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FERMENTATION AND METHANOGENIC CHARACTERISTICS OF LEAFY BIOMASS FEEDSTOCKS IN A SOLID PHASE BIOGAS FERMENTOR

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Abstract

Biomass feedstocks, such as leaf litter, weeds and agro-residues, have been considered as alternative feedstocks to meet rural energy needs in India. Six different types of biomass substrates representing commonly available fresh and dry feedstocks were studied for their decomposition pattern and methanogenic activities in order to arrive at optimum design parameters for solid phase digestion. Broussenetia papyrifera, Parthenium hysterophorus, Synedrella nodiflora *(fresh leaf biomass feedstocks), and paddy straw, underwent a rapid initial decomposition losing 30–40% volatile solids (VS) within 10 d. This decomposition pattern appeared to favour growth and colonization of hydrogenotrophic methanogens in the latter three feedstocks. Stable biogas production was found wherever approximately similar rates of aceticlastic and hydrogenotrophic methanogenic activity were recorded on decomposing biomass feedstocks. Inadequate colonization by aceticlastic methanogens was found to be the main cause of a poor start-up and lower daily gas production rates, especially in the presence of rapid VS destruction. Two dry feedstocks, cane trash and bagasse were found to have an acidogenesis-limited decomposition pattern with <40% VS destruction in 45 d. These results suggested that proper start-up procedures were needed to ensure adequate build-up of aceticlastic methanogens, and the use of a mixed biomass feedstock comprised of fresh and dry biomass had a better chance of stable biogas production, conversion efficiency and gas yield. © 1997 Elsevier Science Ltd.*

Key words: Biomass feedstocks, biogas, methanogen colonization, fermentation pattern.

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INTRODUCTION

India has about two million small-scale biogas plants designed for operation with animal dung slurry, with potential for a total of 12–15 million plants. Alternative biomass feedstocks need to be considered to meet the cooking energy needs of the remaining 60–75 million rural households (Khandelwal, 1990; Ravindranath and Hall, 1995). Tropical countries such as India are endowed with large quantities of potentially fermentable biomass, such as leaf litter, terrestrial and aquatic weeds and agro-residues, which can serve as alternative feedstocks for biogas production (estimated at 1130 million tons y^{-1}, Jagadish, 1997). The existing small-scale biogas plants designed to digest homogenous animal dung slurry are not suitable for biomass feedstocks. The narrow inlet pipe, an inability to handle floating biomass (scum) and the absence of simple techniques to remove digested biomass render these designs unsuitable for continuous operation with herbaceous biomass feedstocks. Therefore new biogas plant designs have to be evolved. Such new designs need to incorporate features which avoid or escape floating of biomass, eliminate feedstock pretreatment, use physico-chemical and fermentation properties of biomass feedstocks to improve digestion and, finally, function satisfactorily in spite of rapid volatile fatty acid (VFA) fluxes released during biomass digestion. Solid state and dry digestion processes (> 10% total solids, TS) that digested biomass feedstocks in the absence of a large proportion of liquid phase and used large inlet ports were reported to overcome problems related to floating, pretreatment and feedstock addition (Ten Brummeler and Koster, 1990). When a small quantity of recycled digestor liquid was sprinkled over the decomposing biomass bed in solid-state stratified

bed (SSB) digestors, VFA-rich pockets were dissipated (Spendlin, 1991; Chanakya *et al.*, 1993; 1995). The SSB digestors permitted use of untreated, large sized biomass feedstocks and avoided particle size reduction. This feature is useful for rural India where grid power for pre-treatment is unreliable. Further, at long solids retention time (SRT, 40 d) pulverizing feedstocks did not increase gas production significantly (Moorhead and Nordstedt, 1993). However, several aspects of the SSB process have remained unclear, especially with respect to decomposition of biomass feedstocks.

