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Studies on biphasic biomethanation of spoiled mango puree

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In a pilot-scale (175 L) study, spoiled mango puree (8.0-12.0% total solids) was subjected to biphasic biomethanation. For this purpose, an efficient phase separation between puree hydrolysis/acidification and its methanogenesis was achieved by using three acidogenic reactors (ARs) in series, operated aerobically at ambient temperature. The hydrolysate coming out of the AR series contained high percentage of volatile fatty acids (VFAs, 15,000-20,000 mg L^{-1}), indicating efficient conversion of sugars. Subsequently, this hydrolysate was fed to two methanogenic reactors (MRs), operated anaerobically at 35±1.0°C. About 30% digestate from MRs was further recycled to ARs for enhancing the efficiency of hydrolytic activity. This arrangement permitted the maintenance of optimum *p*H in ARs at 4.5-5.5 range and an efficient anaerobic digestion in MRs as indicated by *p*H of the digestate in 7.2-7.8 range.

The optimized conditions permitted, (i) an increase in feedstock initially from 2.0 to 10.0 L/d, (ii) methanogenic transformation of 15,000-20,000 mg L^{-1} VFAs, (iii) generation of 100±20 L d^{-1} biogas containing 65% methane initially and 550±25 L d^{-1} biogas with 78% methane upon stabilization over 180 d, (iv) digestate leaving MRs with 600-1200 mg L^{-1} VFAs, and (v) around 90% efficiency in terms of VFAs utilized.

Keywords: Acidification, biphasic methanogenesis, methane-rich biogas, spoiled mango puree

Introduction

Recycling and stabilization of waste through biphasic anaerobic digestion is a better approach compared to aerobic treatment or composting[1]. Solid wastes from fruits and vegetables, by virtue of being renewable, represent a potentially sustainable resource of energy, if they can be biologically converted to methane. In the process, their net CO_2 contribution to the atmosphere would be zero. India produces over 60×10^6 tons of fruits and vegetables annually, of which around 3% are processed[2]. India produces 45% of mangoes on global scale[3], while Jain Irrigation System Ltd (JISL) has been the largest mango processor (80,000 tons annually) in the country. JISL produces around 40,000 tons of mango puree and offers commercial sterility to it before packaging in 210 kg pre-sterilized bags placed in drums for the export. In some of the drums during storage, the puree gets spoiled, presumably for the use of either over-ripe mangoes or under-sterilization. Such puree being unfit for consumption is explored, in the present study, as a feedstock for biogas production through anaerobic digestion, instead of draining it out to cause damage to the ecosystem.

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Materials and Methods

Waste and Inoculum Collection

Spoiled mango puree was provided by Fruit Processing Plant, JISL, Jalgaon, India. To serve as inoculum for the lab scale and pilot scale studies, an anaerobic sludge was obtained from up-flow anaerobic sludge treatment (UASB) digester treating wastewater from an onion dehydration plant located on the same campus.

Analytical Methods for Characterization

The spoiled mango puree and anaerobic sludge were analyzed for *p*H, total solids (TS), volatile solids (VS), organic carbon (OC) and total Kjeldahl nitrogen (TKN) in accordance with Standard Methods[4].

The spoiled mango puree samples were diluted, centrifuged and analyzed for volatile fatty acids (VFA) concentration by gas chromatography (Nucon 5765), using flame ionization detector (FID) and samples (0.5 µL) were injected onto a ZB-FFAP capillary column (Phenomenex) using nitrogen gas as a carrier; temperature at injector was 240°C, at

column 80-230°C and at detector 260°C, and data were obtained by Nuchrome software.

Methane percentage in biogas was determined by GC (Nucon 5765) by injecting biogas samples (0.5 mL) collected after every 24 h interval into a Porapak Q (80-100 mesh) column, eluted with helium as a carrier gas and monitored with thermal conductivity detector (TCD). Temperature at injector was ambient, at column 60°C and at detector 150°C, and data were obtained by Nuchrome software.

