RESEARCH ARTICLE

# High rate biomethanation using spent biomass as bacterial support

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**Methanogenic bacteria were found strongly adhered to several green biomass feedstocks digested in a solid-phase stratified bed (SSB) biogas fermentor, and hence these digested feedstocks were examined for their potential for use as methanogen support for a high-rate biogas fermentor. A 1.1 l experimental down flow fixed bed (DFFB) fermentor was operated at 35°C using synthesized liquid waste. This gave gas production levels up to 6.5 l/l/d. Methanogenic activities measured on such biomass support material exhibited a potential to achieve much higher biogas production. Short-duration thermal and feed shocks were tolerated without their exhibiting typical methanogen washout characteristics. The DFFB fermentor functioned well even at 21 ± 1°C, with gas production rates up to 3 l/l/d, and thus appeared to have potential for producing biogas at small scales and at high rates from various combinations of liquid and solid biomass wastes.**

BIOGAS production process follows two major stages, involving conversion of complex polymers to many intermediates, mainly volatile fatty acids (VFA) – acidogenesis – and $H_2$, which are in turn converted to methane and carbon dioxide – methanogenesis. Methanogenesis is usually the rate-limiting step due to low growth rates of methanogens[1]. Hence, conditions of suspended growth (in tank reactors) result in 0.3–0.8 l biogas/l reactor/day (l/l/d) (refs 2, 3). Acidogenesis (when not rate-limiting, e.g. whey) is 8–10 times faster than methanogenesis[3]. Retaining methanogens for longer periods in the reactor (longer than the liquid) usually overcomes the limitation of low-growth rates of methanogens. Dense bacterial granulation (upflow anaerobic sludge blanket, UASB) and methanogens attached to inert surfaces (fixed-film reactors) enable long retention periods for methanogenic bacteria. These, however, require a great degree of process control to ensure stable operation and retention of methanogenic bacteria[4,5]. Simple fermentation concepts and methanogen support materials are required to achieve high-biogas production rates (> 5 l/l/d) at small-scale operation in the Indian context.

Previous studies on two-phase and solid-phase biogas fermentation indicated that methanogens colonized and adhered strongly to partly-digested herbaceous biomass feedstocks[6–8]. Digesting of biomass feedstock (@30–40 d SRT), exhibited methanogenic activities of 120–800 µl $CH_4$/g biomass/h (4.8–32 ml biogas/g/d)[9] by methanogenic bacteria which was strongly bound to biomass feedstocks and was neither dislodged during maceration nor by subsequent washing with water (ASTRA 1994, unpublished studies). Such biomass was then envisaged as ideal bacterial support in biomethanation of liquid wastes. The potential and characteristics of such methanogenic-bacteria-colonized, digested biomass feedstock used as a bacterial support in a down flow fixed bed (DFFB) fermentor was examined in this laboratory-scale study. This study could also lead to co-fermentation concepts, involving simultaneous digestion of solid and liquid wastes.

## Material and methods

### *Feedstock*

A typical liquid waste, comprising of suspended and dissolved solids, was prepared with known weights of rice flour in 50–100 ml boiling water. This was further diluted with anoxic water and fermentor effluent according to the required strength and fed to the fermentor. Feed rates in increasing steps of 1.5 g total solids (TS/d up to 9 g TS/d) were adopted as presented in Table 1. The TS and volatile solids (VS) content of rice flour samples used were as follows:

| Rice flour | Period used | TS% | VS% |
|---|---|---|---|
| Sample 1 | 1–156 d | 89.85 | 95.70 |
| Sample 2 | 157–210 d | 88.07 | 93.42 |

### *Fermentor design*

A 100 mm diameter, laboratory-scale DFFB fermentor was fabricated with PVC pipe to provide a 1.1 l total volume. This lab-scale DFFB fermentor was initially packed with 400 g digested biomass feedstock (bacterial support) extracted from a field-scale SSB biogas digestor. The biomass feedstock was packed between two corrugated stainless steel meshes to prevent its floating

