Enhancement of Anaerobic Digestion and Dark Fermentation process using Attached Growth system and Chemical Additives

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Abstract

High-energy demand, global warming and shortage of fossil fuels have motivated researchers to investigate on new energy alternatives with higher efficiencies. Anaerobic digestion as a biochemical process for degrading complex organics without oxygen, has been used as a promising technology for waste management systems and the production of energy and mitigation of the greenhouse gas by utilizing the waste for environmental causes.

In this research, a particulate "BioCord" bioprocess technique is used to enhance of the AD process and increase the process performance. Usage of surface as a support media for bacterial growth and creation of biofilm has resulted in many benefits for the AD system such as shortening the operation and hydraulic retention time and increased efficiency. Moreover, additives of vitamins and micronutrients have been used to enhance the metabolic rate of AD process. The lab scale anaerobic biofilm bioreactors were utilized for evaluating the performance of 4 different BioCords (LS_1, LS_2, HS_1 and HS_2). Also, the impact of *BioStreme,* which as a mixture of metals in specific concentrations, and vitamins using a mixture of different groups of vitamins has been experimented as an additive to the system and showed a positive effect on both the biomethane end biohydrogen production.

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Table of Contents

List of tables

List of figures

Acronyms

ACF	Activated Carbon fiber
AF	Anaerobic filter
AD	Anaerobic Digestion
AFBR	Anaerobic fluidized bed reactors
AMPT	Automatic Methane potential test
ASBR	Anaerobic sequencing batch reactor
ATP	Adenosine triphosphate
BAS	Biofilm Airlift Suspension
BOD	Biochemical Oxygen demand
CGW	Cotton gin waste
CM	Cow manure
COD	Chemical Oxygen demand
CSTR	Completely stirred tank reactor
EGSB	Expanded Granular Sludge Blanket
EPS	Extracellular polymeric substances
FID	Flame ionization detector
GC	Gas chromatography
HCB	Hydrogen consuming bacteria
HPB	Hydrogen producing bacteria
HPR	Hydrogen production rate
HRT	Hydraulic retention time
IC	Internal Circulation

MBR	Membrane bioreactor
OFMSW	Organic fraction of municipal solid waste
OLR	Organic loading rate
PAH	Polycyclic aromatic hydrocarbons
PET	Polyethylene Terephthalate
PVC	Polyvinyl chloride
SRT	Solids retention time
TCD	Thermal conductivity detector
TS	Total solids
TSS	Total suspended solids
UASB	Up-flow Anaerobic Sludge Blanket
VFA	VFAs
VS	Volatile solids
VSS	Volatile suspended solids

Chapter 1 Introduction

1.1 Background

AD is the preferred treatment process for organic wastes due to its low nutrient requirements, low biomass yield, and Biogas (CH_4) production. AD processes have been widely applied to various complex feedstock including municipal wastewater sludge, chemical, and agricultural industry wastewaters. In conventional AD, the acid-forming and methane-forming microorganisms are kept together in a single reactor and there is a delicate balance between these two microbial groups, because both groups differ widely in terms of physiology, nutritional needs, growth kinetics, and sensitivity to environmental conditions. However, current conventional AD processes require a hydraulic retention time (HRT) of up to 40 days to achieve the necessary stabilization of organic wastes, which translates to a large footprint (Metcalf and Eddy, 1972).

Notwithstanding the fact that numerous bioreactor configurations and system schemes are currently available for a wide variety of environmental applications such as Up-flow Anaerobic Sludge Blanket (UASB), Expanded Granular Sludge Blanket (EGSB), Biofilm Airlift Suspension (BAS), and Internal Circulation (IC), it remains a major challenge to design and develop a sustainable bioreactor system. These systems are not only capable of integrating functions i.e. biodegradation, biomass-liquid separation, and biomass retention at high suspended solids content while reducing energy demand but also can be easily applied to retrofit existing conventional technologies (Mustafa et al. 2014). The AnBioCord offers numerous advantages over conventional systems including low footprint, decoupling of hydraulic retention time (HRT) from solids retention time (SRT), and high biomass-liquid separation. Additionally, Biofilms in AnBioCord are complex layers of microorganisms that coat surfaces exposed to substrates. The hydrodynamic strength significantly affects the microbial adhesion to the solid-liquid interface by acting as a repulsive or attractive force, thereby influencing the rate of bio-Methane production. Therefore, there is a pressing need to fully investigate the performance of Biofilms in AnBioCord using a novel Biofilm Anaerobic BioCord Bioreactors (AnBioCord) and investigate the hydrodynamics and kinetics of different BioCords to develop a high rate bio Methane yield biofilm AD process. In addition, using micronutrients as supplements (BioStreme) to AD process as another available enhancement method offer many advantages over common systems such as inexpensive additive supplement, no requirement in changing the configuration of the conventional system and lowering the lag phase of the process, with positive effect on Biogas production.

1.2 Problem statement

AD has always been a method for producing alternative energy sources that can be used instead of fossil fuels, production of Biogas by AD process can reduce Carbon dioxide gas emission, and it also has no negative impact on ozone layer depletion and acid rain production. Furthermore the residue from AD process is a rich source for Nitrogen and phosphorus that are used as fertilizers for plant growth, this process can be used for treating a wide variety of wastes from agricultural to municipal waste (Kwietniewska & Tys 2014). However, with all the advantages mentioned the long startup time and low process stability has been the main obstacles for more commercialization of this system. Attached growth systems have been studied for increasing the process efficiency, however majority of studies have addressed the usage of membrane bioreactors, moving bed bioreactors and specific fixed film reactors, no research has been done on BioCords as support media in anaerobic condition in order to increase the process efficiency and Biogas production, Furthermore, addition of micronutrient supplementation as an enhancement method has been tested to boost the AD and dark fermentation process by increasing the micronutrients dosage, required for bacterial growth in order to increase the methane and hydrogen production, respectively.

1.3 Objectives

In this research, the development of new AD systems was undertaken. The specific research objectives are:

1- Development of new anaerobic biofilm BioCord process in collaboration with Bishop Water Technology, ON, Canada, for increasing the process efficiency, stability and decrease the process failure as well as increasing the Biogas production.

2- Improve the existing AD processes using an additive to increase the process efficiency and system stabilization utilizing nutrient supplements containing BioStreme manufactured by Ecolo odor technologies, ON, Canada and vitamin solution.

3- Establishing a new anaerobic process by increasing the efficiency of dark fermentation process by additive usage for the system and evaluating different concentrations of additives for a more effective result in higher hydrogen production.

1.4 Thesis layout

This thesis comprises of six chapters. After an introduction in the first chapter, a comprehensive literature review including microbial characteristics of AD, effective parameters on the process, different waste materials available, reactor configuration, enhancement methods on AD as well as dark fermentation process principles is presented in Chapter 2.

In chapter 3, the detailed description and methodology from experiment on enhancement of AD by utilizing attached media in batch reactors are provided. Also, a comprehensive result and discussion is presented on process efficiency and Biogas production.

Chapter 4, focuses on utilization of additives including BioStreme and vitamin solution to AD process in batch reactors, describing the material and methods and a detailed discussion on the results, in addition a kinetic modelling using the Gompertz modeling equation has been done for understanding the Biogas production.

In chapter 5, the detailed description and methodology from experiment on enhancement of dark fermentation by utilizing micronutrient supplementation in batch reactors are provided. Also, a comprehensive discussion and results are available as well as kinetic modeling on Hydrogen production.

Finally, chapter 6 compiles the major findings of this study and the direction of future work.

1.5 Contribution of thesis

This study provides an insight into AD as a biological process for treatment of wastes, which will produce Biogas and help in waste management procedure. This study aimed at reaching a stable AD process with high process efficiency and Methane production, and to evaluate the impact of additives on AD and dark fermentation process. A new attached media has been used to overcome the challenges of the AD process. This novel biofilm has been previously used in aerobic systems and has been successfully developed, these methods do not require any specific changes to the conventional AD process which, is cost effective and can prevent biomass accumulation and process failure.

Chapter 2 Literature review

2.1 Abstract

Rapid growth in population, increase in expectations and demands in energy requirements have resulted in a sharp increase in production of municipal and industrial waste, climate change and are the start point for global concern in case of fossil fuel deficiency. These are the reasons for searching for novel solutions such as alternative fuel sources, including methods such as AD of biomass, AD is widely applied to various waste streams. In this chapter a comprehensive review has been done on AD for Biogas production including reviews on factors affecting the efficiency, reactor design and methane production process. However conventional method had rather low efficiency, different methods are taking place as a means of increasing methane production and removal efficiency of the process. In this chapter, various effective improvement methods are presented, lastly, dark fermentation in which anaerobic bacteria can be used to produce hydrogen is reviewed in details with a review on effective parameters on this process as well as some technical challenges which can improve hydrogen production are discussed.

2.2 Introduction

The world is highly dependent on fossil fuels for energy provision and all evidence indicate depilation of this energy source, also these energy sources resulted in several environmental issues such as pollution and greenhouse gas emission. Moreover substantial increase in untreated and unmanaged waste creating odor, hygienic issues and detrimental environmental concerns, the rising need of energy year by year, for reducing the independence on fossil fuels are the main reasons for researchers to search for new resources of energy production and waste management (Kwietniewska & Tys 2014). From all the renewable sources such as solar, wind, hydroelectric and nuclear, biomass seems to be the most promising source of energy from the start since this source can be guaranteed, woody biomass has been an important energy source for mankind (Kwietniewska & Tys 2014), using waste as a source of energy isn't novel, animal waste has been used as a fuel source for burning for many years (Lyberatos 2010). There are various methods and technologies that are used for energy production from biomass which are grouped into thermochemical, biochemical and physicochemical conversion processes shown in **Figure 2-1** (Appels et al. 2011). Many alternative energy sources have been studied, nowadays, biological methods for waste management and clean energy production have been widely used, and anaerobic biological processes seem to be promising processes (Li et al. 2011).

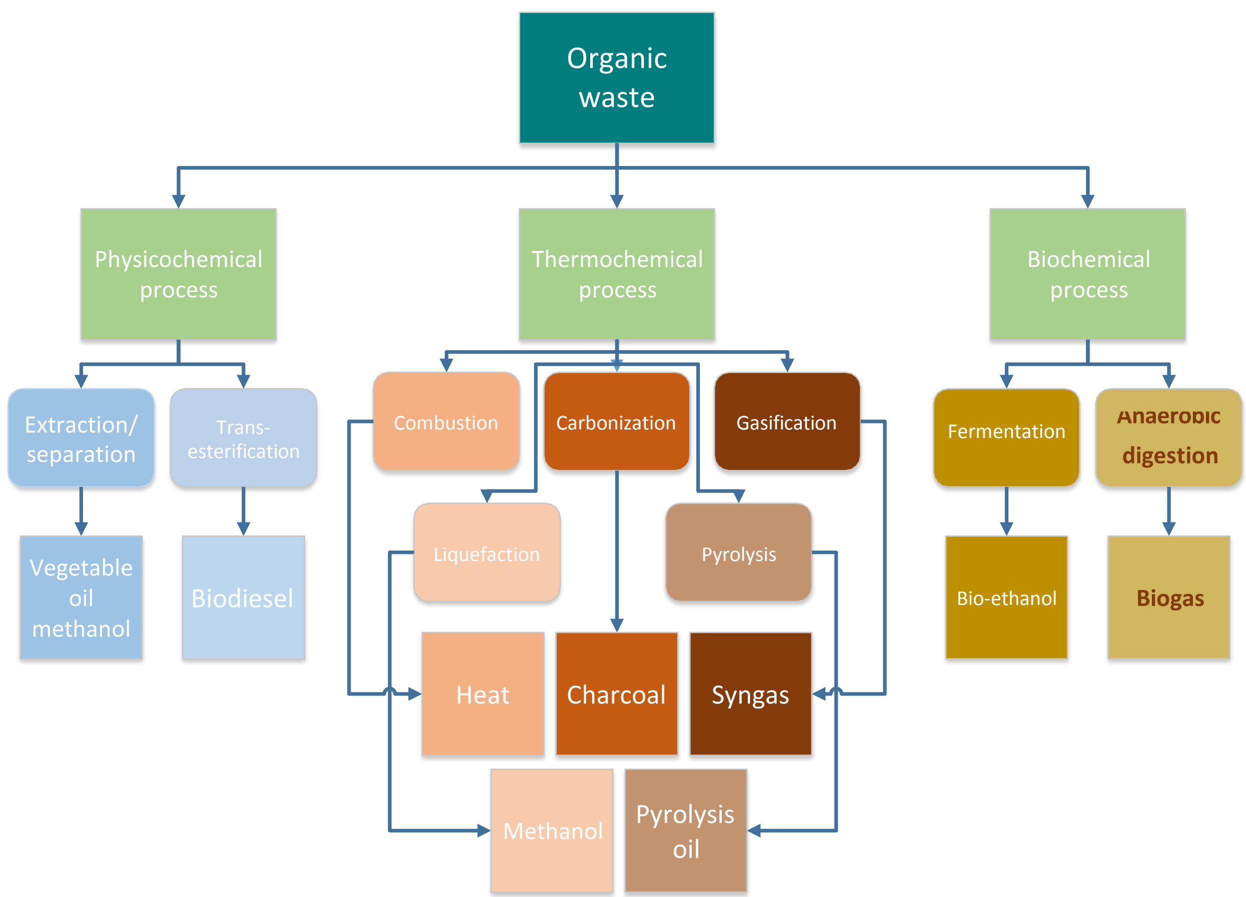


Figure 2-1 Waste conversion processes

AD and dark fermentation are two different biological conversion process that are more in favor because of the reasons indicated below:

1- AD is a anaerobic process that is widely used for organic stabilization of wastes and Biogas production (Li et al. 2011). Production of Biogas by AD can reduce carbon dioxide gas emission and it also has no negative impact on ozone depletion and acid rain production. A rich source of nitrogen and phosphorus is also produced that can be utilized as fertilizers for plant growth (Kwietniewska & Tys 2014).
2- Dark fermentation is another anaerobic process for producing hydrogen and VFAs; this process provides Carbon neutral energy source as an alternative to fossil fuels.

Currently, demand in using biological processes is increasing, improvement methods such as pretreatment, additives and attached growth media can accelerate the anaerobic process and can provide applicable by-products. In this chapter, AD process and dark fermentation will be discussed in details as well as the improvement methods for increasing the efficiencies of the system.

2.3 Anaerobic Digestion

This process is being used for waste stabilization for more than 80 years now (Parkin & Owen 1987), AD is a process that transforms organic matters into gas with 60 to 70% of Methane (CH_4) and 40 to 30% of CO_2 with organic residue in absence of oxygen (Kwietniewska & Tys 2014) shown in **Figure 2-2**, the organic residue consists of high amount of Nitrogen (Li et al. 2011). AD can be categorized into different groups by its operating parameter and design of reactor, for example it can be grouped by temperature or solid content (Li et al. 2011).

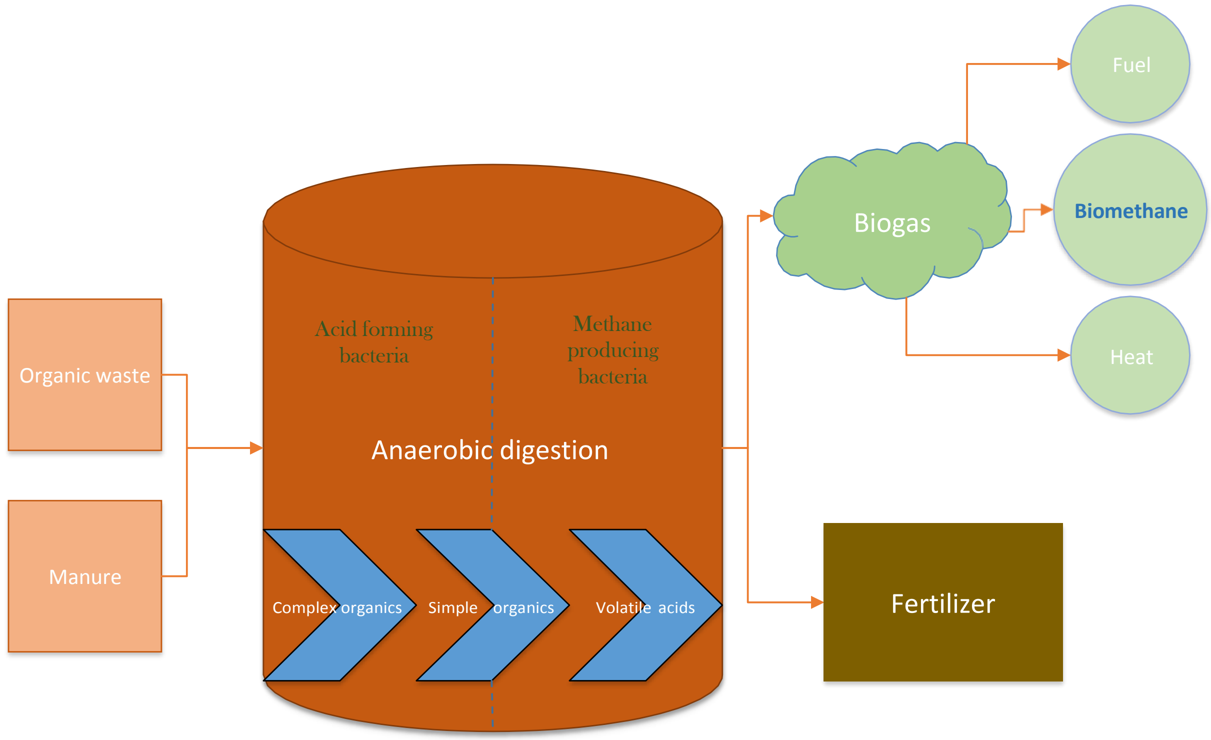


Figure 2-2 AD process configuration

This process consist of four different stages: hydrolysis, acidogenesis, acetogenesis and methanogenesis shown in **Figure 2-3,** however some literatures indicated that AD process has only three stages of hydrolysis, acidogenesis and methanogenesis, but the AD product and results are the same (C. Zhang et al. 2014).

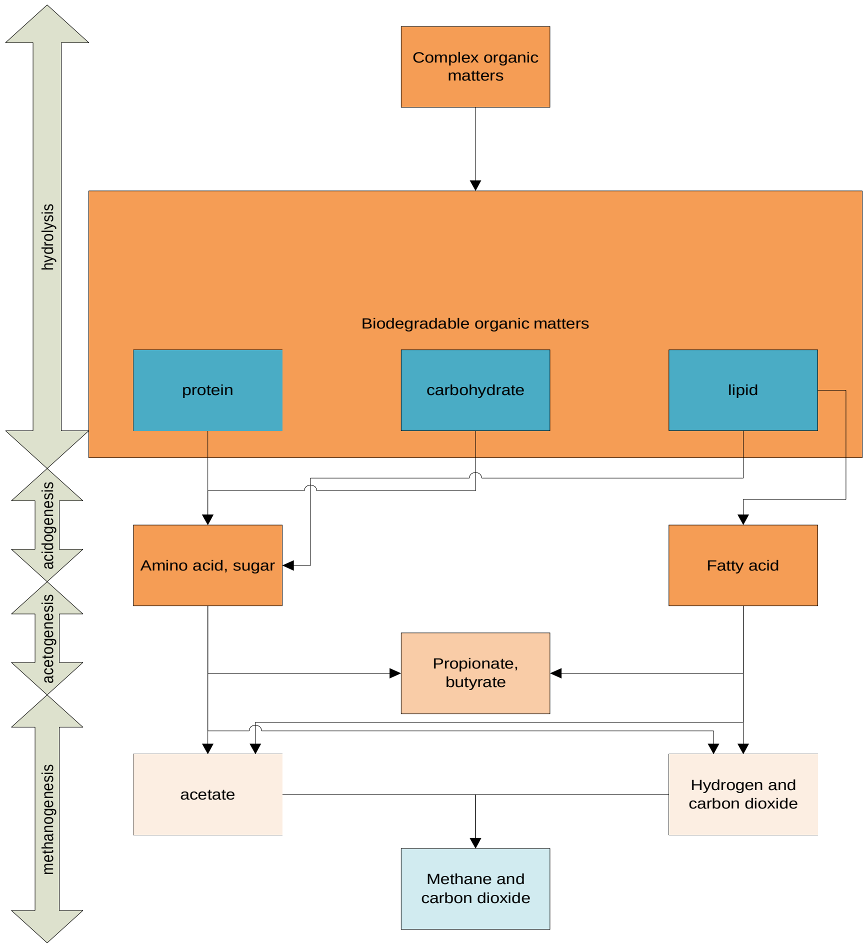


Figure 2-3 AD stages

In the first step (hydrolysis) complex organic matters are broken down to proper sized materials to access the cell walls as energy and nutrient source, this stage is critical since wastewater consists of many insoluble components which can't be utilized by bacteria. This step of the process is attained by extracellular, hydrolytic enzymes produced by bacteria population for this matter (Parkin & Owen 1987). In this step, proteins, lipids and carbohydrates are converted to amino acids, sugars and fatty acids respectively (Li et al. 2011), presence of sufficient extracellular hydrolytic enzymes are crucial for providing the proper contact of bacteria with organics without facing limitation in stabilization rate of total reaction. In the next step, these simpler organics are converted to VFAs, CO_2, H_2, and acetic acids, besides acetic acids, smaller organic acids such as propionic, butyric and valeric acid is produced in the second step, acidification. Hydrogen produced in this step is an energy source that is consumed by methanogenic bacteria for methane formation (Parkin & Owen 1987). In the third stage of the process, acetate, carbon dioxide and hydrogen are digested which are utilized for methane production (C. Zhang et al. 2014), there are two types of bacteria present in this step of the process, 1- Hydrogen-producing acetogenic bacteria that produces hydrogen and 2- hydrogen-consuming acetogenic bacteria which produces acetate. Hydrogen is important for organic consumption and production adjustment, but hydrogen pressure is important, if it departs from the proper range it would inhibit methane production (Parkin & Owen 1987). In the last step of the process, for stabilization, methane and carbon dioxide is produced. Two different groups of methanogens are present; one group uses acetate to produce methane and other group uses hydrogen and carbon dioxide.

Most of AD processes used today are single stage processes, which are dependent on different parameters such as pH, ammonia, nutrients and trace elements therefore it is very important to be in the right range of characteristics for a long term AD process (C. Zhang et al. 2014).

In dark fermentation, the overexploitation of fossil fuels and the understanding that this source of energy isn't endless, which can excessively increase the CO_2 gas emission and can result in energy crisis worldwide, are the reasons for exploring for alternative sources of energy that are more environmentally friendly and renewable (Lyberatos 2010). H_2 can be an alternative energy source that has the potential to produce sustainable fuels, without generating greenhouse gases. This source of energy has higher energy yield compared to other sources such as methane, natural gas and ethanol (Meng et al. 2014). However most of this energy source is produced from fossil fuels, therefore it is very important to provide a more environmentally friendly method for hydrogen production (Meng et al. 2014) and also the main obstacles are the high production and storage cost (Chong et al. 2009). Hydrogen is an odorless, colorless, tasteless and safe gas that produces no pollutants (Chong et al. 2009), it just produces water. There are different methods for hydrogen production such as chemical and electrolysis pathways. In water electrolysis method, water is electrolyzed in to molecular hydrogen and oxygen gases using electric energy, therefore the cost of the electricity input defines the cost of the method. The thermochemical hydrogen methods used are steam reforming, partial oxidation/auto-thermal reforming and gasification of coal and woody biomass (Chen 2006), but their high cost and energy requirement has led the attractions towards biological pathways for hydrogen production (Chong et al. 2009). The biological pathways for Hydrogen production are bio-photolysis of water, photo-fermentation and dark fermentation of organics, dark fermentation has the highest yield with the simplest way of operation (Lyberatos 2010). This process is a biological process that is used for hydrogen production without any lighting sources, many different feeding sources can be used in this process such as stillage, leachate and sludge, a more economical way for this method is to use organic wastes instead of pure cultures since it can also perform as a wastewater treatment process as well as hydrogen production system (Meng et al. 2014). Using mixed cultures can help with higher environmental sustainability as well. However mixed cultures contain both hydrogen producing and hydrogen reducing bacteria, so methods such as pretreatment should be applied to eliminate the hydrogen consuming groups (HCB) and maintaining the hydrogen producing bacteria (HPB). The seed sludge used for dark fermentation is a mixed culture since it can be more resistant to changes in temperature and pH, however their kind of cultures consist both bacteria groups (Meng et al. 2014), dark fermentation mostly takes place in anaerobic condition by anaerobic bacteria, and the most common organic substance for hydrogen production is glucose for acetate production shown in equation 1-1 (Lyberatos 2010):

$C_6H_{12}O_6 + 4H_2O \rightarrow 2CH_3OO^{-} + 2HCO_3^{-} + 4H^{+} + 4H_2$ (Equation 1-1)

Dark fermentation has low efficiency in Hydrogen production since most of its enthalpy is used for VFA formation, Acetate and butyrate are the pathways for hydrogen production, about 33% of glucose is converted to H_2 through acetate pathway and 17% over butyrate pathway. The pathway equations are shown below (1-2), (1-3):

$C_6H_{12}O_6 + 2H_2O \rightarrow 4H_2 + 2CH_3COOH + 2CO_2$ acetate pathway (Equation 1-2)

$C_6H_{12}O_6 \rightarrow 2H_2 + CH_3CH_2CH_2COOH + 2CO_2$ butyrate pathway (Equation 1-3)

2.3.1 Microbial communities

AD is a complex process with multi-step microbial communities that have to be in high synergy for better process performance (Appels et al. 2011). The microorganisms present in this process are classified into four groups of hydrolytic, fermentative, acetogenic and methanogenic bacteria. **Figure 2-4** indicates the carbon flow in AD. In a well performed process most of carbon flow takes place between methanogens and fermentative organisms and only 20 to 30% of it is converted to intermediate products before transforming to methane and carbon dioxide (Birgitte K. Ahring et al, 2003).

