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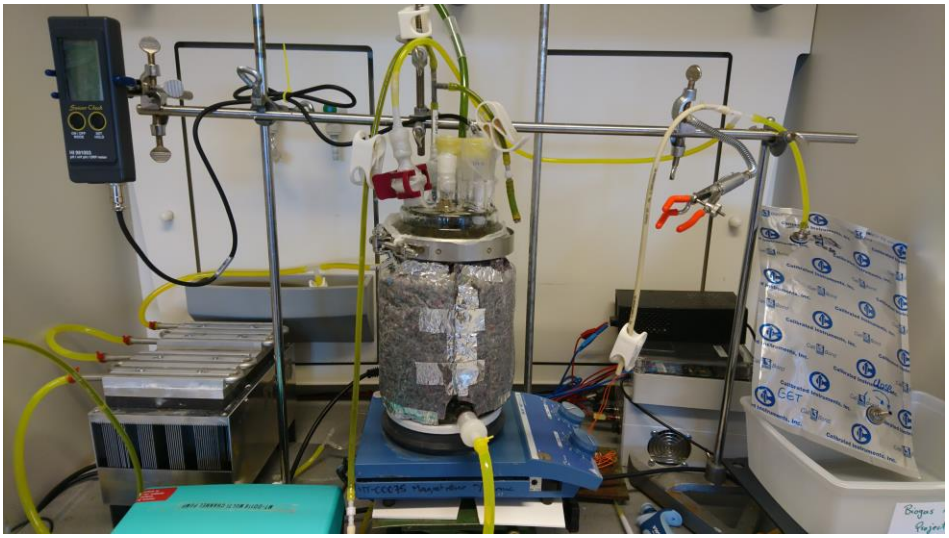
FMH606 Master Thesis 2018

Monitoring and process evaluation of continuous stirred biogas reactor under semi-continuous dosage of $Ca(NO_3)_2$



MT-14-18

Faculty of Technology, Natural sciences and Maritime Sciences
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Title: Monitoring and process evaluation of continuous stirred biogas reactor under semi-continuous dosage of $Ca(NO_3)_2$

Project code: MT-14-18

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Project partner: Yara SA International

Availability: Open

Summary:

Anaerobic digestion is a process typically used for the reduction of the organic load from sludge, with known advantages over other traditional processes being an energy producer in form of biogas. The economic viability of these plants is proportional to the amount of biogas produced by unit of substrate, whereby diverse initiatives have been implemented to increase the effectiveness of AD; among others the improvement of the pretreatment and the design of new reactors. It has been confirmed that the addition of small quantities nitrate ion increases the digestibility of the COD source. The purpose of this study is to replicate the operational conditions of a CSTR with temperature control in lab scale, to evaluate the yield of biogas production and efficiency with the addition of small quantities of $Ca(NO3)_2$, keeping track on parameters as COD, pH and ORP to observe the evolution of the microorganism behavior and the biogas production.

Preface

As the world is moving forward and human energy requirements are increasing, it is our responsibility to study and improve the potential of alternative energies, to bridge the gap that has grown between the comfort of everyday life and the sustainable use of natural resources. A change is to be made before it is too late to fix the consequences.

Thanks to Yara and USN who promoted the development of this project and provided the required equipment and material, my gratitude to professors Rune Bakke and Carlos Dinamarca for their guidance and advise for the successful completion of the thesis, to PHD candidate Michal Sposob and his helpful willing attitude and technical support, and to Mehrdad Torabzadegan for his technical inputs.

Porsgrunn, 10.05.2018.

Diego Carvajal.

Nomenclature

AD	Anaerobic Digestion
COD	Chemical Oxygen Demand
CODt	Total Chemical Oxygen Demand
CODs	Soluble Chemical Oxygen Demand
CO_2	Carbon dioxide
CH_4	Methane
H_2	Hydrogen gas
H^+	Hydrogen ion
HRT	Hydraulic retention Time
H_2S	Hydrogen sulfide
$I_{ph.bac}$	pH inhibition of acetogens and acidogens
LCFA	Long chain fatty acids
NO_3^-	Nitrate ion
N_2	Nitrogen gas
n	Number of samples /replicates
O_2	Oxygen gas
TS	Total solids
VFA	Volatile Fatty Acids
VS	Volatile solids
SRB	Sulphate reducing bacteria
ORP	Oxidation-Reduction Potential
OHPA	Obligate hydrogen producing bacteria

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1 Introduction

Modern society has been dealing for decades with different sorts of pollution affecting the air, soil, and water. Nowadays the harmful effects of these pollutants are becoming evident, from the greenhouse effect, to rivers and hydric sources where animals can no longer survive. Therefor there is a motivation not only to research new alternatives to fulfill the gaps between the human comfort and the handling of organic wastes, but to understand and improve the traditional technologies that are helping to reduce the human carbon footprint.

The greenhouse effect is accumulation of gases in the atmosphere that retains part of the solar energy, emitting radiation in the infrared range which gradually increases the temperature in the planet. The most well-known contributor to this phenomenon is the carbon dioxide, and where the irrational consumption of fossil fuels is the main generation source. On the other hand, the generation of greenhouse gases such as methane are also the product of inadequate disposal of organic wastes.

Landfills and livestock: the landfills now banned in Europe but still openly used in other countries, are spaces where the disposal and accumulation of organic and inorganic compounds offers a perfect environment for the growth of microorganism emitting methane and carbon dioxide to the atmosphere without any kind of control or further benefits. In a similar way the livestock manure on the land fields without any sort of treatment has the same fate.

Wastewaters: The residual water produced by industrial, domestic and agriculture sources have a high concentration of suspended and soluble organic compounds. To avoid the contamination of hydric sources it is required to reduce the organic load by chemical or microbiological ways, producing carbon dioxide and methane, that can be approached in an energy production plant or storage for further applications.

Aiming to reach an adequate treatment of organic waste, different technologies have been implemented, like aerobic treatment of biosolids, or chemical treatment of waste water. These technics don’t allow energy reuse, and some transfers the problem from solid waste to the atmosphere as solid waste incineration.

Bio-methanation has emerged as a sustainable microbiological process capable of handling solid and liquid organic waste in an integral way, as in a controlled environment allows the

growth of microorganism that can reduce the complex organic composite fractions, to produce biogas with high concentration of methane, that can be cleaned and stored in proper conditions afterwards.

As an initiative for reducing the dependence on fossil fuels and ensuring the energy security for economic growth, the European union has targeted for 2030 that at least 27% of the energy consumption must be produced by renewables ways (European Comission, 2017, p. 11).

It is required to diversify the renewable sources of energy, and to research feasible alternatives other than photovoltaic cells, and wind energy. Biofuels has been one of the main discussion topics of diverse commissions, showing benefits in the transformation of biomass to alcohols or biogas, but the alcohol fermentative process has a drawback since the raw material is a potential food source, and the competition of the acquirement might increase the prices, therefor hindering the possibility to use as food source, as an example the main source of bioethanol in USA is the usage of corn (Hill & Hanson, 2017), a potential food source for low income people.

Biomethane can be produced from organic wastes, showing a more sustainable and feasible path to increase the use in public transport, heating systems, and even has been proposed to link biomethane to the natural gas grid.

1.1 Bio-methanation an integral solution

Bio-methanation or most commonly known as anaerobic digestion is a process where microorganism in absence of oxygen use complex organic compounds available in wastewater, food-waste, and agriculture waste, as energy source to live and reproduce, this complex compound is then degraded and lead to the production of digestate and biogas.

The main advantages of this process compared to other waste solutions mentioned before are (Tchobanoglous, Burton, & Stensel, 2014, p. 1061):

-Less energy requirements, as it doesn’t require aeration.

-Higher organic volumetric loadings, 8 times higher than aerobic process.

-Lower biological sludge production by a factor 6-8 times, sludge processing cost is reduced greatly.

-Fewer nutrients required compared to aerobic degradation, since is less biomass produced.

-Methane as product, a potential energetic source.

-Elimination of off-gas air pollution.

1.2 Anaerobic digestion overview

An anaerobic digester is a mixture of diverse sort of microorganism with no requirement of oxygen in a reactor in direct contact with the feed source. The main bacterial population will be determined by the nature of the feedstock, and the balance of organic compounds would vary the proportion of carbohydrates, fat and protein degradation (Hobson, 1993, p. 9). This process occurs in a sequence of 5 steps.

1.2.1 Disintegration.

Disintegration is the initial process in which by the application of mechanical, thermal or biological processes the complex structures are disintegrated to achieve better digestion in the future steps, this can be explained as it can facilitate the release of intracellular substances by rupturing the cell wall and make them more accessible to subsequent microbial actions. The products of this step are carbohydrates, proteins, and lipids (fat, oil, grease) (Zhen, Lu, Kato, Zhao, & Li, 2017, p. 560).

1.2.2 Hydrolysis.

Hydrolysis is the first step in the degradation of large organic compound, and biopolymers by bacterial activity catalyzed by enzymatic reactions excreted by fermentative microorganism, in which the particulate solids are converted into soluble compounds as monosaccharides, amino acids and low chain fatty acids. Anaerobic digester contains between 10^8 – 10^9 hydrolytic bacteria per milliliter, comprising both facultative and obligate anaerobes (Mara & Horan, 2003, p. 394).

The hydrolysis reaction can be simplified as:

$$C_6H_{10}O_4 + 2H_2O \rightarrow C_6H_{12}O_6 + H_2 \qquad \text{(R1.2.1)}$$

1.2.3 Acidogenesis

Acidogenesis is more known as known as fermentation since the products get a carboxylic acid group. The monomers and products from the hydrolysis are degraded further to produce acetate, hydrogen, carbon dioxide, propionate butyrate and valerate. The organic substrate at this step serve as bot the electron donors and acceptors (Tchobanoglous et al.,2014, p. 1062).

There is a high diversity of microorganism in charge of this step, but some of them known as facultative can work in anaerobic and aerobic conditions. These species have an important role since they act as protection for the obligated anaerobic bacteria (methanogenesis) consuming oxygen that might be in solution in the biodigestate (Mara & Horan, 2003, p. 392).

1.2.4 Acetogenesis

Continuing with the reaction chain, the acetogenic bacteria oversee breaking down the intermediate products of acidogenesis and low carbon fatty acids into acetic acid.

