Technical University of Denmark

DTU Environment
Department of Environmental Engineering

MASTER'S THESIS

EX-SITU BIOGAS UPGRADE IN PLUG FLOW REACTORS

Comparison between a control reactor and a reactor filled with packing material

Marianthi Kostoula

Supervisors:

Professor Irini Angelidaki

Researcher Panagiotis Kougias

Home University Professor Gerasimos Lyberatos

September 2016

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Acknowledgements

I would first like to thank my supervisor professor Irini Angelidaki in DTU for accepting my application, trusting me to join the Bioenergy group at DTU Environment in Denmark and for helping me gain all this knowledge in this field.

My special thanks to my master's thesis daily supervisor Panagiotis Kougias for his guidance, his endless patience, his constant support and encouragement during the whole time that I was working for my thesis and his unconditional help for my experimental work. His office door was always open and he consistently allowed this paper to be my own work, but steered me in the right direction whenever I needed him.

I would also like to acknowledge my professor at my home university "National Technical University of Athens" Mr. Gerasimos Lyberatos for supporting me from distance, promoting me as an exchange student and for offering me this great possibility of working in such a wonderful environment. I am gratefully indebted to his for his very valuable help on this thesis.

Many thanks to my officemates and my co-workers who made the working hours in the lab joyful and the after office hours fun and enjoying.

I would also like to thank a lot all my amazing Danes friends who introduced me to this wonderful country and for making me part of their everyday life.

I would really like to thank my best friends from home who were always there supporting me during all the difficult moments that I've been through and helped me to complete my work. Without them this research could not have been successfully completed.

Finally, I would like to express my very profound gratitude to my family who are always by my side for offering me this opportunity, for providing me with unfailing support, love and continuous encouragement throughout my years of study and through the process of researching and writing this thesis. This accomplishment would not have been possible without you. Thank you all.

Marianthi Kostoula.

Abstract

Biogas produced by anaerobic digestion, is mainly used in a gas motor for heat and electricity production. However, after removal of CO_2, biogas can be upgraded to natural gas quality, giving more utilization possibilities, such as utilization as autogas, or distant utilization by using the existing natural gas grid. Biogas upgrading is the process of increasing the concentration of methane in biogas. Although this has been done since several decades at industrial scale via physical and chemical methods, these processes present some financial and environmental disadvantages, therefore new technologies are being developed. Recent studies show that biogas upgrading can be achieved via biological treatment using hydrogenotrophic methanogens fed with H_2 and CO_2. The SYMBIO project at the Department of Environmental Engineering of the Technical University of Denmark is working in this innovative way of biological biogas upgrading, where the hydrogen supplied to the process is obtained by water electrolysis using peak load/excess electricity from wind mills. After some research about in-situ biogas upgrading, ex-situ process was tested, resulting best performance for thermophilic regime, and the gas-liquid mass transfer being the rate-limiting step for efficient hydrogen utilization. The current study presents a new biological method for biogas upgrading into two separate, anaerobic, up-flow UASB biogas reactors one used as a control and one filed with packing material denoted R1 and R2 respectively. They contained an enriched culture of hydrogenotrophic methanogens (inoculum) and were fed via diffusers with a mixture of 62% CH_4, 15% CO_2 and 23% CH_4, while providing the microorganisms with all the necessary nutrients through a liquid fully degassed digestate from manure. Both reactors operated under thermophilic conditions (55^0C) and the methanogens were enriched to convert CO_2 to CH_4 by addition of H_2. Five different periods of various gas feeding and recirculation rates were tested. Enrichment at thermophilic temperature (55^0 C) resulted in CO_2 and H_2 bioconversion rate of 920 $LCH_4/L_{reactor}$day for R2, which was 4% higher than that of R1 (537 $LCH_4/L_{reactor}$day. Biogas upgrading was tested under various operation conditions. The produced biogas had a maximum CH_4 content of 92% at steady-state, at gas feeding flow of 4,3 L/day (HRT=8 hours) and recirculation of 177,6 L/day.

Theoretical background

Need for Renewable Energy

The global scientific community has lately been facing strong concerns with respect to the sustainability of the future of our world due to the appearance of intense climatic changes, the lack of energy resources and the rural development in the coming years. It is scientifically proved that fossil fuel combustion and other land-use human activities cause the release of gases responsible for the greenhouse effect such as carbon dioxide (CO_2), methane (CH_4) and nitrous oxide (N_2O), which are deranging the Earth's climate. [1]

The Intergovernmental Panel on Climate Change (IPCC) Fourth Assessment Report underlined that the world's growing population and therefore per capita energy demand, are leading to the rapid increase in greenhouse gas (GHG) emissions. Above all, in the last 10 years, transport has shown the highest rates of growth in GHG emissions in any sector.[2]

It is widely accepted that solving those problems could be achieved only by combined actions, such as changes in behavior, changes in vehicle technologies, expansion of public transport and introduction of innovative fuels and technologies. It is globally admitted, that a substantial segment of fossil resources used as feedstocks for industrial productions could be replaced by plant-based raw materials (biomass). In the Renewable energy directive (2009/28/EC) biomass is defined as follows: "Biomass means the biodegradable fraction of products, wastes and residues from biological origin from agriculture (including vegetable and animal substances), forestry and related industries including fisheries and aquaculture, as well as the biodegradable fraction of industrial and municipal waste".[3]

The above mentioned concerns have greatly stimulated the interest in renewable energy sources and mainly bioenergy production from biomass. Nowadays, the European Union has special targets for increasing the share of bioenergy to 20% of the total energy consumption, with a share of 10% for renewable fuels in the

overall transport fuel supply. The abundance of biomass makes it one of the world's most important sources of renewable energy and production of biofuels.[4] Therefore, biofuels and more precisely production of biogas is considered to be an emerging alternative energy technology. Biogas is envisioned as a key element in emerging renewable energy strategies in Europe, motivated by the European Union target of achieving 20% renewable energy by 2020. [5]

Biofuels

A biofuel is produced through contemporary biological processes, such as agriculture and anaerobic digestion of organic waste, rather than a fuel produced by geological processes. It can be derived directly from plants or indirectly from agricultural, commercial, domestic, and/or industrial wastes.

Presently, biofuels can be identified as 1st and 2nd generation biofuels. First generation biofuels are produced from raw materials in competition with food and feed industries, a fact that provokes ethical, political and environmental concerns. As a result, the second generation biofuels, also known as advanced biofuels (i.e. from raw materials based on waste, residues or non-food crop biomass) were developed. They gradually obtained an increasing world- wide interest as a likely ‘‘greener” alternative to fossil fuels and conventional biofuels and led to the belief that the use of biomass in bio refinery complexes is expected to ensure additional environmental benefits and implement national energy security, thanks to the coproduction of both bioenergy and high value chemicals. Some examples of biofuels are: ethanol, methanol, biodiesel, bio butanol, syngas, biogas etc. [6]

Biogas

Important issues related to human and animal health and food safety require increasingly sustainable solutions for handling and recycling of animal manure and organic wastes. That’s where biogas from anaerobic co-digestion of animal

manure, combined with pre- and post-treatment technologies, play a very important role.

Biogas is one of the most important biofuels used in the emerging market for renewable energy, because as a clean and CO_2-nutral energy carrier, it can make an important contribution to increase renewable energy's share in energy supply. It is estimated that a major part of the EU-27 renewable energy target by 2020 will be met by bioenergy, at least 25% of which will be biogas. In addition, the global capacity for power generation from commercial biogas facilities will be more than double over the next decade increasing from 14.5 gigawatts (GW) in 2012 to 29.5 GW in 2022. [7]

Biogas production in Denmark and SYMBIO project

The history of biogas production in Denmark is long and not exempt of struggles. The research and establishment of farm scale biogas plants started already in the 70's while the first centralized biogas plant was constructed in 1984. Therefore, with 20 centralized plants and over 35 farm scale plants, the digestion of manure and organic waste is a well-established technological practice in Denmark. The Danish government proposed a target of using 50% of the manure produced in Denmark for renewable energy production by 2020, and it would essentially be met through a strong biogas expansion. [1, 4, 5]

During the last years, the Bioenergy Research Group at the Department of Environmental Engineering of DTU has been working in the **SYMBIO** project, in which the upgrading of the biogas is done via a biochemical process, using hydrogenothophic methanogenic microorganisms. The hydrogen supplied to the biochemical reaction is obtained through hydrolysis of water, carried out using the peak load/excess electricity from wind mills. This hydrogen is added to the biochemical reactor together with the biogas, and thus biologically converting the CO_2 in the biogas into methane. The main two objectives of the SYMBIO project are: 1) biogas upgrading and enhancement and 2) decoupling biogas production from biomass availability.[8]

Within the first objective, H_2 will be combined with the CO_2 in the biogas for biogas production and upgrading. Within the second objective, H_2 and waste-CO_2 from sources such as exhaust gas from combustion gas motors, or from ethanol production can be injected into anaerobic reactors to increase the biogas production and decouple biogas from biomass availability.

In such a process, biogas enhancement and upgrading will be achieved, giving synergistic advantages for both the overall renewable energy system with high share of wind power and for the biogas plants themselves. The effects of hydrogen on the biochemistry and microbiology of the process and the technical solutions for improving hydrogen utilization in the biogas reactors will be studied in order to optimize the conversion of hydrogen to methane.

System analyses and integrated system designs will be conducted to evaluate the environmental and economic impacts and design an optimized renewable energy system decoupled from excess biomass demands. This idea of the current project has never been applied and it offers several advantages:

- Contributes to lower upgrading cost of biogas
- Storage of wind power as methane using the existing natural gas grid/storage
- Possibility for flexible electricity production according to the energy demand variations.

Biogas definition

Biogas is produced after anaerobic digestion of mixtures of corn derived starch, manure, organic waste, grasses and residues. It follows a similar production path worldwide and in the last few years it has been strong implemented in countries with economic subsides for electricity generation from biogas (especially European countries). In some countries, biogas is also used as transportation biofuel, after upgrading to bio methane. [1] Biogas can be produced from nearly all kind of biological feedstock types, within these from the primary agricultural sectors and from various organic waste streams from the overall society. The largest resource is represented by animal manure and slurries from cattle and

pig production units as well as from poultry, fish, fur, etc. In the EU-27 alone, more than 1500 mill. tones of animal manure are produced every year.[9]

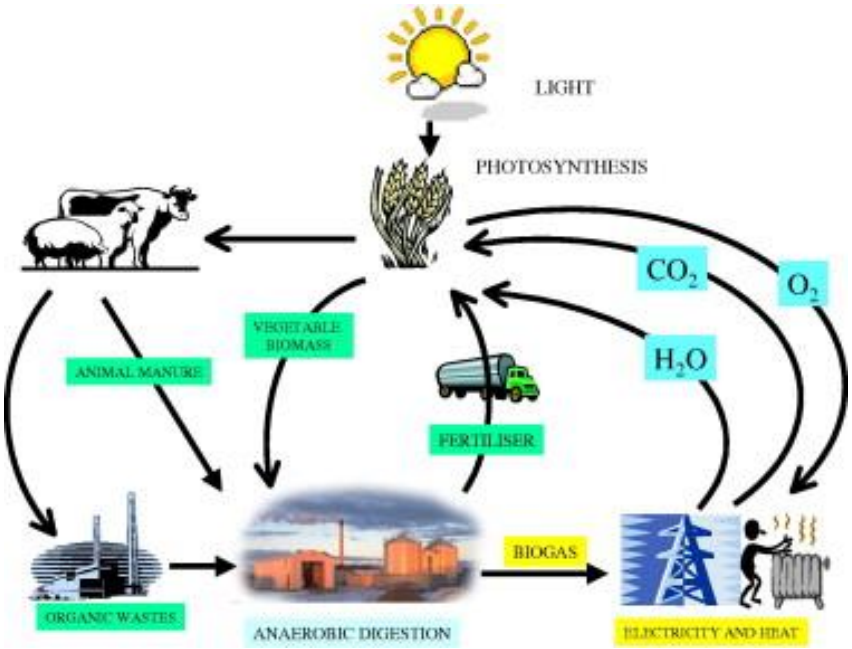


FIGURE 1: SCHEMATIC REPRESENTATION OF THE SUSTAINABLE CYCLE OF ANAEROBIC CO-DIGESTION OF ANIMAL MANURE AND ORGANIC WASTES.

As mentioned above, biogas produced from anaerobic digestion processes and landfill consists mainly of methane (CH_4) and carbon dioxide (CO_2). The substrate used, fermentation technology and collection method can all affect the production and composition of raw biogas. Besides CH_4 and CO_2, raw biogas also contains small amounts of ammonia (NH_3), hydrogen sulphide (H_2S), hydrogen (H_2), oxygen (O_2), nitrogen (N_2) and carbon monoxide (CO). Some of the impurities may have significant negative impacts on the utilization system, such as corrosion, increased emissions and hazards for human health. [10]

Biogas can be produced by anaerobic digestion with anaerobic bacteria, which digest material inside a closed system, or fermentation of biodegradable materials. As it is a renewable energy source, in many cases it exerts a very small carbon footprint. The gases methane, hydrogen, and carbon monoxide (CO) can be combusted or oxidized with oxygen. This energy release allows biogas to be used as a fuel; it can be used for any heating purpose, such as cooking. It can also be used in a gas engine to convert the energy in the gas into electricity and heat. Biogas can be compressed, the same way natural gas is compressed to CNG, and

used to power motor vehicles. In the UK, for example, biogas is estimated to have the potential to replace around 17% of vehicle fuel. It qualifies for renewable energy subsidies in some parts of the world. Biogas can be cleaned and upgraded to natural gas standards, when it becomes bio-methane.[11]

Biogas composition

The composition of biogas varies depending upon the origin of the anaerobic digestion process. Landfill gas typically has methane concentrations around 50%. Advanced waste treatment technologies can produce biogas with 55%–75% methane, which for reactors with free liquids can be increased to 80%-90% methane using in-situ gas purification techniques, as produced, biogas contains water vapor. The fractional volume of water vapor is a function of biogas temperature; correction of measured gas volume for water vapor content and thermal expansion is easily done via simple mathematics which yields the standardized volume of dry biogas.

In some cases, biogas contains siloxanes. They are formed from the anaerobic decomposition of materials commonly found in soaps and detergents. During combustion of biogas containing siloxanes, silicon is released and can combine with free oxygen or other elements in the combustion gas. Deposits are formed containing mostly silica (SiO_2) or silicates (Si_xO_y) and can contain calcium, sulfur, zinc, phosphorus. Such white mineral deposits accumulate to a surface thickness of several millimeters and must be removed by chemical or mechanical means. Practical and cost-effective technologies to remove siloxanes and other biogas contaminants are available.

For 1000 kg (wet weight) of input to a typical bio digester, total solids may be 30% of the wet weight while volatile suspended solids may be 90% of the total solids. Protein would be 20% of the volatile solids, carbohydrates would be 70% of the volatile solids, and finally fats would be 10% of the volatile solids.[9]

Compound	Formula	%
Methane	CH_4	50–75
Carbon dioxide	CO_2	25–50
Nitrogen	N_2	0–10
Hydrogen	H_2	0–1
Hydrogen sulfide	H_2S	0–3
Oxygen	O_2	0–0

FIGURE 2: TYPICAL COMPOSITION OF BIOGAS

Methanogens & Methanogenesis

The special microorganisms whose metabolic activity in anoxic conditions gives as a byproduct methane, are called **methanogens** and belong to the **archaea** classification, which differs from the bacteria. They are obligate anaerobic archaea and can be found in wetlands and in the digestive tracts of animals and humans, affecting the methane concentration of functions like belching or flatulence, and their main role is producing energy from the biosynthesis of methane.[12] The biological production of methane is also called methanogenesis and is performed by methanogenic archaea populations, which are necessary in anaerobic wastewater treatments. Methanogenesis plays a major role in the last step of the anoxic degradation of organic substances, transforming acetate, CO_2, and H_2 to methane (CH_4). There are many different kinds of methanogens, such as the extremophiles who can be found in hot springs, submarine hydrothermal vents or solid rocks of the Earth's crust, deep below the surface, but generally these lithotrophic microorganisms are widely distributed in oxygen-free environments and participate actively in the carbon cycle. [12, 13]

Methanogens' shape can be either spherical (coccoid) or rod (bacilli). They do not form a monophyletic group even though they all belong to Archaea, as there have been described over fifty different species of methanogens. They are unable to function under aerobic conditions because they are anaerobic organisms which need anoxic conditions. By anoxic conditions, it is meant an environment that lacks oxygen as methogens are sensitive even at trace level presence of

oxygen and cannot sustain oxygen stress for a prolonged time.[14] A group of methogens called hydrogenotrophic use as a carbon source, carbon dioxide (CO_2) and as a reducing agent hydrogen (H_2), so these hydrogenotrophic archaea, microorganisms bind CO_2 with H_2 and convert them to methane as follows:

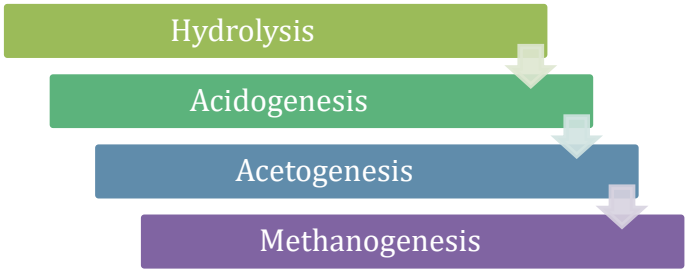
$$CO_2 + 4H_2 \rightarrow CH_4 + 2H_2O \ (1)$$

$$\Delta G^0 = -130{,}7 \ \frac{kJ}{mol}$$

The available hydrogen and some of the CO_2 react to produce methane, by creating an electrochemical gradient across cell membrane which can be used for generating ATP through chemiosmosis, unlike with what happens to plants and algae, which use as a reducing agent, water. In the cell walls of the bacteria it is found a polymer called peptidoglycan, which does not exist in those of Archaea and therefore in methanogens. However, some methogens' cell walls are composed of pseudo peptidoglycan whereas others have minimum one paracrystaloline array (S-layer) made up of fitting together proteins. The production through this way is known as hydrogenotrophic methanogenesis, and it is the CO_2 that accepts the electron and it is reduced to methane. This route contribute around one- third of total methane production in methanogenesis.[15]

Anaerobic Digestion Process

As mentioned above, anaerobic digestion takes place in an oxygen free environment by special microorgansims. The organic biowaste decomposes in four stages, which are shown below:



The general model for degradation of organic material (polymeric substances like carbohydrates, protein, and fats) under anaerobic conditions operates principally with three main groups of bacteria which together convert the organic material to methane, carbon dioxide and water. The fermentative bacteria hydrolyze the polymers to soluble oligomers- and monomers by action of extracellular enzymes. After that the dissolved products are taken up by the bacteria and fermented, forming acetate and other short-chain fatty acids, alcohols, hydrogen and carbon dioxide which are released into the environment. Short-chain fatty acids longer than acetate and alcohols are oxidized by the hydrogen producing acetogenic bacteria, resulting in the formation of hydrogen, acetate, formate and carbon dioxide. The end products from the metabolism of the fermentative and acetogenic bacteria, acetate, formate and hydrogen, are transformed into methane by the methane producing bacteria. This three step model for anaerobic transformation of organic material can be used to give an overall view, but it does simplify things. To have a more adequate model, other groups of bacteria which can play a major role under certain conditions must be considered.

The whole procedure is shown shortly by Figure 3 and explained more below.

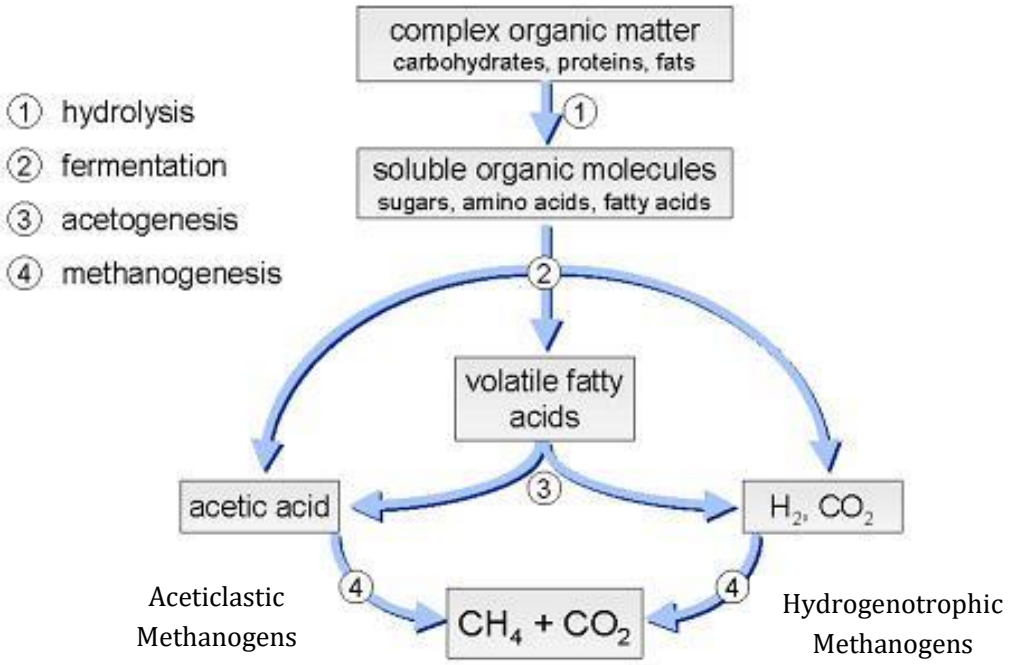


FIGURE 3: ANAEROBIC DIGESTION PROCESS

It is obvious from Figure 4 below that acetate is the most important source of methane in the anaerobic environment, giving rise to approx. 70% CH_4 while the remaining 30% is formed from hydrogen and carbon dioxide. The main part of the acetate and hydrogen is formed directly from the fermentative step of the anaerobic degradation process whereas approx. 30% are produced via intermediates.