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**Table 1.** Fermentor operation pattern

| Period operated (days) | Feed rate g/d | gTS/d | gVS/d | Feeding frequency no./d | Effluent recycle vol (l) | Total feed (l) |
|---|---|---|---|---|---|---|
| 1 | 1.5 | 1.34 | 1.29 | 1 | 0.5 | 0.5 |
| 5 | 3.0 | 2.70 | 2.58 | 2 | 1.0 | 1.0 |
| 16 | 4.5 | 4.04 | 3.87 | 3 | 1.5 | 1.5 |
| 28 | 6.0 | 5.39 | 5.16 | 3 | 1.5 | 1.5 |
| 18 | 7.5 | 6.74 | 6.45 | 3 | 1.2 | 1.5 |
| 9 | 9.0 | 8.09 | 7.74 | 3 | 0.9 | 1.5 |
| 5 | 9.0 | 8.09 | 7.74 | 3 | 0.6 | 1.5 |
| 8 | 5.0 | 4.49 | 4.30 | 2 | 0.2 | 1.0 |
| 4 | 0.0 | | | | | |
| 20 | 3.0 | 2.70 | 2.58 | 2 | 0.5 | 1.0 |
| 8 | 3.0 | 2.70 | 2.58 | 2 | 0.5 | 1.0 |
| 1 | 3.0 | 2.64 | 2.46 | 2 | 0.5 | 1.0 |
| 2 | 1.5 | 1.32 | 1.23 | 1 | 0.3 | 0.5 |
| 2 | 3.5 | 3.08 | 2.87 | 2 | 0.5 | 1.0 |
| 12 | 4.5 | 3.96 | 3.69 | 2 | 0.5 | 1.0 |
| 2 | 0.0 | | | | | |
| 5 | 5.0 | 4.40 | 4.10 | 2 | 0.5 | 1.0 |
| 5 | 0.0 | | | | | |
| 12 | 4.0 | 3.52 | 3.28 | 2 | 0.5 | 1.0 |
| 14 | 6.0 | 5.28 | 4.92 | 2 | 0.5 | 1.0 |
| 33 | 4.5 | 3.96 | 3.69 | 2 | 0.5 | 1.0 |

as well as for easy release of gas. The gas produced daily was collected by downward displacement of water in a 4 l storage vessel.

### *Operation*

The DFFB fermentor was normally operated at 35°C unless for some specific reasons the temperature was altered to introduce perturbations. The DFFB fermentor was fed intermittently (2–3 times daily) as in Table 1. The gas produced daily was measured by the downward displacement of water. It was envisaged that the feedstock thus prepared would represent biogas production from a wide range of effluents and wastes which contain both soluble and particulate fermentable and non-fermentable matter. This liquid feedstock was introduced from the top and the fermentor was immediately sealed. The effluent was collected and stored for recycling and analyses.

### *Biomass used for bacterial support*

The biomass used as bacterial support was derived from digesting a green biomass feedstock which comprised of 75% w/w *Synedrella nodiflora* (a locally-occurring weed) and 25% w/w segregated urban garbage at a SRT of 45 d. At the time of start-up the bacterial support used in DFFB fermentor underwent a short and rapid initial disintegration for 3 d. Particulate material of this support was detected in the effluent during this period. The bacterial support also underwent a gradual disintegration during the study period. As a result of this, it was necessary to replenish the bacterial support after 93 d of operation. About 40% v/v of the original bacterial support was lost through disintegration, washout and possibly some degree of compaction. Additional bacterial support material (200 g) was introduced into the DFFB fermentor to compensate for this loss. This bacterial support material at this stage was mainly of urban garbage origin. The operation was continued in this mode for a period of 210 d with a second cycle of gradually increasing feed rates. After a 210 d study period, the fermentor was dismantled and examined for physical and microbiological characteristics.

### *Start up*

The biomass-packed DFFB fermentor was initially filled with the digestor liquid extracted from a SSB biomass fermentor. During start up, the feed was prepared in recycled effluent and operated at room temperature between 23 and 25°C. After a 5 d operation when residual gas production of the support material fell to low levels, the fermentor was operated at 35°C. A step-wise increase in feed rate was followed as given in Table 1. A particular feed rate was maintained till the daily gas production levels became steady.

### *Physico-chemical analyses*

TS, VS, VFA content, gas composition, pH, etc. were determined according to standard methods[7,10]. The VFA and gas composition were determined by a gas chromatograph[7].

