

## Recovery of acerbic anaerobic digester for biogas production from pomegranate shells using organic loading approach

Kalpana Jain, Pradeep Suryawanshi & Ambalal Chaudhari\*

School of Life Sciences, Kavayitri Bahinabai Chaudhari North Maharashtra University,  
Jalgaon-425 001, Maharashtra, India

Received 19 June 2019; revised 15 July 2019

Anaerobic digestion of pomegranate shells was conducted in 25 L bioreactor operating at  $35 \pm 0.5^\circ\text{C}$ . The digester showed a reasonable amount of biogas ( $0.71 \text{ m}^3/\text{kg VS fed}$ ) and methane (55.7%) with stable pH and acid: alkali profiles when operated at organic loading rate (OLR) from 1.0 to  $3.0 \text{ kg VS/day/m}^{-3}$ . The reactor exhibited stable performance with methane yield of  $0.44 \text{ m}^3/\text{kg VS fed}$  and reduction of 38.5% volatile solids (VS). As organic loading rate increased to  $3.5 \text{ kg VS/day/m}^{-3}$ , accumulation of volatile fatty acid (VFA; 2797 ppm), mainly propionic acid (1617 ppm) was noticeable. The digester turned sour (pH 4.32) with lower biogas ( $2.5 \text{ Ld}^{-1}$ ) and methane (30.80%) production, reflecting the case of overloading. Reversal of organic loading rate from 3.5 to  $3.0 \text{ kg VS/day/m}^{-3}$  gradually restored the upset anaerobic digester to normal profile in 4 weeks as judged from a gradual increase in biogas ( $6.5 \text{ Ld}^{-1}$ ) and methane (58.4%).

**Keywords:** Anaerobic digestion, Methane yield, Organic loading rate (OLR), Propionate, *Punica granatum*, Volatile solids

Pomegranate (*Punica granatum*) is cultivated in the subtropical and temperate regions of the world and India ranked second largest to produce 745 MT<sup>1</sup>. About 52% of pomegranate fruit is processed (322-341 L/ton of fruit) to minimize spoilage and 50% of the total fruit corresponds to non-edible shell<sup>2</sup>. On the contrary, pomegranate shell (PS) contains large amounts of organic matter which is mostly abandoned. Due to their organic nature and composition, PS deteriorates easily leading to foul smell and cause environmental threat. Jain Irrigation Systems Ltd. (JISL) Jalgaon process 50000 tons per annum of pomegranate fruits to produce 18000 tons of juice/juice concentrate and generate 6500 tons of seeds and 21000 tons of shells every year. Of these, seeds are mechanically crushed to extract oil as a feedstock for health<sup>3</sup> and food industry<sup>4</sup>, while de-oiled cake of seeds (DOCS) has been explored as a potential feedstock for the biogas production by anaerobic digestion<sup>5</sup>. Anaerobic digestion (AD) of PS and DOCS appeared as more suitable bio-treatment than alternative combustion and gasification or composting because of the high moisture and organic matter content of the pomegranate shell<sup>5</sup>.

The performance of AD processes is affected

by feedstock characteristics, reactor design, and operation conditions, but OLR represents the actual amount of organic volatile solids to be fed in anaerobic digester each day because it determines the level of biochemical activity and stability in the digesters<sup>6</sup>. Excess of organic volatile solids not being fully degraded by the microbial composition within the digester leads to the accumulation of surface-active by-products that promote foaming<sup>7</sup>, change the amount and composition of VFA<sup>8</sup> ( $>1.5 \text{ g/L}^{-1}$ ) produced by the acidogenic bacteria which alter the bacterial community<sup>9,10</sup>. The mesophilic AD operates OLR in the range from 0.7 to  $7.2 \text{ kg VS/m}^3/\text{d}$  and usually results in (i) souring above the suggested operational range<sup>10</sup>; (ii) greater bacterial diversity; (iii) shift from a *Clostridium* dominated community to a microbial community<sup>11</sup> comprising of *Gamma proteobacteria*, *Actinobacteria*, *Bacteroides*, *Deferribacters*<sup>12</sup>; (iv) proportionately high numbers of *Methanosarcina* and more of syntrophic bacteria<sup>13</sup>; (v) more propionic acid content<sup>14</sup>; and (vi) decrease of the archaea biomass<sup>15</sup>. The most preferred strategy in such circumstances is to operate the bioreactor under low OLR. However, such strategy results in low biogas production compromise process efficiency and economic feasibility.

The improvement in the performance of digester under stress conditions is achieved either through

\*Correspondence:  
E-mail: ambchasls@gmail.com

(i) promoting functional diversity in the microbial community using granular substrate<sup>16</sup>; or (ii) bio-augmentation and (iii) changing the operational conditions to enhance functional diversity and activity<sup>17</sup>. Of these, former approach is extensively studied and demonstrated influence of stress conditions on archaeal and bacterial communities, but more efforts are required for later approach on key operational process indicators<sup>18,19</sup> including, alkalinity, pH, propionic acid: acetic acid ratio and VFA: total alkalinity (V: A) ratio because feedstock composition may not provide a true picture about how new feedstock will affect digester performance at least at biochemical level or microbial level. The organic loading rate is a crucial parameter that represents the biological conversion capacity of the system, affects microbial ecology and characteristics of the operational system. For this, the digester needs to be exposed to a series of OLR changes using a specific feedstock.