There are two groups of aceto-bacteria:

- Obligate hydrogen producing bacteria (OHPA): produce acetic acid, H_2 and CO_2 using the mayor fatty acid intermediates, propionate, butyrate and valerate. They have an important role in breaking down low carbon fatty acids product of the hydrolysis of lipids.
- Homo-acetogens, are strictly anaerobic microorganism that use H_2 and carbon dioxide to produce acetic acid.

1.2.5 Methane-genesis:

Methanogenesis is carried out exclusively by anaerobic microorganism using acetic acid, hydrogen and carbon dioxide to produce methane and carbon dioxide. There are two groups of methanogenic bacteria, depending on the source of carbon used to produce methane:

Acetoclastic methanogens: acetic acid is the most important way to methane production, up to 70% of the total methane is produced by this type of bacteria.

$$CH_3COOH \rightarrow CH_4 + CO_2 \qquad \text{(R1.2.2)}$$

Hydrogen utilizing methanogens: the remaining 30% of methane production is due to the action of microorganism that reduce carbon dioxide, formate, and ethanol using the hydrogen produced in the hydrolysis and aceto-genesis steps (Mara & Horan, 2003, p. 394).

$$CO_2 + 4H_2 \rightarrow CH_4 + 2\,H_2O \quad \text{(R1.2.3)}$$

$$2CH_3CH_2OH + CO_2 \rightarrow CH_4 + 2CH_3COOH \quad \text{(R1.2.4)}$$

The current challenges among researchers has been to improve the methane yield, by easing the access for microorganism and enzymes to complex biopolymers, this include the upgrading of the disintegration processes with physical and thermal methods, and nowadays the addition of substances that in some way would catalyze the hydrolysis step is gaining more popularity.

The objective of this project is to replicate the conditions of a CSTR anaerobic digester using a mix of food waste, biosolids and fish waste in a lab scale to evaluate the yield of biogas per substrate unit and process efficiency, applying calcium nitrate in small concentrations as will be explained later in the experimental part. The application of small quantities of an oxidizer substance is intended to improve the hydrolysis process breaking down more complex substances that without further treatment would remain almost intact through the effluent of the reactor.

2 Related Literature

The organic waste treatment plants have a flow diagram like the shown in figure 2.1, although this study focuses on the improvement of AD, it is important to consider an overview of the inputs and outputs of the process since the design and optimization has a high dependence on the correct measurement and estimation.

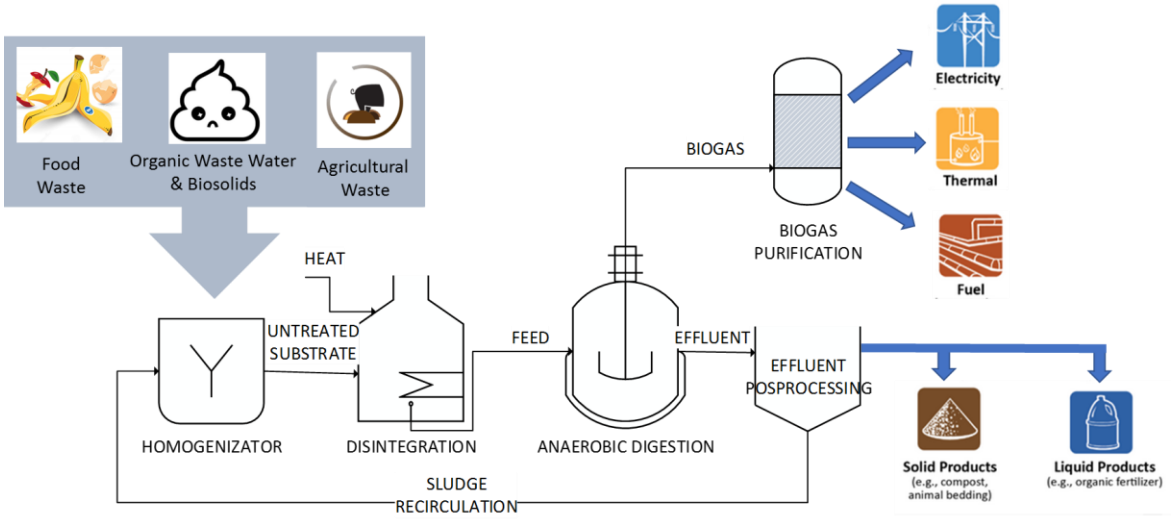


Figure 2.1: Flow diagram of common waste treatment plant.

2.1 To consider in AD

In chemical engineering when designing a plant that requires multiple reactions it is essential to find which will be the "bottleneck" in the process. The slowest reactions will determine the total rate of conversion and the main design parameters for the plant.

AD can be defined in simple words as a chain of reactions performed by microorganisms, in which the reagent is a mixture of biopolymers in organic wastes, the product is biogas, and bio-digestate with lower organic content. In this case the composition of the substrate is the most important for the calculation of the total reaction rate.

Previous studies related the hydrolysis rate to the substrate characteristics:

> When simple organic matter, such as sugars and starch is converted to methane, methanogenesis will be the rate-limiting step, as acidogenesis rates are higher than methanogenesis rates. However, during complex biomass digestion, due to the rigid

structure of plant materials (e.g., straw, wood, corn Stover), hydrolysis will be the rate-limiting step and directly affect (Azman, Khadem, Lier, Zeeman, & Plugge, 2015, p. 2524).

For anaerobic digestion systems, a high solid content is considered when the total solids measurement is above 4%, the digestion of these sort of feedstock dictates the type of equipment required to handle the feed. In the case of this project the feed is considered with high solid contents since the TS value is 7%. Therefor a continuous stirrer tank reactor can handle the process. Batch reactors are used in feedstock with total solids above 15% (Hobson, 1993, p. 175).

Based on the total solids, and volatile solids it is possible in some extend a forecast of the feed quality, for this is required to define the inert COD as organic and inorganic compounds that will remain stable under the influence of microorganism action, and the potential biodegradable COD as the organic portion of the solids that most likely would turn into biogas.

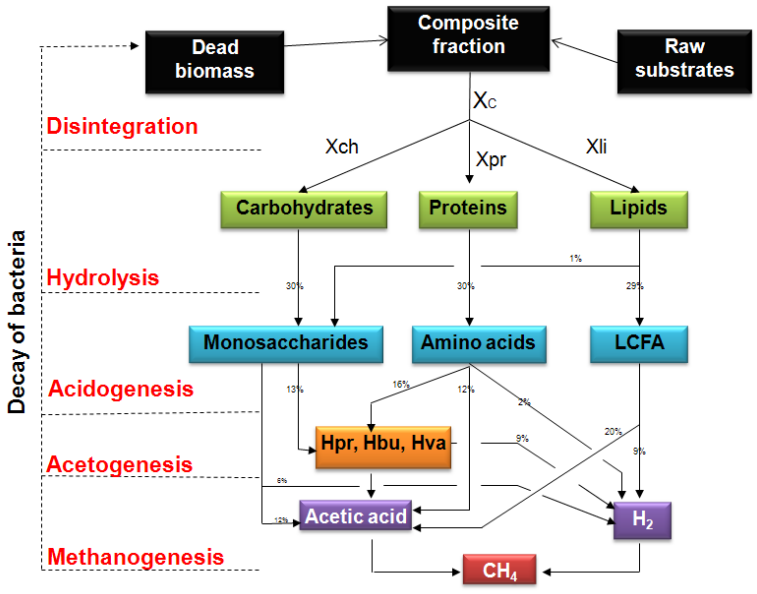


Figure 2.2: Anaerobic Digestion Model (Biernacki, 2014)

Figure 2.2 shows an overview of the reactions that are occurring simultaneously and in perfect equilibrium within the reactor. The composite fraction (X_c) on the top of the sketch, can be interpreted as the potential biodegradable COD in the feed after the disintegration process,

elaborating a simplistic model it can be assumed that the mixture of biopolymers is in fact organic chains in form of carbohydrates (X_ch), proteins (X_pr), and lipids (X_li).

The feed used in this project is a mix between food waste, industrial fish waste and sewage sludge. This substrate contains high concentration of insoluble organic matter and slowly biodegradable suspended solids originated from lipids and proteins (Zouari, 2015, p. 808). The feed provided is already pre-treated with mechanical and thermal processes for the disintegration. Therefore, the most likely path to increase the efficiency without changing the nature of the feed is increasing the hydrolysis rate of the substrate in the reactor.

2.2 Operation parameters

2.2.1 ORP and pH

ORP and pH are common parameters for diverse water treatment plants, from potabilization and demineralization, to waste water plants. The relevance of these parameters is that they are easy to measure and give a quick indication to interpret what is going on in the process.

For wastewaters treatment by microbiology action it is often required to adjust the pH with the addition of chemicals, the allowable range is often from 6.5-8.5. The pH of aqueous systems is typically measured with a pH meter (Tchobanoglous, et al.,2014, p.90).

AD model simplifies the pH dependence of the operation on the concentration of acetate, butyrate, propionate, valerate, and NH_4^+, HCO_3 ions (Batstone, et al., 2002, p. 71). A high concentration in the VFA implies a reduction of the pH obstructing several reactions rates.

The most sensitive microorganism towards pH inhibition are the methanogens, since the uptake of acetate is reduced when pH is lower than 7. expressed in equation 2.1:

$$I_{pH} = \exp\left(-3 * \left(\frac{pH - \mathrm{I_{ph\,ac_{ul}}}}{\mathrm{I_{ph\,ac_{ul}}} - \mathrm{I_{ph\,ac_{ll}}}}\right)^2\right) \quad (2.1)$$ (Batstone, et al., 2002, p. 71)

Where: I_ph_ac_ul = pH where there is no inhibition of methanogenesis (usually 7)

I_ph_ac_ll = pH where there is full inhibition of methanogenesis (usually 6)

Like the methanogenic bacteria, the fermentative microorganism has an optimal range of operation, somewhat less sensitive and functional in a wider range of pH between 4.0 and 8.5; at a lower pH the main products are acetic and butyric acid, while at a pH of 8.0 mainly acetic and propionic acid are produced (Appels, Baeyens, Degre, & Dewil, 2008, p. 759).

The ORP can be measured similarly as pH, by means of a probe in contact with the wastewater, the probe measures the amount of electrical charges of the ions that can be interpreted in millivolts (mV). Depending on the characteristic of the water the value can be positive or negative.

Several microbiological processes operate in a defined range of ORP values, positive values have a higher presence of oxidizing substances as dissolved oxygen, negative values are most probably anaerobic. A wide range of ORP values are shown in table 2.1.

