A Comparison of Performance between Two Anaerobic Biodigesters Configurations for Biogas Production

Una Comparación de Rendimiento entre Dos Configuraciones de Biodigestores Anaerobios para la Producción de Biogás

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Abstract

A comparison of performance was made for the two most common anaerobic biodigesters existing configuration to date, continuously stirred tank biodigester (CSTBR) and plug-flow biodigester (PFBR) to produce biogas and electricity by anaerobic digestion using as feedstock dairy manure feasible for application in rural areas of Latin America to generate renewable energy as electricity and liquid fertilizer, while reducing animal wastewater pollution, greenhouse gases, and odor. The two configuration CSTBR and PFBR systems were installed at bench scale by using dairy manure as feedstock. The results showed that under the same operation conditions HRT 1 day and OLR 19.4 g COD/L day the CSTBR produced more biogas (1.0 L/L d), methane (0.8 L/L d) than the PFBR corresponding values were biogas (0.8 L/L d), methane (0.6 L/L d), and removed more organic matter, 12% COD, 8.7% VS than did the PFBR, 11.5% COD, 8.1% VS. The performance resulted similar when the HRT was increased to 3 days and the OLR decreased to 9.8 g COD/L day. The microbial communities between the CSTBR and PFBR configurations were performed by using pyrosequencing molecular technique. The microbial communities are highly variable with the distribution and abundance of taxonomic groups differing between the samples taken from the two AD configuration systems, more richness of archaeal microbial diversity were present in the PFBR whereas higher bacterial microbial diversity were present in the CSTBR sample. It is recommended the application of CSTBR configuration for AD. However, the PFBR configuration can be improved by intermittent mixing application, that failure because of clogging of solids due to long HRT and SRT, particularly for small PFBR installed in developing countries.

***Index terms*− Anaerobic digestion, biodigester configuration, microbial community, biogas production, biotechnology.**

Resumen

La comparación del rendimiento se hizo para las dos configuraciones de biodigestores comunes existentes a la fecha el tanque continuamente (CSTBR) y el de flujo a pistón (PFBR), para producir biogás y electricidad mediante digestión anaerobia utilizando como sustrato desechos industriales de granjas de leche para su aplicación en áreas rurales, de Latinoamérica y poder general energía renovable como la electricidad y fertilizante líquido, a la vez reducir la contaminación de las aguas residuales, gases de efecto invernadero, y el olor. Dos sistemas de configuración CSTBR y PFBR fueron instalados a escala de laboratorio utilizando como sustrato desechos de lechería. Los resultados muestran que bajo las mismas condiciones de operación HRT 1 día y OLR 19.4 g DQO/L día el CSTBR produce más biogás (1.0 L/L d), metano (0.8 L/L d) que el PFBR cuyos valores correspondientes fueron biogás (0.8 L/L d), metano (0.6 L/L d), y remoción de materia orgánica 12% COD, 8.7% VS que los obtenidos en el PFBR, 11.5% COD, 8.1% VS. El rendimiento fue el mismo cuando el HRT fue incrementado a 3 días y el OLR disminuido a 9.8 g DQO/L dia. La comunidad microbiana entre las configuraciones CSTBR and PFBR fueron realizadas usando la técnica de pirosecuenciación. Las comunidades microbianas son variables con una distribución y abundancia de grupos taxonómicos diferentes entre las muestras de los sistemas de configuraciones, una mayor riqueza de diversidad microbiana de archaeal se encontró en la muestra del PFBR mientras que una mayor diversidad de bacterias se encontró en la muestra del CSTBR. Se recomienda aplicar la configuración CSTBR para la DA. Sin embargo, la configuración PFBR puede ser mejorada aplicando agitación intermitente, que fallan debido a la colmatación de solidos debido a los altos valores de HRT y SRT, especialmente para biodigestores PFBR a pequeña escala instalados en países en desarrollo.

***Palabras clave* − Digestión anaerobia, configuración de biodigesters, comunidad microbiana, biogás.**

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1. INTRODUCTION

Dairy manure disposal represents a major environmental problem in developing countries farms, due to untreated dairy manure and wastewater results in in pollution of waterways, noxious odors and release of methane (CH_4), which is a greenhouse gas with 21 times the global warming potential of carbon dioxide (CO_2) [1]. The use of dairy manure for producing clean and renewable energy through anaerobic digestion (AD) in developing countries would improve human health, the local environment and socioeconomic conditions [2].

The AD bioprocess is a naturally occurring phenomenon where organic matter is biodegraded by a consortium of anaerobic microorganisms with low nutrient requirements and oxygen free environment to break down complex organic wastes and produce biogas consisting primarily in methane (CH_4) and CO_2 mixture, its composition depend on the redox state of the organic carbon [3]. Fig. 1 shows the conversion of organic matter to biogas throughout a series of complex biochemical reactions. It can be identified four stages: The first, Hydrolysis (depolymerization) of organic molecules (lipids, polysaccharides, proteins, and nucleic acids) to the corresponding monomers (fatty acids and glycerol, monosaccharaides, aminoacids, nitrogenous bases). A number of bacterial species have been isolated during this phase including species belonging to the genera *Acetivibrio*, *Bacteroides*, *Clostridium* , and *Coprothermobacter* [4]. The second stage of Acidogenesis, the monomers produced during the hydrolysis are metabolized intracellularly into a variety of short chain fatty acids (SCFA), producing also amines and alcohols to some extent, as well as hydrogen and CO_2. A wide variety of microorganisms have been identified as participating in this stage.

