Effectiveness of the pretreatment methods on mesophilic anaerobic co-digestion of fruit, food and vegetable waste

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Abstract

ABSTRACT

Pretreatment of the fruit, food and vegetable waste materials could enhance the biogas generation in a shorter time along with waste reduction. The aim of the present work was to assess the effectiveness of alkaline, hydrothermal, thermal and ultrasonication pretreatment of fruit, food, and vegetable waste with cow dung for the mesophilic anaerobic co-digestion. The mesophilic anaerobic co-digestion of fruit food and vegetable waste as single substrate with cow dung were performed in a laboratory scale 1L batch digester for 30 days at 40 ± 2 °C temperature. Obtained result show that pretreatment process has enhanced the biogas and biomethane production by 35 % and 44.4 % with 19.89 % TS and 17.30 % VS removal in case of ultrasonication pretreatment (100.45 ml biogas/gVS and 27.92 ml CH₄/gVS.), while slight increase is found with thermal and hydrothermal pretreatment as compared to untreated FFVW. The biogas production is enhanced by 35%, 20.4%, 5%, 6% and biomethane production are enhanced by 44.4%, 22%, 11%, 9.8% in the case of ultrasonication, alkaline, thermal and hydrothermal pretreatment process, respectively. The stable mesophilic anaerobic co-digestion operation was achieved and impacts of the different pretreatment methods on the biogas yield and net energy recovery along with viability of anaerobic co-digestion have been explored.

Highlights

- Effectiveness of alkaline, hydrothermal, thermal and ultrasonication pretreatment on fruit, food and vegetable waste through mesophilic anaerobic co-digestion was investigated.
- Net energy (biogas and biomethane) recovery and economic viability of mesophilic anaerobic co-digestion for fruit, food and vegetable waste was analysed.
- The enhancement in the biogas production by 35%, 20.4%, 5%, 6% through ultrasonication, alkaline, thermal and hydrothermal pretreatment, respectively.
- The enhanced biomethane production are achieved by 44.4%, 22%, 11%, 9.8% through ultrasonication, alkaline, thermal and hydrothermal pretreatment, respectively

Introduction

The gradual increase in the discharge of fruit, food, and vegetable waste (FFVW) have a lethal impact on the environment in many developing countries due to their slow rate of biodegradability and co-digestion reaction (Morales-Polo et al. 2019). Waste generation and energy crisis due to increasing population, industrialization and urbanization are major challenges in the most developing countries in 21st Century (Mondal et al. 2017; Shankar et al. 2017; Rani et al. 2017; Chaurasia and Mondal 2021). FFVW are the major organic component present in the municipal solid waste (MSW) and it is generated from the household activity of food, fruit and vegetable (kitchen refuse), farming, restaurant and commercial activity (Zhang et al. 2019; Al bkoor Alrawashdeh 2019). The relative amount of FFVW in MSW depends on the urbanization and consumption standards of the community (Jain et al. 2015; Pavi et al. 2017). It is estimated that around 33 percent of the FFVW is wasted globally (about 1.3 billion tons per year) and it is increased by 44% by 2025, while India (~ 15%) is the second largest producer of FFVW in the world (Handous et al. 2019; Li et al. 2019). The improper and open disposal of FFVW have adverse effects on the environment by contaminating the water, generation of odour, greenhouse gases emissions, breeding of mosquitos and rodents. The most common FFVW stabilization technology in world-wide practice biological, thermal and thermochemical process that includes landfilling, composting, incineration and anaerobic co-digestion (Balussou et
Landfills technologies has adverse impacts on soil, groundwater, and climate, while incineration of FFVW increases air-pollution by the emission of harmful gases such as oxide of sulphur and nitrogen, and other greenhouse gases in the environment (Hong et al. 2018; Sittijunda and Reungsang 2018). Among these waste stabilization techniques only few are sustainable such as anaerobic co-digestion etc. that being widely used or reported and being commercialized (Akshaya and Jacob 2018; N et al. 2020). Although FFVW or municipal waste stabilization techniques are described in the literatures but recent research studied are focused on the improvement in the resources recovery and waste stabilization along with process development (Ariunbaatar et al. 2014a; Akshaya and Jacob 2018; N et al. 2020). Biological or biochemical waste stabilization techniques such as anaerobic co-digestion along with some other got potential research attention in the last decade in many developing countries due to it is advantages such as energy and resources recovery (value-added products, chemicals etc.) along with waste reduction (Ariunbaatar et al. 2014a; Rodriguez Correa and Kruse 2018; Akshaya and Jacob 2018; Morales-Polo et al. 2019; Shen et al. 2019; N et al. 2020). Anaerobic co-digestion (AD) converts FFVW materials into biogas/biomethane along with quite stabilized organic fertilizer and concurrently treats the residues with reduction in the waste (Akshaya and Jacob 2018; Bayrakdar 2019; Li et al. 2019; Chaurasia et al. 2020a). Anaerobic co-digestion (AD) converts FFVW materials into biogas/biomethane through hydrolysis, acidogenesis, acetogenesis and methanogenesis. In which, hydrolysis is the rate-limiting step for biogas generation reaction because it has higher influence on the degradation efficiency of the feedstock or waste materials (Razaghi et al. 2016; Pham Van et al. 2019). Mesophilic AD (30–50°C) has given preference over thermophilic AD (> 50°C) due to higher rate of organic waste degradation and methane production, while thermophilic AD has some operational disadvantages such as high operational cost, susceptibility to process parameter stability, high risk of inhibition due to sudden environmental changes and complex structural facilities (Wang et al. 2018; Zhang et al. 2019). Therefore, in practice mesophilic AD is preferential process over thermophilic AD for prolonged operation of AD.

