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Anaerobic co-digestion of sewage sludge and food waste

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Abstract

Anaerobic co-digestion of organic matter improves digester operating characteristics and its performance. In the present work, food waste was collected from the institute cafeteria. Two types of sludge (before centrifuge and after centrifuge) were collected from the fluidised bed reactor of the institute treating sewage wastewater. Food waste and sludge were studied for their physico–chemical characteristics, such as pH, chemical oxygen demand, total solids, volatile solids, ammoniacal nitrogen, and total nitrogen. A biomethane potential assay was carried out to find out the optimum mixing ratio of food waste and sludge for anaerobic co-digestion. Results indicated that food waste mixed with sludge in the ratio of 1:2 produced the maximum biogas of 823 ml gVS^{-1} (21 days) with an average methane content of 60%. Batch studies were conducted in 5 L lab-glass reactors at a mesophilic temperature. The effect of different substrate loading rates on biogas production was investigated. The mixing ratio of food waste and sludge was 1:2. A loading rate of 1 g VS L d^{-1} gave the maximum biogas production of 742 ml g^{-1} VS L d^{-1} with a methane content of 50%, followed by 2 g VS L d^{-1} with biogas of 539 ml g^{-1} VS L d^{-1}. Microbial diversity of the reactor during fed batch studies was investigated by terminal restriction fragment length polymorphism. A pilot-scale co-digestion of food waste and sludge (before centrifuge) indicated the process stability of anaerobic digestion.

Keywords

Anaerobic co-digestion, food waste, methanogens, terminal restriction fragment length polymorphism, sewage sludge

Introduction

Anaerobic digestion (AD) is a naturally occurring biological process of microbial decomposition in oxygen depleted environments. During the AD process, organic matter is broken down into simpler chemical components, such as methane (CH_4), carbon dioxide (CO_2), hydrogen sulphide (H_2S), and manure (slurry). The anaerobic metabolism takes place in four steps, namely hydrolysis, acidogenesis, acetogenesis, and methanogens and is by hydrolytic, acidogenic, acetogenic and methanogenicarchaea, respectively (Bouallagui et al., 2004; Verstraete et al., 2009).

Methanogens are either hydrogenotrophs or acetogens involving two different methane-forming pathways, namely a hydrogenotrophic pathway and acetoclastic pathway (Liu and Whitman, 2008). The genus *Methanosaeta* comprises species exclusively using the acetoclastic pathway. Species of the genus *Methanosarcina* are able to use both pathways, while other members of the order *Methanosarcinales* are methylotrophs or hydrogenotrophs. Other methanogenic orders (*Methanomicrobiales*, *Methanobacteriales*, and *Methanococcales*) relevant for the biogas process produce methane only via the hydrogenotrophic pathway.

Among the various micro-organisms present in the reactor, methanogens are most sensitive and difficult to study in culture-based methods. The methanogenic population is studied using a polymerase chain reaction based on a methyl coenzyme M reductase (*mcrA*) gene target as a molecular marker. A study of the methanogenic population gives information about the productive digestion process (Nikolausz et al., 2013; Prabhudessai, 2013; Traversi et al., 2011).

AD technology can be used to handle almost any type of biodegradable organic materials, including wastes such as plant and animal wastes, cow manure, waste paper, grass clippings, leftover food in municipal solid waste, domestic and industrial wastewater, etc. (Austermann, 2007; Global Methane Initiative, 2013). AD technology was originally designed for sewage sludge and animal manure (Patha, 2014). However, sewage sludge and manure are not materials with high potential for AD. Therefore, to increase the efficiency of the process, the digesters can be operated using a co-digestion process by feeding them with two or more types of feedstocks (Vlachopoulou, 2010).

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Sludge is rich in nitrogen and trace elements, but low in biodegradable organic matter (Kim et al., 2011; Lee et al., 2013). Hence biomethane potential (BMP) will be very low. Food waste (FW) is a problematic organic waste and is available in abundance. FW is very rich in volatile solids, which can be easily converted to methane using an anaerobic process. However, it usually has low amount of nutrients (Esteves and Devlin, 2010).

Anaerobic co-digestion is a process where two or more substrates with complementary characteristics are mixed for combined treatment. Co-digestion of FW holds promise owing to a higher methane yield, as well as an accelerated methane production rate (Koch et al., 2015). Co-digestion of FW can utilise the nutrients and bacterial diversities in various wastes to optimise the digestion process (Hartmann and Ahring, 2005). Apparently, it has a synergistic effect that overcomes the imbalance in nutrients and improves biodegradation. This effect results in a higher methane yield compared with AD of a single waste, which increases the organic content inside the reactor, enhances digestate stabilisation, dilutes the potential inhibitory and/or toxic compounds such as ammonia, Na^+, etc. (Alvarez et al., 2009; Chen et al., 2008; Corral et al., 2008; Dai et al., 2013). Apart from the above benefits, diverting organic waste from landfills allows for the production of organic fertilisers and conditioners for agricultural and land use, greenhouse gas emission reductions, and economic benefits (United States Environmental Protection Agency, 2015).

