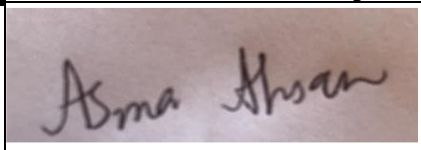
Universitetet i Stavanger

FACULTY OF SCIENCE AND TECHNOLOGY

MASTER'S THESIS

Study programme/specialisation: Master's in environmental engineering	Spring semester, 2019 Open
Author: Asma Ahsan Khan	 …… (signature of author)
Programme coordinator: Roald Kommedal Supervisor(s): Roald Kommedal, Anders Wold	
Title of master's thesis: **The study of biomethane potential from the Anaerobic Digestion and Co-digestion of complex Organic Wastes in Batch and CSTR modes**	
Credits (ECTS): 30	
Keywords: Anaerobic digestion, Co-digestion, batch tests, CSTR, Sludge retention time, Sewage sludge, Fish wastes and Food wastes.	Number of pages: ……69 + supplemental material/other: …….9 pages Total pages……78 Stavanger, 15th June 2019

The Study of Biomethane Potential from the Anaerobic Digestion and Co-digestion of Complex Organic Wastes in Batch and CSTR modes

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2019

Abstract

The world is now exploring economical and environmentally friendly resources of renewable energy. Refined biogas is one of the essential resources of renewable energy that has the potential of substituting some of the fossil fuels. Anaerobic digestion has been recognized as a biochemical method of biogas generation that can transform organic compounds into a sustainable source of energy but possess some drawbacks linked to substrate characteristics. Therefore, anaerobic digestion and co-digestion of various wastes were carried out to assess the biogas yield using batch and semi CSTR systems. In the batch tests, four different waste fractions, i.e., primary and secondary sewage sludge, fish wastes, food wastes, and the industrial sludge were investigated in mono-digestion and co-digestion processes. Different mixture ratios were prepared, and the methane yield (Y_{CH4}=$gCOD_{CH4}/gCOD_{removed}$), the specific methanogenic activity (SMA), and a kinetic parameter (k_h) were determined using the batch digestion assays at mesophilic conditions (35^oC) and possible effect of co-digestion of these wastes was examined. The primary sludge showed the higher (70%) COD conversion to methane than fish sludge and co-digestion caused the lowering of methane yield (60%). But mixing of secondary sludge with food wastes and fish sludge from Steinsvik had greater yield (89%) than individual substrates (59-60%). The starch as positive control gave about 70-80 % methane production showing good biodegradability. Then co-digestion of primary sludge and fish sludge (3:1) was carried out in four CSTR reactors with 15 days, 7.5 days, 5 days and 3.75 days at a constant loading rate of 2.9gCOD/d. Furthermore, the effect of different operational conditions like pH, VFA concentration, hydraulic retention time (HRT), chemical oxygen demand (COD) removal, volatile solid (VS) removal efficiency and biogas or methane production was studied in these reactors. The co-digestion of primary sludge and fish sludge in CSTR showed a stable system at retention times of 15 and 7.5 days throughout the experiment and give higher methane yields (60-100%). The overall system performance was stable in each of the four reactors with different retention times and CSTR proved to be better system for co-digestion than batch reactors.

Keywords: *anaerobic co-digestion, biodegradation assays, biochemical methane potential, Fish wastes, municipal wastewater sludge: primary and secondary sewage sludge, household organic waste, Industrial food waste, potato starch, synergistic effect, batch reactors, Continuously stirred tank reactor.*

ACKNOWLEDGEMENTS

Firstly, I would like to express my deep gratitude to Assoc. Prof. Roald Kommedal, my research supervisor, for his professional guidance, enthusiastic encouragement and useful critiques of this research work. My sincere appreciation always goes to him for providing me learning opportunity to do this project and helping me whenever I needed the most. I would also like to thank Anders Wold, my main advisor for sharing his knowledge and experience and thus keeping my progress on schedule. It would not be possible for me to complete this research work without his continuous advice, technical assistance and kind nature.

I would like to extend my thanks to the engineers of the laboratory of the department especially Liv Margereth Aksland, Hans Kristian Brekken for their help in offering me the resources in running the program.

The completion of this project could not have been accomplished without the support of my fellow classmates and especially my lab-mates, Kobina Quansah, Manju Sapkota and Roberto Taibo for the interesting discussions, working together, and for all the pleasure we have had together in the last two years.

Any attempt at any level can 't be satisfactorily completed without the support and guidance of my parents and friends. I wish to thank my parents for their help, encouragement, best wishes and prayers throughout my study and in all my lifetime. Also, special thanks to my siblings especially Umair for providing me last time prof-reading.

Finally, to my supportive husband Ahsan, my thanks. It was a comfort and relief to know that you were willing to provide management of our household activities while I completed my work. My heartfelt thanks to you, Rafia and Fariah for the love and time we shared together and being always there in my life.

Asma Ahsan Khan
June 2019.

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1. Introduction

Currently developed and developing countries are looking for alternative sources of energy. Particularly in developing countries, significant quantity of waste is being generated from both household as well as industrial activities. On the way to dispose these wastes, some innovative and advanced research plans (Ohnishia *et al.*, 2016), are initiated to transform the waste into consumable energy or some value-added products. All developing countries are facing the huge problem of disposal of diverse municipal solid waste produced from urban centers. To get awareness of the municipal solid waste management, organic fractions produced are identified and being evaluated for recovery of energy (Pagés-Díaz *et. al*, 2015).

Today, most of the primary energy supply in the world is covered by fossil fuels such as oil, coal, and natural gas, which together account for about 81% of the energy demand (Figure 1.1). Present scenarios have shown that due to the negative impacts of fossil fuels on the environment and continuous misuse of the natural resources, the public interest has shifted towards renewable energy sources to provide a sustainable future for energy production. According to the recommendations of the European Union (EU), about 20% of the total energy supply should come from renewable resources by the year 2020 (World energy consumption, Wikipedia).

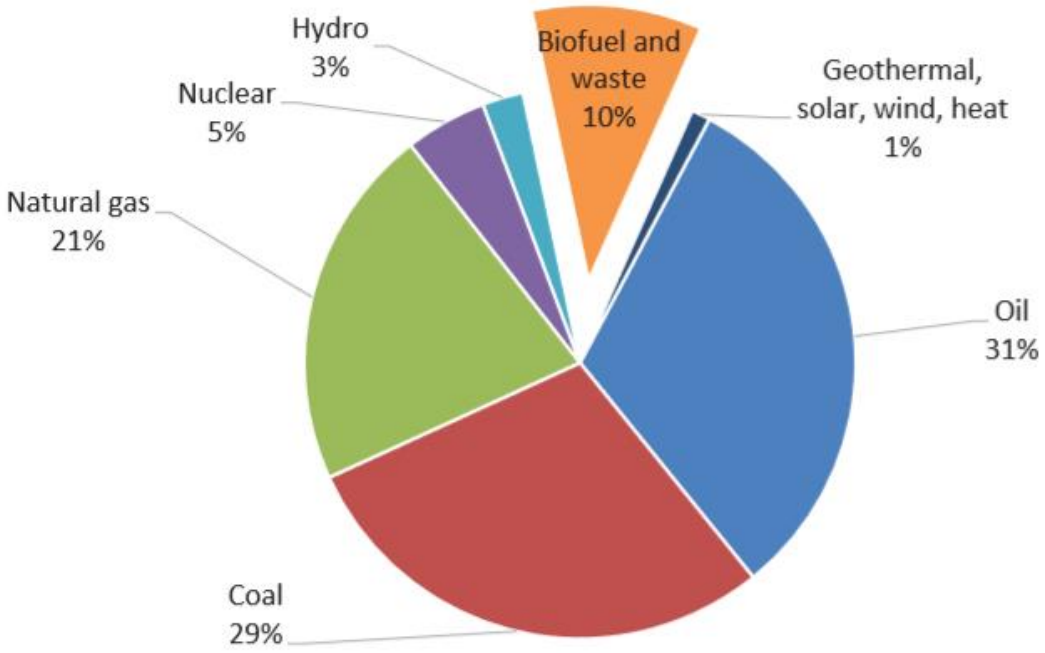


Figure 1.1: World total primary energy supply in 2012 (Pagés-Díaz *et. al,* 2015)

In Europe, biogas production was up to 174 TWh that was about 8% of total renewable energy production in 2015 (Torrijos, 2016). Germany and Sweden are among the largest producers of biogas. It is mainly produced from wastewater sludge, agriculture wastes, households and industrial wastes. Biogas is mainly used for the co-generation of electricity and heat in the European Union (EU), while cooking and lighting are the major utilizing forms in the developing countries (Kampen *et al.,* 2016). Therefore, in the last decades, the use of the anaerobic digestion for treatment of organic solid wastes became increasingly popular. Karagiannidis and Perkoulidis (2009) claimed that this technical development could be explained by three main factors: primarily, regulatory requirements to apply a treatment process in order to dispose of organic solid wastes is more environmental friendly than landfills; Secondly, the opportunity to obtain a renewable alternative fuel and positive net energy production; And thirdly, relatively low process design and operational costs.

In Norway, production of biogas from sewage sludge and other organic wastes has proved to be a reliable way for waste treatment. If we survey the present-day scenario in Norway, the Ministry of Climate and Environment in Norway introduced a new plan for biogas in 2014 (Tormod Briseid, 2015). The aim of this policy was to stimulate the production of biogas from different substrates by using different methods in research and development. It could be achieved by increasing the varieties and amounts of organic wastes used. For this purpose, biowastes, fish sludge and fish wastes were proposed to be added in the digestion of previously used food wastes, agricultural wastes, industrial wastes as well as sewage sludge. The digestate produced during AD is used as fertilizer in agriculture. But there are certain limitations for the digestate obtained from sewage sludge, as it depends on the concentration of heavy metals in the digestate.

Anaerobic digestion is a fermentation process during which the organic material is degraded and then biogas (composed of CO_2 and CH_4 gases) is produced. In nature, this happens usually in environments where organic material is available and redox potential is low (no external electron acceptors). Examples are the stomachs of ruminants, in marshes, sediments of oceans, lakes and ditches, in landfills and municipal sewers. AD is the cost-efficient method in removing biodegradable compounds and widely used to stabilize wastewater sludge and through that reduce organic load before final sludge disposal. It is a waste-to-energy technology and is also used for digestion of other organic wastes, like animal manure, food waste, organic fraction of municipal solid waste and industrial wastewater sludge (Li *et al.* 2015). Nonetheless, anaerobic digestion of single substrates of unbalanced bacterial growth composition is challenging. Consequently, anaerobic co-digestion, the simultaneous digestion of two or more substrates, is a potential option to overcome the drawbacks of mono-digestion and improve the economic performance due to higher methane production.

1.1. Objectives:

This study was the continuation of the project initiated by the municipality of Bergen (project manager), in cooperation with several other institutions including: Aquateam COWI (project supervisor), IVAR IKS, Bergen University College, the University of Stavanger, Norwegian Seafood Centre and Blue Planet. The project title was "*Utilization of waste from marine food production for regional renewable energy*" aiming to optimize the utilization of organic waste from the aquaculture industry. in the Western regions of Norway. The main goals of the project were:

1. Finding the optimal co-digestion ratios for aquaculture waste and municipal waste.
2. Estimate the potential for increasing methane production by utilization of aquaculture waste.
3. Assess nutrient and heavy metals conversion in the during anaerobic co-digestion of fish wastes

Sub-Objectives:

The sub-objectives can be stated as

1. To evaluate the potential of biogas production and process stability when digesting and co-digesting fish wastes, municipal waste and other organic wastes.
2. To investigate the synergistic and competetive effects of co-digestion.
3. What were the inhibiting factors in co-digestion with fish wastes as fish wastes has higher ammonia nitrogen and lipid content?
4. Which of the organic wastes produces highest methane yield Y_{CH4} ($NmL_{CH4}/gVS_{substrate}$) and SMA specific methanogenic activity ($gCOD_{NmLCH4}/gVS_{inoculum}/d$) and $Y_{gCOD/gCOD}$.
5. To understand the influences of co-digestion of fish wastes and primary sludge and operational conditions (sludge retention time) on overall methane yield ($NmL_{CH4}/gCOD_{substrate}$).
6. To compare the performances of batch assays and continuous stirred fed tank reactors (CSTR) in co-digestion and BMP of mixed sludges.
7. To evaluate the best possible hydraulic retention time for co-digestion in CSTR.

2. Literature review and theoretical background

2.1. The basic principles of anaerobic digestion:

Anaerobic degradation also known as digestion can be defined as a complex biological conversion process in the absence of external electron acceptor for instance oxygen as in aerobic processes or nitrate and sulphate as in anoxic processes resulting in the conversion of biodegradable organic matter into mixture of two core end products: biogas and digestant. Biogas produced from AD is a blend consisting by volume generally of methane ($CH_4 \approx 60\%$), carbon dioxide ($CO_2 \approx$

40%), and small bits of hydrogen sulphide (H_2S), nitrogen (N_2), hydrogen (H_2), oxygen (O_2), carbon monoxide (CO), water vapor (H_2O), or other gases and vapors of various organic compounds and digestate is the decomposed substrate, rich in macro- and micro nutrients and therefore suitable to be used as plant fertilizer (McInerney *et al.* 1980). This process is very common to many natural environments and mainly applied today to produce biogas in airproof reactor tanks.

There are four basic chemical and biological stages of anaerobic digestion includes; Hydrolysis and Disintegration, Acidogenesis, Acetogenesis, and Methanogenesis (Appels *et al.,* 2008) as shown in the figure 2.1. The process of digestion begins with the bacterial hydrolysis of the input-materials to break down soluble or insoluble organic polymers such as carbohydrate. After the hydrolysis, acidogenic bacteria convert the monosaccharides and amino acids into carbon dioxide, ammonia, hydrogen and organic acids. After that acetogenic bacteria convert these organic acids into acetic acid with additional ammonia, hydrogen and carbon dioxide (Ueno *et al.*, 2001). Methanogenic bacteria then convert acetic acid and hydrogen to methane and carbon dioxide.

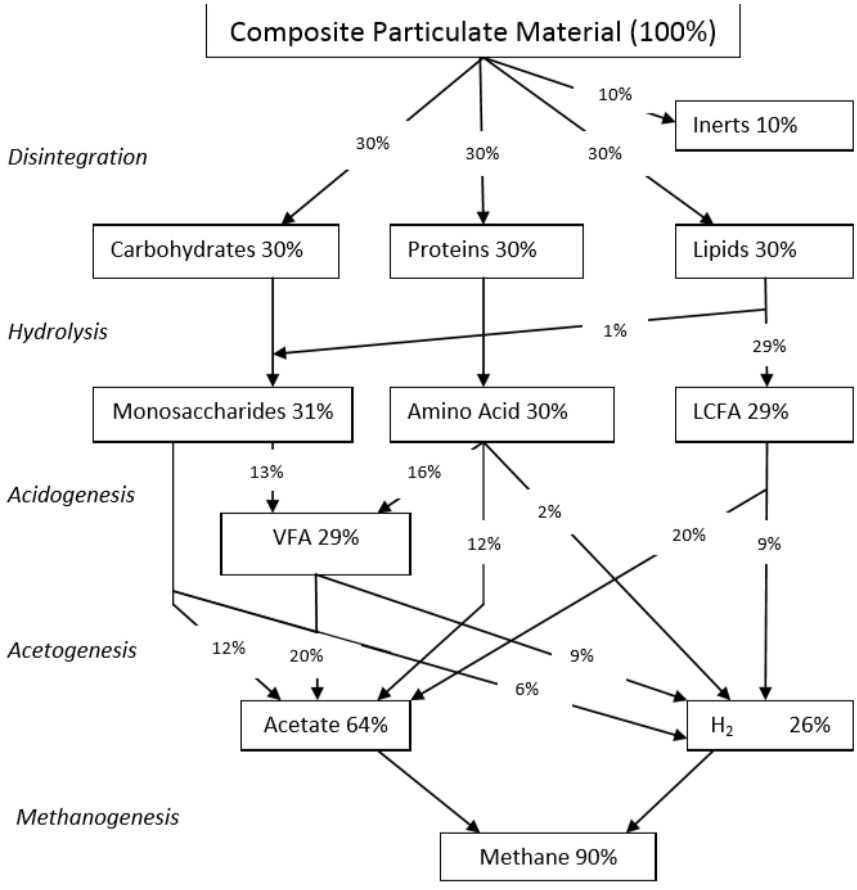


Figure 2.1. showing different steps of anaerobic digestion. COD flux for a particulate composite is comprised of 10% inert and 30% of each of carbohydrates, proteins and lipids (in terms of COD) (Batstone *et al,*.2002).

2.1.2. COD mass balance in AD reactor:

The most useful parameters for evaluating the efficiency of biogas production are the reduction in VS or COD. Typically, most of the studies on AD of organic substrate have been experimented with one-stage mesophilic CSTR or semi-CSTR. In these systems, the stable and profitable methods to enhance CH_4–biogas production and decrease volatile solids or COD depend largely on operational parameters. In anaerobic digestion processes, COD is mostly taken as a control tool for biogas production. COD is preferably used to determine the organic fraction of any sludge sample rather than VS content. This is attained by adding a strong chemical agent to the given wastes sample in an acidic system. In order to monitor performance of reactor, COD mass balance is determined by measuring COD of influent, effluent and COD removed as methane produced. C Figure 2.2 shows COD balance of an anaerobic digestion (Matheri *et al.*,2017) and provides information about efficiency of anaerobic digester.

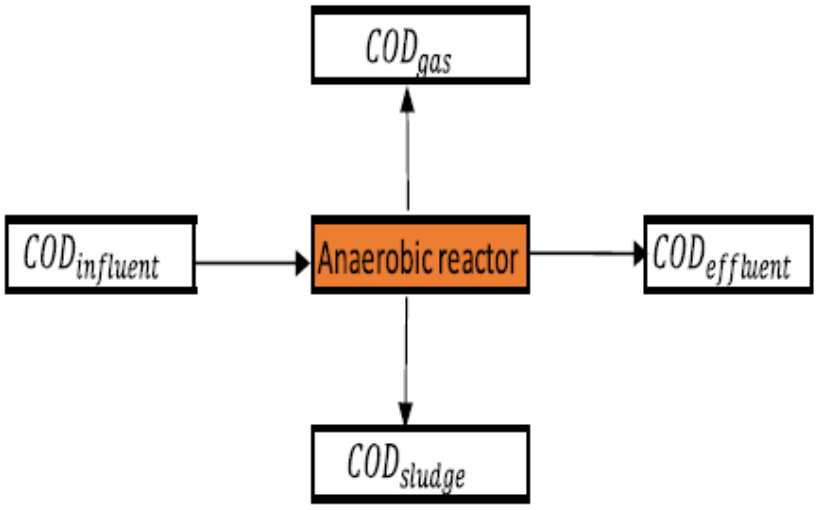


Figure 2.2 COD balance of anaerobic digestion (Matheri *et al.*,2017)

2.1.3. Hydrolysis and Disintegration

Disintegration involves the breakup and dissolution of the organic material and seldom requires any biological processes as the depolymerization enzymes outside the microbe cells use the existing organic material, breaking it into simpler substances. This process is particularly important for the complex wastes like sludges and food wastes as it allows for the lysis of complex organic material. Moreover, it can change the rates of hydrolysis of different composites (Batstone *et al.*, 2002). Hydrolysis is the process during which enzymes excreted by anaerobic bacteria transform complex, solid material into simple and soluble molecules that are permeable through the cell walls and membranes of these microorganisms. During hydrolysis, particulate or soluble macromolecules are converted to its soluble monomers. The most important particulate materials are composed of proteins, carbohydrates and lipids that by the action of enzymes are converted to amino acids, monosaccharides and long chain fatty acids respectively. The enzymes for the process are produced by the bacteria themselves (Henze *et al.*, 2009). The heterotrophic organisms carry

out this process by attaching to the particles surface, produce enzymes in the surrounding area and get energy from soluble products released by the enzymatic reaction. Thus, the bacteria who grow up on the particle should be considered as the effective catalyst and known as fermentative bacteria (Batstone *et al.,* 2002). For this reason, hydrolysis and disintegration are the rate-limiting steps in AD. The monomers produced from hydrolysis are utilized by acidogenic bacteria.

2.1.4. Acidogenesis:

Acidogenesis is also known as fermentation or anaerobic oxidation and occur in the absence of any external electron acceptor or donor. During this process, acidogenic bacteria transform the monomers produced in hydrolysis to number of simpler products. So, they consume LCFA, amino acids and monosaccharides for their growth and further convert them to volatile fatty acids, alcohols, CO_2, lactic acid, H_2, NH_3, H_2S. The nature of products formed during acidogenesis depends on the operational conditions and nature of medium used. In contrast to hydrolysis and acetogenic steps, acidogenesis is faster conversion step in the anaerobic digestion and free energy of the reaction is higher responsible for higher growth rates in acidifying bacteria. That's why souring of reactor occurs sometimes and pH inside the reactor drops because of higher VFA produced during acidogenesis. Consequently, methanogenic activity is inhibited in acidic condition and methane production is reduced or stopped in some cases (Henze *et al.,* 2009).

2.1.5. Acetogenesis:

Acetogenesis involves the intermediary production of acetic acid. At this step, short chain fatty acids are further changed into acetate, H_2 and CO_2 and new bacteria biomass. It's also called homoacetogenesis because the formation of acetic acid might also occur from H_2 and CO_2 produced during acidogenesis. The acetate formation is also oxidation reaction without any internal electron acceptor. Therefore, an additional electron acceptor is required for activity of acetogenic bacteria. The main substrates for this type of fermentation are propionate and butyrate, while commonly used electron acceptors are, H^+ ions or CO_2 resulting in the production of H_2 gas and formate. The products formed are further used by methanogenic bacteria. Therefore, H_2 gas is the inhibitor for their activity. The co-existence of H_2 consuming methanogenic bacteria and H_2 producing acetogenic bacteria is only possible in the certain narrow range of hydrogen or formate amounts. Thus, they also effect the kinetics, modelling and overall methane yields in the anaerobic processes. In a properly operational anaerobic reactor, a balance is retained between the methanogenic and acetogenic activities, i.e., the H_2 produced by acetogens is readily taken by methanogens and biogas is formed (Henze *et al.,* 2009; Batstone *et al.,* 2002).