Biogas production is known to occur largely through a two step reaction system. Polymers in biomass are first hydrolysed and converted predominantly to acetic acid and H_2 (acidogenesis). These intermediates from acidogenesis are converted to biogas by two distinct groups of methanogens (methanogenesis). It has been reported that many simple constituents of herbaceous biomass feedstocks (such as sugars, pectins and hemicelluloses) were rapidly hydrolysed and converted to methanogenic intermediates, mainly VFA. Among many biomass feedstocks $>25\%$ of the added volatile solids (VS) were converted to VFA in 2–8 d (Viswanath *et al.*, 1992; Sarada and Joseph, 1993; Kida *et al.*, 1994; Chanakya *et al.*, 1995). Such VFA fluxes and accumulations have often led to poor start-up and digestor failure. These failures were identified to be largely due to an inadequate initial methanogen population size in relation to the proportion of acidogenic microflora in biomass feedstocks. This imbalance was shown to be overcome when 40% (w/w) of animal dung or anaerobic compost was added as methanogen-rich inoculum to biomass feedstocks (Ten Brummeler *et al.*, 1991). The continuous use of 40% w/w level of inoculum leads to increased digestor sizes and costs and would therefore be undesirable for small-scale application in rural India. The problem of the additional digestor space required was overcome by adopting a careful start-up procedure involving a gradually increasing feed rate and a sufficiently large starting bed of digested biomass (Chanakya *et al.*, 1993; 1995). This suggested that the decomposition rate of biomass feedstocks and the build-up of a balanced population of the required microflora were crucial for the process.

In these studies it was shown that $>94\%$ of biogas was produced by the biomass bed rather than digestor liquid (Chanakya *et al.*, 1993). Furthermore, sprinkling recycled digestor liquid enhanced gas production (Spendlin, 1991; Chanakya *et al.*, 1993). Approximately, 10% of the sprinkled digestor liquid moved slowly through the bed (4–6 h) and prevented VFA build-up. The acetate levels in the digestor liquid fell to low levels once high gas production was achieved (30–45 d, Chanakya *et al.*, 1995). High methanogen populations colonized the digesting biomass feedstocks after a certain SRT (Barlaz *et al.*, 1989; Aldrich, 1993; Chanakya *et al.*, 1995). From these reports we concluded that in SSB digestors methanogen populations would gradually build up on feedstocks to reach a plateau and subsequently fall to low levels in lower regions corresponding to longer SRTs, where methanogen intermediates do not reach. However, in order to scale up the SSB technique, it is necessary to firstly understand the rates and pattern of feedstock decomposition and determine their relation to the rates and nature of methanogen colonization on typical biomass feedstocks. The rapidity and extent of methanogen colonization appeared to be dependent upon the initial composition of biomass substrate and the rate at which it decomposed to produce several methanogen intermediates (e.g. VFA). This phenomenon was utilized to design and operate bag type solid state biogas digestors (Ten Brummeler *et al.*, 1991) and semi-continuously fed SSB biogas digestors (Chanakya *et al.*, 1995) using untreated green or herbaceous biomass feedstocks, such as freshly harvested leaf biomass, partially-dry leaf litter and agro-residues.

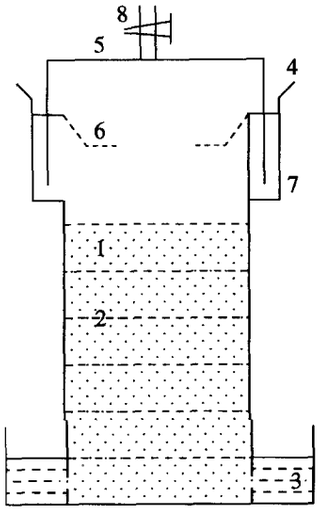
In this study, we attempted to reconstruct the profile of methanogen colonization on several biomass feedstocks by measuring the methanogenic activities at various periods of decomposition in laboratory-scale SSB digestors. An attempt was made to determine the stage of decomposition at which methanogenic activities begin, peak and decline on each of the above feedstocks. It was envisaged that such an analyses would provide a method to determine the optimum SRT for various substrates in SSB digestors and also provide methods to determine optimum submergence time for biomass feedstocks in plug flow like biogas digestors (Chanakya and Jagadish, 1997).

METHODS

Digestor design and operation

The six laboratory-scale SSB digestors used in the study were fabricated using polyvinyl chloride (PVC) based materials conforming to Fig. 1. The total volume was 8l and deducting the volume of the sprinkler gave a working volume of 6l. These digestors were connected to 4l gas collection and storage systems. The gas production was measured by downward displacement of water on a daily basis. These digestors were operated at ambient temperature (26 ± 2°C). In order to effect a rapid start-up, two kinds of methanogenic inocula were utilized (Chanakya *et al.*, 1995). Digested biomass removed from a SSB digestor was placed at the bottom of the digestors to give a bed height of 5 cm. Digestor liquid (2 l) from another SSB digestor was poured into the liquid reservoir. Evaporation losses of

digestor liquid were made up with anoxic tap water. To feed the digestor, the feed hatch was lifted, a nylon mesh was placed on the existing biomass bed and a known weight of feedstock was added without compacting it. The feed hatch was replaced immediately. The digestor liquid (1 l) was recycled through a liquid seal once a day and allowed to sprinkle on the bed. No digested feedstock was removed from the digestors during the study period. These digestors were operated on a weekly-fed basis for a period of 80–107 d, after which the biomass within was destructively sampled to determine its physico-chemical properties and methanogenic activities.



