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ORIGINAL ARTICLE

Enhanced biomethane production by 2-stage anaerobic co-digestion of animal manure with pretreated organic waste

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Abstract
Anaerobic co-digestion (AD) of different organic wastes is a promising technique to enhance clean energy (bioenergy) and manure (slurry) production, reducing stress on the environment. This is an experimental study aimed to investigate 2-stage co-digestion of pretreated organic wastes mixed with fresh animal manure (BD) and digester's operation conditions (digester temperature and pH) to enhance biomethane production. To increase lignocellulose digestibility and biomethane (BM) production, fruit + vegetable waste (FVW) and corn stalks + wheat straw (CR) in ratio (1:1), respectively, were pretreated with inoculum taken from an anaerobic digester of poultry manure at 35 °C. This AD experiment was performed in a fixed dome biodigester with volumetric capacity of 2.3 m^3. Biomethane potential (BMP) tests were conducted for biomass treatments and inoculum used (T_1, T_2, T_i) at 35 °C. In this study, the temperature of biodigester material was measured in mesophilic (30–40°C) and thermophilic (40–50°C) ranges and pH of fresh feed and slurry feed digesters was in optimum methane production range (7.01–7.52). The total daily methane productions from T_1 and T_2 were 125.13 ml/g VS and 104.89 ml/g VS in mesophilic range (30–40°C) while these values were 148.41 ml/g VS and 132.74 ml/g VS in mesophilic range (40–50°C), respectively. The 2nd stage digestion of slurry from fresh feed digester added 39–45% and 35–38% more methane production in T_1 and T_2 respectively. On calibration with BMP tests, experimental data have shown the synergetic effect on methane production and its thermal characteristics promoted by co-digestion of pretreated organic waste and BD. The economic and feasibility analysis proved the biomass co-digestion project viable and adoptable with positive (5.39 $) net return value (NRV), 2.92 years payback time (PBT), and 1.34 benefit cost ratio (BCR).

Keywords Pretreated organic wastes · Animal manure · Anaerobic co-digestion · Methane production · Economic analysis

1 Introduction

Pakistan is an agricultural country, so the country has huge potential for use of biomass to produce bioenergy [1]. Through the effective management and utilization of these energy sources, Pakistan can produce an enormous amount of renewable energy to meet its maximum energy demand [2]. The potential of biogas technology in Pakistan was found high as an alternate fuel source [3].

Anaerobic digestion (AD) is a reliable method of treating organic waste, which is an alternative to waste disposal [4] as well as a renewable energy source [5, 6]. The solid by-product of the anaerobic process (slurry) can be used as an organic agricultural fertilizer due to its nutritional value [7]. Anaerobic digestion is a multi-step biological process that is automatically agitated and depends on operating conditions [8], such as digestibility, pH, carbon to nitrogen (C/N) ratio, feed rate, pot life, and presence of inhibitors. Several studies have been conducted to investigate the environmental benefits of the biogas technology and to evaluate whether execution of biomass digestion technology is sustainable technology as an alternate energy source [9, 10].

The commonly used substrates for anaerobic digesters are livestock wastes, organic segment of municipal solid waste

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(OFMSW), food and vegetable waste, and agricultural crop residues [11]. Biomass is accessible effectively and in a substantial amount in rural zones. The general population of the rural zones will not need to pay cash for the feeding material while adopting of a biogas plant. Pakistan produces around 50 million tons of crop wastes and domesticated animals and poultry liters [12]. These wastes can be used as feeding material for biogas plants. The utilization of such waste gives energy as biogas furthermore minimizes the environmental pollution that is created by the vast amount of waste.

The pretreated substrate structures significantly affect the entire anaerobic digestive process. Anaerobic digestion consists of microorganisms processed on the organic matter of a nutrient in which the complex structure of cellulose and starch is converted into soluble organic compounds. The polymers are transformed into soluble monomers by enzymatic hydrolysis. In addition, temperature and pH ensure good digestion, as microorganisms are sensitive to heat and have favorable pH values. Biomethane production depends on the nature and quality of the substrate, pH, loading rate, toxicity, mixing, availability of nutrients, sludge concentration, digestive size and shape, C/N ratio, shelf life, alkalinity, prime feed total volatile solids (VS), chemical oxygen demand (COD), total solids (TS), and volatile liquids [13]. Biomethane is the end product of organic waste with anaerobic digestion and associated actions of different groups. Biogas is mainly composed of traces of methane (CH_4), carbon dioxide (CO_2), hydrogen sulfide (H_2S), hydrogen (H_2), nitrogen (N_2), and other gases [14].

The sole digestion of OFMSW animal manure, fruit, and plant waste results in lower biomethane production rates, longer digestion times, and slower volatile fatty acids (VFA) than food and vegetable waste [15, 16]. Furthermore, the biogas production from a single mass of pig manure, measured in VS, differs from the product obtained from the anaerobic digestion of poultry or bovine manure of the same mass [17]. On the other hand, the concentration of water-soluble substances (sugar, amino acid, protein, minerals) is higher in agricultural, fruit, and vegetable waste and non-water-soluble substances (lignin, cellulose, hemi cellulose, polyamides) are in less quantity [18]. This confirms that the vegetable wastes have more potential to enhanced methane production. Additionally, non-water substances present in plant waste can be utilized through pretreatment techniques that may help to enhance biomethane production of fruit and crop wastes. In the case of animal manure, the type and age of animal, its feeding, living conditions, and storage time of manure are the main factors affecting the quality and quantity of methane production. Generally, fine ground waste produces more methane due to larger contact area provided to bacteria [19].

Several researchers investigated the anaerobic co-digestion of several solid as well as liquid organic wastes [20–25]. The appropriate anaerobic co-digestion process can give interesting results due to synergetic effects produced by different organic fractions of substrate [26–29], i.e., mixing organic substances can result in the production of mixture with a C/N ratio included in the optimal range 20:1 to 30:1 [30]. The additional advantages of co-digestion are the dilution of potentially toxic elements present in the associated substrates, the regulation of pH and humidity, the increase in the content of biodegradable material, and the increase in bacterial activity. All of these benefits lead to a more stable and efficient digestive process and lead to greater bioenergy production [31].

The biomass digestion plant was developed in order to reduce fuel-wood requirements using bio energy as an alternate fuel energy source. This work was aimed to investigate anaerobic digestion (AD) of animal manure mixed with two different combinations of organic waste from fruit, vegetable, and agricultural crop residues. The co-digestion of precomposted (fruit + vegetable) waste and crop (corn stalk + wheat straw) was mixed with fresh animal manure (buffalo dung) and was carried out to improve biomethane production, biogas composition, and its thermal characteristics. In addition, the slurry from fresh feed co-digesters was re-digestion for complete biodegradation of substrate and to obtain higher total biomethane production. The economic feasibility of biomethane plant was also conducted for profitability and sustainability of plant.

