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Anaerobic Biodegradability and Biomethanation Potential of Fruit-Vegetable Wastes at Sindh, Pakistan

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Abstract

The urban environment of every city of Pakistan has been degraded because of open dumping and burning of organic wastes along with other wastes. The focus of this study was to evaluate the fruit and vegetable wastes for methane generation through biomethanation process. After collection, various parameters such as alkalinity, volatile fatty acids, pH, lignin content, moisture content, total solids, volatile solids, fixed carbon, and elements (C, H, N, O, S) of waste samples were determined by using standard methodology. Anaerobic biodegradability of fruit and vegetable wastes was observed from 54 to 77% and from 59 to 87% along with their methane generation potential in the range of 258-367 NmL /gmVS and 274-407 NmL/gmVS, respectively. Further, the effect of feedstock to inoculum ratio was studied. The result of that showed that lower methane potential at a higher ratio and vice versa was observed. It was concluded that at a lower feedstock to inoculum ratio, fruit as well as vegetable wastes become more feasible for the biomethanation process. The study recommends that the conversion of fruit and vegetable wastes into methane gas by anaerobic digestion plays a significant role to save urban environment of the country.

Keywords: Fruit waste, Vegetable waste, Solid wastes, Biomethane potential, Substrate to inoculum ratio

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Introduction

Increasing the generation of municipal solid waste (MSW) is one of the current burning issues among all other environmental issues like water pollution, soil pollution, wastewater generation, air pollution etc. The main causes of increasing MSW are overgrowing population and urbanization. Proper management of MSW has become a challenging task for developing nations [1]. Nowadays, Pakistan is facing a major challenging issue that is generation of solid waste at an alarming rate. About 32.6 M tones/year with generation and collection rate of 0.43 kg/c/day and 50-60%, respectively is generated because of increasing population in the country [2]. Many socio-economic and environmental issues have been created which are associated with improper collection, transportation, and disposal of waste in Pakistan. Moreover, improper dumping of waste in any open space is frequently observed even along the road and highways sides [3]. There is a lack of properly engineered landfills for disposal of waste. There are few landfill sites in Lahore and Karachi which are not properly designed according to engineering and scientific principles [4].

Depending upon the sources of generation, MSW mostly consists of different inorganic (i.e. metal, glass, ash, dirt dust stones etc.) and organic substances including food wastes (fruit and vegetable wastes), yard wastes, paper wastes etc. Some of them are biodegradable and others are combustible, recyclable, biodegradable etc. The contribution of biodegradable wastes like yard, fruit, and vegetable wastes in MSW is increasing. There are various disposal and treatment methods, for example, landfill, incineration, composting, anaerobic digestion etc, which are globally applied to dispose of and/or to treat MSW to recover energy from it. Municipal landfills, land spreading and feeding animals are methods of disposal for biodegradable wastes in Pakistan [5]. Landfill of fruit-vegetable wastes (FrVW) provides a source of nuisance because of their moisture content, volatile solids, and high biodegradability [6]. Another study suggests that uncontrolled dumping and landfills of FrVW create environmental pollution like water, land, and air pollution [7]. Moreover, high volatile and water contents of FrVW give a generation of heavy odor and plenty of leachates during the collection, transportation, and landfill of these wastes [8].

To overcome these drawbacks of numerous methods, biological methods for treatment FrVW are the most economical and environmentally sound [9]. Various biological methods for different organic wastes such as food wastes and FrVW have been mechanized by some research groups [10]. Biomethanation is anaerobic digestion which has been suggested as one of the alternative biological methods to treat high organic and water content wastes to recover non-conventional energy in terms of biogas as well as to make organic substances more stable [9]. According to Naik et al. [11] biomethanation is a more attractive method than rest of biological methods as by its biogas is generated which contains methane and carbon dioxide. The findings of studies [12] showed that the electricity could be generated by methane and effluent of biomethanation plants contains enough nutrients which are helpful to improve soil fertility as reported by Ahring et al. [13]. Biomethanation is the best solution to treat FrVW as compared to present methods adopted to dispose of the FrVW which are inappropriate and result in environmental pollution [14]. Moreover, a lower operational energy input as well as initial investment cost make biomethanation more important than conventional aerobic composting processes and hence a cleaner and more renewable energy source is produced by biomethanation of FrVW [15]. Before anaerobic digestion, the feasibility of biowastes to produce biogas is analyzed by one of the most popular techniques known as biochemical methane potential test (BMPT). The BMPT provides high quality and quantity data by maintaining a satisfactory degradation profile [16]. In this regard, this study was carried out which focuses to examine the anaerobic biodegradability as well as methane potential of FrVW and to analyze the effect of feedstock-inoculum ratio (FI_r) on methane yield of substrates.