### *Potential methanogenic rates on biomass support*

The potential methanogenic rates on residual bacterial support material were determined in the presence of unlimited methanogenic substrates without pH control by a method similar to that reported previously[9,11]. Biomass support as well as the digestor effluent were sampled from the DFFB fermentor at two different feed rates. Bacterial support in wet state (2 g) or effluent samples (20 ml) extracted from the fermentor were placed in 65 ml vials (in triplicate). These vials were rapidly flushed with biogas immediately to remove traces of oxygen, capped with rubber stoppers and crimp-sealed. Subsequently they were flushed with $O_2$-free nitrogen to lower the methane content in the head space gas. The vials with solid biomass were injected with 2 ml anoxic water to act as a water seal. These vials were fed one of the following methanogenic substrates; 30 mg sodium

acetate in 20 ml anoxic water, 15 ml $H_2$ + 5 ml $CO_2$, no additional substrate (control), in order to determine the aceticlastic, hydrogenotrophic and residual methanogenic activities, respectively. These vials were incubated in upside down position at 22 ± 1°C for 5, 40 and 40 h for hydrogenotrophic, aceticlastic and residual activities, respectively. These short incubation periods used for the assay did not provide opportunities for extensive methanogen multiplication and hence they reflected potential methanogenic activities existing on the support material within the fermentor. The methane content of the head space gas was determined by a GC before and at the end of the incubation period after adjusting head space gas to atmospheric pressure. The net methane produced during the incubation period was determined by difference. These methanogenic rates were obtained as µl $CH_4$/g support (or ml effluent)/h. These values were later converted to ml biogas/g/d (assuming a biogas composition of 60% $CH_4$ and 40% $CO_2$, conversion factor 1 µl/g/h = 0.04 ml/g/d). This facilitated comparison of the performance with other high-rate reactors. Methanogenic activities found on various other biomass feedstocks sampled from laboratory-scale SSB fermentors at various SRTs were also determined in a similar manner and were used for comparison.

### *Feed and temperature shocks*

Temperature shocks, as are likely to arise from equipment and power failure, were introduced for short periods in a few instances (Figure 1 *a*); i) typical thermostat failure leading to high temperature (60°C for about 4 h), ii) no heating which lowered temperature to 24°C for 24 h on two occasions. The resumption of gas production after these shocks was monitored without any other alterations to the operation. Feed shocks were introduced by abrupt reduction in feed rates on two occasions and the rate of change in gas production was monitored. Between 180 and 210 d of operation, the fermentor was operated at room temperature (21 ± 1°C) to determine its performance at ambient temperature.

### *Volatile solid conversion efficiency*

The feed VS to gas conversion efficiency was computed as follows. The mass of biogas (60% $CH_4$, 40% $CO_2$) produced (gas production volume corrected for temperature, pressure and vapour pressure), divided by the mass of VS fed was used to compute VS conversion efficiency. While the conversion efficiency was computed daily, the average weekly values were plotted in Figure 1 *a*.