Lab Scale Studies

A circular, fixed-dome, bioreactor (height 32.0 cm, internal diameter 29.6 cm, 25 L volume) was fabricated from PVC sheet, with an inlet and outlet provision. This was fitted with an agitator to provide uniformity of *p*H and temperature in the digesting slurry (12.5 L mango puree hydrolysate & 12.5 L UASB sludge as inoculum). Hydrolysate was prepared in 10 L capacity plastic acid reactors (ARs). Anaerobic digestion was carried out at 35.0±1.0°C. To conserve energy, frequency of agitation for close contact between microbes from the inoculum and hydrolysate was automated at 50 rpm for 2 min after every 30 min. VFA and methane were analysed by gas chromatography after every 24 h interval.

Pilot Scale Studies

On the basis of waste characterization and lab scale studies (25 L), it was evident that spoiled mango puree degraded very fast as judged from declining VFA concentration, the prime parameter selected for monitoring the performance of biphasic biomethanation. Therefore, pilot scale studies (175 L) were undertaken to explore techno-economic aspects of biogas production. Its experimental set up is schematically depicted in Fig. 1.

Acid Reactors (ARs)

Three 100 L capacity insulated ARs (AR1, AR2 & AR3) were used for the hydrolysis of spoiled mango puree. They were arranged sequentially in such a way that initial substrate was pumped in AR1 and equal quantity of its hydrolysate was sequentially transferred to AR2 and in due course from AR2 to AR3. An average ambient temperature during the entire hydrolysis period was 35±2°C, an optimal for mesophilic digestion[5].

Methanogenic Reactors (MRs)

Two stainless steel (0.56 m internal diameter, 0.75 m height, effective volume 0.175 m^3) MRs were fabricated, having jacket for hot water circulation and insulation for temperature control. The MRs were loaded with hydrolysate from AR3 and inoculum obtained from UASB sludge in 1:1 (v/v) proportion through an inlet at their top. Both the MRs have automatic semi-continuous agitation provision (60 rpm for 5 min h^{-1}) for proper mixing of the digesting mixture. The mixture was allowed to acclimatize for a period of 30 d, during which *p*H of the sludge was monitored daily until stabilization, while hydrolysate from AR3 was fed to it at 8 h interval to minimize overloading. Two floating drum type gas holders were fabricated for the collection of biogas generated in MRs.

Methanogenic Reactor Digestate Collection Tank (MRDCT)

Two 100 L capacity PVC tanks were used for the collection of overflowing digestate effluent from the MRs, which was pumped to either ARs or MRs for recycling.

Parameters Monitored

Data on *p*H of the hydrolysate in ARs and MRs, volume of biogas produced, % methane and VFA in the digestate were monitored daily for 180 d after acclimatization period of 30 d for stabilization of microbial population in the mango puree hydrolysate. Average ambient temperature data was collected daily from Jain Greenhouse meteorology station, while TS and VS in the digestate were monitored once a week. Specific biogas production was calculated by dividing biogas produced (L d^{-1}) to substrate fed (L d^{-1}). During the experimental period, biogas produced was measured daily by a wet type gas flow meter (INSREF, Chennai).

Results and Discussion

Experimental Strategy

The application of biphasic anaerobic digestion (BAD) for treating wastes from dairy, fruits and vegetables and food processing has been convincingly established by several workers[2,6-11]. Their success prompted the authors to use BAD for treating spoiled mango puree in the present study. Similarly, activated sludge has been used as a source of inoculum containing hydrolytic, acidogenic and methanogenic microbial population in BAD of a variety of wastes[12-14]. In the present study also, activated sludge was used as an inoculum. Further, efficiency of digestion was introduced through separation of phases of BAD; it ensured favourable environmental