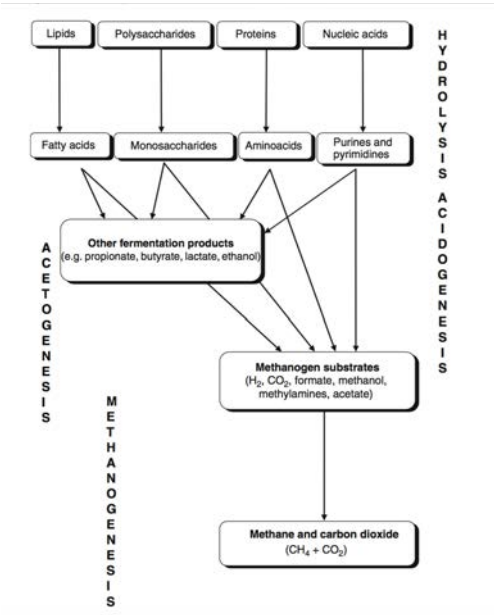

Figure 1: Anaerobic Digestion Pathway [7]

The phyla *Actinomycetes*, *Bacteriodetes*, *Chloroflexi*, *Firmicutes*, and *Proteobacteria* [5]. During the third stage Acetogenesis, acetate and some SCFA is used as a substrate in the methanogenesis pathway of methanogens, other SCFAs need to be converted in acetate or CO_2 and H_2 in order to maximize methane production. A group of bacteria known as syntrophic acetogens are responsible for these conversion, genera sucha a *Syntrophotobacter* and *Smithella* of the order *Deltaproteobacteira* are able to convert propionate to acetate, while the genus *Syntrophomonas* of the phylum *Firmicutes* are capable to converting butyrate and longer chain SFCA [6]. In the final stage of methanogenesis, methanogenic microorganisms (Archaea) generate methane out of acetate (Acetoclastic methanogens) and hydrogen (Hydrogenotrophic methanogens). The methanogens are divided into three groups according to phylogenetic and phenotypic similarity [7]. The first group, methanogens, include the orders *Methanobacteriales*, *Methanococcales*, and *Methanpyrales*, the second and third group includes the order *Methanomicrobiales* and Methanosarcinales, respectively [6, 7]. Commonly recovered hydrogenotrophic methanogens include the genera *Methanobacterium*, *Methanoculleus*, and *Methanospirillum* [8].

1.1. Feedstock

The composition of biogas varies with type of feedstock or substrate, the chemical composition of the substrate is one of the most important parameters that determine the AD bioprocess characteristics. The prevalence of particular microbial species to survive in a competing environment, depending on their ability to grow in the medium with the specific organic and inorganic constituents. Dairy manure is a prime choice for AD because it has a neutral pH and high buffering capacity, contains a naturally occurring mix of microorganisms responsible for anaerobic biodegradation, and provides an array of nutrients, micronutrients, and trace metals nontoxic to the desirable anaerobic population [9, 10].

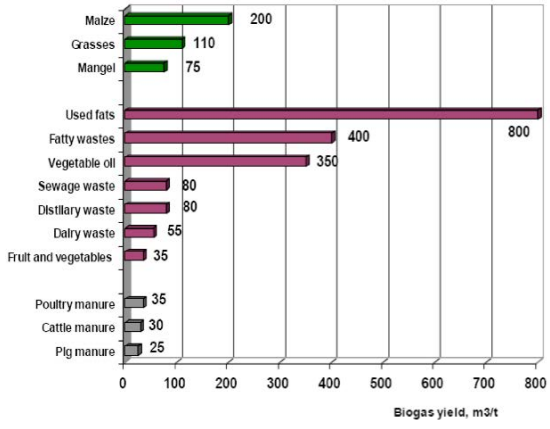

Figure 2: Biogas Yields of Different Feedstocks [12]

However, due to low biogas yield production, co-digestion with other substrates such as fats, oils and grease has been proposed to maximize biogas yield production as it is shown in Fig. 2.

1.2. Biogas Composition

The composition of biogas varies with type of feedstock and operating conditions of the biodigester, an average composition of biogas consists of 50-75% CH_4 and 25-50% CO_2 along with other trace components like water vapor, (H_2O), hydrogen sulfide (H_2S), and ammonia (NH_3), different sources of production lead to different specific compositions (Table 1). Different sources of production lead to different specific composition. The presence of H_2S, CO_2 and H_2O makes biogas very corrosive and requires the use of treatment to minimize these components. CH_4 is the only component of biogas that contributes to the heating value. For instance, 1 $m^3$of biogas at standard temperature and pressure (STP) containing 60% Ch4 has a heating value 21.5 MJ (5.97 kWh of electricity equivalent) compared to 3.58 MJ (9.94 kWh electricity equivalent) per m^3 of pure CH_4 at STP [11].

