_{RB}$COD for utilizing by the organism in biomass. The hydrolysis is a relatively slow process and is thus termed "slowly biodegradable COD" ($_{SB}$COD). The unbiodegradable fraction ($_{UnBio}$COD) is not degraded by any microorganism and is enmeshed into the biomass. The soluble part is small inert molecules and residues from bacteria cell interactions and degradation. The particulate $_{UnBio}$COD is larger complex molecules interacting with the biomass and became one part of the sludge. (Ydstebø., 2010)

Biodegradation of organic matter in anaerobic digestion with determining COD flow is a key factor. One of the most important characteristics of the influent COD is the biodegradability of the organic composite particulate $_{SB}$COD. Distinguishing between available degradable substrate towards the total influent COD is important consider a fraction of the influent COD may not be anaerobically biodegradable $_{UnBio}$COD.

The COD flux for an organic compound trough the process is fractionized into four fractions in the ADM1 model. The particulate composite organics is divided into carbohydrates (30%), proteins (30%) and fats (Lipids 30%). The inert fraction $_{UnBio}$COD consist 10%(Batstone et al., 2002).

2.3 Anaerobic microbiology

Anaerobic degradation pathway of organic matter is a multi step process. These processes are carried out by a large and varied group of microorganisms which live in a symbiotic relationship (HENZE et al,. 2002). This process is both parallel and cross linked reactions with five successive stages: (1) disintegration, (2) hydrolysis, (3) acidogenesis, (4) acetogenesis, and (5) methanogenesis. The anaerobic ecosystem is the result of complex

interactions among microorganisms of several different species. The diversity in pieces, functional relationship and groups of bacteria according to their metabolic reactions are divided into: (i) fermentative bacteria, (ii) hydrogen-producing acetogenic bacteria, (iii) hydrogen-consuming acetogenic bacteria, (iv) carbon dioxide-reducing methanogens, and (v) aceticlastic methanogens (Henze, 2008). A schematic of the reaction steps is given below in Figure 2.2.

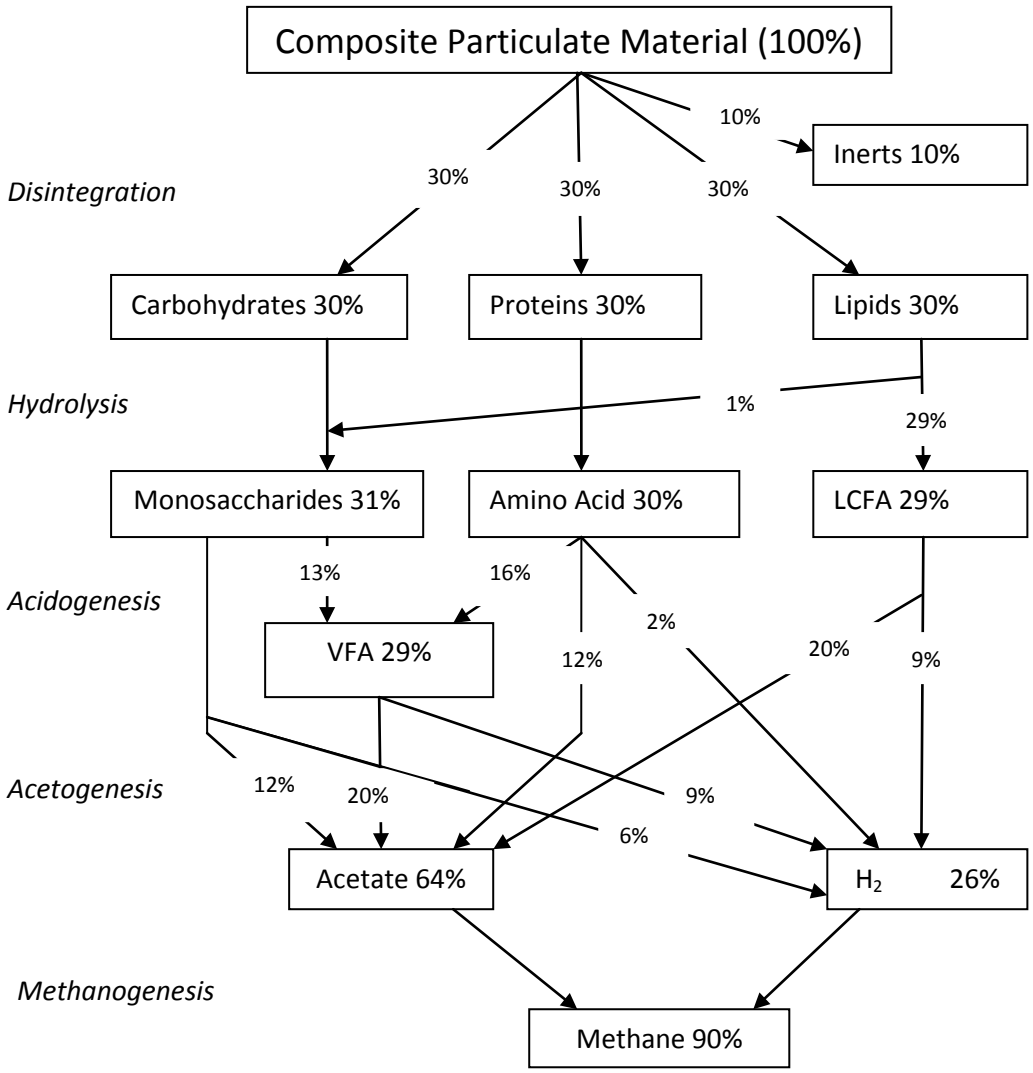


Figure 2.2: COD flux, the chemical oxygen demand flux for a particulate composite comprised of 10% inerts, and 30% each of carbohydrates, proteins and lipids. VFA are represented as propionic acid (10%), butyric acid (12%) and valeric acid (7%) (Batstone et al., 2002).

2.3.1 Disintegration

Disintegration of organic matter is mainly processed by mechanical shear forces and dissolution, the act of breaking up an organic composite compound. Disintegration of the composites material into polymeric constituents is a non-biological process. Extracellular depolymerisation enzymes (EE) act on the pool of available organic material, dividing them into smaller molecular weight products (Batstone et al., 2002).

Every microorganism produces many enzymes and certain enzymes are produced in larger amounts by some organisms. This is excreted into the medium and extracellular enzymes (EE), called exo-hydrolytic or endo- hydrolytic enzyme are digesting insoluble polymers such as starch, cellulose and protein. The products of digestion are then transported into the cell where they are used as nutrient for growth. Enzymes is a biological catalysts and useful because of their high specificity for substrate, specialists on single substrate carbon. In biochemistry, a catalyst is a substance that lowers the activation energy in bioprocesses, thereby increasing the reaction rate. These enzymes can also catalyze reactions that demands energy, converting energy-poor substrates into energy–rich products (Madigan et al,.2009). In table 2.1 the enzyme group hydrolasis and lyases are listed with their subgroups according to converting diversity of carbon substrat.

Group	Sub group													
	1	2	3	4	5	6	7	8	9	10	11	12	13	99
3 (Hydrolases)	Acting on ester bonds (Esterases)	Acting on Glucosidic bonds (Glucosylases)	Acting on ether bonds (Etherases)	Acting on peptide bonds (Peptidases)	Acting on carbon-nitrogen bonds, other than peptide bonds (Amidases)	Acting on acid anhydrides (Anhydrases)	Acting on carbon-carbon bonds (Ketonases)	Acting on halide bonds	Acting on phosphorus-nitrogen bonds	Acting on sulfur-nitrogen bonds	Acting on carbon-phosphorus bonds	Acting on sulfur-sulfur bonds	Acting on Carbon-Sulfur Bonds	
4 (Lyases)	Carbon-Carbon Lyases	Carbon-Oxygen Lyases	Carbon-Nitrogen Lyases	Carbon-Sulfur Lyases	Carbon-Halide Lyases	Phosphorus-Oxygen Lyases								Other Lyases

Table 2.1 The enzyme Lyases and Hydrolases with subgroups interactions (Madigan et al., 2009)

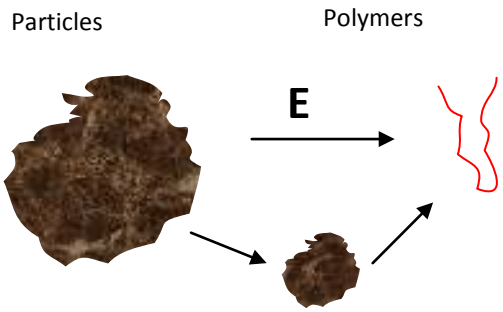
Most organics in wastewater sludge and high loaded industrial sludge waste are polymeric and particulate matter. Depolymerisation by lytic and hydrolytic enzymes acting on polymers is a crucial step into for all degradation processes and fermentation with biogas production. The main EE groups consist of proteins or proteases, cellulases, amylases, glucanases, all these acting on polysaccharides. The other one is lipases who are acting on fats, oils and lipids. Lignin or lignocelluloses do not easily depolymerise and form the 10% inert fraction from organics composites (Kommedal, 2009).

Extracellular depolymerisation enzymes (EE) are important for degrading organics and dead material by this enzymatic activity. Many studies have characterized the activity of exogenous enzyme in sludge for wastewater systems by the enzymatic activity. Batch tests under anaerobic conditions showed increased proteases activity and Vetter et al. (1998)

concluded that released EE provide individual bacteria with a powerful feeding mechanism, especially in typical wastewater (WW) conditions with high surface area and high particulate organic concentrations (kommedal,2003). Not unlike sludge from WW and high concentrated industry-sludge. The cell free enzyme provides an important carbon source which is readily accessible for the acidogenic bacteria due to nutrient for growth (Henze et al, 2008).

This non-biological process mediating the breakdown and solubilisation of complex organic material to soluble substrates is also called hydrolysis through the process. The soluble products from enzymatic degradation of proteins, carbohydrates (polysaccharides) and lipids are monosaccharides, amino acids and long chain fatty acids (LCFA). These products is than serve as substrate for the following process of hydrolysis. The other products of disintegration are the inert`s particulate and inert soluble material (Batstone et al., 2002).

Complex organic composite, the particulate colloids in order of 0.001 - 1 µm or larger particles > 1 µm are disintegrated physically. Disintegration products are dissolved polymers of polysaccharides, proteins and lipids. Due to empirical studies and lack of detailed information disintegration is set to a first order process. Figure 2.3 (Kommedal 2009).



$$r_{dis} = k_{dis} \cdot X_{Composite}$$

k_{dis} = 0.4 to 1.0 1/d for mesophilic and thermophilic, respectively.

$X_{Composite}$ = Organic particulate composite.

r_{dis}= Disintegration rate.

Figure 2.3 Complex organic composite materials and particulate(Kommedal 2009).

2.3.2 Hydrolysis

In anaerobic digestion (AD) the term hydrolysis is used to describe degradation of a defined particulate or macromolecular substrate to its soluble monomers. For particulates, hydrolysis is merely a surface phenomenon, while the process is molecular for smaller macromolecules (biopolymers). During hydrolysis, proteins are hydrolysed to amino acids, polysaccharide to simple sugars and lipids to long chain fatty acids (LCFA) (Henze, 2008). This is performed by heterotrophic microorganisms that attached to particles, produce enzymes in the vicinity of the particle and benefit from soluble products released by the enzymatic reaction. Therefore, the microorganisms growing on the particle surface, rather than the enzyme produced, should be regarded as the effective catalyst (Batstone et al., 2002). Products from hydrolysis are readily accessible for acidogenic bacteria.

The hydrolysis process is very sensitive to temperature and temp-fluctuations. Hydrolysis is generally considered to be the rate-limiting step during AD of complex substrates (Henze, 2008). Investigations by Chandler et al., (1980) and Zeeman et al., (1996) showed that this is not because of lack of enzyme activity but more due to the availability of free accessible surface area of the particles and the overall structure of the solid substrate (Henze et al,2008). Naturally access of nutrients for the bacteria has to go through the enzymatic process by the exohydrolytic enzymes, this activity considered to be the rate-limiting step during AD, consider the sludges content of ca 80% suspended matter (SS) and the main polymers in sludge are proteins, lipids and carbohydrates.

2.3.3 Depolymerisation stoichiometry and kinetics

During the enzymatic hydrolysis process, polymers are hydrolyzed into dissolved readily bio-degradable substrates. Polymer products like amino acid, simple sugars and long chain fatty acids (LCFA) are depolymerized into mono- or short oligomers. These products can pass through the cell walls and be transported into the G-periplasmic space of fermentative bacteria. The cut-off limit for transport through the cell membrane is thought to be around 600 Dalton (Da) g/mole or below 1000 Da based on diffusible cut-off limit for molecules (kommedal, R., 2003). In most cases this is the rate limiting process according to organic composite, the particulate colloids in order of 0.001 - 1 µm or larger. Especial when a high suspended solid concentration with a high SS/COD, the designing of the process is based on this hydrolysis step (Henze et al, 2008).

Stoichiometry and kinetics of depolymerisation are modelled by Michaelis- Menten expressions, or, as in WWTP models, by the Contois model of diffusion limited Monod kinetics. In equation 2.1 below the first order kinetics for depolymerisation (Kommedal., 2009).

(2.1) $r_{hyd} = k_h \cdot X_{polymers}$

Hydrolysis of biopolymers by the action of exo-enzymes is divided into three processes;

- hydrolysis of protein
- hydrolysis of polysacchaarides
- hydrolysis of fats or lipids

Anaerobic digestion models normally use first order kinetics due to the lack of information on biomass (fermenters). Kinetic parameters for biopolymers 2.2, 2.3 and 2.4 are:

(2.2) - $k_{h,polysacc}$ = 10 1/d

(2.3) - $k_{h,proteins}$ = 10 1/d

(2.4) - $k_{h,lipids}$ = 10 1/d

The hydrolysis rate is dependent on biopolymer ability to degrade easily or not, degrade sugar is easier than lignin. The particle size, pH value and temperature are also affecting the rate (Gerber et al, 2008).

2.3.4 Acidogenesis

Acidogenesis or fermentation is generally defined as an anaerobic acid-producing microbial process without an additional electron acceptor (Batstone et al., 2002). During the step acidogenesis, amino acids (AA) and simple sugars, products from hydrolysis which are relatively small soluble compounds, are taken up by bacterial cell membrane and subsequently fermented or anaerobically oxidized (Henze, 2008). Fermentation is an anaerobic conversion of depolymerized products like sugar and amino acids into volatile fatty acids (VFA) or Carboxylic acids (CoA) (Kommedal, 2009). During this growth of fermenters, energy (ATP) is produced directly from an energy-rich intermediate by substrate level phosphorylation (Madigan et al., 2006).

Monomer converts to VFA'a relative fast; the growth rate of fermenters is in the same ratio as aerobic rates, 2-7 1/d. Short chain volatile fatty acids such as acetate (HAc), propionate (HPr) and butyrate (HBu) are main products. Some alcohols (ethanol, propanol and butanol) are formed and also formic and lactic acid under some conditions. Due to the lack of external electron acceptor, the formation of reduced metabolic products is by substrate

internal electron translocation. One part of the molecule fermented is oxidized while another part is reduced. The electrons can also be transferred to cytoplasmic electron acceptors, most often H^+ or pyruvate. The redox balance is achieved by reduction of protons into hydrogen gas, from H^+ to H_2, see figure 2-4.

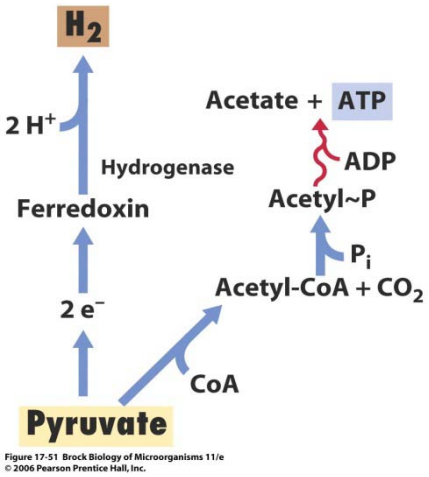


Figure 2.4. Redox balancing in fermentation (Madigan et al,.2009).

In fermentation there must be oxidation-reduction (redox) balance. In a number of fermentation, electron balance is maintained by the production of molecular hydrogen, H_2 . The iron-sulfur protein called ferrodoxin is the electron carrier and catalyzed by the enzyme hydrogenase. Production acetate and ATP (adenosine triphosphate) is synthesized from the hydrolysis of energy-rich intermediate acetyl-phosphate. However, conversion of proteins and polysaccharides into pyruvate forming fermentation products, they all have to be converted through pyruvate (Madigan et al,. 2009).

The large free energy associated with the excreted fermentation products cause the growth yield of acidogens to be low (typically $Y_{X/S}$ of 0.1 - 0.2 g BM/g COD). (Kommedal, R.,2009.) The table 1.1 lists several acidogenic reactions starting from sucrose and generating different amounts of VFA's, HCO_3, H_2 and H^+.

From Table 2.2 it follows that the $\Delta G^{0'}$ of the less energetic acidogenic reactions with sucrose as the substrate strongly depends on the prevailing H_2 concentrations. If H_2 is effectively removed by H_2 scavenging organisms such as methanogens, acetate will be the main end product (Henze, 2008).

Table 2.2 Acidogenic reactions with sucrose as the substrate and the corresponding free energy change ($\Delta G^{0'}$) at 25°C (Henze, 2008)

Reaction	$\Delta G^{0'}$ (kJ/mol)	Eq.
$C_{12}H_{22}O_{11} + 9H_2O \rightarrow 4CH_3COO^- + 4HCO^-_3 + 8H^+ + 8H_2$	-457.5	1.1
$C_{12}H_{22}O_{11} + 5H_2O \rightarrow 2CH_3CH_2CH_2COO^- + 4HCO^-_2 + 6H^+ + 4H_2$	-554.1	1.2
$C_{12}H_{22}O_{11} + 3H_2O \rightarrow 2CH_3COO^- + 2CH_3CH_2COO^- + 2HCO_3^- + 6H^+ + 2H_2$	-610.5	1.3

Acidogenesis is the most rapid conversion step in the anaerobic food chain, with a minimum dobling time in 30 minutes (Batstone et al., 2003). They prefer degradation to acetic acid, since this step results in the highest energy yield for their growth (Mosy, 1983). This can be seen from the table 2.3, by comparing the parameters between acidogenesis and methanogenesis. The $\Delta G^{0'}$ of acidifying reactions is highest of all anaerobic conversions, resulting in a higher bacterial growth rates, and a higher bacterial yields and conversion rates compared to methanogenesis (Table 2.3) (Henze, 2008). Souring of the sludge solution occurs because the products of acidogenesis lower pH and they are produced faster than consumed (kinetic effect).

Table 2.3 Averaged kinetic properties of acidifiers and methanogens (Henze, 2008)

Process	Conversion rate gCOD/gVSS.d	Y gVSS/gCOD	K_S mgCOD/l	μ_m 1/d
Acidogenesis	13	0.15	200	2.00
Methanogenesis	3	0.03	30	0.12
Overall	2	0.03 - 0.18	-	0.12

The amino acid is de-ammonified by anaerobic oxidation. This process is yielding VFA and H_2. In conjunction with the reductive de-ammonification amino acid the other amino acids is consuming the produced H_2. Ammonium (NH_3) is released from both reactions and subsequently acts as a proton acceptor, which can balance the pH drop that would occur when acidic compounds are produced (Henze, 2008).

The acidogenic conversion of amino acids generally follows the Stickland reaction. Clostridia species obtain their energy by fermenting amino acids and only amino acids pair. In this situation, one amino acid is function like an electron donor and oxidised. The other acid is the electron acceptor and reduced. This coupled amino acid fermentation is known as stickland reactions. The product of Stickland reactions are NH_3, CO_2 and a carboxylic acid (CoA) with a number carbon fewer than the amino acid that was oxidized (Madigam et al, 2008).

Acidogenesis is inhibited by free ammonia and LCFA accumulation. This is due to the Stickland reaction. Fermentation of amino-acid (AA) to volatile fatty acid (VFA) and carboxylic acids (CoA) intermediates followed by substrate level phosphorylation through Stickland type coupled oxidation and reduction, figure 2.4. (Kommedal, 2009.)

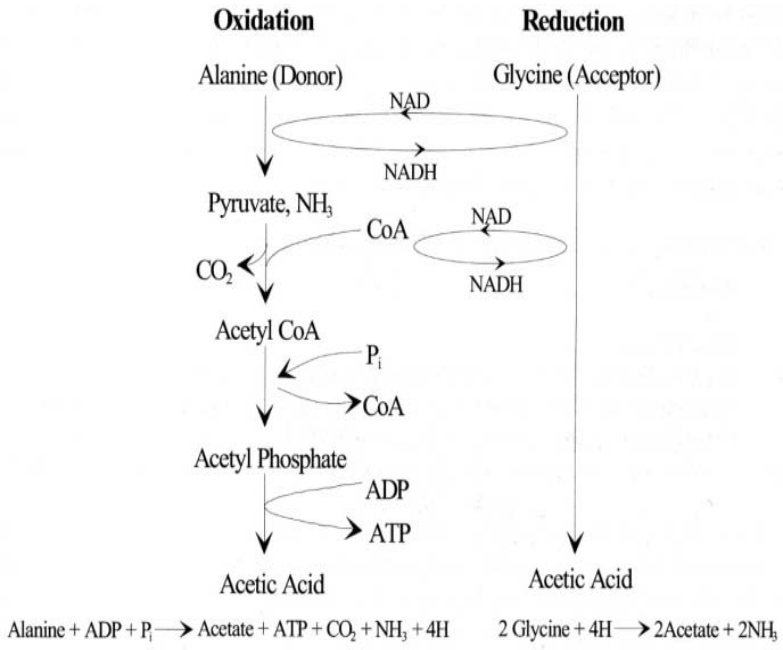


Figure 2.4. Sticland coupled process of alanine and glycine. (Batstone. et al,. 2002)

Stickland reactions occur more rapidly than uncoupled degradation. There are numbers of characteristics of Stickland fermentation of amino acids. The pathway is showed in figure 3-4, and general characteristics are listed below:

1. Amino acid can act as acceptors, donors or both.
2. Acting like e^- donor on carbon to CO_2 and form a carboxylic acid with one carbon shorter than the originals acid, alanine to acetate.

3. Acting as an e^- acceptor and retains carbon atoms to form a carboxylic acid with the same chain length as the original amino acid, glycine to acetate.

4. Histidine cannot be degraded of Stickland reactions. Madigan et al, (2008) is listing up Histidine can be oxidized by the coupled reaction with AA Arginine who is reduced.

5. 10% of total amino acids are degraded by uncoupled oxidation because lack of electron acceptors. This is resulting in hydrogen or formate production.

For modeling the acidogenesis and amino acids this is important. Since given the amino acid mixture of the source protein, the stoichiometric yield of the product can be predicted (Batstone et al., 2002) table 2.4.

Table 2.4 Stickland products (Batstone et al.,2002)

Amino acid	Molecular formula	HAc	HPr	Hbu	HVa	IN	IC	Other	H_2	ATP	Beef flesh[1]	Casein[1]
Arginine	$C_6H_{14}O_2N_4$	0.5	0.5	0	0.5	4	1	0	–1	1	5.40	2.80
Histidine	$C_6H_9O_2N_3$	1	0	0.5	0	3	1	1	0	2	2.40	2.60
Lysine	$C_6H_{14}O_2N_2$	1	0	1	0	2	0	0	0	1	7.20	6.40
Tyrosine	$C_9H_{11}O_3N$	1	0	0	0	1	1	0.882	1	1	2.70	4.30
Tryptophan	$C_{11}H_{12}O_3N$	0	0	0	0	1	1	1.471	2	1	0.90	0.80
Phenylalanine	$C_9H_{11}O_2N$	0	0	0	0	1	1	1.176	2	1	3.60	4.00
Cysteine	$C_3H_6O_2NS$	1	0	0	0	1	1	0	0.5	1	1.50	0.10
Methionine	$C_5H_{11}O_2NS$	0	1	0	0	1	1	0	1	1	2.00	2.50
Threonine	$C_4H_9O_3N$	1	0	0.5	0	1	0	0	–1	1	4.80	3.90
Serine	$C_3H_7O_3N$	1	0	0	0	1	1	0	1	1	5.70	7.60
Leucine/ Isoleucine	$C_6H_{13}O_2N$	0	0	0	1	1	1	0	2	1	14.40	14.20
Valine	$C_5H_{11}O_2N$	0	0	1	0	1	1	0	2	1	6.50	6.70
Glutamine	$C_5H_9O_4N$	1	0	0.5	0	1	1	0	0	2	13.50	19.20
Aspartate	$C_4H_7O_4N$	1	0	0	0	1	2	0	2	2	8.80	6.40
Glycine	$C_2H_5O_2N$	1	0	0	0	1	0	0	–1	0	8.40	3.00
Alanine	$C_3H_7O_2N$	1	0	0	0	1	1	0	2	1	8.40	4.00
Proline	$C_5H_9O_2N$	0.5	0.5	0	0.5	1	0	0	–1	0	4.00	11.40

Basic units of mole product/mole amino acid. 1. Units of mole % total amino acids.

Table D.5: Calculated products from amino acids.

Source	Acetate $f_{ac,aa}$	Propionate $f_{pro,aa}$	Butyrate $f_{bu,aa}$	Valerate $f_{va,aa}$	IN $f_{IN,aa}$	H_2 $f_{h2,aa}$
Casein	0.33	0.07	0.27	0.26	0.008	0.07
Beef flesh	0.53	0.07	0.19	0.19	0.011	0.02

2.3.5 Acidogenesis from LCFA

One of the main group of constitutes is lipids in sludge from different wastes. Lipids are important constituents as they are large structural component of the cell membranes. Lipids can also be carbon and energy reserves. The function of lipids is converting it into lipids to glycerol and long chain fatty acid is via hydrolysis called lipase activity. This process is catalyzed by extracellular (EE) enzymes lipases. Converting the lipids proceeds rapidly compared to the subsequent steps. Glycerol backbone is fermented to acetate through acidogenesis using H^+ as e^- acceptor. The degradation of LCFA is an oxidation reaction with an internal electron acceptor (H^+) (Batstone et al., 2002). Long chain fatty acids (LCFA) are also oxidized to acetyl-CoA. This is done by β-oxidation and electrons are transferred to protons forming H_2, which serve as terminal electron acceptor. Forming Acetyl-CoA is combined with CO_2 to acetate under substrate level phosphorylation. See figure 2.5 (Kommedal, R,.2009)

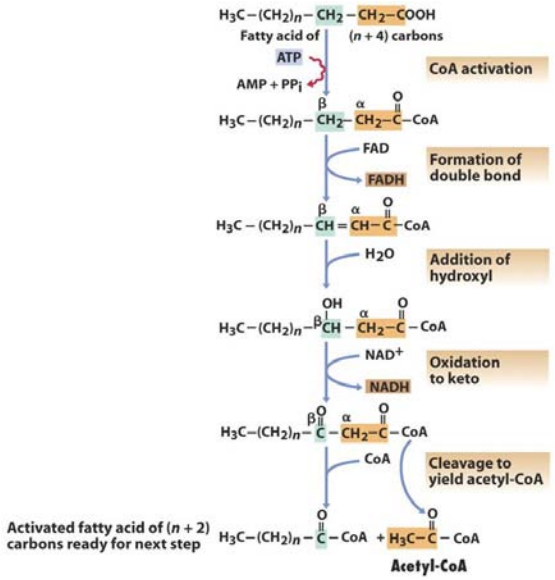


Figure 2.5 Acidogenesis of LCFA(Madigan et al,.2009)

The resulting LCFA are degraded to acetate and hydrogen via activation and ß-oxidation. This ß-Oxidation of LCFA occurs under both mesophilic and thermophilic conditions. Higher temperature promotes growth and biosynthesis of longer fatty acids. At low concentration LCFA my complicate the process by inhibition, but in a well developed process, adaptation occurs and readily degrade the feeds with a high content of lipids. (Batstone. et al,. 2002)

2.3.6 Acetogenesis

Acetogenic bacterial conversion of products derived from the fermentation process. The process has to convert other compounds than acetate, like Co_2 who is reduced into acetate usually with hydrogen gas (H_2) as electron donor. This is done by the acetogens reducing CO_2 by the acetyl-CoA pathway. The most important acetogenic substrates are propionate

and butyrate; they are also key intermediates in AD process. But also lactate, methanol and ethanol and is converted in the same way. Carbon dioxide (CO_2) and even H_2 are converted by homo-acetogens to acetate as shown in Table 2.5 (Henze, 2008).

The formation of acetic acid is when glucose are converted into two molecules of pyruvate with reduction of two molecules of NAD^+. Both molecules of pyruvate are converted to acetyl-CoA or acetylphosphate. The latter molecule is reduced two times to form ethanol. This allows the microorganisms to conserve the energy of acetylphosphate by synthesising ATP. CoA formed is converted to acetylphosphate witch is used as donor for synthesis ATP and 2 moles of ATP per mole glucose is converted by the pathway to pyruvate. One mole of acetic acid formed is assumed to represent a net gain 1 mole ATP. Some microorganisms are able to generate; three mol acetic acid per mol glycose. Further conversions and formation of one mole HAc is done by reduction of two moles CO_2 with further conversion of pyruvic acid to acetyl-CoA. (Ydstebø,L., 2005).

Acetogens is converting the long chain fatty acids being formed during lipase activity. The LCFAs are converted by specific acetogenic bacteria following the β-oxidation in which acetate moieties are split from the aliphatic chain (Table 2.5) (Henze, 2008).The syntrophic organism acetogens and autotrophic methanogens are often found growing together in syntrophic aggregates. Syntrophic reaction in AD is a secondary fermentation. The acetogenic bacteria ferment the products of other anaerobes. The syntrophic reaction is H_2 production linked to H_2 consumption by another. Thereby reducing the diffusive interaction between the two groups making H_2 more efficient (Kommedal, 2009).

The figure shows the syntrophic pathway for butyrate converted into acetate and hydrogen, fig 2.6.

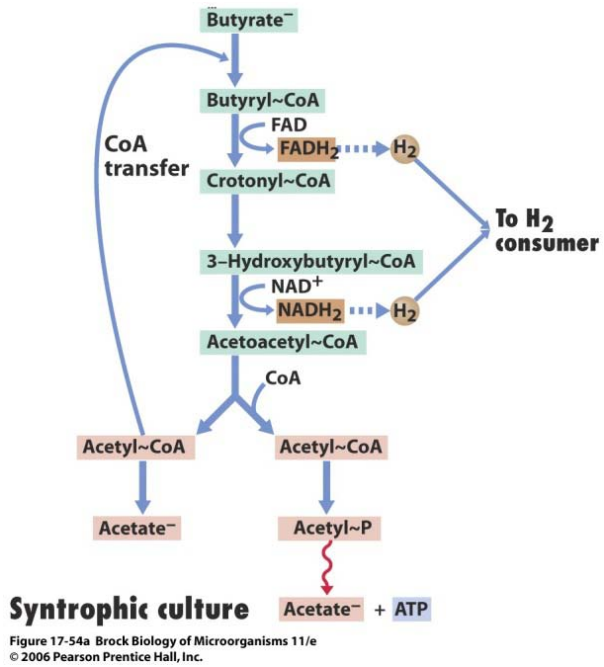


Figure 2.6. Butyrate degradation in syntrophic culture (Madigan et al., 2009).

Table 2.5 Stoichiometry and change of free energy (ΔG°') for some acetogenic reactions at neutral pH and STP (Henze, 2008)

Compound	Reaction	ΔG°(kJ/mole)	Eq.
Lactate	$CH_3CHOHCOO^- + 2H_2O \rightarrow CH_3COO^- + HCO_3^- + H^+ + 2H_2$	-4.2	1.4
Ethanol	$CH_3CH_2OH + H_2O \rightarrow CH_3COO^- + H^+ + 2H_2$	+9.6	1.5
Butyrate	$CH_3CH_2CH_2COO^- + 2H_2O \rightarrow 2CH_3COO^- + H^+ + 2H_2$	+48.1	1.6
Propionate	$CH_3CH_2COO^- + 3H_2O \rightarrow CH_3COO^- + HCO_3 + H^+ + 3H_2$	+76.1	1.7
Methanol	$4CH_3OH + 2CO_2 \rightarrow 3CH_3COOH + 2H_2O$	-2.9	1.8
Hydrogen-CO_2	$2HCO_3^- + 4H_2 + H^+ \rightarrow CH_3COO^- + 4H_2O$	-70.3	1.9
Palmitate	$CH_3\text{-}(CH_2)_{14}\text{-}COO^- + 14H_2O \rightarrow 8CH_3COO^- + 7H^+ + 14H_2$	+345.6	2.0

The acetogenic bacteria are obligate hydrogen producers and their metabolism is inhibited by hydrogen. H^+ serve as internal electron acceptor during regeneration of intracellular electron carriers, like NADH, and their metabolism is thermodynamically inhibited by hydrogen. The thermodynamically conversion of this reaction is showed for propionate in eq.2.5 (Henze, 2008):

$$\Delta G' = \Delta G^{\circ\prime} + RT\ln\frac{[\text{Acetate}] * [CO_2] * [H_2]^3}{[\text{Propionate}]} \qquad (2.5)$$

Studies of this acetogenic conversions have elucidated the required narrow associations between the H_2-producing acetogenic bacteria and the H_2-consuming methanogenic bacteria, thereby resulting the H_2 level in their environment (Henze, 2008).

2.3.7 Interspecie hydrogen transfer

Syntrophic transfer is a situation where two different organisms degrade the substance and conserve energy doing it. The two organisms cannot degrade the substance individually. The process is an direct transfer of the metabolic product (H_2) to the consumer in acetogenesis, and it is called "interspecie hydrogen transfer" (Madigan et al., 2006). Syntrophic transfer reaction in AD is a secondary fermentation, which acetogenic bacteria ferment the products of the other anaerobes organisms. Acetogens and autotrophic methanogens, the syntrophic organisms are found growing and interacting in syntrophic aggregates. This effect makes the diffusive distance shorter for the two groups of species producing H_2 more efficient. The heart of syntrophic reaction is H_2 production by one partner linked to H_2 consumption by another. This interdependence means that the degradation of higher fatty acids and alcohols largely depends on the activity of electron scavenging organisms such as methanogenic bacteria. Microbial association in which a H_2-producing organism only can grow in presence of a H_2-consuming organism is schematic displayed in Figure 2.7 for syntrophic reaction.

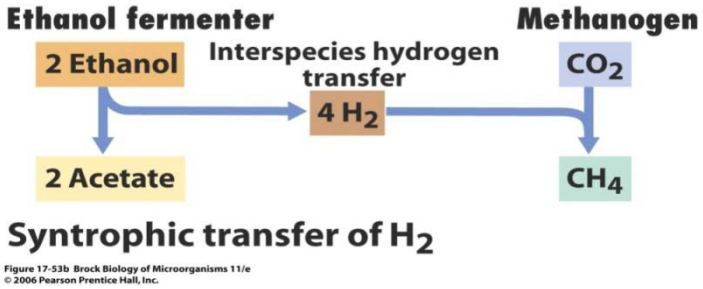


Figure 2.7 Syntrophy with inter species H_2 transfer (Madigan et al., 2006)

The thermodynamics of hydrogen utilising methanogenic and syntrophic acetogenesis reactions are only possible in a narrow range of hydrogen or formate concentrations. It is also influenced by a lower degree of other product and substrate concentrations. This is important for modelling. Due to the thermodynamic limitations are largely determining the parameter for hydrogen inhibition, as well as half saturation coefficients and yields. The limitations are illustrated in Figure 2.8, which shows the thermodynamic yield ($\Delta G'$) for methanogenesis and three AD oxidation reactions.

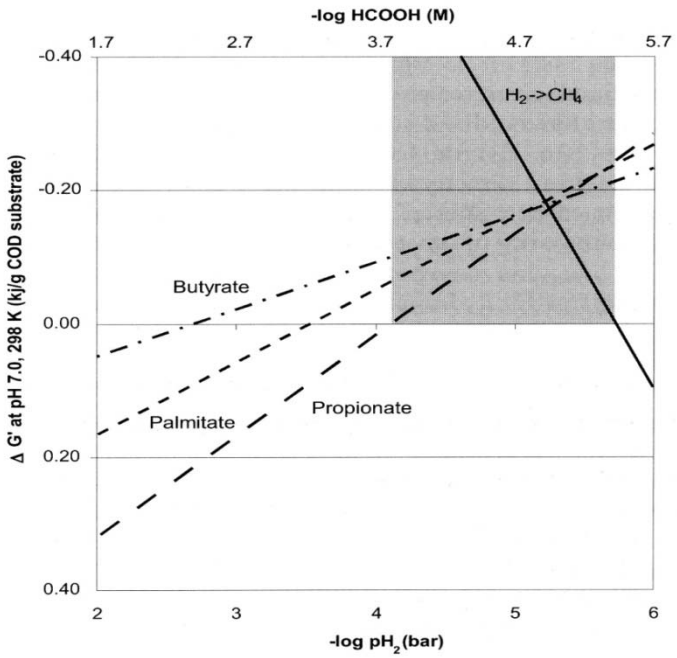


Figure 2.8 Free energy changes as a function of the H_2 partial pressure (Batstone et al., 2002)

The shaded region indicates where methanogenesis and propionate oxidation are simultaneously possible(Batstone et al., 2002). There is an upper limit, set by the acetogens, and a lower limit set by the methanogens of syntrophic thermodynamically transfer of VFA's to methane. The hydrogen concentration must be kept within this so called "hydrogen window", which is in between the partial pressures of 10^{-4} to 10^{-6} bars, otherwise acetogens or autotrophic methanogens will inhibit the process (Kommedal, 2008). The figure 1.3 is showing the digestion conditions for the hydrogen partial pressure is maintained at an

extremely low pressure. Under stabilized digestion conditions the partial pressure is maintained at an extremely low level. This pressure is maintained by an effective uptake of hydrogen by the methanogens and sulphate reducing bacteria. Methanogenic bacteria usually utilize molecular hydrogen in the AD so rapidly that the hydrogen partial pressure drops below the optimal pressure range 10^{-4} to 10^{-6} bars. This is enough to ensure the actual occurrence of the hydrogen producing acetogenic reaction. In a well adopted process and a properly function methanogenic process the hydrogen partial pressure will not exceeds the low range 10^{-4} bar. In this low range of pressure, hence hydrogen concentration the degradation of ethanol, butyrate or propionate becomes exergonic and will yield energy for the acetogens (Henze et al., 2008).

In figure 2.9 the importance of acetate concentration is showed by the acetoclastic methanogenesis as acetate sink by the vertical line. The shaded area shows the space in which all the five reactions, theoretical and simultaneously is possible. The dotted line is the measured hydrogen utilising threshold level for methanogenesis. From the figure 2.9 the thermodynamically possible region is further limited, comparing the limitations to autotrophic methanogenes. For various acetate and hydrogen concentrations, the lines for zero (ΔG′), the thermodynamic yield is displayed in figure 2.9 below.

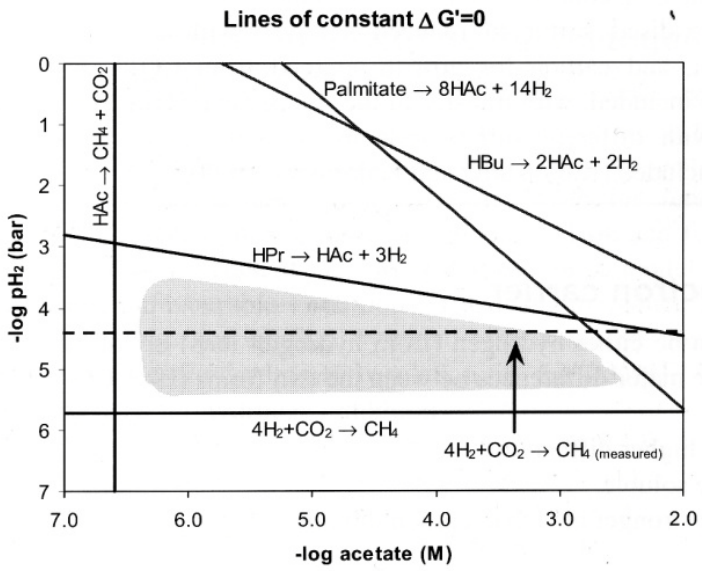


Figure 2.9. Shaded region where all reactions are possible and the threshold level for methanogenisis (Batstone et al., 2002)

From the figure 2.9 the clear effect of increasing acetate concentrations will limit the acetogenesis reaction. The electron carriers H^+ or CO_2 for the syntrophic hydrogen producing acetogenesis, has to be maintained at a low concentration when oxidizing the organic acids. This is due to the oxidation reaction to be thermodynamically possible. However, hydrogen and formate (HCOOH) are consumed by hydrogen utilising methanogenesis organisms by effective uptake (Batstone et al., 2002). Studies carried out on acetogenic conversions have elucidated the required narrow association between the H_2-produsing acetogenic bacteria

and H_2-consuming methanogenic bacteria with interaction and regulation of H_2 level into the environment (Cord-Ruwisch et al.,1988).