2.1.6. Methanogenesis:

This is the final step of AD in which methane, CO_2 and new cell material is formed from acetate, formate and methanol. As stated by Henze *et al.,* (2009) methanogenic bacteria are located at the bottom of the anaerobic food chain, and due to their activity, organic matter does not accumulate in anaerobic environments in greater amounts. Methanogenic Archaea are classified based on the

substrates used for methane production: first are the strictly acetoclastic methanogens which convert the acetate to methane, which constitute the genus *Methanosaeta* and *Methanosarcina*, and second class are the hydrogenotrophic methanogens forming the orders of *Methanobacteriale.* Moreover, the genus *Methanosarcina* is considered a mixotrophic methanogen since they can use either acetate or H_2/CO_2 to produce methane. Among all microbes, methanogens are mostly sensitive to variations in environmental and operational conditions in anaerobic digestion.

In fact, Ziganshin *et al.* (2013) observed the accumulation of the *Methanosarcina* species with the increase in VFA concentrations. As *Methanosarcina* has μ_{max}=0.12 (1/d) and K_s=30mgCOD/L while *Methanosaeta* has μ_{max}=0.71 (1/d) and K_s=300mgCOD/L as illustrated in the figure 2.3. So *Methanosaeta spp* are usually present abundantly in the systems with higher retention times such as in sludge bed systems, anaerobic filters and biofilms and effluent concentrations are expected to be lowest in such systems. Comparatively, *Methanosarcina* has lower affinity with the substrate but they can use variety of substrates and convert them to methane. So *Methanosarcina* are expected to be present in the solid digestors.

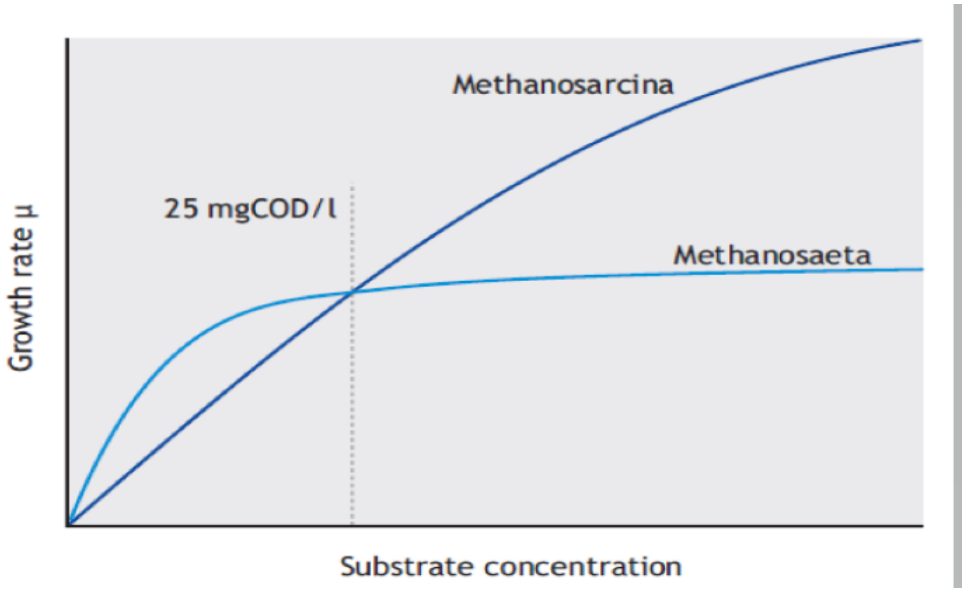


Fig 2.3 showing growth curves of acetogenic *Methanosarcina spp*. and *Methanosaeta spp*. (Henze *et al.,* 2009)

2.2. Anaerobic Co-digestion (AcoD)

Co-digestion means the digestion of two different substrates simultaneously for increasing the digestion efficacy and energy production. In co-digestion main substrate used is the sludge from wastewater while its mixed with lower proportions of other substrates like food wastes and industrial wastes. When mixing these substrates, there can many possible results. It can enhance or decrease the biogas production leading to synergistic or competitive effects. But co-digestion always requires the controlled management of the reactor conditions (Tchobanoglous *et al.*, 2014).

It is considered as a well-established process in Europe, along with Germany and Scandinavia for being the pioneers, having over twenty years of knowledge and experience in the field (Appels *et al*., 2011). The number of co-digestion plants is unremittingly increasing in so many European countries and has become a regular practice largely in the agricultural sector. The advantages of co-digestion take account of better digestibility, enhanced degradation rates, higher digester capacity with enhanced biogas production as well as methane yield arising from the availability of additional nutrients with more efficient utilization of cost sharing and equipment. Primarily, because of the research perception, AcoD focused mainly on mixing substrates which favor positive interactions, i.e. macro and micronutrient equilibrium, moisture balance and dilute inhibitory or toxic compounds (Mata-Alvarez *et al.,* 2000). Further studies have revealed that co-digestion of numerous substrates, like for example, banana peel, plantain peel, spent grains and rice husk, pig waste, cassava peels, sewage and brewery sludge, amongst many others, have resulted in better-quality methane yield by as far as 60 % compared to that achieved from single substrates (Babel *et al.,* 2009). On the other hand, nowadays, because of the industrial viewpoint and the improvement of methane production, it is mainly a result of enhancing the organic loading rate (OLR) mostly than synergisms, in which all kinds of mixtures are used.

In some current practices, co-digestion is majorly used, where two or more than two different substrates (known as co-substrates) are combined in the reactor to increase the organic matter content (increased chemical oxygen demand) and thereby attaining increased rates of biogas production. The yield and composition of biogas mainly depend on the raw materials and type of co-substrate, use of pretreatment methods, etc. The wastes having higher concentrations of lipids and readily biodegradable carbohydrates give indication of higher methane production potential, whereas lignocellulosic materials reflect lower methane concentrations in biogas. Moreover, by co-digesting, the content of heavy metals in the digestate is certainly decreased and thus improves the composition of the digestate to confirm that it can be recycled without any further treatment in the form of biofertilizer. To elude process failures, the best methods used nowadays involve pre-treatment of raw materials. It will include the evaporation of stick-water to increase the solid content, and at same time the hydrolysis of fish material with high protein content. By applying these pre-treatment methods increases the intensity of substrate degradation and efficiency of the process (Henze *et al.,* 2009). Presence of different substrates in wastes can give different biogas yields as shown in the table 2.1. The fats and greases offer the higher yield of biogas per gram of solids used by bacteria than proteins.

Table 2.1 biogas unit production and methane content (Tchobanoglous et al., 2014)

Substrate type	Gas yield per unit solids used (m^3/kg)	Methane content
Fats	1.2-1.4	62-72
Scum	0.9-1.0	70-75
Grease	1.1	68
Proteins	0.7	73

2.3. Benefits of co-digestion

There are normally three factors that favor the co-digestion of different kinds of substrates:

Technical factors: Primary factor is that it's a feasible technological solution that has an alleviating effect on the problem of disposing wastes. So, co-digestion is a better solution to remove complex wastes from collecting system, particularly if a waste is making blockage, bad odors or any kind of damage. Also, it increases the capacity of existing anaerobic digester especially while co-digesting wastes that enhance biogas production of wastewater sludge and thereby increase loading rates of organic solids. Moreover, it offers a reliable exit for wastewater sludges and other organic wastes and expand knowledge of handling wastes. Consequently, if mixed sludges are properly handled as a slurry, would be easier to transport through pipes and need less space than liquid wastes.

Economic factors: By co-digestion, more biogas is typically produced for combined heat and power systems as it increases the availability of nutrients and bacterial diversity in substrate thereby augments the anaerobic digestion. As a result, solids retention time is reduced, and the efficiency of biogas generated is increased owing to a variability of organic material having better nutrients for the anaerobic consortia in a digester (Matheri *et al.,*2017). Furthermore, it reduces the cost of waste treatment, operation and odor management in the anaerobic plants and construction of additional liquid treatment system can be avoided. However, cost proficiency of co-digestion depends on many factors. The most important factors include nature of waste, location and distance from plant, pre-treatment and electricity prices.

Environmental benefits: From environmental point of view, co-digestion decrease land application of organic wastes which provides methane production instead of carbon dioxide storage in carbon sequestering. Thus, it lowers the discharge of greenhouse gases especially methane that is directly related to improving energy recovery from waste materials (Tchobanoglous *et al.,* 2014).

2.4. Substrates for biogas production by AcoD

2.4.1. Fish Sludge

If the fish farming in Norway is considered, major nutrients in the form of nitrogen (27g N) and phosphorus (9g P) are being lost to the sea every year in the form of feed residues and fish sludge (Hamilton *et al.,* 2017). These losses by fish feces are comparable to the nutrient losses by animal manure. This nutrient flow makes current fish farming systems highly unmanageable. Therefore, fish sludge needs to be anaerobically digested or used as a fertilizer (Brød *et al.,* 2017). The biogas production by fish sludge varies with its composition which mainly depends on the type of fish species used, feeding habits, sex, season and finally the health of fish. This waste is considered as a mixture of solid and liquid wastes. The solid matter contains fish tissues and bones while the liquid phase consists of blood-water and stick-water, in which are both proteins and oils in higher amounts. One of the major complications that restrict the usage of this type of sludge is its variable nature. Generally, fish wastes have up to 60 % proteins, up to 20 % fats and varying amount of minerals (calcium and hydroxyapatite from bones and scales). In addition, palmitic acid, monosaturated acids and oleic acid are in abundance in these waste streams (Ghaly *et al*., 2013). Present studies suggest that the digestion and co-digestion of fish waste both have substantial ability for methane production. Researches indicate bio methane potential of 0.2-0.9 CH_4 m^3/kgVS of fish sludge added. Furthermore, fish waste is also used in anaerobic digestion experiments as a sole substrate and as silage, also in co-digestion with cow manure, sisal pulp, and waste from strawberry processing (Achinas *et al.,* 2017). Fish waste co-digested with some food wastes, such as strawberry waste had produced methane yields that could be compared to the yields of other food-waste streams.

Limitations of Co-digestion with fish wastes

The production of biogas by anaerobic of different substrates with diverse properties has beneficial results with other substrates, but waste from fish processing poses a typical technological problem. From fish wastes, increased levels of ammonia (if co-digested with bio-waste rich in proteins), long chain fatty acids (co-digesting with biowastes rich in lipids) and sometimes heavy metals are released during digestion, which prevents the digestion of substrates. Higher concentrations of ammonia can outcome in the accumulation of VFAs (acetic acid as the foremost type in the batch tests). Depending on the reactor type and organic loading rate, this can prevent the process particularly if the substrate is very high in fats and oils. Furthermore, the use of aquaculture wastes leads to the production of higher chemical oxygen demand that can cause the toxicity inside the reactor and inhibition of anaerobic digestion. Nevertheless, previously studied anaerobic digestion of some fish species showed the considerable bio methane production potential when tested in mesophilic and thermophilic environments (Achinas *et al.,* 2017). In some previous researches the combination of acidity and higher concentrations of fats and proteins make the fish sludge difficult to digest as a single substrate because it can cause the production of long chain fatty acids and pH in the digester is lowered. Methane production is possible at pH range between 6.5 to 8.5, while

the optimum levels for methane production is between 7 and 8 (Weiland, 2010). So, the production of volatile fatty acids by fish wastes is challenging and can give poor biogas yields.

2.4.2. Food wastes

Food waste is the most challenging part of municipal waste because of its high moisture content, variability and higher amount of carbohydrates, proteins that can be efficiently converted into methane which is used as energy source while the sludge obtained as fertilizer (Davidsson *et al.*, 2008). Thus, characteristics of food waste and sludge determine the feasibility and the operation parameters for co-digestion. Although several possibilities have been recommended for the managing the food wastes, including incineration, composting, the use of food waste disposal units and AD, but anaerobic co-digestion has attracted the more attention, mainly in strategy making, due to its potential for energy generation. Food waste can either be utilized as only nutrients resource for anaerobic bacteria in a biogas plant or can be mixed with other sludges like fish waste, sewage sludge or septic wastes etc. or can be disposed in dedicated disposers of food. This waste mixture denotes an extremely biodegradable co-substrate, which, if exceeding certain threshold limit, improves the biogas production of the sewage sludge digesters only by increasing the OLR (Salman Zafar, 2018).

2.4.3. Industrial food wastes

Large quantities of food are wasted globally, with a non-small amount being assigned to the industrial as well as production level. One major benefit of industrial food waste is that it is commonly a more homogeneous resource that can be more effortlessly converted into higher value products. Industrial food-waste streams were selected because they generate large amount of predictable food waste streams that are highly consistent and homogenous than domestic food waste streams; such predictability is desirable in order to target the recovery and processing of specific compounds and in case of anaerobic digestion (AD) it allows for onsite consumption of the generated biogas. There are variety of applications available for such food wastes. Anaerobic digestion is a very well-developed technology that permits to produce biogas for energy from food waste. Thus, the appropriate handling of industrial food waste could alleviate the approximately 1.9×10^8tons of CO_2 equivalent emissions currently being produced by the waste. It is noticeable that regardless of the end-product, industrial food waste is an underutilized source that should be placed to a higher value uses (RedCorn *et al.*, 2018).

2.4.4. Primary and secondary sludge

Primary sludge comprises of settleable solids derived from primary settlement tanks. Typically, primary sludge is organic matter containing 17% protein but 27% carbohydrates and has a higher C:N ratio than secondary sludge. Biogas production from primary sludge could be between 0.842 –0.968 Nm^3/kg VS but sewage sludges are in general a poor feedstock for anaerobic digestion because it contains insufficient carbon and too much nitrogen. Secondary sludge (surplus activated sludge) has relatively low degradability, especially that resulting from the operation of activated sludge plants at long sludge ages (Carrrere *et al.*, 2010). The composition of SAS is fundamentally different to that of primary sludge because the activated sludge process results in biomass

composed of microbial and extracellular polymeric substances (EPS). These are dense mix of biopolymers which are comprised of polysaccharides, proteins, nucleic acids, uronic acids, humic substances, lipids and some other polymeric substances as well. That's the reason EPS are comparatively recalcitrant to anaerobic digestion. Various authors (Mininni *et al.,* 2004; Horan and Lowe, 2008) showed that the biogas potential from SAS is relatively high, between 0.767 – 0.868 Nm^3/kg VS, considering that the digestibility of SAS is commonly perceived as poor.

2.4.5. Potato Starch as positive control:

Starch is a polysaccharide composed of many six-carbon sugar (glucose) units connected through 1,4 alpha glycosidic linkage. In nature, it is made by photosynthetic plants mainly as energy storage. It is the most common carbohydrate in human food and is present in large amounts in the common plants like rice, wheat, maize and potatoes. Most commercial starch is made from corn, wheat and potatoes. Commercially, starch is obtained by crushing or grinding starch-containing tubers or seeds and then mixing the pulp with water; then its remaining impurities are removed from resulting paste and then dried (encyclopedia Britannica). Among all categories of starch, potato starch represents 14% of the entire starch manufactured in Europe (Gomand, Waterschoot, Fierens, & Delcour, 2015) and 4% in the remaining world (Basiak, Lenart, & Debeaufort, 2017). Potato starch is considered as a very refined starch, which comprises smallest quantities of proteins and lipids. Moreover, its lower in cost, has a greater swelling power, paste clarity, solubility and viscosity than the starch gained from other natural sources for example wheat, rice or corn. Also, it is rich in certainly degradable, high energy sugars that have considerable potential for fermentation.

2.5. Biochemical methane potential (BMP) tests

Different kinds of methods exist worldwide to determine the BMP of numerous types of sludges. These range from theoretical to experimental tools as shown in the figure 3. A Biochemical Methane Potential (BMP) test is the most used instrument to provide a measure of anaerobic degradability of a given substrate methane yield, the extent of anaerobic activity, reaction-rate kinetics, the influence of inoculum pre-treatments, and the effect of mixing with diverse viscosities because of its high reliability and validity as it is based on conditions that approximate practical AD processes (Jingura and Kamusoko, 2017). Moreover, BMP tests can measure the residual organic material remaining after treatment that can still be used to convert to biogas and the non-degradable part remaining (Moody *et al.,* 2009). The use of BMP tests provides a relatively inexpensive, simple and repeatable method to make comparisons of the anaerobic digestibility and potential biogas potential between different substrates (Owens *et al.,* 1993). The methane potential is expressed in terms of standard temperature and pressure (STP) ml CH_4 per 1 g of VS added (mL CH_4 / g VS) (Hansen *et al.,* 2003). It was also reported that the information determined by BMP tests is helpful to characterize and evaluate the optimal design and performance of the AcoD process. In addition, BMP testing can reveal the possible mechanisms of synergy between the co-digestion mixtures (Ebner *et al.,* 2016). The conventional BMP process is complex and time-

consuming and takes approximately 30–90 days. This length may increase the cost of feedstock storage and management, and the optimal combinations of substrates may be unstable. Even though detailed guidelines for BMP test protocols exist, recent studies have shown that the outcome can vary significantly between laboratories, which indicates the need to further standardize the BMP test protocol. Researchers have suggested various alternative methods to alleviate the drawbacks of traditional BMP traditional BMP measurement.

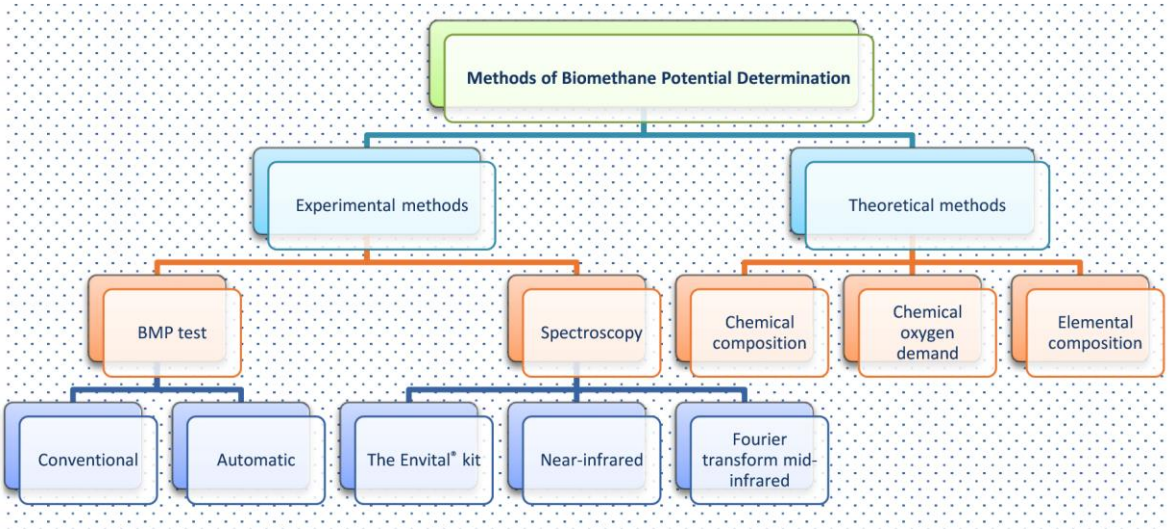


Figure 2.4 Overall summary of biomethane potential tests (Jingura and Kamusoko, 2017)

2.5.1. Quality Check for Inoculum:

The BMP tests are conducted using inoculum from well- functioning anaerobic digester. As described by Angelidaki *et al.*, (2009) the typical values for operational parameters of the digester showing an inoculum of good quality execution are:

- pH: > 7.0 and <8.5
- VFA: < 1.0 gCH_3COOH L^{-1}
- NH_4: < 2.5 gN-NH_4 L^{-1}
- Alkalinity: > 3 g$CaCO_3$ L^{-1}

So, these conditions should be met before using an inoculum as a blank or medium in biomethane potential tests.

2.7. Factors Affecting Performance and Operation

The performance and operation of anaerobic digesters is influenced by many factors. There are mainly three types of factors including loading factors, operational factors and environmental factors. Process loading factors are sludge retention time (SRT), hydraulic loading rate, environmental factors range from temperature, pH, nutrients availability, and the amount of toxic substances; and operational factors like mixing, and the nature of the waste being digested. Moreover, with thorough understanding of these parameters, balanced and healthy anaerobic

system can be achieved. In addition, stable conditions are essential to increase the activity of microorganisms responsible for methane production within the reactor.

2.7.1. Solids Retention Time

The most important parameter affecting the efficiency and successful execution of any biochemical operation is solids retention time (SRT). Because it decides the species of microorganisms that can grow in the anaerobic system and the degree to which different reactions would take place. Figure 2.4 shows characteristic SRT for a variety of anaerobic transformations at 35 °C. SRT is dependent on the temperature of the reaction medium and types of substrates. At lower temperatures, longer SRT are favorable as microbial activity is reduced (Grady *et al.*, 2011). Similarly, different substrates have different retention times in digestion, e. g., hydrolysis of insoluble carbohydrates and proteins to form monosaccharides and amino acids is faster and takes about three days. Comparatively lipids hydrolysis is quite slow and long chain fatty acids formation takes around six days.

Although SRT is the fundamental control parameter but it is usually challenging to determine it precisely in some anaerobic processes. However, SRT can be easily determined in flow-through systems such as anaerobic digesters, where it simply equals the HRT. Hydraulic retention time is the time required for any microbe to digest and consume the given substrate and it should be carefully controlled. The metabolic activities of microorganisms can be inhibited by uncontrolled retention times. The longer HRTs can cause the death of microorganisms due to deficiency of nutrients. Therefore, for industrial-scale applications, shorter HRTs are proposed to reduce the size of the digester and enhance its capacity (Li *et al.*, 2015). Thus, total biogas production and net electrical energy production can be increased by decreasing investment costs.