Table 1: Chemical Composition of Biogas from Different Sources of Production

Component	Household waste	Wastewater treatment plants sludge	Agricultural waste	Waste from agri-food industry
CH_4 % vol	50–60	60–70	60–75	68
CO_2 % vol	30–34	33–19	33–19	26
N_2 % vol	5–0	1–0	1–0	–
O_2 % vol	1–0	<0.5	<0.5	–
H_2O % vol	6 (@ 40 °C)	6 (@ 40 °C)	6 (@ 40 °C)	6 (@ 40 °C)
Total % vol	100	10	100	100
H_2S mg/m^3	0–200	1000–4000	3000–10,000	400
NH_3 mg/m^3		–	50–100	–
Aromatic mg/m^3	0–200	–	–	–
Organochlorinated or organofluorated mg/m^3	100–800	–	–	–

1.3. Factors which Influence Anaerobic Digestion

Since AD is a biochemical process lead by consortia of microorganisms which biodegrade complex high-molecular-weight organic compounds into CH_4, any stress on the system may lead to a change in species types and their relative population levels, which ultimately reflected in the biodigester performance. Hence, the organic substrate added is one of the most important factors affecting AD of organic waste [13].

1.3.1 C/N ratio

The selection of the appropriate organic substrate or inoculum depends on the composition of the organic substrate. The ratio C/N in the range of 20-30 is considered to be optimum for AD. If the C/N ratio is too high, the nitrogen is consumed rapidly by the methanogens to meet their protein requirement and is no longer available to react with the remaining carbon content in the material. As a result the biogas production gets depressed. If C/N ratio is too low, nitrogen is liberated and accumulates in the form of ammonia, which increases the pH of the material. The dairy manure has an average of C/N ratio of 24, to maintain the C/N level of the biodigester material at optimum levels, materials of high C/N ration can be mixed with materials of low C/N ratio [14].

1.3.2 pH

Optimum biogas production is achieved when pH value of the input feedstock is between 6 and 7. During the initial period of DA, organic acids are produced and the pH of the material decreases. As DA continues and the concentration of NH_3 increases, due to the digestion of N, the pH value increases until CH4 gas production stabilizes, the pH remains between 7.2 and 8.2 [15].

1.3.3 Temperature

Different species of methanogenic bacteria growth in three different temperature ranges: phychrophilic (<10°C), mesophilic (20-40 °C) and thermophilic (50-65°C). Outside these narrow ranges of temperature the concerned microbial consortia is not able to survive [16]. The mesophilic temperature considered to be most suitable for AD is 35°C, whereas in the thermophilic digestion 55°C is considered to be ideal. Although thermophilic AD bioprocess is more efficient than the mesophilic, it is more difficult to control and also needs extra energy inputs.

1.3.4 HRT and SRT

Retention time is the duration for which organic (substrate) and microorganism (solids) must remain together in a biodigester to achieve the desired extent of biodegradation. The term hydraulic retention time $HRT=V_{BR}/Q$ (V_{BR} os the biodigester volume and Q, is volumetric flow rate of the substrate) is used to denote substrate retention time, this is the time which an organic material, sought to be anaerobically biodegraded spends in a biodigester from the instant of its entry into the biodigester to its exit. Solid retention time (SRT) is the term to denote microorganisms (solids) in a biodigester. It is the volatile solids content in any substrate which participate in AD. SRT is the duration for which active microorganisms reside in a biodigester [15].

1.3.5 F/M ratio

The food to microorganism ratio is the quantity of substrate and the quantity of microorganisms available to consume that substrate. A lower that adequate F/M ratio will result in a greater percentage of the substrate being converted to biogas. A suitable F/M ratio can be achieved by reducing the HRT to enhance biodigester efficiency, and keeping SRT high, i.e. when the substrate passes through the biodigester

quickly but microorganisms pass through much more slowly. Thus, low F/M ration means that at any given time more quantities of microorganisms are present in a biodigester [16].

1.3.6 Organic loading rate

Organic loading rate, ORL=C/HRT (C is the concentration of the substrate, expressed in volatile solids, VS or chemical oxygen demand, COD) is a measure of the biological conversion capacity of the AD system. This process control parameter is important when the AD is carried out in continuous mode.

Overloading may cause a significant rise in volatile fatty acids concentration, leading to a sharp drop in pH, consequently to system failure. Studies of AD on biowaste in developed countries describe ORL in the range of 48 kg VS/m^3 day for continuous stirred biodigesters, and for non-stirred biodigesters, which are predominant in developing countries, and OLR below 2 kg VS/m^3 day is recommended [17].