Anaerobic digestion of a variety of solid organic substrates of agricultural origin including, pomegranate marc has been reported for biodegradability and biogas production<sup>13,20,21</sup>. Only a few reports indicate recovery of sour bioreactor for biogas production<sup>22</sup>, but none report anaerobic digestion of pomegranate shells. During the anaerobic digestion of pomegranate shells (PS) the digesters repeatedly turned sour; characterized by low pH (6.8), poor acid:alkali ratio<sup>1,5</sup>, reduced rate of biogas generation accompanied by low % methane<sup>23</sup>. Incidentally, the digester that inadvertently turns acerbic during anaerobic digestion of PS has not been studied for recovery of biogas.

## Materials and Methods

### Bioreactor

A circular, fixed-dome biodigester (height 32.0 cm, internal diameter 29.6 cm and 25 L capacity) was fabricated from PVC, with an inlet and outlet provision and fitted with an agitator to provide uniformity of pH and temperature in the substrate slurry and its close contact with a consortium of microbes from the inoculums (Plate 1). To conserve energy, the frequency of agitation was automated at 100 rpm for 3 min after every 30 min and set-up was kept at 35±0.5°C in the temperature-controlled room.

### Substrate for biogas production

PS from the fruit processing plant of JISL, Jalgaon stored in plastic bags was used as a substrate. The

particle size of PS was 1.0-1.5 cm, neither too large to avoid clogging of the bioreactor nor too small for rapid settling and yet permitting easy access for microbes to carry out its digestion. PS was used at 10% total solids (TS) in the study.

### Inoculum

The inoculum comprised of cattle dung (47.5%, v/v) + water (47.5%, v/v) + effluent from the ongoing biogas plant (5%, v/v) operated at 35°C with mixed fruit waste. About 10% of inoculum was used to inoculate 25 L bioreactor.

### Optimized experimental setup

AD was carried out at 35±0.5°C and pH 6.8-8.0 as reported earlier<sup>24</sup>. Initially, PS substrate was added at the organic loading rate (OLR= kg of volatile solids/m<sup>3</sup>/day) of 1.0. This rate was continued until 30 days. AD was attempted at low HRT (30 days) at 35°C. Subsequently, OLR was increased by 0.5 after every 30 days and analyzed for steady increase in microbial count.

### Analytical methods for characterization

Analysis of initial fruit waste and bioreactor slurry for total solids (TS), volatile solids (VS), total organic carbon (TOC), total Kjeldahl nitrogen (TKN), total carbohydrates, lignin, and phosphorus was performed as per standard methods<sup>25</sup>. Microbial analysis was carried out using the method adopted earlier<sup>23</sup>.

Volatile fatty acid (VFA) and methane analysis were done by gas chromatography (Clarus 500, Perkin Elmer, USA), equipped with flame ionization detector (FID) and thermal conductivity detector (TCD). VFAs were analyzed using FID and capillary



Plate 1 — 25 L lab scale fixed dome bioreactor

column (Elite WAX ETR, 30 m × 0.32 mm × 1.0 μM) and helium were used as a carrier gas. The temperature of the detector, injector, and oven was 260°C, 240°C and 80-230°C (programmed), respectively. Methane was analyzed using a stainless-steel packed column (HAYSEP-Q, 80/100 mesh, 9' × 1/8''OD). The temperature of the detector and injector was 100°C and the oven set to 40°C<sup>5</sup>.

The measurement of biogas was carried out after every 24 h using a calibrated gas flow meter of 1 L per revolution capacity based on water displacement method<sup>26</sup>.

## Results and Discussion

Anaerobic digestion (AD) of mixed fruit and vegetable waste into biogas and organic manure production was carried out<sup>27</sup> and unable to throw light on the digestion parameters for the use of PS. Hence, AD of PS (Table 1) was continued for more biogas production.

### Single-phase digestion

In single-phase AD, acid-forming bacteria (acidogens) and methane forming archaea (methanogens) co-exist in the same biological environment. The complex organics in the waste are first acted upon by the hydrolytic, fermentative and acidogenic bacteria present at the top portion of biomass resulting in the production of VFAs, which in turn are acted upon by the methanogens at the bottom, resulting in the production of biogas. For this purpose, AD of PS was continued for more biogas production.

PS is a hard structure, composed of 32.3% TS, of which 95.4% are VS, indicating its biodegradability (Table 1). In PS, C: N ratio was 39.1, which indicated high carbon content and comparatively low nitrogen, rendering it relatively difficult to digest. The analysis

of PS was in accordance as previously reported<sup>23</sup>. Because of the high TS/VS, C/N ratio, the pomegranate shell appeared as the most suitable substrate for anaerobic digestion. Accordingly, the potential of PS for biogas production and methane content was assessed at 10% total solids (TS) in the present study.