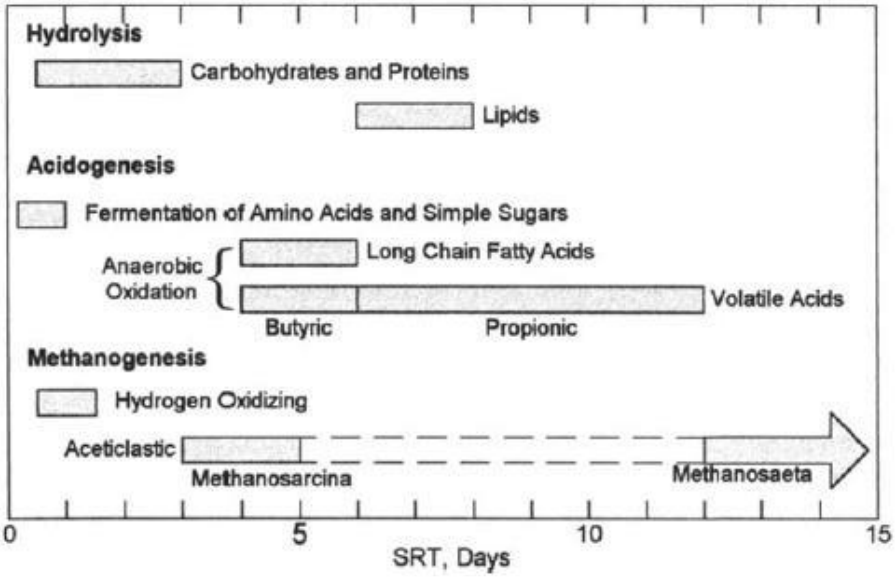


Figure 2.5 Different SRT ranges for some conversions in anaerobic bioreactors (Grady et al.,2011)

2.7.2. pH

Like all biochemical operations, the pH has an important impact in the AD system. If the pH diverges from an optimal value, activity of microorganisms would be decreased because the solubilization of organic matters is greatly influenced (Feng *et al.,* 2015). Thus, availability of substrate as well as enzymatic reactions of microorganisms are dependent on pH (Neshat *et al.,* 2017). During anaerobic digestion, microbial consortia have different optimum pH values, but most of them prefer to grow around neutral pH. Thus, to achieve maximum biogas production, pH should be maintained between 6.8 and 7.2 (Lemmer *et al.,* 2017). This range mostly offers best conditions for the methanogens and is essential to maintain their healthy activity. The activity of acidogenic bacteria is also influenced by pH; however, the effect is less important and mainly changes the types of products produced by them. The higher molecular weight volatile fatty acids are formed at lower pH, especially propionic and butyric acid, but acetic acid formation is favored at higher pH.

2.7.3. Temperature

Like all other biological processes, the performance of anaerobic processes is significantly changed by operating temperature. Selecting and regulating the temperature is important as it controls the activity of microorganisms during anaerobic digestion process. AD is performed at three distinct temperature ranges: at psychrophilic (25°C), mesophilic (around 35°C), and thermophilic (around 55°C) conditions (Rosińska and Karwowska, 2017). The best performance is usually attained by process at 30°C to 40°C for mesophilic or 50°C to 60°C for thermophilic ranges and generally anaerobic processes are designed to function in these ranges. Usually methanogens are believed to show optimum growth at these two temperature ranges. They can grow at lower temperatures as well, but longer retention times are required to counterbalance for the slower specific rates for maximum growth. So, for practical purposes, temperatures in the 20°C to 25°C limits are found to be the lowest temperature in anaerobic systems. Additionally, functioning temperature affects hydrolytic and acidogenic and acetogenic reactions also. But for wastewaters having higher concentration of readily biodegradable organic matter, the impact of temperature on methanogenesis is the key interest. Comparatively, for wastewaters consisting largely of complex organic compounds or particulate materials, the effect of temperature on hydrolysis and acidogenesis will be the most important concern.

2.7.4. Organic loading Rates:

The organic loading rate can be defined as the quantity of organic solids loaded per unit time per unit volume of any wastewater treatment system. OLR is generally considered as essential parameter for achieving optimal microorganism activity (Neshat *et al.,* 2017) in an anaerobic system. So, OLR should be adjusted in optimum range for any system because lower OLR could cause the inefficiency of AD technology. On the other hand, higher OLR increases the diversity of microbial species in reactor, needs less energy for heating systems, and reduces the required

digester volume and cost. Nevertheless, when the OLR is enhanced ahead of certain range, it can cause the greater accumulation of VFA and ethanol, inadequate heat transfer, and unbalanced circulation during mixing.

2.7.5. Carbon to nitrogen ratio:

The carbon to nitrogen (C/N) ratio of organic materials also has great effect overall AcoD process. There is an optimal C/N ratio for each anaerobic system and the substrates having optimum C/N ratio provide enough nutrients for microorganisms to maximize biogas production (Reilly *et al.*, 2016). Higher concentrations of ammonia can be produced in the systems with lower C/N ratio and inhibit microbial growth. On the contrary, higher C/N ratio than optimal leads to formation large amounts of VFAs of in the fermentation process. Thus, keeping a suitable C/N ratio is important in the AcoD technique for maximum methane yields.

2.7.6. Inhibitory and Toxic Materials

The anaerobic systems are very sensitive to inhibition by compounds already present in the wastewater or produced during digestion as reaction intermediates. Inhibition causes a decrease in the maximum specific growth rate of microorganisms, thereby demanding longer SRTs of biochemical operations for the equivalent yields that would be produced in the absence of the inhibitors. If the concentration of inhibitor increases continuously, toxic reaction will cause the killing of microorganisms, results in the failure of entire process. In the previous literature, inhibition and toxicity has been used interchangeably and no clear distinction has been made. So, these terms should not be strictly interpreted. However, it should be accepted that, in most cases, increase in concentration of compound can change the term “inhibition” into “toxicity”. Different inorganic substances can produce an inhibitory reaction; the most important among these are light metal cations, ammonia, sulfide, and heavy metals. Also, sulfate hinders the methane production by giving an alternating electron acceptor and cause the production of dangerous gas. Moreover, soluble sulfide uses an oxygen demand that reduces the amount of COD removed as methane. Many organic compounds are also reported to be inhibitory, particularly to methanogens (Grady *et al.*, 2011) like organic acids, nitrogenous compounds and fatty acids.

Knowledge Gaps

Based on theoretical background, it’s possible to find the knowledge gap. There was not considerable research that had been performed on the co-digestion of fish sludges and sewage sludge using CSTRs. It was not fully established yet whether digesting fish sludge with other sewage sludge has benefits or not on net biogas production. This research was basically conducted to study the advantages and disadvantages of anaerobic co-digestion of fish wastes with other sludges and organic substrates using batch and CSTR systems. For this purpose, several substrates were used to evaluate the methane potential of substrates and comparison was made between digestion and co-digestion in batch assays. Furthermore, the focus was to investigate the optimal

SRT for CSTR reactors for converting organic matter to methane. To accomplish this goal, four personally designed laboratory scale CSTR reactor systems were set up for treatment of various sludge as substrates under mesophilic conditions (35 °C). Thus, steady state was achieved, and optimum operational conditions were investigated. Daily methane production was measured using AMPTS instrument and COD recovery in the form of methane production was calculated.

3. Materials and Methods

3.1. Substrates Collection and Preparation

Fish sludges were brought from Steinsvik and Fister Smolt Hjelmeland, primary sludge (municipal waste), secondary sludge (Biowastes) were taken from IVAR Sentralrenseanlegg Nord-Jæren (SNJ) and food wastes from food truck in Stavanger, were analyzed and utilized in the experiments presented in this study. Septic sludge was from septic tanks in households that do not have sewage (dewatered septic sludge). Industrial wastes from Tine were the wastes from dairy (Dairy waste). We got these sludges from Ivar SNJ where these are treated together with primary and bio sludge. Flash tank is the pulper sludge that has been pretreated with thermal hydrolysis. It was collected from IVAR Grødaland where thermal hydrolysis process is used as a pretreatment method. All these sludges were collected in 1000ml bottles and preserved in the fridge at 8^0C and further used in batch tests.

3.2. Set up of Biomethane potential tests:

3.2.1. Blank Samples:

The inoculum was taken from the digester that was properly functioning at IVAR Sentralrenseanlegg Nord-Jæren (SNJ). For assuring the quality of inoculum, analyses of pH, volatile fatty acids (VFA), ammonium, bicarbonate and total alkalinity were performed at IVAR using Internal methods as shown in the table 3.1. It was reported that the optimal pH for anaerobic digestion is between 6.5-8.0. If pH drops below 6, the activity of the methanogens decreases rapidly so that at a pH of 5.5 they usually inactive (Henze *et al.*,). Therefore, every time BMP test was set up, all these parameters were measured intermittently because the inoculum was always taken from the same source.

Table 3.1 showing the analysis of Inoculum (measured at IVAR)

Analysis	**Amount**	**Method**
Bicarbonate Alkalinity	6100mg/L HCO_3	Internal Method
VFA	423mg HAC/L	Internal Method
pH	7.35	NS 4720
Total Alkalinity	7625mg/L HCO_3	Internal Method

3.2.2. Positive Control:

When performing batch tests, positive control samples were run for the confirmation of BMP test results. Thus, inoculum activity is validated using a standard substrate and experimental yield is

compared with that reported in the literature. Moreover, positive controls also allow verification of gas measurement method. For this purpose, potato starch was used as positive control. However, in the literature microcrystalline cellulose and tributyrin had commonly used as positive controls due to their good performance in AD (Holliger *et al*.,2016). Starch has similar properties as cellulose therefore it was used because of following reasons. First, its structure is well-defined as it is composed of glucose as the monomer, which allows the theoretical BMP to be easily determined as shown below. Second, it is a polymer and involve all biodegradation steps including hydrolysis during AD. Lastly, it was convenient and storable, relatively cheaper, and could be easily purchased as a good quality product (e.g. from Sigma-Aldrich). The use of a mixture of polymers would be an interesting option to validate inoculum activity towards more than one class of biomolecules. For each positive control substance, it is essential to confirm the TS and VS percentage of the product utilized in the tests.

Theoretical methane potential:

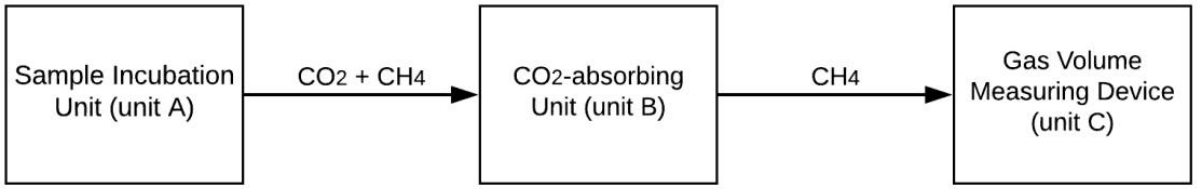


Figure 3.1 shows the flow of biogas in AD process

In an anaerobic reactor inlet COD transformed into gaseous COD can be measurement of methane production: 1 kg of COD can theoretically be transformed into 0.35 m^3 methane at standard conditions (Equation 1) (Henze *et al.*, 2008)

$$V_{CH4} = \frac{\frac{22.4\ l\ CH4}{mole\ CH4}}{\frac{64\ gCOD}{mole\ CH4}} = 0.35 l\ {}^{CH4}/_{gCOD}$$

Equation 3.1 theoretical methane production

Expected methane production values (volume) was calculated using the universal gas law. In the equation, V is defined as volume occupied by the gas in liter, n is the moles of gas, R is the constant 0.082057 atm.L/mole.K, T is the temperature (kelvin) and P is the pressure (atm).

$$V = nRT/P$$

For glucose: $C_6H_{12}O_6+H_2O \rightarrow 3CO_2+3CH_4$
COD/VS=1.07gCOD/gVS
Max BMP= 374ml CH_4/gVS
Expected ThBMP= 336ml/gVS

For starch: $C_6H_{10}O_5+H_2O \rightarrow 3CO_2+3CH_4$

COD/VS=1.18gCOD/gVS
Max BMP= 414 ml CH_4/gVS
ThBMP expected= 370ml/gVS

3.2.3. Gas measurement

Biogas production is measured by different techniques, e.g. by volumetric, manometric, and gas chromatography methods. But each method has discrepancies as continuous measurement of gas can't be measured. Therefore, in the present study advanced method for gas measurement was used using AMPTS instrument.

3.2.4. AMPTS II instrument:

In this study, biochemical methane potential (BMP) tests were used to assess the anaerobic biodegradability and biomethane potential of complex organic wastes as well as the rate of methane production on lab scale reactors. For this purpose, AMPTS II instrument was used for performing batch tests with triplicate positive controls consisting of 15 test vessels.

Preparation of NaOH solution for CO_2-absorption:

For CO_2 measurement, 3M NaOH solution was prepared and following procedure was followed. It is highly alkaline, so preparation was done inside the fume hood using protective gloves. In order to prepare a 3 M NaOH solution the necessary amount of NaOH was weighed and mixed with approximately 75% of the needed volume of distilled water (e.g. 120 g NaOH in 750ml of 1 L water). The solubilization of NaOH produced high heat in water, so small volumes of additional water were added followed by magnetic stirring. When the NaOH is fully dissolved, whole amount of remaining water was added and mixed well. Afterwards, a 0.4 % Thymolphthalein pH-indicator solution was prepared by dissolving 40 mg of it in 9 ml ethanol 99.5% followed by adding 1 ml water. As thymolphthalein is not soluble in water, but it is readily soluble in ethanol. In the last step the NaOH solution containing the pH indicator was prepared, by mixing 5 ml of the 0.4 % Thymolphthalein solution per liter of 3M NaOH solution. The color of the solution from colorless to dark blue as a result of pH indicator Thymolphthalein.

Equipment Functioning:

The instrument setup could be divided into three units as shown in the figure 3.7:

A is a thermostatic water bath consisting of 15 glass bottles (500 ml) as reactors having plastic caps with agitators/motors and short motor cables,

B is CO_2 absorption tray, and

C is gas volume measuring device comprising of water bath package (including water tank, flow cell holder, 15 injection mold flow cells containing magnetic metal pieces, base and protection plate) with plastic glass lid. In the sample Incubation Unit (unit A), up to 15 vials containing small amounts of a sample with anaerobic inoculum were incubated at a required temperature. The medium in each vial was mixed by a slow rotating agitator. The operating conditions, substrate

concentrations and temperature were kept constant while mixing was applied. Mixing also minimizes accumulation of solids and the amount of scum. Biogas was continuously produced inside these anaerobic reactor vessels. In the CO_2-absorbing Unit (unit B), the biogas produced in each vial passes through an individual vial containing an alkaline solution. Several acid gasses fractions, such CO_2 and H_2S, were retained by chemical interaction with NaOH, only allowing CH_4 to pass by the biomethane Gas Volume Measuring Device. As mentioned earlier a pH indicator was added into each vial for controlling the acid binding capacity of the solution. In the Gas Volume Measuring Device (unit C), the volume of CH_4 gas released from unit B was measured using a wet gas flow measuring device with a multi-flow cell arrangement (15 cells). This measuring device was working according to the principle of liquid displacement & buoyancy and can monitor ultra-low gas flows; a digital pulse was generated when a defined volume of gas flows through the device. An assimilated data recognition system was employed to record, present and analyze the results (Bioprocess control. (n.d.).

*Figure 3.**2** showing the three basic components of AMPTS (bioprocess control manual,2018)*

3.3. Initial Batch Tests 1 and 2

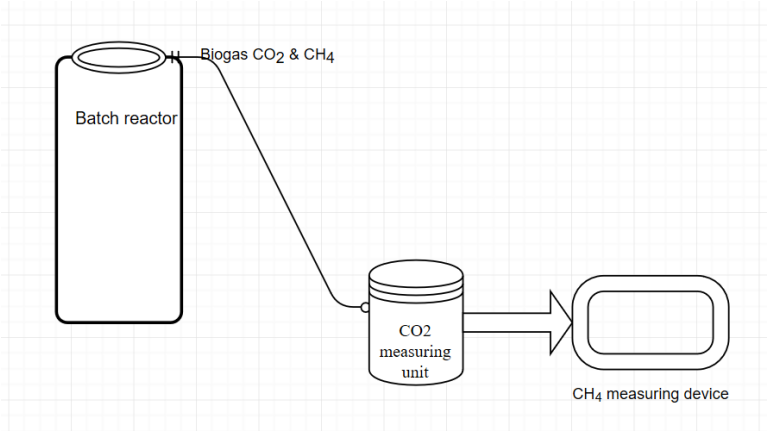


Figure 3.3 showing schematic diagram of batch test set up

3.3.1. Anaerobic Digestion and Co-digestion of Fish Sludge and Primary sludge:

The anaerobic batch tests were done in triplicates at mesophilic conditions (35^0C) in accordance with the given procedure given in AMPTS II instrument manual. Biomethane potential of the dried fish waste from Steinsvik and the primary sludge from IVAR, SNJ was determined in the first two experiments. An inoculum having 2% TS and 1.1% VS was utilized for each of the batch tests. The original volatile solid (VS) ratio of substrate to inoculum was approximately maintained at 1:2 during all the experimental setups. A BMP test was carried out for inoculum to assess the volume of methane produced by the fermentation of blank sample. Ten BMP tests were conducted and each of them were run in triplicates. First three reactors were used as blank with 400 ml inoculum in each of them without adding any substrate, in the next 3 bottles, a start medium containing 400ml inoculum and 2g starch was introduced. The inoculum was added to prepare an optimal growth medium for the substrate in the batch test and potato Starch (Sigma-Aldrich) was used as positive control.

Two batch tests were run simultaneously with AMPTS II instrument. The substrates were analyzed with respect to their VS content in the initial 1.0 batch test setup (Table 1) included reactors with triplicates of control/reference samples (blank), fish waste samples of different VS concentrations and primary sludge samples of different VS loads.

In the batch tests 2 six reactors (4,5,6,7,8,9) were set up as triplicate of a 50% VS/VS mix, while two other reactors (10,11,12,13,14,15) were set up as triplicates of a mix of 25% fish waste and 75% primary sludge. Each reactor with 500ml capacity was filled with 400 mL of inoculum and given amounts of VS of substrate. The substrate proportions in the different batch systems are summarized in Table 3.2 and 3.3. After the setup all the reactors were sealed with rubber septum and metallic cover and then flushed with an anaerobic N_2 gas for some time. During the whole incubation period the reactors were kept in a water bath at 35^0C ($\pm 1^0$C) and were shaken continuously at 50 rpm.

Table 3.2 Batch test of fish sludge and primary sewage sludge

Reactor Numbers	Amount of substrate	Actual conditions
1	Blank	400ml inoculum
2	Blank	400ml inoculum
3	Blank	400ml inoculum
4	2g VS Positive control	2.003g starch+400ml inoculum
5	2g VS Positive control	2.001g starch+400ml inoculum
6	2g VS Positive control	2.001g starch+400ml inoculum
7	2g VS Primary Sludge	53.721g primary sludge+400ml inoculum
8	2g VS Primary Sludge	53.926g primary sludge+400ml inoculum
9	2g VS Primary Sludge	53.565g primary sludge+400ml inoculum
10	3g VS Primary Sludge	77.039g primary sludge+400ml inoculum
11	3g VS Primary Sludge	77.125g primary sludge+400ml inoculum
12	3g VS Primary Sludge	77.951g primary sludge+400ml inoculum
13	2g VS Fish Sludge	2.869g fish sludge+400ml inoculum
14	2g VS Fish Sludge	2.891g fish sludge+400ml inoculum
15	2g VS Fish Sludge	2.85g fish sludge+400ml inoculum

Table 3.3 batch test 2 co-digestion of fish sludge and primary sewage sludge

Reactor Numbers	Amount	Actual amount added
1	3g VS Fish Sludge	4.323g fish sludge+400ml inoculum
2	3g VS Fish Sludge	4.311g fish sludge+400ml inoculum
3	3g VS Fish Sludge	4.299g fish sludge+400ml inoculum
4	1g VS Primary Sludge+1g VS Fish Sludge	26.228gPS+1.443gFS+400ml inoculum
5	Mix: 1g VS Primary Sludge+1g VS Fish Sludge	25.817gPS+1.47gFS+400ml inoculum
6	Mix: 1g VS Primary Sludge+1g VS Fish Sludge	25.906gPS+1.458gFS+400ml inoculum
7	Mix: 1.5g VS Primary Sludge+1.5g VS Fish Sludge	38.829gPS+2.193gFS+400ml inoculum
8	Mix: 1.5g VS Primary Sludge+1.5g VS Fish Sludge	39.990gPS+2.215gFS+400ml inoculum
9	Mix: 1.5g VS Primary Sludge+1.5g VS Fish Sludge	38.274gPS+2.14gFS+400ml inoculum
10	Mix: 1.75g VS Primary Sludge+0.25g VS Fish Sludge	44.968gPS+0.387gFS+400ml inoculum
11	Mix: 1.75g VS Primary Sludge+0.25g VS Fish Sludge	44.247gPS+0.383gFS+400ml inoculum
12	Mix: 1.75g VS Primary Sludge+0.25g VS Fish Sludge	44.858gPS+0.370gFS+400ml inoculum
13	Mix: 2g VS Primary Sludge+1g VS Fish Sludge	52.725gPS+1.459gFS+400ml inoculum
14	Mix: 2g VS Primary Sludge+1g VS Fish Sludge	51.466gPS+1.561gFS+400ml inoculum
15	Mix: 2g VS Primary Sludge+1g VS Fish Sludge	51.478gPS+1.433gFS+400ml inoculum

3.3.2. Batch Tests 3

AcoD of Food wastes, Fish Wastes and secondary sludge:

In the batch test 3, secondary sludge from IVAR, SNJ was used in addition to food wastes and fish sludge from Steinsvik. Food wastes were also collected from IVAR, SNJ Mekjarvik. In the first part of the experiment, 3g VS of each of the sludge was digested and in the 2nd part, co-digestion was carried out using the mixture (1:1 ratio) of the sludges. Lastly, one triplicate sample was run including the mixture of food wastes, fish sludge and secondary sludge each consisting of (1:1:1) ratios as shown in the tables below.