1.3.7 Toxicity

Heavy metals such as Cu, Ni, Co, Cr, Zn, and Pb are essential for bacterial growth in very small quantities, however higher quantities have a toxic effect. Detergents, such as soap, antibiotics, and organic solvents also inhibit the microorganisms. Recovery of biodigesters following inhibition by toxic substances can only be achieved by cessation of feeding and flushing the contents to push the concentration of inhibitory substances to below the toxic level. The presence of pathogenic bacteria and viruses present in the feedstock can pose risk of infection to the workers handling the waste for its AD. Hydrogen pressure plays an important role in the control of the AD, reactions leading from volatile fatty acids and carbohydrates to acetic acid and H_2 are thermodynamically unfavorable under standard conditions, having positive standard free energies [15]. Thus, when the H_2 partial pressure is high, these reactions will not proceed and instead, conventional fermentation occurs, with the result that many reduced organic end products are formed and little substrate is consumed. Under conditions in which the partial pressure of H_2 is 10^{-4} atmospheres or less, the reactions are favorable leading to end products that can be converted to CH_4 [15]

1.3.8 Mixing/Agitation

Mixing is required to maintain fluid homogeneity, hence to process stability, within a biodigester. The objectives of mixing are to combine the incoming substrate with microorganism, to stop the formation of scum, and to avoid pronounced temperature gradients within the biodigester. Very rapid mixing can disrupt the microbial community while too slow a stirring can cause inadequate mixing and short-circuiting. The extent mixing required is also dependent on the content of the digestion mixture [18].

1.4. Biodigester Configurations

The selection of appropriate biodigester configurations is very important for the development of effective AD bioprocess. Biodigester for AD bioprocess may be divided in two categories, depending on the manner in which the microorganisms grow: suspended in the feedstock undergoing fermentation or attached to a solid support (Grady et al 2011). The major suspended growth biodigesters are CSTBR, PFBR, whereas the major attached growth are fluidized bed and packed tower, among others. The major suspended growth AD bioprocess is high-rate where the AD occurs at faster rate, lower HRT (10-15 days) and high SRT. Whereas low-rate the HRT is too high (40-50 days), the HRT and SRT are identical. In developing countries low-rate biodigesters are used by farmers and by the dairy industry for extracting biogas, with poor technical knowledge in their operation and maintenance, while in developed countries larger meat producing and dairies employ more sophisticated high-rate biodigesters (CSTBR and PFBR) for biogas production with high USA-EPA, AgSTAR technical supervision [19].

1.4.1 CSTBR

Complete stirred tank biodigester (CSTBR) is the most common biodigester utilized in AD bioprocess (Fig. 3). In general, CSTBRs are operated at equal HRT and SRT without any internal biomass retention device, hence the microbial population gets washed out from the biodigester along with the effluent (lower-rate), this can be prevented by increasing F/M ratio and SRT and decreasing HRT (without compromising SRT), this is characterized by high-rate biodigester, it leads to the presence of greater concentration of microorganisms in the biodigester.

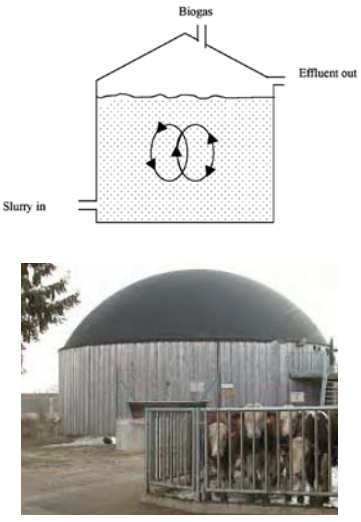

Figure 3: Complete Stirred Tank Biodigester (CSTBR) [12]

Mixing (mechanical, hydraulic, pneumatic) can be continuous of periodically, the treatment efficiency of a CSTBR is further enhanced by heating the biodigester content with a proper temperature control system. The CSTBR can be fed semi-continuously or continuously to the biodigester [18].

1.4.2 PFBR

Plug flow biodigester (PFBR) receive feed at one end of the biodigester and remove from the opposite side (Fig.4). The feedstock is not mixed, and continuous management or internal heating are not required when located in a tropical climate, like in most developing countries. The friction from biodigester walls and the bubbling of the produced biogas result in some mixing of the contents in the feedstock. The solids tend to settle out, resulting in a longer SRT and better degradation of solids in these systems. Some advantages compared with CSTBRs are the simplicity of the design, reduced energy inputs, high stability and cost [20]. Their drawbacks include reduced effective reactor volume due to settling of solids, lower efficiencies for colder feeds due to lack of heating, and difficulties in maintaining uniform biodigester conditions [21].

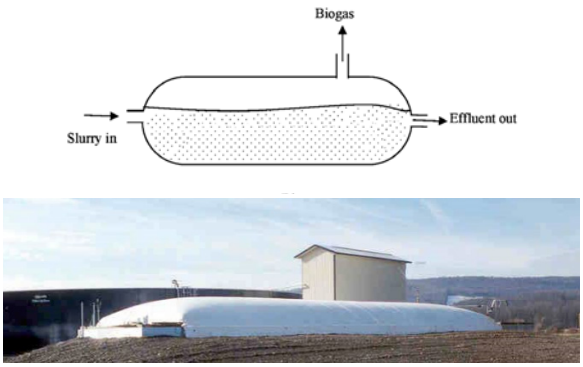

Figure 4: Plug-flow Biodigester (PFBR) [19]

1.5. Microbial Groups and their Interaction

The AD bioprocess is conceptually divided in four phases, each phase is carried through by an array of Bacteria and Archaea. Methanogens belong to the domain archaea, which is distinct from bacteria. Methanogens should not be confused with methanotrophs, which consume methane rather than produce it. Methanogens differ from other bacteria not only by their types of metabolism but also by a number of characteristic features in the composition of their cell constituents. Almost all shapes known in the Eurobacteria can be found in the methanogens (). It has been reported that the operational parameters impact on the microbial diversity of the AD system, if the OLR is too high, the growth and metabolism of the syntrophic bacteira and methahogens, leading to buildup of short chain fatty acids and acidification of the system [22].

