Environ. Eng. Res. 2024; 29(1): 220518
https://doi.org/10.4491/eer.2022.518

pISSN 1226-1025
eISSN 2005-968X

Research

Effect of inoculums type and optimization of inoculum to substrate ratio on the kinetics of biogas production of fruit and vegetable waste

Akanksha Agrawal, Parmesh Kumar Chaudhari, Prabir Ghosh†

Department of Chemical Engineering, National Institute of Technology Raipur, Chhattisgarh, 492010, India

Received September 12, 2022 **Revised** March 10, 2023 **Accepted** April 30, 2023

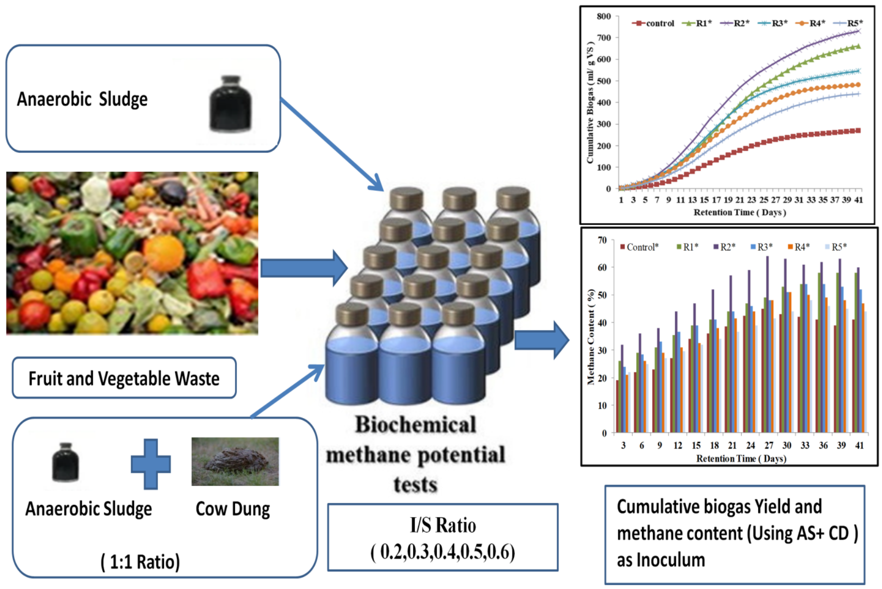
ABSTRACT

Annually 5.6 million tonnes of FVW are produced in India. Anaerobic digestion (AD) holds the potential of generating renewable energy from this organic waste. Therefore, an attempt has been made to optimize inoculum to substrate (I/S) ratio via biochemical methane potential (BMP) of FVW using two different types of inoculums viz. anaerobic sludge and a mixture of anaerobic sludge and cow dung in (1:1) ratio. Different (I/S) ratios selected for the study were 0.2, 0.3, 0.4, 0.5, and 0.6. Results indicated that anaerobic sludge (AS) along with cow dung (CD) at a (1:1) ratio performed better than AS alone. The highest cumulative biogas yield was obtained at (I/S) ratio 0.3 for AS+CD (1:1) ratio, which is equivalent to 468.82 ml/g VS. On the other hand, the biogas yield using only AS was found to be 459.49ml /g VS. The highest methane content was obtained as 64% and 61.2%, for inoculums AS+CD and AS, respectively. Performance analysis portrayed volatile solids (VS) reductions of 74% and 68% for (AS+CD) and AS, respectively. The kinetic parameters of AD were studied by the modified Gompertz model (MGM) and the first-order kinetic model. The experimental data fitted well with the MGM model.

Keywords: Biochemical methane potential, Inoculum source, I/S ratio, Modified Gompertz Model

† Corresponding author
E-mail: prabirg.che@nitrr.ac.in
Tel: +91-7773892359 Fax: +91-771-2254600
ORCID: 0000-0003-3126-0887