Table 3.4 Setup of Batch test 3

Reactor Numbers	Amount	Actual amount added
1	Blank	400ml inoculum
2	Blank	400ml inoculum
3	Blank	400ml inoculum
4	3g VS Positive control	3.262g starch+400ml inoculum
5	3g VS Positive control	3.046g starch+400ml inoculum
6	3g VS Positive control	3.001g starch+400ml inoculum
7	3g VS Secondary Sludge	67.125gSS+400ml inoculum
8	3g VS Secondary Sludge	67.209gSS+400ml inoculum
9	3g VS Secondary Sludge	66.839gSS+400ml inoculum
10	3g VS Food waste	136.026gFW+400ml inoculum
11	3g VS Food waste	136.772gFW+400ml inoculum
12	3g VS Food waste	136.193gFW+400ml inoculum
13	3g VS Fish Sludge	4.308g FS+400ml inoculum
14	3g VS Fish Sludge	4.379g FS+400ml inoculum
15	3g VS Fish Sludge	4.352g FS+400ml inoculum

Table 3.5 Setup of Batch test 3

Reactor Numbers	Amount	Actual amount added
16	Mix:1.5g Fish Sludge+1.5g Secondary Sludge	2.202g fish sludge+ 33.675g SS+400ml inoculum
17	Mix:1.5g Fish Sludge+1.5g Secondary Sludge	2.201g fish sludge+ 33.067g SS+400ml inoculum
18	Mix:1.5g Fish Sludge+1.5g Secondary Sludge	2.209g fish sludge+ 33.067g SS+400ml inoculum
19	Mix:1.5g VS Fish Sludge+1.5g VS Food Waste	2.224g fish sludge+ 68.175g FW+400ml inoculum
20	Mix:1.5g VS Fish Sludge+1.5g VS Food Waste	2.209g fish sludge+ 68.685g FW+400ml inoculum
21	Mix:1.5g VS Fish Sludge+1.5g VS Food Waste	2.194g fish sludge+ 68.686g FW+400ml inoculum
22	Mix: 1g VS Secondary Sludge+1g VS Food Waste+1g Fish Sludge	1.409g fish sludge+ 46.116g FW+22.609gSS+ 400ml inoculum
23	Mix: 1g VS Secondary Sludge+1g VS Food Waste+1g Fish Sludge	1.408g fish sludge+ 45.827g FW+23.011gSS+ 400ml inoculum
24	Mix: 1g VS Secondary Sludge+1g VS Food Waste+1g Fish Sludge	1.408g fish sludge+ 45.882g FW+30.102 gSS+ 400ml inoculum

3.3.3. Batch Test 4

AcoD of septic wastes and fish food wastes:

In the 4th batch test, fish food wastes and septic sludge were collected from IVAR, SNJ Mekjarvik. During the current experiment, in the first 6 flasks, 3g VS of fish food wastes sludge and 2g of septic sludge were digested with positive control and blanks whereas in the last two co-digestion was carried out using the mixture (1:1 ratio) of each of sludges. All samples were run in parallels.

Table 3.6 set up of batch test 4

Reactor Numbers	Amount	Actual amount added
1	Blank	400ml inoculum
2	Blank	400ml inoculum
3	3g positive control	3.003g starch+400ml inoculum
4	3g positive control	3.003g starch+400ml inoculum
5	2g septic wastes	76.830g septic wastes+400ml inoculum
6	2g septic wastes	77.266g septic wastes+400ml inoculum
7	3g fish food wastes	5.534 g fish food wastes+400ml inoculum
8	3g fish food wastes	5.534 g fish food wastes+400ml inoculum
9	Mix:1.5g Fish food wastes+1.5g septic wastes	37.422g Septic sludge+2.049gFish food wastes+400ml inoculum
10	Mix:1.5g Fish food wastes+1.5g septic wastes	37.422g Septic sludge+2.049gFish food wastes+400ml inoculum

3.3.4. Batch Test 5

Anaerobic digestion of Industrial food wastes and fish sludge Hjelmeland :

In the 4th batch test, industrial food wastes and fish sludge were collected from IVAR, SNJ Mekjarvik. Industrial food wastes include food wastes from restaurant, dairy wastes from Tine dairy industry, and pulper. During the current experiment, in the first 14 flasks, 3g VS of each of the fish sludge from Hjelmeland, and 2g of septic sludge were digested with positive control and blanks whereas in the last one co-digestion was carried out using the mixture (1:1 ratio) of each of puler and flash tank sludges. All samples were run in parallels.

Table 3.7 shows the set-up of batch test 5

Reactor Numbers	Amount	Actual amount added
1	Blank	400ml inoculum
2	Blank	400ml inoculum
3	4g VS Positive control	4.007g starch+400ml inoculum
4	4g VS Positive control	4.001g starch+400ml inoculum
5	3g VS Fish Sludge Hjelmeland	30.342g Fish sludge hjelmeland+400ml inoculum
6	3g VS Fish Sludge Hjelmeland	30.342g Fish sludge hjelmeland+400ml inoculum
7	3g VS Food Waste Restaurant	30.258g Food Waste Restaurant+400ml inoculum
8	3g VS Food Waste Restaurant	30.328g Food Waste Restaurant+400ml inoculum
9	3g VS Flash Tank	46.384g Flash Tank+400ml inoculum
10	3g VS Flash Tank	46.287g Flash Tank+400ml inoculum
11	3g VS pulper	32.429g pulper +400ml inoculum
12	3g VS pulper	32.429g pulper r+400ml inoculum
13	2g Tine dairy waste	64.574g Tine dairy wastes+400ml inoculum
14	2g Tine dairy waste	64.553g Tine dairy wastes+400ml inoculum
15	Mix:1.5g Flash Tank+1.5g pulper	23.25g flash tank+16.2g pulper +400ml inoculum

3.4. Continuous Stirred fed Reactors (CSTR):

3.4.1. Sample collection and characterization:

Collected sludge was originally blended by Polytron PT3000 blender to reduce any heavier fragments prior to feeding. Both sludge samples were stored in plastic containers after collecting from sources. Then, transported to the laboratory and preserved at 4˚C for further study were physicochemical analysis and treatment. For dilution purposes, alkalinity was maintained between 5.2 g $CaCO_3$/l by using 0.1M $NaHCO_3$.

3.4.2. Seeding: Seeding of CSTR was done using inoculum collected from municipal sewage treatment plant situated in Mekjarvik. Reactor was fed with mixed sludge (75% primary sludge+25% fish sludge) having suspended solids 0.52 g-TSS/L and 0.45 g volatile suspended solids (VSS) per liter. The generated biogas was studied after 24 hours.

3.4.3. CSTR construction & Operation

Four one L continuous-stirred tank reactors (CSTRs) were operated at mesophilic conditions with a hydraulic retention time (HRT) of 15 days, 7.5 days, 5 days and 3.75 days which were fed once a day throughout the experiment. The temperature was kept at 35 ± 1 °C via build-in water bath. The reactors were stirred with a metallic stirrer powered by an electric motor continuously with 50 rpm. All reactors were fed with a mixture of primary sludge and fish sludge in a ratio of 3:1, based on the VS content of the substrates. Each of them was filled with 750 milliliters of inoculum at the start of the experiment, and initially operated at an organic loading rate (OLR) of 2.9 kg/d for 15 days. The ports were fitted with Tygon laboratory tubes for sludge feeding (inlet) and sludge removal (outlet) as shown in the figure 3.4. The laboratory tubes had fitted plastic tubing clamps for opening and closing and reactors had a rotating shaft for continuous sample mixing. During the tests, the biogas production was monitored continuously from each reactor daily. The bioreactors were incubated for 45 days with continuous feeding daily. In the first week, conditioning of reactor was done to detect stability of the system such as gas leaking, reactor performance and inoculum quality. The inoculum had a pH of 7.35 when the sludge mixture was introduced to the bioreactors. During feeding process the stopper tube connected to unit B was closed using a plastic clamp to prevent entry of gas. Feeding was done with a 100 ml plastic syringe through the feeding inlet. In the first reactor, 50 ml of sample was injected, and 50 ml of sample was withdrawn in the first bioreactor with HRT of 15 days. Similarly, in the second reactor 100ml of sample was injected, and 100 ml of sample was withdrawn in the first bioreactor with HRT of 7.5 days. The 3rd and 4th reactors were operated at 5days and 3.75 days respectively as shown in the table 3.5. A volumetric cylinder was utilized to accumulate the sludge effluent from the outlet tube. The volume in the reactors were maintained at 750 milliliters, and occasionally when the total volume went above 750 ml, a necessary sample volume was extracted. In CSTR, the HRT is the same as SRT. Conductivity and pH were measured daily on the effluent samples. VFA, nitrogen, phosphorus,

total COD were conducted several times during the CSTR running time. The biogas produced was measured by the AMPTS II and the results were presented and measured online by the software system used in bioprocess control.

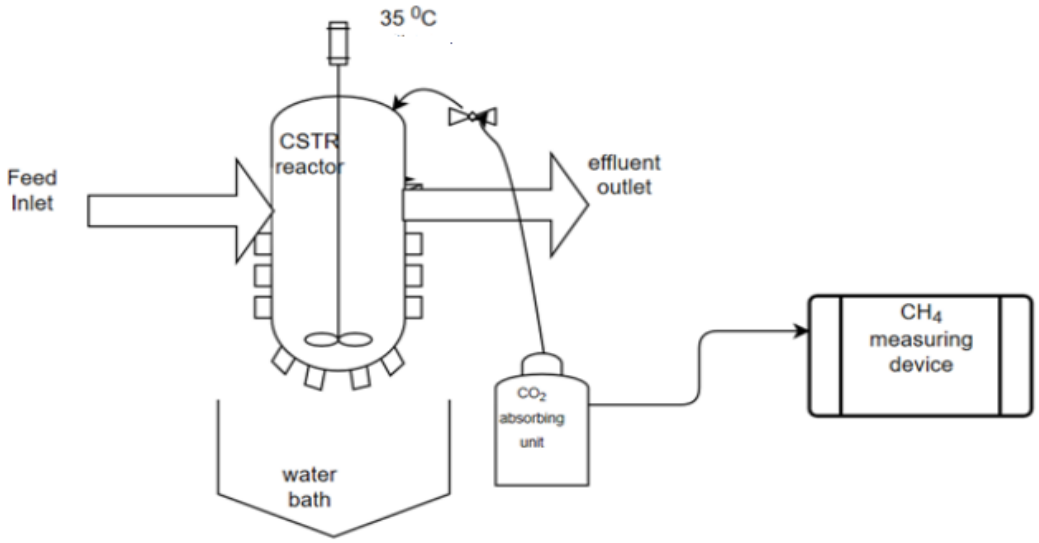


Figure 3.4 showing schematic diagram of CSTR system used

Table 3.5 showing the operational conditions in the CSTRs

Reactors	Reactor Volume (ml)	Feed (ml/d)	OLR (gCOD/d)	HRT/SRT(d)
R1	750	50	2.9	15
R2	750	100	2.9	7.5
R3	750	150	2.9	5
¤4	750	200	2.9	3.75

3.5. Analytical Methods

Before performing anlysis, it was made sure that sample to be analyzed was homogenized by shaking in order to have uniform particle distribution. In some cases, the samples were blended using a blender. Usually, washing and/or diluting was pre-requisite for analysis in some analytical procedures. For this purpose, distilled water was used. In order to characterize sludge before (the inlet sludge), during and after (effluent), several analytical methods were carried out. In some of the cases, the sludge samples were diluted so that the end concentrations would fit the concentration ranges of the test kits. The end concentrations were pre-calculated based on the established sludge characterizations. For measurement of volatile fatty acids and alkalinity, filtration was not used because clogging was expected in this case due to larger particles and their higher concentrations. Therefore, to analyze the dissolved or soluble substances, the centrifugation at 10000 RCF for 15 minutes using Thermo Fisher Heraus Sepatech Biofuge 17RS centrifuge provided a clear supernatant and showed promising results. The analytical methods used are described as follows.

3.5.1. pH Measurement

pH measurement in each reactor was carried out immediately after collecting effluent using TitroLine® 5000 Auto-Titrator. All pH meters were calibrated with standard solutions of buffers with pH 4.01 and 7.00 on a regular basis.

3.5.2. Volatile fatty acids and alkalinity measurements

The volatile fatty acids and alkalinity measurements of the effluents were carried out during 45 days of CSTR running. Normal titrations were carried out using an automatic titration system TitroLine® 5000 Auto-titration (Instrument-teknikk AS, Oslo). TitroLine® 5000 system is made of six main components: valve-cover lid and display; probe; dosing unit; titration tip unit; stirrer; and acid/base bottle. However, a manual titration set-up was also used to validate the results. The VFA analysis followed the 5-point titration procedure (Lahav & Loewenthal, 2000). 15 ml sample of the respective effluent from each reactor was centrifuged using a Thermo Fisher Heraus Sepatech Biofuge 17RS centrifuge. About 5 ml of the centrifuged sample was diluted to 50 ml using deionized water and placed on a magnetic stirrer. The rotation was set to a lower mode to minimize the absorption or loss of carbon dioxide by the solution. Additionally, conductivity and temperature measurements were also conducted (4.4.1). The initial pH was recorded. For titration purposes, 0.065M and 0.1M hydrochloric acid (Sigma-Aldrich) were prepared and used as titrant. In manual titration, the titrant was added through a 50 ml glass burette. Volume of acid added were read at four different pH values approximately around 6.7, 5.9, 5.2 and 4.3. The actual volumes of acids used at respective pH values were noted. Finally, amount of alkalinity as mg $CaCO_3$/L and VFA concentrations were calculated using the software program TITRA 5.

3.5.3. Orthophosphate and total phosphorus measurements

At the end of the experiment in CSTR reactors, nitrogen and phosphorous removal were studied. During this study, the phosphate test kits from Merck Spectroquant were used to perform

orthophosphate and total phosphorus measurements for the sludge samples. A measuring range of 0.05-5.00 mg/l PO4-P was used. During the following procedure, the orthophosphate ions in a sulphuric solution will react with molybdate ions to form molybdophosphoric acid. Also, the ascorbic acid present in the cell will reduce this to phosphomolybdenum blue that can be determined photometrically. As this test only measures orthophostphate, the sludge samples should have been decomposed by digestion before we can measure the total phosphorus concentrations (*more information can be found in the Merck Spectroquant instruction manual*). As stated in the Merck Spectroquant instruction manual, digestion for the determination of total phosphorus was done by pipetting 5 ml of diluted sludge sample into a reaction cell and adding 1 dose of the included reagent P-1K. Afterwards, the reaction cell was then heated at 120 °C for 30 minutes in a Merck TR 620 thermoreactor. Then cooling it to room temperature, 5 drops of reagent P-2K and 1 dose of reagent P-3K was added and the cell was shaken vigorously. The cell was left to stand for 5 minutes and concentration of PO_4-P was determined using a Merck Spectroquant Pharo 300 spectrophotometer. For determination of orthophosphate, the digestion step was excluded.

3.5.4. Ammonium and total nitrogen measurements:

In order to measure ammonium and total nitrogen in the effluent at steady state, ammonium and total nitrogen test kits from Merck Spectroquant were used. A measuring range of 4.0-80.0 mg/l NH_4-N were used was used for ammonium testing, and two different measuring ranges were used for total nitrogen testing: 0.5-15.0 mg/l and 10-150 mg/l. During this process, inside the thermoreactor, organic and inorganic nitrogen compounds were converted to nitrate in the presence of an oxidizing agent. This nitrate reacts with 2,6-dimethylphenol to form 4-nitro-2,6-dimethylphenol which can be measured photometrically to determine the total nitrogen value (*additional information can be found in the Merck Spectroquant instruction manual*). As written in the Merck Spectroquant instruction manual, 10 ml of diluted sample was pipetted into an empty cell. Then 1 level of reagent N-1K was added and the cell was thoroughly mixed. After mixing, 6 drops of reagent N-2K were added and the cell was mixed again. The prepared cell was heated at 120 °C in a thermoreactor for 1 hour. After cooling, 1 ml of sample was transferred from the digested, sample and pipetted into a reaction cell. Also, 1 ml of reagent N-3K was added and the cell contents were mixed. The reaction cell could stand for 10 minutes and then measured by Merck Spectroquant Pharo 300 spectrophotometer.

Ammonium nitrogen usually exists both as ammonium and as ammonia. Ammonium nitrogen is present almost entirely as ammonia in the highly alkaline environment. This ammonia reacts with hypochlorite ions and forms monochloramine. Monochloramine reacts with substituted phenol to form a derivative indophenol that is blue in color and can be determined photometrically to establish the ammonium value (*further information can be found in the Merck Spectroquant instruction manual*). As stated in the Merck Spectroquant instruction manual, 0.10 ml of diluted sample was pipetted into a reaction cell and mixed. To the diluted sample, acid was added until it reached the pH of below 6. It was done to avoid the loss of nitrogen in the form of NH_3 gas. After mixing,a dose of reagent NH_4-1K was added and the reaction cell was shaken vigorously. The cell

was left to stand for 15 minutes and then measured in a Merck Spectroquant Pharo 300 spectrophotometer. Lastly, ammonia concentrations were calculated based on the determined ammonium concentrations and the pH (Equation 1).

3.5.5. Error and accuracy analysis

The WTW Multi 340i, was used for pH and conductivity measurements, provided the following instrument specifications: pH accuracy= ± 0.01 pH, ±1 mV. Conductivity accuracy= ±1% of value. The pH accuracy value can also be applicable for VFA measurements. In addition, the AMPTS II CO_2-absorbing Unit (unit B) had a measured absorption efficiency of >98%. the Gas Volume Measuring Device (unit C) had a measured accuracy of 5% (relative accuracy error) and a precision of 1% (coefficient of variation) (Bioprocess control, u.d.). For measuring COD, nitrogen and phosphates, the cell test kits from Merck Spectroquant provided analytical quality assurance in their instruction manuals as shown in the table below (jenny, 2018).

Table 3.8 showing error and accuracy analysis of methods used

Cell Test	Measuring Range	Unit	Standard deviation of the method	Accuracy of measurement value
COD	50-500	Mg/l COD	±2.0	max ±13
COD	500-10000	Mg/l COD	±31.3	max ±143
Phosphate	0.05-5.00	Mg/l PO_4-P	±0.024	max ±0.08
Ammonium	4.0-80	Mg/l NH_4-N	±0.49	max ±1.9
Nitrogen(total)	10-150	Mg/l N	±1.1	max ±5
Nitrogen(total)	0.5-15	Mg/l N	±0.14	max ±0.6

3.5.7. Analysis of Sludges:

The total solids (TS), volatile solids (VS), and chemical oxygen demand (COD) are definitive analysis. TS is the total of dissolved solids and suspended solids. TS and COD are important to assess anaerobic process efficiency. TS, VS and COD were calculated using standard methods stated below. All the measurements were taken in triplicates and standard error was calculated using the formula

$$\text{Standard error} = \frac{\text{Standard deviation}}{\sqrt{n}}$$

Where n=number of replicates. Standard error was calculated instead of standard deviation because three independent measurements were made in each case.

ANALYSIS 1. DETERMINATION OF TOTAL COD (TCOD) OF SLUDGES BY COLORIMETRIC CLOSED REFLUX METHOD

2g of Fish sludge and other sludges was taken and each of them diluted 100, 200, 400 times. To make sure sludge sample to be analyzed is homogenized shaking was used, or preferably a homogenizer to make sure enough particle and solids distribution was achieved. Using a volumetric pipette, 2 ml of diluted homogenized sludge sample was added to a COD vial, and immediately covered and the vial became hot. In this study, COD test kits were used to carry out the wastewater analysis. These kits have digestion and catalyst solutions that, under managed conditions, react with wastewater samples to be measured. The COD tests kits used were Merck Spectroquant® which were 100 and 1500 mg/l of COD concentration range. The sample was digested in thermo reactor (Model TR 620) at 148°C for two hours using fume hood. Afterwards, COD vial was removed from reactor and allowed to cool in metal test tube rack until room temperature. Tube was swirled a couple of times during cooling. Upon reaching room temperature, test tube was placed in spectrometer cell compartment (Spectroquant Pharo 300), and mark was aligned with orientation mark, thus *COD* was noted. Reading is equivalent to *tCOD*. COD vial was placed in prescribed container (do not empty content as the reagents contain strong acids and may include Hg).

ANALYSIS 2: DETERMINATION OF TOTAL SOLIDS (TS) AND TOTAL VOLATILE SOLIDS (TVS) BY EVAPORATION, COMBUSTION AND WEIGHING

This measurement method was conducted based on the standard method for characterization of wastewater (SM 2540 B, C and E) (Clesceri, Greenberg, & Eaton, 1998). The evaporating dish (porcelain dish) was pre-combusted (pre-ignited) and cooled in desiccator (prepared already) using gloves. The homogenized samples of each sludge were taken in triplicates to have good precision. The tara weight of evaporation dish was weighed on an analytical balance (0.1 mg resolution) and noted as m_{dish}. 25-50g of digestate was weighed out in evaporating dish and mass was recorded as W_{sample}. Gloves were used, and extra care was taken to avoid touching the dishes. This evaporation dish was placed in the evaporating oven Termaks 9000 laboratory drying oven at 95-97°C and left to evaporate overnight. The evaporated residual was dried for 1 h at 103-105°C. Dish was cooled in desiccator to room temperature. Evaporating dish and residual were weighed on analytical balance. Weight was noted and cooled for another 15 minutes in desiccator. Measurements were repeated to check that (dish + residual) has constant weight. TS of sample was calculated as

$$TS\left[\frac{g}{g}\right] = \frac{m_{dish+residual} - m_{dish}}{W_{sample}}$$

After that porcelain dish was placed in Nabertherm muffle oven and sample was combusted for 20-30 min and dish was cooled for a short time in air before transfer to desiccator (until temperature has cooled to the drying temperature; approximate). TVS of sample was calculated as

$$TVS\left[\frac{g}{g}\right] = TS - \frac{m_{dish+ignited\ residual} - m_{dish}}{W_{sample}}$$

Finally, fixed solids residual was removed, and porcelain dish was soaked in soap-water (Kommedal, R., 2017).

4. Results

Sludge for sample inoculation in all experiments were collected from IVAR, SNJ and subjected to biogas potential testing using AMPTS instrument. The results part is divided into 2 sections. In the first part, different substrates, biowaste together with fish waste, potato starch, municipal primary and secondary sludge as well as their mixtures in different ratios were tested. In the second part, co-digestion of primary sewage sludge and fish sludge from Steinsvik were co-digested in CSTR using the same but measuring system was modified for CSTR operation.