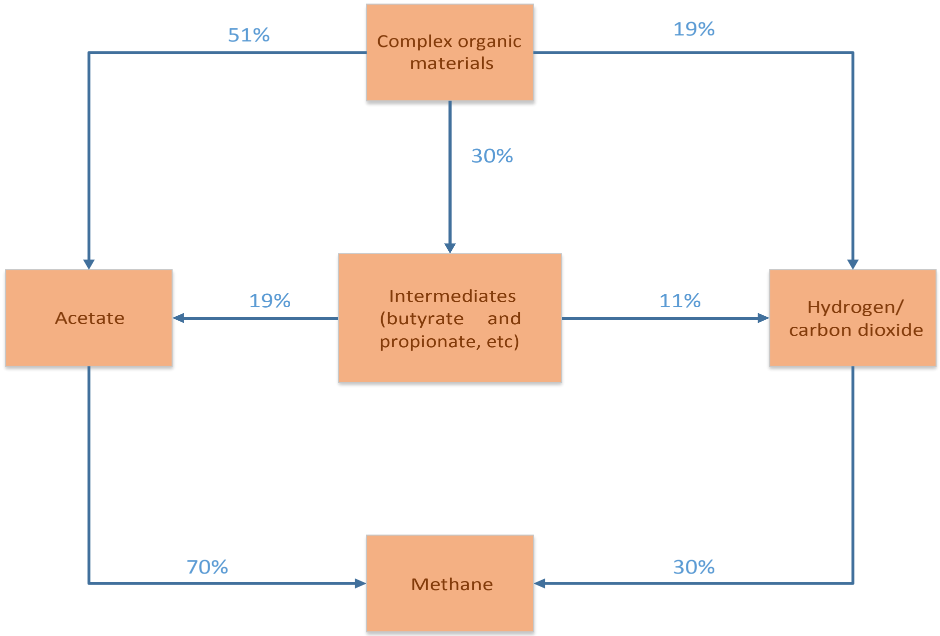


Figure 2-4 Carbon flow in AD with active methanogens

2.3.1.1 Hydrolytic bacteria

To proceed in the process, condensation of complex organics into soluble monomeric and dimeric substrates is done by this group of bacteria. These groups of bacteria are crucial since they produce VFAs (VFA) that are used at the end of process for Biogas production. This step indicates the efficiency of the process (Jain et al. 2015a). One insoluble compound is cellulose which under enzymatic hydrolysis with organisms such as *cellulomonas*, *clostridium*, *thermonospora*, *baceriodes*, *erwinia*, *acetovibrio*, *microbispora* and *Streptomyces* that can produce cellulase by cellulose hydrolyzation.

2.3.1.2 Fermentative bacteria

These groups of bacteria including: *saccharomyces*, *butyrate fermentative*, *butyribacterium* and *clostridium*, consume soluble to produce intermediate products such as VFA, CO_2 and hydrogen which are mostly used for methane production in the last step (Li et al. 2011).

2.3.1.3 Acetogenic bacteria

Acetogenic bacteria are different from acetate forming fermentative bacteria since this group can reduce carbon dioxide for acetate production. They consist of two groups: 1- completely acetogenic such as *acetobacterium*, *sporomusa* and 2- non-acetogenic microorganisms such as *clostridium, ruminococcus* and *eubacterium* (Li et al. 2011). Abundance and diversity of acetogens and importance of acetate for methane production is the reason for potency of AD, however it should be considered that acetogens are hydrogen producer bacteria, so it is very important to have the proper relationship between acetogens (H_2 producers) and methanogens (H_2 consumers) (Jain et al. 2015a).

2.3.1.4 Methane forming bacteria

These groups of bacteria are very sensitive to environmental changes, they produce methane and carbon dioxide by conversion of acidic and acetogenic fermentation, most bacteria groups in this step are rod and sphere shaped (Mustafa et al. 2014), the methane produced is 70% from acetic acid fermentation and 30% from Hydrogen and CO_2 redox reaction (Jain et al. 2015a). In methanogenic phase there are few energy sources such as formic acid, acetic acid, methanol and hydrogen (Parkin & Owen 1987). For this process to reach its optimal level it is important to provide equal speed of decomposition in the sequential steps. As mentioned a balance between acid formers and methane fermenters is substantial for high efficiency. Methane producers are sensitive to environmental condition for example the pH level should be in the range of 6.5 to 8 while if the acid formers grow in a faster pace the pH will drop to an improper level for methane production, therefore keeping a balance in feeding supply, temperature and pH in the system is very important (Jain et al. 2015a).

2.3.2 Feed characterization

In the past, AD process was mostly used for treating animal waste and sewage sludge, but by increasing the need of waste management and renewable energy, AD has been used for various waste sources such as industrial and municipal wastes. Now different feeds can be used in AD, which vary in composition, fluid dynamics and biodegradability and homogeneity. For example, cow and pig manure have about 3 to 12 % of solids content but for chicken manure this number is from 10 to 30%. There are some agro-industrial wastes that may contain less than 1% of solids (Jain et al. 2015a). In feedstock, there might be materials that are unwanted for AD process such as, sands glass and metals or polymeric components. There are different sources of substrates used:

2.3.2.1 Food waste

This source is highly dependent on the eating habit of each place, but overall, total solids and volatile solids are in the range of 18.1 to 30.9 and 17.1 to 26.35 respectively, which indicates that 70 to 80% of food waste is made of water, because of its high-water content, this kind of waste is easily biodegradable. Since this kind of waste has high organic soluble that are easily converted to VFAs in early stages of AD process, it can cause inhibition in methane production because of excessive amount of VFA and pH drop in the system, to overcome this problem co-digesting this stream with another feedstock such as carbohydrate-rich waste or dividing the process into two-phase can be helpful (Li et al. 2011). Co-digesting is common for this waste stream because of its imbalance between trace elements and macronutrients, the trace elements are mostly insufficient while there are excessive amount of macronutrients (C. Zhang et al. 2014). The traditional methods for disposal of food waste, was landfill, incineration and aerobic composting, landfilling has been banned in many countries and incineration uses high amount of energy and it also pollutes the air, using food waste as direct animal feed can also be problematic since it can cause different diseases (C. Zhang et al. 2014).

2.3.2.2 Agricultural waste and energy crops

AD for producing Biogas has various advantages such as flexible use of produced methane as fuel as an alternative for natural gas, there has been many research on using energy crops and residues for CO_2 emission reduction (Demirel & Scherer 2011), This waste has high potential in producing Biogas and it is available at very low cost, the carbohydrates inside this waste are mostly in form of polysaccharides, cellulose and hemicellulose which because of their covalence bonds are not available for fermentation and break down, for this reason, pretreatment methods are used such as dilute acids, steam explosion and lime and ammonia pretreatments (Li et al. 2011). Residues from food crops and energy crops such as wheat, beet and maize have high potential in AD usage, the methane production potential of cellulose containing wastes can only be indicated after proper pretreatment, however co-digesting of this source with other wastes can provide the needed nutrients and increase the process efficiency (Appels et al. 2011).

2.3.2.3 Algae

Recently, new sources have been used for AD process such as microalgae. The algal sink toward the anoxic and aphotic zone of the reservoir and they die which means they become a part of the bottom deposit and degradation occurs which produces excessive amount of phosphorus and ammonium and also releases toxic gases such as H_2S, the process lowers the oxygen which is dangerous for wildlife especially fish species, This source requires a lot of fertilizers and needs management of waste for the residue which AD process can overcome the problems and can balance the nitrogen and phosphorus excessive production. The characteristics of microalgae is highly dependent on physical, chemical and biological factors, the cultivation method and environmental conditions such as light and salination (Kwietniewska & Tys 2014).

2.3.2.4 Organic fraction of municipal solid waste (OFMSW)

This kind of waste is a mixture of different sources from food waste to yard waste, therefore it is highly dependent on the waste collection from place to place considering the season as well. This waste has potential in Biogas production in AD process but its residue is hard to handle and needs to be disposed in landfills or incinerated (Li et al. 2011). The composition of this waste varies with the location, for example in rural areas the waste mostly may contain garden waste, the waste can also change by season considering the difference in lifestyle and culture of each place (Appels et al. 2011).

2.3.2.5 Dairy waste

Dairy industry is very crucial since it provides essential human nutrition from milk, such as cheese, butter and yogurt, the dairy wastewater is produced by washing the equipment and by products of each process, this wastewater consists of mostly lactose, soluble proteins, lipids, mineral salts, this kind of waste includes many organics alongside with other pollutants which can highly pollute the environment if not properly treated (Karadag et al. 2015).

2.3.2.6 Sewage sludge

The sludge produced during wastewater treatment process as a by-product of the chemical, physical and biological treatment processes is very important to be properly treated, using AD process seems to be an environmentally friendly and economical method for treatment of this waste, this process offers the highest methane production yield worldwide (Appels et al. 2011).

2.3.3 Factors affecting the performance of AD process and Biogas generation

2.3.3.1 pH level and VFA concentration

VFA which consist of acetic acid, propionic acid, butyric acid and valeric acid are the main intermediates of the AD process. The VFA inside the AD process can be converted to CO_2 and CH_4 by methanogenic bacteria. These components are not inhibitory by themselves but they can reduce the pH to a certain level, accumulation of VFA can result in pH drop which long chain fatty acids prevent the activity of gram positive bacteria including methanogens (Kwietniewska & Tys 2014) and failure of the system, acetic acid and propionic acid are the most important acids in Biogas production, for a proper process the acetic acid to propionic acid ratio should be less than 1.4 and the acetic acid concentration should be less than 0.8 g/l, there are different methods used for measurement of VFA such as high performance liquid chromatography, gas chromatography or ion exclusion which are quite time consuming and material based which are not suitable for commercial scale. VFA indicates the pH level of the process which is very important. Fermenters need pH range of 4 to 8.5 while methanogens need pH level of 6.5 to 7.2. There are different VFA that are important in each pH level, in low pH acetic and butyric acid is important while in higher pH level acetic and propionic acids have more significant effects (C. Zhang et al. 2014).

2.3.3.2 Temperature range

Intra-cellular proteins thermos stability in microbial communities indicate the system's stability to temperature changes (Amani et al. 2010). This parameter influences the Methane yield and the activity of enzymes and co-enzymes. There are three different ranges of temperature used for bacterial growth: psychrophilic (under 20°C), mesophilic (between 35° to 55° C) and thermophilic (above 55°C) conditions. Thermophilic temperature has higher specific growth rates, high metabolic rate and better rates for destruction of pathogens with higher methane production. Nitrogen degradation and phosphorus assimilation is higher in thermophilic condition, as well as more sensitivity to environment. It is known that the temperature change rate should be less than 1°C/day, higher rates ends in process failure (C. Zhang et al. 2014). Thermophilic conditions are mostly not used since it is tricky to heat up a tank to such high temperatures, in high efficiency systems the energy required is offset by higher gas production, the advantages and disadvantages of each temperature range is indicated in **Table 2-1**. Temperature levels are very important since it can directly affect the growth (Jain et al. 2015b). By combining mesophilic and thermophilic temperature conditions, stability problem in the process can be mainly solved by using mesophilic reactor as a polishing stage (Han & Dague 1997).

Table 2-1 Advantages and disadvantages of temperature ranges

Temperature range	advantages	disadvantages
Psychrophilic (15°C)	Increased net of biomass yield of methanogenic population	Slow process High energy demand for bio-degradation (Appels et al. 2008)
Mesophilic (30-40°C)	Less sensitive to environmental changes	Low Methane yield (C. Zhang et al. 2014)
Thermophilic (50-60°C)	Fast due to increase in biochemical reaction rates Improved dewatering High pathogen destruction	High energy requirement High odor potential (Lettinga et al. 2001)

2.3.3.3 C/N ratio

Except for carbon, nitrogen is also an important source for microorganism to produce cell protein which is important for Biogas production, both carbon and nitrogen are important for AD process since nitrogen helps with building cell structure and carbon helps in providing energy (Jain et al. 2015b), it is important to be in the proper range of C/N ratio, for solid state waste the right range is between 20.1 to 30.1 to prevent excessive production of total ammonia nitrogen and VFAs as intermediates, since they can decrease the methanogenic activity for methane production, however for solid state waste the C/N ratio is dependent on feedstock, but 25.1 seems to be optimal level (Li et al. 2011). For algae as feedstock pH and C/N ratio are completely related. The optimal C/ N ratio is between 20 to 35, lower C/N ratio means protein rich material which results in excessive ammonia production that effects the activity of microorganism and higher amount of VFA production (Kwietniewska & Tys 2014).

2.3.3.4 Organic loading rate

This factor indicates the number of volatile solids fed to the system every day in continuous systems. most systems operate in organic loading rate of 0.5 to 1.6 kgVS/M^3.day (Jain et al. 2015b) Rising the organic loading rate can increase the methane production and can result in smaller reactors and lower cost (Jain et al. 2015b) until reaching the overloading stage which will accumulate acid and the fermentation step will stop. Adding substrate to the system before adaption taking place can cause high VFA production and pH drop in the system which inhibits methane production (Kwietniewska & Tys 2014). OLR fluctuations in the system in short time durations with a wide range can disturb the balance between methanogenesis and acidogenesis stages in the AD process (Amani et al. 2010).

2.3.3.5 Retention time

Retention time is the time needed for degradation of all organics which depends on temperature and composition. The average retention time for mesophilic condition is 15 to 30 days and is shorter for thermophilic condition. There are two different retention times: 1- hydraulic retention time 2- solid retention time (Kwietniewska & Tys 2014).

Hydraulic retention time (HRT)

HRT is the ratio of reactor volume to influent flow rate. Short HRT can cause accumulation of VFAs and bacterial loss which ends in wash out in the system, also longer HRT can result in ineffective utilization of components (Kwietniewska & Tys 2014). HRT may vary from season to season by temperature change or day to day by feedstock change, so the optimal HRT varies with waste composition, temperature and system's details (Buekens 2005).

Solid retention time (SRT)

SRT is a common parameter used for designing in wastewater treatment plants which indicates the amount of residence time for microorganism in the reactor, which is directly related to the growth rate of microorganisms, providing the time for organisms to reproduce themselves, high SRTs will provide the proper time duration for slow growth bacteria enrichment, establishing a more diverse microbial community in the system (Clara et al. 2005).

2.3.3.6 Nutrients

The most important nutrients needed for AD process are C, H_2, O_2, N_2, P and S among all nutrients N_2 and S are the ones that are facing deficiency in feed stocks such as municipal solid waste which means extra supplement should be added (Jain et al. 2015b), nitrogen and phosphorus are the most important ones among all other nutrients, other micronutrients are nickel, cobalt, sodium, selenium, tungsten, magnesium, barium and molybdenum which are mainly sufficient in the wastewater itself (Amani et al. 2010).

2.3.3.7 Ammonia

This material is the product of nitrogen rich compound biodegradation such as protein which is mostly in form of ammonium (NH_4^+) or free ammonia (NH_3). This compound is essential for growth of bacteria however it also can be toxic in high concentrations. It is reported that in C/N ratio under 30, ammonia is in low levels which resulted in low AD performance, however high loading rates can cause VFA accumulation but having the enough ammonia can result in avoidance of VFA inhibition and higher methane production. Except for buffer capacity, ammonia is inhibitory to many bacteria in high concentration, which it diffuses the cell membrane and stops cell function, literature indicates that acetoclastic methanogens are more sensitive to ammonia inhibition compared to the hydrogenotrophic bacteria, so it mostly stops methane production in the system, there are various methods for excessive ammonia removal such as biological nitrogen elimination, stripping or membrane contactors (C. Zhang et al. 2014).

2.3.4 Biogas AD reactors

there are different reactors that can be utilized in AD considering the growth process which can be suspended or attached growth process, in suspended growth systems the microorganisms and substrate can freely move around in the reactor while in attached systems microorganisms intend to grow on a support media surface which provides more surface area for microorganism growth (Mustafa et al. 2014). Below there are reactor types that are mainly used in AD systems:

2.3.4.1 Completely stirred tank reactor (CSTR)

This reactor contains a mixer for an even mixture inside the system, inadequate mixing can result in less stabilized waste, decrease in Biogas production and an uneven distribution of microorganisms, enzymes and substrate (Kaparaju et al. 2008). CSTR reactors can be both in single stage or two stage process, the difference is in separation of the process steps, in two stage process the methanogenesis stage is separated from the acidogenesis step since these stages may have different operational conditions and duration for microorganism growth (Mustafa et al. 2014).

2.3.4.2 Anaerobic sequencing batch reactor (ASBR)

This kind of reactor has a cycle pathway with four sequential steps: feed, react, settle and decant, this reactor has the advantage of higher Biogas production and also high food to microorganism ratio comparing to CSTR reactor however it has less capacity in organic loading rate (Mustafa et al. 2014), this reactor is of interest because of no clarifier requirement and higher retention time for slow growing bacteria community (Bouallagui et al. 2005). By filling the reactor with support media anaerobic sequencing batch biofilm reactor can be introduced, in conventional ASBR there is granular biomass available while in ASBBR there are other support media such as polyurethane foams available for microorganism growth (Karadag et al. 2015).

2.3.4.3 Anaerobic membrane bioreactor (AnMBR)

This reactor is a more novel technology compared to other systems, with advantages such as low sludge production with a complete biomass retention however problems such as membrane fouling which ends in higher energy requirement and lower process performances are the main disadvantages of this reactor which requires more studies (Mustafa et al. 2014).

2.3.4.4 Anaerobic plug flow

These reactors mainly contain an insulated vessel and a heated tank which is mostly made of reinforced concrete, steel or fiberglass without any need of internal mixing, these digesters can operate in different temperature conditions (Karadag et al. 2015).

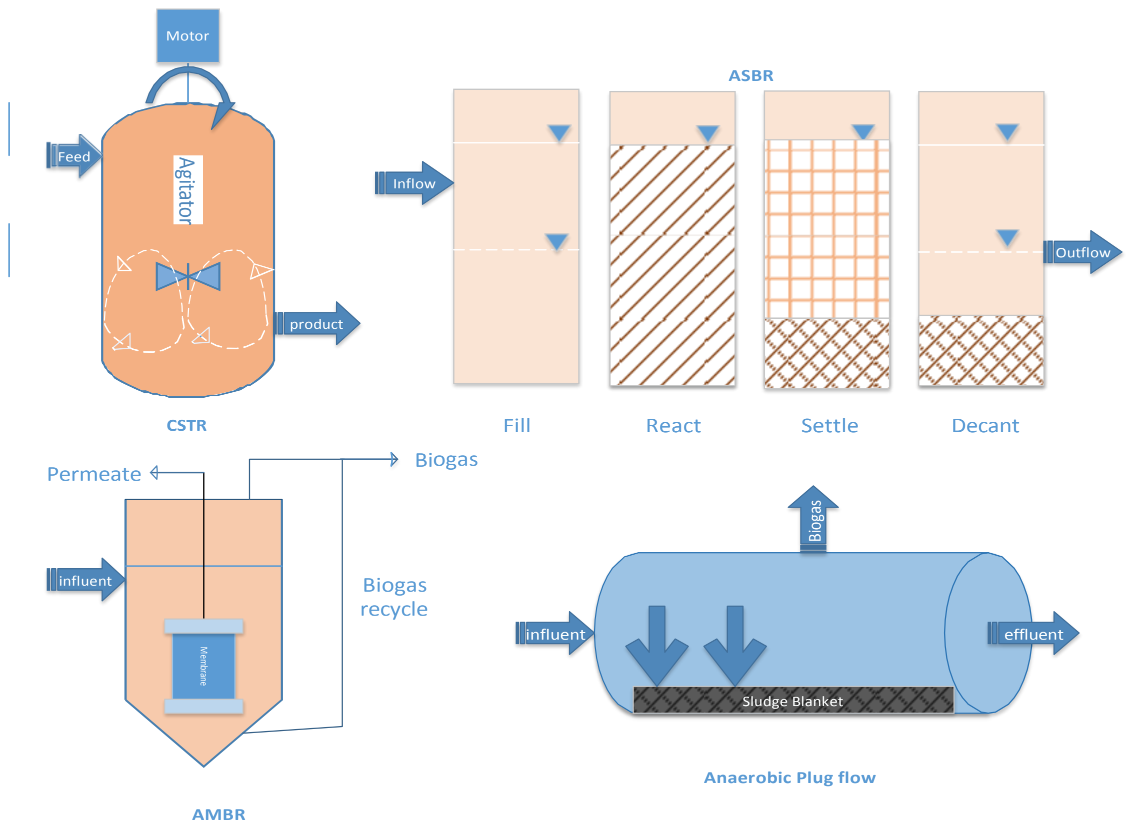


Figure 2-5 Reactors configuration

2.3.4.5 Covered lagoons

These systems contain a large lagoon with a gas tight cover, they mainly operate in ambient temperature and have very long hydraulic retention time of up to 45 days (Karadag et al. 2015).

2.3.4.6 Anaerobic filter

Anaerobic filter is a common biofilm reactor that is mainly effective on low strength wastewaters, the feeding system in this reactor can be single or multiple, the single feeding can be either horizontal or vertical (down-flow or up-flow) (Karadag et al. 2015), this reactor has the advantages of high organic loading capacity, low hydraulic retention time and low sensitivity to shocks in the system, since this reactor is effective for low solid content wastewaters, it is mostly a secondary wastewater treatment and is mostly combined with another treatment process (Mustafa et al. 2014).

2.3.4.7 Up-flow anaerobic sludge blanket (UASB)

This kind of reactor mostly contains dense granules that are produced inside the reactor itself, the wastewater moving from bottom of the reactor towards upper side of the reactor, going through the dense layer of granule inside the reactor (Mustafa et al. 2014). Expanded granular sludge bed (EGSB) is a reactor which is very similar to UASB reactor but the main difference is that it can treat higher liquid velocity compared to UASB reactor (Mustafa et al. 2014).

2.3.4.8 Anaerobic fluidized bed reactors

These reactors are mostly used for industrial wastewater treatment, such as food industries and paper industries, AFBR offers a lot of advantages such as high stability process, high treatment efficiency and high heat and mass transfer rates (Mustafa et al. 2014).

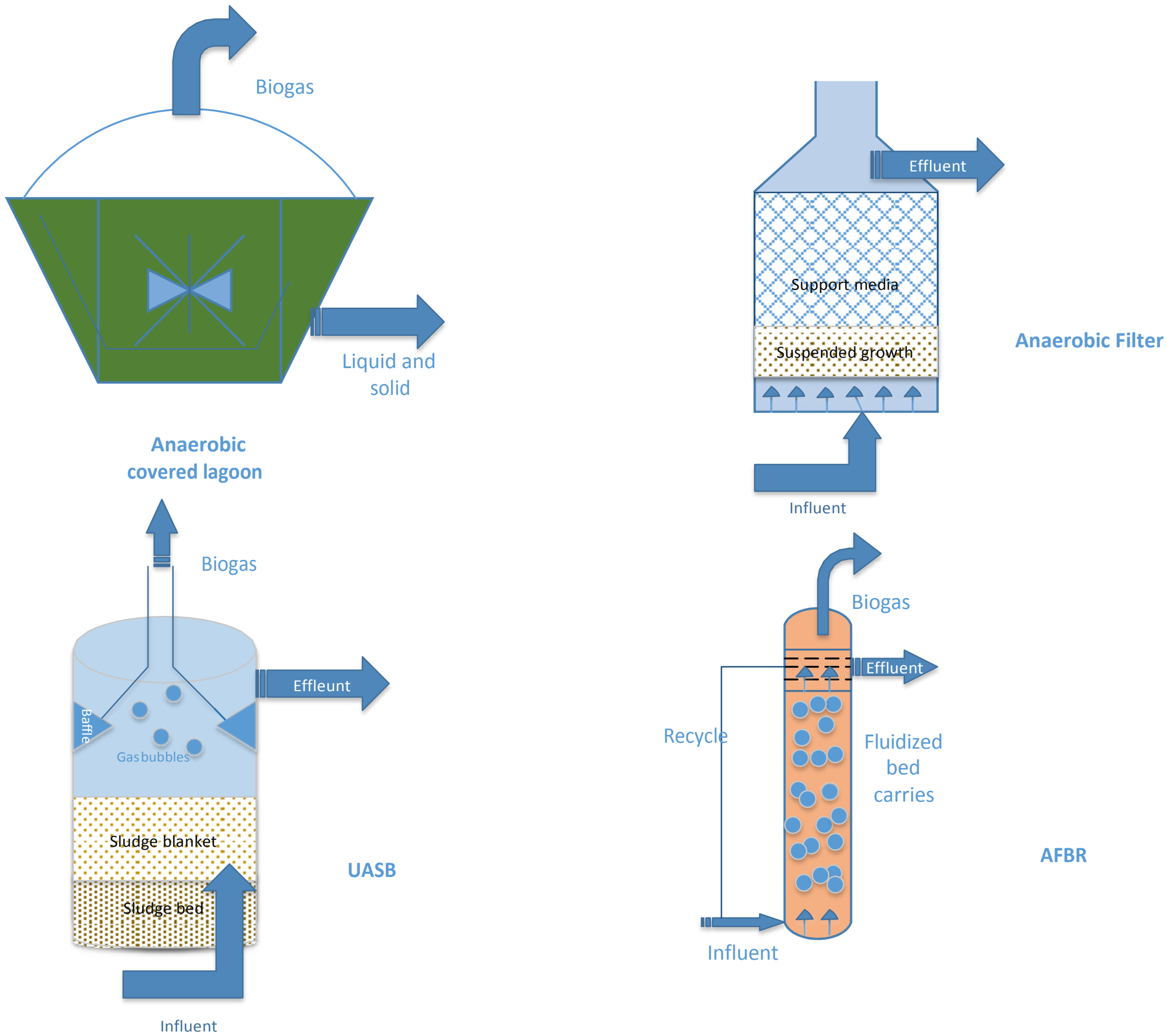


Figure 2-6 Reactors configuration

2.3.5 Improvement methods on Biogas production process

2.3.5.1 Pretreatments

There are various physical, chemical and enzymatic pretreatment that can be used for higher biodegradation of solid organic wastes, many studies have showed different pretreatments such as chemical, mechanical, ultrasound and thermal have been successful in increasing the Biogas production. Pretreatment helps weakening the cell walls for allowing methanogenic bacteria to use the organic material inside the cell, in AD process, hydrolysis step is the rate limiting step, so it is important to enhance this stage for whole process improvement, there are many factors that affect hydrolysis such as feed characterization, structure and particle size, acceleration of this stage can be done by different pretreatment methods (C. Zhang et al. 2014). By physical treatment smaller particles are produced which have higher specific surface area which is beneficial for Biogas production, hydrogen production before methane production can also be an improvement for biodegradation and hydrolysis (Kwietniewska & Tys 2014).

2.3.5.1.1 Mechanical pretreatment

This pretreatment is defined for breaking down the substrate particles into smaller sizes which can increase the specific surface area that can provide better contact between substrate and inoculum, which improves AD process. Larger particle size materials produce less amount of methane because of a decrease in COD degradation. There are different kind of mechanical pretreatments that are used for AD such as sonication, collision, high pressure homogenizer, maceration and liquefaction (Jain et al. 2015a). In various studies, it was mentioned that mechanical pretreatment on municipal solid waste has minor effect on methane production and Biogas composition, Izumi, studied the effect of size reduction on methane production in food waste and found size reduction can increase up to 40% of COD conversion and 28% of methane production, however decreasing the size to less than 0.7mm can cause VFA accumulation, which drops the pH level and results in less methane production (Izumi et al. 2010).