2.3.8 Methanogenesis

The final stage in conversion of organic matter into methane and carbon dioxide is methanogenesis. The group of organisms is known collectively as methanogens and classified as archea, both reduce carbon dioxide using hydrogen as electron donor. They are a group of strict obligate anaerobes. Methanogenesis is also a complex series of biochemical reactions that employ novel enzymatic steps with coenzymes. The coenzymes is divided into two classes, first of them are using acetate to produce methane. By the enzyme, acetoclastic methanogens is de-carboxylate acetate to form CH_4 and CO_2. The second enzymatic converting process, together with organisms oxidize hydrogen with carbon dioxide as the electron acceptor to produce methane (Tchobanogolous et al.,2004).

In this stage the influent COD is finally converted into gaseous. The influent COD is converted into gaseous and leave the liquid phase of the reactor system (Henze, 2008). The most important precursor is acetate (70%), while the remaining 30% is formed from H_2/CO_2 or formate (Angelidaki et al). The methanogens are classified into two major groups:

1. The acetate converting or acetoclastic methanogens
2. The hydrogen utilising hydrogenotrophic or autotrophic methanogens (Table 2.6).

Table 2.6 Most important methanogenic reactions, the corresponding free energy change (ΔG°′) and some kinetic properties (Henze, 2008).

Functional step	Reaction	ΔG°′ kJ/mole	μ_{ma} 1/d	T_d d	K_S mgCOD/l	Eq.
Acetotrophic Methanogenesis*	$CH_3COO^- + H_2O \rightarrow CH_4 + HCO_3^-$	-31	0.12[a] 0.71[b]	5.8[a] 1.0[b]	30[a] 300[b]	2.2
Hydrogenotrophic Methanogenesis	$CO_2 + 4H_2 \rightarrow CH_4 + 2H_2O$	-131	2.85	0.2	0.06	2.3

*Two different methanogenesis belonging to [a]Methanosarcina spec. and [b]Methanosaeta spec.
Table 2.6 lists two types of aceticlastic methanogens with very different kinetic parameters.

Final conversion of acetate and H_2 (or HCOOH) is carried out by two groups of methanogens:

1. Acetoclastic methanogens using acetate as substrate. Equation 2.6.

(2.6) $CH_3COOH \rightarrow CH_4 + CO_2$

2. Hydrogenotrophic or autotrophic methanogens using H_2 as electron donor reducing CO_2 to CH_4. Equation 20.

(2.7) $CO + 4\ H_2 \rightarrow CH_4 + H_2O$

CO_2 is used as electron acceptor during H_2 growth, equation 2.8

(2.8) $4\ H_2 + CO_2 \rightarrow CH_4 + 2\ H_2O$

ΔG = -131 kJ/mole

The energy generation in methanogens is driven by reversed electron transport and ATP-ase, and not the process substrate level phosphorylation. The microorganisms found among methanogens have litoautotrophic and organoheterotrophic growth strategies. They can also grow on H_2 (Hydrogenotrophs) or formate, alcohols, acetate or methylated compounds like CH_3OH (Acetotrophs). The electron acceptor during the hydrogen growth is carbon dioxide. (Kommedal, 2009). In figure 2.10 the pathway of converting and reducing CO_2 to CH_4 with H_2 as electron donor is lined up with different enzymatic steps into the final mineralization methane.

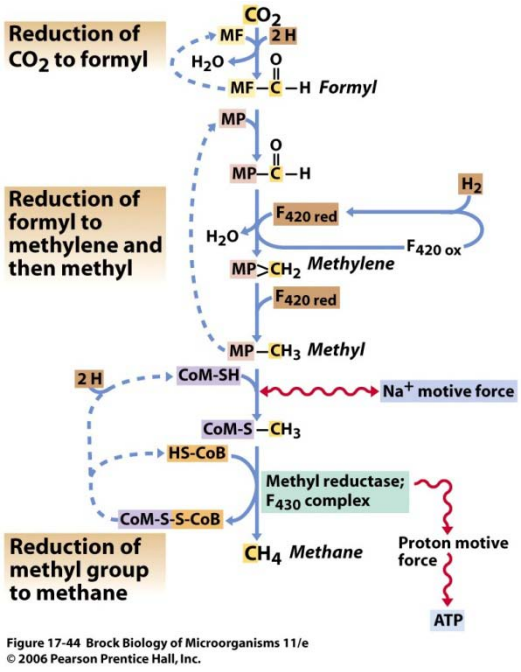


Figure 2.10. Hydrogenotrophy and growth on H_2 (Madigan et al., 2009)

The microorganisms of organoheterotrophic methanogenesis is found in the genera Methanobacterium, methanocaldococcus and methanosarcina. For thermophilic and hyperthermophilic are among the methanopyrales and the genus methanotermus. Common genuses are:

- *Methanobacterium*
- *Methanococcus and thermococcus*
- *Methanomicrobium*
- *Methanospirillium*
- *Methanolobus*

- *Methanosae and* methanosarcina

Methanogens are obligate anaerobes, with a very narrow substrate spectrum. Some can only use certain determined substrates such as acetate, formate, methanol, methylamines and H_2/CO_2 or CO. The growth rate is very low for acetoclastic methanogens using acetate as substrate. Hydrogenotrophic or autotrophic methanogens bacteria using H_2 as electron donor reducing CO_2, have much higher maximum growth rate, with a doubling time of 4-12 hours (Hence et al, 2008). Because of these features and under various conditions the acetogenic reaction step is preceding remarkable stable in high rate anaerobic system.
Two genera utilize acetate to produce methane (Madigan et al.,2000) methanosarcina and methanosaeta. They are dominating each in different acetate concentration.
Methanosarcina has a great growth rate and have a relative wide substrate spectrum and convert likely methylamines, methanol, formate and the regular acetate and H_2/CO_2. They have relatively high μ_{max} and low substrate affinity. While methanosaeta have longer retention time and thereby degrading acetate with kinetic characterized with low μ_{max} and a very high substrate affinity.

In figure 2.11 the acetoclastic methanogens are converting acetate to produce methane. The acetoclastic methanogens is de-carboxylate acetate (acetyl-CaO) via interaction with carbon monoxide dehydrogenase of the acetyl-CaO patway. Then it is transferred to corrinoid enzyme to yield CH_3-corrinoid. From this state, the degradation are going in to the terminal step through the CoM-mediated of methanogenesis. The CO groups is simultaneously oxidising the acetate intermediates to yield the final step to form CH_4 and CO_2 (Madigan et al,. 2009).

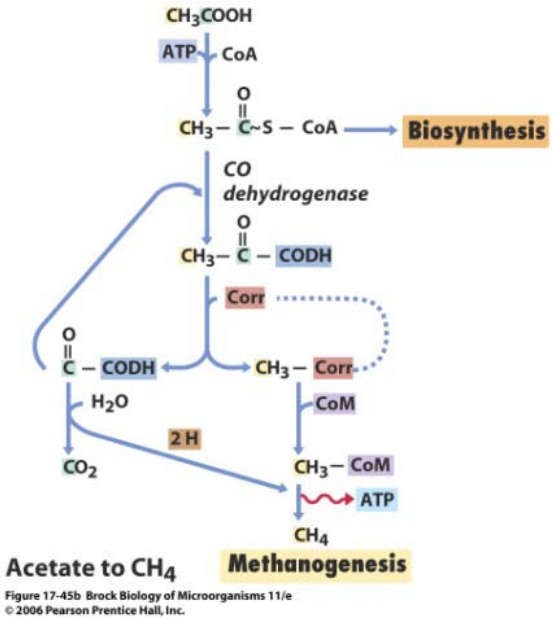


Figure 2.11. Acetate conversion into carbon dioxide and methane. (Madigan et al., 2009)

2.3.9 Bioenergetics of methanogenesis

Acetoclastic and autotrophic methanogens free energies for both are very low. These organisms are known to rely on proton or cation motive force energetics through reversed electron flow in the cell membrane. Maximum growth rates are low (0.3 – 0.5 1/d) as well as the growth yield (0.05 – 0.1 g BM(VSS)/g COD). Methanogenesis is almost always the limiting process during anaerobic sludge digester design. The process step has low growth kinetics require long mean cell residence times in bioreactors. (Kommedal, R., 2009.)

2.3.10 Inhibition and pH

The anaerobic process is limited to a relatively narrow pH interval from approximate 6.0 to 8.5. Process level outside this area can lead to imbalance or AD-failure. There are many factors which influence the pH value. The main compounds that influence on pH are production of organic acids and carbon dioxide. These compounds cause drop in pH level. Another compound is ammonia, with accumulation the pH will increase due to the $CaCO_3$ - reaction and alkalinity concentration. (Kommedal, R., 2009).

There are several other process steps and bacterial consortia that may be inhibited by substrates or products from the anaerobic digestion process and degradation pathway. Other compounds with external toxins from industrial wastes can be an issue as well.

Biostatic inhibition is non-reactive and reversible. Examples one product inhibitions are weak acids and bases such as VFA, NH_3 and H_2S, pH inhibition and cations. The weak volatile fatty acids; acetic (HAc), propionic (HPr), Byturic (HBu), formic (HFa) and valeric (Hva) have pK_a's at 4.7-4.9, and cause pH inhibition when accumulating in the digestion reactor. H_2S may be produced from dissolved sulphates, or other oxidized intermediates. Both sulfide and hydrogen sulfide is toxic to bacteria and largely inhibitory (Batstone et al.,2002). Ammonia may also inhibit the process or main free base in reactor with a high pH and with pK_a level at 9.25.

Biocidal inhibition is related to reactive toxicity and is generally irreversible processes. Long chain fatty acids(LCFA) is one important example. LCFA's, such as Palmitic acids, Stearic acids, etc., inhibit growth irreversibly by competitively inhibit bacterial fatty acid synthesis, uncoupling key reactions in the electron transport chain and change membrane permeability. Other examples are detergents, aldehydes, nitro-compounds, cyanide, azides, antibiotics and electrophiles.

Of the different species and genera susceptible to inhibition, the methanogens are known to be very sensitive towards these effects, and is normally considered to be the first process inhibited in the reaction pathway of anaerobic processes. (Kommedal, R.,2009).

2.3.11 Temperature

The temperature has an effect on all reactions in anaerobic thermodynamics by Vant`t Hoff equation. Oxidative reactions become more favourable at higher temperature in general. There are three major operation ranges defined in anaerobic bioreactors, described in the figure 2.12(Batstone et al,.2002);

1. Psychrophilic 4-15^{o}C
2. Mesophilic 20-40^{o}C
3. Thermophilic 45-70^{o}C

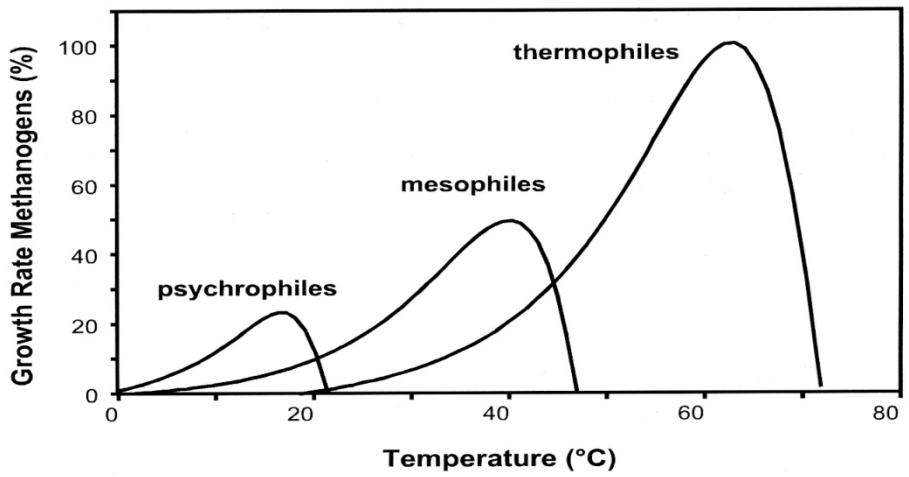


Figure 2.12 Growth rates for the three major class of microorganisms defined in anaerobic digestion processes (Batstone et al.,2002).

The temperature is providing and stimulating interactions for different group of organisms. Anaerobic digestion can operate effectively between these temperatures and for mesophilic the optimum is 35 ^{o}C hence 55^{o}C for the thermophilic. The population can easily drop to zero with increasing temperature for each individual's organisms. Biochemical the temperature can effect the anaerobic digestion reactor in different ways by several mechanisms.

a) Increasing temperature will increase disintegration and hydrolysis rates, predicted by Arrhenius equation.
b) Decrease in reaction rate with increasing temperature above optimum for the microorganisms, >40 ^{o}C for mesophilic and >60 ^{o}C for thermophilic.
c) Change or decrease in thermodynamic yield due to increased growth rates and bioenergetics with increased temperature.
d) Shift in metabolic pathways due to change in thermodynamic yield. Methanogens seems to be the most sensitive group.
e) The death rate increase due to hydrolysis and decay of active organism.

Organic concentration and temperature have greatly effect on design of reactors. At lower temperature slower reaction rates and longer solid retention time (SRT), this leads to larger reactor volumes and lower organic loads. In equation Empirical combined effects of temperature (T) in Kelvin (K) on the minimum solid retention time (SRT) *(Batstone et al,.2002)*:

$$SRT_{\min} = \frac{1}{0.267 \cdot 10^{1-0.015\cdot(208-T)} - 0.015} \qquad (2.9)$$

2.4 Codigestion, monitoring and modelling

The performance of an anaerobic digestion process is much depending on the type and the composition of the organic sludge to be digested. Organic waste with a wide range of characteristics has been successful anaerobic digested, such as sewage sludge, industrial waste, waste from slaughterhouse, fruit and vegetable, manure and agriculture biomass. The effects of codigestion are needed to be investigated further, studies for investigating the overall stability of the process according to the dynamic conditions and input of varies organics. The waste composition into the digesters is of great interest due to the optimizing the overall production of biogas. Sewage sludge has a long tradition of being anaerobic digested for reduce the sludge production volume and stabilisation of the organic sludges. Therefore, anaerobic digesters are in many cases very simply in construction and the process poorly monitored with the result of an process with low organic loading rate (OLR) to avoid overload (Murto, M., et al,. 2003).

Other organic wastes have been introduced for codigestion for improving the biogas yield. This is due to positive synergisms established in the digester and supplying of missing, limiting nutrients in the process (Mata-Alvarez et al., 2000). Codigestion lies in balancing several parameters in the co-substrate mixture. The key is to find some qualities of co-substrate that can be advantageous for use in the biogas process. While other qualities can hinder the degradation seriously (Hartmann et al., 2002).

In general carbon/nitrogen (C/N) ratio have to be balanced. The nutrients content and composition in the sludge, with a wide range of micro and macronutrients are important for all living organisms. This diversity is stimulating the microorganism for high consumption and production of digestion products. Microorganisms is in the multistep AD process is producing VFA, charging the alkalinity and pH in processing and degrading organics into methane and

carbon dioxide. So the intermediate products, the VFA, pH and alkalinity is important processes intermediates parameters for evaluating the co-substrate (Carlson,M., et al,. 2009).

In this study, the investigated system is the digesters at the municipal wastewater treatment plant SNJ. The plant is operating with excess sludge from the WWTP settled sludge and co-digested with external industrial high concentrated food waste. The aim of this investigation was to assess the process with characterization of organic load and the sludge in to the system. Additional analysis of AD process with use of the IWA Model no. 1. (ADM1) and parameter estimation by the software AQUASIM. Take into account the time involved with cost and experimental studies, mathematical models are extreme useful in efficient operation, design and modification of bioprocesses (Ozkan-Yucel et al,. 2010). The ADM1 model of full scale digesters also may use for testing and development of new controlling strategies or compartments. The anaerobic intermediates degradation is reflecting the current metabolic status of the active organisms the system. To be able to monitoring the anaerobic digestion process, the activity of different groups of organisms can be measured indirectly, e.g. by the gas production rate, or by the accumulation of intermediates in the digesters (Gujer et al., 1983). One line sensors and application is not available for monitoring specific microbial populations. Therefore, the model could predict some important unmeasured parameters together with experimental analysis.

For monitoring the process, the most commonly used indicators include volatile fatty acids (VFA), alkalinity, pH and gas production rate. Normally the amounts of hydrogen, methane and carbon dioxide also are measured in the biogas. The process monitoring at the WWTP SNJ, consists of on-line monitoring of the volume of gas produced with percentage, weekly off-line analysis of pH, alkalinity (ALK) and organic content, total solids (TS) and volatile solids (VS) in raw sludge and in digesters. The online analysis of the gas composition is also monitored. The process operation for the digesters is only based on pH limit to be above 6.8.

The ADM1 model was used to simulate full scale anaerobic digestion using dynamic influent data from sludge production and external industrial sludge with use of AQUASIM for parameter estimation. This test was due to validate the steady-state conditions for a period of three weeks. The plant was also subjected with a pulse load for dynamic analysis for two days. During the experiments, the alkalinity, VFA, pH was measured, the gas production rate were monitored and biogas composition. The degradation of organic matter was also measured at steady state and dynamic conditions.

2.5 WWTP and AD process at SNJ

Thickened raw sludge is pumped from settling to a 500 m^3 buffer storage tank. According to design, raw sludge should be fresh before anaerobic processes become active. However the sludge is fast going into putrefaction when storage is prolonged during the processing from settling. The solid content of raw sludge consists in general of approximately 5% solids and 95% water. The influent organic load by the wastewater is not constant and the buffer storage tank is acting like leveller to provide a constant OLR. Fluctuating in COD, solids and VTS fractions are giving the AD process load variations and result in a fluxing biogas production. The organic content and the composition in the raw sludge from settling process is the main sludge in volume and used for codigestion process which can balances the solids contents in the reactors. Industrial food waste, fats and other industrial high-content organic have a high TS content. Total solids content higher then > 10-15% is not unusually in some cases and often diluted with raw sludge to become more available for the transport system. This industrial slurry wastes can be codigested with the sewage sludge for increasing and levelling the TS content and the biogas production at output (Carlson, M., et al,. 2009).

The flow diagram below (Figure 2.13) shows the digestion process plant consists of two anaerobic reactors (digesters), two heat exchangers and two buffer tanks. The raw sludge flow from sedimentation arrives to the buffer tank 1. This buffer tank 1 (500 m^3) serves as storage feeding for the reactors and levelling the influent raw sludge flow. It receives raw sludge (RS) as described above, in addition to external wastewater sludge (biomass sludge), industrial food waste (FWS), slaughterhouse waste and industrial organic waste. The external industrial sludge waste has access to the digesters or buffer tank 1 by pipelines and pumping systems.

Degradation and digestion of organics takes place in the reactors. The sizes of reactors are 3500 m^3 and headspace volume biogas storage inside each reactor of 226 m^3.
When the AD plant receives external industrial sludge, the wastes are pumped into the buffer tank who is the normal procedure and/or directly in to the AD reactors. The function of the buffer tank is important in maintaining a uniform organic and hydraulic load (OLR) for the reactors. To facilitate constant conditions in the digestion process the sludge is pumped to the digesters in a 1 hour cycle at 15m^3/h sequentially and the hydraulic loading per reactor is approximately 180 m^3/d (Osli, K., 2011).
The sludge input to reactors approximately equals the amount of sludge leaving the system. Digested sludge is stored in buffer tank 2 and subsequently dewatered in centrifuges.

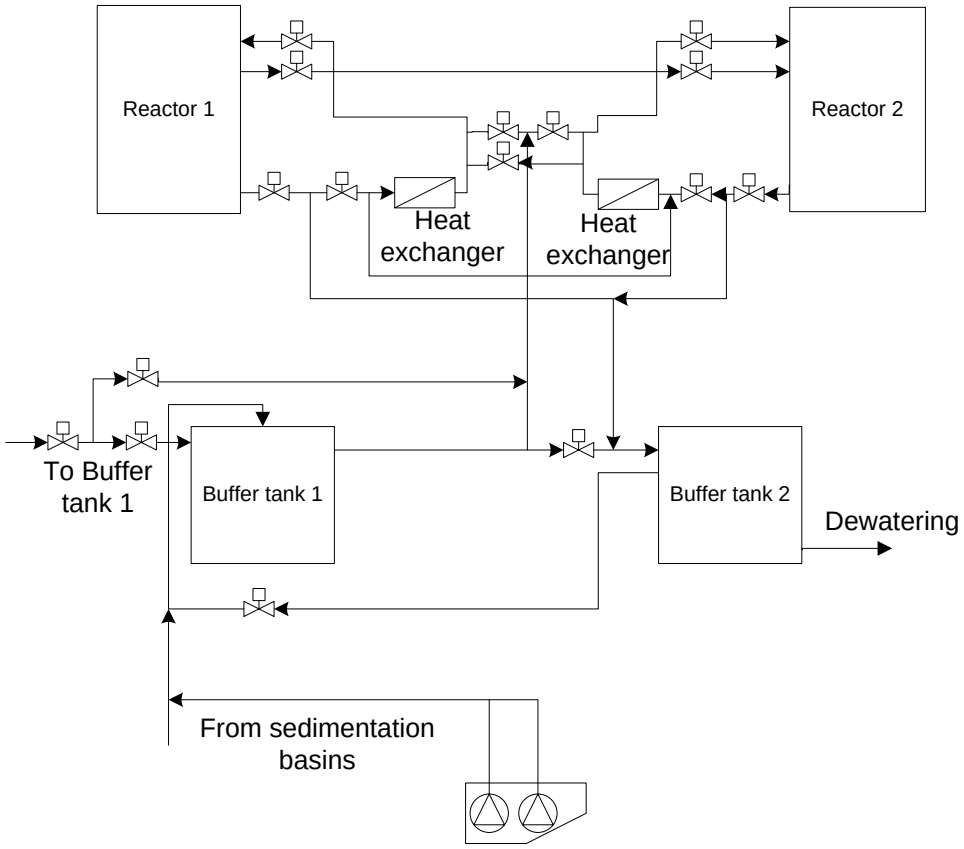


Figure 2.13 Flow diagram of SNJ plant (Popov, J. 2010)

Heat exchangers ensure that the reactors operate at constant mesophilic conditions maintained at 37°C. To hold digester temperature steady despite fluctuations in the temperature of the incoming sludge at 12-15°C, the high-rate digesters are heated in order to increase disintegration, hydrolysis and methanogenesis rates.

Both digesters at SNJ plant are mixed by injecting compressed biogas into the liquid through pipelines diffuser lances. The digesters are mixed in order to improve mass transfer between microorganisms and their substrates and to prevent formation of scum at the water level and sediments at the bottom. The mixing is to maintain a high enough liquid velocity so that all the solids remain in suspension. (Rittmann and McCarthy, 2001)

Sludge processing after centrifugal dewatering the sludge is dried using heat from the biogas burners. The solids content of the product after centrifugal dewatering and thermal drying is about 85% and extruded into small biopellets.

3 Methods

This chapter consist of methods and materials. First chapter is an introduction to the software AQUASIM to give an overview of the structure basic platform for entering parameters. The next chapter is based on the model ADM1, anaerobic digestion no.1. by IWA task group for mathematical modeling of anaerobic digestion process. Here the definitions and expressions with nomenclature, units or dimensions are connected to the model ADM1. This nomenclature, dimensions and expressions are fundamental for the parameter estimation and act like a base of parameters for sampling and experimental studies at the AD process.

Following chapters is containing all experimental work and methods. All measurements and analysis were generated in the laboratory at SNJ and at the laboratory at UIS. In this study several analyses were done. Parameters which were investigated are pH, chemical oxygen demand (COD), measurement and determination of total solids (TS) and volatile solids (VS), and volatile fatty acids (VFAs) and alkalinity (ALK). In this chapter it is explained how the experiments were conducted in detail for later experiments or studies. The names of the instruments and equipment used for these analyses are precisely described.

3.1 Aquasim

AQUASIM is a computer program for the identification and simulation of aquatic systems. The platform model consists of a system with ordinary and/or partial differential equations and algebraic equations. The program is determining and describes the behaviour of a given set of important state variables of an aquatic system. The differential equations for substance transport and for water flow can be selected by the choice of technical or environmental compartments, which can be connected by links. Figure 3.1 visualizes the platform for the system and the dependencies between the four subsystems; 1.variables, 2.processes, 3.compartments and 4. links.

1. Variables
   The basic structure of the AQUASIM model is the system of variables. Variables are objects which are characterized by property with a numerical value. This value may depend on the values of other variables. Different types of variables are distinguished: State variables, program variables, constant variables, real list variables, variable list variables, formula and probe variables. An typical state variable is substrate concentration in reactor and for the constant variables e.g., influent flow rate. The system of variables serves as a databank of variables for the formulation of the other subsystems (Reichert, 1998)

2. Processes

   Two types of processes are distinguished: Dynamic and equilibrium processes. Typical for a dynamic process is growth and decay of microorganism and aeration with oxygen consumption. For equilibrium processes the chemical equilibria buffersystem, pH and alkalinity is classical.

3. Compartments

   The system of compartments is divided into different subsystems. The subsystem is designed to spatially divide the system under investigation. Examples on compartments are different reactors like; mixed reactor (CFSTR) biofilm, plug-flow and rivers and lakes compartments and in this study AD-reactor is the main compartments.

4. Links

   The last subsystem of the AQUASIM model structure is the system of links. The objects of this subsystem are used to connect the compartments to the desired spatial configuration. The links is divided into diffusive and advective links. Mass transfer along with the media, sludge or liquid is advective link and molecular diffusion by Fick`s 1. Law, with gas transfer throught the system is diffusive links. In AD processes the gases CO_2, H_2 and CH_4 are involved with diffusive links. All compartments may be connected in all different combinations and linked by diffusive or advective links (Reichert, 1998).

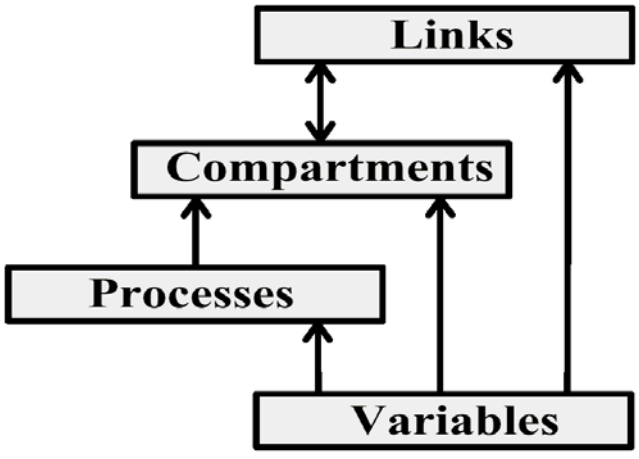


Figure 3.1 Main platform of model structure (Reichert, 1998)

The modelling and parameter estimation with use of Aquasim requires a wide range of input data and parameters for modelling AD reactors in full scale. Dimensions, nomenclature and definitions are important crucial step in modelling of reactors or any compartments.

3.2 State Variables and dimensions in expressions

The use of Aquasim program needs to define a wide range of variables, processes and compartments with defined links. The Anaerobic Digestion Model No. 1 (ADM1) introduces generic nomenclature, units and definitions. This nomenclature, dimensions and expressions from IWA Task Group will be used in this study and integrated into the Aquasim model for parameter estimation. The empirical formula of $C_5H_7O_2N$ represents biomass as in Activated Sludge Model (ASM) series (Batstone et al., 2002) and in this studies of modelling AD reactors.

3.2.1 Units

ADM1 had chosen COD (kgCOD m^{-3} = gCOD m^{-1}) as the chemical component base unit because of its use in wastewater characterisation and measurement in concentrated streams. It's widely used in biogas utilisation industries, the implicit balancing of carbon oxidation state and to enable partial compatibility with the IWA Activated Sludge Models. Molar basis (kmole m^{-3} = M) is used for components with no COD such as inorganic carbon (CO_2 and HCO_3^-) and inorganic nitrogen (NH_4^+ and NH_3). A molar (M) and kgCOD m^{-3} basis was chosen to facilitate $\log_{10}$ conversion (e.g. pH and pK_a) for physic-chemical equation (Batstone et al., 2002).

Table 3.1 Units in ADM1(Batstone et al., 2002)

Measure	**Units**
Concentration	kgCOD m^{-3}
Concentration (non-COD)	kmoleC m^{-3}
Concentration (nitrogen non-COD)	kmoleN m^{-3}
Pressure	bar
Temperature	K
Distance	m
Volume	m^3
Energy	J (kJ)
Time	d (day)

3.2.2 Nomenclature with description of parameters and variables

There are four main parameters and variables: kinetic parameters, stoichiometric coefficients, equilibrium coefficients, dynamic state and algebraic variables.

Table 3.2 Stoichiometric coefficients in model(Batstone et al., 2002)

Symbol	Description	Units
C_i	Carbon content of component I	kmoleC $kgCOD^{-1}$
N_i	Nitrogen content of component i	kmoleN $kgCOD^{-1}$
$V_{i,j}$	Rate coefficient for component I on process j	nominally kgCOD m^{-3}
$F_{product,substrate}$	Yield (catabolism only) of product on substrate	kgCOD $kgCOD^{-1}$

Table 3.3 Equilibrium coefficients and constants (Batstone et al., 2002)

Symbol	Description	Units
H_{gas}	Gas law constant (equal K_H^{-1})	bar M^{-1} (bar m^{-3} $kmole^{-1}$)
$K_{a,acid}$	Acid-base equilibrium coefficient	M (kmole m^{-3})
K_H	Henry's law coefficient	M bar^{-1} (kmole $m^{-3}bar^{-1}$)
pK_a	$-\log_{10}[K_a]$	
R	Gas law constant ($8.314x10^{-2}$)	bar $M^{-1}K^{-1}$(bar m^3 $kmole^{-1}$ K^{-1})
ΔG	Free energy	J. $mole^{-1}$

Table 3.4 Kinetic parameters and rates used in model(Batstone et al., 2002)

Symbol	Description	Units
$K_{A/Bi}$	Acid base kinetic parameter	$M^{-1}d^{-1}$
k_{dec}	First order decay rate	d^{-1}
$I_{inhibitor,process}$	Inhibition function (see K_I)	
$K_{process}$	First order parameter(for hydrolysis)	d^{-1}
k_La	Gas-liquid transfer coefficient	d^{-1}
$K_{I,inhibit,\ substrate}$	50% inhibitory concentration	kgCOD m^{-3}
$K_{m,process}$	Monod maximum specific uptake rate (μ_{max}/Y)	kgCOD_S $kgCOD\_X^{-1}$ d^{-1}
$K_{S,process}$	Half saturation value	kgCOD_S m^{-3}
ρ_j	Kinetic rate of process j	kgCOD_S $m^{-3}d^{-1}$
$Y_{substrate}$	Yield on biomass on substrate	kgCOD_X $kgCOD\_S^{-1}$
μ_{max}	Monod maximum specific growth rate	d^{-1}

Table 3.5 Dynamic state, algebraic variables and derived variables(Batstone et al., 2002).

Symbol	Description	Units
pH	$-\log_{10}[H^+]$	
$p_{gas,i}$	Pressure of gas i	bar
P_{gas}	Total gas pressure	bar
S_i	Soluble component i	kgCOD m^{-3}
$t_{res,X}$	Extended retention of solids	d
T	Temperature	K
V	Volume	m^3
X_i	Particulate component	kgCOD m^{-3}

3.2.3 Dynamic State Variables

Values calculated at a specific time [t] are "dynamic state variables" and are solutions of the set of differential equations, defined by the ADM1 process rates.

Table 3.6 Dynamic state variable characteristic (DAE) system (Batstone et al., 2002)

Name	i	Description	Units	MW	gCOD·$mole^{-1}$	Carbon content (C_i)	Nitrogen content (N_i)
X_c	13	Composite		varies	Varies	varies	Varies
X_{ch}	14	carbohydrates		varies	varies	0.0313	varies
X_{pr}	15	proteins		varies	varies	varies	varies
X_{li}	16	lipids		806	2320	0.0220	0
X_I	24	particulate inerts		varies	varies	varies	varies
S_I	12	soluble inerts		varies	varies	varies	varies
S_{su}	1	monosaccharide's		180	192	0.0313	0
S_{aa}	2	amino acids		varies	varies	varies	Varies
S_{fa}	3	total LCFA		256	736	0.0217	0
S_{va}	4	total valerate		102	208	0.0240	0
S_{bu}	5	total butyrate		88	160	0.0250	0
S_{pro}	6	total propionate		74	112	0.0268	0
S_{ac}	7	total acetate		60	64	0.0313	0
S_{h2}	8	hydrogen		2	16	0	0
S_{ch4}	9	methane		16	64	0.0156	0
S_{IC}	10	inorganic carbon	M	44	0	1	0
S_{IN}	11	inorganic nitrogen	M	17	0	0	1
X_{su-h2}	17-23	biomass		113	160	0.0313	0.00625
S_{cat}		cations	M	varies	0	0	0
S_{an}		anions	M	varies	0	0	0

Process configuration is modelled by inputs with initial conditions time t=0. When a differential algebraic equation (DAE) implementation is used, the state of a system at time [t] is fully defined by the value of these 26 variables in each vessel (Batstone et al., 2002)

3.3 Biochemical processes and structure of reactions in the ADM1

The model includes the three overall biochemical cellular steps; acidogenesis (fermentation), acetogenesis with anaerobic oxidation of organic acids, and methanogenesis. The biochemical cellular steps also include as well the extracellular, partly non-biological disintegration step and an extracellular hydrolysis step (figure 2.2). Three of the processes hydrolysis, acidogenesis and acetogenesis have a number of parallel reactions. Complex composite particulate waste is assumed to be homogeneous, which disintegrates to carbohydrate, protein and lipid particulate substrate (Batstone et al., 2002).

The ADM1 model mainly included to facilitate modelling of waste activated sludge digestion, a disintegration step is thought to precede more complex hydrolytic steps, but is also generally used when the primary substrate can be represented with lumped kinetic and biodegradability parameters for primary sludge and other substrates. The complex particulate pool is also used as a pre-lysis repository of dead biomass. Therefore the disintegration step is intended to include an array of steps such as lysis, non-enzymatic decay, phase separation and physical breakdown or shearing (Batstone et al., 2002).

All steps in extracellular process were assumed to be first order, which is an empirical function reflecting the cumulative effect of a multi-step process. Cellular kinetics is described by three expressions; uptake, growth and decay in table 1.1 and 1.2 in Appendix A.

The key rate equation is substrate uptake, which is based on substrate level Monod-type kinetics. Biomass growth is implicit in substrate uptake and consumption with first order decay was assumed and described with an independent set of expressions. ADM1 and Batstone, etal., chose substrate uptake related kinetics, rather than growth related kinetics to decouple growth from uptake, and allow variable yields.

The structure of the overall biochemical processes is schematically listed due to the reaction model in ADM1 below in figure 3.2.

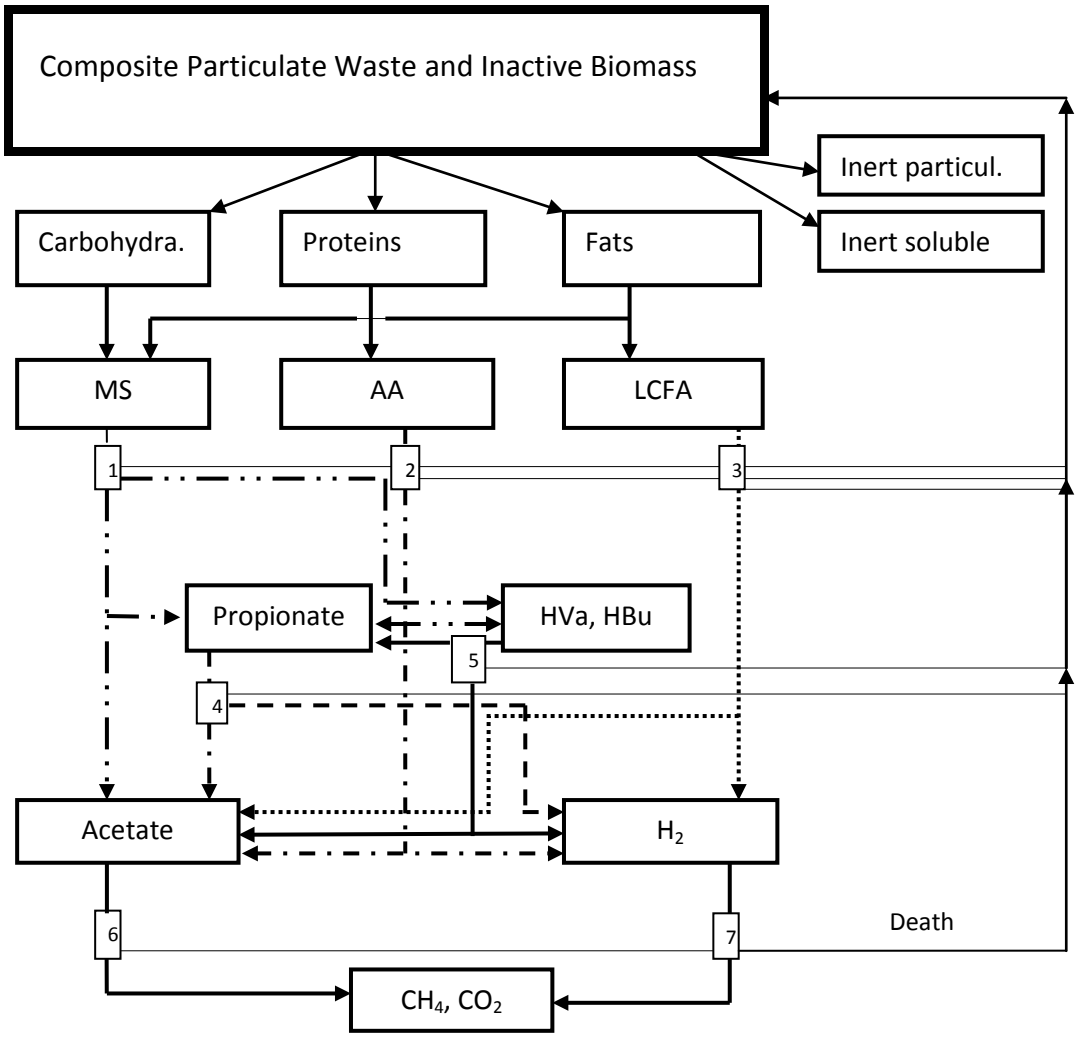


Figure 3.2: The anaerobic model as implemented including biochemical processes (1) acidogenesis from sugars; (2) acidogenesis from amino acids; (3) acetogenesis from LCFA; (4) acetogenesis from propionate; (5) acetogenesis from butyrate and valerate; (6) aceticlastic methanogenesis; and (7) hydrogenotrophic methanogenesis (Batstone et al., 2002).

The structure of describing composite particulate organics is assumed to be homogeneous in the model with the fractions carbohydrate (X_{ch}), protein (X_{pr}) and lipids (X_{li}). With codigestion, this portioned structure of the fractions is characterised due to varying content in different wastes. When modelling with this portioned structure, the content of each fraction of carbohydrate (X_{ch}), protein (X_{pr}) and lipids(X_{li}) are given different degrading rate due to the composition.