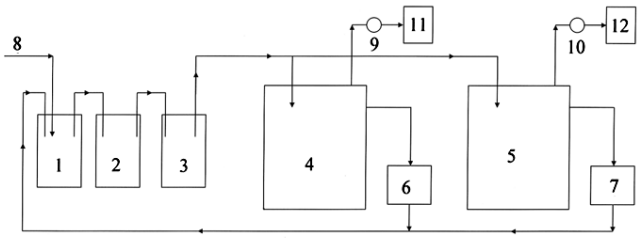

Fig. 1—Schematic presentation for biphasic biomethanation of spoiled mango puree: 1, AR1; 2, AR2; 3, AR3; 4, MR1; 5, MR2; 6 & 7, MRDCT; 8, Spoiled mango puree; 9 & 10: Gas flow meter; and 11 & 12, Gas holders.

conditions for optimal growth of different groups of microbes involved in methane production, so that shorter hydraulic retention time (HRT) of 8 h was attainable[9,13].

Waste Characterization

The characteristics of spoiled mango puree and anaerobic sludge used for biomethanation has been summarized in Table 1. The *p*H of the spoiled mango puree was very low (2.71), which could be inhibitory for both hydrolysis and methanogenesis. Therefore, the MRs were initially fed with spoiled mango puree with an equal volume of anaerobic sludge for 30 d. As a result of mixing these in 1:1 (v/v) ratio, *p*H of the digesting mixture was changed to 6.92, total solids to 8.5%, VS to 83.2%, organic carbon to 48.8%, total Kjeldahl nitrogen to 3.28% and C:N ratio to 14.9. The characterization studies confirmed that spoiled mango puree contained high amount of readily degradable volatile solids concentration (92.7%).

VFA composition of hydrolysate as obtained by gas chromatography is shown in Table 2. Reflecting the efficiency of conversion of sugars in mango puree, the analysis showed the largest contribution (4572 mg L^{-1}) by easily metabolizable acetic acid and decreasing amount of n-butyric acid, n-valeric acid and n-caproic acid, followed by isobutyric acid, isovaleric acid and isocaproic acid. However, the presence of propionic acid as the second largest VFA ingredient (1684 mg L^{-1}) amounting to 21% of the total VFA, was a cause of concern in view of its inhibitory potential for the generation of methane.

Lab Scale Studies

Results from lab scale studies over 90 d after stabilization showed: (i) feed hydrolysate increased from 0.3 to 1.5 L d^{-1}, (ii) reduction in VFA concentration in the digestate from 12,000-5,000 mg L^{-1} to 350-520 mg L^{-1}, and (iii) increase in biogas from 10 to 28 L d^{-1} and methane content from 68 to 82%. These promising results prompted to undertake pilot scale study.

Table 1—Characteristics of spoiled mango puree and anaerobic sludge

Parameters	Spoiled mango puree	Anaerobic sludge
*p*H	2.71	7.55
Total solids (%)	8.40	8.57
Total volatile solids (%)	92.72	73.62
Total organic carbon (%)	62.26	35.33
Total Kjeldahl nitrogen (%)	1.46	5.10

Table 2—Profile of VFA in the spoiled mango puree hydrolysate

Acid	VFA (mg L^{-1})
Acetic acid	4572
Propionic acid	1684
n-Butyric acid	1027
iso-Butyric acid	91
n-Valeric acid	256
iso-Valeric acid	48
n-Caproic acid	178
iso-Caproic acid	14
Total	7870

Pilot Scale Studies

The differences in the behaviour of two parallel sets MR1 and MR2 were marginal; never exceeding 5%. Therefore, the following results are an average of MR1 and MR2. Periodic fluctuation in the *p*H of the hydrolysate ranged between 4.69 to 5.42 and digestate between 7.16 to 7.75. From these figures, it is evident that in spite of spoiled mango puree being acidic in nature, the *p*H of the hydrolysate obtained from ARs was relatively constant, which could be probably attributed to—(i) the alkalinity in the digestate recycled after BAD, and (ii) the dilution due to mixing of the hydrolysate from AR3, freshly fed hydrolysate and digestate obtained from MRs. These profiles gave first indication that *p*H of the hydrolysate was in an acceptable range for an efficient AD.