Pretreatment of substrate such as ultrasonication, thermal, alkaline etc. are the essential to accelerate the AD process for obtaining value-added by-products and increases the biogas/biomethane yield (Li et al. 2017, 2019). Pretreatment of FFVW is additional step required to make AD more preferential waste stabilization techniques due to establishing proper synergism in the digestion medium, breakdown of complex nutrients, provides proper C/N ratio, solubilisation of recalcitrant components and increases the biodegradability of the organic content (Pavi et al. 2017; Akshaya and Jacob 2018; Li et al. 2019). Pretreatment of FFVW is mainly required due to presence of undesirable components such as lignin and complex components because degradation of lignin is difficult due to the presence of 3- phenyl-propane precursor monomers, and it prevent availability of cellulose and hemicellulose to the microorganism as well as slower the hydrolysis rate in the AD process (Chen et al. 2016; Baruah et al. 2018). Thus, pretreatment helps in the degradation of lignin and undesirable components, which ensure the availability of the feedstock to the microbial species along with reduction in the susceptibility of the enzymes towards hydrolysis (Mozhiarasi et al. 2020). So, the pretreatment of the FFVW or feedstock offer more substrate constituent available for the microorganism that enhanced the resources recovery of the process such as improvement in the biogas yield. Different pretreatment methods have different impacts and influence on the overall efficiency and economy of the AD process for the processing of FFVW (Ariunbaatar et al. 2015a; Collard et al. 2015; Chaurasia et al. 2020b). The suitability of a pretreatment method dependent on the composition of the FFVW, while composition of the FFVW waste is vary with geography and site of the FFVW waste generation. Therefore, selection of most effective pretreatment method for the AD process needs to be explored.

Few literature recommended that FFVW should be used for AD in alkaline media to ensure that stable AD performance because alkaline pretreatment lowering the lignin content and enhanced enzymatic hydrolysis (Xiang et al. 2016; Singh et al. 2019). In thermal pretreatment, temperature has the important role to change the rate of biogas
production such as thermal pre-treatment of FW at 120 °C has increases the biogas production by 24% (Ariunbaatar et al. 2015a), whereas thermal pretreatment at 170 °C decreases the rate of biogas production by 8% (Ariunbaatar et al. 2015a; Al bkoor Alrawashdeh 2019). Thermal pretreatment at 80 °C for 1.5 h and thermophilic pretreatment at 50 °C for 6–12 h can increases the biogas yield around 40% (Ariunbaatar et al. 2014b; Al bkoor Alrawashdeh 2019). Ultrasonication pretreatment on cattle manure with food waste and sludge can enhanced the rate of biogas production of 0.85 L CH$_4$/L d$^{-1}$ by 31% as compared to untreated (Verma et al. 2017; Sasmal et al. 2018). The study on cow dung has concluded that the maximum biogas yield (603 L CH$_4$/kg VS) by a mixture of 70% manure, 20% food waste and 10% sewage sludge operating at 36 °C with OLR of 1.2g VS/ L day (Verma et al. 2016; Al bkoor Alrawashdeh 2019). Biodegradability, high volatile solids and moisture content along with low solid content are characteristics of FFVW. These characteristic of FFVW are getting more research interest in last decade for their waste to energy conversion process such as AD and the different pretreatment methods on the feedstock. Very few literatures were available on the comparisons of alkaline, hydrothermal, thermal and ultrasonication pretreatment of FFVW for mesophilic AD process.

The aim of the present study was to investigate the effectiveness of alkaline, hydrothermal, thermal and ultrasonication pretreatment process in order to estimate the biogas/biomethane potential of FFVW through mesophilic AD. Initially, a series of batch experimentation were conducted to optimize the AD process parameter along with pretreatment process. The mesophilic AD operation of FFVW (FFVW are considered as single substrate) with cow dung were performed in a laboratory scale 1L batch co-digester for 30 days at 40 ± 2 °C temperature. Characteristics of the pretreated FFVW, effect of pH on mesophilic AD, effect of pretreatment on the volatile solid (VS) and total solid (TS) removal, biogas and biomethane production and yield, enhancement in biogas/biomethane yield due to pretreatment, process economic analysis including operating cost, net energy analysis has been studied.

**Materials And Methods**

**FFVW as single substrate for AD process:** The FFVW composition depends on the region, culture, season and demographic factors, Therefore, synthetic FFVW was prepared as described in the literature (Ariunbaatar et al. 2014b) to get desirable C/N ratio in the feedstock. The typical composition of FFVW used in this study are described in Table 1.

| Component            | % Wet weight | % Wet weight fraction from other study* |
|----------------------|--------------|----------------------------------------|
| Fruits and fruit peelings | 30           | 25-30                                  |
| vegetables           | 30           | 25-30                                  |
| Egg                  | 20           | 6-10                                   |
| bread                | 10           | 5-12                                   |
| chapatti             | 10           | 5-10                                   |
| Miscellaneous        | -            | 0-25                                   |

* MTT Agrifood Research Finland, 2010
The FFVW sample was brought from the IIT Roorkee, India, campus cafeteria and brought the composition as closest to the literatures (Yong et al. 2015; Nanda et al. 2016; Wang et al. 2018) through content measurement. Initially, sample were sorted, shredded, homogenised to get ~ 5 mm particles size and after that sample was mixed with tap water to achieve desirable TS. These steps are used to enables the FFVW feedstock for passive leaching of soluble sugars and nutrients (Voelklein et al. 2017). FFVW sample was store at 4 °C and cow dung was store at 37 °C overnight and brought at the room temperature for the experimentation to get adopt in mesophilic conditions.

Pre-treatment methods: As per the reported literature each FFVW digester was sterilized by using autoclave at 121 °C (15 Psi) for 30 min prior to the pretreatment. Pretreatment methods such as alkaline, hydrothermal, thermal and ultrasonication were applied separately on each set of FFVW sample along with control (untreated). After that sample were applied for the mesophilic AD experimentations process as reported in literature (Xiang et al. 2016; Singh et al. 2019).

Alkaline pre-treatment: The alkaline pretreatment of FFVW was employed by adding 6N NaOH (2% W/V) with continuous stirring at 200 rpm at room temperature (25 °C) for 0.5 h (Xiang et al. 2016). The pH of FFVW was maintained using citrate-phosphate buffer of neutral pH after that sample was applied to mesophilic AD experimentation to investigate the biogas production potential.