3.3.1 Model Presentation in Matrix Format

The stoichiometry and process rate matrix for ADM1 biochemical reactions, are given in Table 1.1 for soluble components and Table 1.2 for particulate components in Appendix A. For each component, the mass balance within the system boundary can in general be expressed as follows:

Accumulation = Input – Output + Reaction

The input and output terms describes flow across the system boundaries, and depend on physical characteristics of the modelled system. Within the reaction term, there are a number of specific processes, such as growth, hydrolysis, decay, etc. that influences other components. The matrix method represents the reaction terms for each component, subdivided by processes. Moving vertically through the matrix the process index (j) changes; while moving horizontally, the component index (i) changes (Batstone et al., 2002). The process index and description is given in the left hand column, while the component index and nomenclature is given in the topmost row. In the right hand column the process rate (ρ_j) for each process is given, while the remainder of each row is filled with stoichiometric coefficient ($V_{i,j}$) that describe the influence of that row's process on individual components. The overall volume-specific reaction term (r_i) for each component i can be formulated by summing the products of the stoichiometric coefficients in column i and their process rates (Batstone et al., 2002):

$$r_i = \sum_j V_{i,j}\rho_j \qquad \text{Eq. 3.1}$$

For example, the overall rate of reaction for monosaccharide's (r_1) is:

$$r_1 \sum_j V_{i,j}\rho_j = \underbrace{k_{hyd,ch}X_{ch}}_{\text{Hydrolysis of Carbohydrate}} + \underbrace{(1 - f_{fa,li})k_{hyd,li}X_{li}}_{\text{Hydrolysis of Lipidss}} - \underbrace{k_{m,su}\frac{S_{su}}{K_S+S}X_{su}I_1}_{\text{Uptake of Sugars}} \qquad \text{Eq.3.2}$$

The advantage of matrix presentation method is that conversion of COD, nitrogen and carbon continuity can easily checked. The stoichiometric coefficients are after adjustment to consistent units for each row, should add up to zero, as COD, carbon or nitrogen lost from reactants must flow into the products. The matrix format allows comparison of different model and facilitates transforming the model into a computer program (Batstone et al., 2002).

3.4 Laboratory work and analysis

The laboratory work and analysis where conducted for investigating steady state conditions and dynamic conditions. Solids analysis with total solids (TS) and volatile solids (VS) where processed at the plant. COD analysis and volatile fatty acids (VFA) measurements where partly conducted at the laboratory and at the University of Stavanger (UIS) laboratory. In this section the experimental work and analysis are explained and how it was conducted including the names of the instruments and equipments used for these analyses. Methods for wastewater analysis were adapted from standard methods (APHA et al., 2006) according to (Ydstebø, 2008). Sludge samples were taken from the AD reactor 1 and 2, the buffer tank with raw sludge and external sludge like food waste (FWS) using tap points in the process loop into 1000 ml poly ethylene (PEH) bottles.

3.4.1 Solid analysis

Solids refer to matter in suspended or dissolved form in waste and sludges. Solids analysis is important in the control of physical and biological processes in the AD reactors. Performing solids analysis is due to the total content of organic compound for determining COD in total solids (TS) and characterisation of the energy content (Formular 3.3 and 3.4)

(3.3) Total solids (TS) = Suspended Solids (SS) + Dissolved Solids (DS)

(3.4) TS = SS + DS

In short the TS remains after evaporation of water at 103-105°C. SS are non-filterable and DS are filterable through a 1 µm (micrometer) pore-size, GF/C 6 Whatman filters. Determining of the concentration of the total volatile solids (TVS), the procedure is to combust all organic compounds at 550°C and determine the residue after combustion, the total fixed solids (TFS). For determine the concentration of total volatile solids TVS is showed in equation 3.5.

(3.5) TVS = TS - FTS

For determination of total solids, a clean aluminium plate was weighted on an analytical balance with four decimals. The sludge sample was homogenized and added to about 2,5-3 grams and weighed. The sample was dried at 105°C for around 20 min in a Sartorius thermo control oven, program nr.3 to constant weight. After this the plate and solids weight was determined, and the total solids (TS) where found as the difference. To determine volatile solids the plate and sample is combusted at 550°C for 2 hours in Carbolite Furnaces oven. After the combusting in oven the samples were cooled down in a desiccator to room temperature and weight on balance. The volatile solids have combusted and the remaining solids are inorganic (fixed) solids (FS) or the ash.

3.4.2 Measurements of pH, Alk and VFA

The sludge samples should be treated and analysed as fast as possible after sampling time. Sampled sludge should be "regarded and treated like fresh food" to keep the freshness of the sample for optimal measurement. The first measurement of the sludge was pH measurement and second two parallel activities where processed, TS measurement and supernatant production. For effective sludge sample treatment, centrifugation where conducted for supernatant production. The supernatant from the separation process is for preparing the sludge for ALK and VFA titrations by 5-steps titration and for filtered COD and VFA`s measurement. The raw sludge has TS content in a level of 6.0-6.5% and will fast clog and block the filter in vacuum-filtration. Therefore with centrifugation and filtration of the supernatant production where optimized by using two-layers of filters. The vacuum filtration was conducted through a 2 x 1µm filters by vacuum flask using standard methods increased the sample treatment efficiently. From the centrifugation process the filtrated volume of the supernatant produced approximate 60ml dissolved filtered sludge-liquid. This dissolved liquid from the sludge where divided into tree measurements or pre-treatments steps;

1. Alkalinity and VFA`s by 5-points titrations, 20ml.
2. VFA by Ion Chromatograph (IC), 20ml.
3. COD content of dissolved/filtered sludge, 20ml.

Alkalinity and VFA measurements by 5-points titration procedure with calculations has to be processed at once, according to changes in the composition of VFA`s and alkalinity. The procedure according to standard methods described in "analytical methods in the wastewater laboratory" (Ydstebø, 2008). Pre-treated sample for IC analysis of VFA`s has to be cooled down and freeze to prevent change in VFA-compositions. The dissolved COD sample also has to be conserved for later measurement. The conservation of the dissolved sample is done by lowering the pH by adding HCL-acid into the sample down to pH 4.

The procedure on pH measurement was first calibration of the instrument before measurement of the sample, once a day. The instrument was calibrated with two different solution pH: 4.01 and pH: 7.00. at 20°C room temperature. The procedure is an internal program, named calibration mode, and the instrument type is; WTW, Multi 340i, (Wissenschaftlich-technische Werkstatten GmbH). Calibration solutions were premade from the instrument supplier "WTW, Multi 340i".

Procedure of 5-points titrations for alkalinity and VFA analysis with measurement is processed in following steps. By using a graduated cylinder, the filtered sample of appropriate volume 10-50ml and dilute to 50ml. Transfer into an 100ml beaker and put on a magnetic stirrer at low rotation, 60-100 rpm to minimize and avoid CO_2 input or loss.

- Measure the conductivity in the diluted sample or in the original sample. Filtration or centrifugation doesn't affect this activity and measurement.
- Measure the temperature in the sample. Room temperature at 20°C.
- Record the initial pH after stabilizing usually 2-3min.

If the pH is lower than 6.7, add NaOH until pH is about 6.7(+/-) 0.1 read volume by using pipette with 0.1ml volume in the steps until pH 6.7, note the NaOH volume consumption.

a) If the pH is lower than 6.7, add NaOH until pH is about 6.7(+/-) 0.1 read volume by using pipette with 0.1ml volume in the steps until pH 6.7, note the NaOH volume consumption v_1. If the pH is higher than 6.7, start titration with HCL until pH is about 6.7(+/-) 0.1 read volume, also called v_1.
b) The next step is titration with HCL by using an burette until the pH 5.9(+/- 0.1), note the volume v_2.
c) Continue the titration until pH 5.2 (+/- 0.1), note the volume v_3.
d) Step 5 of titration with HCL until 5.9(+/- 0.1), note the volume v_4.
e) Add all numbers, volumes and pH steps into "TITRA-5" and calculate.

Volatile fatty acids (VFAs) and ionic compounds were analysed with Ion Chromatograph (IC) type Dionex ICS-3000 at the University of Stavanger (UIS). The procedure for preparation of samples for VFAs analysis is following:

1. Approximately 45-50ml X 3 of sludge were transferred to conical centrifuge tubes.

2. Samples were centrifuged at 8500 rpm for 15 min. in Thermo Electron LED GmbH Heraeus Scientific Biofuge primo Centrifuge model 75005181. Program nr.4.

3. The supernatant 60-80ml was filtered by vacuum-filtration through a (2 x 0.45) µm (micrometer) and the filtrate was transferred to a 20 ml sampling-beaker.

4. The samples where cooled and freeze down.

5. In general preparing for IC-sampling, dilution of the sample 1:10. High concentrated samples 1:20, typically raw sludge and high concentrated food waste or high organic loaded AD reactors.

6. The steady state sampling, digester sludge was diluted 1:10 and raw waste, food waste sludge 1:20. At the dynamic test, the 5-point titration gave result of high amount of VFA`s, so the dilution of the AD reactor sludge where diluted 1:20 and for raw sludge. Before and after the test was regarded as normal state and diluted 1:10.

7. The diluted sample where transferred into vials, short thread 1,5 ml (32x11,6mm), clear glass,VWR International and filtered with 0.2 µm filters. This is due to the sensitivity of the IC-instrument and the columns. Approximate 1,4-1,7 was filtered into sampler vials for IC analysis using pre-rinsed 0.2 µm syringe filters, "PALL Acrodisc" with Luer Lock.

Stock solutions was made for VFA`s and cations intermediate analysis and measurement. For the acids, 10g/l with dilutions to 50mg/l for; lactate (HLA), propionate (HPR), acetate (HAC), formate (HAC), butyrate (HBU) and valerate (HVA) stock solutions were made. For the cations, sodium (Na^+), calcium (Ca^{2+}), potassium (K^+), magnesium (Mg^{2+}) and ammonium (NH_4^+) , 500mg/l stock solutions were made an diluted to 25mg/l. One blank distilled water and one tap water for measuring void time and response for the column. Tap water used for flushing and cleaning the column before sampling sequences (Ydstebø., L., 2011)

3.4.3 COD analysis in bioprocesses

The COD flow through the system is used to determining the organic load for the anaerobic digestion. Calculating the COD is based on the biodegradable fractions $_{B}COD(_{RB}COD+ _{SB}COD)$. Different substrate C-sources like sludge from wastewater treatment plant (WWTP) and industrial sludges are different in concentration of C-sources and degradability.

The first part is definitions of (COD) Chemical Oxygen Demand and standard method for determining chemical oxygen demand. A stepwise explanation of measurement of COD procedure, from the start to final measurement is explained, with an approach to meet the COD level of measurement and practical working approaches.

When analysing organic matter a strong acidic solution is digesting the organic compound. The oxygen (O_2) is consumed by the process and produced carbon dioxide (CO_2) and water (H_2O). The process is called "chemical oxygen demand" (COD) and has a standard procedure. The strong premade solution is dichromate ($Cr_2O_7^{2-}$) with a known theoretical oxygen demand. Dichromate ($Cr_2O_7^{2-}$) is very strong oxidising agent which is being reduced to Cr^{3+} in acidic solution. With spectrometry chromium species are coloured and absorb in the UV-Vis

region of spectrum. The colour change is proportional to the reduction of chromium, which is proportional to the oxidation of the organic compound. (Ydstebø,L.,2010.)

More precise acknowledge of the method is the amount of a specified oxidant that reacts with the sample and consumed under controlled conditions is defined as COD. Expressed in the term of its oxygen equivalence, the quantity of oxidant that is consumed under the reaction. Specified oxidant in the method, dichromate ion ($Cr_2O_7^{2-}$) is reduced to chronic ion (Cr^{3+}).
The principle is oxidizing organic matter by boiling in a mixture of chromic and sulphuric acids. A sample is refluxed in strongly acid solution with a known excess of potassium dichromate ($K_2Cr_2O_7$). The dichromate ion is oxidizing organic matter as COD when the sample is digested. The changes in chromium species in the reaction from the hexavalent (VI) state to the trivalent (III) state are coloured. The coloured species absorb in the visible region of the spectrum. The dichromate ion ($Cr_2O_7^{2-}$) absorbs strongly in the 400 nm region, where the chromic ion (Cr^{3+}) absorption is much less. The chromic ion absorbs strongly in the 600nm region, where the dichromate has nearly zero absorption. Chromic ion (Cr^{3+}) has a minimum absorption in the region of 400nm. That gives the working absorption maximum at 420 nm. For COD values between 100 and 900mg/L, increase in chromic ion (Cr^{3+}) in the 600nm region is determined. Higher values can be obtained by sample dilution. COD values of 90 mg/L or less can be determined by following the decrease in dichromate ion ($Cr_2O_7^{2-}$) at 420nm. The colour change is proportional to the reduction of Cr species, which is proportional to the oxidation of the organic matter (A. Standard Methods Committee, 1997)

For COD determination by Hach-method includes prepared vials with the necessary reagents for analysis. The determined volume of the sample is 0.2 or 2ml. The sample is added to the vial that is digested at 150 ^{o}C for 2 hours. By UV-Vis, colourimetric determination the sample is processed in a Hach DR-2000 spectrophotometer. The method includes three ranges of COD;

1. 0-150 [mg/L],
2. 0-1500 [mg/L],
3. 0-15000[mg/L].

A zero sample containing distilled water is also prepared for calibration of zero. Dilution of the sample may be necessary in order to match the measuring range.(L.Ydstebø, 2008)

The procedure for sample-preparation with COD`s measurements of the sludge for biodegradable $_{B}$COD and filtered ($_{RB}$COD), the samples were prepared into solutions in following order:

1. Sample PEH bottle of volume 250ml for $_{B}$COD was zero calibrated on the balance.

2. The sludge was homogenized with 4 seconds shaking.

3. Sludge sample where transferred with a course pipette and weight-inn with approximate 2-2.5 g of sludge.

4. Weight of sample was noted from the analytical balance (four decimals) and then diluted into 200 ml distilled water.

5. The sample was then conserved with 2ml acid 4M H_2SO_4, with lowering the pH down to 4. This was done because COD measurement has to be processed later.

6. Samples was cooled down and stored in the refrigerator at 5°C for later measurement.

7. Digestion solution was prepared by HACH (premade) 0-1500mgCOD/l.

8. The COD sample where slowly heated up to room temperature and homogenized by shaking in 4 seconds. NB course particulate is settling fast to the bottom.

9. 2ml of sample was transferred with 1-5ml auto-pipette into the vial with premade solution.

10. The vials with sample solution is digested and boiled in a heater, Hach COD reactor at 150°C for 2 hours, cooled down to room temperature in approximate 20min. and measured using HACH spectrophotometer.

11. The samples were colorimetric determined using HACH DR-2000 spectrophotometer wavelength set at 620 nm, method nr 430 with adjustment for wavelength into 620nm. Use instructions manual for the instrument HACH DR-2000.

12. Calibration of the instrument was conducted by the digested zero sample. Confirm the zero calibration with distilled water of zero wavelengths (nm).

The same procedure was conducted with filtered/dissolved COD, the $_{RB}$COD from the supernatant. Dilution of the samples in order to match the measuring range at 500-1000 mg/l was in many cases necessary. Especially the high concentrated food wastes (FWS) have a high strength concentration of 6000-12000 mgCOD/l. For the traditional sludges,

experience and plant data give the ratio 1-1,5 (mgCOD/mgTS) for approximations of the measurement levels. Calculations and analysis of the sludge samples were conducted following approach;

1. Raw sludge is in normally in the range of 70-80 kgCOD/m^3 and the range of 4,5% VTS. Due to experience from the plant or plant data, the level is mainly in this order.

2. Raw sludge = VTS (%) x 1,5 = COD (mg/l)

3. Raw sludge → 4,5 (%) ~ 4,5 gVTS/l = 45000(mgVS/l)

4. Approximation of COD for raw sludge =
   45000(gVTS) x 1,5(gCOD/gVTS) = 67500(mgCOD/l)

5. Dilution factor of raw sludge: 67500(mgCOD/l)/100 = 675mg/l.

Dilution of raw sludge into 1:100 fit well to the measurement range of 500-1000mg/l. The same approach was conducted for the other sludges. If the digestion solution is turning fast into green colour when adding sample from the original diluted solution , make one more parallel of 1:5 or 1:10 dilutions, depend on the concentration and what kind of sludge you are working with. The approach is as follows for 1:5 or 1:10;

1. Pipette 2,5ml sample into a beaker and dilute with distilled water 10ml (2 x 5ml).

2. Mix the sample gentle and transfer 2ml new sample into Hach vials for measurement.

3. If the reaction turns into dark green again from the 1:5 dilution, split the 2ml pipetation into 1ml sample and 1ml distilled water; 1:10 dilution into the Hach premade digestion solution.

In general raw sludge from settling and filtered $_{RB}$COD has to be diluted 1:5 or 1:10 in addition to diluted original solution. FWS is high concentrated COD and the dilution has to be high to meet the 500-1000mg/l range of measurement. Approximation and calculation with test measurement with several parallels has to be conducted to find the level.

3.5 The yield constant $Y_{X/S}$ and COD relations

During anaerobic treatment the COD is only "re-arranged" in the converting process. Complex organics matter is degraded and broken down into more simple intermediates and mineralized to CH_4 and CO_2. All influent COD ends up in the end-product methane and CO_2, minus the COD that is incorporated in the new biomass (BM). The relationship to Yield $Y_{x/s}$, the ratio $Y_{x/s}$=(biomass produced)/(substrate consumed) is calculated by specific COD from typical AD fractionations like, sugars, lipids and proteins. In acidogenesis of sugars, glucose fermentation can result in different alternative fermentation products. Apart from organic acids (VFA) one other important substrate for AD process is lactate and ethanol. During high concentration load of organics, lactate increasing from being insignificant to the highest organic acid for a transient overload situation (Batstone et al., 2002). This specific COD for different intermediates means that we have different yield constant for different substrate.

Samples are evaluated to determining the organic load. The COD evaluation is one part of this standard method. An example of calculating the COD concentration with a 300ml/l Ethanol C_2H_5OH and 100ml/l glucose $C_6H_{12}O_6$ solution sample is performed in this equations (Ydstebø, L.,2010);

(3.6) COD = gCOD/gEth x Cons.solut(mg/l) + gCOD/gGluc x Cons.solut (mg/l)

COD, the mass of oxygen required for oxidising the organic compound to CO_2 and H_2O.

Oxidising of Ethanol in equation 3.7

(3.7) Reaction Ethanol:

$C_2H_5OH + 3O_2 \rightarrow 2CO_2 + 3H_2O$.

$COD/Ethanol = (3_{mol} x 32_{oxygen/mol})/(1_{mol} x 46_{Ethanol/mol}) =$

COD/Ethanol = 2.08gCOD/gEthanol

Oxidising of Glucose in equation 3.8

(3.8) Reaction Glucose :

$C_6H_{12}O_6 + 6O_2 \rightarrow 6CO_2 + 6H_2O$.

$COD/Glucose = (6_{mol} x 32_{oxygen/mol})/(1_{mol} x 180_{Glucose/mol})$

COD/Glucose = 1.067gCOD/gGlucose

The COD concentration, the organic load in the sample is determined in formula below 3.9

(3.9) COD = gCOD/gEth x Cons.solut(mg/l) + gCOD/gGluc x Cons.solut (mg/l)

COD = 2.08gCOD/gEth x 300(mgEth/l) + 1.067gCOD/gGluc x 100(mgGluc/l)

COD = 731 mg COD/l

The sludge contains complex organic compounds and particles called VSS (volatile suspended solids). The composition of the organic compound is $C_{60}H_{87}O_{23}N_{12}P_1$ for sludge in wastewater. The COD concentration or the organic load can be calculated in the ratio of COD/VSS. (Ydstebø,L.,2010.)

Formular weigth(F_W) of organic sludge showed in formula 3.10.

(3.10) F_W (gVSS) = $C_{60}H_{87}O_{23}N_{12}P_1$ = (60x12)+(87x1)+(16x23)+(12x14)+(1x31)= 1374gVSS

The mass of oxygen (COD) required for oxidising the organic compound ($C_{60}H_{87}O_{23}N_{12}P_1$) to CO_2 and H_2O. Balancing the biochemical reaction give in equation 3.11.

(3.11) $C_{60}H_{87}O_{23}N_{12}P_1 + 62.5O_2 \rightarrow 60CO_2O + 12NH_3 + H_3PO_4 + 24H_2O$.

Calculating the ratio for sludge (gCOD/gVSS) for this composition in equation 3.12.

(3.12) COD/VSS= (62.5mol x 32g COD/mol)/1374gVSS = 1.455gCOD/gVSS. (Ydstebø,L.,2010)

In the model ADM1 the sludge formula is $C_5H_7O_2N$ and the yield constant $Y_{X/S}$ is the ratio of the amount of biomass (BM) as VSS produced against substrate consumed(S). The substrate is, like explained above, divided into main fractions, polysaccharides (carbohydrates), proteins and lipids with intermediates productions throughout the process. In evaluation and modelling systems, distinction has been made between the observed yield and the synthesis yield or “true” yield. The observed BM yield is based on the actual production of BM on substrate consumption. The BM yield and substrate consumed is in this case actually less than the synthesis yield, because of cell loss in concurrent with growth. Yield is given in the literature or from measurement and calculated from following equations of Yield constant $Y_{X/S}$ = (BM)[g]**/**(S)[g] (Tchobanogolous et al.,2004).

True yield ($Y_{X/S}$) and observed yield ($Y_{X/S}^{obs}$) are based on measurements on biomass (BM) produced (VSS)[$^{mg\ VSS}$/l] and substrate consumed (COD)[^{mgCOD}/l], formula 3.13;

(3.13.) The observed Yield constant $Y_{X/S}^{obs}$

$Y_{X/S}^{obs}$ = (VSS)[$^{mg\ VSS}$/l]/(COD)[^{mgCOD}/l]

COD is the oxygen demand in mgCOD/liter and volatile suspended solids (VSS) is mgVSS/liter.

The observed yield ($Y_{X/S}^{obs}$) is then in mgVSS/mgCOD.

For conversion of observed yield into ratio with the dimension (gCOD)/(gVSS) the amount of oxygen consumed in oxidising $C_5H_7O_2N$, the yield constant is determined in this equation 3.14.

(3.14) $C_5H_7O_2N+5O_2 \rightarrow 5CO_2 + NH_3 + 2H_2O$.

From the equation 11., 1mol BM is oxidized in endogen respiration and 5mol oxygen are consumed. Conversion into gCOD/gVSS in this equation 3.15. (Ravndal, K.T.,2009)

(3.15) Convertion into gCOD/gVSS=

$5mol \times 32^{gO}/_{mol} / 1mol(12x5+7+14+2x16)^{gVSS}/_{mol}$ = <u>1.42 gCOD/gVSS</u>

The observed yield constant $Y_{X/S}^{obs}$ is then calculated in equation equation 3.16.

(3.16.) The observed $Y_{X/S}^{obs} = [1.42 (^{mgCOD}/_{mgVSS})] \times [VSS(^{mgVSS}/_{l}]/COD(^{mgCOD}/l)$

In general 1g of polysaccharides (carbohydrate) or protein is approximately equivalent to 1g of COD. The unit are stated in oxygen pr. volume or concentration in gram $gCOD/m^3$. The consumption of dichromate ($Cr_2O_7^{2-}$) is converted into an equivalent oxygen demand. That means the amount of oxygen that which will be consumed if the oxidation process had taken place by using oxygen. (Ydstebø,L.,2010.)
COD gives a fair estimate of the content of organic matter in WWTP and the sludge's. The range may be 90-95% of theoretical oxygen demand by complete oxidation of all organic matter. (Mogens,H.,et al.,Springer 2002)

3.6 Online process parameters

During the period of analyses of AD process, different process parameters were measured online as part of the operational control system and logged for this study. The loading rates from buffer tank to AD reactors (RT1) and (RT2), loading rates of sludge collected from sedimentation basins to the buffer tank, external sludge to buffer tank, out of reactor 1 and 2, food waste to buffer tank, gas produced in reactor 1 and 2, and methane content in the gas were all logged by the process control room.

The raw sludge from settling (RS) into the buffer tank 1 and further into the reactors is the main target of volumetric logging. Additional sludge loading rates from another/external wastes was also logged from controlling systems on plant. (Osli, 2011).

3.7 Full scale preliminary batch testing

From the steady state period it was observed rapid responds on different external sludge loads. From ordinary conditions with normal activity at the plant, the behaviour of the AD reactors where responding visible with high activity and biogas production. The biogas production responded after 20-30 minutes from delivery to the buffer tank. The buffer tank has a volume 500m^3 and consists mainly of raw sludge (RS) from settling WWTP, and the test batch consisting of volume at 25 m^3. That give range of dilution for the high concentrated organic (FWS) in the level of 1:10 to 1:20, in practise the sludge level is approximate 250-350 m^3 so the rate of dilution is more 1:10-14. The feeding rate of RS from buffertank into the digesters is normal in the range 20-25m^3/h. For better stimulating high responds to the batch, it was planned to inject direct into one AD-reactor and the other reactor is acting as the "Stabile one" or reference reactor. Of the figure 3.3 the red line is the test reactor with biogas production from injected batch. The green line is the reference reactor with rather stabile biogas production.

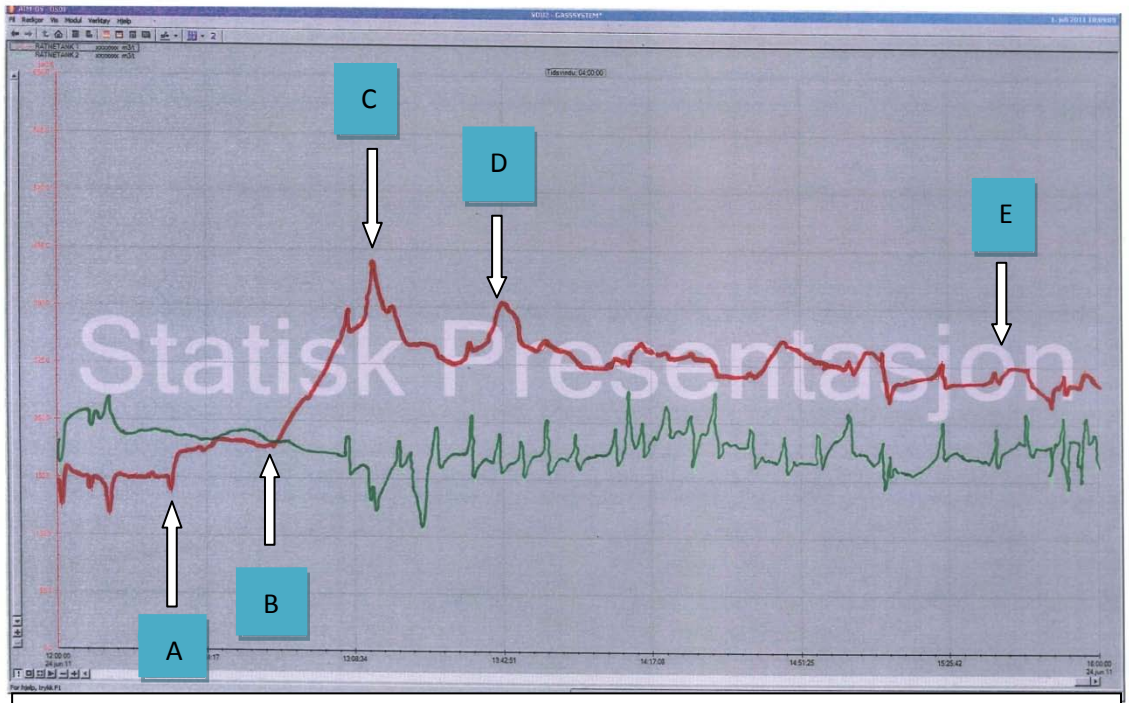


Figure 3.3 Batch test and response of biogas production for further planning.

From the figure 3.3 the injected FWS was pumped into the system at the time 12:30 (A). Approximate 20min later the activity is increasing with a high rate of biogas production (B), the production is continuously increasing to max level of production after 20min (C). After this max-point the production is decreasing with a new peak at a lower level (D). The reference reactor was acting stabile throughout the test period. The high rate test reactor slowly decreased in gas production for 24 hours after batch injection and went into the same level as reference reactor.

The full scale batch test gave very useful information of reaction. The first 30minutes is a very high and intense reaction. The activity in the reactor is high and the sludge sampling should be as fast as possible. A sampling intensity of 5 to max 10 minute in sampling time is necessary to catch the dynamics in the reactor for first 45min. The reaction after 45 minutes is quite stable and decreasing for 24 hours, until stabile production levels for both reactors.

The behaviour of the reactors was observed for 24 hour for planning the sampling intensity in the dynamic test later on for this study. For organization of practical sampling intensity and administration of laboratory work, this test gave very useful information especially within the first hours after injection. This test gave also valuable information of what kind of measurement it was possible to process during the test period.

4 Results

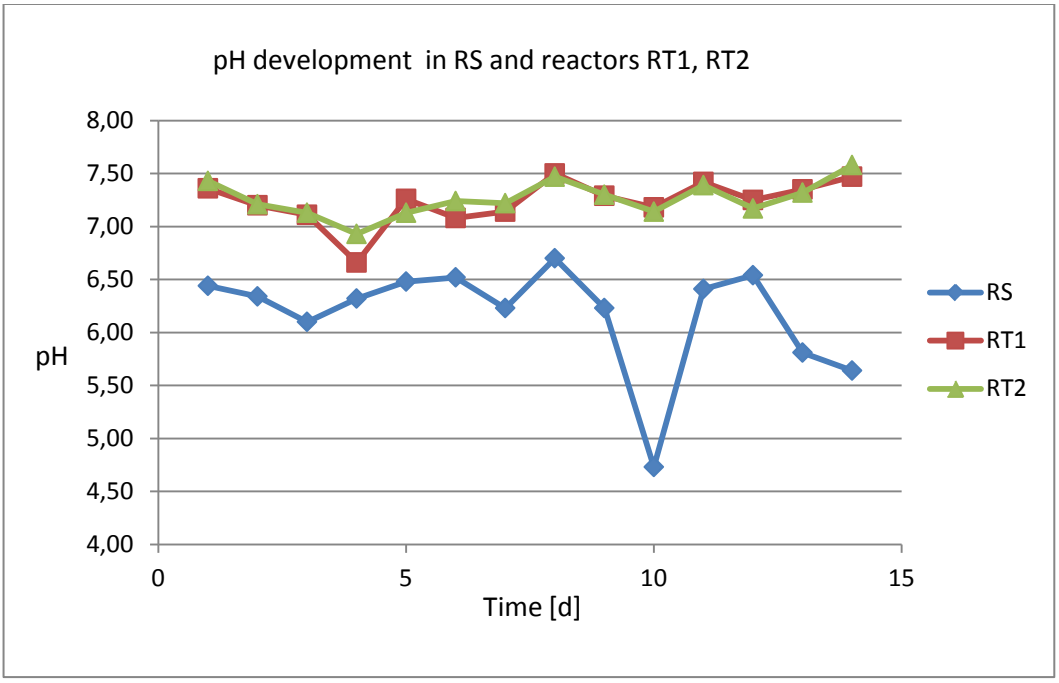
The first section in this part is presenting the result from 14-20 days experimental campaign of ordinary situation at the plant. For the experimental work in this period is called “steady state” period. The next chapter is presenting the dynamic state of a period for 24 hours until 2 days. This was due to the preliminary dynamic batch test with rapid reaction in AD reactor. Finally data from steady and dynamic state were by model ADM1 implemented for parameter estimation and analysis.

4.1 Steady State period

This study of normal behaviour of the AD process was conducted in 03-20 of May 2011. Ordinary raw sludge from settling basins was the main organic load with some high concentrated food waste (FWS) into the system. The raw and FWS sludges where characterized to determine TS, FTS and VTS. COD measurements on biodegradable COD ($_{Bio}$COD) and $_{RB}$COD from the supernatant, with ALK, pH and VFA`s measurements with calculations. The behaviour of the digesters was observed when batch biodegradability assays were carried out with codigestion of raw waste with high concentrated organic food waste almost daily. Digester sludges where analysed for total solids, volatile solids and inerts. Alkalinity and pH measurement with VFA`s analysis.

4.1.1 pH and alkalinity with VFA`s interactions

For the raw sludge (RS) in buffer tank and the digested sludge in reactors RT1 and RT2 the pH was measured throughout the period. Due to settling of solids and precipitation with ferric chloride and the input of FWS the pH is showed for the period in the graph 4.1.



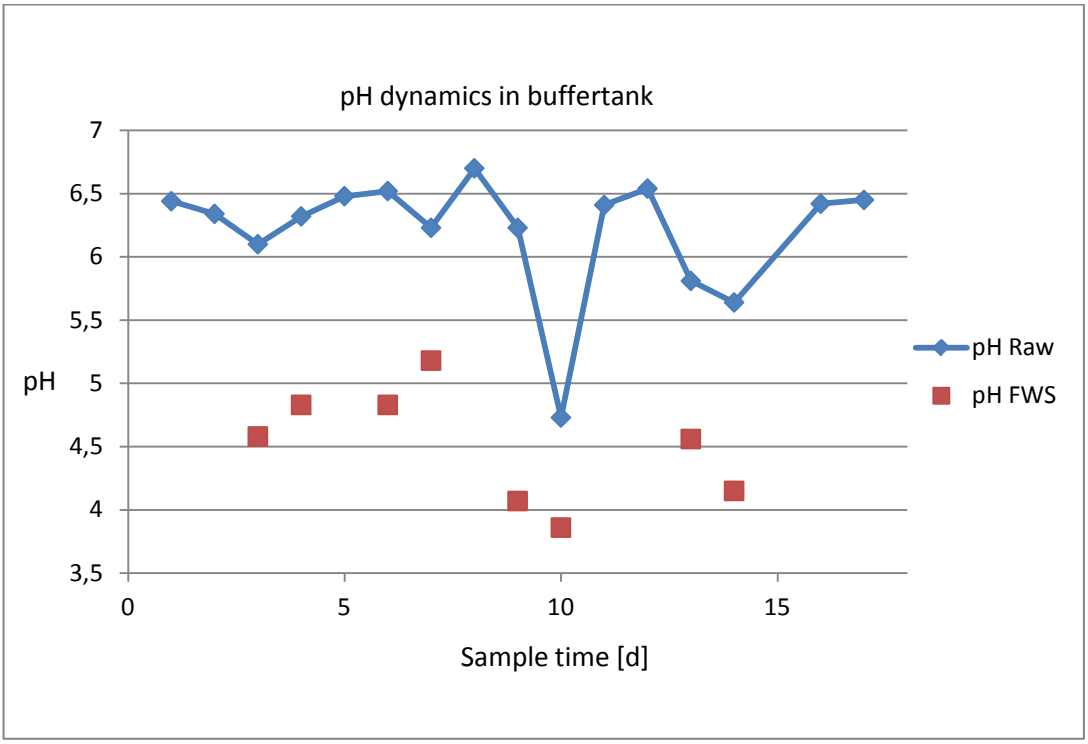
Graph 4.1 pH development in Raw Sludge (RS) and digested sludge RT1 and RT2.

The anaerobic digestion process is sensitive to pH and substances that can be inhibitory. Preferred pH values in AD process are near neutral and pH below 6.8, the methanogenic activity process is inhibited. The high content and production of CO_2 gas in the system, normally 30-35% of the biogas is the principal consumer of alkalinity in the digestion process, therefore a high alkalinity is required to assure pH near 7 in the process. Alkalinity concentration in the range of 3000-5000mg/l as $CaCO_3$ is often a preferred value. (Tchobanogolous et al.,2004).

Observations of fluctuating pH in the buffer tank directed for an extended measure of pH in the steady state period. The behaviour of pH in buffer tank is responding in some way either to raw sludge (RS) or external wastes. Hydrolysis and fermentation from ordinary RS may cause the reactions and pH dynamics in buffer tank. From the graph 4.2, pH dynamics are obvious and the sludges are interacting with incoming food waste sludge (FWS) who enter the buffer tank. The incoming FWS has a low pH due to the processing and pre treatment of the food-waste.

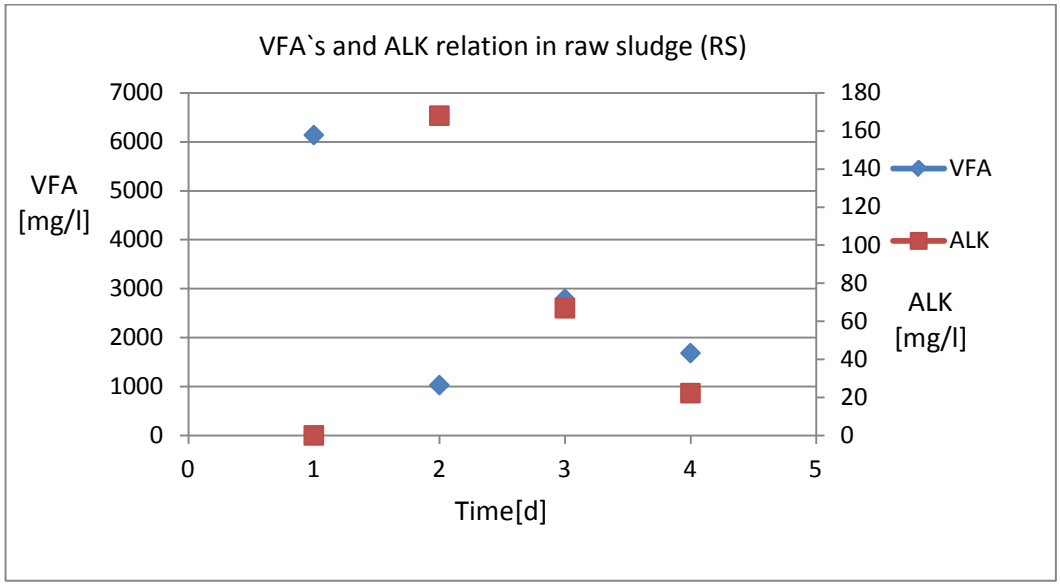
Table 4.1 sample time[d] for the “steady state” according to actual time.

Sample [nr]	1	2	3	4	5	6	7	8	9
Time [d]	03.05 Tues	04.05 Wedn	05.05 Thurs	06.05 Fri	08.05 Sun	09.05 Mon	10.05 Tues	11.05 Wed	12.05 Thurs
Sample [nr]	10	11	12	13	14				
Time [d]	13.05 Fri	14.05 Satur	18.05 Wedn	19.05 Thurs	20.05 Fri				



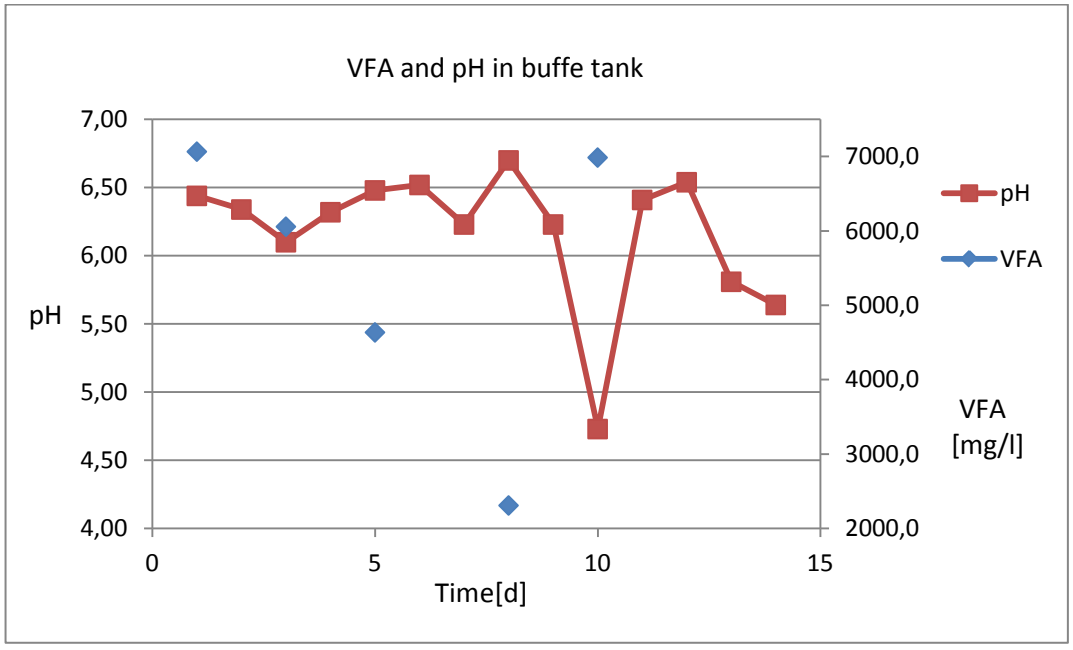
Graph 4.2 pH dynamics and influence of FWS with low pH.