2 Materials and methods

2.1 Experimental site

This study was conducted at Rana Agricultural Farm Jatoi, Pakistan in 2020 to produce biomethane (bioenergy) from co-digestion different fruit + vegetable and crop waste with animal manure and to analyze its physio-thermal characteristics.

2.2 Co-digestion technology

A fixed dome type biogas plant constructed using concrete rings was economical and can be reused after dismantling the biogas plant. The plant consisted of four digesters chambers associated in arrangement having same feeding chambers (Fig. 1). Two digesters were designed to feed fresh material, rest of two were received slurry from fresh feed digester. The objective of slurry feed digesters was to provide extra time to exit slurry for further anaerobic fermentation. Every digester consisted of 8 concrete rings with 4 feet diameter, 3 inches thickness, and 10 inches height. The total volume of every digester is 2.3 m^3 while volume of gas holder was 0.25 m^3. PVC pipes were installed for substrate inlet, biogas, and slurry outlet.

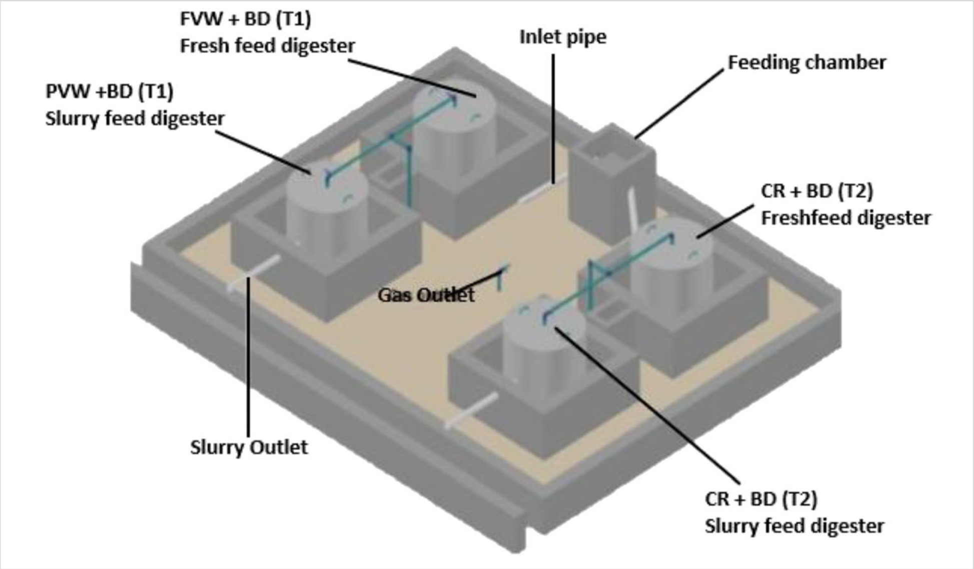


Fig. 1 Three-dimensional view of co-digestion plant with fermentation digester under each treatment

The fermentation chamber was 6 feet deep. The soil was hard and rocky, and RCC boundary wall was constructed around digesters. For isolation of digester, wheat straw was filled in between boundary wall and digester rings. Feeding chamber was at the midpoint of the fresh feed digester (digesters 1 and 3). Feeding chamber (3 × 2 × 1 feet) was made of concrete and blocks 3 feet above the ground to accomplish gravity for feeding materials. Fresh material feed digesters were connected with slurry feed digesters through slurry feed chamber in order to collect slurry outflow.

PVC pipe of diameter 4 inches was fixed 1 foot over the base of fermentation chamber at an angle of 45° for feeding the material from feeding chamber to fermentation chamber. The slurry outlet pipe was fixed 1.5 feet deep in the fermentation chamber. The slurry outlet was fitted at an angle of 15° to keep outlet in the slurry in order to avoid gas leakage. The schematic diagram of constructed biogas plant is shown in Fig. 2.

2.3 Feedstock for co-digestion

The biomass feedstocks used for the production of biogas were fruit + vegetable waste (FVW), crop (corn stalks, wheat straw) waste (CR), and fresh buffalo manure (BD). The fruit and vegetable wastes were collected from food and vegetable market, Rawalpindi while the crop and animal (Buffalo) dung were taken from Koont research farm, PMAS Arid Agriculture University, Rawalpindi.

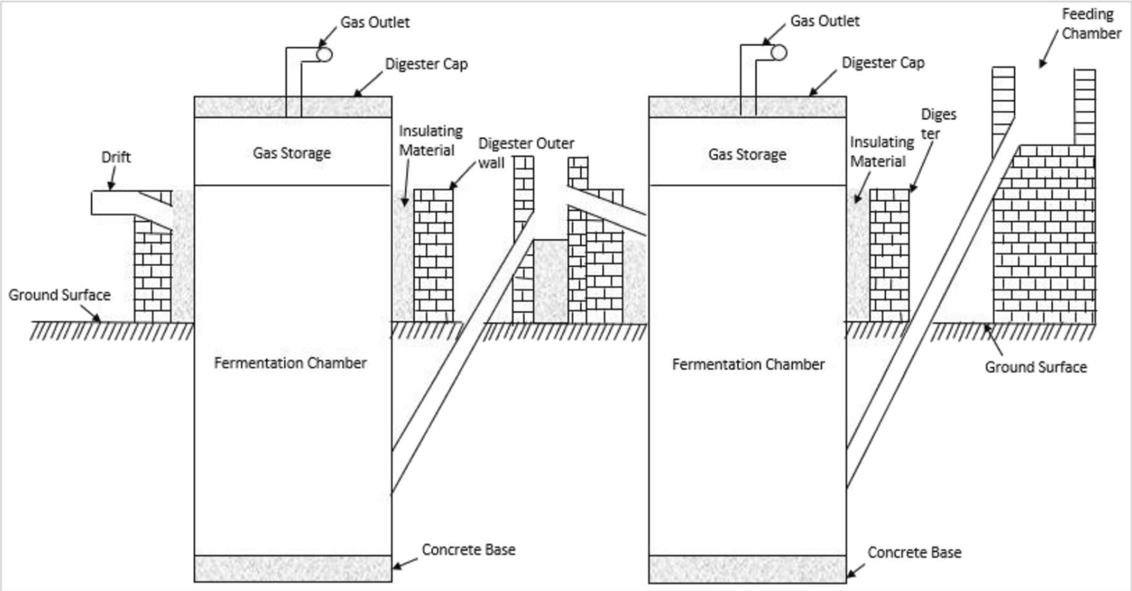


Fig. 2 Sectional view of fixed dome biogas plant

The decomposition of substrate biomass is dependent on feed load, digester temperature, hydro retention time, and pretreatment of biomass [32]. Substrate material should fulfill required nutritional content of microorganism for energy, new cell development, and trace elements and vitamins for microbial enzymes. The C/N ratio should not be too high to eliminate N decency [33]. The optimal C/N ratio is also affected by phosphorous and trace element content [34], decomposition efficiency, and composition of substrate material [35]. Suitable C/N ratio enhances methanogenesis. The C/N ratios of substrates used in this study are shown in Table 1.