Hydrothermal pre-treatment: Hydrothermal pretreatment was performed on the FFVW by autoclaving at 120±2 °C for 0.5 h in closed air sealed batch digester (Ding et al. 2017). After that FFVW sample were cooled down to room temperature. Pretreated residue was collected by filtration, washed 3 times with deionized water and left in freezer for freeze-dried for 24 h overnight in order to freeze the bacterial metabolic activity.

Thermal pretreatment: Thermal pretreatment was carried out on FFVW substrate by kept the FFVW sample in a glass jar with properly sealed in hot water at 80±2 °C temperature for 1.5 h in hot water bath tub (Ariunbaatar et al. 2014b). Further, the sample brought to the room temperature and then applied to the mesophilic AD experimentation.

Ultrasonication: Ultrasonication pretreatment were performed by using a laboratory-scale sonicator instrument (Model UP400S, 400W, Hielscher, Germany). FFVW substrate was sonicated in a beaker continuously stirred with magnetic stirrer, operating at 80 µm amplitude and 24 kHz for 15 minutes in hot water bath (35±2 °C temperature). The ultrasound probe (disruptor) was immersed into the FFVW at a depth of 3.5 cm (Ma et al. 2021). After that FFVW sample was left to cooled down until the room temperature then sample was applied for mesophilic AD process.

Experimental setup: Initially, a series of batch experiments were conducted to optimize the experimental AD parameter. FFVW sample was considered as the single substrate for the mesophilic AD operation. The mesophilic AD of FFVW with cow dung were performed in a laboratory scale 1L batch digester for 30 days at 40±2 °C temperature. The experiments were carried out in a glass bottle batch digester with working volume of 1 litre contained 450 ml FFVW, 150 ml cow dung and 100 ml deionized water, which is FFVW to cow dung (as inoculum) ratio was around 0.3 g-VS/g-VS for each batch co-digester. Each digester was sterilized by autoclaving at 121 °C for 30 minutes before the addition of cow dung as microbical sources. Each digester after the addition of cow dung was sparged with nitrogen gas with flow rate of 30 ml per minute for 15 min to achieved the anaerobic condition. All the digesters were operated under mesophilic condition (40±2 °C) using a thermostatic water bath where temperature was controlled using hotwater circulator. Untreated FFVW digester was taken as a control to investigate the effect of pretreatment on biogas production, while other parameter was kept constant. In the text digesters are symbolized as untreated FFVW (R1), ultrasonication treated (R2), alkaline treated (R3), thermal treated (R4) and hydrothermal treated (R5) as specified in Table 3. Each digester was designed with three ports on the headspace for purging from nitrogen, gas collection unit
via silicon tube and for sample collection through gas syringe for further analysis as presented in Fig. 1. The experiments were conducted in triplicate and experimental data point are obtained at an average value.

**Analytical techniques:** Sample and produced gas was collected and analysed on daily basis. Parameter such as total solid (TS), volatile solid (VS), ash content, density and carbon-nitrogen ratio were measured according to standard protocol of the APHA (2012) (Zhai et al. 2015). The chemical oxygen demand (COD) and alkalinity of the influent and effluent samples were measured by Hanna multiparameter (HI83099, Hanna USA) with their reagent as per standard protocol (APHA, 2012). Conductivity and pH were measured by Hanna digital meter (HI 98311, Hanna USA). The concentrations of carbohydrates and others were analysed by using the UV spectrophotometer (UV-1800, Shimadzu, Japan). The volume of produced biogas was daily measured through a water displacement technique. In which digester was connected to measuring cylinder filled with acidified water saturated with salt using silicon tube. Gas volume was determined by based on the amount of water displaced from the gas collection unit. The biogas and biomethane content was qualitatively analysed using a gas chromatograph (NEWCHROME 6800 gas chromatograph, India, installed with Porapak-Q column) and quantification of the produce gases (biomethane and biogas) were estimated. The injected volume sample was 60 µl and operational conditions were as follows: argon gas as carrier gas (30 ml/min), injector and oven temperature 60 °C, and detector temperature 90°. Calibration of gas chromatography instruments was done before the analysis of sample by using a standard gas composition H₂ 5.18%, CO 4.98%, CH₄ 39.72%, CO₂ 30.06% and N₂ 20.06%.

**Net energy analysis:** The net energy balance was estimated for pretreatment step and extra energy produced due to pretreatment, while capital cost was neglected in this study. The energy based calculation was done as described by (Bouallagui et al. 2004; Ariunbaatar et al. 2014b, 2015a) with some consideration for calculation simplicity as: the net energy balance was made for the 1 ton FFVW (27 m³ digester volume) in term of total energy requirements for the pretreatment step and energy generated in the form of biogas, 0.85 conversion factor for biogas to thermal energy and energy content of biomethane (6.5 kWh/m³). The density of FFVW and inoculum around 1030-1078 Kg/m³. The material for digester and pretreatment is same with thermal conductivity low as 0.022 Wm⁻¹K⁻¹ and ambient temperature around 25 °C. The energy calculation was based on whole digester basis.

**Cost estimation:** Implementation viability of any commercial process is sole depends on the economics of the process. Operating cost of mesophilic AD and pretreatment process includes cost of chemical, electricity, sludge disposal, transportations, fixed cost etc. (Tawk et al. 2020). In the present study, chemical cost and electricity charges were taken in account for preliminary economic evaluation with assumption for 1 ton/m³ FFVW (Eq. 1) (Wang et al. 2021).

\[
\text{Operating cost} = a \times C_{\text{chemical}} + b \times C_{\text{electricity}} + c \times C_{\text{Nitrogen gas}} \quad \text{(Eq. 1)}
\]

Where, \(C_{\text{Chemical}}\) (Kg or L), \(C_{\text{Electricity}}\) (kWh) and \(C_{\text{Nitrogen gas}}\) (Kg) are amount of chemical material, electrical energy and gas require during process, respectively. Whereas, \(a\), \(b\) and \(c\) are coefficient of chemical material price, industrial electricity price and industrial gas price of the market as in the year 2019, respectively.