2.3.5.1.2 Chemical pretreatment

By using strong acids, alkalis and oxidants, organic compounds are destructed since AD needs pH adjustment with alkalinity, it is preferred to use alkalinity pretreatment, solution and saponication occur in these reactions which reduce the particle size and increases the specific surface area, the preferred chemicals for alkalinity pretreatment is NaOH and $Ca(OH)_2$, however NaOH can highly boost the AD process but it is fairly expensive compared to $Ca(OH)_2$, therefore mostly this chemical is used (López Torres & Espinosa Llorens 2008), acid pretreatment is considered suitable for substrates with high level of lignocellulose content, really strong acid usage can result in production of unwanted materials such as furfural and hydroxymethylfurfural, this method is not suitable for carbohydrate rich wastes since it can produce high amount of VFA because of degradation acceleration and accumulation (Jain et al. 2015a). Among chemical methods for pretreatment, ammonia pretreatment is receiving attention since it doesn’t produce a side waste stream because ammonia can be utilized as a Nitrogen source in the process (Zhong et al. 2011).

2.3.5.1.3 Thermal pretreatment

This treatment enhances the dewatering process as well as the digestible handling. A wide range between 50 to 250°C are used to improve AD process, however some articles have showed that heating higher than 70°C can cause accumulation because of new chemical bonds (Jain et al. 2015a), therefore thermal pretreatment can be divided in two groups, 1- heating up to 70◦C or 120◦C which increases the Biogas production up to 20 to 30% 2- heating up to 160°C or 180°C which increases the Biogas production from 40 to 100% , so however it increases the Biogas production much higher but the results may vary in a wide range (Bougrier et al. 2008). Using thermal pretreatment for sludge wastes can breakdown the gel structure and release the intracellular water bound which means this method provides a high level of solubilisation and enhancement of methane production (Bougrier et al. 2007).

2.3.5.2 Co-digestion

Adding a second stream to provide the missing nutrients in the first stream can improve the physicochemical characteristics which increases the process efficiency, this improvement method offers easier sludge handling, inhibition prevention and C/N ratio adjustment. Improving the loading rate by co-digestion can be economically feasible and can result in higher Methane production by nutrient balance (Kwietniewska & Tys 2014). Even in some situations co-digestion can provide the moisture content of the feed, however co-digestion may have some disadvantages such as high cost of slurry transportation and difficulty in combining different waste policies (Mata-Alvarez et al. 2000). (Yen & Brune 2007) have studied on co-digestion of algal sludge and waste paper with high Carbon content, the C/N ratio in algal sludge is mainly low which results in release of excessive amount of total ammonia Nitrogen and VFAs, co-digesting algal sludge with paper waste increased the Methane production by two times, by increasing the C/N ratio to an optimum level. (Macias-Corral et al. 2008) experimented the co-digestion of dairy cow manure (CM), organic fraction of municipal solid waste (OFMSW) and cotton gin waste (CGW), the results showed higher Methane yield up to 172 m^3 Methane/ dry waste per ton in co-digesting of CM and OFMSW compared to single waste treatments, co-digesting of CGW and CM also resulted in 87 m^3 Methane/ dry waste per ton which is higher than individual wastes.

2.3.5.3 Attached growth systems

In cases, startup time of AD takes up to 4 months or more, an important way to provide a more economical process is to shorten the time duration (Escudié et al. 2011), Using granules and carrier materials such as biofilm are two methods of preserving the high biomass concentration and shortening the startup time, there are several important factors for choosing carrier materials and adhesion of microorganisms on them, mostly for microorganism to adhere to carrier material, physicochemical properties such as roughness and low surface energy and microbiological composition of inoculum is important (Habouzit et al. 2011). Most of the materials used as carriers are highly porous and have a high specific surface (Barca et al. 2015). Carrier materials can have different shapes and sizes. They can be granular, cylindrical and spheroidal. Depending on the kind of reactor used in the experiment different sizes with considering the density of the carrier material are in use (Barca et al. 2015). Biofilm process can be divided into three groups: 1-. Moving medium in which biofilms continuously are in motion inside reactors by mechanical, hydraulic or air force such as anaerobic fluidized bed or moving bed bioreactors. 2- Fixed medium systems that consists of motionless media like anaerobic filter and up flow anaerobic sludge blanket Fixed film reactors which may have a problem of clogging that occurs mainly in filters. 3- anaerobic sludge Granulation mostly taking place in UASB reactors. The advantages and disadvantages of biofilm is showed in the table below:

Table 2-2 Advantages and disadvantages of biofilm reactors with particles

Advantages	Disadvantages
High reactor concentration	Difficulty in measuring biofilm thickness
Compact reactor with smaller size	Clearing up of particles because of biofilm overgrowth
Minimization in sludge production	Costly liquid distributers
High biofilm surface area	Long start-up time for biofilm formation

2.3.5.3.1 Biofilm process stages

Development of biofilm is a biological reaction process with different stages as below shown in **Figure 2-7**:

1- Adsorption: materials with different characteristics are adsorbed to organic layer in very short time duration (minutes) of water disclosure.

2- Microbial transportation to surface: the suspended particles in the flow are transported to surface by different ways such as molecular diffusion, turbulent eddy transport, sedimentation and thermophoresis.

3- Adhesion of microorganisms to surface: adhesion is a two-stage process, reversible and irreversible adhesion.

4- Biofilm production: this stage indicates the net material accumulation from cellular and microbial production of extracellular polymers.

5- Biofilm detachment: during the development process, part of biofilm is exfoliated, however sloughing may occur some times which is removal of a massive part of biofilm due to nutrient limitation in lower layers of biofilm (Bryers 1982).

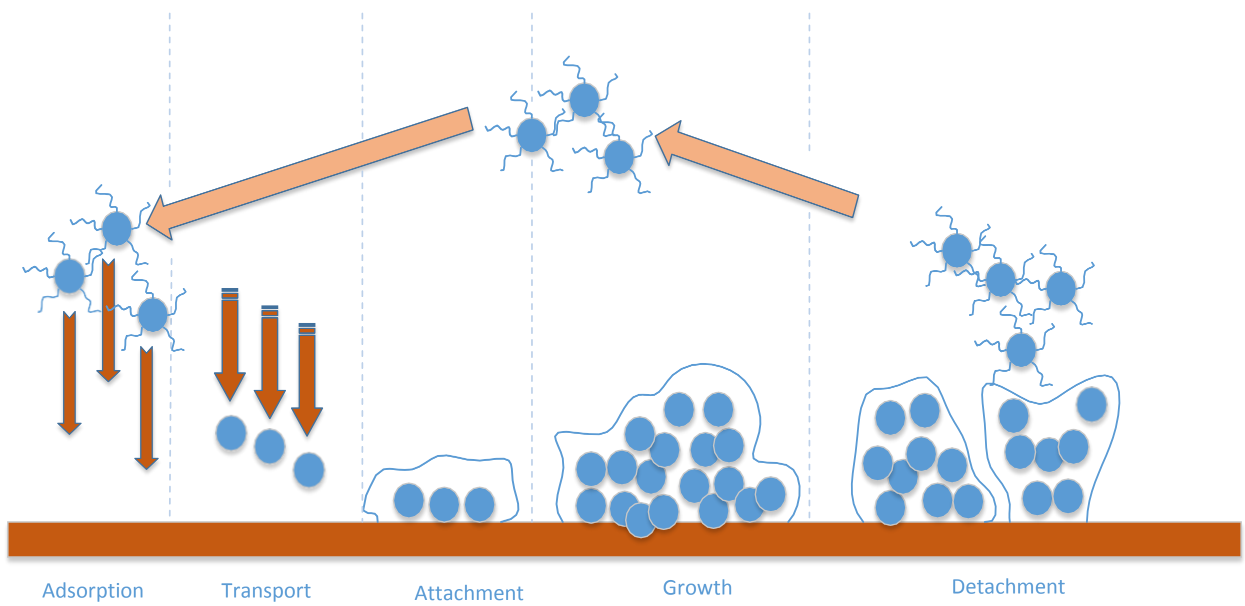


Figure 2-7 Biofilm formation process

2.3.5.3.2 Different biofilm structures

Sludge granulation

The major success in UASB reactors are because of granulation formation, this phenomenon allows higher loading rate compared to conventional process, also granulation decreases the reactor size and operation cost. There are two main reasons for high loading rates, 1- high settling parameters of granules, which are the main reason for uncoupling of hydraulic retention time and solid retention time. 2- granular sludge provide high methanogenic activity, the studies show that acetogenic bacteria in granules are highly connected to hydrogenotrophic methanogens which provide high hydrogen transfer, which results in high degradation rates.

There are different theories on anaerobic sludge granulation separated into three groups of physical, microbial and thermodynamic. Physical theory: in this theory, physical conditions such as liquid and gas up-flow velocity, suspended solid, removal of excessive sludge are the reasons for granulation.

Microbial theory: this theory is based on microorganism characteristics, in this theory physical approaches are also considered, the granulation parameters are based on the microbiology and the reactor conditions such as hydrodynamics.

Thermodynamic theory: in this theory, energy involved in adhesion is the main term in granulation mechanism analysis, hydrophobicity and electrophoretic mobility are the factors that are important in this theory (Hulshoff Pol et al. 2004).

Moving medium

Moving medium as a group of biofilm process can improve the digestion system by increasing the retention time of microorganisms inside the reactor. Moving bed reactors contain biofilms that are attached to the carrier which can freely move inside the reactor (Azizi & Sithebe 2015). For different wastes, various kind of materials can be used as attached media, for example for dairy waste seashell, charcoal, plastic materials, ceramics and natural stones are used (Karadag et al. 2015). Treatment of high strength milk permeate as a dairy waste has been studied in anaerobic moving bed biofilm reactor in mesophilic condition with organic loading rate of 2 to 20 gTCOD/L.day, the results showed 86.3-73.2% of TCOD removal and maximum substrate utilization rate of 89.3 gTCOD/L.day (Wang et al. 2009). A study on polyethylene carriers with different characteristics showed that higher specific surface area in carriers which resulted in higher COD removal of 80%with OLR of 29.59 gCOD/L.day (Chai et al. 2014).

Fixed film medium

Another group of biofilm process is fixed film reactors, such as anaerobic filter, which consists of a vertical filter bed that contains inert materials as support media to ease the interaction of microorganisms and substrate (Switzenbaum 1983). These reactors offer many advantages such as shortening the HRT from 30 to 40 day to few hours, enhanced process performance because of increase in specific surface area, however there has to be careful consideration for choosing the proper carrier material in order to provide a long life of fixed film, the materials used should be non-biodegradable, available in market and inexpensive; nylon, PVC (Polyvinyl chloride) and clay pipes have been used for years now (Yadvika et al. 2004), the main problem for fixed film reactors are is the excessive accumulation of biomass in the reactor because of long time period for running the experiment (Escudié et al. 2011). There have been many studies on utilizing fixed film medium for AD, (Vartak et al, 1997), experimented on two different attached media, limestone gravels and polyester mattings and a combination of both in two different temperature ranges of psychrophilic (10°C) and mesophilic (37°C), comparing them to conventional system without support media, the results showed significant methane production increase and maximum reduction in VS and COD in mesophilic condition as well. A study was done on cane molasses stillage in fixed film reactor packed with a plastic medium, with loading rate of 14.2 -20.4 kgCOD/m^3.day and HRT of 3.3 to 2.5 days, the results showed 85 to 97% of BOD and 60 to 73% of COD removal with Biogas production of 6.5-8.4 m^3/m^3.day (Bories et al. 1988). experiment on treatment of distillery spent wash treatment was done in anaerobic fixed film reactor using different media such as charcoal, coconut coir and nylon fibre, the results showed higher Biogas production up to 7.2 m^3/m^3.day with 64% COD removal without any pre-treatment of substrate using coconut coir as support material (Acharya et al. 2008). (Kennedy et al. 1988) studied on treatment of landfill leachate, both in up-flow blanket filter and down-flow stationary film reactor which achieved 97% of COD removal in both systems, inorganic heavy metals were accumulated in both reactors, coated the biofilm support in down-flow stationary film reactor and concentrated in reactor sludge bed and filter media on up-flow blanket filter reactors.

2.3.5.4 Additives

AD is a strong way of stabilization of sludge resulting from waste water treatment plants. Biological anaerobic treatment of wastewater's working principle is based on a mixture of diverse types of bacteria functioning in a mixed culture. The performance of the process is strongly dependent on the balance of AD fundamentals, because of low yield of the anaerobic processes in contrast to aerobic pathways, the concentrations of nutrients required for the procedure are lower. If AD process is showing poor performance without any obvious reason, deficiency of trace metals may be the problematic matter that needs to be checked, underestimation of importance of trace element requirement in commercialized level may be a crucial complication (Demirel & Scherer 2011).

Some of the most important factors affecting the bacterial growth and resulting in optimization of the process are as following; retention time, proper pH and temperature, proper feeding and sufficient number of nutrients, reliance of anaerobic fermentation and microbial growth is very high to availability of the proper level of nutrient supplements (Demirel & Scherer 2011).

Addition of nutrients may solve the problem of the process with high concentrations of VFAs. It can also affect the metabolic rate of the digestion and by increasing the substrate degradation rate, it may result in lower reactor sizes and costs (Parkin & Owen 1987).

Except for nutrients needed for AD process there are different light and heavy metals that are required for AD process, the light metals such as (Na, K, Mg, Ca and Al) and heavy metals such as (Cr, Co, Cu, Zn and Ni) which are important for enzyme synthesis and activity although even metals can cause inhibition in the AD process (C. Zhang et al. 2014). Free metals such as iron, nickel, cobalt, molybdenum, selenium and tungsten are essential for groups of methanogenic bacteria such as: *Methanosarcina barkeri; Methanospirillum hungatii; Methanocorpusculum parvum; Methanobacterium thermoautotrophicum, and Methanobacterium wolfei; Methanococcus voltae, and Methanococcus vanielli, and Methanococcoides methylaten* (Demirel & Scherer 2011), Using different metals can increase the Biogas production by utilizing in enzyme structure of bacteria. Magnesium, calcium, sodium and potassium may be toxic to the process, high concentrations of calcium results in excessive Carbonate and phosphate production which limits the mass transfer occurring in biomass, high concentrations of heavy metals such as copper, zinc, chromium and nickel in substrate results in non- biodegradable heavy metal accumulation which will have negative effect on process (Kwietniewska & Tys 2014), heavy metals can cause enzyme function and structure disruption, however the level of inhibition depends on the chemical form of the metal, pH and redox potential. Inhibition by heavy metals mostly occur in treatment of municipal sewage and sludge or industrial wastewater. There are many substrate sources that lack in metal concentrations therefore, addition of metal elements is studied (C. Zhang et al. 2014). Below different additives are discussed in details:

2.3.5.4.1 Enzymes

There are different contaminants that need to be degraded in wastes such as hydrocarbons, phenols, acids, esters and alcohols that can be biodegraded easily, although there are some compounds such as polychlorinated biphenyls (PCBs) and polycyclic aromatic hydrocarbons (PAHs) that are less biodegradable, therefore for biodegradation these compounds should interact with enzymatic systems (Gianfreda & Rao 2004), adding enzymes to AD process can shorten the digesting time, increase the digestibility of the sludge and decrease the cost of disposal, using enzymes can increase the release of extracellular polymeric substances (EPS) and also can convert resistant elements to biodegradable ones (Yang et al. 2010). Extracellular polymeric substances consist of a wide range of oxidoreductases and hydrolases, these enzymes can transform polymeric substances to partially degraded or oxidized compounds that can easily be utilized by cells (Gianfreda & Rao 2004). Using enzymes as an additive in food waste treatment through AD allowed higher organic loading rate in the process (Romano et al. 2009), for example using *Aspergillus sp. A-1* as an enzyme for treatment of citrus peels improved the AD process and also pretreating the peels with fungus enzymes increased the organic loading rate up to 50% (Akao et al. 1992).

2.3.5.4.2 Light metals

Light metals such as sodium, potassium, calcium and magnesium like any other micro nutrient are essential for microbial growth and specific growth rate, they are present in the influent in moderate concentrations, however increase in their concentration can result in process inhibition and slow growth in the system (Chen, Jay J. Cheng, et al. 2008).

Calcium

Calcium is important for growth of specific methanogenic groups such as *Methanosarcina sp. strain TM-I, methanococcus voltae* and *Methanosarcina barkeri* with different concentrations (Murrayt & Zinder 1985), however excessive calcium addition may interrupt sludge retention, granule growth and stability in the system (Thiele et al. 1989), high calcium content increases the product of carbonate and phosphate which will reduce methanogenic activity, loss of nutrients and buffer capacity (Chen, Jay J. Cheng, et al. 2008), there are few studies on effect of calcium on AD, (Yu et al. 2001) experimented on effect of calcium on UASB reactor, the results showed that concentrations between 150 to 300 mg/L can boost the process by enhancement of the three stages of granulation. Calcium Carbonate can also enhance the biomass activity unless the biomass is scaled, since it will be less active because of mass transfer limitation (Chen, Jay J. Cheng, et al. 2008).

Magnesium

Magnesium concentration up to 300 mg/L does not highly effect the AD, however increasing the magnesium to a certain level can increase the growth of certain methanogenic bacteria such as *Methanosarcina thermophila TM1* and *a Methanosarcinae*-dominated UASB reactor (Chen, Jay J. Cheng, et al. 2008), formation and growth of cells for *M. thermophila TM-1* has shown to be affected by the magnesium concentration, optimal level of Mg^{2+} of 30 Nm was indicated and without any magnesium no growth has been reported (Schmidt & Ahring 1993).

Sodium

Food industry wastes produce high sodium concentration substrates; sea food wastewaters may reach sodium concentrations up to 12 g dm^{-3} , however methane production will face difficulty in Sodium concentrations of 10-14 g dm^{-3} (Soto, Mendez and Lema, 1993), for VFA degrading bacteria, sodium is more inhibitory to propionic acid-utilizing bacteria than to acetic acid-utilizing bacteria (Chen, Jay J. Cheng, et al. 2008).

Potassium

Low Potassium concentrations result in performance improvement in mesophilic and thermophilic temperature ranges, however increase in concentration will inhibit the process mostly in thermophilic temperature ranges, it is observed in different studies that potassium is inhibitory to acetate producing methanogens, combination of other metals such as Sodium, Calcium and Aluminum can reduce the Potassium toxicity in the process (Chen, Jay J. Cheng, et al. 2008).

2.3.5.4.3 Heavy metals

Heavy metals can be a part of active site of enzymes and can directly participate in catalysis. Effective concentrations of these metals can benefit the protein and nucleic acid molecular structure and indirectly affect the biological pathway controlling by enzymes and membranes (Vallee and Ulner, 1972). Chromium, Iron, Cobalt, Copper, Zinc, Cadmium, and Nickel are the main heavy meals available in the wastewater streams in high concentrations, the important characteristic of heavy metals is that they are not biodegradable and can produce aggregates in the system that can cause toxicity and process failure (Chen, Jay J. Cheng, et al. 2008). Mainly methanogens are more affected by heavy metal high concentration compared to acidogens, however there are some trophic groups and microorganisms available in the process that are more influenced by toxicity of heavy metals than methanogens (Chen, Jay J. Cheng, et al. 2008). Heavy metals can have different forms in the AD process, they can be in form of sulfide, Carbonate or hydroxide, they can participate in sorption to solid fraction or intermediate formation, however only soluble, free forms of heavy metals are toxic (Chen, Jay J. Cheng, et al. 2008). Sensitivity to heavy metal toxicity in acidogens and methanogens are as shown respectively: Cu > Zn > Cr > Cd > Ni > Pb and Cd > Cu > Cr > Zn > Pb > Ni (Lin 1993).

2.3.5.4.4 Vitamins

There is very few studies on addition of vitamins to AD process, but it has been stated that adding vitamins can improve the resistant of the process to biotic and abiotic changes and shocks in AD (Muller and Muller, 2012). (Angelidaki et al. 1990) studied on adding bentonite as an additive to oil in thermophilic AD, the results showed that bentonite added to the process aided in oil having less inhibitory effects on start-up time and to the digestion process, it was observed that more than 80% of the oil was degraded in few hours after feeding when bentonite is added to the system.

2.3.6 Biogas

Biogas is nearly 70% methane and almost 30% CO_2, the composition of this gas is shown in **Table 2-3** (Kwietniewska & Tys 2014), this gas is 20% lighter than air and can't be converted to liquid in normal temperature, by removing the CO_2 the remaining compressed gas can be used for stationary applications and transportation, also CNG which is enriched Biogas. there are different methods for Biogas enrichment in order to remove the CO_2 and H_2S and water, the easiest and cheapest method is to used pressurized water as an absorbent liquid, which absorbs the H_2S and CO_2 by the down going water, methane can be collected from the vessel in top of the reactor but the down side of this method is high water requirement (Jain et al. 2015b).

Table 2-3 Biogas composition

Biogas elements	content
Methane	50-75%
Carbon dioxide	25-50%
water	6-6.5%
Oxygen	0.9-1.1%
Nitrogen	3.9-4.1%
Hydrogen	-
Hydrogen sulfide	<0.1-0.8%
Trace elements	-

The Biogas composition indicates the stability of the system; the process is stable when the products are only CH_4 and CO_2 but if H_2 and CO are also in the final gas composition then there is a disturbance in the system, mass balance helps with methane production measurement while energy balance helps with maintaining the proper temperature level. In COD mass balance, 90% of the COD is converted to methane and the other 10% is used for biomass inside the reactor, in theory a rate of 0.35 m^3 CH_4/kg COD is considered for estimation of methane production, the products of anaerobic degraded organic materials are the most oxidized form of Carbon (CO_2) and the most reduced form which is CH_4 in Biogas, the amount of Biogas produced in digesters are always lower than the theoretical values which is because of 1- a part of substrate is used for biomass synthesis, 2- some of the substrate never gets used for producing effluent and 3- here might be a lack of nutrients (Jain et al. 2015b).

Chapter 3 Enhancement of AD by using particulate growth systems

3.1 Introduction

Sharp boost in population and urbanization has resulted in higher living standards and energy demand (Minghua et al. 2009) which are the main reasons for waste production expedition (Abarca et al. 2013). Various environmental challenges from waste by the community and the waste characterization, requires different methods for recovering and reusing the materials in waste which is known as waste management (Demirbas 2011). The usage of proper waste management strategy is a method for Biogas production which can be a versatile carrier for taking over the conventional energy sources such as fossil fuels for generating electricity, heat and vehicle fuels (Weiland 2010). Biogas is environmentally friendly and efficient energy because of its low hazardous pollutants. The Biogas is a product of AD process as a clean energy source which by proper functioning can result in providing partial energy demands, resource conservation and protection of environmental sources (Yadvika et al. 2004), however it mostly contains only 55 to 65% of methane. By upgrading the Biogas, the product which is called methane rich Biogas can also be used for chemical and material production (Appels et al. 2011).

AD (AD) is a promising technology as a waste management system that produces energy and reduces the greenhouse gas by utilizing the waste, moreover the product of AD process named digestate is capable for agricultural use as fertilizer (Adu-gyamfi et al. 2012a). AD as a widely used waste treatment method, converts organic materials present in the waste to Biogas in an oxygen free environment, by complex microorganism community (Gong et al. 2011). There are multiple factors that have impact on AD such as bacterial community, contact of microorganisms and substrate, retention time and mass transfer (Karim et al. 2005). With all the disadvantages of

AD process such as slow start-up, short Biogas production duration and toxicity (Ye et al. 2005); (Gong et al. 2011), its efficiency mainly in Biogas production can be highly enhanced by microbial community engineering. Methane formation occurs in four connected stages namely, hydrolysis, acidogenesis, acetogenesis and methanogenesis which are linked to each other because of sequential work of each microbe (Adu-gyamfi et al. 2012b) in which the microbial community of AD process consists of acidogenic, acetogenic and methanogenic bacteria that the later community plays an important role in methane production since this group has higher sensitivity to environmental changes, therefore efficient AD process requires development in methanogenic bacteria (Mustafa et al. 2014).

Aside from microbial fouling (Langer et al. 2014a), using biofilms as a fixed media increases the retention time of microorganisms in the system which boosts the number of methanogens in reactor which is a possible approach for higher Biogas production. For example, Jonatan (Andersson & Björnsson 2002) studied the effect of straw as a biofilm carrier on AD and demonstrated that it produces higher Biogas and also has higher COD removal of 50 – 73% at different OLR of 2.4-24gCOD/L.day comparing to suspended plastic carriers. Usage of biofilms results in higher efficiency in the degradation of organic matters which is an outcome of aggregation of microorganisms, by that the biomass increases and more efficient degradation occurs (Langer et al. 2014b), Biomass wash out, a common problem in AD systems can also be addressed by using biofilms as fixed media. These advantages offered re due to EPS synthesis by microbial cells that offer protection for biofilm and can increase the stability of the system (Langer et al. 2014b)(Sutherland 2001), hence this is why suspended cultures are more affected by environmental conditions such as temperature, pH, nutrition concentration and toxicity comparing to fixed media. For instance, David (Martinez-Sosa et al. 2011) studied treatment of municipal wastewater using submerged membrane bioreactor which indicated over 90% of COD removal efficiency with less pathogens and toxicity in the effluent. Kuan (Show & Tay 1999) studied the influence of support media in an anaerobic filter reactor which indicated 78% removal compared to 57% of COD removal in suspended growth system. Biofilm characteristics is categorized into two groups of granular and fibrous biofilms. Granular biofilms are more likely to result in clogging that defines excess head loss and frequent backwash (Wang et al. 2005). In contrast fibrous biofilms seem to have the capability to overcome this problem (Gong et al. 2011). As mentioned, one of the advances available for AD process is improving its waste stabilization and solid reduction by understanding the microbiology process in the system. Possible solution is using attached media in reactor, because of slow growth of methanogenic bacteria, reactors have to provide long contact time for substrates and enzymes with bacterial communities. Therefore, for a better fermentation, there is a need for long solid retention time, on the other hand, it is more economical to have short hydraulic retention times. Conventional anaerobic reactors lack the ability to separate SRT from HRT and attached media is a solution to this problem. Configuration/ application of using biofilm in treating wastes originates from trickling filter from 1980s. Attached growth systems have several advantages over suspended growth systems such as less maintenance and required energy as well as compactness of reactors.

Biofilm process can be divided into two groups: 1- Moving medium in which biofilms continuously are in motion inside reactors by mechanical, hydraulic or air force such as anaerobic fluidized bed or moving bed bioreactors. 2- Fixed medium systems that consists of motionless media like anaerobic filter and up flow anaerobic sludge blanket Fixed film reactors have a problem of clogging that occurs mainly in filters.

Moving medium as the first group of biofilm process can also improve the digestion system by increasing the retention time of microorganisms inside the reactor. Moving bed reactors contain biofilms that are attached to the carrier which can freely move inside the reactor. As mentioned before attached media reactors have smaller footprint and are highly resistant to shock loads. Moving bed reactors and anaerobic fluidized bed reactors, both contain moving mediums (Azizi & Sithebe 2015). There have been many studies on AFBR reactor, these reactors provide high purification capacity with no problem of clogging or sludge retention, and microorganisms grow on small particles because of large available surface area provided by the carriers. These reactors are compact and rather small. Biofilm thickness produced in these reactors are dependent on turbulence of Biogas produced in the system (Heijnen et al. 1989), (Buffiere et al. 1998) studied treatment of red wine distillery wastewater using a down flow fluidized bed with ground particles as support media and achieved 85% of TOC removal in OLR of 4.5 kgTOC/m^3.day and Methane production of 64% of 6.8 L/L.day.