These fluctuating conditions of the pH in raw sludge are predicting the state of acid/base or alkalinity in buffertank. Due to the hydrolysis and fermentation, the process in the buffer tank is producing VFA`s. In the graph 4.3 the alkalinity and VFA`s concentration is measured and calculate by 5-pionts titration method. In general we can see high acids concentration and a low alkalinity in the buffer tank. The high concentration of VFA is due to the ongoing hydrolysis and fermentation processes in raw sludge with consumption of alkalinity. The alkalinity concentration is measured to be low and in a level of 0-168 mg ALK/l as HAc (acetic acid) in the buffer tank in the measured period.



Graph 4.3 The concentration of VFA`s and the low alkalinity in raw sludge, buffer tank.

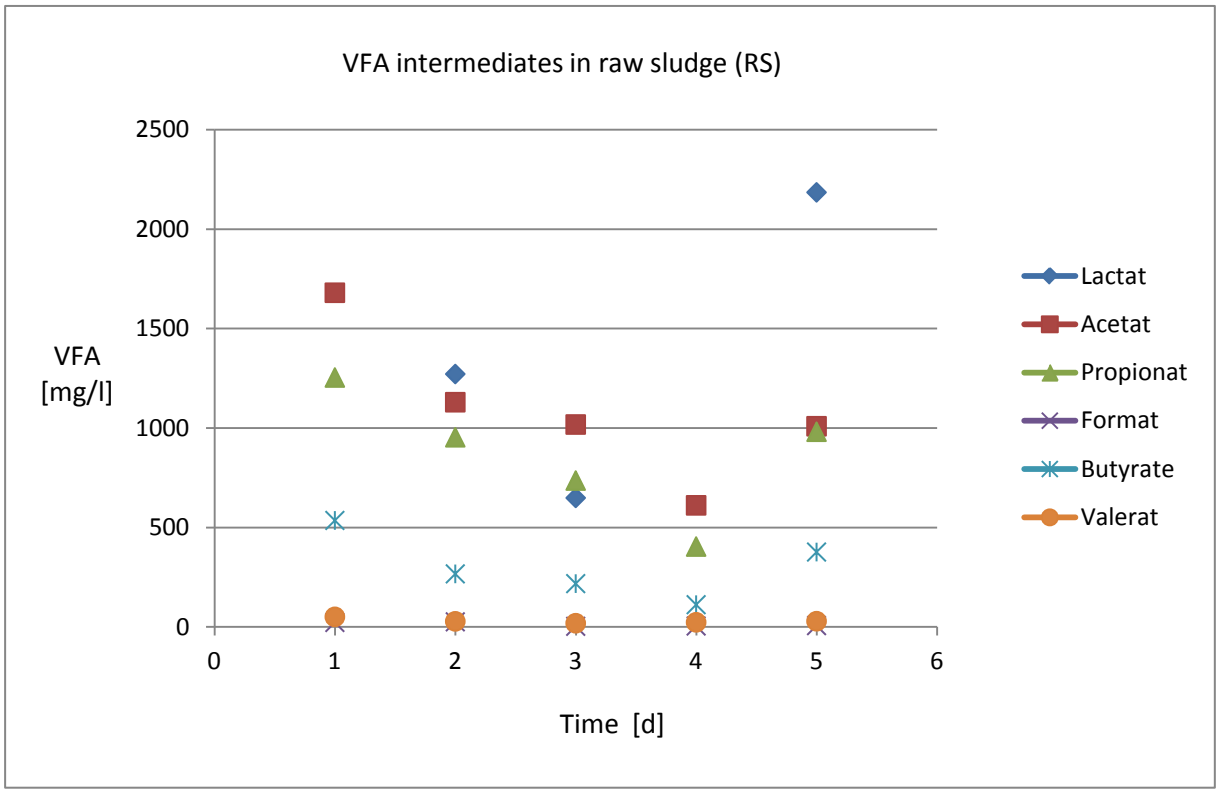
This measurement of 5-point titrations gave results for more extended measurements of pH and VFA`s in buffer tank and analysis of raw sludge (RS). The pH relations and dynamics have the same tendency in graph 4.4 and 4.3 with process interactions that is fluctuating the pH due to the acid/base relations. The pH in graph 4.4 is showing sensibility of VFA`s concentrations.



Graph 4.4 The concentration of VFA`s and pH dynamics in raw sludge, buffertank.

The extended sampling of dynamics in the RS or buffertank was to explore more of the ongoing process in the first stages of sludge treatment after settling. The buffer tank is designed mainly for hydraulic loadings and process kinetics and degrading processes are truly underestimated for this compartment. The fluctuating VFA`s in graph 4.4 is describing the similar interaction and dynamics in graph 4.5. The volatile fatty acids are divided into

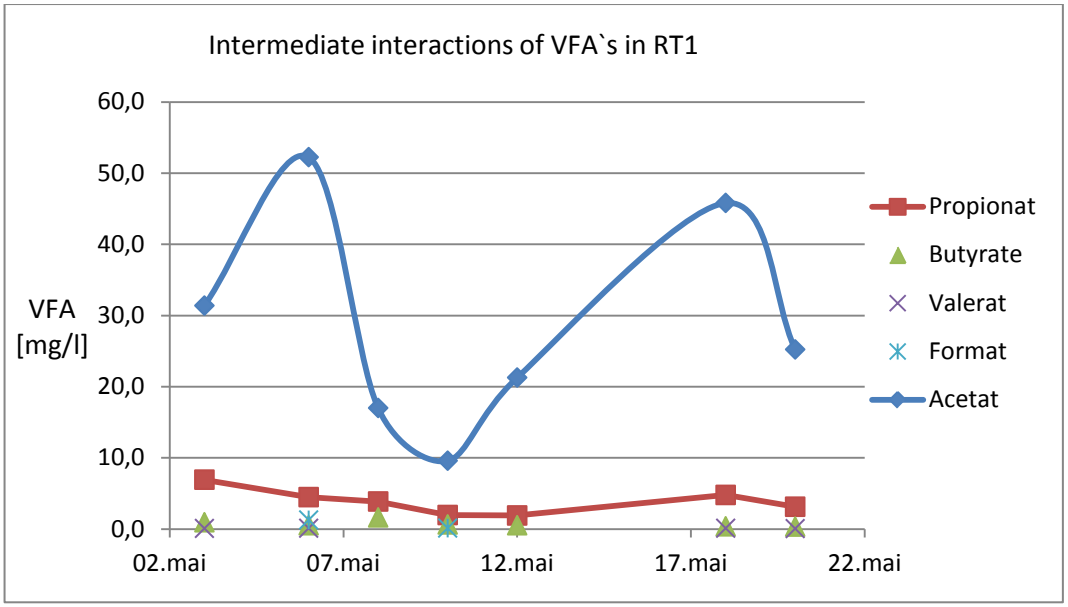
fractions with their concentration by Ion Chromatograph (IC) type Dionex ICS-3000 at the University Of Stavanger (UIS). The IC- measurement in steady state period, showed in graph 4.5.



Graph 4.5 Intermediate concentrations of the VFA`s dynamics in raw sludge, buffertank.

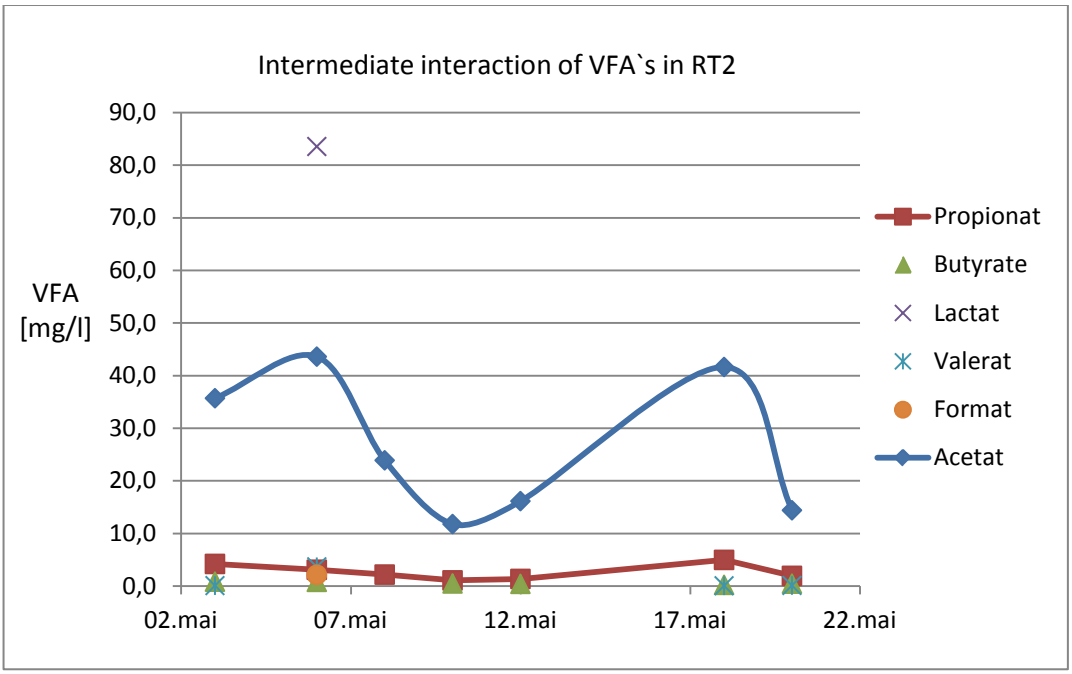
Due to the intermediate measurement and IC-analysis the level of lactic, acetic and propionate acids is in the range of 400-2200 mg/l and formate, butyric and valeric acid is present but in a lower concentration. Based on experience and from operation of AD reactors the level of VFA`s is normally in the range of 0.1-2.5(g/l) as HAc with GC-analysis under clearly stable conditions. From the graph 4.5 the concentration in buffer tank of VFA`s is in the range of 500-1500mg/l HAc for the raw sludge. The amount of VFA`s in the raw sludge is due to the depolymerisation process of polysaccharides, fats and proteins. Different groups of microorganism are capable to process hydrolysis and fermentation with converting complex composite organics (Angelidaki.,et al.,TheBiogasProcess).

Concentration of VFA in the reactors RT1 and RT2 is in a lower state with fluctuating conditions. The measurement of the intermediates is presented in graph 4.6 and 4.7.



Graph 4.6 Intermediate dynamics during the sampled period.

The sampled period and measurement are days with high organic load from FWS. The load from buffer tank was relatively stable for the sampled period of May 2011.



Graph 4.7 Intermediate dynamics of VFA`s during the sampled period.

For both reactors the level of Acetate (HAc) is fluctuating from 9-11.8 mg/l to 44-52 mg/l. For the other intermediates Propionate (HPr), Lactate (HLa), Butyrate (HBu), Valerat (HVa) and Formate (HFa) the levels of concentration is low, from zero to 6.9 mg/l for Propionate (HPr).

4.1.2 Mineralization and cations

The ion concentration in sludge solution is due to the state of process in the actual compartment. In table 4.2 the average concentration for sodium (Na^+), ammonium-nitrogen (NH_4-N), potassium (K^+), magnesium (Mg^{2+}) and calcium (Ca^{2+}) is presented for the period. In AD fermentation process the complex organic composite is undergoing a degradation and mineralization process. The level and concentration of cations are lined up for the compartment; buffer tank (RS), and the digestion reactors RT1 and RT2 in table 4.2.

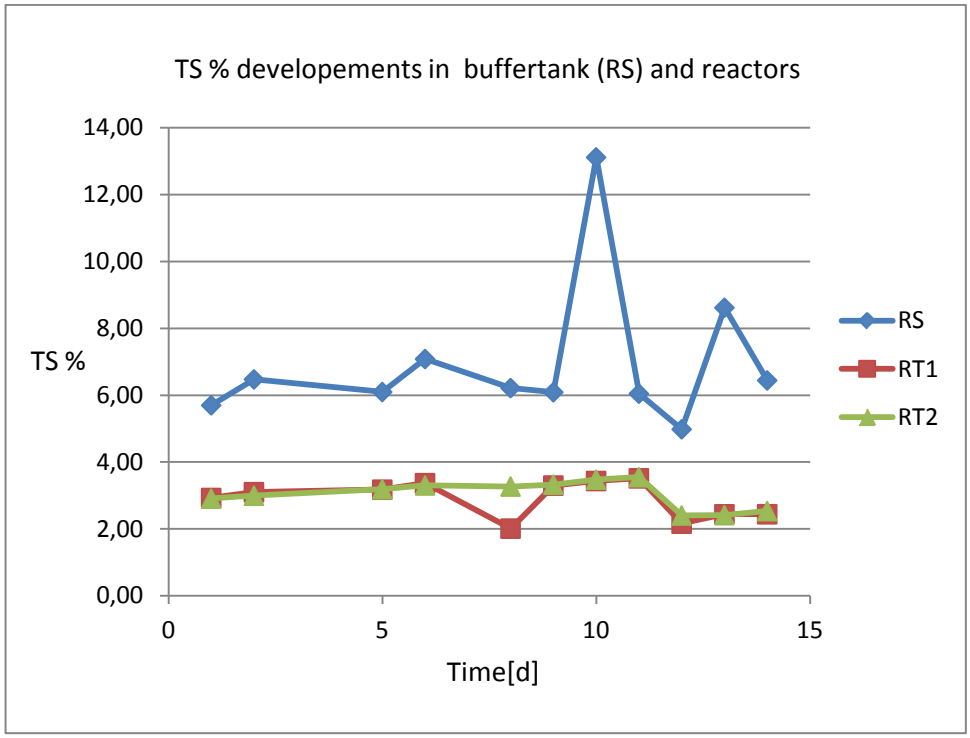
Table 4.2 Cations concentration in compartments

Cations	RS [mg/l]	RT1 [mg/l]	RT2 [mg/l]
Na	736	840	816
NH4-N	216	692	687
K	141	255	254
Mg	130	105	103
Ca	172	80	78

The raw sludge (RS) in buffer tank is containing the minerals sodium (Na^+), ammonium (NH_4-N) and potassium (K^+) with a level of ion concentration under the digested sludge in RT1-2 respectively. The level of NH_4^+ is the most interesting due to the various organic loads and the characteristic of the organics. Reported average influent concentration of ammonium into WW-AD reactors is in the level of 70-130mg/l (NH_4-N)(Mogens., et al.,2008). Another author give the level of ammonia concentration from full scale test with concentration of NH_4^+ in supernatant in order of 500-600mg/l. Stating the toxicity as total ammonium concentration the author reported a toxicity concentration range 1500-3000mg/l (NH_4-N) at a pH above 7.4. The level of 3000mg/l is stated toxic at any pH. (Tchobanogolous et al.,2004).

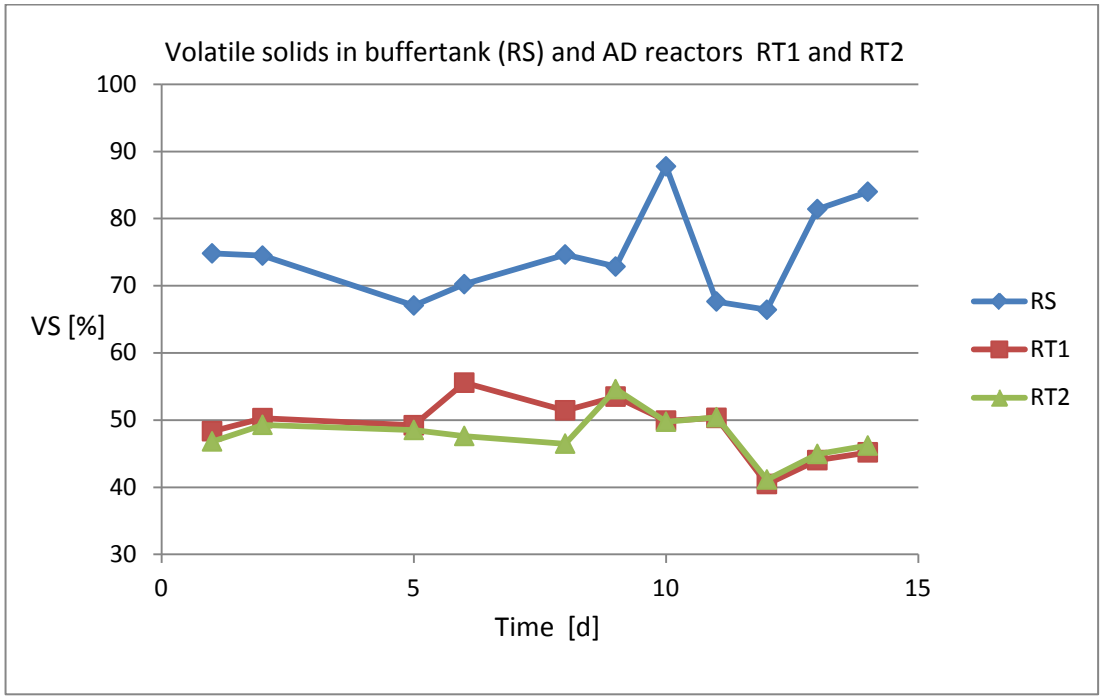
4.1.3 Solids analysis

The reduction of complex organic materials was measured through the fraction of volatile solids. VS in the waste that are degraded in AD process is reduced in level of 50-65% depends on solids digestion time (SRT). Following in graph 4.8 the behaviour of TS fractions in the compartments, the buffer tank and how TS is reduced in the AD-reactors



Graph 4.8 Total solids in the raw sludge and reactors over the sampled period.

The volatile fraction (VS) is degraded and reduced throughout the anaerobic digestion. From the graph 4.9, the content of volatile solids is reduced from approximate 74% VS to 48% VS in RT1-2 reactors.



Graph 4.9 Volatile solids[%] and development for the compartments.

4.1.4 COD mass balance

The COD balance is used to account for the COD conversion during fermentation. The COD conversion and COD loss in AD reactor is accounted for methane production. By stoichiometry then COD equivalent of methane can be determined. The amount of oxygen needed to oxidize methane to carbon dioxide and water is the specific COD equivalent for methane.

$$CH_4 + 2O_2 \rightarrow CO_2 + 2H_2O$$

The COD_{CH4} per mole is 2(32g O_2/mole)=64 g O_2/mole CH_4, and at standard conditions (0°C at 1 atm) the volume of methane can be determined from the general formula under. General formula for converting and calculating V = $1m^3(biogas)$ into $\left[\frac{g\ COD}{m^3 biogas}\right]$ ratio factor.

$$V = \frac{n \cdot R \cdot T}{P_{atm}}$$

The AD-reactors at SNJ operate with processes temperature T=37-40 °C and with a slightly under pressure at P_{atm}=0.67 partial pressure. The calculation is as follows;

$$\frac{n_{CH4}}{m^3} = \frac{P_{atm}}{R \cdot T} \rightarrow \frac{0.67 \cdot 101 \cdot 10^3 Pa}{8.314 \frac{Pa \cdot m^3}{mole \cdot K} \cdot 313K} = 26 \frac{mole\ CH4}{m^3 biogas} \cdot 2 \frac{mole O_2}{mole\ CH_4}$$

The calculated value for processes temperature T=40^{o}C and with a partial pressure at P_{atm}=0.67 converted to Pascal, the amount of gas is V = 26 litre. The COD_{CH4} per mole is 2(32g O_2/mole) =64 g O_2/mole CH_4, so the CH_4 equivalent of COD converted is 26litre/64 g O_2/mole CH_4= 0,4liter CH_4/gCOD.

$$52 \frac{mole\ O_2}{m^3 biogas} \cdot 32 \frac{g\ O_2}{mole\ O_2} = 1664\ \frac{g\ COD}{m^3 biogas}$$

The measured biogas production online can be converted into COD equivalent for overall COD balance by this calculations. Due to the purpose of checking calculations and COD balance for whether COD entering the reactor equals to COD converted into biogas plus COD leaving the reactor. In general the COD balance for an AD reactor can be expressed in following COD-balance (Kommedal,R., 2011).

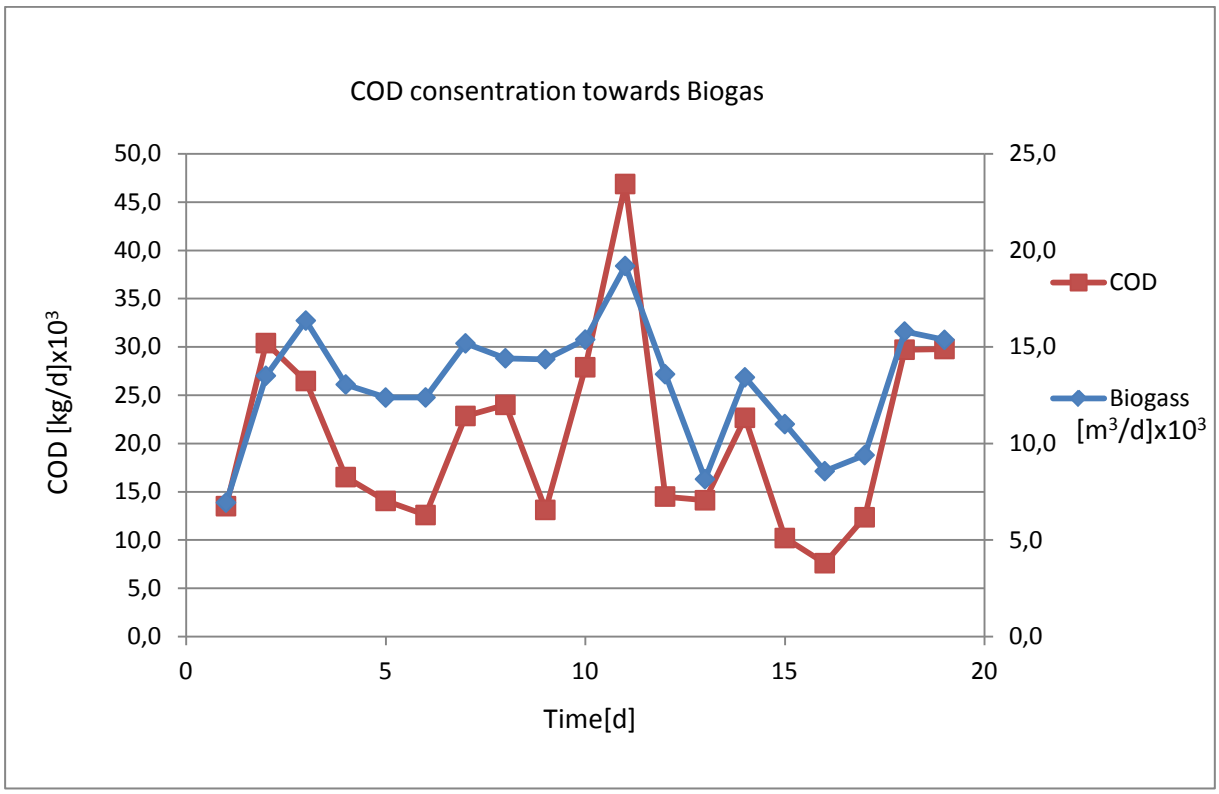
$$COD_{into\ reactor} = COD_{out\ with\ effluent} + COD_{converted\ to\ methane}$$

Table 4.3 COD balance over measured period.

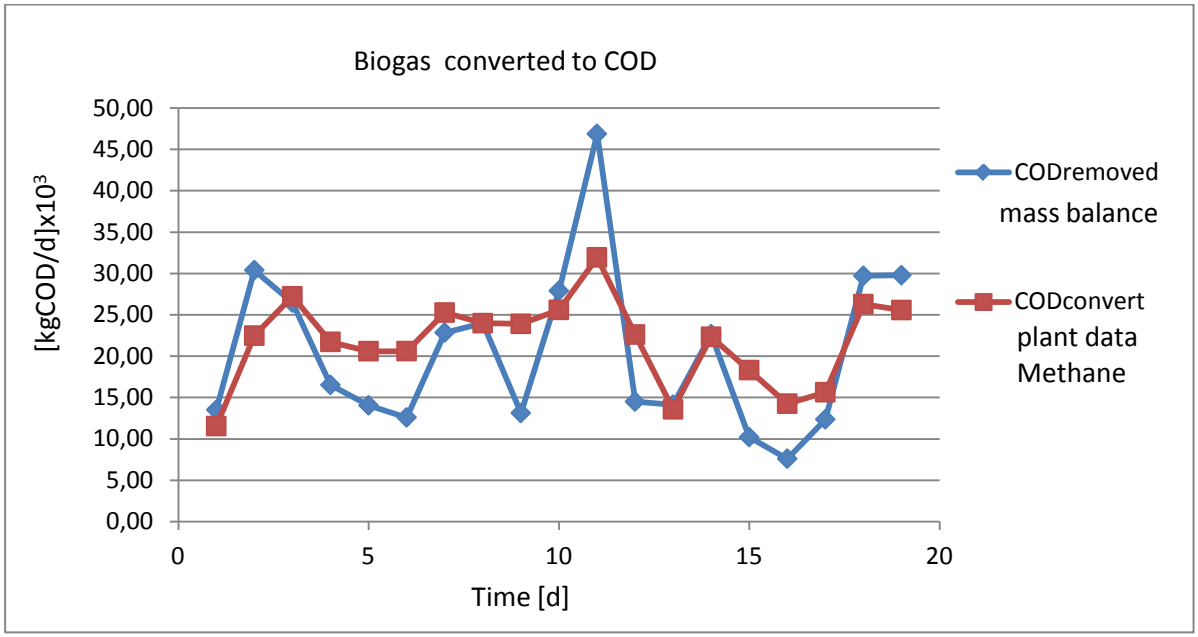
Average plant data		Average measured values			Calculation	
Sludge Flow	Biogas Flow	TotCOD	$COD_{influent}$	$COD_{effluent}$	$COD_{removed}$	$COD_{Methane}$
[m^3/d]	[m^3/d]	[KgCOD/m^3]	[KgCOD/d]	[KgCOD/d]	[KgCOD/d]	[KgCOD/d]
365	13076	85	31025	10585	20440	21758

The table 4.3 the COD balance is based on average measured values and data from controlling computer system at the plant. The calculated value of $COD_{Methane}$ into biogas is calculated by the conversion factor 1664 gCOD/m^3Biogas, (1.66kgCOD/m^3Biogas).

The COD relations to biogas are showed in Graph 4.10 and obviously the COD is related to the degraded COD. Some peaks and levels is due to further discussion but the tendency is the degraded COD is in the level of the factor 1664 gCOD/m^3Biogas below the online measured biogas production. In graph 4.11 biogas productions is converted to COD and compared with measured and analysed COD.



Graph 4.10 COD concentration and biogas produced online data.



Graph 4.11 Biogas productions converted into COD from measured and plant online data.

4.1.5 VS reduction and organic loads

The amount of volatile solids reduction in AD process, or conversion of the termed "slowly biodegradable COD" ($_{SB}$COD), "all the organics" is important to determine. The degree of stabilization obtained is often measured by the %-reduction of VS. The volatile reduction is closely related to solids retention time (SRT) or time of AD-digestion, normally in the range of 15-20 days. Calculation of VS reduction is based on measurement and the average values is listed up in table 4.4 for the compartment buffer tank (RS) and the reactors AD-sludge with treated sludge (Tchobanoglous et al., 2003).

Table 4.4 Measured average values in week 18-19-20.

Sampled Solids	**Total solids %** **[TS]**	**Volatile solids %** **[VS]**	**Fixed solids %** **[FS]**
Raw sludge (RS)	7	74.7	25.3
Digested sludge (AD)	3	48.4	51.6

For untreated raw sludge (RS) and digested-(AD)sludge, the total volatile solids is reduced and converted during digestion. When calculating the VS%-reduction, the FS (ash) content is assume to be conservative and the weight of FS into the reactor is equal to that being

removed. For this calculation it is assumed that (1) the weight of the fixed solids in the digested AD-sludge equals the weight of fixed solids in the raw sludge and (2) the volatile solids are the only constituents of the untreated sludge lost during digestion. The method for determine VS%-reduction is calculated by this approach from the book (Tchobanoglous et al., 2003)

1. Calculation of FS (%) in raw (undigested) sludge;

$$25.3\% = \frac{0.253\ \text{kg} \cdot 100}{0.253\ \text{kg} + 0.747\text{kg}}$$

2. Calculate the VS, X (kg) in AD treated sludge, then FS in RS sludge;

$$48.4\% = \frac{0.253\ \text{kg} \cdot 100}{0.253\ \text{kg} + \text{X kg}}$$

$$\text{X} = \frac{0.253\text{kg} \cdot 100}{48.4\text{kg}} - 0.253\text{kg} = 0.2697\ \text{kg to VS}$$

3. Weight of VS in AD-sludges is then determined;

VS (kg) = 0.253 + X = 0.5227 kg

4. Percent reduction in total suspended solids (TS):

$$\text{R}_{\text{TS}} = \frac{(1 - 0.5227)\text{kg}}{1\ \text{kg}} \cdot 100 = 47.7\%$$

5. Percent reduction in volatile solids (VS):

$$\text{R}_{\text{VS}} = \frac{(0.747 - 0.2697)\text{kg}}{0.747\ \text{kg}} \cdot 100 = 63.9\%$$

The weight of the digested solids based on 1 kg of TS, raw sludge and determined in step 3, and computed to 0.5227 kg. The digestion time or solid retention time (SRT) values for

complete mixed reactors is given in table 4.5. AD reactors are temperature sensible and the level for mesophilic conditions is approximate 37-40^{o}C.

Table 4.5 Estimated volatile solids reduction, VS[%] in AD reactors.

Digestion time SRT [d]	Volatile solids reduction [%]
30	65.5
20	60.0
15	56.0

(Tchobanoglous et al., 2003)

4.1.6 Calculation of SRT and organic loading rate (OLR)

Typical values for solids retention time (SRT) for completely mixed high rate AD reactors, are in the range of 10-20 days in practice. At a lower SRT and limit of 10 days, is sufficient to ensure an adequate safety factor against a washout of the methanogenic population. Effect on volatile solids reduction for SRT above 15 days are relatively incremental small with reactor temp at 35^{o}C (Tchobanoglous et al., 2003). Loading factors on to the AD reactors are important for design and analysis of steady and dynamic state. The level of loading factors and method is various but the two most favoured are based on;

A) The mass of volatile solids added per day [kgVS/d], per unit volume (m^3) of AD-reactor capacity, OLR [kgVS/m^3d].

B) The mass of volatile solids added to the digester each day per mass of volatile solids in the AD-reactor. OLR [$kgVS_{in}$ /d]/[$kgVS_{out}$ /d].

The most used and preferred method is the first method A. Volatile solids and COD loading rates is from solids analysis in wwtp laboratory SNJ. Analysis of TS, VS and COD has given average values for further calculations. The quantity of volatile solids is measured to be 55 kgVS/m^3 and 85kg_{Bio}COD /m^3, respectively. The plant consist of two AD compartments at V=3500 m^3. Values for AD-digestion is measured and mentioned in table 4.2 above. The sludge contains approximate 93 percent moisture and has specific gravity of 1.02. (Tchobanoglous et al., 2003)

The daily volume flux of sludge for this calculation is average value from the data control system at the plant Q = 182 m^3/d. Measured COD is from solids analysis and determined to be 85 kgCOD/m^3 as an average value and computed in following expressions steps;

1. Determination of $_{Bio}$COD loading and $COD_{effluent}$ by measured and plant average data values.

   COD concentration into reactor;
   $_{Bio}$COD [kgCOD/d] = 85kgCOD/m^3 · 182 m^3/d = 15470 kgCOD/d

   COD concentration out of AD reactor
   $COD_{effluent}$[kgCOD/d] = 28.6kgCOD/m^3 · 182 m^3/d = 5205 kgCOD/d

2. Solids retention time SRT or "time of digestion" and check of digester volume capacity ;

$$\mathrm{SRT} = \frac{\mathrm{V}}{\mathrm{Q}} = \frac{3500\mathrm{m}^3}{182\ \mathrm{m}^3/\mathrm{d}} = 19.23\ \mathrm{d}$$

3. Volumetric removal rate is computed by average COD in AD reactors as follow:

$$\mathrm{kg}\frac{\mathrm{COD}}{\mathrm{m}^3}\cdot\mathrm{d} = \frac{15470\ \mathrm{kgCOD/d}}{3499.9\ \mathrm{m}^3} = 4.42\ \mathrm{kgCOD/m}^3\cdot\mathrm{d}$$

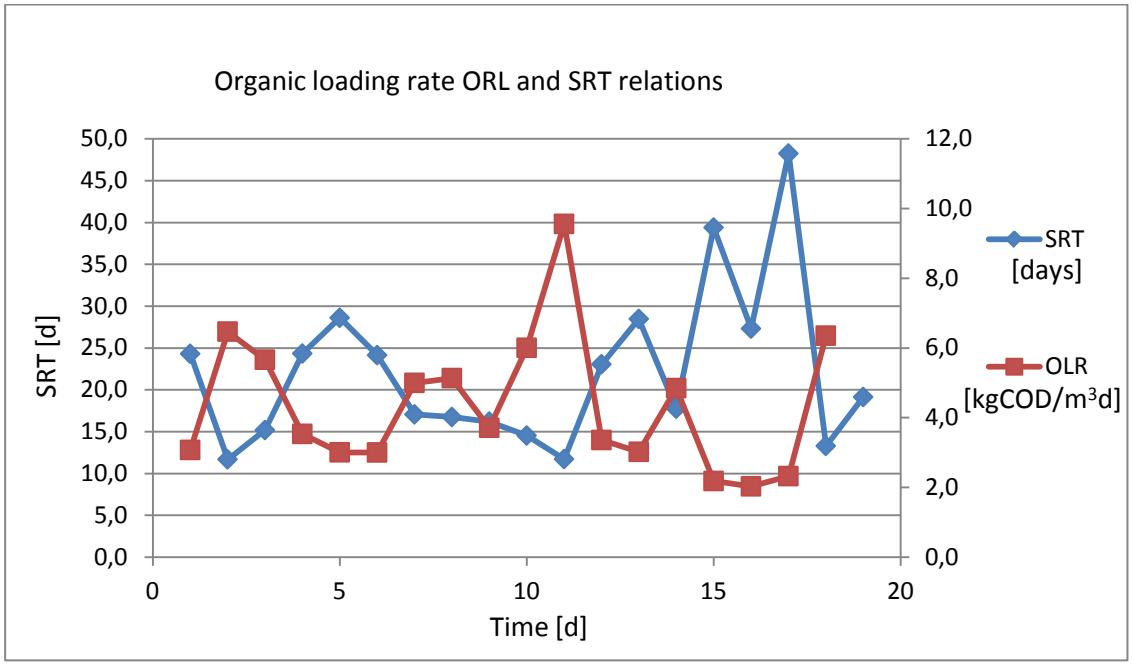
4. The organic loading rate (OLR) based on $_{Bio}$COD [kgCOD/d] loadings is calculated:

$$\mathrm{OLR}\left[\frac{\mathrm{CODin}\cdot\mathrm{Flow}}{\mathrm{Volume}}\right] = \frac{85\mathrm{kg}\frac{\mathrm{COD}}{\mathrm{m}^3}\cdot\frac{182\mathrm{m}^3}{\mathrm{d}}}{3500\mathrm{m}^3} = \frac{4.42\ \mathrm{kgCOD}}{\mathrm{m}^3}\cdot\mathrm{d}$$

5. The organic loading rate (OLR) based on volatile solids [kgVS/d] loadings is calculated;

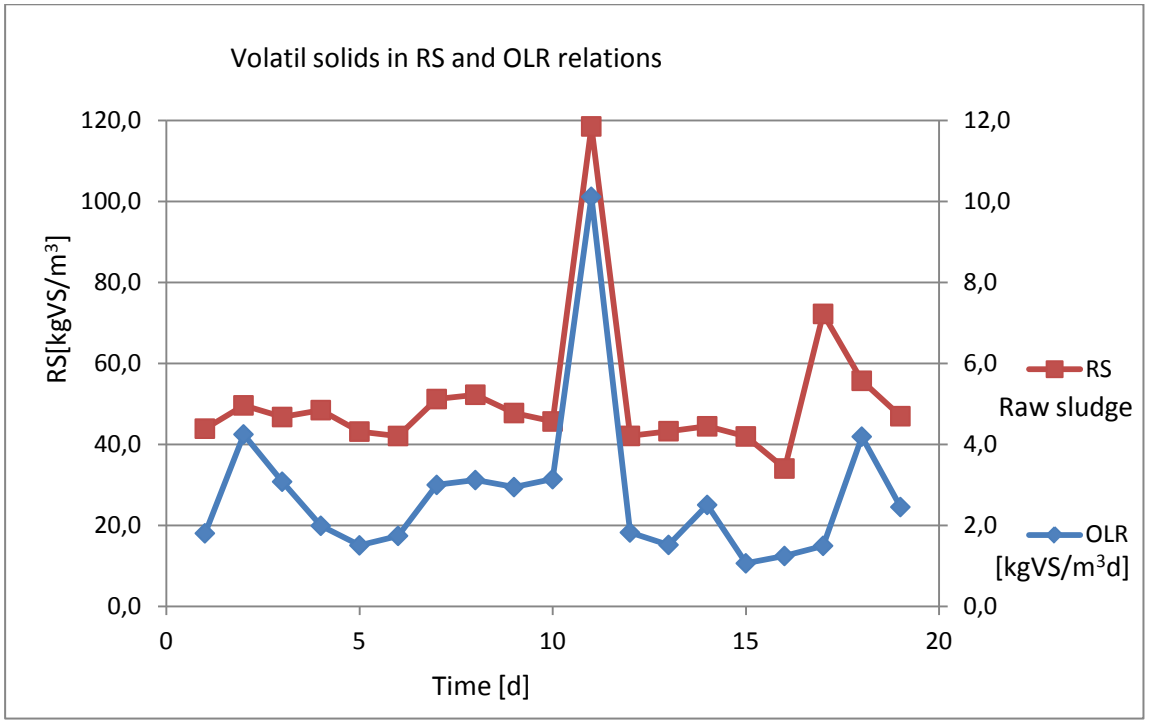
$$\mathrm{OLR}\left[\frac{\mathrm{VS}\cdot\mathrm{Flow}}{\mathrm{Volume}}\right] = \frac{55\mathrm{kg\ VS/m}^3\cdot 182\mathrm{m}^3/\mathrm{d}}{3500\mathrm{m}^3} = 2.9\ \mathrm{kgVS/m}^3\cdot\mathrm{d}$$

From the calculation above the graph 4.12 are a graphical presentation of the solids retention time (SRT) or the digestion time with relations to organic loadings based on COD.



Graph 4.12 SRT and organic loading rate is fluctuating with the load.

The organic loading rate OLR is in the range from 2-6 [kgCOD/m^3d], with fluctuating behavior and peaks up to 9.6 [kgCOD/m^3d]. The OLR is an important factor for processing the anaerobic digestion. Waste flows with different organic concentration give AD reactors various conditions for AD processing. Analysis of the organic load is important for design and utilization of the reactor capacity. At the graph 4.13 the VS content in raw sludge is predicting the level of OLR.



Graph 4.13 The raw sludge (RS) with VS concentration OLR dynamics.

From this analysis of steady state conditions the OLR is fluctuating from 1.1- 4.2 [kgVS/m^3d] and the relation to VS concentration in RS is obviously and closely related. The relation is the loading factor and the mass of volatile solids added per day [kgVS/d], per unit volume (m^3) of AD-reactor capacity, OLR [kgVS/m^3d]. Normally and stabilized AD processes is in the range of 1.6-4.8 [kgVS/m^3d] for OLR (Tchobanogolous et al.,2004).

4.1.7 Calculating the mass balance

Calculation based on solids analysis during the "steady state" period mean values.

Table 4.6 Mass balance over measured period.