**Results And Discussion**
**Characteristics of FFVW and cow dung:** For the stable operation of AD and biogas yield required essential AD parameter must be within optimum range for the AD process (Wang et al. 2018). FFVW and cow dung are characterized before pretreatment process and on daily basis during experimentations as shown in the Table 1. The FFVW had a TS of 16.63 % and moisture 83.36 %, which is in a ratio of 5. The C/N ratio in FFVW is 17.95 and in cow dung 20.71 which is in optimum range (15-30) as given in many literatures (Ariunbaatar et al. 2014b). FFVW and cow dung had protein content around 16 and 13 % of TS, carbohydrates 25 and 60 % of TS and COD is 215 and 112 g/L, respectively. The characteristics of FFVW as shown in the Table 2, and characteristic of FFVW feedstock in the digester as shown in Table 3, clearly indicate that the FFVW could be a potential substrate for mesophilic AD process (Ariunbaatar et al. 2014c). VS present in the cow dung around 82% of TS can be assume that mostly contained microbial species (Shah et al. 2015; Akshaya and Jacob 2018).

Table 2 Characteristics of substrate (FFVW) and inoculum (cow dung).

| S. No. | Parameter                  | Unit         | FFVW | Cow dung |
|-------|----------------------------|--------------|------|----------|
| 1     | Total solid (TS)           | %            | 16.63| 15.99    |
| 2     | Moisture                   | %            | 83.36| 84.00    |
| 3     | Volatile solid (VS)        | %            | 9.58 | 13.11    |
| 4     | Ash content                | %TS          | 42.36| 18.01    |
| 5     | Total suspended solid (TSS)| %TS          | 63.70| 78.28    |
| 6     | Volatile suspended solid (VSS)| %TSS | 86.08| 92.21    |
| 7     | Density                    | Kg/m$^3$     | 1.11 | 1.07     |
| 8     | pH                         | -            | 5.66±.01| 7.12     |
| 9     | Alkalinity                 | mg/L as CaCO3 equivalent | 950-1050 | 4000-4100 |
| 10    | Carbohydrate (Glucose)     | %TS          | 25±1 | 60±1     |
| 11    | Protein                    | %TS          | 16±1 | 13±1     |
| 12    | COD                        | g/L          | 215.51| 112.72   |
| 13    | Soluble COD                | g/L          | 70.73 | 41.13    |
| 14    | Total organic carbon       | %TS          | 47.39| 43.69    |
| 15    | Total organic nitrogen     | %TS          | 2.64 | 2.11     |
| 16    | C/N                        | -            | 17.95| 20.71    |

**Characteristics of FFVW feedstock in the untreated and pretreated batch co-digester:** Mesophilic AD is a complex and multiphase process that would simultaneously digest substrates in order to produce the substrate for the next phase reaction. This can be achieved by a stable AD process and proper microbial growth. Thus, it is important to maintain the essential parameter in the correct range during AD operation, to ensure the appropriate AD operation. All the co-digester has TS in the range of 14-15 %, VS in the range of 12-13 % and carbohydrates in the range of 33-35 % of TS, respectively. The density of co-digester ranges between 1030-1078 g/m$^3$ with 85-86% of moisture content and COD ranges between 1530-1620 mg/L. So, the most of the essential AD parameter as shown in Table 3, are within optimum range for appropriate AD operation (Neves et al. 2009; Morales-Polo et al. 2019; Li et al. 2019). The pH of
the mesophilic AD co-digester ranges between 6.25-6.32 except R3 whose pH was 7.6 due alkaline pretreatment (introduction of 6 N NaOH). It indicates pH of co-digester is near to optimum pH range for AD (Zhai et al. 2015), while FFVW show slight alkalinity and that may control a possible acidification during the digestion (Bouallagui et al. 2004), whereas inoculum has high pH that help to maintaining stability during the mesophilic AD (Sittijunda and Reungsang 2018). The C/N ratio in the range of 18 to 19, which is in optimum range and it provide essential nutrients for the growth of anaerobic bacteria as well as maintaining stability during digestion process (Li et al. 2011; Jain et al. 2015; Yong et al. 2015). The ash content was initially around 42 % of TS in FFVW and 18% of TS in cow dung, while after pretreatment it is in range of around 7-14 % of TS in both that is close to desirable range for the AD (Ariunbaatar et al. 2014c; Nanda et al. 2016; Voelklein et al. 2017). FFVW has slightly higher ash content due to the presence of eggshells, impurity (soil) and inorganics in FFVW sample.

### Table 3 The characteristics of untreated and pretreated batch digester.

| S. No. | Parameter          | Units | R1 (FFVW + Cow dung) | R2 (FFVW+ Cow dung) | R3 (FFVW + Cow dung) | R4 (FFVW+ Cow dung) | R5 (FFVW+ Cow dung) |
|--------|--------------------|-------|----------------------|---------------------|---------------------|---------------------|---------------------|
| 1      | Total solid (TS)   | %     | 14.35                | 14.20               | 14.02               | 14.03               | 14.03               |
| 2      | Moisture           | %     | 85.64                | 85.79               | 85.97               | 85.97               | 85.96               |
| 3      | Volatile solid (VS)| %     | 13.12                | 13.14               | 12.4                | 12.11               | 12.11               |
| 4      | Ash content        | %TS   | 8.607                | 7.5                 | 11.55               | 13.67               | 13.67               |
| 5      | Density            | Kg/m3 | 1.07                 | 1.03                | 1.03                | 1.03                | 1.03                |
| 6      | pH                 |       | 6.29                 | 6.26                | 7.63                | 6.30                | 6.31                |
| 7      | Alkalinity         | mg/L  | 1600-1700            | 1550-1650           | 1950-2050           | 1450-1550           | 1500-1600           |
|        |                    | as CaCO₃ |                   |                     |                     |                     |                     |
| 8      | Carbohydrate       | %TS   | 34±1                 | 35±1                | 34±1                | 33±1                | 35±1                |
| 9      | Protein            | %TS   | 15±1                 | 15±1                | 14±1                | 14±1                | 14±1                |
| 10     | COD                | g/L   | 161.98               | 158.36              | 153.26              | 156.09              | 157.56              |
| 11     | Soluble COD        | g/L   | 55.04                | 52.96               | 50.69               | 53.15               | 53.61               |
| 12     | C/N                |       | 18.47                | 18.28               | 18.04               | 18.05               | 18.05               |