Materials and Methods

Preparation of Feedstock and Inoculum

In this study, FrVW and buffalo dung (BD) were used as feedstock and inoculum respectively. About 5-10 kg of each fruit and vegetable waste was collected from fruit shops and vegetable markets of Hyderabad city correspondingly. After collection, samples were dried in oven until achieving constant weight [17]. Then cutting and shredding of represented samples were carried out manually using scissors and thus such type of practice continued until the size of sample became less than or equal to 2 mm [18]. After that, samples were stored at 4 ^{o}C in plastic bags for later determination of their feasibility to generate methane potential. For inoculation, fresh BD was collected.

Feedstock and Inoculum Characteristics

Standard methods were used to determine various characteristics of sample such as FS (fixed solids), VS (volatile solids), TS (total solids) and MC (moisture content) [19]. The ultimate analysis (as % of dry basis) of feedstock as well as inoculum was analyzed according to the BBOT23122013 method. The percentage of C (Carbon), H (Hydrogen), N (Nitrogen) and S (Sulphur) was determined with the help of a CHNS analyzer, while subtraction method was used to calculate O (oxygen) percentage [20]. LC (Lignin Content) and CC (Cellulose Content) of samples were determined by a method suggested by Kelly et al. [21]. The pH, TA (Total Alkalinity) and VFA (Volatile Fatty Acids) of feedstocks, inoculum, and effluent at the end of BMP tests were evaluated according to the standard methods [19].

Batch Assays Preparation

The degradation of VS present in biomass produces methane that is measured by adopting the BMP test. In this test, incubation of a small amount of biomass with the source (inoculum) of active methane producing microbes proceeds to yield biogas that contains methane and carbon dioxide. In this study, BMP tests were performed with a semi-automatic BMPT system which was locally designed and fabricated (Fig. 1). It works according to the same principle as the conventional and fully automatic BMPT system.

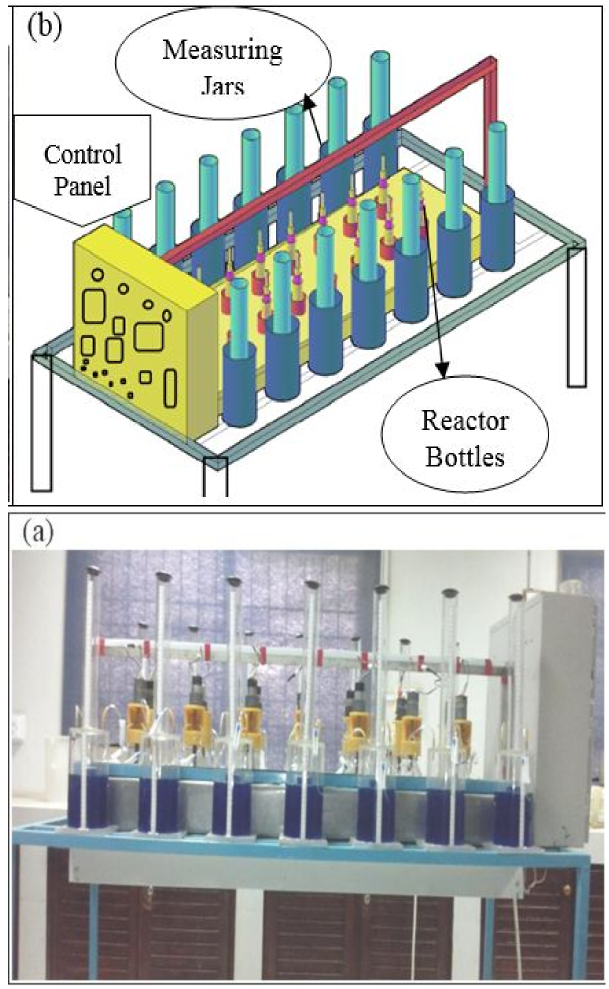

Figure 1. **(a) Semi-automatic biochemical methane potential test system (SABMPTS) (b) three-dimension view of SABMPTS.**

A total of fourteen reactor bottles were equally installed from both sides of SABMPTS along with gas measuring jars as shown in Fig. 1. Each reactor bottle was filled up to 400 mL each one was filled with tap water along with feedstocks at different FI_{rs} as described in Table 1. The FI_{rs} were prepared by increasing and decreasing the VS contents of feedstocks and inoculums, respectively (Table 1).