Biochemical Reaction	ORP (mV)
Nitrification	+100 to +350
BOD degradation with free molecular oxygen	+50to +250
Biological phosphorus removal	+25 to +250
Denitrification	+50 to -50
Sulfide formation	-50 to -250
Biological phosphorus release	-100 to -250
Acid formation(fermentation)	-100 to -225
Methane production	-175 to -400

Table 2.1: ORP values according biochemical process (Gerardi, 2007, p. 2).

2.2.2 Temperature

In the kinetics of microorganisms for substrate consumption, the temperature must be considered since it is vital for the definition of parameters as the specific reaction rate. This relationship is given by the van't Hoff-Arrhenius equation 2.2 (Tchobanoglous, et al.,2014, p.31).

$$\frac{d(\ln k)}{dT} = \frac{E}{R^2} \quad (2.2)$$

Where:
k = reaction rate constant at T
T = temperature, K
E = activation energy
R = ideal gas constant ($8.314\ J/mol * K$)

According to equation 2.3 the hydrolytic activity increases until an optimum temperature, after which rapidly decreases (Veeken & Hamelers, 1999, p. 253). The temperature also influences the interaction between mass diffusion coefficients.

The microorganism culture can be defined by the range of temperature operation as mesophilic (30-45°C) or thermophilic (45-60°C). Variation in the temperature can cause a reduction in the biogas production. In the case of mesophilic conditions, the microorganism allowed temperature fluctuation tolerance is ±3 (Azman Samet et al., 2015, p 2544).

2.2.3 Ammonium

Bacteria requires sources of nitrogen for synthesis of cell constituents, nitrogen in ammonium form is one of the most important nutrient for the microbiological growth, it had been found that many carbohydrate-fermenting bacteria and methanogenic bacteria uses ammonia as source of nitrogen (Hobson, 1993, p. 34).

Half reaction for ammonium ion assimilation for bacterial cell synthesis is described as:

$$\frac{1}{5}CO_2 + \frac{1}{20}HCO_3^- + \frac{1}{20}NH_4^+ + H^+ + e^- \rightarrow \frac{1}{20}C_5H_7O_2N + \frac{9}{20}H_2O$$

(R2.2.1) (Tchobanoglous, et al.,2014, p.580)

The proper ratio of carbon and ammonia is fundamental for the operation of an anaerobic digester, C/N ratios ranges between 20:1 and 30:1 has been proposed to be ideal. In cases of high C/N ratio the nitrogen will be quickly consumed and will act as the reagent limit since the growth rate is limited, and the actual culture cannot process the remaining carbon. On the opposite side lower C/N ratio may end up as inhibition of the whole process since free ammonia (strong base) will be present raising the pH (R. Kigozi, Muzenda, & Aboyade, 2014, p. 2).

A research on the effect of the application of ammonia as TAN in AD in a range of 0 to 4.5 g/l, found that concentrations lower than 1.54 g/l were beneficial. While from a concentration of 3.78 g/l the inhibition of methanogenesis increased (Sheng, et al., 2013, p. 209).

2.2.4 VFA

Volatile fatty acids play an important role in the AD process, being the substrate for methanogen bacteria. And therefore, having the suitable tools for measuring is possible to use

them as a reference for a proper operation. Quantifying VFAs allows the identification of changes in the process faster than pH or VS since the variation of carboxylic acid concentrations can be observed in hours or in a few days, while parameters such as pH and VS require several days or even weeks until it is possible to observe any change (Ahring, Samdberg, & Angelidaki, 1995, p. 559).

An unbalanced operation would occur when the production of VFA is higher than the consumption, this can lead to unwanted accumulation and reach levels that reduce the pH of the operation, these variations are generally due to changes in the organic load. The toxicity of high concentrations of VFA has been investigated in several scientific articles, however the drop in pH is responsible for the operational instability and its consequent inhibition for methanogenesis (Ahring et al., 1995, p.560).

2.3 Effect of oxidizers in AD

To increase the hydrolysis rate, it would be desirable to increase the enzymes activity, this advantage can be obtained under aerobic and anoxic environments compared to anaerobic. The ASM No. 2 considers hydrolysis rate for anaerobic conditions to be 10% of the rate at aerobic conditions, while for anoxic conditions this rate is 60% of aerobic condition (Goel, Mino, Satoh, & Matsuo, 1998, p. 2081).

This achievement of higher hydrolysis rates can be obtained by two means, the addition of low levels of oxygen which is called micro aeration, and the addition of aqueous solution of calcium nitrate. Both methods previously found to improve bio-gasification enhancing the degradation of not easily accessible organic matter by improving hydrolysis without disturbing strictly anaerobic microorganism.

2.3.1 Micro-Aeration

It is well known that the exposure of strictly anaerobic microorganisms to oxygen can inhibit their growth rate. As a result, we can expect a higher concentration of carbon dioxide in the biogas, or the complete instability of the operation in the reactor.

This effect of oxygen is represented in the Inhibition expression 2.3:

$$I_{O2} = \frac{K_{O2}}{K_{O2} + S_{O2}} \qquad (2.3)$$

Where:

K_{O2}=Dissolved oxygen Inhibitory Constant

S_{O2} = Dissolved oxygen concentration

However, it has been demonstrated that the addition of small amounts of dissolved oxygen, may be beneficial in AD cultures, increasing the production of biogas, among other reasons the organic matter has greater solubilization with small amounts of oxygen (Botheju, Lie, & Bakke, 2009, p. 191).

The addition of oxygen in the form of air to the feed of the reactor, must be done considering the level of diffusion that oxygen has in liquids, in this case waste water, for this reason it is required that the supply is made in the form of small bubbles with the help of a proper diffuser to enhance oxygen-liquid contact. The smallest the bubble the higher the Surface/Volume ratio which means higher contact surface, the insufficiency in micro aeration is detrimental for the development of strictly anaerobic or facultative microorganisms (Zhu, Lü, Hao, He, & Shao, 2009, p. 2049).

The facultative microorganisms are of great interest in this application since they use this oxygen for their functions in various mechanisms, providing a defense system for strictly anaerobic bacteria and reducing in a way the chance to inhibit the methanogenesis process. The effect of the aerobic and anaerobic operation is evident in the development of the microorganism, comparing key parameters as yield of biomass and half saturation constants in the models ADM-1 and ASM- 2 (Botheju et al.2009, p.193).

Analysis of the bacterial community structure at the end of AD under micro aerated conditions reported a rise to the relative abundance in bacteria associated with hydrolysis, compared to anaerobic conditions (Fu, Wang, Shi, & Guo, 2016, p. 528). The same report concludes that under micro aerobic conditions methanogenic microorganisms was changed to acclimatize the micro aerobic conditions.

2.3.2 Nitrate in aqueous solution

The benefits of micro aeration have been well-known documented, nevertheless in practical operation systems might turn in further inconveniences, since the most economical way of

providing oxygen is air, higher concentrations of nitrogen in the biogas can be expected, on the other hand it would require a robust system of safety control system due to the possibility of accumulating undesired concentrations of oxygen in the headspace leading to increase explosion risks (Norway Patent No. EP 2 457 878 A1, 2012, p. 3).

To replicate the advantages of the micro-aeration in AD, it is necessary that the addition of an oxidizing agent that has no secondary toxicity effects. It was found that the addition of nitrate in low concentrations was convenient to increase the production of biogas, without affecting the methane concentrations. Nitrate is an electron acceptor when added in sub-inhibitory levels, can provide enough electrons to stimulate the AD process without disturbing it (Norway Patent No. EP 2 457 878 A1, 2012, p. 1) since hydrolysis is enhanced due to production of extracellular enzymes by bacterial growth.

Studies points that high levels of nitrite and nitrate influent are toxic to the entire microbial ecosystem, leading to a global inhibition of methanogens similar as dissolved oxygen in equation (2.3) (Akunna, Bizeau, & Moletta, 1992, p. 831). On the same trend, other research studied the effects of different nitrate concentration finding a breaking point where the production of biogas got negatively affected with nitrite concentrations over $1\ g\ /\ L$ in the reactor (Sheng, et al., 2013, p. 209). Nevertheless, the authors also reported an increase in the biogas yield in the nitrate range of nitrate concentrations from 100 to 500 mg/L compared to the test without nitrate. Such inhibition occurs due to carbon deficiency carried during the reaction (Andalib, Nakhla, McIntee, & J.Zhu, 2011, p. 13):

$$24NO_3^- + 5C_6H_{12}O_6 \rightarrow 12N_2 + 30CO_2 + 18H_2O + 24OH^-$$

(R2.3 1)

As the percentage of nitrate influent decreases, denitrification is likely to happen due to electron channeling to denitrifier microorganism. But in a very low range of nitrate addition methanogenesis will dominate in the system. Researches on the Suppression of methane production by nitrate concluded that the main mechanism involved was inhibition of methanogenesis by denitrification intermediates rather than competition for substrate as it reduces acetate methanogenesis almost completely (Roy & Conrad, 1999, p. 49).

Similarly, to micro-aerated systems, it has been shown that in an attached growth denitrifier bacteria tend to grow along the outer surface of media providing a shield effect against low

concentration of oxidants, while methanogenic microorganism accumulates in the inner part (Andalib et al., 2011, p.6).

In other words, the addition of nitrite required to improve the biogas yield must be high enough to enhance the hydrolysis process by increasing the activity of enzymatic action produced by facultative microorganism that use the nitrate as an electron acceptor, but low enough to ensure that the free nitrate and denitrification intermediates don't inhibit the methanogenesis. The nitrate added to the initial degradation steps leads to more biomass in form of anoxic species that later can be degraded anaerobically.

2.3.3 Additional benefits of nitrate

As an additional benefit the application of calcium nitrate to AD, enhances the biogas quality as it reduces the concentration of H_2S, hypothetically by the competition of the substrate for denitrifier Sulphate reducing bacteria (SRB) (Stoeck, Filker, Breiner, Wendel, & Doppelbauer, 2017, p. 52). By these means reducing the corrosiveness and odors, and therefore the maintenance of equipment.

2.3.4 Nitrate dosage

It is required to stablish a correlation between the feed properties and the quantity of nitrate required, in this project this estimation is based on patent (Norway Patent No. EP 2 457 878 A1, 2012, p. 7).

The unit determining the capacity to consume O_2 upon decomposition of organic matter is the COD. The COD level measured may be used to determine the amount of nitrate to be added. The mass ratio of nitrate and oxygen is 1.55. This ratio is used to express nitrate in terms of chemical oxygen demand (COD).

