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PROCESS SIMULATION AND EXPERIMENTAL INVESTIGATION OF BIOFUEL
PRODUCTION IN A HIGH RATE ANAEROBIC DIGESTION PROCESS

by

HAIDER AL-RUBAYE

A DISSERTATION

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MISSOURI UNIVERSITY OF SCIENCE AND TECHNOLOGY

In Partial Fulfillment of the Requirements for the Degree

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PUBLICATION DISSERTATION OPTION

This dissertation consists of the following three articles that have been submitted for publication as follows:

Paper I, pages 6-52, Process Simulation of Two-Stage Anaerobic Digestion for Methane Production. Published in BIOFUELS BY TAYLOR AND FRANCIS, 2017.

Paper II, pages 53-99, The Impact of Hydraulic Retention Time and Operating Temperature on Biofuel Production and Process Wastewater Treatment, submitted to CHEMICAL ENGINEERING AND PROCESSING-PROCESS INTENSIFICATION.

Paper III, pages 100-135, The Pre-acidification gas impact on upgrading the biogas produced in expanded granular sludge bed reactor, submitted to JOURNAL OF CHEMICAL ENGINEERING.

This dissertation follows formatting rules as set forth by the Missouri University of Science and Technology.

ABSTRACT

The non-hazardous waste management hierarchy of the US EPA calls for “Reduce, Reuse, and Recycle” (or the three R’s) of wastes. The anaerobic digestion process is one of the most important methods that used to treat the wastes and at the same time generate energy out of it. The anaerobic digestion process generates a mixture of methane and carbon dioxide gases which is known as biogas.

The biogas composition is about 50-70% methane and 10-30% of carbon dioxide and trace amount of other gases like hydrogen and hydrogen sulfide. This biogas can be used in power generation, heating systems and in combined heating and powering systems. Also, it could be upgraded to improve its quality and make is utilized in all equipment used for natural gas with a minimal adjustment due to the lower BTU contents for methane gas.

Three papers were written and submitted regarding the biogas production and the liquid waste water treatment. The first paper focuses on developing a process modeling simulation by aspen plus for the anaerobic digestion process and on conducting a sensitivity analysis to investigate the parameters that could upgrade the biogas quality. The second paper focuses on the effect of hydraulic retention time of the substrate on the biogas production. This investigation conducted in a two-stage high rate expanded granular sludge bed reactor under different variables like substrate concentration, organic loading rate, and operating temperature. The third paper focuses on upgrading the biogas quality and quantity in a two-stage expanded granular sludge bed reactor by investigating the effect of injecting the pre-acidification gas (the first stage), which is mainly produced a gas mixture consist of hydrogen and carbon dioxide) into the second stage (the expanded granular sludge bed reactor).

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SECTION

1. INTRODUCTION

One of the biggest challenges facing nations today is the growing demand for energy and how to supply it and the need for potable water. Conventional sources of fossil fuel-based energy are being depleted, and are primarily responsible for air pollution. While there have been many technological developments to harness energy from renewable sources, their intermittent availability, so their use for power generation is not wide spread.

A variety of socio-economic reasons have increased the focus in the United States on utilizing renewable energy. Since the industrial revolution, the presence of greenhouse gases in the environment has risen to record levels due to society's' widespread use of fossil fuels. Additionally, fossil fuel reserves are not replenished as quickly as they are used for energy generation.

Eco-contamination due to fuel spills have a significantly negative impact on the environment, affecting the food-chain, ground-water compositions, and those industries which rely on these resources (i.e., fishing, tourism, etc.). Also, due to the global economy, import and export of fossil fuels have also affected national security and international peace [1]. It is therefore imperative that efforts are made to develop and use sustainable energy to meet socio-economic infrastructure (logistics, utilities, transportation industries), and make use of locally available renewable energy to supplant or replace traditional fossil fuel-based processes.

Anaerobic digestion (AD) is an excellent source of renewable energy, which efficiently uses organic based resources including wastewater and helps improve water quality. Anaerobic digestion is defined as the biological decomposition of carbon-based

organic materials occurring in an oxygen-free environment where microorganisms convert complex organic polymers into soluble monomers, while generating “biogas” composed of methane and carbon dioxide. Biogas can be used for heating, cooking, power generation, or utilized in combined heating and power generation equipment. Biogas consists of approximately 70% methane and 30% carbon dioxide gases. Biogas can be utilized in all equipment designed to use natural gas with minimum adjustments for the lower energy content of biogas [2]. Anaerobic digestion has demonstrated potential for both industrial and domestic wastewater treatment processes [3]. Table 1.1 shows a typical biogas composition [4].

The anaerobic digestion process has been known for a long time. The first system was reported to be built in India in the eighteenth century and then utilized in England to produce fuel for street lamps. After that, researchers discovered the relation between the amount and quality of the biogas produced and the organic material contained in wastewater. In the twentieth century, anaerobic digestion was utilized in many places around the world as a wastewater treatment method and as an energy fuel source [5].

Over time, many different anaerobic digester configurations have been developed. Some of the conventional configurations included the conventional stirred tank reactor, which is a simple mixing tank to maintain a uniform temperature and organics substrate distribution. Another more complicated design included an internal medium like an anaerobic fluidized bed reactor, or a packed bed digester. Also, these configurations are classified by the feed introduced into the reactor. Some of these batch reactors feed wastewater at the beginning of the process and include no input or output streams except for the produced biogas output. Disadvantages of the batch reactor includes its footprint

and the long processing time to begin producing biogas [6]. The continuous reactor design feeds substrate into the system with continuous effluent stream flow out. The main disadvantage of the continuous biogas reactor is the possibility of the fresh substrate by passing the gas production process and leaving in the effluent stream.

Lastly, an important type of anaerobic digestion reactor configurations is the flow-through reactor, where the substrate passes through a liquid or solid medium. This type of reactor, called the high rate anaerobic digestion reactor, requires a much smaller footprint and less time to produce biogas. Furthermore, this type of digester utilize granular biomass particles. A primary advantage of this type of reactor is the capacity for granular biomass bed that expands inside the reactor and thus, improves the hydrodynamic mixing of the microorganisms and the substrate. Consequently, the contact time between the microorganisms and the substrate increases, resulting in increased biogas production as well.

The anaerobic digestion process consists of four major steps, with each step being controlled by a specific type of microorganism with the limiting step dependent on the type of substrate fed to the system. These steps include: 1) Hydrolysis, 2) Acidogenesis, 3) Acetogenesis, and 4) Methanogenesis.

Table 1.1. Composition of the biogas produced from the anaerobic digestion process

Typical Composition of Biogas		
Compound	Chemical Formula	Composition%
Methane	CH ₄	50-75
Carbon Dioxide	CO ₂	25-50
Nitrogen	N ₂	0-10
Hydrogen	H ₂	0-1
Hydrogen Sulfide	H ₂ S	0-3

Hydrolysis is the first step, and involves extracellular enzymes to break down a complex substrate of sugars, fats, and proteins into simpler molecules like glucose, long chain fatty acids, and amino acids. This step may be the rate-limiting step for a non-degradable substrate. The second step is the acidogenesis, where all the monomers produced from the previous step are converted into volatile fatty acids including acetic acid, butyric acid, propionic acid, with hydrogen and carbon dioxide as side products. The third step is acetogenesis, where intermediate products from previous step are converted into acetic acid, hydrogen, and carbon dioxide. The last step is methanogenesis, where methanogenic bacteria convert the acetic acid, hydrogen, and the carbon dioxide into methane. This step is controlled by two types of bacteria including acetoclastic methanogens (converts acetic acid into methane and carbon dioxide) and hydrogenotrophic bacteria (converts hydrogen and carbon dioxide into methane) [7]–[9]. Many factors and operating conditions are pivotal to efficient anaerobic digestion. Each of these parameters take on specific values for optimal operation that maximize the biogas production, notably, temperature, pH, hydraulic retention time (HRT), solid retention time (SRT), substrate concentration, and the percentage of substrate solids.

Three papers which describe the research conducted in this work are presented in this dissertation. Paper I discusses the development of a process simulation model using a commercial process simulation package (Aspen TM 8.6) for the anaerobic digestion process. Different parameters including substrate concentration, substrate types, hydrogen gas injection, and operating pressure were manipulated to assess their impact on the biogas production rate and the relative amount of methane generated. Paper II describes the experimental investigation of hydraulic retention time (HRT) and the substrate

concentration on the production rate and composition of biogas. This investigation was conducted at two different temperature ranges, namely, the mesophilic (34-36°C) and thermophilic (50-55°C) temperature ranges. Paper III discusses the effect of injecting the gas mixture produced in the first stage (pre-acidification) into the second stage (expanded granular sludge bed reactor), at a certain rate to investigate its effect on the overall biogas production rate and its composition running at two temperature ranges (mesophilic and thermophilic)

PAPER

I. SIMULATION OF TWO-STAGE ANAEROBIC DIGESTION FOR METHANE PRODUCTION

Published in Biofuels Journal by Taylor and Francis, 2017

ABSTRACT

A process simulation model was developed for the anaerobic digestion (AD) process used for biogas generation. Aspen Plus software was used for this purpose. The developed model predicts the production of biogas from any substrate at any given process condition. This model was validated against a variety of industrial data on anaerobic digestion. The four steps (Hydrolysis, Acidogenesis, Acetogenesis, Methanogenesis) with a total of forty six reactions that represent the AD process were simulated with appropriate kinetics. Sensitivity analysis was implemented for 5, 10, 20 and 30% substrates concentrations to increase the methane share in the biogas by studying the effects of hydrogen addition, HRT and pressure. The developed model is flexible and predicts enhancement of methane composition by hydrogen injection qualitatively and quantitatively.

1. INTRODUCTION AND BACKGROUND

One of the biggest challenges facing the world today is the ever-growing demand for energy and how to meet this demand reliably and affordably. Traditional sources of energy are getting scarce, being labeled as pollutants, or are socially unacceptable; at the same time, new renewable sources are not economically viable on their own.

Most industrial countries are heavily dependent on fossil fuels to operate their power plants, so have started funding projects and research to generate renewable energy

as an alternative (or resilient) energy source. Fossil fuels could pose a threat to national security, especially if it is imported from a foreign source.

Furthermore, alternative energy sources may reduce pollution and contributions to greenhouse gas emissions and, thus, global warming [1]. Two types of studies have been investigated in the anaerobic digestion (AD) process: experimental and theoretical studies.

1.1 EXPERIMENTAL INVESTIGATION

Historically, the septic tank was considered the simplest type of anaerobic digestion [1,2]. The produced biogas was typically used for heating and cooking purposes in Assyria and Persia during the tenth and sixteenth centuries respectively [3,4]. The first anaerobic digestion process was built at a leper colony in India in 1859. There were AD plants for sewage treatment in England in 1895 that produced biogas for street lamps in Exeter. The AD process has been improving rapidly since the 1960s. The anaerobic filter plant was developed in 1969 [5] and the high rate up flow anaerobic sludge blanket reactor was developed in the Netherlands in the late 1970s [2]. Luo et al. utilized hydrogen gas to upgrade the biogas and increase the composition of methane in it. The methane share increased to about 22% after the hydrogen addition, while the carbon dioxide composition decreased to about 15% [6].

Kaparaju et al. conducted an experimental investigation to optimize biogas production by using two anaerobic digesters in series, and it has been proved that using serial digesters could improve biogas production by about 10% [7]. Ghorbanian et al. [8, 9] studied the impact of hydraulic retention time (HRT) on biogas production by using an expanded granular sludge bed reactor (EGSB). The results from Ghorbanian et al. showed that the removal efficiency for chemical oxygen demand (COD) and biogas production rate

increased by 33–42% and 22–32%, respectively, as HRT increased by approximately five to six times at a fixed organic loading rate (OLR). The effect of supplemental hydrogen on biogas enhancement and substrate removal efficiency has also been studied. The experimental data demonstrated that biogas quality enhanced by 10–20% depending on the hydrogen injection rate; in addition to that, energy yields increased by 33–42% and COD removal efficiency remained constant at about 98% [10].

Nunez and Martinez [11] worked on anaerobic treatment of slaughterhouse wastewater in EGSB. The reactor was inoculated with a granular sludge from an anaerobic reactor of a brewery factory. The EGSB was 1.4 m high and 0.044 m in diameter. The results showed that when HRT is increased, the removal efficiency will increase by 65–80% while increasing the organic loading rate at a given HRT will not have any significant effect on COD removal efficiency. Methane production will increase due to OLR increase. Pakarinen et al. [12] investigated the effect of organic loading rate and retention time on hydrogen production in a continuous stirred tank reactor where, besides methane production, hydrogen production from energy crops through dark fermentation has been shown to be possible, where the methanogenic microorganisms could be used for hydrogen production when there are short HRT from three to five days and a higher OLR up to 10 kg VS/m³.day.

Fujita et al. [13] studied the effect of biogas production by adding rich carbon content material (corn stover) to swine manure. The animal manure has a very high nitrogen composition compared to the carbon content in it. C: N ratio should be about 4:1, where carbon represents the energy source for the microorganisms and nitrogen is used for microbial growth to make it more efficient. Having high nitrogen concentration will cause

an inhibition of the anaerobic digestion process. Adding the corn stover will enhance biogas productivity, thus more than 50% of the carbon content in the corn stover will be utilized. Biogas production will be higher than digesting either swine manure or corn stover alone.

1.2 THEORETICAL INVESTIGATION

Theoretical studies involve building a simulation model for the whole process using one of the simulation software codes such as Aspen Plus or Aspen Hysys. The most important advantage of this type is the ease of conducting a sensitivity analysis and changing variables, which can be made in a shorter time than that needed in a real plant. Most companies recommending this type of research as it does not require a huge investment unlike the actual setup and it gives accurate results in a shorter time. Peris [14] used an Aspen Plus simulation code for a complete AD process. This process consists of two stages (two AD reactors) to implement the optimal conditions of each step in each one. All AD reactions introduced in the simulation would occur in both stages. The feedstock used for this process was biomass, which basically consists of carbohydrates, proteins, and lipids. Serrano assumed that the substrate (feed) is already hydrolyzed, so the proposed model described only the last three steps for the AD, which makes his model unfit for other applications. Rajendran et al. [15] developed a process simulation model using Aspen Plus to simulate the AD process. In this model, the intermediary metabolisms of all four phases of the anaerobic fermentation were involved. There were some challenges that made the model inconsistent, especially the mass balance of the reactions that had been introduced in the model.

2. ANAEROBIC DIGESTION STEPS

The AD process can be defined as a series of reactions that take place in a multistep process by which microorganisms break down the biodegradable organic materials in the absence of oxygen. This process can be used for industrial and domestic purposes to manage waste and produce fuel. This fuel consists mainly of methane (50-70%), carbon dioxide (30-50%), and a small amount of other gases, called biogas [8,9]. Biogas has the ability to operate in most of the equipment designed for the natural gas with a minimal modification as methane has a lower BTU content. Table 1 shows the composition of biogas produced from the AD process [4,10,16]. The AD process has many advantages [1,2], such as low energy consumption, low production of biological solid wastes, the ability to work independently without any feed for long intervals, low nutrient and chemical requirements for the process, high COD removal rates at high loading rates, improvement to dewaterability, production of energy gases, and odor free end products. Many types and configurations for AD have been developed such as digester tanks, anaerobic filters, anaerobic fluidized reactors, anaerobic baffled reactors, up-flow anaerobic sludge beds, and hybrid reactors [3,8]. These configurations have been developed to reduce digestion time and required land space, thus increasing biogas production and organic loading rate.

These processes occur through microorganisms called anaerobes, which biochemically convert the organic materials presented in the wastewater into methane, carbon dioxide, and biomass, which is in general 'biogas'. The steps for the anaerobic process are shown in Figure 1. In anaerobic biotechnology, microorganisms obtain the required energy through a series of metabolic reactions in which oxidized organics and/or

hydrogen are utilized to provide energy for biomass cell growth. Anaerobic biodegradation treatment consists of four major steps: hydrolysis, acidification, acetogenesis, and methanogenesis [8–10].

The first step can be simply defined as the conversion of the complex undissolved organic substances like complex polymers (fats, cellulose, proteins) into smaller (soluble) monomers like long-chain fatty acids, amino acids, and glucose by extracellular enzymes. This step is considered as the limiting step for the entire process and it is affected by pH, biomass concentrations, and presence of the organic substrates.

Thirteen reactions have been set in the hydrolysis step and are based on their extent. The second step converts the products from the first step (monomers) into volatile fatty acids, propionic acid, acetic acid, hydrogen, and carbon dioxide.

Table 1. Composition of the biogas

Typical composition of biogas		
Compound	Molecular formula	Percentage
Methane	CH ₄	50–75
Carbon dioxide	CO ₂	25–50
Nitrogen	N ₂	0–10
Hydrogen	H ₂	0–1
Hydrogen sulfide	H ₂ S	0–3
Oxygen	O ₂	0–0

The acidifying bacteria has a very high pH tolerance, so they can handle a low pH (pH value less than 4). In the third step, the intermediates from the previous step are converted into carbon dioxide, hydrogen, and acetate. This step is endergonic, which is achieved by syntrophic coupling between acetogenic bacteria and hydrogen consuming methanogenic bacteria.

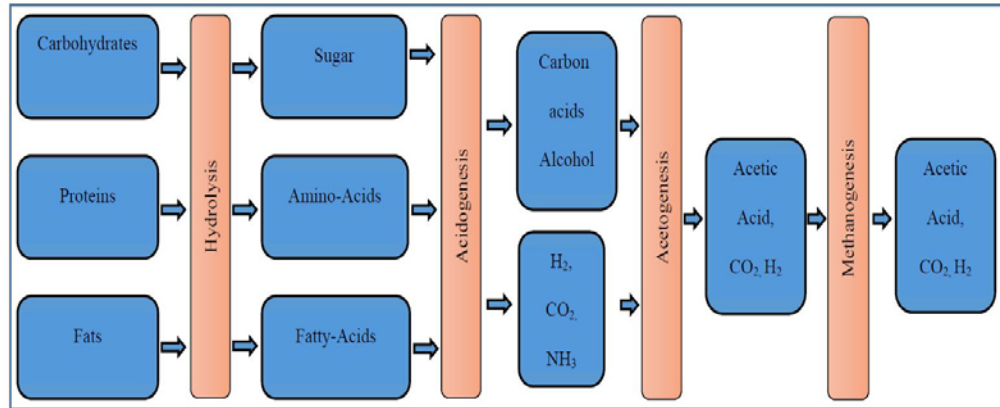


Figure 1. Anaerobic digestion process [3,18,19]

The fourth step converts the intermediate products from the acetogenesis into methane gas and carbon dioxide. The two types of bacteria responsible for this conversion are acetoclastic methanogens, which convert the acetate, and hydrogenotrophic methanogens, which convert the hydrogen produced from the acidification and the acetogenesis steps by autotrophic oxidation of hydrogen. The chemical reaction kinetics of the last three steps were already included in the model. There are factors that affect anaerobic digestion and biogas production. These factors when changed above or below their optimum values might act as inhibitors by causing fluctuations in the biogas production. One of these factors is temperature. There are three temperatures ranges: psychrophilic (15-25 °C), mesophilic (32-42 °C), and thermophilic (50-65 °C) The last type has been considered as the best for maximizing biogas production, although it is hard to maintain steady state temperature for the process as it consumes more energy than the other two types.

Also, pH is a crucial factor, where the optimum value lies in the range 6.8-8.5. This factor might be the reason for splitting the process of AD into two stages, as each stage will work on a different pH value. The carbon: nitrogen ratio (C:N), which should be about

30:1, is essential for the process. Carbon represents the energy source for the microorganisms while nitrogen is used for microbial growth to make it more efficient.

Furthermore, HRT and SRT are essentials operating parameters. HRT could be defined as the time spent by the solids inside the reactor while SRT will be the ratio of the solids maintained inside the reactor to the solids leaving the reactor. In many conventional reactors, these two values would remain the same, except in high rate AD, where the value of the SRT will be much higher than the HRT. There are many factors that could affect the AD, such as the dry content, pressure, and ammonia content. Table 2 shows the optimum values for all factors [3,17].

The AD process can be optimized for either a high solid content, which is commonly used in Europe, or a low solid content. The main advantage of the high solid technique is the small volume size of the reactor due to use of less water in the system [1].

Wet digestion has been applied in more applications, especially in the United States. There are two methods to introduce the feedstock into the AD: the ‘batch and continuous processes’. For the first method, the digestion process occurs in a batch reactor where there is no output or input and the four AD steps take place in one tank [1].

Table 2. Optimum parameter values for anaerobic digestion [17]

Parameter	Hydrolysis/ Acidogenesis	Methanogenesis
Temperature (°C)	25–35	Mesophilic: 30–40, Thermophilic 50–60
pH value	5.0–6.0	6.7–7.5
C:N ratio	10–45	20–30
Redox potential	400 to -300 mV	Less than -250mV
C:N:P:S ratio	500:15:5:3	600:15:5:3
Trace elements	No special requirements	Essential: Ni, Co, Mo, Se

Normally an agitator should be equipped with the batch reactor process to provide a uniform temperature and concentration distribution.

Such a system can be described simply as a stirred tank reactor. It will be filled with the fresh substrates all at once and left to degrade anaerobically without any interference until the end of the cycle phase.

The batch process requires more precise measurement and monitoring equipment to function optimally. In the biogas industry, most commercial biogas production plants use the conventional continuous process, which is considered as the second method in introducing the feed, either as a single or a double stage AD process.

In most of the cases, the feedstock is loaded into the reactor either once or several times a day. Introducing the feedstock continuously could cause a short circuiting of the feed, which means that fresh load would flow directly out of the reactor if mixing is too high or the input and output tubes are located improperly. The digester is usually single stage; although some are built in pairs, they do not function as a stage separated process. Usually, digesters are equipped with a preparation tank, where various substrates are mixed together and prepared for loading, which also serves as a buffer tank. In many cases, a post-treatment tank could be added (also called a post fermenter) [3,17]. The applications of the biogas will be for heating purposes and electricity. About 10% of the biogas will be used to maintain the temperature of the digester.

3. ANAEROBIC DIGESTION MODELS

There are many models that describe the anaerobic digestion kinetics. Some of these models focus on the inhibitions in the process [3] while other models describe the AD process [20]. Anaerobic digestion model no.1 (ADM1) is considered the most

important model for AD, which considers that the substrate introduced to the system as a feed will consist of carbohydrates, proteins, and fats. This model consists of two types of reactions: the biochemical reactions and physico-chemical reactions. For the first type, the enzymes, whether intracellular or extracellular, will serve as the catalyst.

A disintegration step has been included in this model, which converts the biomass into inert carbohydrates, proteins, and lipids by breaking the chemical structure of the biomass, thereby affecting the biogas production rate. This step and the hydrolysis step are controlled by the extracellular enzymes. Acidogenesis, acetogenesis, and methanogenesis are also included in this model. All of the above steps are operated by microorganisms. The second type of reactions in the ADM1 are the physico-chemical reactions, which include the acid-base reactions and the gas-liquid transfer. These reactions do not involve the microorganisms, except solid precipitation, which is not included in the physicochemical reactions. The second model, by Angelidaki [21], includes inhibitors like free ammonia, volatile fatty acids, acetate, and long-chain fatty acids. This model does not include the temperature and hydrogen inhibitions, but it could be used to predict the process performance and to assist in the operation of biogas plants that utilize a complex mixture of wastes.

4. ASPEN MODEL DESCRIPTION

Most industrial companies are focusing on the process simulation modeling as it is an approach to save time and monetary investments and replicates plant operations accurately. The selection of the property package, NRTL (non-random, two liquid model) in this case, is the first step in the simulation process. The reason for choosing this package is its ability to calculate activity coefficients and mole fractions. Anaerobic digestion model

no. 1 proposes that the substrate feed rate will consist of carbohydrates, proteins, and fats. Introduction of material components will be the next step and it will be followed according to the ADM1 model. Some of the properties, especially the thermodynamic properties like heat of formation and specific heat of the standard liquid volume, cannot be estimated by Aspen Plus. Hence, assumptions have been done, using some of the components that have known thermodynamic properties, as these components have a chemical structure like the components needed to specify their properties. Such assumptions would not affect the biogas production rate, as it depends only on the kinetics of the reactions [3,14]. There are 13 reactions for the hydrolysis step.

Due to insufficient information about the hydrolysis reactions, a stoichiometric reactor will be used because it does not require many inputs to the model, but requires the extent of reaction [15,20,21]. The produced monomers from the first step go through a series of calculator blocks to calculate the rate of reactions in the AD, which are basically written by Fortran code. It is programmed to calculate the products from each reaction. Amino acids pass through an amino acid calculator block where they get converted to several VFA components. These components pass through several VFA calculator blocks such as the valeric acid block and the propionic acid block, then through the methanogenesis block to calculate the amount of produced biogas and hence the rate of the reactions.

The next three steps of AD were conducted in a continuous stirred tank reactor (kinetic reactor). The reactions of these steps are based on kinetics and all the required inputs will be specified from ADM1 and the comprehensive model. Table 3 shows the kinetic constants that were used in the simulation. First, a heater was selected to maintain

the required temperature for the whole system based on the temperature ranges favorable for the AD process. A heat exchanger was employed to heat the feed stream instead of introducing the feed at the required temperature directly to calculate the energy required by the system to maintain the required temperature (the thermophilic range 50-65 °C). Despite the high energy that is consumed by the process, the biogas production rate will be higher [3,14,18].

Table 3. Kinetic constants used in aspen model [20,21]

Group	μ_{\max} (d ⁻¹)	K_s (g/L)	K_{s,NH_3} ^e (g/L)	K_i (g/L)	K_i ^b (g/L)
Carbohydrate enzymatic	1	–	–	0.33 (VFA) ^a	–
Protein enzymatic	1	–	–	0.33 (VFA) ^a	–
Glucose acidogens	5.1	0.50 (glc)	0.05	–	5.0 ^c
Lipolytic	0.53	0.01 (GTO)	0.05	–	5.0 ^c
Long-chain fatty acid degraders	0.55	0.02 (ol.)	0.05	–	5.0 ^d
Amino acid degraders	6.38	–	–	–	–
Propionate degraders	0.49	0.259 (HPr)	0.05	0.96 (HAc)	5.0 ^c
Butyrate degraders	0.67	0.176 (HBt)	0.05	0.72 (HAc)	5.0 ^c
Valerate degraders	0.69	0.175 (Val)	0.05	0.40 (HAc)	5.0 ^c
Methanogens	0.60	0.120 (HAc)	0.05	0.26 (HAc)	5.0 ^c

The heater was connected to the stoichiometric reactor (the hydrolysis step in the AD, pre-acidification tank in the experimental setup). The intermediate products exit the stoichiometric reactor and feed the main reactor that is a continuous stirred tank reactor (CSTR). The last three steps for the AD are held in the main reactor represented by a reactor with a recycle stream to maintain the uniform temperature and mass distribution for the high rate digesters.

More than thirty three kinetic reactions will be held in the CSTR reactor. The reaction rates will be calculated by the calculator blocks in Aspen. FORTRAN code is used for these calculations [14]. At this point, the AD process is completed with two streams exiting the reactor. One is the gas stream, which has the biogas and other gases in a trace amounts; the other is the liquid stream, which goes through a splitter to split a part of it as

recycle that connects with the feed stream. In the experimental setup, two pumps were used: the feed pump, which pumps the liquid from the pre-acidification tank to the main reactor; and the second pump, which pumps a specific amount of liquid from the effluent stream to the feed line as a recycle, the rest being discharged. The model calculates all the components that exit the reactor even when its concentration is less than 10-20. This being negligible, a component splitter separates these components from the biogas. A flash separator separates the water from the biogas stream by decreasing the temperature of the stream and then a gas filter separating the hydrogen component, as hydrogen molecules are the smallest among gas molecules, to circulate it in the process again. The Aspen Plus process model is shown in Figure 2. Simulating such a process is really challenging as it is not like an ordinary set of reactions that occur within a chemical process to get products once the optimum conditions are available. The AD process entirely depends on the activity of the granular biomass that has the microorganisms inside the reactor, which comes from effluent obtained from different plants like food manufacturing companies and cow dung with anaerobic sludge plants. The delicacy and the softness of the biomass granules and its high sensitivity to the environment are the biggest challenge in the AD process.

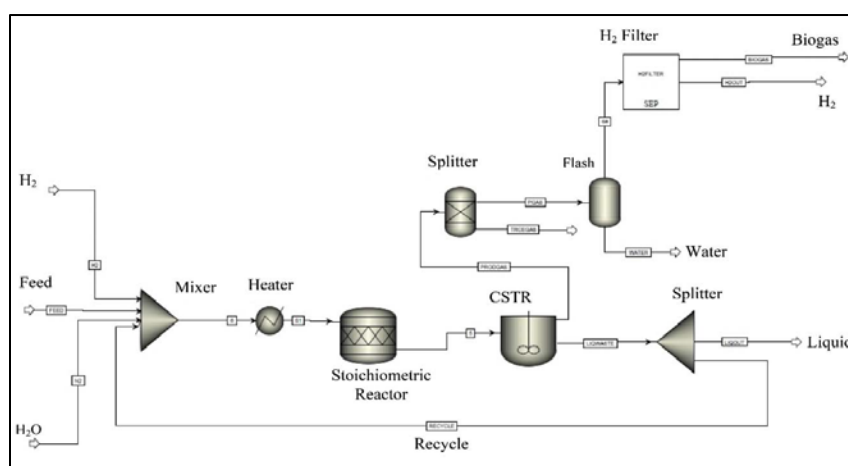


Figure 2. Aspen plus model for anaerobic digestion process

5. RESULTS AND DISCUSSION

The biggest challenge in developing the AD simulation model by Aspen Plus is that it requires a specific analysis for the feed to run the simulation properly. The anaerobic digestion process is held by the microorganisms, so their activity cannot be simulated in Aspen and only the kinetics and the reactions would be specified in it.

5.1 PROCESS MODELING VALIDATION

Validating any proposed simulated model is essential for making it widely applicable. This can be done by comparing the results simulated from the model with results developed from experimental setups that are working under similar conditions. In this paper, results developed from the model had been compared with experimental data to check the validity of the model: three cases have been used in this validation. It is important to mention that this model cannot predict the activity of the microorganisms in the AD process, hence, kinetics and chemical reactions can be deduced.