As mentioned before, diary waste is considered a complex wastewater because of lipids, proteins and carbohydrates present inside it. (Rafael Borja & Banks 1995) experimented treating of ice cream wastewater in mesophilic condition in OLR of 15.6 gCOD/L.d and 8 hours of HRT, in an AFBR reactor using saponite mainly consisting of magnesium silicate as its support media. This reactor resulted in 94.5% of COD removal and 61% of methane production from 0.33 L/gCOD removed of gas production.

Zeolite as a common moving media seems to be a perfect biofilm for AFBR reactor because of its high specific surface area, (Fernández et al. 2007) studied the usage of zeolite as support media for AFBR reactor with OLR of 20 gCOD/L.day that resulted in 90% COD removal with methane production yield of 0.29 LCH_4/gCOD consumed.

Moving bed bioreactors with a moving medium are compact reactors that are used for municipal and industrial wastewater for COD removal, nitrification and denitrification. (Sheli & Moletta 2007) studied treatment of vinasses in a mixed moving bed biofilm reactor and found out that in OLR of 1.37 to 4.62 gsCOD/L.day with HRT of 2.8 to 6.3 day, the reactors achieved 68 to 92% of COD removal, however increasing the OLR could result in a drop in the removal efficiency. (Rodgers et al. 2004) Rodgers Used MBR reactor with plastic biofilm media in mesophilic condition for treating whey wastewater. The COD removal efficiency reached 89% in HRT of 1 day and OLR of 11.6 kgCOD/m^3.day, by decreasing the HRT to 0.6 day and increasing the OLR to 15.2 kgCOD/m^3.day, COD removal decreased to 81%. The overall Methane yield was 333.4 LCH_4/kgCOD removed with 63% of methane. Milk permeate which is high in fat, protein, lactose and mineral salts has been experimented by (Wang et al. 2009) in a AMBR reactor, Wang found that a 87-73% COD removal can be achieved in OLR of 2 to 20 gTCOD/L.d with Methane yield of o.341 LCH_4/gTCOD removed. The second group of biofilm process is fixed film reactors, as mentioned one of the fixed film reactors is anaerobic filter, which consists of a vertical filter bed that contains inert materials as support media to ease the interaction of microorganisms and substrate. This reactor is well suited for treating soluble wastes, without any need of recycle with a rather low solid production, Anaerobic Filter reactors requires power for pushing the liquid to have an upstream flow, and these reactors are mainly satisfactory for low strength wastes with low organic loading rates (Switzenbaum 1983),(Rodgers & Zhan 2003). (Young & McCarty 1969) studied on anaerobic filter reactor filled with stones as its support media for treating synthetic wastewater with concentration below 6000 mg COD/L with organic loading rate of 26.5 to 212 LB COD/d.1000cu.ft, the liquid detention time was 4.5 to 7.2 hours. In OLR of 26.5 LB

COD/d.1000cu.ft, and HRT of 7.2 hours the reactors had the highest COD removal of 93%, by increasing the OLR, COD removal efficiency dropped to lower than 60%, in the highest rate.

Borja (R. Borja & Banks 1995) also studied anaerobic filter (AF) and anaerobic fluidized bed reactor (FBR) for treating palm oil mill effluent (POME) waste, both reactors worked in organic loading of 10 gCOD/L.day and 6 hours residence time and resulted in 90% of COD removal, by increasing the OLR to 40 gCOD/L.day, both reactors showed lower COD removal efficiencies, however FBR had better results with 78% of COD removal. AF reactor did not operate in organic loading higher than 20 gCOD/L.day. This is probably because POME waste is considered a high strength waste with high COD and SS concentration and anaerobic filter is mostly suitable for low strength wastewaters. According to (Ruiz et al,1997) slaughterhouse waste which is considered a medium to high concentration waste can be treated using anaerobic filter reactor and a UASB reactor, results of the experiment shows 90% of COD removal with OLR of 1 to 5 kgCOD/m^3.day for UASB reactor but higher OLRs only achieved 60% COD removal. AF reactor contained PVC raschig rings as its supporting media, this reactor showed poor results compared to UASB reactor and in OLRs higher than 5 kgCOD/m^3.day destabilizing and washout occurred in the system. Overall, the highest Methane production of 53% of 1.34 m^3/m^3.day (total Biogas production) occurred with HRT of 1.2 day at OLR of 6.58 kgCOD/m^3.day with COD removal of 93% in UASB reactor. The highest methane production of 33% of 1.06 m^3/m^3.day (total Biogas production) resulted with HRT of 0.6 day and OLR of 8.36 kgCOD/m^3.day with COD removal of 67% in AF reactor.

As mentioned before, UASB reactor is another fixed film reactor which is more preferable compared to AF since it has more ability in treating higher strength wastes and also no need of specific support media, however less requirement on using media may be a disadvantage as well since it means there is a need for a sludge with high settling properties. (Wiegant & Lettinga 1985) Reported that thermophilic AD process in a UASB reactor for treating sugar wastewater results in a 26.9 m^3/day Biogas production with 57% for methane in OLR of 49.3 kgCOD/m^3.day.

AD process is suitable for Dairy waste which is very rich in carbohydrates but it contains low suspended solids, (Gavala & Kopsinis 1999) found out that by using UASB reactor with OLR of 6.2 gCOD/L.day, COD removal up to 98% can be achieved however increasing the OLR can decrease the removal efficiency by more than 10%. This specific attached media reactor is suitable for different wastes with various strengths. (Lu et al. 2015) Experimented treatment of starch wastewater using UASB reactor at an OLR of 4 gCOD/L.day which achieved 82 to 98% of COD removal and methane production of 0.33 LCH_4/gCOD removed, however increasing HRT more than 3 hours caused VFA accumulation, the optimal HRT was 6 hours. There are also studies on treatment of landfill leachate, (Kennedy 2000) experimented using sequencing batch and continuous flow UASB reactors with OLR of 0.6 to 19.7 gCOD/L.day, the sequencing batch had COD removal of 71 to 92% with HRT of 24, 18 and 12 hours, the continuous reactor resulted in 77% to 91% of COD removal with the same HRTs.

Table 3-1 shows various studies taken place on fixed film and moving bed reactors in anaerobic condition.

Table 3-1 Literature review of attached and moving media

	reactor	type of feed	media	HRT	OLR	Temp (°C)	COD removal	reference
fixed film reactors	anaerobic hybrid filter	cassava starch wastewater	nylon fibre	5.4 days	0.5-4 gCOD/L.day	>25°	94%	(Chaiprasert et al. 2003)
	anaerobic filter	ice cream wastewater	filter pack	1.28 days	35 gCOD/L.day	35°	80%	(Hawkes et al. 1995)
	UASB	leachate from food waste	granular sludge	0.44 days	15.8 gCOD/L.day	37°	96%	(Shin et al. 2001)
	UASB	dairy waste	granular sludge	6 days	6.3 gCOD/L.day	35°	98%	(Gavala & Kopsinis 1999)
	UASB	wastewater with starch	granular sludge	6 hrs	4 gCOD/L.day	35°	82-98.7%	(Lu et al. 2015)
	UASB	as the sole Carbon source	granular sludge	36 hrs	6.6 gCOD/L.day	35°	73%	(Dinsdale et al. 1997)
moving bed reactors	moving bed biofilm reactor system	landfill leachate	bio carriers made of organic polymer mixed with Nano-sized inorganic ingredients	4 days	4.08 gCOD/L.day	35°	91%	(Chen et al. 2008)
	moving bed biofilm reactor system	landfill leachate	bio carriers made of organic polymer mixed with Nano-sized inorganic ingredients	1 days	7.66 gCOD/L.day	35°	92%	(Chen et al. 2008)
	moving bed biofilm reactor system	landfill leachate	bio carriers made of organic polymer mixed with Nano-sized inorganic ingredients	2.5 days	6.27 gCOD/L.day	35°	89%	(Chen et al. 2008)
	anaerobic fluidized bed bioreactor(AnFBR)	primary sludge from municipal wastewater treatment	zeolite	4 days	19 gCOD/L.day	37°	68%	(Andalib et al. 2014)

anaerobic fluidized bed bioreactor(AnFBR)	primary sludge from municipal wastewater treatment	zeolite	3.5 days	29 gCOD/L.day	37°	88%	(Andalib et al. 2014)
AFBR	thin stillage	zeolite particles	3.5 days	29 gCOD/L.day	37°	80%	(Andalib et al. 2012)
AnFBR	primary sludge	high-density polyethylene	2.2 days	18 gCOD/L.day	37°	62%	(Wang et al. 2016)

The aim of this study was to evaluate the performance of four new different fibrous biofilms, manufactured by Bishop Water technology, to introduce a new fixed film media with higher efficiency and economically favourable with higher methane production. Therefore, reactors with different attached media were set up. The objective of this experiment was to determine the biofilm with highest removal efficiency and methane production.

3.2 Materials and methods

3.2.1 Feeding and seed sludge

For the start-up, the AD reactors were inoculated with the seed sludge and the degassing process occurred for 4 days until Biogas production from each reactor was detectable and stabilized. The seed sludge as the inoculum for this experiment was collected from a mesophilic AD system which experimented on biofilms in continuous mode process. The fresh cow manure was collected from farm and was stored in 4°C for further usage, **Table 3-2**, **Table 3-3** indicate the biomass and wastewater characteristics. Before feeding, the substrate was filtered for reduction of large particles to minimize the chance of clogging in reactors. The system was fed with substrate as an experiment, then the reactors were sealed with caps and nitrogen was injected for providing an oxygen free environment. The biofilms were attached to tubes inside reactors for minimizing the movement of media in the system.

Table 3-2 Biomass characterization

	Mesophilic biomass
TS (mg/L)	57873.3±4562
VS (mg/L)	39593.3±2468
TCOD (mg/L)	48800±1420
SCOD (mg/L)	11500±760
pH	7.76±0.02

Table 3-3 Feed characterization

Cow manure	Feed
TS (mg/L)	89245±5200
VS (mg/L)	62120±2315
TSS (mg/L)	75240±3505
VSS (mg/L)	54420±3890
TCOD (mg/L)	68900±6500
SCOD (mg/L)	17400±1530
pH	8.34±0.05
Alkalinity (mg $CaCO_3$/L)	4417.5±520

3.2.2 Biofilm carrier

The legit biofilm holds factors such as high specific surface area, stability, resistance to acid and base, no biodegradability, light weight, strong in environmental conditions and economically feasible to be used in commercial scale. There are various biofilms that have been used as attached media according to literature (Gong et al. 2011).

BioCords manufactured by bishop water technology, Made of a cord covered with rings of thread, both made of polymers, have been used in aerobic condition and were successful in wastewater treatment, these attached media are available in different styles and constructions to suit specific waste streams which are simple to install, in this study 4 BioCords named BioCord

HS_1, BioCord HS_2, BioCord LS_1 and BioCord LS_2 with different specifications indicated in **Table 3-4** are evaluated in anaerobic condition.

Table 3-4 Biofilm characteristics

biofilm number	specific surface area	Accessible surface area	length	application
BioCord HS_1	$2.4m^2/m$	$0.14\ m^2$	6 cm	Ideal for wastewater with high suspended solids
BioCord HS_2	$2.4m^2/m$	$0.14\ m^2$	6 cm	
BioCord LS_1	$1.2m^2/m$	$0.14\ m^2$	10 cm	Biological treatment for low and medium concentration
BioCord LS_2	$1.6m^2/m$	$0.14\ m^2$	8 cm	

3.2.3 AD experimental setup and operation

Five groups of 3 reactors were conducted in the experiment, the first group was set as the control system without any biofilm media, and the other four groups contained one specific kind of BioCord attached to the tube inside reactors. The schematic of reactors is shown in **Figure 3-1** which consists of 4 main sections, the reactors were experimented in 35°C constant temperature, as shown in **Figure 3-1**, and the incubation system was connected to motor controller for determination of agitators. For Biogas production measurement, the system is connected to automatic methane potential test (AMPTs), which contains CO_2-fixing unit which are vails containing alkaline solution that retains other gases except CH_4 that allows to pass through to gas volume measuring device, that digital pulses are produced by certain amount of gas flow which goes through the device and at last control and analysis unit displays the results. (Bioprocess Control Sweden manual, 2016)

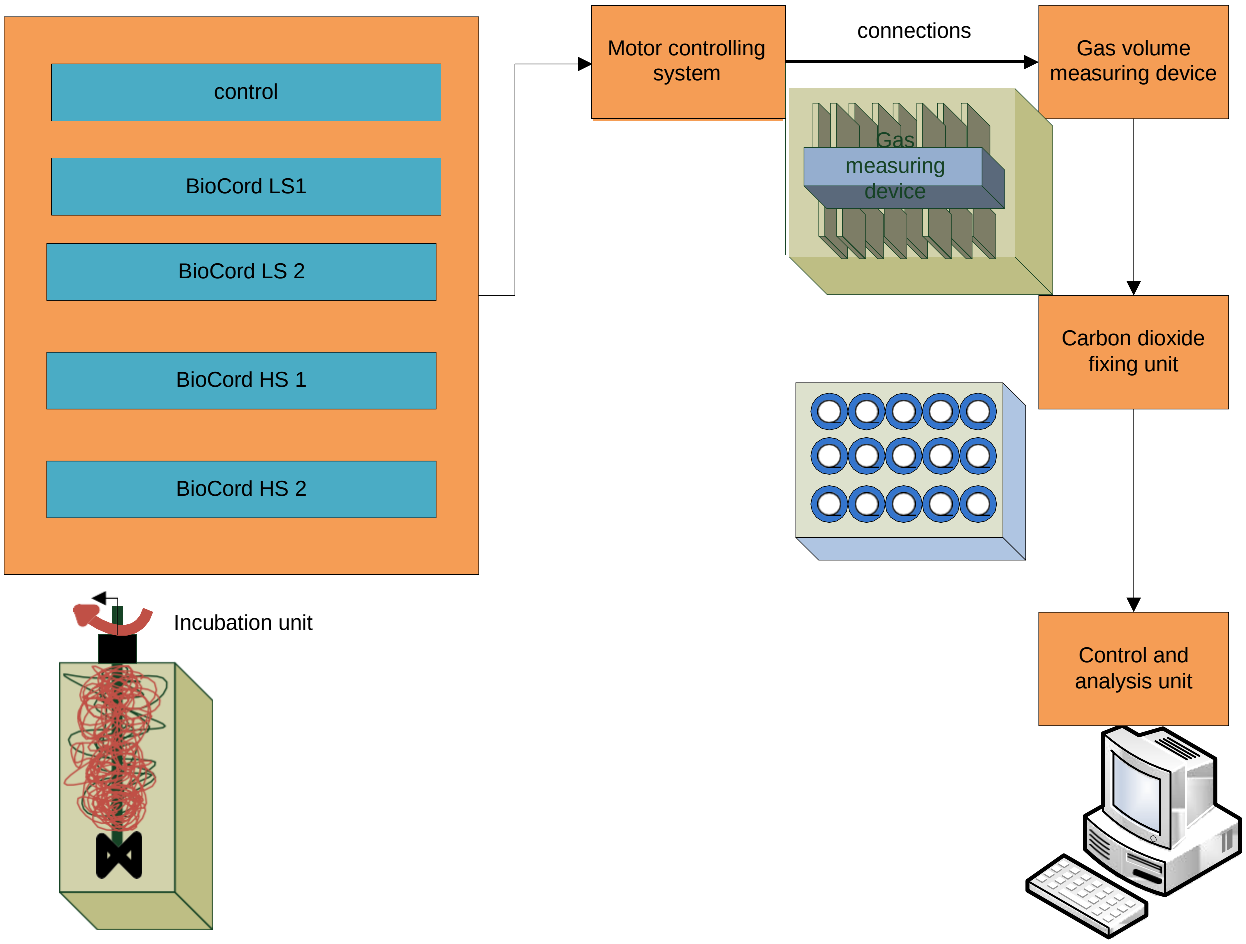


Figure 3-1 Schematic diagram of the experimental setup

3.2.4 Analysis

Anaerobic batch reactors were used to conduct methane production potential and rate by using biofilm as an effective pathway for microorganism growth. The amount of substrate and the biomass is measured by considering food to microorganism ratio. Five series of samples from feed and substrate were collected and firstly diluted and filtered through 0.45 µm, analysis was achieved using standard methods for wastewater examination, total COD and soluble COD, solids (TS, VS, TSS and VSS) and alkalinity in influent and effluent were measured per the standards, pH was measured using pH-meter.

Produced Biogas was measured by the bioprocess control system which automatically analyzed methane production of each reactor using the automatic methane potential test system. The measuring data were downloaded on daily basis from the device.

3.3 Results and discussion

3.3.1 Anaerobic treatment performance

AD is considered a likely preferable technology for recovering energy through waste treatment, however its disadvantages has limited the application of this process. A biofilm as an improvement method can optimize methane production as well as the removal efficiencies. Nonetheless selection of the right attached media for each process is the key challenge. For instance Wei Jia Gang (Gong et al. 2011) studied three different biofilm carriers (activated carbon fibre (ACF), polyvinyl alcohol fibre and glass fibre) in anaerobic digesters and indicated that ACF had the most promising results with the highest methane production and removal efficiencies. In another study (Martí-herrero et al. 2014) PET (Polyethylene Terephthalate) rings were used as biofilm carriers and resulted in 44% more Biogas production compared to the reference condition.

In this study four types of BioCords have been used for evaluation of AD process, since the reactors were maintained at nearly the same VS and pH range, BioCords performance are comparable, the organic loading rate was fixed on 2.8 gCOD/L during long term mesophilic experiment for all 15 batch reactors, the food to microorganism ratio (F/M) was 0.68 mgCOD/mgVSS.day in the beginning of process and decreased by deficiency of substrate in time. The total performance is indicated in the **Table 3-5** for all groups of experiment.

Figure 3-2, **Figure 3-3** indicate influent and effluent COD and COD removal rate in the batch bioreactors with different BioCords with each group’s methane production. The influent COD concentration for all 5 groups varied from 68700 to 69000 mg/L with average of 68900 mg/L. During the process, COD removal rates increased and resulted in decreased COD concentration in effluents, however removal efficiencies indicate that BioCord LS_2 obtained the highest amount of COD removal with 88 % compared to the other three groups of BioCords and to control system with 78 % removal, measurement of TCOD and SCOD removal efficiencies confirmed the precession of BioCord LS_2. Also, as shown in **Table 3-6** BioCord LS_2 has the highest mass balance. The removal of COD as pollutant showed that reactors containing BioCord LS_2 had higher rates of degradations which resulted in higher Methane production rates, an adequate range of removal efficiencies in anaerobic reactors for degrading organic materials is dependent on the quality of methanogenic bacteria which can efficiently convert VFAs to methane and CO_2 in an oxygen free environment (Gong et al. 2011).

Table 3-5 Total performance of all experimental groups

	control system without attached media		system with BioCord HS1		system with BioCord HS2		system with BioCord LS1		system with BioCord LS2	
OLR	2.8 gCOD/L.d									
samples	influent	effluent	influent	effluent	influent	effluent	influent	effluent	influent	effluent
TSS (mg/L)	61133.33±3089	48633.3±1747	57166.67±8503	46133.3±7539	61966.67±4841	46266.7±4129	60333.3±550	44333.3±1250	57766.7±520	37900±2364
VSS (mg/L)	43200±1992	34500±1802	40333.3±5479	32533.3±5937	44200±3637	33100±2343	42266.7±305	31033.3±1607	42266.7±453	29000±1374
TCOD (mg/L)	54300±1300	39500±770	45500±1340	45600±4100	52430±2177	44330±5271	47300±1665	38370±4550	47630±3000	37300±2700
SCOD (mg/L)	11200±7800	9100±880	11200±960	11500±12	12870±655	10600±98	12070±13	11130±10	11800±98	8900±920
pH	7.81±0.005	7.99±0.04	7.8±0.02	7.92±0.03	7.81±0.01	7.92±0	7.8±0.01	7.93±0.02	7.78±0.01	7.9±0.01
Alkalinity (mgCaCO3/L)	3420.53±332	2899.61±213	3974.41±752	4335.62±817	4469.92±177	3719.94±259	3208.49±632	3591.66±103	2998.97±492	3382.11±757

Table 3-6 COD analysis of all experimental groups

	CH4 (mL/d)	TCOD in(mg/L)	TCOD eff(mg/L)	COD removal eff	TCOD in (g/d)	TCOD out (g/d)	TCOD for CH4(mg/L)	VSS in(mg/L)	VSS out(mg/L)	VSS removal eff	CH4 (L/gCOD$_{consumed}$)	Theoretical CH4 (mL/d)	CH4 (L/day)	Measured CH4/Theoretical CH4	% COD mass balance
control	870.90	68900	15300	78%	1.378	0.307	2204.8	75240	34500	54%	1137.37	18760	0.871	0.046	81%
HS1	1048.27	68900	11500	83%	1.378	0.230	2653.8	75240	32533.33	57%	1278.37	20090	1.048	0.052	87%
HS2	1067.27	68900	10600	85%	1.378	0.213	2701.9	75240	33100	56%	1281.45	20405	1.067	0.052	89%
LS1	1042.33	68900	11130	84%	1.378	0.223	2638.8	75240	31033.33	59%	1263.00	20219	1.042	0.052	88%
LS2	1141.57	68900	8600	88%	1.378	0.172	2890.0	75240	29000	61%	1325.20	21105	1.142	0.054	92%

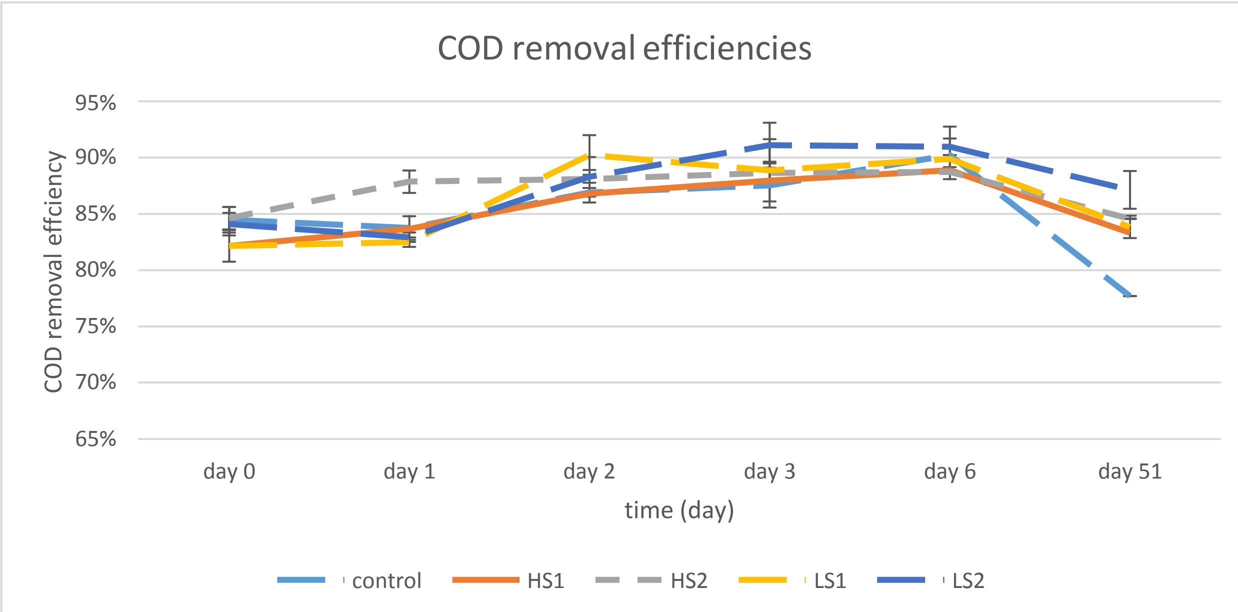


Figure 3-2 COD removal and of all groups in experiment

The variation of influent ammonia effluent and removal efficiency of NH_4-N was investigated. The influent NH_4-N for all 15 reactors varied from 2200 to 3000 mg/L with an average of 2500 mg/L. results showed that reactors had removal rates around 22% which indicates that little ammonia was utilized by bacteria and nitrification was not remarkable, however BioCord LS_2 had higher nitrification rates, resulting in less ammonia concentration in effluent. Results showed that higher COD removal can be obtained by using biofilm, while it isn't very specific to say which BioCord had higher ammonia removal efficiency. The pH always varied in a quite narrow range which signified the minimum amount of VFAs being accumulated inside reactors, in the acidogenesis stage of AD, VFA are produced following by conversion of these acids to acetate. This intermediate product (VFA) causes microbial stress which is the reason for instability of anaerobic process by overloading that can cause VFA repletion which is the reason for pH decline. Carbohydrates are hydrolysed to sugar following by a fermentation stage that produces VFAs, and proteins are also degraded to VFAs, decrease in pH can kill the methanogenic bacteria and prevent the Methane production (Elbeshbishy & Nakhla 2012). The narrow pH variation indicated that BioCord LS_2 had an approximately more stable process than the other three BioCords, alkalinity values as shown in **Table 3-5** have oscillation but the average pH value of 7.7 to 8 marks an approved buffering capacity was available for the process.