The dosing of nitrate added to the reactor can be calculated by equation 2.4, it must be calculated as fraction of COD in the feed:

$$q(NO_3)[mg/d] = Q(input)[ml/d] * COD\left[\frac{g}{ml}\right] * 1.55 * \mu \quad (2.4)$$

μ = Fraction of COD as nitrate.

3 Material and methods

A CSTR is used laboratory scale, replicating the operational conditions of a full-scale AD. The study is performed in two stages: Phase 1 operation with 0% nitrate addition, and phase 2 operation with 0.2% of nitrate addition according to equation 2.4.

Measurements of the physicochemical properties of the effluent were made four times per week, while substrate analysis was made once a week. The measured properties are: CODt, CODs, TS, VS, NH_4^+, NO_3^-, pH and VFAs. Biogas production was collected on daily basis, by removing the volume of gas accumulated in storage bags; composition analysis (CH_4 and CO_2) was carried out at least once a week for a period of 9 weeks.

The substrate used is composed of sludge from water treatment plants of 9 municipalities around Drammen region, industrial fish waste, domestic septic and food waste. These components were mixed and thereafter processed in a thermal hydrolysis pre-treatment with residence time of 20 minutes and 170 degrees Celsius. Activated sludge from a biogas plant in Drammen is used as inoculum. The substrate flow was stablished accordingly to an HRT of 20 days, the temperature of the process is set at 35°C. Samples taken during the experiment are kept refrigerated at 4°C to inhibit any microbiology degradation that can alter the measurements.

3.1 Equipment

The high total solids of the feed indicate that a feasible reactor for this project is a CSTR. The glass reactor has a working volume of 1 litter and 0.2 litter of headspace, is continuously stirred at 500 RPM. The reactor is an adaptation from an electrochemical cell with a multiport cover made by Gamry Instruments.

Figure 3.1: Reactor overview (Gamry Instruments, 2017), 1. Reactor body and external jacket, 2. Multiport cover.

On the cover it has three entrances and three adapted hoses for the streams, effluent, feed and biogas as shown in figure 3.1, the biogas hose is linked to the headspace of the reactor to ensure only gas will flow through it. A titanium body pH / ORP / Temperature electrode is adapted, the electrode is immersed in the bio-digestate, as the feed and the effluent hoses. The reactor has an external glass jacket for heat exchange.

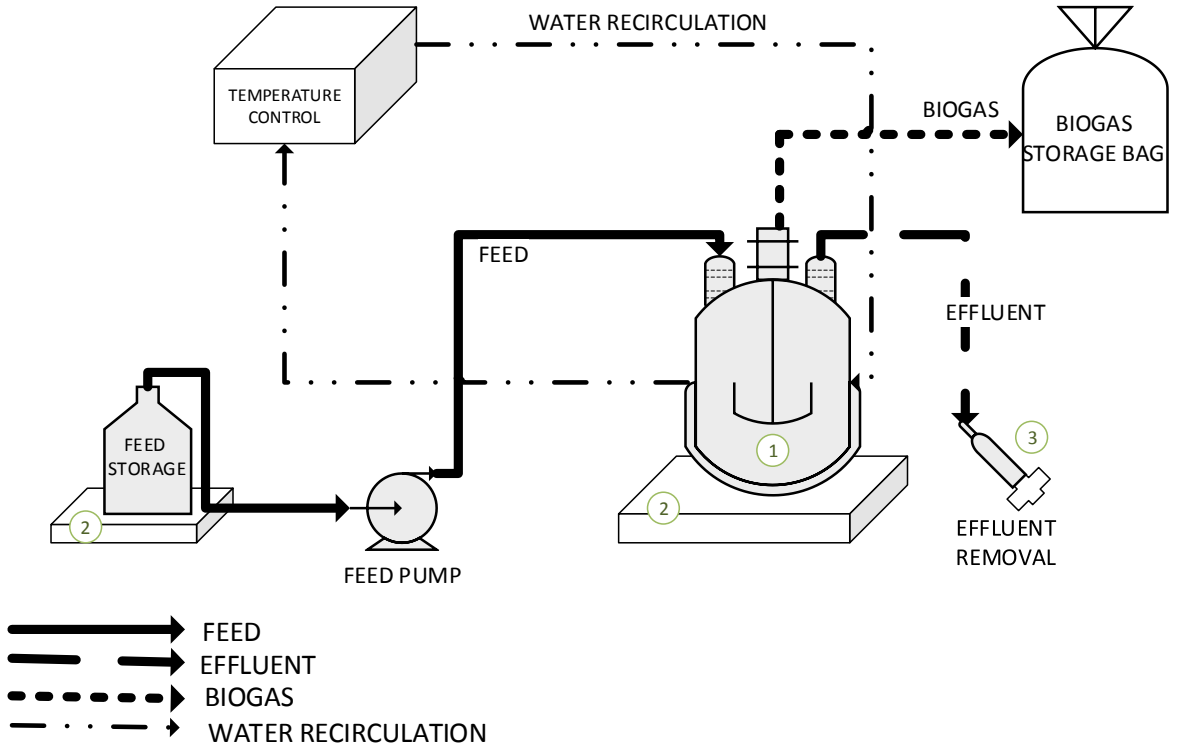


Figure 3.2: Anaerobic digester diagram flow: (1) CSTR, (2) magnetic stirrer, (3) manual effluent extractor.

The substrate inlet has been adapted to provide feed manually, or with a pump. In this specific project during the weekdays the inlet has been done manually with a syringe and on weekends with a pump, due to high concentration of solids (and therefore high viscosity) it is difficult to set a continuous flow by the low diameter hoses.

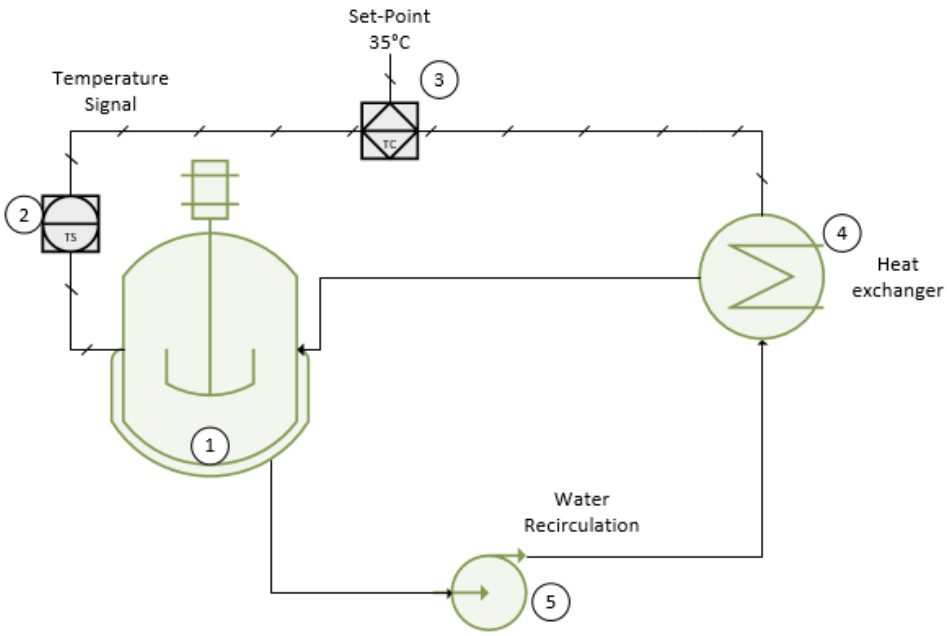


Figure 3.3: CSTR temperature control loop: (1) Reactor, (2) Temperature sensor, (3) PI controller, (4) Peltier thermoelectric cooler, (5) Water recirculation pump.

The system has a temperature PI control running under LabVIEW software, with proportional bandwidth of 17.5, and integral gain of 0.01. The fluid used as refrigerant is tap water, it flows with the action of a metering pump through 8 mm plastic hoses and the reactor jacket to keep the temperature of the process at 35 $\pm$ 0.1°C. The heat exchanger unit is capable of cooling or heating the fluid according to the requirements of the control.

3.2 Experimental plan

3.2.1 Reactor Set-up, 3 weeks

In the first week of the project the reactor was filled with water in both tank and the jacket to test any possible leak in the joints, thereafter pressurized nitrogen gas flowed through the head space to expose any possible gas leak. These tests were carried out to ensure that the content of the reactor would not interact with atmospheric air and so to keep anaerobic conditions, on the other hand the fluid volume in the jacket is intended to remain constant to avoid unnecessary opening and refilling.

The reactor was operated for two weeks until it reaches a biogas production steady state.

3.2.2 Phase 1: Operation with 0% nitrate addition, 2 weeks

After the reactor reach a stable state of biogas production and effluent COD, the Phase 1 of operation started. The feed added to the reactor is diluted with distilled water to easy the flow through the hoses, and to keep a total COD in average of 74 ±1.9 g/L.

The feed load was equal during the different phases of the project with 50 mL feed /day, during weekdays the inflow was added manually with the sample taken from the fridge to avoid any degradation or contamination, during the weekends the feed was storage in a 400-mL glass recipient constantly stirred at 800 RPM to ensure proper homogenization and to avoid clogging of the feed hose, it was pumped with a metering pump controlled with an on-off timer.

3.2.3 Phase 2: operation with 0.2% nitrate addition, 3 weeks

With average of total COD of 74g/L, using equation 2.4 to calculate de BPO dosage, with μ = 0.2%.

$$q(NO_3)[mg/d] = 50[ml/d] * 74\left[\frac{mg\ COD}{ml}\right] * 1.55 * \frac{0.2}{100} = 11.47\ mg\ NO_3/d$$

For practical purposes the same amount of BPO is added in each substrate supply, nevertheless the feed nitrite load is analyzed every week.

3.3 Feed characteristic

The substrate characteristics used as feed are shown in table 3.1. The collection of the sample had a high quantity of lumps, therefore it was indispensable to guarantee its previous homogenization, and later addition of distilled water to guarantee uniform characteristics throughout the experiment and facilitate fluidity within the system.