The major problem with the conventional wastewater treatment plants (WWTPs) is the management of its sludge. The sludge that is generated has great energy potential and can be used for an onsite energy generation purpose. It is a challenging, yet achievable goal to make WWTPs energy neutral when wastewater facilities are designed and operated for this purpose, through a combination of energy efficiency best practices and energy production technologies (Lynne et al. 2013). The energy contained in wastewater and sludge has been estimated to exceed the energy needed for treatment by a factor of 10 (Lauren, 2011). The major approaches that have been used to improve the sustainability of WWTPs is energy efficiency improvement by onsite energy generation in the form of electricity from combustion of biogas and sometimes in the form of heat and fuel, which offsets fossil fuel demands (Mo and Zhang, 2013). This can be achieved through one of several processes, such as combustion, gasification, and AD, in order to produce electricity. In the last couple of decades, AD of sewage sludge has been regarded as the most appropriate technology for renewable energy recovery and nutrient-rich fertiliser production in a sustainable manner (Sharholy et al., 2008).

The main objective of this study was to investigate the co-digestion of sewage sludge and FW at lab-scale and at pilot-scale, and to study the methanogenic diversity during the anaerobic co-digestion process.

Material and methods

Characterisation of organic wastes

FW was collected from the institute cafeteria at Birla Institute of Technology and Science (BITS) Pilani K K Birla Goa Campus. The main components of the FW included uneaten food and food preparation leftovers from the kitchen, such as cooked pulses, cooked rice, noodles, cooked vegetables, and raw leafy vegetables/salad.

Sludge was taken from the wastewater treatment plant of the institute. Two types of sludge were collected, one before centrifuge (BC) and the other after centrifuge (AC). FW and sludge were studied for their physico–chemical characteristics, such as pH, chemical oxygen demand (COD), total solids (TSs), volatile solids (VSs), NH_3-N, and Total Kjeldahl Nitrogen (TKN) as per standard methods (APHA, 1995).

Inoculum source

Effluent from the existing anaerobic pilot plant treating FW in our institute was used as the inoculum. The effluent was brought in the laboratory in a closed container and was monitored for gas production. It was pre-incubated at 32 °C until it reached the endogenous respiration stage and was then used for the BMP assay. The inoculum used had a pH of 7.4 and a VS content of 23.2 g L^{-1}.

Biomethane potential (BMP) assay

BMP assay was carried out for each individual substrate as well as for the mixed substrates to find out the optimum ratio of mixing of FW and sludge for anaerobic co-digestion. The BMP assay was performed using serum bottles according to Angelidaki et al. (2009). Bottles were prepared by adding the substrate, inoculum, and basal medium containing $NaHCO_3$ (5 g L^{-1}) to a final volume of 100 ml. The mixed substrate having FW and sludge was added to the vials in various ratios 1:1, 1.5:1, 2:1, 1:1.5, and 1:2 to a final concentration of 1 gVS L^{-1}. The vials were sealed and the headspace flushed with N_2 gas. The assay was carried out in replicates.

The biogas accumulated in the bottle headspace was measured using a water displacement method and analysed using gas chromatograph (GC) Chemito GC 7610 (Thermofisher Scentific, Mumbai, India) equipped with thermal conductivity detector (TCD) detector and hydrogen as the carrier gas. A packed stainless steel column was used, having solid support of a spherocarb, length 2 m; diameter 1/8th. The GC oven temperature was programmed to increase from 60 °C to 120 °C at the rate of 5 °C per min. The temperatures of injector and detector were at 150 °C and 183 °C, respectively.

Loading rate determination

The effect of increasing the substrate loading rate on biogas production was investigated using single phase 5-L lab-scale glass reactors at a mesophilic temperature on a semi-batch mode with one cycle per day. The working volume of the reactor was 5 L. The mixing ratio for the two substrates was decided based on the results obtained from BMP assay study. Inoculum was taken from the outlet of the biogas digester working on the FW. Stirring in the reactor was done with the help of a magnetic stirring system.

At the end of the cycle, the required volume was withdrawn and fresh substrate was added to start the next cycle. Various loading rates tested were: 0.5, 1.0, 2.0, 4.0, and 7.0 gVS L d^{-1}. Loading was increased gradually from 0.5 to 7 gVS L d^{-1}. When the biogas production was stabilised for a given loading rate, only then the next loading rate was started.

Biogas production was measured and methane content was analysed using GC. Samples were taken at various time intervals and were analysed for pH, COD, TS, VS, ammonia, TKN, and methanogen diversity.

Community composition and activity of methanogens during co-digestion using terminal restriction fragment length polymorphism (T-RFLP)

Samples that were collected during the loading rate study were also analysed for diversity of methanogens using T-RFLP.

DNA isolation from samples. Samples were collected from the reactor at different intervals during the loading rate study; 500 µL of the sample was measured and centrifuged at 10,000 r min^{-1} for 10 min. Pellet was mixed with Lysis buffer and glass beads and processed for DNA isolation using a modified HiPer Soil DNA kit (HiMedia) protocol. Microbial cells were lysed by bead beating and a detergent lysis protocol, as per the HiPer Soil DNA kit. The total DNA was eluted in 20.0 µL of nuclease free water. A quality assessment of DNA was done by agarose gel electrophoresis.