### Evaluation of pH on mesophilic AD process: Literature shows that pH could affects the activity of acidogenic and methanogenic microorganisms as well as stability of the AD process (Ding et al. 2017). The pH variation with co-
digestion time (days) of the different digester are as shown in the Fig. 2. Initially, all the digester has pH (~6.3) within the optimum range, except the R3 digester, which has pH 7.6 due to alkaline treatment. Whereas pH of all the digester start decreasing with the progress of the co-digestion process. At 7th day of fermentation R2, R4 and R5 has pH ~6, while R1 has pH ~5.9. On the other hand, R3 decrees the pH ~6.9 from 7.6 at 10th day of fermentation. This was considered appropriate for methanogenesis process and it mainly due to organic acid generation substantially during the high solid reaction phase (Yang et al. 2015). Generally, rapid accumulation of organic acid inhibits the AD process and lower the biogas yield, but the pH profile and biogas/biomethane production profile does not show it (Zhai et al. 2015). After 7th days (10th days in case of R3) pH was increases then decreases and same pattern was repeated until the saturation point of fermentation (30th days) process. Such results are prominently due to change in activity of intermediate enzyme production and metabolic pathways, while when pH starts to increase possible due to the consumption of VFAs and formation of CO₂/H₂ (Yang et al. 2015). Thus, pH of the whole AD process was lies between 5.8 to 6.32 (in case of R3 5.76-6.18). This indicates the digester has pH close to optimum range of pH for methanogenesis and it can also conclude that all the digester has almost stable AD operation for the enhanced biogas/biomethane yield. At some point of co-digestion, the pH of digester in case of R4 (except R3) digester was below the optimum pH due to the accumulation of undissociated VFAs, carbohydrates, proteins, and fats by hydrolysing microorganisms (Ma et al. 2021; Wang et al. 2021). For the stable AD operation pH was controlled by adding NaHCO₃ and NaOH in the digester. From the biogas/biomethane experimental data show that comparatively small amount of biogas produced across all the digester at outside of optimum pH, while higher amount of biogas produced at optimum pH (5.6-7.3) during 13th-20th days of fermentation. The maximum biogas produced (380 ml/days) in R3 digester at pH ~7 on 13th days, while minimum biogas produced (315 ml/days) in R1 digester at pH ~5.8. In case of R2 achieved maximum biogas production at pH ~5.9 during 13th-20th days of fermentation, whereas R4 has maximum biogas production on13th days of fermentation at pH ~5.6. On the other hand, the maximum biomethane production in most of the digester achieved on 13th days of co-digestion at pH ~7 in R3, at pH ~6 in R2 and at pH ~5.6 in R4 digester, respectively. In which maximum biomethane (116 ml/days) in case of R2 and lowest (103 ml/days) in case of R5 at pH ~5.9.

The obtained results suggest that optimum pH range protects the methanogenic bacteria from deactivation, and methanogens work effectively in optimum pH range (Yang et al. 2015; Amare et al. 2019). TS, VS, soluble COD and/or carbon content, nitrogen and the ratio between them (C/N ratio) are directly associated to the pH and an indicator of a correct development of acidogenesis (Ding et al. 2017). During the process, slight rise and drop of alkalinity was seen due to microbial metabolic activity, intermediate alkalinity is due to undissociated VFA compound formation (Singh et al. 2019). Beside this the accumulation of VFAs (insoluble macromolecular such as carbohydrates, proteins, and fats) by hydrolysing microorganism (Chen et al. 2016). During the process slight variation in the pH was observed. It may occur due to bicarbonate concentration, alkalinity of the system and CO₂ produced during the mesophilic AD.

**TS and VS removal:**

All the digester of FFVW feedstock has TS (14-15%), volatile solids (85-92% of TS) as shown in Table 3, are found in the optimum range of AD and its well agree with literature (Raposo et al. 2011; Shah et al. 2015).

The TS and VS removal across all the digester with the progress of the mesophilic AD are presented in Fig. 3(a) and 3(b). From the obtained experimental result shows that maximum TS removal was observed in the R2, R4 and R5 digester round ~16.6 %, while lowest in case of R1 (14.2 %) and R3 (14.8 %) digester. TS and VS are essential nutrient along with other parameter for stable AD operation and improved biogas/biomethane production. Thus, it can conclude that ultrasonication (R2), thermal (R4) and hydrothermal (R5) has positive impacts on TS removal and
suitable for stable AD operations as compared to untreated digester (R1). Maximum VS removal was achieved in case of R4 digester around 21.2 %, followed by R5 around 21.2 %, R2 and R3 around 19.5 %, while lowest in case of R1 digester around 17.3%. Obtained data shows that thermal pretreatment is highly suitable among another pretreatment for maximum VS removal as compared to untreated digester (R1). Obtained data clearly indicate that pretreatment enhanced the TS and VS removal and also enhanced the corresponding biogas/biomethane yield in comparison of untreated FFVW. It is well known fact that the extent of TS and VS removal give the corresponding biogas/biomethane yield as well as energy recovery (Voelklein et al. 2017; Ma et al. 2021). These results also reveal that solubilisation of complex nutrient and the extent of the TS and VS removal has the equivalent extent of the biodegradability (Xiang et al. 2016).