Graphical Abstract



1. Introduction

Fruit and vegetable waste (FVW) are extensively grown and consumed across the globe. India is the second largest producer of fruit and vegetable. However, 30-40% of the production is squandered annually, which is equivalent to a net worth of Rs. 13300 crores. In India, FVW production is about 5.6 million tonnes annually [1] and the maximum amount of waste is produced by the southern region of India [2]. FVW is generated during harvesting, transportation, storage, marketing, and processing. Currently, in most of the developing and developed countries, FVW is disposed of in the outskirts of the cities which adds a burden to the sanitary landfill and creates a negative impact on the environment. Landfilling is practically not feasible due to scarcity of the land and allied numerous disadvantages such as release of the greenhouse gases (GHG) and groundwater contamination [3, 4]. Therefore, this approach is highly discouraged which, thrust on searching for a viable solution that might address the current problem of waste management and energy crisis. In addition, the Swachh Bharat ("Clean India") mission, which was launched in 2014, and the revised Solid Waste Management Rules 2016 legally bind urban authorities to plan, design, implement, and monitor MSW management in such a way that resource recovery and waste minimization should be the priority. Among the different alternative technologies available for waste management, like incineration, gasification, pyrolysis, and anaerobic digestion (AD), AD appears to be lucrative and environmentally friendly technology to reduce the volume of waste and resource recovery in the form of methane and digestate [5, 6]. The immense amount of organic waste produced across the globe holds the substantial potential of generating biogas up to 4000 Mm^3 annually which has an energy potential of around 86,000 TJ per year [7]. Renewable energy generated from biomass such as agricultural waste, FVW, organic fraction of municipal solid waste (OFMSW), and animal waste can be considered as green energy which can contribute to reduce our dependency on fossil fuels [8]. AD is widely applied in countries like China, India, Thailand, Philippines, Korea, Switzerland, USA, and Germany. Biogas generated as a result of AD is a colourless, odorless, lighter-than-air form burnt with a bright-blue flame and consists of 50%-75% CH_4, 30%-60% CO_2, and 0-3% H_2S with trace amounts of nitrogen and hydrogen [9, 10]. The density of biogas is 0.83 g/L with an approximate octane number is 110. The combustion temperature of biogas is 700℃, and the flame temperature is 870℃. However, the composition of the biogas varies with respect to the nature of the substrate, and type of the fermentation method deployed [11]. The digestate produced at the end of the AD is rich in N, P, and K which can be used as a biofertilizer in the agriculture field [7]. Furthermore, the presence of high moisture and VS content in FVW, confirms its suitability for the AD. It also results in a higher quantity of biogas as compared to other waste such as sludge [12]. However, a significant amount of active inoculums, which consists of a complex community of bacteria that catalyze a series of interdependent biochemical processes (hydrolysis, acidogenesis, acetogenesis, and methanogenesis), is essential for an effective digestion process [9]. To determine the effectiveness of AD, a case study of the optimization of inoculum to substrate ratio is vital and fascinating. The methane potential of the substrate depends upon the organic content of the substrate, the quantity of the inoculum used, reactor configuration, and environmental conditions within the digester. Therefore, before commencing AD, the biomethane potential (BMP) of the substrate should be evaluated. BMP is the test used for the determination of the extent of biodegradability of the waste [13]. In this method, inoculum and substrate were injected into a BMP unit under optimal conditions such as pH and temperature to identify the gas production and methane content [14]. The addition of inoculum is necessary for the batch reactors to start reactions [15]. The ratio of VS of inoculum to initial volatile solids (VS) of feedstock at the time of commencement of the batch digestion is known as (I/S) ratio and is important to provide an optimum quantity of the inoculum to ensure the effective performance of the BMP test [16]. (I/S) ratio plays a crucial role in the anaerobic digestion of FVW processes and also in the degradation of VS of the organic solid particles [14]. It has been reported that the methane yield of a substrate may vary according to the amount and composition of the substrates utilized. Furthermore, the type and quantity of inoculum used in the BMP test and their cultivation conditions control the entire AD. Inoculums contain active microbial consortia required for the AD of the organic matter. It varies according to the different types of substrates as the amount of the VFA produced and ammonia produced at the end of the hydrolysis of complex carbohydrates, protein, and other organic substances respectively to the buffer medium [17]. In a single-stage digester, the main challenge is to prevent the accumulation of the VFA inside the seed particles beyond their assimilative methanogenic capacity. This can be prohibited by raising the amount of inoculum to overcome irreversible acidification during start-up [18, 19]. Each substrate has an acceptable (I/S) ratio depending on the amount of volatile fatty acids (VFA) produced and the capacity to buffer VFA accumulation during the anaerobic process, [20]. Therefore, before initiating the continuous digestions process, it is critical to adjust the (I/S) ratio for a highly biodegradable substrate [21]. Hence, BMP is a simple way to measure methane yield from organic waste and compare potential methane yield between different samples subjected to the test in a short duration [22]. The inclusion of the appropriate dose of inoculum enhances the synergistic relationship between microbial consortia which in turn enhances the biogas yield of the substrate [9]. However, among other considerations, the inoculum-to-substrate ratio and the nature of the substrate play a vital role in effective AD [23]. According to Raposo et al. [24], an adequate amount of inoculum addition speeds up the onset of active methanogenesis and helps overcome digester inhibition. As per the study carried out by [16], methane content in the biogas was enhanced with the increase in the (I/S) ratio from 1 to 4. The (I/S) ratio is defined as the initial ratio of VS in the inoculum to the VS in the feedstock during the start of a batch digestion experiment [25]. The (I/S) ratio was reported as a very important parameter in batch anaerobic digestion [26]. It entails the addition of appropriate inoculum to the substrate to supply the necessary bacteria to initiate the reaction. Every substrate has a specific (I/S) ratio based on the amount of VFA present and the ability to buffer the accumulated VFA during digestion. Lower (I/S) ratios could hinder the induction of the enzyme necessary for anaerobic digestion whereas greater (I/S) ratios could be harmful [25]. Anaerobic digestion's lag phase may also be impacted by the (I/S) ratio [27]. Initially, anaerobic digestion was started arbitrarily by applying inoculum. Therefore, it is necessary to ascertain the optimal amount of inoculum that will be needed for the best biogas yield from varied substrates. Furthermore, literature shows that scientific information on the optimum (I/S) ratio for maximum biogas yield from FVW is scarce or non-existent. Therefore, the present study aims at evaluating the optimal (I/S) ratio which is essential for the biodegradation of FVW via biochemical methane potential (BMP) test using anaerobic sludge and a mixture of anaerobic sludge and cow dung in (1:1) ratio. Kinetic models like the First order model (FOM) and Modified Gompertz Model (MGM) were also applied to determine the influence of (I/S) ratios on the biogas yield.

2. Material and Methods

2.1. Materials

2.1.1. Fruit and vegetable waste

Fruit and vegetable waste, used as feedstock was procured from the local vegetable market. It consists of Banana (20±5%), Apple (10±5%), Sapodilla (10±5%), Spinach (10±5%), Cabbage leaves (10±5%), Cauliflower leaves (10±5%), Eggplant (10±5%), Potato (10±5%), Bottle gourd (5±3%) and Ridged gourd (5±3%). The ratio depicts the quantity of waste likely to be generated in the local market on that day. The vegetable and fruit waste were washed repeatedly to get rid of any impurities present. The waste was mechanically pulverized using a domestic mixer grinder to reduce the particle size. It was used immediately for the experiments.

2.1.2. Inoculum

Two different types of inoculums namely anaerobic sludge and a mixture of cow dung and anaerobic sludge in a 1:1 ratio was used in the present study to assess their influence on biomethane production. Anaerobic sludge was procured from an anaerobic digester treating dairy residue in a mesophilic regime, whereas the fresh cow dung was collected from the local dairy. Degasification of inoculum was done before its use to deplete residual biodegradable organic matter. The inoculums were stored at 4ºC till

Table 1. Characteristics of Inoculums and Substrate

Parameter	FVW	Anaerobic Sludge (AS)	Cow dung (CD)	AS+ CD (1:1)
pH	4.8	7.62	6.9	6.33
Moisture content (%)	93%	94%	77%	84%
Total solids (TS) (%)	4.6%	5.76%	7.6%	6.68%
Volatile solids (VS)(%TS)	92.75	65.98	83.17	88.12
Carbon (C) (%)	44.6	19.45	24	26
Nitrogen (N) (%)	2.40	2.54	2.1	1.68
C/N	18.58	7.65	11.42	15.45
Total Alkalinity (mg $CaCO_3$/L)	740	3000	3250	3600

Table 2. Initial Characteristics of the Substrate and Inoculums at Different (I/S) Ratio

Parameters	Control	Control*	R_1	R_1^*	R_2	R_2^*	R_3	R_3^*	R_4	R_4^*	R_5	R_5^*
pH	7.62	6.7	5.25	5.6	5.45	5.8	5.65	5.76	5.8	6.2	6.2	6.4
TS	4.47	4.6	4.98	5.2	5.16	5.4	5.44	5.6	5.97	6.0	6.18	6.2
VS	3.8	4.04	4.335	4.47	4.7	4.86	5.06	5.15	5.56	5.6	5.75	5.8
VS (% TS)	85.01	87.82	87.04	85.96	91.08	90	93.01	91.96	93.13	93.34	93.04	93.54

*indicates the digester's fed with (AS+CD)

further use. Characteristics of the inoculums and substrates are presented in Table 1.

