



Sustainable Production of Biogas in Large Bioreactor under Psychrophilic and Mesophilic Conditions

Ankur Choudhary¹; Ashish Kumar²; Tanvi Govil³; Rajesh Kumar Sani⁴; Gorky⁵; and Sudhir Kumar⁶

Abstract: This study reports the sustainability of a large semicontinuous anaerobic reactor (3,000 L) for biogas production from food wastes for a period of 324 days. The methane potential ranged from 418.3 to 13.9 mL CH₄/g VS_{added} during the experiment. An average 54.8% reduction was observed in biogas production during the winter season compared with summer. Results showed that food wastes could be utilized in a low-temperature range (0°C–15°C) as well as a high-temperature range (15°C–27°C). The total microbial community analysis revealed mixed consortia representing acetotrophic, hydrogenotrophic, and methylotrophic archaea (e.g., *Methanobacterium*, *Methanosarcina*, and *Methanospirillum*), indicating a syntrophic pathway for methane production. The bacterial community was well represented by two major phyla: Proteobacteria (31.3%) and Bacteroidetes (14.92%). The highest maximum specific biogas production (R_m) and maximum biogas production potential (A) were 134.5 mL CH₄/g VS/day and 34.1 L CH₄/g VS for modified Gompertz and first-order equations, respectively. The methane production data were modeled, and showed substantial agreement with experimental results; however, the first-order equation had the best agreement with the experimental data ($R^2 = 0.99$). DOI: 10.1061/(ASCE)EE.1943-7870.0001645. © 2019 American Society of Civil Engineers.

Author keywords: Anaerobic digestion; Organic fraction of municipal solid waste; Psychrophilic temperature; Methane potential; Stability.

Introduction

Generation of municipal solid waste (MSW) is one of the major problems for developing and developed countries across the world. India generates enormous amounts of MSW: per capita MSW generation in India is 400 g/day (Nandan et al. 2017). Approximately 90 million tons of MSW was generated in 2009, and it is expected to increase to 300 million tons by 2047 (Gupta et al. 2015). Due to its improper management, solid waste pollutes the environment and is detrimental to human health and safety. Food waste (FW) is an integral part of MSW which contains approximately 40%–45% of the organic loadings (Nandan et al. 2017). Unmanaged FW is a

considerable cause of vermin attraction, release of harmful gases, and odor problems. However, residual FW is a source of renewable energy and can produce biogas through anaerobic digestion (AD) as one of the options (Rao et al. 2000). FW is an attractive substrate for AD because of its low total solids (TS) and high content of soluble organics, which make it readily biodegradable. Additionally, FW offers low-cost alternatives for methane production and is abundantly available (David et al. 2018). Anaerobic digestion, which occurs naturally in the absence of oxygen, can degrade a vast variety of complex wastes, in addition to generating combustible biogas, which can be used as an energy source (David et al. 2018; Heinrichs et al. 1990; Izumi et al. 2010; Kim et al. 2006; Kondusamy and Kalamdhad 2014; Iisu and Rittmann 2016; Rao et al. 2000). A stable anaerobic reactor can produce energy that could be used for cooking and lighting purposes. It also aids the generation of nutrient-rich bioslurry, in addition to reducing contamination of water, air, and soil (Alvarez et al. 2006; Tafdrup 1995). AD of any substrate is a temperature-dependent mechanism. Most studies have been conducted in warmer climates and at low altitude (with temperatures in the mesophilic range) because these areas are more favorable for biogas generation (Anjum et al. 2017; Awasthi et al. 2018; Capson-Tojo et al. 2017; Guerrero and Bevilaqua 2015; Khoshnevisan et al. 2018; Dingnan and Zhang 2016; Oleszkiewicz and Poggi-Valardo 1997). Nevertheless, there are certain places at intermediate or higher altitudes, i.e., $\geq 1,000$ m above mean sea level (MSL) where reactors are subjected to cold climatic conditions, and such reactors face drawbacks in terms of stability (Alvarez et al. 2006; Alvarez and Lidén 2008; Massé et al. 2003). The temperature significantly varies in winter and summer, which in turn affects the biogas generation rates. In a study of the digestion of cattle dung, the biogas generation rate varied from 0.03 m³ kg⁻¹ feed in summer to 0.007 m³ kg⁻¹ feed in winter (Kanwar and Guleri 1994). Other studies also have reported a linear decrement in methane production as the temperature decreased

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over the range 10°C–23°C. Because of this, even after 100 years of development and optimization of the process for cattle dung, the acceptability of this particular technology in making available biogas as energy for heating and cooking in the colder and hilly areas of any country in the winter season is a matter of great concern (Kashyap et al. 2003).

Various designs have been suggested and practiced for such geological and climatic conditions in the past. Khadi and Village Industries Commission (KVIC) Janta and Deenbandhu AD plants are among the most successful and globally accepted plants (Singh et al. 1997). Although these plants have been well propagated in India and neighbouring countries, they have failed to succeed in areas with elevation $\geq 1,000$ m above MSL due to problems such as high construction cost, the requirement for skilled labor, undulating topography, rocky strata, heavy rainfall, snowfall, and, most importantly, low temperature in winters. Between 1980 and 2000, a number of studies were conducted at higher altitude ($\geq 1,000$) to test the biogas potential of conventional organic wastes such as cattle manure, night soil, sludge, and wastewater (Jensen et al. 2018; Kalia and Kanwar 1989; Kalia and Kanwar 1996; Kalia and Singh 1998; Kalogo et al. 2001; Podmirseg et al. 2016; Puñal et al. 2001). To the best of our knowledge, no study has been conducted so far of a pilot-scale semicontinuous reactor at psychrophilic and mesophilic variable temperature ranges in which FW is used as a substrate in the hilly terrain region of Himachal Pradesh, India. AD of FW is not similar to the conventional AD of cattle manure and wastewater (Rao et al. 2000). The AD process of FW needs to be understood at low-temperature ranges. Therefore, the objectives of this study were to test the feasibility of AD in a single-phase reactor fed with FW at psychrophilic and mesophilic temperature and to decipher the microbial communities involved in the digestion of FW. AD at psychrophilic temperatures is driven by a complex microbiome acclimatized to psychrophilic temperatures. The key microbial communities (hydrolytic, aceto/acidogenic, syntrophic, and methanogenic) of the reactor were analyzed based on the metagenomic data. Furthermore, no literature is available which used kinetic and mathematical models for low mesophilic and psychrophilic temperatures in continuously varied temperature and compared them for an extended duration. Two mathematical models and one kinetic model were used to predict the cumulative methane and behaviors of the anaerobic semicontinuous reactor. These models used the results obtained from a pilot-scale anaerobic reactor.