TS and VS degradation plays a vital role in mesophilic AD performance. From the Fig. 3(a) and 3(b), it has been observed that the initial rate of TS removal was rapid due to microbial activity and degradation of substrate was higher after that it attained equilibrium. This can be attributed to the fact that, initially TS was available for microbes and most of substrate was having VS, the proportion of which decreased at higher rate but as the time lapsed, the proportion of VS decreased and the rate of reduction of TS moved towards equilibrium (Zhang et al. 2019). Similar types of trends were observed in Fig. 3(b) and it can be concluded that the amount of biogas/biomethane produce is directly proportional to the amount of volatile content present in the substrate (Ding et al. 2017). The yield in this is slightly lower than reported literature is possible due to the inhibition or interruption of some undesirable components on the mesophilic AD process that may present in the feedstock (Ariunbaatar et al. 2014a, b; Shah et al. 2015; Yong et al. 2015; Michalopoulos et al. 2019; Li et al. 2019). Apart from these, this study shows that the stable mesophilic AD operation of FFVW as well as shows that the food and vegetable waste can be treated efficiently in the AD process along with resources recovery with waste reduction.

**Biogas production:** The daily biogas production (DBP) and cumulative biogas production (CBP) profile for all the FFVW digester are illustrated in Fig. 4(a) and 4(b). The maximum DBP was achieved in R3 (380 ml) digester followed by R2 (370 ml), R4 (345 ml), R5 (340 ml), while minimum DBP in R1 (330 ml) digester. This shows that pretreatment has improved the biogas production as compared to untreated FFVW digester and lower DBP in case of R4 and R5 possibly due to severe acidification of the reaction system caused by the fast accumulation of organic acids (Wu et al. 2016). The maximum pretreatment effects were observed in case of R3 (alkaline) and lowest impacts in case of R5 (Hydrothermal). From the Fig. 4 (a), it was observed that the biogas production started from the 4th day of fermentation and attained the maximum biogas production around 13th day and lasted around 25th days of fermentation. As the results suggest that acetogenic and methanogenic activity of microbes was maximum when VFA started to convert into CH$_4$ and CO$_2$ (Stabnikova et al. 2008). The maximum CBP production were achieved in the case of R2 (1320 ml, 100.45 ml/gVS) followed by R3 (1180 ml, 94.77 ml/gVS), R4 (1030 ml, 85.05 ml/gVS), and R5 (1040 ml, 85.87 ml/gVS) digester, respectively, while lowest in the case of R1 (980 ml, 74.69 ml/gVS) digester. This clearly indicates that pretreatment has positive impacts on biogas production and the has the maximum DBP and CBP enhancement in case of ultrasonication pretreatment (R2), while lowest enhancement in case of thermal (R4) and hydrothermal (R5) as compared to untreated FFVW (R1). The lower pretreatment impacts in R4 and R5 are may be due to generation of organic acid trigger severe acidification and low pH would stimulate acidogenic activity and inhibit methanogenic activity (Akshaya and Jacob 2018).

The CBP was maximum in case of R2 (1320 ml) followed by R3 (1180 ml), R4 (1030 ml) and R5 (1040 ml), while lowest in case of the R1 (untreated FFVW) digester (980 ml). The CBP was enhanced by pretreatment on FFVW as R2, followed by R3, R4 and R5 digester by 35 %, 20.4 %, 5 % and 6 %, respectively, in comparisons of R1 (untreated) digester. From the CBP curve as shown in Fig. 4(b), it was observed that the biogas production started from the 4th
day of fermentation and curve plateau (Fig. 4(b)) was obtained 15th-20th days of fermentations. This is possible due to the H₂ and CO₂ formation start with 3rd to 7th day and contributed in the formation of biomethane gas (Ariunbaatar et al. 2014a). The maximum DBP and CBP were observed in between 10th-15th days of fermentation across all digester due to acetogenic and methanogenic activity of microbes and at this stage VFA started to convert into CH₄ and CO₂ and its conversion contributed to the maximum biogas production (Ariunbaatar et al. 2015b). All the experimental results obtained in this study were summarized in Table 4. Obtained results indicated that the fruit, food and vegetable waste can be treated efficiently through AD process along with proper disposal of waste materials that is necessary for the sustainable development.

**Methane production:** The daily methane production (DMP), cumulative methane production (CMP) profile from the FFVW through the mesophilic AD process are shown in Fig. 5(a) and 5(b). The DMP and CMP curve profile are having similar as in nature of variation as found in case of CBP and DBP. The maximum CMP and DMP were attained in the R2 (367 ml, 27.92 CH₄/gVS) digester, R3 (310 ml, 25 CH₄/gVS) digester, R4 (282 ml, 23.28 CH₄/gVS) and R5 (279 ml, 23.03 CH₄/gVS) digester, while lowest in the case of R1 (254 ml, 19.35 CH₄/gVS) digester. Obtained data reveal that pretreatment on FFVW has improve the mesophilic AD performances and the maximum pretreatment impacts were observed in case of R2 and R3, while less impacts in case of thermal (R4) and hydrothermal (R5). Thus, it can conclude that the alkaline and ultrasonication pretreatment were most suitable on FFVW for mesophilic AD process for the recovery of the energy (Dong et al. 2010; Wu et al. 2016). It was shown that methane production was trigger on 7th days and lasted on 25th days of fermentation process., while achieved the highest peak on 13th days of fermentations. The CMP are enhanced by 44.4 %, 22 %, 11.1 %, 9.8 % in case of R2, R3, R4, R5 digester respectively, as compared by R1 digester.

Obtained experiments data for the biogas and biomethane production as VS removal show pretreatment enhanced the performance of mesophilic AD and have high impacts in case of R2 and R3 and has negative impacts in case of R4 and R5 digester. The possible reason in case of ultrasonication (R2) treatment is the cleavage of the intermolecular ester linkages between hemicelluloses and lignin, while in case of alkaline (R3) treatment is removal of acetyl groups and uronic acid substitutions in hemicelluloses that increases the accessibility of the carbohydrates to enzymatic hydrolysis (Chen et al. 2016; Baruah et al. 2018). On the basis of VS removal, the highest biogas yield is in ultrasonication pretreated digester (R2) 100.45 ml biogas/gVS and methane yield is 27.92 ml CH₄/gVS with 27.80 % TS and 16.89 % VS removal, whereas lowest biogas yield is in untreated digester (R1) of 74.69 ml biogas/gVS and methane yield is 19.35 ml CH₄/gVS with 14.22 % TS and 17.30 % VS removal. Methane and biogas production and yield were compared in the Table 4 and 5 for the entire process with percentage removal of VS and TS.