The model simulated two reactors: a stoichiometric reactor used for the reactions from the hydrolysis step and a continuously stirred tank reactor (CSTR) for acidogenic, acetogenic, and methanogenic steps. For validation, as shown in Figure 3. The three feed cases (as per respective literatures) were considered: Case 1 cattle manure, composition of the manure was taken from Budiyo [22]; Case 2 cow manure from Snertinge biogas plant, Germany [7]; Case 3 wastewater generated from industrial and agricultural activities [10]. The task is to apply similar conditions for each case and compare their results with the results obtained from the experimental data.

For Case 1, cow manure has been used as feed which is predominantly fiber (lignin, cellulose, and hemicellulose) [22]. This manure was used as substrate with a loading rate

of 0.33 L/day at HRT of 15 days. According to this study, 49.89% of methane was produced, calculated per gram of cattle manure which falls in range with the simulated result of 46.25%.

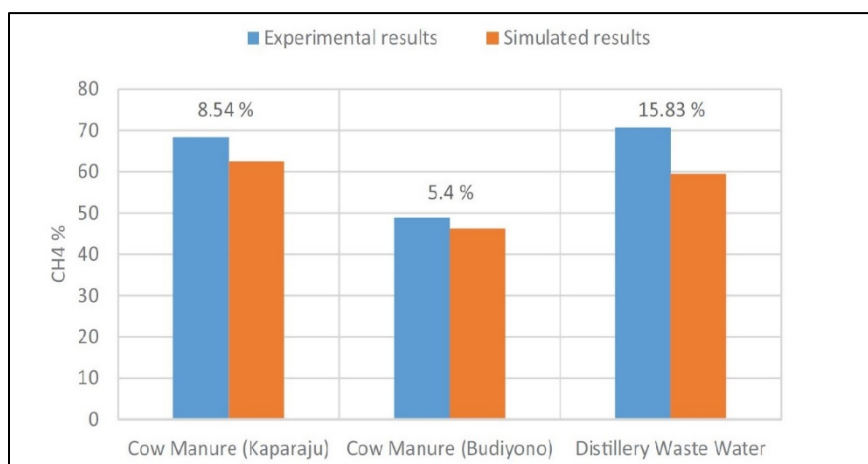


Figure 3. Aspen model validation with experimental data

In Case 2, to optimize biogas production, Kaparaju demonstrated the comparison of one-step CSTR with that of the two phase system with two methanogenic reactors connected in series.

Results shows that serial digestion, with combined working volume of 5 L and 15 days HRT, could improve conversion efficiency. According to the model, CH₄% was 62.52% and from the serial digestion of the two reactors at thermophilic range (55 °C), CH₄% was 68.36%.

According to Case 3, 70.7% CH₄ was obtained at 3.0 g COD/L.day by seeding a 60 L anaerobic expanded granular sludge bed (EGSB) with 45 L of active biomass (no dilution). From the Aspen plus, model, simulations reported CH₄% value to be 59.51%. These cases depicting their deviations from experimental and simulated results are shown in Figure 3. Table 4 shows the waste water composition for the three studied cases.

Table 4. Composition of substrates used as feed in the aspen

Components	Case 1	Case 2	Case 3
Glycine	0.083	–	–
Cellulose	0.204	–	–
Hemicellulose	0.086	–	–
Glucose	0.257	–	0.53
Triolein	0.015	–	0.09
Acetic acid	–	0.45	–
Protein	–	–	0.3
Propionic acid	–	0.265	–
Valerate	–	0.097	–
Butyrate	–	0.079	–
Iso-butyrate	–	0.053	–
Iso-valerate	–	0.053	–
Inerts	0.355	–	0.08

5.2 EFFECT OF SUBSTRATE FEED RATE ON METHANE GAS

Biogas production rate depends on influent feed rate and concentration of the substrates. We prepared different concentrations for the substrates of 5, 10, 20 and 30% of carbon content at different volumetric flow rates taken from the literature [8,23] as shown in Figure 4.

At a constant feed rate for the substrate, the methane rate increases with the increase in substrates concentration and that the accumulated methane rate increases with the increase in feed rate. Figure 5 shows that at constant feed rate, the composition of the methane gas produced from the AD decreases with increasing substrates concentration, as increasing the organic concentration beyond a specific point will cause an inhibition of the entire process. This is in line with the experimental investigations in the literature.

For different substrates concentrations, the methane gas that is produced will slightly decrease with the increase of feed rate, while residence time and hydraulic retention time for the AD process will decrease, causing a discharge for some of the unreacted substrate material without reaction to produce methane.

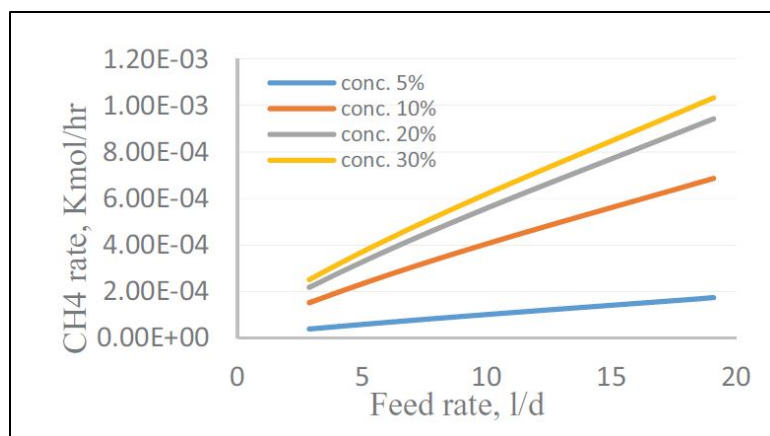


Figure 4. Methane component rate as a function of flowrate for different substrate concentrations

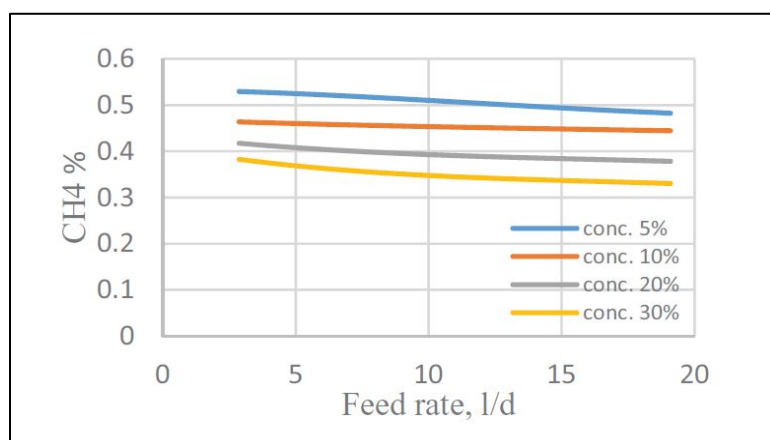


Figure 5. Methane composition as a function of flowrate for different substrate concentration

This conclusion can also be deduced from the literatures that studied the effect of HRT on biogas production [7, 8, 10, 13, 23–30].

5.3 EFFECT OF HYDROGEN INTRODUCTION ON METHANE PRODUCTION

Ghorbanian et al. [9] stated that introducing a specific amount of hydrogen gas could enhance the methane gas composition in the biogas from 71 to 89% and reduce the carbon dioxide from 29 to 11%. Thus, biogas was produced which was predominantly methane.

Methane has the potential to be a reliable resource for hydrocarbons production like butanol or other types of renewable fuels. The amount of hydrogen gas added should be the same amount of hydrogen generated from the pre-acidification step and from the acidogenic and acetogenic steps. Luo and Angelidaki [31] used the hydrogen to upgrade the biogas that contains methane about 40-75% and carbon dioxide about 25-60% to an upgraded biogas of about 90% methane.

Upgrading the biogas into something similar to natural gas has many advantages, as it could increase the BTU content for the biogas and make the biogas more flexible for use in more applications. Also, the unreacted hydrogen in the biogas would form a gas mixture with the methane and would improve the combustion properties for the biogas, making it almost applicable in all-natural gas equipment [6].

Finally, upgrading the biogas will make its transportation easier by using the existing natural gas grid. Introducing or using hydrogen to increase the composition of methane gas in the anaerobic digestion process would be the most appropriate method as it is cheaper than other methods like water washing or pressure swing adsorption. In the current model, a hydrogen gas stream was introduced to the process where a sensitivity analysis had been conducted to study the effect of hydrogen introduction to the process on the biogas production and methane gas composition.

Experimentally, once the hydrogen gas was introduced to the system, hydrogenotrophic methanogens microorganisms will consume this hydrogen with the carbon dioxide and transform them into methane. The methane composition in the biogas will start increasing until it reaches its maximum. After that, increasing the hydrogen gas will cause an inhibition of the process as the acidity level increases and the pH value

decreases and causes deactivation of the hydrogenotrophic methanogens. Therefore, the methane composition will start decreasing as shown in Figure 6 for one specific volumetric flow rate. Figure 7 shows the effect of hydrogen on the methane production rate: for a specific value it will reach the maximum; after that, the methane rate will decrease.

The trends in Figure 6 and 7 are compatible with the literature from Ghorbanian [9] and Luo et al. [6,23,31]. There also seems to be a difference in methane composition and production rate, which can be spotted when plotted against the feed rate from Figure 8; methane composition was higher when hydrogen gas was introduced than those values without hydrogen introduction.

Figure 9 shows the relation between the methane production rate and the substrate feed rate with and without hydrogen introduction, where both increase as they represent the accumulation production rate of methane, but the one for the addition of hydrogen will be higher than those without the addition.

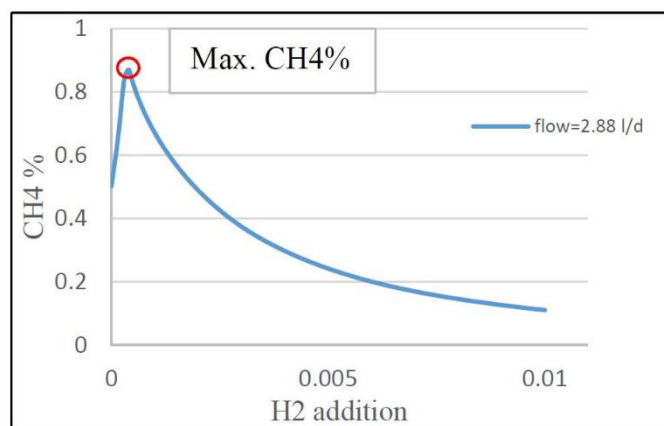


Figure 6. Relation of CH₄ composition with H₂ introduced to the system at a specific volumetric flowrate

Rates were calculated by conducting a sensitivity analysis for the hydrogen addition to the process for four substrates concentrations, where the accumulated methane rate would increase with increasing the flow rate of the feed.

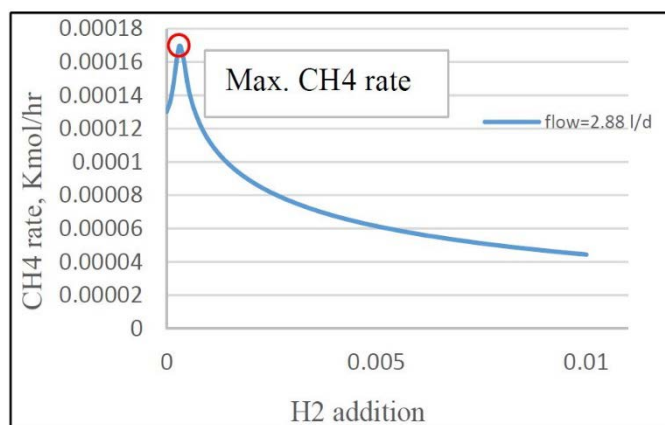


Figure 7. Relation of CH₄ rate with the H₂ introduced to the system at a specific volumetric flowrate

A sensitivity analysis was conducted to estimate the maximum methane composition and production rate for different substrates concentrations and flow rates, as shown in Figures 10-13. This value (i.e. of hydrogen) will increase with increasing the concentration of the feed substrate and the feed rate.

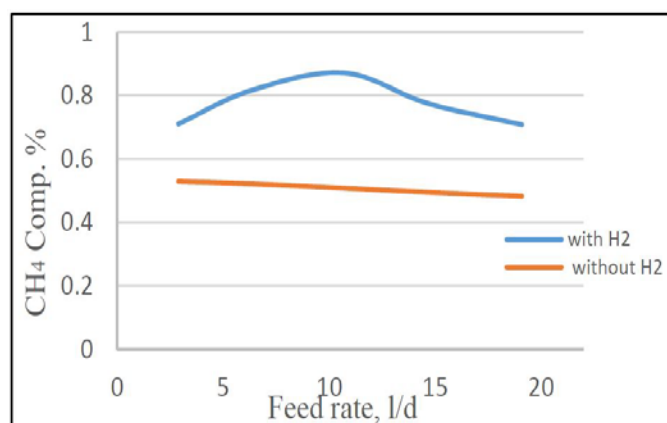


Figure 8. Effect of the H₂ addition on CH₄ composition

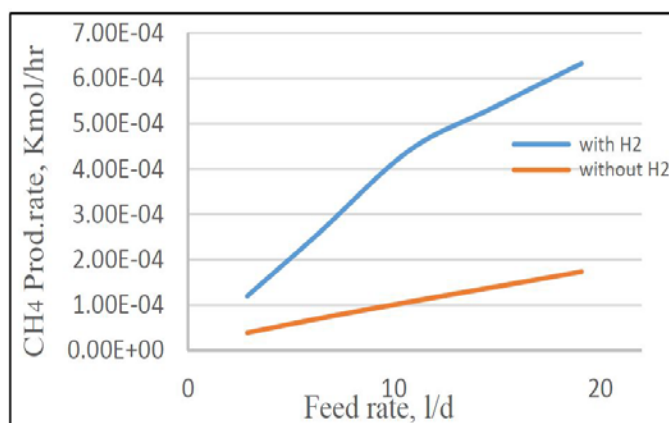


Figure 9. Effect of the H₂ addition on CH₄ production rate

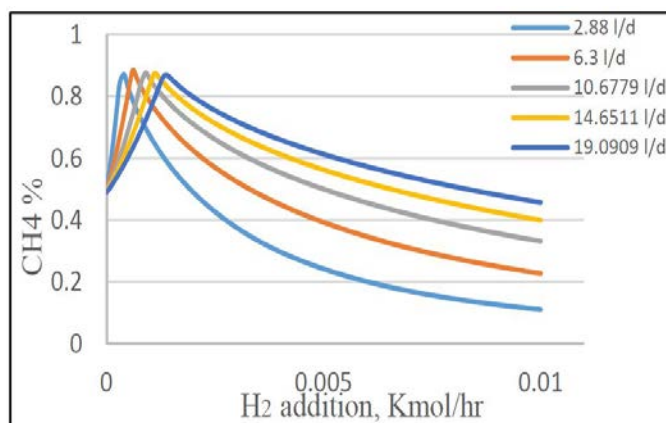


Figure 10. Sensitivity analysis for different feed rates at 5% substrate concentration

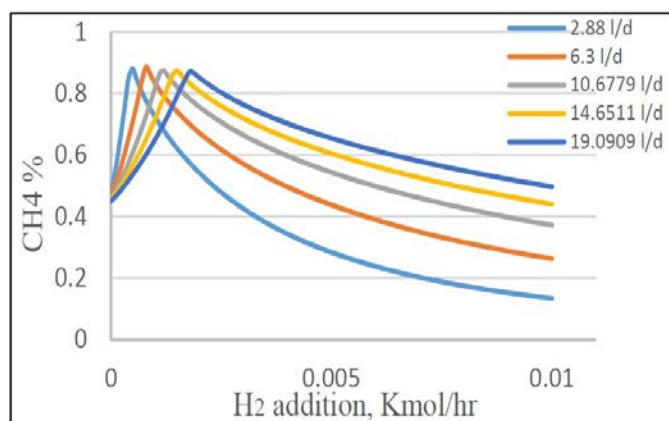


Figure 11. Sensitivity analysis for different feed rates at 10% substrate concentration

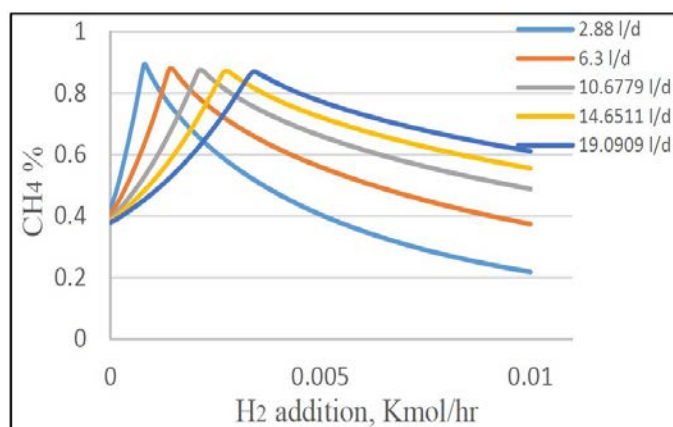


Figure 12. Sensitivity analysis for different feed rates at 20% substrate concentration

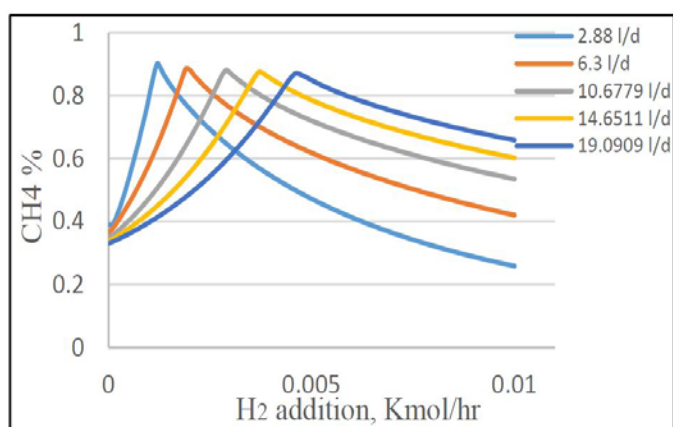


Figure 13. Sensitivity analysis for different feed rates at 30% substrate concentration

Figure 14-Figure 17 show that the methane rate could be maximized by adding the required amount of hydrogen gas to the process. The amount of hydrogen gas required to maximize the methane rate would increase with increasing the concentration of the feed substrates. A 30% increase in methane composition is achieved to reach about 85-90% CH₄ as a total maximum composition for the produced methane based on the substrates concentrations and the feed rate.

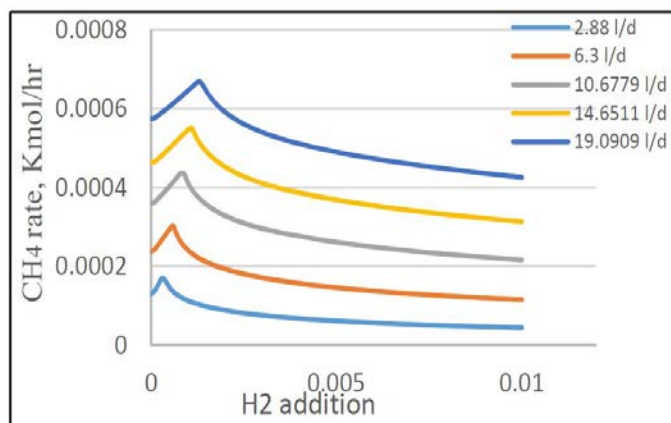


Figure 14. Sensitivity analysis for different feed rates at 5% substrate concentration

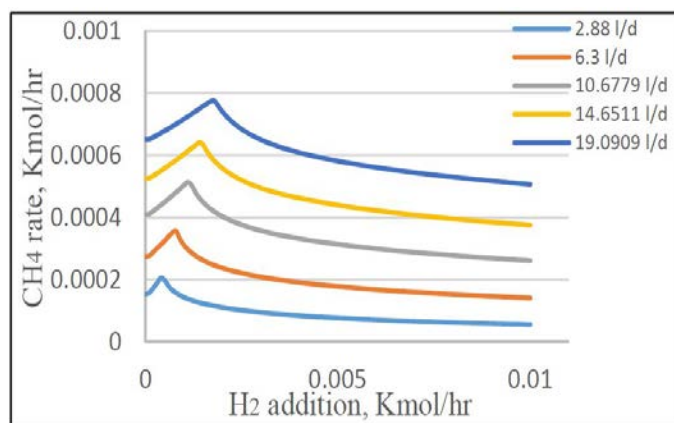


Figure 15. Sensitivity analysis for different feed rates at 10% substrate concentration

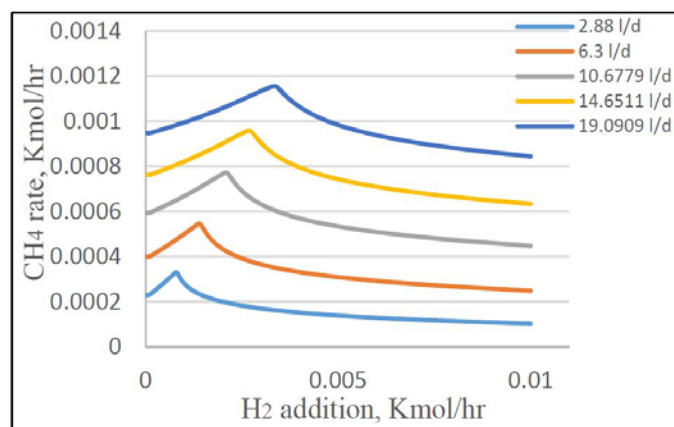


Figure 16. Sensitivity analysis for different feed rates at 20% substrate concentration

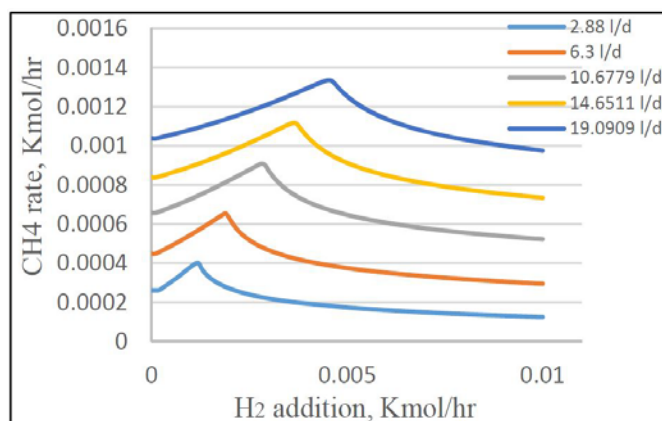


Figure 17. Sensitivity analysis for different feed rates at 30% substrate concentration

5.4 EFFECT OF PRESSURE ON METHANE PRODUCTION

Operating pressure is a very important factor that could affect the anaerobic digestion process and hence methane production, so a sensitivity analysis was conducted to check the contribution of pressure. Figure 18-Figure 21 show the sensitivity analysis to study the effect of operating pressure on methane composition.

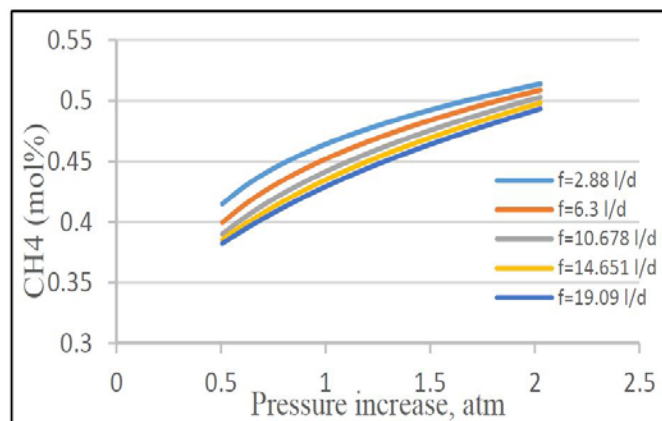


Figure 18. Sensitivity analysis (effect of pressure) on methane composition for different feed rates at 5% substrate concentration

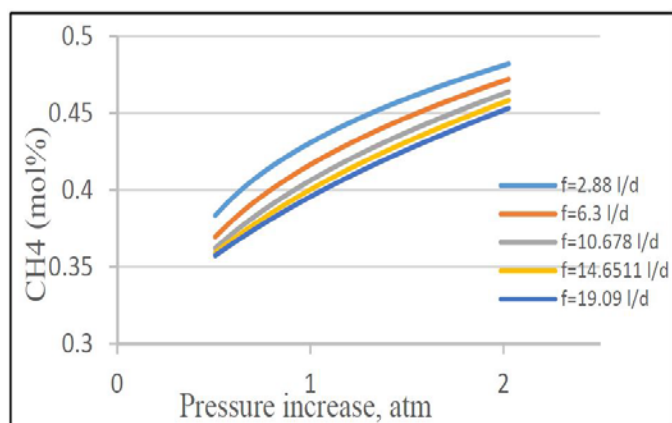


Figure 19. Sensitivity analysis (effect of pressure) on methane composition for different feed rates at 10% substrate concentration

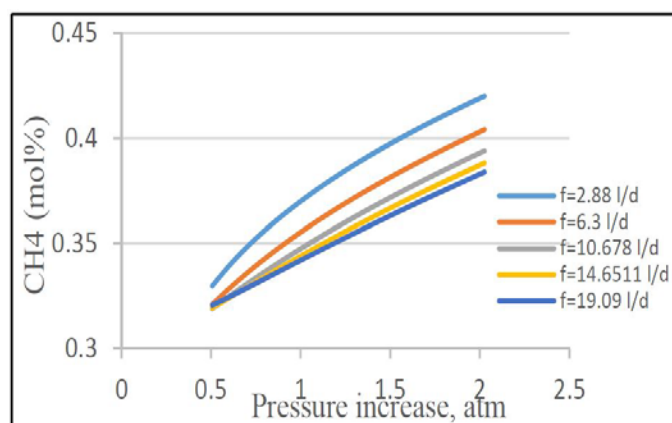


Figure 20. Sensitivity analysis (effect of pressure) on methane composition for different feed rates at 20% substrate concentration

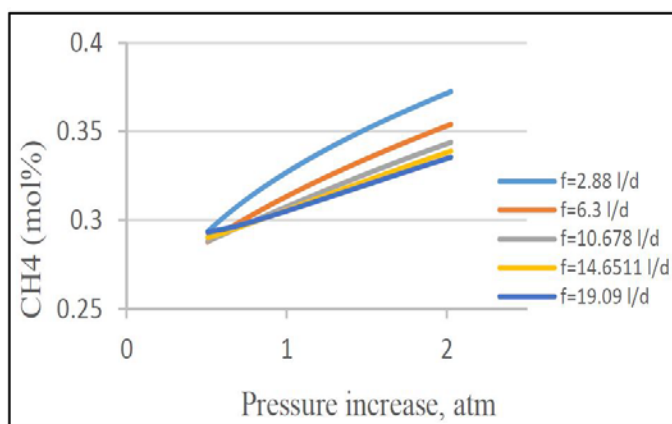


Figure 21. Sensitivity analysis (effect of pressure) on methane composition for different feed rates at 30% substrate concentration

It can be observed that the methane composition will increase with an increase in the pressure of the process, but it will decrease as the feed rate increases at a specific pressure value, due to the short residence time that the feed would spend inside the reactor. These results are already mentioned under the effect of feed rate on methane production. Figure 22-Figure 25 show the accumulated methane production rate would slightly increase with increasing the pressure, as the solubility in the liquid phase of the digester of some compounds depends on its pressure. The trends in Figures 22-25 are compatible with the literature [3,20,31–34].

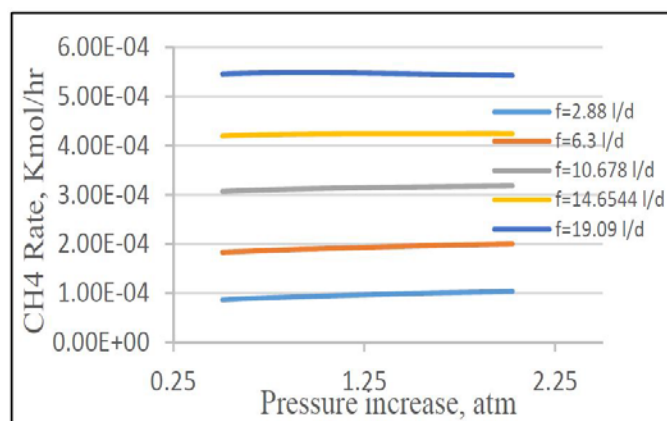


Figure 22. Sensitivity analysis (effect of pressure) on methane production rate for different feed rates at 5% substrate concentration

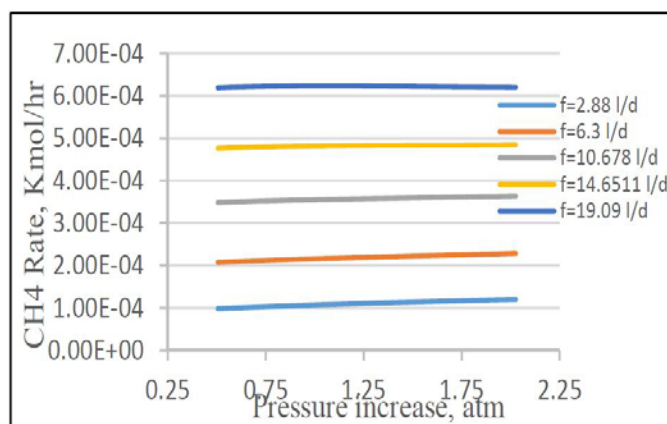


Figure 23. Sensitivity analysis (effect of pressure) on methane production rate for different feed rates at 10% substrate concentration

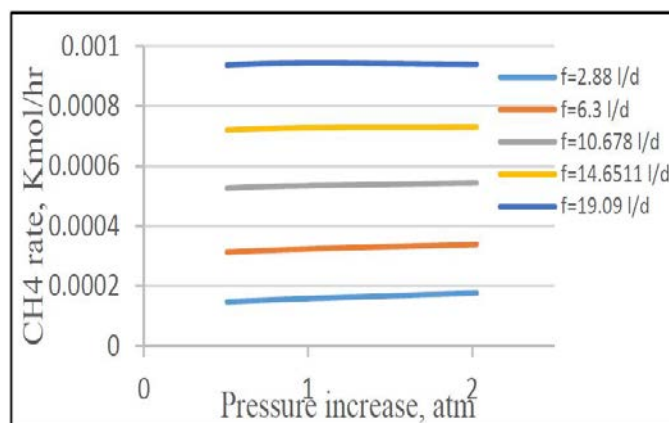


Figure 24. Sensitivity analysis (effect of pressure) on methane production rate for different feed rates at 20% substrate concentration

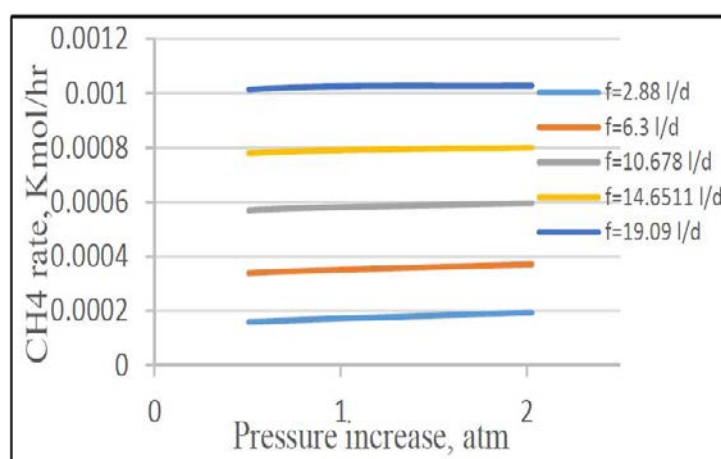


Figure 25. Sensitivity analysis (effect of pressure) on methane production rate for different feed rates at 30% substrate concentration

6. CONCLUSION

An Aspen Plus model has been developed to represent the anaerobic digestion process. It is designed to represent the four steps that form the anaerobic digestion process. A stoichiometric reactor was used to represent the hydrolysis step and thirteen reactions were introduced. The rest of the anaerobic digestion steps were represented in a kinetic reactor and thirty three chemical reactions were defined in it. Component separators were used to separate the components that were close to zero.