2.2. Methods

2.2.1. Experimental setup

Biochemical methane potential (BMP) was conducted in triplicate in anaerobic batch mode for a hydraulic retention time of 41 days. PET bottles of 500 ml capacity with a working volume of 300 ml were converted to BMP assay with inlet and outlet arrangement for the biogas measurement. The pictorial diagram of the digester is presented in Fig. 1. The temperature of the BMP assay was maintained at 37ºC using a water bath. BMP of the substrate was evaluated in the batch mode for two different types of inoculums and for different (I/S) ratios, which are control (only inoculum), R_1 = 0.2, R_2 = 0.3, R_3 = 0.4, R_4 = 0.5 and R_5 = 0.6. Anaerobic digesters were fed with the desired amount of the substrate and inoculums as per a predefined ratio and then sealed tightly. These digesters were then purged with nitrogen gas for about 45 seconds to ensure anaerobic conditions inside the reactor. The initial characteristics of the mix of substrate and inoculum at different (I/S) ratios are presented in Table 2. Each reactor was shaken manually once a day to ensure homogeneous mixing. The anaerobic digestion was carried out until the cumulative biogas production was observed less than 1%. The volume of the biogas was measured daily using the water displacement method daily. Each batch was conducted in triplicate to avoid human errors. To avoid inhibition of the biogas production due to rapid acidification which may occur due to the high biodegradability of the FVW, about 0.5 g of sodium bicarbonate was added as a buffering agent to each reactor for adjusting pH and alkalinity [28, 29]. The methane composition of the biogas was measured using gas chromatography (Nucon 5700) equipped with a Porapak Q column and hydrogen gas was used as carrier gas with a flow rate of 30 ml/min. Calibration of gas chromatography instruments was done before the analysis of the sample by using a standard gas composition of H_2-5.18%, CO-4.98%, CH_4-39.72%, CO_2-30.06%, and N_2-20.06%.

2.2.2. Analytical methods

The pH of the sample was measured using a hydrogen ion-sensitive electrode using the portable pH meter (Systronics Model No.335). Total solids (TS), volatile solids (VS), pH, and alkalinity were measured per APHA [30]. Volatile fatty acids were measured as per the method described by Chatterjee et al. [31].

The efficiency of the reactors was evaluated based on VS removal rate (%). This can be calculated as the ratio between the amount of VS reduced during the digestion period and VS added to the reactor as mentioned in Eq. (1).

Fig. 1. Experimental setup for BMP assay

$$VS_{removal} = \frac{(VS_{added} - VS_{digestate})}{VS_{added}} \quad (1)$$

2.2.3. Kinetic modelling

Various kinetic models have been used to study the anaerobic digestion (AD) process. In the present study, two of them have been used namely the First-order model and the Modified Gompertz Model (MGM model). The MGM model is based on the assumption that biogas increases to its peak exponentially whereas the first-order model is based on the assumption that biogas was increased to its peak linearly [32]. The validity of the models was determined using the (R^2) value. The first-order model is given by Eq. (2) and the MGM model is given by Eq. (3).

First order model:

$$Y(t) = Y_0\,(\,1 - \exp(-k.t)) \quad (2)$$

Modified Gompertz Model:

$$Y(t) = Y_0\, exp\left\{-\exp\,[\frac{R_m.e}{Y_0}(\lambda - t)] + 1\right\} \quad (3)$$

where Y(t) = cumulative biogas at digestion time t days (mL/g VS),
Y_0 = ultimate biogas production potential (mL/g VS),
k = first order rate constant for biogas appearance (1/day),
Rm = maximum biogas production rate (mL/g VS/day),
λ = lag phase period or minimum time to produce biogas (days),
t = cumulative time for biogas production (days), and
e = mathematical constant (2.718).

Out of the above constants and variables, all values are known except first-order rate constant k which was calculated by transforming Eq. (2) in the form of Eq. (4).

$$ln \frac{(Yt - Y0)}{Y0} = kt \quad (4)$$

Plot between $ln \frac{(Yt-Y0)}{Y0}$ and time t gives the value of k.

3. Results and Discussion

3.1. Effect of Inoculum Type and (I/S) Ratio on Biogas Yield

Biogas production is dependent on the nature of the substrate, (I/S) ratio, and the source of inoculum. The inclusion of inoculum speeds up the activity of microbes and helps overcome the lag period of the digester [31]. The rate of biodegradation, lag time, and chances of degradation of substrate relies on the population of the microbes present inside the digester [32]. Moreover, it also helps in overcoming the inhibition due to VFA and ammonia by maintaining the synergy among the individual substrate in the reactor [33]. To initiate anaerobic digestion, the inoculum was applied arbitrarily. However, to maximize biogas yield, there should be an optimum dose of inoculum which varies with the nature and amount of substrate. Therefore, there is a pressing need to determine the optimum (I/S) ratio for the anaerobic digestion of FVW.

In the present study, two different types of inoculums and the effect of different (I/S) ratios on biogas production were investigated. The variation of the daily biogas yields at different (I/S) ratios using AS and AS: CD (1:1) is depicted in Fig. 2(a) and 2(b), respectively. From the graph, it is evident that the biogas yield of the substrate was enhanced due to the inclusion of inoculum as it helped to activate the microbial activities in the reactor in the initial stage [34]. There was a clear difference in the initial volume of biogas recorded in digesters R_1, R_2, R_3, R_4, and R_5 when compared with the control reactor.

Increasing the (I/S) ratio from 0.2 to 0.3 increased the substrate utilization rate and the highest biogas was recorded for (I/S) ratio of 0.3 using anaerobic sludge along with cow dung which is equivalent to 15.67 ml/g VS on day 28, attributed to the high C/N ratio. On the other hand, when only anaerobic sludge was used, the highest biogas was recorded as 18.08 ml/g VS on day 22, however, the cumulative biogas yield at (I/S) ratio 0.3 was lower than the cumulative biogas yield using (AS+CD). This is attributed to the improved nutritional balance in the feedstock and diluted toxic compounds in the reactor, positive synergism, and active microbes in the digesting medium [33, 34]. The biogas production increased gradually during the first few days of operation due to the presence of easily biodegradable matter in the substrate [34] after which it got declined for all the reactors due to acid accumulation, and the presence of the limited amount of substrate available for bioconversion. Beyond three weeks, the rate of biogas production dropped due to possible acidification which may occur in the digester. Furthermore, nearly 80% of the bioconversion occurred during this digestion period.