The characteristics of feedstock and inoculum used to enhance biological lignocellulos degradation (composting) are given in Table 1. Moisture content (MC) of feedstock material was determined by oven dry method at 105°C until no further weight change [36]. Organic matter (OM) of substrate and inoculum was measured by the dried sample ignition in a muffle furnace @ 550°C for 4 h. Total nitrogen (N) and organic carbon (C) were measured by Kjeldahl and Turin methods, respectively [37]. TS, VS, and F_{li} of each substrate were measured according to standard methods [38]. F_{pr} in each organic substrate was measured by standard method [38], whereas F_{ch} was measured by subtracting the sum of protein and lipids from VS contents [39].

2.4 Pretreatment of feedstock

Pretreatment of feedstock matter was performed to kill the pathogenic microorganism, remove unbiodegradable matter, concentrate organic matter, and feed preparation [40]. The sorted fruit, vegetable, and crop wastes were chopped to a size of 150–200 mm [41] by using mills, blenders, screws, and rotating knives. The moisture of crop wastes was measured < 15% (8–11%). Equal proportion (w/w) of chopped fruit and vegetable wastes were mixed uniformly and composted in an underground pit (4 cubic feet) for about 2–3 weeks. A similar method was used for composting of crop waste. The inoculum was taken from an anaerobic digester of poultry manure at 35 °C. Biological pretreatment [40] was carried out to enhance substrate biodegradation and more methane production with reduction in particle size [42].

2.5 Experiment for co-digestion

The experiment was performed during April 1, 2020, to May 25, 2020. Treatments T_1 and T_2 were carried out simultaneously under similar ambient conditions in FVW+BD and CR+BD digesters (Fig. 1). Initially, 500 kg feeding substrate material was filled directly into fermentation chamber and covered with a lid. The retention time of 10 days was given after filling substrate material. After retention period, 25 kg of each feedstocks (FVW, CR and BD) was fed with 50 kg of water. The treatments used in this study were T_1 (FVW + BD + water) and T_2 (CR + BD + water) with the feeding ratio (Table 2). In order to get maximum methane production from unit mass of feedstock, the slurry of fresh feed digester was further used as feeding material for slurry feed digester.

2.6 Studied characteristics

2.6.1 pH of digestive material

Five subsamples were taken from the different locations in the digester and mixed thoroughly to get a representative sample from all these materials with uniform sample characteristics. The pH of substrate and digestion material samples was measured using pH meter (CPC 411).

Table 1 Examining the characteristics of substrate and inoculum

Characteristic	BD	FVW	CR	Inoculum
Moisture content (% MC)	82.7 (0.3)*	73.5 (0.5)	9.8 (0.1)	88.3 (0.4)
Organic matter (% OM)	76.3 (1.8)	81.1 (2.1)	91.2 (0.5)	69.5 (0.9)
TS (g/kg) wet	109.6 (0.6)	155.7 (0.5)	104.2 (0.8)	141.3 (0.8)
VS (g/kg) wet	89.1 (0.7)	113.6 (0.4)	82.7 (0.5)	84.9 (0.5)
pH	7.05 (0.06)	-	-	7.67 (0.03)
C (% of TS)	42.2 (0.05)	44.1 (0.3)	48.5 (0.05)	29.35 (0.05)
N (% of TS)	4.12 (0.01)	0.52 (0.09)	0.62 (0.01)	1.57 (0.02)
C/N	10.24	84.8	78.23	18.7
F_{ch} (g/g) dry	0.18	0.32	0.21	0.19
F_{pr} (g/g) dry	0.24	0.19	0.32	0.26
F_{li} (g/g) dry	0.018	0.02	0.022	0.02

*Standard error ($n = 3$), *TS* total solid, *VS* volatile solid, *C* organic carbon, *N* total nitrogen, F_{ch} F_{pr} F_{li} carbohydrates, protein, and lipids fractions

Table 2 Co-digestion treatments and their description

Feedstock	Treatment	Mixing weight	Mixing ratio (w/w)
Fruit + vegetable waste (FVW)	T_1	FVW = 25 kg	1:1:2
Crop (corn + wheat) waste (CR)	(FVW + BD) + H_2O	BD = 25 kg	
Animal manure (BD)		Water = 50 kg	
	T_2	CR = 25 kg	1:1:2
	(CR + BD) + H_2O	BD = 25 kg	
		Water = 50 kg	

2.6.2 Temperature of digestion chamber

Temperature variations in fermentation digesters were recorded by digital temperature sensors [43] installed inside the digester at three layers (2-feet layer) of fermentation material and outside to record ambient temperature. The measured ambient temperature data was verified with the temperature data collected from a sub-station of Pakistan Meteorological Department (PMD) located at University main campus. The temperature readings of digesters were taken every 10 min, six times a day (0:00 to 24:00 O'clock), and recorded by data logger [44]. The net degree hour temperature (NDH) was used to investigate the effect of inorganic amendments on the composting temperature and to evaluate how these temperatures variate with the progress of waste digestion process [45].

$$\begin{aligned}\mathrm{NDH}\left(^{\circ}\mathrm{C\ h\ day}^{-1}\right) = {} & 4\left(T_{0\text{–}4\mathrm{h}} + T_{4\text{–}8\mathrm{h}} + T_{8\text{–}12\mathrm{h}} + T_{12\text{–}16\mathrm{h}} + T_{16\text{–}20\mathrm{h}} + T_{20\text{–}24\mathrm{h}}\right) \\ & -4\left(T_{\mathrm{a}0\text{–}4\mathrm{h}} + T_{\mathrm{a}4\text{–}8\mathrm{h}} + T_{\mathrm{a}8\text{–}12\mathrm{h}} + T_{\mathrm{a}12\text{–}16\mathrm{h}} + T_{\mathrm{a}16\text{–}20\mathrm{h}} + T_{\mathrm{a}20\text{–}24\mathrm{h}}\right)\end{aligned} \tag{1}$$

where NDH is net degree hour temperature in the composting pit adjusted with ambient temperature (°C h day^{-1}) and $T_{\mathrm{i}\text{–}4\mathrm{ih}}$ and $T_{\mathrm{ai}\text{–}4\mathrm{ih}}$ are mean compost temperature and mean ambient temperature measured after every 10 min in 4 h interval (°C).

2.6.3 Biomethane production

The amount of biomethane production (BM) under different co-digestion treatments and gaseous compositions (CH_4, CO_2, O_2, and H_2S) from each digester was measured by a portable biogas analyzer (GA5000, Geotech). For the first month, the biogas composition was analyzed twice a day and later once a day until the end of experiment. The daily burning time (Bt) for collected gas was measured using standard gas burner. The calorific values of biomethane burning were measured by bomb calorimeter [46].