Feed		STD
CODt (g/L)	73.95	±1.9
CODs (g/L)	12.08	±1.3
NH4-N(mg/L)	1232	±63
pH	6.8	±0.28
TS (g/Kg)	64.1	±4.6
VS (g/Kg)	41.7	±3.3

Table 3.1: Substrate characterization, n = 10

The biogas plant uses as feed a combination of 25% fish oil waste, 5% of food waste and 70% of communal sludge on a weigh base, therefore it is expected high COD related as proteins and lipids, on the other hand it is expected that near 70% of the total COD has a high potential to be transformed to biogas according to VS and TS values (Table 3.1).

3.3.1 Feed VFAs

The profile composition of VFA of the feed is shown in Figure 3.4

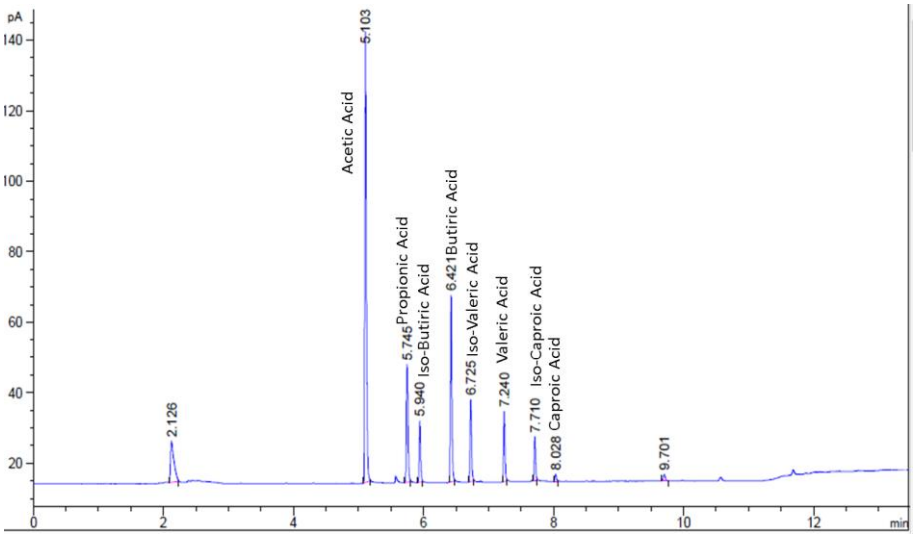


Figure 3.4: Substrate volatile fatty acids profile.

In figure 3.5 the higher composition of VFA with approximately 60% of the total is acetic acid.

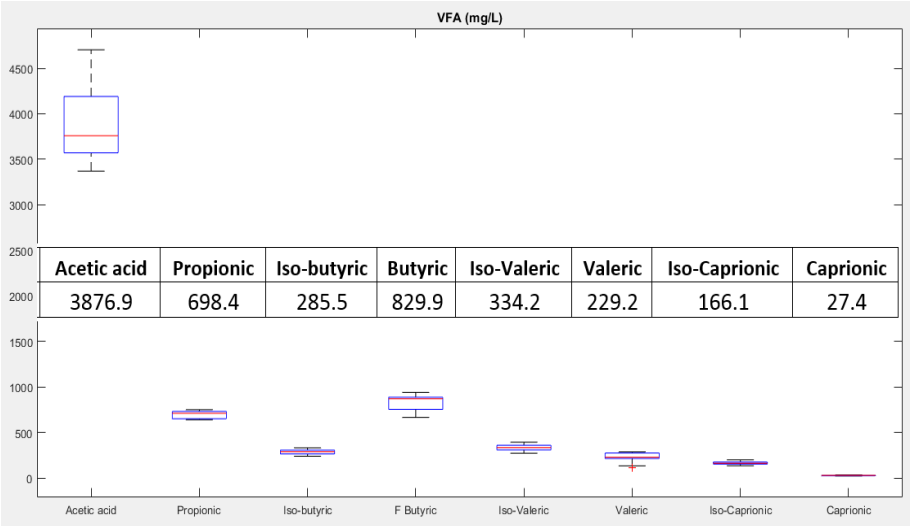


Figure 3.5: Boxplot of VFA concentration of the Substrate.

3.4 Chemical analysis

Gas composition: The sample is taken in 20 mL syringes, using a Teflon block valve to keep isolated the biogas from the air. The gas composition is analyzed using a multiple gas chromatograph model SRI 8610C with a thermal conductivity detector with two columns. The oven temperature is constant at 80°C and the gas carrier is helium (SRI Instruments, 2016).

VFA: The samples are centrifuged at 20000 rpm for 15 minutes, then filtered at 0.45 µm with GxF mullti-layered Acordisc PSF syringe filters. The effluent sample is diluted 1:5 in volume and 1.35 mL is mixed with 0.15 mL formic acid 0.65 M in a 1.5 mL GC sample vials. The analysis was carried using gas chromatography Hewlett Packard 6890.

COD, NO_3-N, NH_4^+-N: the samples are previously centrifuged, filtered and diluted. Afterwards analysis was carried by procedure base on Spectroquant® Pharo 300 user manual (Spectroquant, 2014).

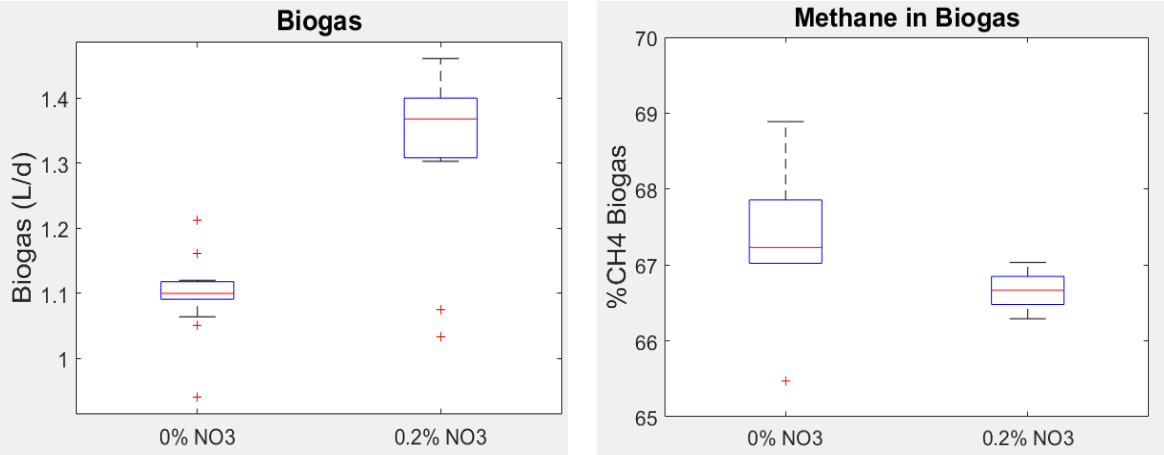
TS-VS: The analysis was carried according to US-standards 2540 B and 2540 E, respectively. (Eaton, Clesceri, & Greenberg, 1995).

4 Results

The results are presented by comparing the two phases of the project. The use of boxplot serves as an additional resource in the comparison since it provides a broader picture regarding the statistical review and data management. It is important to mention that the NO_3^- supply was carried out in a semi-continuous manner and there was no increase of this ion concentration during phase 2 in the effluent.

4.1 Biogas production

Figure 4.1 a) shows the dispersion of the daily biogas production during phase 1 and 2, biogas production rates increased in average 22% with the addition of calcium nitrate from 1.10 to 1.35 L/d in average. In figure 4.1 b) it can be observed that methane concentration has been reduced in less than 1% without affecting the benefits of increased biogas production rate, giving in average of 67% methane content in biogas.

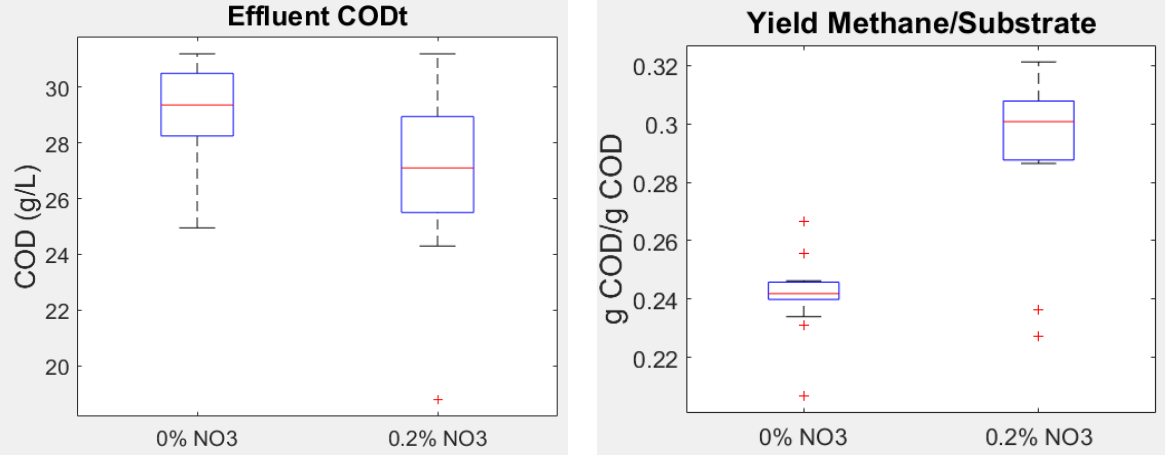


a) b)

Figure 4.1: Boxplot comparing the operation of AD with 0% and 0.2% of NO_3^- a) Biogas production rate, b) Methane concentration in biogas.

4.1.1 COD mass balance

Figure 4.2 a) shows a reduction of the effluent COD by 8% indicating a higher transformation of the feed COD to methane, consequently increasing the yield of methane up to 0.294 g COD CH_4/g COD feed (Figure 4.2 b).



a) b)

Figure 4.2: Effect of addition of 0.2% of NO_3^- (as COD in the substrate) a) Effluent COD b) Methane yield by influent COD

An overview of the COD mass balance of the system is shown in figure 4.3, which includes the COD available in the substrate, the COD that is transformed into methane, and the COD of the effluent, finally the actual COD removal.