The results showed that methane composition in the biogas decreased on increasing the feed rate due to the decrease in residence time that the biomass needs to convert to methane. The accumulated methane production rate in the biogas increased with the increasing feed rate. These results match the literature.

A sensitivity analysis was conducted to study the effect of hydrogen gas introduced in the process and its effect on methane gas production. It showed that the methane gas composition increases until it reaches a maximum value at specific hydrogen rate. The continuously increasing hydrogen rate decreases the methane composition due to the increase in the acidity level, which causes inhibition to the AD process.

The amount of hydrogen gas required to maximize the methane composition in the biogas and the accumulated rate of methane increases, as the flow rate and concentration of the substrates increases. Methane gas composition increases by about 30-40 % by introducing hydrogen gas into the system. Furthermore, at a specific feed rate, the methane rate increases with the increase of substrates concentrations, while the composition of the methane gas slightly decreases. Sensitivity analysis was conducted to check if an increase of pressure affects biogas production. It affects solubility in the liquid phase of the digester and, hence, increases degradation and biogas production.

APPENDIX

CHEMICAL REACTION EQUATIONS, VARIABLE DEFINITIONS, AND
CALCULATIONS' FORTRAN CODE USED FOR THE ASPEN SIMULATION

CHEMICAL REACTIONS DEFINED IN ASPEN MODEL

The following script is the input file that will generate an Aspen Plus simulation used in this work. This script will generate the steady-state model that rigorously simulates Anaerobic Digestion Process.

Table 1. Amino acid reactions

Rxn No	Reactants	Products
1	GLYCINE + HYDROGEN \longrightarrow	ACETI-AC(MIXED) + NH3(MIXED)
2	THREONIN + HYDROGEN \longrightarrow	ACETI-AC(MIXED) + 0.5 ISOBU-01(MIXED) + NH3(MIXED)
3	HISTIDIN + 4 WATER + 0.5 HYDROGEN \longrightarrow	FORMAMID(MIXED) + ACETI-AC(MIXED) + 0.5 ISOBU-01(MIXED) + 2 NH3(MIXED) + CO2(MIXED)
4	ARGININE + 3 WATER + HYDROGEN \longrightarrow	0.5 ACETI-AC(MIXED) + 0.5 PROPI-01(MIXED) + 0.5 ISOVA-01(MIXED) + 4 NH3(MIXED) + CO2(MIXED)
5	PROLINE + WATER + HYDROGEN \longrightarrow	0.5 ACETI-AC(MIXED) + 0.5 PROPI-01(MIXED) + 0.5 ISOVA-01(MIXED) + NH3(MIXED)
6	METHIONI + 2 WATER \longrightarrow	PROPI-01(MIXED) + CO2(MIXED) + NH3(MIXED) + HYDROGEN(MIXED) + CH4S(MIXED)
7	SERINE + WATER \longrightarrow	ACETI-AC(MIXED) + NH3(MIXED) + CO2(MIXED) + HYDROGEN(MIXED)
8	THREONIN + WATER \longrightarrow	PROPI-01(MIXED) + NH3(MIXED) + HYDROGEN(MIXED) + CO2(MIXED)
9	ASPARTIC + 2 WATER \longrightarrow	ACETI-AC(MIXED) + NH3(MIXED) + 2 CO2(MIXED) + 2 HYDROGEN(MIXED)
10	GLUTAMIC + WATER \longrightarrow	ACETI-AC(MIXED) + 0.5 ISOBU-01(MIXED) + NH3(MIXED) + CO2(MIXED)
11	GLUTAMIC + 2WATER \longrightarrow	2 ACETI-AC(MIXED) + NH3(MIXED) + CO2(MIXED) + HYDROGEN(MIXED)
12	HISTIDIN + 5 WATER \longrightarrow	FORMAMID(MIXED) + 2 ACETI-AC(MIXED) + 2 NH3(MIXED) + CO2(MIXED) + 0.5 HYDROGEN(MIXED)
13	ARGININE + 6 WATER \longrightarrow	2 ACETI-AC(MIXED) + 4 NH3(MIXED) + 2 CO2(MIXED) + 3 HYDROGEN(MIXED)
14	LYSINE + 2 WATER \longrightarrow	ACETI-AC(MIXED) + ISOBU-01(MIXED) + 2 NH3(MIXED)
15	LEUCINE + 2 WATER \longrightarrow	ISOVA-01(MIXED) + NH3(MIXED) + CO2(MIXED) + 2 HYDROGEN(MIXED)
16	ISOLEUCI + 2 WATER \longrightarrow	ISOVA-01(MIXED) + NH3(MIXED) + CO2(MIXED) + 2 HYDROGEN(MIXED)
17	VALINE + 2 WATER \longrightarrow	ISOBU-01(MIXED) + NH3(MIXED) + CO2(MIXED) + 2 HYDROGEN(MIXED)
18	PHENYLAL + 2 WATER \longrightarrow	BENZENE(MIXED) + ACETI-AC(MIXED) + NH3(MIXED) + CO2(MIXED) + HYDROGEN(MIXED)
19	TYROSINE + 2 WATER \longrightarrow	PHENOL(MIXED) + ACETI-AC(MIXED) + NH3(MIXED) + CO2(MIXED) + HYDROGEN(MIXED)
20	TRYPTOPH + 2 WATER \longrightarrow	INDOLE(MIXED) + ACETI-AC(MIXED) + NH3(MIXED) + CO2(MIXED) + HYDROGEN(MIXED)
21	GLYCINE + 0.5 WATER \longrightarrow	0.75 ACETI-AC(MIXED) + NH3(MIXED) + 0.5 CO2(MIXED)
22	ALANINE + 2 WATER \longrightarrow	ACETI-AC(MIXED) + NH3(MIXED) + CO2(MIXED) + 2 HYDROGEN(MIXED)
23	CYSTEINE + 2 WATER \longrightarrow	ACETI-AC(MIXED) + NH3(MIXED) + CO2(MIXED) + 0.5 HYDROGEN(MIXED) + H2S(MIXED)

Table 2. Acidogenesis reactions

Rxn No	Reactants	Products
1	DEXTROSE + 0.1115 NH ₃	0.1115 C ₅ H ₇ NO ₂ (MIXED) + 0.744 ACETI-AC(MIXED) + 0.5 PROPI-01(MIXED) + 0.4409 ISOBU-01(MIXED) + 0.6909 CO ₂ (MIXED) + 1.0254 WATER(MIXED)
2	GLYCEROL + 0.04071 NH ₃ + 0.0291 CO ₂ + 5e-005 HYDROGEN	0.04071 C ₅ H ₇ NO ₂ (MIXED) + 0.94185 PROPI-01(MIXED) + 1.09308 WATER(MIXED)

Table 3. Acetogenesis reactions

Rxn No	Reactants	Products
1	OLEIC-AC + 15.2359 WATER + 0.482 CO ₂ + 0.1701 NH ₃	0.1701 C ₅ H ₇ NO ₂ (MIXED) + 9.02 ACETI-AC (MIXED) + 10.0723 HYDROGEN (MIXED)
2	PROPI-01 + 0.06198 NH ₃ + 0.314336 WATER	0.06198 C ₅ H ₇ NO ₂ (MIXED) + 0.9345 ACETI- AC (MIXED) + 0.660412 METHANE (MIXED) + 0.160688 CO ₂ (MIXED) + 0.000552 HYDROGEN (MIXED)
3	ISOBU-01 + 0.0653 NH ₃ + 0.5543 CO ₂ + 0.8038 WATER + 0.0006 HYDROGEN	0.0653 C ₅ H ₇ NO ₂ (MIXED) + 1.8909 ACETI-AC(MIXED) + 0.446 METHANE(MIXED)
4	ISOVA-01 + 0.0653 NH ₃ + 0.5543 CO ₂ + 0.8044 WATER	0.0653 C ₅ H ₇ NO ₂ (MIXED) + 0.8912 ACETI-AC(MIXED) + PROPI-01(MIXED) + 0.4454 METHANE(MIXED) + 0.0006 HYDROGEN(MIXED)
5	LINOLEIC + 15.356 WATER + 0.482 CO ₂ + 0.1701 NH ₃	0.1701 C ₅ H ₇ NO ₂ (MIXED) + 9.02 ACETI-AC(MIXED) + 10.0723 HYDROGEN(MIXED)
6	PALM + 10 WATER + 2 CO ₂ + NH ₃	C ₅ H ₇ NO ₂ (MIXED) + 6.5 ACETI-AC(MIXED) + 12 HYDROGEN(MIXED)

Table 4. Acid-base reactions

Rxn No	Reactants	Products
1	WATER	H ⁺ (MIXED) + OH ⁻ (MIXED)
2	NH ₃ + H ⁺	NH ₄ ⁺ (MIXED)
3	ACETI-AC	ACETATE(MIXED) + H ⁺ (MIXED)
4	CO ₂ + WATER	H ₂ CO ₃ (MIXED)
5	H ₂ CO ₃	H ⁺ (MIXED) + HCO ₃ ⁻ (MIXED)
6	HCO ₃ ⁻	H ⁺ (MIXED) + CO ₃ ⁻² (MIXED)
7	H ₂ S	H ⁺ (MIXED) + HS ⁻ (MIXED)

Table 5. Hydrogen reaction

Rxn No	Reactants	Products
1	2 CO ₂ + 4 HYDROGEN	ACETI-AC(MIXED) + 2 WATER(MIXED)

Table 6. Methane reaction

Rxn No	Reactants	Products
1	ACETI-AC + 0.022 NH ₃	0.022 C ₅ H ₇ NO ₂ (MIXED) + 0.956 METHANE(MIXED) + 0.088 WATER(MIXED) + 0.934 CO ₂ (MIXED)

Lists of variables with their definitions and calculator block FORTRAN codes

Table 7. List of variables for amino acid calculator block

Variable	Info flow	Definition
VOLFLOW	Import var	Stream-Var Stream=5 Substream=MIXED Variable=STDVOL-FLOW Units=cum/hr
T	Import var	Block-Var Block=REACTOR Variable=TEMP Sentence=PARAM Units=C
SER	Import var	Mass-Flow Stream=5 Substream=MIXED Component=SERINE Units=kg/hr
LEU	Import var	Mass-Flow Stream=5 Substream=MIXED Component=LEUCINE Units=kg/hr
ISO	Import var	Mass-Flow Stream=5 Substream=MIXED Component=ISOLEUCI Units=kg/hr
VAL	Import var	Mass-Flow Stream=5 Substream=MIXED Component=VALINE Units=kg/hr
ASP	Import var	Mass-Flow Stream=5 Substream=MIXED Component=ASPARTIC Units=kg/hr
GLY	Import var	Mass-Flow Stream=5 Substream=MIXED Component=GLYCINE Units=kg/hr
GLU	Import var	Mass-Flow Stream=5 Substream=MIXED Component=GLUTAMIC Units=kg/hr
ALA	Import var	Mass-Flow Stream=5 Substream=MIXED Component=ALANINE Units=kg/hr
PRO	Import var	Mass-Flow Stream=5 Substream=MIXED Component=PROLINE Units=kg/hr
ARG	Import var	Mass-Flow Stream=5 Substream=MIXED Component=ARGININE Units=kg/hr
HIS	Import var	Mass-Flow Stream=5 Substream=MIXED Component=HISTIDIN Units=kg/hr
LYS	Import var	Mass-Flow Stream=5 Substream=MIXED Component=LYSINE Units=kg/hr
TYR	Import var	Mass-Flow Stream=5 Substream=MIXED Component=TYROSINE Units=kg/hr
TRYP	Import var	Mass-Flow Stream=5 Substream=MIXED Component=TRYPTOPH Units=kg/hr
PHE	Import var	Mass-Flow Stream=5 Substream=MIXED Component=PHENYLAL Units=kg/hr
CYS	Import var	Mass-Flow Stream=5 Substream=MIXED Component=CYSTEINE Units=kg/hr
MET	Import var	Mass-Flow Stream=5 Substream=MIXED Component=METHIONI Units=kg/hr
THR	Import var	Mass-Flow Stream=5 Substream=MIXED Component=THREONIN Units=kg/hr
PCONT	Import var	Mass-Flow Stream=5 Substream=MIXED Component=H+ Units=kg/hr
KIN1	Export var	React-Var Block=AMINOACI Variable=PRE-EXP Sentence=RATE-CON ID1=1
KIN2	Export var	React-Var Block=AMINOACI Variable=PRE-EXP Sentence=RATE-CON ID1=2
KIN3	Export var	React-Var Block=AMINOACI Variable=PRE-EXP Sentence=RATE-CON ID1=3
KIN4	Export var	React-Var Block=AMINOACI Variable=PRE-EXP Sentence=RATE-CON ID1=4
KIN5	Export var	React-Var Block=AMINOACI Variable=PRE-EXP Sentence=RATE-CON ID1=5
KIN6	Export var	React-Var Block=AMINOACI Variable=PRE-EXP Sentence=RATE-CON ID1=6
KIN7	Export var	React-Var Block=AMINOACI Variable=PRE-EXP Sentence=RATE-CON ID1=7
KIN8	Export var	React-Var Block=AMINOACI Variable=PRE-EXP Sentence=RATE-CON ID1=8
KIN9	Export var	React-Var Block=AMINOACI Variable=PRE-EXP Sentence=RATE-CON ID1=9
KIN10	Export var	React-Var Block=AMINOACI Variable=PRE-EXP Sentence=RATE-CON ID1=10
KIN11	Export var	React-Var Block=AMINOACI Variable=PRE-EXP Sentence=RATE-CON ID1=11
KIN12	Export var	React-Var Block=AMINOACI Variable=PRE-EXP Sentence=RATE-CON ID1=12
KIN13	Export var	React-Var Block=AMINOACI Variable=PRE-EXP Sentence=RATE-CON ID1=13
KIN14	Export var	React-Var Block=AMINOACI Variable=PRE-EXP Sentence=RATE-CON ID1=14
KIN15	Export var	React-Var Block=AMINOACI Variable=PRE-EXP Sentence=RATE-CON ID1=15
KIN16	Export var	React-Var Block=AMINOACI Variable=PRE-EXP Sentence=RATE-CON ID1=16
KIN17	Export var	React-Var Block=AMINOACI Variable=PRE-EXP Sentence=RATE-CON ID1=17
KIN18	Export var	React-Var Block=AMINOACI Variable=PRE-EXP Sentence=RATE-CON ID1=18
KIN19	Export var	React-Var Block=AMINOACI Variable=PRE-EXP Sentence=RATE-CON ID1=19
KIN20	Export var	React-Var Block=AMINOACI Variable=PRE-EXP Sentence=RATE-CON ID1=20
KIN21	Export var	React-Var Block=AMINOACI Variable=PRE-EXP Sentence=RATE-CON ID1=21
KIN22	Export var	React-Var Block=AMINOACI Variable=PRE-EXP Sentence=RATE-CON ID1=22
KIN23	Export var	React-Var Block=AMINOACI Variable=PRE-EXP Sentence=RATE-CON ID1=23

Table 8. List of variables for butyric acid calculator block

Variable	Info flow	Definition
VOLFLOW	Import var	Stream-Var Stream=5 Substream=MIXED Variable=STDVOL-FLOW Units=cum/hr
HACFLOW	Import var	Mass-Flow Stream=5 Substream=MIXED Component=ACETI-AC Units=kg/hr
NH3	Import var	Mass-Flow Stream=5 Substream=MIXED Component=NH3 Units=kg/hr
LCFAFLOW	Import var	Mass-Flow Stream=5 Substream=MIXED Component=OLEIC-AC Units=kg/hr
PCONT	Import var	Mass-Flow Stream=5 Substream=MIXED Component=H+ Units=kg/hr
NH4	Import var	Mass-Flow Stream=5 Substream=MIXED Component=NH4+ Units=kg/hr
T	Import var	Block-Var Block=REACTOR Variable=TEMP Sentence=PARAM Units=C
H2	Import var	Mass-Flow Stream=5 Substream=MIXED Component=HYDROGEN Units=kg/hr
BUTYFLOW	Import var	Mass-Frac Stream=5 Substream=MIXED Component=ISOBU-01
KINETIC	Export var	React-Var Block=ACETOGEN Variable=PRE-EXP Sentence=RATE-CON ID1=3

Table 9. List of variables for dextrose calculator block

Variable	Info flow	Definition
VOLFLOW	Import var	Stream-Var Stream=5 Substream=MIXED Variable=STDVOL-FLOW Units=cum/hr
GLUFLOW	Import var	Mass-Flow Stream=5 Substream=MIXED Component=DEXTROSE Units=kg/hr
LCFAFLOW	Import var	Mass-Flow Stream=5 Substream=MIXED Component=OLEIC-AC Units=kg/hr
NH3	Import var	Mass-Flow Stream=5 Substream=MIXED Component=NH3 Units=kg/hr
NH4	Import var	Mass-Flow Stream=5 Substream=MIXED Component=NH4+ Units=kg/hr
PCONT	Import var	Mass-Flow Stream=5 Substream=MIXED Component=H+ Units=kg/hr
T	Import var	Block-Var Block=REACTOR Variable=TEMP Sentence=PARAM Units=C
KINETIC	Export var	React-Var Block=ACIDOGEN Variable=PRE-EXP Sentence=RATE-CON ID1=1

Table 10. List of variables for glycerol calculator block

Variable	Info flow	Definition
VOLFLOW	Import var	Stream-Var Stream=5 Substream=MIXED Variable=STDVOL-FLOW Units=cum/hr
GTOFLOW	Import var	Mass-Flow Stream=5 Substream=MIXED Component=GLYCEROL Units=kg/hr
NH3	Import var	Mass-Flow Stream=5 Substream=MIXED Component=NH3 Units=kg/hr
PCONT	Import var	Mass-Flow Stream=5 Substream=MIXED Component=H+ Units=kg/hr
NH4	Import var	Mass-Flow Stream=5 Substream=MIXED Component=NH4+ Units=kg/hr
LCFAFLOW	Import var	Mass-Flow Stream=5 Substream=MIXED Component=OLEIC-AC Units=kg/hr
KINETIIC	Export var	React-Var Block=ACIDOGEN Variable=PRE-EXP Sentence=RATE-CON ID1=2

Table 11. List of variables for linoleic calculator block

Variable	Info flow	Definition
VOLFLOW	Import var	Stream-Var Stream=5 Substream=MIXED Variable=STDVOL-FLOW Units=cum/hr
LINOFLOW	Import var	Mass-Flow Stream=5 Substream=MIXED Component=LINOLEIC Units=kg/hr
NH3	Import var	Mass-Flow Stream=5 Substream=MIXED Component=NH3 Units=kg/hr
NH4	Import var	Mass-Flow Stream=5 Substream=MIXED Component=NH4+ Units=kg/hr
T	Import var	Block-Var Block=REACTOR Variable=TEMP Sentence=PARAM Units=C
PCONT	Import var	Mass-Flow Stream=5 Substream=MIXED Component=H+ Units=kg/hr
KINETIC	Export var	React-Var Block=ACETOGEN Variable=PRE-EXP Sentence=RATE-CON ID1=5

Table 12. List of variables for methane calculator block

Variable	Info flow	Definition
VOLFLOW	Import var	Stream-Var Stream=5 Substream=MIXED Variable=STDVOL-FLOW Units=cum/hr
HACFLOW	Import var	Mass-Flow Stream=5 Substream=MIXED Component=ACETI-AC Units=kg/hr
NH3	Import var	Mass-Flow Stream=5 Substream=MIXED Component=NH3 Units=kg/hr
LCFAFLOW	Import var	Mass-Flow Stream=5 Substream=MIXED Component=OLEIC-AC Units=kg/hr
PCONT	Import var	Mass-Flow Stream=5 Substream=MIXED Component=H+ Units=kg/hr
NH4	Import var	Mass-Flow Stream=5 Substream=MIXED Component=NH4+ Units=kg/hr
T	Import var	Block-Var Block=REACTOR Variable=TEMP Sentence=PARAM Units=C
H2FLOW	Import var	Mass-Flow Stream=5 Substream=MIXED Component=HYDROGEN Units=kg/hr
KINETIC	Export var	React-Var Block=METHAN Variable=PRE-EXP Sentence=RATE-CON ID1=1

Table 13. List of variables for olie calculator block

Variable	Info flow	Definition
VOLFLOW	Import var	Stream-Var Stream=5 Substream=MIXED Variable=STDVOL-FLOW Units=cum/hr
LCFAFLOW	Import var	Mass-Flow Stream=5 Substream=MIXED Component=OLEIC-AC Units=kg/hr
NH3	Import var	Mass-Flow Stream=5 Substream=MIXED Component=NH3 Units=kg/hr
NH4	Import var	Mass-Flow Stream=5 Substream=MIXED Component=NH4+ Units=kg/hr
PCONT	Import var	Mass-Flow Stream=5 Substream=MIXED Component=H+ Units=kg/hr
T	Import var	Block-Var Block=REACTOR Variable=TEMP Sentence=PARAM Units=C
KINETIC	Export var	React-Var Block=ACETOGEN Variable=PRE-EXP Sentence=RATE-CON ID1=1

Table 14. List of variables for palmeric calculator block

Variable	Info flow	Definition
VOLFLOW	Import var	Stream-Var Stream=5 Substream=MIXED Variable=STDVOL-FLOW Units=cum/hr
PALMFLOW	Import var	Mass-Flow Stream=5 Substream=MIXED Component=PALM Units=kg/hr
NH3	Import var	Mass-Flow Stream=5 Substream=MIXED Component=NH3 Units=kg/hr
NH4	Import var	Mass-Flow Stream=5 Substream=MIXED Component=NH4+ Units=kg/hr
T	Import var	Block-Var Block=REACTOR Variable=TEMP Sentence=PARAM Units=C
PCONT	Import var	Mass-Flow Stream=5 Substream=MIXED Component=H+ Units=kg/hr
KINETIC	Export var	React-Var Block=ACETOGEN Variable=PRE-EXP Sentence=RATE-CON ID1=6

Table 15. List of variables for propionic calculator block

Variable	Info flow	Definition
VOLFLOW	Import var	Stream-Var Stream=5 Substream=MIXED Variable=STDVOL-FLOW Units=cum/hr
HACFLOW	Import var	Mass-Flow Stream=5 Substream=MIXED Component=ACETI-AC Units=kg/hr
TNH3FLOW	Import var	Mass-Flow Stream=5 Substream=MIXED Component=NH3 Units=kg/hr
LCFAFLOW	Import var	Mass-Flow Stream=5 Substream=MIXED Component=OLEIC-AC Units=kg/hr
PCONT	Import var	Mass-Flow Stream=5 Substream=MIXED Component=H+ Units=kg/hr
T	Import var	Block-Var Block=REACTOR Variable=TEMP Sentence=PARAM Units=C
NH4	Import var	Mass-Flow Stream=5 Substream=MIXED Component=NH4+ Units=kg/hr
PROPFLOW	Import var	Mass-Flow Stream=5 Substream=MIXED Component=PROPI-01 Units=kg/hr
H2	Import var	Mass-Flow Stream=5 Substream=MIXED Component=HYDROGEN Units=kg/hr
KINETIC	Export var	React-Var Block=ACETOGEN Variable=PRE-EXP Sentence=RATE-CON ID1=2

Table 16. List of variables for valeric calculator block

Variable	Info flow	Definition
VOLFLOW	Import var	Stream-Var Stream=5 Substream=MIXED Variable=STDVOL-FLOW Units=cum/hr
HACFLOW	Import var	Mass-Flow Stream=5 Substream=MIXED Component=ACETI-AC Units=kg/hr
TNH3FLOW	Import var	Mass-Flow Stream=5 Substream=MIXED Component=NH3 Units=kg/hr
LCFAFLOW	Import var	Mass-Flow Stream=5 Substream=MIXED Component=OLEIC-AC Units=kg/hr
PCONT	Import var	Mass-Flow Stream=5 Substream=MIXED Component=H+ Units=kg/hr
NH4	Import var	Mass-Flow Stream=5 Substream=MIXED Component=NH4+ Units=kg/hr
T	Import var	Block-Var Block=REACTOR Variable=TEMP Sentence=PARAM Units=C
H2	Import var	Mass-Flow Stream=5 Substream=MIXED Component=HYDROGEN Units=kg/hr
KINETIC	Export var	React-Var Block=ACETOGEN Variable=PRE-EXP Sentence=RATE-CON ID1=4

Table 17. Calculator block FORTRAN code for amino acids

```

A = 0.00000001
AA1 = ARG + HIS + LYS + TYR + TRYP + PHE + CYS + MET + THR + SER
AA2 = LEU + ISO + VAL + GLU + ASP + GLY + ALA + PRO
AA = AA1 + AA2
IF (AA .EQ. 0.) THEN
AA = AA + A
ENDIF
IF (VOLFLOW .EQ. 0.) THEN
VOLFLOW = VOLFLOW + A
ENDIF
IF (PCONT .EQ. 0.) THEN
PCONT = 0.0000001
ENDIF
PH = - ALOG10 ((PCONT)/(VOLFLOW))
c PH = 6.5
IF ( PH .LT. 5.5 ) THEN
S = ((PH - 5.5) / ( 5.5 - 4 ))
ENDIF
IF (S .LT.0) THEN
S = -S
ENDIF
R = ((-3.)*(S**2.))
Q= ( 2.7182818284**(R))
N=( 1. / (1. + 0.3 / ( AA / VOLFLOW)))
T = T+273.15
TO = 55 + 273.15
Z = 70 * EXP (-(-14143.72619 / 8.314) * (1/T - 1/TO))
L = ( 1. / (3600. * 24. )) * Z
K = L*N*Q
KIN1 = K
KIN2 = K
KIN3 = K
KIN4 = K
KIN5 = K
KIN6 = K
KIN7 = K
KIN8 = K

```



```

KIN9 = K
KIN10 = K
KIN11 = K
KIN12 = K
KIN13 = K
KIN14 = K
KIN15 = K
KIN16 = K
KIN17 = K
KIN18 = K
KIN19 = K
KIN20 = K
KIN21 = K

```

Table 18. Calculator block FORTRAN code for butyric acid

```

A = .00000001
TNH3FLOW= NH3 + NH4
IF (HACFLOW .EQ. 0.) THEN
  HACFLOW = HACFLOW + A
ENDIF
IF (LCFAFLOW .EQ. 0.) THEN
  LCFAFLOW = LCFAFLOW + A
ENDIF
IF (VOLFLOW .EQ. 0.) THEN
  VOLFLOW = VOLFLOW + A
ENDIF
IF (TNH3FLOW .EQ. 0.) THEN
  TNH3FLOW = TNH3FLOW + A
ENDIF
IF (PCONT .EQ. 0.) THEN
  PCONT = 0.0000001
ENDIF
IF (BUTYFLOW .EQ. 0.) THEN
  BUTYFLOW = A
ENDIF
T=T+273.15
T0=55+273.15
H2I = 0.00003 + 0.000001 * ( T - T0 )
I = ( 1. / ( 1. + ( H2 / VOLFLOW ) / H2I ) )
Z = 30 *EXP(-(-17043.8653/8.314)*(1/T-1/T0))
L = (1. / ( 3600. * 24. ) ) * Z
U = .176 + .01*(T-T0)
N = ( 1. / ( 1. + U / ( BUTYFLOW / VOLFLOW ) ) )
M = ( 1. / ( 1. + .05 / ( TNH3FLOW / VOLFLOW ) ) )
O = ( 1. / ( 1. + ( HACFLOW / VOLFLOW ) / .72 ) )
P = ( 1. / ( 1. + ( LCFAFLOW / VOLFLOW ) / 5. ) )
PH = - ALOG10((PCONT)/(VOLFLOW))
PH = 6.5
IF ( PH .LT. 5.5) THEN
  S = (( PH - 5.5 ) / (5.5 - 4. ) )
  IF ( S .LT. 0.) THEN
    S = -S

```

```

ENDIF
R = ((-3.)*(S**2.))
Q =(2.7182818284**(R))
ELSE
Q = 1
ENDIF
K = L * N * M * O * P * Q * I
      KINETIC=K

```

Table 19. Calculator block FORTRAN code for dextrose

```

A = .00000001
TNH3FLOW= NH3 + NH4
IF (VOLFLOW .EQ. 0.) THEN
VOLFLOW = VOLFLOW + A
ENDIF
IF (LCFAFLOW .EQ. 0.) THEN
LCFAFLOW = LCFAFLOW + A
ENDIF
IF (TNH3FLOW .EQ. 0.) THEN
TNH3FLOW = TNH3FLOW + A
ENDIF
IF (GLUFLOW .EQ. 0.) THEN
GLUFLOW = GLUFLOW + A
ENDIF
T = T+273.15
T0=55+273.15
IF (PCONT .EQ. 0.) THEN
PCONT = 0.0000001
ENDIF
PH = - ALOG10((PCONT)/(VOLFLOW))
PH = 6.5
IF ( PH .LT. 5.5) THEN
S = (( PH - 5.5 ) / (5.5 - 4. ) )
IF (S .LT. 0.) THEN
S= -S
ENDIF
R = ((-3.)*(S**2.))
Q =(2.7182818284**(R))
ELSE
Q = 1
ENDIF
Z = 70*EXP(-(-35616.457/8.314)*(1/T-1/T0))
L = ( 1. / ( 3600. * 24. ) ) * Z
P = 0.5 + 0.025 * ( T - T0 )
N = 5.1 * ( 1. / ( 1. + P / ( GLUFLOW / VOLFLOW ) ) )
M = ( 1. / ( 1. + .05 / ( TNH3FLOW / VOLFLOW ) ) )
O = ( 1. / ( 1. + ( LCFAFLOW / VOLFLOW ) / 5. ) )
K = L * N * M * O * Q
      KINETIC=K