The gas measuring jars related to reactor bottles filled up to 50% of their capacity with 3 M of NaOH solution to absorb CO_2. About 0.5 g of $NaHCO_3$ was added in all reactors to avoid a drop in pH. Then all reactors were dipped into hot water tanks up to half of their height and connected in parallel with gas measuring jars. After that, nitrogen gas was purged to expel oxygen gas from reactors. Duplicate experiments of feedstocks were performed at various FI_{rs} upto 40 days. The BMP tests were carried out at a mesophilic temperature which is favorable condition for methanogenic bacteria [22].

Table 1. **Preparation of sample for batch assay.**

Waste Types	Reactor Bottles	Feedstocks	FIrs
	R1	FrW1	30:70
	R2	FrW2	40:60
Fruit wastes	R3	FrW3	50:50
	R4	FrW4	60:40
	R5	FW5	70:30
	R6	VW1	30:70
	R7	VW2	40:60
Vegetable wastes	R8	VW3	50:50
	R9	VW4	60:40
	R10	VW5	70:30

Anaerobic Biodegradability

Anaerobic biodegradability (ABD) in terms of percentage was estimated by equation (1) [23]. In which, Exp.MP stands for experimental methane potential (NmL/gmVS) which was obtained using equation (2) and Theo.MP represents theoretical methane potential of feedstock (Nml/gVS) which was determined with the help of Bushwell and Mueller equation (3) [24]. In equation (2), $VS_{(fs)}$, $VS_{(ino.)}$ and $VS_{(fs\ \&\ ino.)}$ stand for volatile solid of feedstock, inoculum and both feedstocks along with inoculum, respectively. Whereas, the subscripts a, b, c, d and e used in equation (3) represent the mole fraction of C, H, O, N and S correspondingly. The whole biodegradable substance gives CH_4, CO_2, NH_3 and H_2S as assumed by equation (3) [25]. Further, a modified form [26] of equation (3) was used as equation (4) to calculate Theo. MP of feedstock and inoculum.

$$ABD = Exp.MP/Theo.MP \times 100 \quad (1)$$

$$Exp.MP = [VS_{(fs\ \&\ ino.)} - V_{(ino.)}]/VS_{(fs)} \quad (2)$$

$$C_aH_bO_cN_dS_e + XH_2O \longrightarrow YCH_4 + ZCO_2 + dNH_3 + eH_2S \quad (3)$$

Where;

$$X = (4a - 2c + 3d - 2e)/4 \quad (i)$$
$$Y = (4a + b - 3d - 2e)/8 \quad (ii)$$
$$Z = (4a - b + 2c + 3d + 2e)/8 \quad (iii)$$

$$Theo.\ MP = (930C + 2790H - 350O - 600N - 175 - S)/ C + H + O + N + S \quad (4)$$

Volatile Solid Reduction

The volatile solid reduction (VSR) in percentage was determined by using equation (5), where $VS_{(in)}$ and $VS_{(out)}$ denote the percentage of VS before and after BMP tests respectively. The VSR represents how much quantity of VS is converted into methane yield as the methane production is the function of the presence of VS in the substrates.

$$VSR = (VS_{in} - VS_{out})/VS_{in} \quad (5)$$

Results and Discussion

Composition and Characteristics of Waste Sample

The composition of substrates including fruit waste (FrW) and vegetable waste (VW) is shown in Fig. 2 (a) and (b), respectively. The share of different components of FrW generated in the summer season is determined as 35%, 25%, 20%, 10% and 10% by mango peels & discarded, watermelon discarded, melons discarded, sapodillas discarded, and papayas, respectively as mentioned in Fig. 2(a). The physical composition of VW generated in the summer season is contributed by various components at equal proportions as illustrated in Fig. 2(b). Substrate composition plays an important role in AD and is directly associated with biodegradability [27].