2.6.4 BMP test

Biomethane potential (BMP) tests were carried out for three different organic wastes. The main characteristics of used organic wastes in terms of total solids (TS), volatile solids (VS), carbohydrates fraction (F_{ch}), protein fraction (F_{pr}), lipids (F_{li}), etc. are presented in Table 1. These BMP tests were conducted on each combination of organic waste (mentioned as test indexes T_1 and T_2). An additional BMP test (mentioned as test index T_{i}) was also conducted for the inoculum to identify the volume of methane production by the digestion of organic solids present in the anaerobic sludge. In total, three BMP tests were performed and each of them in triplicate. The mass of BD, CR, FVW, inoculum, and Na_2CO_3 used to perform the BMP tests is presented in Table 3. Representative samples of co-digestion materials were collected according to waste sampling methodology [41]. The samples were ground and sieved to have a homogenous material and particle size ranging between 1 and 2 cm.

Table 3 Mass (g) of substrate, inoculum, and Na_2CO_3 used in BMP tests

BMP test	CD	BD	FVW	Inoculum	Na_2CO_3
T_1	38.81 (0.35)*	32.72 (0.3)	-	150.23 (0.55)	0.35 (0.05)
T_2	-	32.72 (0.3)	28.23 (0.3)	150.1 (0.6)	0.30 (0.01)
T_{i}	-	-	-	150.15 (0.35)	0.10 (0.01)

*Standard error (n = 3)

BMP tests were performed under a controlled and reproducible condition in a 1000-ml glass bottle GL 45 (Schott Duran, Germany). Each bottle was partially filled with inoculum and a substrate according to the VS content ratio equal to 2; tap water was added up to a 500-ml bottle volume. A small amount of Na_2CO_3 powder (0.1–0.6 g) were also added to maintain optimal pH. Each bottle was sealed with a 5-mm-thick silicone disc and a plastic screw cap (Schott, Duran Germany). Bottle shakers KL-2 (Edmund Bushler, Germany) was used to shake all bottles for about 30 min at 80 rpm speed and were immersed up to half of their height in 35 °C hot water by 200-watt (A-763) submersible heaters (Hagen, Germany). To collect methane, test bottles were connected with inverted 1000-ml glass bottles containing 2% NaOH alkaline solution. Daily biogas production was recorded through volume of NaOH solution displaced from the measure bottle. The CO_2 content in the biogas did not affect the volumetric measurement of methane due to its dissolved nature in alkaline NaOH solution.

2.6.5 Slurry physical and biological analysis

The physical and biological characteristics were evaluated. The bacteriological groups, salmonella [47], whole coliforms, and fecal coliforms were measured [48] and were studied and expressed in the amount of colony-forming unit per gram of compost (CFU/g compost).

2.6.6 Statistical analysis

Physical and chemical characteristics of substrate (feedstock) and digestion chamber material were measured in triplicate and standard deviation was determined. The effect of co-digestion treatments on studied parameters was analyzed through statistical analysis (ANOVA) according to completely randomized design (CRD). Mean difference was acknowledged at < 0.05 significance level using SPSS-24 [49].

2.7 Economic and feasibility analysis

Economics of co-digestion technology is based on project site and local conditions of the study area with different outcomes. Production of biomass digestion requires technology, raw materials, quality check, and production costs, which are the major determinants of the technology used [50]. Generally, economic analysis indicates the economic feasibility of the project. It is based on technology used, digestion material, skills of labor, and investment cost [51]. The economic factors for total fixed and variable cost were measured on the basis of local prices and market conditions. The economic analysis was performed (Table 4) by calculating three main economic factors: net return value (NRV), payback time (PBT), and benefit cost ratio (BCR).

3 Results and discussion

3.1 pH variation in digestion process

pH was one of the most important parameters for the stability of AD system, which could affect the activity of acidogenic and methanogenic microorganisms [53]. The change of pH for fresh feed and slurry feed digesters of T_1 (FVW + BD) and T_2 (CR + BD) during the fermentation process is indicated in Fig. 3. Initially, pH in fresh feed and slurry feed digesters of both treatments significantly increased until the 40th day and later minor changes in pH were observed. The initial pH of fresh feed and slurry feed digesters of T_1 and T_2 was (7.07 and 7.06) and (7.02 and 7.01), respectively. pH was gradually increased to 7.4 and 7.3 in fresh feed and slurry feed digesters until 40°C and remained steady during the later reaction time; this was probably because the organic acids were generated substantially during the high solid reaction system. The rapid accumulation of organic acids could result in serious inhibition and lower biogas yield. The highest pH value (7.52) was measured in the T_1 (fresh feed digester) and lowest (7.37) in T_2 (slurry feed digester).

According to previous studies carried out, pH affects the rate of biogas production as microbes involved operate within a neutral pH. The pH was found in range (7.2–7.3) for two substrates used, cow dung and water lettuce [54]. Lee et al. [55] indicated that the optimum range of pH for methanogenesis in AD was 6.5 to 8.3, and pH in the reaction system reached more than 6 was considered appropriate for the methanogenesis process. In the following days, the pH of B2, B3, and B3 first

Table 4 Economic indicators for the feasibility of biomass co-digestion technology

Parameter	Significance	Measurement equipment
Net return value (NRV)	The net values (cost) obtained after the deduction of total production cost.	$\mathrm{NRV} = \sum_{t=0}^{n} \left(C_\mathrm{b} - C_\mathrm{p}\right)(1+I)^{-t}$
Payback time (P_t)	The total number of years required to recover project's total investment.	$\sum_{t=1}^{P_\mathrm{t}} \left(C_\mathrm{b} - C_\mathrm{p}\right)(1+I)^{-t} = 0$
Benefit-cost ratio (BCR)	The ratio of total worth of benefits to the total worth of cost.	$\mathrm{BCR} = \frac{\sum_{t=1}^{n} C_\mathrm{b}(1+I)^{-t}}{\sum_{t=1}^{n} C_\mathrm{p}(1+I)^{-t}}$

Source: [52]. C_b and C_p are benefits and production cost, respectively, $(C_\mathrm{b} - C_\mathrm{p})^{-t}$ is net cash in a year (t), n is project life, and I is the cut-off discount rate