1 Biomass bed
2 Nylon mesh
3 Liquid reservoir
4 Liquid recycle port
5 Feed hatch
6 Sprinkler
7 Liquid seal
8 Gas outlet

Fig. 1. Sketch of the laboratory scale solid-phase stratified bed fermentor with its operating components.

Feedstocks used and feed rates

Conventional Indian biogas plants operating on dung are designed for a gas production rate (GPR) of 0.5 m^3 biogas m^{-3} digestor volume d^{-1} (expressed as $m^3\ m^{-3}\ d^{-1}$ or $l\ l^{-1}\ d^{-1}$, Rajabapaiah *et al.*, 1979). We screened six commonly found biomass residues of rural India (three dry and three green, Table 1). An attempt was made to determine which of these feedstocks could give a GPR of 0.5 $l\ l^{-1}\ d^{-1}$. The feed rates were chosen based on past experience to give a daily biogas output of at least 3l (Chanakya *et al.*, 1995). Assuming that > 50% TS would be converted to gas, the fermentors were fed *ca* 40 g total solids (TS, 35–38 g VS wk^{-1}) once a week [except cane trash — a 25% VS destruction was assumed and 80 g TS wk^{-1} was fed, Fig. 2(a, b)].

In order to avoid VFA build-up, no feeding was done in the second week. In spite of this, in the case of green biomass feedstocks, a fall in pH of the digestor liquid (6–6.2) was observed during the fourth week. Feedstock addition was avoided during the fourth week. The pH of digestor liquid was measured before feedstock addition in all cases. Except in the case mentioned above, pH of the digestor liquid was always > 7 and it allowed continued weekly feeding. This start-up procedure was expected to allow adequate time for the build-up of the required methanogenic flora on biomass feedstocks (Chanakya *et al.*, 1995). After 4 weeks, digestors were fed once a week. Allowing for fluctuations in the moisture content of the freshly harvested green substrates, the actual VS fed to the fermentors were close to the desired rates [Fig. 2(a, b)].

Table 1. Feedstocks used and performance characteristics under pseudo steady-state conditions in SSB digestors

No	Performance indices	Units	Dry feedstocks			Green feedstocks		
			Paddy straw	Bagasse	Cane trash	*Synedrella*	*Parthenium*	Paper mulberry
1	Fresh weight at feeding	g	50	50	100	200	200	200
2	Onset of steady-state[a]	d	38	40	63	56	49	70
3	Avg. feed rate at SS[b]	g TS d^{-1}	5.65	5.79	10.90	6.01	5.62	6.01
4	Avg. feed rate at SS[b]	g VS d^{-1}	4.53	5.25	10.20	4.84	4.59	4.98
5	Avg. VS content of feed[b]	% TS	80.0	90.7	93.6	80.5	81.7	82.9
6	VS destruction at SS	% VS	56.5	37.1	49.8	68.1	78.1	85.5
7	VS destruction at SS[c]	g d^{-1}	2.56	1.95	5.08	3.30	3.58	4.26
8	Steady-state gas[d]	l/d	1.01	2.42	2.32	1.58	1.40	5.15
9	Bed volume at SS[e]	l	2.91	3.33	5.31	2.07	2.61	4.73
10	Gas production rate[f]	$l\ l^{-1}\ d^{-1}$	0.36	0.73	0.44	0.77	0.54	1.09
11	Gas per unit VS lost	$l\ g^{-1}$	0.39	1.24	0.46	0.48	0.39	1.21
12	Theoretical gas yield[g]	$l\ d^{-1}$	2.66	2.03	5.28	3.43	3.73	4.43
13	Avg. VS content after 25 d	% VS, wet wt	11.5	12.7	17.9	9.4	6.9	3.4

[a]SS = Pseudo steady-state: where VS destruction as well as gas production reached plateau (Figures 2 and 3). [b]Averaged from feeding of 28/35 d to end of study period. [c]% VS lost at SS × VS fed d^{-1} at SS. [d]Average daily gas production of five consecutive weeks after SS was reached. [e]Bed volume (l) at the beginning of pseudo steady-state period. [f]GPR = gas production rate corrected for actual digester volume used by biomass (*e). [g]g VS lost × biogas density under study conditions (0.96 $g\ l^{-1}$); = (or 1.04 $l\ g^{-1}$; 60% CH_4, 38% CO_2, 2% H_2; 710 mm Hg, 27°C, 4% water vapour). Dry feedstocks: paddy straw (*Oryza sativa*), bagasse and cane trash (*Saccharum officinarum*). Green feedstocks: *Synedrella nodiflora*, *Parthenium hysterophorus*, *Broussenetia papyrifera* (paper mulberry).