The effect of temperature at which the AD is carried out on the microbial community is important, whereas the majority of AD systems are operated at mesophilic temperatures; the use of psychrophilic and thermophilic digestion systems has increased in the last years. The composition of the microbial communities in psychrophilic AD systems have similar composition to those in mesophilic AD systems, with fermentative members of the *Bacteriodetes* and syntrophic members of the *Proteobacteria* being predominant [23]. However, Thermophilic AD offer a greater pathogen reduction, decreased retention time. And higher rate of biogas production compared with mesophilic AD (Iranpour, 2002). It have been reported that the bacterial communities in thermophilic AD are *Thermoabaerobacte*r, *Anaerolinea*, and members of the phylum *Thermotogae* [24].

In light of the above, a few studies have examined a comparison of performance between two most common anaerobic biodigesters configuration CSTBR and PFBR to biogas production and electricity using as feedstock dairy manure. In addition, biogas arises from the activities and syntrophic interactions of a consortium of anaerobic bacteria and methanogens, following the advances in lasts years, nucleic acid-based molecular methods are able to identify microorganisms by the DNA sequences of their ribosomal RNA (rRNA) genes, without the need to isolate the microorganisms. Other molecular tools are now commonly used such as sequence-based techniques, clone libraries and pyrosequencing, this method is prevalently applied in metagenomics to analyze community structures of AD habitats. Pyrosequencing is one of the most popular high-throughput sequencing technologies. By eliminating the need for construction a clone library, which can be technically challenging for characterize anaerobic microbial communities. This study also explores a sequence-based pyrosequencing to characterize the microbial community of AD samples within each of biodigesters CSTBR and PFBR.

2. MATERIALS AND METHODS

2.1. Experimental Procedure and Analytical Methods

Fresh flushed dairy manure was collected from the Miedema dairy farm in Circleville, OH, USA. The manure produced at the barns is flushed twice a day and the liquid flowed finally into a lagoon. The feedstock was prepared by diluting the stream directly obtained from the lagoon with water at a ration 1:2 (w/w) and screening through a 0. 5 mm opening and stored at (-10 oC) and thawed before to use, several manure samples were used during the experiments. The experiments consisted in two biodigester configurations, one CSTBR and a PFBR consisted of three CSTBRs connected sequentially. Each biodigester had a volume of 4.5 L (14 diameter,

30 cm in height), mechanically mixed at 30 rpm, maintained at an operating temperature 25±1 °C. To start the AD the biodigesters were inoculated with seed sampled from the lagoon, and operated in a batch mode until biogas production from each biodigester was identified. Afterwards (14 days) the system was switched to continuous mode. The biogas was produced for each biodigester was recorded using water displacement [25], once the system reached steady state, at this point the biogas and liquid were sampled. The biogas samples were analyzed for CH_4 and CO_2 content, chemical oxygen demand (COD), VS, pH alkalinity, and VFA concentration. VS, COD, alkalinity, ammonium, and total N were analyzed according standard methods [26]. VFA were analyzed using a Diones DX-500 system (Sunnyvale, CA USA) as described by (Hu) . CH_4 and CO_2 content of the biogas were determined using a gas chromatograph (Series 350, Gow Mac Instruments, Co., Leigh Valley, PA) with thermal conductivity detector, The temperature of the injection port and the packed column (HayesepQ, Supelco, Bellefonte, PA) was 20°C, and that of the detector was 40°C. Helium at a flow rate of 60 mL/min was used as carrier gas. The statistical analysis of the selected analyses, were analyzed by Statistical Analysis System program 9 (SAS institute Inc., NC, USA), for each groups ANOVA was applied, a probability value less than 5% ($p<0.05$) was defined as statistically significant.

2.2. Cloning of 16S rRNA and Construction of Gene Clone Library

Examination and comparison of microbial community in two biodigester CSTBR and PFBR using pyrosequencing. Genomic DNA was extracted from both sludge A and sludge B using soil DNA isolation kit (Norgen Biotek Corporation, Canada) and protocol as recommended by the manufacturer. Extracted DNA was used in the subsequent PCR amplification with a domain Bacteria-specific forward primer 8f (5'-AGAGTTTGATYMTGGCTC-3') and a reverse primer 1490r (5'-GGTTACCTTGTTACGACTT-3'). Archaea 16S rRNA genes were amplified with a domain Archaea-specific forward primer 1f (5'-TCYGKTTGATCCYGSCRGAG-3') and reverse primer 1100r (5'- TGGGTCTCGCTCGTTG-3').