Material and Methods

Experimental Setup

The experiments were conducted at Jaypee University of Information Technology (JUIT), Wagnaghat, Himachal Pradesh, India, located at an altitude of 1,544 m above MSL. Experiment setup consisted of a cylindrical reactor (3,000 L) made of PVC and a cylindrical gas holder with a capacity of 2,000 L (Fig. 1). The gas holder was inverted in the reactor to hold the gas produced in the reactor during AD of FW.

Substrate and Inoculum

Food waste for the present study, collected from the JUIT cafeteria, was used as a substrate. About 3 kg food waste was collected every day from Monday to Saturday and stored at 4°C. The waste did not include any peelings of raw vegetable or fruit because only cooked and processed food was included in the experiment. The waste was completely mixed and shredded thoroughly to an average particle

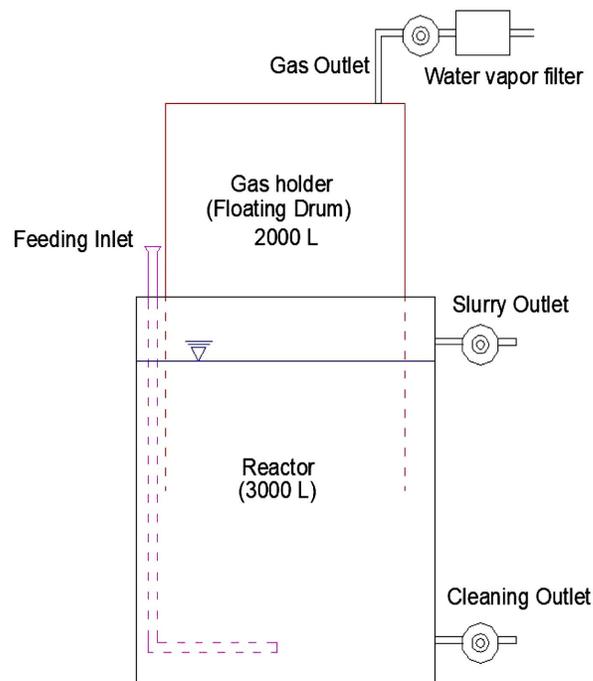


Fig. 1. Image of an outdoor 3,000-L anaerobic semi-continuous reactor at the Jaypee University of Information Technology in Himachal Pradesh.

size of 1–2 mm. Cow dung available in the nearby village was used as inoculum in the anaerobic reactor.

Reactor Operating Conditions

The reactor was started on March 10, 2017, and systematic feedings of FW were made and the system was allowed to stabilize for about 50 days before taking any samples. Gas measurements were carried out from May 1, 2017 to March 24, 2018. Safley and Westerman (1994) found that the organic loading rate (OLR) is dependent on temperature. They also proposed an equation from which a suitable OLR can be calculated based on the various temperature ranges. They suggested a suitable OLR range of 0.08–0.24 g VS/L · day⁻¹ at 10°C. In the present study, the reactor was run at three different OLRs, i.e., 0.08, 0.07, and 0.04 g VS_{added}/L · day⁻¹ based on the rise and fall of ambient temperature. Gas was measured based on the daily increment in height of the gas holder. The volume of biogas generated was calculated by multiplying the area of the gas holder by the daily increment in height of the gas holder.

Analytical Method

The experiment analyzed the following parameters: temperature, pH, TS, volatile solids (VS), total organic carbon (TOC), total Kjeldahl nitrogen (TKN) (Jackson 1985), and alkalinity. The total organic carbon content of the substrate was estimated by the method proposed by Nelson and Sommers (1982). Nitrogen content in MSW and agricultural waste samples was assessed by the Kapp (1984). Alkalinity was estimated as per the Kapp (1984) method. Composition of biogas was determined using a Biogas 5000 Geotech gas analyzer (QED Environmental System, Coventry, UK).

Microbial Population Structure Analysis

To study the microbial diversity in the consortium, samples were centrifuged at 14,000 g for 10 min. DNA was extracted from

the cells using a QIAamp DNA Mini Kit (Qiagen, Valencia, California) as per the manufacturer's protocol. The purity and concentration of genomic DNA were tested using a Thermo Scientific NanoDrop 1000 spectrophotometer (Wilmington, Delaware). Microbial diversity analysis was performed using Illumina sequencing by Research and Testing Laboratory (Lubbock, Texas). Briefly, universal 16S primers were applied for eubacterial and archaeal microbial diversity analysis. For eubacterial microbial diversity, DNA samples were amplified for sequencing using a forward primer 515F 5'GTGCCAGCMGCCGCGGTAA, and a reverse primer 806R 5'GGACTACHVGGGTWTCTAAT. For archaeal microbial diversity, the primers used were 517F 5'GACYTAAAGSRNCCGTAGC and 909R 5TTTCAGYCTTGCGRCCGTAC. A single-step polymerase chain reaction (PCR) reaction with Qiagen HotStarTaq master mix (Qiagen, Valencia, California) was carried under the following thermal profile: 95°C for 5 min; 35 cycles of 94°C for 30 s, 54°C for 40 s, and 72°C for 1 min; and 1 cycle of 72°C for 10 min and 4°C hold. One of the flow cell binding sites was cleaved, giving amplified products in one direction. Similar steps were repeated to obtain amplified products with the other flow cell binding site. Amplification products were visualized with eGels (Life Technologies, Grand Island, New York) and were pooled in equimolar amounts. Each pool was selected in two rounds using Agencourt AMPure XP (Beckman Coulter, Indianapolis, Indiana) at a 0.7 ratio for both rounds. Size-selected pools then were quantified using a Qubit 2.0 fluorometer (Life Technologies) and loaded on an Illumina MiSeq (San Diego, California) 2 × 300 flow cell. The Illumina MiSeq read these sequences one by one, and each pool was identified as a unique sample within the flow cell lane. The obtained sequencing results were compared with the already-sequenced microorganism and the samples were identified.