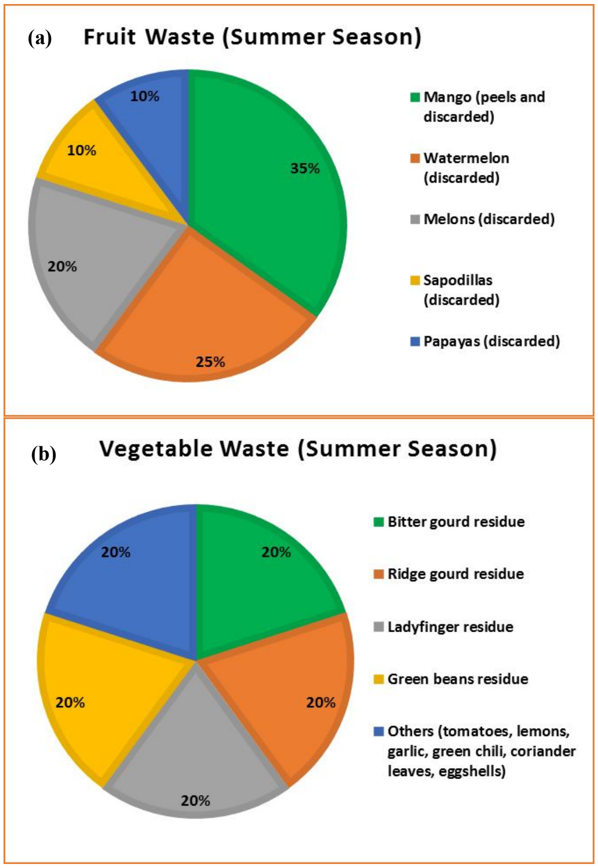

Figure 2. **Composition of (a) fruit waste and (b) vegetable waste sample for BMP test.**

Table 2. **Characteristics of substrate samples and inoculum.**

Parameters	Substrate		Inoculum
	FrW	***VW***	***BD***
Proximate analysis			
MC (%)	92.47	94.87	85.65
TS (%)	7.53	5.13	14.35
VS (% of TS)	87.45	90.82	83.42
FS (% of TS)	12.55	9.18	16.58
Ultimate analysis			
C (% of TS)	42.15	41.33	40.08
H (% of TS)	0.09	5.68	5.19
O (% of TS)	40.91	37.87	33.04
N (% of TS)	3.21	4.76	3.95
S (% of TS)	1.09	1.18	1.16
C/N (% of TS)	13.13	8.68	10.15
Other characteristics			
LC (% of VS)	12.09	9.67	7.21
CC (% of VS)	16.5	23.5	19.5
pH	4.05	4.7	6.85
TA (mg $CaCO_3$/L)	200	225	1225
VFA (mg CH_3COOH/L)	552	354	630

The results of various characteristics including percent of C, O, N, S, H, MC, TS, FS, VS, CC, LC, C/N ratio, pH, TA and VFA of substrate and inoculum were determined and given in Table 2.

BMP Results and ABD

Figure 3 represents the cumulative methane production of substrates at various FIrs. In all batch assays of the experiment different methane yields were observed. The cumulative methane for VW at various FIrs of 30:70, 40:60, 50:50, 60:40, and 70:30 were 407.0, 384.5, 347.7, 317.1, and 274.2 in mL/g VS, respectively. Similarly, the co-digestion of FrW showed the same trend of cumulative methane generation which was comparatively lower than the VW co-digestion ratios. The methane determined for FrW at different FIrs of 30:70, 40:60, 50:50, 60:40, and 70:30 were 367.3, 330.6, 303.4, 286.6, and 257.5 mL g^{-1} VS, respectively. Maximum methane yield of 407.0 mL g^{-1} VS and 367.3 mL g^{-1} VS was determined at a co-digestion ratio of (30:70) for VW1 and FrW1, respectively (Fig. 3).