2.3. Pyrosequencing

DNA samples extracted from both sludge A and sludge B were sent for high- throughput sequencing by a sequencing company (Axil Scientific, Singapore).

A region of approximately 469 bp encompassing the V3 and V4 hypervariable regions of the 16S rRNA gene was targeted for sequencing. After pooling of equal amount of PCR products from each sample, their sequencings were performed with the Illumina MiSeq platform (San Diego, CA, USA) using the 250PE protocol [27,28].

3. RESULTS AND DISCUSSION

The characterization of the influent feedstock properties used to prepare anaerobic digestion experiments are shown in Table 2. The average COD and VS of the feedstock prepared are within the range of the stream leaving the dairy farms, generally influent concentration 10 to 30 g COD/L [29]. Although, the feedstock B is stronger than feedstock A, both feedstock already contained VFAs due to natural biodegradation organic matter of the fresh dairy manure that occur after excreted by the animals. Alkalinity content around 1 g/L has been reported in discharges of liquid manure in other dairy farms [30].

Table 2: Composition of the Materials as Feedstock Used in Anaerobic Digestion Experiments

Parameter	feedstock A*	feedstock B*
Temperature (°C)	25.2±0.2	25.1±0.2
pH	7.3±0.2	7.2±0.3
COD (g/L)	19.4±3.1	29.3±7.1
VSS (g/L)	13.4±2.1	20.9±2.6
Ammonia (mg N/L)	109±10	183±3
Total N (mg N/L)	410±20	540±25
Alkalinity (g $CaCO_3$/L)	1.1±0.2	1.7±0.4
VFA		
Propionate (mg/L)	10.7±0.5	20.3±1.5
Butirate (mg/L)	6.0±0.9	13.0±0.6
Valerate (mg/L)	1.0±0.3	2.2±0.2

* Average of three analysis

3.1. AD Performance Comparison between the CSTBR and PFBR Configuration Systems

Table 3 presents a comparative relationship between the anaerobic digestion performance between a CSTBR and PFBR configuration bioreactor systems. The tests were running for the same HRT by combining influent flow rate (Q) and working volume (V) and the feedstock A and B showed in Table 2. The first test run for both configurations CSTBR and PFBR under the same HRT (1 day) and OLR was 19.4 g COD/L day. The results shows that the CSTBR produced more biogas (1.0 L/L d), methane (0.8 L/L d) than the PFBR biogas (0.8 L/L d), methane (0.6 L/L d) and removed more organic matter, 12% COD, 8.7% VS than did a PFBR, 11.5% COD, 8.1% VS. This performance resulted similar, for the second run, although the HRT was increased three times, the OLR decreased to 9.8 g COD/L day, almost to half value of the first run. The effect of OLR (C*Q/V) in combination with HRT (V/Q) is important to biogas production. It has been reported that a pilot scale down flow fixed film reactor at an HRT of 4.9 days and at an OLR of 13 g COD/L day, achieved 75% COD removal and 0.28 L CH_4/g $COD_{removed}$, by increasing the HRT to 6.6 days and reducing the OLR to 8.3 g COD/ l day the COD removal slightly increased 1% and the methane production rose to 0.33 CH_4/g $COD_{removed}$

[31]. It is reported from the literature [32, 33] that a CSTBR performed better than a PFBR configuration, respectively (Table 3), in agreement with our study. Albeit there have been few studies conducted comparison among biodigesters configuration for biogas production to assess the effectiveness of the used system, at least with respect to the superiority of any particular technology. In this study, a comparison of biodigester performance between CSTBR and PFBR configuration of biodigesters were explored by using the livestock AD database published by AgSTAR, which provides basic information on projects of anaerobic biodigesters on livestock farms in the USA that currently operating including and those that have been shut down [19]. EPA-USA, AgSTAR program stimulate farmers in the US to the potential to capture biogas from livestock wastes using AD in biodigesters for electricity generation, greenhouse emissions reduction and recovering the methane from the biogas to generate a cost-effective renewable energy, such as electricity, heating or transportation fuel [34].

In order to make such comparison, it was input the following information: Current project, localization, biodigester type CSTBR and PFBR, dairy livestock, numbers of cows, biogas production, electricity generated, methane emission reduction and the system designers in the Agstar anaerobic program. The programme outcome was biogas generated (cu ft/day) by the two biodigesters configuration CSTBR and PFBR for the same number of animals (2000). A better performance was achieved by using the CSTBR than the PFBR configuration. The values of biogas produced (170,206 cu ft/day), electricity generated (3,354,762 kWh/yr) and CH_4 emission reductions (5,572 metric tons CO_2/yr) were higher than the respective values for the PFBR configuration, biogas produced (87,000 cu ft/day), electricity generated (353,000 kWh/yr) and CH_4 emission reductions (332 metric tons CO_2/yr), respectively, as shown in Table 4. The CSTBR may generate at least twice biogas, 10 times electricity and 17 times CH_4 emission reductions as rather than the PFBR.