The percentage of VS in the hydrolysate from AR3 was 90% and in digestate emerging from MRs was 60-65%. Although this difference in the range of 23-35% appears apparently poor, in reality it is not so due to continued periodic feeding as against complete loading in a single lot. In the latter case, due to complete loading in a single lot, substantial difference

in % VS indicates poor efficiency of digestion; while in the former case, due to periodic feeding, difference in % VS was not much, reflecting efficient digestion stabilized in a narrow VS range.

It is evident from Fig. 2 that biogas production as well as % methane content in it increased as a function of an increase in VFA concentration. Methane content of the biogas varied between 65.5-78.2%. This range was considerably high as compared to % methane in the biogas produced during continuous process (63-65%, unpublished data). Fig. 2 also shows: (i) gradual and continued increase in biogas production with time, (ii) constant maintenance of higher percentage of methane in the biogas, and (iii) constant maintenance of concentration of VAF.

Biomethanation by BAD comprises of three steps, *viz.*, hydrolysis, acidification and methanogenesis, leading to generation of methane and carbon dioxide[15]. In the first stage, spoiled mango puree was hydrolyzed and fermented by rapidly growing hydrolytic bacteria to volatile fatty acids (VFA). In the second stage, VFA are further converted to acetate, hydrogen and carbon dioxide by acidogenic bacteria for further utilization by the methanogens, which in third step yield methane and carbon dioxide. This symbiosis between three steps was maintained for efficient degradation of spoiled mango puree. This was feasible presumably due to adequate microbial seeding and efficient mass transfer[13].

It is clear from Fig. 3 that fed VFA was significantly utilized in the formation of methane-richbiogas, leaving little unutilized VFA in the digestate. Whenever VFA in the digestate of MR was more than 1000 mg L^{-1}, there was a slight decrease in biogas generation as well as its percentage of methane. The % unutilized VFA in the digestate, therefore, could be one of the key parameters to determine frequency and quantum of the feed material.

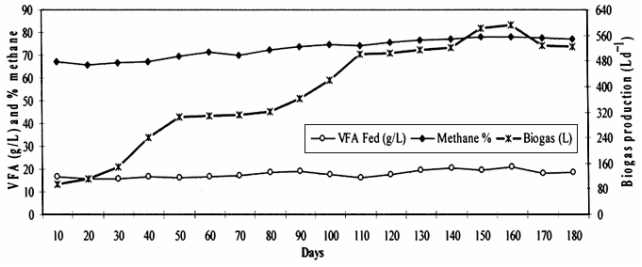

Fig. 2—Profile of VFA fed, biogas production and methane content in MRs

It has been observed that, at increased loading rates, two phase digestion has shown stable *p*H as against single phase system[16]. However, our observations are at variance to this finding as we observed an increase in specific biogas production up to 10 L d^{-1} fed hydrolysate, beyond which resulted in the accumulation of VFA in the digestate and eventually decreasing biogas production and % methane. In fact, to avoid accumulation of VFA, care was exercised to feed 3.3 L hydrolysate 8 h^{-1}, so that VFA level remained below 1000 mg L^{-1} in the digestate and, especially, propionic acid concentration was not allowed to reach toxic limit of 5000 mg L^{-1} to inhibit methane production rate as found ealier[17]. To ensure unimpeded flow of substrate through periodic feeding as against continuous feeding[18], accumulation of propionic acid should be arrested for continuous methane production without disturbing the microbial symbiosis. This required to accord focus for constant GC monitoring the utilization status of propionic acid, main inhibitor during methane production. It is clear from Fig. 4 that, in the present study, there was controlled digestion of propionic acid to keep it at harmless level. The concentration of propionic acid was well below the 1000 mg L^{-1} in both the hydrolysate and digestate.