The mass balance of the system can be represented by the following expressions:

$$COD_{in} * Q = COD_{ef} * Q + COD_m \quad (4.1)$$

$$COD_m = G * \gamma/\rho \quad (4.2)$$

Where:

COD_{in} = COD of the substrate (g/L)

COD_{ef} = COD of the effluent (g/L)

COD_m = COD of methane produced (g/d) calculated at 35 °C

Q = Feed flow (L/d)

G = Biogas flow (L/d)

‘γ= methane concentration in biogas (%)

‘ρ = methane COD 0.4 gCOD/L at 35°C

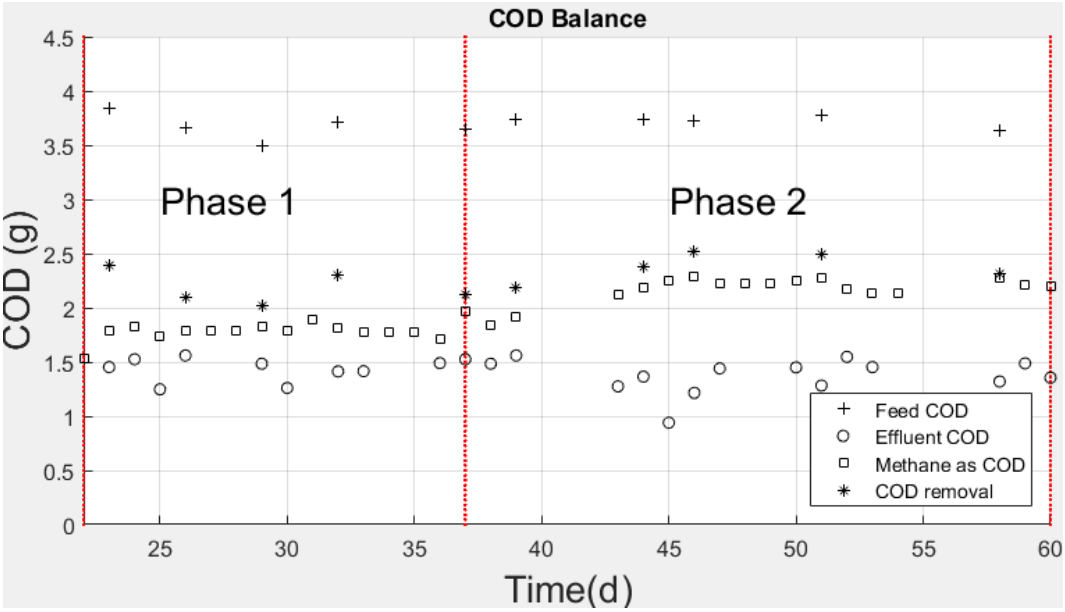


Figure 4.3: COD mass balance.

According to the measurements observed in figure 4.2 a) the average COD_{ef} decreased from 28.74 g/L to 26.5 g/L, knowing that the value of γ is 67% is possible to solve the previous equation the results are shown in table 4.1.

Phase	CODin (g/d)	CODef (g/d)	CODm (g/d)	CODrem (g/d)
I	73.95 ± 1.9	1.44 ± 0.11	1.84 ± 0.09	2.23
II		1.33 ± 0.19	2.26 ± 0.19	2.35
Variation		-8%	23%	5%

Table 4.1: COD balance, comparison between phase I and II. In: Influent; ef: effluent; m: methane; rem: removal. n phase 1 = 9, n phase 2 = 16.

According to table 4.1 it can be observed that the COD removal increased in average 5% in phase 2, reaching a value of 2.38 g COD/d, slightly higher than the COD of the methane produced 2.26 g COD/d, showing a reasonable correlation between the two measurements.

4.1.2 Biogas timeline

The reactor operation was characterized by a constant HRT of 20 days. Biogas production increased in average 0.24 L/d during phase 2 (Fig. 4.4).

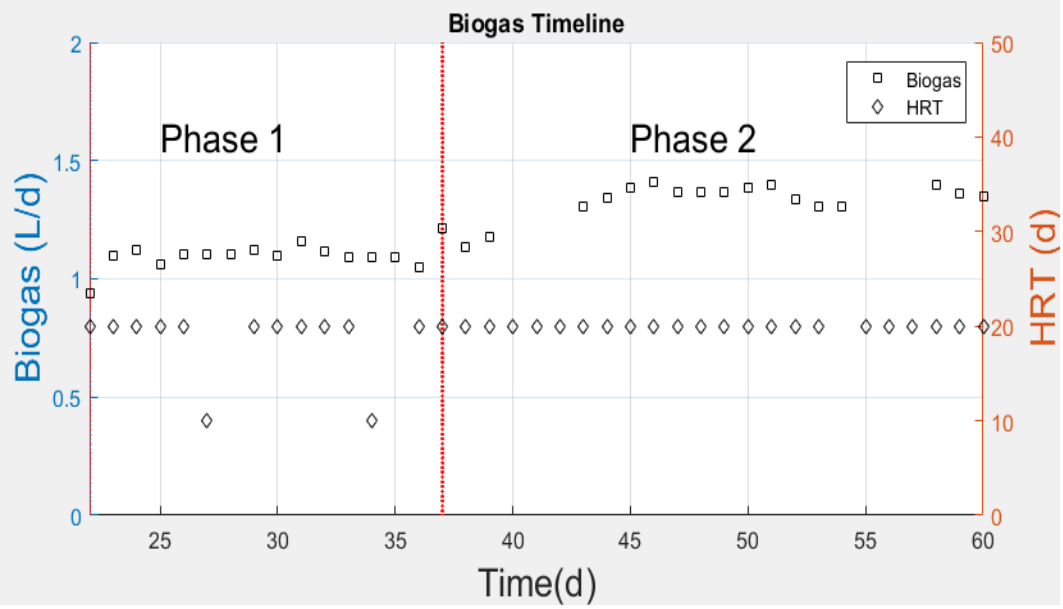


Figure 4.4: Biogas production and hydraulic retention time (HRT).

4.2 Ammonium

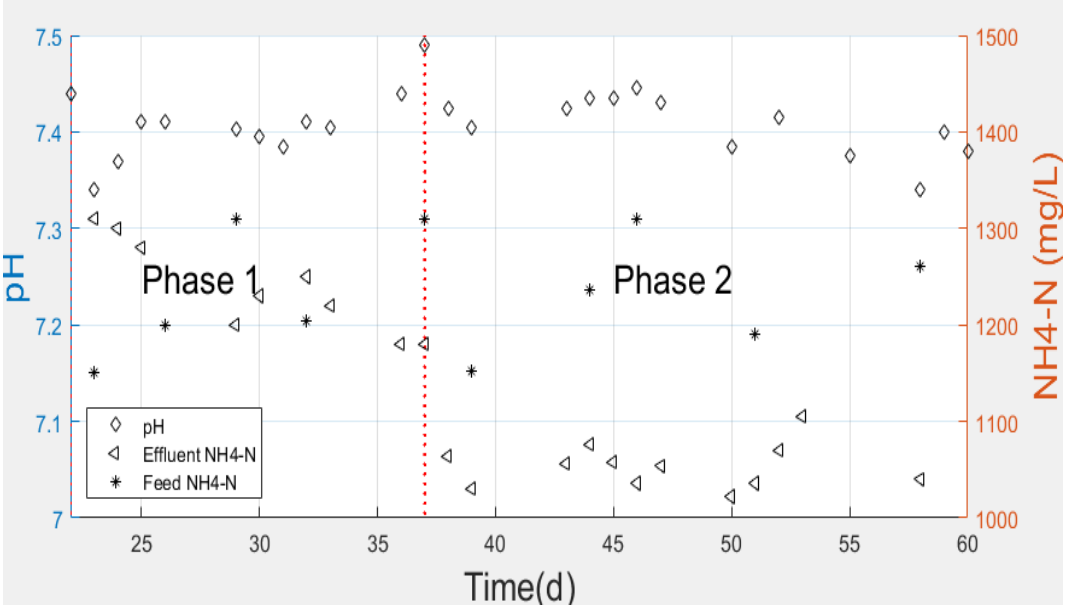


Figure 4.5: Ammonium ion concentration vs pH of the biodigestate

PH varied in the range of 7.3 to 7.5 keeping an average of 7.4 in both phases.

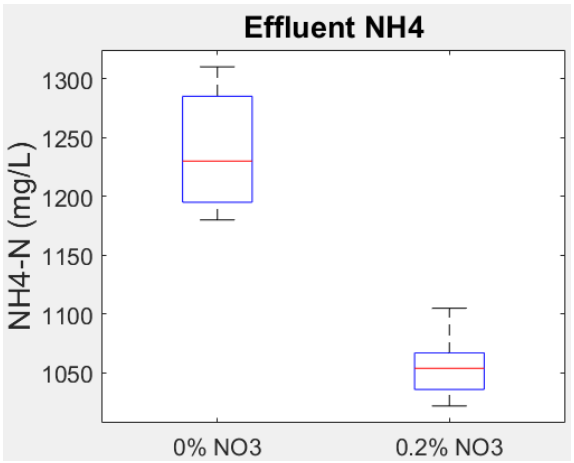


Figure 4.6: NH_4-N concentration boxplot comparison before and after addition of 0.2% NO_3^- (as COD in the substrate).

The ammonium ion concentration showed a fast reduction of 15% immediately the day after the first addition of calcium nitrate, reaching a steady state concentration from 1240 to 1053 mg NH_4^+-N/L.

4.3 Effluent soluble COD

Effluent CODs decreased 28% compared to phase 1, from 1.76 to 1.27 g/L; Unlike the ammonium ion reduction, the CODs took 8 days to reach and steady state.