### AD of PS

An average HRT of slurry inside the bioreactor varied in tropical Indian climate from 30-50 days, as against 100 days in a cold climate, whilst shorter HRT faced the risk of wash-out of the active bacterial population, and longer HRT required a large volume of the digester, necessitating more capital cost. In the present study, methanogenesis was attempted at low HRT (30 days) at 35°C without compromising the fermentation process<sup>23</sup> which is in agreement with the previous study<sup>2</sup>.

Initially, AD of PS at an organic loading rate (OLR) of 1.0 produced more than 80% acetic acid (AA) to serve as substrate for the methanogens, about 8% propionic acid (PA), 3% butyric acid (BA) as the major intermediates. This trend continued in the composition of these VFAs as a function of the OLR and digestion period. While, % TS, % VS, pH, alkali: acid ratio, biogas production, and methane content were monitored to understand their inter-relationship as a function of OLR. It is evident from (Table 2) that the volume of biogas and its methane content were increasing; OLR of 1.0 was gradually increased to 3.5 with steep changes in pH.

Since AD is carried out by a consortium of microorganisms and its success depends on various factors like temperature, pH, acid: alkali ratio, HRT, C:N ratio, *etc.* It has been observed that the rate of methanogenesis may decrease if the pH is lower than 6.3 or higher than 7.8<sup>28</sup>. Monitoring of these factors was necessary to understand as to why the production of biogas and % methane content decreased.

### Effect of pH

The pH affects the growth of microbes during AD and hence pH of the bioreactor was maintained within 6.8-7.8 range at an increasing OLR as suggested by Yadvika *et al.*<sup>29</sup> Accordingly, the initial pH of the slurry subjected to AD was 6.8. Gradually, it started rising as judged from the pH of effluent (digested slurry) and stabilized in 7.1-7.4 range at 0.025-0.076 kg VS fed/day (*i.e.* OLR in the range of 1.0-3.0). At higher OLR (3.5 or 0.089 kg VS fed/day), the pH of

Table 1 — Chemical composition of pomegranate shells

Parameter*	
pH	6.1±0.2
Moisture (%)	67.7±2.4
Total organic carbon (%)	40.9±2.2
Total carbohydrates (%)	30.5±3.5
Total lignin (%)	29.4±2.8
Total Kjeldahl nitrogen (%)	1.1±0.8
Volatile solids (%)	95.4±3.2
C:N ratio	39.1±2.5
Total phosphorus (%)	2.1±0.6

\*Analysis reported on dry weight basis and an average of 10 estimates; ±SD

Table 2 — Relationship between OLR, pH, acid: alkali ratio, biogas, and methane production

OLR (kg VS/day/m <sup>3</sup> )	VS fed/day (kg)	pH	Acid: Alkali ratio	Biogas production/kg VS fed (m <sup>3</sup> /kg VS)	CH <sub>4</sub> production/kg VS fed (m <sup>3</sup> /kg VS)
1.00	0.025	7.42±0.17	4.22	0.55±0.10	0.25±0.004
	0.025	7.30±0.22	3.87	0.63±0.08	0.32±0.004
	0.025	7.33±0.37	3.48	0.59±0.14	0.31±0.007
	0.025	7.20±0.12	4.62	0.67±0.14	0.34±0.007
2.00	0.051	7.18±0.17	3.55	0.55±0.04	0.30±0.002
	0.051	7.41±0.34	4.93	0.57±0.05	0.36±0.003
	0.051	7.39±0.28	4.64	0.61±0.06	0.32±0.003
	0.051	7.28±0.19	4.76	0.67±0.05	0.30±0.002
3.00	0.076	7.21±0.19	4.27	0.67±0.03	0.36±0.002
	0.076	7.37±0.13	3.85	0.70±0.03	0.39±0.002
	0.076	7.03±0.10	3.81	0.72±0.04	0.41±0.002
	0.076	7.40±0.13	4.00	0.78±0.05	0.44±0.003
3.50	0.089	6.86±0.72	3.14	0.60±0.04	0.29±0.002
	0.089	5.35±0.15	1.70	0.54±0.05	0.18±0.002
	0.089	5.16±0.72	1.31	0.43±0.04	0.16±0.001
	0.089	5.04±0.53	1.90	0.41±0.05	0.13±0.002
3.00	0.076	5.60±0.77	2.76	0.55±0.06	0.19±0.002
	0.076	6.42±0.29	3.43	0.63±0.06	0.26±0.003
	0.076	7.21±0.16	3.74	0.74±0.05	0.38±0.001
	0.076	7.26±0.13	4.03	0.76±0.05	0.44±0.003

Each parameter was monitored at 24 h interval and weekly averages are given with ±SD

the effluent gradually dropped to 5.0, indicating unacceptable OLR, since nothing else was done to the digester, except the OLR was increased to 3.5. The main reason for the steep decline in pH was the rapid acidification of the waste. Therefore, the OLR of the waste was restored to 3.0, which eventually restored the pH slowly to 7.2. Whether, the stable pH profile up to 3.0 OLR and its sudden decline to 5.0 at 3.5 OLR had any impact on acid: alkali ratio was then analyzed.