Polymerase Chain Reaction (PCR) amplification of the mcrA gene. The *mcrA* gene was amplified by PCR using the primer set Mlas - F- GGT GGT GTM GGD TTC ACM CAR TA– 3 and McraR - 5- CGT TCA TBG CGT AGT TBG GRT AGT- 3). The Mlas - F primer was labelled at the 5' end with HEX (Hexachloro-fluoresceine) fluorescent dye. The PCR mixture was 0.5 µL of each primer (10 mM), 5 µL of the PCR buffer, 1 µL of dNTP (2.5 mM), 0.5 µL of Taq polymerase (2.5 U μL^{-1}), and double-distilled water for a final reaction volume of 25 µL. PCR was performed at 95 °C for 15 min; 30 cycles of 95 °C for 30 s, 56 °C for 45 s, 72 °C for 30 s, and 72 °C for 10 min. PCR products were checked on 2% agarose gels and purified with the geneO-Spin PCR product purification kit (gene Ombio Technologies, India) according to the manufacturer's protocol.

Restriction digestion and desalting of digested products. Following PCR, 5 µL of PCR products were digested with 0.5 U of HpaII restriction enzymes (Fermentas) for 3 h at 37 °C in a 50 µL reaction volume. Digestion digests were separated on 3% agarose gels in a 1X TBE (Tris/Borate/EDTA) buffer containing ethidium bromide (EtBr), and visualised under ultraviolet (UV) light. A total of 45 µL of the digested DNA was desalted using the following procedure: 2.5 µL of 125 mM EDTA and 1/10 volume of 3M Sodium Acetate, pH 5.2 was added to 45 µL of digested DNA. Further, a 2.5 volume of ice cold ethanol was added into the tubes and mixed well. Tubes were then centrifuged at 12 000 r min^{-1} for 20 min at 18 °C. Supernatant was decanted without dislodging the pellet. Pellet was then washed with 60 µL of 70% ethanol twice by centrifuging at 12,000 r min^{-1} for 20 min at 18 °C. The pellet was then dried at 37 °C for 30 min.

Sample preparation and loading. Hi-Di Formamide (9.7 µL) from Applied Biosystems, Mumbai was added to the dried pellet. Each sample was also added with 0.3 µL GenScan 500 LIZ Internal Size Standard. This mixture was denatured at 95 °C for 3 min and immediately chilled on ice before loading. The samples were then subjected to electrophoresison, the 3130 Genetic Analyzer using the FA_36_POP-7™ run module andG5 dye set. (Applied Biosystems, Mumbai)

GeneMapper data analysis. GeneMapper software based analysis was performed for fragment analysis after completion of the capillary electrophoresis. Output from automated sequencers is in the form of an electropherogram, with peaks representing fluorescently labelled T-RFs detected over time in relation to the size standard. The duration and intensity of the fluorescent signal from T-RFs is reflected in the area and height of each peak detected, respectively. Software specific to each sequencing unit collects data from each run. The ABI 3730 capillary sequencer operates GeneMapper v3.5 (Applied Biosystems, Mumbai), which performs the functions of both GeneScan and Genotyper. The T-RFs for each sample run should be closely examined and the entire run evaluated for the average number of T-RFs detected per sample and the number of T-RFs contained in the various size classes.

Each T-RF was assumed to be an individual operational taxonomic unit (OTU). T-RFs presence–absence was further used for the statistical analysis. The similarity between the sampling sites was analysed using cluster analysis.

A Dendrogram was constructed based on the Bray-Curtis distance measure or index of dissimilarity with unweighted pair group method with arithmetic mean (UPGMA) (Bray and Curtis, 1957; Michener and Sokal, 1957).

Pilot scale AD

Pilot scale AD of FW and sewage sludge was carried out in a horizontal plug flow reactor at BITS Pilani K K Birla Goa Campus. The pilot reactor has a total working volume of 60 m^3 and runs on FW as a substrate. The addition of substrate was stopped until the gas production reached the endogenous respiration. The feeding was resumed with the addition of 80 kg of FW per day (71.2 kg VS d^{-1}) for 1 week. Mixing inside the reactor was done for 20 min every day by recirculation of the slurry from the outlet of the reactor to the inlet of the reactor. After 1 week the substrate quantity was increased to 160 kg d^{-1} (142.4 kg VS d^{-1}) for 1 week. It was then increased to 240 kg d^{-1} (213 kg VS d^{-1}) for 1 week and subsequently to 320 kg FW d^{-1} (284.8 kg VS d^{-1}). This was the total FW available per day on which the biogas reactor was working. After 1 month of the reactor running on FW, 2 m^3 dof^{-1} the sewage sludge (BC) was added to the reactor. Analysis was carried out for the substrate that was added. Outlet characteristics were monitored for pH, COD, TS, VS, NH_3-N, TKN, biogas volume, and methane content.

Results and discussion

Substrate characterisation

The FW used in this study, which was collected from the institute cafeteria, was uniformly homogenised using mixer and grinder.

Table 1. Characteristics of various sample FW, sludge BC, and AC.