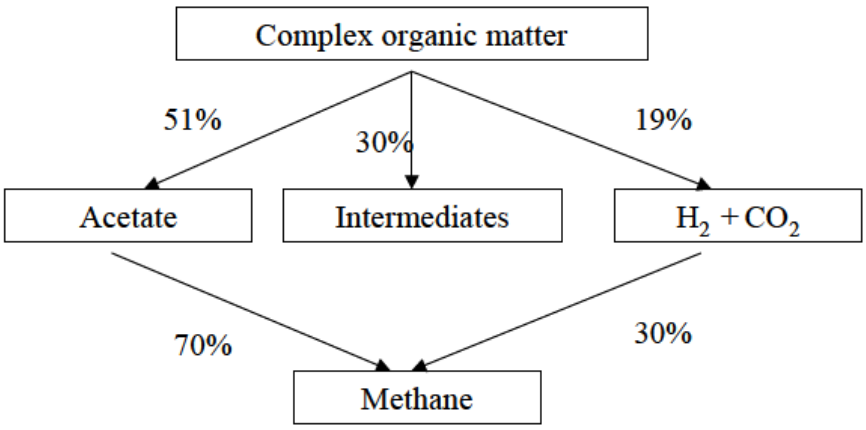


FIGURE 4: THE CARBON FLOW IN ANAEROBIC DEGRADATION OF COMPLEX MEDIUM.

Hydrolysis

The first step of the anaerobic digestion process is the hydrolysis reaction, where the particulate organic substrate is transformed into liquefied monomers and polymers via an extracellular process, done by enzymes released by the fermentative bacteria . Specifically, organic waste is mainly composed of proteins, carbohydrates and fats, which are hydrolized and transfomed into amino acids, monosaccharides and fatty acids respectively. The necessary enzymes are produced by:

- **Proteolytic bacteria**, which produce proteases, that catalyze the hydrolysis of proteins into amino acids
- **Cellulitic and Xylanollitic bacteria**, which produce cellulases and xylanases that degrade cellulose and xylan to glucose and xylose, respectively
- **Lipolytic bacteria**, which produce lipases that convert lipids to glycerol and long chain fatty acids.[16]

Acidogenesis

During this step, the amino acids and sugars produced during the hydrolysis step, are transformed into volatile fatty acids (VFA), alcohols, CO_2 and Hydrogen.

Also ammonium is released from the amino acids. The long-chain fatty acids are converted during the next step. Both, oxidized (such as acetate) and reduced (such as propionate and butyrate) compounds are produced during this step. Mostly acetate and hydrogen are produced directly when the reactor is operating in stable conditions, but when the reactor is overloaded (high concentration of acetate or hydrogen or pH extremes), the process goes towards more production of more reduced forms, such as propionate, butyrate and ethanol. Specifically, acidogenic bacteria transform the products of the first reaction into short chain volatile acids, ketones, alcohols, hydrogen and carbon dioxide. The principal acidogenesis stage products are propionic acid (CH_3CH_2COOH), butyric acid ($CH_3CH_2CH_2COOH$), acetic acid (CH_3COOH), formic acid ($HCOOH$), lactic acid ($C_3H_6O_3$), ethanol (C_2H_5OH) and methanol (CH_3OH), among other. From these products, the hydrogen, carbon dioxide and acetic acid will skip the third stage, acetogenesis, and be utilized directly by the methanogenic bacteria in the final stage.

Acetogenesis

During the acetogenesis, the compounds produced in the fermentation, are oxidized to acetate by obligate hydrogen producing acetogens (therefore this stage also produces hydrogen). They are "obligate" hydrogen producers, since there are no other electron acceptors than free hydrogen ions, which can use the electrons released during the oxidation. Here, also long-chain fatty acids are degraded to acetate by a process called oxidation. Throughout this step, hydrogen concentration is crucial, since "The free energy of reaction for fatty acid oxidation is positive at standard conditions and therefore the reaction needs very low hydrogen concentrations to achieve a negative free energy (and thereby yield energy for anabolism)". [17]

Methanogenesis

The next step of anaerobic digestion (AD), methanogenesis, which consumes hydrogen, helps to keep hydrogen concentration in balance. Even so, the proper range of hydrogen concentration for both processes is very narrow. Concentration of acetate also plays a role in this equilibrium, but since it is produced at a lower stoichiometrically level, it is not the limiting factor. As a result of this, the transfer of H_2 between producers and consumers is critical. Under specific conditions other microorganisms can be present, deviating the main path of the process.[18] The most important methane precursor is acetate (70%), while the remaining 30%, is formed from H_2/CO_2 or formate. The methanogenic bacteria are divided into two main groups: the aceticlastic methane bacteria, degrading acetate, belonging to the genera *Methanosarcina* and *Methanosaeta* (formerly *Methanothrix*), and the hydrogen consuming methanogens (hydrogenotrophic methanogens) of which an array of genera exists. A number of *Methanosarcina* species can transform hydrogen as well as acetate. Substrates of less quantitative importance for methanogens are: methanol, methylsulfides, methylamines and some higher alcohols. Methanogenesis is regarded as the motive force of the anaerobic degradation as it is an energy producing process under standard conditions, as opposed to some of the other processes in the anaerobic degradation. Furthermore, it is the terminal step required for complete mineralization.

Parameters affecting the anaerobic process.

Anaerobic digestion is a microbiological process, so several factors affect the process performance. Among others, the most important are nutrients, temperature, pH level and some inhibitory factors that are described below.

Nutrients

The chemical composition of the cellular material highly reflects which nutrients a microorganism needs. Hydrogen, nitrogen, oxygen and carbon are the main ingredients in organic material. Sulphur is necessary for synthesis of the amino

acids, cysteine and methionine. Phosphorus is found in nucleic acids, phospholipids, ATP, GTP, NAD and FAD. Potassium, calcium, magnesium and iron are required as cofactors for enzyme activity and as components in metal complexes. Sulphide and ammonia are the normal sulphur and nitrogen sources for the microorganisms in the anaerobic reactor, but also organic compounds such as amino acids and urea can supply the microflora with these nutrients. Ammonia which is present in high concentrations in especially chicken manure and mink droppings will be inhibitory if it is present in much higher concentrations than necessary. Pig manure can also contain high concentrations of ammonia, but this depends on the content of solids. These 10 elements must be present in concentrations around 10-4 M. In addition to the ten macro-nutrients a number of other elements should be present in small amounts (below 10-4 M). Especially Ni and Co are important for growth of anaerobic organisms. Nickel is necessary for activating factor F430 (a co-factor involved in the methanogenesis), but in high concentrations nickel can be inhibitory for fermentative as well as methanogenic bacteria. Addition of iron can stimulate the precipitation of phosphates which could otherwise have precipitated important trace metals, and thereby stimulate the process. Calcium, magnesium, sodium and potassium can in high concentrations be inhibitory, but can result in a stimulation of the fermentation if they are added in low concentrations. Improved digestion by addition of the above mentioned monovalent and divalent cations in concentrations between 0.01 M and 0.005 M has been shown. An important fact is that there is a synergistic and antagonistic connection between the cations. Sulphide and phosphate can have an influence on the concentrations of the metal ions in the liquid phase via precipitation reactions. Generally, in domestic animal wastes and manure the micro and macro nutrients which are important for the anaerobic degradation are present.[19]

Temperature

Choice of temperature and control of the level in question are of crucial significance for anaerobic digestion. Temperature has influence on both, physicochemical parameters and the microbiological processes. Regarding the

first, increasing temperature will decrease viscosity (improving mass transfer processes), increase diffusivity (improving the gas-liquid transfer rate), and change the thermodynamic equilibriums (acid-base dissociation coefficients and gas liquid equilibrium). Solubility of gases decreases when increasing temperature, it increases solubility of solids (increasing availability of solids) and fats are melted (emulsified). Most experiments with anaerobic digestion have been done in the mesophilic (30-40◦C) and in the thermophilic (50-60°C) temperature range. The thermophilic process provides a number of advantages compared with the mesophilic:

- reduction of the residence time in the plant
- good destruction of pathogenic organisms
- improved possibility for separation of solid matter from the liquid phase
- better degradation of long-chain fatty acids
- less biomass formation compared with the product formation
- improved solubility and availability of substrates

And essential disadvantages are,

- larger degree of instability
- demand of larger amount of process energy
- larger risk of ammonia inhibition

The most essential advantage of thermophilic operation of joint biogas plants is the destruction of pathogens, which is improved compared to mesophilic operation. Process optimization has allowed development of more continuous feeding regimes, and with thermophilic conditions, sufficient sanitation of the biomass occurs. However, control must always be maintained on the period of time between additions of undigested material and removals from the reactors to insure adequate sanitation time. Even though the investigations with thermophilic pure cultures and co-cultures have shown an optimum temperature near 60°C, it can be wise to keep a lower temperature (52-58°C) in thermophilic biogas plants. This insures a safety margin compared with the negative effects a temperature increase above 60°C can give. Temperature influences the toxicity of ammonia: Toxicity increases with increasing

temperature and can be relieved by decreasing the process temperature. However, when decreasing the temperature to 50°C or less, the growth rate of the thermophilic microorganisms will drop drastically, and a risk of washout of the microbial population can occur due to a growth rate lower than the hydraulic residence time. A well-functioning thermophilic reactor can either be loaded to a higher degree or operate at a lower retention time than a mesophilic reactor. The background of this is that the growth rates for thermophilic organisms are higher than for the comparable mesophilic species. Experiments indicate that at high loading or low retention times, a thermophilic digestion gives a larger gas yield and a higher transformation than a mesophilic digestion. All other parameters in the two reactors are generally similar. The viscosity of the digesting compounds is inversely proportional to the temperature. The substrate is more liquid at high temperatures and the diffusion of dissolved material is thus facilitated. The solubility of various components (NH_3, H_2, CH_4, H_2S, and VFA) also depends on the temperature. This can be of great significance for material which have an inhibiting effect on the process.[10]

There are mainly three microbial operating temperature regimes (psycrophilic, mesophilic and thermophilic methanogens). There is an optimum temperature for the growth rate in each regime. The growth of methanogens in the three temperature regimes is shown in Figure 5 below[11]:

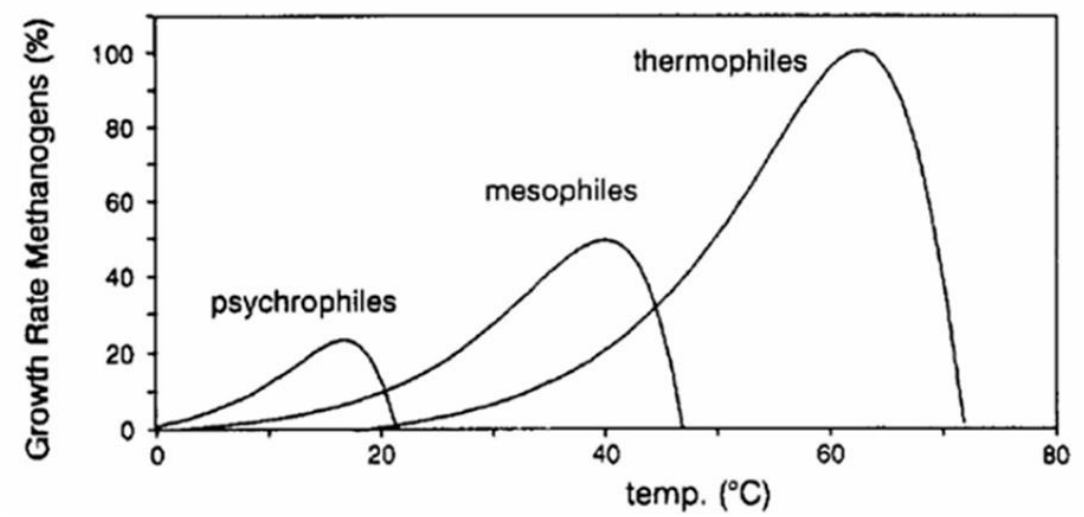


FIGURE 5: RELATIVE GROWTH RATE OF PSYCHROPHILIC, MESOPHILIC AND THERMOPHILIC METHANOGENS.

pH

Methane formation is limited to a relatively narrow pH interval from approx. 5, 5 to 8, 5. Most methanogens have a pH optimum between 7 and 8 while the acid forming bacteria often have a lower optimum. Apart from the influence of the pH on the growth of the microorganisms, pH can affect other factors such as dissociation of important compounds (ammonia, sulphide, organic acids) of importance for the anaerobic digestion process. Optimal pH for mesophilic biogas reactors is between 6,5 and 8,5 and the process is severely inhibited if pH is below 6 or above 8,5. The solubility of carbon dioxide in water becomes smaller at increasing temperature. The actual pH value in thermophilic biogas reactors is therefore also generally higher than in mesophilic plants as dissolved carbon dioxide forms carbonic acid by reaction with water. No specific investigations of the significance of this phenomenon exists. The pH in anaerobic reactors is mainly controlled by the bicarbonate buffer system. Therefore pH in biogas plants depends on the partial pressure of CO_2 and the concentration of alkaline and acid components in the liquid phase. Ammonia produced during degradation of proteins, or ammonia in the feed stream, can e.g. result in an increase in pH. [19, 20]

Process Imbalance and Inhibitors.

As explained previously, since the process that is being studied is biochemical, number of factors may affect the stability of the system. The four main parameters that could lead in a process imbalance are as follows:

- Hydraulic overloading: microorganisms have not enough time to grow and are washed out of the reactor.
- Organic overloading: biomass cannot degrade all the substrate that is fed to the reactor.
- Inhibition of the process: some compounds in certain concentrations could cause the inhibition of the process.
- Sudden changes in process parameters.

Thereby, it will be necessary the control of all these parameters through several variables that show the imbalance of the anaerobic digestion.

Hydraulic overloading arises when important bacteria cannot grow fast enough and are therefore washed out of the reactor. Especially acetate and propionate-degrading organisms grow slowly and can easily be washed out. Hydraulic overloading arises in practice if the efficient reactor volume is diminished, e.g. due to bad stirring, accumulation of sludge or sand or by a sudden increase in the volume of substrate pumped to the reactor (combined hydraulic/organic overloading).[21]

Organic overloading arises when more substrate is fed to a reactor than the microorganisms can degrade in their normal balanced fashion, e.g. when extra substrate is added or when the VS content of the substrate is increased. The slowest processes of the overall degradation will act as bottlenecks, the substrates of which will accumulate in the reactor.

Toxic material added to the reactor can be compounds found in the original material fed to the reactor or substances produced during the anaerobic process. This can happen e.g. by incorporation of protein rich industrial wastes, which will produce large amounts of ammonia, or by incorporation of waste containing fat which can produce toxic concentrations of long-chain fatty acids (LCFA). The anaerobic process will react differently dependent upon the reason for the process imbalance. The response of the process to toxic material will depend on several things, including which microorganisms are poisoned and thereby which link in the metabolic chain is affected. For example, addition of heavy metals leads to an accumulation of VFA, indicating that the fermentative bacteria are less sensitive than the acetogenic and methanogenic bacteria. Alternatively poisoning with LCFA resulted in essentially no accumulation of VFA, which points to the fact that the fermentative bacteria were sensitive to long-chain fatty acids. Acetogenic and methanogenic bacteria are however also sensitive to LCFA. Sudden changes. Even if conditions of the digestion process are changed within normally acceptable limits, these changes can have serious effects if they are introduced too sudden (e.g. feed rate are not increased gradually, ammonia

loading suddenly increased). Also sudden variations in temperature and pH may be crucial. To register the imbalance in the microbial process it is necessary to measure important intermediates and terminal products during the process. This can take place either by measurements in the gas or in the liquid phase. Liquid phase measurements have until now been limited due to time consuming analyses and the presence of interfering compounds in the waste, making extensive sample preparation necessary. Among the disadvantages of analyzing the liquid phase is that it can be difficult to obtain a representative sample from the reactor, i.e. that the stirring has to be efficient and the substrate homogenous - which is often not the case for biogas reactors. With gas phase measurements, chemical and physical conditions as mass transfer between liquid and gas will have to be considered. The advantage of gas phase measurements is that sampling is easy and sample preparation demands are few. Finally, the composition of the gas will be the same in the entire headspace of the reactor.[22]

Biocidal inhibition

Anaerobic process can be inhibited by certain toxic compounds that could exist either in the influent or in the by-products that are produced during the digestion. Sometimes the microorganisms can be adapted to these conditions, but in other cases these conditions can be toxic and affect their functions. Some of the inhibitors are presented as follows:

- *Ammonia:* this compound is produced when the nitrogenous matter is degraded and it seems that methanogens activity is the most affected of the microorganisms. Since the substrate, inoculum, environmental conditions and acclimation varies from one process to another, ammonia allowable concentration ranging from 1,7 to 14 g/l.
- *Sulfide:* it is produced when the sulfate that is in the influent stream is metabolized by the sulfate reducing bacteria.
- *Heavy metals:* The most important compounds in this inhibitor's group are chromium, iron, cobalt, copper, zinc, cadmium and nickel. The main problem is that they cannot be degraded so they will be accumulated.

- *VFAs:* They are formed after hydrolysis of complex organic compounds and its accumulation can inhibit the process. The concentration of these compounds can increase due to the drop in the methanogens activity leading in the process imbalance.
- *Organic compounds*: They can be accumulated due to the low solubility that they have in water and also because of the adsorption that can take place to the surfaces of sludge solids.[23]

Biostatic inhibition

This is a kind of reversible inhibition that affects the intracellular conditions of redox potential, pH and total salts. Biostatic inhibition is caused by free acid and bases (for example, VFAs, H_2S or NH_3), salts, and pH changes. Acetogenic, hydrogen utilizing, and especially aceticlastic microbes are particularly susceptible to biostatic inhibition. The main kind of biostatic inhibition is pH inhibition. When pH is outside the range for energy-limited microbes such as acetogens and methanogens, the energy available for anabolism decreases and directs it towards maintenance. Moreover, if the partial pressure of hydrogen is too high, VFA concentration will also increase slowing down the production of acetic acid and causing the dropping of pH as well.

Product inhibition

An increase in products concentration can cause a drop in the energy available from catabolism. An example of products inhibition is the hydrogen inhibition in the acetogenesis step. Specifically, product overloading might cause the process imbalance leading in an increase in gas production followed by a sudden drop of it, compare to the volume of gas that is expected. Methane content will also be decreased while carbon dioxide concentration will be higher due to the poor activity of hydrogen utilizing methanogens.

Anaerobic Technology for Bioenergy Production

The choice of the appropriate anaerobic digestion technology, depends on the objective of the digestion. A general classification of the anaerobic reactors is low (or standard)-rate and high rate reactors. The difference between both is the retention of the microorganisms (SRT). The continuous stirred tank reactor CSTR with no biomass retention/recycling are classified as low-rate (HRT=SRT=12-40 days). A detailed classification of the different types of anaerobic reactors is presented in figure below:

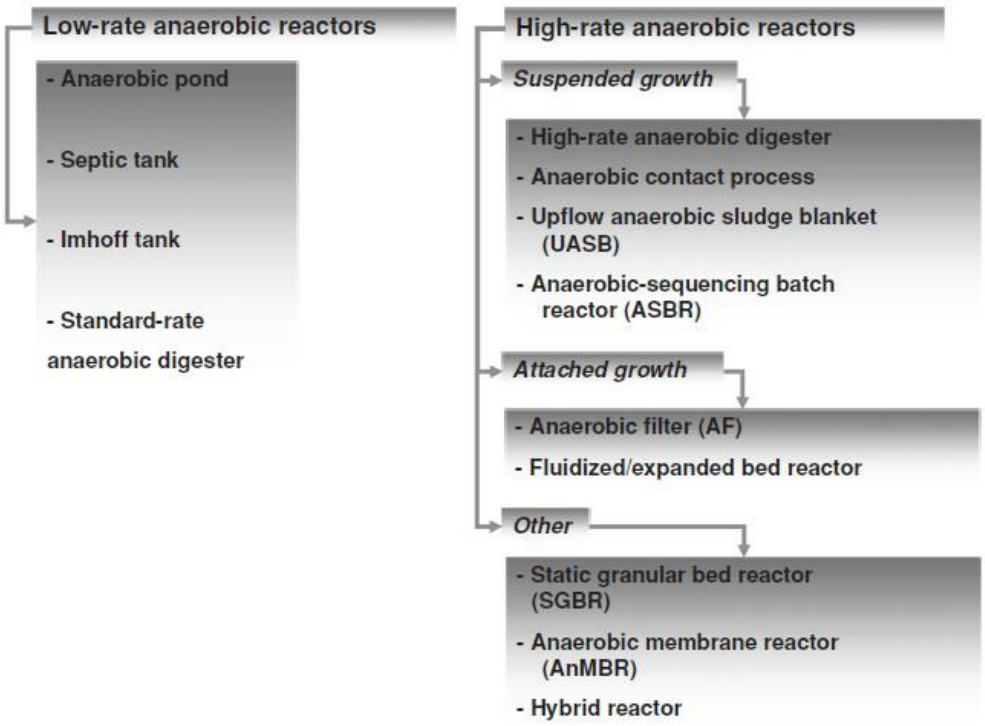


FIGURE 6: CLASSIFICATION OF DIFFERENT TYPES OF ANAEROBIC DIGESTERS

A key factor in the design of bioreactors for bioenergy production, is to decouple the hydraulic retention time (HRT) from the solids retention time (SRT). In this way, preventing wash out of slow-growing anaerobes, and at the same time allowing reduction of reactors volumes. The high-rate reactors have biomass retention in the reactor, either by packed bed/biofilm, granules, or sludge

recycling. The high-rate reactors have SRT >>HRT, and HRT is from several hours to a few days.[24]

Up-flow Anaerobic Sludge Blanket Reactor (UASB)

The UASB was first used by Lettinga and coworkers in the 1970s in Netherland to treat sugar rich substrate.[11] UASB is a system where the biomass grows in granular form, which means that the microorganisms form aggregates, leading in a high SRT and OLR. The characteristics of the sludge largely depend on the conditions during the start-up period as well as the wastewater treated and the inoculums used. In this configuration, certain hydraulic and organic loading conditions are manipulated in order to promote the growth of biomass granules with good settling characteristics. In this way the HRT and SRT are highly decoupled. This technology is suitable for biogas production out of high-strength soluble feed stream. [25]

As can be seen in Figure 7 below, apart from the sludge bed and the up-flow of wastewater, UASB contains a three phase separator in order to avoid the granules release, getting also the separation of the gas produced from the liquid phase. Recirculation could exist, either of the gas or the liquid, to facilitate the movement of the granules and achieving a better contact between biomass and substrate. The gas bubbles can also help out in this aim.