It can be noticed that when the PFBR configuration was modified to Mixed Plug Flow biodigester (MPFBR), for the same operation conditions the performance of MPFBR overcome the CSTBR, respectively, biogas produced (242,000 cu ft/day), electricity generated (3,705,360 kWh/yr) and CH_4 emission reductions (12,5,559 metric tons CO_2/yr). Other comparison made for less number of animals (1,900; 1200; 800 and 600) confirm that the CSTBR configuration performance is better than the PFBR. Although, it is hard to compare biodigester systems operated in different farms, not only because of the possible differences in the anaerobic feedstock characteristics, but also because of the differences in operating parameters such as: temperature, pH, mixing conditions, OLR, HRT, SRT, VFAs content, inoculums type and the composition of the AD effluent. Nevertheless, a comparison was made between the two anaerobic systems with the data available in the literature.

Table 3: Comparison of Performance between CSTBR and PFBR and with Similar Configurations in the Literature

Test*	CSTBR	PFBR
Q(L/day)†	2	6
V (L)	2	6
OLR (g COD/L day)	19.4	19.4
HRT (day)	1	1
Biogas Production (L/L day)	1.0±0.05	0.8±0.05
CH4 Production (L/L day)	0.7±0.03	0.6±0.02
COD (Removed %)	12.1±0.9	11.5±0.8
VS (Removed %)	8.7±0.6	8.1±1.2
Q (L/day)†‡	1	2
V (L)	3	6
OLR (g COD/L day)	9.8	9.8
HRT (day)	3	3
Biogas Production (L/L day)	0.63±0.01	0.58±0.03
CH4 Production (L/L day)	0.4±0.01	0.35±0.02
COD (Removed %)	24.9±1.5	20.1±2.7
VS (Removed %)	17.5±.1.0	14.2±2.0
Reference	[10]*	[11]**
OLR (g COD/L day)	10	4.5
HRT (day)	6	15
Biogas Production (L/L day)	0.88	0.18
CH4 Production (L/L day)	0.6	0.045
COD (Removed %)	-	-
VS (Removed %)	-	-
*Three runs for each test: †: feedstock A;‡: feedstock B; at an operating temperature 25±1.2Oc		
CSTR-CSTR=Continuously stirred biodigester;OLR= 10 gCOD/ L day; T=35°C		
** PFR=NMAD, No mixed anaerobic digester; OLR=3.16 g VS/L day		

3.2. A Comparison of the Microbial Community between the CSTBR and PFBR Configuration Systems

The microbial communities are highly variable with the distribution and abundance of taxonomic groups differing between the samples taken from the two AD configuration systems CSTBR and PFBR. Despite being the same feedstock composition and OLR and HRT, had different Bacterial and Archaeal composition. On the basis of operational taxonomic unit (OUT) composition both the CSTBR and PFBR samples shared the same dominant OUTs (Table 5). The CSTBR sample had higher proportion sequences classified to the phylum.

Table 4. Biogas Production Comparison between CSTBR and PFBR in the USA

Project Name	City, State (4)	Digester Type	Year Operatio	Dairy	Biogas Generation Estimate (cu_ft/day)	Electricity Generated (kWh/yr)	Methane Emission Reductions (metric tons CO2E/yr)	System Designer(s)
Meadowbrook Dairy Digester[(b)]	El Mirage, CA	PFBR	2004	2.000	87.000	353.000	332	RCM Int. LLC
Norm-E-Lane, Inc. (NEL) Digester[(a)]	Chili, WI	M-PFBR	2008	2.000	242.000	3.705.360	12.559	DVO, Inc.
Yippee! Farms Digester[(1), (a)]	Mount Joy, PA	CSTBR	2013	2.000	170.206	3.354.762	5.792	RCM Int. LLC
Twin Birch Dairy Digester[(2), ©]	Skaneateles, NY	PFBR	2003	1.900	100.000	420.480	2.776	Anaerobics, Inc.
Crave Brothers Farm Digester[(3),(a)]	Waterloo, WI	CSTBR	2007	1.900	223.000	1.801.515	3.598	Clear Horizons, LLC
Emerling Farms Digester[(a)]	Perry, NY	PFBR	2006	1.200	110.400	1.305.240	6.504	RCM Int. LLC
Nelson Boys Dairy, LLC Digester[(a)]	Swanton, VT	M-PFBR	2007	1.200	132.000	1.400.000	2.659	DVO, Inc.
Penn England Farm Digester[(a)]	Williamsburg, PA	CSTBR	2006	800	50.000	1.261.440	2.269	Environmental Fabrics
Langerwerf Dairy Digester[(a)]	Durham, CA	PFBR	1982	750	30.000	300.000	1.119	RCM Int. LLC
Pennwood Farms Digester[(b)]	Berlin, PA	CSTBR	2011	600	30.363	395.259	1.376	RCM Int. LLC & Others
Hillcrest Saylors Farm Digester[(a)]	Rockwood, PA	PFBR	2007	600	49.054	1.200.000	1.852	Shawn Saylor
CSTBR=Complete mix; Horizontal Plug Flow=PFBR; Mixed Plug Flow=MPFBR; (1)=Co-digestion food wastes/Pocess water;(2)=Co-digestion=Process water; (3)=Co-digestion= Dairy Processing Water ; Biogas end Uses: (a)=Cogeneration; (b)= Electricity; (c)=Electricity; Boiler/ Furnace fuel, (4) Project Type=Farm Scale								