Physico-chemical analyses

After a period of 80–107 d, the fermentor operation was stopped, the fermentors were drained of digester liquid for 5–10 min and the biomass bed within was pushed out without causing compaction. The fresh weight and average height of each layer of biomass (a week's feed separated by nylon mesh) were determined sequentially from the top-most layer downwards. The bulk density and compaction pattern were determined from this data.

Methanogenic activity assay

The first four layers of biomass from the top and three to four alternate layers from the remaining were chosen to determine their methanogenic activities. These biomass samples (2 g, two to three replicates depending upon availability) were placed in 65 ml glass vials, flushed rapidly with biogas, sealed with rubber caps and crimped. Subsequently, all these vials were flushed with O_2-free nitrogen, 2 ml anoxic water was injected to provide a water seal and vials were placed upside down for incubation ($24 \pm 2°C$). The methanogenic activities were measured under conditions of unlimited substrate without pH control (Sorensen and Ahring, 1993). Biomass samples in these vials were fed with one of the following substrates, 42 mg acetic acid (as sodium acetate in 21 ml water), 10 ml H_2 plus 5 ml CO_2 and no additional substrate (control) to determine the aceticlastic, hydrogenotrophic and residual methanogenic activities, respectively. These vials were incubated for 48 h (residual and aceticlastic) or 4 h (hydrogenotrophic), depending upon the assay required. These incubation times were chosen to ensure that very little growth of bacteria occurred during the incubation period. After the required incubation period, the volume of the head-space gas was determined at atmospheric pressure. The methane content in the head-space gas (at atmospheric pressure) was measured before and after the incubation period. From this the net methane produced was determined as $\mu l\ CH_4\ g^{-1}$ wet solids h^{-1}. Methanogenic activities expressed as $\mu l\ CH_4\ g^{-1}$ VS h^{-1} may be obtained from the VS content (Table 1).

TS and VS destruction

The entire mass of each layer (remaining after sampling for methanogenic activity) was used to determine TS and VS content by standard procedures (APHA, 1975). The TS and VS content were determined for every week's feed for all feedstocks. From the difference between the mass of TS/VS fed and TS/VS remaining in each layer in the digestor, the TS and VS destruction were determined as a function of digestion time. When the