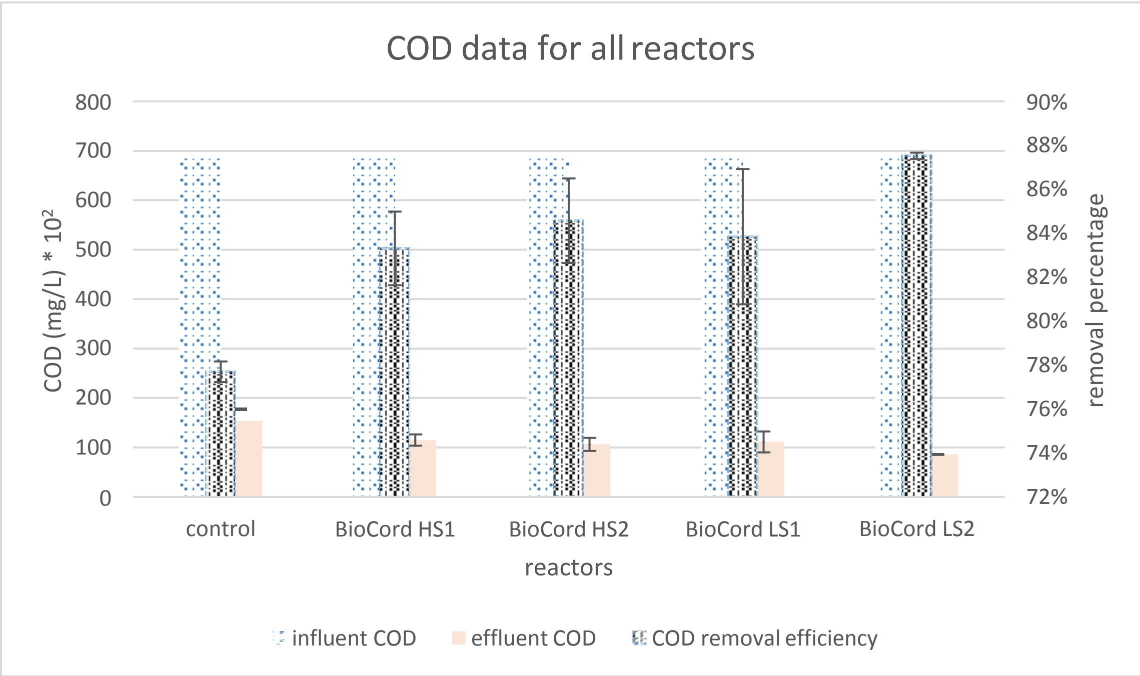


Figure 3-3 COD removal percentages

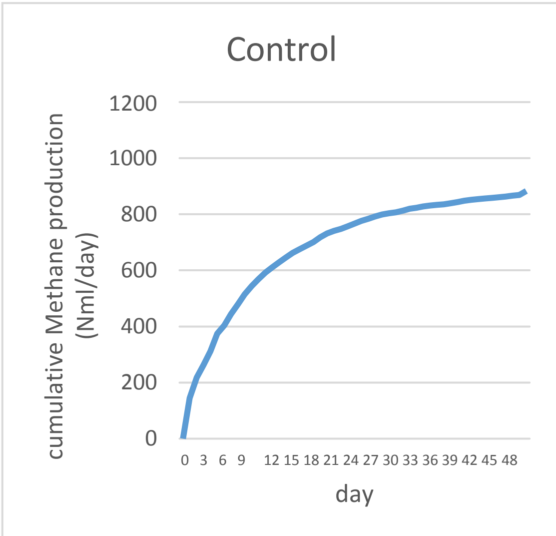
3.3.2 Methane production

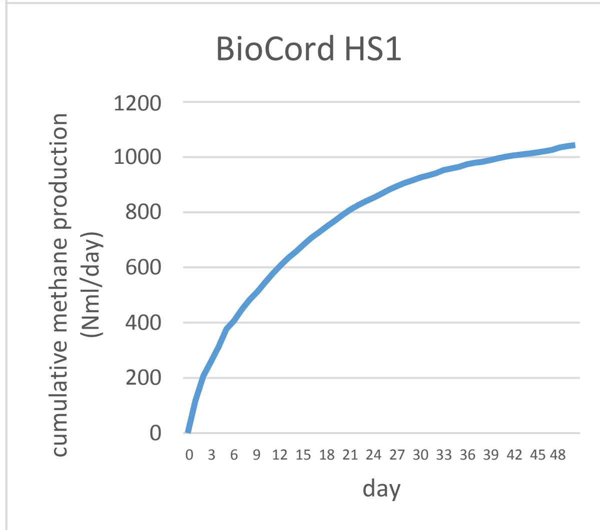
The production of methane and its advantage as high potential energy source is the most important aspect of anaerobic wastewater treatment, at beginning of the experiment nitrogen was used to exclude the air for providing an Oxygen free condition. The reactors run for 3 days before feeding for releasing all the potential methane production, to exclude these amounts of methane produced from total methane measurement. After the degassing process, while the methane production reached a stabilized status, reactors were fed with fresh cow manure and experiment started as a batch system, the accumulative methane production has been monitored for 55 days, an initial increase was indicated for all reactors for about two days, while methane production for BioCord

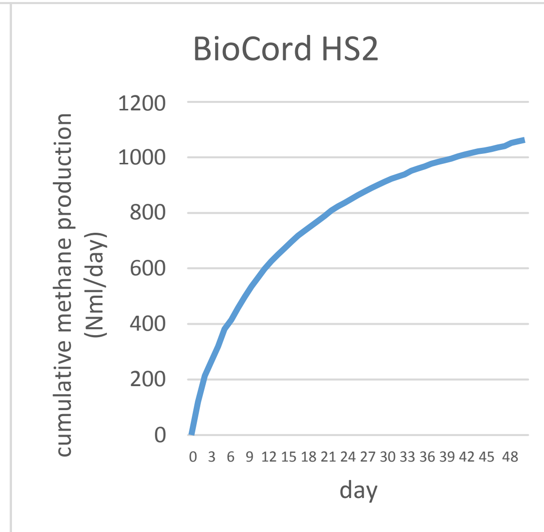
LS_2 was considerably higher than other BioCords, before reaching a stabilized behaviour. In the first 40 days, the values of Methane production had an increasing path, then it started to decrease because of deficiency of substrate. The methane production carried out from batch reactors containing BioCord LS_2 has shown higher than the ones with BioCord HS_1, BioCord HS_2 and BioCord LS_1, reporting a 26 mL/d for the first 39 days and 7 mL/d for the remaining 18 days' efficiency on an average basis for the whole experiment. With respect to Methane yield an average 0.015 LCH_4/gCOD removed were obtained for BioCord LS_2 from day 0 to 51 which is about 10 % higher than the control system and approximately 5 to 10 % higher than the other 3 BioCords. Application of BioCord HS_1, BioCord HS_2 and BioCord LS_1 seemed to have minor effect on increasing methane production thus, less positive results for methane production was indicated from these BioCords.

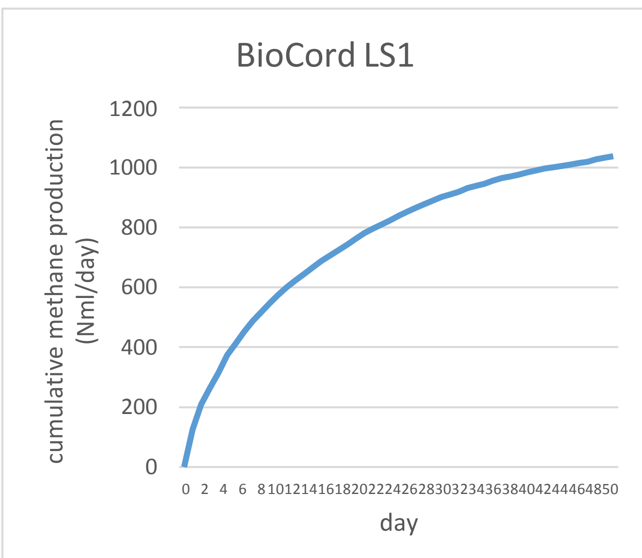
The results mentioned indicated that for methane production shown in **Error! Reference source not found.**, it was likely that BioCord implementation as attached media could improve AD process, also lower standard deviation marks higher stability in reactors containing BioCord LS_2, hence significance of choosing proper attached media is indicated for treatment of cow manure. The initial results demonstrated that BioCord LS_2 should be the most effective BioCord among the 4 tested biofilms for AD process.

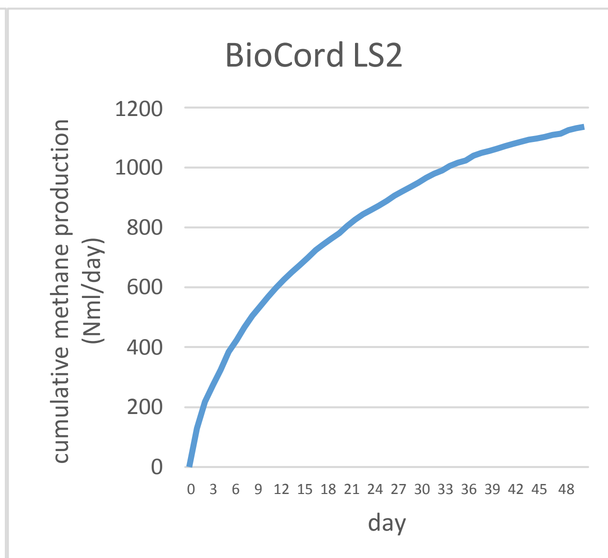
Figure 3-4 Cumulative Methane production for experimental groups


Control
cumulative Methane production (Nml/day)
1200
1000
800
600
400
200
0
0 3 6 9 12 15 18 21 24 27 30 33 36 39 42 45 48
day


BioCord HS1
cumulative methane production (Nml/day)
1200
1000
800
600
400
200
0
0 3 6 9 12 15 18 21 24 27 30 33 36 39 42 45 48
day


BioCord HS2
cumulative methane production (Nml/day)
1200
1000
800
600
400
200
0
0 3 6 9 12 15 18 21 24 27 30 33 36 39 42 45 48
day


BioCord LS1
cumulative methane production (Nml/day)
1200
1000
800
600
400
200
0
0 2 4 6 8101214161820222426283032343638404244464850
day


BioCord LS2
cumulative methane production (Nml/day)
1200
1000
800
600
400
200
0
0 3 6 9 12 15 18 21 24 27 30 33 36 39 42 45 48
day

3.3.3 Performance evaluation of BioCord LS_2 as attached media

The initial results of experiment indicated high potential for BioCord LS_2 as the preferable attached media in AD treatment of cow manure. For brighter vision on application of this BioCord, nitrogen and alkalinity was measured, also biofilms were weighed for indication of attached VSS. **Figure 3-4**, **Error! Reference source not found.** indicate the total VSS removal efficiencies for all groups of experiment. The aim was to have a more specialized look on preference of this biofilm. The results show that BioCord LS_2 had about 5 times more attached biomass with negligible standard deviation shown in **Figure 3-5** which indicated higher amount of waste measurement in a more stabilized environment, moreover reactors containing BioCord LS_2 tend to keep their methane production in a more consistent trend during a longer-term time period compared to other three BioCords.

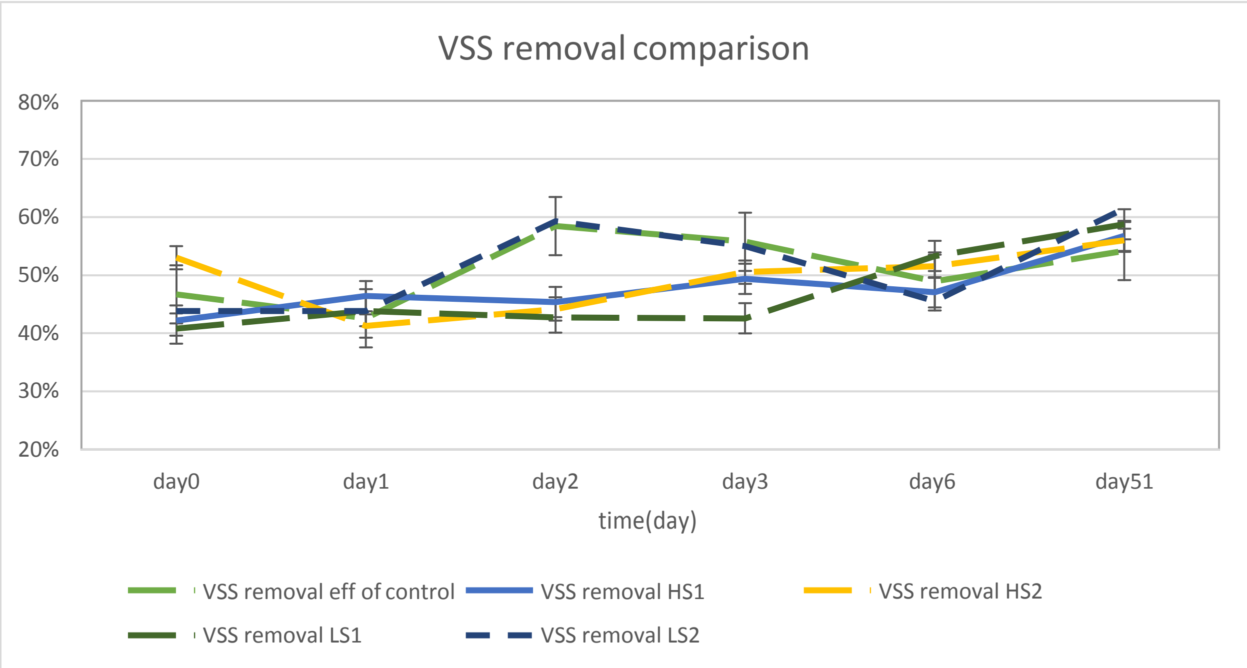


Figure 3-4 VSS removal efficiencies

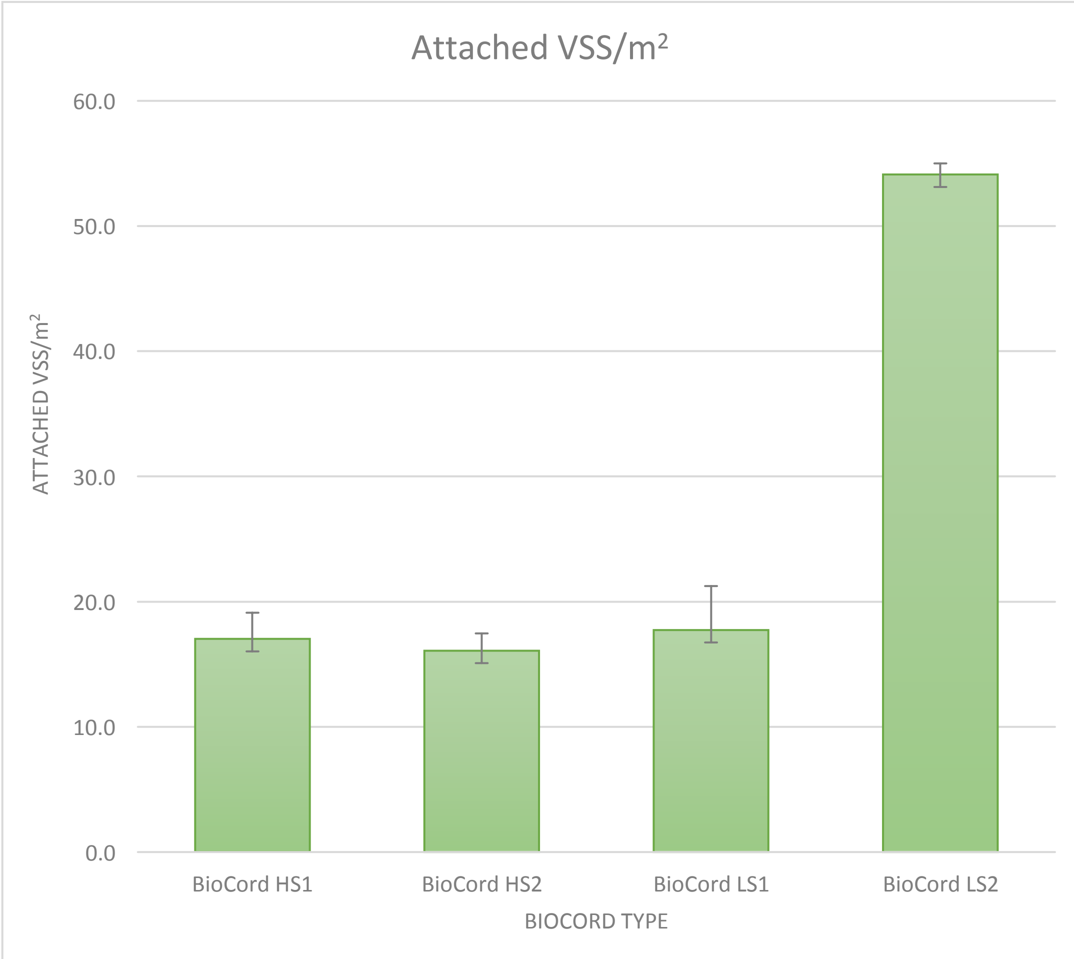


Figure 3-5 Attached biomass to BioCords

3.4 Conclusion

This experiment emphasizes the importance of using BioCords as attached media in anaerobic condition. The results showed that reactors containing BioCord LS_2 as biofilm carrier had superior performance compared to other BioCords evaluated and compared to control system. the experiment indicated that:

- BioCord LS2 had higher amount of Methane production, almost 10 to 15% higher than other BioCords and 30% higher than the conventional system.

- This BioCord showed higher removal efficiency in comparison with the control system.
- Addition of BioCord as an attached media didn't negatively affect the process by causing clogging due to biofilm formation.
- These BioCords are available in market and are economically feasible without any need of major change in the configuration of the system.

Chapter 4 Enhancement of AD process using micronutrient supplement addition “BioStreme “

4.1 Introduction

In order to provide humans and animals with required energy and to support a safe food provision and to reduce the fossil fuel usage and pollution caused by this source (LIN, 2008), novel handling and recycling methods for organic material and animal waste is used, AD with Biogas production is one such method for taking care of this matters (Holm-Nielsen et al. 2009).This process can be used for reducing the pollution caused by different industries as well as offsetting the usage of operation by fossil fuels. AD offers many advantages from low sludge production to energy recovery but the main obstacles for commercialization are long startup time and low process stability. The problems are often caused by difference in physiology, nutrition and growth kinetics in methane and acid forming bacteria inhibition caused by different substances (Chen, Jay J Cheng, et al. 2008). This process is a common process used for treating different groups of organic materials separated in 5 groups of: 1- sewage sludge 2- animal manure 3- food industry waste 4- agricultural residues and crops 5- organic fraction of municipal solid waste. This process has been used for many years however, improving the economic aspects as well as increasing the Biogas production is attracting a lot of attention, utilizing methods such as pretreatment and co-digestion has been studied more compared to introducing additives to the system (Romero-Guiza et al. 2016). There has been studies on additives with different approaches such as 1-low concentration nutrient supplementation 2-high concentration inhibitors adsorbing 3- buffer capacity improvement and 4- substrate biodegradability enhancement, however results of many studies are not comparable since there are difference in substrate, enzyme dosage and digester configuration (Romero-Guiza et al. 2016), for a proper AD in commercialized level, process stability is in high importance. Therefore paying attention to nutritional requirements is required, since nutritional deficiency, results in an unstable process increasing the chance of failure (M_Kayhanian,1995). For a well-performed AD process, nutrients are required in right ratio and concentrations in substrate, the nutrients can be grouped into macro and micro nutrients, nutritional deficiency may cause incomplete bioconversion of organic materials which results in process failure, methanogenic bacteria require a wide variety of mineral nutrients for a strong growth (M_Kayhanian, 1995). As mentioned limitation in micro and macronutrients can be the main reason for poor performance in AD, process stability is a major problem for AD systems which leads to unstable methane production. Therefore, there has been studies on AD process to overcome the AD instability problem and on effective results of adding nutrients in order to increase the Methane production and process stability by maintaining a proper pH level and preventing the VFA accumulation. For instance a study on energy crops treatment supplemented with micro and macro were experimented in single stage continuous reactor, the nutrient supplement increased the methane yield and also it shorten the hydraulic retention time (Nges & Björnsson 2012), in a similar experiment (Zhang et al. 2011) investigated on piggery waste with nutrient supplement and found out higher methane yield and lower levels of VFA accumulation comparing to its control system, nutrients micronutrients are components existing in biomass which are important for buffering role, micronutrients on the other hand, are essential for many enzymatic reactions for methane production (Romero-Guiza et al. 2016), different groups of methanogens such as *Methanosarcina barkeri; Methanospirillum hungatii; Methanocorpusculum parvum; Methanobacterium thermoautotrophicum, and Methanobacterium wolfei; Methanococcus voltae, and Methanococcus vanielli, and Methanococcoides methylaten* (Demirel & Scherer 2011) are influenced by free metal ions as micro-nutrients such as nickel, iron and cobalt, there has been more attention on deficiency of trace elements, despite of the input liquid from different animal wastes, it is assumed that different wastes such as organic wastes, kitchen and slaughterhouse waste consist of sufficient trace elements and micronutrients but this assumption has been under question recently (Schattauer et al. 2011), In literature adding different micronutrients such as Co, Fe, Mo, Ni and Se have been studied (M_Kayhanian,1995).

The macronutrients in AD process are carbon, nitrogen phosphorus, potassium and sulfur, each and every macronutrient has its own specific function and influence on this process:

Carbon is the primary source for energy and is the main source in bacterial cell structure. Since most of organic materials are rich in Carbon source then Carbon is not considered to restrict the process, by defining the ratios of C/N, C/P and C/K, the nutritional requirement can be found. Nitrogen is also a primary nutrient, this nutrient is essential for synthesis in microbes, in different forms such as reduced form which is required for protein synthesis. Phosphorus is less essential compared to carbon or nitrogen but it is still important for nucleic acid synthesis. Potassium is required for increasing the permeability of cell walls aiding in nutrient transportation. Sulfur can be present in forms of non-reduced (Sulfate) or reduced-form (Sulfide) in the AD process, the non-reduced form, sulfates are considered inhibitory to methanogenesis stage, methanogens are only capable of using reduced-form of sulfur, sulfide, effecting the methanogenesis growth, however sulfides are required by different enzymes, H_2S gas production and sulfide precipitation by heavy metals are the negative points of this nutrient (M_Kayhanian,1995).

Different metals as micronutrients can have different advantages such as providing elements for important enzymatic reactions preventing sulfide toxicant actions and stimulating biomass (Oleszkiewicz and Sharma, 1990), heavy metals such as Cr, Cu and Zn are highly effective on acidogenesis and methanogenesis stages of AD process, many of these metals are essential for activity and growth of microbial communities, the exact amount of trace elements required are different in various sources of wastewater (Fermoso et al. 2009). Cobalt as one of micronutrients is essential for specific enzymes such as Carbon monoxide dehydrogenase. Copper, as an additive haven't resulted in any specific effect however this nutrient has been present in different methanogenic strains. Iron is an essential micronutrient and one of the most predominant elements for its conductive characteristics and low cost (Romero-Guiza et al. 2016), it offers various advantages such as activating different enzymes such as pyruvate-ferredoxin oxidoreductase, and discharging extracellular polymers, different forms of Iron is known to accelerate the AD process, in a study by (Y. Zhang et al. 2014), addition of Iron to activated sludge process was investigated rather than pretreatment since it is more cost effective, in this study Iron was added as powder and scrap to the reactor, all forms of Iron increased the methane yield but the highest increase was for reactor containing rusty scraps of Iron with 30% of enhancement. molybdenum is important for formate dehydrogenase however, concentrations of this metal inhibits the production of important sulfides, this metal is crucial for methane fermentation, there has been various concentrations used in different studies but the optimum range of Fe for methanogenesis stage is between 280 mg/m^3 to 50.4 mg/m^3 (Demirel & Scherer 2011). Nickel is essential for the compounds called F factor 430 in cells which is present in all methanogenic bacteria, Nickel can also be the only source for energy, (Williams et al. 1986) investigated on adding Ni as $NiCl_2$ to poultry waste digester, it was shown that utilizing Ni additive increased the Biogas production in early stages after addition, although it didn't necessarily increased the methane content in Biogas, in another study, nickel addition was studied on cattle dung treatment, with an optimum range of 4.2-6.2 µg Ni/g of dry matter in cattle dung, both methane content and Biogas production increased (Dar, 1987), however increasing the Ni concentration more than the limitation point it causes inhibition to methanogenesis stage (Demirel & Scherer 2011). Selenium and Tungsten are both components present in formate dehydrogenase, Tungsten has also been reported to have essential effect on degrading propionate (Zellner & Winter 1987). Zinc similar to copper is present in high concentrations in methanogenic bacteria, however this compound is a part of different enzymes, it is not known to be essential for AD process (M_Kayhanian, 1995). Ni, Co and Fe are the most common nutrient supplement studied in AD process since they are essential for *Carbon-monoxide dehydrogenase, Acetyl-CoA decarbonylase, methyl-*H_4 *STP:HS-CoM methyltransferase, methyl-CoM reductase* and other enzymes that are important in acetoclastic methanogenesis pathways (Romero-Guiza et al. 2016), also these metals are known to be essential in acetotrophic pathway for Methane producing bacteria in which acetate is oxidized to Carbon dioxide and Hydrogen which is continued by hydrogenotropic methanogenesis (Zhu & Tan 2009), **Table 4-2**, **Table 4-3** indicate studies on different light and heavy metals as nutrient supplement to AD process with stimulatory and inhibitory ranges of each. Enzymes have been used as pretreatment to complex organics such as lignocellulosic and lipid rich materials, however enzymes can also be added to AD process as nutrient supplement, adding enzymes to AD process can shorten the digesting time, increase the digestibility of the sludge and decrease the cost of disposal, using enzymes can increase the release of extra cellular polymeric substances (EPS) and also can convert resistant elements to biodegradable ones (Yang et al. 2010), enzymes have high capability to act under toxic and various environmental conditions, they can operate in conditions which different microorganisms, predators and inhibitors are present, because of their smaller size and higher solubility and mobility they have easier access to substrates compared to microbes (Romero-Guiza et al. 2016) . In study co-digestion of grease trap and sewage sludge was investigated with addition of lipase enzyme, at first adding grease trap to sewage sludge decreased the biodegradability however by addition of lipase to the system, the obstacle was completely overcome, moreover it increased the methane production yield, the optimum range of lipase enzyme addition was between 0.33% to 0.83% (v/v) of enzyme (Donoso-Bravo and Fdz-Polanco, 2013), **Table 4-1** shows the overall advantages and disadvantages of different additives.

Based on (Romero-Guiza et al. 2016)

Table 4-1 Advantages and disadvantages of different additives

additives	Elements	Methane yield	Stability	advantages	disadvantages
Micronutrients	Fe	High	Moderate	Higher waste solubilization Biomass stimulation	Precipitation and clogging risk
	Ni	Low	High		
	Co	Low	high		
Enzymes	-	high	low	Increased methanogenic activity Increased hydrolytic activity Higher waste solubilization	Costly Difficult monitoring

Temperature is a very important parameter in concentration of micronutrient supplement, higher temperature levels require higher dosage of supplements since higher temperatures have less nutrient bioavailability (Romero-Guiza et al. 2016). (Uemura 2010), studied AD treatment of organic solid waste from kitchen in CSTR reactor in both mesophilic and thermophilic conditions with retention time of 30 days, both reactors had severe failure because of acidification. The mesophilic system recovered by addition on nutrient supply but not the thermophilic system, indicating that thermophilic reactor required higher concentrations of supplements.