Table 5: Comparison of Taxonomic Composition of Microbial Communities

	CSTBR	PFBR
Bacteiral phylum	**Pyrosequencing**	
Proteobacteria	25%	22%
Firmicutes	8%	9%
Chlorobi	1%	<1%
Bacteriodetes	27%	15%
OP8	2%	1%
Chloroflexi	3%	5%
Spirochaetes	12%	2%
Candidate division WWE1	-	-
Thermotogae (Meotoga infera)	1%	25%
Synergistetes	1%	1%
Cloacamonas	7%	9%
Actinobacteria	3%	<1%
Caldiserica	2%	1%
Others (<1% of total composition)	8%	8%
Class of Proteobateria		
Deltaproteobacteria	11%	5%
Alphaproteobacteria	3%	2%
Gammaproteobacteria	3%	5%
Betaproteobacteria	5%	8%
Archaeal order and genus		
Methanosarcinales	80%	66%

Continue Table 5: Comparison of Taxonomic Composition of Microbial Communities

	CSTBR	PFBR
Bacteiral phylum	**Pyrosequencing**	
Methanosaeta	80%	66%
Methanomicrobiales	8%	4%
Methanospirillum	-	2%
Methanoculleus	-	2%
Thermoplasmata	2%	12%
Methanomassillicoccus	2%	12%
Methanobacteriales	-	2%
Uncultured archaeon		
AY835427	5%	-
Uncultured archaeon		
DHVE6a	-	9%
Others (<1% of total composition)	5%	7%

Proteobateria 25% while the PFBR sample achieved 22% in the, while CSTBR sample has a greater proportion of Bacteriodetes 27% to 15%. Bacteriodetes are involved in the conversion of simple sugars and carbohydrates into SCFAs during the acidogenesis phase of the AD [17] and thus their abundance in the CSTBR data set was expected. The most abundant phylum Thermotogae occurred in the PFBR sample (25%) whereas in the CSTBR sample only 1%, another difference observed with the class Deltaproteobateria was more prevalent in the CSTBR sample (11%) than

in the PFBR (5%). The Fermicutes represented in the proportion in both samples (8% CSTBR, 9% PFBR). Whereas the Betaproteobacteria was less abundant in the CSTR sample (5%) than in the PFBR (8%). The others bacterial phylum was unclassified samples represent about 8% in both samples CSTBR and PFBR, respectively. This results are similar to that reported in the literature [35]. The archaeal diversity presented in Table 4 was limited to the methanogenic class Methanosarcinales and the genus Methanosaeta represented the most abundant presence 80% in the CSTBR sample and just over 66% in the PFBR sample, similar results were reported by other researchers [36]. The CSTR sample had higher proportion of Methanomicrobiales 8% than in the PFBR sample, but Methanospirilium and Methanoculleus (2%) only were represented in the PFBR sample and no in the CSTBR. There were more predominance of Thermoplasmata genus Methanomassillicoccus in the PFBR sample (12%), but just 2% in the CSTBR sample. Also the Methanobacteriales and unclassified genus represented only 5% in the CSTBR sample, whereas 7% in the PFBR sample, these results suggested that more richness of archaeal microbial diversity were present in the PFBR sample than in the CSTBR sample, one explanation may be that in the CSTR system a washout of the anaerobes may occur during the AD bioprocess. Conversely, higher bacterial microbial diversity were present in the CSTBR sample than in the PFBR sample.

4. CONCLUSION AND RECOMENDATIONS

A comparison of AD performance of two system configuration CSTBR and PFBR at bench scale were assessed by using dairy manure as feedstock. It was found that under the same operational conditions (feedstock, HRT, OLR, temperature lower range mesophilic) the CSTBR produced more biogas (CH_4) and remove more organic matter (COD, VS) than the PFBR. Another comparison assessment of biodigester performance between CSTBR and PFBR configurations were explored by using the livestock AD database of anaerobic biodigesters on livestock farms in the USA, A better performance was achieved by using the CSTBR than the PFBR configuration, the values of biogas produced, electricity generated, and CH_4 emission reductions were higher than the PFBR configuration values, respectively. In addition, a comparison of the microbial community between the CSTBR and PFBR were performed by using pyrosequencing molecular technique. The microbial communities are highly variable with the distribution and abundance of taxonomic groups differing between the samples taken from the two AD configuration systems CSTBR and PFBR. Despite being the same feedstock composition and OLR and HRT, had different Bacterial and Archaeal composition, While both the CSTBR and PFBR samples shared the same dominant OUTs. The results suggested that more richness of archaeal microbial diversity were present in the PFBR sample than in the CSTBR. Conversely, higher bacterial microbial diversity were present in the CSTBR sample than in the PFBR.

In light of the results, it is recommended the application of CSTBR configuration for AD. However, the performance of the PFBR configuration can be increased by intermittent mixing application, especially it is recommended for small scale PFBR configuration installed in developing countries that failure because of clogging due to long HRT and SRT.

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