Parameters/samples	FW	Sewage sludge	
		BC	AC
pH	6 ± 0.10	7.3 ± 0.10	7 ± 0.10
TS (%)	26.89 ± 1.89	1.49 ± 0.03	15.89 ± 0.16
VS (%)	23.81 ± 1.30	1.37 ± 0.03	13.18 ± 0.10
VS/TS	0.89 ± 0.03	0.92 ± 0.00	0.83 ± 0.00
COD (mg g^{-1})	307.54 ± 35.01	24.00 ± 4.00	188.42 ± 26.08
NH_3-N (mg L^{-1})	120.71 ± 12.69	122.08 ± 3.36	418.13 ± 87.57
TKN (%)	0.67 ± 0.01	0.17 ± 0.00	0.63 ± 0.01
Moisture (%)	73.11 ± 1.45	98.51 ± 0.03	84.11 ± 0.16

±sign indicates standard error.
AC: after centrifuge; BC: before centrifuge; COD: chemical oxygen demand; FW: food waste; TKN: Total Kjeldahl Nitrogen; TS: total solid; VS: volatile solid; NH3-N: Ammoniacal nitrogen.

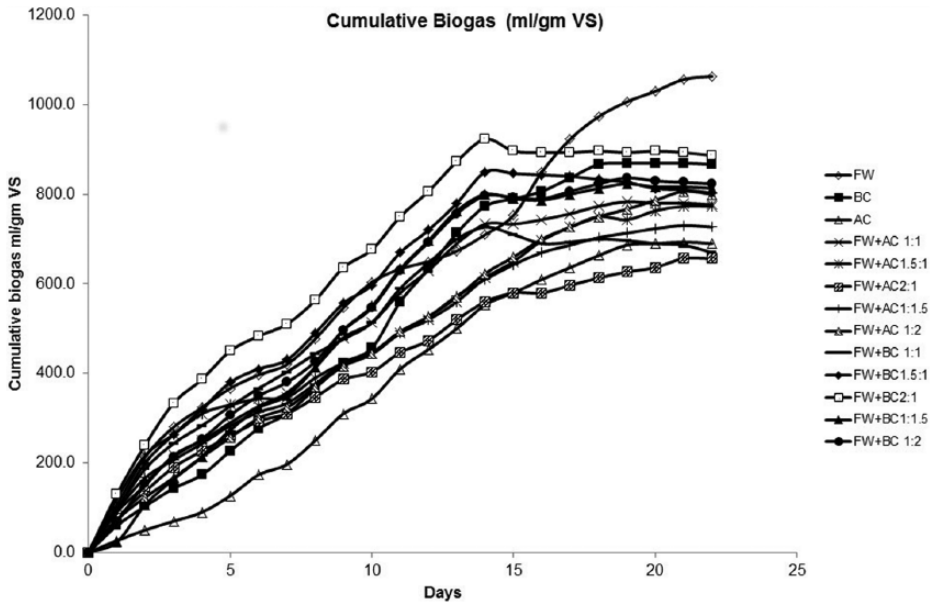

Figure 1. Shows the cumulative biogas production of FW, sludge BC, AC, and co-digestion of various ratios (the values are the average of duplicate measurements and subtracted for negative control values).

It was then used for characterisation. The composition of the FW, sludge BC and AC is shown in Table 1. VSs content of FW, sludge BC and AC were 23.81%, 1.37%, and 13.18%, respectively. Total initial COD was about 307 mg g^{-1} (wet weight) for FW, 24 mg g^{-1} for sludge BC, and 188 mg g^{-1} for sludge AC.

BMP assay

BMP assay was carried out as a batch reaction in 125 ml serum vials. Results indicate that FW alone gave the maximum biogas production, followed by a co-digestion ratio FW+BC 2:1. Least biogas was produced from the AC sludge (Figure 1). Among the co-digestion ratios, least biogas was produced in a FW+AC 2:1 ratio.

After considering the percentage of methane content in biogas, it was observed that FW+BC at a ratio of 1:2 gives the highest methane production (methane 492 ml g^{-1} VS, biogas 823 ml g^{-1} VS). Methane production from FW+BC in the ratio 1:1.5 was also very close to 1:2 (490 ml g^{-1} VS, biogas 800 ml g^{-1} VS) (Table 2). However, when it comes to pilot scale digestion, with a mixing of ratio 1:2, one-quarter times more sludge can be handled for biogas production. The VS degradation efficiency was almost similar at the ratios 1:2 (85%) and 1:1.5 (83%). This proves that co-digestion with a proper mixing ratio gives more biogas and at the same time can handle and take care of two different waste streams.

Loading rate determination

The co-digestion of FW+BC sludge was studied in 5-L lab-scale semi-batch glass reactors at a mesophilic temperature. The effect of increasing the substrate loading rates on biogas production was investigated. The mixing ratio for the two substrates was decided based on the results obtained from the BMP assay study, i.e. FW+BC sludge in a 1:2 ratio. The effect of increasing substrate loading rates on biogas production was investigated using

Table 2. Biogas (ml) values at the end of digestion, with an average methane content (% and volume) measured on four occasions, along with VSs reduction efficiency (%).