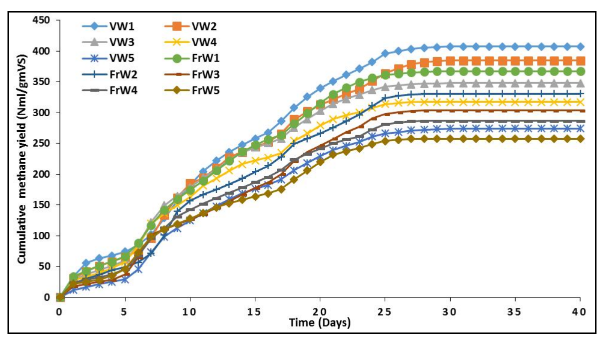

Figure 3. **Methane generation of substrates**

A significant improvement in methane production by co-digestion of rice straw and buffalo dung at 30:70 was also concluded [~~40~~ 28]. Another study conducted by Zhang et al. [29] also optimized a 30:70 ratio with C/N balance for co-digestion of cotton stalks and goat manure. At different mixing ratios, the flow rate of methane generation by FrW and

VW during 40 days of digestion was also assessed and shown in Fig. 4.

Many peaks of methane flow rate were noticed for the co-digestion of VW, which yielded peak values of 28.1 (day 9), 38.6 (day 8), 37.5 (day 7), 37.1 (day 7), and 28.3 mL (day 6) for the FIrs of 30:70, 40:60, 50:50, 60:40, 70:30 ratios, respectively as mentioned in Fig. 4. A similar trend was also observed for the co-digestion of FrW, whereas peak values of 29.1 (day 7), 39.7 (day 10), 36.3 (day 7), 29.1 (day 6), and 27.1 mL (day 7) were determined for the FIrs of 30:70, 40:60, 50:50, 60:40, 70:30 ratios, respectively as represented in Fig. 4.

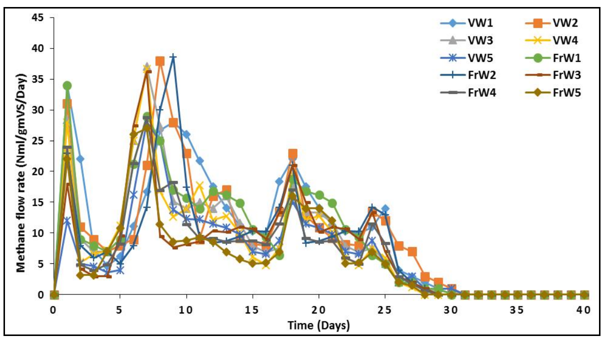

Figure 4. **Flow rate of methane generation of substrates**

It has been analyzed that the daily methane flow rate declined after the 23 days of co-digestion process, due to the degradability and composition of substrate was not as same as the initial composition. The VW was easily disintegrated under anoxic conditions because of lettuce and softy in nature (less cellulose content) as compared with FrW. The FrW like pomegranate, banana, cantaloupe and watermelon take time to degrade in anaerobic conditions. Findings showed that all mixing ratios of VW with BD significantly improved the methane generation and reduced the lag phase. The BMP test directly correlated to biodegradability percentage and calorific value. In an earlier study by Korai et al. [31], the highest methane potential and degradability of VW were reported as 0.40 L/gmVS and 60.1%, respectively which is low in comparison to co-digestion. The present study findings assessed that methane production of substrates significantly increased when mixed at FIrs of 30:70. The Theo.MP of substrates and inoculum is given in Table 3.

Table 3. **Theo.MP of substrate samples and inoculum.**

Substrates	Coefficients of chemical formulas					Theo.MP *(Nml/gmVS)*
	a	*b*	*c*	*d*	*e*	
FrW	103	3	75	7	1	474.41
VW	94	153	64	9	1	467.31
Inoculum (BD)	92	142	57	8	1	444.61

The Theo.MP of FrW, VW and BD were obtained as 474.41 NmL/gmVS, 467.31 NmL/gmVS and 444.61 NmL/gmVS respectively as represented in Table 3. Whereas Exp.MP and ABD of substrates at various FIrs were also determined as given in Table 4.

The Exp.MP of FrW and VW was obtained in the range of 257.5-367.3 Nml/gmVS and 274.2-407.0 NmL/gmVS respectively. The highest ABD of FrW (77.42%) and VW (87.09%) was obtained at FIrs of 30:70 as given in Table 4. While the FIrs of 70:30 of both substrates gave the lowest ABD, that is 54.28% (FrW) and 58.68% (VW) as represented in Table 4. From these results, it is revealed that 30:70 is optimized FIrs among all others.