Light metals	substrate	Stimulatory concentration	Inhibitory concentration	reference
Calcium	Synthetic wastewater contacting glucose	7000 mg/L	-	(Jackson-Moss & Duncan 1991)
	Synthetic wastewater/acetic acid	200 mg/L	2500-4000 mg/L	(Kugelman & Mccarty 1965)
	Lactose	>120 mg/L	<120 mg/L	(Huang et al, 1995)
	Synthetic wastewater	150-300 mg/L	<300 mg/L	(Yu et al. 2001)
Magnesium	Synthetic wastewater (alpha medium)	720 mg/L	-	(Ahring et al. 1991)
	Synthetic wastewater	300 mg/L	400 mg/L	(Schmidt & Ahring 1993)
Potassium	Synthetic wastewater/ acetic acid	>400 mg/L	<400 mg/L	(Kugelman & Mccarty 1965)
	Mesophilic coffee waste	>1200 mg/L	<1200 mg/L	(Fernandez & Forster 1993)
	Thermophilic coffee waste	>200 mg/L	<200 mg/L	(Fernandez & Forster 1993)
Sodium	Synthetic acetate broth	350 mg/L	-	(Khan & Ashraf 1988)
	-	-	3500-5500 mg/L (moderately) 8000 mg/L (strongly)	(Mccarty 1964)

Table 4-2 light metal concentration effect on AD process literature review

Table 4-3 Heavy metal concentration effect on AD process literature review

Heavy metals	substrate	Stimulatory concentration	Inhibitory concentration	reference
	N/A	81 mg/L	Different ratios of acetic, propionic and butyric acid	(Lin 1993)
	N/A	440 mg/L	Different ratios of acetic propionic and butyric acid	(Lin 1993)
Nickel	4 mg/L	35 mg/L	Glucose based synthetic wastewater Sucrose	(Altaş 2009)
	N/A	1600 mg/L	containing wastewater	(Li & Fang 2007)
	7.5 mg/L	2 mg/L	Glucose-based synthetic wastewater	(Altaş 2009)
Zinc	4.5 mg/L	N/A	Sucrose-based wastewater	(Chen, Jay J. Cheng, et al. 2008)
	N/A	1600 mg/L	Sucrose containing wastewater	(Li & Fang 2007)
	N/A	3000 mg/L	Sucrose containing	(Li & Fang 2007)
Chromium	2 mg/L	27 mg/L	wastewater Glucose based synthetic	(Altaş 2009)
	15 mg/L	60 mg/L	wastewater Sucrose based wastewater	(Chen, Jay J Cheng, et al. 2008)

Cobalt	<19 mg/L	160-320 mg/L	Growth medium with dextrose, peptone and yeast extract as Carbon	(Gikas 2007)
	0.2 mg/L	N/A	source Synthetic wastewater	(Kida et al. 2001)
Molybdenum	<0.05 mg/L	N/A	Basal synthetic medium containing propionate	(Worm et al. 2009)
Tungsten	<0.04 mg/L	N/A	Basal synthetic medium containing propionate	(Worm et al. 2009)
Iron	<0.3 mg/L	N/A	Basal synthetic medium containing propionate	(Worm et al. 2009)

4.2 Material and methods

4.2.1 Feeding and seed sludge

In the startup phase, the reactors were inoculated with anaerobic seed sludge from Humber treatment plant (ON, Canada) for almost 4 days for degassing process until the Biogas production from each reactor reaches a stationary phase therefore it won't influence the Biogas production from the substrate fed to the system. Four different experiments were done on different concentrations of nutrients, both synthetic wastewater and real raw anaerobic sludge collected from the Humber treatment plant were utilized as feeding substrate, all the reactors were run in batch mode, therefore they were fed once at the beginning of the experiment and sparked with nitrogen gas and sealed with caps for providing the anaerobic condition, the nutrient solutions were mixed with the substrate prior to feeding process. **Table 4-4**, **Table 4-5** and **Table 4-6** indicate the biomass, real and synthetic feed respectively.

Table 4-4 Mesophilic biomass properties

	Mesophilic biomass
TS (mg/L)	4620
VS (mg/L)	2795
TCOD (mg/L)	3700
SCOD (mg/L)	2500
pH	7.07

Table 4-5 Real feed characterization

	Feed
TS (mg/L)	6895
VS (mg/L)	5145
TSS (mg/L)	6025
VSS (mg/L)	5015
TCOD (mg/L)	9000
SCOD (mg/L)	5000
pH	6.3
Alkalinity (mg $CaCO_3$/L)	2452

Table 4-6 Synthetic feed compositions

Feed Comp.	**CH3COOH (mL/LF)**	**NH4Cl (g/LF)**	**K2HPO4 (g/LF)**	**MgSO 4·7H2 O (g/LF)**	**$CaCl_2 \cdot 2H_2O$ (g/LF)**	**Yeast (g/LF)**	**$NaHCO_3$ (g/LF)**
Con.	9.5-38	0.93	0.1	0.03	0.03	0.03	6.2-24.8
Feed Comp.	Trace element (mL/LF)						
Con.	1						
Trace element	**FeCl2·4H2 O**	**MnCl2·4H2 O**	**H3BO3**	**ZnCl2**	**CuCl2**	**AlCl3**	**CoCl2·6 H2O**
Con. (mg/L)	2000	500	50	50	30	50	50
Trace element	**NiCl2**						
Con. (mg/L)	50						

4.2.2 BioStreme characteristics

Supplying different macro and micronutrients is essential to many AD processes since it has been determined that limitation in nutrients cause poor process performance, addition of different nutrient supplements are known to increase the methane production and inhibit the accumulation of VFAs which is one of the main reasons of process failure (Romero-Guiza et al. 2016). BioStreme used in this experiment was manufactured by Ecolo odor technology (ON, Canada) is a mixture of different light and heavy metals in water solution with different concentrations as shown in **Table 4-7**. Besides BioStreme solution a mixture of different vitamins was added as a solution to the process as shown in **Table 4-8**.

Table 4-7 Biosteme composition

Format	Formula
Water	H_2O
Cobalt Sulphate,	$CoSO_4.H_2O$
Ferrous sulphate, heptahydrate	$FeSO_4.7H_2O$
potassium chloride	KCl
Manganese Sulphate	$MnSO_4.H_2O$
Molybdic acid	MoO_3
Nickel (II) Chloride	$NiCl_2.6H_2O$
Zinc sulphate	$ZnSO_4.1H_2O$
sulphuric acid	
Selenius acid	H_2SeO_3
Sodium Tungstate	$Na_2WO_4 2H_2O$
Vanadyl Sulphate	$VO.SO_4.5H_2O$
Vitamin "Boron"	$Na_2B_8O_{13} 4H_2O$

Table 4-8 Vitamin solution composition

component	vitamin	note
Niacin amide	B_3	Water-like solution
Pantothenic	B_5	Water-like solution
D-biotin	B_7	Water-like solution
Folic acid	Group of B	Bright yellow solution
Palmitate	Group of A	Milky solution
Ascorbic acid	C	Water-like solution

4.2.3 AD experimental setup and operation

4 series of experiment were conducted using different concentrations of BioStreme and vitamin solution in different time periods, all 4 series of experiment were operated in mesophilic (37°C) condition as shown in the schematic in **Figure 4-1**, the incubation system was connected to motor controller for determination of agitators. For Biogas production measurement, the system is connected to automatic methane potential test (AMPTs), which contains CO_2-fixing unit which are vails containing alkaline solution that retains other gases except CH_4 that allows to pass through to gas volume measuring device, that digital pulses are produced by certain amount of gas flow which goes through the device and at last control and analysis unit displays the results (Bioprocess Control Sweden, 2016).

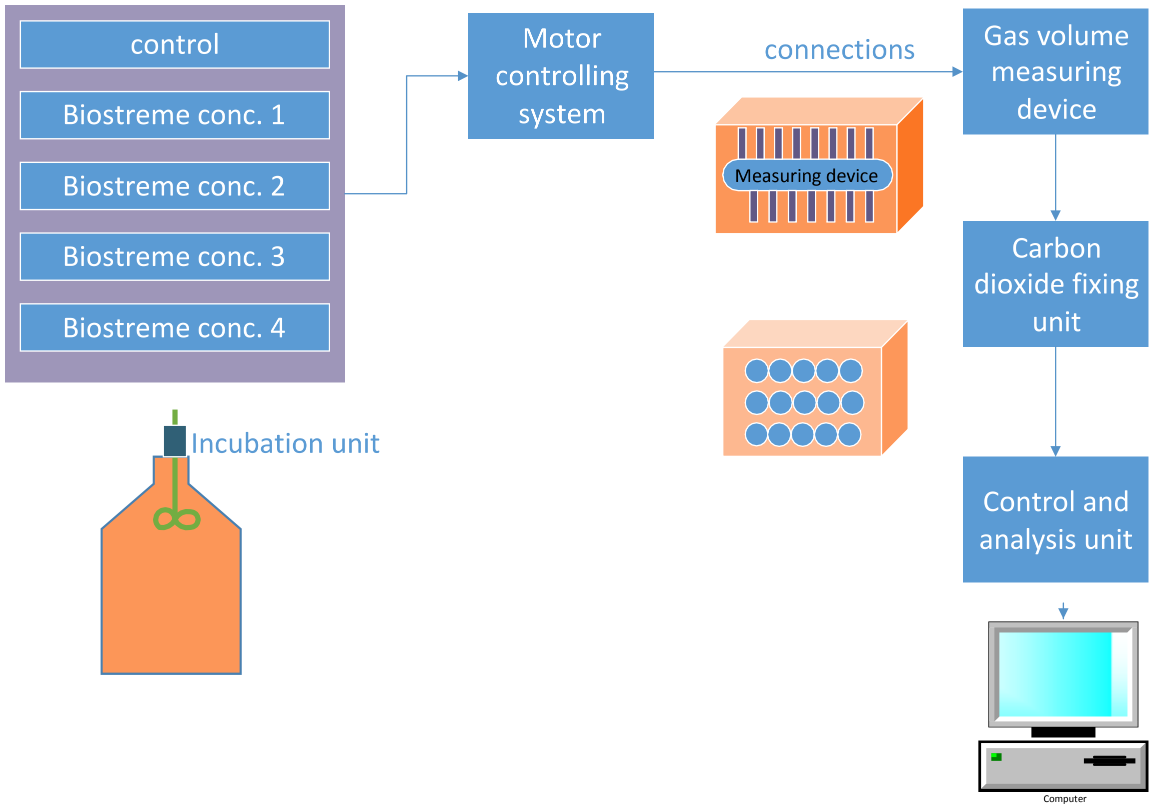


Figure 4-1 Experimental setup configuration

4 series of experiment BioStreme and vitamin solution concentrations are categorized in **Table 4-9**:

Table 4-9 BioStreme and vitamin solution concentrations in experiments

experiment	BioStreme conc.	Vitamin conc.
1	10 ppm 20ppm 50ppm	-
2	20 ppm 50 ppm 100 ppm	-
3	100 ppm	100ppm of BioStreme + 50 ppm of vitamin
	200 ppm	200ppm of BioStreme + 50 ppm of vitamin
4	100 ppm	100ppm of BioStreme + 100 ppm of vitamin
	200 ppm	200ppm of BioStreme + 100 ppm of vitamin
	300 ppm	300ppm of BioStreme + 200 ppm of vitamin

4.2.4 Analysis

Anaerobic batch reactors were used to conduct methane production potential and rate by using biofilm as an effective pathway for microorganism growth. The amount of substrate and the biomass is measured by considering food to microorganism ratio. samples from reactors were collected and diluted and filtered through 0.45 µm, analysis was achieved using standard methods for wastewater examination, total COD and soluble COD, solids (TS, VS, TSS and VSS) and alkalinity in influent and effluent were measured per the standards, pH was measured using pH-meter. The VFA concentration in final samples were measured using gas chromatography (GC) device. Produced Biogas was measured by the bioprocess control system which automatically analyzed methane production of each reactor using the automatic methane potential test system. The measuring data were downloaded on daily basis from the device.

4.2.5 Kinetic modeling

Mathematical modeling of microbial growth has been conducted to measure various parameters, such as the specific growth rate and lag time to investigate microbial growth under different physical and chemical conditions. There are many sigmoidal models available. In this study Gompertz model has been used for modeling which could be applied to microbial growth. The logistic models consider the rate of gas production to be equivalent to microbial activity, as represented by the amount of gas already produced, and to the feeding concentration. The Gompertz model assumption is that the rate of gas production is corresponding to the microbial activity, but the proportionality parameter decreases with time, following first-order kinetics, which can be attributed to the decrease in efficiency of the fermentation rate with time (Altaş 2009). In this study, first cumulative methane production of each case was determined by time, then the Gompertz model was applied to measure the methane production potential (*A*), maximum rate of methane production (μ_m), and the duration of the lag phase (λ).

$Y(t) = A*Exp[-Exp(\mu*e/A(\lambda-t) +1)]$ (Equation 4-1)

4.3 Results and discussion

4.3.1 Operational conditions and AD performance

Micronutrient supplements were added to the substrate in order to increase the process performance and meet the basic nutrient requirements for AD process, the amount of nutrients added was increased by experiment, to analyze the selected micronutrients such as Fe, Ni and Mo which are known to be beneficial to AD process, the concentration increased in order to influence the Methane production, mostly anaerobic sludge contains sufficient amount of nitrogen and phosphorus, however micronutrients such as iron, nickel and cobalt may not be existent in a form available for utilization even though the total concentrations may appear sufficient (Zitomer et al. 2008).

Table 4-10 is the experimental results showing the removal efficiency for COD and VSS and at each experiment with different levels of micronutrients added in the substrate prior to feeding, the resulting experimental durations were from 10 to 45 days for different nutrient concentration levels, since different concentrations, had different timing in affecting the anaerobic reactor performance, the startup time for almost all systems were between 1 to 3 days before starting to produce Biogas.

4.3.2 BioStreme Dosage effect

Several BioStreme dosage were tested, with and without the presence of vitamin, all experiments were carried out with the same inoculum and substrate (real and synthetic waste stream) from the same origin but taken at different times, which can have crucial effect on the results of the experiment. In the first experiment, 3 concentrations of 10, 20 and 50 ppm of BioStreme were mixed with synthetic wastewater prior to feeding and were experimented in triplicates with a control system and a control containing trace-elements for comparison means. Increase in concentration of BioStreme increased the COD removal and VSS removal efficiency, the control system resulted in 62% and 39% of COD and VSS removal while reactors containing 50ppm of BioStreme solution had 90% and 72% of COD and VSS removal respectively, the time duration was only 10 day since after this time the Biogas production reached its stationary phase and no activity was observed in reactors, In the second experiment 3 concentrations of 20, 50 and 100 ppm of BioStreme were studied mixed with synthetic wastewater as well containing to control systems, with and without trace element, in this experiment the average COD removal was lower than the first experiment, which is caused by the reasons mentioned or it may be caused by personal and laboratory errors and differences between two time periods, despite of the differences, increasing the concentration of supplement addition to 100 ppm did not highly effect the COD removal while the COD removal of the reactors containing 20, 50 and 100 ppm of BioStreme were 68, 67 and 66%, however they were all higher compared to the control with only 54% of COD removal efficiency. In the third experimental study, other than BioStreme, vitamin solution was also added to reactors, except for control system, two groups of reactors were spiked with 100 and 200 ppm of BioStreme and 2 other groups were injected with 100ppm and 200ppm of BioStreme with 50ppm of vitamin mixed in substrate, in this study real raw anaerobic wastewater was used as feedstock. The results showed a low difference in COD removal comparing to the conventional system, however the VSS removal was as high as 66% in both reactors with 200ppm of BioStreme with and without vitamin, while the control system had 49% of VSS removal. Mixture of vitamin and BioStreme in this experiment didn’t specifically showed the effect of vitamin on AD process performance, therefore in the fourth experiment other than BioStreme and mixture of BioStreme and vitamin, vitamin concentration was also studied solely, in this experiment real wastewater was fed as substrate, the results for COD and VSS removal had fairly low difference however the results show that high concentration of BioStreme between 100 and 300ppm increases the removal efficiencies, however there is not a linear relation between increasing the concentration over 100 and higher removal efficiencies, vitamin addition had positive results on COD removal but it showed poor results in VSS removal comparing to the conventional process.

Table 4-10 Removal efficiency of different micronutrient concentration addition

experiments	Time duration (day)	BioStreme concentration added (ppm)	Vitamin concentration added (ppm)	COD removal efficiency	VSS removal efficiency
1	10	10		88%	68%
		20	-	90%	66%
		50		90%	72%
2	28	20		68%	74%
		50	-	67%	69%
		100		66%	71%
3	45	100		91%	61%
		200		88%	62%
		100	50	83%	66%
		200	50	86%	66%
4	25	100	-	92%	45%
		200	-	90%	52%
		300	-	91%	48%
		-	100	90%	37%
		-	200	92%	39%
		100	100	89%	37%
		300	200	91%	47%

4.3.3 Process stability

Process stability is defined by VFA, VFA to alkalinity ratio and pH, using two different substrate sources for series of experiment shows differences in these parameters, however adding nutrient supplement in different concentration to both synthetic and real waste streams didn’t result in process failure because of inhibition caused by excessive metal ion concentration in BioStreme, with a value of less than 0.5 for VFA to alkalinity ratio for all reactors in series of experiment shown if **Figure 4-2** which indicated there has been no process inhibition or acidification, and also the pH value range of 7.3 to 7.9 shown in **Table 4-11** during operation shows that there has been a stable process, on the other hand in the first two experiment using synthetic wastewater, all reactors were stable but the reactors containing nutrient supplement produced Biogas for a longer period of time compared to the control system.

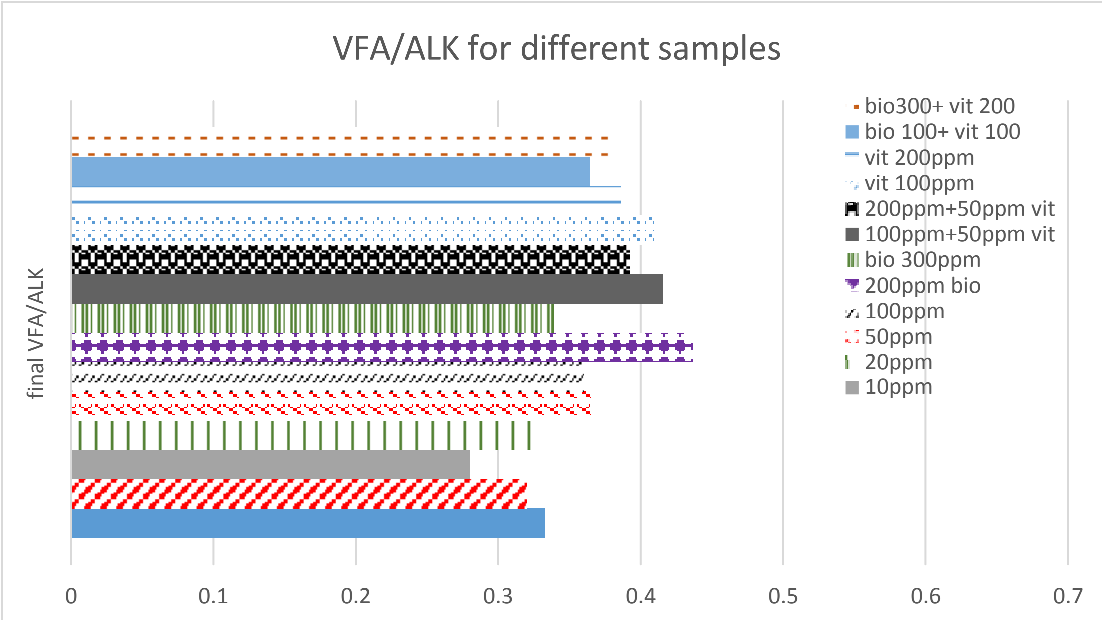


Figure 4-2 VFA/ALK for different nutrient concentration

Table 4-11 VFA/ALK, pH and VFA conc. for different supplement concentrations

experiments	Time duration (day)	BioStreme concentration added (ppm)	Vitamin concentration added (ppm)	VFA Conc. g/l	pH value	VFA/Alk.
1	10	-		2.03	7.53	0.25
		10	-	1.99	7.50	0.28
		20		2.24	7.52	0.33
		50		2.60	7.53	0.4
2	28	-		2.42	7.66	0.34
		20	-	2.51	7.70	0.32
		50		2.67	7.70	0.33
		100		2.22	7.72	0.3
3	45	-		2.77	7.35	0.41
		100		2.69	7.39	0.40
		200	50	2.71	7.39	0.42
		100	50	2.85	7.36	0.43
		200		2.67	7.34	0.39
4	25	-	-	2.84	7.37	0.33
		100	-	2.71	7.47	0.39
		200	-	3.33	7.41	0.45
		300	-	2.56	7.39	0.34
		-	100	3.00	7.44	0.41
		-	200	2.91	7.45	0.39
		100	100	2.73	7.41	0.36
		300	200	2.86	7.42	0.38

4.3.4 Methane production

BioStreme addition was tested in order to evaluate the potential effect of micronutrients on removal efficiencies and its contribution to the total methane produced, **Error! Reference source not found.** show the cumulative methane production for batch tests. In regards to the application of micronutrient addition to AD process, for synthetic feedstock methane production increased with all BioStreme concentration addition compared to control system, however utilizing real wastewater resulted in diverse effect of nutrient on methane production, addition of BioStreme solely in concentrations higher than 100 ppm, resulted in higher methane production, while vitamin addition didn't follow any specific trend as shown in the **Error! Reference source not found.**, there are dosages of solutions that adversely affected methane production, mixture of 200 ppm of BioStreme and 50 ppm of vitamin resulted in the highest methane production compared to other mixtures used, the figures show that addition of metals didn't negatively affect the lag time of methane production.

4.3.5 Effect of BioStreme and vitamin solution on methanogenic activity

The cumulative methane production of different concentrations of BioStreme and vitamin has been conducted in **Error! Reference source not found.**, the cumulative methane production values are available in **Table 4-12**, showing the difference in methane production content and lag times of different experiments, also as shown certain nutrient dosages boosted the methane production, for understanding the effect of nutrient supplement on methanogenic activity, the experimental data were fitted to Gompertz model equation (4-2) using non-linear regression in excel.

$$Y(t) = A*Exp[-Exp(\mu*e/A(\lambda-t) +1)] \quad (4\text{-}2)$$

Table 4-12 Gompertz modeling results for nutrient concentrations

Exp.		cumulative Methane production	A(Nml)	μ (d^{-1})	λ (day)	λ(hr.)
1	control	137	126.9	123.5	0.12	2.88
	10s	141.3	131.77	113.03	0	0
	20s	140.4	134.8	118.14	0.1	2.4
	50s	154.3	132.76	110.99	0.09	2.16
2	control	159.6	141.4	139.7	0.12	2.88
	20s	189.6	167.4	121.6	0.02	0.48
	50s	187.7	171.4	121.3	0.01	0.24
	100s	192.5	175.4	75.8	0	0
3	control	1651.9	1743.78	89.22	4.46	107.04
	100s	1799.6	1832.18	104.31	4.4	108
	200s	1736.5	1854.51	62.7	2.66	63.84
	100+50	1579.9	1608.9	78.4	2.93	70.32
	200+50	1826.8	1838.06	94.91	4.32	103.68
4	control	1476.6	1404.8	164.9	4.54	108.96
	100ppm	1390.8	1440.59	145.75	3.95	94.8
	200ppm	1408.3	1450.57	152.23	3.54	84.96
	300ppm	1468.1	1473.94	153.58	3.6	86.4
	100vit	1350.8	1443.5	104.74	4.43	106.32
	200vit	1503	1695.1	110.62	2.74	65.76
	100ppm+100ppm	1511	1565.9	150.9	4.5	108
	300ppm+200ppm	1382.8	1421.5	157.03	4.2	100.8

Since the experiments had different time durations, the parameters and cumulative methane productions are different, for the first experiment the values of methane production potential (*A*, mL), maximum specific methane production rate (μ, 1/day), and lag-phase duration (ƛ, h) were showing that:

- In each experiment addition of BioStreme and vitamins to the system, increased the methane production potential compared to the conventional system.
- Addition of supplements positively affected the process lag time by decreasing the time needed for starting the methane production.
- Increasing the dosage of the BioStreme and addition of vitamin didn't necessarily boost the potential in methane production.

The results show that in the first two experiment using synthetic feed, different concentrations of BioStreme, increased the Biogas production potential and decreased the lag phase period, however in the 3rd and 4th experiment using real waste as feedstock and mixture of BioStreme and vitamin showed few fluctuations, but overall it resulted in higher Biogas production potential and lower lag phases, 100ppm BioStreme with 50 ppm vitamin mixture had lower Biogas production potential compared to control system, which may be because of clogging occurred in Biogas measurement tubes during the experiment.

4.4 Conclusion

The results show that micronutrient addition to AD process, enabled stable operation and relatively higher methane production, the addition of selected nutrients to synthetic wastewater as a batch process, increased the process performance, however it seems like real wastewater already contained the required nutrients, however continuous addition of nutrient to the process rather than a batch process may increase the process performance and methane yield in a more obvious way.

Chapter 5 Enhancement of dark fermentation process using micronutrient supplement addition (BioStreme and Vitamin solution)

5.1 Introduction

Nowadays, the world energy needs are provided highly by fossil fuels which will eventually result in fossil fuel deficiency, other than that utilization of this energy source, highly increases the emission by COx, NOx, SOx, ashes, tars and other organic compounds (Das 2001), In order to overcome the problems caused by overexploitation of fossil fuels, new energy sources have been developed, hydrogen as one of the cleanest with high energy conversion has attracted a lot of attention, there are different methods available for hydrogen production from chemical to physical and biological methods, biological methods have more of an interest since they are environmentally friendly with less need of energy requirement, (LIN 2008). hydrogen is the most abundant element available and the lightest element, it is a safe energy source to people and environment, today because of sharp increase in population and higher energy demands, utilizing hydrogen as an alternative energy source is a satisfying option (Das 2001), however the main problem right now is that most of the hydrogen utilized it produced from fossil fuels, production of hydrogen from biological methods are exiting ways to develop the potential of hydrogen production, there are different biological methods such as bio-photolysis, indirect bio-photolysis, photo-fermentations, and dark-fermentation (Levin et al. 2004). dark fermentation as one of the methods have high potential both to decrease the waste disposal problems and to produce high potential energy sources from waste, this process is a biological process that transforms organic substrates to Biogas containing mostly hydrogen and carbon dioxide by strictly anaerobic bacteria and also facultative anaerobes in absence of Oxygen (Lyberatos 2010) in different temperature ranges, from mesophilic condition to hyper-thermophilic temperatures (Levin et al. 2004), There are different pathways for hydrogen production in dark fermentation process, in one pathway acetic acid is the end product, in which four moles of H_2 is produced per 1 mole of glucose used, but in another pathway butyrate is the end product which 2 moles of hydrogen is produced per 1 mole of glucose used (Levin et al. 2004). Different substrate sources can be used for this process however, carbohydrate, cellulose and starch based feedstock are most preferred (Lyberatos 2010).

with all the advantages that this process offers, dark fermentation produces a mixture of mostly H_2, CO_2 but also contains CH_4, CO and H_2S in less amounts, the main challenge is the gas composition (Levin et al. 2004), also dark fermentation can be used to produce hydrogen and treat different waste streams, seed sludge that can be derived from different sources such as sewage sludge, anaerobically digested sludge, acclimated sludge, compost, animal manure and soil (Lyberatos 2010) has a wide variety of microorganism community present which may be beneficial for easier adaption to environmental changes and shocks, and also to increase the substrate degradation but on the other hand, waste streams contain both H_2-consuming and H_2- producing bacteria at the same time, therefore different enhancement methods such as pretreatment from physicochemical to biological can be used to eliminate the H_2- consuming organisms (Meng et al. 2014).