Average RS data		Average sludge values into AD and out		
Sludge Flow	VS Flow	Tot.Org.flux	$VS_{redu\ 48.4\%}$	$VS_{Destr\ 63.9\%}$
[m^3/d]	[kgVS/m^3]	[KgVS/d]	[KgVS/d]	[KgVS/d]
365	55	20075	7258	12817

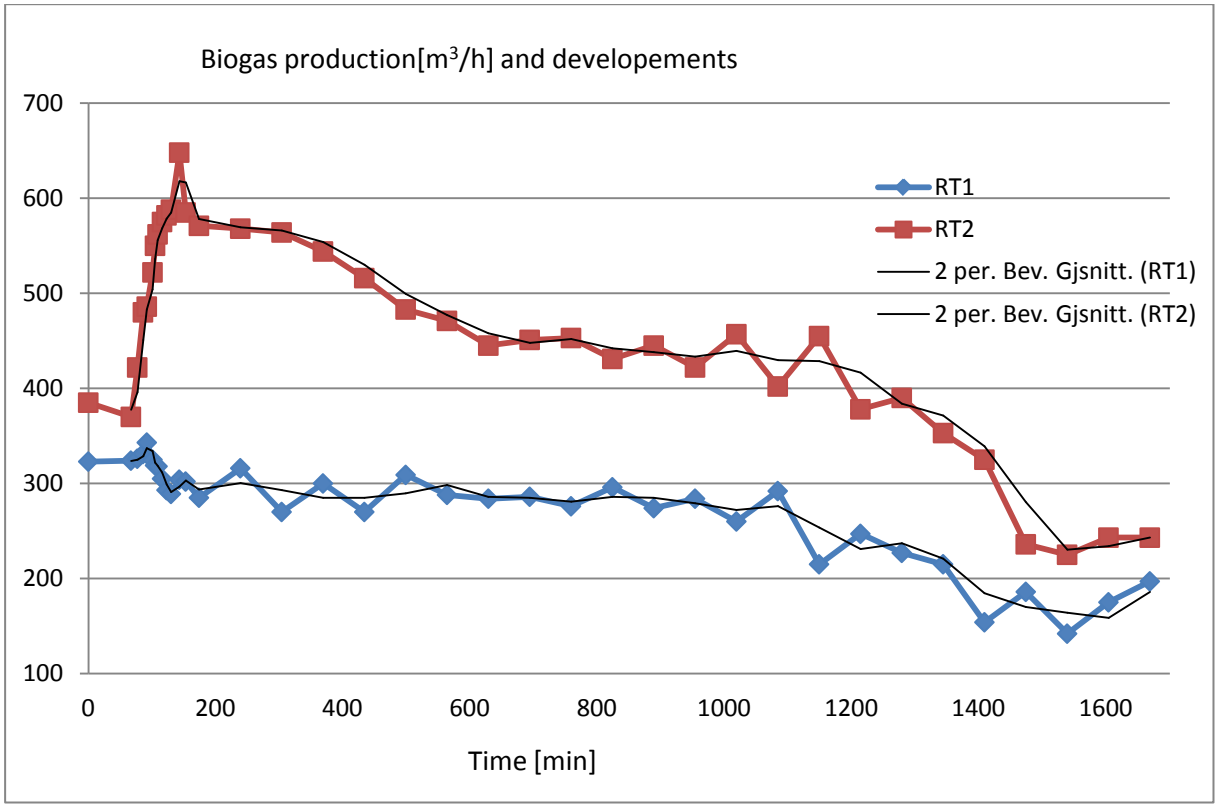
Note Raw sludge is RS.

The average COD/VS was measured to 1.65, and CH_4 production 0.35[m^3/kg COD], then organics converted into biogas are; 12817 [KgVS/d] x 1.65 [COD/VS] x 0.35[m^3/kg COD] = 7402 CH_4 [m^3/d], with 67% methane content the biogas is calculated to 11048[Nm^3/d].

4.2 *Dynamic State period*

For this test case, the study of more dynamic behaviour of the AD process was conducted in August 2011. The background experience from the ordinary “normal State” situation with a preliminary test gave valuable information about the development in reactors. The goal for this injection test is analysis the dynamic responds and behaviour on tested reactor. In practice one reactor is dedicated to be the test reactor and the other is reference reactor.

The forced pulse in this test was injection of 39 tons FWS direct into the AD reactor (RT2). The other AD reactor (RT1) is reference reactor with standard conditions with respect to normal loading rate for the reactors. The same conditions from “stead state period” were achieved for optimal comparative measurement. Standard raw sludge from settling basins was the main organic load to the reactors in 48 hours before testing. The raw sludges (RS) and injection batch where characterized to determine TS, FTS and VTS. COD measurements on biodegradable COD ($_{Bio}$COD) and $_{RB}$COD from the supernatant, with ALK, pH and VFA`s measurements with calculations. The behaviour of the digesters was observed trough the online biogas sampling. The injected batch was codigested with standard sludge and intensively sampled the first 75 minutes from injection. Than normal cycle were established for both digesters. Under this intense period high activity in reactor was expected. In graph 4.14 the biogas dynamics presented.



Graph 4.14 Biogas dynamics from the injected pulse load over the sampled test period.

After the first 75minutes the reactor was producing and degrading organics in high rate. The reactors were observed further for 24 hour for catching developments in the degrading

process. The sampling intensity was decided to carry out with high frequent sampling time of the sludge for the first 1.5 hours. The sampling time was approximate 5-10 minutes in the most intense experimental period. This was due to the preliminary test and observations. The next period the sampling frequencies were lowered down due to the more stable decreasing conditions. This approach to catch the sludge sample where interesting intermediate dynamics are processing is of high interest. The test reactor was intensively analysed for total solids, volatile solids and total COD ($_{Bio}$COD) and soluble COD ($_{RB}$COD). Alkalinity and pH measurement with VFA`s analysis by 5-points titration and IC analysis was conducted. The raw sludge (RS) in buffer tank and the digested sludge in reference reactor RT1 were measured throughout the experimental period for reference values.

The injected pulse load of 39 tons (FWS) was analysed and characterized due to the organic load potential. The table 4.7 is presenting analysis of FWS at the SNJ laboratory with mean values from the "steady state" measurement. External analysis from Eurofins due to characterisation of the solids into polysaccharides, protein and lipids (fats) were conducted with the results of solids analysis for the sludge.

Table 4.7 Food waste sludge analysis of the injected batch with external laboratory analysis.

Lab	pH	TS[%]	TS[g/l]	VS[%]	VS[g/l]	$_{Bio}$COD[g/l]	COD/VS
SNJ	4,47	28,5±0,2	283±2	93,0±0,2	263,2±0,5	382,8±3,9	1,45
Eurofins		29,2	290	93,5	273	360	1,32
SNJ$_{mean}$	4,5	26,9±0,2	267±2	93,4±0,3	250±0,7	352,3±6,4	1,41

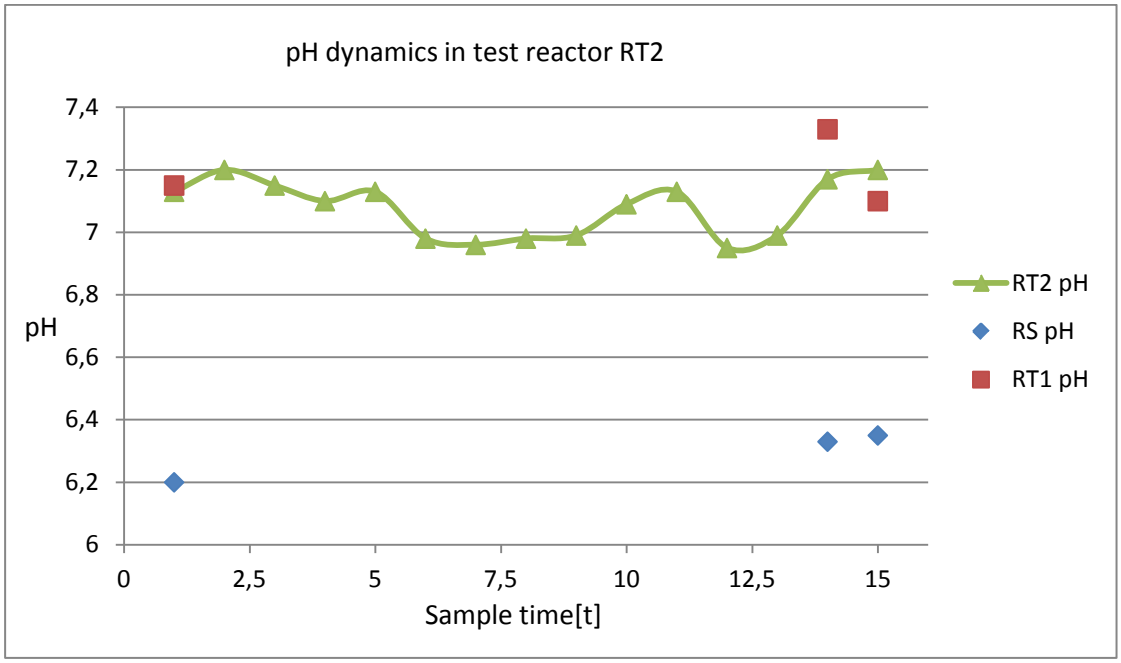
The composition of total solids (TS) in FWS was analysed and characterising into polysaccharide, proteins, lipids and inerts. The average content by characterising constituents gave this values; polysaccharides 34.4[%], proteins 27.1[%] and lipids 31.3[%], inert is measured to be 7.2[%]. The table 4.8 is presenting actual sampling in real time for the whole test period. Max biogas production is at time 13:23 (MBiog) and the normal sequence cycling (NSecv) is re-established at 13:33. The sample nr 15 was conducted the day after, approximate 24hours.

Table 4.8 Exact sampling time for the test period. The "Batch" was injected 12:07.

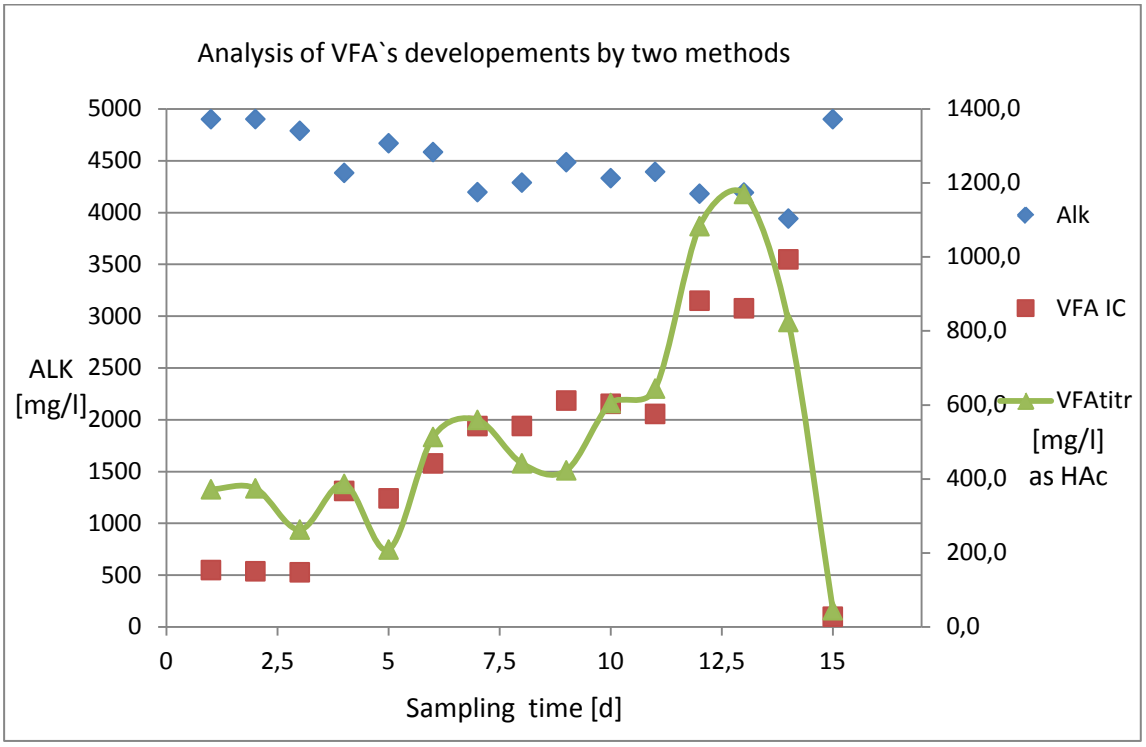
Sample [nr]	1	2	3	4	5	6	7	8	9
Time [t]	11:00	12:07 Batch	12:17	12:26	12.32	12:41	12:45	12:49	12:56
Sample [nr]	10	11	12	13	14	15			
Time [t]	13:03	13:10	13:23 MBiog	13:33 NSecv	14:54	12:30 +24h			

4.2.1 Dynamics in pH, ALK and VFA`s

The injected test batch is measured to 4.47pH and the average measurement is 4.5 from the first period. The measurement of pH in the compartments buffer tank (RS), and digesters RT1 and RT2 in test period is presented in graph 4.15. The RS have a normal low pH from 6.2-6.35 and the reference reactor RT1, the pH 7.15 and end up with 7.1.

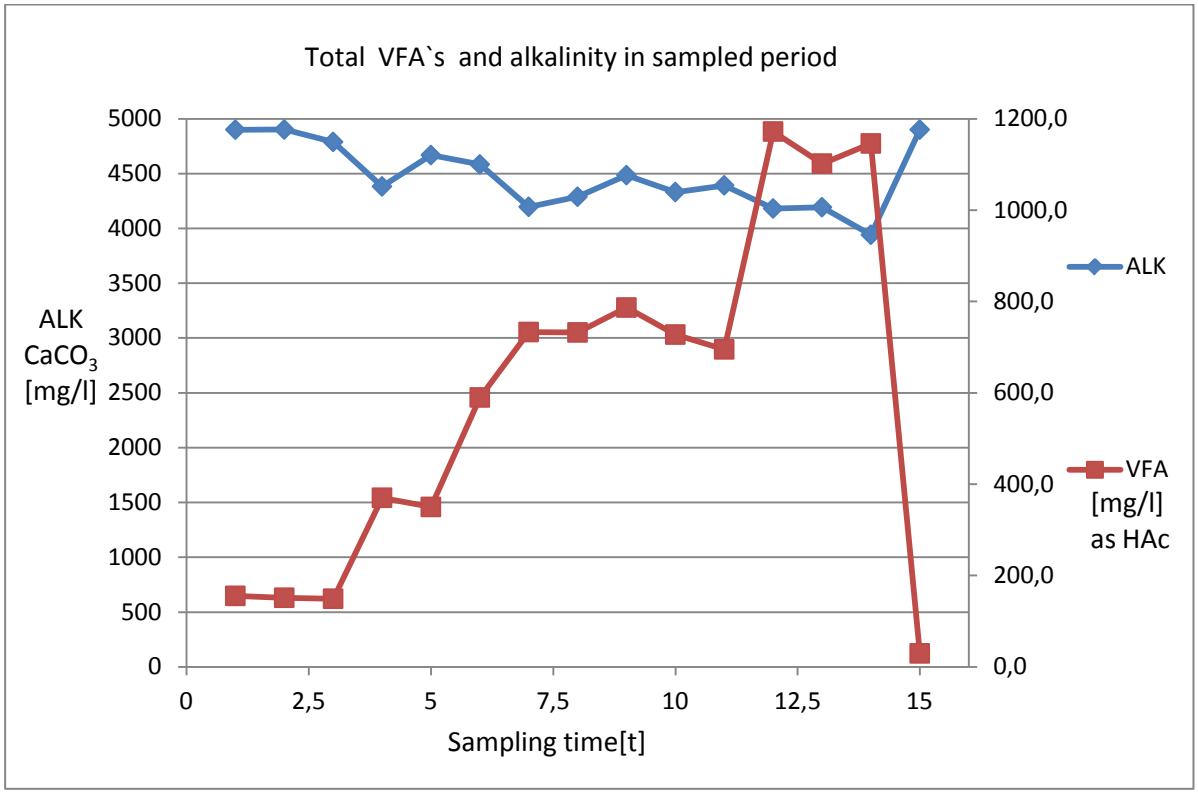


Graph 4.15 Developments of pH in test with reference measurement in RS and RT1.



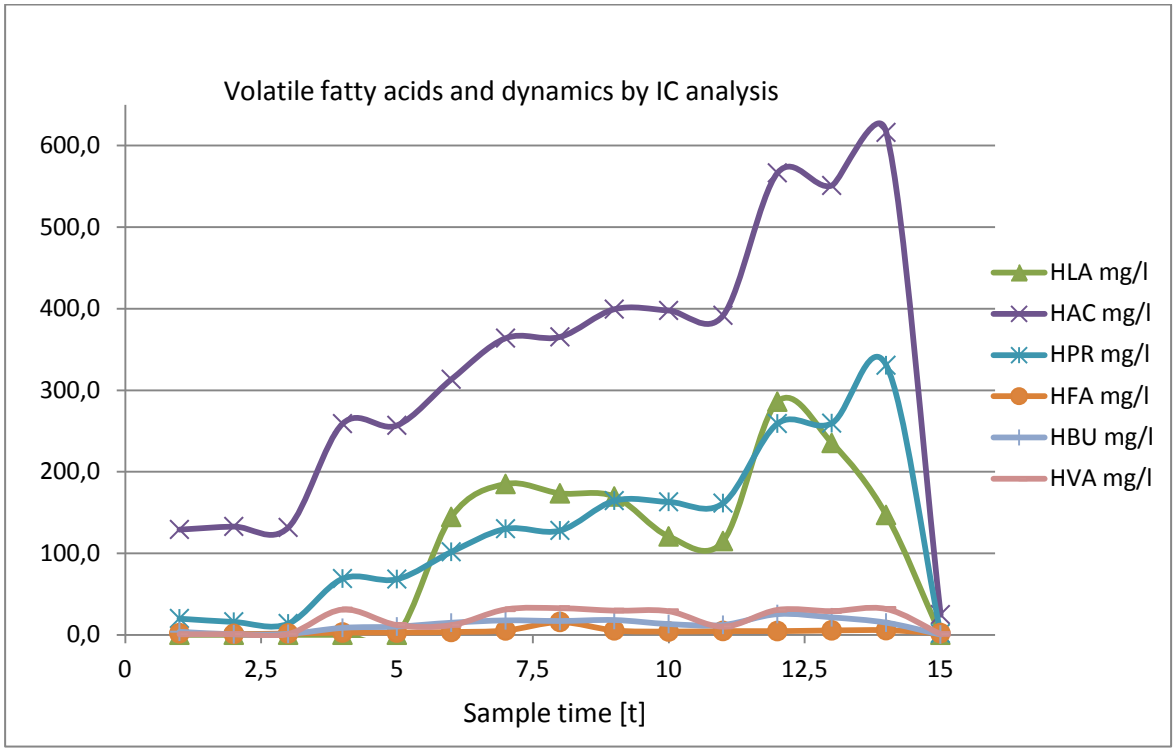
Graph 4.16 Increasing concentration of VFA`s and decreasing ALK in RT2

In graph 4.16 increasing volatile fatty acids are developed with decreasing ALK during the test. With use of two methods for analysis, the 5-point titration with calculations by "TITRA-5" and IC-analysis the reliability of measurement is increased. The 5-point titration method is fluctuating compared with IC-analysis. The VFA`s levels is increasing from 209 mg/l until peak point at 1170 mg/l as HAc with 5-points titration approach. The IC-analysis has the value 146 as start concentration and 993 mg/l as HAc at maximum. In graph 4.16-17 the decreasing alkalinity in test period are presented from time before injection with the start concentration 4900[mg/l] as $CaCO_3$ decreasing until sample time [14] the concentration is 3941 [mg/l] as $CaCO_3$ the batch pulse into the reactor until the process stabilized.



Graph 4.17 The amount of VFA`s concentration and alkalinity development in RT2.

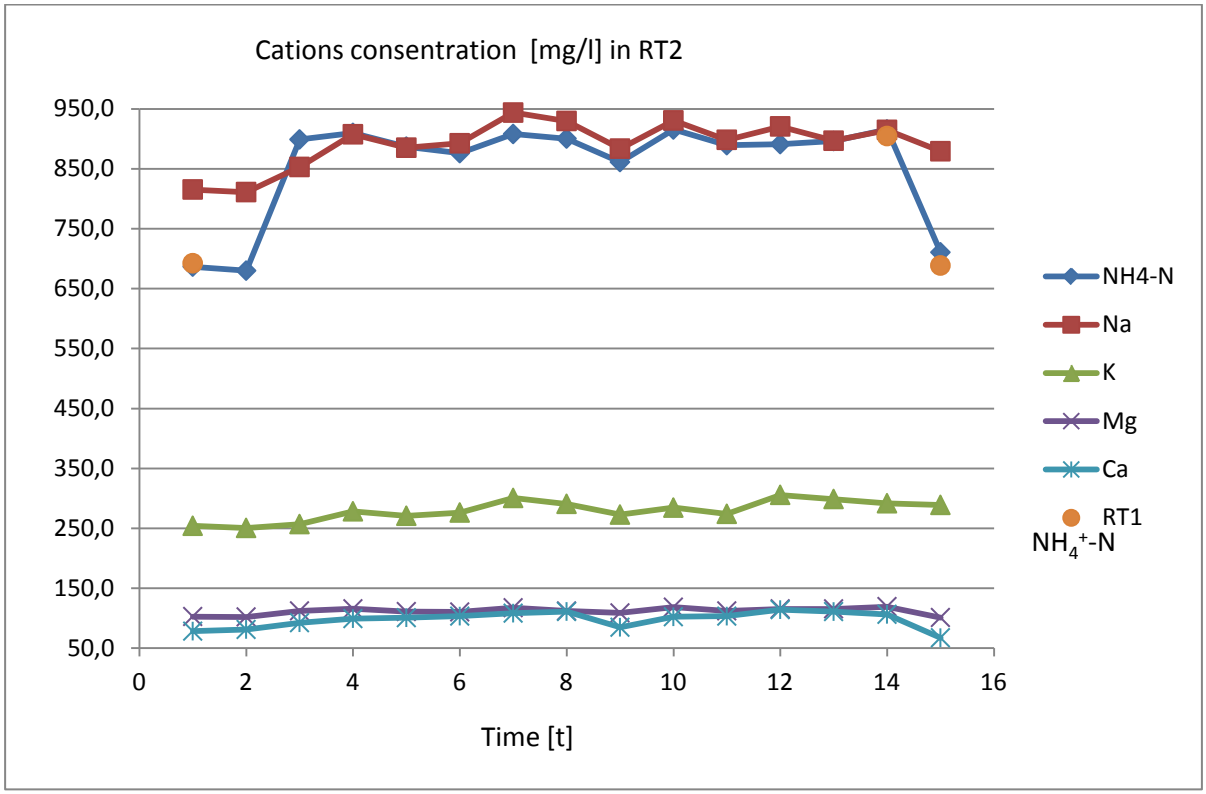
The graph 4.17 is also describing the increasing total VFA`s with an high rate from time of injecting the test batch. Intermediate dynamics is presented in graph 4.18 where interactions between acids and organisms are present. The level of Acetate (HAc) is increasing from approximate 130 mg/l to peak at 616 mg/l. For the other two active intermediates acids, Propionate (HPr) and Lactate (HLa), the levels are increasing from injection time until peaks for HPr at 330 mg/l and HLa at 286 mg/l. For Butyrate (HBu), Valerat (HVa) and Formate (HFa) the levels of concentration is lower, from 0.5 mg/l at injection time until 33 mg/l for Valerat (HVa).



Graph 4.18 Intermediate concentrations and dynamics throughout the test period.

4.2.2 Mineralization and cations

The solids precipitation is the complexing interactions between cations and anions in neutral solid form. Important solids precipitants is calcium carbonate $CaCO_3$ and calcium phosphate $CaPO_4$, magnesium carbonate $MgCO_3$ and other phosphate complexes like “struvite” $MgNH_4PO_4$ and “newberyite” $MgHPO_4$. The metal sulphide precipitants, particularly FeS and Fe_2S_3 is important to measure when $Fe^{2/3+}$ is added to precipitate the sludge. Sulfate redusing bacteria is competing with the methanogens for COD and can be toxic in very low concentration, less than 20mg/l. The two most important solids complexes is $CaCO_3$ from Ca^+ and magnesium precipitates when added $Mg(OH)_2$ for pH adjusting in process.

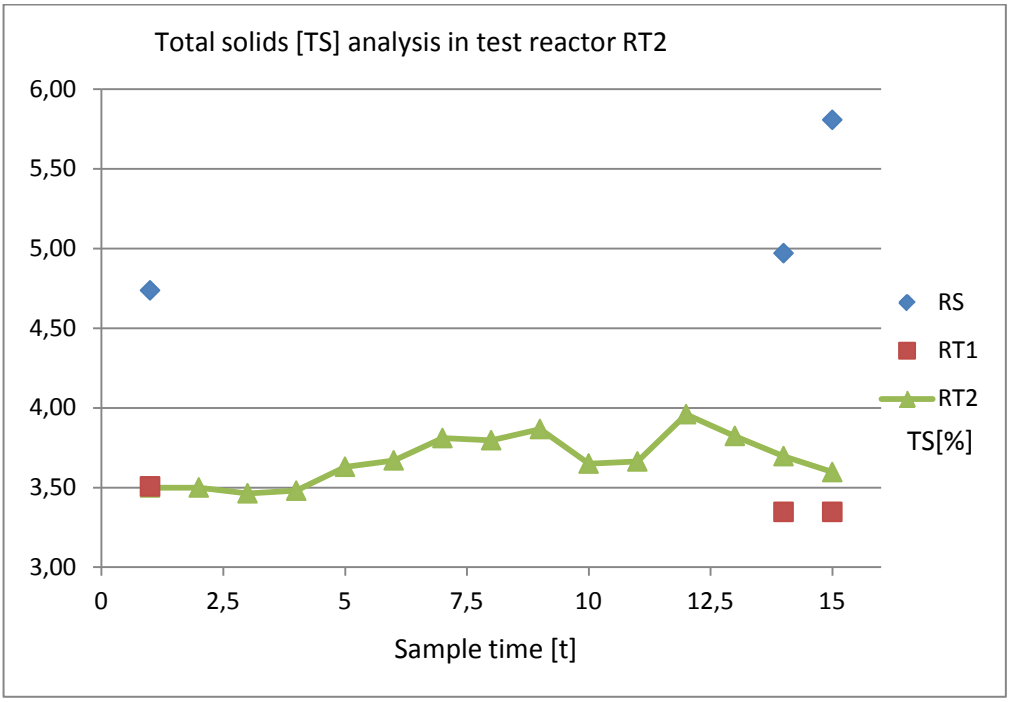


Graph 4.19 Concentration of cations in test reactor RT2, with reference values RT1.

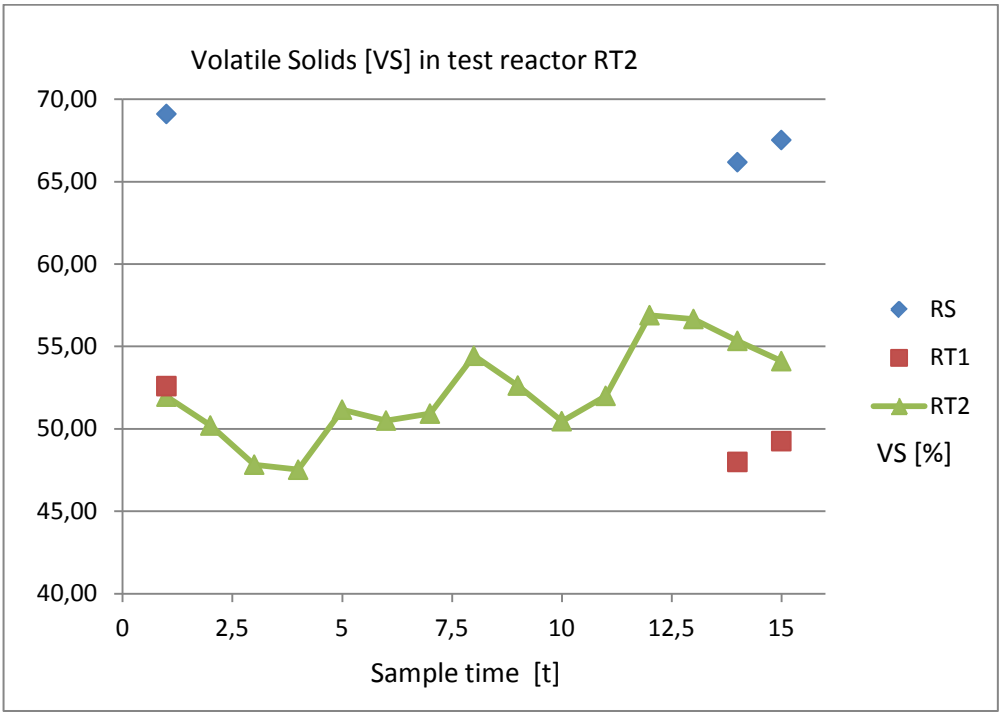
Concentration of cation is presented in graph 4.19 with reference measurement of NH_4^+-N in reactor RT1. Ammonium is rising from 680mg/l to maximum 930 mg/l in the test. Sodium increasing from 811 mg/l to 944mg/l and K^+ has a level of 300mg/l with Ca^{2+} and Mg^{2+} in a lower concentration from 80-120 mg/l.

4.2.3 Solids developments in test period

The development of TS in reactor during the test is presented in graph 4.20 with reference measurements of RS in buffer tank and reference reactor RT1. The samples from the start of the test have almost equal values for RT1 and RT2 before injecting the batch. The level was 3.5[%] TS which is normal level. The level was increasing with some dynamics at sample time [10] and [11] with further development to maximum level of 3.96[%] TS. Maximum TS content is correlates with the time of maximum biogas production. At the end of test period, the sample time values at [14] and [15] found higher TS in test reactor RT2 than RT1. The values in RT2 was decreasing from; 3.7-3.6 [%] and for RT1 was stable at 3.35 [%]. The buffer tank or the raw sludge was at normal conditions with increasing values, from 4.7-5.8[%] in total solids concentration.



Graph 4.20 TS developments in RT2 with reference values for RT1 and RS



Graph 4.21 VS developments in RT2 with reference values for RT1 and RS

In the preliminary test the degradation and converting of volatile solids VS into biogas was noted and analysed. Due to the observations the VS development was surprising in dynamics. Of the graph 4.21, already from sampling before the injected batch, the VS content was decreasing from 52[%] to the lowest point 47.5 [%] in measurements. The VS level increased with some dynamics in the period at sample time [8-12]. Maximum VS[%] point at sample time [12] content of 57 [%] VS. ,with the .after injected batch test we

assumed some dynamics Maximum VS content is correlating with maximum biogas production like TS at sample time[12]. At the end of the test period, the sampled values at [14] and [15] compared with the reference reactor RT1 was higher. The levels for test reactor showed RT2 with 55.3 [%] and RT1 at 48 [%]VS .

Table 4.9 Measured values in RS, reference RT1 and test reactor RT2.

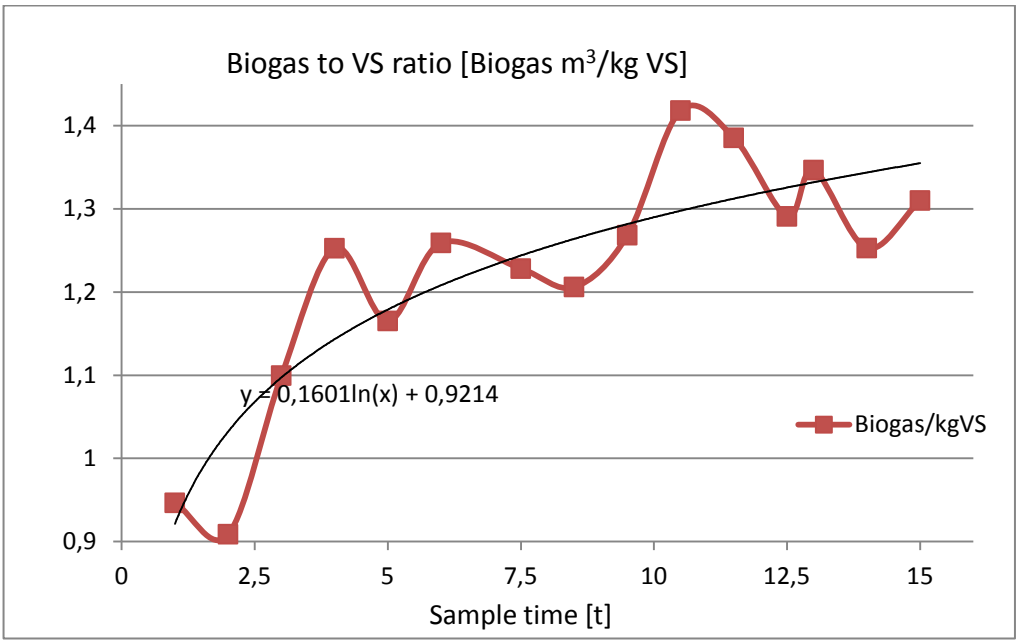
Sampled Solids Sample nr [1]$_{Initial}$	**Total solids % [TS]**	**Volatile solids % [VS]**	**Fixed solids % [FS]**
Raw sludge (RS)	5	69	31
AD sludge RT1	3,5	53	47
AD sludge RT2$_{test}$	3,5	52	48
Sample nr [14]$_{4\ hour}$	**[TS]**	**[VS]**	**[FS]**
Raw sludge (RS)	4,97	66,2	33,8
AD sludge RT1	3,35	48	52
AD sludge RT2$_{test}$	3,7	55,3	44,7
Sample nr [15]$_{24\ hour}$	**[TS]**	**[VS]**	**[FS]**
Raw sludge (RS)	5,8	67,5	32,5
AD sludge RT1	3,5	52,6	47,4
AD sludge RT2$_{test}$	3,6	54,1	45,9

The values in table 4.9 is measurement and solids analysis of the compartments, buffer tank (RS), RT1 the reference reactor and reactive “test” reactor RT2. The initial measurement is indicating standard conditions for the compartments, with TS at 5% in raw sludge and 3.5 in digested sludge (RT1-2). The measurement at the end of test**,** sample nr [15]$_{24hour}$,the values are normalized after test conditions with TS at 5.8% in raw sludge and 3.5-3.6 in digested sludge (RT1-2). Sample nr [14]$_{4hour}$ is a sample with high organic load and converting rate with more deviating values from ordinary conditions. TS values in the reactors are 3.35 for reference RT1 and 3.7 at RT2 test reactor. The VS levels in RT1 are 48% and 55.3% volatile solids in test reactor RT2.

4.2.4 Biogas respond to organic load

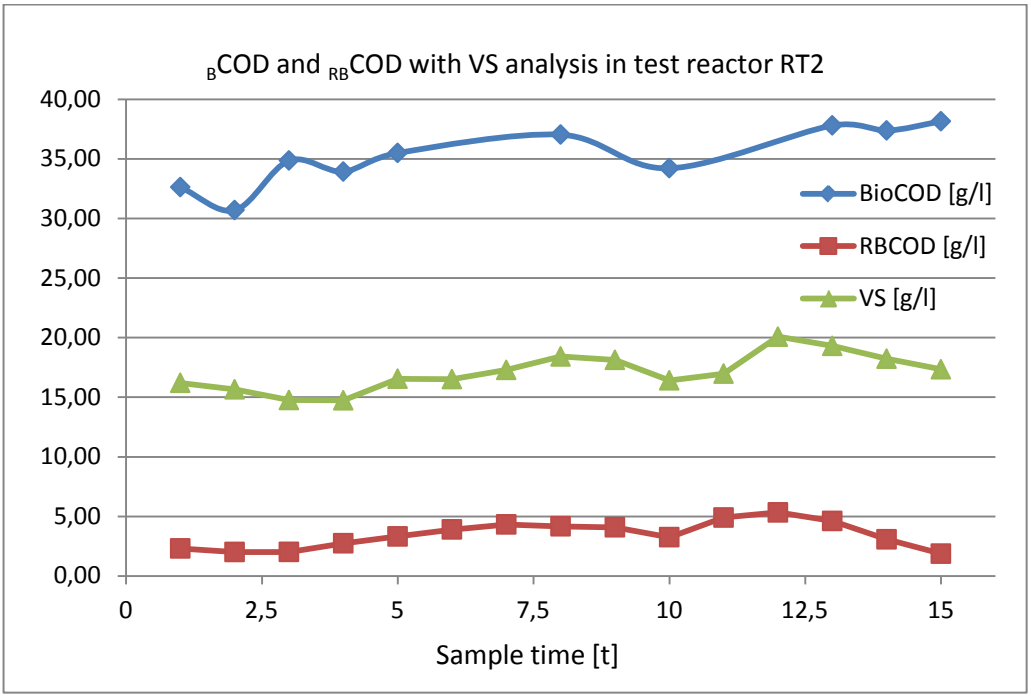
From graph 4.22 the biogas produced per kilo volatile solids is graphically presented for the first part of the test period. The graph is presenting initial conditions until maximum or high(rate)dynamics for the reactor. The time in this graph is deviating from previous scales; the sampling time here is connected to biogas production in real time. So, sample time nr. [15] is from initial time until 3 hours later at 14:54.

The biogas production is monitored to find increasing gas production at the VS concentration in reactor during the test. With max biogas production, 648 Nm^3 was produced and the VS content was measured to 23.0±0.3 [gVS/l]. The average VS % during the test was measured to be 52.2±0.9.



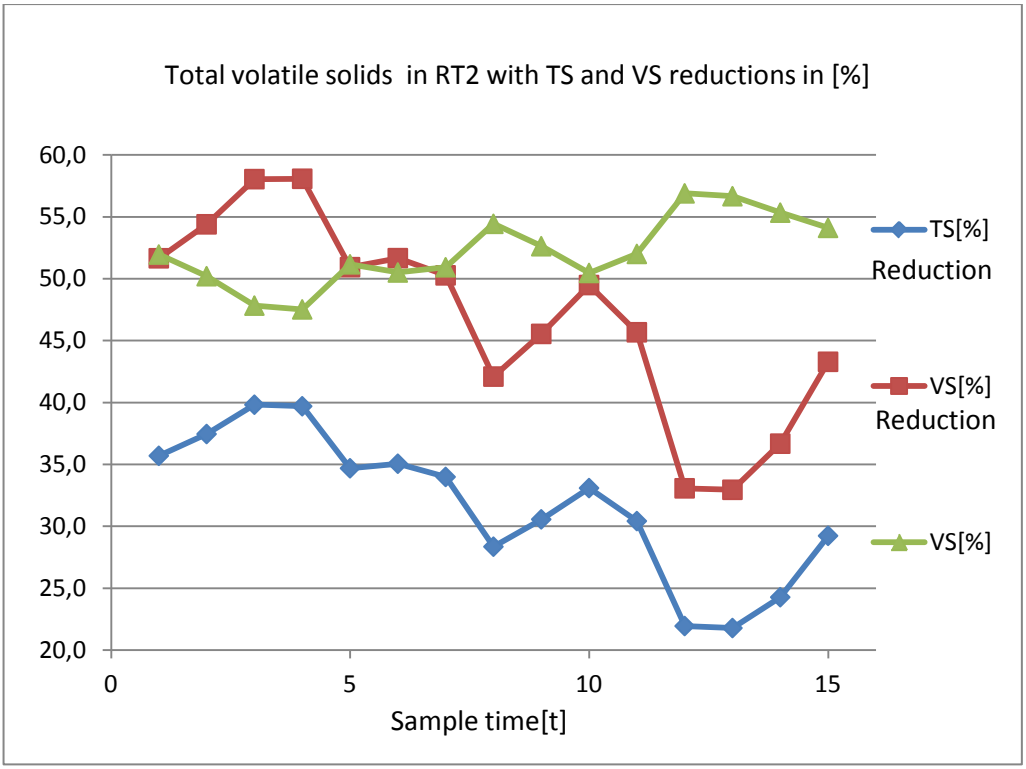
Graph 4.22 Biogas productions from VS concentration in test reactor RT2.

Observation of the increasing gas production showed high dynamics throughout the intensive injection period. The production continued after maximum with same decreasing dynamics until stabilized high production 3 hours after injection.



Graph 4.23 Volatile solids and biodegradable COD development in test period.

Measured biodegradable $_{Bio}$COD and $_{RB}$COD the soluble fraction is presented in graph 4.23. The VS concentrations are also plotted for visualization the relationship to COD.



Graph 4.24 Reduction of TS and VS percentages in tested reactor with total VS developments during the sampled time.