Table 4. **Exp.MP and ABD of substrates at different FI ratios.**

Substrates	FI ratios	Exp.MP (NmL/gmVS)	ABD (%)
Fruit wastes	FrW1 (30:70)	367.3	77.42
	FrW2 (40:60)	330.6	69.69
	FrW3 (50:50)	303.4	63.95
	FrW4 (60:40)	286.6	60.41
	FrW5 (70:30)	257.5	54.28
Vegetable wastes	VW1 (30:70)	407.0	87.09
	VW2 (40:60)	384.5	82.28
	VW3 (50:50)	347.7	74.41
	VW4 (60:40)	317.1	67.87
	VW5 (70:30)	274.2	58.68

Relation between ABD and VSR

The relation between ABD and VSR of different co-digestion mixing ratios of the VW and FrW is illustrated in (Fig. 5a and 5b).

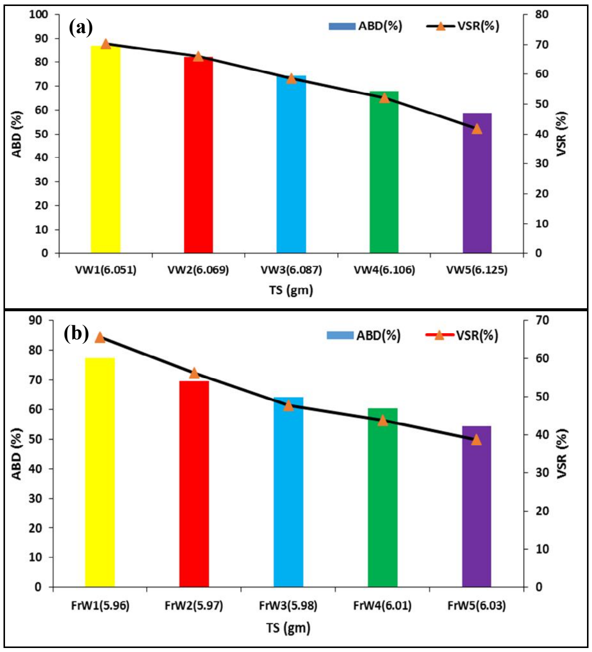

Figure 5. **Volatile soid reduction of (a) vegetable and (b) fruit waste samples at different S/I ratios**

For all co-digestion ratios, it is observed that there was a directly proportional increase to decrease relation between ABD and VSR. The methane yield enhanced when the rate of reduction of ABD and VSR increased. Thus, ABD reflects the material utilization and conversion of the VSR [26,28,32]. From all different mixing ratios such as 30:70, 40:60, 50:50, 60:40 and 70:30, the ABD was determined to be ranging from 88.2 to 65.1% for VW and ranging from 77.2 to 53.4% with FrW. A similar trend was also determined for the VSR which had considerably lower than ABD. The VSR ranged from 67.1 to 48.2% for VW and ranging from 64.5 to 38.7% for FrW (Fig. 5).

Literature finds that substrate composition like LC is an important factor during the digestion process. It is very hard to degrade because of possessing tougher intermolecular bonding. For the substrate that has a higher percentage of LC, the degradation can take a long duration and generate lower methane potential [33,34]. The LC of VW, FrW and BD substrates were determined and the findings are indicated in Table 2. As expected, the FrW was higher LC as compared with VW and BD. FrW contained 12.09 % LC, whereas VW and BD contained 9.67 % and 7.21%, respectively. Moreover, the maximum ABD and VSR degradation occurred at a ratio of 30:70 from both feedstock co-digestions. It means the same quantity of manure (buffalo dung) was mixed, thus balanced the C/N ratio. Therefore, similar ABD and VSR degradations were observed from a 30:70 ratio than other mixing ratios. The balanced C/N decreases the risk of ammonia inhibition and further increases the methane production rate as reported by Comino et al. [35]. In the light of lower LC and balanced C/N ratio of VW with BD results data confirms the ABD and VSR leads to higher methane production.

Stability of Digestion Process

The pH, alkalinity (TA), VFA and VFA/TA ratio at the end of BMP tests from co-digestion mixing ratios of VW and FrW are shown in Table 5.