The observed biogas was in descending order for reactor $R_2>R_1>R_3>R_4>R_5>$ control for both types of inoculums. Other researchers [35, 36] also reported the highest biogas yield at an (I/S) ratio of 0.2 for the OFMSW and mixed sludge. Neves et al. [37] reported a significant increase in the biogas yield at an (I/S) ratio greater than 0.2. They used granular sludge along with food waste. At (I/S) ratio between 0.2 to 0.4, rapid accumulation of VFA was reduced which enhanced methane yield. It is further noted that with the increase in the percentage of inoculum beyond the optimum (I/S) ratio of 0.3, i.e., higher inoculum content and lower substrate concentration, the biogas production declines which is due to the limited amount of substrate as the biogas production comes primarily from the substrate [38]. Moreover, it also leads to an increase in the digester volume [39]. The results obtained from the experiment are in accordance with [33, 34].

3.2. Cumulative Biogas Production

Cumulative biogas variation over the digestion period is depicted in Fig. 3(a) and 3(b). The result pointed out that the highest cumu-

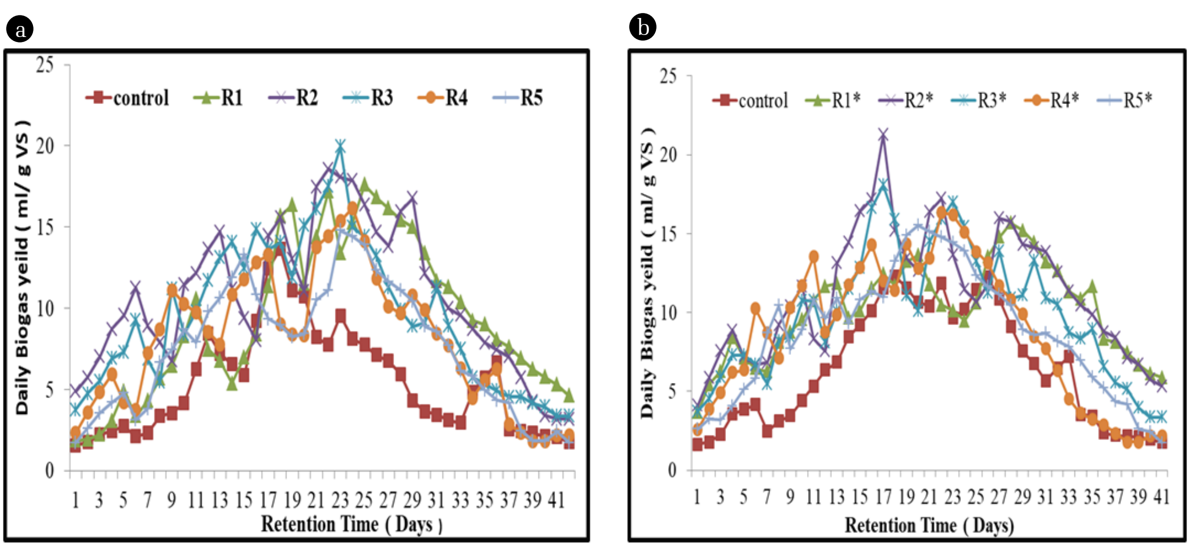


Fig. 2. Daily biogas variation at different (I/S) ratio (a) using AS (b) using AS+CD

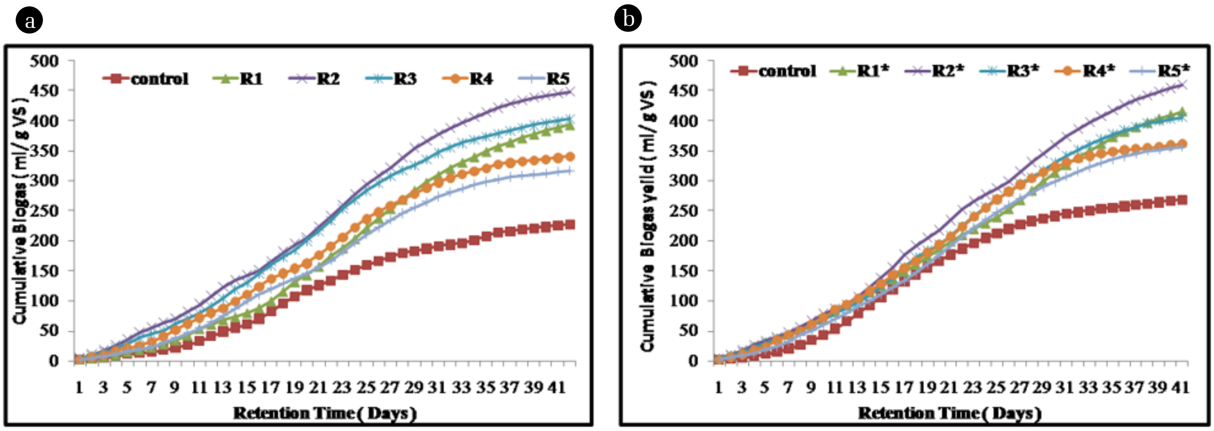


Fig. 3. Cumulative biogas variation at different I/S ratios (a) using AS (b) using AS+CD

lative biogas production was obtained for (I/S) ratio of 0.3 and with anaerobic sludge mixed with cow dung in (1:1) proportion. This is equivalent to 468.82 ml/g VS, whereas the same ratio yielded the highest biogas as 459.49 ml/g VS when anaerobic sludge was used alone. The generic similar trend of cumulative biogas production was observed same for both the inoculum AS and (AS+ CD).

3.3. Methane Content at Different Inoculum to Substrate Ratio

The variation of methane content at different (I/S) ratios for different inoculums over a digestion period is depicted in Fig. 4(a) and 4(b). Methane content in the reactors ranged from 19.5% to 61.2%. Methane production shows a consistent trend throughout the digestion period. However, it is characterized by the initial lag phase followed by a subsequent more rapid increasing phase and finally a stabilization phase. The highest methane concentration was noted for (I/S) ratio 0.3 using anaerobic sludge along with cow dung in (1:1) proportion which is equivalent to 64% followed by R_2, R_1, R_3, R_4, R_5, and Control which is equivalent to 58%, 54%, 51%, 48%, and 45%. On the other hand, the methane concentration using anaerobic sludge as a source of inoculum was found to be 61.2%, 56.12%, and 52.14%, 49%, 46.54%, and 45.6 % for R_2, R1, R_3, R_4, R_5, and Control, respectively. With the increase in the (I/S) ratio beyond 0.3, methane production begins to decline, this may be due to accumulation of the volatile fatty acids which results in a pH drop. Due to the drop in pH, the methanogenic bacteria are unable to convert the organic acids to methane and thus, leading to lower methane production. Similar results were obtained by [40]. The results displayed by different ratios are in accordance with the literature [4]. Low methane yield in the reactors is possibly due to the accumulation of organic acid, thus inhibiting the methanogenic activity of the methanogenic microbes [39]. Also, among the two different types of inoculums used, the mixture of anaerobic sludge with cow dung at a 1:1 ratio gave 11.32% higher methane yield as compared to the anaerobic sludge. This may be attributed to the fact that cow dung contains a higher