Sample	Biogas (ml g^{-1} VS)	Methane (%)	Methane (ml g^{-1} VS)	VS reduction (%)
FW	1063.3 ± 14.5	58.8 ± 5.1	625.4 ± 14.5	86.7 ± 0.8
BC	866.7 ± 5.0	58.6 ± 5.1	507.5 ± 5.0	82.7 ± 0.8
AC	690 ± 12.5	55.9 ± 8.6	385.9 ± 12.5	81 ± 0.2
FWBC 1:1	670 ± 8.5	60.4 ± 5.1	404.8 ± 8.5	86.2 ± 2.6
FWBC 1.5:1	803.3 ± 19.0	60.4 ± 3.4	485 ± 19.0	85.2 ± 1.9
FWBC 2:1	886.7 ± 13.5	53 ± 2.4	469.9 ± 13.5	87.7 ± 2.9
FWBC 1:1.5	813.3 ± 4.0	60.3 ± 4.6	490.6 ± 4.0	82.8 ± 0.2
FWBC 1:2	823.3 ± 6.0	59.8 ± 5.1	492.1 ± 6.0	84.8 ± 1.2
FWAC 1:1	776.7 ± 1.5	58.4 ± 5.8	453.7 ± 1.5	84 ± 1.0
FWAC 1.5:1	773.3 ± 27.5	53 ± 7.0	409.9 ± 27.5	87.1 ± 1.6
FWAC 2:1	656.7 ± 18.0	58.6 ± 6.1	384.6 ± 18.0	86.3 ± 1.4
FWAC 1:1.5	726.7 ± 0.0	57.5 ± 6.4	417.5 ± 0.0	86.2 ± 0.1
FWAC 1:2	800 ± 5.5	58.9 ± 6.2	471.1 ± 5.5	85.3 ± 0.4

±sign indicates standard error.
AC: after centrifuge; BC: before centrifuge; FW: food waste; TS: total solid; VS: volatile solid.

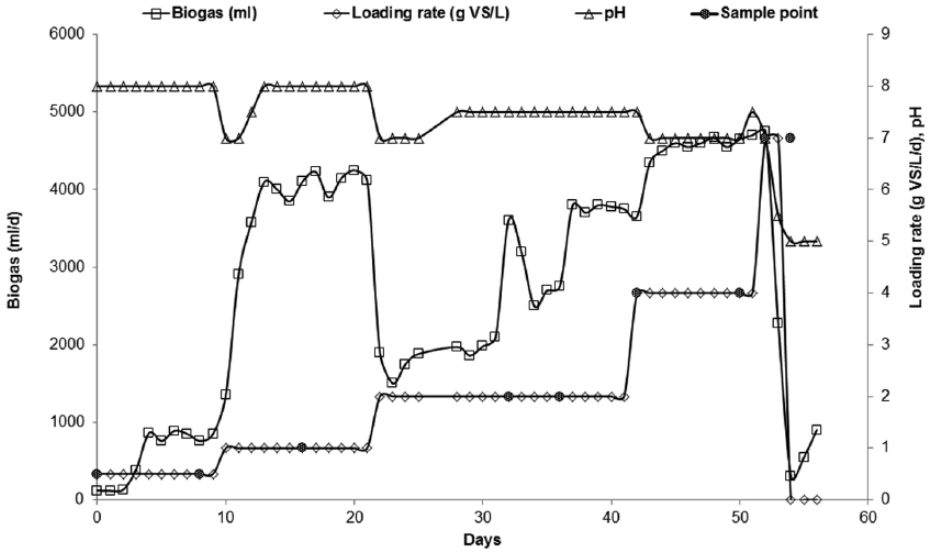

Figure 2. Different loading rate study in a 5 L reaction vessel.

the same mixture. A loading rate of 1 g VS L^{-1} d^{-1} gave the maximum biogas production of 742 ml g^{-1} VS L^{-1} d^{-1} with a methane content of 50%, followed by 2 g VS L^{-1} d^{-1} with biogas of 539 ml g^{-1} VS L^{-1} d^{-1} (Figure 2; Table 3). The biogas production, methane content, as well as pH, decreased comparatively when the loading rate was increased to 4 g VS L^{-1} d^{-1}, and further to 7 g VS L^{-1} d^{-1}. Biogas production drastically went down leading to reactor instability and complete failure when the loading rate was increased to 7 g VS L^{-1} d^{-1}. From Figure 2 it can be observed that loading rate of 1 g VS L^{-1} d^{-1} gave the maximum amount of biogas. Increasing the loading rate beyond 2 g VS L^{-1} d^{-1} was not possible with a 5 L reactor volume capacity, because it would bring down the hydraulic retention time (HRT) below 10 days with the available substrate characteristics. With the loading of 1 g VS L^{-1} d^{-1}, the HRT is 20 days, which seems to be the minimum time period essential for the substrate to get converted to CH_4 and CO_2.

T-RFLP mcrA gene

Electropherograms generated after GeneMapper analysis. T-RFs having size less than 50 bases were eliminated from the analysis as they might result from primer-dimers. The fluorescent signal threshold was set to 50, as per the standards, to minimise the background signal and signals arising from ssDNA non-specific amplicons or fragments.

Methanogenic bacterial richness. The methanogenic bacterial richness obtained from different fragments and elucidated in the T-RFLP technique, wherein each fragment is considered to arise from different methanogenic bacterial strains. Methanogenic bacterial richness ranged from 4 to 66, with an average of 33.77 using the T-RFLP technique. The highest richness was observed in sample 4 (66 T-RFs, loading rate of 2 g VS L^{-1}); while the lowest richness was observed in sample 3 (04 T-RFs loading rate of 1 g VS L^{-1}) (Figure 3).