Table 5. **Characterization of effluent at the end of BMP tests.**

Substrate	pH	TA (mg $CaCO_3$/L)	VFA (mgCH_3COOH/L)	VFA/TA Ratio
VW1	7.0	2445	634	0.259
VW2	7.45	2463	578	0.235
VW3	7.35	2377	632	0.266
VW4	7.2	2246	721	0.321
VW5	6.9	2045	546	0.267
FW1	7.1	2134	574	0.269
FW2	7.3	2168	465	0.214
FW3	7.1	2035	456	0.224
FW4	6.9	1946	672	0.345
FW5	6.85	1987	623	0.314

In AD, various bacteria and archea existed and they had a very close relationship. The archea (methanogens) are very sensitive. They need to survive in suitable conditions and work optimally when a pH ranges from 6.8 to 7.2 [36]. High or low pH values are observed as process imbalances in the digester. High pH value results in an increase in ammonia formation and low pH would cause results increase in VFA concentration [37]. The pH and VFA are the two main parameters in AD for determination. The existence of various VFAs like formic acid, acetic acid, butyric acid, and propionic acid are mostly observed during digestion. All these have direct organic acid products, but combined VFA concentration is the key factor of metabolic level in addition to the pH value. Findings indicated that pH and VFA corresponded with methane yield in co-digestion. Consequently, pH value was proportionate with methane yield, while the total VFA was inversely proportionate [41 29]. As an operating temperature beyond the limit causes the faster hydrolysis of the substrates and results in the accumulation of VFA which inhibits the methane yield [38]. To examine the stability of AD process, VFA/TA ratio is also an important factor, and it should be less than 0.5. But if the ratio exceeds the limit of 0.5 it is considered an indication of instability of the digester [39].

In the co-digestion of VW, the average results of pH, TA, and VFA ranged from 6.9 to 7.45, 2045 to 2463 mg $CaCO_3$/L, 546 to 721 mg CH_3COOH/L, respectively. The same trend of composition was also observed in the co-digestion of FrW. The average results of pH, TA, and VFA ranged from 6.85 to 7.3, 1946 to 2168 2463 mg $CaCO_3$/L, and 456 to 672 mg CH_3COOH/L, respectively as given in Table 5. Moreover, the VFA/TA ratio of co-digestion of VW and FrW with BD at different mixing ratios are graphically illustrated in Fig. 6. In all BMP sample reactors, the VFA/TA ratio was observed to be less than 0.5, thus no inhibition was analyzed in the anaerobic digestion process.

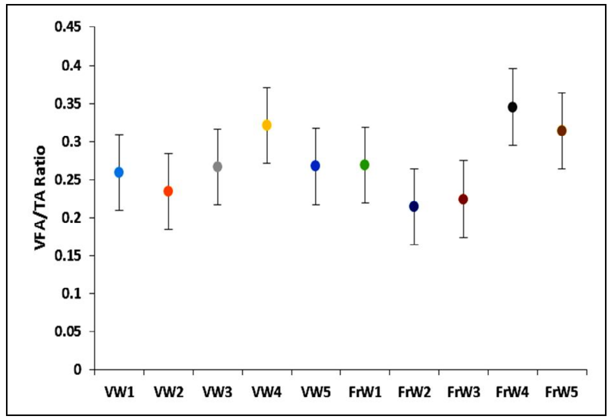

Figure 6. **Stability of digestion process**

Conclusion

The study was carried out to improve the production of methane from VW and FrW with BD through anaerobically co-digestion. The co-digestion substrate ratios of VW and FrW with BD were treated as 30:70, 40:60, 50:50, 60:40, and 70:30. Results indicated that all mixing ratios have increased the production of methane and stabilized the co-digestion process. The maximum methane of 407.0 mL g^{-1} VS and 367.3 mL g^{-1} VS was analyzed from a ratio of 30:70, respectively. A similar optimized ratio (30:70) from VW and FrW effectively increased the production of methane means that the carbon-nitrogen fraction in the co-digestion process was greatly balanced, but VW to BD produced a 9.75% higher percentage of methane compared to FrW to BD. However, compared with an optimal ratio of co-digestion, VW generates a higher volume of methane than FrW due to its high organic content and easy biodegradability. The results and findings of the study lead to recommend that fruit and vegetable wastes should be used as energy sources in terms of methane potential, rather than dumped in an open environment.

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Conflict of Interest

The authors declare no competing interests.

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