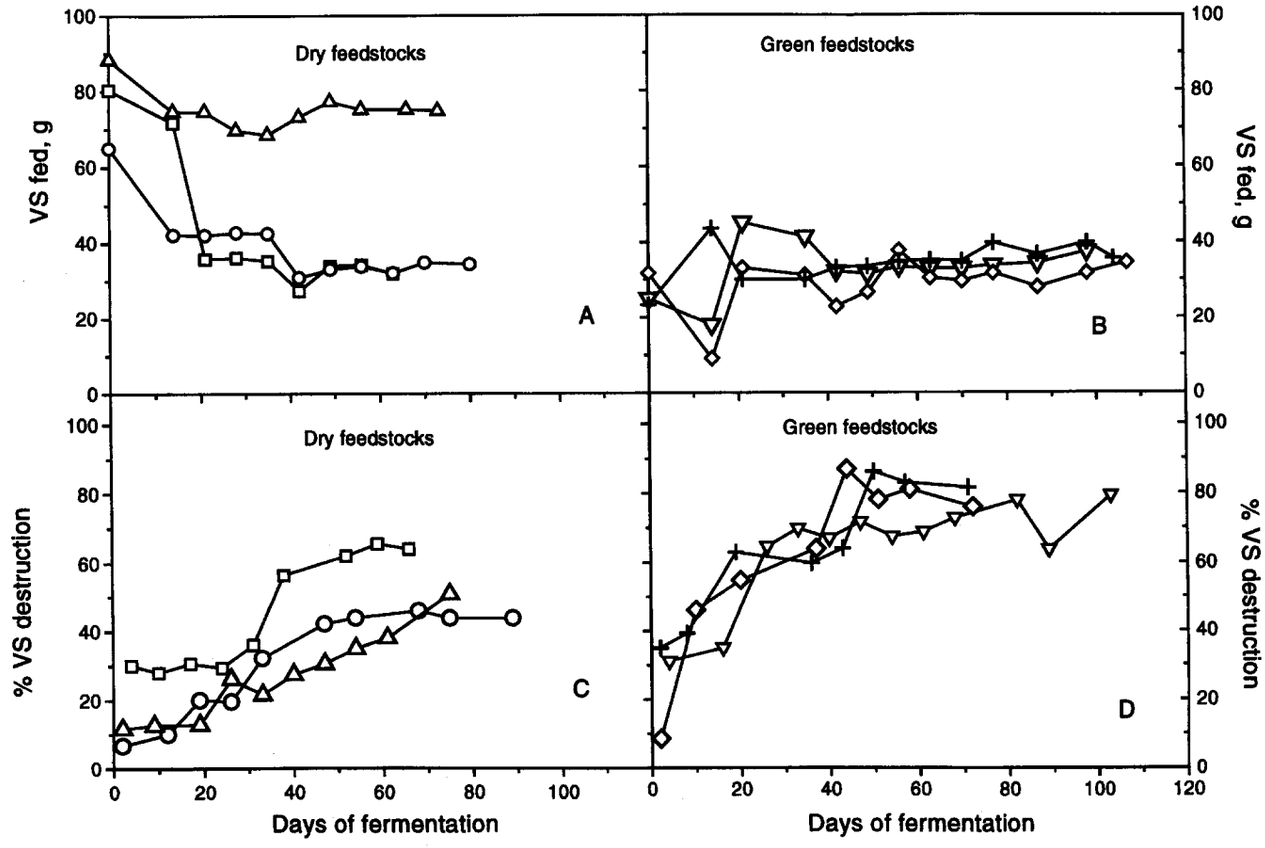


Fig. 2. Volatile solids (VS) fed and VS destroyed during operation of SSB fermentors (dry feedstocks: □ = paddy straw, ○ = bagasse, △ = cane trash; green feedstocks: ▽ = *Synedrella*, ◇ = *Parthenium* and + = paper mulberry).

GPR as well as the extent of VS destruction reached a plateau, the digestors were considered to have reached pseudo steady-state operation. These values were used to analyze the performance (under pseudo steady conditions as shown in Table 1).

RESULTS AND DISCUSSION

Solids decomposition pattern

Dry feedstocks (paddy straw, bagasse and cane trash) decomposed slowly, resulting in a 20–30% VS destruction in 25 d, while green feedstocks decomposed rapidly, resulting in a > 55% VS destruction in the same period [Fig. 2(c, d)]. All three green feedstocks and paddy straw exhibited a typical pattern of a rapid initial decomposition (30–45% VS in 10 d) and reached saturation within 35–50 d. The results of *in situ* decomposition rates measured for green substrates in pilot SSB digestors (Chanakya *et al.*, 1995) were found to be similar to that obtained in this laboratory study. The rate and extent of decomposition achieved in this study were comparable to that achieved with pulverised biomass feedstocks under submerged decomposition (Tong *et al.*, 1989). These observations showed that SSB digestion was comparable to slurry-based fermentation with regard to VS destruction for green biomass feedstocks. The rapid initial VS destruction was reported to be caused by the removal of many simple fractions of biomass, such as ethanol soluble substances, hot water and hot oxalate soluble pectins and some hemi-cellulosic material from green feedstocks (leaf biomass) subject to decomposition in SSB digestors (Chanakya *et al.*, 1995). It was observed earlier that paddy straw contained a significant proportion of such easily decomposable fractions, which partially explains the rapid initial VS destruction (ASTRA, 1995, unpublished studies). However, further work is needed to explain its recalcitrance for 3 weeks and subsequent decomposition [Fig. 2(c)]. On the other hand, bagasse and cane trash decomposed gradually and underwent a 45% VS destruction in 45 and 70 d, respectively. These results suggested that under SSB digestion, bagasse and cane trash were likely to be limited by low hydrolysis (or acidogenic) related factors and hence required a longer solids retention time (SRT) to achieve better conversion efficiencies.