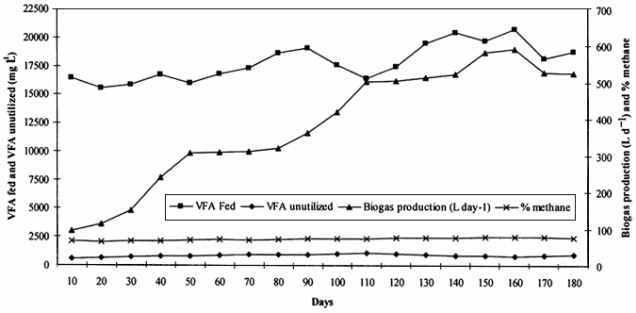

Fig. 3—Profile of VFA fed, VFA unutilized with respect to biogas production and % methane

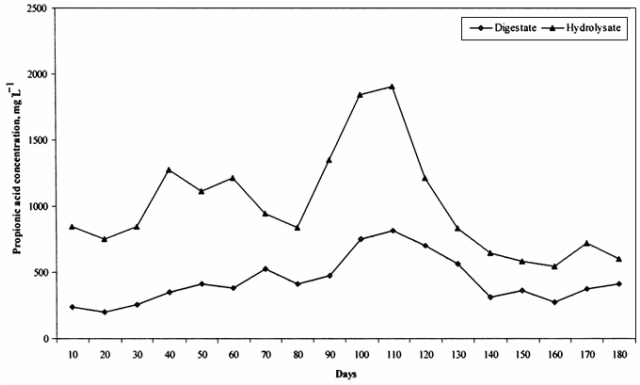

Fig. 4—Profile of propionic acid concentration in spoiled mango puree hydrolysate and digestate as a function of digestion period

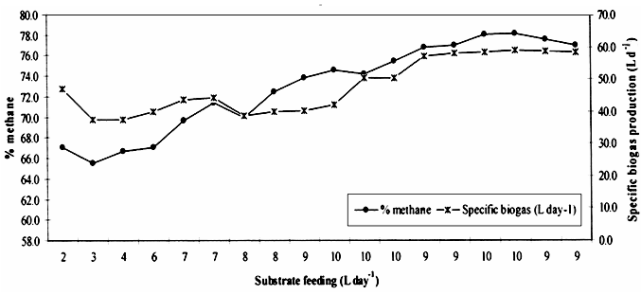

Fig. 5—Profile of specific biogas production and % methane with respect to hydrolysate fed

Fig. 5 shows that spoiled mango puree hydrolysate gave the maximum production of 59.1 L d^{-1} biogas containing 78.2% methane and 90-95% efficiency of digestion. Since, the efficiency of VFA digestion ranged between 90-95%, it can be concluded that not only propionic acid, but other fatty acids too were utilized in methane production, again indicating that desired synergy between different microbial groups was involved[19]. While lipid or VFA-rich substrates are reported to yield 80-82% methane content in biogas[21], present set up has permitted 78-80% methane content through BAD system, which has been successfully used earlier for fruit and vegetable wastes[6], agro-industrial residues[20] and food waste[7,8,14]. Moreover, it is claimed that contrary to other techniques, BAD of organic waste has advantages like: (i) controlled degradation, (ii) reduction in biomass, (iii) freedom from foul odour, and (iv) faster recovery of methane as renewable energy. The present study too has substantiated these merits of BAD.

Conclusion

In the present study, BAD of spoiled mango puree has been successfully explored at 175 L scale by periodic feeding of puree hydrolysate for stabilized *p*H, moderate level of propionic acid and synergy between different microbial species to attain 90-95% efficiency in terms of VFA utilization by consistent production of 78-80% methane in biogas, at 59.1 L d^{-1} specific biogas production over the period of 180 d.

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