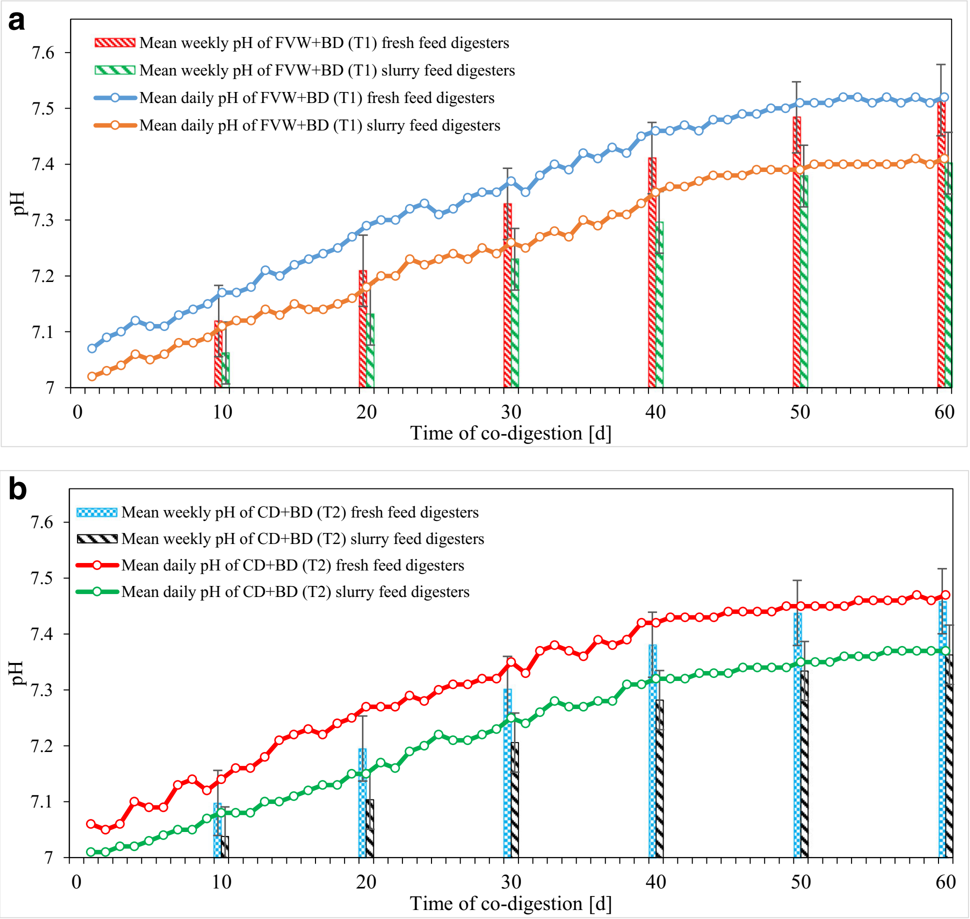


Fig. 3 Effect of ambient temperature and substrate on pH in fermentation digester of **a** FVW + BD and **b** CR + BD treatments

decreased but then increased, and it remained at about 8 at the end of reaction. It only took 4 days for the pH of B3 to recover from the minimum value of 6.7 to 7.8. Westerholm et al. [56] reported that AD process would remain stable and methane yield would keep normal at the pH value of 7.9 when dealing with organic municipal waste. Liu et al. [53] predicted optimum methane yield at pH (> 7) for anaerobic digestion of organic fraction of municipal solid waste. They observed that the optimal values of pH are 7.10 and 7.21 under mesophilic and thermophilic temperature, respectively. Finally, methanogens prefer a pH environment between 7 and 7.5 as reported by Schnurer and Jarvis [42], although there are several biogas plants operating at pH of 8 [57].

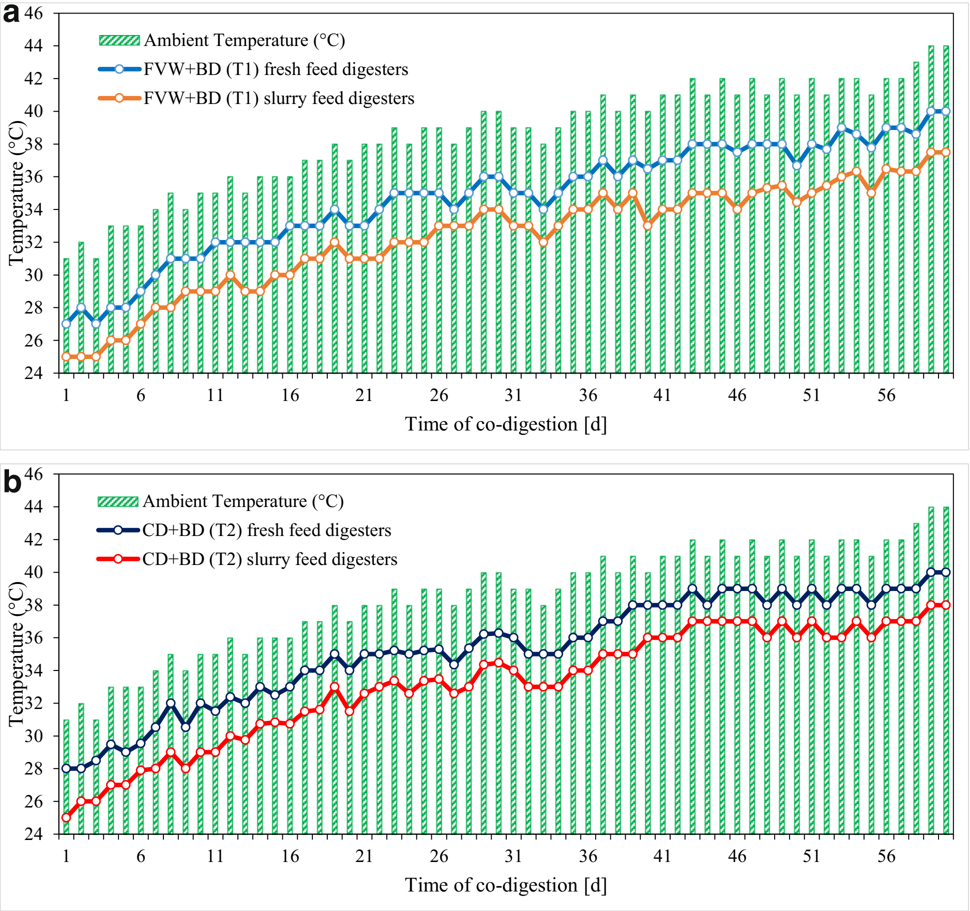
3.2 Temperature of digestion chamber

Temperature is a key parameter of anaerobic digestion and is difficult in digesters that operate without heating. The change could have disastrous consequences for microbial communities and their performance. However, this can be an interesting parameter for adjusting the yield, energy efficiency, or stability of bioreactors. Seasonal fluctuations in heat have been accepted as the main environmental factor affecting the fermentation products of agricultural biogas. The effect of seasonal temperature fluctuations on the operating digesters and biogas production is shown in Fig. 4. The temperature of digestion material from fresh feed and slurry feed digesters were recorded under T_1 (FVW + BD) and T_2 (CR + BD). We always found the temperature of fresh feed digester 3–5 °C below ambient temperature, while temperature in slurry feed digesters was lower as compared to fresh feed digesters. Throughout the experiment, we found temperature of all digesters (fresh feed + slurry feed) 20 °C. The growth rate of methanogens was temperature reliant and precise low at low temperature (< 20 °C) [58–61]. The favorable climatic conditions for the anaerobic digestion process are with ambient temperature is between 20 and 25 °C [62]. On the other hand, in some mountainous regions, spring is warm and winter cold. The low temperatures (< 20 °C) are not suitable for biogas production [63–65].