```

Table 20. Calculator block FORTRAN code for glycerol

```

A = .00000001
TNH3FLOW= NH3 + NH4
IF (VOLFLOW .EQ. 0.) THEN
  VOLFLOW = VOLFLOW + A
ENDIF
IF (LCFAFLOW .EQ. 0.) THEN
  LCFAFLOW = LCFAFLOW + A
ENDIF
IF (TNH3FLOW .EQ. 0.) THEN
  TNH3FLOW = TNH3FLOW + A
ENDIF
IF (GTOFLOW .EQ. 0.) THEN
  GTOFLOW = GTOFLOW + A
ENDIF
IF (PCONT .EQ. 0.) THEN
  PCONT = 0.0000001
ENDIF
L = ( 1. / ( 3600. * 24. ) )
N = 0.53 * ( 1. / ( 1. + .01 / ( GTOFLOW / VOLFLOW ) ) )
M = ( 1. / ( 1. + .05 / ( TNH3FLOW / VOLFLOW ) ) )
O = ( 1. / ( 1. + ( LCFAFLOW / VOLFLOW ) / 5. ) )
PH = - ALOG10((PCONT)/VOLFLOW)
PH = 6.5
IF ( PH .LT. 5.5 ) THEN
  S = (( PH - 5.5 ) / ( 5.5 - 4. ) )
  IF ( S .LT. 0. ) THEN
    S = -S
  ENDIF
  R = ((-3.)*(S**2.))
  Q =(2.7182818284**(R))
ELSE
  Q = 1
ENDIF
KINETIC = L * N * M * O * Q

```

Table 21. Calculator block FORTRAN code for linoleic

```

A = .00000001
TNH3FLOW= NH3 + NH4
A = .00000001
TNH3FLOW= NH3 + NH4
IF (LINOFLOW .EQ. 0.) THEN
  LINOFLOW = LINOFLOW + A
ENDIF
IF (VOLFLOW .EQ. 0.) THEN
  VOLFLOW = VOLFLOW + A
ENDIF
IF (TNH3FLOW .EQ. 0.) THEN
  TNH3FLOW = TNH3FLOW + A

```

```

ENDIF
IF (PCONT .EQ. 0.) THEN
  PCONT = 0.0000001
ENDIF
T = T+273.15
T0=55+273.15
Z = 10 *EXP(-(-21472.7308/8.314)*(1/T-1/T0))
L = ( 1. / ( 3600. * 24. ) ) * Z
O = ( LCFAFLOW / VOLFLOW ) / 5.
N = ( 1. / ( 1. + .02 / (LINOFLOW/VOLFLOW)+O))
M = ( 1. / ( 1. + .05 / ( TNH3FLOW / VOLFLOW ) ) )
PH = - ALOG10((PCONT)/VOLFLOW)
PH = 6.5
IF ( PH .LT. 5.5) THEN
  S = (( PH - 5.5 ) / (5.5 - 4. ) )
  IF ( S .LT. 0.) THEN
    S= -S
  ENDIF
  R = ((-3.)*(S**2.))
  Q =(2.7182818284**(R))
ELSE
  Q = 1
ENDIF
K = L * N * M * Q
KINETIC=K

```

Table 22. Calculator block FORTRAN code for methane

```

A = .00000001
TNH3FLOW = NH3 + NH4
IF (NH3 .EQ. 0.) THEN
  NH3 = NH3 + A
ENDIF
IF (VOLFLOW .EQ. 0.) THEN
  VOLFLOW = VOLFLOW + A
ENDIF
IF (HACFLOW .EQ. 0.) THEN
  HACFLOW= HACFLOW + A
ENDIF
IF (LCFAFLOW .EQ. 0.) THEN
  LCFAFLOW = LCFAFLOW + A
ENDIF
IF (TNH3FLOW .EQ. 0.) THEN
  TNH3FLOW = TNH3FLOW + A
ENDIF
IF (PCONT .EQ. 0.) THEN
  PCONT = 0.0000001
ENDIF
IF (H2FLOW .EQ. 0) THEN
  H2FLOW = A
ENDIF

```

```

T = T+273.15
T0=55+273.15
Z = 16 *EXP(-(-29136.6801/8.314)*(1/T-1/T0))
L = ( 1. / ( 3600. * 24. ) ) * Z
X = ( 1. / ( 3600. * 24. ) ) * 35.
V = .12 + .0075*(T-T0)
N = ( 1. / ( 1. + V / ( HACFLOW / VOLFLOW ) ) )
U = 0.00005 + 0.00000215* ( T - T0)
S = ( 1. / ( 1 + U / ( H2FLOW / VOLFLOW ) ) )
M = ( 1. / ( 1. + .05 / ( TNH3FLOW / VOLFLOW ) ) )
W = .26 + .00046*(T-T0)
O = ( 1. / ( 1. + ( NH3 / VOLFLOW ) / W ) )
P = ( 1. / ( 1. + ( LCFAFLOW / VOLFLOW ) / 5. ) )
PH = - ALOG10((PCONT)/VOLFLOW)
PH = 6.5
Q = (1.+2.*10.**(.5*(5.-6.)))/(1.+10.** (PH-6.)+10.** (5.-PH))
R = (1.+2.*10.**(.5*(6.-7.)))/(1.+10.** (PH-7.)+10.** (6.-PH))
K = L * N * M * O * P * R
      KINETIC=K

```

Table 23. Calculator block FORTRAN code for oliecc

```

A = .00000001
TNH3FLOW= NH3 + NH4
A = .00000001
TNH3FLOW= NH3 + NH4
IF (LCFAFLOW .EQ. 0.) THEN
  LCFAFLOW = LCFAFLOW + A
ENDIF
IF (VOLFLOW .EQ. 0.) THEN
  VOLFLOW = VOLFLOW + A
ENDIF
IF (TNH3FLOW .EQ. 0.) THEN
  TNH3FLOW = TNH3FLOW + A
ENDIF
IF (PCONT .EQ. 0.) THEN
  PCONT = 0.0000001
ENDIF
T = T+273.15
T0=55+273.15
Z = 10 *EXP(-(-21472.7308/8.314)*(1/T-1/T0))
L = ( 1. / ( 3600. * 24. ) ) * Z
O = ( LCFAFLOW / VOLFLOW ) / 5.
N = ( 1. / ( 1. + .02 / (LCFAFLOW/VOLFLOW)+O) )
M = ( 1. / ( 1. + .05 / ( TNH3FLOW / VOLFLOW ) ) )
PH = - ALOG10((PCONT)/VOLFLOW)
PH = 6.5
IF ( PH .LT. 5.5) THEN
  S = (( PH - 5.5 ) / ( 5.5 - 4. ) )
  IF ( S .LT. 0.) THEN

```

```

S= -S
ENDIF
R = ((-3.)*(S**2.))
Q=(2.7182818284**(R))
ELSE
Q = 1
ENDIF
K = L * N * M * Q
      KINETIC=K

```

Table 24. Calculator block FORTRAN code for palmeric

```

A = .00000001
TNH3FLOW= NH3 + NH4
A = .00000001
TNH3FLOW= NH3 + NH4
IF (PALMFLOW .EQ. 0.) THEN
  PALMFLOW = PALMFLOW + A
ENDIF
IF (VOLFLOW .EQ. 0.) THEN
  VOLFLOW = VOLFLOW + A
ENDIF
IF (TNH3FLOW .EQ. 0.) THEN
  TNH3FLOW = TNH3FLOW + A
ENDIF
IF (PCONT .EQ. 0.) THEN
  PCONT = 0.0000001
ENDIF
T = T+273.15
T0=55+273.15
Z = 10 *EXP(-(-21472.7308/8.314)*(1/T-1/T0))
L = ( 1. / ( 3600. * 24. ) ) * Z
O = ( LCFAFLOW / VOLFLOW ) / 5.
N = ( 1. / ( 1. + .02 / (PALMFLOW/VOLFLOW)+O))
M = ( 1. / ( 1. + .05 / ( TNH3FLOW / VOLFLOW ) ) )
PH = - ALOG10((PCONT)/VOLFLOW)
PH = 6.5
IF ( PH .LT. 5.5) THEN
  S = (( PH - 5.5 ) / (5.5 - 4. ) )
  IF (S .LT. 0.) THEN
    S= -S
  ENDIF
  R = ((-3.)*(S**2.))
  Q=(2.7182818284**(R))
ELSE
  Q = 1
ENDIF
K = L * N * M * Q
      KINETIC=K

```

Table 25. Calculator block FORTRAN code for propionic

```

A = .00000001
TNH3FLOW= NH3 + NH4
IF (HACFLOW .EQ. 0.) THEN
  HACFLOW = HACFLOW + A
ENDIF
IF (LCFAFLOW .EQ. 0.) THEN
  LCFAFLOW = LCFAFLOW + A
ENDIF
IF (VOLFLOW .EQ. 0.) THEN
  VOLFLOW = VOLFLOW + A
ENDIF
IF (TNH3FLOW .EQ. 0.) THEN
  TNH3FLOW = TNH3FLOW + A
ENDIF
IF (PCONT .EQ. 0.) THEN
  PCONT = 0.0000001
ENDIF
IF (PROPFLOW .EQ. 0.) THEN
  PROPFLOW = A
ENDIF
T = T+273.15
T0=55+273.15
Z = 20 *EXP(-(-18108.108/8.314)*(1/T-1/T0))
L = (1. / ( 3600. * 24. ) ) * Z
H2I = 0.00001 + 0.000000325 * ( T - T0 )
I = ( 1. / ( 1. + ( H2 / VOLFLOW ) / H2I ) )
U = .259 + 0.01 * (T-T0)
N = ( 1. / ( 1. + U / ( PROPFLOW / VOLFLOW ) ) )
M = ( 1. / ( 1. + .05 / ( TNH3FLOW / VOLFLOW ) ) )
O = ( 1. / ( 1. + ( HACFLOW / VOLFLOW ) / .96 ) )
P = ( 1. / ( 1. + ( LCFAFLOW / VOLFLOW ) / 5. ) )
PH = - ALOG10((PCONT)/VOLFLOW)
PH = 6.5
IF ( PH .LT. 5.5) THEN
  S = (( PH - 5.5 ) / (5.5 - 4. ) )
  IF (S .LT. 0.) THEN
    S= -S
  ENDIF
  R = ((-3.)*(S**2.))
  Q =(2.7182818284**(R))
ELSE
  Q = 1
ENDIF
K = L * N * M * O * P * Q * I
KINETIC=K

```

Table 26. Calculator block FORTRAN code for valeric

```

A = .00000001
IF (HACFLOW .EQ. 0.) THEN
  HACFLOW = A
ENDIF
IF (LCFAFLOW .EQ. 0.) THEN
  LCFAFLOW = A
ENDIF
IF (VOLFLOW .EQ. 0.) THEN
  VOLFLOW = A
ENDIF
IF (TNH3FLOW .EQ. 0.) THEN
  TNH3FLOW = A
ENDIF
IF (PCONT .EQ. 0.) THEN
  PCONT = 0.0000001
ENDIF
IF (VALEFLOW .EQ. 0.) THEN
  VALEFLOW = A
ENDIF
T = T+273.15
T0=55+273.15
H2I = 0.00003 + 0.000001 * ( T - T0 )
I = ( 1. / ( 1. + ( H2 / VOLFLOW ) / H2I ) )
Z = 30 *EXP(-(-17043.8653/8.314)*(1/T-1/T0))
L = (1. / ( 3600. * 24. ) ) * Z
U = .175 + .01*(T-T0)
N = ( 1. / ( 1. + U / ( VALEFLOW / VOLFLOW ) ) )
M = ( 1. / ( 1. + .05 / ( TNH3FLOW / VOLFLOW ) ) )
O = ( 1. / ( 1. + ( HACFLOW / VOLFLOW ) / .4 ) )
P = ( 1. / ( 1. + ( LCFAFLOW / VOLFLOW ) / 5. ) )
PH = - ALOG10((PCONT)/VOLFLOW)
PH = 6.5
IF ( PH .LT. 5.5) THEN
  S = (( PH - 5.5 ) / ( 5.5 - 4. ) )
  IF ( S .LT. 0.) THEN
    S= -S
  ENDIF
  R = ((-3.)*(S**2.))
  Q =(2.7182818284**(R))
ELSE
  Q = 1
ENDIF
K = L * N * M * O * P * Q * I
KINETIC=K

```


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II. THE IMPACT OF HYDRAULIC RETENTION TIME AND OPERATING TEMPERATURE ON BIOFUEL PRODUCTION AND PROCESS WASTE WATER TREATMENT

ABSTRACT

Wastewater from breweries contains high concentration of organic and inorganic compounds, which rank them among the top pollution generating industries. Running wastewater treatment in two – stage anaerobic digestion process is known for enhanced stability and organic removal. Equivalent organic loading rates (OLRs) can be achieved by running high strength chemical oxygen demand (COD) at slower flowrate of low COD strength at higher flowrate.

A pilot scale of two – stage expanded granular sludge bed reactor was fabricated and used to investigate different variables that have an essential contribution to the wastewater treatment. Hydraulic retention time (HRT), pH, temperature, and COD strength are the most effective process variables. Actual process (brewery) wastewater of 20, 30, and 40 g COD/L had been used as a substrate. The tests were run under two temperature ranges.

For the mesophilic range, all three COD strengths were used and for the thermophilic range, only one COD strength was used. Under mesophilic condition (36oC), results show that COD removal efficiency and biogas production rate increased by 1-6 % and 30-40 %, respectively, as HRTs increased by four to five times, while maintaining a constant OLR (2, 3, 4, 5, 6, 7, and 8 g COD/L.day).

Results imply that for equivalent OLRs, better reactor performance is achieved when running high-concentration COD at slower rate compared with a lower – concentration at higher rate. This also implies a diffusion limiting process where higher molecular weight organics, such as complex proteins and fats, likely are passed through

the reactor faster their metabolism rate in the granular biomass for digestion. At higher temperature (50°C), under thermophilic condition and 20 g COD/L strength, results showed that COD removal efficiency and biogas production rate increased by 4% and 30-40 %, respectively, compared to mesophilic range, while maintaining a constant OLR (2, 3, 4, 5, 6, 7, and 8 g COD/L.day). This implies the higher and stronger population of anaerobes present under thermophilic condition rather than mesophilic condition.

Keywords: Anaerobic Digestion, Hydraulic Retention Time, Organic Loading Rate, Chemical Oxygen Demand

1. INTRODUCTION

For developing contraries, coal, natural gas, and crude oil (fossil fuels) considered the cheapest sources for energy. But, it also considered as the main resources for the greenhouse gases (GHG) which has a tremendous effect on the global warming. Biofuels can be an essential alternative fuel that could help reducing the climate change and pollution due to use the fossil fuel. Besides that, designing a bio-refinery that could help changing the organics and biomass feed stocks in to usable liquid and gas fuel and selling biofuels in a market dominated by low – cost fossil fuels are key challenges to establish vigorous biofuel industry [1]–[3].

Due to the enormous growth in the brewery and distillery industries, massive quantities of process wastewater (WW) have been discharged to sewer systems without any treatment. This wastewater contains many organic, inorganic, and solid compounds that pollute the environment [4]. The anaerobic digestion process (AD) as a treatment for wastewater has been used since the 19th century [5], [6]. It has been used for biogas production as well [7], which can be useful for heating and power generation [8][9]. Liquor

industries generate a large amount of wastewater that is discharged to the sewer system [10]–[13]. This wastewater contains enormous amounts of carbon which can be assessed by measuring the chemical oxygen demand (COD). COD is the amount of oxygen required to oxidize the wastewater in a specific volume [11].

Environmental agencies and government-issued regulations can reduce the discharge of these pollutants to natural water resources and start imposing charges on companies that continue discharging without any pollutant-reducing treatment [14]. For example, in the production of wine, an anaerobic digestion process as a treatment for the wastewater before discharging it to the sewer system would be beneficial. In the United States, anaerobic digester (AD) can be used to treat food wastes as a co-digestion besides the WW treatment. There are two main types of AD, conventional and high-rate [9], [15]. The conventional AD (e.g., a continuous stirred tank reactor [CSTR]) can operate as a batch reactor where all four steps that represent the AD process occur in one stage, so there is no input and the only output would be the biogas stream. This CSTR system can also run as a continuous process. Another type of the continuous AD is the high-rate AD process which, as the name implies, generates a higher rate of biogas than the conventional type. A common part in the high-rate AD systems is the internals, which are either support internals for the microorganisms like natural zeolite in the anaerobic fluidized reactor (AFR) [11], or a particulate sludge called the biomass granular particles where the microorganisms reside [5], [9], [16].

Many reactor configurations have been developed such as anaerobic filters (AF) [17], anaerobic baffled reactors (ABR), and anaerobic fluidized bed reactors [18]. The last type of high-rate anaerobic digesters is called the flow-through reactors, in which AD takes

place with biomass granular particles that contain the methanogens required for biogas production.

The up-flow anaerobic sludge blanket (UASB) was the first reactor of this type and was developed more than 40 years ago. This reactor was designed to run with biomass granules to utilize diverse types of high concentration and most soluble wastewaters. Recently, the expanded granular sludge bed reactor (EGSB) was developed, which is considered as a modified version of the UASB. The advantages of EGSB are the bed expansion, which escalates the hydraulic mixing between the biomass and the substrates, and also the presence of the recycle stream [19], [20], which enhances the process stability, especially under high organic loading rates (OLRs).

The EGSB reactor can separate the newly formed sludge from the mature granules by utilizing the upward velocity; therefore, it can be used to treat high-concentration wastewater. Since the microorganisms live in the biomass granular particles inside the reactor, the AD process depends on the diffusion of the wastewater into the biomass where the reactions occur to consume the organic content and generate the biogas, and this should happen under a low suspended solids level.

Various investigations have been conducted on the AD. Ghorbanian [4], [14] studied the impact of the hydraulic retention time on the biogas rate and composition. He found that for an equivalent organic loading rate (OLR), running an AD of high strength COD at a lower rate was much more beneficial than running a low strength COD at a higher rate, which also resulted in longer retention time, higher biogas production and higher COD removal rate [4].

Montalvo [11] studied the red wine wastewater treatment by an anaerobic fluidized bed reactor using zeolite particles. A COD removal efficiency over 80% was achieved, and the volatile fatty acids were less than 400 mg/L. Budiyo [21] also studied the impact of the slaughterhouse WW on biogas production and found that the WW has the potency for producing a total amount of biogas of 2.472 m³/m³.

For simulation, Serrano [22] developed the first model for the anaerobic digestion process using Aspen Plus. The model considered the substrate to be hydrolyzed and thus is comprised of three steps that are separated into two stages to account for the optimum pH value for each step. Al-Rubaye [23] recently developed a full model for the AD process using Aspen Plus. This model has been designed to accommodate diverse types of substrates under mesophilic and thermophilic temperature ranges. The mesophilic temperature range is the operating temperature for the AD and its range is between 30-38 °C, while the thermophilic temperature range is between 48-55 °C.

The AD steps are hydrolysis, acidogenesis, acetogenesis, and methanogenesis, and each one involves microorganisms to carry out the step.

The hydrolysis step can be defined as a conversion of the organic polymers (undissolved) like proteins, fats, and sugar into smaller compounds, such as amino acids, long chain fatty acids, and glucose. This conversion takes place by extracellular enzymes and mainly depends on the pH of the substrates and the presence of the organic compounds [16].

The second step is acidogenesis, which simply converts the monomers into volatile fatty acids such as propionic acid, butyric acid, and acetic acid.

The third step is acetogenesis, where the volatile fatty acids get converted into acetic acid, hydrogen, and carbon dioxide [14].

The last step is the methanogenesis. In this step, there are two types of microorganisms. The acetoclastic methanogens convert acetates and carbon dioxide into methane, and the hydrogenotrophic methanogens convert hydrogen and carbon dioxide into methane [24].

The literature available neither explained the effect of utilizing different high COD concentrations, HRTs, and OLRs [11], nor studied the temperature ranges effect in a high rate AD reactor. In the present investigation, the effect of the hydraulic retention time (HRT), high COD concentration on biogas production with a wide range OLRs and substrate concentration was investigated, running under mesophilic and thermophilic temperature ranges on the same reactor configuration, which will give a better understanding for the temperature factor. A distillery WW with different COD strengths (20, 30, and 40 g COD/L) was utilized in an expanded granular sludge bed reactor. Both the biogas production rate and the biogas composition were studied.

2. MATERIALS AND METHODS

2.1 EXPERIMENTAL SETUP

Figure 1 shows the process and instrumentation diagram (P&ID) for the anaerobic digestion process. An expanded granular sludge bed reactor was designed, built, and utilized for wastewater treatment. The design was done by using Solid Works software and tested with Star CCM+ to check the validity of the design before proceeding with building the setup, which is shown in Figure 2. It consists of two stages: the pre-acidification stage, where the hydrolysis and acidogenesis steps occur, and the main reactor, where

acetogenesis and methanogenesis take place to produce biogas. The reason for conducting the treatment in two stages is to increase the stability of the process and optimizing the conditions of the process, where the growth rate for the acidogenesis is higher than the acetogens. That occurs if the OLR increases, eventually accumulation of the volatile fatty acids (VFAs) will occur in the process, causing inhibition [4], [5], [22], [23]. The VFAs like butyric acid, propionic acid, and acetic acid are the intermediate products after the acidogenesis step.

A distillery WW, the residue from the distillation process, was picked. A couple of analyses were conducted to estimate the organic matter in the WW expressed by the COD. After determining the required COD strength, three different COD concentrations of WW were used (20, 30, and 40 g COD/L). The WW at a specific COD concentration was pumped to the first part of the process which is in a 55-gal plastic tank, before it was introduced to the pre-acidification (PA) stage.

The flowrate in the pre-acidification tank was controlled by a floating valve installed inside the PA tank. The PA tank is a stainless steel tank with a volume of 33 gal, and 18 gal was the active volume used for the wastewater.

A mixer was installed in the PA tank to maintain uniform temperature and mass distribution along with an immersed heater to provide heat to the reactor. A heavy-duty pH sensor from Omega was connected to a pH controller to maintain the required pH level by adding 1 N of sodium hydroxide (NaOH) using a Milwaukee MC122 pH meter with an automatic peristaltic pump. In the pre-acidification (PA) reactor, large polymers or molecules were degraded. Besides that, the pH, nutrients, and temperature were adjusted to the required levels.

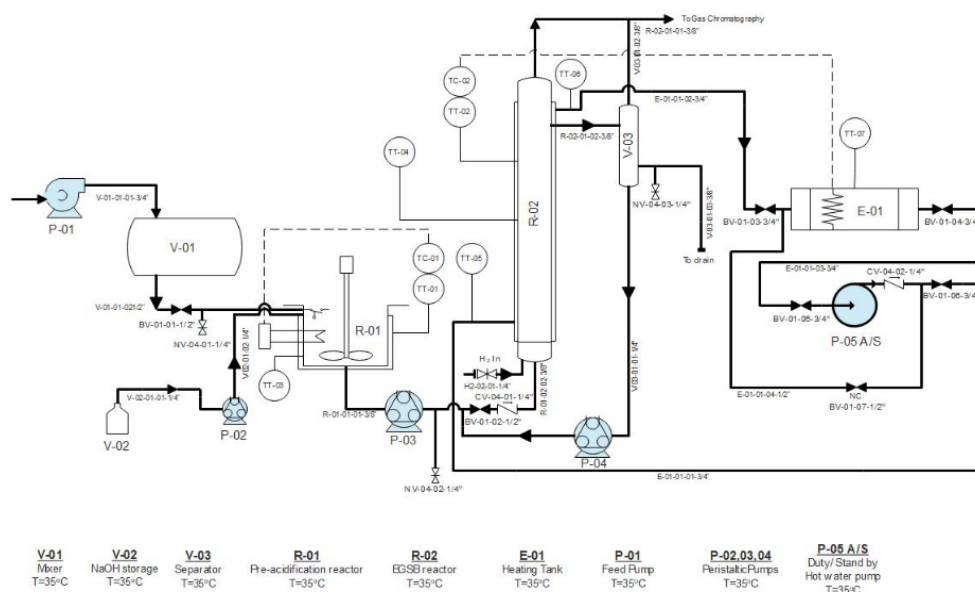


Figure 1. Process and instrumentation diagram for the anaerobic digestion process

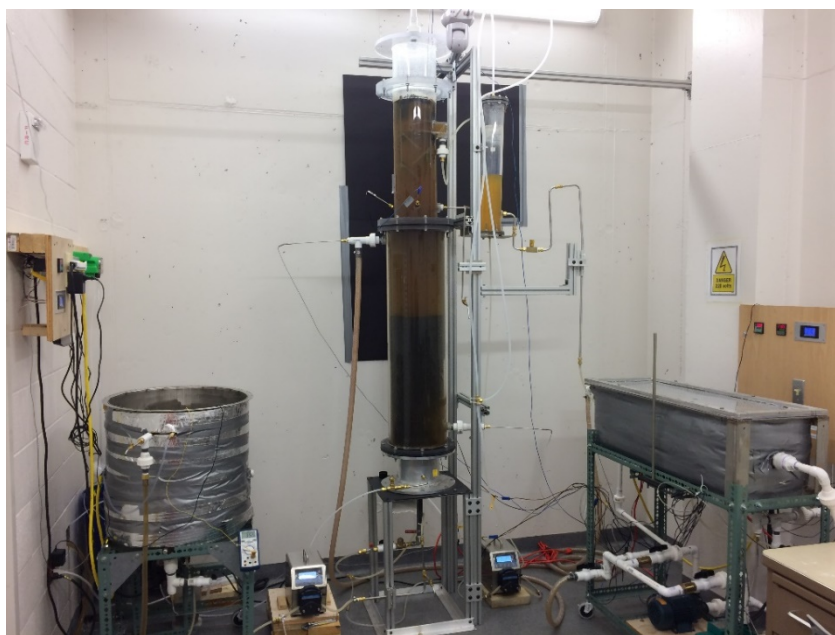


Figure 2. Two-stage anaerobic digestion process

Before running the system, the WW was kept in the PA for 24-48 hrs before it was pumped into the main reactor using the BT600s basic variable speed peristaltic pump (Golander). Processed wastewater from PA was pumped based on a range of an organic loading rate (OLR) varying from OLR 2.0 g COD/L per day to OLR 9 g COD/L per day.

The EGSB is 12 gal in volume and it consisted of three main parts, the aluminum plenum, which is the lower part where the WW first is introduced to the EGSB, a T-shaped pipe installed in the plenum to distribute the liquid evenly in the reactor, and a gas diffuser installed inside the plenum to inject nitrogen gas into the reactor to ensure there is no trapping of air inside in order to maintain a condition conducive for the AD process to occur. A liquid distributor was attached at the top of the plenum containing about 171 holes of 2 mm ID to support the biomass particles and distribute the substrate wastewater uniformly inside the reactor.

Above the plenum is the main part of the EGSB, where the biomass granular particles reside. The main part of the reactor was made of an acrylic material with a diameter of 7.5 in. and 64 in. in height, surrounded by a jacket with a diameter of 11 in., about 1.5 in. of clearance was the annular space where hot water flow to maintain a constant temperature in the reactor. The hot water jacket was used to maintain the temperature inside the reactor, as it further helps to avoid the direct contact of the heating element with the biomass particles. The active volume of the reactor was about 12 gal.

The upper part of the main reactor, the gas-liquid-solid separator, was also made of acrylic materials. The main objective of this part was to separate the generated biogas from the effluent and retain the biomass inside the reactor. The generated gas flowed to the top of the reactor and was stored in a tank, while the liquid effluent flowed out of the reactor

to the small acrylic column, where part of the effluent was recycled back to the main reactor, and the rest was discharged to the sewer system.

The second peristaltic pump circulated the recycle stream at a rate 30% of the main flow-rate. Lastly, a water heating tank with a volume of 23 gal was used to provide heated water to maintain the temperature of the EGSB reactor. Two centrifugal pumps (one was working while the other one was on standby) at $\frac{1}{2}$ horsepower were connected to a timer power switch to make each pump run for 30 min at a time. A temperature controller was installed with the heating tank to provide the required heat to the reactor. A data logger (TC-08, Pico Technology) was used to monitor and record the temperature in the process. It connected to eight thermocouples distributed along the process to monitor the temperature variation. One thermocouple was installed in the pre-acidification tank, and six thermocouples were installed on the main reactor.

These eight thermocouples were used to measure temperatures for the biomass, the substrate, the effluent, the hot water inlet (to the jacket), and the hot water outlet (of the reactor). One thermocouple was used to measure the temperature in the heating tank. The thermocouple types were J and T.

The pressure inside the reactor was monitored using an Omega pressure transducer PX-304 with the indicator DP-350. Nutrient medium and mineral base were added on a regular basis to maintain the activity of the biomass. It contained mineral base I, mineral base II, nutrient base, and a buffer base. Table 1 shows the composition of the nutrients added to the EGSB to maintain the activity of the biomass granular particles.

Table 1. Nutrient medium composition

MEDIUM	COMPONENT	Quantity (mg/mL)
MINERAL BASE I	Cobalt (Co)	0.062
	Iron (Fe)	1.126
	Manganese (Mn)	0.0139
	Boron (B)	0.0044
	Zinc (Zn)	0.0119
	Molybdenum (Mo)	0.0020
	Nickel (Ni)	0.0062
	Selenium (Se)	0.0104
	Copper (Cu)	0.0026
MINERAL BASE II	Calcium (Ca)	5.4
	Magnesium (Mg)	2.36
NUTRIENT BASE	Nitrogen (N)	13.9
	Phosphorus (P)	11.4
	Sulphur (S)	6.76
BUFFER BASE	Sodium Bicarbonate (NaHCO ₃)	40

2.2 WASTEWATER AND GRANULAR BIOMASS PARTICLE CHARACTERISTICS

The biomass used in the process was obtained from Anheuser-Busch Beverage Company with a particle diameter of 2~5 mm. A couple of analyses were done on the biomass, such as volatile suspended solids VSS, total solids TS, total dissolved solids TDS, and the total suspended solids TSS. The protocol followed for these analyses was made by U.S. Geological Survey (USGS, 1989) [14].