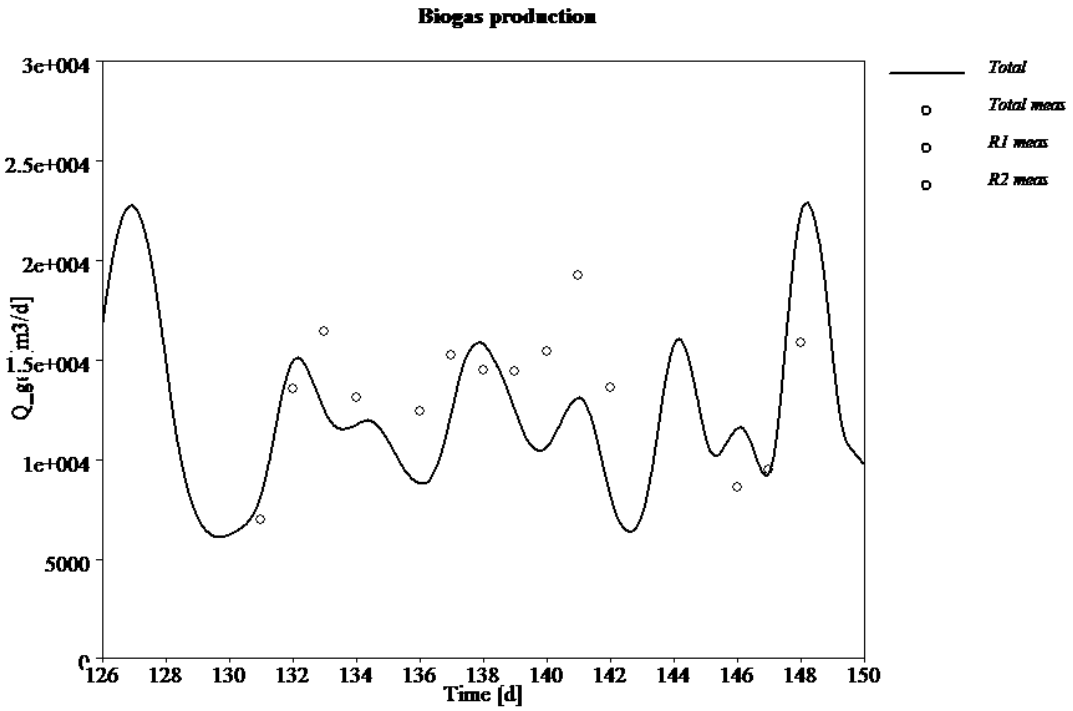
The development of TS, VS reduction is plotted in this graph 4.24 for presenting the decreasing in [%] reduction of solids due to the dynamic test. The approach for this analysis is presented in calculations in chapter 4.1.2 and the dynamics is reflecting fluxing reductions. For TS the solids is decreasing from 40-22% and for the VS the reduction from 58-31%. The volatile solids in reactor is a part of the COD content in reactor and the average COD/VS ratio is calculate to 1.81 [gCOD/gVS] for the injected batch.

4.3 Modelling and parameter estimation.

To explore and investigate the microbiological interactions who take place in the digestion process, simulation are used. Comparison of experimental results with model calculations is an important method for testing theories in natural sciences. Modelling of biological systems is a widely used tool in operating and optimizing different biological waste treatment plants. AD processing of organic matter is a well known technology and widely used treatment of high concentrated sludges from industry and agriculture. Diversity of papers and studies of scientific work reflect the large number of topics within this area. (Mata-Alvarez, Macè and Llabrès.2000).

IWA`s model Anaerobic Digestion Model No.1 (ADM1) and software AQUASIM (Reichert, P., 1998) for numerical simulation is one approach for full scale simulation and calibration of the digestion process.(Ozkan-Yucel,U.G., and Gökcay,C.F.,2010)

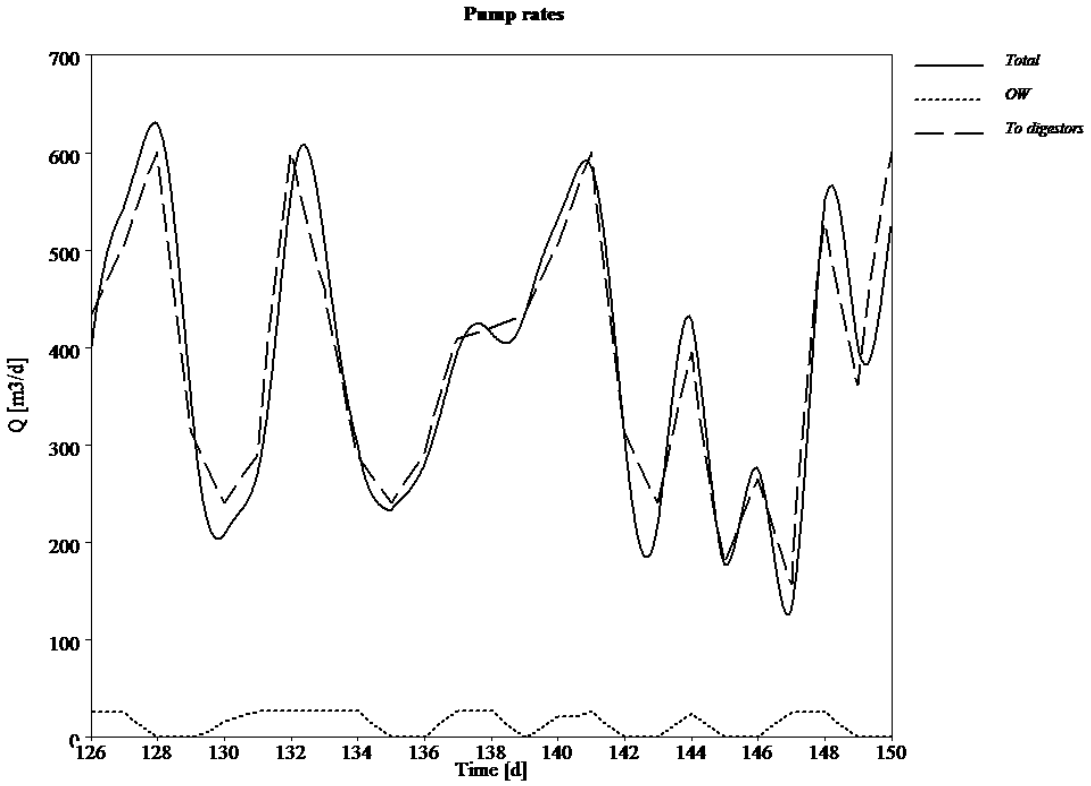
The reactors were modelled in 150 days with organic load from settled sludge (RS) and external high COD content waste. The largest fraction in volume was “food waste sludge” (FWS), but other fractions like fats, sludge from biological WWTP`s and diverse ensilages from fish and food industries was included in the model. In graph 4.25 the estimated biogas production is presented during the extended modelling period. To create “steady state” the monitored and measured period were copied and added up to 150 days.



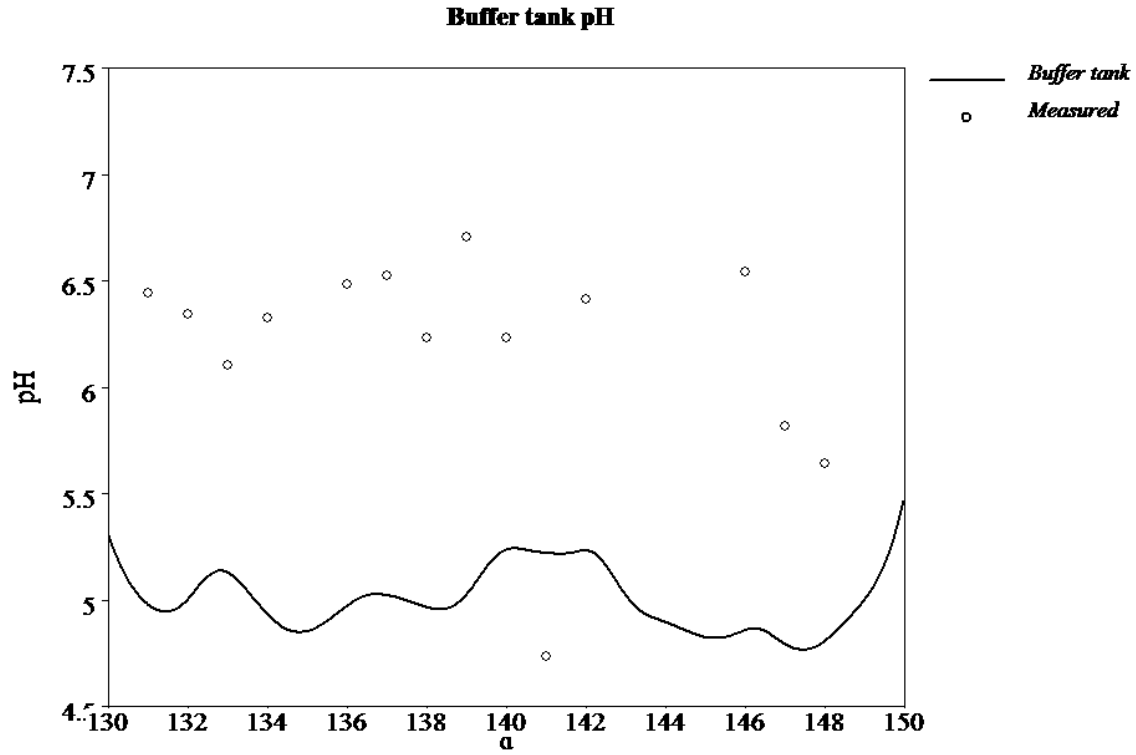
Graph 4.25 Modelling and estimation of biogasproduction.

The biogas production is fluxing from low production at 7000 [Nm^3/d] to maximum at 23000[Nm^3/d]. The monitored values are fluctuating in the level 7500-20000[Nm^3/d]. These variations are connected to the flow into the system and by graph 4.26 the influent flow rates are presented. The pump rates is defined with the tags; “Total”, “To digesters” and “OW”. Total volume flow into buffertank or into the modelled system is “Total”. Further flow of total sludge from buffer tank into digesters is the tag “To digesters”. The total amount of different external sludges into the system is the label OW.

All fractions is in the dimension volume flux [m^3/d] and total flow into system is fluctuating from 140- 640[m^3/d]. And for OW the levels in from 25-35 volume flux [m^3/d].

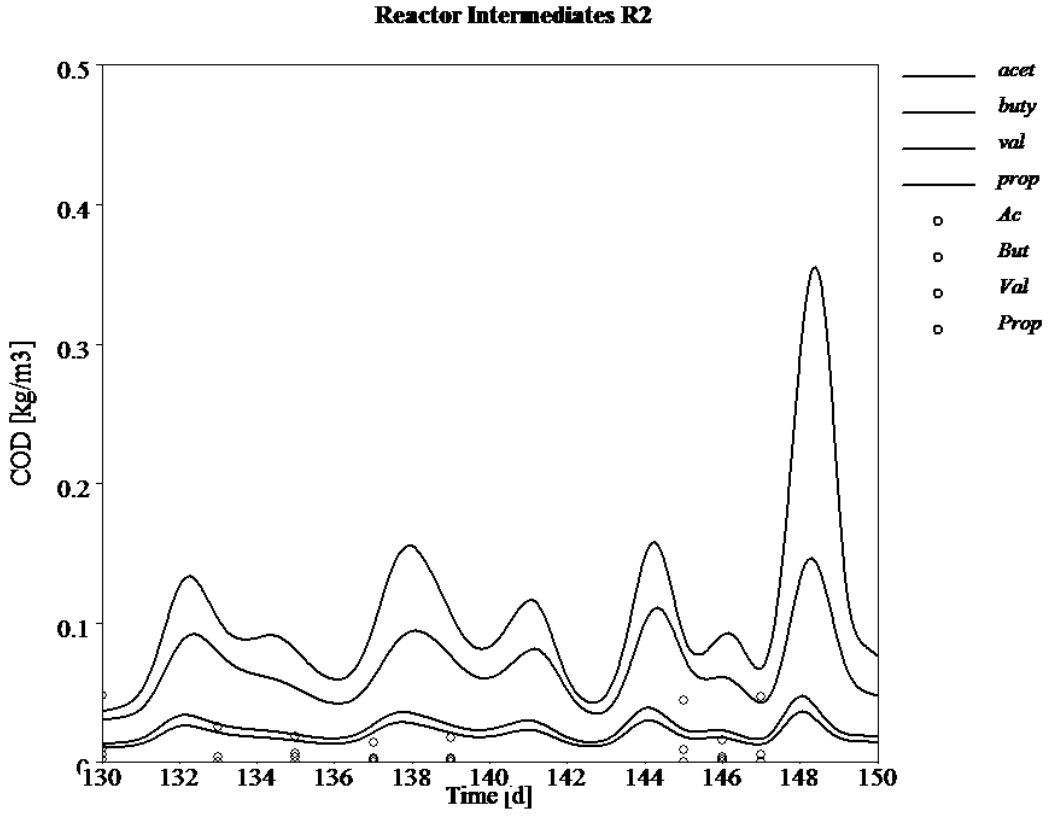


Graph 4.26 Influent volumes to buffer tank, digesters and organic waste loads.



Graph 4.27 Modelled pH in buffer tank.

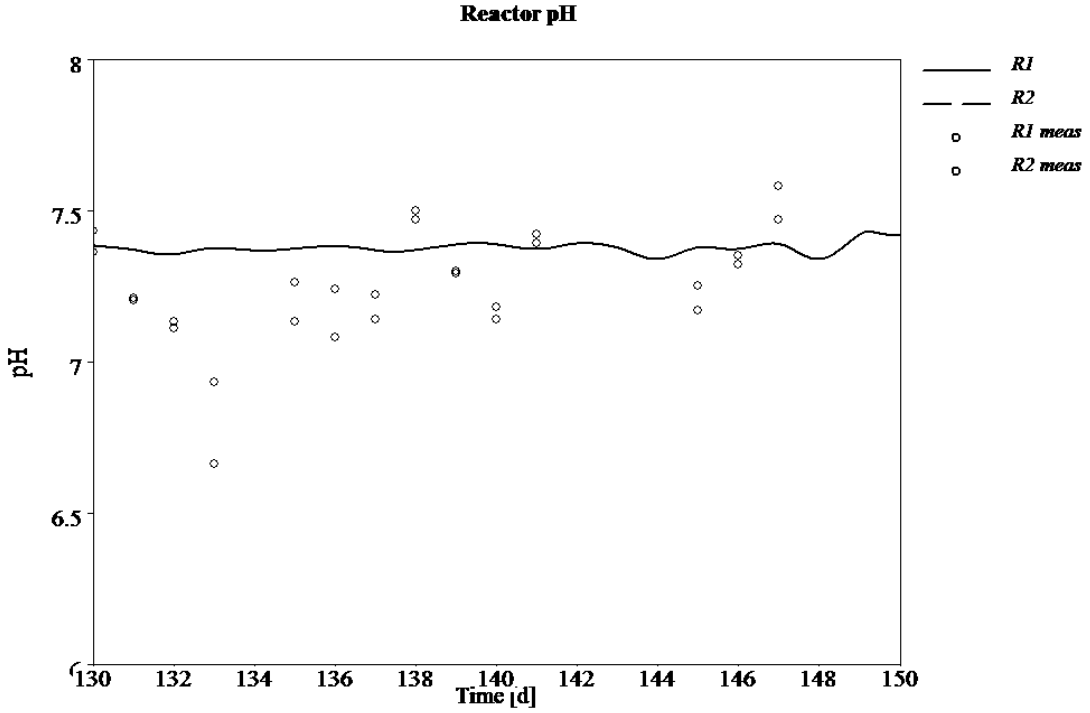
The modelled pH in buffer tank is presented in graph 4.27 and is in a low level from 4.7 to 5.5 at the end. Measured values are in the average level at pH 6.3 and showing high dynamics in the model. The intermediate in buffer tank is presented in graph 5.28 with high activity for acetate (HAc), propionate (HPr) and lower concentration for valerate (HVa) and butyrate (HBu).



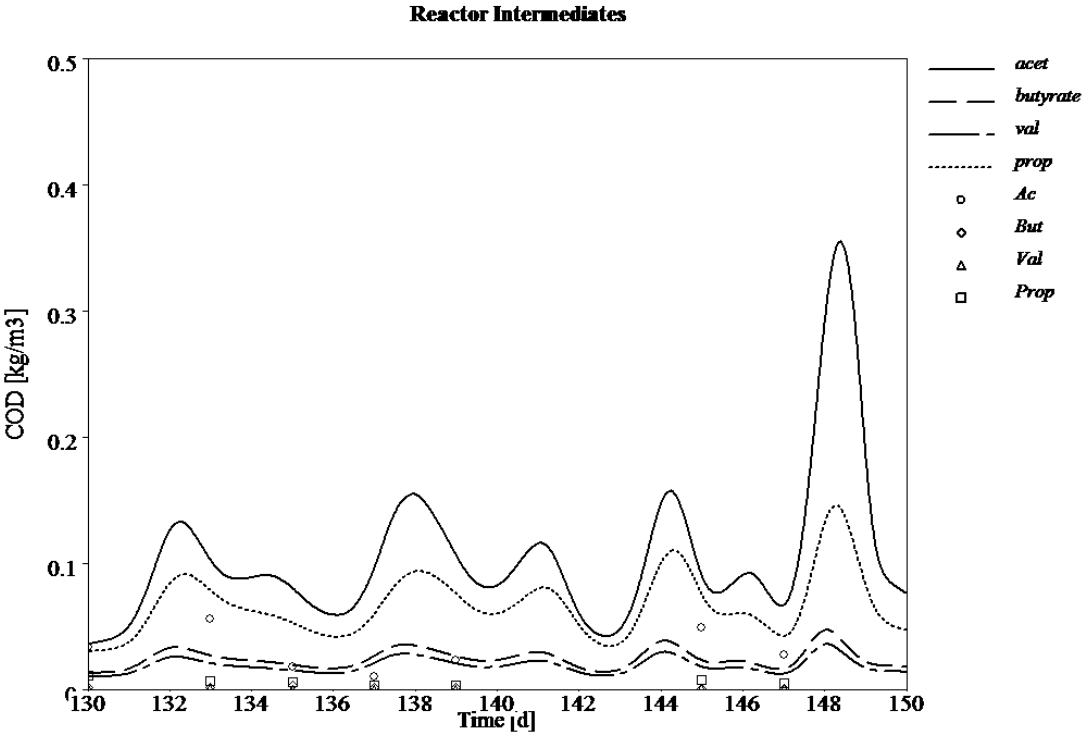
Graph 4.28 Modelled intermediate interactions in buffertank (R2).

The VFA`s interactions in reactors is presented in graph 5.30 with high activity for acetate (HAc), propionate (HPr) and lower concentration for valerate (HVa) and butyrate (HBu). The structure and developments in both models graph 4.28 and 4.30 are showing the dynamics for the intermediates. Similarities are visible and measured data are general in lower concentrations. The pH in AD reactors is showing high stability due to high dynamics by the measured values.

The reactor pH is presented in graph 4.29 and modelled pH is 7.4 with more fluctuating values by measurement from 6.7 to 7.6.



Graph 4.29 Modelled pH values and measured in periode.



Graph 4.30 Modelled interactions by the intermediates.

5 Discussion

First chapter is discussing the “normal” steady state conditions during the sampling period in May 2011. The second chapter will focus and discuss the dynamic test in 26-27.08.2011 and the third session is the ADM1 model parameter estimations and evaluations.

5.1 Experimental analysis and evaluation

In general the investigation of the reactors under “steady and dynamic state” was challenging. Methods and experimental approach have the fundamentals in water science and technology and have being developed for many years. Sample treating and providing the sludge for further measurement was the most challenging and time consuming.

It is an important work due to reliable measurement and analysis. By observation when conducting solids analysis, the sludge was containing living organisms and continuously converting and degrading the organic matter in the sampled bottles. The measurement has to be conducted and treated straight after sampling otherwise the result will be unreliable and poor. The experience of this project was to conduct the solids analyses as fast as possible and not store the sludge for more than 24-36 hours, at 5^{o}C storage temperature.

Comparison of systems is in general normal approach and method for investigating a system. The trap is when relying on other system and analysis without background research of methods and analysis. The conditions are numerous and the wastes within a wide range of organic content, especial when it comes to codigestion with manure (Hartmann et al,. 2005), industrial high content wastes (Alatriste-Mondragon et al., 2006).

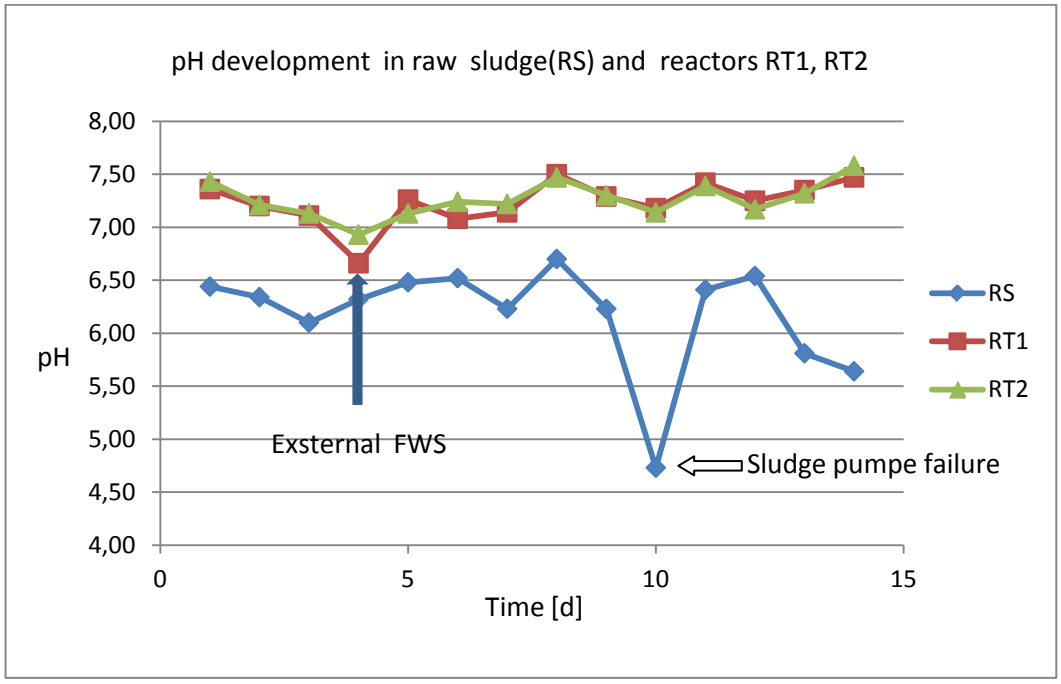
The conclusion is to conduct solids analysis based on standard methods with detailed description of the approach. Then the analysis is based on facts from the system and reliable values due to the influent condition and treatment system. The important issue with comparison is not drawing general conclusions and the need of evaluating each case separately. The waste has unique portion of the constituents polysaccharides, proteins and lipids, especially when for industrial and agriculture wastes.

The composition of total solids (TS) in FWS was analysed and characterising into polysaccharide, proteins, lipids and inerts. The average content by characterising constituents gave this values; polysaccharides 34.4[%], proteins 27.1[%] and lipids 31.3[%], inert is measured to be 7.2[%].

5.2 pH, alkalinity and VFA`s at normal operation

The steady state or "ordinary conditions" survey period were conducted under fairly stabile conditions. Some sludge pumps from settling failed and together with high organic loading during sampled days the sludge system got a "relatively" high dynamic situation. The reactors were acting stabile throughout the sampled period of 22 days with period of high biogas production. Dynamics in pH and alkalinity was observed in RS, RT1 and RT2 due to organic loadings and operation of the AD-plant.

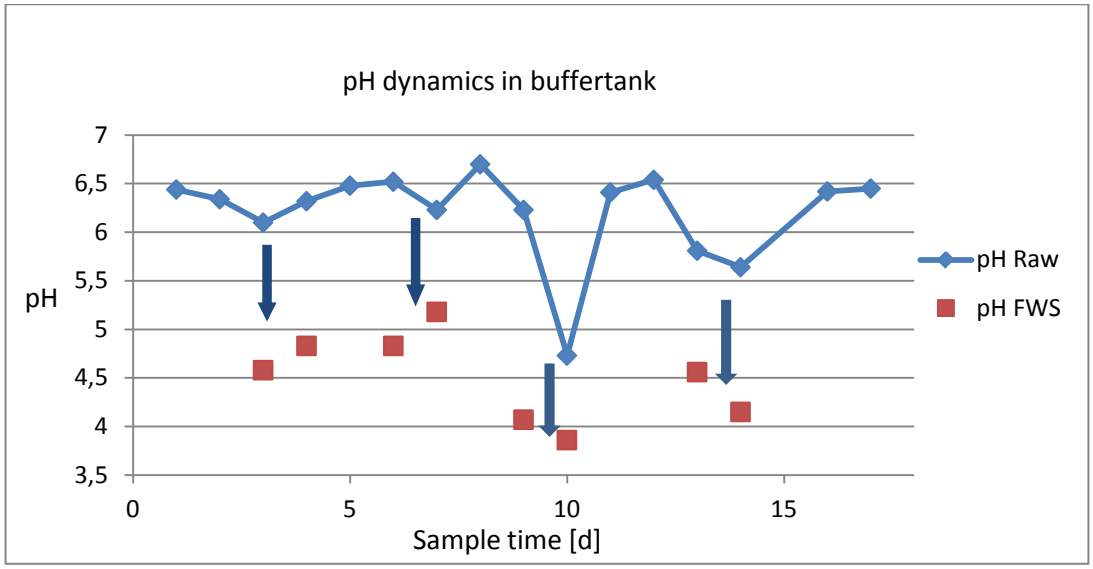
The pH and Alkalinity is ordinary measurement on raw sludge (RS) and digested sludge RT1-2, with a frequency once a week. It is manual laboratory labour and results are ready 2-3 hour after sampling time once a week. Normal pH levels according to plant data approximate 7.0 -7,4 for the digestion. According to literature the AD-process are limited to a narrow pH-interval from approximate 6.0-8.5, pH values outside these levels can lead to imbalance. Optimum for methanogens is between 7 and 8 and the acidogenic microorganism often has a lower optimum.
There are many factors that influence the pH in AD process. In general organic acids and carbon dioxide will cause decreasing in pH values, while accumulation of ammonia will contribute to an incensement of pH (Angelidaki.,et al.,TheBiogasProcess).



Graph 5.1 pH development in Raw Sludge (RS) and digested sludge RT1 and RT2.

Normal condition for pH behaviour was measured with some deviation, especial in RS in sampling day 10, graph 5.1. In the AD reactors the pH level was fluctuating from 6.7-7.6, with smaller drops especially at sample day (4). At this sample time high COD content, food waste sludge (FWS) was delivered to the plant with low pH, approximate 4.5-5.

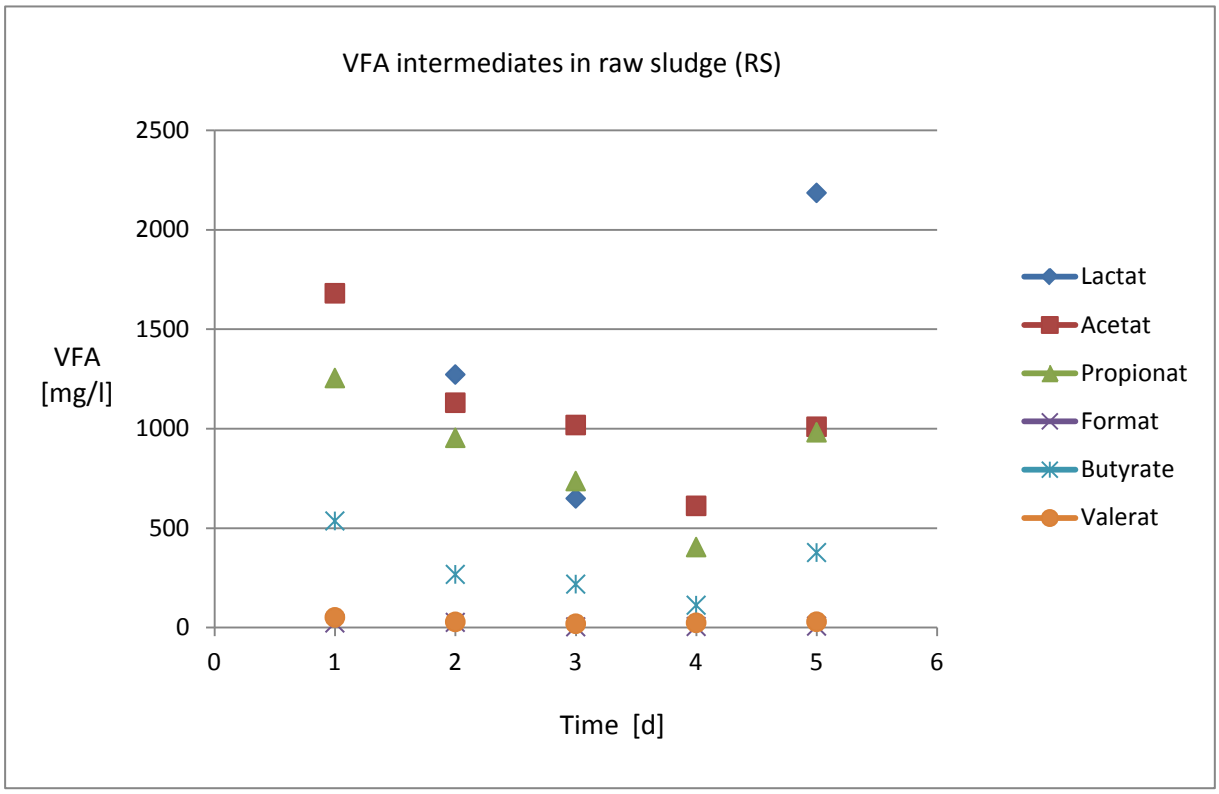
These dependencies of pH drop at deliveries of high concentrated FWS are visible and replicated throughout the sampled period. In graph 5.2 the pH is decreasing in level in the area of input of high content waste to the buffer tank.



Graph 5.2 pH dynamics and influence of FWS with low pH.

Sharp pH drops is usually associated with generating volatile fatty acids in sludge treatment system. In buffer tank the RS content of volatile fatty acid is fluctuating along in which state process are in. From the graph 5.5 the VFA`s concentration in raw sludge indicate conversion process are preceding in the compartment.

The amount of VFA`s in the raw sludge is due to the depolymerisation process of polysaccharides, fats and proteins. Different groups of microorganism are capable to process hydrolysis and fermentation with converting complex composite. The raw sludge in buffer tank is being hydrolysed and fermented along with the retention time in buffer, which is approximate 14-16 hours. The sludge temperature is low compared with the digesters, the level was measured from 17-22^{o}C, and the conversion rate of first order kinetics. The hydrolysis process is very sensitive to temperature and temp-fluctuations. Hydrolysis is generally considered to be the rate-limiting step during AD of complex substrates (Henze, 2008). The high concentrated food waste and industrial wastes has VFA`s concentrations is in various levels of concentrations depending on the pretreatment of the sludge or waste. With substrate mainly as particulate compounds, the hydrolysis or methanogens process is the slowest and control the overall rate. In the graph 5.5 the concentration and the present of acids is an indicator of the processes.



Graph 5.5 Intermediate concentrations of the VFA`s dynamics in raw sludge, buffer tank

From IC analysis the concentration of VFA`s was measured from 400-2200 mg/l. The highest level was lactic then acetic and propionate acids with the range of 400-2200 mg/l and formate, butyric and valeric acid is present but in a lower concentrations. From experience of operating AD reactors the level of VFA`s is in the range of 100-2500 mg/l as HAc with GC-analysis under clearly stable conditions.

The concentrations of VFA's in buffer tank is normally high when FWS is injected to the sludge and the analysis had shown presence of lactic, acetic, propionic, butyric, and valeric acid. Based on analysis and previous studies it can be noted that the fermentation and acidogenesis process is present in the buffer tank. The data obtained for VFA's showed generation of all volatile acids in buffer tank. From the studies analyses conducted it was found that the raw sludge entering the buffer tank has 3-5 times lower values of VFA compared to the sludge leaving the buffer tank which indicates fermentation takes place in the buffer tank.(Popov, J., 2010).

The pH in AD reactors has a dynamic attitude due to the organic loadings graph 5.2. RT1 remained between 6.66 and 7.47 in graph 5.1 and the pH range in reactor RT2 was between 6.93 and 7.67 hence we can conclude that the reactors is operating under stabile pH conditions. The biogas production in AD reactor is by Henry law related to the buffering capacity with dissolved carbon dioxide (CO_2) into the solution. The biogas consists in general of 30-35% CO_2 and contributes to the solution in digesters with alkalinity (ALK) by bicarbonate through the bicarbonate buffering system. A high ALK in the system is necessary

to keep the pH in a level of optimal conditions depends on the characteristic of the substrate. Normal operation conditions are in the level of 3000-5000 mg/l as $CaCO_3$. For organic waste and sludge digestion sufficient ALK is produced by breakdown of protein and amino acids to produce NH_3, which react and combines with CO_2 and H_2O to form alkalinity as ammonium bicarbonate $NH_4(HCO_3)$. The portion of CO_2 in the biogases was 33-35% indicates together with $NH_4(HCO_3)$ in the system contained adequate buffering capacity.

Calcium and magnesium ion`s are present in solution in different concentration. Beside ammonium bicarbonates these ions are also examples of buffering substances for the AD-process. The concentration of ions in sludge solution is due to the state of process. In the table 4.1 the average concentration for sodium (Na^+) in RT1-2 are approximate equal and in the level of 828 mg/l.

The level of toxic concentration is in the range of 3500-5500mg/l for sodium and 2500-4500mg/l for potassium and calcium. The compound magnesium and ammonium-nitrogen (NH_4-N) are moderate inhibitory at concentration 1000-1500mg/l (Mg^{2+}) and 1500-3000 mg/l (NH_4-N). The authors Gopalakrishnan et al., 2000 measured the level of ammonia concentration from full scale test with concentration of NH_4^+ in supernatant in order of 500-600mg/l. The presented values in table 5.1 are in general low, but analysis of ammonium-nitrogen (NH_4-N) is in a level of attention.

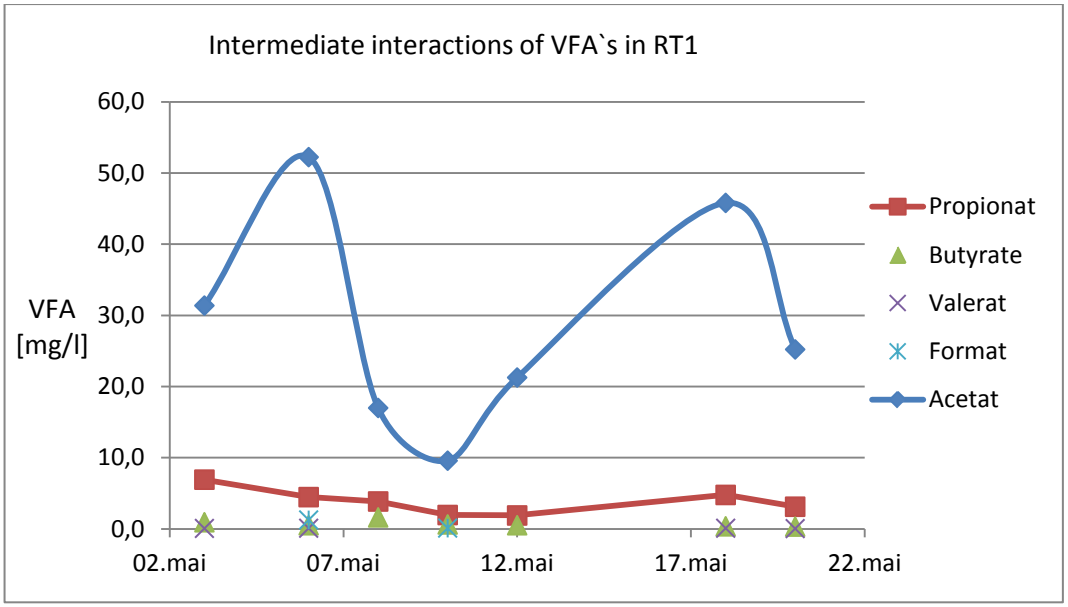
Table 5.1 Cations concentration in compartments.

Cations	RS [mg/l]	RT1 [mg/l]	RT2 [mg/l]
Na	736	840	816
NH4-N	216	692	687
K	141	255	254
Mg	130	105	103
Ca	172	80	78

Reported experimental data with inhibition caused by ammonia for the methanogenic population the level of 2500mg/l[NH_4] was reported (Kiely., at al., 1997). Ammonia and alkalinity concentrations were following each other under this laboratory test, with a high pH 7.8. The experiment indicate the relationship by breakdown of protein and amino acids to produce NH_3, which react and combines with CO_2 and H_2O to form alkalinity as ammonium bicarbonate $NH_4(HCO_3)$. Total ammonium concentration from other tests is toxic for the methanogens organisms in a concentration range of 1500-3000mg/l (NH_4-N) at a pH above 7.4. The level of 3000mg/l is stated toxic at any pH (Tchobanogolous et al.,2004).

The analysis of VFA's in reactors RT1 and RT2 showed the presence of the acids, acetic, lactic, propionic, butyric, valeric and formic acids. By the IC-analysis the concentrations of the intermediates can be discussed and evaluated. By 5-points titration`s we discovered high

acids consumptions during the titrations and pointed out samples with high activity. The analysis of VFA`s is indicated fermentation and acidogenesis process is present and producing VFA's in the reactors RT1 and RT2. The concentration of acetic acid "Acetate" (HAc) in RT1 and RT2 is fluxing along with the state of organic loading. The levels are in the low range at 9-11.8 mg/l and 44-52 mg/l at the high level. Compare with the RS in buffer tank the level of VFA`s concentration is 10-30 times higher, from 666-1680mg/l (HAc).



Graph 5.6 Intermediate dynamics during the sampled period.

For the other intermediates Propionate (HPr), Lactate (HLa), Butyrate (HBu), Valerat (HVa) and Formate (HFa) the levels of concentration is low, from zero to 6.9 mg/l for Propionate (HPr). The level in buffer tank "upstream" has for the intermediate Propionate (HPr) a range from 404-1255mg/l , and that is approximate 50-125 times higher rate in buffer tank. The same situation with (HBu), Valerat (HVa) and Formate (HFa) the levels of concentration is low the AD reactors and approximate 50-100 times lower, and for butyrate even more.

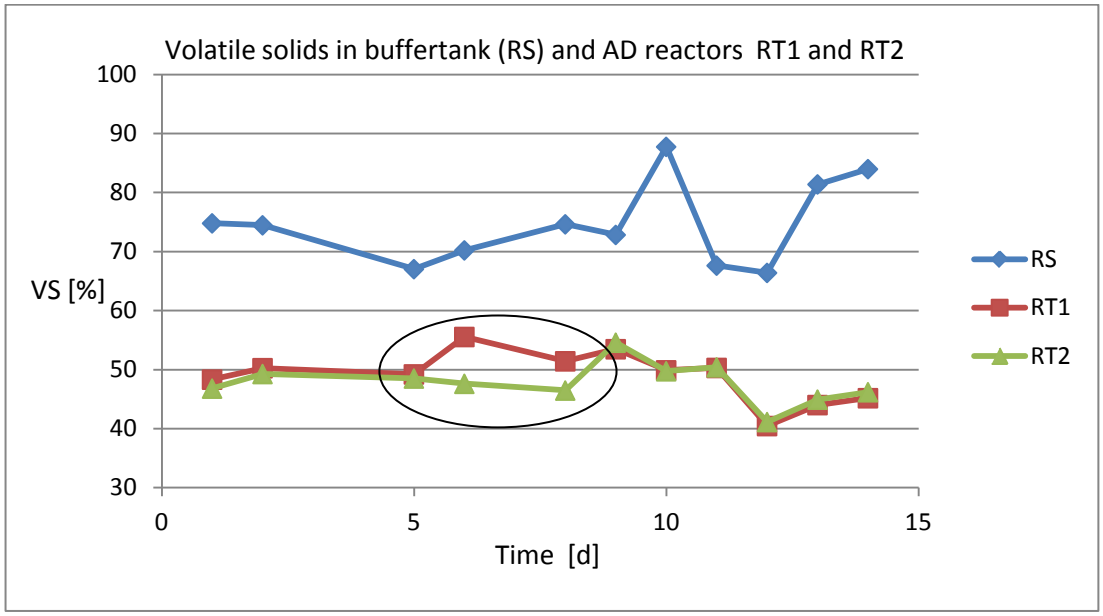
Lactic acid was measured at an interesting high values in the buffer tank (RS) and completely disappeared in reactors. The sampled time[d] 2,3 and 5 was days with high loads of organic waste as FWS. For lactate (HLa) the level was measured to 1272, 650 and 2185 mg/l respectively. This variation and fluxing values may indicate a high speed process for lactate degradation and studies has indicated most of the monosaccharides substrate may degrade via lactate. (Skiada et al., 2000);(Romli et al.,1995). Lactate is degraded very quickly and is therefore seen primary during transient overload conditions in acidification reactors. The behaviour during concentration overload is the lactate increases from being insignificant to the highest organic acid (Batstone et al., 2002). The same attitude was obtained in graph 4.7 in reactor RT2 with 83,5mg/l for HLa. In Raw sludge (RS) graph 5.5 the concentration of lactate has a peak-concentration at 2185 mg/l, which is 2 times higher than acetate concentration.