4.1. Initial characterization of sludges:

Tables 4.1 shows the initial substrate characterization. The total solids (TS), volatile solids (VS), and chemical oxygen demand (COD) were standard analysis. The COD/VS ratio was determined, which is specific COD and is typically reliable with the characteristics of the given substrate or sludge. But determination of COD for the solid heterogenous sludge was quite difficult and open to uncertainty. Therefore COD/VS ratio found for some of the sludges were different than expected. For example, secondary sludge from IVAR has 1.95 gCOD/gVS that is quite higher than normally expected. Similarly, fish sludge from Fister Hjelmeland had 0.61gCOD/gVS that was lower than normally expected for fish sludge. Apart from these results, specific COD ratio determined for other sludges was within typical ranges reported for these sludges. Highest ratio was obtained for food wastes ($2gO_2$/gVS) that could be explained by the fact that food wastes contain more energy content than primary, secondary and fish sludges. These sludges used to have higher content of fiber and due to its less degradability, are not contributing to the specific COD measurement of the substrate. Moreover, if we compare the VS/TS content in the given sample, all types of food wastes (food wastes, fish food wastes. Food waste restaurant had higher percentage of VS/TS ratio as compared to fish sludge and secondary sewage sludge showing the higher amount of organic matter present in the food wastes than their percentages in sludges. Also, food wastes tend to have higher amount of lipids and other biopolymers present indicating the more VS content because lipids had higher specific COD (1.86gCOD/gVS) content as compared to other biomolecules. Flash tank is the pulper wastes that had been thermally hydrolyzed as a pre-treatment, as a result fraction of biodegradable COD increased. It might be the reason flash tank has slightly specific COD than pulper.

Table 4.1 showing initial characterization of sludges

Sludge Type	TS (%)	VS (%)	COD (g/kg)	VS/TS (%)	COD/VS g/g
Primary sludge IVAR (batch tests)	4.50±0.01	3.95±0.04	62±2	87	1.54
Primary sludge IVAR (CSTR)	3.68±0.02	3.42±0.03	47±3	93	1.36
Secondary Sludge IVAR	5.65±0.02	4.6±0.02	90±2	81	1.95
Fish Sludge (Steinsvik)	91.3±0.1	72.30±0.02	995±5	79	1.38
Fish Sludge (Fister hjelmeland)	10.16±0.02	9.94±1.56	61±3	98	0.61
Food wastes	2.2±0.01	2±1	40±4	91	2
Mixed Sludge CSTR	5.15±0.02	4.45±0.04	58±3	86	1.30
Food Waste Restaurant (FWR)	10.25±0.03	9.94±0.02	148±2	97	1.49
Flash tank	7.41±0.02	6.45±0.01	120±1	87	1.86
Pulper	11.42±0.04	9.27±0.03	164±1	81	1.77
Fish food wastes	62.4±0.05	56±1	928±1	90	1.65
Septic wastes	3.0±0.1	2.6±0.1	46±2	87	1.77
Tine dairy wastes	3.4±0.2	3.1±0.1	31±3	91	0.99

4.2. Batch Tests 1 and 2:

AD and Co-AD of Fish Sludge and primary sewage sludge:

The aim of this experiment was to determine the potential for biogas production in mono-digestion and co-digestion processes for fish and primary sludges. Fish sludges and the primary municipal solid wastes from IVAR, SNJ were mono-digested and co-digested at a loading rate ratio of 1:0, 0:1, 1:1, 1:3 respectively. Additionally, blank samples and positive controls using 2 g starch were also run in triplicates.

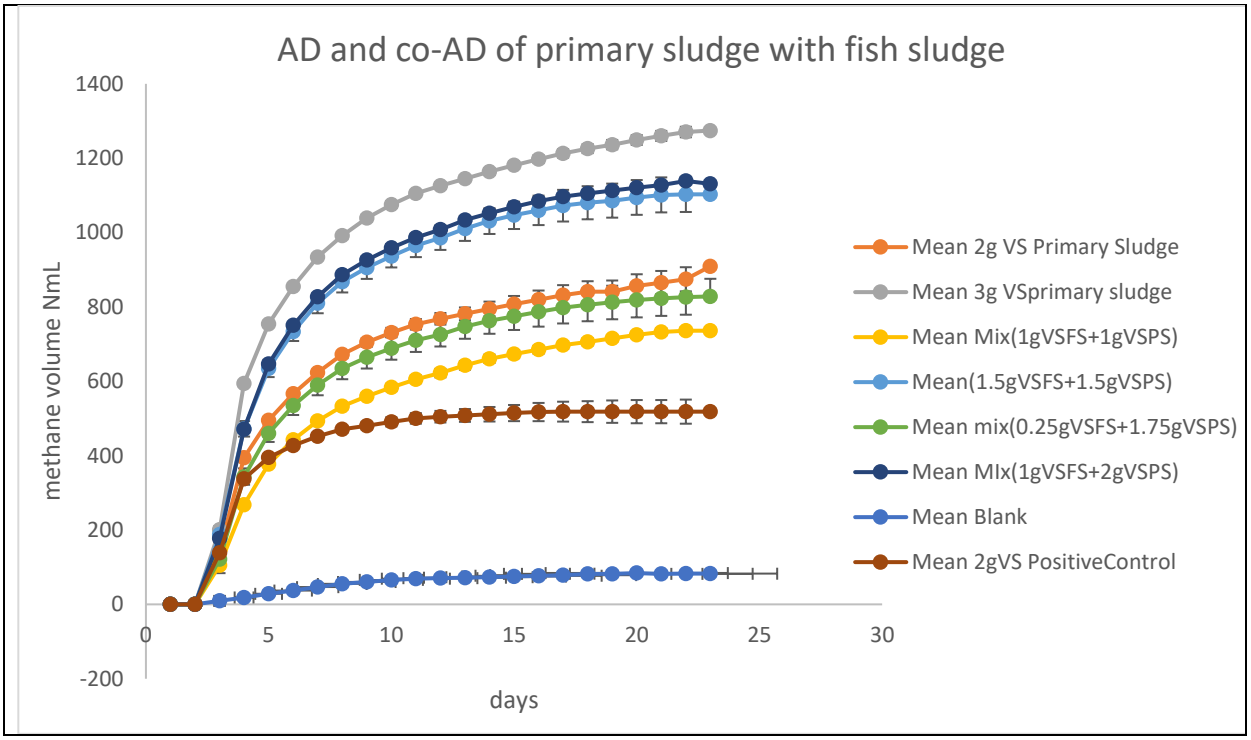


Figure 4.1 shows the total methane production for primary sludge together with fish sludge

Each curve corresponds to the average of three parallels of the specific substrate. The figures show the average total methane production for each sample meaning the methane produced in the blanks has not been subtracted. As indicated in the figure 4.1, the primary sludge has the highest production of methane per gVS of the substrate when compared with the other mono-digested and co-digested samples, while fish wastes lower the methane production when co-digested with primary sludge having same amount of VS. This is because primary sludge has higher COD/VS ratio and in mixed samples also, higher ratio of primary sludge in (3:1 PS+FS) is producing more methane than 1:1 PS+FS.

Also, further analysis of primary sludge and fish sludge (Steinsvik) were carried out at local laboratory Eurofins and proteins, carbohydrates and lipids were estimated as shown in the table 4.2 below.

Table 4.2 shows initial analysis of primary sludge and fish sludge from Steinsvik

Sludge type	NH_4-N	Total Nitrogen	Proteins	Carbohydrates	pH	lipids
Fish sludge (Steinsvik)	8.6gNH_4/g VS	61gN /g VS	318g /g VS	526g/g VS	5.1	121 g//g VS
Primary sludge (IVAR)	4.8gNH_4/g VS	24.05g /g VS	109g /g VS	506g /g VS	5.4	175 g//g VS

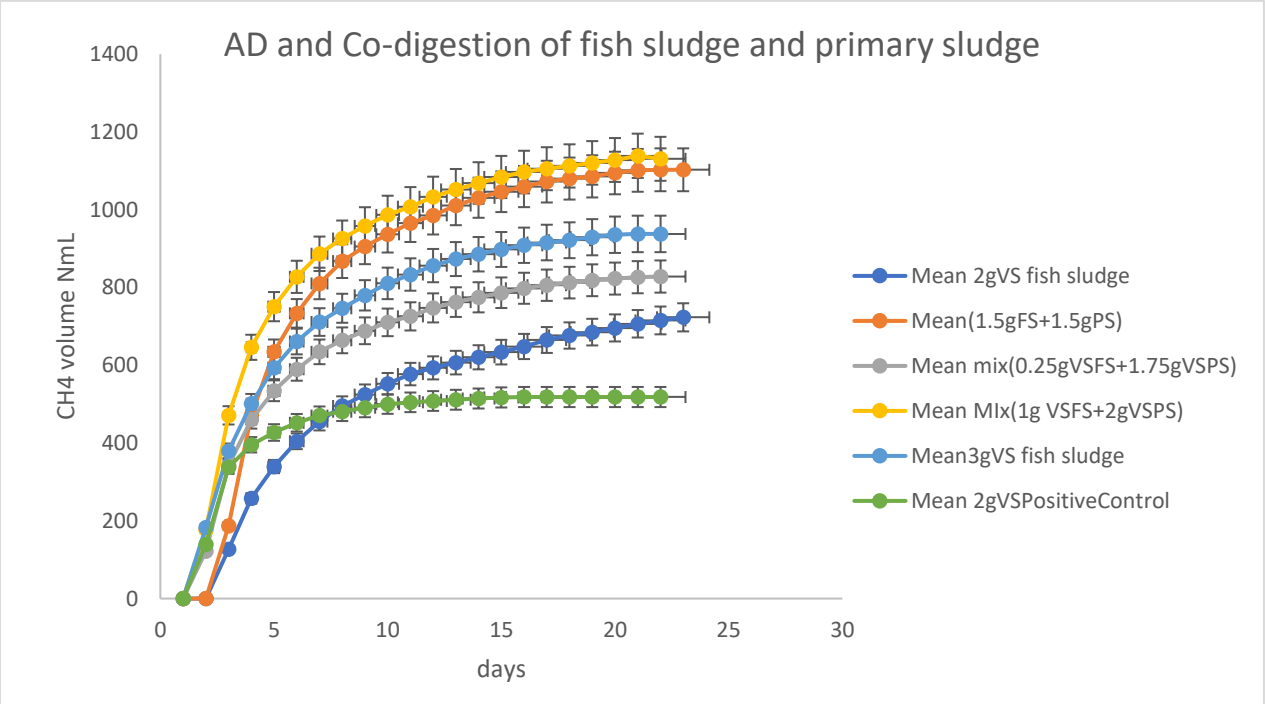


Figure 4.2 showing CH_4 production by fish sludge and co-digestion

It's clear from the figure 4.2 that co-digested samples of fish sludge and primary sludges are producing higher methane as compared to that produced by sole fish sludge samples with same VS content. This is clear from the table 4.1 that fish sludge has lower COD/VS ratio.

Rate of Reaction:

The flowrate of the methane production (Figure 3) in batch test 1.0 is the methane production rate given in ml/d. All series, except the blank, experienced a decrease in the flowrate after 3 days. The series with the highest loading (3g VS) of primary sludge showed the highest production rate while lowest rate was found for 2g fish sludge. The test was terminated at day 21 when the methane production of all series entered a methane production phase like that of the blank.

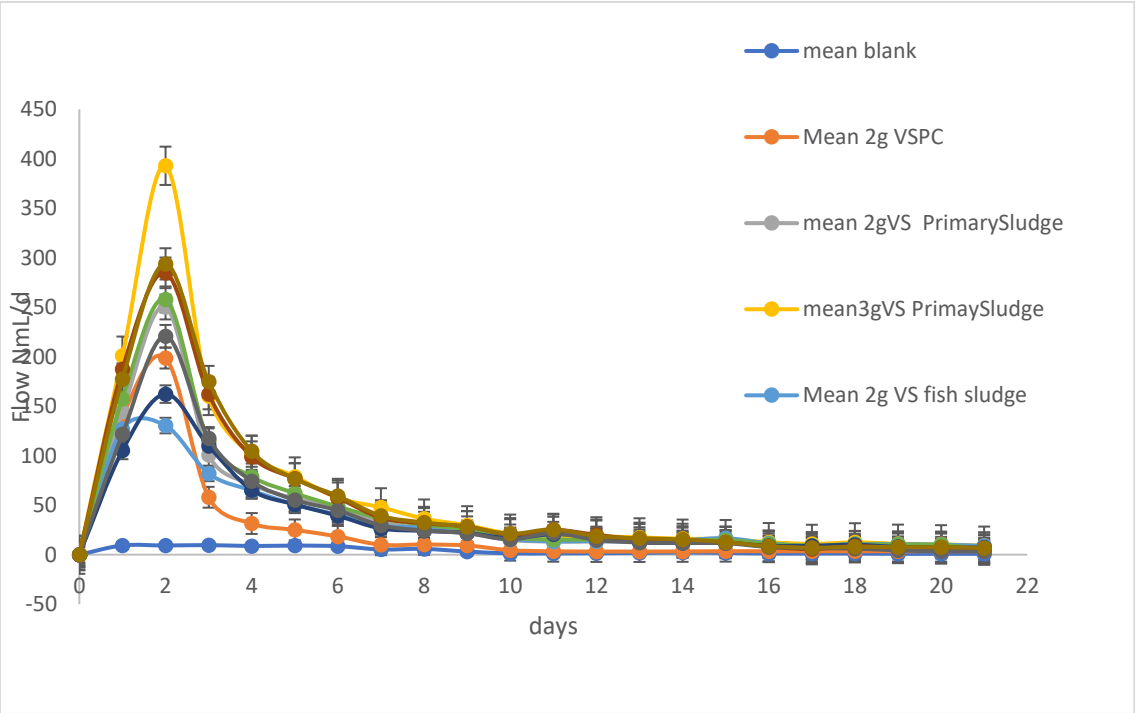


Figure 4.3 showing the average daily flow rate of methane in batch test 1

4.3. Batch Test 3:

AD and Co-AD of Fish Sludge, food wastes and secondary sewage sludge:

Afterwards, the next experiment was performed to determine the efficiency of biogas production in mono-digestion and co-digestion processes for fish wastes, food wastes and secondary sewage sludges. Secondary sewage sludge is referred to as bio sludge as shown in the figure 3. Fish sludges, food wastes and the secondary municipal solid wastes from IVAR, SNJ were4.4mono-digested and co-digested at a loading rate ratio of 1:0, 0:1, 1:1, 1:1, 1:1:1 respectively in the 3rd batch test.

Rate of reaction: The flowrate of the methane production (Figure 4.5) in batch test 3 is the methane production rate given in ml/d. All series experienced a decrease in the flowrate after 3 days. The series with the highest loading (3g VS) of mixed sludge (1gfood wastes+1g biosludge+1g fish sludge) showed the highest production rate while lowest rate was found for 3g bio sludge. The test was terminated after 25 days when the methane production of all series entered a methane production phase like that of the blank.

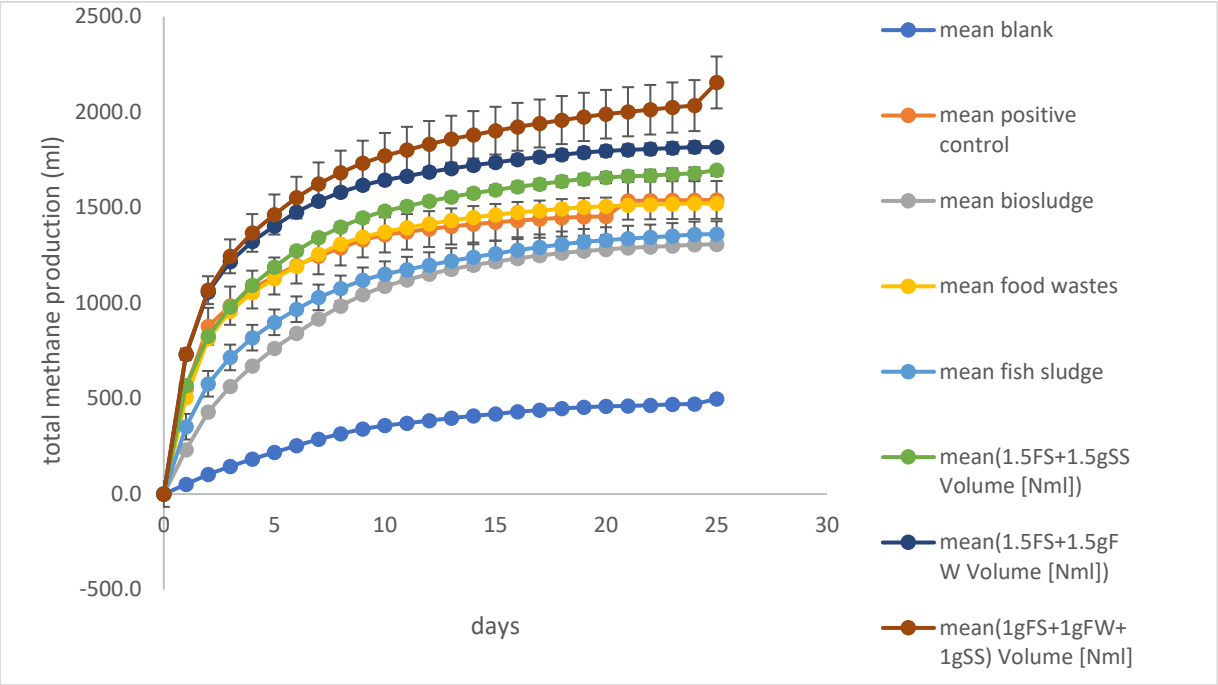


Figure 4.4 shows methane production by AD of food wastes, bio sludge and fish sludge

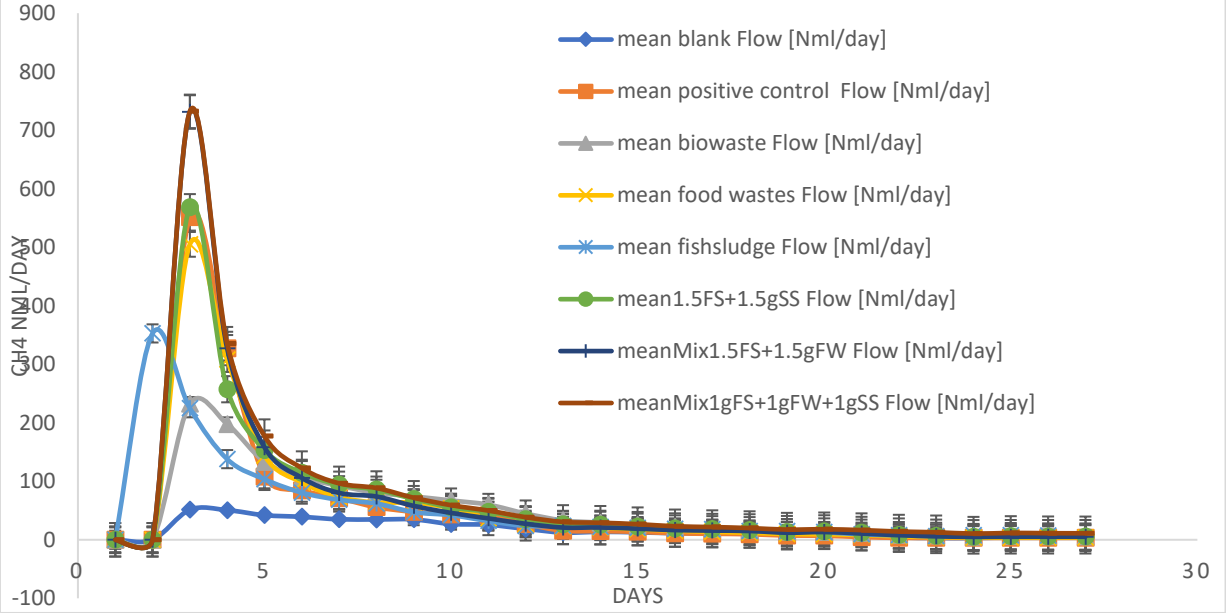


Figure 4.5 shows the reaction rate in AD of food wastes, biowastes and fish sludge

Estimation of hydrolysis constant(k_h):

Results from BMP tests were used to find further data on the given substrate like the hydrolysis rate since the hydrolysis is limiting the anaerobic digestion process, the results obtained are given in the table 4.3. By using the first part of the experimental curve made for the determination of the total methane production of a given substrate (e.g., the initial five days of methane production curves), the hydrolysis constant k_h (day^{-1}) for first order hydrolysis model was defined:

$$\frac{dS}{dt} = -k_h S$$

where, S is the biodegradable substrate, t the time and k_h the first order hydrolysis constant. By separating and integrating the variable and considering the relation between the biodegradable substrate and the methane produced, it can be written as:

$$ln\frac{B_\infty - B_t}{B_\infty} = -k_h t$$

Where B_∞ is the amount of ultimate methane production and where B_t is the methane produced at a given time, t. Then the first order hydrolysis constant (k_h) was obtained from the slope of the linear curve obtained. This constant is distinctive of a given substrate and gives knowledge about the time expected to produce a given ratio of the ultimate methane potential (Mace *et al*. 2003). The example of this model application for food wastes is shown in the figure 4.5 b below.

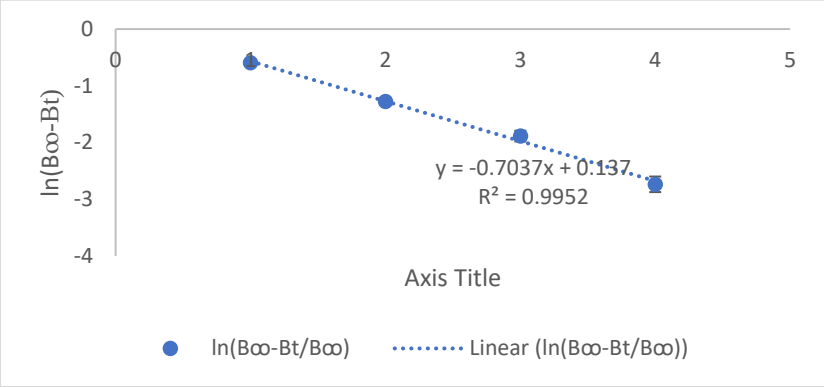


Figure 4.5b showing the 1st order model for k_h calculation for food wastes

Biomethane potential of substrates:

Methane yield describes metabolic activity in an anaerobic system and can be determined according to following equation:

$Y_{CH4} = V_{CH4}/gVS$ [$NmL_{CH4}=gVS_{substrate}$]

where Y_{CH4} is methane yield, V_{CH4} is the accumulated volume of methane (NmL) produced, and VS is the grams of volatile solids in the substrate added to the reactors (g). The biomethane potential yield in terms of $mLCH_4/gVS$, $mlCH_4/gCOD$ and gCOD/gCOD were determined as shown in the table 2. The background methane production from the inoculum obtained from blank tests (where no substrate was added), was deducted from the methane production obtained in the original substrate assays. The blank tests were carried out in triplicates as well for statistical importance.