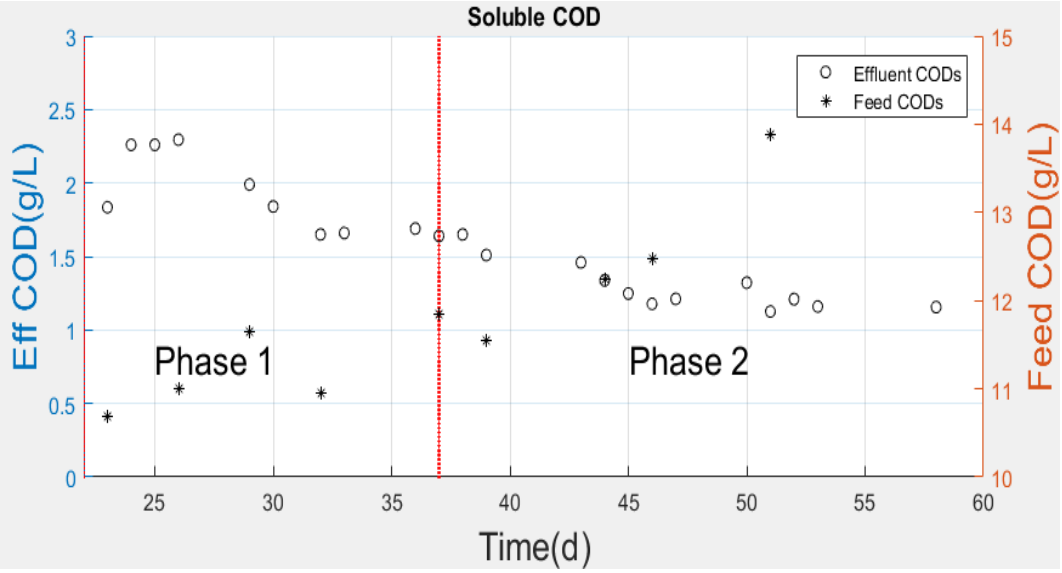


Figure 4.7: Evolution of CODs before and after of addition of 0.2% of NO_3^- as COD in the substrate

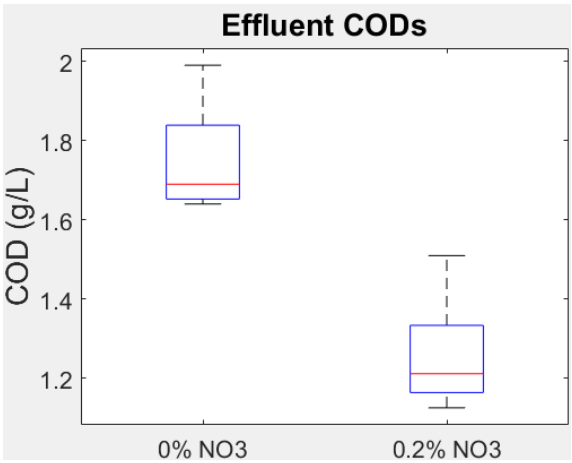


Figure 4.8: Boxplot Effluent CODs comparing phase 1 and 2 of the project.

The CODs uptake rate during the period of day 38 to 45 of operations exhibits a trend that fits nearly to a second-grade polynomial of the form $r_{sp2} = -5.09*t^2 + 374.4*t - 5283$, with a value of $r^2 = 0.9$. The feed CODs had an increasing trend along the experiment.

4.4 Other parameters

4.4.1 VS TS Ratio

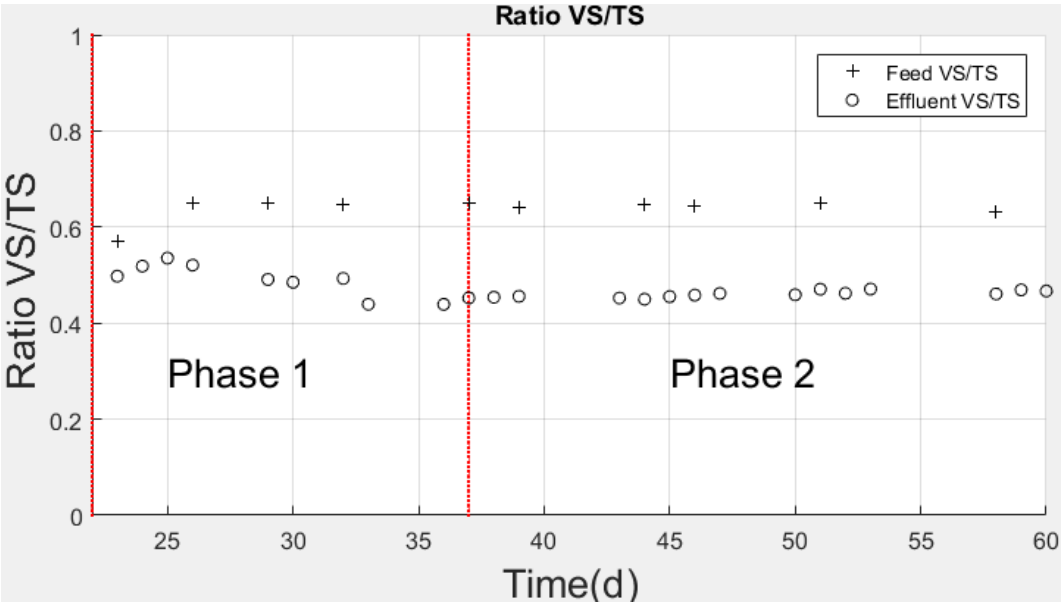


Figure 4.9: Vs/Ts Ratio of the feed and effluent

According to figure 4.9 is possible to see that in average the VS/TS ratio is higher during phase 1 of the project, suggesting a higher transformation of volatile solids to biogas.

4.4.2 VFA of biodigestate

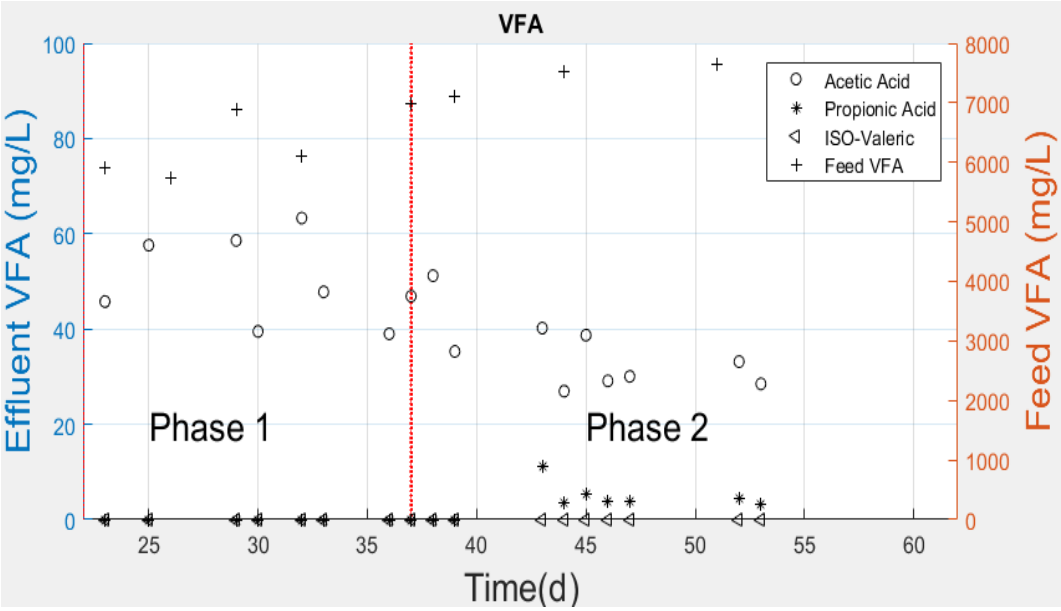


Figure 4.10: VFA evolution during phase 1 and 2

The VFA concentration of the effluent was reduced by 20% in phase 2 as shown in figure 4.10, and the feed VFA shows an increasing trend during time.

4.4.3 Reactor ORP

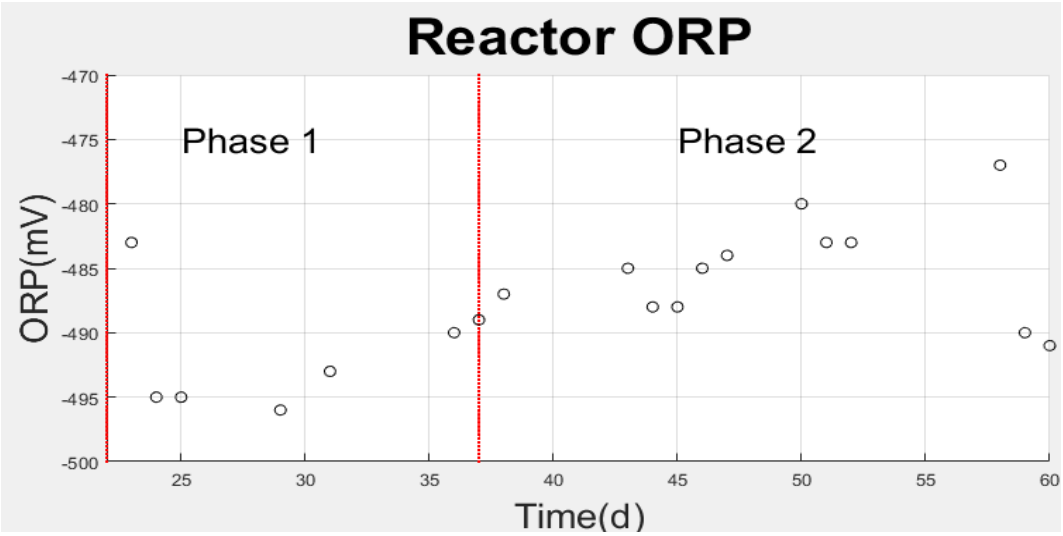


Figure 4.11: Oxidation-Reduction Potential during test

ORP measurement increased from -493mv to -485mv after the addition of calcium nitrate according to figure 4.11, this change was expected due to the oxidizing properties of the nitrate ion, nevertheless the operation range is still optimum for the methanogenic bacteria.

4.4.4 Resume

Effluent	Phase 1	Phase 2	Variation
CODt (g/L)	28.74	26.5	-7.8%
CODs (g/L)	1.76	1.27	-27.8%
NH4-N(mg/L)	1240	1053	-15.1%
VFA(mg/L)	52.3	41.8	-20.1%
pH	7.4	7.4	-
ORP (mv)	-491.6	-485.1	-1.3%
VS/TS	0.5	0.47	-6.0%
Biogas	**Phase 1**	**Phase 2**	**Variation**
Production Rate (L/d)	1.1	1.34	21.8%
% Methane	67.3	66.7	-0.9%
Yield (CH4 COD/Feed COD)	0.241	0.294	22.0%

Table 4.2 Comparison of parameters in phase 1 and phase 2

5 Discussion

5.1 COD removal and Biogas rate

As shown in figure 4.2 a) the reduction of the effluent COD in phase two, it becomes evident that there is a higher degradation of biopolymers mixed in the biodigestate, which previously ended up in the effluent without being hydrolyzed, a basic interpretation of this result suggests an increase on the hydrolysis rate, this leads to the increase of accessible substrate sources for fermentative bacteria.

A comparison between the COD of the methane and the COD removal shows a difference of 0.39 g and 0.08 g in phase 1 and 2 respectively and the reduction trend of the COD in the effluent is not as evident as the methane production increase, making a brief analysis of the data is possible to relate this difference due to the higher dispersion of measurements in phase 2 of the project. On the other hand, the high HRT makes the COD reduction slower.