Kinetic and Mathematical Modeling

Three mathematical models and one kinetic model were used to evaluate the performance and kinetic parameters. These parameters are very important for the design of anaerobic reactors for determination of leachate production from landfills in cold climates. In addition, these parameters also can be used as input parameters in various models to calculate greenhouse gases (GHGs) from open dumping. The first-order kinetic model (FOM) was used to determine the maximum biogas yield (P) and the apparent hydrolysis rate constant (K_h) [Eq. (1)]. The hydrolysis rate constant is a decisive limiting factor in anaerobic fermentation of various wastes such as activated sludge, waste water, and so forth, which governs the process significantly (Donoso-Bravo et al. 2010)

$$P_0 = P\{1 - \exp[-K_h t]\} \quad (1)$$

The modified Gompertz model (MGM) is widespread in multiple fields. Generally, it is used to predict methane production, and more often for the production of hydrogen in the field of anaerobic digestion. This equation has been used by various authors to predict cumulative methane, maximum methane production potential, and specific maximum biogas production and lag phase (Donoso-Bravo et al. 2010). The equation is

$$P_0 = P \exp\left\{-\exp\left[\frac{R_m e}{P}(\lambda - t) + 1\right]\right\} \quad (2)$$

where P = maximum biogas production potential (L/g VS); R_m = maximum specific biogas production (L/g VS/day); λ = lag phase or latency (days); t = time of biogas accumulation (days); and P_0 = cumulative methane production (L/g VS).

The transfer function, also known as the reaction curve-type model (RCM), also is used for curve fitting [Eq. (3)]. This function is used primarily for control purposes. This function considers that any practice can be analyzed as a system which receives various inputs and produces outputs. This model also is used in the anaerobic digestion process in a few cases (Donoso-Bravo et al. 2010)

$$P_0 = P \left\{ 1 - \exp\left[\frac{-R_m(t - \lambda)}{P}\right] \right\} \quad (3)$$

The logistic function is globally used to fit the predicted methane produced with the experimentally observed methane in the anaerobic fermentation process. This function is used not only in the estimation of methane during the anaerobic digestion process but also for the methane production from the leachate produced in landfills. The main assumption made during the fitting process is that the methane production is proportional to the amount of methane already produced, the rate of maximum methane production, and the capacity of methane production (Donoso-Bravo et al. 2010). The present study used a modified version of the logistic function (MLF) for curve fitting and determination of different parameters

$$P_0 = \frac{P}{1 + \exp\left\{\frac{4R_m(\lambda - t)}{P} + 2\right\}} \quad (4)$$

Eqs. (1)–(4) were used to fit the experimental data of this semi-continuous anaerobic digestion study to determine some important kinetic and operational parameters. These are crucial for the design of a semicontinuous anaerobic reactor and the ideal operation of large-scale anaerobic plants that treat food waste in semicontinuously varied ambient temperatures in hilly regions of India. The solutions to Eq. (1)–(4) were found using IBM SPSS Statistics version 20 data editor.

Results and Discussion

The reactor was operated anaerobically for 324 days on FW at variable organic loading rates and compositions of food wastes (Table 1). The food waste carbon/nitrogen (C/N) ratio, a key parameter for the experimental design, was 11.5. C/N ratios of approximately 8–20 were reported in various studies of food waste biogas reactors (El-Mashad et al. 2004; Jiang et al. 2018; Sánchez et al. 2001; Zhang et al. 2007).

Fig. 2(a) depicts the time variation of cumulative biogas and methane gas generated in the anaerobic reactor during the experiment. At the end of the experiment, a total 65,270.6 L biogas and 38,461 L CH₄ was produced. The composition of biogas [Fig. 2(b)] measured was on average 58.9% ± 1.4% CH₄, 39.7% ± 2.9% CO₂, 368 ppm H₂S, and negligible O₂.

Methane yield ranged from 13.9 to 418.3 mL CH₄/g VS_{added} during the entire period of experimentation [Fig. 2(c)]. However, an average of 102.1 mL CH₄/g VS_{added} methane yield was recorded

Table 1. Characterization of food waste

Day	Moisture content (MC)	TS (%)	VS (%)	VS/TS	C (%)	N (%)	C/N
Monday	80.4	19.6	12.3	62.9	42.0	3.3	12.8
Tuesday	86.7	13.3	11.8	89.1	44.2	3.8	11.6
Wednesday	76.9	23.2	16.8	72.3	46.2	5.3	8.7
Thursday	71.7	28.3	11.9	42.0	43.5	2.9	14.9
Friday	67.5	32.5	21.2	65.1	49.7	4.4	11.3
Saturday	78.9	21.1	15.7	74.5	44.8	4.2	10.6