From the Table 4, it can be concluded that pretreatment of feedstock prior to digestion enhanced the performance of mesophilic AD, among all the pretreatment process ultrasonication pretreatment is found that best suited for biogas and methane production, while slight enhancement was achieved in the case of thermal and hydrothermal pretreatment of FFVW. The low yield of biomethane in this study as compared to the almost similar literature is possible due to the FFVW may some contaminants or inhibition effects on the mesophilic AD process (Stabnikova et al. 2008; Michalopoulos et al. 2019). These results show the experimental viability of the mesophilic AD process for the proper disposal of fruit, food and vegetable waste along with biogas recovery. It also shows that the recovery of the resources incentive along with waste reduction in the environment (Akshaya and Jacob 2018; Sittijunda and Reungsang 2018).

**Table 4 Summary of mesophilic AD experimental results**
Comparison of different pretreatment methods: The effectiveness of pretreatment on the FFVW for the mesophilic AD process for the recovery of biogas and biomethane yield and TS and VS removal obtained in this study are summarised in the Table 4. The pretreatment effects on the extra biogas and methane yield are shown in the Fig. 6, and it clearly revel that the maximum biogas and methane yield was found in R2 (ultrasonication) i.e. 100.45 ml biogas/g VS and 27.92 ml CH$_4$/g VS respectively, followed by R3, R4 and R5, while lowest in case of untreated FFVW digester. The maximum biogas production in case of ultrasonication pretreatment of FFVW is possible due to the pretreatment process facilitate the feedstock disintegration by high frequency sound waves will rupture the cell membrane and solubilizing more FFVW nutrient with increased hydrolysis of FFVW (Quiroga et al. 2014). It also increased the solubilisation of organic solids such as soluble COD at mesophilic temperatures. The other possible reason for such results due to the collapse of cavitation bubbles in the slurry that alter the chemical structure by creating free radicals and it leads to increases the microbial activity as well as biogas yield (Elbeshbishy and Nakhla 2011; Marañón et al. 2012). Thermal and hydrothermal pretreatment on FFVW enhanced the biogas production by 5%, 6% and biomethane production are enhanced by 11%, 9.8%, respectively, as compared to control. This is the very less significance (~5-10 %) performance difference was found in the case of thermal and hydrothermal pretreatment even both have the almost similar mesophilic AD performance for biogas and methane yield. This is possible due to the temperature treatment such thermal and hydrothermal pretreatment could have induced the sporulation of some hydrolytic microbes and inhibition of the others microbes (Balussou et al. 2012; Handous et al. 2019). The other possible reason for this is due to the temperature treatment damage the cell walls and rendered the FFVW substrate degradable for mesophilic AD process, while hydrothermal pretreatment of FFVW increased the AD performance due to penetration of the biomass and hydrate cellulose with removal of hemicellulose and some part of lignin (Baruah et al. 2018). This study found that the increasing the temperature for the AD process does not enhanced the either digestion or resources recovery. So, the mesophilic AD must be operating in the 30-50 ranges for higher resources recovery. Alkaline pretreatment on FFVW enhanced the biogas production by 20.4 % and biomethane production by 22 % as compared to control. The alkaline pretreatment on FFVW offer, removal of lignin, improve substrate digestibility, high saccharification, to achieve reduced sugar yield and stable performance for mesophilic AD (Zhai et al. 2015). The obtained results along with the above discussions clearly revel that the ultrasonication pretreatment on FFVW has potential for the resources recovery from such types of waste materials along with waste reduction.

Net energy analysis: As very few studies reported on FFVW mesophilic AD process for net energy recovery analysis even very less literature available for the comparisons of various pretreatment methods for FFVW for the AD or the biological process (Balussou et al. 2012; Tawk et al. 2020). In this study we explored the comparison and effectiveness of pretreatment methods for the AD process along with net energy analysis. The net energy analysis results for the pretreatment methods based on the whole digester (assuming very low heat loss through digester) are
shown in the Table 5. The procedure adopted for the net energy calculated as: in ultrasonication energy used in ultrasonicator and mixing instruments, in alkaline only mixing instruments was used, in thermal and hydrothermal the energy used in heating (Tawfik et al. 2020; Wang et al. 2021).

The extra biogas produced was considered for net energy analysis and energy generate by extra biogas production is enough for apply the pretreatment was investigated. From the Table 5, it reveals that ultrasonication and alkaline process has the positive net energy for pretreatment, while thermal and hydrothermal has the negative net energy. Therefore, in this study it is found that the higher temperature is not suitable to treat FFVW feedstock for mesophilic AD process for the resources recovery and waste reduction. The AD process or biological process must operate around the close range (35-40 °C) of the temperature of the living system.

| Units | Ultrasonication (R) | Alkaline (R3) | Thermal (R4) | Hydrothermal (R5) |
|-------|---------------------|---------------|--------------|-------------------|
|       | Mixing & Ultrasnicator 0.25 h | Mixing 0.25 h | Heating 1.5 h at 80 °C | Heating 0.5h at 120 °C |
| Energy required kWh/ton FFVW | 5±0.2 | 2.5±0.2 | 72±2 | 54±2 |
| Extra biogas produced m³/ton FFVW | 34.2±0.8 | 19.6±0.6 | 4.2±0.4 | 5.6±0.4 |
| Extra energy produced kWh/ton FFVW | 187.6±4.1 | 108.15±3.2 | 23.17±2.2 | 30.9±2.2 |
| Net energy kWh/ton FFVW | 182.6±4.1 | 105.65±3.2 | -48.83±2.2 | -23.1±2.2 |