Table 4.3 showing yield in terms of mL CH4/gVS, ml CH4/gCOD and yield in terms of gCOD/gCOD

Sludge Type	BMP (mL CH_4/gVS)	BMP (mL CH_4/gCOD)	BMP (gCOD/gCOD)	Hydrolysis constant(k_h) day^{-1}
Positive control	259±2	217±2	0.62±0.00	0.83
Primary sludge	414±15	245±2	0.70±0.01	0.73
Fish sludge (Steinsvik)	318±1	207±1	0.59±0.03	0.64
Mix 50% (primary sludge+ fish sludge)	353±9	214±3	0.61±0.06	0.7
Mix 75% (primary sludge+25% fish sludge)	386±5	231±4	0.66±0.01	0.71
Positive control	351±10	295±4	0.84±0.01	0.7
Secondary sludge	267±6	137±2	0.39±0.04	0.57
Food wastes	404±5	202±1	0.58±0.03	0.7
Fish sludge	286±2	207±4	0.59±0.01	0.63
Mix 50% (Fish Sludge+ Secondary Sludge)	390±1	234±1	0.67±0.00	0.60
Mix 50% (Fish Sludge+ Food wastes)	456±3	270±3	0.77±0.05	0.7
Mix: 33.3% (Secondary Sludge+ Food Waste+ Fish Sludge)	550±2	311±1	0.89±0.04	0.66

4.4. Batch Test 4:

AD and Co-AD of Fish food wastes and septic sludge:

In the batch test 4, fish food wastes and the septic wastes from IVAR, SNJ were mono-digested and co-digested at a loading rate ratio of 1:0, 0:1, 1:1. Additionally, blank samples and 2g starch samples were also run in duplicates. The experiment was run at similar conditions as the first two batch tests.

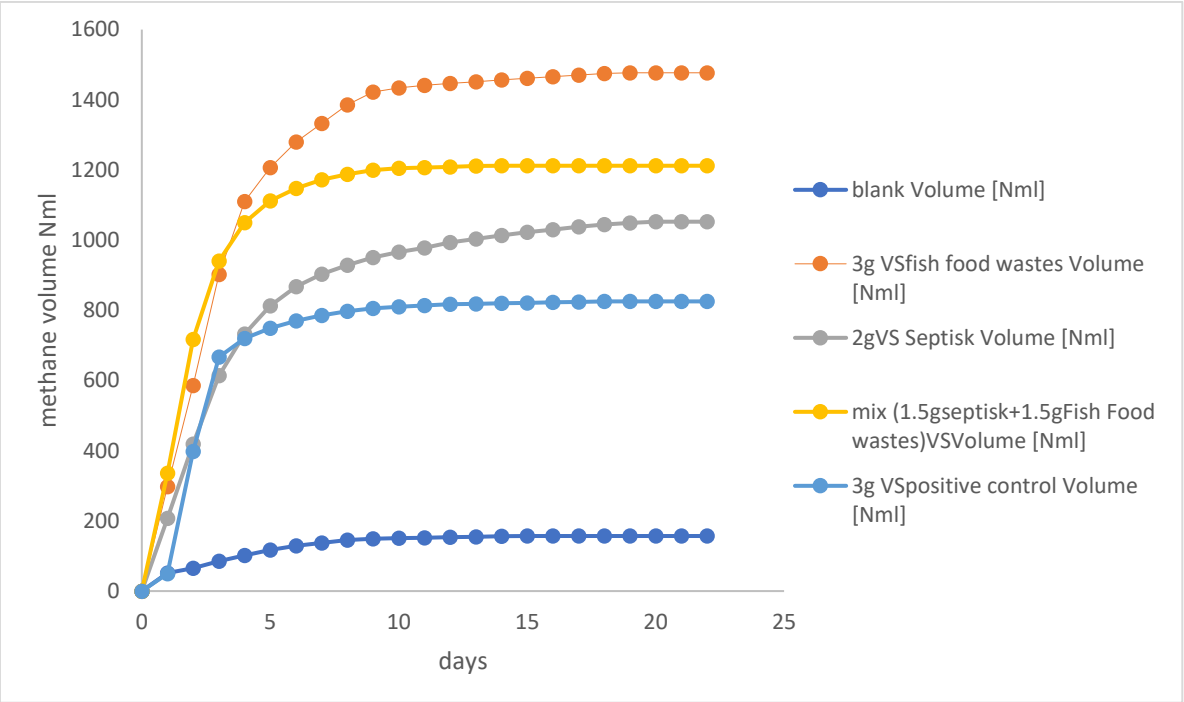


Figure 4.6 shows methane production by AD of fish food wastes and septic sludge

Fish food wastes and septic sludge had a larger particle size and they should have pre-treated. So many of the flasks stop producing biogas after few days and it was possible to measure biogas for only one of the parallels for each of the sludge type. Nevertheless, if we compare the ultimate methane production of different wastes, fish food wastes and septic wastes had highest methane production while mixture of both sludges has lower production. The septic was taken as 2g VS while food wastes were taken as 3g, but the BMP/g VS were almost same 433 ml/gVS= fish food wastes and septic wastes=450ml/gVS respectively.

Rate of reaction:

The flowrate of the methane production given in ml/d (Figure 6) in batch test 4 showed the different results than ultimate methane production rate. This is because different VS amounts were taken. The test was terminated after 23 days when the methane production of all series entered a methane production phase like that of the blank.

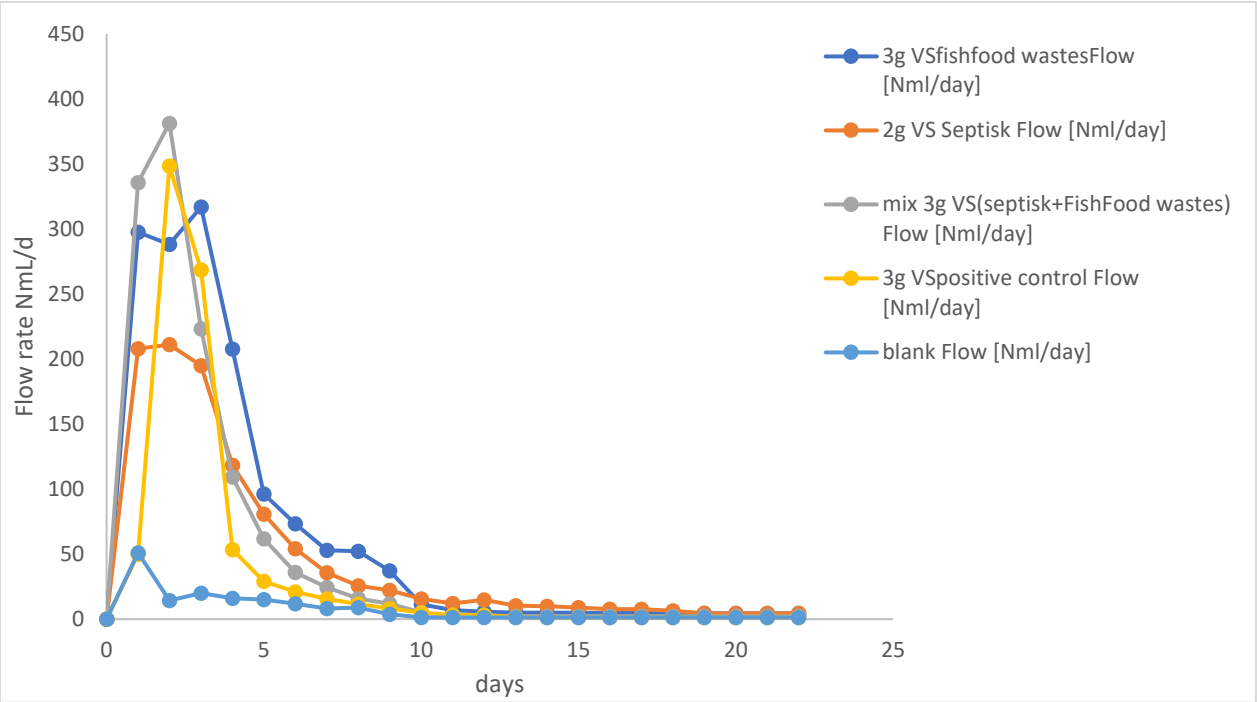


Figure 4.7 shows the flow rate of methane for AD of fish food, septic waste and their mixture

Table 4.4 showing yield in terms of mL CH_4/gVS, ml CH_4/gCOD and yield in terms of gCOD/gCOD

Sludge Type	BMP (mL CH_4/gVS)	BMP (mL CH_4/gCOD)	BMP (gCOD/gCOD)	Hydrolysis constant(k_h) day^{-1}
Positive control	275	231	0.66	1.097
Fish food wastes	440	267	0.76	0.74
Septic wastes	448	253	0.72	0.67
Mix: 50% (fish food wastes+ septic wastes)	352	206	0.65	0.84
Positive control	285	242	0.7	0.92
Fish sludge Hjelmeland	116	190	0.54	0.71
Food waste restaurant	121	81	0.23	0.78
Tine dairy wastes	660	667	1.9	0.75
Pulper	442	227	0.65	0.93
Flash tank	375	202	0.6	1.02
Mix: 50% (flash tank+biopulver)	434	240	0.7	0.89

4.5. Batch Test 5:

AD of restaurant food wastes, pulper, flash tank, Tine dairy wastes and fish sludge Hjelmeland

In the batch test 5, restaurant food wastes, pulper, flash tank, Tine dairy wastes and fish sludge Hjelmeland were anaerobically treated in biomethane potential tests. Also, blank samples and 4g starch samples were also run in duplicates. The experiment was run at similar conditions as the previous batch tests. Co-digestion was carried out with only pulper and flash tank wastes (1:1) as shown in the figure 4.8.

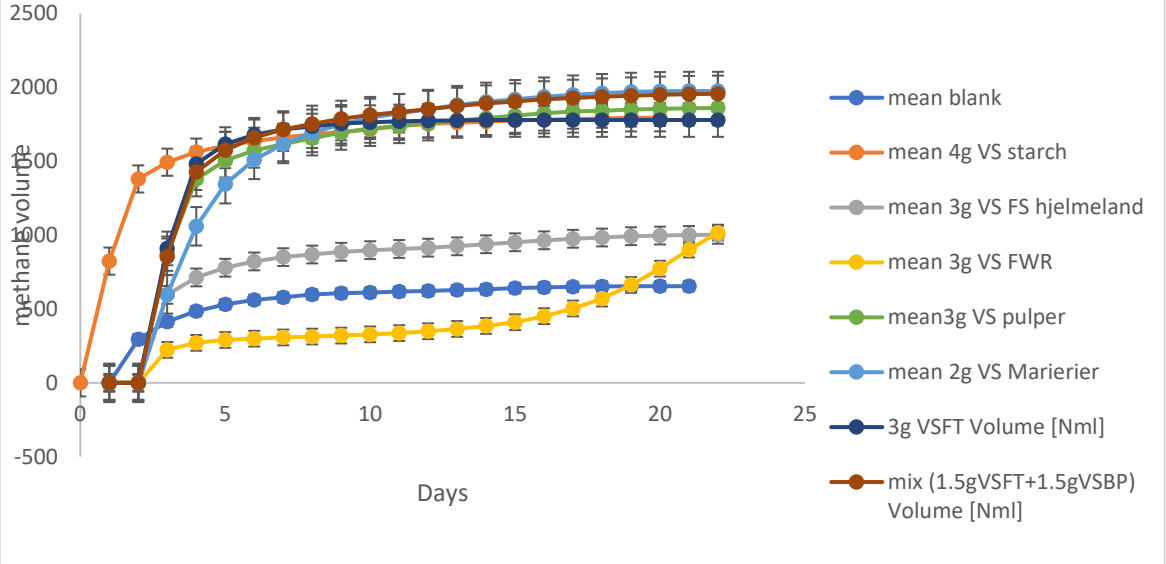


Figure 4.8 shows the total methane production for different sludges in batch test 5

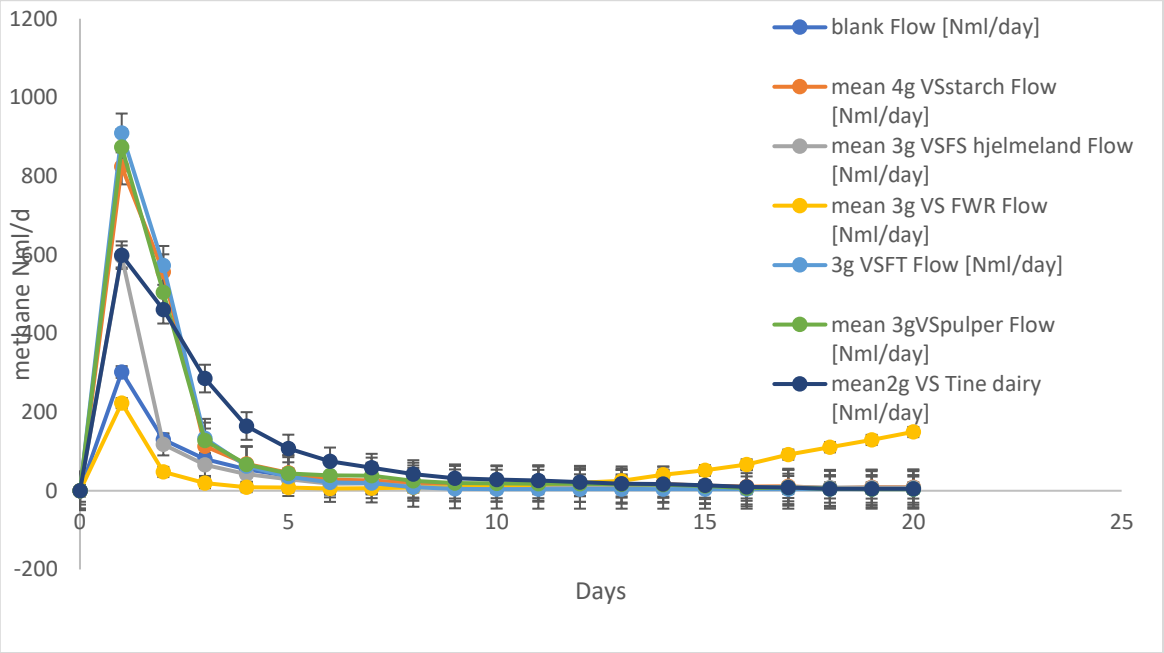


Figure 4.9 shows the total methane production for different sludges in batch test 5

4.5. Effects of co-digestion on BMP yield:

In the present research, anaerobic digestion of different organic substrates and their mixtures were carried out. The results obtained from batch tests are summarized in the figure 4.10. The biomethane potential of the co-digested mixtures are calculated from the yield of the single substrates by considering the VS of each substrate. Co-digestion of different substrates are supposed to cause synergistic or antagonistic effects. The synergism is the enhanced biogas yield for blended samples over the average of the sole substrates. Similarly, indication of antagonism or competitive interaction is by reduced methane yield in the co-digestion samples if contrasted with their individual substrates. But it's not always the case because volatile solids are not the ultimate parameters, and it could be due to variable COD in the sludge samples because COD is controlling the outcome of anaerobic digestion. The samples with reduced methane potential could be due to higher unbiodegradable COD.

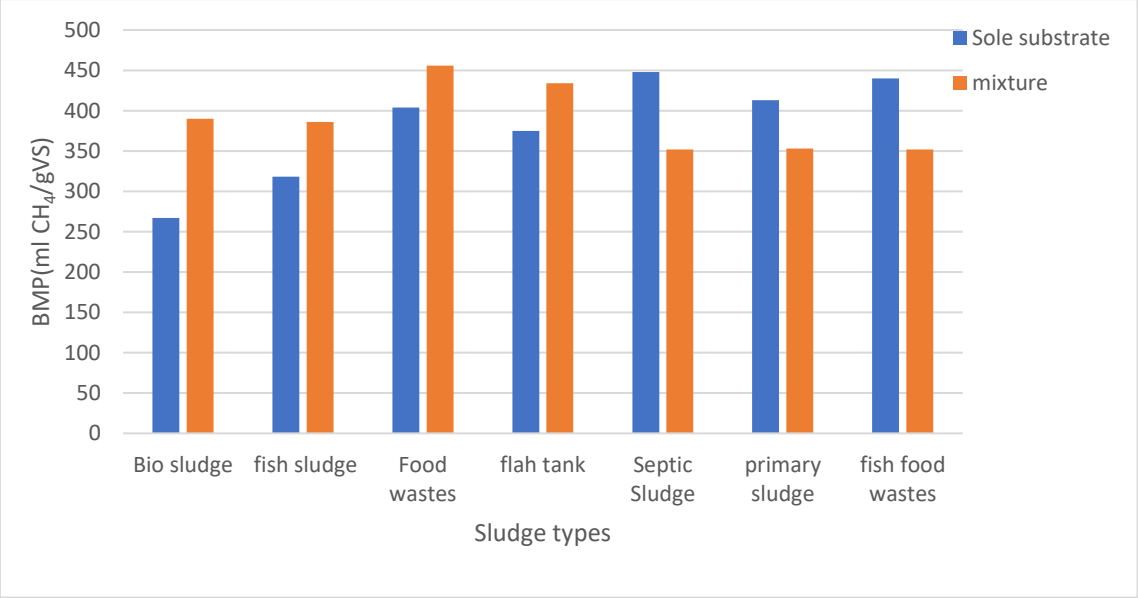


Figure 4.10 shows the increased and reduced BMP of different mixtures of sludges

4.7. Co-digestion in semi-continuous CSTR reactors:

The effect of mixed sludge interactions found in the batch fermentation tests were further investigated by performing semi-continuous experiments in CSTR. A CSTR reactor is efficient for COD removal and high methane production. During this research, the four lab scale reactors of 1000ml were constructed to evaluate the mesophilic anaerobic treatment of mixed sludge (75%primary sludge+25% fish sludge). The volume of sludge was adjusted to 750ml in all of them. Thus, all reactors were run at 35˚C temperature and OLR of 3.86 g COD/L.d with four different hydraulic retention times. Retention time was changed from first reactor at 15days, 2nd at 7.5 days, 3rd at 5 days whereas fourth at 3.75 days.

4.7.1. Conditioning of CSTR reactors:

In the first 7 days all four reactors were run at similar conditions to attain the steady state, i.e. 50 ml of influent was injected and 50ml of effluent was pushed out from the outlet while keeping the same volume at 750ml. For this purpose, inoculum was added to the reactors and feed consisting of mix sludge (75%primary sludge+25% fish sludge) was injected every day. During this phase acclimatization of bacteria in the reactors occurred and small amount of biogas was produced in each of the reactors. Accumulation of VFA were higher upto 2000mg/L although alkalinity was higher too. We can refer to this phase as lag phase as bacteria were adapting to the conditions in the reactors.

After one week, when all the reactors were producing nearly same amount of the gas, they were operated at different solid retention times. For reducing the solid retention time in the 2nd, 3rd and 4th reactors, alkalinity water was used with 0.1M sodium bicarbonate.

4.7.2. Daily methane production in CSTRs:

Figure 4.11 shows the daily methane production in all the four reactors. In the reactors with retention times 15 and 7.5 days, daily methane production was higher for 45 days mostly ranging from 800-1000ml/d. The 3rd reactor has 5 days HRT and had intermediate values of gas ranging from 600-800 ml/d. Whereas the 4th reactor had a lowest gas production ranging from 400-600 ml/d.