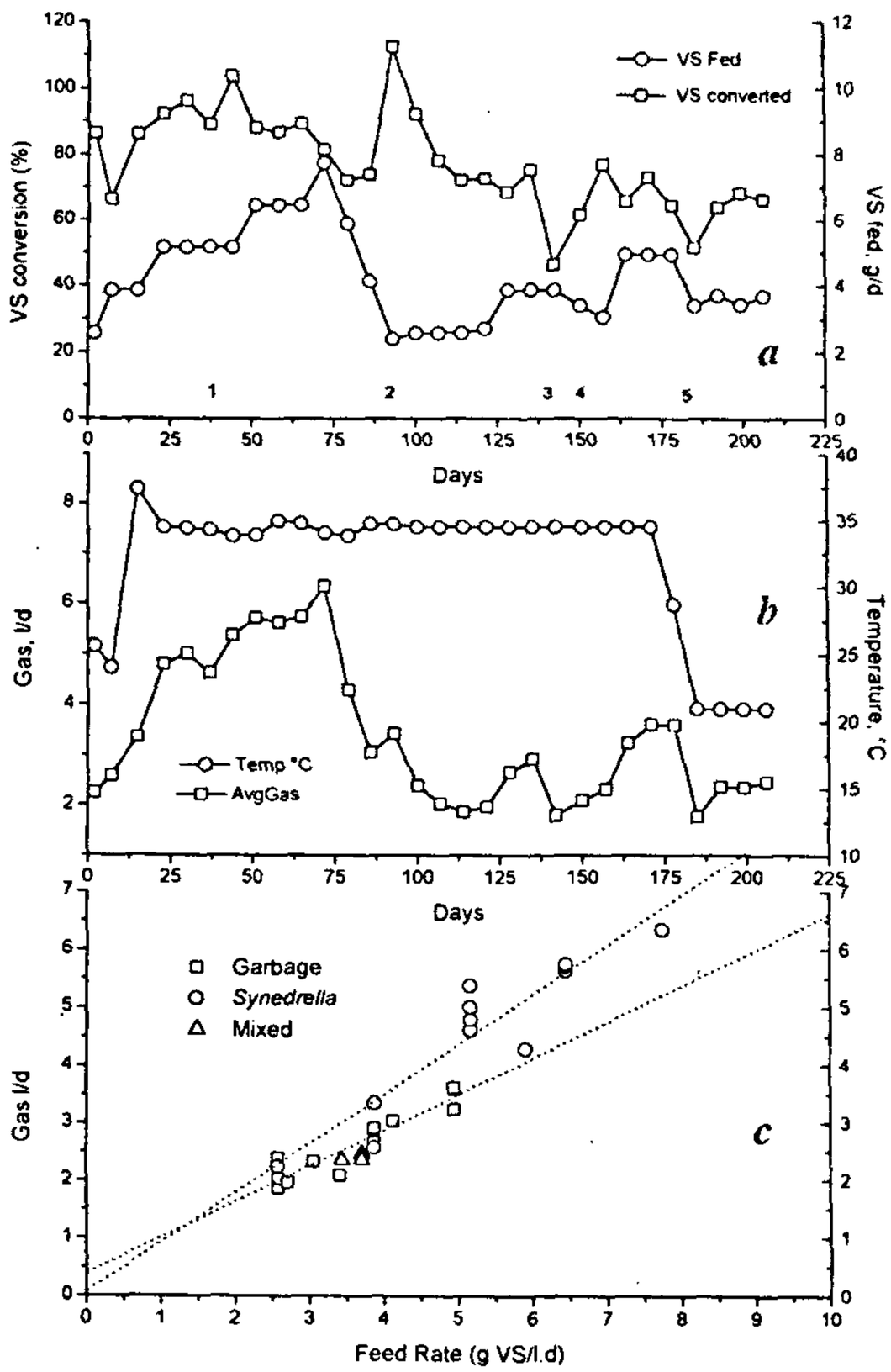


**Figure 1.** Operation and performance characteristics of laboratory-scale DFFB fermentor using digested biomass as methanogenic support (values are 7 d average). Numerals indicate the following feed/operation shocks. 1, Reactor reaching 60°C for 4 h due to thermostat failure; 2, 200 g additional bacterial support added; 3, 4 and 5, No feed addition for 2, 5 and 3 days, respectively. □, garbage as support; O, *Synedrella*, garbage as support; Δ, garbage + *Synedrella* at 22°C.

## Results and discussion

### *Daily gas production*

The gas production pattern (Figure 1 *b*) in relation to a step-wise increase in feed rate (Figure 1 *a*) as well as a gradual replacement of the recycled effluent by anoxic water was studied (Table 1). It was observed that up to a daily feed rate of 7.74 g VS/l/d the gas production rose to support conversion rates of 6.4 g VS as gas with an overall conversion efficiency of 85% (Figure 1 *a*). At each step increase (1.5 g) in feed rate, there was a lag in corresponding rise in gas production rate, the slope of which corresponds to about 0.2 g/d. This indicates that while restarting operation or at start-up, the feed rates need to be enhanced in steps of 0.2 g/l/d to ensure

complete conversion of substrates (Figure 1 *a*, *b*) during start up. Following this gradual rise in gas production, the VS converted to gas, measured on a daily basis, exceeded the VS fed on certain days. This indicated that a part of the feedstock was retained in the fermentor and was subsequently decomposed to biogas.

### *Potential volatile solid conversion and gas production rates*

The VS converted to gas at different feed rates showed a linear trend (Figure 1 *c*). The gas production rates rose to high levels in response to increased feed stock addition until a daily feed rate of 6.4 g VS/l/d. At higher feed rates, the gas production rates appeared to level off indicating this to be the maximum feed rate possible. At a feed rate of 7.74 g VS/l/d the fermentor was stable as long as 60% of the effluent was recycled. Thus this set up could support a feed rate of about 7.74 g VS/l/d corresponding to a biogas production rate of 16.25 ml/g/d (406 µl $CH_4$/g/h) by the support material used. An attempt was made to reduce the dependence on the extent of effluent recycled. When the extent of effluent was reduced to 40% v/v, the fermentor became unstable and the effluent gradually became acidic (pH 6.6). At this stage the fermentor was opened to determine the cause of failure, and this was found to be due to depletion of bacterial support within the DFFB fermentor.