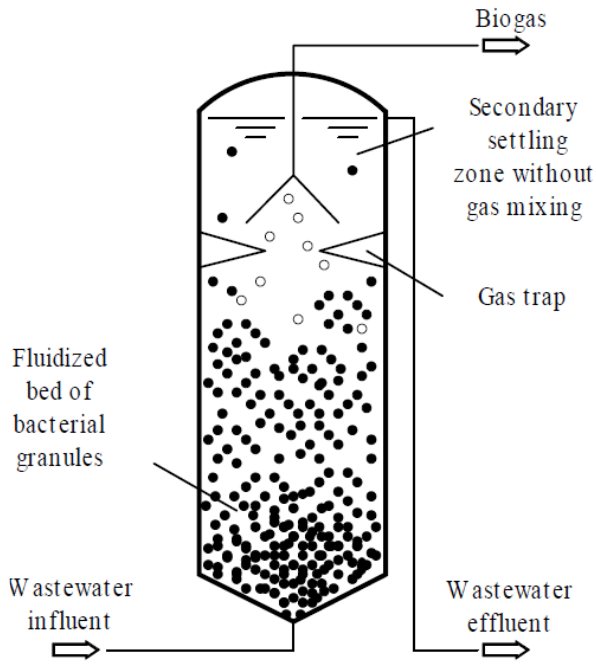


FIGURE 7: DIAGRAM OF AN UP-FLOW ANAEROBIC SLUDGE BLANKET REACTOR

More specifically, a characteristic feature of the UASB reactor is that no inert carrier is needed. Instead immobilization is achieved via the micro-organisms ability to self-immobilize on each other, thus forming dense aggregates, granules, which are kept in the reactor due to their relatively high density. This self-immobilization process can be initiated and partly controlled through the proper choice of liquid flow conditions and gas production rate. The granular sludge in the UASB reactor typically consists of nearly spherical particles of 1 to 5 mm in diameter. These particles (granules) consist of dense packets of the mixture of microorganisms responsible for the anaerobic degradation. The exact mechanism of granulation is still only partly understood, and successful operation of a UASB reactor thus depends on a number of factors including the specific substrate used, source of inoculum, operating conditions and waste water composition. The UASB reactor is far the most widely used high rate anaerobic system for treatment of wastewaters. As mentioned above (Figure 7: Diagram of an up-flow anaerobic sludge blanket reactor UASB reactor has four major components: a sludge bed, a sludge blanket, a gas-solids separator/trap and a secondary settling compartment. The sludge bed is a layer of granulated biomass settled at the bottom of the reactor. [26]The sludge blanket is a suspension of sludge particles mixed with gas produced in the process. Influent wastewater

enters the reactor at the bottom and is biologically degraded in both the sludge bed and the sludge blanket. In the UASB reactor linear flow velocity applied is approx. 1 to 2 m/hours. Produced gas is separated from the liquid by the gas-solids separator. A gas free secondary settling zone is created in the top compartment, where most of the sludge particles that have entered this zone (carried out of the bed by gas convection) will settle back to the reactor, while the rest, i.e. the smallest, are washed out with the effluent. Compared with other advanced anaerobic systems such as anaerobic filters and fluidized bed reactors, the UASB process is able to retain a high concentration of biomass with a high specific activity and to handle high organic loading rates with good COD removal. Furthermore, very efficient mixing can be achieved, as opposed to the fixed film systems where channels and inactive zones might develop. Capital costs for the UASB process are lower than those for other anaerobic processes since the separation of gas, liquid and solids takes place entirely in the reactor and no support medium for bacterial attachment is required. In addition, the process does not have the clogging problem of attached growth systems or the high-energy requirements of fluidized and expanded bed reactors. The primary draw-back is that the mechanism of forming granules is not fully understood, requiring experience or tests before applying the process on new types of waste waters, to ensure that granules will form. Various types of organic material in wastewater, both soluble and partially soluble, have been treated in UASB reactors. They include wastewater from the sugar industry, potato processing, apple processing, fruit and vegetable processing, brewery, alcohol distillery, slaughterhouse, meatpacking, paper mill, soft drink, starch and yeast industry. For soluble waste types, high organic loading as well as high hydraulic loading may be applied whereas moderate loading (approx. 0.5 kg COD/m^3/day) has to be used for wastewaters containing some amount of particles. [26-28]

To sum up, some of the advantages of this system are the less investment, compare to other anaerobic reactors such as bio filter, and the high COD removal, that goes up to 60%. In contrast, a long start-up period is required, during which, some microorganisms could be washed out.[29]

Expanded Granular Sludge Bed Reactor (EGSB)

A newer type of granular bed reactor is the EGSB reactor. This type of reactor is characterized by having an expanded form of granular sludge, obtained as a result of ultrahigh flows through the reactor. Linear flows in the EGSB reactor are 3 to 10 m/h, and can be as high as 15 m/h (compared to 1-2 m/h in an UASB reactor).[30] The system is relatively robust against suspended solids. The influent suspended solids are washed out through the granular bed and leave the reactor with the effluent. The suspended solids, due to the short retention time in the reactor, do not reach to be hydrolyzed to any significant degree. However, the EGSB reactor seems to be efficient to remove soluble organic matter, due to the good contact between the influent organic matter and the granular biomass. The EGSB reactor seems to be particularly useful at lower temperatures and low strength wastewaters, where the flow induced mixing compensates for the reduced gas mixing when biogas production is low, on which the UASB reactor is more dependent. [31]

Aim of the project

The aim of the project is the development of an innovative system for ex-situ biogas upgrading. It will be based on the comparison of two different configurations of reactors to perform ex-situ hydrogenotrophic methanogenesis. The establishment of steady operational conditions were a prerequisite to start the process. The goals of this project were:

- The investigation of the effect of using packing material in the reactor.
- The determination of key parameters affecting the biogas upgrading process, such as the determination of the most suitable values for parameters such as gas recirculation and gas flow rate.

Outline of the thesis

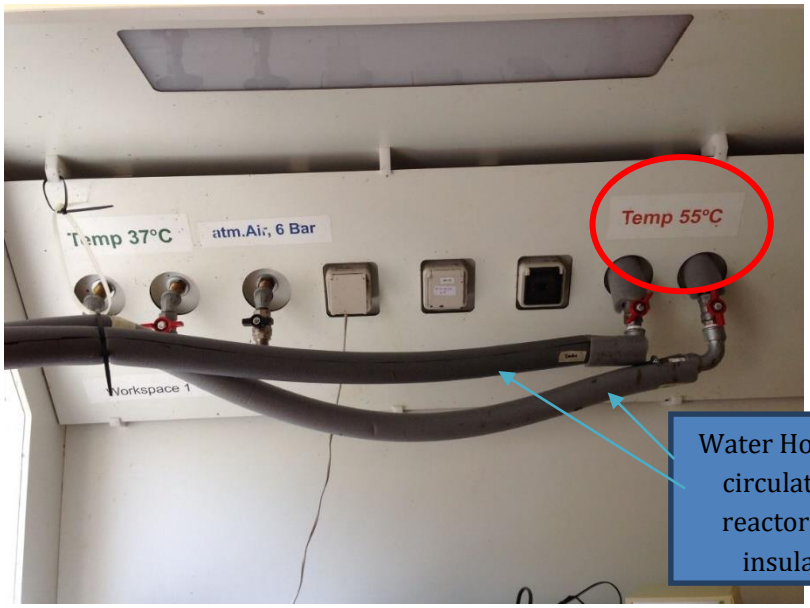
In attempt to provide some answers to the questions arisen, experiments were carried out to determine the best operating conditions. Two up-flow UASB reactors, denoted as R1 and R2, were used, one as a control reactor and one filled with packing material, respectively. In these experiments, thermophilic conditions were tested, which according to previous work done were proven to be the most efficient. The project was divided in five periods of various hydraulic retention times (HRT) and gas recirculation rates in order to define the highest methane production. The comparisons made were between the different HRT and recirculation rates considering the performance of each reactor. Moreover, the impact of packing materials on the performance of the reactor was studied. The process was monitored at daily basis and samples for microbial analyses were taken regularly throughout the process. In this work, most suitable HRT and gas recirculation rate were determined and it was shown that packing material could increase the yield of methane.

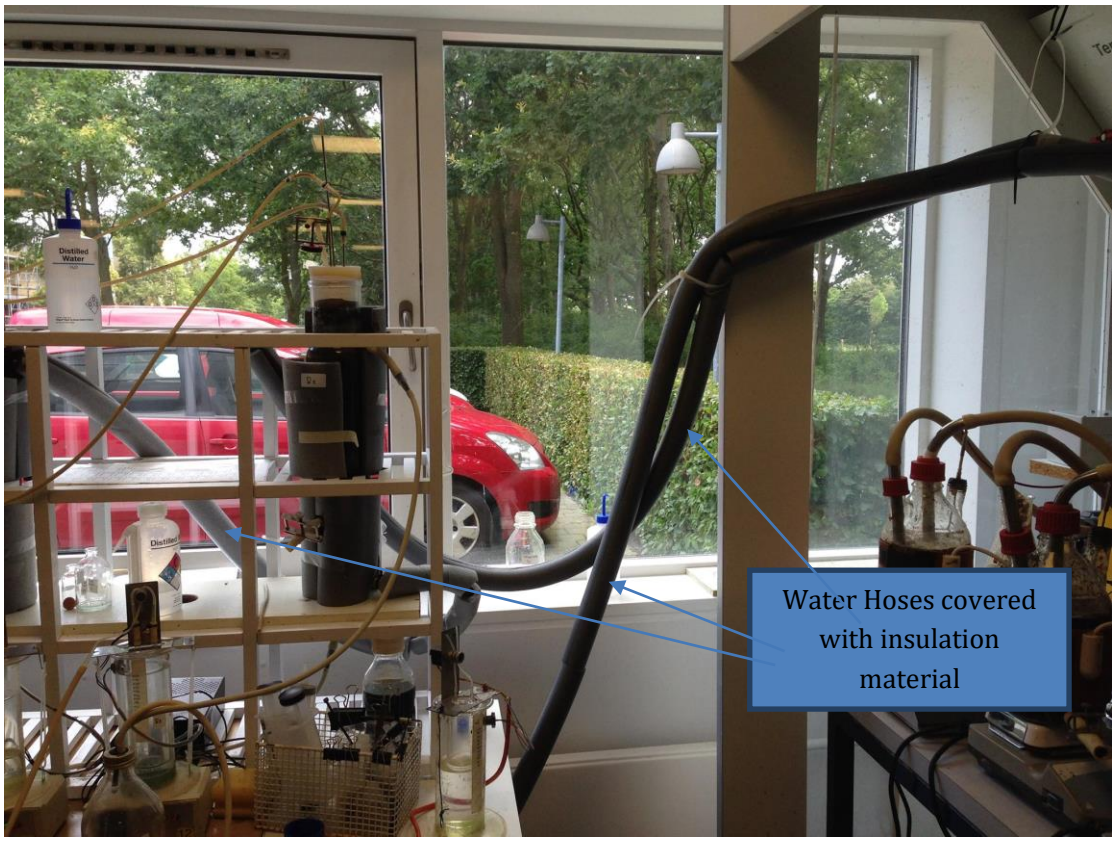
Materials and Methods

At the beginning of the project, a period of about two months was needed so as to collect all the necessary materials and equipment and to assemble the experimental setup. The calibration of each piece of equipment (pumps, gas meters) was also made during the period above.

Setup description

The setup consisted of two bench-scale, cylindrical, anaerobic, up-flow UASB reactors. Each reactor had a working volume of 1,4 L and was surrounded by an empty space, where warm water was circulating at 55^{o}C. They had one output on the bottom, which served as entrance for the liquid feeding, one output in the middle for the liquid sampling (pH, VFA, temperature), two outer outputs for the water circulation and one lid (rubber stop) on the top, where all the tubes were connected. The first reactor, R1, was empty and the second, R2, was filled with packing material, which was safely held between the working volume limits (1,4 L) by two small safety nets, one placed on the bottom and one on the upper part in the inside of the reactor. Two large hoses were permanently and firmly connected with the lab's water supply (55^{o}C) and with the reactors serving water circulation. The difference in pressure caused by two taps which were one open and one closed ensured the circulation of the water. Both reactors and water hoses were covered with insulating material to prevent heat loss and thus maintain the temperature stable. The configuration described is depicted in the following pictures:

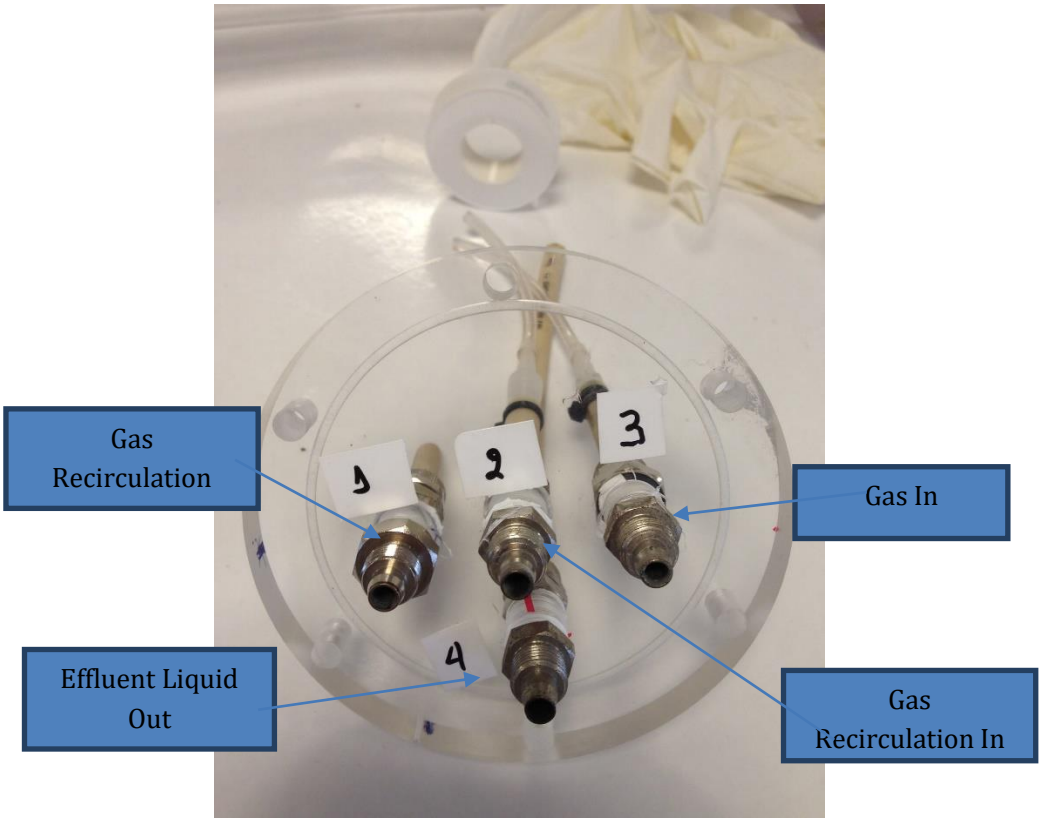




The lid of each reactor consisted of four different sockets:

1. One for the tube of the gas recirculation outlet
2. One for the tube of the gas recirculation inlet
3. One for the tube of the gas feeding (CO_2, CH4, H2)
4. One for the tube of the liquid outlet.

The last tube was placed in order to keep the liquid working volume at exactly 1,4 L. Its length touched exactly the liquid surface at the volume of 1,4 L so when the volume exceeded this point it went out through that tube, maintaining the volume of the reactor constant. The lid is shown in the picture below:



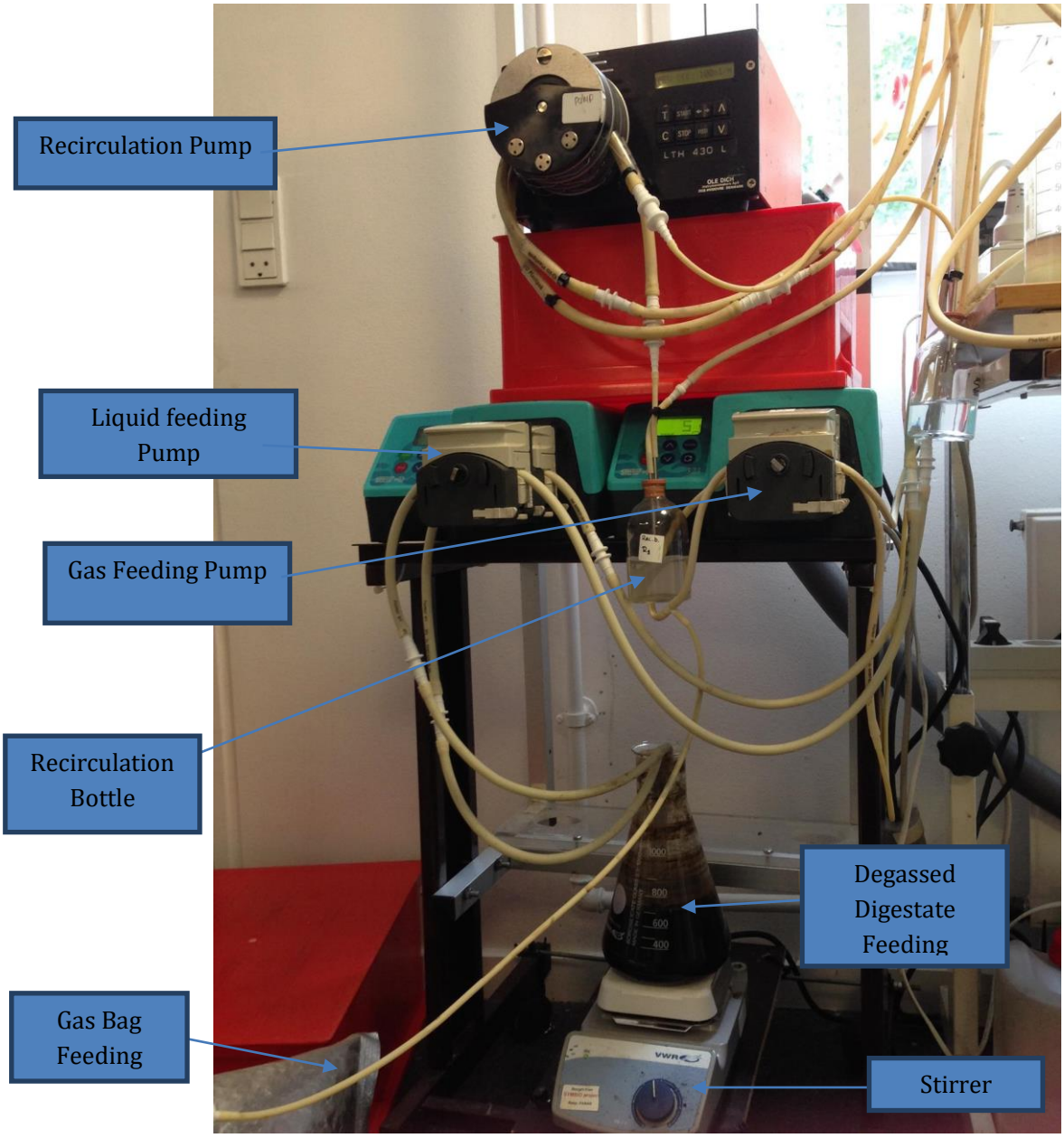
Once the lid was completed and the tubes were assembled, the lid with the tubes were placed on the top of each reactor and sealed with a rubber stop. Tubes 1 (recirculation out) and 3 (gas feed) were connected with diffusers, placed at the inside bottom part of each reactor to distribute the gas through an up-flow motion.

The gas feeding tubes, were connected with the gas feeding pump, which was the same for both reactors and afterwards they were connected to the gas bag. A sampling output was placed in between to check the composition of the gas that was passing by at any moment.

The recirculation tubes (inlet and outlet) of each reactor, were connected to a recirculation bottle flashed with nitrogen, to achieve anaerobic conditions. Then

they were connected to the recirculation pump which was also the same for both reactors.