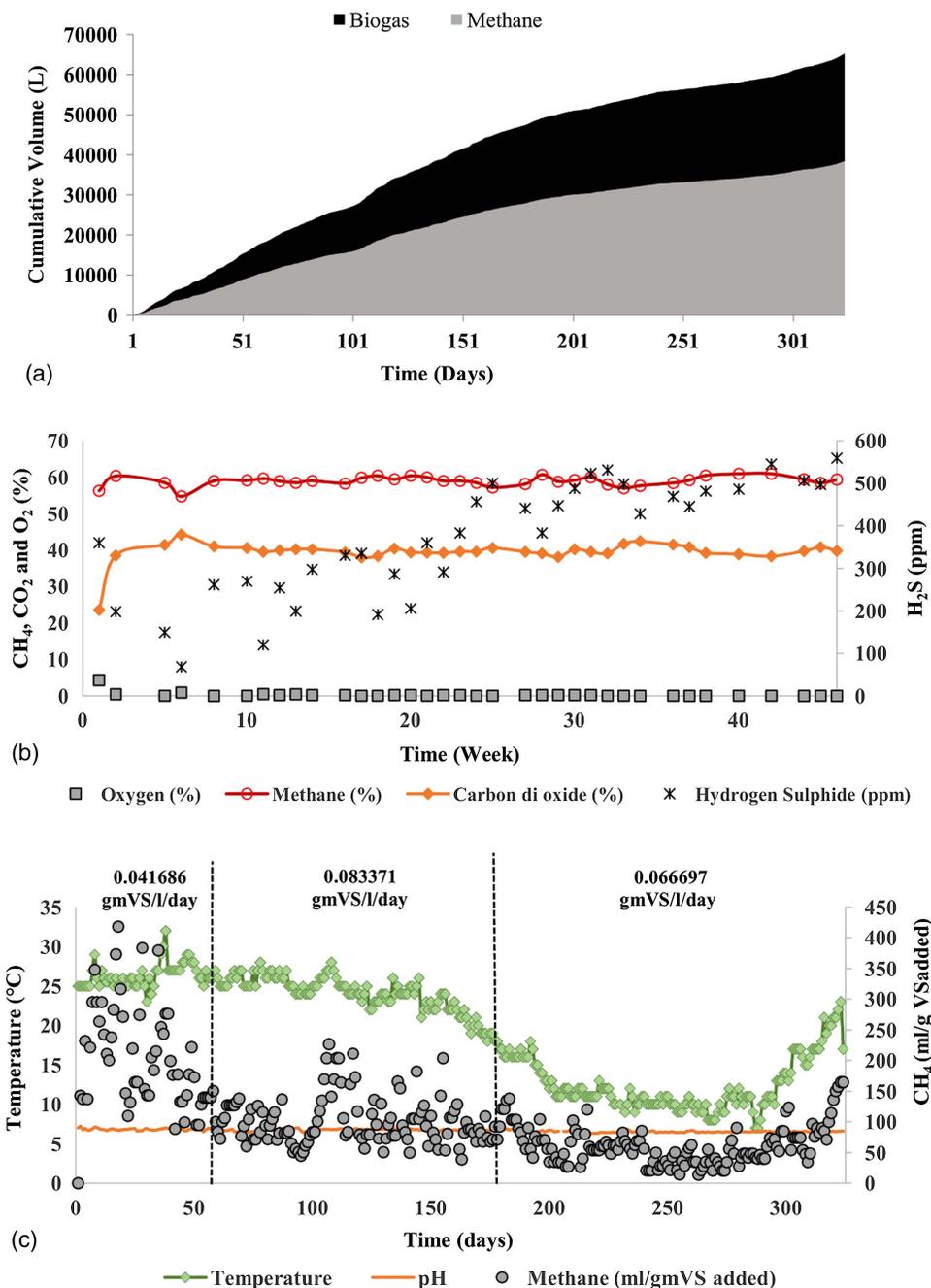


Fig. 2. (a) Temporal variation of cumulative biogas and methane; (b) composition of biogas; and (c) variation of methane with pH and ambient temperature.

for the whole experiment. These results are in accordance with those of Alvarez et al. (2006), who reported methane production in the range 6.4–33.6 mL CH₄/g VS_{added}.

An average reduction of 54.8% in methane production occurred in winter compared with summer. Similar to our study, a drastic change (77%) in biogas yield with a change in seasons was reported by Kanwar and Guleri (1994). The temperature during the digestion has a decided influence on the stability of the activity of enzymes and coenzymes, methane yield, and quality and quantity of digestate (Alvarez et al. 2006; Hill et al. 2001; Kalia and Kanwar 1998; Kanwar and Guleri 1994; Marín et al. 2018; Nozhevnikova et al. 1999; Safley and Westerman 1994; Zhang et al. 2014). The data average monthly temperature and methane generation are plotted

in Fig. 3. There was a linear relationship between these two factors, with $R^2 = 0.93$.

Stability Analysis

Various authors (Alvarez et al. 2006; Hill et al. 2001; Kalia and Kanwar 1998; Kanwar and Guleri 1994; Marín et al. 2018; Nozhevnikova et al. 1999; Safley and Westerman 1994) reported that low mesophilic temperature during digestion has a decisive influence on the stability of the digester. Moreover, the temperature may govern the activity of enzyme and coenzymes, methane yield, and quality and quantity of digestate (Zhang et al. 2014). In the present study, the digester was run at an average temperature of 22.65°C

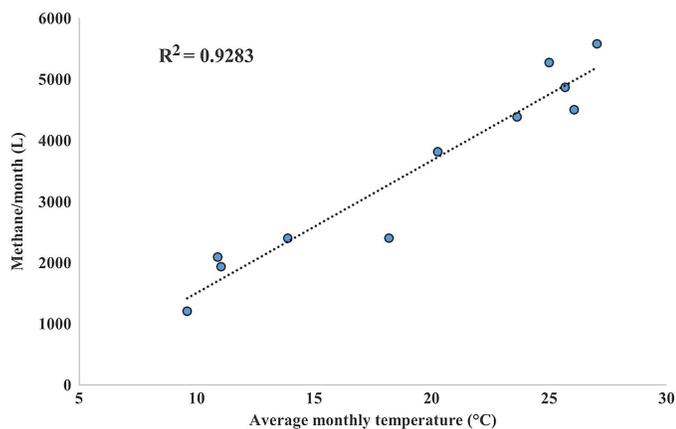


Fig. 3. Correlation between average monthly temperature and methane production per month.

(May 2017–September 2018) and psychrophilic temperature of 12°C (October 2017–March 2018). The stability of any reactor depends upon various factors such as temperature, pH, alkalinity, volatile fatty acids (VFA), and VFA:alkalinity ratio. Fig. 4 shows the variation of total alkalinity and VFA:alkalinity ratio with time. During this period, the stability of the reactor was analyzed. The maximum value of alkalinity of the system was 1,186.7 mg CaCO₃/L; however, the lowest value was 546.7 mg CaCO₃/L, and total VFA ranged from 371.9 to 1,040.8 mg/L. The VFA:alkalinity ratio often was less than 0.8 (stability baseline), however, occasionally this ratio increased to a maximum of 1.13, inhibiting the reactor (Callaghan et al. 2002). This is one of the limitations of single-stage anaerobic reactors. The desirable pH was maintained by the addition of CaCO₃ in the reactor.