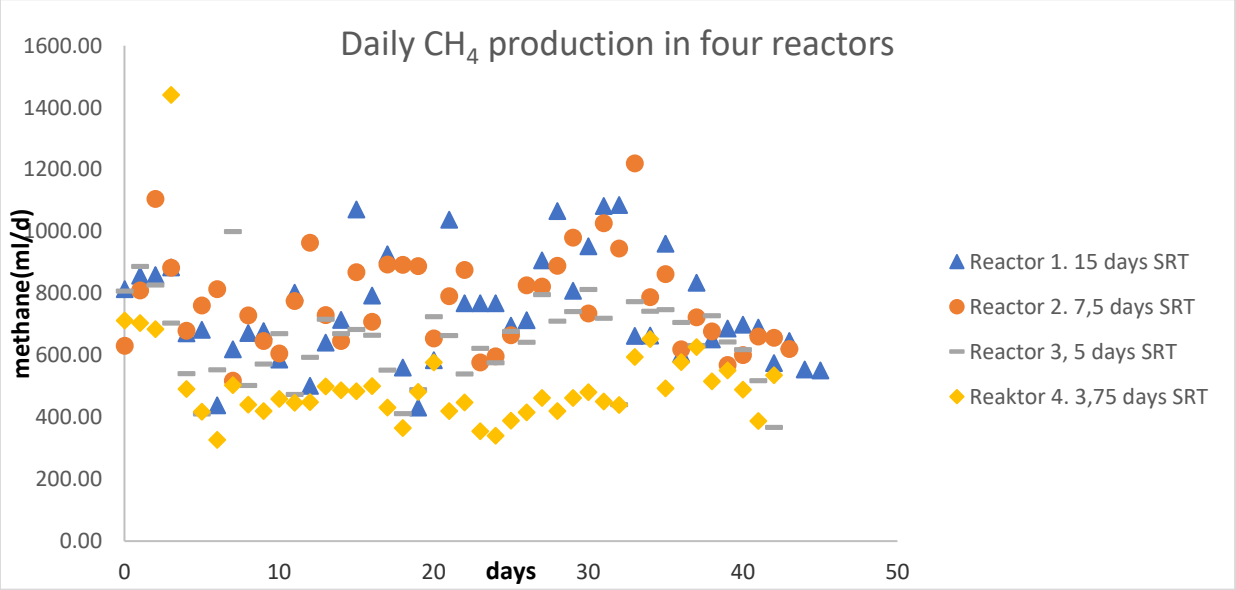


Figure 4.11 showing methane production per day in the four reactors

4.7.3. COD removal in the CSTR:

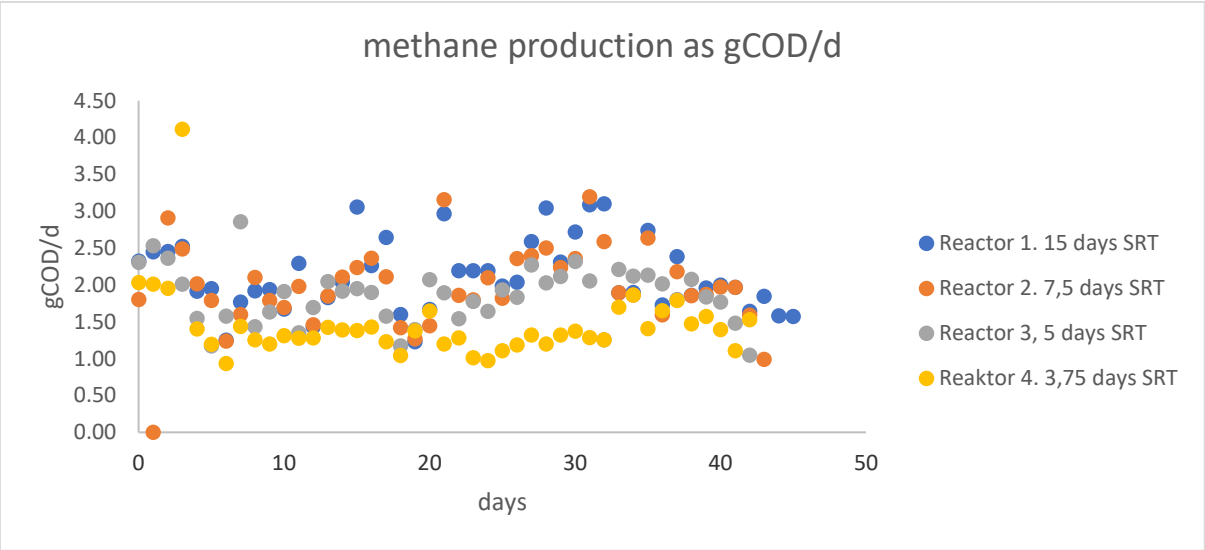
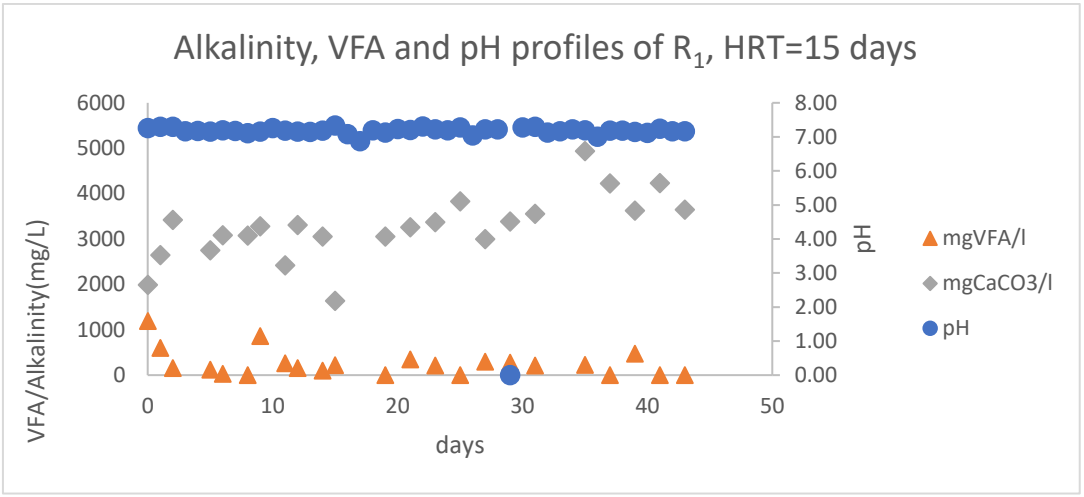


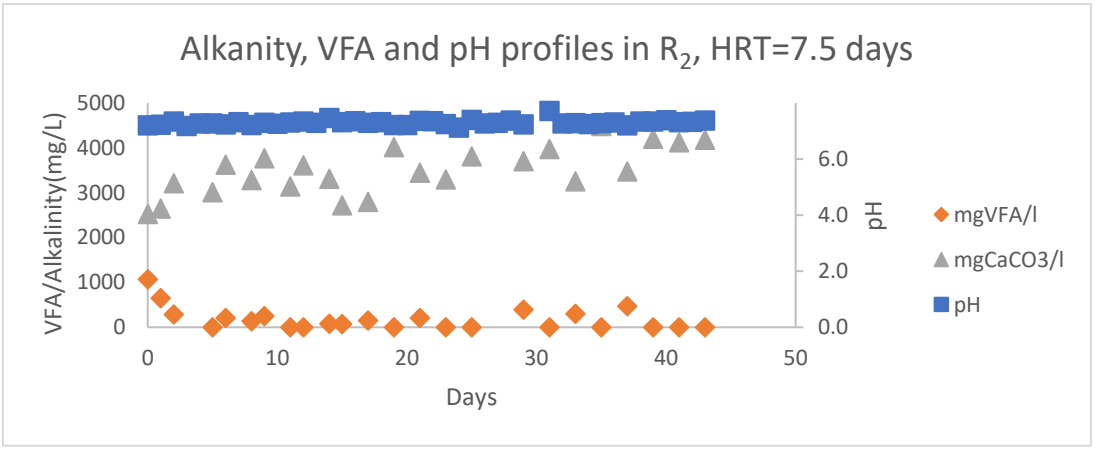
Figure 4.12 showing gCOD converted to methane per day in the four reactors

The inlet COD was 2.9g/d for all the four reactors. So, COD removal for first 3 reactors were quite high. In the first and 2nd reactors, the COD removed as CH_4 was between 60-100% and 50-100% respectively. The 3rd reactor showed the 40-90% COD removed whereas lowest COD (30-65%) was recovered as CH_4 was observed for the 4th reactor with lowest HRT as indicated in the Fig. 4.12.

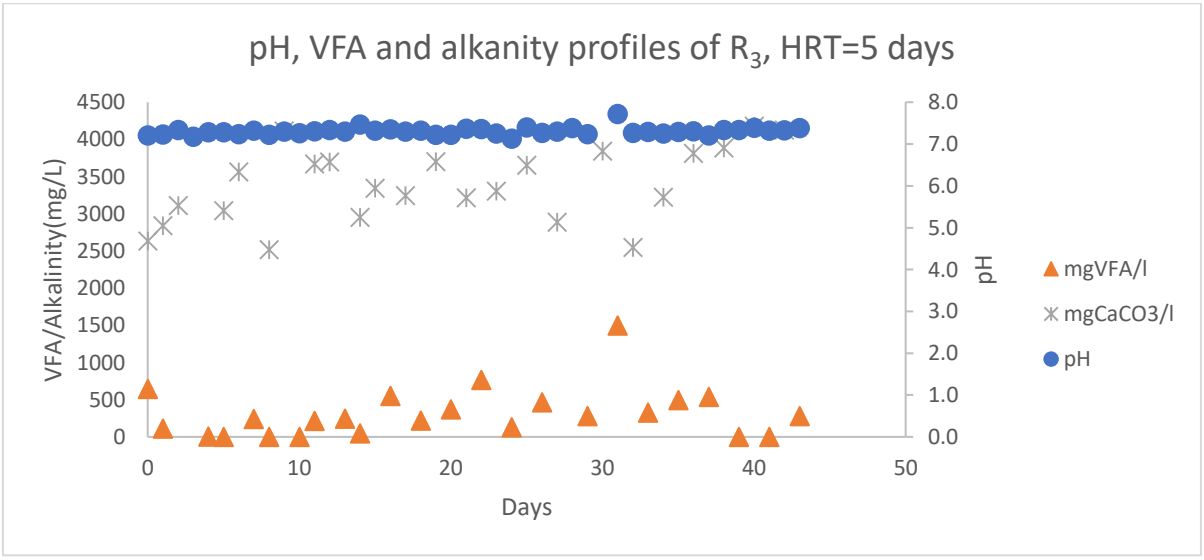
4.7.4. pH, Alkalinity, and VFA Variability:

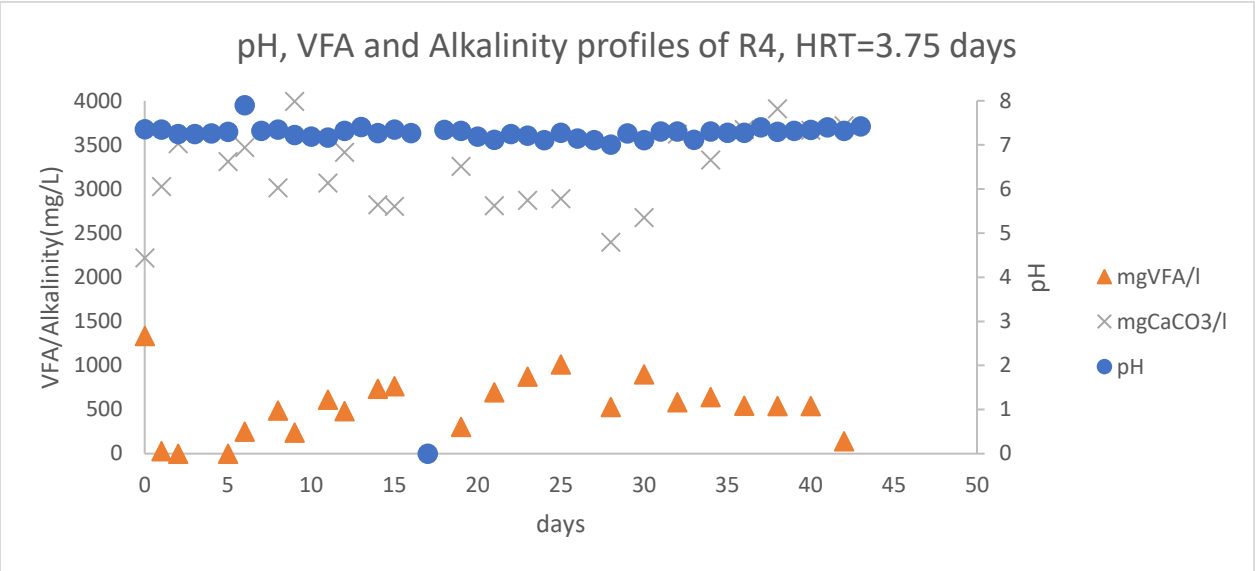
Figures below (4.13a, 4.13b, 4.14a and 4.14b) shows the variability of pH, alkalinity and volatile fatty acid of R_1, R_2, R_3 and R_4 as function of time. The concentrations are expressed as mg acetic acid/l of total VFA and mg $CaCO_3$/l of alkalinity. It was observed the pH in all the four reactors were between 6.8 and 7.7 showing the stability of the system. The first two reactors showed the 200-500 mg/L VFA as average. Moreover, the consumption of VFA was observed in some of the days with their zero-concentration showing higher alkalinity. However, in the last reactor with HRT=3.75 days there were higher concentration of VFA ranging between 400-1500 mg acetic acid/L. Nevertheless, enough alkalinity was present in the reactor 4 as well that contributes to the stability of system and production of biogas.





Figures 4.13a and 4.13b showing the alkalinity, VFA and pH profiles of first two reactors





Figures 4.14a and 4.14b showing the alkalinity, VFA and pH profiles of 3rd and 4th reactors

4.7.5. Hourly rate of reaction:

At different times, hourly rate of reaction was also observed as shown in the figure 4.15. The reactor with 15 days HRT showed highest rate in the beginning while its rate drops quickly after 12 hours. Comparatively, rates of reaction in the reactors with 7.5- and 5-days HRT showed a steady rate throughout 24 hours. The lowest rate was observed for 3.75 days throughout the day.

Furthermore, the figure 4.16 shows the total volume of CH_4 accumulated in the four reactors. First reactor with longest HRT was producing maximum biogas while fourth with shortest HRT was producing lowest. The two with intermediate HRTs were behaving almost similarly and producing intermediate volume of gas.

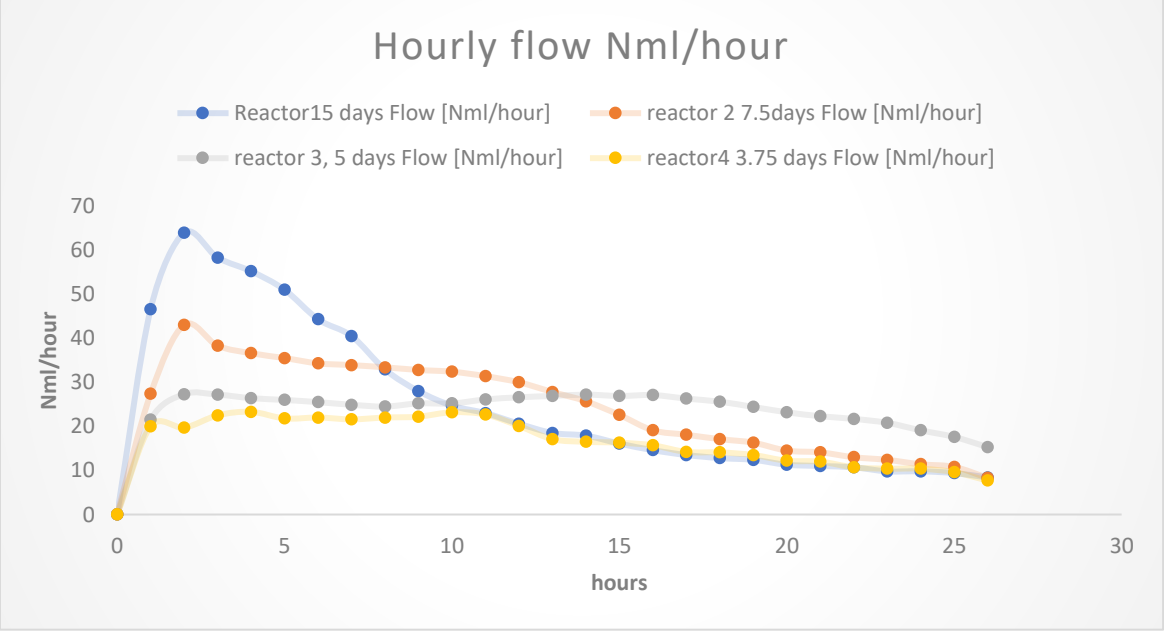
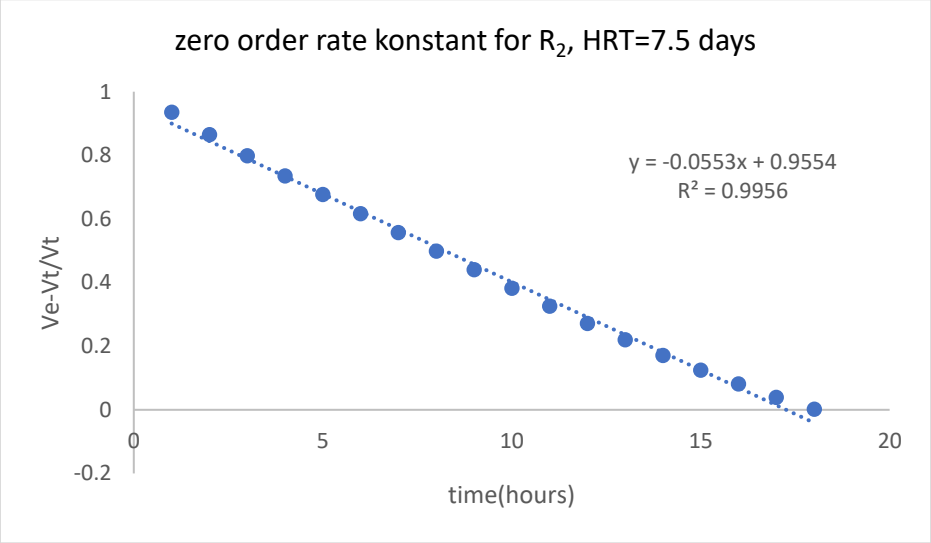


Figure 4.15 indicating the methane production per hour during the whole day after single feeding

Kinetics of reaction:

The zero order, first order and 2nd order equations were applied to determine the kinetics of reaction. The best fit was obtained with zero order with a R^2=0.99 and k_0, k_1 and k_2 values were determined. As substrate concentrations were in excess specifically in the first few hours, so system kinetics were independent of substrate concentrations, rather it was depending on other factors like HRT etc as shown in the figure 4.15 (b). This is in accordance with the situations in CSTRs. The slope of curve gives the k_0.



The graph 4.15 b showing estimation of k_0 for reactor 2

Hourly methane production:

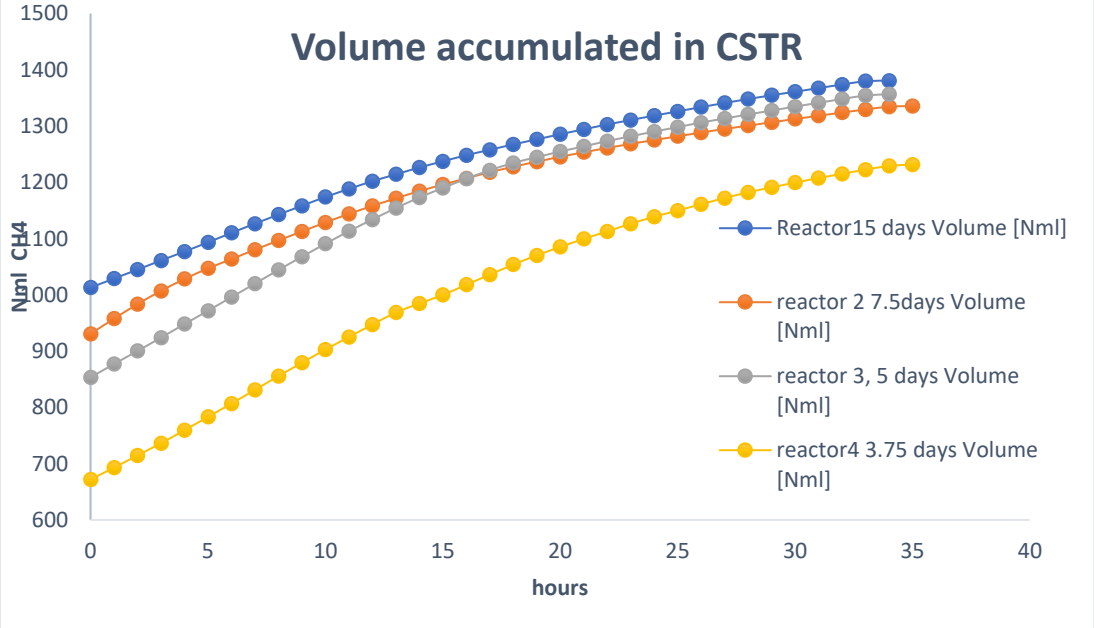


Figure 4.16 indicating the volume of methane accumulated during one day after feeding

Effect of feeding on methane production:

Figure 4.17 shows the effect of feeding on the methane accumulated in the four reactors during 2 days of feedings. After 2nd feeding an exponential increase of methane was observed until it enters a stationary stage.

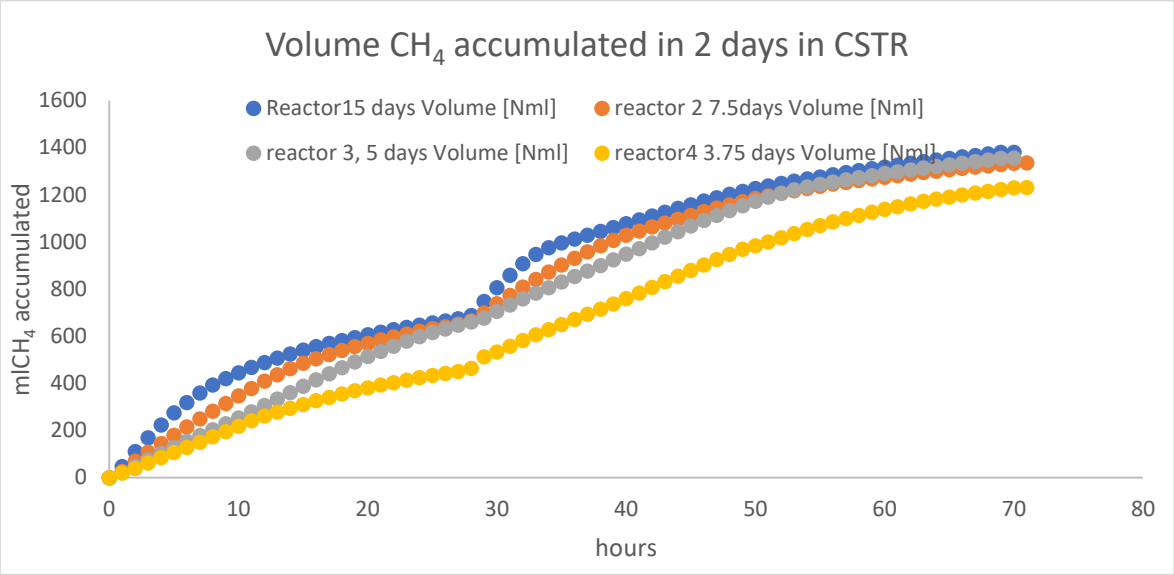


Figure 4.17 indicating the volume of methane accumulated during two days after successive feedings

4.7.6. Nutrients availability and COD Removal:

After 45 days of operation of reactors, steady state was assumed to be achieved. At that time nutrients availability was measured. For this purpose, total Nitrogen, NH_4-N and phosphate concentrations as well COD in the effluents and feed were measured as shown in the table 4.5.