The whole pump system is depicted below:

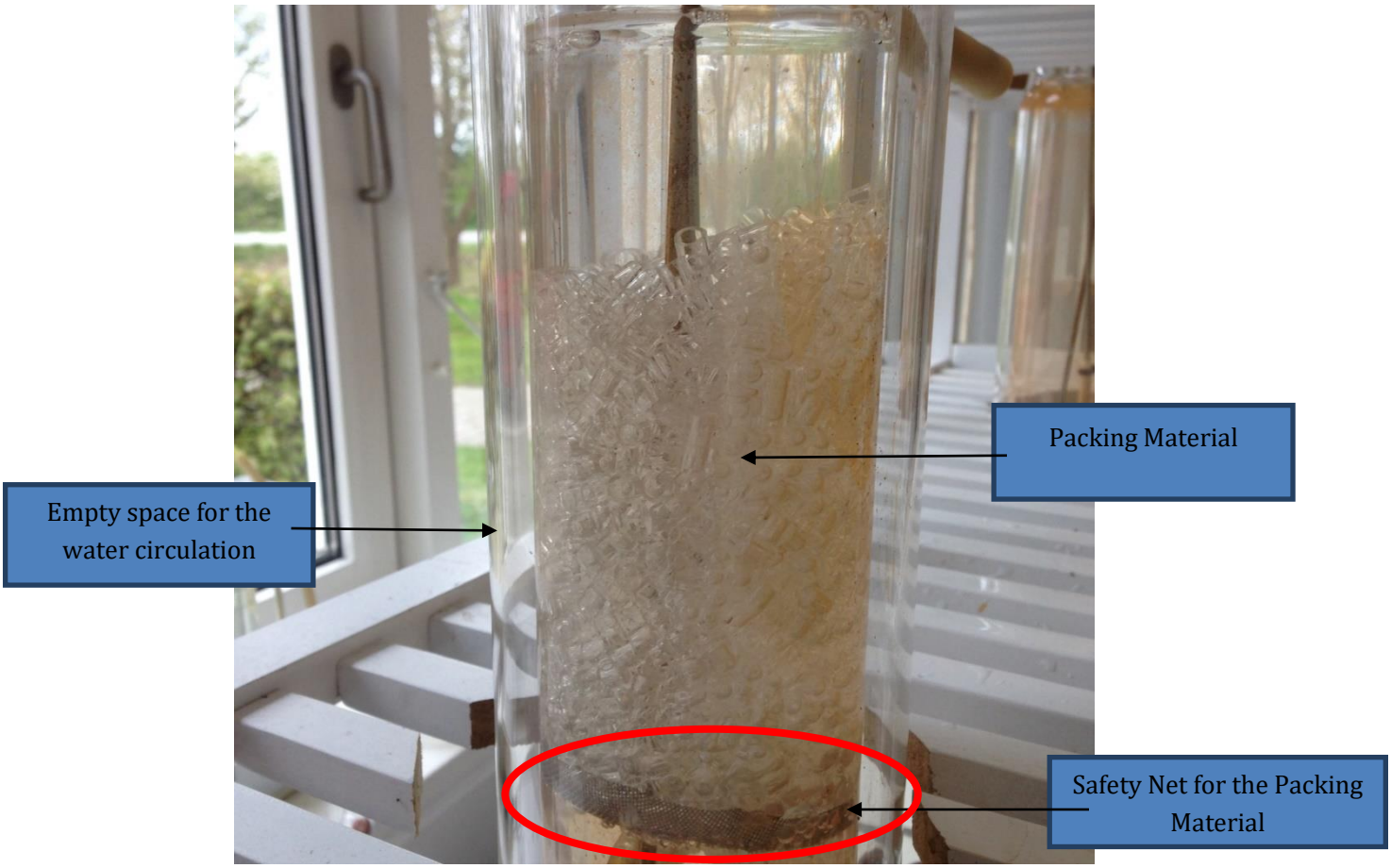


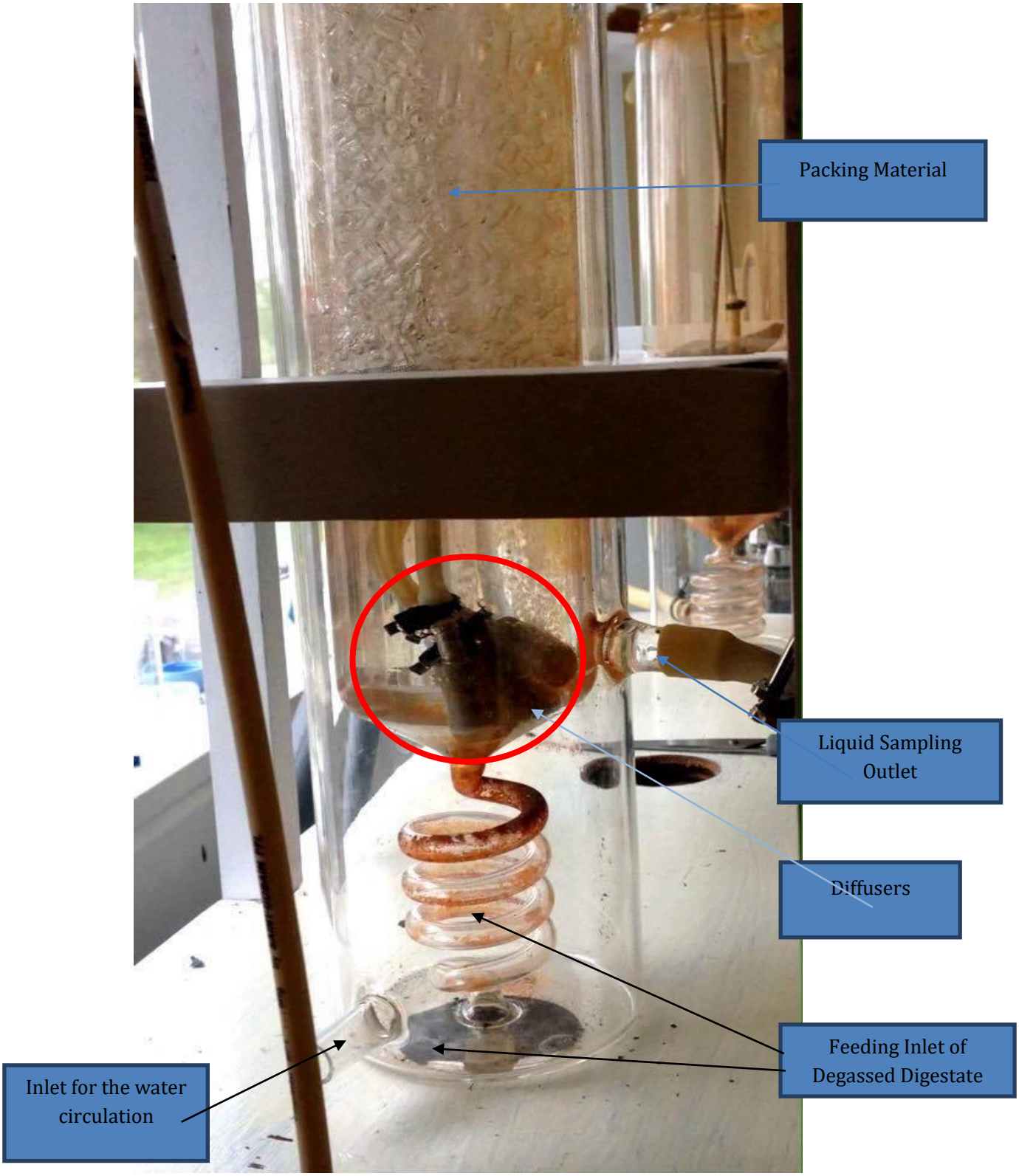
Finally, the liquid outlet tube was connected to an effluent bottle, flashed with nitrogen and to a gas meter, one for each reactor. A sampling outlet was placed in each tube for the gas measurements. The volume of the gas produced in a certain time was measured using a water displacement method (gas meter) in each

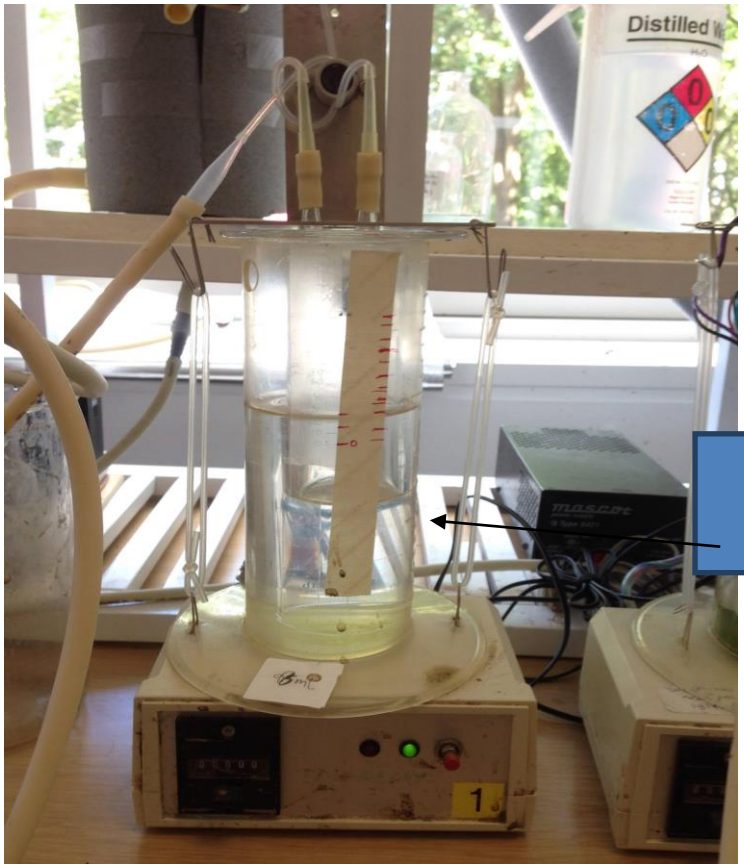
reactor. In this method, the gas entered the gas meter and displaced the same amount of water in the water column. It contained an optic sensor at the level of 100 mL. Consequently, when the water reached the level of the sensor, it sent a signal to the auto valve, and the gas was released, at the same time that one number was increased in the counter.

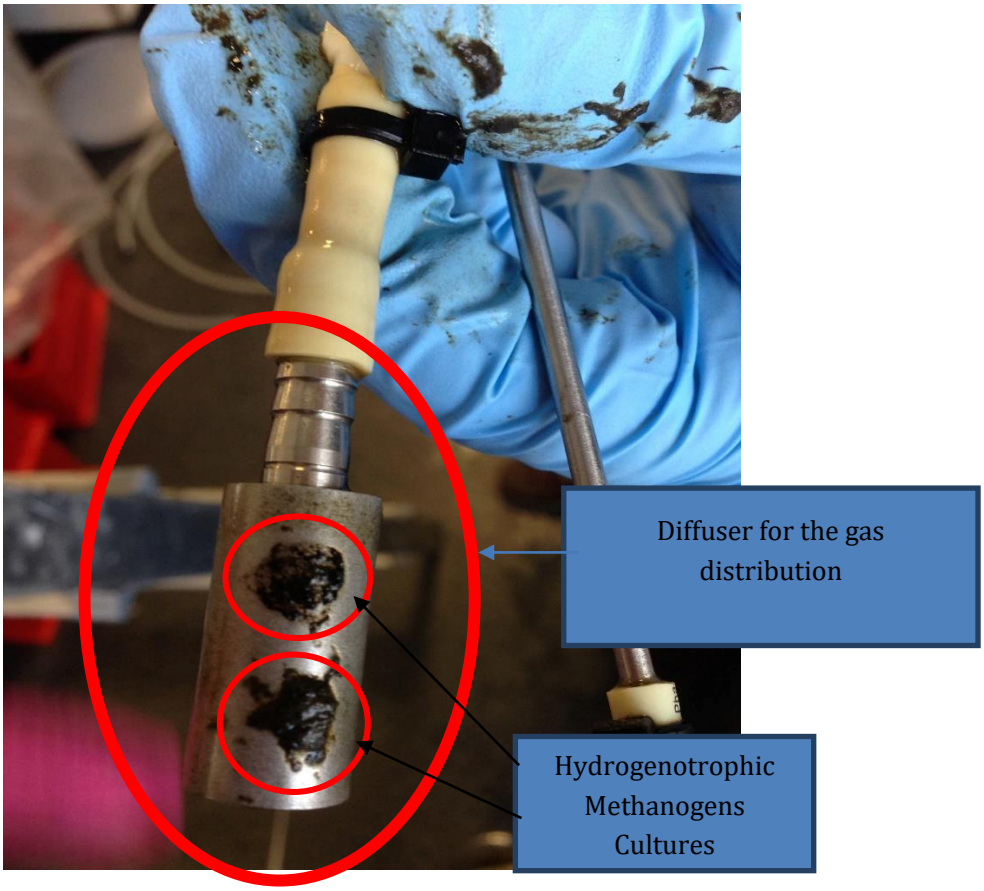
On the outside bottom part of each reactor there was an outlet, which was used for the feeding of the degassed digestate. The outlet of each reactor was connected with a tube which was connected to the feeding pump and the tubes were finally placed inside a beaker full of the feeding digestate. Continuous stirring was applied to the beaker. The feeding pump and the stirrer were connected with timers, in order to control the stirring, the feeding time and the feeding quantity. The gas bag was placed next to the setup inside a carton box in order for it not to be easily accessible, for safety reasons.

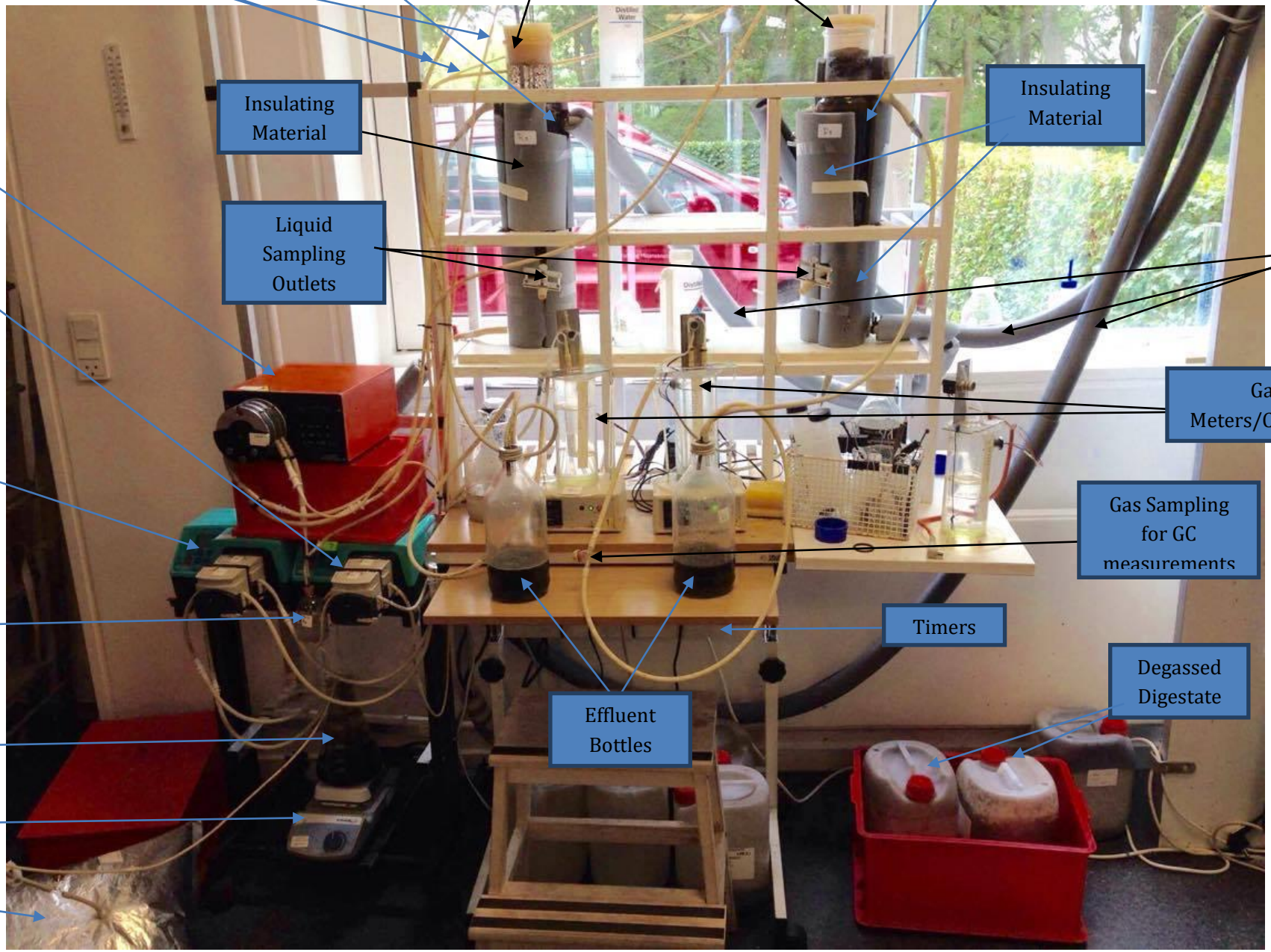
The whole setup described, is shown in the pictures below:




Packing Material
Liquid Sampling Outlet
Diffusers
Feeding Inlet of Degassed Digestate
Inlet for the water circulation


Distilled
Gas Counter/Meter


Diffuser for the gas distribution
Hydrogenotrophic Methanogens Cultures


Connecting Tubes
Reactor 2 (R2) Filled with Packing Material
Rubber Stop Lids
Reactor 1 (R1) Control
Insulating Material
Insulating Material
Recirculation Pump
Liquid Sampling Outlets
55°C Water hoses, insulated
Gas Feeding Pump (CO_2, CH_4, H_2)
Gas Meters/Counters
Liquid Feeding Pump
Gas Sampling for GC measurements
Recirculation Bottle
Timers
Degassed Digestate
Effluent Bottles
Feeding Digestate
Stirrer
Gas Bag

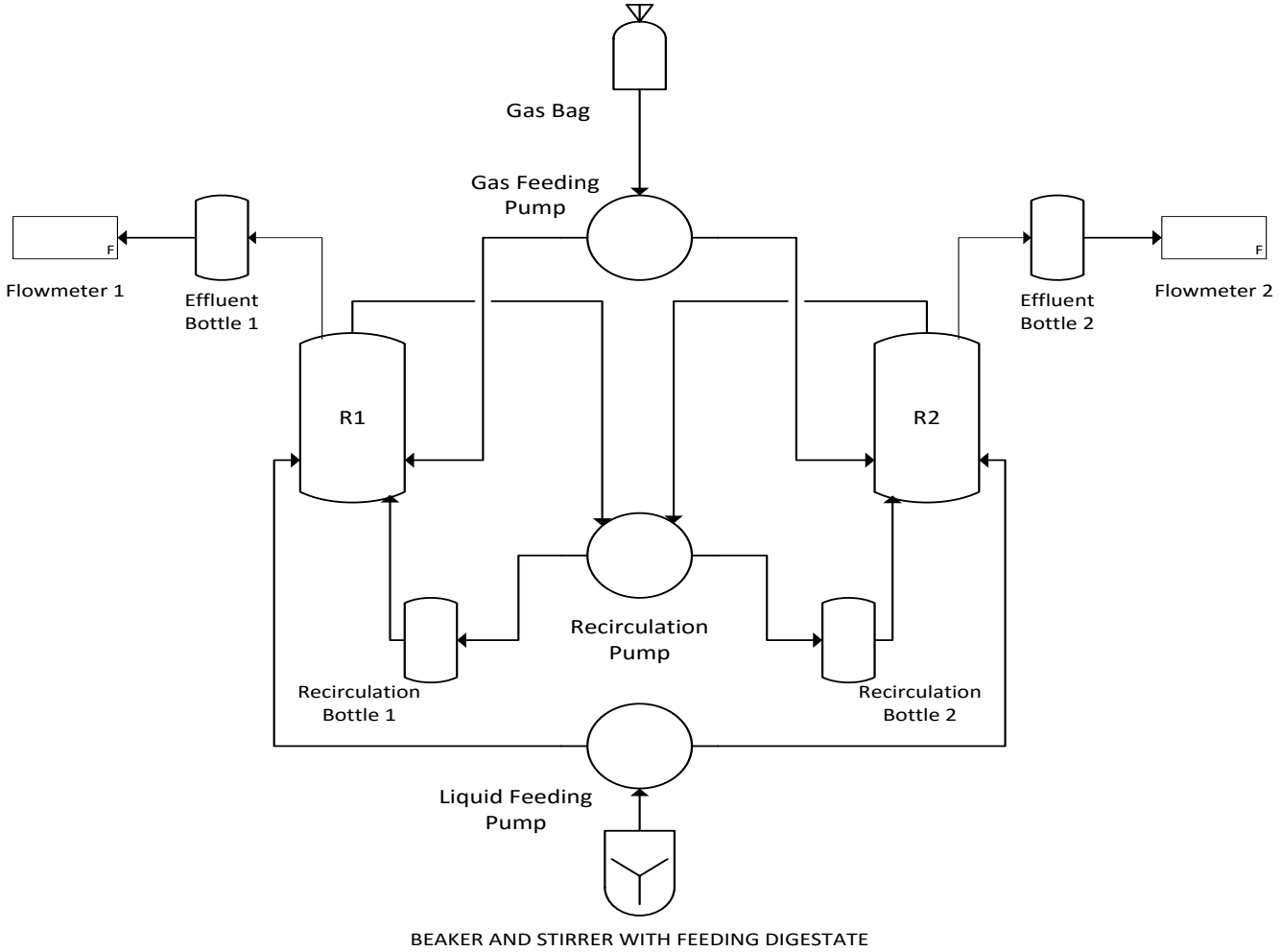


FIGURE 8: FLOW CHART DIAGRAM OF THE EXPERIMENTAL SETUP

Characterization of degassed digestate and inoculum

The degassed digestate was used came from a biogas plant in Hashøj, which treats cattle manure and food waste. Then, it was put in an incubator operating at 55°C for more than two months to completely degrade, therefore, it was almost 100% degassed. It was used only for providing nutrients to the hydrogenotrophic culture. It passed through a strainer to remove any solids and was placed in large plastic containers of 5 L. They were all put in a freezer of -20°C, except for the one being used each time, which was stored in a fridge. This digestate was fed to ensure that the enriched hydrogenotrophic culture from the inoculum would get all the necessary nutrients. The characteristics of the digestate can be seen in the table below:

TABLE 1: CHARACTERISTICS OF THE DEGASSED DIGESTATE

Parameter	Unit	Average Value
pH	-	9,6
Total solids (TS)	g/L	23,1
Volatile solids (VS)	g/L	12
Total Kjeldahl nitrogen (TKN)	g/L	3,6
Ammonium nitrogen (NH4)	g/L	4,9
Total volatile fatty acids (VFA)	mg/L	136,6
Acetate	mg/L	64,9
Propionate	mg/L	39,9
Iso-butyrate	mg/L	17,9
Butyrate	mg/L	2
Iso-valerate	mg/L	11,3
Valerate	mg/L	0,3
n-hexanoate	mg/L	0,3

As it can be seen in table 4, the value of the pH is high (9,6) for thermophilic conditions, so it was acidified with orthophosphoric acid and the new pH achieved was around 8.