3.3 Biomethane production

The amount of daily biogas production was measured and an accumulative biomethane production for a duration of 10 days of substrate retention period. Fresh feed and slurry feed

Fig. 4 Measurement of temperature in fermentation digester of **a** FVW + BD and **b** CR + BD treatments



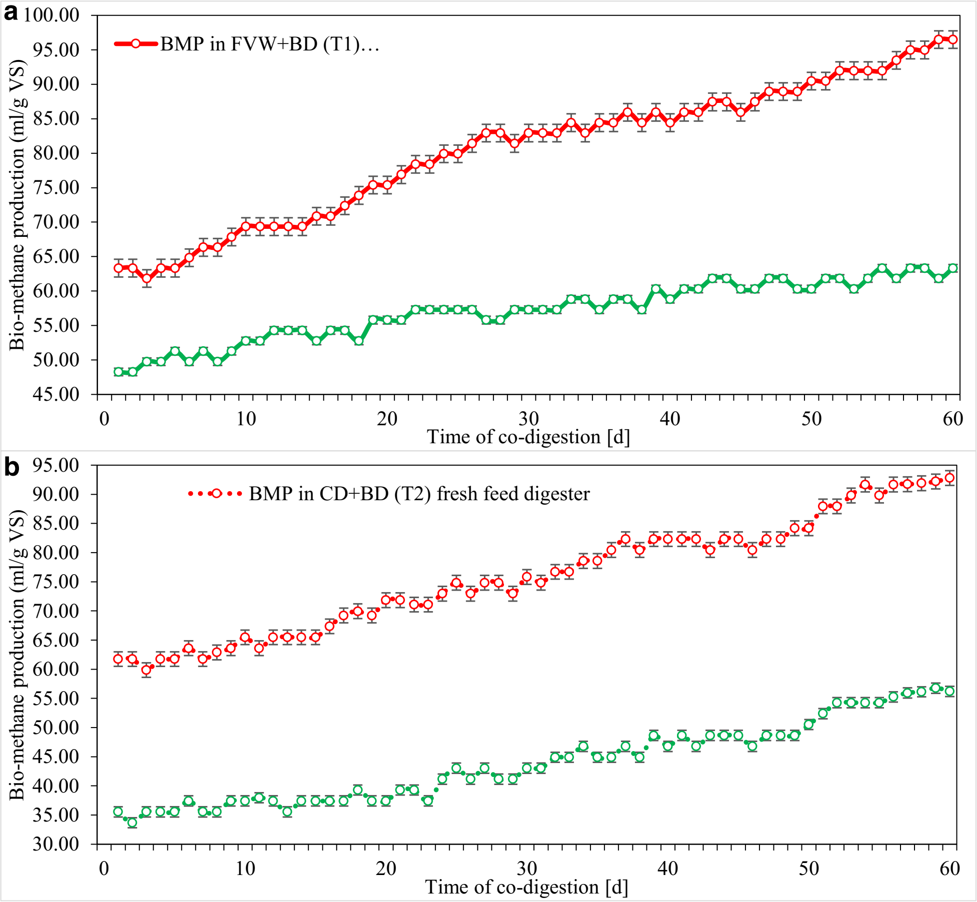
digesters of T_1 (FVW + BD) and T_2 (CR + BD) produced 316.6 ml/g VS (2.1 m^3), 241.22 ml/g VS (1.6 m^3), 308.7 ml/g VS (1.65 m^3), and 177.74 ml/g VS (0.95 m^3) biogas respectively in the context of mesophilic anaerobic digestion. Due to the effect of temperature, gas production was better in quantity and quality at higher temperatures. During the acetogenic process, methanogenic bacteria consume more carbon dioxide and form a methane-rich gas in a thermophilic state. The thermophilic anaerobic digestion process is mainly preferred to mesophilic digestion to increase methanogenic bacteria by increasing biophase production. This is because the high digestive temperatures kill more pathogenic bacteria and increase the methanogenic bacteria to produce a greater yield of methane. In general, methanogenic bacteria are more stable in the thermophilic range than in the mesophilic range.

Figure 5 shows the effect of fermentation temperature in mesophilic and thermophilic ranges on methane production in fresh feed and slurry feed digesters of T_1 (FVW + BD) and T_2 (CR + BD). The average total methane productions (fresh + slurry digesters) were 125.13 ml/g VS (0.83 m^3) and 104.89 ml/g VS (0.56 m^3) from T_1 and T_2 in mesophilic range (30–40°C) while these values were 148.41 ml/g VS (0.98 m^3) and 132.74 ml/g VS (0.71 m^3) in mesophilic range (40–50°C), respectively. Growth rates of microorganisms and interspecies transfer of hydrogen at thermophilic temperatures lead to rapid decomposition rates, increased solids destruction, and increased methane production. Therefore, the thermophilic operation offers the advantages of a short degradation time, good pathogen reduction, high gas production, and good sludge separation.

Various studies have been carried out to enhance methane yield and the stability of the anaerobic digestion process. Co-digestion of macroalgae and sugar industry waste was performed in batches in an up-flow bioreactor, which resulted in a maximum of 375 ml of biogas with 40% methane content and 114 ml/g VS of biogas with 75% methane content, respectively [66]. Additionally, it has been reported that 152 ml/g VS and 198.85 ml/g VS methane yields were obtained from the co-digestion of sheep dung with corrugated paper and the co-digestion sheep dung with office paper, respectively [29].

The anaerobic mono-digestion of cow dung obtained from Katsina Modern Abattoir produced 400 ml of biogas [67]. Sagagi et al. [68] investigated the biogas production rates for pineapple, orange, pumpkin, and spinach wastes as 0.97 m^3, 0.61 m^3, 0.37 m^3, and 0.27 m^3 respectively. The effect of pretreatment depends on the substrate and the type of

Fig. 5 Effect of digester temperature on biomethane production from fresh feed and slurry feed digesters of **a** FVW + BD and **b** CR + BD treatments



pretreatment method. Pretreatment methods include mechanical treatment, alkaline treatment, oxidative treatment using ozone, microwave radiation, and thermal treatment [69]. The complex structure of plant tissue and its inherent forces do not allow them to easily become biogas due to the abundance of nitrogen, creating a higher concentration of volatile fatty acids that stop methanogenesis and result in lower methane production [70, 71]. Thus, the best route to improve biogas production is the anaerobic co-digestion (AD) process [72].