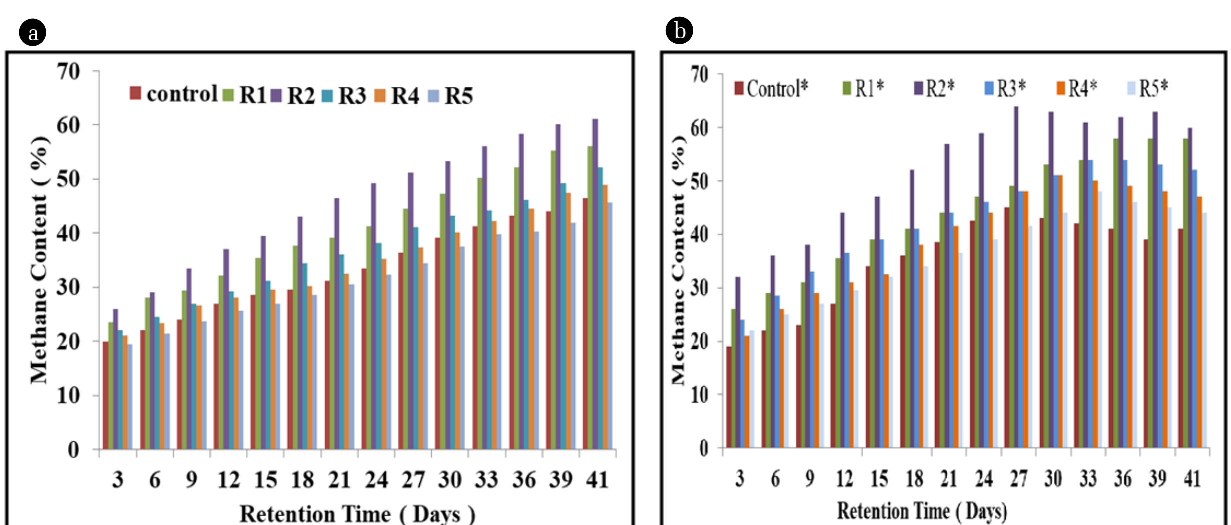


Fig. 4. Methane content at different (I/S) ratios (a) using AS (b) using AS+CD

population of methanogenic archaea which boosts the biogas yield. Moreover, the high pH of the inoculum (CD+AS) helped in adjusting the natural buffer capacity of the digester, thus reducing the risk of VFA inhibition. Almomani et al. [41] obtained methane production of 297.99 ml/g VS for anaerobic co-digestion of agricultural solid waste (ASW) and cow dung (CD) at a ratio of 60:40 almost 31% higher than the (20:80) ratio.

4. Process Efficiency

4.1. pH Variation at Different (I/S) Ratio

During anaerobic digestion, complex organic substances are converted into organic acids. These acids lower the pH of the reactor and offset the methane production if not consumed by the methanogenic bacteria instantly. The pH of the anaerobic digestion should be maintained between 6.8 to 7.3 for the best methanogenic activity [11]. Fruit and vegetable waste having an initial pH of 4.8 was fed to the bioreactors, the residual pH of the reactors was measured at the end of the HRT, and it was found to be 7.2 which was slightly higher than the optimal pH. pH is a crucial parameter that governs all the biochemical reactions during anaerobic digestion viz. hydrolysis, acidogenesis, acetogenesis, and methanogenesis. pH of the reactors should lie within the ideal range of methanogenesis so that methanogens can flourish and produce methane. The initial pH of the reactor without any addition of alkali lies between 5.25 to 7.62. However, almost similar trend of pH was noticed for all the sets of BMP assay as depicted in Fig. 5(a) and 5(b). To start with anaerobic digestion, the pH of all reactors was brought to near neutral with the help of the addition of strong alkali, sodium bicarbonate (5N). However, during the initial stage of operation, the pH of the reactors got decreased significantly due to the formation of organic acid. The pH of the reactors fluctuated between 6.2 to 7.6. The presence of carbohydrates, cellulose, and hemicellulose in FVW affects the pH of the system through hydrolysis. Hydrolysis and acidification of carbohydrates and celluloses results in a pH drop due to higher hydrolysis rates in the initial stage, while the hydrolysate of the proteins and complex organic matter could buffer the system with a slight change in pH [42]. In addition, the pH of the reactors having higher (I/S) ratio depicted stable pH change and it was between 6.5 to 7.6. This may be due to synergistic effects between individual substrates of the composite waste. Furthermore, a lower concentration of the substrate decreased the hydrolysis rate at the initial stage which regulated the hydrolysis and acidification processes. Thereafter, the pH gradually elevated and reached an approximate equivalence of about 7.0 on day 8, possibly due to the consumption of organic acids.

4.2. VFA/Alkalinity Ratio

Due to the high biodegradability of fruit and vegetable waste, high VFA is produced which is mainly responsible for the instability of the reactor. For stable operation of the digester, the alkalinity should range from 2400-5000 mg $CaCO_3$/L [10, 11]. Alkalinity was maintained at 3500 mg/L. VFA/Alkalinity ratio is one of the indicators of the digester's stability which should be maintained below 0.4 [11]. VFA/Alkalinity ratio ranged between 0.25-0.35 for all set-ups during the entire digestion period which indicates that the digester was completely stable and steady throughout the operation. VFA/Alkalinity ratio at different (I/S) ratios using AS and (AS+CD) is depicted in Fig. S1 (a) and S1 (b) respectively, as supplementary materials.