### *Life of the biomass support*

The DFFB fermentor used in this study was packed with bacterial support made of anaerobically digested biomass feedstock as mentioned earlier. Previous studies had shown that such materials underwent 50–70% VS destruction in typical SSB digestors after 45 d SRT (ref. 12) and were colonized by various methanogenic bacteria[8,9]. In this study we found that significant levels of methanogenic activities were already present on various digested biomass feedstocks examined (Table 2). Many cellulolytic bacteria colonized such biomass feedstocks at later stages[8,12,13]. Biomass feedstocks were hence expected to undergo a slower secondary decomposition. This in turn would result in its slow disintegration when used as bacterial support in DFFB fermentors and would require periodic replenishment. However, there were no indices to estimate this rate from any of the previous work.

When the DFFB fermentor showed signs of failure at high feed rates after a 93 d operation, the fermentor was opened to determine the health and nature of the bacterial support. It was observed that a significant quantity of this biomass support had disintegrated and biomass remaining in the fermentor occupied only about 60% of the original packed volume. An additional quantity of 200 g digested biomass feedstock was added (wet weight basis). This permitted resumption of the DFFB fermentor and high rate gas production. When the fermentor was dismantled after a 210 d operation, 300 g wet biomass (@12.5% TS) was recovered from a total of 600 g used. From the difference in mass of bacterial support used and recovered, it was calculated that 300 g of biomass support disintegrated during a 210 d operation (average 1.4 g/d). Therefore we conclude that the bacterial support needs to be replenished at this rate to maintain a high conversion rate (ignoring possible changes in quality of bacterial support).

At a packing density of < 400 g/l, no fouling or flow problems were encountered. At a higher packing density (data not shown), significant bacterial growth as well as feed residue was encountered on the upper surface of biomass support which impeded flow. It is hence concluded that a packing density of < 400 g/l (wet basis, c.12.5% TS) was optimum.

### *Methanogenic activity on biomass support*

From the measurements of methanogenic activities on a few potential bacterial support materials (digested biomass feedstocks, Table 2), it is clear that even at 22°C about 3.6 and 6.6 l/l/d biogas is possible through aceticlastic and hydrogenotrophic routes, respectively in a reactor set up as used in this study (assuming 400 g support/l and 60% $CH_4$). Previous work on fermentation of biomass feedstocks in SSB digestors[7-9] showed that most of the methanogenesis occurred on decomposing biomass feedstock itself. Further, these methanogens were strongly bound to decomposing biomass feedstocks and were not easily dislodged by mashing in the presence of water or a mild detergent (ASTRA, 1994, unpublished study). These results indicate that a wide variety of digested green and dry biomass feedstocks are suitable for use as methanogen support.

From the levels of methanogenic activities obtained (Table 3), it was clear that the biomass support used was responsible for the high rates of biogas production rather than the digestor liquid. The potential methano-

**Table 2.** Methanogenic activities* on a few typical biomass feedstocks

| Type of feedstock | SRT (d) | Aceticlastic µl/g/h | Aceticlastic ml/g/d | SRT (d) | Hydrogenotrophic µl/g/h | Hydrogenotrophic ml/g/d |
|---|---|---|---|---|---|---|
| Parthenium (fresh) | 71 | 224 | 8.96 | 71 | 696 | 27.84 |
| Paper mulberry (fresh) | 40 | 224 | 8.96 | 56 | 299 | 11.97 |
| Synedrella (fresh) | 40 | 209 | 8.36 | 46 | 408 | 16.33 |
| Sugarcane trash (dry) | 41 | 204 | 8.16 | 41 | 267 | 10.67 |

*µl $CH_4$/g/h or ml biogas/g/d at 22°C.