The sum of the cumulative methane productions from fresh feed digester and slurry feed digester respectively is presented in Fig. 6. After every 10 days interval, the cumulative methane production from T_1 was 6.4–17% higher than T_2. At the 20th and 30th days, T_1 (FVW + BD) showed 17% and 16.8% more methane production than T_2 (CR + BD). This high methane production could be due to the multiple digestion allowing substrate more digestion time to substrate material. Figure 5 shows that re-digestion of slurry from fresh feed digesters of both treatments added 48–64 ml/g VS and 35–57 ml/g VS more methane into total methane production. In another study, organic municipal waste and fruit and vegetable waste were digested with increasing composition rates, which resulted in

Fig. 6 Total biomethane production from co-fermentation of organic waste

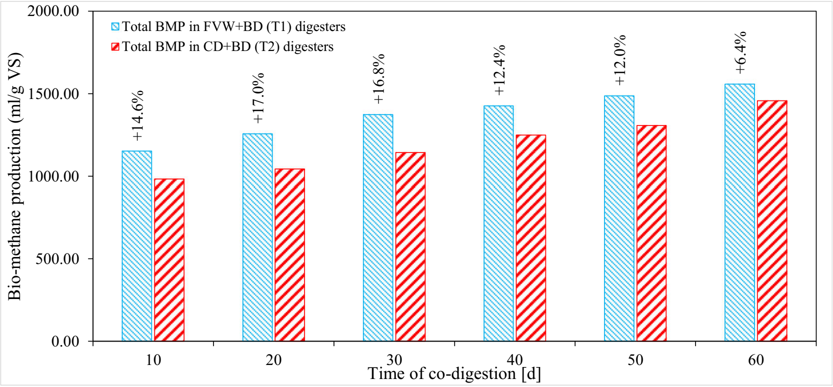


Table 5 Examining composition and thermal characteristics of biogas production

Treatment	Biogas composition (%)				Burning time (min/kg)	Calorific value (J/m^3)
	CH_4	CO_2	O_2	H_2S (ppm)		
T_1 (FVW + BD)	39.65 (0.1)*	29.55 (0.2)	3.5 (0.05)	67.5 (0.3)	97 (0.5)	27.2 (0.3)
T_2 (CR + BD)	39.71 (0.2)	30.66 (0.1)	3.4 (0.01)	69.3 (0.3)	94 (0.3)	26.9 (0.2)

*Standard error (n = 3)

a 141% and 43.8% increase in the methane yield with respect to their mono-digestion, respectively [73].

The data of the biogas composition and thermal values obtained in the experiment are shown in Table 5. The biogas composition obtained from both co-digestion treatments did not show significant difference. The daily biogas production and their burning times were noted. In this experiment, the average burning times were measured as 96–97 min/kg. The energy emitted (kcal/kg) while burning of biogas was significantly affected by the biogas composition. The calorific values obtained from the T_1 and T_2 were 27.2 J/m^3 and 26.9 J/m^3, respectively. Lijó et al. [74] conducted experiments on co-digestion of fruits and agricultural wastes. They measured calorific values of biogas between 25.9 and 30 J/m^3 depending on the percentage of methane in the gas.

3.4 BMP test

Biomethane potential tests T_1 and T_2 were carried out for two categories of organic waste combination of substrates taken from fruit + vegetable waste, crop residue, and animal wastes under controlled and reproducible conditions. These tests concentrated on the influence of co-fermentation of various substrates on methane production (Fig. 7). The highest methane production was obtained in test T_1, consistent to the mixture categorized by the greater percentage of OFMSW. Interestingly, the combined digestion of the two substrates takes over 60 days, which is faster than pure substrates digestion in around 80 days. This is due to mixed properties, i.e., greater buffering capacity compared to pure substrates [75], a lower effect of inhibitory factors such as ammonia compared to pure BM, and a better balance between carbon content and nutrients [76].

These differences in the amount of methane production rates are due to the biological degradation of the first substrates before the start of the experiments and the passage of the second substrates through the digestive systems of the animals. The organic substrate has sufficient buffer capacity. Ammonia in animal manure can improve the biological process by providing the necessary buffer capacity [77]. Cow manure also contains enzymes and a large number of microorganisms that can make the biological process faster and more efficient, as the enzymes help to consume fewer biodegradable components of VWF and CR organic solid waste such as cellulose [78]. In this study, the specific methane productions agreed with the values as reported in literature [79–83].

3.5 Slurry biological analysis

The slurry samples from fresh feed and slurry feed digesters were evaluated for physical and biological characteristics. Slurry from both digesters showed significant quantity of OM that can be utilized as biofertilizer in agriculture. The C/N ratios measured were still above as measured in pure animal manure. The indicator organism in slurry such as fecal coliforms (E. coli) and total coliforms and pathogenic microorganisms such as Salmonella spp. were monitored in the endmaterials obtained. All the composts showed extremely low contents of total and, especially, fecal coliforms (E. coli),

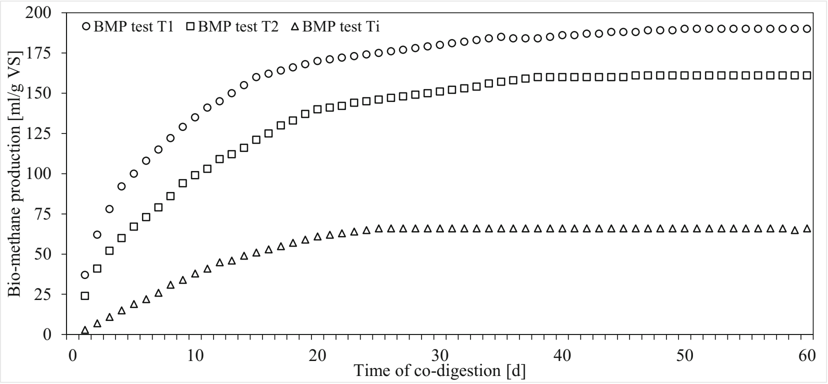


Fig. 7 Cumulative biomethane productions from BMP tests

Table 6 Physical and microbial characteristics of slurry obtained from co-digestion plant

Characteristics	Fresh feed digester	Slurry feed digester
Physical characteristics		
Moisture content (% MC)	89.5	91.6
Organic matter (% OM)	72.9	56.8
C/N	35.7	18.6
Microbial group (CFU g^{-1} slurry)		
Salmonella	ND	ND
Total coliforms	2.16×10^2	1.72×10^2
Fecal coliforms (E. coli)	1.39×10^1	0.92×10^1

ND not detected in 250 ml of slurry

while Salmonella was not detected (Table 6), satisfying the limits established for the sanitary aspects of digested biowaste by different European legislations and guidelines [84].

3.6 Economic analysis

The purpose of the economic analysis was to determine the parameters that significantly influence the feasibility of the project within the expected range of variations. The plant feasibility was evaluated with certain economic indicators (Table 7). The total annual cost of production for biogas plant was calculated as 4942.98 $. The ratio of total variable cost to total production cost that was measured as 9.06 indicated that > 90% of the total production cost was used as total variable cost. Moreover, the high total variable cost of co-digestion technology was the main reason for high total production cost. Labor cost has the biggest share in total variable cost of biomethane production. The gross return value of the production was calculated by multiplying the annual briquette production by their respective prices. Initially, the prices were collected in local currency in Pakistan rupees (PKR as Rs) and then converted into international currency American dollar (USD as $) by multiplying with conversion factor as 1USD = 165 PKR. The conversion factor is the average of currency exchange rate during fiscal year 2020. The total return value (TRV) value was found as 5124.8 dollars per year for briquetting production.