4.3. Volatile Solids Reduction

Biogas yield and VS reduction are the two parameters that are taken into account to assess the reactor's performance and efficiency. VS reduction of 74% was achieved for the reactor R_2 and it was observed that with the increase in the (I/S) ratio, the VS removal efficiency of the reactor decreases. The VS reduction for R_1, R_3, R_4, and R_5 was observed as 66, 67.5%, 64%, and 61.5%, respectively using anaerobic sludge and CD. However, a higher % of VS reduction was recorded for the reactor having anaerobic sludge and cow dung as inoculum. The reduction in VS was recorded as 62%, 66%, 58%, 57% and 54% for R_1, R_2, R3, R4, and

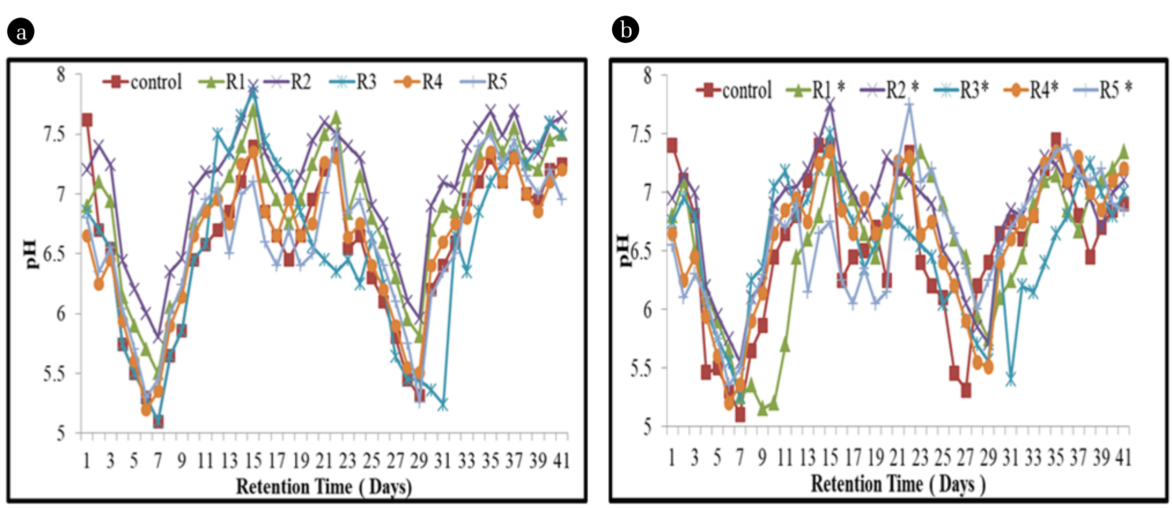


Fig. 5. pH variation at different (I/S) ratios (a) using AS (b) using AS+CD

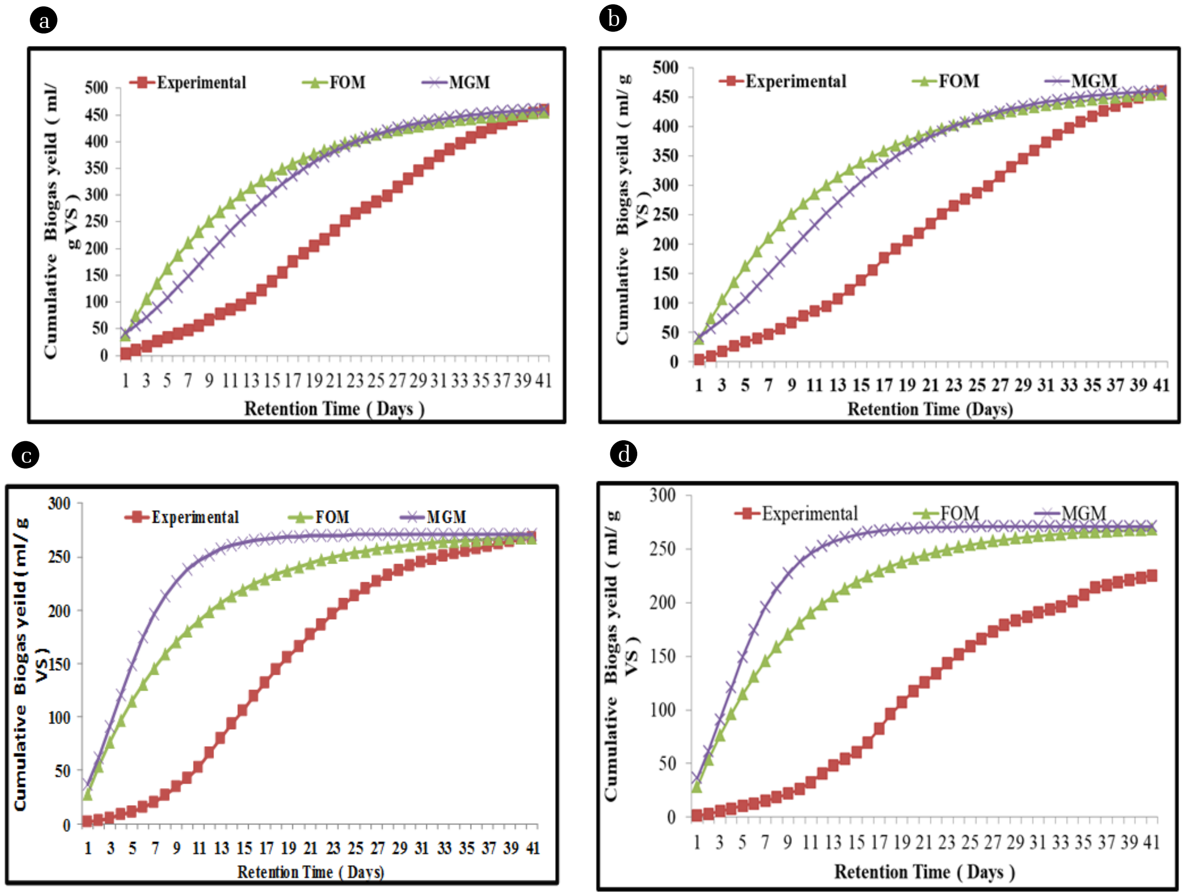


Fig. 6. Comparison of different models with experimental values for R^2 (a) using AS+CD (b) using AS only (c) for control reactor using AS+CD (d) for control reactor using AS

R_5, respectively using anaerobic sludge as inoculum. The results are found in accordance with the literature [37].

5. Kinetic Modelling for AD of FVW

The parametric values of k, R^2 and R_m values at optimal (I/S) ratios and for control reactors using AS and AS+CD are provided in the Table S1 as supplementary material. The parametric values of k and corresponding R^2 and Rm values for different (I/S) ratios are given in the supplementary material (Table S1). The experimental value of cumulative biogas at the end was taken as Y_0. Fig. 6(a), 6(b), 6(c), and 6(d) depict the comparison of the observed and predicted biogas yield at optimal (I/S) ratio and control condition for AS and AS+CD using the first-order kinetic and modified Gompertz model. An almost similar trend was observed for all the (I/S) ratio using AS+CD in a (1:1) ratio. Cumulative yields throughout the experiment duration are plotted against the cumulative yield obtained from the output of kinetic models adopted. It is evident that both the first-order model and the modified Gompertz equation have been able to predict the cumulative yield without major deviation from the experimental data. The value of R^2 indicates the best fitting of the statistical models. The highest value of biogas production (K) was found to be 0.1063 /d obtained for the (I/S) ratio 0.3 using (AS+CD) and 0.0855 /d for AS, which is also characterized by maximum specific biogas yield as 21.26 and 18.57 mL/g VS/day respectively.