For the biomass granular particles, the VSS was 63,914 mg VSS/L, the TSS was 358 mg/L, and the TDS was 2,399 mg/L. The particle size and the pH were 2-5 mm and 6.9-7.2, respectively. The wastewater was collected from a distillery Company in Missouri. Simply, it was the stillage waste obtained after a distillation process and was mixed with water during the cleaning of the distillation vessel. Similar analyses were done for the wastewater to check its characterization before treatment. The VSS), TSS), TDS, and the

pH were 25 mg/L, 1,118 mg/L, 70,626 mg/L, and 3-4, respectively. All these analyses were conducted based on the protocols from the USGS report.

A laboratory spectrophotometer Hach Model DR3900 was used to measure the COD concentration, the volatile fatty acids, the alkalinity, total nitrogen, total ammonia, phosphorus, and sulfate. The vials used for these analyses were TNT 823, TNT 872, TNT 870, TNT 828, TNT 833, TNT 845, and TNT 865, respectively. The COD concentration of the wastewater was about 90 g COD/L, which was diluted with tap water before being fed into the reactor to get the required COD strength.

For the biogas production rate measurement, a water displacement method was used to measure the amount of generated biogas. Its composition was tested by two methods: an analytical protocol by using potassium hydroxide protocol by Young et al [17], and by using gas chromatography (Varian CP-3800).

2.3 REMEDIATION PROCESS AFTER INHIBITION SITUATION

Due to the inhibition, the alkalinity level inside the reactor dropped down to less than 100 mg CaCO_3/l , while it was above 1500 mg CaCO_3/l at a steady state process. According to literature, the ratio of the VFA concentration to the alkalinity of the process should be below 0.5 to confirm the stability of the process according to the literature [1][25]. The remediation process is a way of harvesting the liquid medium of biomass particles and replacing it with fresh water. This process will be accompanied with injecting a sodium bicarbonate solution to increase the alkalinity of the system [4], [14]. The fresh liquid medium used is harvested from an existing biomass that is kept as a standby. The HRT for injecting the liquid medium inside the reactor should be low enough to retain all the microorganisms and the biomass inside the reactor.

The alkalinity of the process is usually monitored every day in addition to the COD and the VFA from the effluent stream. The pH is also monitored every day and a sodium hydroxide solution 1 N was added gradually when needed to increase the pH level inside the reactor. Once the pH level reaches about 7.0, the alkalinity is above 1500 mg CaCO₃/L, and both COD and VFA for the effluent are about 2000 g COD/L, and 500 mg CH₃COOH/L, respectively, and the reactor is ready for wastewater treatment again.

3. RESULTS AND DISCUSSION

3.1 OPERATING UNDER MESOPHILIC TEMPERATURE RANGE

The main advantages of using the high-rate anaerobic digestion are the small size of the footprint and the high organic loading rate that could be achieved. COD strengths of 20, 30, and 40 g COD/L were used.

Based on that and on the reactor's volume, nine different OLRs (2, 2.5, 3, 4, 5, 6, 7, 8, and 9 g COD/L.d) were tested under the mesophilic temperature range (PA reactor's temperature was 35 °C and the EGSB was running at 36 °C). Based on experimental experience and the literature [26]–[28], the selection of the COD should be based on the VSS concentration of the biomass granular particles, where the ratio of the COD to the VSS should be less than 1, which makes the AD system operate in a stable situation. The increase in the OLR should be less than 50% of the previous value so that it does not cause any shock to the biomass [29].

In the PA reactor, some of the organic matter was converted into volatile fatty acids (VFAs), like acetic acid, propionic acid, and butyric acid [14]. The percentage of conversion in the PA can be calculated by using a formula called the degree of the pre-acidification, as shown in equation (1) [30]:

$$\text{Pre-acidification degree (PA)} = \left(1 - \frac{COD_{PA}}{COD_{in}}\right) * 100 \quad (1)$$

Where

COD_{in} is the COD concentration of the feed introduced into the pre-acidification tank and COD_{PA} is the remaining COD concentration after the pre-acidification stage. Due to the ongoing flow of the substrate into and out of the PA reactor based on the organic loading rate of the system, the wastewater had a variable retention time between 5-24 days. As for the OLR of 2 g COD/L.d, the retention time in the PA reactor was 24 days, while for OLR of 8 g COD/L.d, the retention time was about 7 days.

The HRT applied on the PA reactor will be based on the flowrate of the substrate into the EGSB reactor. Table 2 shows the pre-acidification degrees and the hydraulic retention time for all the COD strengths tested. Figure 3 shows the relationship between the HRT for the EGSB and PA% in the PA reactor. The PA% for the high COD strength (40 g COD/L) was the highest based on the HRT, as the substrate spent longer time in the PA reactor than the (30 g COD/l).

The PA% for 30 g COD/L strength was higher than that obtained for the 20 g COD/L. That means more volatile fatty acids were produced for the 40 g COD/L than were produced for the 30 g COD/L, and the volatile fatty acids formation for the 30 g COD/L was higher than that produced for 20 g COD/L. The HRT will be connected to the volumetric flowrate of the substrate that flows from the PA reactor to the EGSB. The higher the HRT, the lower the flowrate of the substrate and the more VFAs produced in the PA reactor. Using two-stage AD will be more beneficial in stabilizing the system, especially if there is an OLR shock, which could cause an inhibition in the system as the acetogens grow at a lower rate than the acidogens [30].

Table 2. Pre-acidification degree and the hydraulic retention time for COD strengths 20, 30, and 40 mg COD/L

COD=20 g COD/l		COD=30 g COD/l		COD=40 g COD/l	
HRT	PA%	HRT	PA%	HRT	PA%
10	32.482	15	50.323	20	63.741
6.66	27.587	10	51.283	13.33	63.836
5	27.413	7.5	50.347	10	63.359
4	26.456	6	51.157	8	63.789
3.33	23.2	5	51.522	6.66	63.965
2.85	27.512	4.285	49.862	5.7	63.872
2.5	25.582	3.75	49.841	5	63.852

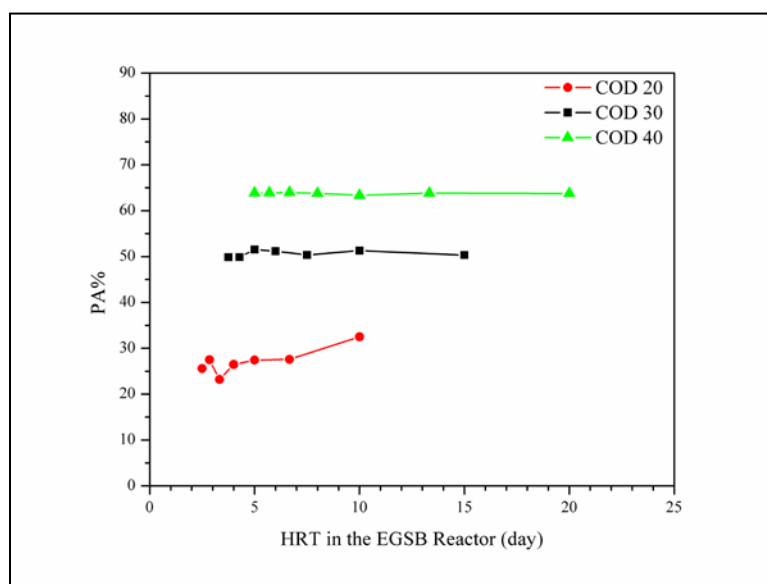


Figure 3. Pre-acidification degree in PA reactor relation with hydraulic retention time (HRT)

Table 3, Table 4, and Table 5 show the substrate concentration based on the COD concentration for different organic loading rates for the influent (i.e., influent is the substrate feed into the PA reactor), the PA (i.e., the substrate feed out of the PA tank), and the effluent (i.e., the remaining substrate out of the system) streams' substrate.

From Tables 3, 4, and 5, it can be shown that the effluent COD concentration does not follow a specific trend with the OLR increase for all the COD strengths that were tested.

That means the removal efficiency also does not change with the increase in organic loading rate and it will remain within a close range, but it got improved with the COD strength increase, which is similar to what was found in the literature. For example, Ghorbanian [4] in his investigation found that the total chemical removal efficiency will increase significantly with increase the hydraulic retention time but not with the organic loading rate changing.

Table 3. COD concentration for the AD streams and the removal efficiency for (20 mg/L) COD strength

OLR, g COD/L.d	20 g COD/l				
	HRT, day	Influent, mg COD/L	PA, mg COD/L	Effluent, mg COD/L	R%
2	10	20,000	13103.67	1601	91.995
3	6.66	20,000	14482.67	1583.41	92.08
4	5	20,000	15557.33	1512.7	92.43
5	4	20,000	14517.33	1558.17	92.209
6	3.33	20,000	15360	1472.54	92.6373
7	2.85	20,000	14497.67	1213.333	93.9333
8	2.5	20,000	14883.67	1279	93.605

Table 4. COD concentration for the AD streams and the removal efficiency for (30 mg/L) COD strength

OLR, g COD/L.d	30 g COD/l				
	HRT, day	Influent, mg COD/L	PA, mg COD/L	Effluent, mg COD/L	R%
2	15	30,000	14903	2084.667	93.05111
3	10	30,000	14367	2159	92.80333
4	7.5	30,000	14055.33	2230	92.56667
5	6	30,000	14615	1629.25	94.56917
6	5	30,000	14655	1394.75	95.35083
7	4.28	30,000	14386.25	1231.25	95.89583
8	3.75	30,000	14167.25	1646	94.51333

Table 5. COD concentration for the AD streams and the removal efficiency for (40 mg/L) COD strength

OLR, g COD/L.d	40 g COD/L				
	HRT, day	Influent, mg COD/L	PA, mg COD/L	Effluent, mg COD/L	R%
2	20	40,000	14503.67	1406	96.485
3	13.33	40,000	14465.67	1588	96.03
4	10	40,000	14656.33	1619.667	95.95083
5	8	40,000	14484.33	1346.333	96.63417
6	6.66	40,000	14414	1517	96.2075
7	5.71	40,000	14451.33	1592	96.02
8	5	40,000	14459.33	1380.667	96.54833

pH level in the PA tank was adjusted to 4.9-5.2 by adding sodium hydroxide 1N. As it seen in Figure 4, for COD strength of (30 g COD/L), the pH value for the effluent didn't affect that much by the increase in the OLR. The pH of the effluent was about 7.0~7.4 for the OLRs from 2-8 g COD/L.d, but under the highest OLR (9 g COD/L.d), the pH value decreased ~5.5 as the rate of the acidogenic microorganisms crossed higher than that required for the acetogens.

The VFA concentration will be higher and cause a drop in the pH level inside the EGSB, resulting in an inhibition inside the main reactor, This trend is similar to what found in the literature [11].

As shown in Figure 5, for COD strength 30 g COD/L, the removal efficiency was increased with the increase in HRT; however, with higher OLR (i.e., OLR= 9 g COD/L.d), the removal efficiency decreased. For the 30 g COD/L, the removal efficiency was increased from 93% to 97% for OLR from 2 g COD/L.d to 7 g COD/L.d respectively; then, it decreased to 95% at OLR 8 g COD/L.d.

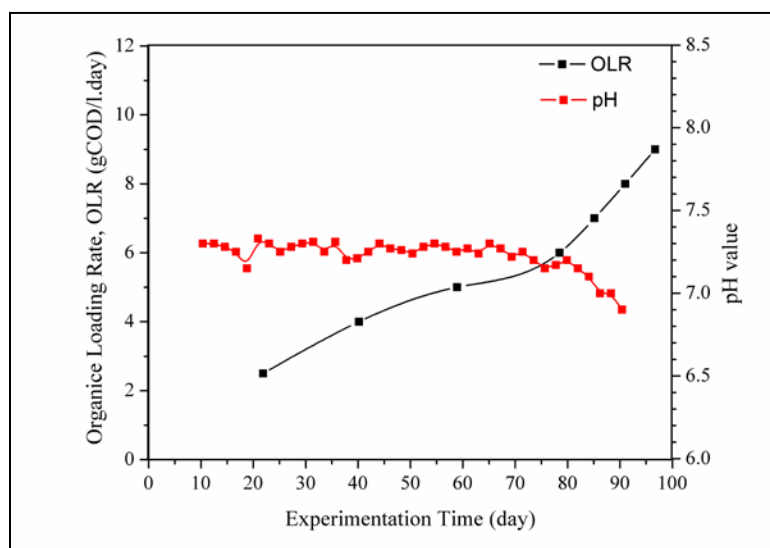


Figure 4. Effluent pH variation with the organic loading rate at different HRTs for COD strength of 30 g COD/L

At OLR 9 g COD/L.d, the removal efficiency decreased dramatically to 64%. This decrease was mainly due to the insufficient residence time required by the microorganisms to consume the organic content inside the substrate as it decreased from 12 days at the lowest OLR 2 g COD/L.d to 3 days at OLR 9 g COD/L.d.

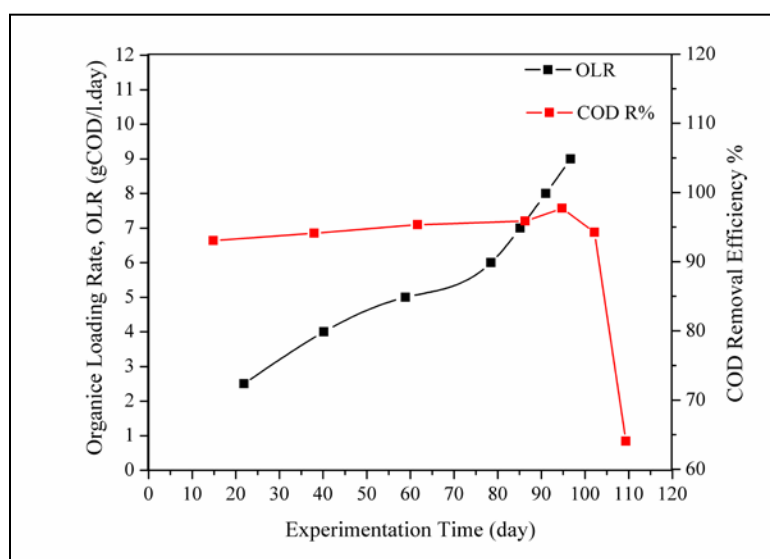


Figure 5. COD removal efficiency variation with the organic loading rate within the experimentation time for COD strength of 30 g COD/L

In Figure 6, for a COD strength of 30 g COD/L, the COD concentration in the PA reactor shows a decrease when the organic loading rate increased. As the OLR increased from 2.5 g COD/L.d to 8 g COD/L.d, the COD from the PA decreased from 14903 g COD/L to 14167 g COD/L in about 96 days. At OLR 9 g COD/L.d, the COD in the PA increased up to 20755 g COD/L. This sudden increase caused an inhibition to the EGSB system as the residence time was too short for the PA reactor to convert the COD into VFA. The amount of the VFA injected into the EGSB was higher than the ability of the acetogens to convert it into the acetate [29][31].

Figure 7 shows the COD for the effluent stream decreased from 2085 g COD/L at OLR 2.5 g COD/L.d to 1650 g COD/L at OLR 8 g COD/L.d. This is due to the acclimatization time required by the microorganisms to stabilize. At OLR 9 g COD/L.d, the COD for the effluent stream increased from 1650 g COD/L to 10350 g COD/L, due to the low residence time in the EGSB reactor required to assimilate the organic matter and the increase in the acidity of the system which causes inhibition to the granular biomass particles.

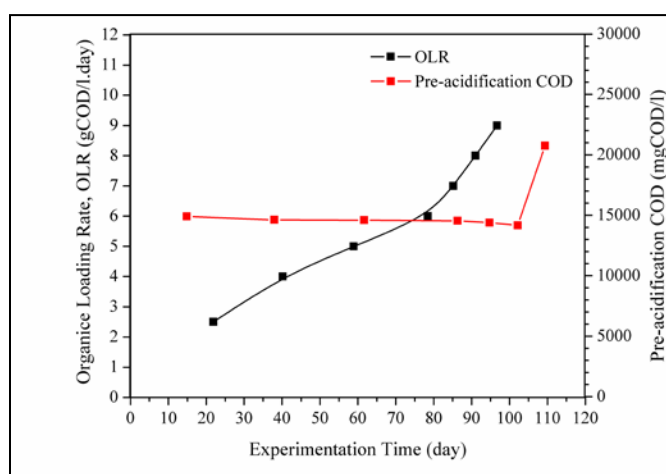


Figure 6. Variation of the pre-acidification COD with the organic loading rate within the experimentation time for COD strength of 30 g COD/L

Figure 8 shows the VFA for the effluent, which is an essential parameter used to check the stability of the process. When the OLR increases from 2.5 g COD/L.d to 8 g COD/L.d, the volatile fatty acids represented by the acetic acid (CH_3COOH) concentration were between 250-300 mg $\text{CH}_3\text{COOH/L}$. At OLR 9 g COD/L.d, the VFA concentration raised from 261 mg $\text{CH}_3\text{COOH/L}$ to about 2600 mg $\text{CH}_3\text{COOH/L}$.

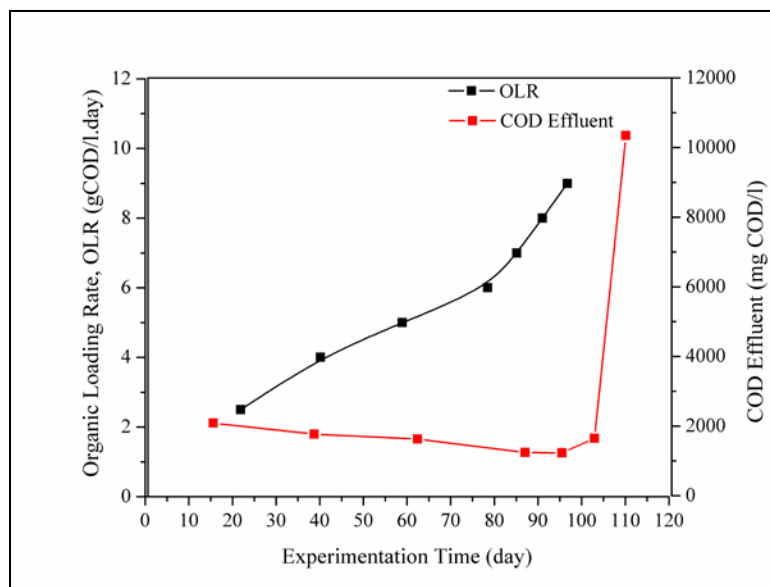


Figure 7. Variation of the effluent COD with the organic loading rate within experimentation time

This behavior was attributed to the VFA overfeeding problem; and the bacterial population had reached their process feeding limit. The higher the VFA formation, the higher the consumption of the COD input. This is demonstrated from Figure 8, Table 2, and Table 5. For the 40 g COD/L, the pre-acidification degree which represents the highest amount of the COD converted in to the VFA (about 63%), and the COD removal efficiency for the same COD strength (40 g COD/L) was also the highest (about 96%).

The treatment of the industrial wastewater will be accompanied by biogas production [32], [33]. The biogas will be produced by the acetoclastic methanogens, which

feed on the acetic acid generated from the acetogenesis step. Besides this, the hydrogen gas produced from the acidogenesis and acetogenesis steps will be converted into methane by the hydrogenotrophic methanogens.

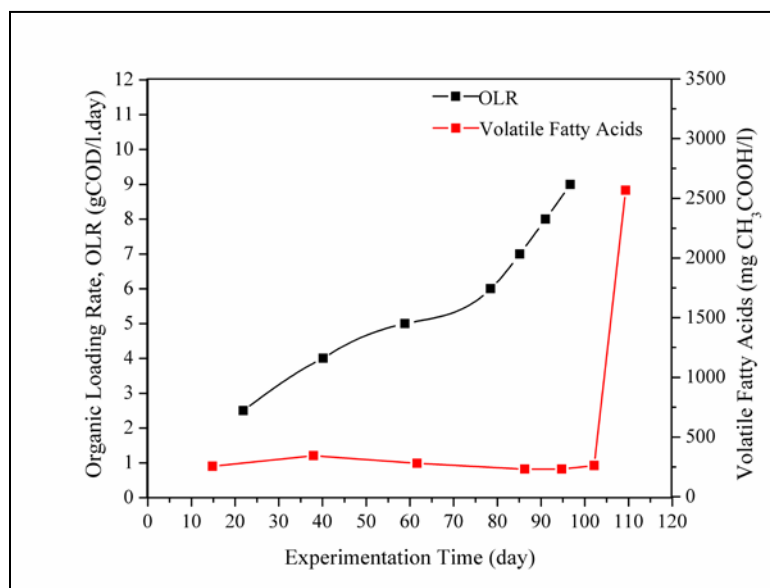


Figure 8. Variation of the effluent volatile fatty acids with the organic loading rate within the experimentation time

Figure 9 shows the biogas production rate along with the OLR through the experimentation time. It can be noted that the biogas production will keep increasing from 10.8 average gal/day at OLR 2.5 g COD/L.d to 23.26 gal/day at OLR 8 g COD/L.d, so the biogas production almost doubled with the increase in OLR within about 92 days. For the next OLR (9 g COD/L.d), the biogas production rate decreased to about 20.2 average gal/day due to the overfeeding situation. When the amount of substrate flow through the EGSB was higher than the ability of the microorganisms to convert all the substrate, the acidity of the reactor will increase and cause an inhibition to the system.

At a steady state process, the production rate of the methane was found to increase with increasing OLR until reaching the maximum, where the HRT inside the reactor was

found to be the same as the time required by the microorganisms to produce biogas at the maximum rate.

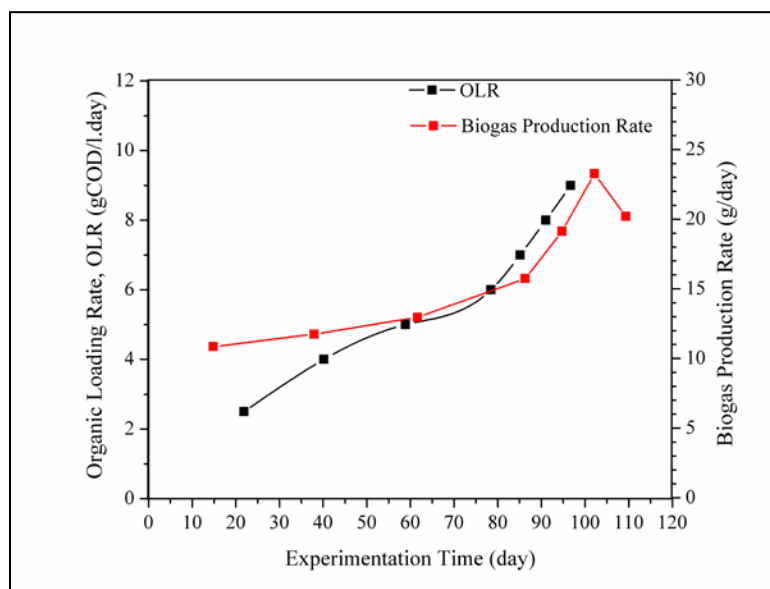


Figure 9. Variation in the biogas gas with the organic loading rate within the experimentation time

Once the OLR increased above that, the HRT was shortened and affected the microorganisms, causing an inhibition of the process. As the inhibition started in the reactor, a remediation process was required to reactivate the microorganisms available inside the biomass particles, as they were over-feeding. Figure 10 shows the progress in pH variation along with the alkalinity level in the remediation time after the inhibition.

Three different COD strengths, 20, 30, and 40 g COD/L, have been tested under an equivalent organic loading rate 2, 2.5, 3, 4, 5, 6, 7, and 8 g COD/L.d. The liquid effluent and the gas composition have been analyzed.

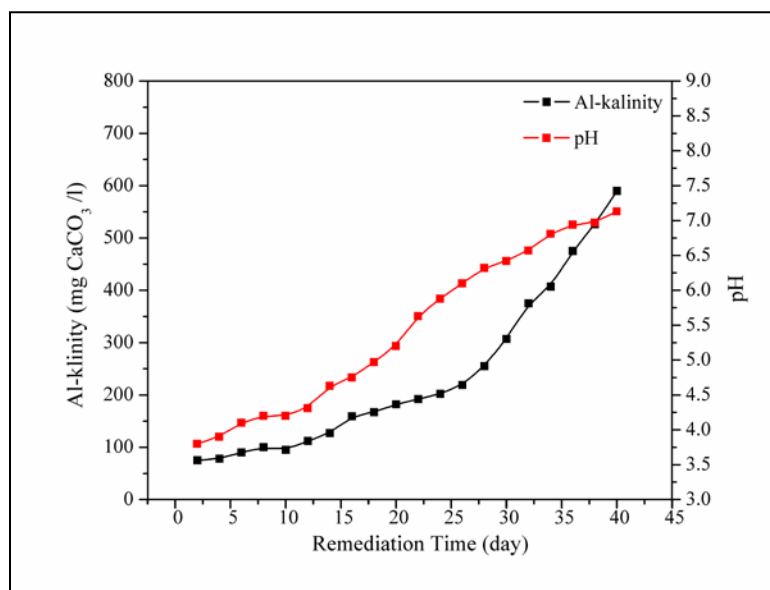


Figure 10. Variation of the pH and the alkalinity level within the remediation time

Figure 11 shows the removal efficiency increased with increasing the HRT for each OLR applied, so for a COD of 20 g COD/L, the removal efficiency would be about 90% at OLR of 3 g COD/L.d while for a COD strength of 40 g COD/L and the same OLR, the removal efficiency was 96%. This applies for all the OLRs, and it is caused by the higher hydraulic retention time that lets the substrate spend a longer time in the reactor, and thus more COD is consumed.

From Figure 12, it can be shown that the biogas production rate increased with an increase in the organic loading rate and at the same time, the lower COD strength had a higher biogas production rate than the higher COD strength [29].

For an equivalent COD strength 20 g COD/L, at OLR of 3 g COD/L.d, the biogas production rate was 9.6 gal/day but at OLR of 8 g COD/L.d, the production rate increased up to 23.3 gal/day since the wastewater flow inside enhanced and had more organics to

digest in the reactor. Besides that, low COD strength WW requires less time for digestion, and it will be easier for the microorganisms to assimilate it than the higher COD strength.

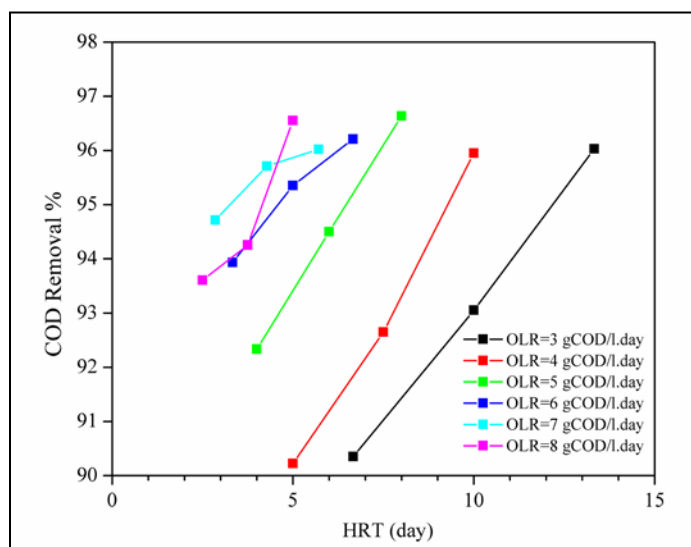


Figure 11. COD removal efficiency variation with the hydraulic retention time under different OLRs

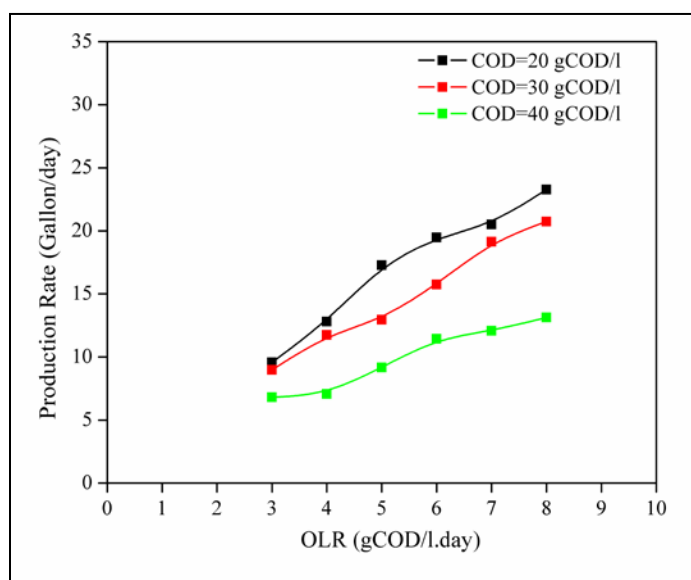


Figure 12. Biogas production rate variation with the organic loading rate for different COD strengths

3.2 OPERATION UNDER THERMOPHILIC TEMPERATURE RANGE

Running an anaerobic digestion system under mesophilic temperature range is rarely found for expanded granular sludge bed reactor and couple of researchers investigated and implemented that [4], [34], [35]. Furthermore, running anaerobic digesters under thermophilic temperature range 50-60 °C were only implemented on batch reactors [26]. The main challenge in operating the high rate AD reactor under thermophilic temperature range is how to maintain the temperature of the system [36]. At thermophilic temperature range, the methanogens growth will be faster than that at mesophilic temperature range and the methanogens production time will be between 10-15 days [37] while for the mesophilic methanogens, it would be between 20-30 days. The thermophilic temperature range will be narrow between 50-60 °C. The transition from mesophilic to thermophilic temperature range can't be at once, the increase in temperature should be at a very low rate (0.5-2) °C/day.

The methanogens would be very sensitive that could be easily shocked and inhibited if it got heated too fast and hence, it affect the biogas production rate. The heating rate for the system was about 0.5 °C/d to heat the system up from 36 °C (mesophilic temperature range) to 55 °C (thermophilic temperature range) to overcome all the chances of inhibition or instability due to the temperature increase.