#### Effect of acid: alkali ratio

As per (Table 2), acid: alkali ratio was in the range of 3.5 to 4.9 throughout the digestion when the pH profile was stabilized in 7.1-7.4 range. By supplementing 0.089 kg VS/day (3.5 OLR), there was a sudden fluctuation in this ratio, first to 1.7 and then to 1.3. However, it improved slightly to 1.9 as a function of the digestion period, indicating either overloading or more time required for digestion, presumably due to the lower microbial population in the digester to handle the increased OLR. It normalized to 4.0 gradually over for 4 weeks. Thus, there was a noticeable change in pH on the acid: alkali ratio which was in line with the previous report<sup>30</sup>.

#### Effect of OLR

Biogas production rate being dependent on OLR, its production and % methane had shown a similar trend of pH and acid: alkali ratio. Increase in OLR from 1.0 to 3.0, no doubt increased biogas production from 0.55 to 0.78 m<sup>3</sup>/kg VS fed, with methane production from 0.25 to 0.44 m<sup>3</sup>/kg VS fed. However, as soon as OLR was increased from 3.0 to 3.5, there was a sharp decline in biogas production from 0.78 m<sup>3</sup> to initially 0.60 m<sup>3</sup> and subsequently to 0.41 m<sup>3</sup>/kg VS fed, with an associated decrease in methane from 0.44 m<sup>3</sup> to first 0.29 m<sup>3</sup> and finally to 0.13 m<sup>3</sup>/kg VS fed, indicating hydraulic overload (wash-out of microorganisms) as a result of insufficient buffering capacity in the digester, giving reduction in methanogenic activity as observed<sup>27</sup>. Therefore, OLR was reset to 3.0 to reverse the unfavourable trend at 3.5 OLR. It took 4 weeks for the trend in biogas production and its methane content to stabilize (Table 2). The bioreactor efficiency (kg of VS fed and its conversion to methane) as a function of OLR reported earlier was optimal at 1.5 OLR using PS as feedstock<sup>23</sup>.

#### Profile of microbes in AD

The control of pH seems essential during increased OLR and particularly when a stable microbial

population has not been established. The accumulation of VFAs altered microbial composition due to more PA and other VFAs at the cost of reduction of AA. The sharp decline in pH during the digestion of PS and a decrease in methane production (Table 2) agree with the inhibition of methanogens more than the acidogens<sup>27,31</sup>.

Meegoda *et al.*<sup>32</sup> suggested that at shorter HRT, the production of VFA could exceed the utilization rates, leading to the digester failure. In our opinion, the overloading of bioreactor may not necessarily be the result of resident time; instead, it was due to a lack of critical microbial population, resulting in the accumulation of VFAs, thereby further inhibiting the microbial population or its activity associated with methane production. This is evident from our observation that at the optimal OLR 3.0 (0.76 kg VS fed), when C:N ratio was 20±2, it was not a case of overloading. However, when C:N ratio was 39:1, it caused sour digester, reflecting overloading, as also observed in the past that the microbial population was indeed affected and appeared genuine in the present study<sup>5</sup>. While DOCS yielded 65-68% methane content, PS gave 53-63% methane, possibly due to the presence of tannin, alkaloids, flavonoids and terpenoids in the PS, which probably arrested microbial growth and digestion. This was further corroborated by facultative and anaerobic TVC in

DOCS digest *vis-à-vis* PS digest (Table 3). At OLR 3.0, the bioreactor showed a continuous increase in facultative and anaerobic CFU, biogas (0.78 m<sup>3</sup>/kg VS fed) and methane (0.44 m<sup>3</sup>/kg VS fed). Therefore, digestion efficiency (kg of VS fed and its conversion to methane) as a function of OLR was optimal at 3.0 for methanogenesis.

From (Table 3), it is distinct that while DOCS promoted the growth of facultative as well as strict anaerobes, the same was at a significantly lower level. It is no wonder therefore that an increase in kg VS fed on 0.076 to 0.089 drastically altered all parameters relevant for biogas and methane production.

#### Profile of VFAs

Ordinarily, propionate concentration is low due to its rapid turnover rate by either propionate-utilizing organisms<sup>33,34</sup> or degradation of propionic acid by a syntrophic association of acetate-, CO<sub>2</sub>- and H<sub>2</sub>-producing microbes. However, high propionate concentration seems to be inhibitory for the methane-forming organisms suggesting that propionate metabolism appears to play a crucial role when bioreactors are subjected to overload conditions, causing disturbances in its ecosystem. Hence, it is essential to restore the environmental conditions conducive for methanogens to function for anaerobic digestion.