**Cost analysis:** There are very few studies reported on cost estimation of biogas generated through mesophilic AD process or biological process. In this study, chemical cost and electricity charges were taken in account for preliminary economic evaluation for biogas generation through AD process (Balussou et al. 2012; Tawfik et al. 2020). All the calculation was done for 100 kg (1 ton) FFVW and cost of biogas generated through mesophilic AD are reported in Table 5. Cost analysis were done to analyse the feasibility and economic viability of mesophilic AD. The lowest cost was found in biogas generated through ultrasonication pretreatment as 38 USD/m³, while maximum cost found in the case of thermal pretreatment (44.9 USD/m³). The cost calculation reveal that the mesophilic AD has good economic viability and potential to be explored for scale-up or being commercialize in near future but it requires more research on some aspects to explored the AD process (Tawfik et al. 2020; Wang et al. 2021).

| Units | Ultrasonication (R) | Alkaline (R3) | Thermal (R4) | Hydrothermal (R5) |
|-------|---------------------|---------------|--------------|-------------------|
|       | Mixing & Ultrasnicator 0.25 h | Mixing 0.25 h | Heating 1.5 h at 80 °C | Heating 0.5h at 120 °C |
| Energy required kWh/ton FFVW | 5±0.2 | 2.5±0.2 | 72±2 | 54±2 |
| Extra biogas produced m³/ton FFVW | 34.2±0.8 | 19.6±0.6 | 4.2±0.4 | 5.6±0.4 |
| Extra energy produced kWh/ton FFVW | 187.6±4.1 | 108.15±3.2 | 23.17±2.2 | 30.9±2.2 |
| Net energy kWh/ton FFVW | 182.6±4.1 | 105.65±3.2 | -48.83±2.2 | -23.1±2.2 |
Pretreatment methods | Cost of FFVW + inoculum (USD) | Biogas yield (ml/gVS) | Cost (USD/m³)
--- | --- | --- | ---
Untreated (R1) | 0.0073311 | 74.69 | 98.1
Ultrasonication (R2) | 0.0078351 | 100.45 | 78
Alkaline (R3) | 0.0080577 | 94.77 | 85
Hydrothermal (R4) | 0.0084399 | 85.05 | 99.2
Thermal (R5) | 0.0090111 | 85.87 | 104.9

**Discussions:** The obtained results in this study shows that the ultrasonication pretreatment has the FFVW or waste biodegradation of 16.89 % TS and 19.44 % VS results high biogas and methane yield of 74.69 ml biogas/gVS and 19.35 ml CH₄/gVS respectively. The highest methane content is observed in R2 digester. However, thermally and hydrothermally pretreated digester achieved higher volatile content removal (except ultrasonication) but still have the low yield of methane content and average yield of biogas. The mesophilic AD on FFVW occurred in a stable manner for all pre-treatment process and initially there is no methanogenesis due to the accumulation of VFA was detected. The possible reason of the lower resources recovery in this study due to the very simple design and less parameter were selected as there is no continuous mixing throughout the AD process, no any inorganic ions and no any specific microbes were used to check the feasibility and economic viability of the AD process (Zhai et al. 2015; Zhang et al. 2019; Al bkoor Alrawashdeh 2019). Anyway, this study shows that the mesophilic AD of FFVW feedstock has the potential for improving the resource recovery along with waste reduction for the sustainable development of the society. This study found that pretreatment methods of FFVW feedstock also play a vital role to enhance the energy generation and process efficiency of AD process for the production of renewable energy from the waste materials.

**Conclusions:** The results obtained in this study implicates that the FFVW or similar feedstock can be explored for the resources recovery along with waste reduction. In this study, stable mesophilic AD operation were achieved on the FFVW with significant biogas or energy recovery along with reduction of the waste in the environment. Effectiveness and comparisons of pretreatment methods on the FFVW through mesophilic AD found that the pretreatment has positive impacts on biogas and methane yield, among these ultrasonication treatment (R2 digester) has achieved the highest yield 100.45 ml biogas/gVS and methane yield is 27.92 ml CH₄/gVS with 27.80 % TS and 16.89 % VS removal, while lowest in the case of untreated (R1 digester). The obtained results in this study reported in Table 4, which slightly lower than some reported literatures indicate that the contamination or inhibition effects of fruit, food and vegetable waste on the process. Higher ash content may be one of them or possible of present of some components of feedstock has negative impacts on the process. The other possible reason for this is due to other studied are conducted under different feedstock composition or different operating conditions. Hence, it is difficult to compare their performance preciously. However, apparently it seems that the performance of the mesophilic AD in the present study with FFVW, in terms of biogas and biomethane yield and energy efficiency are well agree with many previously reported similar studied (Bouallagui et al. 2005; Hendriks and Zeeman 2009; Shen et al. 2013; Sun et al. 2013; Xiao et al. 2013; Kondusamy and Kalamdhad 2014; Zhang et al. 2014; Nalakath et al. 2018; Keskin et al. 2019). From the overall analysis of this study, it has been concluded that, ultrasonication and alkaline pretreatment methods are found suitable for FFVW waste treatment and disposal. Beside this, this study show that the FFVW can be treated efficiently in the mesophilic AD process by further exploring some aspects of the AD process. Further, improvement in the treatment of FFVW waste through the AD or biological process by exploring the pure culture or changing the com
position of waste feedstock. Alternatively, changes in pretreatment conditions were able to stimulate the growth of different microbial guilds able to operate methanogens in a more efficient way are need to be more explored.

**Declarations**

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**Declaration of Competing Interest:** The authors affirm that they don't have any conflict of interest and competing financial interests that possibly will have influence on the work presented in this paper.

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**Figures**

![Figure 1](image)

Figure 1

Schematic diagram of the mesophilic anaerobic co-digestion digester setup.
Figure 2

Variation of pH over time (days) in the mesophilic AD.

Figure 3

(a) Total solid (TS) removal v/s time. (b) Volatile solid (VS) removal v/s time.
Figure 4

(a) Daily biogas production (DBP) comparison. (b) Cumulative biogas production (CBP) comparison.

Figure 5

(a) Daily biomethane production (DMP) comparison. (b) Cumulative biomethane production (CMP) comparisons.
Figure 6

Comparisons of biogas and methane yield.

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