During the heating interval, the substrate was flowing at an organic loading rate of 2 g COD/L.d (HRT of ten days) and the biogas production rate was monitored. As it shown in Figure 38, the biogas was slightly increased with temperature increase at a constant OLR at 20 g COD/L strength. When the reactor was running at 36 °C, the biogas production rate was about 5.6 gal/day and after it reached about 50 °C, the production rate increased to

about 7.9 gal/day. This increase was not completely stable, but it had some fluctuations as the biomass was very sensitive in the temperature transition from mesophilic to thermophilic range. To remediate this fluctuation, temporarily fresh substrate coming from the PA reactor should be decreased and increase the recycle stream to maintain the equivalent OLR. 30% of the flowrate in to the EGSB reactor was recycled normally.

The temperature mapping can be shown in Figure 13, for the whole EGSB reactor under thermophilic temperature range for a time duration from 0-4000 min. All the thermocouples were measuring a steady temperature for the inlet and outlet hot water streams, also the temperature inside the reactor itself was approximately steady for the biomass granular particles and for the substrate liquid.

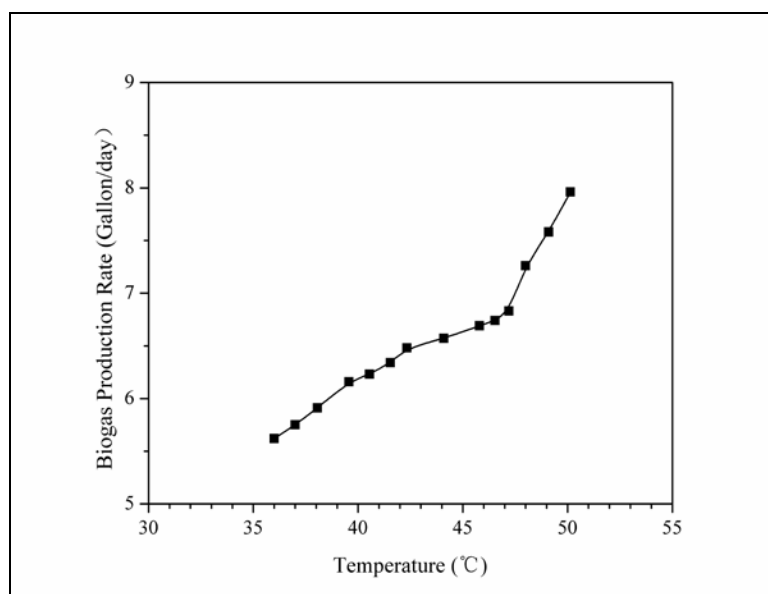


Figure 13. Biogas production rate with temperature increase

The temperature mapping can be shown in Figure 14, for the whole EGSB reactor under thermophilic temperature range for a time duration from 0-4000 min. All the thermocouples were measuring a steady temperature for the inlet and outlet hot water

streams, also the temperature inside the reactor itself was approximately steady for the biomass granular particles and for the substrate liquid. And the temperature inside the PA reactor was also steady at 41°C.

The temperate of the effluent slightly increased even when the temperature mapping for all the other thermocouples were steady, this increase is due to the increase in the biogas production increase with the time. The biogas bubbles in the reactor enhanced the mixing of the substrate in the EGSB reactor and therefore, it will increase the temperature at the top of the reactor especially that no heating system was exist at the top of the reactor where the effluent flow out of the system.

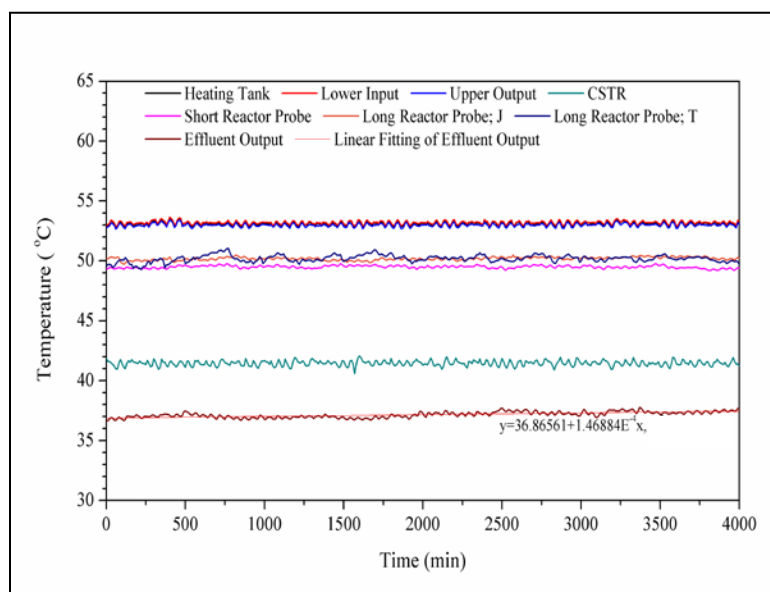


Figure 14. Temperature mapping in two-stage anaerobic digestion (EGSB)

The pH values for the effluent was little higher than that of the mesophilic temperature range, as the solubility of carbon dioxide decreases with temperature increase, so less amount of carbonic acid would be in the system. Furthermore, the production rate under thermophilic range was higher than that at mesophilic range.

For substrate concentration of 20 g COD/L, running at a range of OLRs 2, 3, 4, 5, 6, 7, and 8 g COD/L.d. Figure 15 shows the biogas production rate under mesophilic and thermophilic temperature range at an equivalent OLR values. So at OLR 2 g COD/L.d, the biogas production rate was about 3.325 gal/day under mesophilic temperature range, while it was about 8.57 gal/day under thermophilic temperature range. Also, at OLR 3 g COD/L.d, the biogas production rate was 9.582 gal/day under mesophilic temperature range while it was about 19.37 gal/day under thermophilic range. At OLR 4 g COD/L.d, the production rate for the biogas was about 12.804 gal/day under mesophilic range while it was about 24.14 gal/day under thermophilic temperature range. At OLR 5 g COD/L.d, the biogas production rate was about 17.278 gal/day while at thermophilic range was about 33.91 gal/day. Next, at OLR 6 g COD/L.d the biogas production rate was about 19.457 gal/day at mesophilic range while it was about 40.673 gal/day at thermophilic range. At OLR 7 g COD/L.d the biogas production rate was 20.504 gal/day under mesophilic range while it was about 46.825 gal/day under thermophilic range. At the last OLR (8 g COD/L.d), the biogas production rate was about 20.726 gal/day and 50.174 gal/day under mesophilic and thermophilic temperature ranges, respectively. so the biogas increased about 90-100 % along the OLRs in the thermophilic range more than that at mesophilic range, this is due to the highly growth rate of the methanogens under thermophilic range than mesophilic range which is compatible to what found in the literature [26], [37].

Also as it shown in Figure 16, the COD removal efficiency for the system running under thermophilic range was found to be little higher than that at mesophilic due to the higher growth rate at thermophilic that make the methanogens consume all the organic matters especially that some of the VFA would form in the second stage of the AD process

too. Table 6 shows the data collected from running the AD system under thermophilic temperature range. The biogas composition produced under thermophilic temperature range was found to be almost similar to the biogas produced when the system was running under mesophilic temperature range.

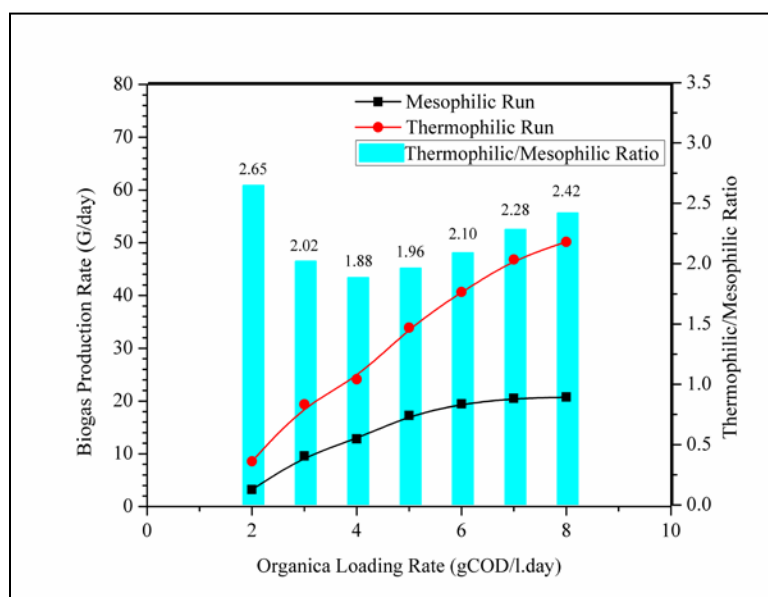


Figure 15. Biogas production rate under mesophilic and thermophilic temperature range and the ratio of biogas increase in thermophilic range to the mesophilic range

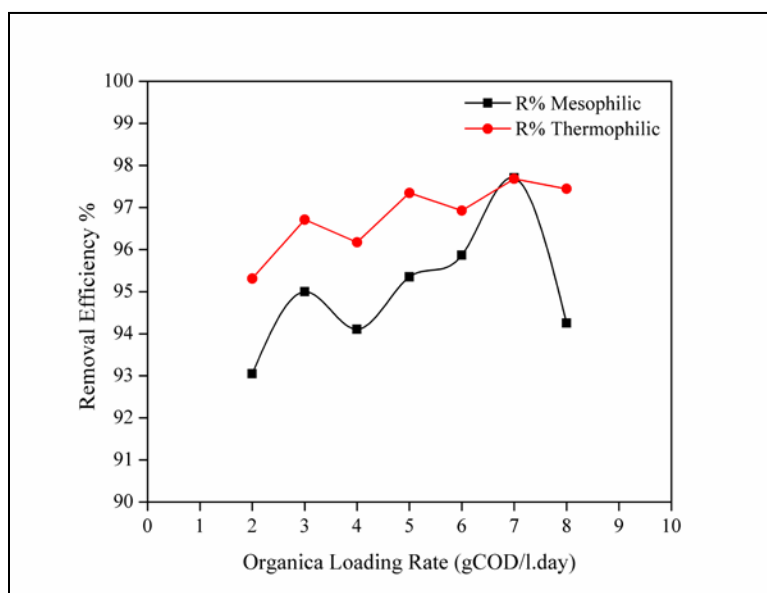


Figure 16. COD removal efficiency with the organic loading rate under mesophilic and thermophilic temperature range

Table 6. Substrate concentrations in AD system running under thermophilic temperature range

OLR, g COD/L.d	20 g COD/L				
	HRT, day	Influent, g COD/L.day	PA, g COD/L.day	Effluent, g COD/L.day	R%
2	10	20,000	13205	937.6	95.312
3	6.66	20,000	13508	657.6	96.712
4	5	20,000	14327	765	96.175
5	4	20,000	14.517	530.6	97.347
6	3.33	20,000	13925	614.6	96.927
7	2.85	20,000	14271	463	97.685
8	2.5	20,000	14832	511	97.445

4. CONCLUSION

A pilot scale of expanded granular sludge bed reactor was designed and built for industrial wastewater treatment, where 20, 30, and 40 g COD/L substrate strengths were used for treatment. The tests were run under two temperature ranges. For mesophilic range, all three COD strengths were used and for thermophilic range, only one COD strength was used. Under mesophilic condition (36°C), results showed that COD removal efficiency and biogas production rate increased by ~1-6% and ~30-40%, respectively, as HRTs increased by approximately four to five times, while maintaining a constant OLR (~2, 2.5, 3, 4, 5, 6, 7, and 8 g COD/L.day).

Results imply that for equivalent OLRs, better reactor performance is achieved when running high-concentration COD at a slower rate compared with a lower concentration COD at a faster rate. This also implies a diffusion limiting process where higher molecular weight organics, such as complex proteins and fats, likely are passed through the reactor faster than their metabolism rate in the granular biomass for digestion. At higher temperature (55°C), under thermophilic condition and 20 g COD/L strength,

results showed that COD removal efficiency and biogas production rate increased by ~4% and ~90-100%, respectively, compared to mesophilic range, while maintaining a constant OLR (~2, 3, 4, 5, 6, 7, and 8 g COD/L. day). This implies the higher and stronger population of anaerobes present under thermophilic condition rather than mesophilic condition.

APPENDIX**STANDARD OPERATING PROCEDURE FOR THE HIGH RATE EXPANDED
GRANULAR SLUDGE BED REACTOR**

1. PURPOSE

Standard Operating Procedures (SOPs) are issued to specifically instruct students in areas of responsibility (Handling anaerobic digestion system), Work Instructions, appropriate specifications and required records. SOPs outline procedures, which must be followed to claim compliance with GLP principles or Safety Statutory rules and regulations.

2. SCOPE

This module provides information on the start-up, operation and control of a digester, and sets forth the reasons why digesters fail. Included in this module are standards for best operating procedures and safe operation of digesters.

3. DEFINITIONS

AD	Anaerobic Digestion, the biodegradation of organic material in the absence of oxygen
BOD	Biological Oxygen Demand – the oxygen demand that bacteria use whilst decomposing biologically available organic matter, where if high BOD organic materials enter a watercourse, they rob the aquatic life of dissolved oxygen during this process
CAPEX	Capital Expenditure
CCP	Critical Control Point featured in a HACCP plan
CH ₄	Methane
COD	Chemical Oxygen Demand – a test commonly used to indirectly measure the amount of organic compounds in water. Most often applied in the waste water industry in determining the financial charges to be imposed on trade effluent discharges to sewer. COD is expressed in milligrams per litre indicating the mass of oxygen consumed per litre of solution. COD determines the amount of organic pollutants found in solutions as a means of measuring water quality
CO ₂	Carbon dioxide
DM	Dry Matter
HACCP	Hazard Analysis & Critical Control Points – a planning tool to identify hazards and corresponding preventative measures in food and pharmaceutical production which has been adapted to ensure safe methods & identify intervention points in the treatment of biodegradable waste streams
HRT	Hydraulic Retention Time – the time in which a substrate is held in a digester
K	Potassium, used as a nutrient in fertiliser (potash)
KWh	Kilowatt hour – the amount of power consumed/generated over a period of one hour
Mesophilic	Temperatures of AD process operation around 35°C
N	Nitrogen, used as a nutrient in fertiliser
Opex	Operating expenditure
OLR	Organic Loading Rate (mass/ volume. time) - an important parameter affecting microbial ecology
pH	Potential hydrogen - a measure of the activity of the solvated hydrogen ion
P	Phosphorus, used as a nutrient in fertiliser
SOP	Standard Operating Procedure – a “how to” step by step approach reduced to written documentation
Substrate	An organic feedstock which is fed into an anaerobic digester
TS	Total Solids
TDS	Total Dissolved Solids
TSS	Total Suspended Solids
T/Yr.	Tonnes per year
Thermophilic	Temperatures of AD process operation around 55°C
VFA	Volatile Fatty Acids – the first degradation product prior to methane generation – the carbon and hydrogen chains shorten, the shortest being H ₂ & P ₂ O – Driving Innovation in AD acetate

4. RESPONSIBILITIES

Research Operator (Student): To ensure that the process for starting a digester and every aspects of digester operation are in control. Suggest potential reasons for digester malfunction. Work with industries to develop best operating practices for their digester and to understand the safety issues when operating a digester.

5. SPECIFIC PROCEDURE

5.1 Version control and naming convention

All controlled documents need to be dated and/or versioned. Some need to be named in a systematic way as well, especially if they belong to a series or set of documents e.g. SOPs and HAZOP.

5.2 Other considerations

Where appropriate, the following information should be on the document:

- Effective date and expiry date or next review date to be maintained. It may be necessary to also include date issued and date printed.
- The document is confidential
- State “Draft” or “Final” as appropriate
- Document identification e.g. a title, department name
- It is necessary to include signature and date of Author, Reviewer and Authorizer e.g. for SOPs, protocols. It may be more convenient to have a separate signature sheet. Include designation or title of signatories.
- Copyright of “Missouri University of Science and Technology”
- If a revision of the control document, state reason for change and list changes.

- Referencing - Wherever reference is made to another controlled document, you may use the instruction “see/refer insert Document Title”. The version number may be excluded.
- There should be sufficient detail, clearly expressed, to enable a trained person to perform the procedure without supervision.
- There should also be sufficient detail to enable a trained person to use the document to train others to perform the task.
- The use of flow diagrams may be useful, especially in complex procedures.

5.3 Storage and archiving

Controlled documents should be stored in an area or room restricted to authorized individuals only. If the controlled documents are part of essential documents, they should be part of the Trial/Research Master File and archived appropriately. Old versions of controlled documents must be archived as well.

6. INTERNAL AND EXTERNAL REFERENCES

This section is used to list all controlled internal references and external references referred to within the text of the SOP only.

Internal References

- P&ID
- HAZOP document

7. Introduction (The Anaerobic Digestion Process)

7.1 Background

Anaerobic digestion is a complex biochemical reaction carried out in a number of steps by several types of microorganisms that require no oxygen to live. This reaction produces biogas, which is primarily composed of methane and carbon dioxide.

7.2 The Anaerobic Digestion Process

In an anaerobic environment, specialized microorganisms break down complex organic matter (carbohydrates, proteins, and fats) into molecules with a smaller atomic mass that are soluble in water (sugars, amino acids, and fatty acids). Methane and carbon dioxide are the primary end products of this process, which is known as biogas. Table 1 lists the typical composition of biogas. More importantly, anaerobic digestion stabilizes the slurry in the digester.

The overall conversion process of complex organic matter into methane and carbon dioxide can be divided into four steps, namely hydrolysis, acidogenesis, acetogenesis and methanogenesis. It should be pointed out that some researchers combine the acidogenesis and acetogenesis steps and make it a three step conversion process.

Table 1. Typical Composition of Biogas Gas (volumetric percent) Source: Becky Larson

Biogas component	Composition of biogas (%)
Methane (CH ₄)	45-65%
Carbon Dioxide (CO ₂)	30-40%
Hydrogen Sulfide (H ₂ S)	0.3-3%
Ammonia (NH ₃)	0-1%
Moisture (H ₂ O)	0-10%
Nitrogen (N ₂)	0-5%
Oxygen (O ₂)	0-2%
Hydrogen (H ₂)	0-1%

In an anaerobic digester, the four processes occur simultaneously. When an anaerobic digester performs properly, the conversion of the products of the first three steps into biogas is virtually complete, so that the concentration of these products is low at any time.

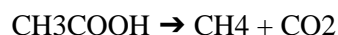
Step 1: Hydrolysis: In anaerobic digestion, hydrolysis is the essential first step, as biomass is normally comprised of very large organic polymers, which are otherwise unusable.

Through hydrolysis, these large polymers, namely proteins, fats and carbohydrates, are broken down into smaller molecules such as amino acids, fatty acids, and simple sugars. While some of the products of hydrolysis, including hydrogen and acetate, may be used by methanogens later in the anaerobic digestion process, the majority of the molecules, which are still relatively large, must be further broken down in the process of acidogenesis so that they may be used to create methane.

Step 2: Fermentation or Acidogenesis: Acidogenesis is the next step of anaerobic digestion in which acidogenic microorganisms further break down the biomass products after hydrolysis. These fermentative bacteria produce an acidic environment in the digester while creating ammonia, H_2 , CO_2 , H_2S , shorter volatile fatty acids, carbonic acids, alcohols, as well as trace amounts of other by-products. While acidogenic bacteria further breaks down the organic matter, it is still too large and unusable for the ultimate goal of methane production, so the biomass must next undergo the process of acetogenesis.

Step 3: Acetogenesis: In general, acetogenesis is the creation of acetate, a derivative of acetic acid, from carbon and energy sources by acetogens. Acetogens catabolize many of the products created in acidogenesis into acetic acid, CO_2 and H_2 , which are used by methanogens to create methane.

Step 4: Methanogenesis: Methanogenesis constitutes the final stage of anaerobic digestion in which methanogens create methane from the final products of acetogenesis as well as from some of the intermediate products from hydrolysis and acidogenesis. There are two general pathways involving the use of acetic acid and carbon dioxide, the two main products of the first three steps of anaerobic digestion, to create methane in methanogenesis:



While CO₂ can be converted into methane and water through the reaction, the main mechanism to create methane in methanogenesis is the path involving acetic acid. This path creates methane and CO₂, the two main products of anaerobic digestion.

8. Procedure (Start-up)

Before starting the system, Study Emergency protocols of SOP, HAZOP and P&ID documents completely. Follow SOP.

8.1 Background

Getting any biological system to operate requires careful attention to a number of parameters. While those parameters that assure a good start up are not technically difficult, failure to follow the correct procedure or to properly monitor the start-up methods result in either a long delay in the anaerobic digestion start-up process or a total failure of the system to produce biogas.

- The system will run from various timelines starting from 2days to 3months period. The system must be monitored all the time, and should take care nothing goes wrong.

This anaerobic digestion system is divided into three units. Unit one consists of substrate storage tank with pump, pH buffer solution container with pH transfer pump and pre-acidification reactor. Unit two consists of pre-acidification transfer pump anaerobic reactor with recirculation pump and effluent buffer tank. Unit three consists of hot water system with circulation pumps.

- Make sure container V-01, container V-02, Pre-acidification Reactor R-01, Anaerobic Bioreactor R-02, Buffer tank V-03, and hot water tank E-01 are clean.

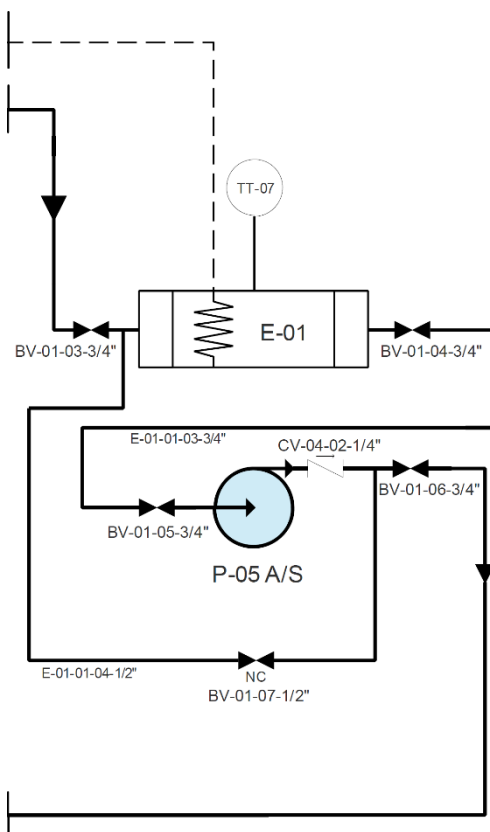
Pre-start-up analysis:

- Carry out substrate (feed waste water) analysis tests such as COD, VFA, TDS, TSS, VSS, Alkalinity, pH, Total Kjeldahl Nitrogen, Phosphorous, Sulphate, and Carbon content.
- Maintain calculation book and record each and every values of the tests carried out which will be used in calculation of OLR, HRT and for maintaining COD to VFA ratio in the substrate.
- Carry out COD, pH, VFA, TDS, TSS, and VSS tests for the biomass and record it.
- Calculate COD (Substrate) to VSS (Biomass) ratio and decide the OLR, which gives HRT of the substrate in the reactor.
- After calculating COD, add necessary dilution factor (Use only RO water, don't use tap water). Make sure your COD value is in the detection range of HACH system, it will be easy to detect COD concentration in the substrate.
- Prepare pH buffer solution using NaOH pellets and RO water. Use 40 grams of NaOH pellets in 1 litre of RO water to make it 1N solution. Store the solution in container V-02.

Procedure for each test is provided in the **annexure-1**, please follow it.

8.2 Procedure for start-up hot water system (Node-3)

If in case of power failure or short or temperature out of control or spillage, follow respective sections in emergency protocol.



Step 1: Make necessary connection for hot water system E-01 (electrical connections, thermocouples, Pumps P-05 A/S)

Step 2: Make sure valves (BV-01-03, BV-01-04, BV-01-05, BV-01-06, BV-01-07) are closed.

Step 3: Fill the hot water tank with RO water only. (Up to the marked line). Switch on the controller which is regulated through thermocouple set on the hot water system and set the temperature to 35°C, set upper limit and lower limit for the controller.

Step 4: Study timer programming manual. Program the timer for switching pumps P-05_A and P-05_S every 30 minutes.

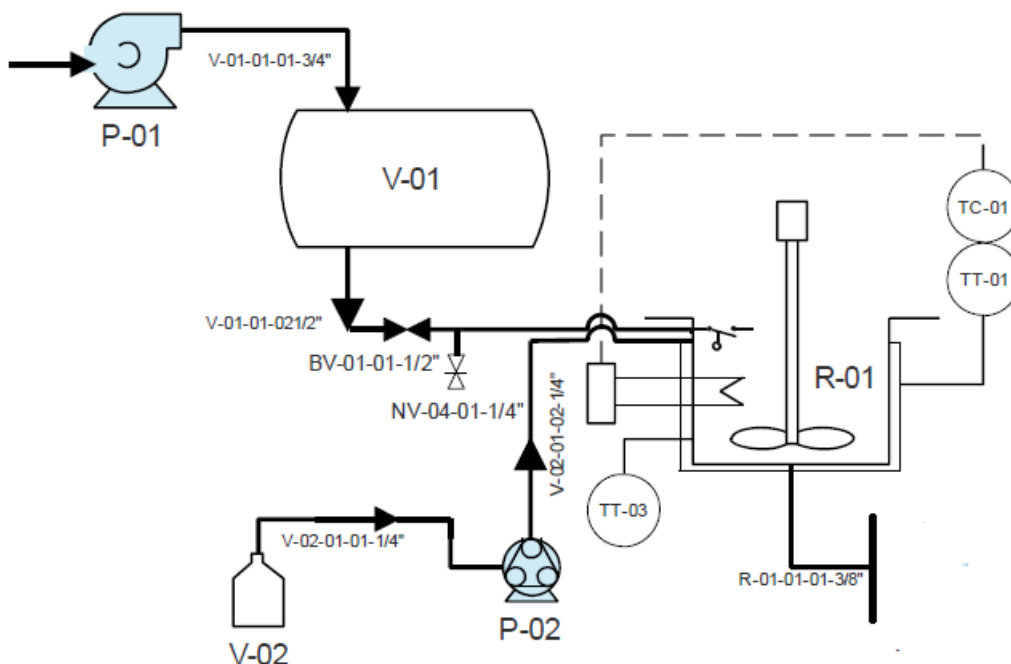
Step 5: Open valves (BV-01-04, BV-01-05, and BV-01-07), switch on the pump (P-05 A/S), which will start to recirculation of water inside the tank.

Step 6: Switch on the heater. Allow the water to achieve set temperature. After reaching the set temperature open these valves (BV-01-06 and BV-01-03) simultaneously close these valves (BV-01-07).

Step 7: Switch the controller setting to the thermocouple mounted on the reactor R-02 (TC-02) from the thermocouple of E-01 (TC-07).

8.3 Procedure for start-up Acidification Reactor (Node-1)

If in case of power failure or short or temperature out of control or spillage, follow respective sections in emergency protocol.



Step 8: Check substrate concentration in the barrel.

Step 9: Make sure the V-01 is clean and the valve (BV-01-01) is closed.

Step 10: Make necessary connection for feed pump P-01. I.e. Electrical connections, connection between barrel and V-01, check before turning it on.

Step 11: Turn on the pump, start filling the tank V-01 (Total Capacity is 50 gallons) up to 10 gallons, turn off the pump and remove all the connections.

Step 12: Connect RO water to the V-01 through plastic tube.

Step 13: As per the required concentration add RO water to achieve it. (i.e. 3X diluted, use RO water for dilution) fill the V-01 up to 40 gallons. Disconnect the tubing.

Step 14: Make necessary connections between R-01, V-01, P-02 and check for electrical connections.

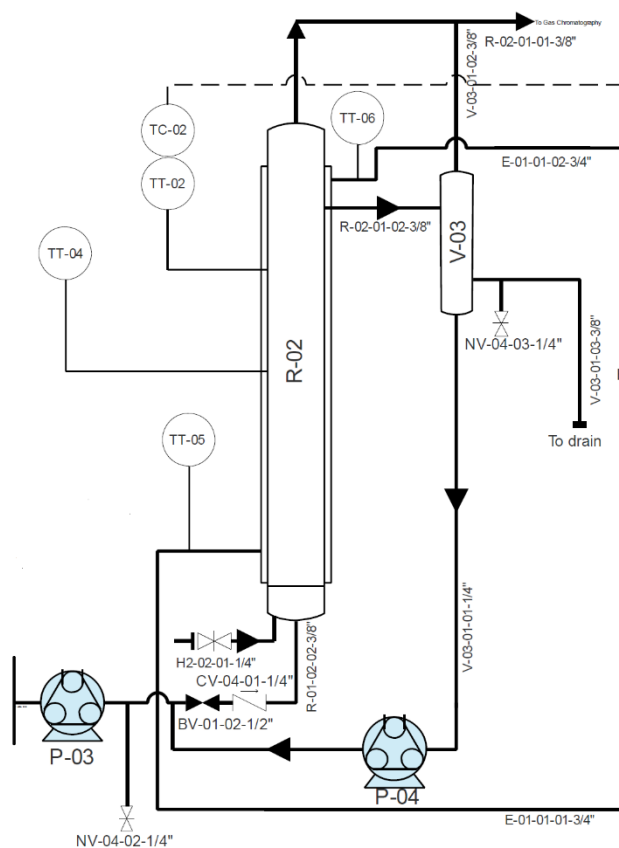
Step 15: Start filling the substrate to reactor R-01 and check the fill volume as it reaches the fill volume it will stop automatically by the float value.

Step 16: Switch on the agitator, switch on the pH sensor automatically pH will be adjusted by drawing the buffer solution into the reactor R-01. Set the pH required (i.e. 6-7). Make sure to maintain the enough amount of buffer solution in the V-02 container.

Step 17: Check for the connection between heater and thermocouple (TC-01). Set the temperature required to achieve (i.e. 35°C), set upper limit and lower limit for the controller. Allow it to reach steady state temperature.

8.4 Procedure for start-up Anaerobic Bioreactor (Node-2)

If in case of power failure or short or temperature out of control or spillage, follow respective sections in emergency protocol.



Step 11: Make necessary connections between P-03, R-02, V-03, and P-04. Check all temperature sensor & transmitter connections. Check jacket connection from hot water system E-01.

Step 12: Add biomass ($3/4^{\text{th}}$ of the reactor), into the reactor by removing top portion of the reactor R-02. Assemble the reactor back. Check for tightness of fitted portion.

Step 13: Pass Nitrogen gas regulated through regulator on the cylinder and needle valve (H2-02-01). Make sure Nitrogen bubbles off from the water, to make sure no oxygen is present in the reactor R-02.

Step 14: After step 7 is completed hot water system will be ready to use. The pump P-03 can be started after step 13.