Table 3 — Comparative profiles of the digested slurry of DOCS *vis-à-vis* PS operated at various OLR

Week	DOCS			PS		
	pH	Facultative (cfu*) × 10 <sup>5</sup>	Anaerobic (cfu*) × 10 <sup>5</sup>	pH	Facultative (cfu*) × 10 <sup>5</sup>	Anaerobic (cfu*) × 10 <sup>5</sup>
OLR (kg VS/day/m <sup>3</sup> ) 3.0 and VS fed/day 0.076 kg						
1	7.42±0.12	118	155	7.21±0.19	99	89
2	7.38±0.27	94	110	7.37±0.13	65	60
3	7.45±0.31	127	134	7.03±0.10	74	70
4	7.44±0.17	130	141	7.40±0.13	80	88
OLR (kg VS/day/m <sup>3</sup> ) 3.5 and VS fed/day 0.089 kg						
1	7.62±0.54	122	148	6.86±0.72	77	59
2	7.68±0.25	108	176	5.35±0.15	51	37
3	7.43±0.41	136	152	5.16±0.72	33	28
4	7.28±0.48	144	136	5.04±0.53	24	16
OLR (kg VS/day/m <sup>3</sup> ) 3.0 and VS fed/day 0.076 kg						
1	7.66±0.22	119	162	5.60±0.77	27	19
2	7.72±0.17	126	151	6.42±0.29	48	35
3	7.81±0.31	135	159	7.21±0.16	67	63
4	7.88±0.36	131	163	7.26±0.13	71	69

\*cfu = colony-forming units; DOCS, the de-oiled cake of seeds

Although several investigators have observed the toxic effect of propionate under unbalanced AD<sup>35</sup>, the extent of inhibition has not been understood<sup>36</sup>. In this regard, it is clear from (Fig. 1) that as OLR 3.0 increased to 3.5 (*i.e.* 0.089 kg VS fed), acetic acid (AA) content decreased sharply from 83 to 22.6%, while propionic acid (PA) increased sharply from 7.8 to 58.1% in total VFAs produced. This finding was found by earlier reports<sup>15,18</sup>. To clarify the precise role of PA, the ratio of acetic acid with other VFAs was calculated (Table 4). Accordingly, at OLR 3.0, AA: PA was in the range of 9.2-11.6; AA: BA, 20.2-34.7; AA:

IBA, 50.5-65.9; AA: VA, 66.9-119.0; AA: IVA, 27.8-47.8; AA: CA, 105.1-352.6 and AA: ICA, 170.1-413.2. However, at OLR 3.5, the ratio of acetic acid with other VFAs decreased significantly, resulting in bioreactor failure. Thereafter, the effect of OLR 3.5 continued in the first week even after reducing the OLR to 3.0. In the subsequent 3-4 weeks, bioreactor gradually recovered with steady ratios as before, as reflected from biogas production as well as methane content in it. A similar approach was earlier suggested that change in operational conditions can increase the functional diversity, and performance of the digesters<sup>14</sup>.

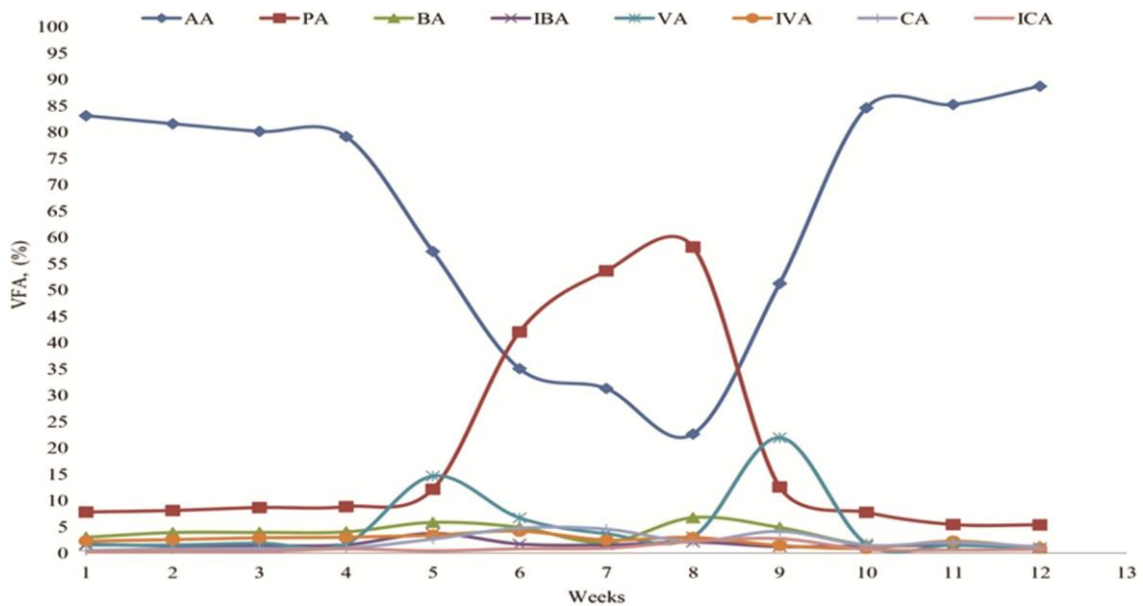


Fig. 1 — Profile of volatile fatty acids during anaerobic digestion of PS at (35±0.5°C)