The composition of the gas feed can be seen in the table below:

TABLE 2: GAS MIXTURE COMPOSITION

Gas Mixture Composition	%
CH_4	23
CO_2	15
H_2	62

The gas mixture was stored in a 150 bar pressure bottle and for safety reasons it was fed to the reactors through a pump from a gasbag of 20L, which was replaced with new gas mixture every day.

The substrate used as initial inoculum was taken, as mentioned, before from a running thermophilic hydrogenotrophic biogas reactor, since the culture needed to be active. The characteristics of this substrate can be seen below:

TABLE 3: CHARACTERISTICS OF THE HYDROGENOTROPHIC INOCULUM

Parameter	Unit	Average Value
pH	-	9,2
Total solids (TS)	g/L	25,5
Volatile solids (VS)	g/L	17,8
Total volatile fatty acids (VFA)	mg/L	587,4
Acetate	mg/L	482
Propionate	mg/L	63
Iso-butyrate	mg/L	12,6
Butyrate	mg/L	5
Iso-valerate	mg/L	24
Valerate	mg/L	0
n-hexanoate	mg/L	1

Analytical Methods

The analytical methods that were used during the process were the following: Analysis of pH, total solids (TS) and volatile solids (VS), Total Kjeldahl Nitrogen (TKN) and ammonia nitrogen according to *Standard Methods for the Examination of water and Wastewater.*[32] Volatile Fatty Acids (VFA) were determined by gas chromatograph (GC Shimadzu) with flame ionisation detector (FID). Methane content in biogas was determined by using a gas chromatograph (GC Shimadzu) equipped with a Porapak 60/80 molecular sieve column and a flame ionisation detector (FID). The most important methods are described below.

Determination of methane in batch reactors (Gas Chromatography)

Gas chromatography (GC) is used to analyze concentration of particular gases that can be vaporized without decomposition. A known volume of gaseous is injected into the column and the carrier gas (hydrogen in this case) takes the sample through it. The gaseous compounds being analyzed interact with the walls of the column, where it can be found the stationary phase. This causes each compound to elute at a different time, known as the retention time of the compound. The comparison of retention times is what gives the results in a chromatography.[33]

Methane, carbon dioxide and nitrogen content were detected by gas chromatography, by using the GC-biogas TCD and specifically the instrument GC Shimadzu 5, methane. A thermal conductivity detector was the one that perceived each compound that had been separated along the column. Gas samples of 0.5 ml were taken from the headspace of each reactor and injected into the chromatograph. Each sample was analyzed in triplicate.[34]

- This Gas Chromatography was only used for the measurement of high level methane content ~ 5-100 %.
- A plastic syringe equipped with blue needles was used which was tighten with Teflon tape.

- The lock was strictly opened only when the gas was injected and closed immediately afterwards.
- Because the Gas Chromatography is very sensitive, it was needed to practice until getting almost the same result from the same sample (<5% difference).

The Gas Chromatography for methane measurement was equipped with a Porapak 60/80 molsievecolumn (6 ft. long and 3 mm in inner diameter) and a flame ionization detector (FID). Nitrogen was used as carrier gas with a pressure of 2.0 kg/cm^2. The injection temperature was set to 110^{o}C. The detector and oven temperature was 160^{o}C. The retention time for methane was around 50 seconds. Standard gas used for measurement was a mixture of 30% N_2, 40% CH_4 and 30% CO_2. Every time that methane content was measured, it was necessary to generate standard curves by analyzing standard gases, so a linear regression was obtained. Thereby, it was possible to account for variations within the Gas Chromatography.

Preparation:

- **Standard preparation**: Firstly, the standard gas bag, which contents 30% N_2, 40% CH_4 and 30% CO_2 was taken. A 118 ml glass bottle, a rubber septum, and an aluminous lid were used. The glass bottle with standard gas was filled like this: the rubber septum was inserted and the aluminous lid was tightened. Two blue needles were placed through the rubber septum. The gas stream was let through one of the needles and the air inside the bottle was streamed out of the other needle. After 15 seconds the gas-through needle was removed and the pressure was let to equilibrate. The pressure from the bottle's watch was read, and was written down to the card on the bottle.
- Check of the syringe: The pressure lock of the syringe was closed, the plunger was moved to the maximum scale of the syringe and then let go. If the plunger of the syringe goes back itself, it can be used. If not, it may

need more Teflon tape for the plastic syringe or for the glass syringe to remove the pressure lock, change to another syringe, and try again.

- The septum should be changed before the beginning of the GC.

Procedure:

1. The power of the Gas Chromatography was turned on, the heater and the display were activated and the computer was powered up.
2. The gas flow was adjusted. The two layer adjustable plastic screws and the air flow and hydrogen flow screws, needed to be adjusted until the air flow watch would go to 0.4 kg/cm^2 and H_2 flow watch to 1 kg/cm^2 . Then, the carrier 2 should be adjusted to 2.0 kg/cm^2.
3. When the fire was successfully ignited, the H_2 flow screw should be lowered to 0.5 kg/cm^2.
4. In the computer a custom method was created and the Stop Time was 40, 60 or more minutes. Here it could only run one chromatogram containing all the methane peaks.
5. **Measurement of standard gas**: The pressure lock of the syringe should be closed. The needle was injected into the standard gas bottle through the rubber septum and then the pressure lock was opened. The syringe with the standard gas was flashed for several times and 0.2 ml were being kept inside the syringe. Then, the pressure lock was closed. On top of the GC machine, the needle was injected into port 2 by opening the pressure lock, injecting the gas inside port 2 and closing the pressure lock at once, while immediately the start on Ch. 1 at the CBM-102 box was pressed. The direction of the needle was kept vertically. The standard was injected at once around 10 times and the difference shouldn't be more than 5 %.
6. **Measurement of samples**: For each sample 3 injections of 0, 5 ml took place and if the difference was more than 5 %, they were made again. For every 10 sample, a control (standard) was injected. If it differ more than +5 %, the 10 previous samples was rejected and injected again.

7. **Calculation:**

CH_4 (%) = (peak area value of biogas sample)/(peak area value of standard sample)*30%

CH_4 mmol (n) = p * v / R * T

n = mol

p = pressure atm.

v = volume ml

R = gas constant 0,0821 l * atm / mol * K

T = temperature Kelvin

CH_4 mmol = 1 atm * 0,2 ml * 1000 mmol/mol / 0,0821 l * atm / (mol * K) *1000 ml/l * 295,16 K

CH_4 mmol = 0,00825 mmol in 0,2 ml ~ 41,3 mmol/l

Determination of pH

The term **pH** (=pondus hydrogenii) was proposed by the Danish Scientist SPL.Sørensen to express small concentrations of hydrogen ions. The pH is a measurement of the ions of hydrogen that exist in the wastewater treated. This parameter was analyzed by using the PHM 92 placed in the Bioenergy lab in DTU Environment. PHM 92 was connected to the Gel pH electrode (pHC3105-8, Radiometer analytical). The pH measuring range was 2-10 and the temperature range was from 0°C to 60°. The electrode is filled with a gel containing KCl.

The laboratory samples were collected in bottles completely filled and tightly closed. Measurement of pH should be performed as soon as possible after sampling, preferably within 5 minutes, so as to avoid shifting in temperature that would cause additional errors. A temperature difference of 2-5°C will be, in most cases, acceptable. pH meter was calibrated before each measurement by using buffers with a pH of 4, 7 and 10.

The pH check of the liquid phase on the inside of each reactor as well as the VFA sampling were carried out, as mentioned earlier, twice per week just before the feeding of the reactors to eliminate possible errors and obtain reliable results

regarding the inoculums' state. The value of pH should be around 8,5 so that the process is not inhibited. Two plastic, transparent syringes of 100 ml each were used, one for each reactor. Each syringe was connected to the sampling exit tube of the reactor. The metallic tube stop was released and some amount of inoculum was flashed several times in order for the syringe to obtain the temperature of the sample and for the sample to be as homogenous as possible. Then, 50 ml were taken with the syringe and 20ml of it were placed in a bottle, which was secured with a special lid and stored in a freezer for possible future usage, 10 ml were put in a small vial for the VFA testing and the rest 20 ml, were emptied in a beaker where temperature and pH values were checked. The pH of the digestate was also measured twice per week, by putting the beaker on a stirrer for at least 10 minutes in order to mix well the content and by using the pH meter afterwards.

Determination of volatile fatty acids (VFA)

The concentration of volatile fatty acids (VFA) was measured one or two times per week to ensure that the process is balanced since an accumulation of this compound might be due to a decontrol in the anaerobic digestion. The samples for VFA analysis were taken from the reactors following the procedure mentioned above (pH), they were prepared following the below points and then the content of each vial was analyzed by chromatography in the GC-10. Samples are injected into GC Shimadzu GC-2012 equipped with a FID. The compounds are separated along a capillary column and VFA concentration is determined by a linear calibration curved obtained by calibration standards and adjusted by the injection standard.

Sample Preparation:

A sample of 3 ml was taken from each one of reactors R1 and R2 and was put into two separate bottles of 20 ml. Afterwards, 0,4 ml of orthophosphate acid 34% was added in each sample and was slightly mixed. Due to the foam formation, 2

ml of each sample were taken and put in two Eppendorf vials. The Eppendorf vials were securely closed and put for centrifugation diametrically in the Eppendorf minispin table centrifuge. Duration of centrifugation was set to 10 minutes at 13.400rpm. Two samples of 1ml of supernatant liquid were taken from each Eppendorf and added to 4 separate glass vials. Afterwards, 100μL of internal standard, 4-methyl-valeric acid, was added to each vial. Finally, each vial was sealed and all of them were stored in the freezer until running the VFA testing.

The linear ranges of the compounds are shown in Table 4

TABLE 4: LINEAR RANGES OF THE COMPOUNDS

Acetate:	1-100 mM	On procedure for validation (expected 6,1- 6050 mg/l)
Propionate:	1-100 mM	On procedure for validation (expected 7,4-7408 mgl)
Iso-butyrate:	1-100 mM	On procedure for validation (expected 8,8-8810 mg/l)
Butyrate:	1-100 mM	On procedure for validation (expected 8,8-8810 mg/l)
Iso-valerate:	1-50 mM	On procedure for validation (expected 5,1-5100 mg/l)
Valerate:	1-50 mM	On procedure for validation (expected 5,1-5100 mg/l)
Hexanoic acid	1-50 mM	On procedure for validation
Ethanol:	1-50 mM	On procedure for validation
Butanol:	1-50 mM	On procedure for validation

After taking the prepared samples off the freezer and letting them liquefied again, the liquid phase is injected into GC Shimadzu GC-2010 equipped with a FID (flame-ionization-detector). The compounds is separated by a capillary column (ZB-FFAP, 30 m, 0,53 mm I.D x 1,0 μm). The VFA concentration is determined by a linear calibration curve obtained by calibration standards and adjusted by the injection standard.

The supernatant should be very clear, so that the needle at the GC would not clog. If the supernatant was not clear, the sample should be centrifuged again until the sample becomes clear. A small glass tube inside the GC vial may be needed if the amount is too low. The sample must be acidified with ortho-phosphoric acid to

rich pH below 2.0, in order to ensure the complete unionization of the VFA. That means that the VFAs (acetate, propionate, butyrate, iso-butyrate, valerate, iso-valerate and hexanoic acid) will be in their acidic form and saturate the basic sites on the analytical column. To acidify sample, as mentioned above, there should be added 50µl of a 34 % H_3PO_4 per ml sample. But depending on the samples buffer capacity, maybe the acid should be stronger.

Preparation of calibration standards and internal standard:

Injection standard: 100 ml 1,1 mM 4-Methyl valeric acid

A 100 ml measuring flask is filled with 50 ml de-ionized water and 15 ml acetone. Then, 605 µl 4- Methyl valeric acid (purity 98 % Merck) were added and the solution needed to be shaken gently. De-ionized water was added till the 100 mL mark. The solution was transferred to a 100 ml bottle and was labeled with content description, production date and expiration date. The injection standard solution was always been kept at 4°C.

Stock solutions:

A stock solution containing 50 mM of all VFA`s.

A stock solution containing 20 mM of all 6 VFA.

A 500 ml measuring flask was filled with approximately 480 ml distilled water. The amount of Na-propionate (this is the only salt used) was weighed and been added to the water. The measuring flask was stirred by a magnetic stirrer and into the liquid funnel the bolded amount of each acid was added.

The measuring flask was filled till the mark with distilled water + magnets volume. On top it was added 30 µl/ml of a 17 % ortho-phosphoric acid ~ 1,5 ml/50 ml and was stirred for 2 hours.

TABLE 5: STOCK SOLUTION PREPARATION

	Mw g/mol	Conc. (mM)	Stock (mL)	Density (g/mL)	VFA Addition pr. stock solution (µL)	Purity (%)	Boiling point (°C)
Acetate	60,05	50/20	500	1,05	1430 / 572	100	118

Propionate*	74,08	50/20	500	0,99	2,402 g / 0,9606 g		141
Iso-butyrate	88,1	50/20	500	0,95	2318 / 927	90	155
Butyrate	88,1	50/20	500	0,96	2294 / 918	99	164
Iso-valerate	102,13	50/20	500	0,94	2716 / 1086	99	177
Valerate	102,13	50/20	500	0,94	2716 / 1086	99	186
Hexanoic acid	116,16	50/20	500	0,93	3186	98	203
Ethanol	46,08	50/20	500	0,80	1500	96	78.5
1-Butanol	74,12	50/20	500	0,81	2299	99,5	116

Preparation of standards.

All dilutions were made with 0,5 % H_3PO_4 and in 50 ml measuring flask.

TABLE 6: DILUTION FACTORS FOR THE STOCK SOLUTION PREPARATION

mM std.	From stock solution	Dilution factor
100	-	1
75	100	1,33
50	100/50	2/1
20	100/50/20	5/2,5/1
10	100/50/20	10/5/2
5	100/50/20	20/10/4
2	100/50/20	50/25/10
1	100/50/20	100/50/20

GC setup: For Ethanol, Butanol and Volatile fatty acids

- Injection mode and volume: split less, 1µL
- Injection port was 150^{o} C.
- Detector temperature was 230^{o} C.
- Initial temperature for column oven was 70^{o} C., hold time 3.5 min.
- Temperature rate was 20^{o} C/min. till 230^{o}C and hold time 3.5 min.
- Column flow was 2.77 ml/min
- Runtime was 15 min. /sample, approx.
- Capillary column ZB-FFAP, 30 m, 0, 53 mm I.D x 1, 0 am

Calculations:

In the post run-program, GC Solution, parameters were set up for calculating standard and samples. The area ratio was calculated by the formula: area std. or sample / area internal STD. The calibration curve was area ratio against concentration.

Supplement:

Preparation of propionate from solid matter, which was one of the 7 acids in the solution:

- Propionate (s): solubility 37g/100 ml
- Stock solution: 100 mM
- Mw Na-propionate = 96,06 g/mol
- Weight of Na-propionate:
  g/Mw = M*V/1000 → g = Mw * M * V/ 1000 →g = 96, 06 g/mol * 0, 1 mol/l * 500 ml / 1000ml/l → g = 4.803 g Na-propionate (s) for 500 ml

Determination of total solids (TS) and volatile solids (VS)

Total solids are a measure of the suspended and dissolved solids in water and therefore their analysis is important regarding the control of biological and physical wastewater treatment processes and wastewater effluent limitations. On the other hand, volatile solids are those solids lost on ignition at 550°C and they give a rough approximation of the amount of organic matter present in the solid fraction of wastewater.[11] The VS content describes the content of organic material in the waste, and is defined as the amount of matter in a dried sample lost after one hour at a temperature of approx. 55°C in air. The method relies on the fact that most organic materials ignite and combust at this temperature, while most inorganic compounds require higher temperatures.

The **standard method** used to determine the total solids as well as the suspended solids is shown below:

- Put the crucibles in the oven at 550 °C for one hour, cool and weigh (M1).
- Place an amount of sample on the crucibles and weigh again (M2).
- Dry the sample in the oven at 105 °C for 24 hours, cool and weigh (M3).

- Put the sample in the oven at 550 °C for one hour, cool and weigh (M4)

Measurements were done in triplicate and the mean was used. The following equations were used to determine the TS/VS ratio in the samples:

$$TS = \frac{M3 - M1}{M2 - M1} \qquad and \qquad VS = \frac{M3 - M4}{M2 - M1}$$

Ash, which was also determined, is totally composed of inorganic compounds that were not burned during the glowing of the sample. As a result, ash is calculated as the difference between total solids and volatile solids.

Determination of hydrogen (H_2)

The determination of hydrogen was conducted by using the Mikrolab GC and following the procedure described below:

- Headspace gas samples of 0,5 ml were taken from the reactors and were injected into the Mikrolab GC. The injector and detector temperature was 90°C. The temperature program was hold isothermal at 80°C.
- The samples were taken with a 1 ml syringe equipped with luer lock and a blue needle. Analysis of gas in batch experiments has to be done by using a syringe equipped with luer lock, for gas tight syringe system.
- Samples should be analyzed within short time after sampling. Samples taken with a syringe can be stored for analysis within 30 minutes, providing the syringe needle is inserted into a rubber stopper, in any other case gas will diffuse from the syringe.
- If the injection hole was not reached, the sample were no longer representative and were discharged.

The quantification range was 1%-100% v/v hydrogen (10.000-1.000.000 ppm) and the detection limit was 0.6 %v/v hydrogen (6000 ppm).

The equipment and apparatus used for the analysis:

- Mikrolab Aarhus a/s. biogas gas chromatograph coupled with a thermal conductivity detector and packed columns for compound separation.
  Front column: Molsieve 5A 60/80, 4.5m x 3mm ID
  Back column: Moliseve 5A 80/100, 6" x 1/4"
- Disposable syringes and blue needles
- Sampling syringe (1 ml) with luer lock, gas tight
- Standard gas from AGA a/s, containing 100% hydrogen.

The gas standard was purchased as a pure gas from AGA a/s and a calibration curve was constructed by injecting standard gas mixture volumes of 0,2 ml, 0,5 ml and 1,0 ml in duplicates. Samples and controls were injected in volume of 0,5 ml. The control sample had to be analyzed in triplicates, estimate the mean and record the result.

Computational methods

The results from the methane and hydrogen Gas Chromotographers, (GC) were given in percentages for N_2, CH_4 and CO_2 and for H_2 respectively. That's why the first step was a sum of N_2, CH_4, CO_2 and H_2 and then normalization out of 100% of the results for each reactor. After that, from the total normalized results, the percentage of N_2 was deducted, and then the results were normalized again without the N_2.

According to equation (1) below and the setup's mass balance, theoretically when the whole amount of CO_2 is converted to CH_4 by using H_2, there will always be a 2% of H_2 that doesn't react so is never converted. That happens because of the following:

$$4H_2 + CO_2 \rightarrow CH_4 + 2\,H_2O \quad (1)$$

Input:

62% H_2
15% CO_2
23% CH_4

V= 1, 4 L

Gas Flow Rate for the **first period**= 3 (ml/min)*(60*24) = 4.320 ml/day

That means:

H_2: 62/100*4.320=2.678,4 ml/day

CO_2: 15/100*4.320=648 ml/day

CH_4: 23/100*4.320=993, 6 ml/day

According to the equation (1) above:

H_2/CO_2=4/1

Namely, 648 ml/day of CO_2 reacts with 4*648=2.592 ml/day of H_2 in order to be converted to CH_4. However, the feeding gives 2.678,4 ml/day of H_2.

That's why 2.678,4 - 2.592= 86, 4 ml/day of H_2 will never be converted and will move to the setup's output or 86, 4/4320=0, 02 *100 %= 2% of H_2 cannot react with CO_2.

Output:

H_2: 86,4 ml/day (non converted)

CH_4: 993,6 ml/day from the gas feeding and 648 ml/day converted (CO_2/CH_4=1/1)

CH_4 total: 993,6+648=1641,6 ml/day

CO_2: 0 ml/day

Theoretical Total Output: 86,4+1641,6= 1.728 ml/day

The calculations for the output gases were made as follows:

The normalized without N_2 value for each gas (CH_4, CO_2 and H_2) was multiplied by the total gas value from the gas counter of the setup and then divided by 100. Finally, for the **H_2 conversion efficiency (100%)** the theoretical value of the converted amount of H_2 was divided by the real output H_2 value calculated above and multiplied by 100% to get the final percentage.

For the mass balance the calculations were made as follows:

Each gas counter (of R1 and R2) recorded every day the actual gas production inside the reactors. This value was written down and used for the mass balance calculations. From that value it was deducted the liquid feeding which was 50 ml/day at first and then 100 ml/day. Therefore, that was the actual gas production value, that could be called ***a***. The without N_2 normalized value for each gas was multiplied by quantity ***a*** and then divided by 100, in order to get the rate of each gas (ml/day). For the final H_2 rate, by the above value the non converted 86,4 (ml/day) were deducted.
All of the above were made every day for each gas and each period for all the different gas feedings and these values were used for all the diagrams.

Experimental description – Ex situ biogas upgrade

The biogas inside the reactor is not only produced by the degradation of the substrate but it is also prepared in a gas bag and it is injected in the reactor through the diffuser. The biogas in the gas bag is composed of 23% of methane (CH_4), 15% of carbon dioxide (CO_2) and 62% of hydrogen (H_2). The hydrogen is used to upgrade the biogas since the latter reacts with the carbon dioxide. The hydrogenotrophic methanogens convert the hydrogen contained in the biogas into methane and therefore the produced biogas would be enriched in methane (upgrading).