Under normal stable conditions the principal products of fermentation are acetate HAc, hydrogen (H_2), CO_2 and propionate (HPr) and butyrate (Hbu). The intermediate propionate and butyrate are fermented further and converted into hydrogen, CO_2 and acetate. These are the final fermentation products and are the precursors for the methanogenesis and methane formation.

High concentration of acetate and intermediates in the raw sludge and low VFA`s concentration in the reactors RT1 and RT2 are indicating a stable pool of acetogenesis converting intermediates into acetate and H_2. The methanogens organisms in the reactors are final degrading acetic acid and hydrogen into methane. Analysis of these data indicates degradation process in all compartments, the reactors; with hydrolysis, acidogenesis, acetogenesis and methanogenesis are present in the reactors. In the buffer tank the high level of VFA`s is strong indication of fermentation process.

5.3 Organic load and VS reduction

Volatile solids in the compartments buffer tank (RS) and the reactors RT1 and RT2 is over the sampled period in Mai 2011 showing dynamic developments. The raw sludge is fluctuating from 66-88% in VS content with the peak point at time; day 10, the failure of sludge pump from sedimentation. This caused an increase of TS in the sludge system with measured values on this day at; 13.12±0.05[%]. Interesting observation is that the reactors is not affecting visible on TS or VS at this sampled day 10.



Graph 5.9 Volatile solids[%] and development for the compartments.

The graph 5.9 is showing volatile solids fractions (VS) in raw sludge (RS) and reduced in AD reactors RT1-2. In buffer tank the VS content was 74.7±0.3[%] and in reduced form in RT1; 49±1[%] and RT2; 47.8±0.9[%] in VS content. There are some deviations in VS at the pointed area in sample day 6 and 7. The reactors are following each other in VS content perfectly,

but this days is deviations due to delayed sampling and sludge solids analysis. In the solids analysis the samples is degrading further in the 1000 ml poly ethylene (PEH) bottles. The digestion temperature is high and the process is ongoing. Under the measurement and solid analysis, the bottle pressure was increasing and released due to the biogas development and reduction of organic matter. Together with high strength FWS the raw sludge is feeding the reactors with high content of VFA`s and occasionally the feeding sequence, when injecting FWS. Then the situation of VFA`s feed to dedicated reactor is high concentration in of VFA`s, reference to graph 5.5 and 4.7 with occasionally high lactic acid concentration at 83.5 mg/l.

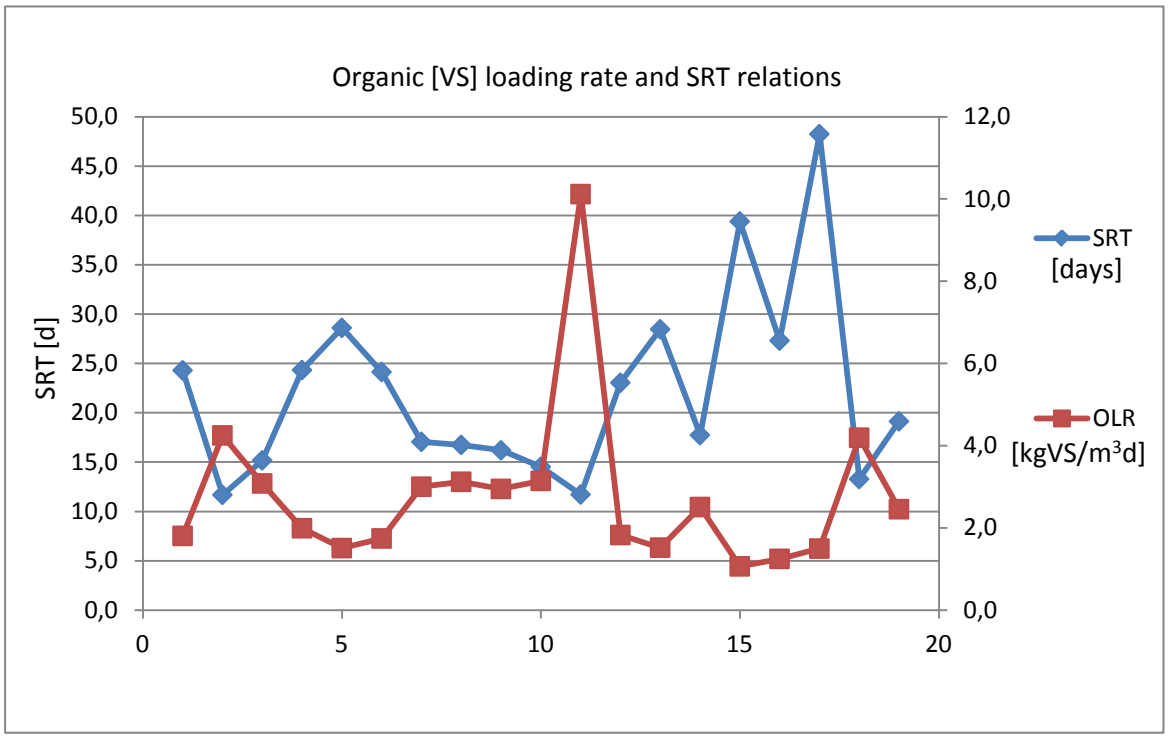
So it is recommended for "Solid analysis" to handle and measure the same day and be aware sampling time ref. sequence feed to reactors, to avoid deviations in measurement.

Table 5.2 Average solids analysis values, measured in week 18-19-20.

Sampled Solids	**Total solids %** **[TS]**	**Volatile solids %** **[VS]**	**Fixed solids %** **[FS]**
Raw sludge (RS)	7±0.06	74.7±0.3	25.3
Digested sludge RT1	2.9±0.9	49±1	51
Digested sludge RT2	3±0.05	47.8±0.9	52.2
Solids reduction RT1	49[%]	40[%]	
Solids reduction RT2	51[%]	42[%]	

TS and VS reduction and destruction is based on calculations in chapter 4.1.2. The VS[%] in RT1 and RT2 reactors is quite close and reliable. The TS [%] in RS is high due to the average measurement and this day 10 with 13.12[%] TS. If we skip this day the average is down to 6.4 in TS[%]. The AD reactions hydrolysis, acidogenesis, acetogenesis and methanogenesis are directly related to SRT. An increase or decrease in SRT has high impact on each AD process step.

Of the graph 5.10 the relations between organic loads and solids retention time (SRT) is visible and developed in opposite direction. The situations with high OLR is given low SRT and with opposite course. The level of the "volatile loading factor" OLR is for the sampled period varying from 1.1-4.3 [kgVS/m^3d] except from the peak from the "pump failure" day with two times higher solids concentration, approximate 13 TS[%]. The values for SRT are in the range from 11.7- 48 days from the graph, and the average SRT 22[d]. The highest values of SRT is the low feeding rate into digesters approximate 75-100m^3/d for one AD-reactor.



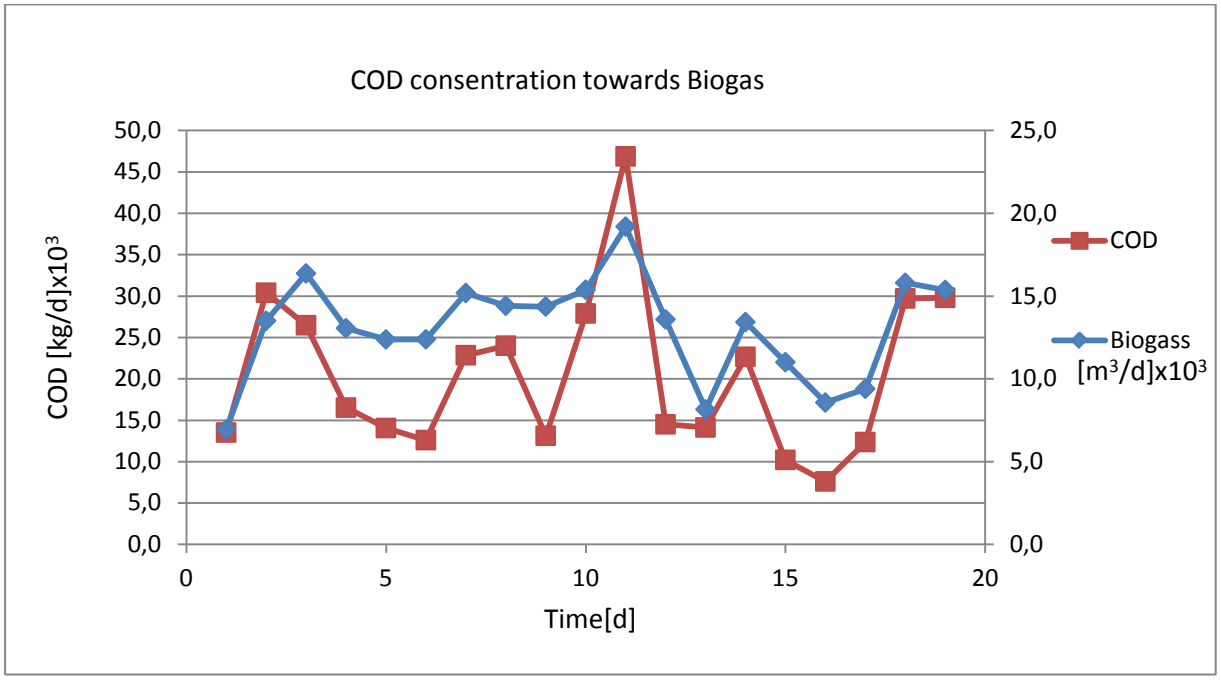
Graph 5.10 OLR rate developments with some dependencies towards the SRT

The same course pattern of COD load [kgCOD/m^3d] is measured for the same period with the same SRT behavior, see graph 4.12 in previous chapter. The organic load is an important operation parameter for capacity utilization of the AD reactors. Solid analysis is the fundament with characterization of polysaccharides, protein and lipids for complete analysis of the organic load. The microorganism in reactor is degrading the substrate due to the kinetics and the substrate composition.

The C/N relation (carbon/nitrogen) is one factor due to the composition of the organic load to be aware of. The relation effect the capacity of reactor by the content of polysaccharides, protein and lipids. The carbon source, the mono- and polysaccharides is the energy source for the organisms in reactor. Nitrogen content is regulating the growth and may limit the population with low access of N. With high content of Nitrogen, with protein degradation, there is a risk for accumulating NH_4^+ which can be toxic in high concentrations. In general the C/N relation factor 20 is recommended for codigestion and levels below 15 and over 30 are levels which affect the capacity of reactor (Carlsson et al.,2009)

5.4 The COD balance and Biogas conversion

In graph 5.11 the COD relations to biogas are graphical presented and due to COD concentration the relationship with biogas produced is the converted COD. Deviations in measurement of total COD or $_{B}$COD, give the measurement discrepancy to match the produced biogas. Obviously the COD is related to the degraded COD and some peaks and levels is due to further discussion but the tendency is the degraded COD is in the level of the factor 1664 gCOD/m^{3}Biogas below the online measured biogas production.

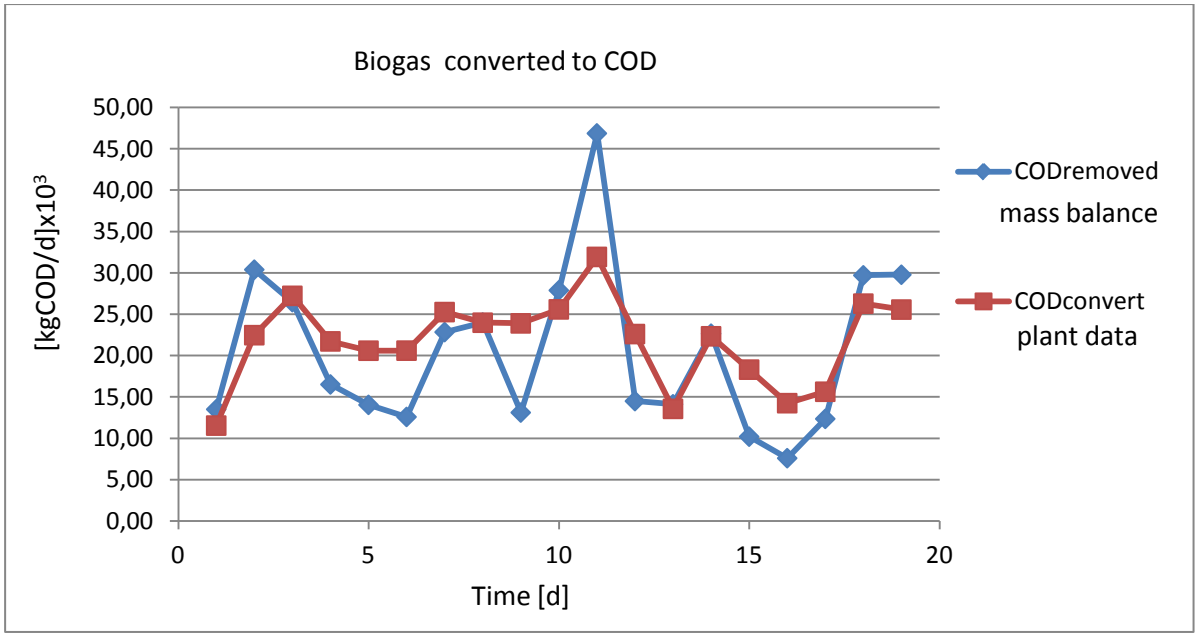


Graph 5.11 COD concentration and biogas produced online data.

COD balance is based on average values from solids analysis and data from computer system at the plant. The table 4.1 in chapter 4 the calculated value of $COD_{Methane}$ into biogas is calculated by the conversion factor 1664 gCOD/m^{3}Biogas, (1.66kgCOD/m^{3}Biogas). From the table $COD_{influent}$ =31025 and $COD_{effluent}$ is 10585 and the difference is then the removed ; $COD_{Removed}$ =20440. The calculated and COD from produced biogas at the plant is $COD_{Methane,}$ and calculated by the conversion factor 1664 gCOD/m^{3}Biogas to $COD_{Methane}$= 21758. This is deviating with difference COD = $COD_{Methane}$ - $COD_{Removed}$ = 21758-20440 =1318 kgCOD/d, which is calculated to 2.4% discrepancy.

In the COD balance there is average data which is levered due to optimizing the values for better analysing the average data. In graph 5.12 the biogas productions is converted to COD and compared with measured and analysed COD. The measurement is in the level of the produced or converted biogas data ($COD_{Methane}$). From the graph the level of $COD_{removed}$, from the COD balance, in general is in a level of under the biogas/$COD_{methane}$ at the plant. At sample day 10, with peak COD load 47000[kgCOD/d] it was measured high COD content in influent, both settled sludge and FWS, the COD was high. $COD_{Methane}$ and the biogas production increased but in a lower degree. The rate of 1.5 higher COD from the

measurement is high compare with rest of the measurement is in the level under the $COD_{methane}$. Measurement is a part of the analytic approach and it forces us to increase for better measurement for more reliable values. Systematic measurement over longer periods and improvement of the practical approach for conducting measurement is one important step.

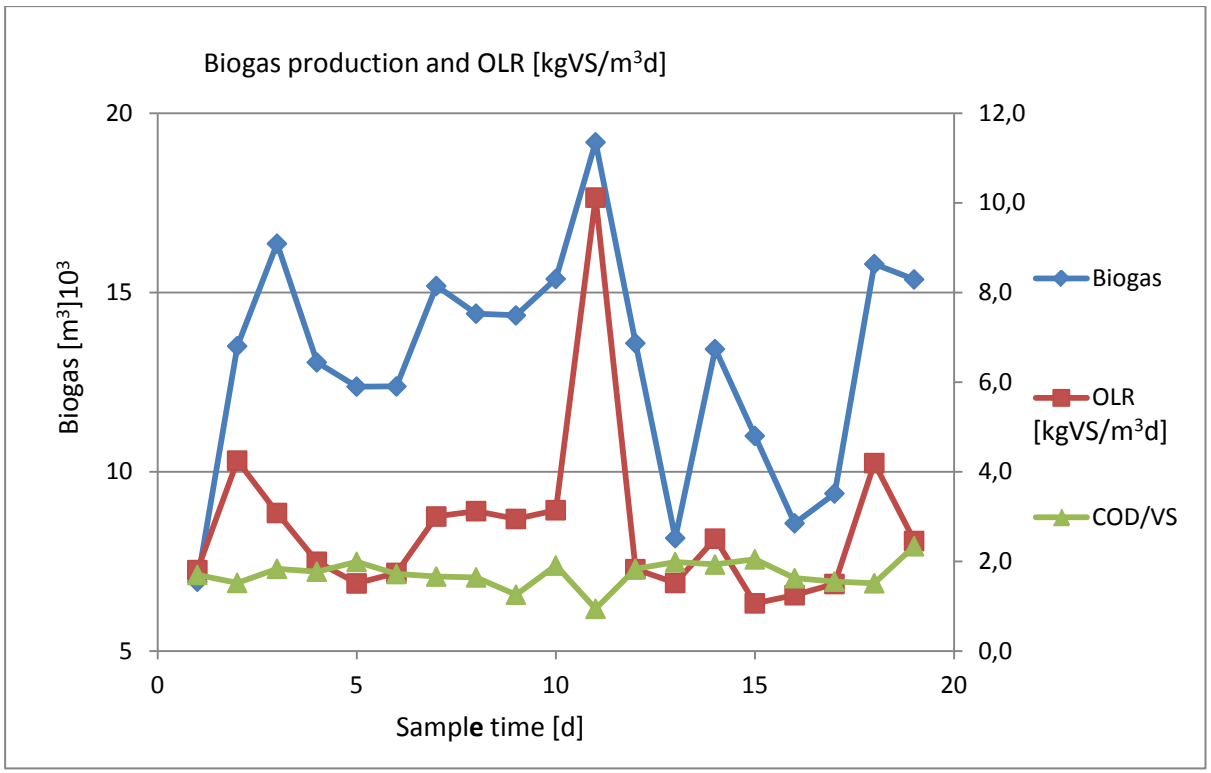


Graph 5.12 Biogas productions converted into COD from measured and plant online data.

The gas production or $COD_{Methane}$ cannot be used to alone for evaluation of the AD process. Rapid process changes in gas production are depending on feedings and the composition of the load. The capacity or process has to be correlated and related to the biogas potential, in terms of specific gas yield;

1. Biogas [Nm^3/gCOD]
2. Biogas [Nm^3/gVS]

This correlation and relationship for biogas production related to organic load for different wastes is crucial for operating AD reactors with codigestion. The organic loading rate [kgVS/m^3d] is presented in graph 5.13 with actual biogas production. The curve for OLR is following the gas production and confirms the relation. There are peak values in both graphs at the sample day 10 which was expected from previous analysis. The actual biogas production at this point is increasing in with a high rate. From 15000 m^3/d which is an level over normal production and increasing up to 19000 m^3/d. At this point day 10 the TS into buffer tank was measured to 13[%] which is approximate two times more organic loads into the reactor. From the graph the OLR rate is increasing from 3-11 and give rise to the biogas production. The average biogas production in this sample period is 13000 Nm^3.



Graph 5.13 OLR with biogas dynamics and COD/VS ratio for comparative analysis.

COD/VS ratios from this analysis is based on the measurement of $_{B}$COD (Total COD) the "biodegradable COD" from sampled and measured RS in buffer tank. The VS fraction is based on the same analysis with respect to the solids content. From the graph the COD/VS ratio have a fluctuating behaviour due to the measurement, and the level from 0.9-2.3 is calculated. The average value is calculated to be COD/VS = 1.65

Average mass balance evaluation from previous measurement and study, are in the range of 70-80[kgCOD/m^3] for the raw sludge (RS) in buffer tank. Sampled average COD flux for the steady state period table 4.3 was in higher activity with 85[kgCOD/m^3].

From average measurement 13000 Nm^3 biogas was produced and by the conversion factor 1664 [gCOD/m^3Biogas], (1.66kgCOD/m^3Biogas) the calculated value is 21580 [kgCOD/d]. The average solid flux into the reactors 365 [m^3/d], times the organic load 51,1 [kgVS/m^3]; average VS in to the reactor in the sampled period;

1. Produced biogas and converted to COD by [1.66kgCOD/m^3Biogas]

$$\mathrm{COD/VS} = \frac{\frac{(13076\ \mathrm{Nm3/d\ X\ 1.664})\mathrm{kgCOD}}{\mathrm{Nm3Biogas}}}{\frac{365\mathrm{m3}}{\mathrm{d}}\ \mathrm{X}\ 51.1\ \frac{\mathrm{kgVS}}{\mathrm{m3}}} = 1.17$$

2. The COD/VS ratio by the measured average values, $_{Bio}$COD=84.7 [kgCOD/m^3], and the Volatile solids VS = 51.1[kgVS/m^3]

$$\mathrm{COD/VS} = \frac{84.7\left[\frac{\mathrm{kgCOD}}{\mathrm{m3}}\right]}{51.1\left[\frac{\mathrm{kgVS}}{\mathrm{m3}}\right]} = 1.65$$

The deviation in COD/VS ratios between the calculations is truly deviation in the methods and measured $_B$COD, the high content in COD cause to dilute the sludge sample in the range of 100-200 times. The diluted sludge is then homogenized and shaked for optimal measurement. The particles in the solution are sinking and the fats are floating to the top. This situation makes it difficult to make homogeneous measurement with reliable values. For VS measurement it is discussed above under chapter 5.2 but in short context, the sludge is changing in composition during measurement, volatile components is evaporating and the process is not stopped until the microorganism are in very small population or cooled down to 2-3 ^{o}C.

5.5 Dynamic test

The dynamic behaviour of the AD process was conducted in 26 August 2011. The experience from situation with a preliminary test gave valuable information about the developments in reactors. The situations with two equal reactors are perfect to conduct full scale testing and experiments. One reactor is dedicated to be the test reactor and the other is reference reactor. For this injection test, analysis and dynamic responds or behaviour of reactor with interactions of the intermediates was of high interest.

The injected batch of 39 tons with FWS was pumped direct into the AD reactor (RT2). The other AD reactor (RT1) was reference reactor with normal loading rate at 15m^3/h. The injection pumping rate was calculated to 26m^3/h, which is higher rate than normal for ordinary conditions. The raw sludges (RS) and injection batch where characterized and analysed for TS, FTS and VTS measurements. COD measurements on biodegradable, $_{Bio}$COD and $_{RB}$COD from the supernatant, with ALK, pH and VFA`s measurements with calculations. The measurement of VFA`s and ALK was conducted with 5-point titration and IC analysis. With IC the cations also was measured.

5.5.1 COD dynamics

The reactor increased biogas production momentarily from normal production at 360 Nm^3 with rapid rise to maximum at 648 Nm^3 in 1 hour and 16 minutes. The rapid start was due to the content of $_{RB}$COD fraction from $_{Bio}$COD; 383 [gCOD/l] which is easily converted into biogas. The measured and calculate value is 48% of $_{Bio}$COD; 383 [gCOD/l] = 183 $_{BRB}$COD [gCOD/l] consumed. In table 5.1 COD values is presented with increasing values for $_{Bio}$COD throughout the period. For $_{RB}$COD the development is at maximum "MBiog" gas production. The slowly biodegradable particulate $_{SB}$COD is still in a high level, compared with initial conditions and the rate of gas production.

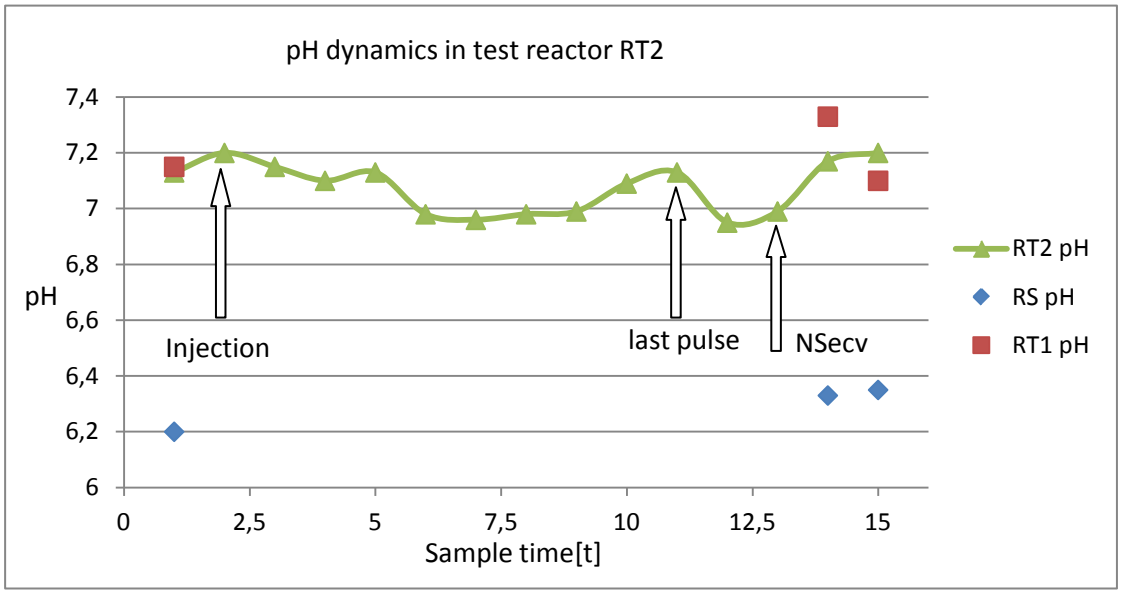
Table 5.3 COD developments in RT2 for the test period. The "Batch" was injected 12:07.

Sample [nr] Time [t]	1 11:00	2 12:07 Batch	3 12:17	4 12:26	5 12.32	6 12:41	7 12:45	8 12:49
$_{Bio}$COD[g/l]	32.6	30.7	34.9	33.9	35.5			37.05
$_{RB}$COD[g/l]	2.3	2.01	2.01	2.74	3.31	3.9	4.3	4.2
Sample [nr] Time [t]	9 12:56	10 13:03	11 13:10	12 13:23 MBiog	13 13:33 NSecv	14 14:54	15 12:30 +24h	
$_{Bio}$COD[g/l]		34.2			37.8	37.4	38.1	
$_{RB}$COD[g/l]	4.1	3.25	4.9	5.3	4.6	3.1	1.9	

Note; This sample number [nr] is corresponding to all sample time [t] in the dynamic test and the x-axis of all the presenting graphs.

5.5.2 Alkalinity, pH and volatile fatty acids dynamics

Strait before injecting the FWS the pH was measured for both reactors to 7.15. In graph 5.14 the pH dynamics are starting at injection point. The pH slowly decreases until stabilization at 6.96. Then the pH start to rise again into pH 7.13 with a new decrease in value down to 6,95 and ends up with normal operation pH at 7.20.

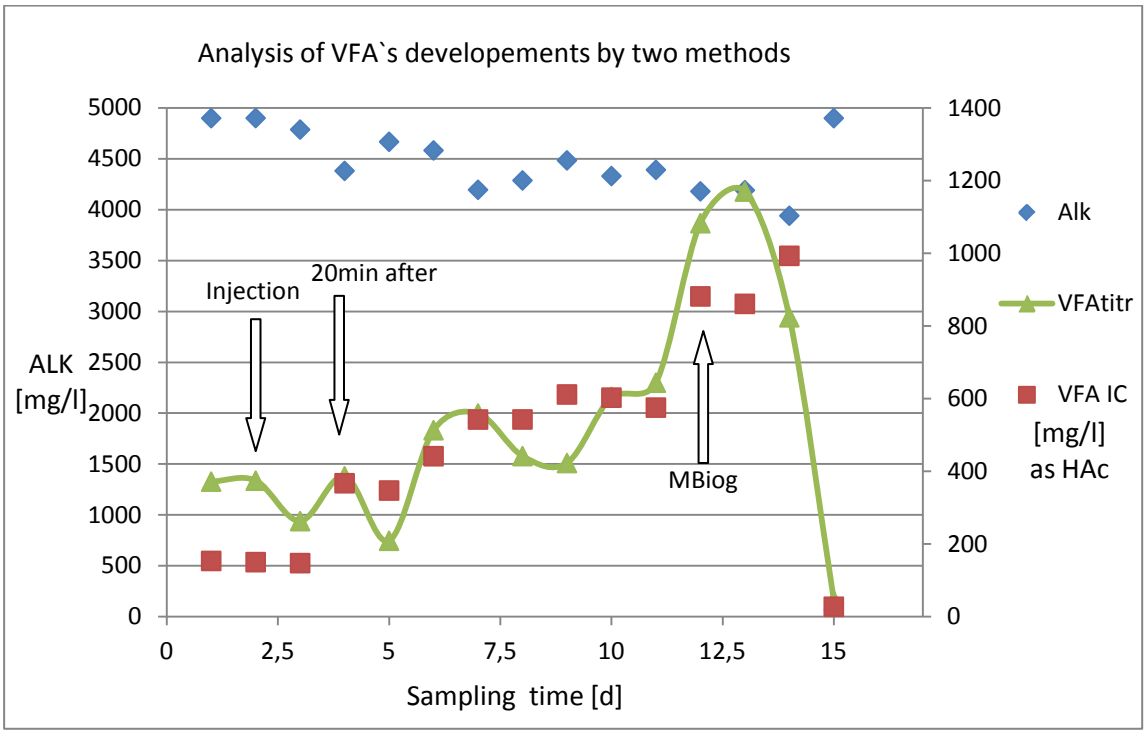


Graph 5.14 pH dynamics over test period with reference pH in RT1 and RS.

The pH for RS is from 6.2-6.35 which is a normal level compare with steady state period. The average pH was calculated to 6.2 for the steady state. In the reference reactor RT1, the pH was measured with higher value compared with RT2. At the sample time [14] the difference pH was calculated to 0.16 units. The injected reactor is responding to the waste pulse. The pH dynamics is in the level of 0.2 units in difference from minimum to maximum pH values. In steady state period the pH difference in most dynamic reactor where 0.84 pH units, with level from 6.66-7.5 for comparison. For this pulse test the pH was within the standard deviation of this parameter for normal operation. After injection the pH starts to decrease slowly until pH stabilization. At this level, the time for the first pulse injecting was done. The average injection rate was 26m^3/h and 67% of the batch was injected at sample time [8], the ph was stabilized and starts to increase due to organic loadings thereby with VFA`s conversions and consumption. The last 33% of the FWS was injected by the "last pulse" at sample time [11], with a new drop in pH. This last pulse was due to practical pumping capacity and viscosity of FWS. After this last injection of rest sludge the AD process was put to normal conditions for sequential circulation and inpumping cycle (NSecv).

From the graph 5.15 the alkalinity slowly decrease with increasing VFA`s. The alkalinity is at high concentration 4900 [mg/l] as $CaCO_3$ due to the breakdown of the constituents protein and amino acids to form ALK as $NH_4(HCO_3)$. The principally consumer of ALK is the gas

content of CO_2 in the reactor. The CO_2 concentration is increasing during the test, from normal operation 33% CO_2 and 67% methane to 40% CO_2. The same observation has being seen under ordinary conditions (Oslid., 2011) and under preliminary test. In general, study of reactor behaviour in test 2010, showed level down to 39% (Popov,J.,2010)



Graph 5.15 Increasing concentration of VFA`s and decreasing ALK in RT2.

The level of ALK 4900 [mg/l] as $CaCO_3$ give the reactor status as high buffered system, normal level in stabile established reactors is from 2000-5000 mg/l. The reactor were stressed with high load and the alkalinity decreased to 3900 [mg/l] as $CaCO_3$, that is ALK consumption of approximate 1000[mg/l] as $CaCO_3$. This is a significant number but for the reactor and the microbial system the process is well established with a high alkalinity. The VFA`s increased during the first sampling period in a high rate. The test batch were containing high amount of VFA`s due to the pretreatment process for the FWS with high temperature at 100°C under 2 bar pressure. Before this sterilisation of the waste, hydrolysis and fermentation is taking place and lowering the pH to 4.47. When injecting this batch, soluble $_{Rb}$COD is giving the degraders acidogenesis god conditions. Normally content in buffer tank with FWS was in the level from 2-7000mg/l as HAc in “Steady state” period. At injection test the level in RS was 3600 mg/l as HAc with 5-point titration method and 2700 mg/l as HAc at IC- analysis.

Measurement of low concentrations in RT2 before injection is due the well established methanogens in reactors. The VFA`s concentration begin to increase after 20 minutes and continue increase to maximum(5-point.Titr.) at sample time [13] 13:33, approximate 1hour and 26 minutes after injection. Then the process was put back to normal sequence (NSecv)

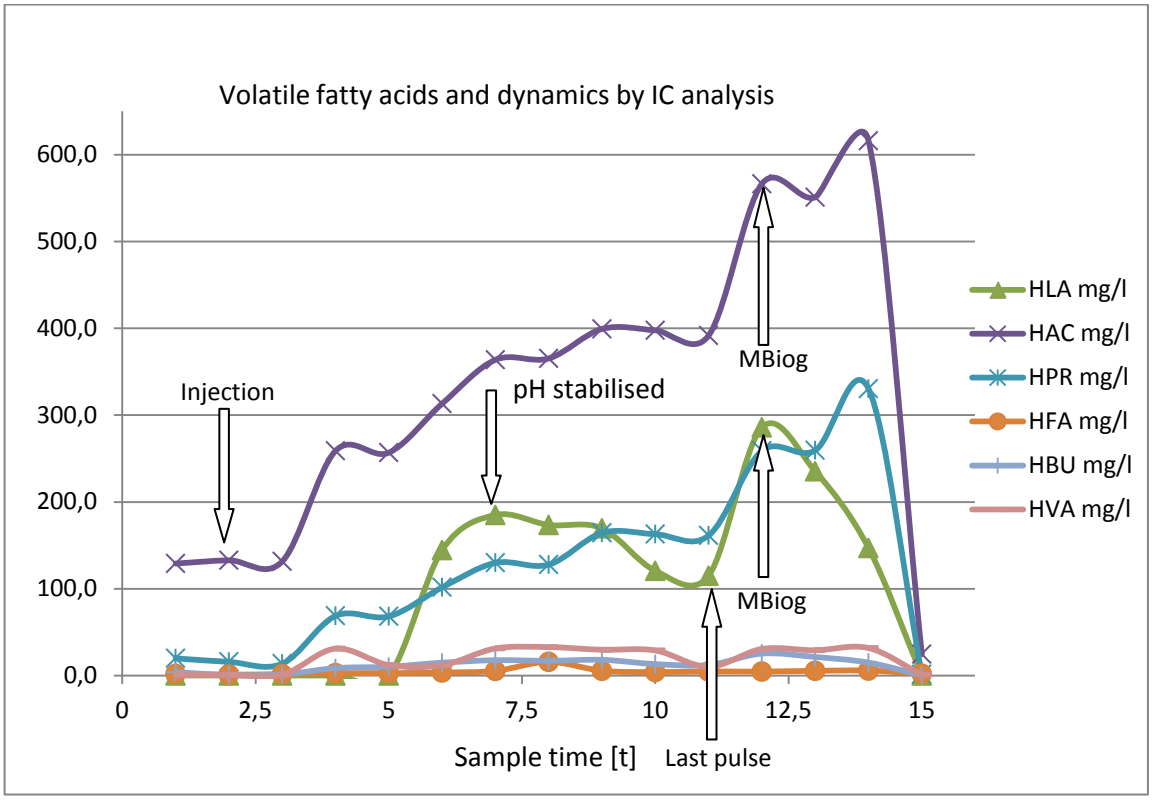
and the start level was 374 to max; 1170 mg/l as HAc(5-pointTitra) and for IC- analysis; 150-880 mg/l as HAc. Maximum for IC in graph 5.15 was at sample time [14] 14:53 with the concentration 993 mg/l HAc. There was some deviation between the two methods of VFA`s analysis. The start and max points, concentrations has more fluxing values at 5-point`s method. In general the values is reflecting the same developments of VFA`s and the values is in the same area, except from measurement at sample time [11],[12] for 5-point`s method. The values are 200-300 mg/l higher than IC-measurement and IC is more thigh in dynamics. So, the IC measurement seems to be more reliable method for exact evaluation of the numbers to draw a conclusion.

At sample time [12] (MBiog) maximum biogas production is received. At this stage the conversion of intermediates into methane and carbon dioxide is produced by the population methanogenesis. The methanogenesis is bacterial conversion of acetic acids and hydrogen.

The monitored parameters returned to more normal but high level after 10 hours for the injected reactor. In the graph 4.14 the plateau for RT2 is several degrees higher in production than RT1 for over 20 hours. Biogas production was then slowly decreasing back to equal levels approximate 24-26 hours for RT2. That means it took 24 hours for the effect of injected pulse to totally absorb by the system.

5.5.3 IC analysis of intermediates

The change in ALK, pH and VFA`s concentration was significant and the values is reliable compared with steady state and monitored parameters at ordinary conditions. At the steady state the pH was under the set limit 6.8. But the high alkalinity is preventing reactor failure due to acidification. ALK level at actual situation was 6000-7000mg/l as $CaCO_3$. At dynamic state ALK is under 5000 mg/l as $CaCO_3$ and still defined high in ALK. Measurement by IC of VFA`s in Graph 5.16 is presenting the concentrations of the intermediates. Dynamics is starting 10 minutes after injection with increase of acetic acid.



Graph 5.16 Volatile acids measured by IC for the different intermediate concentrations.

The biogas production is reacting spontaneous on the pulse load, the rate of injecting is due to the pump capacity and was estimated to 26m^3/h for the test. When approximate 4-6 m^3 is loaded at time [3] 12:17 the pH is reacting and ALK is already decreasing. HAc is increasing with a high rate, HPr and Hva is also increasing in concentrations. Propionic acid is increasing steady until [14] 14:53 at 330mg/l HPr and the dynamic very similar to HAc but in two times lower concentration. At time [5] 12:32 Lactic acid is responding 25 minutes after start injecting. Approximate 10-15 m^3 have being injected and HLa is responding in high rate until sample time [7] 12:45, 38 minutes after injection. At this point the HLa is getting in to a plateau for a while and start to decrease until the “last pulse”. This dynamics is direct reflecting dynamics at pH in graph 5.14, lactate is a key-parameter and most of the monosaccharides may degrade via lactate (Batstone et al., 2002). The pK_a for HLa is relatively low 3.08, which give a strong effect on pH values as seen in graph 5.14. The “last pulse” is one more time given substrate for the acidogens and convert monosaccharides and

amino acids into intermediates HAc, HPr and HLa. This three main acids is clearly increasing in concentration with some dynamics until sample time [14] 14:53 with strong indication on pH decrease.

Acidogenesis is the acid phase process with a rapid growth of acidogenic microorganisms and the rate is μ_{max}= 2-7[d^{-1}]. The product from this phase is mainly VFA`s as acetic, propionic, butyric acids. Some other acids is produced under special conditions like lactic and formic acids and some alcohols such ethanol, propanol and butanol. The fraction of energy associated with the excreted fermentation products cause the remaining energy for growth to be limited. And therefore the growth Yield is low; Y = 0.1-0.2[gVSS/gCOD]

The degradation of HLA is very quickly and is therefore seen primarily during transient overload conditions in acidification reactors. Under high concentrated load the lactate increases from being insignificant to the highest organic acid in terms of COD (Batstone et al., 2002). Similar approach in modelling of AD-reactor where conducted with glucose first fermentation into lactate which was further converted into acetic and propionic acids. The consumption and degradation of lactic acid is very rapid compared to other reactions, resulting in low lactic concentrations except for very high loads conditions or batch operations (Skiadas et al,.2000). Summarising literature research for lactic acid seems to appear most frequently during batch operations during unstable growth and fluxing conditions, and will effect pH due to the low pK_a of 3.88 (Ydstebø,L., 2005).