Gas production pattern

All the substrates studied except cane trash resulted in pseudo steady-state operation within 30–70 d after start-up (Fig. 2Fig. 3). The gas production levels under pseudo steady-state conditions were better than the desired level of $0.5\,l\,l^{-1}\,d^{-1}$ in all but two substrates, paddy straw and cane trash (Table 1). These results indicated that in SSB digestors, physical or chemical pre-treatment was not needed for many biomass feedstocks to achieve the desired GPR. Under pseudo steady-state conditions, in four feedstocks (paddy straw, cane trash, *Parthenium* and *Synedrella*), two indicators of VS conversion efficiency, l gas g^{-1} VS destruction or the theoretical gas yield vs observed gas yield, were unsatisfactory (Table 1). These indicators suggested that in these four feedstocks the process was limited by methanogenesis related problems.

Changes in bulk densities

The bulk densities achieved by various substrates as a function of digestion period is presented in Fig. 4(a, b). All the substrates studied underwent compaction to acquire bulk densities in the range of 450–600 kg m^{-3} (wet basis). Among green feedstocks, the gain in bulk density was initially rapid when compared to dry feedstocks. When the total volume of the decomposing biomass was deter-

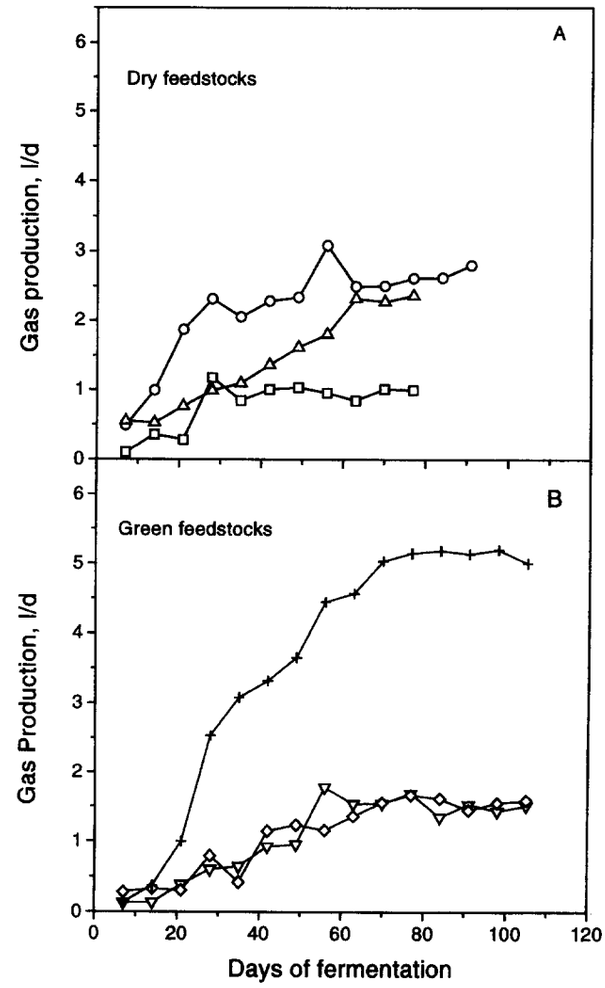


Fig. 3. Daily gas production rates observed with various biomass feedstocks (a) dry and (b) fresh feedstocks (dry feedstocks: □ = paddy straw, ○ = bagasse, △ = cane trash; green feedstocks: ▽ = *Synedrella*, ◇ = *Parthenium* and + = paper mulberry).

mined, it was observed that with all the substrates (except cane trash and paper mulberry, Table 1), only about half the fermentor volume was occupied by decomposing feedstock. This suggested a potential to double the feed rate used, i.e. upto 9–10 g VS l^{-1} d^{-1} under steady-state operation (Table 1), along with a higher GPR, especially when such increases in bulk density are matched by corresponding increases in methanogenic activities.

Methanogenic activities on biomass substrates

It was envisaged that the potential methanogenic activities of digesting feedstocks would gradually increase, reach a plateau and then sharply decline. The period at which a decline in methanogenic activity occurred could then be considered as optimum SRT for a specific feedstock, by which time significant VS would already have been destroyed. This would then ensure the complete conversion of transported methanogenic intermediates to gas