6. Conclusions

For enhanced biogas production, mixed sludge has been proven to be the most suitable co-substrate for the valorization of the FVW. It was proved to be the best over the anaerobic sludge for higher biogas production. Anaerobic co-digestion of anaerobic sludge (AS) with fruit and vegetable wastes (FVW) is beneficial and offers a major promising solution for maximizing methane production. In the present study, anaerobic co-digestion of FVW and (AS+CD) at various mixing ratios was performed to obtain the best mixing ratio for optimal methane production. The highest methane yield was obtained at a mixing ratio of 30:70 (AS+CD) to FVW - 468.82 ml/g VS added), which was higher than the mono-digestion of the FVW (270.94 ml/g VS added). When the (I/S) ratio was increased beyond 0.4, the biogas yield started declining, which is attributed to an imbalance in the nutrients. The minimum cumulative biogas yield was recorded at a mixing ratio of 60:40 (359.95 mL/g VS added). When AS was used in conjunction with CD gave the best process stability and resulted in the highest biomethane production. An increase in the inoculum-to-substrate ratio beyond 0.5 reduced the biogas output which is mainly because of the higher OLR of the feedstock. pH and alkalinity of the digester play a vital role in the performance of the digester which needs to be monitored and regulated. The inclusion of cow dung helped in regulating the pH of the digester. Experimental data was validated with the First order model and Modified Gompertz Model and the results fit well with the MGM model. However, the techno-economic analysis could reveal the potential of the proposed methodology for scale up, a further study on pilot scale digester is recommended. Cost-benefit analysis can be performed based on the data obtained. The comparison of the various studies with the present study is illustrated in Table S2.

Conflict of Interest

The authors declare that they have no conflict of interest.

Author Contribution

A.A. (PhD Student) conceived, designed, conducted the study, and drafted the manuscript. P.K.C. (Associate Professor) supervised, resourced, and validated the work. P.G. (Associate Professor) supervised, suggested, revised, and edited the manuscript. All authors read and approved the final manuscript.

References

1. Viturtia AM, Mata-Alvarez J, Cecchi F. Two-phase continuous anaerobic digestion of fruit and vegetable wastes. *Resour. Conserv. Recycl.* 1995;13:257-267. https://doi.org/10.1016/0269-7483(89)90130-4
2. Melikoglu M, Lin CS, Webb C. Analysing global food waste problem: pinpointing the facts and estimating the energy content. *Cent. Eur. J. Eng.* 2013;3:157-164. https://doi.org/10.2478/s13531-012-0058-5
3. Zhu B, Gikas P, Zhang R, Lord J, Jenkins B, Li X. Characteristics and biogas production potential of municipal solid wastes pre-treated with a rotary drum reactor. *Bioresour. Technol.* 2009;100:1122-1129. https://doi.org/10.1016/j.biortech.2008.08.024
4. Lin J, Zuo J, Gan L, et al. Effects of mixture ratio on anaerobic co-digestion with fruit and vegetable waste and food waste of China. *J. Environ. Sci.* 2011;23:1403-1408. https://doi.org/10.1016/S1001-0742(10)60572-4
5. Vijay VK, Kapoor R, Trivedi A, Vijay V. Biogas as clean fuel for cooking and transportation needs in India. *Adv. Bioprocess Technol.* 2015; 257-275. https://doi.org/10.1007/978-3-319-17915-5_14
6. Nguyen PH, Kuruparan P, Visvanathan C. Anaerobic digestion of municipal solid waste as a treatment prior to landfill. *Bioresour. Technol.* 2007; 98:380-387. https://doi.org/10.1016/j.biortech.2005.12.018
7. Khalid A, Arshad M, Anjum M, Mahmood T, Dawson L. The anaerobic digestion of solid organic waste. *Waste Manage.* 2011; 31:1737-1744. https://doi.org/10.1016/j.wasman.2011.03.021
8. Weiland P. Biogas production: current state and perspectives. *Appl. Microbiol. Biotechnol.* 2010;85:849-860. http://dx.doi.org/10.1007/s00253-009-2246-7
9. Aybek A, Üçok S. Determination and evaluation of biogas and methane productions of vegetable and fruit wastes with Hohenheim Batch Test method. *Int. J. Agric. Biol. Eng.* 2017; 10(4):207-215. http://doi.org/10.25165/j.ijabe.20171004.2864
10. Boulanger A, Pinet E, Bouix M, Bouchez T, Mansour AA. Effect of inoculum to substrate ratio (I/S) on municipal solid waste anaerobic degradation kinetics and potential. *Waste Manage.* 2012; 32:2258-2265. https://doi.org/10.1016/j.wasman.2012.07.024
11. Chatterjee B, Mazumder D. Anaerobic digestion for the stabilization of the organic fraction of municipal solid waste: A review. *Environ. Rev.* 2016;24:426-459. https://doi.org/10.1139/er-2015-0077
12. Agrawal A, Chaudhari PK, Ghosh P. Anaerobic digestion of fruit and vegetable waste: a critical review of associated challenges. *Environ. Sci. Pollut. Res.* 2022;4:1-26. https://doi.org/10.1007/s11356-022-216437
13. Raposo F, Fernández Cegrí V, De la Rubia MA, et al. Biochemical methane potential (BMP) of solid organic substrates: evaluation of anaerobic biodegradability using data from an international interlaboratory study. *J. Chem. Technol. Biotechnol.* 2011;86: 1088-1098. https://doi.org/10.1002/jctb.2622
14. Labatut RA, Angenent LT, Scott NR. Biochemical methane potential and biodegradability of complex organic substrates. *Bioresour. Technol.* 2011;102:2255–2264. https://doi.org/10.1016/j.biortech.2010.10.035
15. Liu G, Zhang R, El-Mashada HM, Dong R. Effect of feed to inoculum ratios on biogas yields of food and green wastes. *Bioresour. Technol.* 2009;100:5103–5108. https://doi.org/10.1016/j.biortech.2009.03.081
16. Yoon YM, Kim SH, Shin KS, Kim CH. Effects of substrate to inoculum ratio on the biochemical methane potential of piggery slaughterhouse wastes. *Asian-Australas. J. Anim. Sci.* 2014;27:600. https://doi.org/10.5713%2Fajas.2013.13537
17. Feng L, Li Y, Chen C, et al. Biochemical methane potential (BMP) of vinegar residue and the influence of feed to inoculum ratios on biogas production. *BioResources* 2013;8:2487-2498. http://dx.doi.org/10.15376/biores.8.2.2487-2498
18. Neves L, Oliveira R, Alves MM. Influence of inoculum activity on the biomethanization of a kitchen waste under different waste/inoculum ratios. *Process Biochem.* 2004;39:2019-2024. https://doi.org/10.1016/j.procbio.2003.10.002
19. Veeken A, Hamelers B. Effect of temperature on the hydrolysis rate of selected biowaste components. *Bioresour. Technol.* 1999; 69:249-254. https://doi.org/10.1016/S0960-8524(98)00188-6
20. Kalyuzhnyi S, Veeken A, Hamelers B. Two-particle model of anaerobic solid-state fermentation. *Water Sci. Technol.* 2000; 41:43–50. https://doi.org/10.2166/wst.2000.0054
21. Lesteur M, Bellon-Maurel V, Gonzalez C, et al. Alternative

methods for determining anaerobic biodegradability: a review. *Process Biochem.* 2010;45:431-440. https://doi.org/10.1016/j.procbio.2009.11.018