During this fluxing conditions when injecting “last pulse” in graph 5.16 the lactic acid indicated max biogas production “Mbiog” at sample time [12] 13:23 , 1hour 16minutes after the batch was injected. HLa concentration is peaking rapidly up to 286 mg/l and decreases in high rate. This behaviour and observation of HLa is very similar to other studies with high loaded systems there lactic acid is generated and consumed rapidly and therefore rarely detected in acidogenic processes.

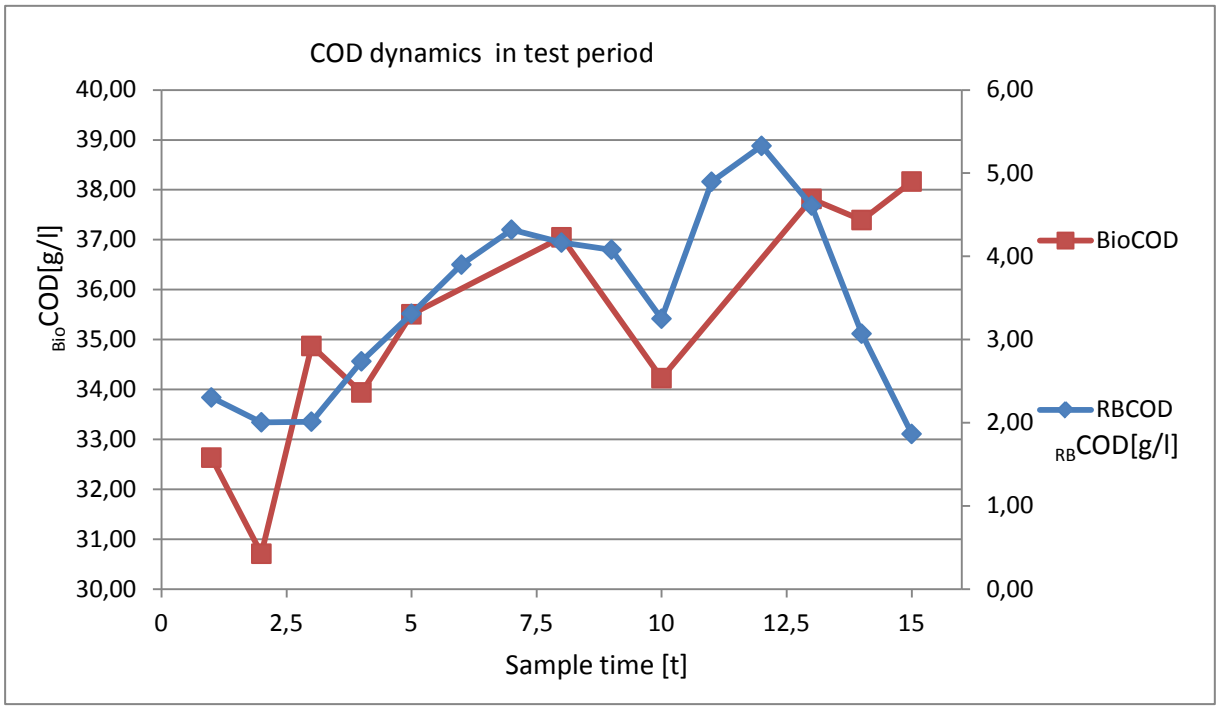
Generation of lactic acid is the solution to the problem of finding an acceptor for the H-atoms removed in the pathway to pyruvate. Lactic acids are the most common product in fermentation of sugar. When pyruvic acid is reduced to lactic acid $NADH_2$ is oxidised to NAD^+ with lactic acid as electron acceptor by Embden-Mayerhof pathway (glycolysis). This glycolysis pathway is a biochemical process in which glucose is fermented yielding ATP and by the lactic acid bacteria has the stoichiometry and free-energy yields;

1. Glucose → 2 $lactate^-$ + $2H^+$, Free-energy yields for lactate: -196kJ.

Propionic acid generation in test is fluxing but in a lower degree than HLa and are following the dynamics for acetic acid development in graph 5.16. Elevated levels of propionic are often associated with surge and high organic loadings. For propionic acids the preferred substrate for HPr-forming bacteria is lactic acid, so these organisms can grow on the major end product from lactic acids fermentation. The main pathways for propionic acid formation

from lactic acids are; the acrylate pathway and the succinate-propinate phatway. (Ydstebø,L., 2005).

The major acid, acetic acid, generated during the test, is increasing from 133 mg/l at time [3] 12:17 when the pH is starting to decrease. The HAc continues to increase with a high rate until 566 mg/l at maximum biogas production, sample time [12] 13:23. At this point both acetate and lactate is increasing further in concentration until sample time [14] 14:53. From this point, unfortunately the measurement of AD-sludge was decreased in sampling intensity. The form and shape of the propionic and acectic acids developments compare with the biogas production plateau in graph 4.14 is accounted for continuously VFA`s dynamics due to the level of conversion into methane. This interaction and conversion is of course decreasing due to the lowered gasproduction. After 20 hours the major drop in intermediates interactions occurs with decrease in biogas production. It took approximately 24 hours from injection the pulse load before the effect was fully absorbed by the system. The dynamics and COD development are presented in the graph 5.17 . The $_{Bio}$COD feedings are increasing due to the rate of injecting the batch. Easily biodegradable $_{RB}$COD are in low concentrations due to the fast conversion by the degraders.



Graph 5.17 $_{Bio}$COD feedings and $_{RB}$COD developments in AD reactor.

5.5.4 Kinetics and process indicators

Conversion of injected batch and the intermediate interactions have different conversion rates due to the pathway for degradation and the substrate composition. The acetogenesis is degrading the fermentation products (from acidogenesis) to acetic acid for being converted further by the methanogenesis. Acetogenic organisms have a growth rate slightly higher than the methanogens, with μ_{max}= 0.5-0.8 [d^{-1}] , but lower than the acidogenesis

which is in the rate of μ_{max}= 2-7[d^{-1}]. The acetogenesis require continuously removal of H_2 in order to grow otherwise the organisms may be inhibited. In the fermentation process there must be redox balance and molecular H_2 is the major electron donor. (Madigan et al., 2009).

The degradation of higher organic acids by oxidation step is conducted without internal electron acceptor by the acetoclastic methanogens. The microorganisms are oxidizing the acetic acid and are required to utilize an additional electron acceptor such as hydrogen ions (H^+) and CO_2 to produce hydrogen gas H_2 and formate. Hydrogen and formate is consumed by the methanogens and the electron-carriers must be kept in a low concentration (Batstone et al.,2002). Syntrophic acetogenesis and hydrogen utilizing methanogenesis is reducing CO_2 in reactions and are only possible in a narrow range of H_2 and formate concentration. The growt rate is low μ_{max}= 0.3-0.5 [d^{-1}] with long retention time for the methanogens is required. Growth yield is also very low as the majority of the energy in the substrate is converted into biogas with a growth yield of Y =0,05-0.1 [gVSS/gCOD] (Ydstebø,L., 2005).

H_2 concentration in the test was not measured but still of major interest, due to the narrow concentration for optimal process. In general the pressure maintained in reactors is stimulating the methanogens for optimal conversion. At high H_2 concentration the acetogenic reaction are not favourable thermodynamically unless the concentration of the H_2 or partial pressure are very low $pH_2 < 1.10^{-4}$. In AD reactors the hydrogen utilizing methanogens maintain a low level of H_2 , allowing acetogenesis, while directing fermentation towards propionic or lactic acid. H_2 generated in fermentation is normally not enough for sufficient inhibition of acetogenesis and affect the conversion products. H_2 production in acetogenesis may inhibit acetogenesis by “product inhibition” and depends on subsequent removal of H_2 for the reaction to proceed. In general for the test there was conversion of propionate and lactic acid.

H_2 gas is related to many complicated interaction between conversion processes of complex organic constituents in solution and liquid-gas phase in the reactor. The utilization of hydrogen produced by the acidogens and other anaerobe microorganisms is the interspecies hydrogen transfer by the methanogens. This regulation, or in fact the methanogens organisms serve as a hydrogen sink that allow the fermentation to proceed. If high loading occurs the methanogens do not utilize the hydrogen fast enough, propionate and butyrate fermentation will be lowered with accumulation of VFA`s in the reactor and with decreasing pH as result. Generation of lactic acid seems to occur in high loaded systems with reduction of pH as one of the result. There is no generation of H_2 by lactic acid, however conversion of lactic acid to acetic acid generates hydrogen. Generation of propionic consumes hydrogen and lactic acid seems to be the preferred substrate for propionic acid generating organisms and could be the source of propionic acid generated in these systems. If the pathway succinate-propionate approach is used, H_2 is consumed in generation of propionate from lactic acid. (Ydstebø,L., 2005).

For this injection test the accumulation of intermediates are significant and similar behaviour is observed and discussed in several studies. The transfer of hydrogen plays an

important role in the overall regulation of the anaerobic AD-process and especially in the oxidation of VFA`s. Hydrogen production and accumulation can give important and additional information about the microbiological limitations and degradation pattern that cannot be concluded from the VFA`s accumulation alone. Concentration of dissolved hydrogen more than 40 nM or partial pressure for H_2 low $pH_2 < 1.10^{-4}$ is crucial for regulating the COD flow during mineralization. Higher concentration will direct the electron flow from the methanogens to the production of electron sinks such as butyrate, propionate, lactate or ethanol. The hydrogen dependent obligate syntrophic acetogens organisms degrading propionate and butyrate will be inhibited at elevated hydrogen concentrations. On this background, hydrogen accumulation is a good indicator of imbalance between some of the most sensitive microbiological groups in the AD-process. Monitoring of the H_2 concentration online will give valuable information of VFA`s dynamics and inhibitions processes of the organisms and increase the basic understanding of the metabolism. Due to the diversity and complicated dynamics of hydrogen in conversion processes makes hydrogen inadequate as a single control parameter. Use of hydrogen parameter should always be conducted together with other process parameters, cause reactor dynamics and variability on substrate flux into the system (Bjørnsson et al., (2001).

Degradation of lipids, the main group of constitutes in sludge from different wastes is lipids. Lipids are important constituents as they are large structural component of the cell membranes. Industrial food wastes with high content of fats or lipids are mostly in the form of triacylglycerols. The degradation by the AD-process is first hydrolysis to glycerol and long fatty acids (LCFA) by extracellular enzymes called lipases. The steps are regarded as very rapid processes and LCFA is degraded to HAc and hydrogen via activation and β-oxidation. LCFA can be inhibitory at low concentrations and detoxification of the LCFA β-oxidizing organisms are by activation by acryl-CoA to LCFA-CoA. The inhibition mechanism is an adsorption on the cell surface and factors like cell surface area to LCFA concentration ratio, pH may have influence. Heavy inhabitation is irreversible and recovery by decreasing influent LCFA concentrations. The aceticlastic methanogens is probably the most inhibited organisms, but all organisms are inhibited into varying degrees (Batstone et al., 2002)

LCFA can complicate the process by inhibition but adaptions may also occur and a well developed process will readily degrade high content lipids feed. In adopted cultures the efficient LCFA degradation will be able to remove LCFA as fast as they are released from the hydrolysis of lipids. Therefore, the lipids content in feeds are of high interest due to LCFA inhibition. In the injected batch the composition of total solids (TS) in FWS was analysed and characterising into polysaccharide, proteins, lipids and inerts. The average content by characterising constituents gave this values; polysaccharides 34.4[%], proteins 27.1[%] and lipids 31.3[%], inert is measured to be 7.2[%].

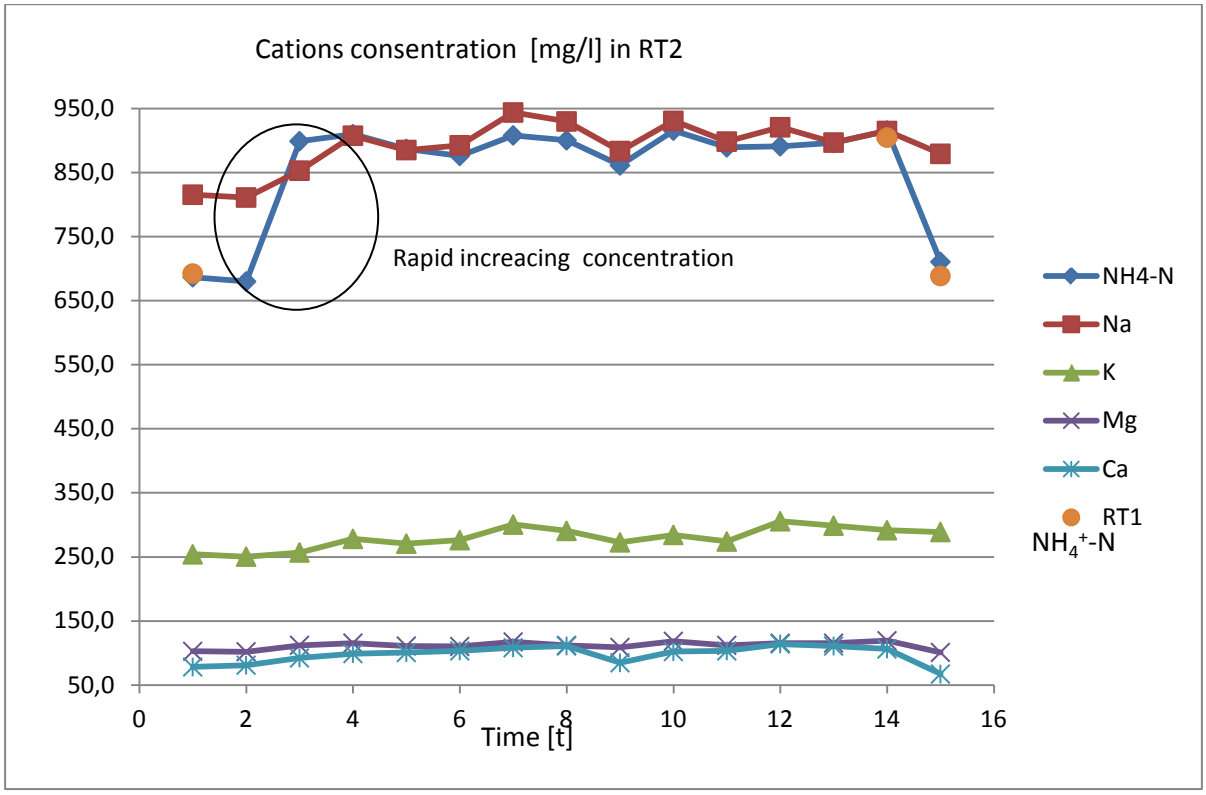
The AD process seems to be very well adopted and degradation of lipids are processing well, but fats content in sludge for industrial food waste can be very high in content and precautions has to be taken.

It is well accepted that also the VFA`s concentrations can serve as efficient process parameters for detecting imbalances. However in which way or what controlling strategies for the different VFA`s should be include is various. In studies under overload conditions with high VFA dynamics, ammonia and long chain fatty acid`s forced inhabitation was conducted. The studies shows all VFA`s, except for valerate, increased rapidly and propionate was the slowest acid due to recovery back to reference level. Propionate gave the best indication of when the biogas process had reetablished. This kinetic of propionate was in good agreement with the study showing the propionate degraders are the slowest growing and most sensitive VFA`s degrading organisms in AD process (Nielsen, et al., 2006).

For the injected batch the purpose was to explore and examination of the behaviour of the AD reactor. There was god response to the pH, ALK, VFA`s and biogas production. The gas production seems to proceed well under and after the injected batch. The biogas production was high and the VFA`s interactions were significant for acetic, propionic and lactic acids. Valerate was present with the highest concentration for the VFA`s in trace concentrations level.

5.5.5 Mineralisation and toxic compounds

The mineralisation from organic compound to Na^+, Ca^{2+}, Mg^{2+}, K^+, NH_4^+ by the AD-process are of high interest. The ammonium, calcium and magnesium are of highest interest due to contribution to alkalinity. As discussed previous, breakdown of protein and amino acids in the raw sludge is feeding ammonium bicarbonate into the digesters and give the AD-sludge buffercapacity. The main consumer of ALK is CO_2 from methanogens organisms degrading acetate.



Graph 5.18 Cations analysis by IC. NH_4^+ and Na^+ at higher concentration in the test.

The levels in steady state period are lower especially for NH_4^+ and Na^+. The concentrations during the injection test are mainly the same for the cations Ca^{2+}, Mg^{2+} and K^+. For the minerals and ions NH_4^+ and Na^+, the levels are higher in concentration during the test. For Na^+ the level approximate 50-100 mg/l higher in concentration compare with the "steady state" period. The level before injection is equal to the steady state concentration for sodium and after injection it is rising approximate 100mg/l. For NH_4^+ it is more interesting due to the possibility of inhibition. The level for the injected batch is approximate 200 mg/l higher concentration compared with steady state. From time injection the batch the ammonium concentration increased relatively rapid and stabilized on this high level approximate 950 [mg/l NH_4^+] during the test.

Ammonia inhibition can occur in much higher concentrations, levels from 1500-300[mg/l NH_4^+] but the NH_3 "free ammonia" can be toxic in much lower concentrations. Ammonia is a

weak base and dissociates in water to form NH_4^+ an hydroxyl ions and the amount of free ammonia is a function of temperature and pH. At pH 7.5 and with digester sludge temperature 30-35 ^oC, 2-5% of the ammonium [NH_4^+] will be as free ammonia [NH_3] in solution. The toxicity threshold for ammonia has been reported to be 100mg/l as [NH_3-N]. The measured level is not in a very high and the free ammonia is calculated to be 47.5mg as [NH_3-N], this value is regarded to be evaluating with the background of the method and approach of injecting the batch is not the normal operation.

Anaerobic fermentation processes have been reported with higher threshold toxicity level than 100mg/l as [NH_3-N]. Levels from 3000 mg/l as [NH_4-N] above 7.4 and even long term acclimatized cultures can process in the range of 5-8000 mg/l as [NH_4-N] (Tchobanoglous et al., 2004)

Degradation of protein rich sludge like FWS is contributing with higher levels of NH_4-N into the sludge solution, approximate 30%. Settled sludge from the WWTP is contributing with the basic level of ammonium into the sludge system, with breakdown of protein and amino acid conversion.

In general parameter as gas production, composition, pH, VFA`s concentration and degrading, VS destruction. Most of these indicators are suitable for detecting gradual changes. However the pH, VS destruction and gas composition are often to slow for optimal detection of sudden changes. pH changes are small in highly buffered systems, as often seen in reactors with high ammonia loads and even in severely stressed systems(Ahring, et al., 1995). The same evaluation is discussed in (Bjørnson., et al., 2000) The VFA`s concentration were superior for indicating overload of the microbial populations. Alkalinity and pH proved to be good monitoring parameters. However the possibility of using the pH as a process indicator is strongly dependent on the buffering capacity.

5.6 Discussion of parameter estimation and ADM1 model

ADM1 modelling by the software AQUASIM was conducted for 150 days to perform a steady state. The sampled and monitored process parameters are fluctuating in values, which give high dynamics in results. Measured dynamics under ordinary conditions (first period in May 2011) is very high. Flow rates are fluctuating and the biogas production is responding to this behaviour. In graph 4.25 the gas production is due to these fluxing conditions reflecting an average level of biogas production which is reliable and within the same level as monitored values.

The pH and VFA`s interactions in the buffer tank and reactors is also reflecting the high dynamics for the model. Compared with the measured values there are high dynamics. In the buffer tank (R2) graph 4.27 the modelled pH level is in general slightly higher than the

monitored and measured. The estimation is in a level higher than expected and presented in the range of 6.4 for a average value. These levels could be due to the definition of the compartment in the model Aquasim. The definition of buffer tank compartment has same process definitions and conditions as the reactors. Temperature and intermediate interactions gives dynamics similar to the reactors.

The pH and VFA`s in reactors is more within the expected dynamics. The levels for pH are slightly higher than measured. Intermediates is reflecting the high dynamics and showing high activity for HAc and HPr and lower concentration for HBu and HVa. Butyrate and valerate interactions are by the model presenting low activities which is the general level in measurement.

Modelling high dynamics is complex. The result are showing high dynamics even with "input of some regulation" for the model (Kommedal., 2011). The results are deviating and analysis based on this model has to be further investigated for conclusions. In general some of presented data can be of interest. The model result for the compartment buffer tank is due to attention. The result here shows the increasing fermentation process only by change one parameter. The model estimated buffer tank temperatures equal to the reactors.

Modelling of high dynamics in steady state is complex. Monitored data for input values is important to evaluate before accessing the program. The numbers and values have to be evaluated by analysis of historical data from the plant. Longer period and more stable periods should be preferred for creating the steady state in modelling.

6 Conclusion

6.1 Ordinary conditions "Steady state"

The definition of "steady state" is more for an issue for the modelling part with ADM1 and Aquasim program. The ordinary conditions "Steady State" at the AD-reactor is showing high activity with dynamics due to the organic loading. The organic load from settled sludge, high content food waste (FWS) and on other biological WWTP sludges was measured for the buffer tank to be (RS) was monitored to 365[m^3/d] with the COD flux 85[kgCOD/m^3]. In this period the dynamics for the system was high and analysis of RS in buffer tank is stating this conditions. The highest VFA concentration was measured to be 2200[mg/l] Hla which is characterised as an intermediate in high loaded systems. The VFA`s concentration was measured from 2000-7000 [mg/l] as HAc. The ALK was low and in trace levels as respected, levels from zero to 168 [mg/l] as $CaCO_3$.

Solids analysis on raw sludge in buffer tank (RS) showed TS influent sludge at 7 [%] and VS 74.7 [%]. This condition together with VFA's concentrations and pH dynamics strongly indicates active depolymerisation and fermentation in the buffer tank. The status for this compartment is "buffer tank" and designed due to fluctuating hydraulic loadings. In this case, analysis of organic loads has giving this compartment another status due to the purpose of hydraulic levelling, organic loads and biogas production. The function and strategy for this compartment has to be reconsidered.

Mass balance over the system was calculated based on solids analysis over the AD reactors. Total solids was 3 [%] and VS; 48.4 [%] with total COD removed of 20440 [kgCOD/d]. The average monitored biogas production was 13076 [Nm^3/d] which is converted to $COD_{converted}$= 21758[kgCOD/d] in this period. The concentration of volatile solids into the AD was measured to 20075 [KgVS/d] with VS destruction at 63.9% ; 12817[KgVS/d] VS reduction. Converted into biogas by the factors; 1.65 [COD/VS], 0.35[m^3/kg COD] give 7402 CH_4 [m^3/d] in methane production, and with 67% CH_4 content the biogas is calculated to 11048[Nm^3/d].

Monitored average biogas production was 13076 [Nm^3/d], compared with 11048[Nm^3/d] from solids give the difference 2028 [Nm^3/d] and is 15% deviation. This failure is assumed to be the measurement of total COD which was in a high concentration with high rate of dilution. Biogas production rate for monitored; 1.02[$_{Biogas}Nm^3/kgVS_{Destruction}$] and measured calculated rate; 11048[Nm^3/d]/ 12817[KgVS/d]= 0.86[$_{Biogas}Nm^3/kgVS_{Destruction}$].

Organic loading rate (OLR) was fluctuating from 1.1-4.2 [kgVS/m^3d]. This is high variations and is reflecting the conditions of feed sludge (RS) from buffertank, with fluxing rate of organic loading and concentration. Considering this variations, the stability of reactor is relative god but dynamics is significant for the measured values of pH, volatile fatty acids (VFA`s) and alkalinity (ALK). The reactor is definitive in state of dynamics. The results are high variations in biogas production and conversion process into methane. These flexing conditions are creating some processing problems and should be leveled for more smother and stable processing.
The interactions of VFA`s, pH and alkalinity in reactor was measured for acetate to 40-50[mg/l] and for one peak of lactic acid at 83.5 [mg/l]. Lactic acid (HLa) is indicating and stating the dynamic conditions. The levels are indicating the amount of population of the degrader's methanogens due to the low concentration in the reactor compared with RS. Alkalinity is relatively high with fluctuations in concentrations from 4000-6000 [mg/l] as $CaCO_3$. These high levels are due to the protein and amino acid degradation from the contribution in biomass/settled sludge and FWS.

Toxicity and inhibition compounds was evaluated and found in low levels. Ammonium concentration measured to 690 [mg/l NH_4-N] and free ammonia calculated to 27.6[mg/l NH_3] in analysis.

The AD reactor is acting stable and robust considered the fluctuating organic load conditions OLR at 4.2. Biogas production is due to the OLR and operating under fluxing conditions, which is a challenge for further gas processing.

6.2 Dynamic State

The dynamic behaviour of AD process was conducted in RT2. RT1 was reference reactor. Developments in reactor were investigated by injection 39 tons FWS direct into the AD reactor RT2. The raw sludges (RS) and injected batch where characterized for TS, FTS and VTS determination and presented in table 6.1. COD measurements for biodegradable COD ($_{Bio}$COD) and $_{RB}$COD from the supernatant with ALK, pH and VFA`s was conducted. The behaviour of the digesters was observed trough the online biogas monitoring. The injected batch was codigested with standard sludge for 1hour and 25 minutes, before returning back to normal sequencing cycles.

Biogas production reacted momentarily with increased rate to maximum at 648 Nm^3 in 1 hour and 16 minutes. The rapid start assumed to be conversion of $_{RB}$COD fraction from $_{Bio}$COD; 383 [gCOD/l] which is easily processed into biogas. The measured and calculate value is 48% from $_{Bio}$COD 383 [gCOD/l]; which is 183[gCOD/l]. The high value is not surprising due to the collection and pretreatment of FWS. High enzymatic and disintegrations processes with acid and fermentation processes has already started in previous sludge-collecting and handling steps. The pH is low from 3.5 and average at pH 4.5. The preprocessed sludge is sterilized with high temperature, 100^oC under 2-3 bar pressure. This last step of pretreatment is even hydrolysis the FWS before AD processing.

Development on pH was decreasing from 7.2-6.95 with alkalinity dynamics from 4900-3900[mg/l] as $CaCO_3$, the CH_4 % was lowered from 67% to 60% during the injection test. VFA`s concentrations gave high responds to the injected batch, acetic acid (HAc), propionic (HPr) and lactate showed high dynamics. Accumulating of VFA`s was significant and the dynamics reliable.

HAc from 374 to max; 1170[mg/l] as HAc(5-pointTitra) and for IC- analysis; 150-880 [mg/l] as HAc. Propionic acid has dynamic very similar to HAc, in two times lower concentration with maximum 330mg/l HPr at sample time [14]. Lactic acid (HLa), the levels are increasing from injection time until peak for HLa at 286 mg/l. Lactic acid is an stress load indicator and the maximum point was [12] at maks biogas production. HAc and HPr was still accumulating and building up concentration. Maximum concentration for these acids has to be measured with an extended sampling for 4-6 hours after intensive sample period with a sample frequency one in hour.

For Butyrate (HBu), Valerat (HVa) and Formate (HFa) the levels of concentration is lower, from 0.5 mg/l at injection time until 33 mg/l for Valerat (HVa).

The reactor was acting stable due to the accumulation of VFA`s and decrease in pH. Decrease in pH result in increased amounts of non-dissociated fatty acid, which further intensifies the possibility of inhibition. The reactor was not inhibiting by accumulation of acids and the pH exceeds 6.95 at the lowest with alkalinity relatively stable and not under 3500[mg/l]as $CaCO_3$. On these analysis the reactors has high stability and considering the fluctuating loads, the AD process are dynamic with high intermediate interactions.

Propionate and higher VFA`s are important parameters to monitor in AD-processes, since they reflect the imbalance between the microbial groups involved in the degradation. Further degradation of these compounds can only proceed after the removal of hydrogen from the process. One line monitoring of dissolved hydrogen accumulation in the liquid is an appropriate method of process controlling. Use of hydrogen parameter should always be conducted together with other process parameters, cause reactor dynamics and variability on substrate flux into the system (Bjørnsson et al., (2001). One line pH measurement is relatively straight forward to monitor and strongly recommended. Combinations of VFA`s and biogas production, especially analysis of acetate, propionate and lactate are effective group of indicators (Kanokwan et al.,2010). The individual VFA`s online monitoring systems are under further developments for an industrial prototype and more research is required due to the robustness for full scale operations.

The ammonium, calcium and magnesium are of highest interest due to contribution to alkalinity. to Na^+, Ca^{2+}, Mg^{2+}, K^+, NH_4^+ by the AD-process are of high interest. Toxicity level of free ammonia is calculated based on measurement and evaluated. The level for the injected batch is approximate 200[mg/l] higher concentrations compared with steady state and ammonium concentration increased relatively rapid and stabilized on this high level approximate 950 [mg/l NH_4^+] during the test. Free ammonia is calculated to be 47.5[mg/l] as NH_3 and 30[mg/l] is normal conditions. The injected test batch released low free ammonia concentrations and concluded not toxic.

LCFA inhibition by the mechanism of adsorption to cell surface is well known inhibition process. The toxicity of lipids is depending on how fast the hydrolysis proceeds compared to further fermentation. In a well adapted reactor high concentrations of lipids can be degraded. The lipids fraction is containing high energy content and is almost completely degraded with a high gas yield. The fats are of interest in codigestion but precautions have to be taken. Further characterisation of the waste, with modelling the amount of polysaccharides, proteins and lipids has to be conducted with analysis.

The level of lipids in FWS was measured to 31.3[%] and reactor is responding to injection with high gas yields and high intermediate interactions. Fermentation further is well adapted in the reactor and inhibition by LCFA is not occurring.

6.3 Modelling and parameter estimation

ADM1 modelling by the software AQUASIM was conducted for 150 days. Two month of monitored plant data was the basic for steady state analysis.

ADM1 was implemented and applied to the monitored data from the plant. The model simulation indicated that biogas measured at SNJ is in the average level and results gained by simulation also indicate fermentation and acidogenesis in buffer tank due to the enhanced process with higher temperature.

The biogas production obtained by the addition of food waste sludge (FWS) is attributed to the higher biodegradability of food waste. The high content of polysaccharides, protein and lipids in the waste is the main reason for the increased biogas production. Approximate 40% of gas production is due to the external high COD content sludge. Therefore continue investigation and modelling for enhanced processing are important due to optimizing codigestion.

For “steady state” sampling of low dynamic conditions, sampling time is an important issue. Organic loads into the system are during daytime. Bioprocesses are reacting due to these organic loadings with high dynamics. Early in the morning or late afternoon could be a better timing for more stable plant data.

The high dynamics in the model is due to the rapid conversion by the first steps and rates. The model was not able to stabilize the process and with characterising the contents of polysaccharides, proteins and lipids the model got even higher in dynamics.

The implemented data was by the model ADM1 and Aquasim estimated and analysed. The result is deviating and further investigations have to be conducted on the steady state model. Especial the first steps in model has to be evaluated due to kinetics.

6.4 Further work and investigations

Further work and improved process monitoring with process analysis combined by ADM1 modelling is required for further optimizing AD process capacity at SNJ. Issues for further developments of AD modelling and biogas production is;

- Degree of fermentation analysis upstream buffer tank.
- High load intermediates interactions, toxicity and inhibition.
- One line monitoring strategy and developments.
- Process data dumps into XL format for optimizing investigations.
- Process calibration and validation for overall AD process.
- Optimizing gas treatment downstream. Organic load and volume analysis.

The function of buffer tank and strategy for enhanced pretreatment has to be evaluated. Initial conversion processes, like defragmentation of organic particles, hydrolysis and fermentation of hydrolysis products should be investigated further. Processes in buffer tank are easy to enhance by increasing temperature and volume. Following investigations and treatment should be considered;

- Evaluation of dynamics in buffer tank, solids retention time (SRT) and total volume.
- Investigation and evaluation of dynamics in buffer tank due to increase disintegration and hydrolysis and fermentation in raw sludge.

- Installing heat exchanger for enhanced pretreatment of raw sludge and overall AD process.

7 Appendix A

Appendix A

Table A.1: Biochemical rate coefficient ($V_{i,j}$) and kinetic rate equations (ρ_j) for soluble components (i=1-12; j=1-19) (Batstone et al., 2002)

Component →	i	1	2	3	4	5	6	7	8	9	10	11	12	Rate (ρ, kg COD m^{-3} d^{-1})
j ↓ Process ↓		S_{su}	S_{aa}	S_{fa}	S_{va}	S_{bu}	S_{pro}	S_{ac}	S_{h2}	S_{ch4}	S_{IC}	S_{IN}	S_I	
1 Disintegration													$f_{d,xc}$	$k_{dis}X_c$
2 Hydrolysis Carbon		1												$k_{hyd,ch}X_{ch}$
3 Hydrolysis of Prot			1											$k_{hyd,pr}X_{pr}$
4 Hydrolysis of Lipid		$1-f_{fa,li}$		$f_{fa,li}$										$k_{hyd,li}X_{li}$
5 Uptake of Sugars	-1					$(1-Y_{su})\,f_{bu,su}$	$(1-Y_{su})\,f_{pro,su}$	$(1-Y_{su})\,f_{ac,su}$	$(1-Y_{su})\,f_{h2,su}$		$-\sum_{i=1-9,11-24} C_i\nu_{i,}$	$-(Y_{su})\,N_{bac}$		$k_{m,su}\frac{S_{su}}{K_S+S_{su}}X_{su}I_1$
6 Uptake of Amino Acids	-1			$(1-Y_{aa})\,f_{va,aa}$		$(1-Y_{aa})\,f_{bu,aa}$	$(1-Y_{aa})\,f_{pro,aa}$	$(1-Y_{aa})\,f_{ac,aa}$	$(1-Y_{aa})\,f_{h2,aa}$		$-\sum_{i=1-9,11-24} C_i\nu_{i,}$	$N_{aa}-(Y_{aa})N_{bac}$		$k_{m,aa}\frac{S_{aa}}{K_S+S_{aa}}X_{aa}I_1$
7 Uptake of LCFA		-1						$(1-Y_{fa})\,0.7$	$(1-Y_{fa})\,0.3$			$-(Y_{fa})\,N_{bac}$		$k_{m,fa}\frac{S_{fa}}{K_S+S_{fa}}X_{fa}I_2$
8 Uptake of Valerate				-1			$(1-Y_{va})\,0.54$	$(1-Y_{va})\,0.31$	$(1-Y_{va})\,0.15$			$-(Y_{va})\,N_{bac}$		$k_{m,va}\frac{S_{va}}{K_S+S_{va}}X_{c4}\frac{1}{1+S_{bu}/S_{va}}I_2$
9 Uptake of Butyrate					-1			$(1-Y_{bu})\,0.8$	$(1-Y_{bu})\,0.2$			$-(Y_{bu})\,N_{bac}$		$k_{m,bu}\frac{S_{bu}}{K_S+S_{bu}}X_{c4}\frac{1}{1+S_{va}/S_{bu}}I_2$
10 Uptake of Propionate						-1		$(1-Y_{pro})\,0.57$	$(1-Y_{pro})\,0.43$		$-\sum_{i=1-9,11-24} C_i\nu_{i,}$	$-(Y_{pro})\,N_{bac}$		$k_{m,pro}\frac{S_{pro}}{K_S+S_{pro}}X_{pro}I_2$
11 Uptake of Acetate								-1		$(1-Y_{ac})$	$-\sum_{i=1-9,11-24} C_i\nu_{i,}$	$-(Y_{ac})\,N_{bac}$		$k_{m,ac}\frac{S_{ac}}{K_S+S_{ac}}X_{ac}I_3$
12 Uptake of Hydrogen									-1	$(1-Y_{h2})$	$-\sum_{i=1-9,11-24} C_i\nu_{i,}$	$-(Y_{h2})\,N_{bac}$		$k_{m,h2}\frac{S_{h2}}{K_S+S_{h2}}X_{h2}I_1$
13 Decay of X_{su}														$k_{dec,Xsu}X_{su}$
14 Decay of X_{aa}														$k_{dec,Xaa}X_{aa}$
15 Decay of X_{fa}														$k_{dec,Xfa}X_{fa}$
16 Decay of X_{c4}														$k_{dec,Xc4}X_{c4}$
17 Decay of X_{pro}														$k_{dec,Xpro}X_{pro}$
18 Decay of X_{ac}														$k_{dec,Xac}X_{ac}$
19 Decay of X_{h2}														$k_{dec,Xh2}X_{h2}$
		Monosaccharides (kgCOD m^{-3})	Amino Acids (kgCOD m^{-3})	Long chain fatty acids (kgCOD m^{-3})	Total Valerate (kgCOD m^{-3})	Total Butyrate (kgCOD m^{-3})	Total Propionate (kgCOD m^{-3})	Total Acetate (kgCOD m^{-3})	Hydrogen gas (kgCOD m^{-3})	Methane gas (kgCOD m^{-3})	Inorganic Carbon (kmoleC m^{-3})	Inorganic Nitrogen (kmoleN m^{-3})	Soluble inerts (kgCOD m^{-3})	Inhibition factors: $I_1 = I_{pH,aa}I_{IN,lim}$ $I_2 = I_{pH,aa}I_{IN,lim}I_{h2,h2}$ $I_3 = I_{pH,aa}I_{IN,lim}I_{h2,ac}$

Table

Component → i		13	14	15	16	17	18	19	20	21	22	23	24	Rate
j	Process ↓	X_c	X_{ch}	X_{pr}	X_{li}	X_{su}	X_{aa}	X_{fa}	X_{c4}	X_{pro}	X_{ac}	X_{h2}	X_I	(ρ_j, kg COD.m^{-3}.d^{-1})
1	Disintegration	-1	$f_{ch,xc}$	$F_{pr,xc}$	$F_{li,xc}$								$f_{xI,xc}$	$k_{dis}X_c$
2	Hydrolysis Carbon		-1											$K_{hyd,ch}X_{ch}$
3	Hydrolysis of Prot			-1										$K_{hyd,pr}X_{pr}$
4	Hydrolysis of Lipid				-1									$K_{hyd,li}X_{li}$
5	Uptake of Sugars					Y_{su}								$k_{m,su}\frac{S_{su}}{K_S+S}X_{su}I_1$
6	Uptake of Amino Acids						Y_{aa}							$k_{m,aa}\frac{S_{aa}}{K_S+S_{aa}}X_{aa}I_1$
7	Uptake of LCFA							Y_{fa}						$k_{m,fa}\frac{S_{fa}}{K_S+S_{fa}}X_{fa}I_2$
8	Uptake of Valerate								Y_{c4}					$k_{m,c4}\frac{S_{va}}{K_S+S_{va}}X_{c4}\frac{1}{1+S_{bu}/S_{va}}I_2$
9	Uptake of Butyrate								Y_{c4}					$k_{m,c4}\frac{S_{bu}}{K_S+S_{bu}}X_{c4}\frac{1}{1+S_{va}/S_{bu}}I_2$
10	Uptake of Propionate									Y_{pro}				$k_{m,pr}\frac{S_{pro}}{K_S+S_{pro}}X_{pro}I_2$
11	Uptake of Acetate										Y_{ac}			$k_{m,ac}\frac{S_{ac}}{K_S+S_{ac}}X_{ac}I_3$
12	Uptake of Hydrogen											Y_{h2}		$k_{m,h2}\frac{S_{h2}}{K_S+S_{h2}}X_{h2}I_1$
13	Decay of X_{su}	1				-1								$k_{dec,Xsu}X_{su}$
14	Decay of X_{aa}	1					-1							$k_{dec,Xaa}X_{aa}$
15	Decay of X_{fa}	1						-1						$k_{dec,Xfa}X_{fa}$
16	Decay of X_{c4}	1							-1					$k_{dec,Xc4}X_{c4}$
17	Decay of X_{pro}	1								-1				$k_{dec,Xpro}X_{pro}$
18	Decay of X_{ac}	1									-1			$k_{dec,Xac}X_{ac}$
19	Decay of X_{h2}											-1		$k_{dec,Xh2}X_{h2}$
		Composites (kgCOD m^{-3})	Carbohydrates (kgCOD m^{-3})	Proteins (kgCOD m^{-3})	Lipids (kgCOD m^{-3})	Sugar degraders (kgCOD m^{-3})	Amino acids degraders (kgCOD m^{-3})	LCFA degraders (kgCOD m^{-3})	Valerate and Butyrate degraders (kgCOD m^{-3})	Propionate degraders (kgCOD m^{-3})	Acetate degraders (kgCOD m^{-3})	Hydrogen degraders (kgCOD m^{-3})	Particulate inerts (kgCOD m^{-3})	Inhibition factors: $I_1 = I_{pH}I_{IN,lim}$ $I_2 = I_{pH}I_{IN,lim}I_{h2}$ $I_3 = I_{pH}I_{IN,lim}I_{NH3,Xac}$

8 Referances

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