Table 4.5 shows the amount of nutrients at steady state

Reactors	Total Nitrogen(g/kg)	Phosphates (g/kg)	NH_4-N	COD(g/kg)
Feed	10.7	0.3	0.40	58
R_1	1.6	0.15	0.44	28.32
R_2	1.13	0.12	0.37	13.5
R_3	4.9	0.095	0.27	12.6
R_4	4.9	0.082	0.14	5.84

It is clear from the table that nitrogen and phosphates were present in the enough amounts in the first two reactors. That's according to our findings because the methane yields were higher in these reactors also. Also, COD removal was also checked. Reactor with higher retention times had higher

COD, it might be because more substrate concentration and better growth of microorganisms takes place in these reactors. That's why VS and TS were in greater amounts in these reactors as shown below in table 4.6.

Table 4.6 shows the TS removal at steady state

Reactors	TS (%)	TVS(%)	TS removal (%)	TVS removal (%)
Feed	5.15	4.45	-	-
R_1	2.7	1.84	48	59
R_2	1.1	0.56	78.7	87
R_3	1.28	0.7	75	84
R_4	0.95	0.46	82	90

5. Discussion

The discussion part has mainly three parts. The first part is discussion on batch tests while second part is discussion on CSTR. After that there is comparison of two reactors and last part is about the application of SRT on digester in IVAR.

5.1. Discussion on batch operation:

The important results of the BMP tests were the curves made from total methane production (Figs. 4.1, 4.3, 4.5) where on the X-axis, the time in days and on Y-axis the corresponding total bio-methane production is presented. As stated by Esposito *et al.,* (2012) usually these curves are reverse L shaped that represents the maximum theoretical bio-methane production that could be obtained from the given substrate considered. These graphs could be divided into 3 main regions:

- preliminary phase;
- intermediary phase;
- final phase.

These steps could be well explained by different stages of anaerobic digestion. The earliest phase is the hydrolysis step in which polymeric fragments are degraded through the action of exo-enzymes to smaller molecules which can cross the cell barrier. This situation can be compared to the typical growth curves of microbes where the initial phase is lag phase (adaptation phase), and bacteria need time to adapt to the conditions provided, the larger or complex the solid particles are, the lower the bio-methanation rate is in the initial phase since the first step of the anaerobic digestion, i.e. breakdown and hydrolysis, would require a longer time to complete, showing this step as the rate controlling step of the entire process. Acidogenesis and methanogenesis is expected to occur after hydrolytic interactions in the exponential phase of the curves.

The second types of curves are the rates of reaction during anaerobic digestion and it depends on several factors (Esposito *et al.,* (2012)). The most important factor affecting the rate is the amount and types of solids present in anaerobic systems. As time proceeds during batch tests, the concentration of solids left decreases as well as the bio-methanation rate. This behavior is well described by curves as shown in the figures 4.2, 4.6 and 4.7. Also, the bigger and more complex the organic solid particles are, the lower the bio-methanation rate is during the initial phase since the first step of the anaerobic digestion, i.e. disintegration and hydrolysis, takes a longer time to complete, and thus lowering the rate of the whole process. Thirdly, biodegradability of substrate has also profound effect on the rate. These curves can be divided into two parts. The first part corresponds to breakdown of readily biodegradable COD and rate of reaction is maximum during this phase. Afterwards when the rate of biogas suddenly lowers showing methane production by using slowly biodegradable COD. This could be observed by the curves of positive control in each of the figures 4.1-4.7.

However, presence of inhibitors can decrease the daily methane production rate than anticipated and or even of a bio-methanation rate equal to zero. These inhibitors can cause a decrease in pH because the microbial species that contribute to the anaerobic digestion process are specifically

sensitive to pH. Hence, when the pH in the system is far from the normal pH range, the biomethanation rate is lower than usual (Frunzo *et al*, 2012).

COD balance

It is believed that there is no COD destruction in anaerobic reactors as in aerobic reactors, but COD is only rearranged. The complex organic matter is broken-down into simpler intermediates and after few stages, mineralized into CH_4 and CO_2. COD that entered the system ends up in the end-product CH_4 as well as the COD that is assimilated into new bacterial biomass. Therefore, a complete mass balance could be achieved by using COD as a basic factor. Consequently, in anaerobic systems the COD is generally taken as a control tool (Henze *et al*., 2009):

$COD_{in}=COD_{out}$

This COD balance was calculated as BMP yield in terms of $gCOD_{CH4}/gCOD_{removed}$ for all the substrates and their mixtures. Depending on biodegradability and amount of solids present, maximum yield was obtained for starch and mixture of solids consisting of secondary sludge, food wastes and fish sludge as shown in table 4.3.

Based on above discussion, firstly, we would explain the bio-methanation rate of starch referred to as positive control. The positive control analyses give an idea of the inoculum reaction towards "standard" substrates. Starch is biodegradable and in bio-available form, so in both figures 4.1 and 4.3, starch had higher rate in the beginning. However, the total methane production of primary sludge, fish sludge and mixed sludges were higher because after first few days the lower concentration of starch was left in positive control. Also, the starch is readily biodegradable substance and its consumed in the medium easily. This phenomenon could be clearly observed in the bio-methanation curves as well as in flow rates curves in each of the five batch tests (figure 4.1-4.6). Moreover, this fact could also be explained by hydrolysis constant k_h of starch which is higher (0.83 day^{-1}) as compared to other substrates indicating the higher biodegradability of standard substrate used.

During first 2 batch tests, as indicated in the figure 4.1, the primary sludge had the highest production of methane when compared with the other mono-digested and co-digested samples. This is clear from specific COD of the primary sludge that is 1.56 gCOD/gVS of the primary sludge, whereas fish wastes lower the methane production when co-digested with primary sludge having same amount of VS. This difference can be attributed to the fact that fish wastes has lower Specific COD=1.38gO_2/gVS that contributes to lowering of methane volume for co-digested samples as compared to primary sludge with same amount of VS content. Moreover, even if the samples might have same VS content, their COD would be different, and COD is the main driver of the methane production in batch assays. So, we can say in case of primary sludge, the higher COD leads to greater production of methane. Secondly, fish wastes might have higher unbiodegradable COD that is causing the lowering of biogas production in the mixed samples. Therefore, addition of fish sludge to primary sludge can lead to antagonistic effect if compared with sole primary sludge samples as described in the figure 4.10.

In the 3rd batch test, lowest biomethane volume was observed for secondary sewage sludge although COD/VS=1.95gO_2/gVS for secondary sludge was quite high and yield obtained was 0.39gCOD/COD that was lowest among all the substrates. Similarly, food wastes had highest COD/VS=2gO_2/gVS but the $Y_{gCOD/gCOD}$=0.58 was not as high as expected. However, when these sludges were co-digested with fish sludges, $Y_{gCOD/gCOD}$ was higher up to 0.7. Even when secondary sludge was co-digested with food wastes and fish sludge, maximum yield $Y_{gCOD/gCOD}$ =0.89 was obtained. This could be due to the synergistic effect caused by diversity of substrates present in the three sludges. This effect was observed in the previous research as well. Food wastes when mixed with other sludges like fish waste, sewage sludge indicates a highly biodegradable co-substrate, which, improves the biogas production of the secondary sludge digesters just by increasing the OLR (Salman Zafar, 2018). Additionally, the synergistic effects might appear from the contribution of additional alkalinity, trace elements, or any other improvement which one sludge by itself might be deficient and could result in an increase in substrate biodegradability and therefore overall methane potential.

Later in the 4th batch, fish food wastes and septic sludge were producing almost same volume of methane. This could be clear from the ratio (COD/VS=1.65 and 1.77) content of the fish food wastes and septic sludge that contributes to similar yield of biogas. Also, when we observe the rate of reaction in the figure 4.7, the mixture of fish food wastes and septic sludge had highest rate of reaction in the beginning when compared with their individual rates. Thus, co-digestion is enhancing hydrolysis and disintegration rates (k_h=0.84 day^{-1}) but overall yield of biogas was lower than individual samples. However, for this batch test series all the reactors were not producing biogas after 14 days. Some of the samples stopped after few days. This might be since fish food wastes and septic sludge had larger substrate size, were highly insoluble and not in bio-available form. So, they could not produce biogas for longer period. These errors could be avoided by performing the experiment after grinding or pre-treatment of the sludges.

Finally, in the 5th batch tests 4g starch was tested and highest (kh=0.89) value was obtained showing that higher substrate concentration could increase the biodegradation rate and rate of methane production as well. It was hypothesis that thermal hydrolytic processes can increase the biodegradable COD and consequently more methane production but it's not the case. If we compare the yields of pulper and flash tank wastes (table 4.4), pulper had higher methane yield than pulper and mixing did not have any significant effect on the yield as shown in the fig. 4.8. Moreover, Tine dairy wastes had highest methane production, although COD/VS was lowest. This might be because COD measurement of sludges had greater uncertainty and could have some error in the COD/VS value. Also, Tine wastes might have higher percentage of biodegradable COD leading to higher biogas yield.

5.2. Discussion on CSTR:

5.2.1. Effect of SRT (sludge retention time) on daily methane production:

This study compares the HRT of different CSTR reactors utilizing same substrates for biogas production while retaining the OLR equivalent in all of them. Organic Loading Rate (OLR) indicates the amount of kilograms of organic solids loaded per m^3 of digester volume and unit of time. HRT correlates the reactor volume and the daily feed volume by describing the average time the solid particles spend in the biogas digester. It is usually presumed that the longer the SRT (sludge retention time), more the biodegradation of organic matter, but it's not always the case because AD require optimum SRT. Both retention Time (HRT) and OLR affect the biogas process (Sherieff *et al.*, 2016).

If we compare the methane production and COD removed at different retention times, maximum methane production (60-100%) was achieved at 15 days and 7.5 days. While at 3.75 days methane production was half of that produced at longer retention times (Fig. 4.11, 4.12). This is because the chosen SRT must always exceed the minimum SRT linked with the microorganisms responsible for a biochemical conversion. The minimum SRT is the value below which a group of microorganisms is unable to grow in a suspended growth reactor. Moreover, it affects the range of microorganisms that can grow in a bioreactor, as well as their activity, in that way influencing effluent quality. As mentioned earlier, substrate used for feeding of CSTR consisted of primary sludge and fish sludge. Fish sludge had higher concentration of proteins and carbohydrates (table 4.2). It was reported in literature (Grady *et al.*, 2011) that hydrolysis of particulate carbohydrates and proteins to form monosaccharides and amino acids is relatively rapid reaction and takes about three days under thermophilic conditions. As biogas was being produced in our reactors even at shorter SRTs like 3.75 and 5 days. However, the hydrolysis of lipids to produce long chain fatty acids (LCFA) and other products is a much slower reaction that does not mostly occur for SRT values less than around six days (Grady *et al.*, 2011). That's why VFAs were present in the reactor with shorter retention time throughout the experiment and their concentrations were higher (Fig 4.14b) than reactors with longer ones. Moreover, that hydrolysis might not be rate limiting in this case, but the methanogenesis is the overall rate limiting step in the process of biogas production from the particulate substrates. The reason for this can be 1) Low methanogenic biomass concentration (low biomass density); 2) atrial inhibition due to NH_3, H_2S, pH, or other potential partially inhibiting factors.

The pH and VFA levels during the experiment showed a properly functioning process (Figures 4.13, 4.14) in each reactor. The pH remained between 6.8 and 7.8 in all four reactors demonstrating a healthy AD process. Liu *et al.* (2008) reported that the optimum pH for higher biogas yield is 6.5–7.5. Although different anaerobic microorganisms require different favorable pH values, most of them would rather survive in neutral pH conditions. It is especially essential for anaerobic activities because the methanogens are influenced to a larger extent than the other microorganisms. Additionally, the VFA accumulation throughout the experiment remained within the optimal concentrations (<1500 mg/L) described in the literature for CSTR reactors, indicating a stable AD system. As expected, small rise in the VFA levels was seen after every two days. But it was reestablished toward lower levels after each increase. However, a slight decrease in pH was

observed in the 4th reactor in some days due to increased production of higher molecular weight VFAs but pH was never less than 6.8 demonstrating a good buffering system in the reactor.
Finally, nutrients availability was checked at steady state after 45 days of experiment. The macronutrients(P) and nitrogen(N) were available in all the reactors especially in the first two reactors. Macronutrients play a vital role in the growth and metabolism of anaerobic microorganisms. They can also act as buffering agents balancing C:N ratio, or as central part of enzymes involved in the methane production. Therefore, it was reported that presence of P and N could improve digestion process (Hinken *et al.*,2008; Shrerer *et al.*,2009). Moreover, during AD, nutrients such as ammonia and phosphorus are released from nitrogenous and/or phosphorous rich organic matter, which offers the possibility to recover the nutrients from digestate.

5.2.2. Effect of SRT (sludge retention time) on rate of reaction:

The hourly rates of reactions were compared at different SRTs (Fig 4.14, 4.15 and 4.16) and the reactor with a longest retention time had a highest rate of reaction in the first 12 hours but its rate decreases rapidly after that. Comparatively, the reactors with retention times of 7.5 and 5 days had moderate production of biogas throughout the 24 hours and rate of reaction did not change significantly after first 12 hours. The reactor with 3.75 days SRT had slowest rate indicating the lowest production of gas. When we compare the two successive feedings in the reactors, since at the end of the first feeding bacteria were lacking substrate and biogas production was low, 2nd feeding cause the exponential production of gas.

The rate of reaction can be best explained by studying the microbial dynamics in the anaerobic systems. In fact, Ziganshin *et al.* (2013) observed the accumulation of the *Methanosarcina* species with the increase in VFA concentrations. *Methanosarcina* has μ_{max}=0.12 (1/d) and K_s=30mgCOD/L while *Methanosaeta* has μ_{max}=0.71 (1/d) and K_s=300mgCOD/L as illustrated in the theoretical background section 2.5 figure 2.3. Methanosarcina has a μ_{max} of 0.12 1/d. At SRT = 3.75, the μ-b_h must be higher than 0.27 1/d. Hence, Methanosarcina cannot be sustained in the reactor at this low SRT as it needs at least 1/(μmax-bh) to survive the hydraulic washout (which is at least about 10 days). Also; with the long time required to wash out these species, a new steady state would have required more than 3 SRT's, If we compare the above situation to our reactors, the 4th reactor had SRT lower indicating that dominant species in the reactor with 3.75 days were *Methanosaeta* as they outcompete *Methanosarcina* at lower SRTs. Similarly, in case of R_1 and R_2 where longer SRTs and substrate concentrations were higher, *Methanosarcina* would dominate as shown in the figure 4.15. In contrast, in R_3 with 5 days retention time might have mixed concentrations of both species due to intermediate concentrations of acetate but methanosaeta will dominate here also due to lower retention times. This result is in accordance with previous findings which stated that *Methanosaeta* and *Methanosarcina* dominate as a function of the volatile fatty acids and ammonia concentration in the digester medium. Karakashev *et al.* (2005) reported that *Methanosaeta* outcompete in the medium with lower VFA and ammonia levels whereas *Methanosarcina* dominate when the VFA or the ammonia concentration are in higher conceentrations, maybe due to their morphology. Thus, changes in the sludge composition can alter

the digester medium, such as VFA and ammonia concentration, and therefore drift to changes in the methanogenic composition. For example, the addition of a co-substrate that increased the ammonia levels (fish sludge in our case) leading to a shift in methanogens, usually dominating the *Methanosarcina* but at higher SRTs [Lin *et al.*, 2012; Xia *et al.,* 2012].

5.3. Comparison of batch and CSTR performance:

The use of batch fermentation tests has some limitations. For example, no information is provided regarding process efficiency, stability, or instability under continuous long-term operation. It's not easy to compare CSTR performance and batch test data because the batch phase is a highly dynamic process, starting with a single load much greater than that experienced during a typical CSTR feeding event. So, it can cause the inhibitory levels of VFA forming during the early days of batch digestion and even a sharp decrease in pH. Moreover, CSTR has some advantages over batch reactors as maximum growth rate can be constantly achieved at steady state conditions by controlling the loading rate and temperature, while in batch it is not possible to reach the steady state as the concentrations of the components are constantly changing with the digestion time (Zhang *et al., (*2016)). Also, in semi continuous system, depending on the waste composition, the biodegradation, and the activity of the biomass, the process can be operated using different retention times. Additionally, effect of different parameters like organic loading rate, the hydraulic and solid retention time, the formation of specific metabolic intermediates, optimization of feed composition, the microbial community dynamics, and structure, etc. can be investigated in a semi-continuous operation which is not possible in a closed system like batch (Pagés-Díaz *et. al*, 2015). As a result, when complex solid substrates must be treated, the use of a continuously stirred tank reactor (CSTR) is recommended. As in this study if methane yield obtained from co-digestion of mixed sludge (75%primary sludge+25% fish sludge) in batch and CSTR were compared, batch had 66% COD removed as methane in 20 days, while in case of CSTR with 15 days SRT daily recovery of methane were more than 80% most of the times. So, in case of sludge with high NH_4 concentration, CSTR is generally recommended. The overall system performance was stable CSTR reactors with different retention times and CSTR proved to be better system for co-digestion than batch reactors

5.4. Application of SRT to anaerobic digester at IVAR:

The current digester at IVAR is working at 15 days SRT. But as found during this study, the reactor operated at 7.5 days were producing biogas in similar concentrations and inhibitions were not found. As VFA concentrations were quite low at 7.5 days. So, it is possible to use 7.5 days SRT instead of 15 days, as it would be economically favorable and require relatively less energy. Moreover, primary sludges should be co-digested with fish sludge (Steinsvik) in semi continuous CSTR mode as fish sludge has higher amount of nutrients, proteins and polysaccharides that would contribute to higher production of biogas at shorter retention times and would generate more energy.

6. Future Research

Further research is needed in the anaerobic digestion of fish waste in order to verify the use of fish as a sole or co-substrate in biomethane production. One of the best ways to co-digest fish waste is with food and sewage waste. Due to limited research in this field, experimental data is very limited. The food wastes and sewage sludge have greater potential for energy production by using dry or wet residues in anaerobic digestion for biomethane production. So, fish wastes if co-digested with secondary sewage sludge and food wastes in semi-fed CSTRs would be useful research area on lab or pilot scale plants.

The main challenges in biogas production using AcoD technology are investigating inhibiting substances, parameter calibration and characterization, the dynamic behavior of microorganisms, and characterizing the organic constituents. Various studies have been done to minimize the problems encountered in biogas production through AcoD technology. However, the process stability and optimization still require further investigation and more knowledge is needed about how microbial population is affected by the addition of a co- substrate, in terms of kinetics, stability and yields.

Additionally, industrialization of biogas production will require suitable mass balance mathematical models. The existing models cannot evaluate the complex properties and the conversion process of the biomass effectively. The effects of sulfur, phosphorus, nitrogen and heavy metals in the sludges also need to be studied in the future for AcoD models. Investigation of all these matters is essential to develop a universal model for the AcoD process. Moreover, effect of these substances are also important because the sludge after anaerobic treatment is used as fertilizers.

Finally, these CSTR experiments can be done with different sludge mixtures, different organic loading rates, solid retention times and at higher temperatures to find the optimum conditions for achieving the maximum methane yields. Also, OLR can be increased gradually in the reactor and observe the effects of increasing and decreasing OLRs on overall yield of methane. Alternatively, CSTR in series can also be a good choice for future results because as the literature shows that the biomethane yield could be enhanced by serial CSTRs configuration functioned under co-digestion and mono-digestion. Also, it will be interesting to perform the microbial community analysis, as microbial dynamics in anaerobic environments is strongly affected by change in environmental and operational conditions.

7. Conclusion

Based on the results obtained from the research we can make the following conclusions:

- The starch showed the average BMP of 72% of the theoretical yield indicating good inoculum activity and starch a good standard substrate in anaerobic digestion in all batch tests.

- The anaerobic digestion of batch testing of fish waste from Steinsvik (72% TS, 995 g/kg COD) gave a BMP of 59% in batch test 1.0 and 2.0. Primary sludge from IVAR SNJ (3.95% VS, 62 g/kg COD) achieved a BMP of 70% in batch test 1.0. Co-digestion of both these sludges give 66% BMP and no significant effect was observed in co-digestion.

- Co-digestion of food waste (2 % VS, 40 g/kg COD), fish sludge from Steinsvik with active sludge from IVAR SNJ (4.6% VS, 90 g/kg COD) achieved a BMP of 89% in batch test 2.0 that is greater than yields obtained from single substrates.

- Co-digestion of the flash tank wastes (6.45% VS, 120 g/kg COD) and pulper (9.27% VS, 164 g/kg COD) from Grødaland achieved a BMP of 70% in batch test 5.0 that was higher than their individual BMPs.

Anaerobic treatment of the fish waste from Steinsvik and the primary sludge from IVAR SNJ, using a daily fed stirred tank reactor at 15-day SRT and 7.5, gave a 65-100% and 60-95% methane yield for 45 days. The results from the batch tests and the continuous daily fed stirred tank reactor were different with respect to methane yield. The co-digestion of primary sludge and fish sludge in CSTR showed a stable system at retention times of 15 and 7.5 days throughout the experiment and give higher methane yields (60-100%). The overall system performance was stable CSTR reactors with different retention times and CSTR proved to be better system for co-digestion than batch reactors. Further research can be carried out on anaerobic treatment using CSTR systems with different sludge mixtures, different organic loading rates, solid retention times and at higher temperatures to find the optimum conditions for achieving the maximum methane yields.

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Abbreviations

ADM1	Anaerobic Digestion Model No.1
AMB	Acetoclastic Methanogenic Bacteria
AMPTS	Automatic Methane Potential Test System
COD	Chemical Oxygen Demand
DAF	Dissolved Air Flotation
EGSB	Expanded Granular Sludge Blanket
DO	Dissolved Oxygen
HRT	Hydraulic Retention Time
IVAR	*Interkommunalt Vann Avløp og Renovasjon*
LCFA	Long Chain Fatty Acid
OLR	Organic Loading Rate
SBR	Sequencing Batch Reactor
SRB	Sulphate Reducing Bacteria
SCFA	Short Chain Fatty Acid
SRT	Solid/Sludge Retention Time
STP	Standard Temperature and Pressure
TN	Total Nitrogen
TP	Total Phosphorous
UASB	Up-flow Anaerobic Sludge Blanket
VFA	Volatile Fatty Acid
VSS	Volatile Suspended Solid
WWTP	Wastewater Treatment Plant