Microbial Community Structures of Reactor

Bacterial Diversity

Bacteria from 15 phyla, 32 classes, 55 orders, and 82 families were identified, but most did not exceed 1% of all identified sequences (Fig. 5).

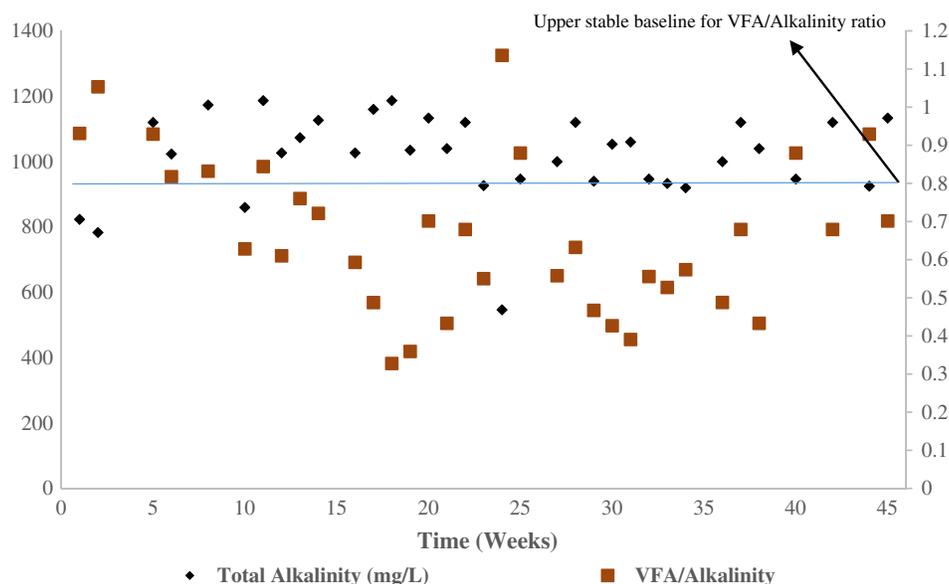


Fig. 4. Stability parameters of the reactor.

The majority of sequences in the food digested at the phylum level belonged to Proteobacteria (31.3% of the sequences in the final phase), whereas the relative abundance of Bacteroidetes, Chloroflexi, Firmicutes, and Spirochaetes was 14.92%, 6.1%, 4.07%, and 1.36% of all the bacteria reads, respectively. Among Proteobacteria, Gammaproteobacteria, which is a well-known organic acid (acetate, propionate, and butyrate)- and sugar-utilizing class of Proteobacteria (Cabrol et al. 2017) were identified as the most dominant class, at 27.23%. Furthermore, Proteobacteria often are facultative or obligate anaerobes, and thus can tolerate a range of oxic conditions. Therefore, it is postulated that the Proteobacteria contribute to homeostasis of the anaerobic environment, and hence to the stability of the strictly anaerobic microbiota (Moon et al. 2018). Among the next most abundant phyla, Bacteroidetes, Bacteroidia and Cytophagia occupied the sample consortia. Within the phyla Chloroflexi and Firmicutes, Anaerolineae and Clostridia classes dominated, respectively. The strictly anaerobic family of Bacteroidaceae has the ability to metabolize a variety of organic compounds including lipids, proteins, cellulose, and sugars (Khan et al. 1980). Thus, in our kitchen waste digesters, they probably played an important role in converting protein and amino acids to acetate and ammonia. Furthermore, the literature has reported a predominance of Firmicutes, Bacteroidetes and Proteobacteria from digesters fed with protein-rich substrates such as casein or bovine serum albumin (Tang et al. 2005). In most biogas reactors, the mean ratio of Bacteroidetes to Firmicutes is 1:5.6–6.0 (Güllert et al. 2016). A decreased population percentage of Bacteroidetes is known to reduce the initial hydrolysis rate. In contrast to this, Bacteroidetes dominated in our reactor, with the ratio of Bacteroidetes to Firmicutes of 3.6:1. This resulted in the stable hydrolytic performance of our biogas reactor.

At the order level, Pseudomonadales and Bacteroidales, with a total of 37.05%, represented the consortia, indicating the prevalence of syntrophic acetogenesis. At the genus level, the presence of *Smithella*, *Syntrophus*, *Syntrophorabdus*, and *Syntrophobacter* confirmed this. Syntrophic bacterial communities convert propionate and butyrate to acetic acid in the acetogenic phase. The members of Syntrophobacterales (e.g., *Syntrophobacter*) degrade organic compounds into end products such as acetate and carbon dioxide. However, this is not a major pathway for methane