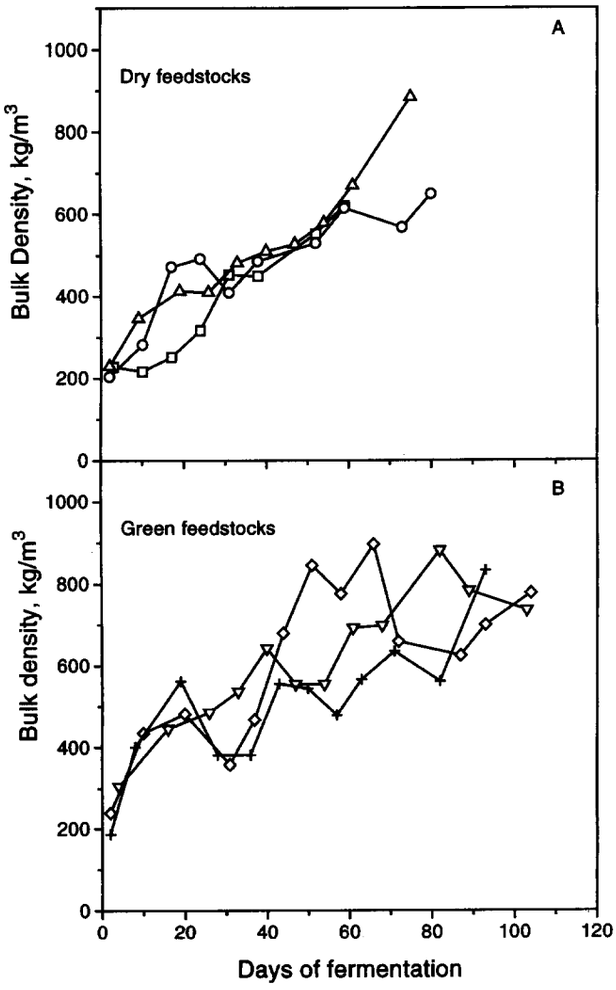


Fig. 4. Bulk densities acquired by decomposing biomass feedstocks at various SRTs in solid-phase stratified bed fermentors (dry feedstocks: □ = paddy straw, ○ = bagasse, △ = cane trash; green feedstocks: ▽ = *Synedrella*, ◇ = *Parthenium* and + = paper mulberry).

(Kang and Weiland, 1993). By measuring the potential methanogenic activities on various feedstocks digested for different SRTs in a SSB digestor, we examined whether such an approach was possible.

The potential methanogenic activities measured were greater than residual methanogenic activities for periods varying between 20 and 60 d SRT in all substrates, except cane trash [Fig. 5(a–f)]. This suggested that a high level of methanogen population developed on these five feedstocks. The presence of such high activities in older layers, without accompanying VS destruction (Table 1), suggested the continued availability of several methanogen intermediates, possibly transported from other layers above. These results showed that the biomass feedstocks underwent a transition from methanogen-deficient to methanogen-rich zones with digestion time. In the SSB digestors, the succession was quicker when compared to that occurring in land fills (Barlaz *et al.*, 1989). However, the peak methanogenic activity did not always decay to residual levels as envisaged before. Only in the case of paper mulberry were higher aceticlastic as well as hydrogenotrophic activities sustained between 34–60 d and declining to residual levels after 85 d SRT.

In the case of paddy straw, *Synedrella* and *Parthenium*, characterized by a low VS to gas conversion (Table 1) and a high initial VS destruction, hydrogenotrophic activity was predominant and occurred earlier than the onset of aceticlastic activity [Fig. 5(a,d,e)]. This suggested that high levels of H_2 occurred in these layers which in turn represented incomplete/perturbed decomposition. These results showed that the above three feedstocks required lower feed rates at start-up (to build aceticlastic activity), a greater mass of starting bed and an improvement in the sprinkling technique to transport/dissipate methanogen intermediates that were rapidly produced in the early stages.

In slowly decomposing feedstocks, cane trash and bagasse, marginally higher hydrogenotrophic and aceticlastic activities (over residual) were observed [Fig. 5(b, c)], which suggested that, in spite of liquid recycle, very low levels of methanogen intermediates were rendered available to more digested feedstocks in layers below. In the case of bagasse, where most of the VS destruction resulted in complete conversion to biogas (in mass terms, Table 1), it indicated a need to enhance the extent of acidogenesis (mainly hydrolysis) to obtain a better GPR. It was difficult to extend this conclusion to cane trash, where consistent steady-state operation could not be obtained and required further investigations. The hydrogenotrophic (0.70–9.1 ml CH_4 g^{-1} VS h^{-1}) and aceticlastic (0.31–6.56 ml CH_4 g^{-1} VS h^{-1}) rates obtained were comparable to equivalent values reported for other sources of high methanogenic activities (Wu *et al.*, 1991; Yu and Pinder, 1993).