More precisely, both cylindrical, anaerobic, up-flow UASB reactors had a working volume of 1,4 L each and were performed under thermophilic conditions (55^{o}C). As mentioned above, two gas diffusers were used to distribute the gas mixture,

one for the feeding of the gas and one for the gas recirculation. The reactors were closed by rubber stops with one output for the recirculation flow, two inputs (gas outflow and recirculation flow) and one output of the liquid phase. They were fed with digested slurry (fully degassed digestate) and a gas mixture. Feeding (gas and liquid) pumps, recirculation pumps, gas meters, recirculation bottles, timers, stirrers and effluent bottles were set up.

In the first stage, all the materials and equipment for the two reactors were assembled and calibrated. Both reactors were connected with the pumps and the heating water. Then the setup was tested with water and air so that it could be checked for any leakages. During this stage, some leaks were detected, but since it was difficult to locate the exact points and fix them, this part of the experiment lasted almost two months. All the pumps were also tested as well as the heating water to be around 55^{o}C.

In the beginning, each reactor was being fed with 50 ml of degassed digestate once per day, but due to the very frequent sampling, it was increased to 100 ml per day divided in 50 ml twice per day, for each reactor (timer set every 12 hours, 50 ml/min for 15 seconds at 94 rpm).

There were five different periods for the experiment that concerned the HRT and the recirculation rate, as it can be seen in table 1 below:

TABLE 7: EXPERIMENTAL PERIODS

Period	Recirculation (L/day)	Gas feed (L/day)	Gas HRT (h)
I	177.60	4.32	8
II	236.16	4.32	8
III	236.16	5.76	6
IV	295.20	5.76	6
V	295.20	7.20	4

The values for the gas feed were suitably converted according to the calibration of the available pumps.

The two reactors were initially loaded with inoculum that was obtained from a previous experiment that was running in the lab, and it could be described as an enriched hydrogenotrophic culture with a very active population, conducted in a thermophilic biogas reactor. [35] A trial period was operated in order to observe the reactors and obtain steady state, during which some problems were encountered like the formation of foam inside the gap space on the top of the reactors. The problem was solved by flashing with some nitrogen several times during the day.

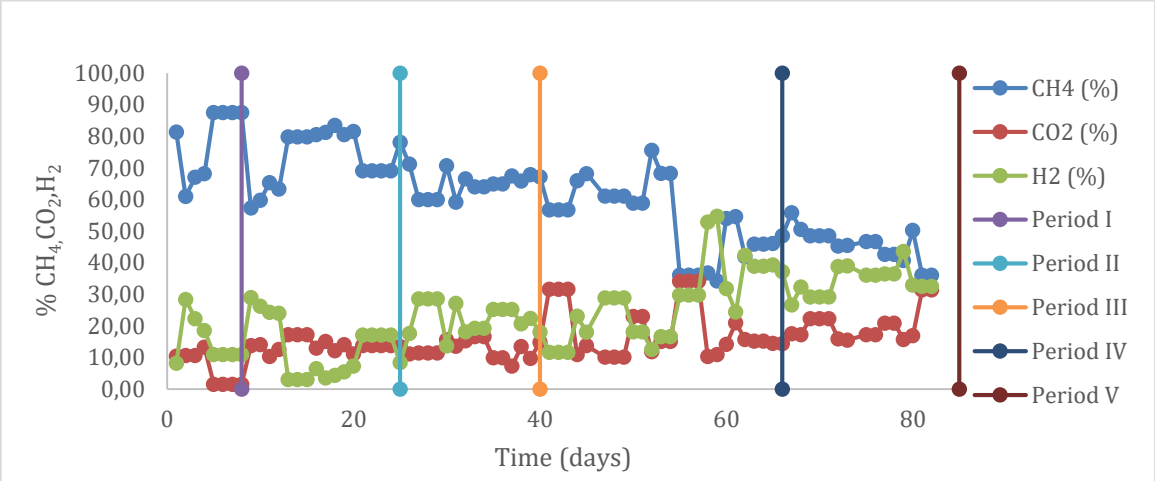
After all the preliminary steps were completed, the main experimental work was ready to begin. The experimental procedure followed was the same and is described below:

1. Check of the reactor setup
2. Check if the heating water is working properly
3. Check the effluent bottles of the setup and empty them when needed
4. Empty the recirculation bottles every day

The gas sample was always taken before changing the effluent bottles, so that there wouldn't be any gas leakages, which may affect the testing results.

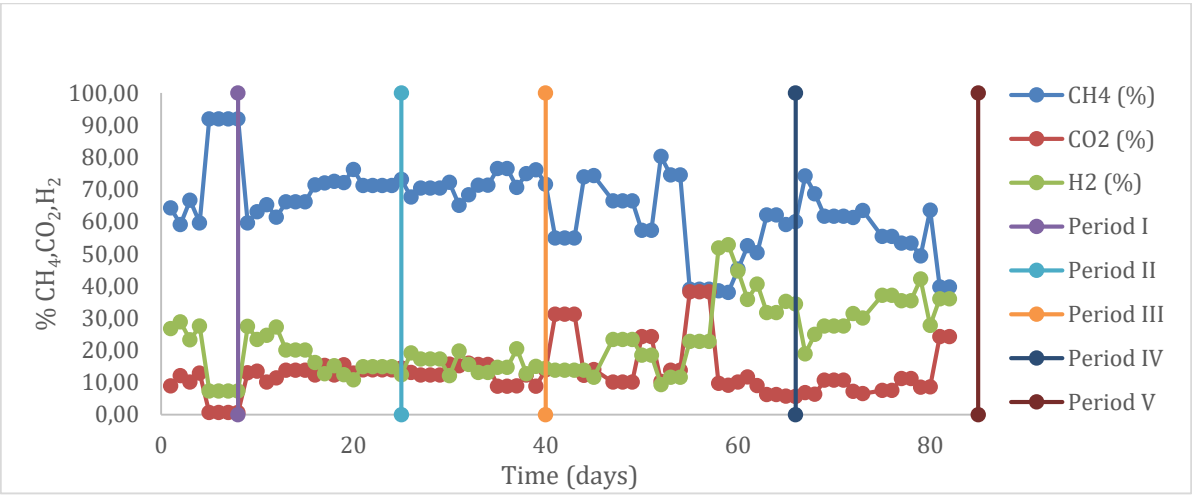
Results and discussion

In this section, the graphs that gather the performances of the reactors under various operating conditions are presented. The rates that are shown below in the graphs below, were calculated per working volume of 1,4 L for each reactor.

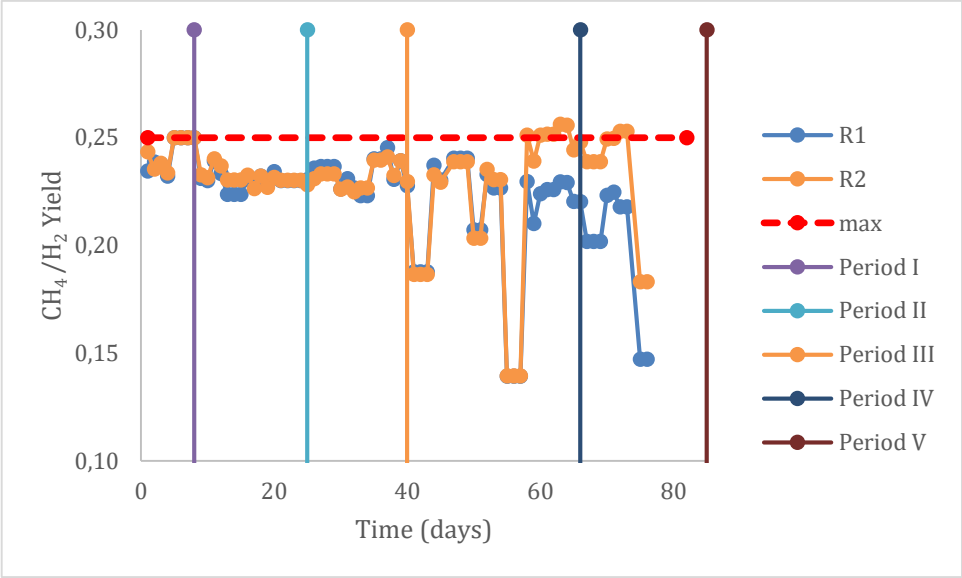


GRAPH 1: % ACTUAL GAS (CH_4, CO_2, H_2) IN THE HEADSPACE OF R1

In Graph 1 and Graph 2, the actual gas composition of the headspace of each reactor throughout the experimental period is presented. It is obvious that the methane amount is the highest as it is the one produced, while CO_2 and H_2 are relatively low during the whole experimental process as they react and are consumed.



GRAPH 2: % ACTUAL GAS (CH_4, CO_2, H_2) IN THE HEADSPACE OF R2



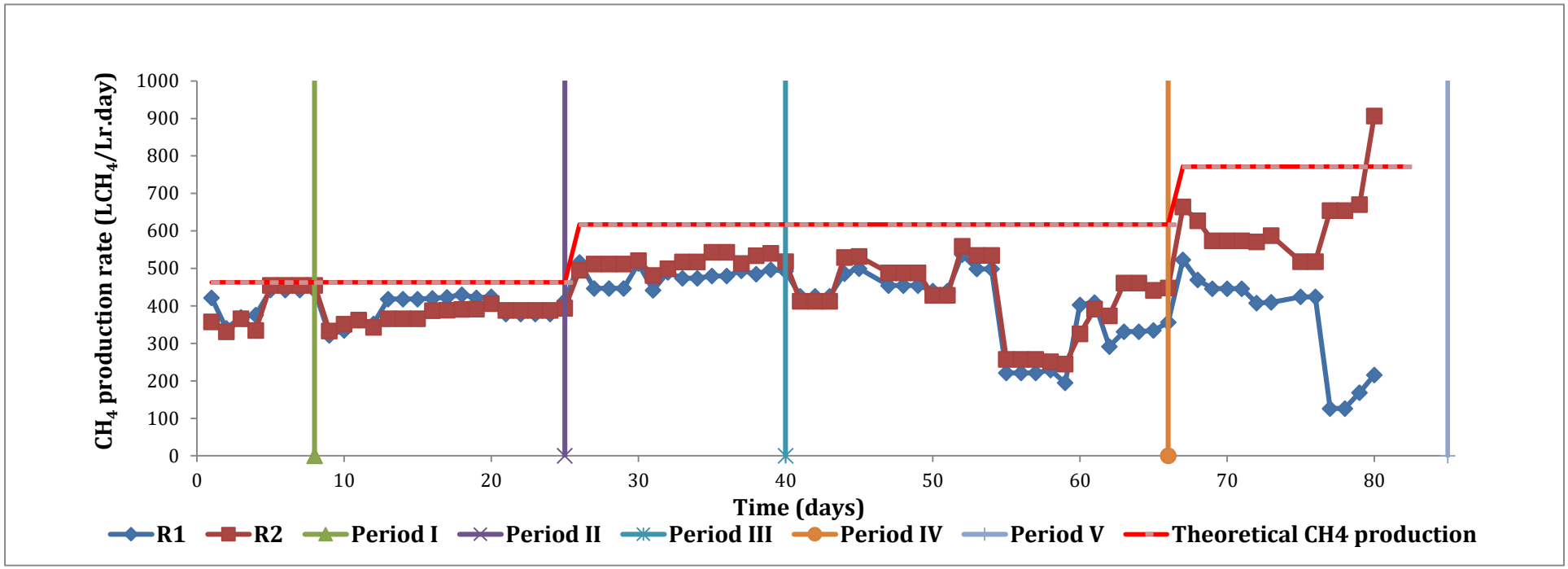
GRAPH 3: METHANE YIELD (CH_4/H_2)

In Graph 3 the methane yield is presented and it was calculated as the amount of CH_4 (L/Lr.day) produced per H_2 (L/Lr.day) consumed. Theoretically, the molar ratio of CH_4 production rate to H_2 consumption rate is 0,25. Nevertheless, the measured ratio was higher than 0,25 during some of the operational days. That may be explained by the excess CH_4 production from residual organic matter contained in the inoculum. However, the methane contribution originating from residual organic matter in the inoculum was eliminated throughout the experimental process. The CO_2:H_2 ratios were around 0,25 during the steady-states of periods I, II, III, IV and V. That shows that the consumed H_2 was almost stoichiometrically converted to CH_4.

From the graph below Graph 4, the methane production of the reactors can be obtained. It can be seen, that during the first days of each period methane production decreases. The configurations needed some time until reaching steady state condition and that could explain the early decrease. After a few days, there is an increase of methane production in each period, so the configurations are working better and methane is produced. Specifically, it can be observed that during period I there is a higher production (around 442 ml $CH_4/L_{reactor}$day) than period II (400 ml $CH_4/L_{reactor}$day). Moreover, for period III there is an even higher methane production than the two previous periods (500 ml $CH_4/L_{reactor}$day). This

can be explained by the fact that the gas feeding was increased, so the reactors managed to obtain a better performance. Periods IV and V are really unstable, probably due to some problems with the configuration such as foaming production. This fact can be explained due to a problem of air entering. It was day 55, when foam production was observed. The foam entered the gas recirculation line and blocked the diffusers. When this situation was noticed, there was no other possibility than to open the reactors, install new diffusers and clean them. After closing them again, they were washed with the feeding gas with a high flow for one day. Even so, some air remained, until it was washed out from the reactor and the concentration of methane started increasing again. However, during the last period (V) there is a substantial increase of CH_4 production (600 ml $CH_4/L_{reactor}$day) for R2. The second episode of air entering occurred due to clogging of the effluent line, which resulted in over-pressure inside the reactor and a minor reactor cracking. This was first noticed around day 83 and it was immediately corrected (sealed again) but most probably it occurred some days before, when the decrease of the methane concentration and increase of the nitrogen concentration were observed. Concerning the two reactors, it can be observed that R_2, which is filled with packing material, is performing better than the control reactor R_1 during all the periods (I-V). It was expected to observe that result, as the packing material was supposed to help at the better breaking down of the gas bubbles, so the microorganisms were able to utilize them better and be more efficient. The last experimental period (V) presented the best results in methane production. Therefore, the optimum conditions seen for R2 were for the gas feeding 7,2L/day (HRT: 4 hours) and for the recirculation rate 295,2L/day. If the results above are compared to the theoretical value of the methane that could be produced (red line) according to the mass balance of the setup the results are lower than expected. This can be explained by the fact that the gas composition of the feeding changes during the day. Specifically, the pH is changing and therefore the balance between CO_2 and bicarbonate/carbonate also changes, so the CO_2 in the calculations refer only to the gas phase and not to the liquid. Moreover, since the dissolved H_2 in the liquid phase of the reactor was not measured we cannot assume precisely the theoretical methane that could be produced. In order to do so in the mass balance the theoretical conversion of VFA

to CH_4 equivalents should be taken into account. Finally, after a period the acetate concentration was accumulated inside the reactor, indicating homoacetogenesis process (i.e. utilisation of H_2 for acetate formation). If taken into account all the above then the red line should match the achieved CH_4 production rate. Generally, the microbial culture performs differently due to the sudden condition changes to restore the balance. The procedure followed, is extremely sensitive to any change, as the feeding for the micro-organisms is gas, so it could be easily affected by any minor factor.



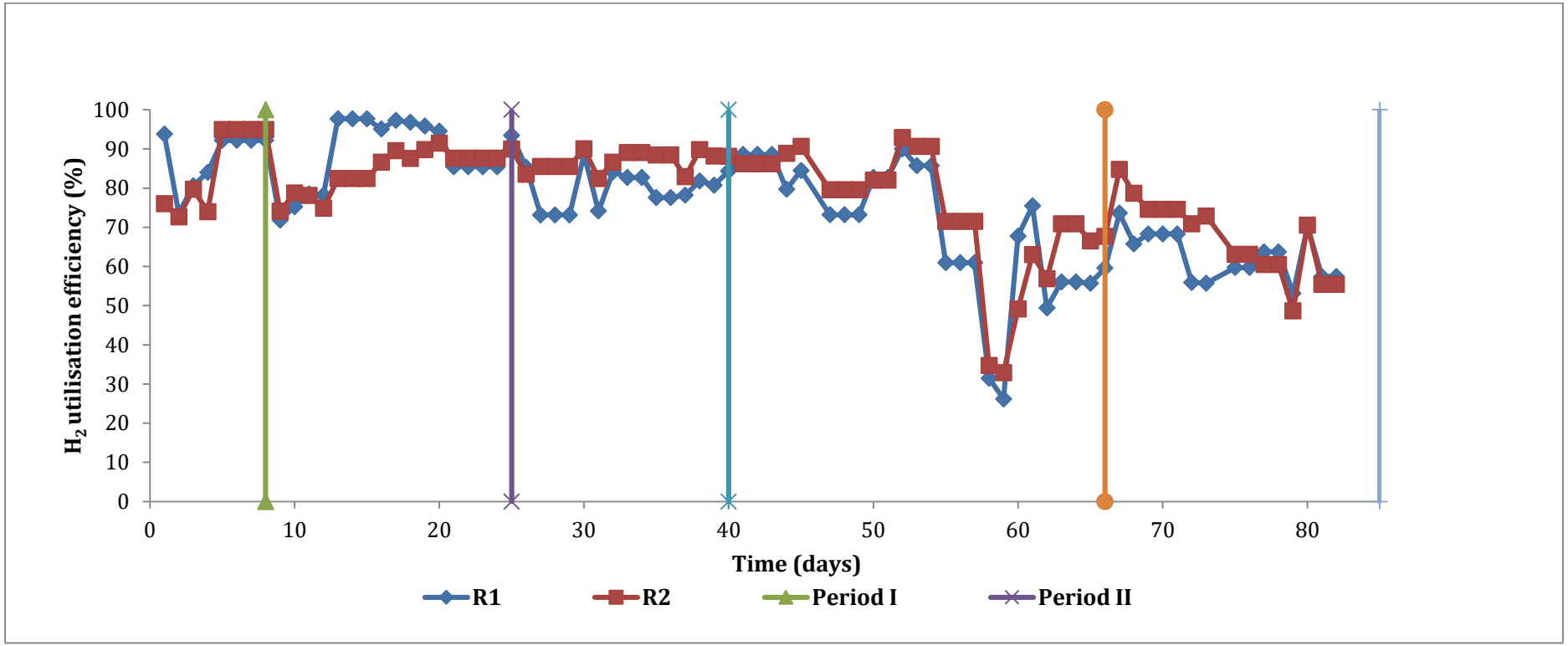
GRAPH 4: CH_4 PRODUCTION RATE FOR R1 AND R2

In the graph below Graph 5: h_2 utilization efficiency (%) for r1 and r2Graph 5, the % conversion of H_2 is presented. It was calculated as:

1-[actual H_2 production/ (hydrogen in-theoretical unconverted H_2 in)]*100%.

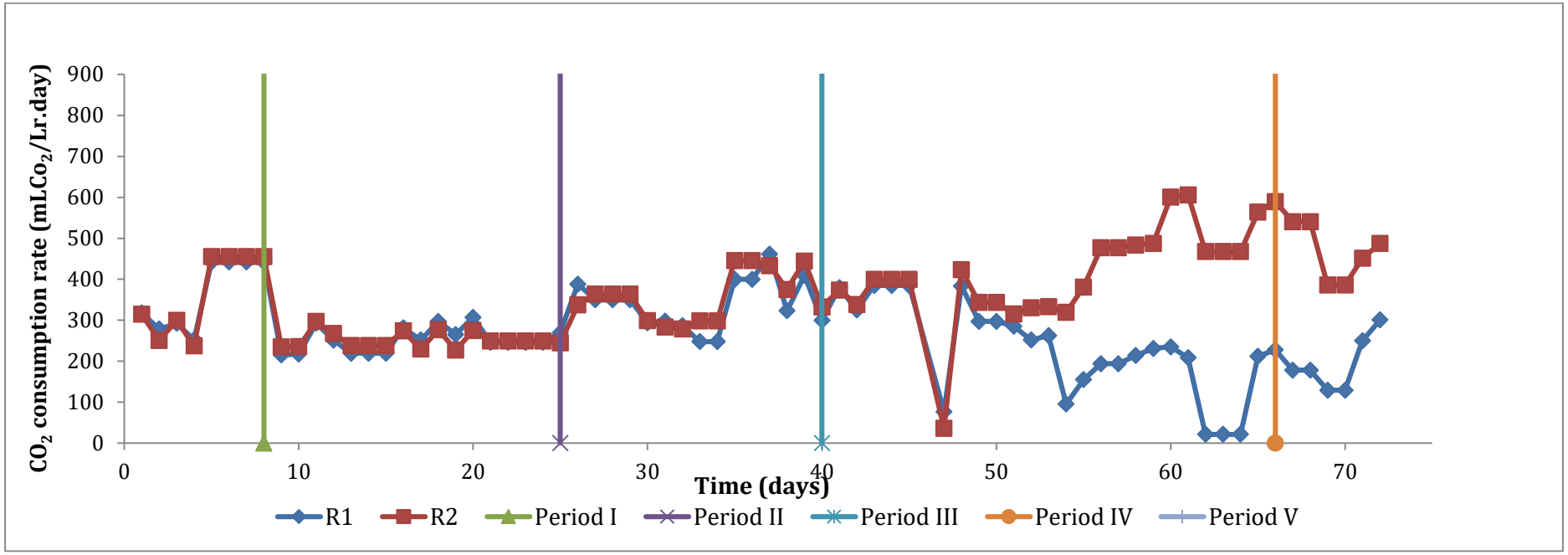
The percentage of the hydrogen utilization efficiency is generally stable, except during the last two periods (IV and V). It is sustained high (around 90%) throughout the first three periods and there is a decrease after day 55, probably due to the reasons mentioned before (foaming production and cleaning of the reactors). Comparing the efficiency of the two reactors, a better performance of R_2 can be seen. That could be explained by the addition of the packing material that seems to improve the performance of the reactor and agrees with the previous results. R2 shows a high and stable H_2 utilization efficiency, but no great changes are observed when the operation conditions change (gas feeding rate, gas recirculation rate). On the other hand, R1 presented a temporary improvement in hydrogen conversion during period II after increasing the gas recirculation rate, but during period III it drops again, showing that there was possibly an inhibition of the hydrogen utilization. It could also be extracted that during the last two periods when the gas retention time was lowered significantly compared to the initial operating conditions the reactors needed more time to obtain steady state conditions.