There has been studies on different factors to understand the limiting factors in dark fermentation process, some studies have focused on activity of hydrogen producing enzymes however there hasn’t been any absolute evidence for this matter, metal ions are known to be effective on cell growth operating as enzyme factors, the most important metals that effect the dark fermentation process are Mg, Na, Zn and Fe in which magnesium had the highest influence by activating over 10 enzymes such as hexokinase, phosphofructokinase and phosphoglycerate kinase (Chong et al.

2009), also there has been study on effect of Iron in different concentrations on hydrogen production in dark fermentation process which resulted in higher H_2 production (Zhang et al. 2005) also in another study it was found that in lower than ambient temperature more concentrations of Iron is needed in order to activate the Hydrogenase by bacteria for oxidation of reduced ferredoxin to produce molecular hydrogen (Zhang & Shen 2006).

In this study, the effect of BioStreme as a nutrient supplement which is a mixture of different heavy and light metals in water solution, and also vitamin solution in different concentrations was investigated on hydrogen production in dark fermentation process.

5.2 Materials and methods

5.2.1 Seed sludge and substrate

anaerobically seed sludge from Humber treatment plant, ON, Canada was used as the seed, the sludge was preheated to 70°C for 30 minutes prior to use, the Error! Reference source not found. shows the characteristics of seed sludge, 15 reactors were experimented in parallel, the systems were seeded with 500 ml of sludge and started up in a batch mode with the feed containing glucose with the composition: glucose 32 g/l, $NaHCO_3$ 2-16 g/l, $CaCl_2$ 140 mg/L, $MgCl_2.6H_2O$ 160 mg/L, NH_4HCO_3 600 mg/L, $MgSO_4.7H_2O$ 160 mg/L, Urea 500-2000 mg/L, Na_2CO_3 124-300 mg/L, $KHCO_3$ 156 mg/L, K_2HPO_4 15-20 mg/L, H_3PO_4 250-1500 mg/L and trace solution 500 mg/L, therefore they were fed once at the beginning of the experiment and sparked with nitrogen gas and sealed with caps for providing the anaerobic condition, the nutrient solutions were mixed with the substrate prior to feeding process

Table 5-1 Biomass characterization

	Mesophilic biomass
TS (mg/L)	6867
VS (mg/L)	1955
TCOD (mg/L)	4550
SCOD (mg/L)	2500
pH	5.5

5.2.2 BioStreme Concentration

Supplying different macro and micronutrients is essential to many AD processes since it has been determined that limitation in nutrients cause poor process performance, addition of different nutrient supplements are known to increase the methane production and inhibit the accumulation of VFAs which is one of the main reasons of process failure (Romero-Guiza et al. 2016). BioStreme used in this experiment manufactured by Ecolo odor technology, ON, Canada, is a mixture of different light and heavy metals in water solution with different concentrations as shown in Error! Reference source not found.. Besides BioStreme solution a mixture of different vitamins was added as a solution to the process shown in **Table** .

Table 5-2 BioStreme composition

Format	Formula
Water	H_2O
Cobalt Sulphate,	$CoSO_4.H_2O$
Ferrous sulphate, heptahydrate	$FeSO_4.7H_2O$
potassium chloride	KCl

Manganese Sulphate	$MnSO_4.H_2O$
Molybdic acid	MoO_3
Nickel (II) Chloride	$NiCl_2.6H_2O$
Zinc sulphate	$ZnSO_4.1H_2O$
sulphuric acid	
Selenius acid	H_2SeO3
Sodium Tungstate	$Na_2WO_4.2H_2O$
Vanadyl Sulphate	$VO.SO_4.5H_2O$
Vitamin "Boron"	$Na_2B_8O_{13}.4H_2O$

Table 5-3 Vitamin solution composition

component	vitamin	note
Niacin amide	B_3	Water-like solution
Pantothenic	B_5	Water-like solution
D-biotin	B_7	Water-like solution
Folic acid	Group of B	Bright yellow solution
Palmitate	Group of A	Milky solution
Ascorbic acid	C	Water-like solution

5.2.3 Batch setup and operation

15 lab scale batch systems were operated in 37°C for 7 days, with different BioStreme concentrations, as the schematic in Error! Reference source not found. shows, the incubation system was connected to motor controller for determination of agitators. For Biogas production measurement, the system is connected to automatic methane potential test (AMPTs), which contains CO_2-fixing unit which are vails containing alkaline solution that retains other gases except CH_4 that allows to pass through to gas volume measuring device, that digital pulses are produced by certain amount of gas flow which goes through the device and at last control and analysis unit displays the results (Bioprocess Control Sweden n.d.). by the previous batch experiments

conducted, volume of substrate and seed were calculated based on a substrate to biomass (S°/X°) ratio of 1.2 gCOD/gVSS using the equation below (5-1) (Nasr et al. 2014):

$$S°/X° = \frac{Vsub(L)*TCODeq(g/L)}{\text{[illegible]}} \quad \text{(Equation 5-1)}$$

In which V_{sub} and V_{seed} represent the substrate and seed volume respectively, $TCOD_{eq}$ is the TCOD equivalent for different ratios of missing volumes of waste-stream. The initial pH of all bottles was adjusted to 5-5.5 using HCl.

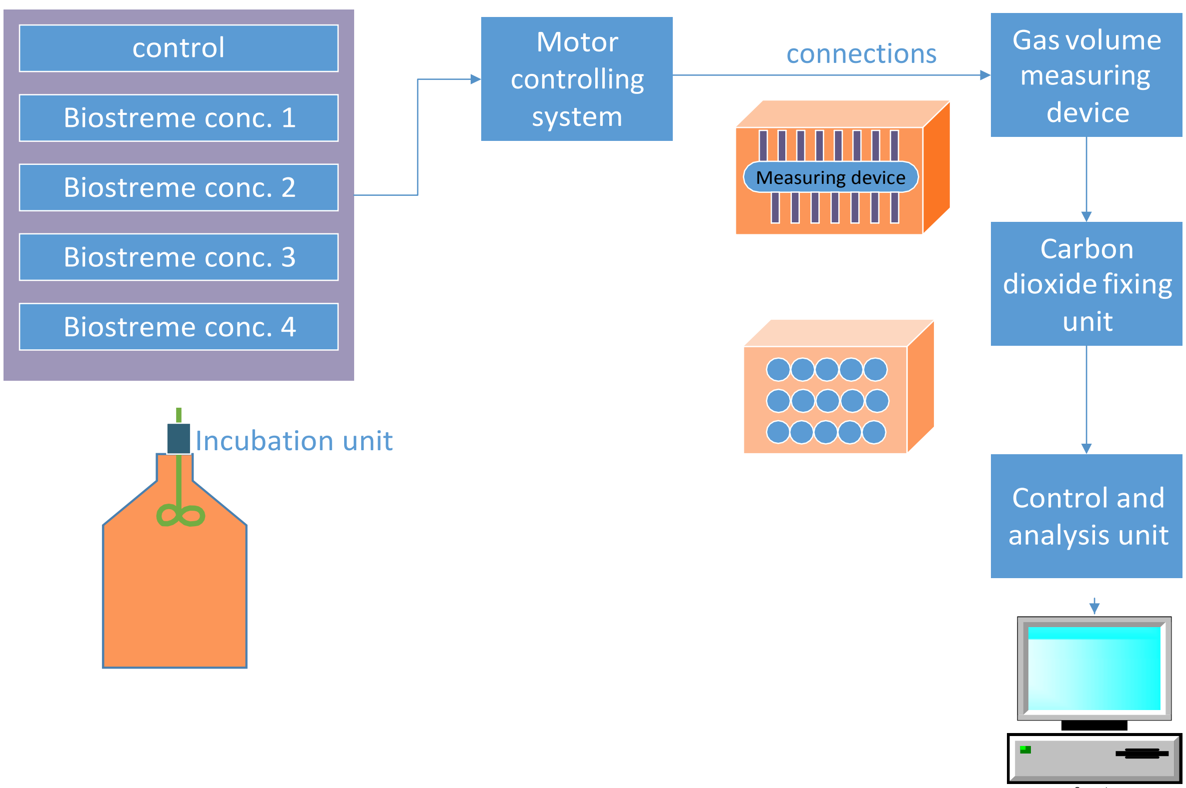


Figure 5-1 Experimental system configuration

4 different concentrations of BioStreme and vitamin were used as shown in Error! Reference source not found., with a control system for comparison means.

Table 5-4 Different concentrations of BioStreme and vitamin used in each experiment

Reactor groups	BioStreme conc. (ppm)	Vitamin conc. (ppm)
control	-	-
1	100	-
2	200	-
3	300	-
4	100	100

5.2.4 Analysis

The Biogas production composition including hydrogen, methane and CO_2 was measured by AMPTS device and the Hydrogen production was determined by a gas chromatograph SRI 8610C equipped with a thermal conductivity detector (TCD) and a molecular sieve column, nitrogen was used the carrier gas, the temperature for column and TCD was set 80°C. the concentration of VFAs were also analyzed using a gas chromatograph with a flame ionization detector (FID) using a fused silica column MXT-WAX, for this measurement helium is used as the carrier gas, the temperature of the column and detector were set at 200°C, total COD and soluble COD, solids (TS, VS, TSS and VSS) and alkalinity in influent and effluent were measured per the standards, pH was measured using pH-meter.

5.3 Result and discussion

5.3.1 Process performance and operational conditions

Micronutrient supplements were added to the substrate in order to increase the process performance and meet the basic nutrient requirements for dark fermentation process, the amount of nutrients addition was increased, to analyze the selected micronutrients such as Fe, Ni and Mo which are known to be beneficial to anaerobic process. The concentration increased in order to influence the hydrogen production, mostly anaerobic sludge contains sufficient amount of Nitrogen and phosphorus, however micronutrients such as Iron, Nickel and Cobalt may not be existent in a form available for utilization even though the total concentrations may appear sufficient (Zitomer et al. 2008).

Table 4-5 shows the results for COD and VSS removal efficiency in each BioStreme and vitamin dosage which was mixed with the substrate prior to feeding, the experiment duration was 48-72 hours.

5.3.2 BioStreme dosage effect

Several BioStreme dosages were tested with or without the presence of vitamin, all experiments were carried out using the same source of substrate and inoculum, in the experiment 3 different concentrations of BioStreme 100, 200 and 300ppm and also a mixture of 100ppm of BioStreme and 100ppm of vitamin were tested, all experiments run in triplicates with a control system for comparison means. As shown, increase in the concentration of BioStreme and also adding vitamin increased the COD and VSS removal of the system, however the COD removal for all reactors were rather low without any known reasons, addition of nutrient supplement in any concentration has positive effect on both COD and VSS removal compared to the conventional system.

Table 5-5 Removal efficiencies of different experiments

Reactor type	BioStreme conc. (ppm)	Vitamin conc. (ppm)	COD removal	VSS removal
control	-	-	14%	24%
1	100	-	39%	33%
2	200	-	46%	44%
3	300	-	40%	44%
4	100	100	55%	49%

5.3.3 process stability

Process stability is defined by VFA, VFA to alkalinity ratio and pH, , adding nutrient supplement in different concentration to synthetic waste streams didn't result in process failure because of inhibition caused by excessive metal ion concentration in BioStreme, with a value of less than 1.8 for VFA to alkalinity ratio for all reactors in series of experiment shown in **Error! Reference source not found.** which indicated there has been no process inhibition or acidification, and also the pH value range of 4.5 to 5.5 shown in Error! Reference source not found. during operation shows that there has been a stable process except for one of the control reactors that had a pH value of 3 which resulted in process failure and VFA acclimation in the system, the data from this reactor was eliminated from the study, VFA concentration ranges from 0.5 to 0.9 g/l for all reactors shown in Error! Reference source not found. and **Figure** .

Table 5-6 VFA, pH and VFA/ALK ratio of each experiment

Reactor type	BioStreme(ppm)	Vitamin(ppm)	VFA (mg/L)	pH	VFA/ALK
control	-	-	925.5	4.4	1.4
1	100	-	806.3	4.9	1.2
2	200	-	1016.6	5.0	1.2
3	300	-	1045	4.8	1.8
4	100	100	940	5.1	1.1

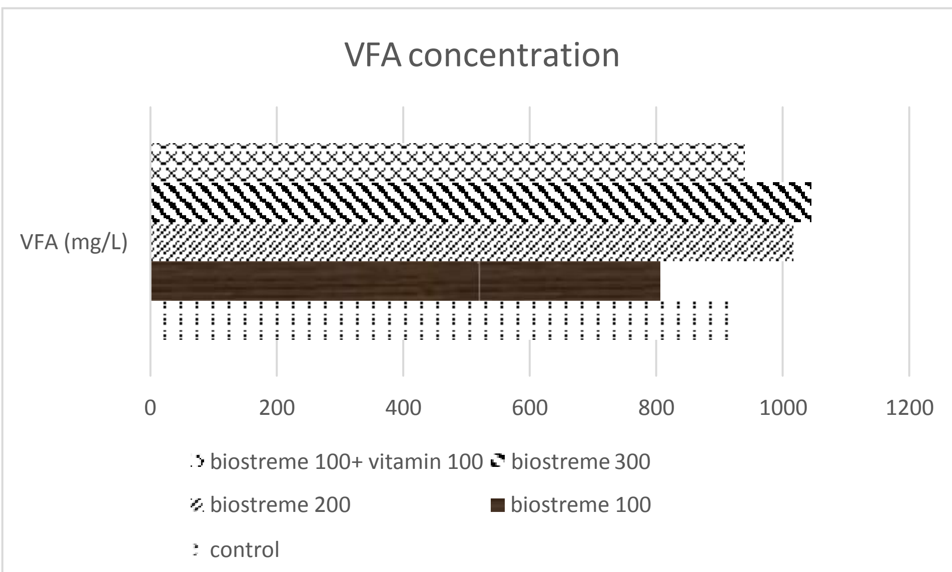


Figure 5-2 VFA concentration of each experiment

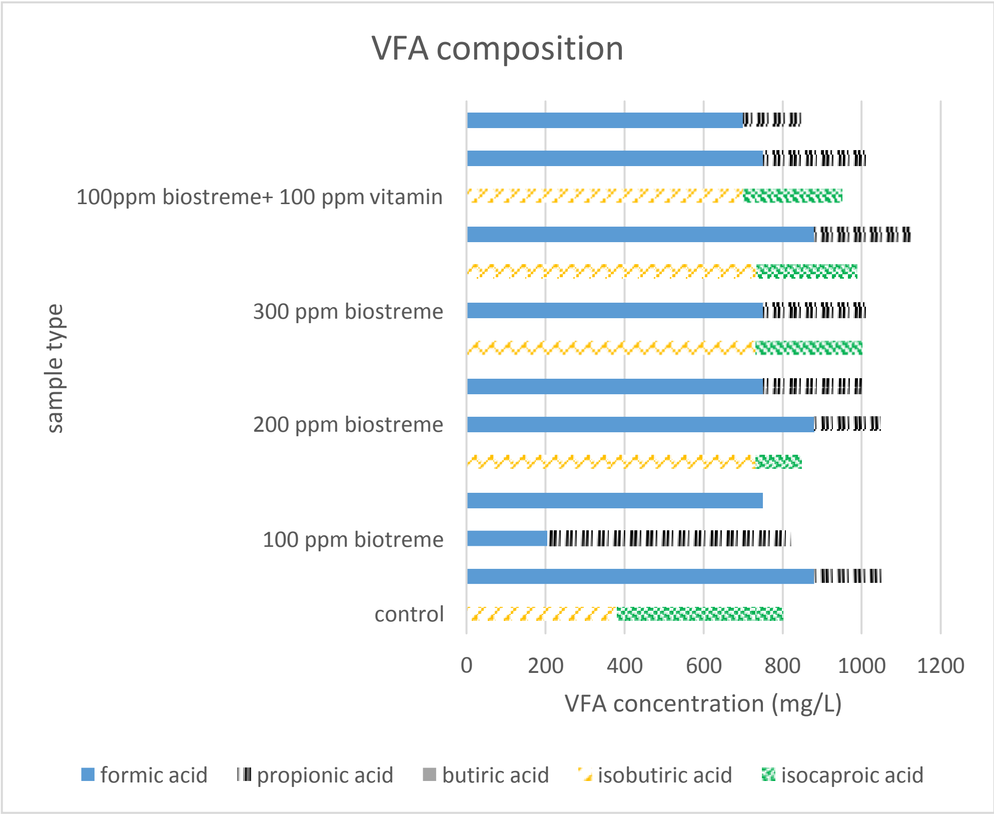


Figure 5-3 VFA composition of each experiment

5.3.4 Hydrogen production

as mentioned through the dark fermentation process, hydrogen is produced in this experiment, hydrogen production was monitored in order to indicate the optimal dosage of BioStreme and vitamin added to the process, hydrogen production activity (A_h) was measured to understand the efficacy degree of different nutrient dosages on hydrogen production.

A_h (%)= H_m/H_c*100% (Equation 5-2)

where H_m denotes the amount of Hydrogen produced in 48 to 72 h, using BioStreme and vitamin dosed to feedstock. H_c denotes the amount of hydrogen produced at 48 to 72h by the control (LIN 2008).

Figure 5-4 shows the hydrogen production from control system and micronutrient supplemented systems, to indicate the effect of nutrient supplementation, the cumulative hydrogen production was retained from the total Biogas production amount with CO_2 being eliminated by CO_2 fixing unit. Differences in hydrogen production is observed using different dosages of BioStreme and vitamin. For different dosages of BioStreme solely, the similar trend was observed, however increasing the concentration of micronutrient addition resulted in less methane production. Error! Reference source not found. shows the relation between hydrogen activity (A_h) and micronutrient concentration, as indicated A_h is dependent to micronutrient dosage, all A_h values for all dosages of supplements exceeded 100% meaning stimulation in hydrogen production occurred and no metal toxicity happened in any concentration, however increasing the BioStreme concentration or addition of Vitamin solution to the process decreased the hydrogen production activity.

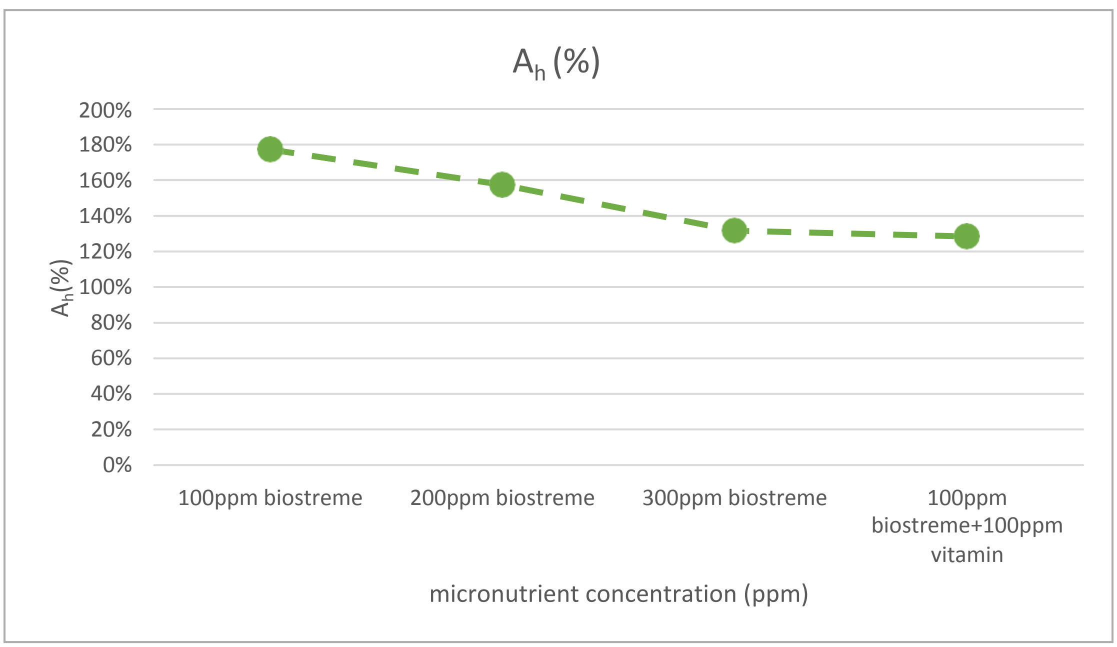


Figure 5-3 Effect of micronutrient dosage on A_h

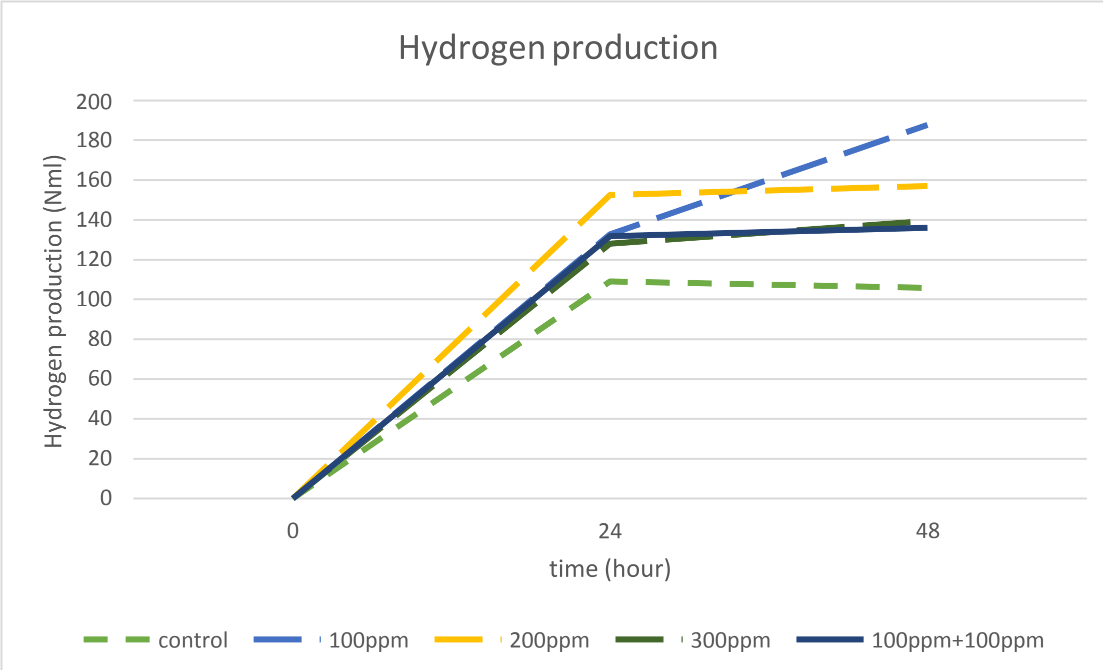


Figure 5-4 Cumulative Hydrogen production for each experiment

5.3.5 Kinetic analysis

The hydrogen production potential, maximum hydrogen production rate (HPR) and lag phase time were elucidated using the modified Gompertz equation (5-3), that has been used to describe the progress of cumulative hydrogen production obtained from a batch experiment

$H(t)=P*\exp(-\exp((R_m*e/P)*(\lambda-t)+1))$ (Equation 5-3)

where H(t) is the cumulative hydrogen production (mL), P is the hydrogen production potential (mL), R_m is the maximum hydrogen production rate (mL/h), e is the 2.71828, λ is the lag-phase time (h), t is the time (h). The cumulative hydrogen production of different concentrations of BioStreme and vitamin has been conducted in **Figure 5-4**, the cumulative hydrogen production values are available in Error! Reference source not found., showing the difference in hydrogen production content and lag times of different dosages, as shown higher nutrient dosages boosted the hydrogen production, for understanding the effect of nutrient supplement on methanogenic activity, the experimental data were fitted to Gompertz model using non-linear regression in excel.

Table 5-7 Kinetic parameters for each experiment

	cumulative Hydrogen production	P(Nml)	R_m(Nml/h)	Y(h)
control	109.9	109.5	60	6
BioStreme 100ppm	187.7	160.18	60	4
BioStreme 200ppm	166.6	161.91	60	4.4
BioStreme 300ppm	139.4	133.87	60	5
BioStreme 100ppm+ vitamin 100ppm	136	133.8	60	5

Since all dosages were experimented in the same time duration results can be compared with each other, the hydrogen production potential was BioStreme 200ppm> BioStreme 100ppm> BioStreme 300ppm> BioStreme 100ppm+ vitamin 100ppm> control which shows that increasing the dosage higher than 300ppm and addition of vitamin to nutrient supplement decreases the potential in hydrogen production, however all systems supplemented with BioStreme and vitamin had higher hydrogen production and lower lag phase compared to the control system. At the end of experiment, when the hydrogen production decreases, the hydrogen content of each process was measured using gas chromatography and the results showed that all reactors containing BioStreme had higher hydrogen content compared to control system, in addition the reactors supplied with 100 and 200ppm of BioStreme had twice hydrogen production than control system, however with increasing the dosage and adding the vitamin the hydrogen percentage decreased.

5.4 Conclusion

The results show that addition of micronutrient to dark fermentation process, enabled higher process efficiency and Biogas production with higher hydrogen content, the process was done as a batch system with only one spike of micronutrient along with feeding, however continuous feeding with micronutrient addition may result in higher process performance compared to batch system.

Chapter 6 Conclusion and future work

This chapter summarizes the major findings of this thesis along with the direction of future work

6.1 Conclusions

In this thesis, the aim was to evaluate enhancement methods for boosting AD process for higher Biogas production and removal efficiency as well as higher stability in performance, two main enhancement methods have been developed, the main findings are:

- Attached media usage in AD process increased the Methane production and process performance, four different BioCords were used, and all four groups resulted in higher efficiency compared to the conventional system.
- BioCord LS_2 showed the highest results with 30% higher Methane production compared to the control system and 10-15% higher than other BioCords.
- BioCord LS_2 had the highest COD and VSS removal of 88% and 61% respectively comparing to other attached media groups as well as the control system.
- Usage of BioCord as an improvement method is a rather easy and inexpensive way since there is no need for change in reactor configuration.
- Another method for improving AD is supplement addition, usually the process lacks in the micronutrient concentration, however using the proper number of additives is critical since inhibition may occur with excessive amount of added elements.
- Addition of supplement named BioStreme and vitamin solution increased the COD and VSS removal efficiency, moreover increasing the concentration of additives seems to boost the process performance.