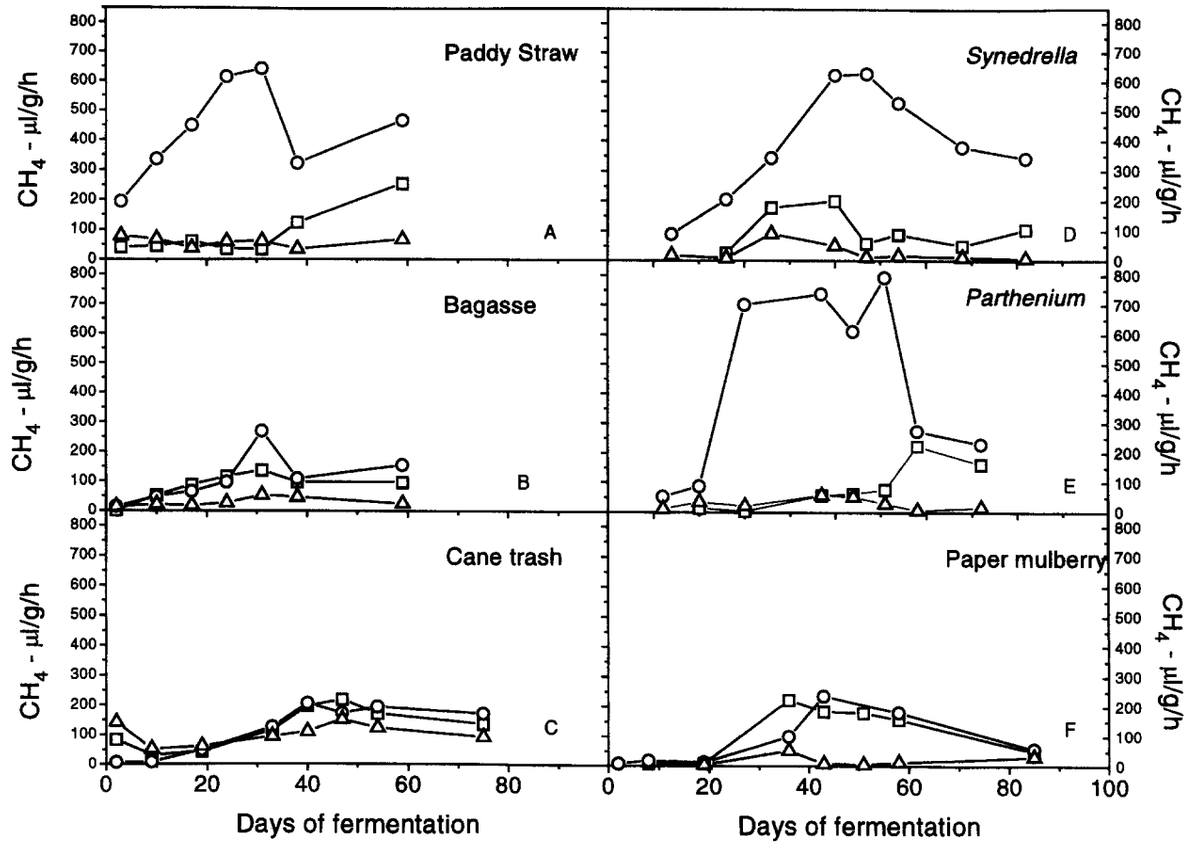


Fig. 5. Methanogenic activities measured on decomposing biomass feedstocks removed from the SSB fermentor at various SRTs (□ = aceticlastic, ○ = hydrogenotrophic and △ = residual).

CONCLUSIONS

Many of the typical biomass feedstocks available for biogasification in rural India can be fermented in a solid-phase stratified bed fermentor to effect a > 45% VS conversion. In SSB digestors, many biomass feedstocks initially undergo a stage of rapid VS destruction under methanogen-deficient conditions. This is followed by a transition to a methanogen-rich situation capable of rapid conversion of methanogen intermediates produced elsewhere. The SSB technique requires a careful start-up but has the potential to overcome many problems associated with small-scale biogas production from biomass. It is possible to operate biogas plants at gas production levels comparable to, or better than, conventional dung based plants ($0.5\,l\,l^{-1}\,d^{-1}$). Potential exists to improve this performance even further by increasing the feed rates and by finding suitable mixes of green and dry biomass feedstocks.

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