Step 15: After reaching steady state temperature in the reactor R-01. Start pump P-03 as per calculations set required flowrate. (Retention time in the R-01 24-48 hrs).

Step 16: Allow the reactor to attain steady state.

Step 17: After reaching steady state, reactor will have overflow which will be collected in buffer tank V-03, where part it is recycled back to the reactor R-02 from pump P-04 (i.e. 30% of flowrate of pump P-03) and rest of overflow is sent as effluent.

Step 18: *Hydrogen addition to the reactor, Hydrogen is added through regulator on the cylinder and needle valve (H2-02-01), flow is controlled through rotameter on the line. Hydrogen addition is based on the calculation, please make sure there won't any accumulation of the hydrogen gas in the reactor.

Step 19: Top of reactor as liquid, solid, gas separator from which liquid is separated and sent to the V-03. The biogas generated is sent out and volume of gas generated is calculated by volume displacement method (manually) part of it is analysed in gas chromatography for gas composition. Biomass solids will retain inside the reactor a fabric strainer installed inside the reactor to retain.

9. EMERGENCY PROTOCOLS

- Check for emergency shower location in the lab
- Check for eye shower location in the lab
- Check for fire hydrant location in the lab
- Check for emergency push buttons in the lab
- Check for all the drain point location in the lab
- In case of electrical short or fire accident, report the incident to the physical facility or university police. Even though you controlled it.
- Analyse the root cause for the failure and make sure it does not repeat again.

9.1 Spillage Protocol: In case of spillage (substrate, NaOH, RO water, Biomass) turn off the power sources near the spillage area

- Wet mop the area and push all the spillage into nearest drain point.
- Dry mop the area. If in case of uncontrollable spillage or leak of RO water from the source.
- Disconnect all the electrical connections near spillage (push the emergency red button).
- Call physical facility Ph: (573)341-4252
- Call university police Ph: (573)341-4300

9.2 Electrical Short protocol: In case of electrical short.

- Unplug the all the cables from the sockets
- In case short circuit which causes fire, use the hydrant to suppress the fire. If it goes out of control press the fire emergency button (push the emergency red button).
- Call physical facility Ph: (573)341-4252
- Call university police Ph: (573)341-4300

9.3 Fire protocol: In case of fire emergency.

- If the fire can be controlled by you, then use the hydrant near you to suppress the fire and inform the physical facility or university police about the incident.
- If fire cannot be controlled by you, push the fire emergency button and report the incident to physical facility & university police.
- Call physical facility Ph: (573)341-4252
- Call university police Ph: (573)341-4300

10. CHANGE HISTORY

Where the SOP is the initial version:

- SOP Rev. No: Record the SOP and version number
- Effective Date: Record effective date of the SOP or “see page 1”
- Significant Changes: State, “Initial version” or “new SOP”

- Previous SOP Rev. No.: State “NA”. Where replacing a previous SOP:
- SOP Rev. No: Record the SOP and new version number

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III. THE PRE-ACIDIFICATION GAS IMPACT ON UPGRADING THE BIOGAS PRODUCED IN EXPANDED GRANULAR SLUDGE BED REACTOR

ABSTRACT

Two-stage anaerobic reactors are being widely used in the organic waste management industry. In these reactors, up to one-third of the chemical oxygen demand (COD) content and the volatile solid of the organic waste are naturally pre-acidified in a first stage pre-acidification (PA) or pre-digester holding tank and then fed to a second stage digester for conversion to methane. Traditionally, all the generated gases from the PA tank are vented to the atmosphere. Hydrogen and carbon dioxide are the main gases generated in the PA tank. A pilot scale two-stage anaerobic expanded granular sludge bed reactor was fabricated and used to investigate the impact of the PA gas injection into the second stage. The gas from the PA reactor was captured and stored in storage tank.

A brewery wastewater with Chemical Oxygen Demand (COD) of 20 g COD/L was used as the substrate. The tests were run under two temperature ranges and five organic loading rates (~2, 3, 4, 5, and 6 g COD/L.day). For mesophilic range, the biogas production and energy yield increased by 10-90% and 40-130%, respectively, from without PA gas injection case compared to with PA injection case. This indicated the value of capturing the PA gas and utilizing it in enhancing the energy yield of the anaerobic digester. For thermophilic range, the biogas production and energy yield increased by 12-40% and 90-140%, respectively, from without PA gas injection compared to with PA injection case. For each OLR, the gas production and energy yield were 90 to 160% more in thermophilic range than the mesophilic range for the cases with and without the PA gas injection. This clearly implies that higher temperature range has a significant and positive impact on

energy yield in a digester. One of the important findings was the amount of the PA gas injected into the EGSB reactor should be less than 50% of the theoretical calculated hydrogen gas based on ethanol substrate assumption

1. INTRODUCTION

Due to the high fines imposed by the government environmental agencies for discharging wastewater into the natural water ponds, most of the industrial companies-built wastewater treatment plants in situ to treat the wastes generated, whether solids or liquids. These wastes contain organic matters that could be degraded and consumed in the anaerobic digestion process, resulting in an eco-friendly effluent being discharged to the environment[1].

The wastewater discharged from breweries, for example, has a high quantity of sugar (glucose) and a less amount of proteins and lipids. It could be treated in wastewater treatment plants so the effluent which is coming out of the treatment unit is not harmful to the environment and at the same time, these wastes would be a good source of energy [2]–[4][5]. Two-stage anaerobic reactors are being widely used in the organic waste management industry. These reactors consist of fermentation and acidification steps in the first stage and acetogenesis and methanogenesis steps in the second stage. In this process, up to one-third of the chemical oxygen demand (COD) and the volatile solids in the organic waste were naturally pre-acidified in a first stage preacidification (PA) (or sometimes it is called pre-digester's holding tank) and then fed into a second stage digester for methane production. Traditionally, all the generated gases from the PA tank vented to the atmosphere. Hydrogen and carbon dioxide are the main gases generated in the PA tank. The gas released to the atmosphere causes a heat dissipation from the system as these are

warm gases. The biogas, the final product of the anaerobic digestion, is typically composed of 50-70% methane, 30-50% carbon dioxide; and some trace gases such as hydrogen sulfide, carbon monoxide, and hydrogen[1], [6], [7].

By increasing the quality and the energy yield of the biogas, it can be used as a fuel in road vehicles and generators or conditioned to specifications that can be combusted produce electricity. The higher the methane content in the biogas, the higher the energy yield in the form of heat and electricity.

The biogas composition depends on the nature of the substrate fed to the anaerobic digester, in addition to other factors like the operating temperature and the reactor configuration. Different methods can be used to enhance the methane composition in the biogas. Most of these methods require setup and power to operate. Some of these methods might also be expensive to build. These techniques will remove the carbon dioxide from the biogas, so it could affect the biogas rate. Some examples of these methods are electric swing adsorption [8], polymeric membrane [9], CO₂ removal by water washing technique[10], and chemical treatment [11].

In the literature, several researchers investigated upgrading the biogas product in the AD process[2], [12]. Al-Rubaye, et.al., [12] developed a process modeling simulation for the anaerobic digestion using Aspen plus software and used different feed stocks (substrates) with various concentrations. Also, Al-Rubaye conducted a sensitivity analysis to investigate the effect of injecting hydrogen gas into the anaerobic digester. Results showed that methane composition in biogas was between 88-90 % based on the substrate concentration. Ghorbanian, et.al, [13] studied the impact of injecting supplemental hydrogen gas into an expanded granular sludge bed reactor (EGSB). Ghorbanian's system

was composed of two stages. In the first stage, the hydrolysis and acidogenesis steps occurred in one tank called pre-acidification. The second stage included the other two steps (acetogenesis and methanogenesis), which were held in the EGSB reactor. It has been found that the methane composition in the biogas was enhanced (10-20%) by injecting hydrogen gas at two different rates below the theoretical generated amount of hydrogen under mesophilic temperature range (i.e. 35°C). Angelidaki and Luo [14] also investigated the effect of injecting hydrogen gas on methane composition in biogas. They used a continuous stirred tank reactor of 1 L volume and ran the system under mesophilic and thermophilic temperature ranges.

The thermophilic temperature range was found to be more effective in producing a high percentage of methane (about 90%). Many anaerobic digester configurations have been used for wastewater treatment and biogas production, they are classified as conventional and high rate anaerobic digesters. The expanded granular sludge bed reactor (EGSB), an AD reactor configuration classified under the high rate anaerobic digesters, is an updated version of the up flow anaerobic sludge blanket (UASB) [15]. It is equipped with a recycle stream for the effluent that helps in bed expansion and enhances the mixing inside the reactor. The residence time of the substrate in the sludge zone within the EGSB will be longer due to the bed expansion, therefore, more biogas will be produced. The methane share in biogas could be maximized and utilized in all the equipment and devices designed for natural gas with a minimal adjustment in the equipment used due to the lower energy content of the biogas. The carbon dioxide removal from the biogas could be done by different methods as mentioned before, however, using these techniques might also lose quantities of methane gas with the carbon dioxide. Few literature references were found

regarding the biogas enhancement, and they either used a batch anaerobic digester configuration like Angelidaki et.al [14], [16], or used a supplemental pure hydrogen into the system like Ghorbanian et.al[13].

In this paper, the gas from the PA reactor, which is a gas mixture of hydrogen and carbon dioxide generated from the pre-acidification tank was captured and stored in a storage tank. The PA gas that contains hydrogen gas was injected into the expanded granular sludge bed reactor. This injection was at a rate of 50% of the theoretical hydrogen gas calculated. This was done to estimate the amount of hydrogen generated from the PA tank and to investigate the impact of the PA gas on the biogas rate/composition produced in the EGSB reactor. This investigation was carried on under mesophilic and thermophilic temperature ranges for substrate concentration of 20 g COD/L.

2. MATERIALS AND EXPERIMENTAL METHOD

2.1 THE EXPERIMENTAL SETUP

Utilizing the carbon dioxide and converting it into methane gas is beneficial. It can be done by reacting hydrogen gas with the carbon dioxide using the hydrogenotrophic methanogens. The PA gas mixture can be captured from the first stage of the anaerobic digestion (the pre-acidification stage) where theoretically about 50-60% of the generated gas is hydrogen and the rest is carbon dioxide [14], [17]. This reaction can be considered as one of the advantages of having a two-stage anaerobic digestion instead of only one stage, as a sudden loading shock could lead to concomitant increase in the acid production, which could not be matched by a like increase in methanogenesis [18], as the acidogens grow at a rate higher than the acetogens. this could cause an accumulation of the volatile

fatty acids (VFA's) in the system and thus, the pH would drop and cause inhibition in the process [19]–[22].

Using the PA gas mixture injection method to maximize the methane composition in the biogas could enhance the biogas quantitatively and qualitatively, as the methane gas has specific volume three times larger than carbon dioxide [13]. Also, it will help reduce the heat dissipation of the warm gases out of the system, by enhancing the mixing inside the system via the PA gas bubbles passing through the biomass. A two-stage anaerobic digestion process was built to study the impact of PA gas (hydrogen and carbon dioxide) injection on the biogas production. Process and instrumentation diagram for the two stages anaerobic digestion process is shown in Figure 1.

The pre-acidification tank is 33 gal total volume (active volume is about 18 gal) made of stainless steel material and equipped with a stainless-steel mixer to maintain a uniform temperature and mass distribution. A Milwaukee pH controller instrument with MP810 dosing pump was installed to pump sodium hydroxide (NaOH) to adjust the pH of the substrate. Nutrient supplements were also added into the PA tank to keep the microorganisms active.

The PA reactor was insulated to avoid heat loss and was equipped with a submersible controlled heater to maintain the required temperature. To capture the PA gas that was produced in the PA reactor, two vacuum pumps (Focal Flux, Max pressure delivery 35 psi) 12V DC were connected in parallel and were energized by a timer switch that ran the pumps for 30 seconds every 90 minutes.

The PA gas that vacuumed from the PA reactor was stored in a 5 gallon steel tank rated up to 250 psig. The PA storage tank was connected to a pressure transducer (Omega pressure transducer model PX304 with transducer indicator DP-350).

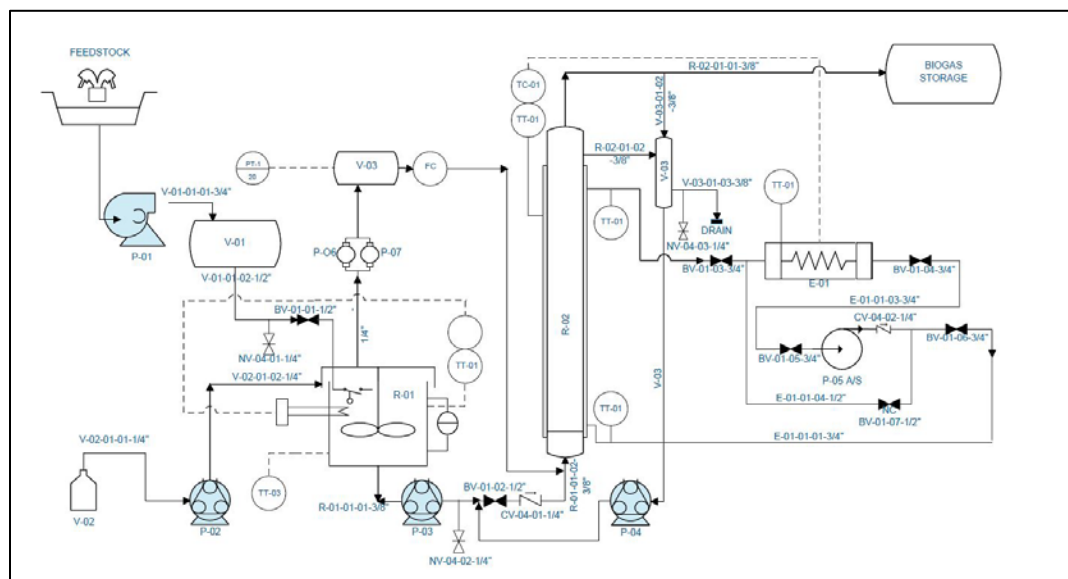


Figure 1. Process and instrumentation diagram (P&ID) for the two-stage anaerobic digestion process

The PA gas then flow through a mass flow controller into the bottom of the EGSB reactor. Two BT300S basic variable speed peristaltic pumps were used with pump heads (YZ15). The first one was used to pump the substrate (volatile fatty acids) from the PA reactor to the EGSB reactor and the second one was used to control the recycle stream of the effluent back into the system again at a specific ratio.

The lower part of the EGSB where the substrate first got introduced into the system through a gas sparger, is made of aluminum material. Above that was a plexiglass distributor with 171 holes (2mm dia) to distribute the substrate's flow uniformly in the reactor and support the biomass granular particles which were above the distributor. The section above the aluminum plenum was the main part of the EGSB where the biomass

granular particles were located. It consisted of two concentric columns, the outer one is 11" I.D. and the inner one 7.5" I.D. Both columns were made of acrylic material.

Hot water at a specific temperature was pumped through the annular space to maintain constant temperature inside the reactor as the biomass granular particles cannot be exposed to a direct heat by a submersible heater besides, the temperature will not be uniform along the reactor (temperature gradient). The upper part of the EGSB was the Gas-Liquid-Solid separator. The generated biogas then passed through to the top part while the liquid goes out to the side of the separator and the solids (biomass) are retained inside the reactor. The liquid effluent from the digester flowed to a small column where a recycle stream was connected to the second peristaltic pump that recycled part of the effluent (about 30% of the fresh feed rate). The rest of the effluent was discharged to the sewer system.

The other main part of the AD system was the heating system used to maintain the temperature of the EGSB. It consisted of a 23 gallon stainless-steel tank. In which a heater of 4500 watts was installed, connected to a temperature controller which maintained a specific temperature for heating the EGSB reactor. The hot water was pumped into the annular space between the two columns in the EGSB reactor by using two centrifugal pumps connected in parallel and plugged to a timer switch to switch between the pumps every 30 minutes. Each pump had a one-way check valve to maintain the direction of the hot water flow. Figure 2 and Figure 3, show the two stages AD and the PA gas capturing system with the PA reactor, respectively.

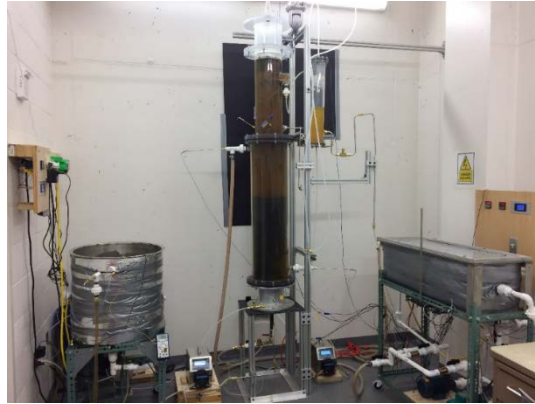


Figure 2. Two-stage anaerobic digestion process

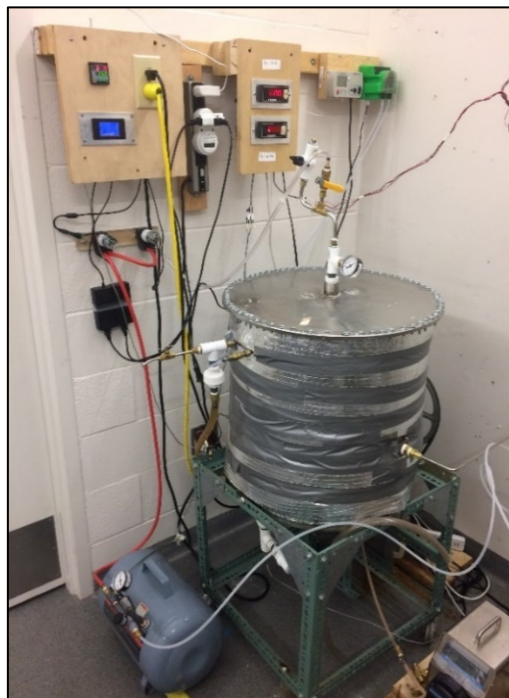


Figure 3. Pre-acidification reactor with hydrogen gas capturing system

2.2 MEASURING AND ANALYSIS DEVICES

Different parameters were monitored to check the stability progress of the reactor, like process temperature, pH level, biogas production, chemical oxygen demand, volatile fatty acids, methane composition in biogas, and biogas production rate.

For process temperature, ten thermocouples were used to monitor the temperature along the process. Two thermocouples were connected to the temperature controllers of the

PA reactor and the heating tank and the other eight were connected to a data logger, distributed as is shown in Table 1. A temperature data logger (Pico data logger of eight channels, TC-08) was used. A mass flow controller (MFC) from Brooks (SLA5850) was used to control the PA gas flow into the EGSB reactor. Two display digital panels were used to monitor current, voltage, power, and energy used for the two heaters.

Table 1. Thermocouples distribution in the AD system

TC Number	Description	Thermocouple Type
1	The heating tank	T
2	Jacket inlet hot water	T
3	Jacket outlet hot water	T
4	The PA reactor (CSTR)	T
5	Inside the EGSB, Liquid temperature	J
6	Inside the EGSB, Biomass granular particles temperature	J
7	Inside the EGSB, biomass granular particles temperature	T
8	Effluent outlet temperature, Top	T

For the effluent analysis, a laboratory spectrophotometer (Hach DR3900) was used to measure chemical oxygen demand (TNT823 vial), Ammonia (TNT vial 833), Total Nitrogen (TNT vial 828), Phosphorous (TNT vial 845), Total Al-Kalinity (TNT vial 870). The Volatile Fatty Acids (VFA's) (TNT vial 872), Sulfate (TNT vial 865), and Phenols (TNT vial 868). For the gas analysis, two different ways were used. The first was an analytical method based on the absorption of the carbon dioxide by the potassium hydroxide (0.5N) [23] and the second method used for gas analysis was gas chromatography (Varian Cp-3800, TCD/FID configuration) with fused silica capillary column (100m*0.25mm*0.5 μ m).

Measuring the biogas production rate was done by a water displacement method. It consists of a glass container filled with water (12 gal) and connected to the reactor. The biogas was collected daily and a gas sample was taken in a gas sample bag for analysis purposes and the rest was burned. Figure 4 shows the biogas rate measurement by a water displacement method.



Figure 4. Biogas rate measurement by water displacement method

2.3 BIOMASS GRANULAR PARTICLES

The common factor between the EGSB and the UASB are the granular biomass particles utilized in the reactors. They are porous granules that have the microorganisms (acidogens, acetogens, and methanogens) inside.

The biomass granular particles used in this research were collected from Anheuser-Busch. Several analyses had been conducted on the biomass granular particles, such as total solids (TS), total dissolved solids (TDS), total suspended solids (TSS), and volatile suspended solids (VSS). The analysis protocols used were quoted from the United States Geological Survey (USGS).

For the biomass, the TDS was about 2000 mg/L. The TSS was 79000 mg/L and the VSS was 78000 mg/L. The pH of the biomass medium was about 7.5 before adding into the reactor. Literature proposed that the biomass granular particles be composed of several layers with methanotrix aggregates as nucleation in the center[24], [25]. The mechanism that was used to let the substrate flow into the biomass particle was diffusion. According to the mechanism, the degradable substrates were diffused inside the biomass particles where the acidogens, acetogens, and methanogens reside. Any solid particles in the substrate were treated and removed before introducing the substrate to the AD system, therefore, using the supernatant wastewater was important as the solid particles could clog the pores of the biomass granular particles in the reactor.

The biomass were acclimatized for 20 days at low organic loading rate of the substrate, in order to prevent any shock from occurring to the microorganisms which reduce the biogas production rate and affect its composition [5], [26].

2.4 EXPERIMENTAL PROCEDURE

The wastewater used for the AD process was collected from a distillery company and it was basically the stillage waste of a distillation process. The collected wastewater was filtered to get rid of the large solid particles. A sample of the wastewater was taken and diluted at least six times with tap water, then it was analyzed through the spectrophotometer for COD, sulfate, phosphorous, alkalinity, ammonia, nitrogen, VFA's, and phenols concentrations.

The raw wastewater used had a total COD of 90 g COD/L and it was diluted to obtain the required concentration of 20 g COD/L for the experiment. The selection of COD strength was based on the VSS of the biomass granular particles and the availability of the

wastewater quantities. The ratio of the COD to the VSS was less than 1 in order to maintain the stability of the reactor. Table 2 shows the characteristics of the wastewater that was used in the anaerobic digestion system.

The EGSB reactor was seeded with about 30 liters of biomass of 78,000g VSS/L. Only one COD strength (20 g COD/L) was tested for the hydrogen impact. The 20 g COD/L substrate was stored in a 55 gallon drum and it was connected to the PA reactor.

Table 2. Characteristics of the wastewater used in the anaerobic digestion system

COD, mg/L	VFA, mg/L	Sulfate, mg/L	Phosphate, mg/L
20,000	3239.4	180	17.1
Phenols, mg/L	Al-Kalinity, mg/L	Ammonia, mg/L	Nitrogen, mg/L
12.73	0	0.5	40.7

The feed to the reactor flowed by gravity and it was controlled in the PA reactor by a floating valve. In the PA reactor, the active volume of the PA was 18 gallon. Before injecting the substrate into the main reactor, it was kept in the PA reactor about 24-48 hrs and then injected into the EGSB. The nutrients concentration level, temperature, and pH of the substrate were adjusted in the PA reactor as was mentioned before.

The substrate remained in the PA before it was injected into the EGSB. The retention time in the PA was variable based on flowrate in and out of the PA reactor, constant active volume inside the PA reactor, and the change in the organic loading rate for the substrate at 20 g COD/L. A mixer was installed inside the reactor with an assumption proposed that the substrate was homogeneous and any specific amount of the substrate flow out of the PA reactor spent the same residence time inside the PA reactor to get the same substrate quality.

Table 3 shows the hydraulic retention time of the substrate inside the pre-acidification tank. It is important to mention that for the startup, the organic loading rate was 2 g COD/[L.day] and it kept running for almost 20 days for microorganisms' acclimation. The temperature in the PA reactor was about 35-40 °C. For the EGSB, two different temperature ranges were tested, mesophilic (33-36) °C and thermophilic (50-52) °C, respectively.

To maintain and monitor the stability of the system, the COD, VFA, and pH for the effluent were checked every 24-48 hrs. Nutrient/ Mineral/ Buffer medium was added on a regular basis to the EGSB to stabilize the microorganisms and keep them active. A list of the nutrients medium composition is shown in Table 4.

To estimate the theoretical rate of hydrogen gas produced in the PA reactor, an assumption was made that the substrate was composed of only ethanol compound as it is difficult to analyze the actual substrate feedstock into its main compounds.

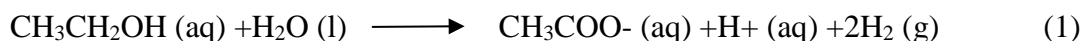
Table 3. Retention time in the PA reactor and the hydraulic retention time in the EGSB reactor per each OLR for 20 g COD/L substrate strength

Organic Loading Rate (OLR), g COD/L. Day	Flowrate, gal/day	PA Reactor Retention Time, Day	EGSB Hydraulic Retention Time, Day
2.0	0.833	24	10
2.5	1.04	21	8
3.0	1.25	17	6.66
4.0	1.666	13.2	5
5.0	2.08	10.56	4
6.0	2.5	8.8	3.34
7.0	2.916	7.5	2.85
8.0	3.333	6.6	2.5

Calculating the theoretical hydrogen generated from the PA reactor was based on the chemical reaction in equation (1). The PA gas mixture contained a hydrogen gas of

about 45-60% while the rest was carbon dioxide. It was injected into the system at a rate below the theoretical hydrogen calculated (about 50% of the theoretical values) based on the Ethanol degradation.

The chemical reaction used, based on the assumption, is in (1).



To calculate the amount of hydrogen generated theoretically in the PA reactor, the degree of pre-acidification (PA%), the COD strength of the feed substrate, and the substrate flowrate in to the system should be known. A simple example could explain the procedure. If the PA% of the substrate after the PA reactor was, for example, 30% of a substrate concentration of 10 g COD/L, and the substrate flowrate was 1 L/hr, then the amount of pre-acidified substrate would be 3 g COD/L. The pre-acidified substrate was then divided by the molecular weight of ethanol (46 g/g mole) to give about 0.065 mole of ethanol pre-acidified. According to the chemical reaction equation (1), one mole of ethanol will produce 2 moles of hydrogen, which means $2 \times 0.065 = 0.13$ mole of hydrogen gas produced. Multiplying 0.13 mole hydrogen by its molecular weight makes the total hydrogen produced 0.26 g/L. since the density of hydrogen gas is about 0.09 g/L, thus $0.26 / 0.09 = 2.9$ L of hydrogen produced per one liter of substrate. Finally, multiply the produced hydrogen rate into the particular fresh substrate flow to calculate the hydrogen produced based on the substrate flowrate (2.9 L H₂/hr).

Based on this example, Table 5 shows the amount of hydrogen gas produced based on the substrate flowrate, the PA%, and the COD strength used in this investigation. So, for each OLR's value, there is a corresponding value of hydrogen gas rate from the PA reactor that should be injected into the EGSB reactor. The hydrogen gas flowrate was

within a gas mixture from the PA reactor which was controlled by a mass flow controller, as mentioned previously. The hydrogen gas composition in the PA gas mixture was about (45-60) % according to the gas analysis done by gas chromatography. An average value of PA% was considered for the calculations.

The PA% selected was about 27% as an average value for the degree of acidification in the PA reactor. Only 50% of the theoretical hydrogen calculated value was taken for each OLR to prevent any possible shock to the microorganisms in the biomass granular particles. So, the PA gas mixture rate injected in to the EGSB reactor, which contained about (45-60) % hydrogen, was injected at a rate of half of the theoretical hydrogen calculated.

Table 4. Nutrient, mineral, and buffer concentrations

	Component	Quantity Needed, (mg/ml)
Mineral Base I	Nickle(Ni)	0.0062
	Copper(Cu)	0.0026
	Zinc(Zn)	0.0119
	Iron(Fe)	1.126
	Cobalt(Co)	0.062
	Manganese(Mn)	0.0139
	Selenium(Se)	0.0104
	Molybdenum(Mo)	0.002
	Boron(B)	0.0044
Mineral Base II	Magnesium(Mg)	2.36
	Calcium(Ca)	5.4
Nutrient Base	Phosphorous(P)	11.4
	Nitrogen(N)	13.9
	Sulfur(S)	6.76
Buffer Base	Sodium Bicarbonate (NaHCO ₃)	40

Table 5. Calculated hydrogen rates (theoretical) and actual H₂ injection from the PA reactor for each organic loading rate

OLR, g COD/L.d	HRT, day	Total Flow, ml/min	Fresh Stream, (70%), ml/min	Recycle Stream, ml/min	PA%	Theo. H ₂ Generated, ml/min	50% Theo. H ₂ generation	Actual H ₂ injection in PA gas
2	10	3.125	2.187	0.937	27	11.413	5.706	3.423
3	6.66	4.687	3.281	1.406	27	17.119	8.559	5.135
4	5	6.25	4.375	1.875	27	22.826	11.413	6.847
5	4	7.812	5.468	2.343	27	28.532	14.266	8.559
6	3.33	9.375	6.562	2.812	27	34.239	17.119	10.271
7	2.85	10.93	7.656	3.281	27	39.945	19.972	11.983
8	2.5	12.5	8.75	3.75	27	45.652	22.826	13.695

2.5 INHIBITION AND INSTABILITY REMEDIATION PROTOCOL

The AD system was subjected to many factors that could cause an inhibition to the system. The biogas production rate is the important key to monitor the system stability. If the biogas rate suddenly dropped, that means either there was an OLR shock because of the low hydraulic retention time (HRT), or there was a fluctuation in one or more of the operating conditions.

The amount of the VFA concentration in the effluent coming out of the system should be at a certain level. As a stability indicator, the ratio between the volatile fatty acids to the total alkalinity (VFA/Total alkalinity) should be about 0.5 or less[21]. Adjusting the total alkalinity was done by adding sodium bicarbonate (baking soda). So, for example if the VFA concentration in the effluent was 250 mg/L, then the total alkalinity required should be at least about 1000 mg/L, and since the baking soda has an alkalinity of $50/84=0.6$ g alkalinity/g NaHCO₃ then, 1000 mg/L of baking soda will add 600 mg/L alkalinity to the system.