- Increasing the concentration of BioStreme up to 300 ppm increased the cumulative methane production, however addition of vitamin showed fluctuation in the results.
- Addition of BioStreme to the process enhanced the kinetics of the process, it increased the methane production potential and it decreased the lag time, the results were boosted by increasing the dosage.
- Since addition of BioStreme had beneficial effect on methane production form AD process it has been tested to indicate whether it can boost the hydrogen production using the dark fermentation process, with the same anaerobic bacteria groups.
- Addition of BioStreme and vitamin solution increased the hydrogen production and the process performance both in removal efficiency and the system stability.
- Despite of an increase in process performance in AD process by addition of higher dosage of supplement, increasing the concentration of BioStreme and vitamin to dark fermentation process for hydrogen production decreased the process performance and hydrogen production.
- 100 ppm of BioStreme seem to be the optimal range of BioStreme addition with twice as much hydrogen content compared to the control system.

6.2 Direction of future work

Considering the positive effect of enhancement methods on batch AD and dark fermentation process for methane and hydrogen production respectively, future works can be further experimented for improving the processes, several facts can be pointed out:

- Performing continuous AD process, to evaluate the process performance with BioStreme added substrate.

- Addition of BioStreme to substrate several hours prior to feeding to provide the time for adaption and also for the bacteria to utilize the trace elements for boosting the hydrolysis stage.

Bibliography

Abarca, L., Maas, G. & Hogland, W., 2013. Solid waste management challenges for cities in developing countries. *Waste Management*, 33(1), pp.220–232. Available at: http://dx.doi.org/10.1016/j.wasman.2012.09.008.

Acharya, B.K., Mohana, S. & Madamwar, D., 2008. Anaerobic treatment of distillery spent wash - A study on upflow anaerobic fixed film bioreactor. *Bioresource Technology*, 99(11), pp.4621–4626.

Adu-gyamfi, N., Rao, S. & Hobbs, P.J., 2012a. Bioresource Technology Optimizing anaerobic digestion by selection of the immobilizing surface for enhanced methane production. *BIORESOURCE TECHNOLOGY*, 120, pp.248–255. Available at: http://dx.doi.org/10.1016/j.biortech.2012.06.042.

Adu-gyamfi, N., Rao, S. & Hobbs, P.J., 2012b. Bioresource Technology Optimizing anaerobic digestion by selection of the immobilizing surface for enhanced methane production. *BIORESOURCE TECHNOLOGY*, 120, pp.248–255.

Ahring, B.K. et al., 1991. Effects of cations on Methanosarcina thermophila TM-1 growing on moderate concentrations of acetate: production of single cells. *Applied Microbiology and Biotechnology*, 35(5), pp.686–689.

Akao, T. et al., 1992. The methane fermentation of Citrus unshu peel pretreated with fungus enzymes. *Bioresource Technology*, 41(1), pp.35–39.

Altaş, L., 2009. Inhibitory effect of heavy metals on methane-producing anaerobic granular sludge. *Journal of Hazardous Materials*, 162(2-3), pp.1551–1556.

Amani, T., Nosrati, M. & Sreekrishnan, T.R., 2010. Anaerobic digestion from the viewpoint of microbiological, chemical, and operational aspects — a review. *Environmental Reviews*, 18(NA), pp.255–278.

Andersson, J. & Björnsson, L., 2002. Evaluation of straw as a biofilm carrier in the methanogenic stage of two-stage anaerobic digestion of crop residues. *Bioresource Technology*, 85(1), pp.51–56.

Angelidaki, I., Petersen, S.P. & Ahring, B.K., 1990. Effects of lipids on thermophilic anaerobic digestion and reduction of lipid inhibition upon addition of bentonite. *Applied Microbiology and Biotechnology*, 33(4), pp.469–472.

Anon, 1995_-_M_Kayhanian_-_Pilotscalehighsolidsthermophilicanaerobicdigestion[retrieved-2017-05-29].pdf.

Anon, Attached film media performance in psychrophilic anaerob[retrieved-2016-11-09].pdf.

Anon, Ruiz Treatment of slaughterhouse wastewater in a UASB reactor.pdf.

Appels, L. et al., 2011. Anaerobic digestion in global bio-energy production: Potential and research challenges. *Renewable and Sustainable Energy Reviews*, 15(9), pp.4295–4301. Available at: http://linkinghub.elsevier.com/retrieve/pii/S1364032111003686.

Appels, L. et al., 2008. Principles and potential of the anaerobic digestion of waste-activated sludge. *Progress in Energy and Combustion Science*, 34(6), pp.755–781.

Azizi, S. & Sithebe, N.P., 2015. Use of Electro-Chemical Process for Waste Sludge Generated from Moving Bed Bioreactor. *International Journal of Environmental Science and Development*, 6(8), pp.585–589. Available at: http://www.ijesd.org/index.php?m=content&c=index&a=show&catid=68&id=1017.

Barca, C. et al., 2015. Anaerobic biofilm reactors for dark fermentative hydrogen production from wastewater: a review. *Bioresource Technology*, 185, pp.386–398. Available at: http://linkinghub.elsevier.com/retrieve/pii/S0960852415002424.

Biofilms, A.A., 1995. (A)? =. , 45(c), pp.212–218.

Bioprocess Control Sweden, AMPTS II & AMPTS II Light Automatic Methane Potential Test System Operation and Maintenance Manual.

Biotech, E., *No Title*,

Bories, A., Raynal, J. & Bazile, F., 1988. Anaerobic digestion of high-strength distillery wastewater (cane molasses stillage) in a fixed-film reactor. *Biological Wastes*, 23(4), pp.251–267.

Borja, R. & Banks, C.J., 1995. Comparison of an Anaerobic Filter and an Anaerobic Fluidized Bed Reactor Treating Palm Oil Mill Effluent. *Process Biochemistry*, 30(6), pp.511–521.

Borja, R. & Banks, C.J., 1995. Response of an anaerobic fluidized bed reactor treating ice-cream wastewater to organic, hydraulic, temperature and pH shocks. *Journal of Biotechnology*, 39(3), pp.251–259.

Bouallagui, H. et al., 2005. Bioreactor performance in anaerobic digestion of fruit and vegetable wastes. *Process Biochemistry*, 40(3-4), pp.989–995.

Bougrier, C., Delgenès, J.P. & Carrère, H., 2008. Effects of thermal treatments on five different waste activated sludge samples solubilisation, physical properties and anaerobic digestion. *Chemical Engineering Journal*, 139(2), pp.236–244.

Bougrier, C., Delgenès, J.P. & Carrère, H., 2007. Impacts of thermal pre-treatments on the semi-continuous anaerobic digestion of waste activated sludge. *Biochemical Engineering Journal*, 34(1), pp.20–27.

Bryers, J.D., 1982. Processes Governing. , XXIV, pp.2451–2476.

Buekens, A., 2005. Energy recovery from residual waste by means of anaerobic digestion technologies. *The future of residual waste management in Europe 2005*, pp.1–15.

Buffiere, P., Moletta, R. & Elmaleh, S., 1998. ANAEROBIC DIGESTION OF WINE DISTILLERY WASTEWATER IN DOWN-FLOW FLUIDIZED BED. , 32(12), pp.3593–3600.

Chai, S. et al., 2014. Anaerobic treatment of winery wastewater in moving bed biofilm reactors. *Desalination and Water Treatment*, 52(10-12), pp.1841–1849.

Chen, W., 2006. Biological hydrogen production by anaerobic fermentation by.

Chen, Y., Cheng, J.J. & Creamer, K.S., 2008. Inhibition of anaerobic digestion process: A review. *Bioresource Technology*, 99(10), pp.4044–4064. Available at: http://linkinghub.elsevier.com/retrieve/pii/S0960852407001563.

Chen, Y., Cheng, J.J. & Creamer, K.S., 2008. Inhibition of anaerobic digestion process: a review. *Bioresource technology*, 99(10), pp.4044–64. Available at: http://www.sciencedirect.com/science/article/pii/S0960852407001563 [Accessed July 9, 2014].

Chong, M. et al., 2009. Biohydrogen production from biomass and industrial wastes by dark fermentation. *International Journal of Hydrogen Energy*, 34(8), pp.3277–3287.

Clara, M. et al., 2005. The solids retention time - A suitable design parameter to evaluate the capacity of wastewater treatment plants to remove micropollutants. *Water Research*, 39(1), pp.97–106.

Dar, H., 1987. Response of a Cattle Dung Methane Fermentation to. *Control*, 22, pp.261–268.

Das, D., 2001. Hydrogen production by biological processes: a survey of literature. *International Journal of Hydrogen Energy*, 26(1), pp.13–28.

Demirbas, A., 2011. Waste management , waste resource facilities and waste conversion processes. *Energy Conversion and Management*, 52(2), pp.1280–1287. Available at: http://dx.doi.org/10.1016/j.enconman.2010.09.025.

Demirel, B. & Scherer, P., 2011. Trace element requirements of agricultural biogas digesters during biological conversion of renewable biomass to methane. *Biomass and Bioenergy*, 35(3), pp.992–998.

Donoso-Bravo, A. & Fdz-Polanco, M., 2013. Anaerobic co-digestion of sewage sludge and grease trap: Assessment of enzyme addition. *Process Biochemistry*, 48(5-6), pp.936–940.

Elbeshbishy, E. & Nakhla, G., 2012. Batch anaerobic co-digestion of proteins and carbohydrates. *Bioresource Technology*, 116, pp.170–178. Available at:

http://linkinghub.elsevier.com/retrieve/pii/S0960852412006578.

Escudié, R. et al., 2011. Control of start-up and operation of anaerobic biofilm reactors: An overview of 15 years of research. *Water Research*, 45(1), pp.1–10.

Fermoso, F.G. et al., 2009. Metal supplementation to UASB bioreactors: from cell-metal interactions to full-scale application. *Science of the Total Environment*, 407(12), pp.3652–3667.

Fernández, N. et al., 2007. Real evidence about zeolite as microorganisms immobilizer in anaerobic fluidized bed reactors. *Process Biochemistry*, 42(4), pp.721–728. Available at: http://www.sciencedirect.com/science/article/pii/S135951130600451X.

Fernandez, N. & Forster, C.F., 1993. A study of the operation of mesophilic and thermophilic anaerobic filters treating a synthetic coffee waste. *Bioresource Technology*, 45(3), pp.223–227.

Gavala, H. & Kopsinis, H., 1999. Treatment of dairy wastewater using an upflow anaerobic sludge blanket reactor. *Journal of agricultural …*, 73, pp.59–63. Available at: http://www.sciencedirect.com/science/article/pii/S0021863498903912.

Gianfreda, L. & Rao, M.A., 2004. Potential of extra cellular enzymes in remediation of polluted soils: A review. *Enzyme and Microbial Technology*, 35(4), pp.339–354.

Gikas, P., 2007. Kinetic responses of activated sludge to individual and joint nickel (Ni(II)) and cobalt (Co(II)): An isobolographic approach. *Journal of Hazardous Materials*, 143(1-2), pp.246–256.

Gong, W.J. et al., 2011. Selection and evaluation of biofilm carrier in anaerobic digestion treatment of cattle manure. *Energy*, 36(5), pp.3572–3578. Available at: http://dx.doi.org/10.1016/j.energy.2011.03.068.

Habouzit, F. et al., 2011. Influence of support material properties on the potential selection of Archaea during initial adhesion of a methanogenic consortium. *Bioresource Technology*, 102(5), pp.4054–4060.

Han, Y. & Dague, R., 1997. Laboratory Studies on the Temperature-Phased Anaerobic Digestion of Domestic Wastewater Sludge. *Wat. Env. Res.*, 69(6), pp.1139–1143.

Heijnen, J.J. et al., 1989. Review on the application of anaerobic fluidized bed reactors in waste-water treatment. *The Chemical Engineering Journal*, 41(3), pp.37–50.

Holm-Nielsen, J.B., Al Seadi, T. & Oleskowicz-Popiel, P., 2009. The future of anaerobic digestion and biogas utilization. *Bioresource Technology*, 100(22), pp.5478–5484.

Hulshoff Pol, L.W. et al., 2004. Anaerobic sludge granulation. *Water Research*, 38(6), pp.1376–

1389.

Izumi, K. et al., 2010. Effects of particle size on anaerobic digestion of food waste. *International Biodeterioration & Biodegradation*, 64(7), pp.601–608. Available at: http://www.sciencedirect.com/science/article/pii/S0964830510001344.

Jackson-Moss, C. a & Duncan, J.R., 1991. The effect of aluminium on Anaerobic digestion. *Biotechnology Letters*, 13(2), pp.143–148.

Jain, S. et al., 2015a. A comprehensive review on operating parameters and different pretreatment methodologies for anaerobic digestion of municipal solid waste. *Renewable and Sustainable Energy Reviews*, 52, pp.142–154. Available at: http://www.sciencedirect.com/science/article/pii/S1364032115007388 [Accessed August 13, 2015].

Jain, S. et al., 2015b. A comprehensive review on operating parameters and different pretreatment methodologies for anaerobic digestion of municipal solid waste. *Renewable and Sustainable Energy Reviews*, 52, pp.142–154. Available at: http://linkinghub.elsevier.com/retrieve/pii/S1364032115007388.

Kaparaju, P. et al., 2008. Effects of mixing on methane production during thermophilic anaerobic digestion of manure: Lab-scale and pilot-scale studies. *Bioresource Technology*, 99(11), pp.4919–4928.

Karadag, D. et al., 2015. A review on anaerobic biofilm reactors for the treatment of dairy industry wastewater. *Process Biochemistry*, 50(2), pp.262–271. Available at: http://linkinghub.elsevier.com/retrieve/pii/S1359511314005832.

Karim, K. et al., 2005. Anaerobic digestion of animal waste: Effect of mode of mixing. *Water Research*, 39(15), pp.3597–3606.

Kennedy, K., 2000. Treatment of landfill leachate using sequencing batch and continuous flow upflow anaerobic sludge blanket (UASB) reactors. *Water Research*, 34(14), pp.3640–3656.

Kennedy, K.J., Hamoda, M.F. & Guiot, S.G., 1988. Anaerobic Treatment of Leachate Using Fixed Film and Sludge Bed Systems. *Journal Water Pollution Control Federation*, 60(9), pp.1675–1683.

Khan, A.H. & Ashraf, M.Y., 1988. Effect of sodium chloride on growth and mineral composition of sorghum. *Acta Physiologiae Plantarum*, 10(3), pp.257–264.

Kida, K. et al., 2001. Influence of Ni2+ and Co2+ on methanogenic activity and the amounts of coenzymes involved in methanogenesis. *Journal of Bioscience and Bioengineering*, 91(6), pp.590–595.

Kugelman, I.J. & Mccarty, P.L., 1965. Cation toxicity and stimulation in anaerobic waste treatment. *Water Pollution Control Federation*, 37(1), pp.97–116.

Kwietniewska, E. & Tys, J., 2014. Process characteristics, inhibition factors and methane yields of anaerobic digestion process, with particular focus on microalgal biomass fermentation. *Renewable and Sustainable Energy Reviews*, 34, pp.491–500. Available at: http://dx.doi.org/10.1016/j.rser.2014.03.041.

Langer, S. et al., 2014a. Dynamics of biofilm formation during anaerobic digestion of organic waste. *Anaerobe*, 29, pp.44–51. Available at: http://dx.doi.org/10.1016/j.anaerobe.2013.11.013.

Langer, S. et al., 2014b. Dynamics of biofilm formation during anaerobic digestion of organic waste. *Anaerobe*, 29, pp.44–51.

Lettinga, G., Rebac, S. & Zeeman, G., 2001. Challenge of psychrophilic anaerobic wastewater treatment. *Trends in Biotechnology*, 19(9), pp.363–370.

Levin, D.B., Pitt, L. & Love, M., 2004. Biohydrogen production: Prospects and limitations to practical application. *International Journal of Hydrogen Energy*, 29(2), pp.173–185.

Li, C. & Fang, H.H.P., 2007. Inhibition of heavy metals on fermentative hydrogen production by granular sludge. *Chemosphere*, 67(4), pp.668–673.

Li, Y., Park, S.Y. & Zhu, J., 2011. Solid-state anaerobic digestion for methane production from organic waste. *Renewable and Sustainable Energy Reviews*, 15(1), pp.821–826.

LIN, C., 2008. Heavy metal effects on fermentative hydrogen production using natural mixed microflora. *International Journal of Hydrogen Energy*, 33(2), pp.587–593.

Lin, C.-Y., 1993. Effect of heavy metals on acidogenesis in anaerobic digestion. *Water Research*, 27(1), pp.147–152.

López Torres, M. & Espinosa Llorens, M. d C., 2008. Effect of alkaline pretreatment on anaerobic digestion of solid wastes. *Waste Management*, 28(11), pp.2229–2234.

Lu, X. et al., 2015. Operation performance and granule characterization of upflow anaerobic sludge blanket (UASB) reactor treating wastewater with starch as the sole carbon source. *Bioresource Technology*, 180, pp.264–273. Available at: http://dx.doi.org/10.1016/j.biortech.2015.01.010.

Lyberatos, I.N.G.A.G., 2010. Biohydrogen Production from Biomass and Wastes via Dark Fermentation : A Review. , pp.21–39.

M??ller, K. & M??ller, T., 2012. Effects of anaerobic digestion on digestate nutrient availability and crop growth: A review. *Engineering in Life Sciences*, 12(3), pp.242–257.

Macias-Corral, M. et al., 2008. Anaerobic digestion of municipal solid waste and agricultural waste and the effect of co-digestion with dairy cow manure. *Bioresource Technology*, 99(17), pp.8288–8293.

Martí-herrero, J. et al., 2014. Bioresource Technology Improvement through low cost biofilm carrier in anaerobic tubular digestion in cold climate regions. , 167, pp.87–93.

Martinez-Sosa, D. et al., 2011. Anaerobic submerged membrane bioreactor (AnSMBR) for municipal wastewater treatment under mesophilic and psychrophilic temperature conditions. *Bioresource Technology*, 102(22), pp.10377–10385. Available at: http://dx.doi.org/10.1016/j.biortech.2011.09.012.

Mata-Alvarez, J., Macé, S. & Llabrés, P., 2000. Anaerobic digestion of organic solid wastes. An overview of research achievements and perspectives. *Bioresource Technology*, 74(1), pp.3–16.

Mccarty, P.L., 1964. Anaerobic Waste Treatment Fundamentals.

Meng, Y., Yeong, T. & Ching, J., 2014. A review of sustainable hydrogen production using seed sludge via dark fermentation. *Renewable and Sustainable Energy Reviews*, 34, pp.471–482.

Minghua, Z. et al., 2009. Municipal solid waste management in Pudong New Area , China. *Waste Management*, 29(3), pp.1227–1233. Available at: http://dx.doi.org/10.1016/j.wasman.2008.07.016.

Murrayt, P.A. & Zinder, S.H., 1985. Nutritional Requirements of Methanosarcina Strain TM-1. *Analysis*, 50(1), pp.49–55.

Mustafa, N. et al., 2014. Anaerobic digestion of municipal wastewater sludges using anaerobic fluidized bed bioreactor. *Bioresource technology*, 172(December), pp.461–6. Available at: http://www.sciencedirect.com/science/article/pii/S0960852414013406.

Nasr, N. et al., 2014. Biohydrogen production from pretreated corn cobs. *International Journal of Hydrogen Energy*, 39(35), pp.19921–19927.

Nges, I.A. & Björnsson, L., 2012. High methane yields and stable operation during anaerobic digestion of nutrient-supplemented energy crop mixtures. *Biomass and Bioenergy*, 47, pp.62–70.

Oleszkiewicz, J.A. & Sharma, V.K., 1990. Stimulation and inhibition of anaerobic processes by heavy metals-A review. *Biological Wastes*, 31(1), pp.45–67.

Parkin, B.G.F. & Owen, W.F., 1987. Fundamentals of anaerobic digestion of wastewater sludges. , 112(5), pp.867–920.

Rodgers, M. & Zhan, X.-M., 2003. Moving-medium biofilm reactors. *Reviews in Environmental Science and Biotechnology*, (2003), pp.213–224.

Rodgers, M., Zhan, X.-M. & Dolan, B., 2004. Mixing Characteristics and Whey Wastewater Treatment of a Novel Moving Anaerobic Biofilm Reactor. *Journal of Environmental Science and Health, Part A*, 39(8), pp.2183–2193. Available at: http://www.tandfonline.com/doi/abs/10.1081/ESE-120039383#.Ve9Cl5fpV9g.

Romano, R.T. et al., 2009. The effect of enzyme addition on anaerobic digestion of Jose Tall Wheat Grass. *Bioresource Technology*, 100(20), pp.4564–4571.

Romero-G??iza, M.S. et al., 2016. The role of additives on anaerobic digestion: A review. *Renewable and Sustainable Energy Reviews*, 58, pp.1486–1499.

Schattauer, A. et al., 2011. Abundance of trace elements in demonstration biogas plants. *Biosystems Engineering*, 108(1), pp.57–65.

Schmidt, J.E. & Ahring, B.K., 1993. Effects of magnesium on thermophilic acetate-degrading granules in upflow anaerobic sludge blanket (UASB) reactors. *Enzyme and Microbial Technology*, 15(4), pp.304–310.

Sheli, C. & Moletta, R., 2007. Anaerobic treatment of vinasses by a sequentially mixed moving bed biofilm reactor. *Water Science and Technology*, 56(2), pp.1–7.

Show, K.Y. & Tay, J.H., 1999. Influence of support media on biomass growth and retention in anaerobic filters. *Water Research*, 33(6), pp.1471–1481.

Soto, M., M??ndez, R. & Lema, J.M., 1993. Sodium inhibition and sulphate reduction in the anaerobic treatment of mussel processing wastewaters. *Journal of Chemical Technology & Biotechnology*, 58(1), pp.1–7.

Sutherland, I.W., 2001. Biofilm exopolysaccharides: A strong and sticky framework. *Microbiology*, 147(1), pp.3–9.

Switzenbaum, M.S., 1983. Anaerobic fixed film wastewater treatment. *Enzyme and Microbial Technology*, 5(4), pp.242–250.

Thiele, J.H. et al., 1989. Ecoengeneering high rate anaerobic digestion systems: analysis of improved syntrophic biemethanation catalysts. *Biotechnol Bioeng*, 35, pp.990–999.

Uemura, S., 2010. Mineral requirements for mesophilic and thermophilic anaerobic digestion of organic solid waste. *Int. J. Environ. Res.*, 4(1), pp.33–40.

Wang, R.C., Wen, X.H. & Qian, Y., 2005. Influence of carrier concentration on the performance and microbial characteristics of a suspended carrier biofilm reactor. *Process Biochemistry*, 40(9), pp.2992–3001.

Wang, S. et al., 2009. Performance and kinetic evaluation of anaerobic moving bed biofilm reactor for treating milk permeate from dairy industry. *Bioresource Technology*, 100(23), pp.5641–5647. Available at: http://dx.doi.org/10.1016/j.biortech.2009.06.028.

Weiland, P., 2010. Biogas production: Current state and perspectives. *Applied Microbiology and Biotechnology*, 85(4), pp.849–860.

Wiegant, W.M. & Lettinga, G., 1985. Thermophilic anaerobic digestion of sugars in upflow anaerobic sludge blanket reactors. *Biotechnology and Bioengineering*, XXVII, pp.1603–1607.

Williams, C.M., Shih, J.C.H. & Spears, J.W., 1986. Effect of nickel on biological methane generation from a laboratory poultry waste digester. *Biotechnology and Bioengineering*, 28(11), pp.1608–1610.

Worm, P. et al., 2009. Decreased activity of a propionate degrading community in a UASB reactor fed with synthetic medium without molybdenum, tungsten and selenium. *Enzyme and Microbial Technology*, 45(2), pp.139–145.

Yadvika et al., 2004. Enhancement of biogas production from solid substrates using different techniques - A review. *Bioresource Technology*, 95(1), pp.1–10.

Yang, Q. et al., 2010. Enhanced efficiency of biological excess sludge hydrolysis under anaerobic digestion by additional enzymes. *Bioresource Technology*, 101(9), pp.2924–2930.

Ye, F.X., Chen, Y.X. & Feng, X.S., 2005. Advanced start-up of anaerobic attached film expanded bed reactor by pre-aeration of biofilm carrier. *Bioresource Technology*, 96(1), pp.115–119.

Yen, H. & Brune, D., 2007. Anaerobic co-digestion of algal sludge and waste paper to produce methane. *Bioresource Technology*, 98(1), pp.130–134. Available at: http://linkinghub.elsevier.com/retrieve/pii/S0960852405005365.

Young, J.C. & McCarty, P.L., 1969. The anaerobic filter for waste treatment. *Journal - Water Pollution Control Federation*, 41(519), p.Suppl:R160+.

Yu, H.Q., Tay, J.H. & Fang, H.H.P., 2001. The roles of calcium in sludge granulation during UASB reactor start-up. *Water Research*, 35(4), pp.1052–1060.

Zellner, G. & Winter, J., 1987. Growth promoting effect of tungsten on methanogens and incorporation of tungsten-185 into cells. *FEMS Microbiology Letters*, 40(1), pp.81–87.

Zhang, C. et al., 2014. Reviewing the anaerobic digestion of food waste for biogas production. *Renewable and Sustainable Energy Reviews*, 38, pp.383–392. Available at: http://dx.doi.org/10.1016/j.rser.2014.05.038.

Zhang, L., Lee, Y.W. & Jahng, D., 2011. Anaerobic co-digestion of food waste and piggery wastewater: Focusing on the role of trace elements. *Bioresource Technology*, 102(8), pp.5048–5059.

Zhang, Y. et al., 2014. Enhanced high-solids anaerobic digestion of waste activated sludge by the addition of scrap iron. *Bioresource Technology*, 159, pp.297–304.

Zhang, Y., Liu, G. & Shen, J., 2005. Hydrogen production in batch culture of mixed bacteria with sucrose under different iron concentrations. *International Journal of Hydrogen Energy*, 30(8), pp.855–860.

Zhang, Y. & Shen, J., 2006. Effect of temperature and iron concentration on the growth and hydrogen production of mixed bacteria. *International Journal of Hydrogen Energy*, 31(4), pp.441–446.

Zhong, W. et al., 2011. Comparison of chemical and biological pretreatment of corn straw for biogas production by anaerobic digestion. *Renewable Energy*, 36(6), pp.1875–1879. Available at: http://dx.doi.org/10.1016/j.renene.2010.12.020.

Zhu, X. & Tan, X., 2009. Metalloproteins/metalloenzymes for the synthesis of acetyl-CoA in the Wood-Ljungdahl pathway. *Science in China, Series B: Chemistry*, 52(12), pp.2071–2082.

Zitomer, D.H., Johnson, C.C. & Speece, R.E., 2008. Metal Stimulation and Municipal Digester Thermophilic/Mesophilic Activity. *Journal of Environmental Engineering*, 134(1), pp.42–47.