Table 4 — The ratio of acetic acid with other VFAs at various OLR

Week	AA:PA	AA:BA	AA: IBA	AA:VA	AA: IVA	AA:CA	AA: ICA
3.0 OLR (kg VS/day/m <sup>3</sup> ) and 0.076 kg VS fed/day							
1	11.61±4.18	34.75±2.06	50.50±1.80	66.89±18.64	47.82±13.68	352.68±18.41	413.24±17.46
2	10.63±2.26	22.59±6.01	65.94±2.28	119.01±13.10	34.58±10.63	160.37±18.62	220.99±12.82
3	9.43±1.57	20.84±3.35	56.43±5.99	81.25±17.62	28.17±3.79	167.14±27.91	257.19±13.42
4	9.25±1.84	20.20±4.00	51.27±5.90	68.32±22.30	27.79±6.83	105.15±15.95	170.10±18.37
3.5 OLR (kg VS/day/m <sup>3</sup> ) and 0.089 kg VS fed/day							
1	4.80±0.70	11.16±4.62	15.57±2.19	12.58±22.24	17.12±2.71	31.45±6.08	149.76±6.42
2	1.02±0.84	8.57±5.10	26.39±19.85	8.49±6.89	9.56±18.29	15.47±9.38	54.89±4.20
3	0.59±0.10	32.39±26.41	22.30±9.23	11.23±6.80	18.29±10.52	8.18±3.65	40.44±8.42
4	0.39±0.14	12.31±13.74	15.18±8.96	9.73±7.62	8.82±5.21	17.69±17.47	25.83±6.60
3.0 OLR (kg VS/day/m <sup>3</sup> ) and 0.076 kg VS fed/day							
1	8.54±8.58	32.50±13.50	41.22±17.02	13.26±14.53	37.91±23.20	38.16±17.36	59.49±6.99
2	11.99±4.28	85.14±17.08	64.66±12.95	97.59±18.76	117.80±27.22	73.35±16.80	187.96±12.21
3	16.86±4.56	90.73±27.50	60.94±19.69	112.99±18.79	64.62±17.53	45.70±12.90	184.82±16.98
4	16.83±2.10	101.31±16.82	93.30±16.32	133.66±14.35	167.19±14.92	144.08±12.27	217.05±14.29

AA, Acetic acid; PA, Propionic acid; BA, Butyric acid; IBA, Iso-Butyric acid; VA, Valeric acid; IVA, Iso-Valeric acid

Thus, in the light of unfavourable VFA ratios at OLR 3.5 and gradual reversal of OLR to 3.0 restored acetic acid (AA) to 85.2% and propionic acid (PA) to 5.41%, suggest that PA at lower level is essential to restore functional methanogenic activity possibly syntrophic interactions between hydrogenotrophic methanogens and bacteria of the digester<sup>36,37,38</sup>. VFAs being intermediates in the metabolic pathway of methane production, at higher than permissible concentrations, VFA induces feedback inhibition and cause microbial stress that ultimately leads to digester failure. The present study suggests that digester failure during AD of PS due to high OLR could be advantageously utilized to recover the biogas production.

The calculations of VFA ratios indicated that (i) higher concentrations of propionate affected the degradation of all VFAs, (ii) the range of VFAs considered than the absolute value of any single VFA, and (iii) more reliance on respective ratios rather than absolute values. The consideration of the above facts suggests the success or failure of AD. Alternative hypotheses on the role of propionate in this regard may also be worth mentioning: (i) a toxicity of alcohol increases, with chain length, one might expect higher degree of inhibition with longer-chain-length fatty acids; (ii) substrate inhibition is probably caused by toxicity of un-dissociated form of propionic acid<sup>39</sup> and its effect on bacterial membrane; (iii) at high propionate concentrations, high amount of sodium may also contribute to inhibition<sup>40,41</sup>; (iv) branched-chain fatty acids (IBA, IVA, and ICA) may have some role, not understood as yet.

#### Overcoming digester failure

The performance of AD could be improved by (i) optimizing the OLR on which other operational parameters like pH and acid: alkali ratio depend, (ii) satisfying the nutritional requirements of microbes, using pre-optimized biological cum chemical additive such as compost at 20±2 C:N ratio, (iii) manipulating the feed proportions, (iv) re-circulating the digested slurry (washed-out microbes) back into the bioreactor, (v) bioaugmenting select anaerobes for growth on branched-chain fatty acids and (vi) modifying the design of the existing biogas plants. In the present study, sour digester was set right by optimizing the OLR, which restored essential pH range, acid: alkali ratio for optimal biogas production, and more methane content.