**Table 3.** Methanogenic rates measured at 22°C on biomass support (Expressed as ml biogas (@60% $CH_4$)/g fresh biomass (@11% TS)/d)

| Source of methanogenic activity and feed rates | Biogas production rate | | | |
|---|---|---|---|---|
| | Aceti-clastic (1) | Hydrogen-otrophic (2) | Residual (3) | Total (1+2) |
| *Feed rate 3g/l/d* | | | | |
| Effluent | 0.06 | 0.11 | 0.11 | 0.17 |
| Biomass | 4.2 | 7.56 | 0.14 | 11.76 |
| Feed rate 4.5 g/l/d | | | | |
| Effluent | 0.09 | 0.22 | 0.04 | 0.31 |
| Biomass | 7.15 | 13.54 | 0.95 | 20.69 |

For effluent read as ml/ml effluent/d, to obtain values as µl $CH_4$/g/h multiply by 25.

genic rates (Table 3, at 22°C) when compensated for 400 g/l bacterial support and a 35°C operating temperature would be much higher than the maximum gas production rates (6.4 l/d, Figure 1 *c*) obtained in this study. Even when gas production rates began to fall at high feed rates (7.74 g VS/l/d), only low levels (< 1 g/l) of unconverted VFA was detected in the effluent. These observations indicated that the bacterial support system in this study was limited by the availability of methanogenic substrates. High levels of unconverted VFA (> 1.5 g/l) were recorded only when effluent recycling rate was reduced.

It was envisaged that when these materials were used as bacterial support in DFFB biogas fermentors, the potential aceticlastic and hydrogenotrophic methanogenic activities would increase as a result of high substrate availability and concomitant bacterial growth. However, although we find an increase in methanogenic activity with increased feed rates (Table 3), the methanogenic activities (at 4.5 g/l/d) are in a similar range as measured for other feedstocks before being used in DFFB fermentor (Table 2). It may therefore be concluded that the aceticlastic methanogenic activity is likely to be saturated at about 225 µl/g/h (9 ml biogas/g/d, at 22°C and conditions employed). Further, although high hydrogenotrophic activities are possible (Tables 2 and 3), it may not be desirable to have high levels of hydrogen in the fermentor because it results in several perturbations.

### *Starvation and temperature shocks*

A 24, 48 or 96 h interruption in feedstock addition did not create serious imbalances in subsequent fermentor performance. The fermentor recorded pre-stressed levels of gas production within 72 h of resuming normal feed rates. A temperature shock (60°C for 4 h or 22°C for 1 d) gave a similar response as in the case of feed shocks (Figure 1 *a*). These two preliminary observations show that biomass support provides a significant level of protection against such shocks.

Conversion efficiencies at ambient temperatures (21 ± 1°C) were reduced to levels in the range of 60–70% of VS added. The lowered gas production was not accompanied by reduced pH or increased VFA in effluent (souring). This indicated that the cause was a lowered rate of acidogenesis and inadequate HRT rather than inadequate methanogenic activities. Gas production rates up to 3 l/l/d corresponding to a feed rate of *3.69 g VS/l/d were recorded. These rates are reasonably* high and indicate potential to operate such reactors at ambient temperatures and longer hydraulic retention time.

### *Conversion efficiencies*

During the initial phase, up to 93 d, very high conversion efficiencies (Figure 1 *a*, *c*) were recorded. These conversion efficiencies have been recorded at HRT values in the range of 8–16 h and compare well with other high rate fermentor operations reported[14]. These conversion efficiencies and rates have been possible only at a high level of effluent recycling. Alternative techniques to overcome/reduce the dependence on effluent recycling needs to be found in future studies.

## Conclusions

Anaerobically digested biomass is a simple support for methanogens. A wide variety of biomass feedstocks can act as methanogen support and these supports can be used in high rate down flow fixed bed biogas fermentors and have to be replenished at the rate of $3.6 \times 10^{-3}$ g/g support used/d. The technique described in this study has the potential to handle both solid (garbage or biomass) and liquid wastes (effluents) simultaneously.


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# MEETINGS/SYMPOSIA/SEMINARS

**International Conference on Scientific Computing and Modelling**

Date: 9–11 July 1998
Place: Calcutta

The topics include: High performance computing in science and industry; Computational fluid dynamics and applications; Operational research and industry/networking/web computing; Large scale nonlinear systems; Stiff system computation.

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**International Conference on Polymer Characterization (POLYCHAR-7)**

Date: 5–8 January 1999
Place: Denton, Texas

The theme includes: Predictive methods, Polymerization, Polymer liquid crystals, Mechanical properties and performance, Dielectric and electrical properties, Interfaces, Rheology and processing.

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