Table 3. Characteristics of sludge inside the 5 l reactor and methane content during the loading rate study.

Sample	Day	Loading (g VS L d^{-1})	TS (%)	VS (%)	NH_3-N (mg L^{-1})	Total *N* (%)	COD (mg L^{-1})	Methane (%)
1	0	0.5	0.56	0.54	441.00	0.85	1028	34.26
2	8	0.5	0.98	0.60	674.33	1.07	1238	57.33
3	16	1	1.65	1.08	714.00	1.02	6678	48.92
4	32	1	1.18	0.73	639.33	1.09	3528	50.69
5	36	2	0.90	0.56	641.67	1.10	2998	49.99
6	42	2	1.07	0.76	515.67	1.11	2738	51.16
7	50	4	1.22	0.97	518.00	1.17	5188	57.86
8	52	4	1.40	1.14	466.67	1.08	6888	49.37
9	54	7	1.72	1.44	590.33	1.56	10808	35.71

COD: chemical oxygen demand; TS: total solid; VS: volatile solid.

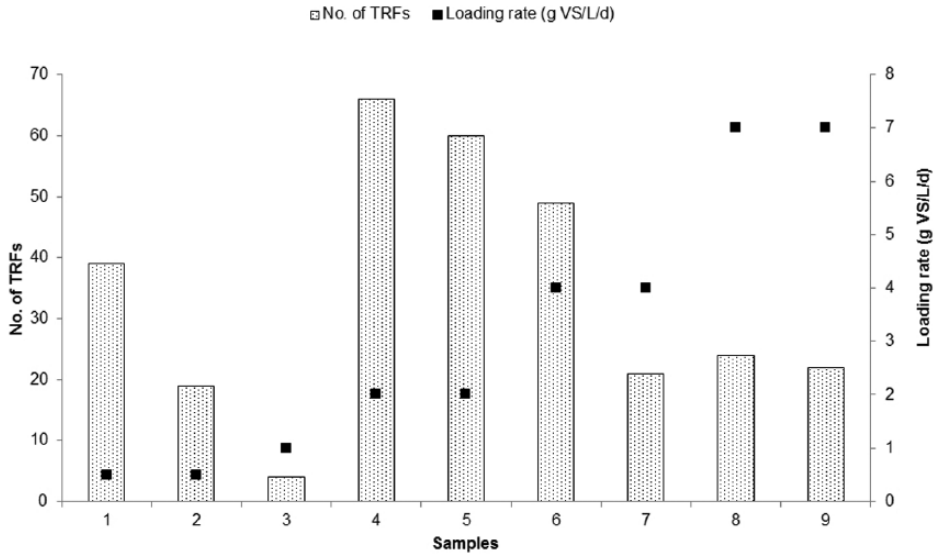

Figure 3. Methanogens richness obtained and elucidated by the T-RFLP technique.

A Dendrogram was constructed based on a Bray–Curtis distance measure or index of dissimilarity with Unweighted Pair Group Method with Arithmetic Mean (UPGMA) (Figure 4). The statistics was done using Paleontological statistics (PAST) software (http://folk.uio.no/ohammer/past/). (The Bray–Curtis dissimilarity is bound between 0 and 1, where 0 means the two sites have the same composition (that is they share all the species), and 1 means the two sites do not share any species.)

From Figure 4 it can be seen that methanogenic species in all the samples are nearly 35% diverse and 65% similar. Species at a low loading rate are clustered together (sample 1, 2, and 3) and species at a high loading rate are clustered together (samples 4–9). Sample 1 and 2 were taken at the start and end of the loading rate 0.5 g VS L, respectively. Species in both samples share around 65% similarity. In sample 3, where the T-RFs richness was only 4, the diversity is very less and it is related to species samples 1 and 2. This suggests that only a specific group of methanogens from the previous stage got enriched during the loading rate of 1 g VS^{-1}. Samples 4 and 5 have 80% similarity among them and both share 73% similarity with sample 6. Samples 8 and 9 are 85% similar and share 74% similarity with species from sample 7. The diversity among species from sample 4, 5, and 6

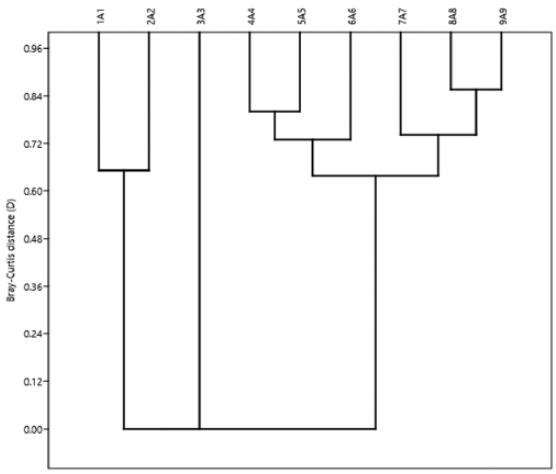

Figure 4. Cluster analysis of methanogens based on the diversity of *mcrA* genes showing Bray–Curtis similarity distance measures with UPGMA using PAST 3.0 software.