#### Conclusion

The optimal performance of anaerobic digester (AD) is crucial to the end-users and community for subsequent resource conservation and environmental protection. However, process instability, too low or too high loading rate, and slow recovery after digester failure and specific requirements for waste composition (DOCS *vis-à-vis* PS) need a careful strategy. In this study, the effect of a gradual increase in OLR on the digestion of pomegranate shells was investigated. The results show that increased OLR (3.5) leads to the souring of digester due to gradual changes in acid:alkali ratio, pH and VFA concentrations. Reversing the OLR (3.0) to the lower side stabilized and recovered the upset digester to normal. OLR of 3.0 in the present study provides (i) insight into changes in microbial community that occur during the period of digester upset; and (ii) proper guidance for operation of anaerobic digester using pomegranate shells (PS) as a feedstock component. Thus, the study suggests that organic loading rate (OLR) for any new or specific feedstock should be investigated during anaerobic digestion and maintained at an appropriate level to protect the functional microbial system.

#### Acknowledgement

Authors are grateful to Late Padmashree Dr. B. H. Jain, Chairman, JISL, Jalgaon for providing state-of-the-art R & D facilities to conduct the research work. Authors are also thankful to DST, New Delhi for financial support under FIST program to the School of Life Sciences.

#### Conflict of Interest

All authors declare no conflict of interest.

#### References

- 1 Saxena M, *Handbook of horticulture statistics* 2014, Ministry of Agriculture, Govt. of India, New Delhi, (2014) 1.
- 2 Sreekumar S, Sithul H, Muraleedharan P, Azeez JM & Sreedharan S, Pomegranate fruit as a rich source of biologically active compounds. *Biomed Res Int*, (2014) (doi 10.1155/2014/686921).
- 3 Badyal A, Sharma V, Kour N & Singh SK, Anticancer efficacy of methanolic extract of some medicinal plants from Jammu region, Jammu & Kashmir, India. *Indian J Biochem Biophys*, 53 (2016) 51.
- 4 Viuda-Martos M, Fernandez-Lopez J & Perez-Alvarez JA, Pomegranate and its main functional components as related to human health: A review. *Compr Rev Food Sci Food Safety*, 9 (2010) 635.