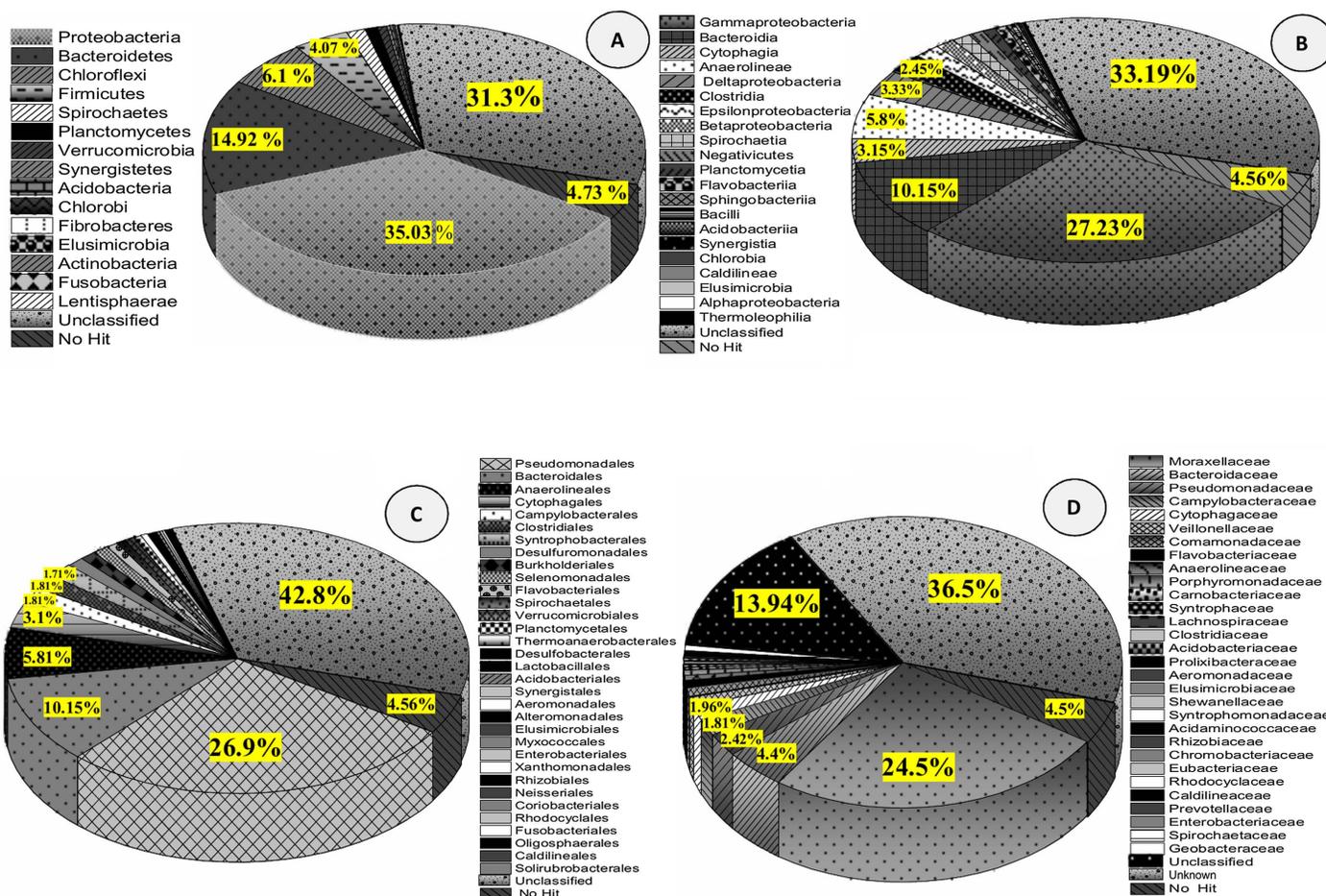


Fig. 5. Bacterial taxonomic assignments at various levels (A = phylum, B = class, C = order, and D = family).

production in most ADs because syntrophic acetogens are less competitive than acetoclastic methanogens (because of high affinity for acetates). However, under conditions that inhibit acetoclastic methanogens, such as high ammonia concentration and high operation temperature, this pathway is important to biogas production. Consequently, a high concentration of ammonia might have inhibited acetoclastic methanogens. In this study, hydraulic retention time (HRT) varied from 20 to 22 days, and long HRT favors acetogenic pathways. When *Methanosaetaceae* are not present, acetate oxidation is the dominant methanogenic pathway (Karakashev et al. 2006). Our sample lacked *Methanosaetaceae* in its consortia (0%). The rest of the consortia comprised aerobic or facultative anaerobic Proteobacteria, e.g., *Acinetobacter* (24%), *Pseudomonas* (3%), *Cytobacter* (3%), *Geobacter* (2%), and *Arcobacter* (2%), which likely were involved in organic substrate degradation and oxygen consumption for the maintenance of anoxic environment (Rui et al. 2015).

Archaeal Diversity

In the investigated biomass, Euryarchaeota, which includes all known methanogens, was the predominant archaea phylum, accounting for 85% of total sequences, followed by Crenarchaeota at 1.4%. Interestingly, 13.6% of archaea phyla were unclassified (Fig. 6). The predominant classes within Euryarchaeota were Methanomicrobia (65%) and Methanobacteria (20%), and the predominant orders within Euryarchaeota were acetotrophic and methylotrophic Methanomicrobiales (39.7%), Methanobacteriales (20.7%), and Methanosarcinales (18.3%). At the genus level,

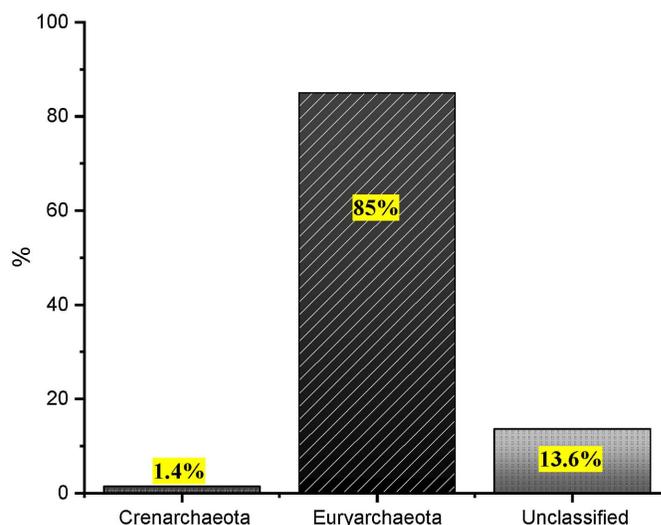


Fig. 6. Dominance of archaeal phyla in Sample D.