with that of species from samples 7, 8, and 9 is 36%. As the loading rate was increased from 0.5 g VS L^{-1} to 7 g VS L^{-1}, the shift in the species was observed. It was previously observed that hydrogenotrophic methanogens, such as genus *Methanosarcina*, genus

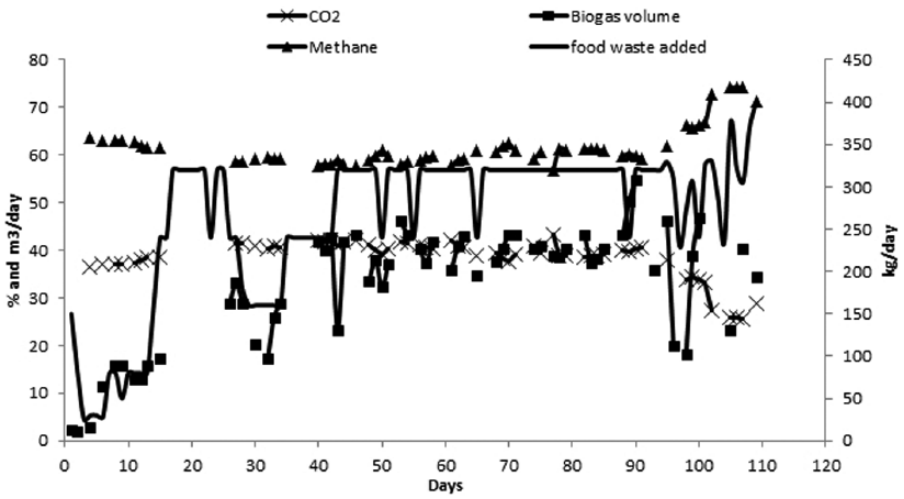

Figure 5. Biogas production and composition during pilot scale co-digestion studies.

Methanoculleus predominate in the reactors with higher organic loading rate (OLR), whereas, *Methanosaeta* represented the predominant acetoclastic methanogens in reactors with lower OLR (Nikolausz et al., 2013). It might be possible in this case that the genus *Methanosarcina* is dominating other species at a higher organic loading rate. Similar results were reported by authors from our laboratory with the reactor running on FW as a substrate at higher OLR (Prabhudessai, 2013). Genus such as *Methanosarcinaceae* has lower substrate affinity with a maximum substrate utilisation, can also occur in the reactors working at high OLR (Yu et al., 2005).

Pilot scale anaerobic co-digestion of FW and sewage sludge

The pilot scale co-digestion study was carried out for total of 109 days in a horizontal plug flow reactor with 60 m^3 capacity. The reactor was loaded with a gradual increase in FW concentration from 1 kg VS m^{-3} to 4.7 kg VS m^{-3} over a period of an initial 4 weeks (Figure 5). After that, the loading was maintained at constant rate of 4.7 kg VS m^{-3} for the next 4 weeks. The sewage sludge was added from day 64.

During the first 4 weeks, a gradual increase in biogas production was observed. The methane percentage was in the constant range of 60%–64%. The percentage of CO_2 was observed to increase gradually during the first 4 weeks, from 36% to 40%, after which it was stabilised at 40%.

Slurry of the reactor at the outlet was analysed during the study on a regular basis. pH of the slurry at the outlet remained constant at 7.2. TKN was found to fluctuate between 0.14% to 0.28%.

In the pilot scale study, after the start of the addition of sludge, there was an increase in biogas production, but significantly less as the mixing was at a ratio of 1:0.34 (on the basis of VS). With FW of 320 kg d^{-1}, the retention time was 120 days. To achieve the ratio of 1:2 FW + BC sludge, the volume of the sludge to be loaded was 11.66 m^3 d^{-1}. With this loading rate, the HRT would come down to 5 days leading to souring of the reactor (Bouallagui et al., 2004), incomplete digestion of the substrate, and subsequently leading to failure of the reactor. To keep the HRT at the optimum time, the addition of the sludge was maintained at the rate of 2 m^3. This would give a HRT of 26 days.

Even with the low loading of sludge, as the co-digestion time increased, the methane percentage in the biogas increased from 60% to 73%, and at the same time the CO_2 content gradually decreased to 26%. After day 90, the addition of FW was not constant owing to its availability and also the composition of FW available varied each day. The sludge addition was kept constant at 2 m^3 d^{-1}. Owing to this, there was variation in the volume of biogas produced. The biogas produced was supplied to the institute cafeteria for the purpose of cooking.

Ammonia nitrogen in the slurry increased during the initial FW addition from 880 mg L^{-1} to 1090 mg L^{-1}. Once the sludge was added, the ammonia in slurry started decreasing continuously up to 500 mg L^{-1} (Figure 6). A similar trend was observed with TS, VS, and COD at the outlet (Figures 7 and 8). A decrease in the content of TS, VS, and COD after the addition of sludge indicates the increase in the digestion efficiency and stability of the reactor.

AD of sludge alone at WWTPs is not an efficient process owing to less biogas and methane yield. Septic tanks are common in India, from which the sludge is removed with the help of a tanker lorry. This sludge can be used for biogas production in co-digestion with the FW that is available in enormous quantities generated by hotels and restaurants (Koch et al., 2015).