- 5 Suryawanshi PC, Kirtane RD, Chaudhari AB & Kothari R, Conservation and recycling of pomegranate seeds and shells for value addition. *J Renew Sust Energy*, 1 (2009) 013107.
- 6 Chen Y, Rößler B, Zielonka S, Wonneberger AM & Lemmer A, Effects of organic loading rate on the performance of a pressurized anaerobic filter in two-phase anaerobic digestion. *Energies*, 7 (2014) 736.
- 7 Gomez E, Martin J & Michel CF, Effects of organic loading rate on reactor performance and archaeal community structure in mesophilic anaerobic digesters treating municipal sewage sludge. *Waste Manag Res*, 29 (2011) 1117.
- 8 Zealand AM, Roskilly AP & Graham DW, Effect of feeding frequency and organic loading rate on biomethane production in the anaerobic digestion of rice straw. *App Energy*, 207 (2017) 156.
- 9 Barber WP, Anaerobic digester foaming: causes and solutions. *Water*, 21 (2005) 45.
- 10 Wijekoon KC, Visvanathan C & Abeynayaka A, Effect of organic loading rate on VFA production, organic matter removal and microbial activity of a two-stage thermophilic anaerobic membrane bioreactor. *Biores Technol*, 102 (2011) 5353.
- 11 Ros M, Franke-Whittle IH, Morales AB, Insam H, Ayuso M & Pascual JA, Archaeal community dynamics and abiotic characteristics in a mesophilic anaerobic co-digestion process treating fruit and vegetable processing waste sludge with chopped fresh artichoke waste. *Biores Technol*, 136 (2013) 1.
- 12 Vincent NM, Wei, Y, Zhang J, Yu D & Tong J, Characterization and dynamic shift of microbial communities during start-up, overloading and steady-state in an anaerobic membrane bioreactor. *Int J Environ Res Public Health*, 15 (2018) 1399.
- 13 Rincon B, Borja R, Gonzalez JM, Portillo MC & Saiz-Jimenez C, Influence of organic loading rate and hydraulic retention time on the performance, stability and microbial communities of the one-stage anaerobic digestion of two-phase olive mill solid residue. *Biochem Eng J*, 40 (2008) 253.
- 14 McMahan KD, Zheng D, Stams AJM, Mackie RI & Raskin L, Microbial population dynamics during start-up and overload conditions of anaerobic digesters treating municipal solid waste and sewage sludge. *Biotechnol Bioeng*, 87 (2004) 823.
- 15 Demirel B & Yenigun O, Two phase anaerobic digestion processes: A review. *Chem Technol Biotechnol*, 77 (2002) 743.
- 16 Fernandez AS, Hashsham SA, Dollhopf SL, Raskin L, Glagoleva O, Dazzo FB, Hickey RF, Criddle CS & Tiedje JM, Flexible community structure correlates with stable community function in methanogenic bioreactor communities perturbed by glucose. *Appl Environ Microbiol*, 66 (2000) 4058.
- 17 Sarker S, Lamb J J, Hjelme DR & Lien KM, A review of the role of critical parameters in the design and operation of biogas production plants. *Appl Sci*, 9 (2019) 1915.
- 18 Gunaseelan VN, Biochemical methane potential of fruits and vegetable solid waste feedstocks. *Biom Bioener*, 26 (2004) 389.
- 19 He Q LL & Peng X, Early warning indicators and microbial mechanisms for process failure due to organic overloading in food waste digesters. *J Environ Eng*, 143 (2017) 04017077.
- 20 Kang H & Weiland P, Ultimate anaerobic biodegradability of agro-industrial residues. *Biores Technol*, 40 (1992) 245.
- 21 Qu W, Pan Z, Zhang R, Ma H, Chen X, Zhu B, Wang Z & Atungulu GG, Integrated extraction and anaerobic digestion process for recovery of nutraceuticals and biogas from pomegranate marc. *Trans Am Soc Agric Biol Eng*, 52 (2009) 1997.
- 22 Li Y, Abraham C, Nelson MC & Chen P, Effect of organic loading on microbiota in a temperature-phased anaerobic digestion (TPAD) system co-digesting dairy manure and waste whey. *Appl Microbiol Biotechnol*, 99 (2015) 8777.
- 23 Kirtane RD, Suryawanshi PC, Patil MR, Chaudhari AB & Kothari RM, Optimization of organic loading rate for different fruit wastes during biomethanization *J Sci Ind Res*, 68 (2009) 252.
- 24 Chae KJ, Jang A, Yim SK & Kim IS, The effects of digestion temperature and temperature shock on the biogas yields from the mesophilic anaerobic digestion of swine manure. *Biores Technol*, 99 (2008) 1.
- 25 Clesceri LS Greenberg AE & Eaton AD, *Standard methods for the examination of water and wastewater*, (American Public Health Association, Washington, DC, USA) 2000, 893.
- 26 Kirtane RD, Suryawanshi PC, Chaudhari AB & Kothari RM, Studies on biphasic biomethanation of spoiled mango puree. *Int J Biotechnol*, 9 (2010) 308.
- 27 Alvarez R & Liden G, Semi-continuous co-digestion of solid slaughter house waste, manure and fruit and vegetable waste. *Renew Energy*, 33 (2008) 726.
- 28 Verma S, *Anaerobic digestion of biodegradable organics in municipal solid wastes*. Ph.D. Thesis, Columbia University, 2002.
- 29 Yadavika, Santosh, Sreekrishnan TR, Kohli S & Rana V, Enhancement of biogas production from solid substrates using different techniques- a review. *Biores Technol*, 95 (2004) 1.
- 30 Gerardi MH, *The microbiology of anaerobic digesters* (Wastewater Microbiology Series Wiley-Interscience, New York, USA) 2003, 99.
- 31 Kaparaju P & Rintala J, Anaerobic co-digestion of potato tuber and its industrial by-products with pig manure. *Res Conserv Recyc*, 43 (2005) 175.
- 32 Meegoda J N, Li B, Patel K & Wang L B, A Review of the processes, parameters, and optimization of anaerobic digestion. *Intl J Env Research and Public Health*, 15 (2018) 2224.
- 33 Pind PF, Angelidaki I & Ahring BK, Dynamics of the anaerobic process: effects of volatile fatty acids. *Biotechnol Bioeng*, 82 (2003) 7911.
- 34 Boone DR & Bryant MP, Propionate-degrading bacterium, *Syntrophobacter wolinii* sp. nov. gen. nov, from methanogenic ecosystems. *Appl Environ Microbiol*, 40 (1980) 626.
- 35 Barredo MS & Evison LM, Effect of propionate toxicity on methanogen-enriched sludge, *Methanobrevibacter smithii* and *Methanospirillum hungatii* at different pH values. *Appl Environ Microbiol*, 57 (1991) 1764.
- 36 Boone DR & Xun L, Effects of pH, temperature and nutrients on propionate degradation by a methanogenic enrichment culture. *Appl Environ Microbiol*, 53 (1987) 1589.
- 37 Hori T, Haruta S, Ueno Y, Ishii M & Igarashi Y, Dynamic transition of a methanogenic population in response to the concentration of volatile fatty acids in a thermophilic anaerobic digester. *Appl Environ Microbiol*, 72 (2006) 1623.
- 38 Castellano-Hinojosa A, Armato C, Pozo C, González-Martínez A & González-López J, New concepts in anaerobic digestion processes: Recent advances and biological aspects. *Appl Microbiol Biotechnol*, 102 (2018) 5065.



- 39 Li Y, Yang G, Li L & Sun Y, Bioaugmentation for overloaded anaerobic digestion recovery with acid tolerant methanogenic enrichment. *Waste Manag*, 79 (2018) 744.
- 40 van Lier JB, Grolle KCF, Frijters CTMJ, Stams AJM & Lettinga G, Effects of acetate, propionate and butyrate on the thermophilic anaerobic degradation of propionate by methanogenic sludge and defined cultures. *Appl Environ Microbiol*, 59 (1993) 1003.
- 41 Li X, Huang J, Liu Y, Huang T, Maurer C & Kranert M, Effects of salt on anaerobic digestion of food waste with different component characteristics and fermentation concentrations. *Energies*, 12 (2019) 3571.