If the two graphs are compared, it can be seen that they both show the same behavior for each period operating, so the results agree.



GRAPH 5: H_2 UTILIZATION EFFICIENCY (%) FOR R1 AND R2

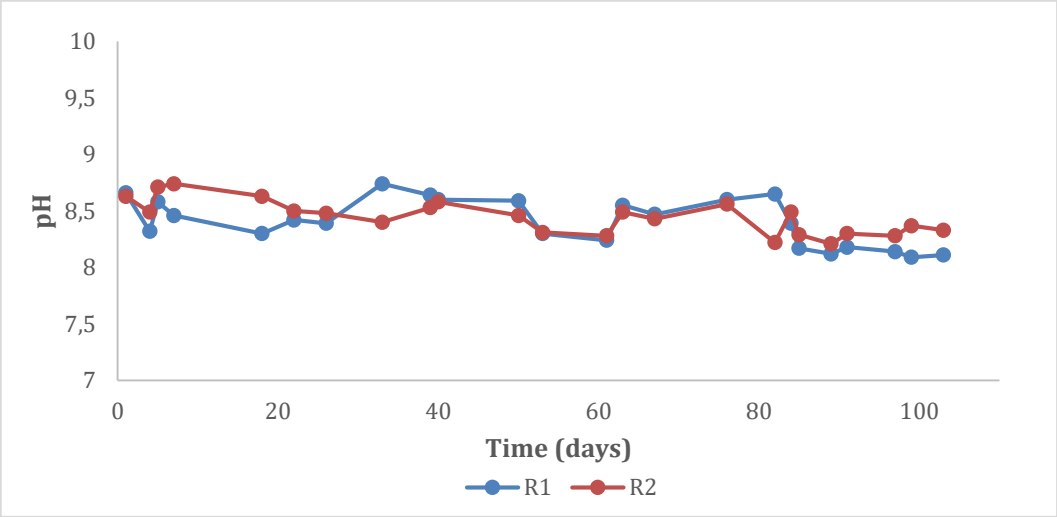
In the graph below (Graph 6), the CO_2 consumption rate is presented for both reactors R1 and R2. This rate was calculated as the CO_2 converted (CO_2 in- CO_2 out produced inside the reactor) per liter reactor.



GRAPH 6: CO_2 CONSUMPTION RATE FOR R1 AND R2

The consumption rate is almost the same for both reactors during the first four periods and it is maintained stable. However, during period V, R2 shows a high consumption rate in contrast with R1 which has a sharp decrease. That probably occurred because of the change in the gas retention time during period V, which affected positively R2 with the packing material. R2 performed better than R1 by consuming higher amounts of CO_2, which means producing larger amounts of CH_4. The data that is missing after day 75 is due to a problem with the gas counters.

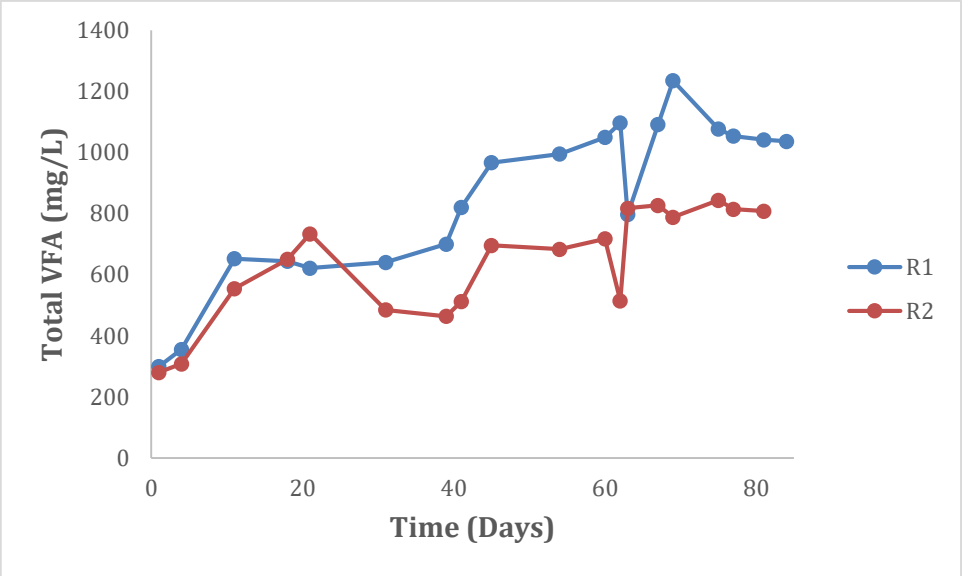
For the complete analysis of the performance of the reactors, values of pH and VFA are necessary. The graphs below show the pH values, the Total VFA and the detailed VFA for each one of the reactors. A comparison is also made between the pH values and the Total VFA to extract some information regarding the operating system and confirm the theoretical expectations. Process where microorganisms are involved need to have a good control of the environmental factors, this is the reason why pH values should be maintained around 8 in order for its activity not to be inhibited. Furthermore, both pH and volatile fatty acids analysis are used to determine if there is an imbalance in the process.



GRAPH 7: PH VALUES FOR R1 AND R2

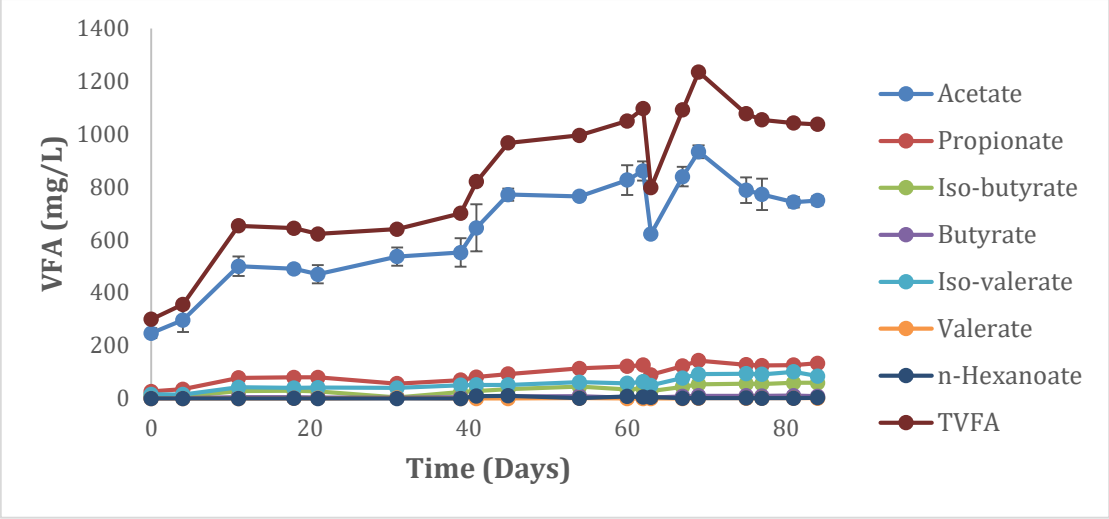
As it can be observed from Graph 7 the pH values for both reactors were relatively stable around 8,5. During the last periods, the pH stabilized at a lower range of around 8,2 which indicates that more methane is produced and this is probably a result of higher CO_2 consumption. Therefore, the intense presence of CO_2 can justify the pH drop. That's probably caused due to the changes made during the last period (V) when the gas feeding (HRT) and the recirculation rate were the highest of the whole procedure. This stable and relatively low pH value suggests that there is no inhibition of hydrogenotrophic methanogens and therefore methane comes mainly from the hydrogen consumption. The rest of the hydrogen is probably utilized to produce acetate through homoacetogenesis, which could contribute to the production of acetate in the reactors noticed in the

graphs below. The stability of the pH indicates that accumulation of ammonia didn't occurred during the process.

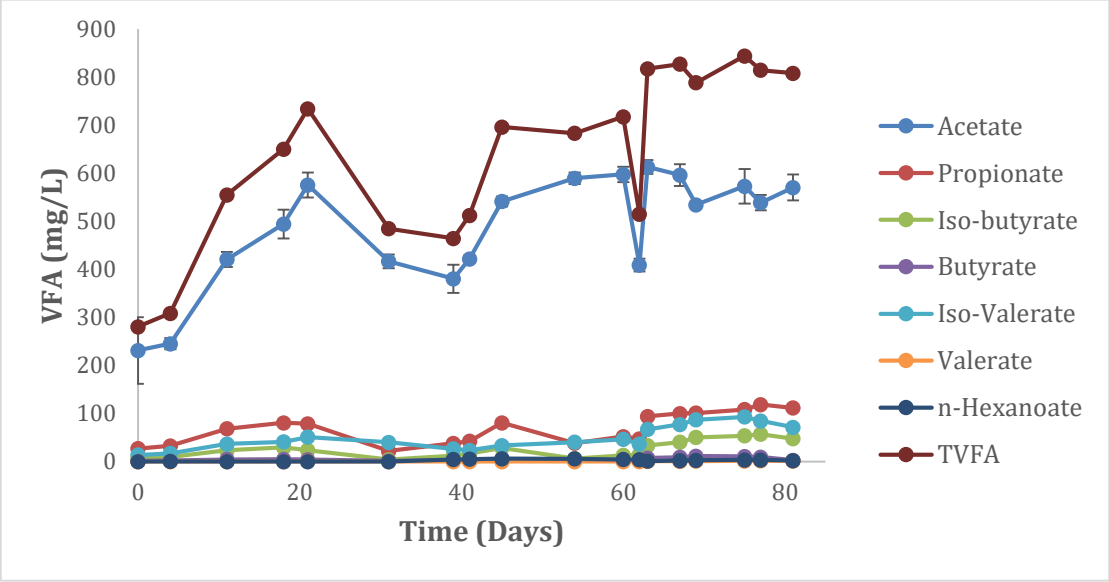


GRAPH 8: TOTAL VFA IN R1 AND R2

As it can be seen Graph 8, the total VFA in both reactors increases almost constantly throughout the procedure, except for a decrease around day 31 and a sudden drop on day 63. The TVFA concentration obtained in period V (HRT= 4 hours) increased significantly with respect to periods I-IV (HRT=8 and HRT=6 hours), which could indicate that retention time was not enough for an optimal degree of substrate degradation. There were obtained 1.235 mg/L and 844 mg/L of volatile fatty acids in the inoculum of R1 and R2 respectively, which determine the increasing profile of VFA production with the increasing of the various retention times. The optimal bacterial activity in period V was also reflected in the acetate concentration, which reached the maximum value during period V (933 mg/L for R1 and 573 mg/L for R2). Finally, the TVFA in R1 are higher than in R2 so it can be concluded that R2 performs a better methane production.

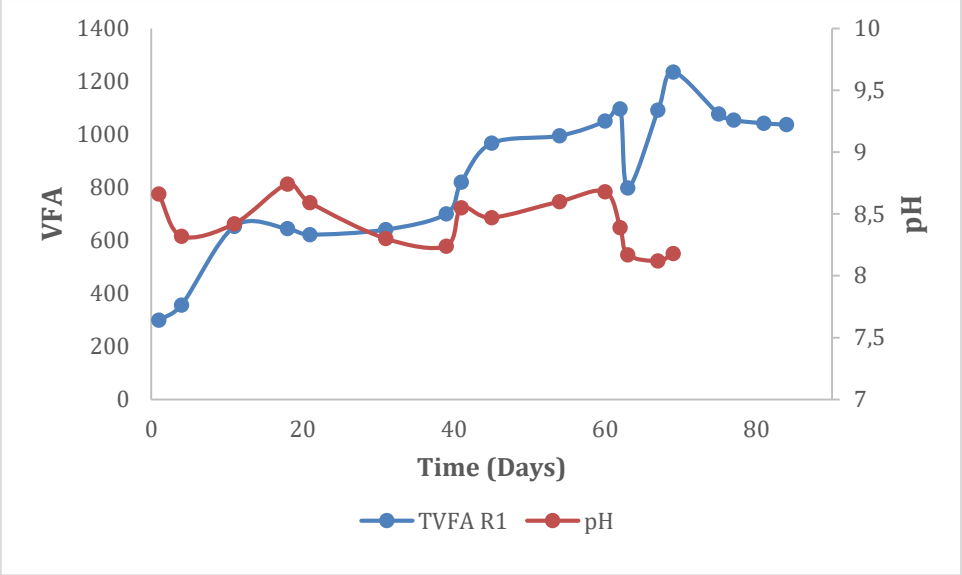


GRAPH 9: DETAILED VFA FOR R1

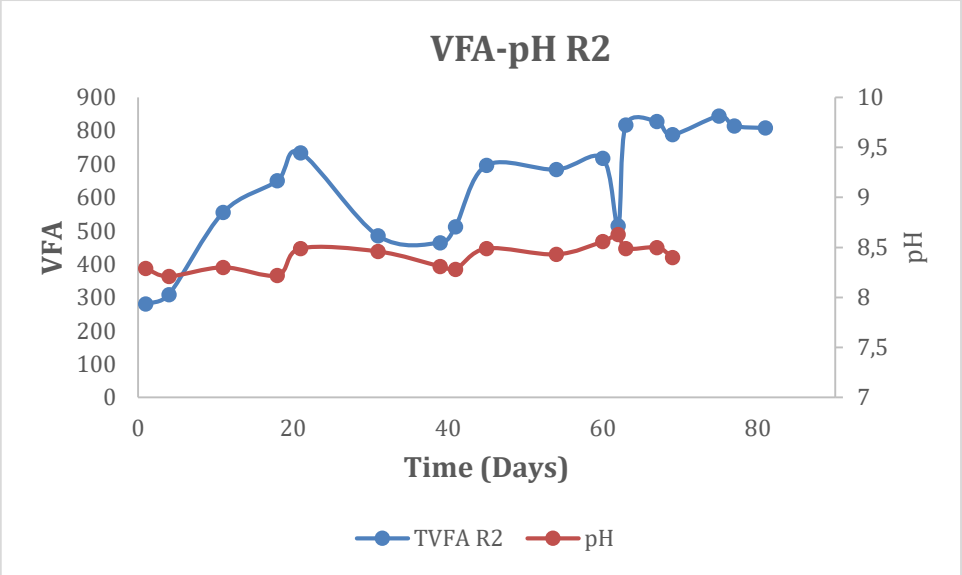


GRAPH 10: DETAILED VFA FOR R2

Out of the graphs of individual VFA for each reactor, it is deduced that most of the VFA in the reactors is in the form of acetate (calculating this percentage with the raw data, it is in average 76%). The concentrations of each compound present similar behavior with acetate, which has the main changes. The reactor was fed with digestate that had 136,6 mg/L TVFA which is a very low concentration. From the individual VFAs it can be seen that the highest increase was due to the acetate.



GRAPH 11: COMPARISON BETWEEN VFA AND PH OF R1



GRAPH 12: COMPARISON BETWEEN VFA AND PH FOR R2

Graph 11 presents the results for pH and VFA for the first reactor (R1). It is observed that total concentration of VFA increases after a sudden change in the reactor and go back to a lower value when steady state conditions are achieved again. That is because in this point the process is imbalanced and VFA production raise. The pH was around 8,5 during the whole experimental procedure.

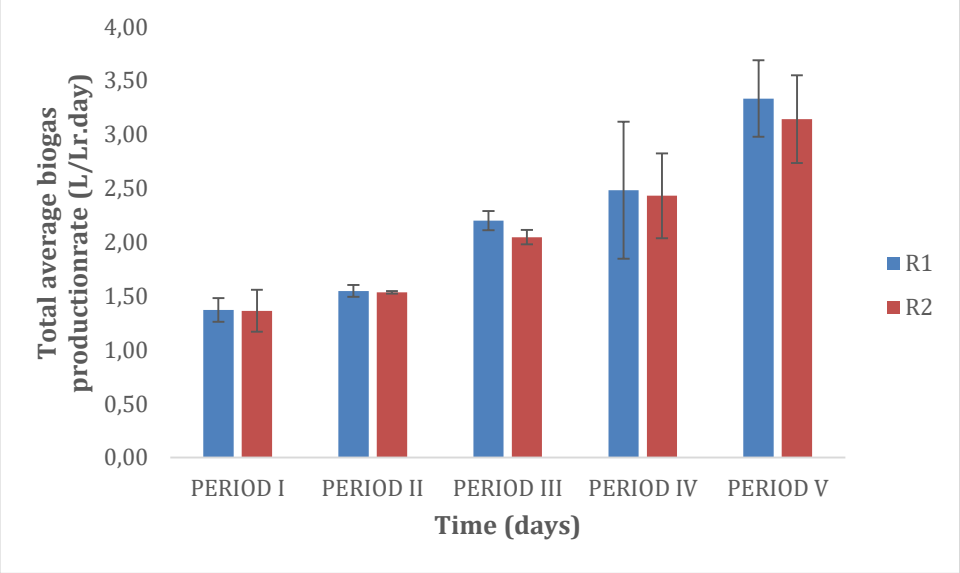
As seen in Graph 12 the concentration of VFA in this reactor (R2) is quite unstable which means that during the whole digestion there was probably

accumulation of this compound leading to a quite unbalanced process. In contrast, the pH remains quite stable during the whole time in reactor R2. However, around day 23 a sudden rise of pH is observed for both reactors and that can be explained because of the reaction between the hydrogen and the carbon dioxide. According to Le Châtelier's principle, changing the concentration of one compound, the equilibrium will shift to the side that would reduce that change because the chemical system will attempt to oppose the change affected to the original state of equilibrium. Therefore, when considering equation 2 below, if CO_2 in the liquid phase reacts with the hydrogen, HCO_3- will be converted to CO_2 so the pH will increase due to the loss of protons.

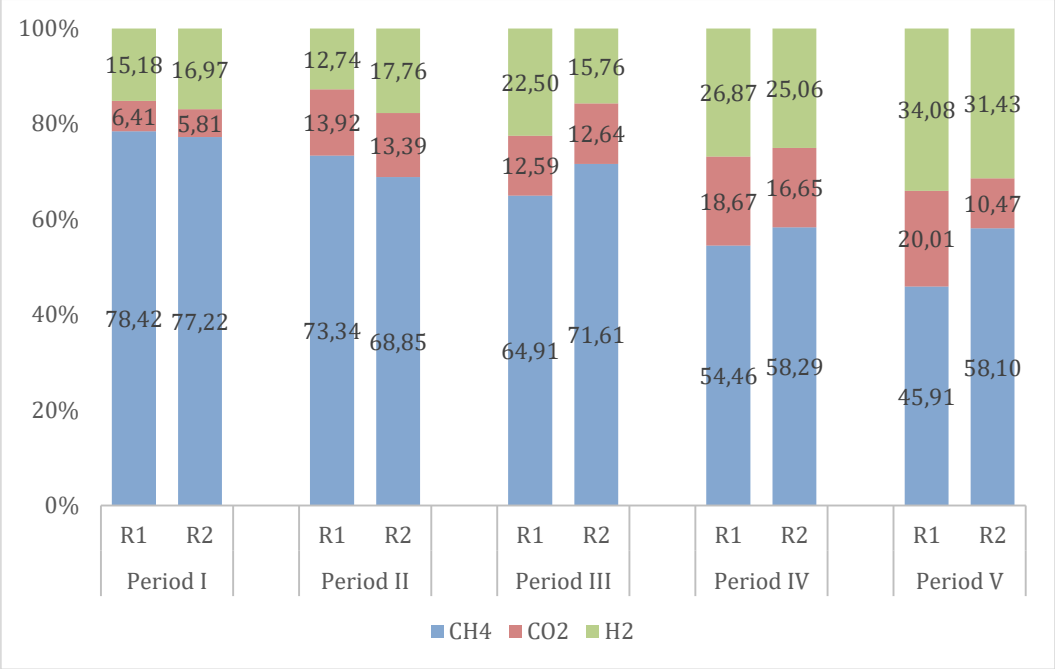
$$CO_2 + H_2O \leftrightarrow HCO_3^- + H^+ \quad (2)$$

The higher level of pH observed, resulted in a slight increase in VFA concentration, but afterwards it went back rapidly to normal values.

Finally, as seen in Graph 13 and Graph 14, the total average biogas production is better in R1 than in R2 during all the experimental periods. However, if the amounts of each gas are closely observed in Graph 14, it can be seen that the methane production (which is the gas produced) is higher mainly in R2. Therefore, in order to extract safe conclusions about the reactors the detailed amounts of each gas should be studied.



GRAPH 13: TOTAL AVERAGE BIOGAS PRODUCTION RATE OF EACH PERIOD FOR R1 AND R2.



GRAPH 14: AVERAGE % COMPOSITION OF CH_4, CO_2 AND H_2 FOR EVERY EXPERIMENTAL PERIOD.

In the tables below, the various performances of each reactor under different operation conditions, during the five periods of the experiment after achieving steady state conditions, are presented. The data of Table 8 and Table 9 present average values in steady state conditions for each period. As mentioned before, no substrate was fed in this reactor but a mixture of methane, carbon dioxide and hydrogen was injected in order to simulate the upgrading of a biogas that has not been produced in the reactor itself.

- **Reactor R_1 (control)**

TABLE 8: PERFORMANCE OF REACTOR R_1 UNDER DIFFERENT OPERATION CONDITIONS.