22. Fernandez B, Porrier P, Chamy R. Effect of inoculum-substrate ratio on the start-up of solid waste anaerobic digesters. *Water Sci. Technol.* 2001;44:103-108. https://doi.org/10.2166/wst.2001.0191
23. El-Mashad HM, Zhang R. Biogas production from co-digestion of dairy manure and food waste. *Bioresour. Technol.* 2010; 101:4021-4028. https://doi.org/10.1016/j.biortech.2010.01.027
24. Raposo F, Borja R, Rincon B, Jimenez AM. Assessment of process control parameters in the biochemical methane potential of sunflower oil cake. *Biomass Bioenergy.* 2008;32:1235-1244. https://doi.org/10.101.6/j.biombioe.2008.02.019
25. Mareno-Andrade I, Buitron G. Influence of the origin of the inoculum on the anaerobic biodegradability test. *Water Sci. Technol.* 2004;49:53-59. https://doi.org/10.2166/wst.2004.0017
26. Li D, Chen L, Liu X, et al. Instability mechanisms and early warning indicators for mesophilic anaerobic digestion of vegetable waste. *Bioresour. Technol.* 2017;245:90-97. https://doi.org/10.1016/j.biortech.2017.07.098
27. Lavagnolo MC, Girotto F, Rafieenia R, Danieli L, Alibardi L. Two-stage anaerobic digestion of the organic fraction of municipal solid waste–Effects of process conditions during batch tests. *Renew. Energy* 2018;126:14-20. https://doi.org/10.1016/j.renene.2018.03.039
28. Zhang C, Su H, Baeyens J, Tan T. Reviewing the anaerobic digestion of food waste for biogas production. *Renew. Sustain. Energy Rev.* 2014;38:383-392. https://doi.org/10.1016/j.rser.2014.05.038
29. Cabbai V, Ballico M, Aneggi E, Goi D. BMP tests of source selected OFMSW to evaluate anaerobic codigestion with sewage sludge. *Waste Manage.* 2013;33:1626-1632. https://doi.org/10.1016/j.wasman.2013.03.020
30. APHA. Standard methods for examination of water and waste water, 17th ed. American Public Health Association;1989. Washington.
31. Hashimoto AG, Varel VH, Chen YR. Ultimate methane yield from beef cattle manure: effect t of temperature, ration constituents, antibiotics, and manure age. *Agric. Wastes* 1981; 3:241-256. https://doi.org/10.1016/0141-4607(81)90011-1
32. Raposo F, Borja R, Martín MA, Martín A, De la Rubia MA, Rincón B. Influence of inoculum–substrate ratio on the anaerobic digestion of sunflower oil cake in batch mode: process stability and kinetic evaluation. *Chem. Eng. J.* 2009; 149:70-77. https://doi.org/10.1016/j.cej.2008.10.001
33. Habiba L, Hassib B, Moktar H. Improvement of activated sludge stabilisation and filterability during anaerobic digestion by fruit and vegetable waste addition. *Bioresour. Technol.* 2009; 100:1555-1560. https://doi.org/10.1016/j.biortech.2008.09.019
34. Elsayed M, Andres Y, Blel W. Anaerobic co-digestion of linen, sugar beet pulp, and wheat straw with cow manure: Effects of mixing ratio and transient change of co-substrate. *Biomass Convers. Biorefin.* 2022; 1-10. https://doi.org/10.1007/s13399-021-02229-8
35. Vats N, Khan AA, Ahmad K. Effect of substrate ratio on biogas yield for anaerobic co-digestion of fruit vegetable waste & sugarcane bagasse. *Environ. Technol. Innovation.* 2019;13:331-339. https://doi.org/10.1016/j.eti.2019.01.003
36. Ahmed B, Tyagi VK, Khan AA, Kazmi AA. Optimization of process parameters for enhanced biogas yield from anaerobic co-digestion of OFMSW and bio-solids. *Biomass Convers. Biorefin.* 2020;1-12. https://doi.org/10.1007/s13399-020-00919-3.
37. Neves L, Oliveira R, Alves MM. Influence of inoculum activity on the bio-methanization of a kitchen waste under different waste/inoculum ratios. *Process Biochem.* 2004;39:2019-2024. https://doi.org/10.1016/j.procbio.2003.10.002
38. Owamah HI, Ikpeseni SC, Alfa MI, et al. Influence of inoculum/substrate ratio on biogas yield and kinetics from the anaerobic co-digestion of food waste and maize husk. *Environ. Nanotechnol. Monit. Manag.* 2021;16:100558. https://doi.org/10.1016/j.enmm.2021.100558
39. Sri Bala Kameswari K, Kalyanaraman C, Porselvam S, Thanasekaran K. Optimization of inoculum to substrate ratio for bio-energy generation in co-digestion of tannery solid wastes. *Clean Technol. Environ. Policy* 2012;14:241-250. http://dx.doi.org/10.1007/s10098-011-0391-z
40. Dhamodharan K, Kumar V, Kalamdhad AS. Effect of different livestock dungs as inoculum on food waste anaerobic digestion and its kinetics. *Bioresour. Technol.* 2015;180:237-241. https://doi.org/10.1016/j.biortech.2014.12.066
41. Almomani F, Shawaqfah M, Bhosale RR, Kumar A, Khraisheh MAM. Intermediate ozonation to enhance biogas production in batch and continuous systems using animal dung and agricultural waste. *Int. Biodeter. Biodegrad.* 2017;119:176-187. https://doi.org/10.1016/j.ibiod.2016.11.008
42. Dinsdale RM, Premier GC, Hawkes FR, Hawkes DL. Two-stage anaerobic co-digestion of waste activated sludge and fruit/vegetable waste using inclined tubular digesters. *Bioresour. Technol.* 2000;72:159-168. https://doi.org/10.1016/S0960-8524(99)00105-4