Conclusion

Co-digestion BMP of the sewage sludge with FW indicated that a desired mixing ratio of 1:2 was optimum for co-digestion of FW and sludge, respectively. A loading rate was determined in a 5-L reactor and found to be optimum at 1 g VS L d^{-1}. In the methanogenic diversity studies during the loading rate using T-RFLP, it was found that hydrogenotrophic methanogens might be predominating in the co-digestion. Co-digestion was further

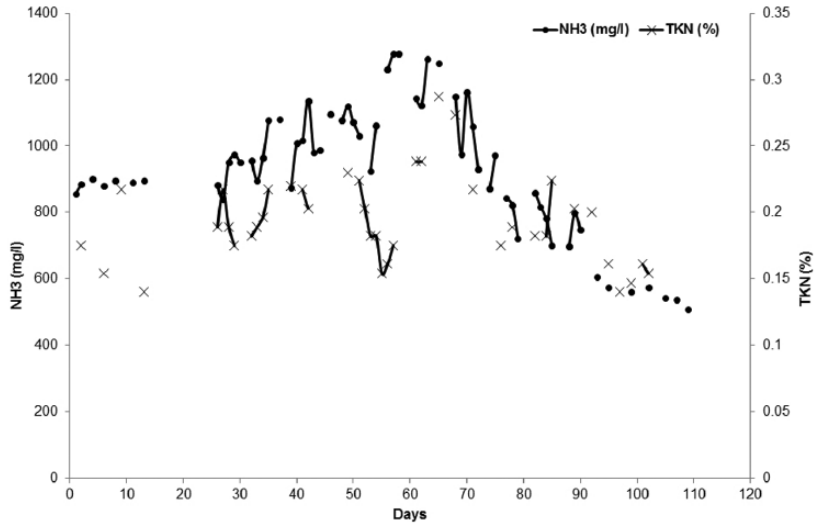

Figure 6. NH_3-N and TKN values of the effluent over time (in days) during the pilot scale co-digestion studies.

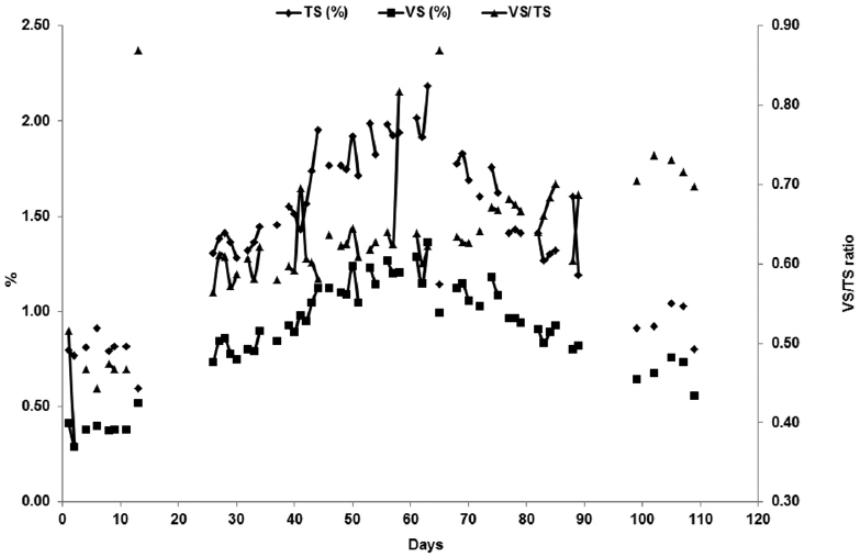

Figure 7. TS, VS, and VS/TS of the effluent over time (in days) during the pilot scale co-digestion studies.

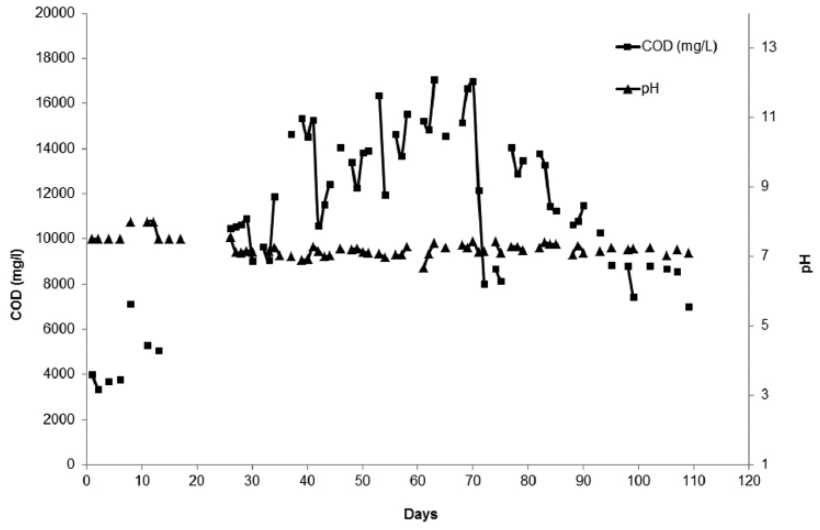

Figure 8. COD and pH of the effluent over time (in days) during the pilot scale co-digestion studies.

successfully carried out at a pilot scale in a 60 m^3 reactor. The co-digestion process was successfully applied to handle the sewage sludge as well as municipal kitchen waste, along with the energy production.

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