On the same trend of the COD reduction it is possible to observe a similar behavior in the average of the VS/TS ratio, suggesting a higher transformation of volatile solids to biogas.

Finally, as observed in figures 4.7 and 4.10 the soluble COD and VFA concentration are increasing along the time, which suggest that the feed is being hydrolyzed along the operation time, even though it has been carefully storage at low temperature to reduce microbiological degradation, the higher concentration of readily biodegradable COD to the reactor can increase slightly the gas production.

5.2 Microorganism kinetics

According to ADM model (Batstone, et al., 2002) the rate of hydrolysis r_h follows a first order behavior expressed by:

$$r_h = k_h * X_i \quad (5.1)$$

Where:

K_h = hydrolysis first order constant (1/d)

X_i = Particulate COD (Carbohydrates, Lipids, Proteins)

A roughly estimation of r_h can be obtained from the table 4.1 as the value of COD_{rem} meanwhile the particulate COD can be obtained as the effluent COD which give us hydrolysis constants:

$K_{h1} = 1.55\ d^-$

$K_{h2} = 1.77\ d^-$

Which results in a hydrolysis rate increase of 15%, this coincides with (Norway Patent No. EP 2 457 878 A1, 2012) and is reaffirmed by the direct correlation of the COD removal and the methane production increase. Nevertheless, there is a difference between the methane produced and the removal of COD is approximately 5%, showing a higher COD removal than methane production, the reason for this difference can be related to dissolved inorganic impurities in the feed that may influence the COD analysis.

Based on the studies of micro-aeration model (Botheju, Lie, & Bakke, 2009, p. 193), and making a parallel of effects of dissolved oxygen and dissolved NO_3, one can expect a higher growth rate of microorganism and therefore a higher concentration of biomass consequently, the increase of enzymatic activity for the hydrolysis process.

5.2.1 CODs variation

A higher increase in hydrolysis would be related to an increase in the CODs, however, due to the kinetics of the microorganisms involved in the fermentation, a higher biomass concentration would increase the uptake of substrate, this can be expressed as a Monod expression by:

$$R_i = k_{m\_i} * X_i * \frac{S_i}{k_{s\_i} + S_i} \quad (5.2)$$

Where:

R= rate of uptake by microorganism

K_m = maximum uptake rate ($\frac{Kg\ COD\ of\ substrate}{Kg\ COD\ of\ biomass * day}$)

Ks = half saturation constant ($\frac{Kg\ COD\ of\ substrate}{m^3}$)

S = Substrate concentration ($\frac{Kg\ COD\ of\ substrate}{m^3}$)

X = Biomass ($\frac{Kg\ COD\ of\ substrate}{m^3}$)

i = index for, sugars, amino acids or LCFA

The reduction of the CODs might be interpreted as a better uptake of substrate in the form of monosaccharides, amino-acids and LCFA according to expression 5.2 it can be directly related to the increase of the biomass mentioned before.

5.3 Methane concentration

In phase 2 the concentration of methane was reduced by less than 1% against the methane concentration of the biogas in phase 1 of the project without overshadowing the higher biogas production; in the case of an increase in nitrate supply in the AD, a greater proportion of carbon dioxide can be expected due to anoxic kinetics and substrate consumption by denitrifying microorganisms (Sheng, et al., 2013).

According to the methane concentration it is possible to relate the increase in the biogas to greater accessibility of methanogenic bacteria to substrates suitable for the transformation to methane, and to a lesser extent the increase in denitrifying biomass due to the low increase of carbon dioxide concentration.

5.4 Ammonia concentration

A higher assimilation of ammonia due to microorganism activity is related to a higher concentration of fermentative biomass. A higher yield for the transformation of CODs into biomass is expected as the nitrate stimulates the anoxic growth of biomass.

The reduction in the concentration of ammonia during phase 2 observed in figure 4.5 suggests the relevance to keep track of the NH_4^- concentration, since higher growth rates of biomass or a sudden reduction in the ammonia concentration in the feed can affect the supply of N for the generation of new biomass, by reducing the NH_4^+ concentration in the biodigestate even though the nitrite added also served as nitrogen source.

5.5 ORP pH and temperature

the pH remained on average at 7.4 ± 0.1 which suggests that the sludge has a good buffer capacity, this property provided a good environment condition for methanogenic bacteria. The variation of ORP was in the order of 1.3%, the increase was expected by the addition of an oxidizing substance such as calcium nitrate, nevertheless the ORP value was on the normal operation range for methanogenesis in both stages.

The variation in temperature was of magnitude of ±0.3 °C. These results suggest that the addition of Nitrate didn't have a negative effect on the methanogen culture.

5.6 Suggestion for further improvements

To improve the automation of the process the installation of two additional pumps. One for the supply of calcium nitrate, and another for the evacuation of the effluent as the design of the reactor makes it difficult to remove it by overflow, it is advisable to use hose diameter higher than 6mm if high concentration of solids is expected.

To elaborate a better interpretation of the reduction of CODs after the addition of NO_3, it is recommended to analyze which kind of compounds constitute the soluble phase of the effluent since the reductions in the concentration of the VFA do not compensate for 5% of what was reduced, suggesting a reduction in monosaccharides, LCFA, or amino acids; the use of HPLC could be of great help in this sort of classification and to stablish a better correlation of NO_3^- addition and substrate characteristics.

6 Conclusions

The use of calcium nitrate at low concentrations in substrates with high concentration of solids have demonstrated the enhancement of biogas production in AD. The nitrate ion added to the substrate in a ratio of 0.2% (NO_3 as COD: substrate COD) has increased the biogas generation by 22% without altering the methane concentration it is also linked to an increase of the COD removal of the AD, suggesting an increase of the hydrolysis rate compared to strictly anaerobic operation conditions and a higher transformation of the biopolymers into methane. As a result, at the industrial level, it could represent a higher profitability due to the increase in energy production in the form of biogas per unit of raw material, coupled with this the obtaining of an effluent with lower concentration of solids that facilitates its subsequent post-treatment.

The consumption of the ammonium ion during the operation with nitrate is interesting since it is relevant for the biomass growth. On the other hand, it can be beneficial in plants where ammonium accumulation is a problem due to the source of the substrate or the recirculation of sludge.

Process control parameters such as pH and ORP did not vary significantly, which suggests a practical indicator to avoid possible inhibitions by changes in the concentration of VFA that affect the pH or harmful concentrations of NO_3 in the biodigestate.

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Appendices

Appendix A – Topic description

Title: Monitoring and process evaluation of continuous stirred biogas reactor under semicontiguous dosage of Ca(NO3)2.

USN supervisors: Carlos Dinamarca, Rune Bakke, Michal Sposob

External partner: YARA International

Task background:

Sludge treatment by anaerobic digestion may be the best method to obtain both energy and nutrients recovery, thus complying with the future "circular economy". A key challenge is to boost methane yield leading to higher quality fertilizer and fewer expenses in treating reject water downstream. Nitrate dosage is a well-proven way to achieve this, but more efforts are required in dosage implementation and evaluation parameters, especially under transient conditions. The project intends to gradually optimize overall biogas process by semi-continuous dosage of Ca(NO3)2. Focus will be given to the evaluation of process parameters such as changes in the redox potential to achieve optimum methane yield and overall process stability.

Task description:
-Experimentation by a continuous stirred reactor with temperature control and semiautomated dosage of Ca(NO3)2
-Routinely chemical analysis: TCOD, SCOD, TS, VS, NH4+, NO3-, pH, VFAs
-Data collection and analysis
-Participation in project meetings
- Writing final report

Student category: EET student
Practical arrangements: Work will be carried out at USN.

Appendix B – Calculation nitrate

Based on the complete oxidation of glucose to CO_2 and H_2O:

$$C_6H_{12}O_6 + 24OH^- \rightarrow 6CO_2 + 18H_2O + 24e^- \quad (1)$$

The half reaction can be balanced with oxygen or nitrate as an electron acceptor:

$$4.8NO_3^- + 24e^- + 28.8H^+ \rightarrow 2.4N_2 + 14.4H_2O \quad (2)$$

$$6O_2 + 24e^- + 24H^+ \rightarrow 12H_2O \quad (3)$$

The full reaction in each case is:

$$C_6H_{12}O_6 + 4.8NO_3^- + 4.8H^+ \rightarrow 6CO_2 + 2.4N_2 + 8.4H_2O \quad (4)$$

$$C_6H_{12}O_6 + 6O_2 \rightarrow 6CO_2 + 6H_2O \quad (5)$$

The mass ratio between nitrate and chemical oxygen demand (COD) is found by:

$$\frac{4.8\ moles\ NO_3^-}{6\ moles\ O_2} * \frac{\frac{62\ mg\ NO_3^-}{mole\ NO_3^-}}{\frac{32\ mg\ O_2}{mole\ O_2}} = 1.55 \frac{mg\ NO_3^-}{mg\ O_2} \quad (6)$$

The dosing of nitrate added to each reactor must be calculated as fraction (or percentage, %) of COD in the feed (COD load), and can be calculated by equation **Error! Reference source not found.**:

$$q(NO_3)[mg] = Q(input)[m^3] * COD\left[\frac{mg}{ml}\right] * 1.55 * \mu \quad (7)$$

In the present work Calcium nitrate (CN) is the substance carrying the nitrate $Ca(NO_3)_2$. The amount of CN to be added is obtained by:

$$\frac{1\ mole * 164 \frac{mg\ CN}{mole\ CN}}{2\ moles\ NO_3^- * 124 \frac{mg\ NO_3^-}{mole\ NO_3^-}} = 1{,}32 \frac{mg\ CN}{mg\ NO_3^-} \quad (8)$$

This ratio was divided by the density of CN to find the volume of CN

$$\frac{1{,}32 \frac{mg\ CN}{mg\ NO_3^-}}{1800 \frac{mg\ CN}{ml\ CN}} = 7.33 * 10^{-4} \frac{ml\ CN}{mg\ NO_3^-} \quad (9)$$

CN is dissolved in a 45% solution in the biogas potential optimizator® (BPO)

$$\frac{7.33 * 10^{-4} \frac{ml\ CN}{mg\ NO_3^-}}{0.45 \frac{ml\ CN}{ml\ BPO}} = 1.63 \frac{\mu l\ BPO}{mg\ NO_3^-} \quad 10$$

The influent nitrate found from equation 7 can then be multiplied by the ratio from equation 10 to find the amount of biogas optimizator® to be added.