Generally, project feasibility is based on net return value (NRV). A positive NRV value indicates the acceptance of projects for continuing the investment for the future and negative NRV is rejection [85], while projects with zero NRVs make investors indifferent [86]. In this study, the NRV was measured as 5.39$ and confirmed that the biomass co-digestion project is profitable and feasible for sustainable

Table 7 Techno-economic feasibility analysis for anaerobic co-digestion plant

Cost factor	Rate (Rs./unit)	Quantity (units/day)	Annual cost (in 000)	
			PKR	USD
Feedstock (kg)				
Animal manure	2.5	50	22.81	0.14
Fruit and vegetable waste	3	25	27.38	0.17
Crop residue (corn + wheat)	10	25	91.25	0.55
Biomass transportation	10		182.50	1.11
Labor (h)	125	4	182.50	1.11
Feedstock preparation				
Chopping and mixing (kWh)	15	5	27.38	0.17
Composting of waste (day)	50	1	18.25	0.11
Human labor (h)	125	4	182.50	1.11
Total variable costs ($ ha^{-1})			*734.56*	*4.45*
Construction cost (10 years)	300,000		30.0	0.182
Equipment and accessories	215,000		43.0	0.26
Depreciation	10% of purchased cost		7.30	0.04
Shelter for briquetting plant	1% of purchased cost		0.73	0.004
Total fixed costs			*81.03*	*0.49*
Total production costs			*815.59*	*4.94*
Total return value (TRV) @ 125 Rs/kg of compressed gas			1095.0	5.12
Net return value (NRV)			279.41	5.39
Payback time (PBT)			2.92	
Benefit cost ratio (BCR)			1.34	

USD* was measured under the conversion rate of 1 USD = 165 PKR waste management. This result is in line with Gwavuya et al. [87] that the small sizes of biogas plant in Ethiopia were more profitable than the large sizes. Kabir et al. [88] showed that under assumption with subsidy, biogas users in Bangladesh obtain better financial results compared to assumption without subsidy. Walekhwa et al. [86] measured the positive net present values of 4500$, 7000$, and 9500$ for 8 m^3, 12 m^3, and 16 m^3 plants, respectively, which showed that biogas systems were economically viable in Uganda.

The payback time is the number of years required to recover project investment, usually compared with economic period of the project. The lesser the payback time, the more feasibility level of the project. Huiru et al. [89] calculated 9.3 years payback time for anaerobic digestion plant fed with canteen food waste. However, the same project took 6.5 years for food waste biogas project as reported by Xu et al. [90]. In our research study, the payback time is about 3 years which means 33% investment return per year. This is because we use all local material in the construction of waste digestion plant and the location was suitable for the easy availability organic waste and animal manure. In Pakistan, Ansari et al. [91] measured the payback period of the project which was 5.35 years. Benefits from biogas plants covered all costs.

A project feasibility is based on BCR ≥ 1 [92]. In this research, BCR was calculated as 1.34 which indicated that the project is acceptable to organic waste digestion. The measured values of BCR proved the acceptability of biogas production technology because the studied economic indicators evaluated in the economic feasibility analysis were found positive. In another study in Pakistan [93], households with a rudimentary biogas plant can save around Rs. 3550/month on fuel, LPG, and manure costs. Further they can save Rs.600 by the replacement of chemical fertilizers with an organic slurry. Engler et al. [94] demonstrated that the economic value of energy alone is not sufficient to cover the cost of the project if it does not provide environmental benefits in the form of credit, such as reduced economic value and odors, by reducing pests and weed seeds or other environmental issues have been considered. According to Abbas et al. [95], the BCR of biogas plants was > 1 at all levels. The 6-m^3 BCR of the biogas plants was only 1.56 for fuel replacement, which only allowed for power generation. There are return rates of around 18% per 6 m^3 of similarly designed facilities in Pakistan [95]. The adoption of biogas technology is more beneficial for families who buy all their firewood. Households that use dung for combustion benefit more from households that collect firewood using biogas technology.

4 Conclusion and recommendations

This study investigated the effects of operating conditions, type of substrate, and multiple-stage co-digestion on enhanced biomethane production. The pretreated fruit + vegetable waste (FVW) and corn stalks + wheat straw (CR) in ratio (1:1) mixed with fresh animal manure (BD) were used in 2-stage co-digestion. The inoculum was taken from an anaerobic digester of poultry manure at 35 °C. This AD process was carried out by using a fixed dome type biodigester with the capacity of 2.3 m^3. To calibrate experimental data, three biomethane potential (BMP) tests were also conducted for selected biomass treatments at 35°C. The total daily methane productions from fresh feed and slurry digesters under T_1 and T_2 were 125.13 ml/g VS (0.83 m^3) and 104.89 ml/g VS (0.56 m^3) in mesophilic range (30–40°C) while these values were 148.41 ml/g VS (0.98 m^3) and 132.74 ml/g VS (0.71 m^3) in mesophilic range (40–50°C), respectively. The cumulative total methane productions (fresh feed and slurry feed digester) with 10 days interval in T_1 were 6.4–17% higher than T_2. The 2nd stage digestion of slurry from fresh feed digester added 39–45% and 35–38% more methane production in T_1 and T_2 respectively. Experimental data was calibrated with BMP tests, showing the synergetic effect on methane production and its thermal characteristics promoted by co-digestion of pretreated organic waste and BD. The techno-economic and feasibility analysis was conducted to evaluate the economic benefits of adopting biodigestion technology for co-digestion of organic waste. The biomass co-digestion project was proved to be a viable and environmentally friendly technology with zero carbon emission. The project feasibility was confirmed with positive (5.39 $) net return value (NRV). Economic analysis indicated 2.92 years payback time (PBT) to recover all the investment cost for this project with 1.34 as benefit to cost ratio (BCR). Such techno-economic analysis could be replicated with similar socio-economic characteristics for better understanding in adoption of biogas/alternate energy resources.

The following suggestions are necessary to enhance adaptation of this bioresources technology: technical knowledge of farmers towards biogas technology should be improved. For this, farmers need capacity building through technical training at farmers level, strengthening infrastructure of TEVTA (Technical Training Institute). Additionally, agricultural extension department must extend their services, and biodigestion technology must be included in extension services. This can help to incline rate of adoption and farmer's perceptions regarding financial benefits of biogas technology because addition in income increases the chances of adaptation. Local agricultural departments and public agencies should offer economic incentives in terms of subsidies of soft loans.

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Author contributions Rana Shahzad Noor conceived the conceptualization of study, design and development of the experiment, data collection, formal analysis, investigation, methodology, visualization, writing an original draft, reviewed, and write-up editing. Aziz Ahmed contributed in development of research project, data collection, and formal analysis. Irfan Abbas and Rabeea Noor worked on manuscript review and editing, Fiaz Hussain and Muhammad Umair contributed in data collection and methodology. Yong Sun reviewed the research methodology and manuscript draft as internal reviewer for the manuscript.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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