Period	I	II	III	IV	V
Gas injection rate $L/(L_{reactor}day)$	3	3	4	4	5
Gas retention time (h)	8	8	6	6	4
Recirculation rate (L/day)	178	236	236	295	295
Biogas production rate $L/(L_{reactor}day)$	1,37±0,11	1,55±0,05	2,20±0,09	2,48±0,64	3,33±0,36
Yield (CH_4/H_2)	0,22	0,20	0,19	0,13	0,07

- **Reactor R_2 (with packing material)**

TABLE 9: PERFORMANCE OF REACTOR R_2 UNDER DIFFERENT OPERATION CONDITIONS

Period	I	II	III	IV	V
Gas injection rate $L/(L_{reactor}day)$	3	3	4	4	5
Gas retention time (h)	8	8	6	6	4
Recirculation rate (L/day)	178	236	236	295	295
Biogas production rate $L/(L_{reactor}day)$	1,36±0,19	1,53±0,01	2,05±0,07	2,43±0,39	3,14±0,41
Yield (CH_4/H_2)	0,23	0,20	0,21	0,17	0,18

Conclusions

The present study proposed and demonstrated a method for biogas upgrading in two UASB anaerobic reactors one filled with packing material and one used as a control reactor. Both reactors operated in thermophilic conditions and had obvious biomethanation potential from H_2 and CO_2. Biogas upgrading in the thermophilic anaerobic reactor filled with packing material (R2) showed the highest CH_4 production rate (906 $L/L_{reactor}day$), which was obtained during period V (day 80) with the gas feeding as high as 7,2 L/day (HRT=4 h) and the recirculation rate 295,2 L/day. For R1, the highest methane production rate (537 $L/L_{reactor}day$) was reached during period IV (day 52) with the gas feeding at 5,76 L/day (HRT=6 hours) and the recirculation rate at 295,2 L/day. In the thermophilic conditions of the experiment, the production of VFA increased with the gas feeding up to a production of 1.235 mg/L and 844 mg/L for R1 and R2 respectively, for 4 hours of retention time (gas feeding: 7,2 L/day and recirculation 295,2 L/day). The yields obtained can be considered as optimal observing the high values that were reached the value 0,25 for CH_4/H_2 during period I and V. The acidogenic process in the working conditions studied was mostly stable, considering the parameters pH, acetate concentration and VFA. The comparison of the two reactors configurations resulted in a total better performance of R2, regarding the concentration of methane in the effluent and the hydrogen conversion efficiency. The performance of R2 was generally better

than R1 regarding the production of methane, due to the packing material which helped at the constant aceticlastic methanogenesis that contributed to the methane production, and to the fact that no particular inhibiting influence was noticed (meaning that the pH influence on the VFA accumulation was constantly the same). However, at the end of the experiments when the pH tended to stabilize at around 8,3 , the performance of the reactors, regarding the methane production tended to differ a lot. The degassed digested that was used as a nutrient provider was a suitable waste to be treated in a UASB reactor, because it was a liquid waste coming from manure and provided to the microorganisms whatever was necessary. Moreover, it was a way to control the pH inside the reactor if anything went wrong, meaning that if for example the pH rises inside the reactor it can be fixed by acidifying the liquid feeding. It also helped with the whole monitoring as it was completely degassed and tended to minimize aceticlastic methanogenesis inside the reactor, so the image of the gas production in the headspace of the reactors was clear. Although, aceticlastic methanogenesis is positive in the way that it also produces CH_4, the drawback is that it also produces CO_2. This means that extra H_2 has to be fed in order to get rid of CO_2 produced from aceticlastic methanogens, and thus resulting in extra monitoring. In total, ex-situ biogas upgrading was successfully upgraded until a high level of 92% through this technology but didn't reach 94% of methane content in the biogas introduced that had 23% of methane. However, in the last stage, when the gas flow composed by hydrogen, methane and carbon dioxide was increased to 7,2 liters per day and the recirculation rate to 295,2 L/day, the process was optimized, for R2 which had the packing material. Thus, either a further increase in the gas recirculation or utilization to enhance the hydrogen conversion to methane could be developed in a subsequent study. These main findings are useful for the future industrial application of the biogas upgrading via hydrogenotrophic methanogenesis.

Recommendations for the future

Some important factors influencing hydrogen utilization are the configuration of the reactor and the gas-liquid contact time. This last point can be achieved through keeping high recirculation flows of the gas. For these reasons, more research in the area of finding optimum reactor configurations that increase the gas-liquid mass transfer is needed, as well as, a further study of various gas feeding flows and recirculation rates for the UASB configuration. The effects of organic loading rate, alkalinity, and other parameters on the ex-situ biogas upgrading process need also to be studied. Another interesting future work could be to do a systematic testing whether pH has significant effect on ex-situ biogas upgrading using fully degassed digested manure. By trying to add different concentration of buffer compound (for example, phosphate buffer) to the digested manure, so different reactors are running at different pH controls, and compared with the reactor that has no pH control.

Moreover, it would be a real future perspective to measure the activity of the micro-organisms inside the reactors in order to find and characterize their microbial culture and achieve to measure their activity by using the real time PCR method. In that way, it could also be achieved to fully characterize the genome of these methanogens and find out which metabolic pathway they follow.

It is worth mentioning, that the CH_4 amount in the feeding gas was used because this study was interested in the possibility of taking biogas from a production unit and connect it with such a production line. That could also result in a connection of this artificial biogas with the natural gas pipelines.

Finally, since Denmark has a high wind energy production, it would be useful to find applications for this energy in various fields. The idea is to use this energy to obtain hydrogen through the water electrolysis and then utilize the gas in the biogas upgrading as it is already been done for the SYMBIO project.

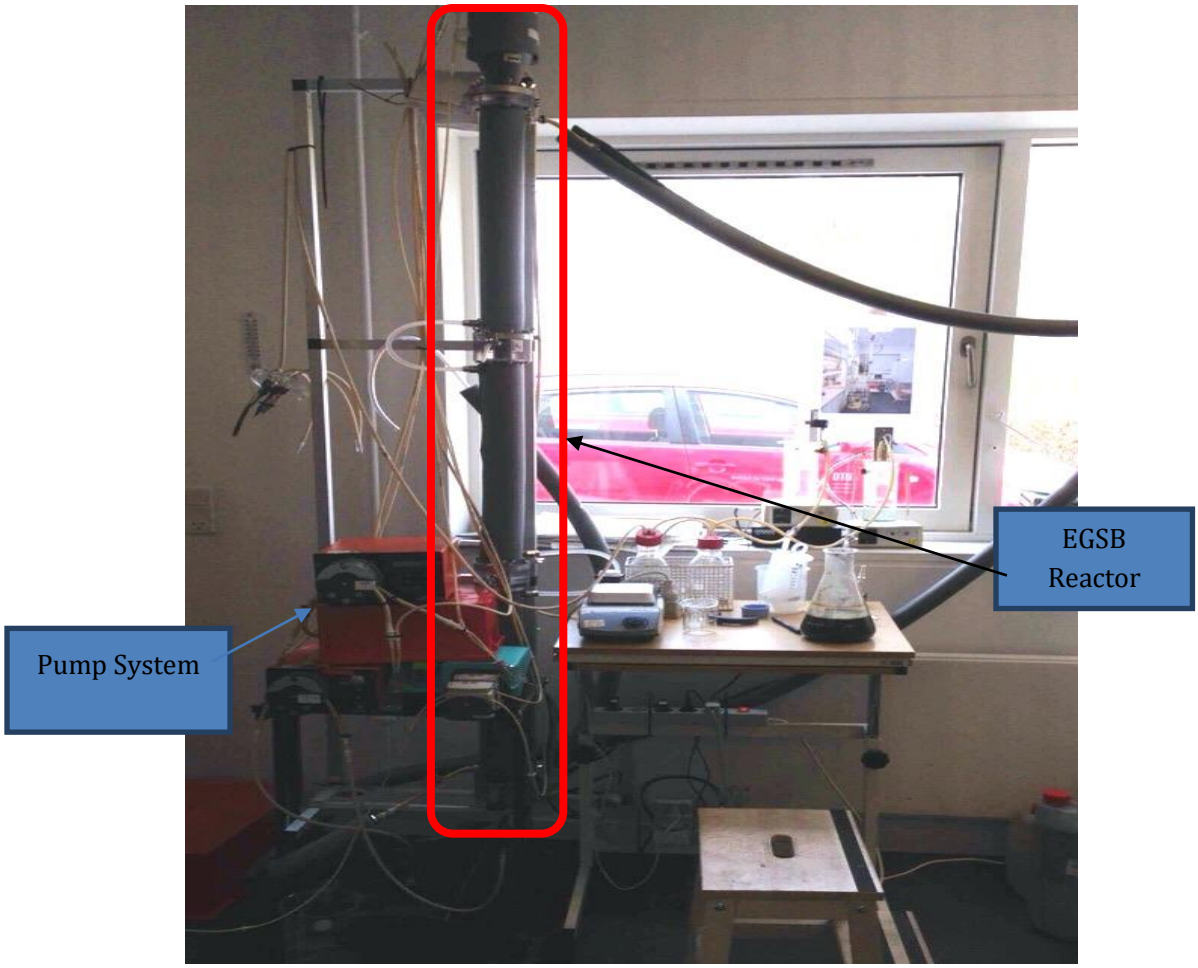
Appendix

Additional Experiment

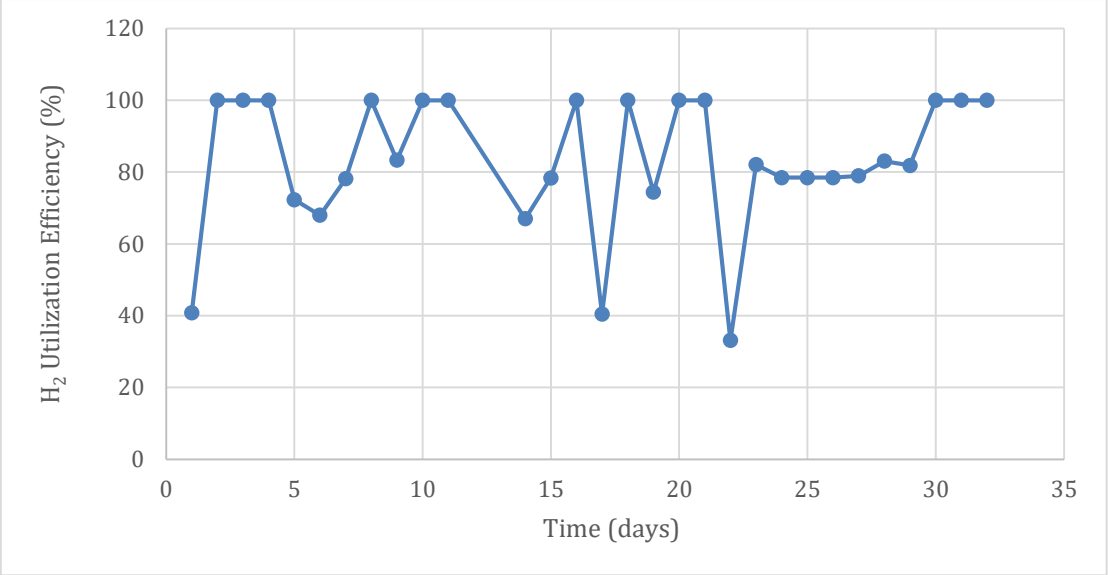
EGSB Reactor

A lab-scale, custom-made, cylindrical, up-flow, expanded granular sludge bed (EGSB) reactor was used in this project for the extension of the biogas upgrading study. It had the same characteristics with the control UASB reactor except for its geometrical characteristics. It had a very small diameter (around 2 cm) and a high length (around 140 cm), in order to achieve the same working volume of 1,4 L , while giving the methanogens more time to digest the gases . The whole configuration was set up in the same way and operated under the same conditions as the ones used in the UASB reactors. The inoculum inside the reactor, the degassed digestate feeding and the gas feeding were the same and the same operating principle was followed throughout the experimental procedure.

The EGSB reactor run for a period of one month with gas HRT of 8 hours and gas recirculation of 7,4 L/h or 177,6 L/day. The setup is depicted below:

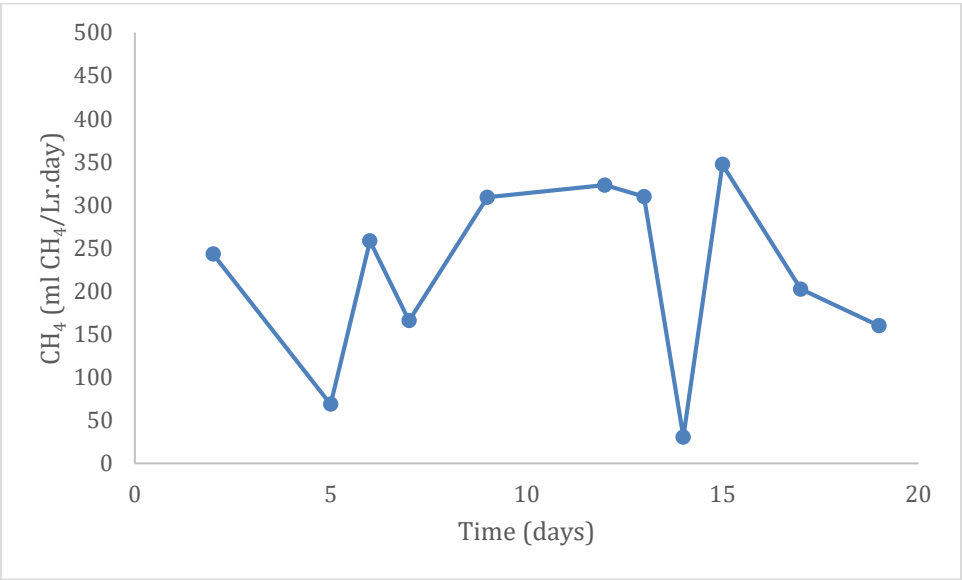


Unfortunately, the configuration faced many issues. There were leakages detected in various parts of the reactor throughout the whole process because of high H_2 partial pressure that causes crackings, so air was coming inside the reactor. Therefore, the monitoring was very difficult and the results couldn't be enough objective. Moreover, methanogenic cultures were developed on the diffusers, causing gas clogging. The initial inoculum had a few microorganisms that were sensitive to sudden changes of their environment. Finally, the process was very sensitive due to the feeding that was a gas mixture and its composition was easily affected inside the gas bag. However, some interesting results came out which are presented below.



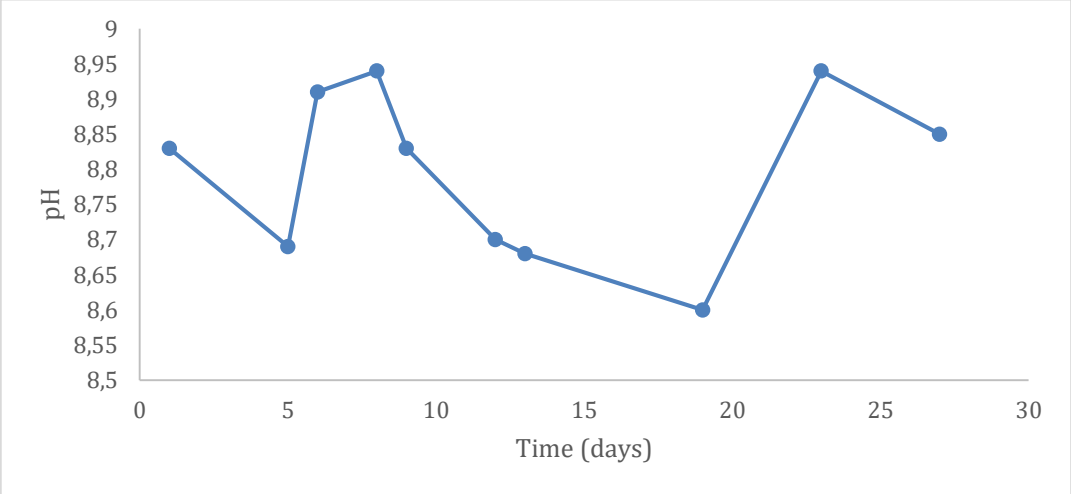
GRAPH A: H_2 UTILIZATION EFFICIENCY

As it can be seen in Graph A above, the utilization efficiency of H_2 reached significant levels. Although the procedure obtained steady state, due to the issues mentioned above, the data gathered display a rather unstable experimental procedure. However, the configuration was able to regain balance quickly and reach an optimum level of hydrogen utilization efficiency of 100%. Therefore, since the hydrogen was efficiently utilized, methane was produced by following the hydrogenotrophic methanogenesis path in relatively high rates. However, as it can be seen below, the methane production rate is not as high as expected, so probably some inhibitory factors caused unbalance to the procedure.



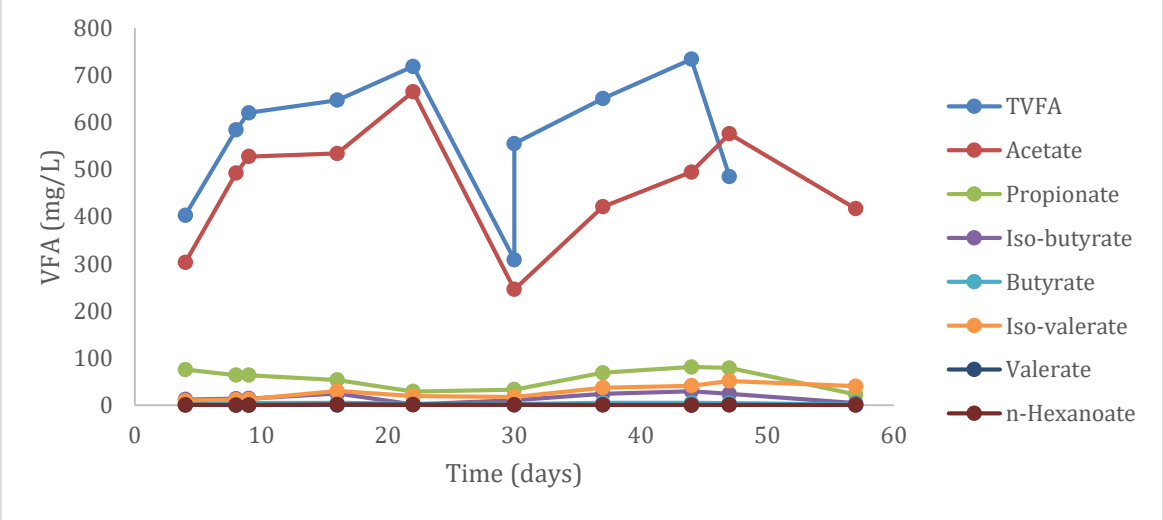
GRAPH B: METHANE PRODUCTION RATE

The methane production rate can be described as unstable and with many changes during the procedure. This is probably caused due to the many factors that caused process imbalance to the reactor and were mentioned previously. The higher CH_4 production rate that was achieved was 347 ml $CH_4/L_{reactor}day$, around day 15. The steady state is approached at some points, but then easily lost and regained once more.



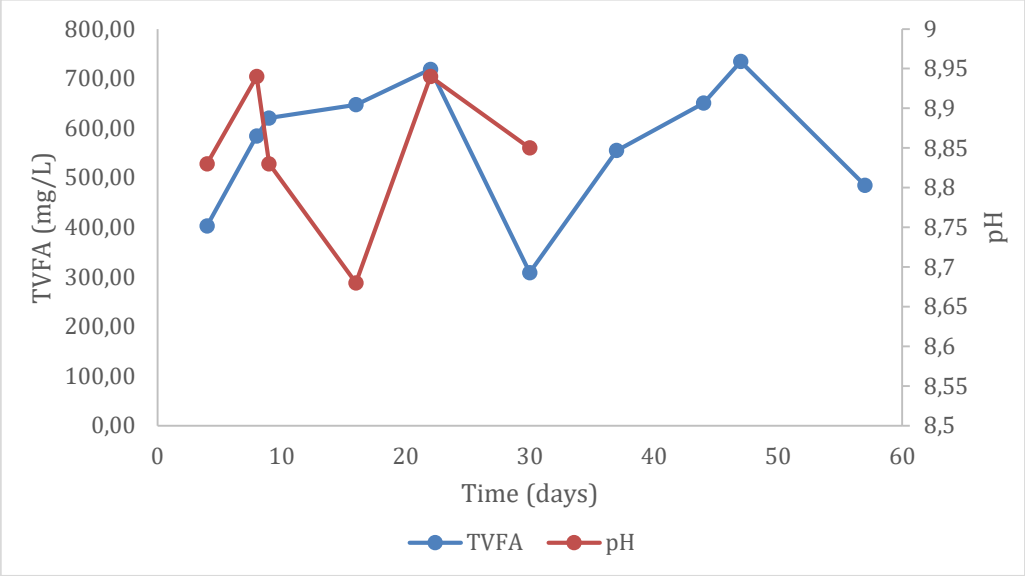
GRAPH C: PH VALUES

According to Graph C, the pH values were in the range of 8,6-8,9. This is a relatively high pH which indicates process inhibition and the pH values are relatively unstable throughout the whole process.



GRAPH O: TOTAL VFA AND DETAILED VFA CONCENTRATIONS.

The Total VFA concentration indicates an increasing during the experimental period mainly caused by the acetate concentration which is relatively high. The graphs show a rather unstable period with lots of changes.



GRAPH P: TOTAL VFA CONCENTRATIONS AND PH VALUES

The comparison between the VFA and pH values show that during the first days both TVFA and pH were increased but after that the TVFA drops suddenly while the pH increases until they meet each other. Finally, the pH follows the TVFA dropping until day 30 that it was monitored.

The whole experiment indicates some interesting points like the high hydrogen utilization efficiency and should be retried in the future using better materials in order to endure the high pressures and be made with various operating conditions, as a higher gas feeding and recirculation rate. The constant pH and VFA sampling could lead to some useful results about the reactor's behavior and about the microorganisms inside it.

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