Methanobacterium was prevalent, which along with *Methanosarcina* (20%) and *Methanospirillum* (18%) made up most of the sample (Fig. 7). *Methanosarcina* are favored under high acetate concentration, whereas low concentration of acetate favors acetoclastic methanogens such as *Methanosaeta* (Smith and Ingram-Smith 2007). Our reactor was open and had variations

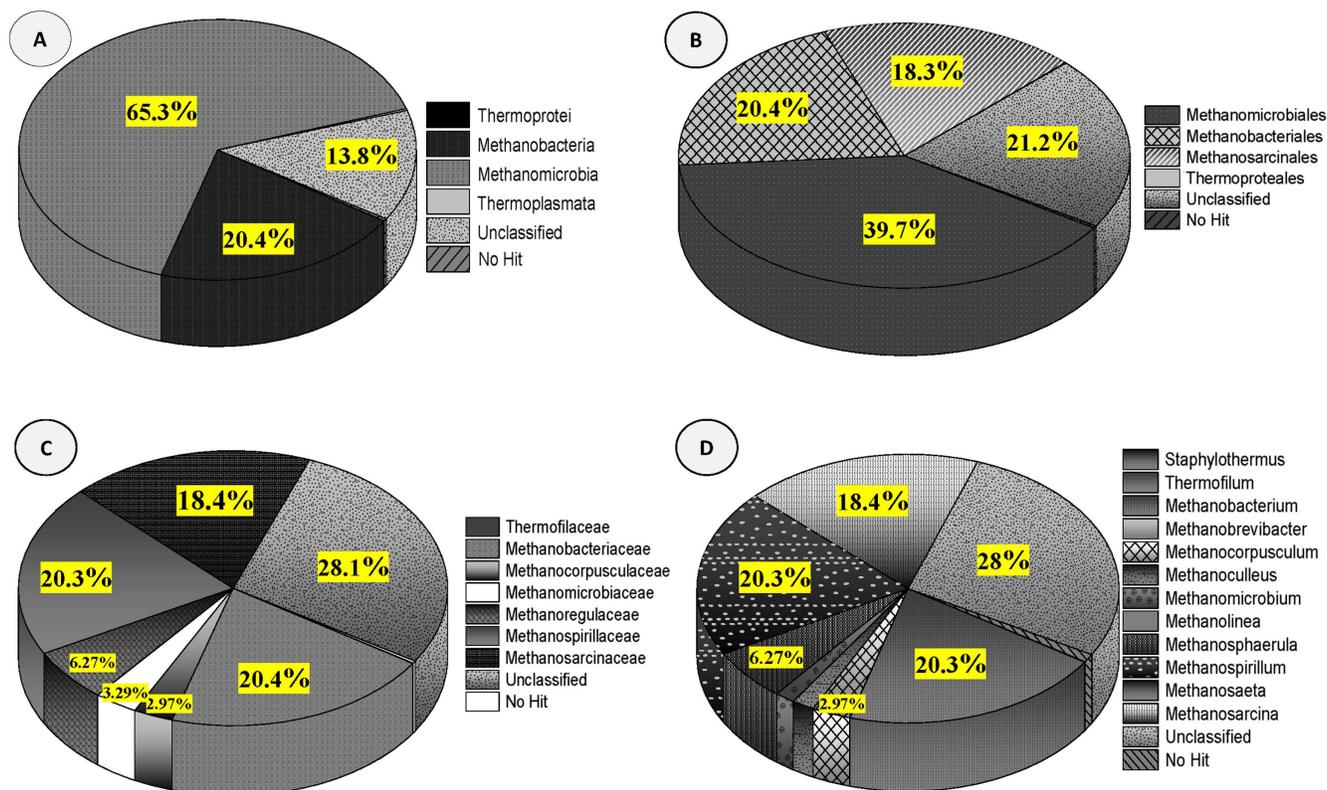


Fig. 7. Archaeal taxonomic assignments at various levels for Sample D (A = class, B = order, C = family, and D = genus).

of temperatures throughout the period of study. Under these conditions, *Methanosarcina* outcompetes other archaeal genera, because *Methanosarcina* can withstand extreme temperature fluctuations. *Methanobacterium* is an acetogenic bacterial genera that converts the acid-phase products into acetates and hydrogen which may be used by methanogenic bacteria. Therein, methane is produced either from acetate by acetoclastic methanogens or from CO_2/H_2 by hydrogenotrophic methanogens. Whereas *Methanosarcina* is a potential acetoclastic methanogen, *Methanosphaera* and *Methanospirillum* represent hydrogenotrophic bacteria. The main hydrogenotrophic microorganisms participating in the anaerobic processing of fruit and vegetable wastes are *Methanosphaera* (Wang et al. 2014). Thus, the presence of mixed consortia of these entire genera representing acetotrophic, hydrogenotrophic, and methylotrophic archaea in balance indicates the equal role that these genera play in methane production during anaerobic processing of kitchen digests. This syntrophic cooperation is essential for the proper functioning of both H_2 -producing syntrophic and methanogenic archaea, because it affects their metabolism. Methanogenic microorganisms consume hydrogen and decrease the partial pressure of this gas, which stimulates acetogenic bacteria activity (Świątczak et al. 2017). Overall, the bacterial domain was extremely diverse. In contrast, the archaeal community comprised comparatively less phylogenetically diverse groups, represented by at most nine genera. This probably is because only limited phylogenetically methanogenic groups exist in the world, and these archaea account for a small proportion of the microflora in anaerobic reactors (Guo et al. 2015).

Mathematical and Kinetic Modeling

The FOM, MGM, RCM, and MLF were selected to determine the most accurate fitting model for FW in semicontinuously varied