During inhibition remediation process, the acidic liquid medium inside the EGSB should be replaced. A liquid mixture of nutrients medium/ water was injected into the

reactor at low organic loading rate, this would help get rid of the undigested substrates and adjust the pH level. The water/nutrients solution would continue to be injected till the pH of the effluent reach about 7.3 and the VFA/alkalinity is less than or equal 0.5. If the VFA concentration doesn't decrease to the required level or if the biomass got sensitive then, the pre-acidification stream injected into the second stage should be paused and let only the recycle stream to be active till the microorganisms retrieve their own activity.

3. RESULTS AND DISCUSSION

3.1 MESOPHILIC TEMPERATURE INVESTIGATION

The EGSB reactor was operating under two different temperature ranges, Mesophilic and thermophilic temperature ranges which were ($\sim 36^{\circ}\text{C}$) and ($\sim 50^{\circ}\text{C}$) respectively.

The substrate COD concentration was 20 g COD/L used for a range of organic loading rates (2-8) g COD/L.day. The first stage of the AD system (PA reactor) has the hydrolysis and acidogenesis steps, was running under $\sim 38^{\circ}\text{C}$, where the volatile fatty acids (VFAs) formed in the acidogenesis step. The second stage composes of the acetogenesis and methanogenesis steps. Having the AD system in two stages maximizes the acid formation and optimizes the methane gas production [27]. All the nutrients required for the system were added in this stage.

The pH for the substrate was also adjusted to the required level ($\sim 5-5.5$) by adding sodium hydroxide 1N. The VFAs formed were within the range (25-28 %) for both temperature ranges, which were close to results found in the literature [13], [14], [22]. The pre-acidification percentage was calculated by equation (2)

$$\text{Pre-acidification degree (PA)} = \left(1 - \frac{COD_{PA}}{COD_{in}}\right) * 100 \quad (1)$$

Where

PA % is the pre-acidification percentage, COD_{PA} is the COD concentration from the PA substrate stream, and COD_{in} is the COD concentration from the influent stream (substrate feed to the PA tank).

During the PA reaction, hydrogen and carbon dioxide were produced. In previous literature, hydrogen-carbon dioxide gas mixture generated in the PA reactor wasn't captured [13]. Calculating the theoretical hydrogen produced from the PA reactor requires the substrate to be analyzed to its main components.

Since there is no clear chemical formula for the substrate used in the system, the substrate was assumed to consist of only ethanol compound to calculate the theoretical hydrogen generated from the PA reactor. The PA gas mixture contained about 40-60 % hydrogen gas and the rest was carbon dioxide based on the gas analysis by the gas chromatography instrument.

The pH values during the investigation are shown in Figure 5. The samples tested for the pH were from the effluent coming out of the EGSB reactor. For the experiment with no PA gas injection, the pH values were between (7.0-7.4) for the OLRs from (2-7) g COD/L.day. For the next OLR (8 g COD/L.day), the pH started to drop slightly below that, and it dropped further for the following OLR (9 g COD/L.day).

The pH drop occurred due to the insufficient residence time inside the reactor for the microorganisms to feed on the substrate. Also, the growth rate of acetogens is slower than that of the acidogens. This would increase the acidity level in the reactor (VFA concentrations) represented in low pH level, causing inhibition in the system.

For the experiment of the PA gas injection from the PA tank under mesophilic temperature range, the pH values were slightly higher than the case of no gas injection. pH levels were about (7.4-7.65) for the OLRs from (2-6) g COD/L/day, which were a little higher than that in the experiment with no PA gas injection. This was due to the fact that all the carbon dioxide in the substrate (carbonic acid) was utilized with the hydrogen gas from the PA tank by the hydrogenotrophic methanogens, which is similar to what was found in the literature [13]. Consuming the hydrogen gas and the carbon dioxide led to more biogas production with higher methane composition. More than 70% of the biogas generated would be by the acetoclastic methanogens while the contribution of the hydrogenotrophic methanogens would be about 30% for the biogas production [28]

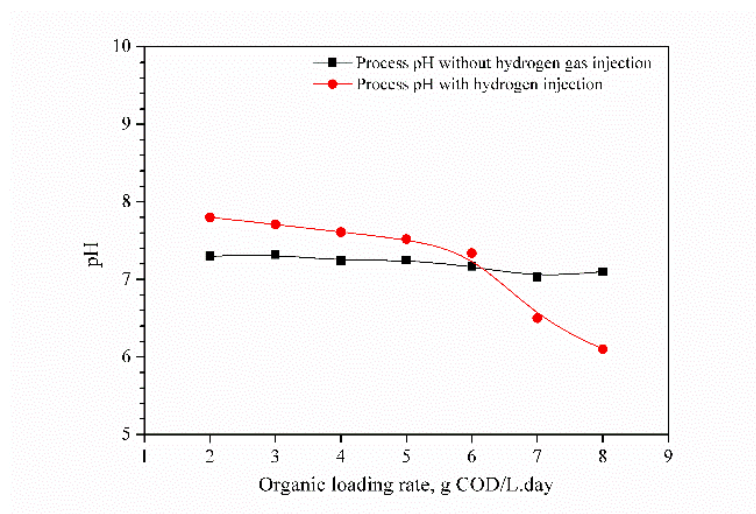


Figure 5. pH value ranging inside the EGSB reactor under mesophilic temperature range

For the case of PA gas injection, the pH drop after OLR 6 g COD/L.day was due to the excess amount of PA gas injected into the system, which means the amount of hydrogen injected was more than the required for the hydrogenotrophic methanogens to consume. That excess hydrogen led to an increase in the acidity level and eventually caused an inhibition in the system. As shown in Table 6, during the hydrogen gas injection under

mesophilic temperature range (36°C), the COD for the influent, the PA, and the effluent streams were monitored, along with the VFAs concentrations.

Fluctuating or low values for the PA% were due to either a very short residence time in the PA tank which prevented the reactor from making a sufficient amount of VFAs. Also, it might have resulted from using a different batch of waste water, causing a disturbance to the acidogens. The COD concentration for the effluent, especially for the last three OLRs, increased gradually. This increase was due to two reasons. The first, the residence time for the substrate inside the reactor was decreased, so more effluent with higher COD concentration was coming out. The second, the amount of the hydrogen gas in the PA gas mixture injected was higher than the normal consumption rate required by the hydrogenotrophic methanogens to convert to methane.

Table 6. Chemical oxygen demand (COD), volatile fatty acids, and pre-acidification percentages for each OLR tested under mesophilic temperature range

Mesophilic Range (36 °C)								
OLR	COD _{in}	COD _{PA}	COD _{Effluent}	R%	VFA _{in}	VFA _{PA}	VFA _{Effluent}	PA%
2	20000	14835	659	96.705	2200.333	8520.12	112	25.825
3	20000	14584	657.33	96.713	2468	9117.5	129.333	27.08
4	20000	14474	545	97.275	2000	7240.5	134.76	27.63
5	20000	14588.	550	97.25	1997	7385.8	151.666	27.05
6	20000	14465.	605	96.975	1837.33	6633.7	172.78	27.671
7	20000	14496	684	96.58	1804.26	6550.9	209.89	27.52
8	20000	15380	1307	93.465	1713.58	7424.1	804.61	23.1

The biogas production rate was monitored to check the processes' performance and stability while injecting the PA gas mixture into the system under two different temperature ranges. For the first case with no PA injection, different OLRs were tested (2, 3, 4, 5, 6, 7, and 8 g COD/L.day). The biogas production rate increased with OLR increase. So for the

OLR 2 g COD/L.day, the biogas production rate was 3.235 gal/day and the methane composition was about 72.67%. Next, OLR was 3 g COD/L.day, the biogas production rate was 8.1 gal/day and the methane composition was 72.66%. The third OLR was 4 g COD/L.day and the biogas production rate was about 10.852 gal/day and the methane composition was about 71.8%. For OLR 5 g COD/L.day, the biogas production rate and the methane composition were 17.278 gal/day and 70.1%, respectively. The next OLR was 6 g COD/L.day and the biogas production rate and the methane composition were 19.457 gal/day and 69.47%, respectively. The biogas production rate and the methane composition for OLR 7 g COD/L.day were 20.504 gal/day and 69.29%, respectively. The last OLR tested was 8 g COD/L.day and the biogas production rate was 20.726 gal/day and the methane composition was about 69.1%. According to the mentioned data above, the biogas rate increased with the increase of the organic loading rate (No evidence for inhibition situation found in the system besides that, the pH level in the system was almost stable). Also the biogas production rate increased with increase of the hydraulic retention time at equivalent organic loading rate, which are consistent with what found in the literature [29]–[31].

For the experiment with the PA gas injection that contains about 60% hydrogen gas, the biogas production rate increased with the OLRs increase, but it started decreasing after that. So, for OLR of 2 g COD/L.day, the biogas production rate was about 6.125 gal/day and the methane composition was about 89.2%. The next OLR was 3 g COD/L.day and the biogas production rate was 9.582 gal/day while the methane composition was 88.3%. The biogas production was about 14.304 gal/day and the methane composition was about 88.1% for the OLR 4 g COD/L.day. Next OLR was 5 g COD/L.day and the biogas

production rate was about 19.237 gal/day, while the methane composition was about 87.9%. For the OLR 6 g COD/L.day, the biogas production rate was the maximum of 21.362 gal/day while the methane composition was 87%. The production rate for the biogas for the next OLRs (7 and 8 g COD/L.day) were about 15.919 gal/day and 10.291 gal/day, respectively. The drop in the biogas production rate for OLR 7 g COD/L.day and 8 g COD/L.day was due to the excess PA gas injected which contains about 60% hydrogen gas, accompanied with a low recorded pH value. This means selecting an injection rate of 50 % of the theoretical hydrogen into the AD reactor was higher than the system's need, as it caused an inhibition in the system which was showed as a decrease in the biogas production rate and a drop in the pH level of the effluent. Based on that, the OLRs considered and plotted were up to 6 g COD/L.day where the max biogas production rate occurred.

From the results explained above, the PA gas rate injected into the EGSB reactor should be less than 50% of the theoretical calculated hydrogen rate. In Figure 6, the biogas production rate under mesophilic temperature range (for the case of PA gas injected into the EGSB reactor) was found to be higher than the case of no PA gas injected. This occurred as the carbon dioxide and the hydrogen were converted by the hydrogenotrophic methanogens into methane gas. As is mentioned in the literature, the hydrogenotrophic contribution in the biogas production was about 30% while the rest is counted for the acetoclastic methanogens [2], [28]. Since the methane has a specific volume three times more than the hydrogen gas, then the biogas production rate besides the methane composition was improved.

For the energy yield, Figure 7 shows the energy yield in KJ/L based on the biogas production rate and methane composition. The energy content (volumetric) for the methane is about 40 KJ/L. According to the results, the energy yield of the biogas increased with the increase of the organic loading rate.

The energy yield for the case of PA gas injection (i.e. hydrogen gas injection) was found to be higher than the case of no gas injected. For OLR of 2 g COD/L.day, the energy content was about 355 KJ/L and 826 KJ/L for the cases of no PA gas and with PA injection, respectively.

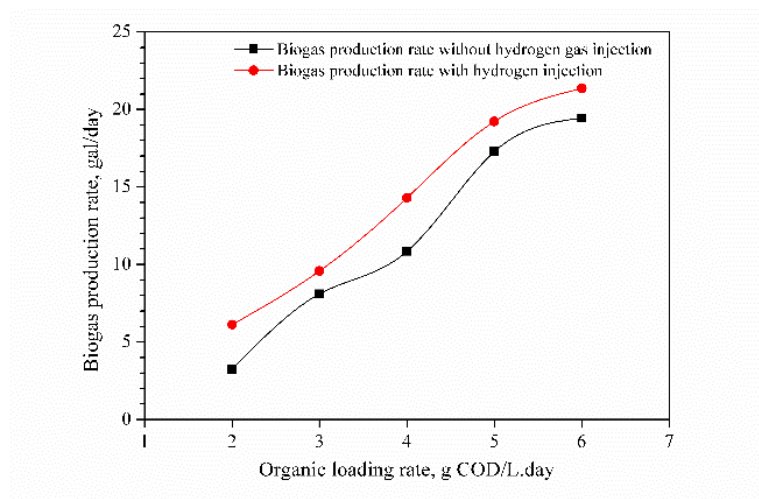


Figure 6. Biogas production rate with the organic loading rate for the AD system for with and without hydrogen injection

The energy yield was increased about 130% when injected the PA gas. For the next OLR of 3 g COD/L.day, the energy yield was 890.2 KJ/L and 1279.2 KJ/L for the cases of without and with PA gas injection, respectively.

The energy yield increased about 44% when the PA gas was injected into the EGSB reactor. The energy yields for OLR of 4 g COD/L.day were about 1178 KJ/L and 1905 KJ/L for the cases of no PA and with PA gas injection, respectively, which means an energy

yield increase of 62% when the PA gas was injected into the system. For the OLR 5 g COD/L.day, the energy yield for no PA gas and with PA gas injection were about 1831 KJ/L and 2557 KJ/L, so the case of PA gas injection improved the energy yield to about 40%. The last OLR tested was OLR 6 g COD/L.day, the energy yield was 2044 KJ/L for the case of no PA gas injection and about 2810 KJ/L for the case of PA gas injection, which means the energy yield was improved about 38% when the PA gas was injected into the system.

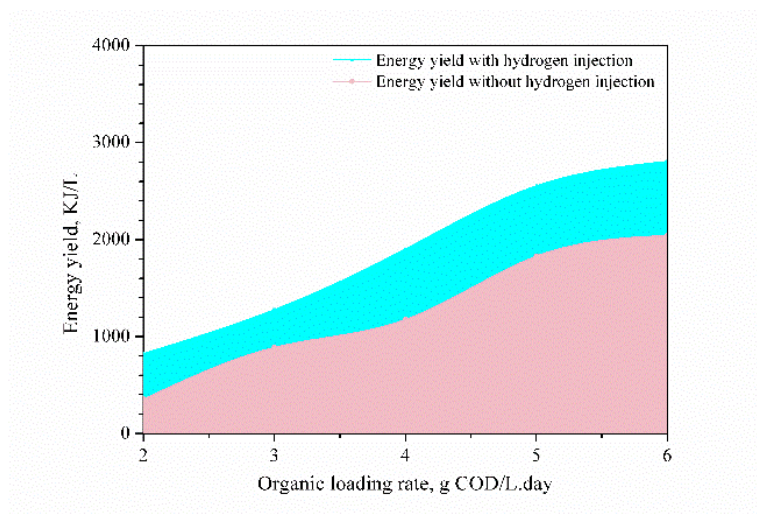


Figure 7. Energy yield of biogas product with the organic loading rate of the substrate

3.2 THERMOPHILIC TEMPERATURE INVESTIGATION

Running the anaerobic digestion system under thermophilic temperature was challenging, since the temperature transition from the mesophilic to the thermophilic range should be gradually increased at a rate not more than 2 °C/day for heating from 35 °C to 40 °C and then about 1 °C for heating from 40 °C to 50 °C. The microorganisms are very sensitive under thermophilic temperature, so the COD, VFA, Alkalinity, pH, and the biogas production rate should be monitored every day. If there are any unusual readings for the

parameters mentioned above, then the fresh feed that flows into the system should be paused and allow only the recycle line to run. Similar to mesophilic temperature range investigation, one substrate concentration was used (20 g COD/L).

The organic loading rates tested in the system were 2, 3, 4, 5, 6, 7, and 8 g COD/L.day. Similar to the mesophilic investigation, an assumption was made that the substrate consisted only of ethanol compound. The amount of PA gas injected into the system was 50% of the theoretical amount calculated. The amount of the PA gas mixture injected into the system was based on the organic loading rate.

The pH of the system under thermophilic temperature range was found to be a little higher than the case of mesophilic range (both cases were with PA gas injection). This was due to a couple of reasons. First, as the temperature was increased, the solubility of the carbon dioxide in liquid decreased (carbonic acid) and, second, the hydrogenotrophic methanogens converted most of the carbon dioxide in the system with the hydrogen gas into methane gas. As shown in Figure 8, the pH values for the effluent of the EGSB reactor were slightly decreased from 7.3 to 7.1 for the case of mesophilic temperature range with no PA gas injection. For the case of thermophilic temperature range with no PA gas injection, the pH values were also slightly decreased from 7.41 to 7.2. These higher pH values for the case of thermophilic range were due to the decrease in the solubility of the carbon dioxide in the substrate (carbonic acid).

For the case of mesophilic temperature investigation with PA gas injection, the pH values was slightly decreased from 7.8 to 7.34 for the organic loading rate from 2 to 6 g COD/L.day. For the following organic loading rates (i.e. 7 and 8 g COD/L.day), the pH values were dropped to 6.1. This occurred due to the high amount of hydrogen gas injected

within the PA gas mixture which was above the ability of the hydrogenotrophic methanogens to convert it into methane gas, this remaining hydrogen accumulated in the system and caused a sudden drop in pH values (i.e. an inhibition status occurred) [32].

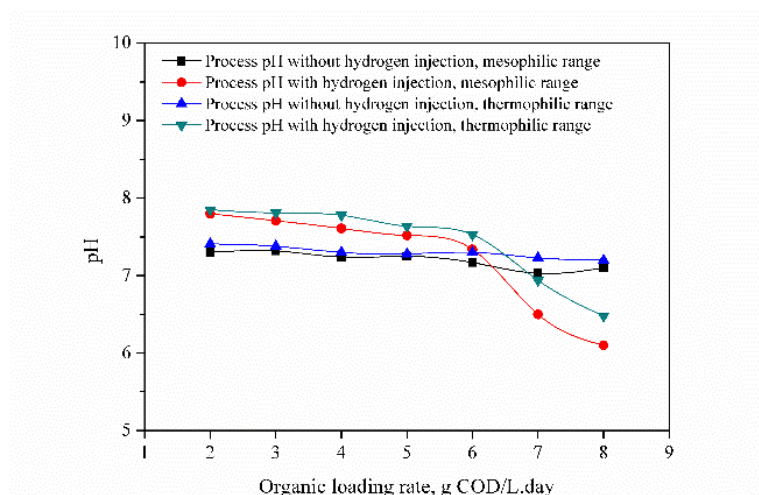


Figure 8. Effluent pH level for four different cases (with and without PA gas injection under mesophilic and thermophilic temperature ranges)

At thermophilic temperature range, the pH for the case of PA gas injection was slightly decreased from 7.85 to 7.53 for the organic loading rate of 2 to 6 g COD/L.day. For organic loading rate 7 and 8 g COD/L.day, the pH values decreased to 6.94 and 6.48, respectively.

As is shown in Table 7, the chemical oxygen demand, volatile fatty acids, the removal efficiency, and the pre-acidification degree were recorded for each organic loading rate tested under the thermophilic temperature range for the case of PA gas injection.

For the organic loading rate 7 and 8 g COD/L.day, the chemical oxygen demand for the effluent stream increased, which is the same trend that occurred in the mesophilic temperature range case.

This increase in the COD concentration was due to hydrogen gas accumulated inside the system, as the hydrogen accumulated due to the PA gas injection at a rate higher than the rate of consumption by the hydrogenotrophic methanogens.

Table 7. Chemical oxygen demand (COD), volatile fatty acids, and pre-acidification percentages for each OLR tested under thermophilic temperature range

Thermophilic Range (50°C)								
OLR	COD _{in}	COD _{PA}	COD _{Eff}	R%	VFA _{in}	VFA _{PA}	VFA _{Effluent}	PA%
2	20000	14872	721	96.395	1884.8	7379.95	122.51	25.64
3	20000	14794	678.51	96.607	1769.7	6824.95	130.24	26.03
4	20000	14571	653.21	96.733	1832.7	6776.63	135.64	27.14
5	20000	14652	691.75	96.541	1789.3	6716.59	141.94	26.74
6	20000	13925	674	96.63	1855.4	6128.78	154.52	30.37
7	20000	14271	701	96.495	1791	6274.30	230.47	28.64
8	20000	14513	1521	92.395	1823.6	6671.44	937.34	27.43

In addition to that, for both cases (without PA gas injection and with PA gas injection), the trend of COD concentration for the effluent with the organic loading rate run under the thermophilic range was similar to the effluent COD from the case of mesophilic temperature range, but was a little lower as the microorganisms were sensitive to the high temperature. This was applied on the volatile fatty acids also. The biogas production rate was the main parameter monitored to investigate the effect of injecting the PA gas mixture into the EGSB reactor under both mesophilic and thermophilic temperature ranges.

As is shown in Figure 9, the biogas production rate increased with increasing organic loading rate. For the case of PA gas injection under mesophilic temperature range, the biogas production rate was increased from 6.125 gal/day at OLR of 2 g COD/L.day to about 21.362 gal/day at OLR of 6 g COD/L.day. For the case of PA gas mixture injected

in thermophilic temperature range, the biogas production rate was increased from 11.91 gal/day at OLR of 2 g COD/L.day to about 51.712 gal/ day at OLR of 6 g COD/L.day. So the percentage of biogas production increased about 94% at OLR 2 g COD/L.day and about 120% increase for OLR 3 g COD/L.day. For OLR 4 g COD/L.day, the biogas production percentage increase was about 127%. For OLR 5 g COD/L.day, the biogas production rate was increased about 126% higher when injecting the PA gas at thermophilic temperature range. For the last OLR (6 g COD/L.day), the biogas production rate increased about 140% for the case of PA gas injection under thermophilic temperature range.

Also, two more curves were plotted in Figure 9 which shows the biogas production rate with no PA gas injection under both mesophilic and thermophilic temperature ranges. So, based on the presented data, it can be concluded that the biogas production will be significantly increased when the PA gas is injected into the EGSB reactor under thermophilic temperature range. This also applies even for the case of no PA gas injection under thermophilic temperature range. Thus, the contribution of the PA gas injection into the EGSB would improve the biogas qualitatively and quantitatively but, it leans more towards the qualitative aspect, while for the temperature effect, it will significantly enhance both the quantity of the biogas and its quality. The methane compositions along the investigation were nearly similar to the values estimated at mesophilic temperature range. All the results obtained above followed the same trend found in the literature [17], [33], [34].

The energy yield of the biogas produced when the PA gas mixture was injected under both mesophilic and thermophilic temperature ranges is shown in Figure 10. The

energy yield of the biogas reached the highest value when biogas was generated, in the case of PA injection at thermophilic temperature range.

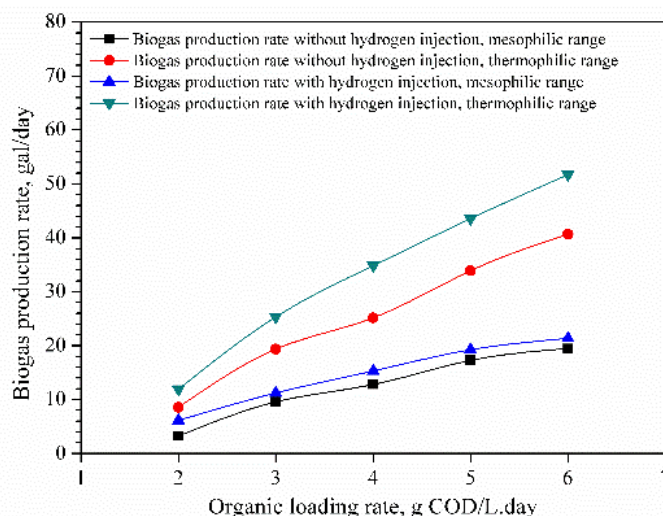


Figure 9. Biogas production rates for four different cases (with and without PA gas injection under mesophilic and thermophilic temperature range)

The energy yield was improved about 75% for the case of PA gas injection under thermophilic temperature range for OLR 2 g COD/L.day. For OLR 3 g COD/L.day, the energy yield was enhanced about 122% at thermophilic temperature range. The next OLR was 4 g COD/L.day and the energy yield was improved about 118%. Also, the energy yield was increased about 103% at OLR 5 g COD/L.day. Lastly, the energy yield was improved about 117% at OLR 6 g COD/L.day.

The temperature mapping inside the reactor played a significant role in detecting the stability with the EGSB reactor and hence, it could become an early detector in the case of inhibition occurring in the system. As it shown in Figure 11, when the pH values for the effluent were almost within the acceptable range (7.4-7.8), the temperature of the substrate, the biomass and the effluent inside the EGSB were stable at 50°C and 36°C, respectively.

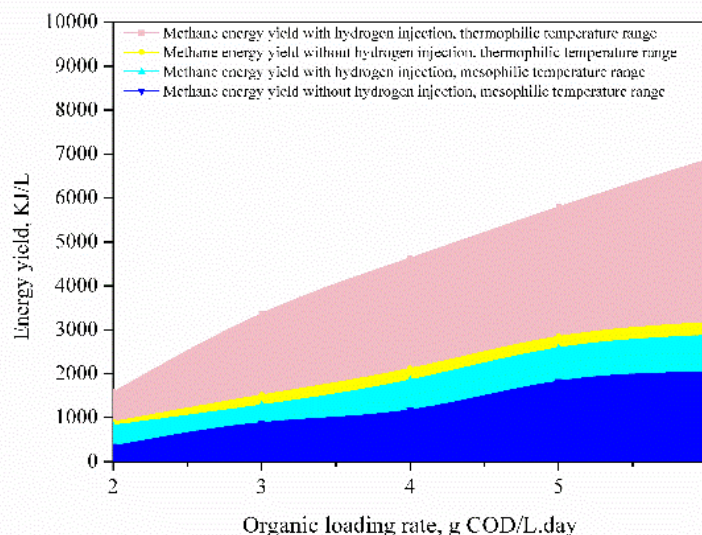


Figure 10. Energy yields for the generated biogas under four different cases (with and without PA gas injection under mesophilic and thermophilic temperature range)

When the pH of the substrate inside the EGSB was intentionally manipulated to be lower than the optimum value, the temperature profile inside the EGSB reactor was disturbed and recorded fluctuated values. This could be noticed in the fluctuation in the temperature reading in the middle of Figure 11. This occurred as the microorganisms were acclimatized to a certain pH level and when it was changed, it caused a disturbance in their activity and affected the biogas production.

This disturbance in biogas production (a decrease in the production rate) affected the heat transfer distribution in the reactor, as the biogas bubblers helped in maintain a uniform temperature inside the reactor and any disturbance caused a non-uniformity of the temperature profile. So, based on the results found, the temperature mapping could be an early inhibition detector and can detect if there is any instability in the system before it was detected by the regular pH probe.

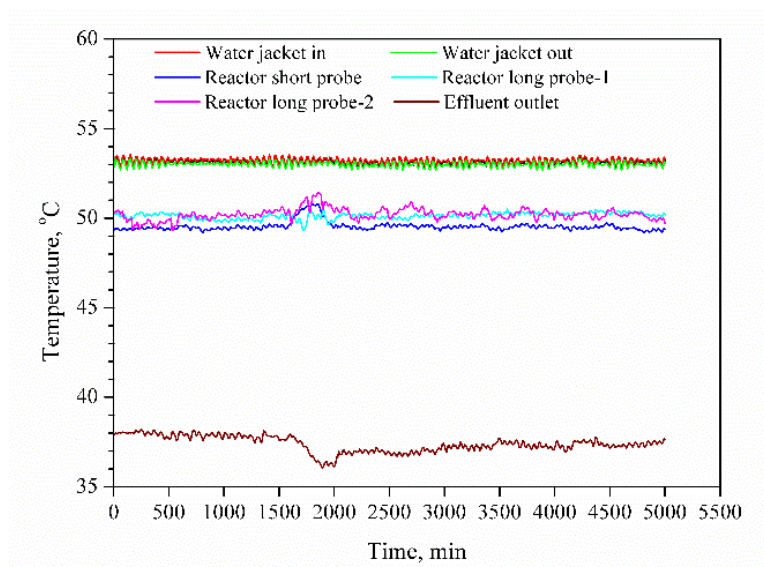


Figure 11. Temperature mapping inside the EGSB reactor

4. CONCLUSION

A pilot scale two-stage anaerobic expanded granular sludge bed reactor was fabricated and used to investigate the impact of pre-acidification (PA) gas injection into the second stage. Traditionally, all the generated gases from the PA tank are vented to the atmosphere. The gas from the PA reactor was captured and stored in a storage tank. Hydrogen and carbon dioxide are the main gases generated in the PA tank.

A brewery wastewater with Chemical Oxygen Demand (COD) of 20 g COD/L was used as the substrate. The tests were run under two temperature ranges and five organic loading rates (~2, 3, 4, 5, and 6 g COD/L.day).

For mesophilic range, the biogas production and energy yield increased by 10-90% and 40-130%, respectively, from without PA gas injection compared with PA injection. This indicates the value of capturing the PA gas and utilizing it in enhancing the energy yield of the anaerobic digester.

For thermophilic range, the biogas production and energy yield increased by 12-40% and 90-140%, respectively, from without PA gas injection compared to with PA injection. For each OLR, the gas production and energy yield were 90 to 160% more in the thermophilic range than the mesophilic range. This clearly implies that higher temperature range has a significant and positive impact on energy yield in a digester. An important fact found was that the amount of PA gas injected into the EGSB reactor should be less than 50% of the theoretical calculated hydrogen value based on the ethanol substrate assumption.

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SECTION

2. CONCLUSIONS

The process simulation model of the AD showed an upgrading for the methane composition of the biogas due to the injection of hydrogen gas into the process. The methane gas percentage increased from about 70% to about 90%. The AD model developed in Aspen gives an objective to find the optimum temperature, reactor size, and substrate flowrate to achieve an optimum process. The AD model can use various types of wastewaters to be tested in different operating conditions in order to find the optimum and maintain the system running at high performance.

The impact of the hydraulic retention time in an expanded granular sludge bed reactor was tested. Three different substrate concentrations were used at mesophilic temperature range (34-36°C). The results showed that at an equivalent organic loading rate, for a better process performance, it is better to run a high substrate concentration at lower rate compared with lower substrate concentration flows at higher rate. Some of the amino acids and the fats takes longer time to get assimilated. The AD system then ran at thermophilic temperature range (49-50°C). The biogas production was increased about 100% when the system ran at thermophilic range compared with the biogas production rate at mesophilic temperature range.

The important point that learnt was the possibility to upgrade the methane composition of the biogas in-situ. This was done by capturing the hydrogen gas generated from the first stage of the AD (pre-acidification stage). The hydrogen gas was injected at specific rate and increased with the increase of the substrate flow. The biogas quality got enhanced about 88% methane instead of 70% for the case of no gas hydrogen gas injection.

The biogas production rate and the energy yield of the biogas got enhanced for the case of hydrogen gas injection when the system was running at thermophilic temperature range. This enhancement was due to the escalation in the microbial growth of the microorganisms when the system operated at thermophilic temperature range

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VITA

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