ambient temperatures in hilly terrain. Fig. 8 summarizes the model parameters and compares their fitting accuracy, and the parameters of each model are listed in Table 2. These models consist of several kinetic and mathematical parameters, such as lag phase (λ) or latency. The lag phase is the minimum expected time necessary to initiate methanogens to start the anaerobic digestion process. In this study, the lag phase was 1 day; this indicates that methanogens rapidly initiated the digestion of FW. RCM predicted 2.3 days; however, MGM and MLF predicted a negative lag phase ($\lambda < 0$), which has no physical meaning. Donoso-Bravo et al. (2010) used primary and secondary sludge from a wastewater treatment plant for anaerobic digestion and reported a negative lag phase. They concluded that these models are not suitable for the prediction of the lag phase where work is methane-generation oriented (Donoso-Bravo et al. 2010). On the other hand, R^2 is a parameter by which the fitting accuracy between observed and predicted values can be evaluated. The R^2 values for the FOM, MGM, RCM, and MLF were 0.99, 0.99, 0.99, and 0.98, respectively. All the models had a significant correlation between observed and simulated values. Additionally, in the case of the FOM and RCM at $t = 0$, methane generation is almost negligible, but a key issue for the MGM and MLF is the shape of the simulated curves. In the very beginning of these two curves, i.e., at $t = 0$, the models predicted that the methane generation is not zero, which is practically impossible and has no physical meaning. In the case of the MGM, when λ is negligible and $t = 0$, P converges to $P_0/(\exp(\exp))$ which is always greater than 0, i.e., positive, but when $\lambda > 0$ and $t = 0$, P converges to zero (Donoso-Bravo et al. 2010). Therefore, this study focused on the value of R_m , P , and P_0 considering that for methane generation the lag phase is negligible or zero. A similar condition occurred for the MLF. Overall, the FOM was the most accurate model, with $K_h = 0.007 \text{ day}^{-1}$, $R^2 = 0.99$, $P = 32.9 \text{ LCH}_4/\text{g VS}$, and $R = 134.5 \text{ LCH}_4/\text{g VS/day}$. The MGM was the best

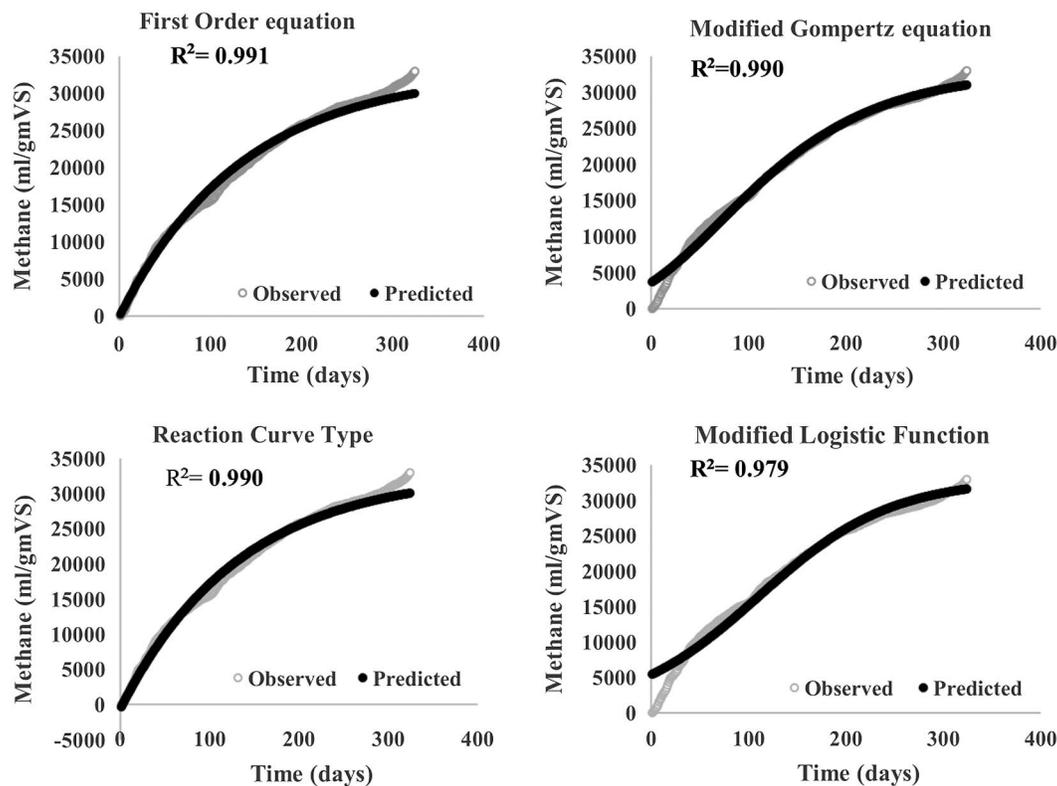


Fig. 8. Comparison of mathematical and kinetic models.

Table 2. Coefficients and fitting accuracy of models

Model parameter	FOM	MGM	RCM	MLF
P^a (L CH ₄ /g VS)	34.1	32.9	33.9	32.1
R (mL CH ₄ /g × VS/day)	N/A	134.5	-248.3	121.8
K (day ⁻¹)	0.007	N/A	N/A	N/A
λ (days)	N/A	0	2.32	0
R^2	0.99	0.99	0.99	0.97
$(P_0)^a$ (L CH ₄ /g VS)	29.9	31.0	30.0	31.6

^aCumulative methane production.

mathematical model. No significant difference was found in the values of R_m , P , and P_0 . Moreover, the MLF predicted P_0 with the least deviation, 4.1%, and all models had an average deviation of 7%.

Conclusion

Experiments in a pilot-scale (3,000-L) semicontinuous anaerobic reactor were conducted for 324 days at varied temperatures. The reactor was fed with food waste at varying OLRs. The production of methane had a linear dependency ($R^2 = 0.93$) on the average monthly temperature. The effect of daily temperature variation, pH, and VFA was analyzed with respect to biogas production rate, i.e., methane yield. The analysis of microbial community composition at low temperature revealed a wide phylogenetic diversity of the hydrolytic and methanogenic populations. In this anaerobic digester, Proteobacteria dominated the bacterial community, whereas Methanomicrobia dominated the archaeal community, with the overall emergence of a highly active psychrotolerant although still mesophilic biomass. For AD in Himachal Pradesh, aspects that deserve more substantial studies include the required environmental conditions in Himachal Pradesh (HP) due to extensive temperature

fluctuations in day and night. The results of the present study will be helpful for researchers working in the field of landfill design parameters such as the production of leachate, greenhouse gas emissions from scientific and nonscientific dumping, and developing large-scale AD in the low-temperature range without a heating system. The stability analysis of the anaerobic digester indicated that AD of FW is feasible in a low mesophilic temperature range.

Data Availability Statement

Some or all data, models, or code generated or used during the study are available from the corresponding author by request. These include the experimental data related to the feeding, daily biogas generation, temperature, and stability; details of the four models used in the analysis; and the experimental data related to the microbial population structure.

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