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SHORT COMMUNICATION

Effect of Fluctuating Temperature Regime on Psychrophilic Anaerobic Digestion of Nightsoil

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ABSTRACT

The effect of temperature fluctuation on anaerobic digestion of nightsoil using 10 °C adapted inoculum was studied. The digester was subjected to repeated temperature cycling of 10 °C and 30 °C. The total biogas, CH_4 and H_2S content,volatile fatty acids and microbial counts were compared with control digesters. No significant deleterious effect was noticed during initial temperature shock of one week. However, repeated exposures reduced the counts of hydrogenotrophic methanogens. There was no effect on the content of CH_4 and H_2S in the biogas.

1. INTRODUCTION

Human waste disposal in an innoccuous form in highly populated and developing countries, such as India is an evergrowing problem. The improper disposal of waste causes a serious threat of organic pollution to the environment and also several infectious diseases are bound to occur in epidemic proportions due to the contamination of drinking water resources[1]. The problem is more aggravated at low temperature and high altitude regions, such as Himalayan regions of India, where no proper human waste disposal method is in practice. Even, Antarctica which is known for its pristine environment, is not an exception in this regard. At present, human waste is either incinerated or physically transported out of the continent.

Although, aerobic degradation of organic waste is considered to be efficient, anaerobic digestion appears to be more suitable in view of the generation of biogas which can be used for maintenance of digester temperature (in addition to better hygiene) with least human intervention. Low temperature causes deleterious effect on anaerobic digestion because of relatively longer generation time of anaerobic bacterial populations and lower biochemical activity, resulting in the decrease of biogas yield and digester failure. Earlier studies[2-5] revealed that nightsoil could be digested at low temperature. The digestion of nightsoil at 10 °C was carried out using an adapted inoculum from cow-dung digesters[6].

The low temperature regions of tropical countries are bound to encounter fluctuation in diurnal and seasonal temperatures. Moreover, the low temperature adapted inoculum has the chance of exposure to ambient temperatures (15°–37 °C) during transportation to the installation site which may be deleterious to them. Effect of fluctuating temperature on anaerobic digestion has been studied[7-9]. However, detailed studies on nightsoil

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are not available. It has been recommended to maintain the digester temperature constant (within +1 °C) because temperature variation has deleterious effect on microbial population[10]. In view of the above, the present study was undertaken to assess the effect of temperature fluctuation (between 10 °C to 30 °C) on anaerobic nightsoil digestion and its effect on the amount and composition of biogas, microbial population and other parameters.

2. MATERIALS & METHODS

2.1 Inoculum

The adaptation of nightsoil slurry for inoculating the digester was carried out as reported earlier[6].

2.2 Digestion of Nightsoil

Nightsoil digestion was carried out in conical flasks (2 l) with a working volume of 1.8 l. The flasks were initially filled with adapted inoculum and the feeding was done in semicontinuous mode using 1:1 (w/v) diluted nightsoil slurry. The digesters were fed daily with a hydraulic retention time of 25 days. The control digesters in duplicate were maintained constantly at 10 °C and 30 °C in environmental chambers. The test digesters were subjected to the cyclic process of incubation at 10 °C and 30 °C with one week interval. Experiments were continued for eight weeks.

2.3 Analysis

Biogas production was recorded daily, whereas CH_4 content of biogas, p*H* of the slurry and amount of volatile fatty acids (VFA) were recorded weekly. Volatile fatty acids and CH_4 contents were quantified using Shimadzu gas chromatograph fitted with flame ionisation detector using free fatty acid phase (FFAP) column. H_2S was monitored using thermal conductivity detector and porapaq Q column.

2.4 Bacterial Counts

Sulphate reducing bacteria (SRB) as well as methanogenic bacteria were enumerated by most probable number (MPN) method as described by Pfenning[11], *et al.* and Balch[12], *et al.* Morphological characterisation of methanogens was carried out using fluorescence microscope.

3. RESULTS & DISCUSSION

Biogas production at 1 °C varied from 1000 ml to 1410 ml per week and increased approx. three to four folds at 30 °C. Maximum biogas production from nightsoil at 30 °C was attained during fourth week. At 10 °C, although peak of biogas production was not very pronounced, maximum gas was observed during seventh week of digestion. The digester subjected to temperature fluctuation produced maximum biogas during third week at 30 °C (7300 ml) as shown in Fig. 1. Hashimoto[13], *et al.* also noticed the peak of biogas production during fermentation of nightsoil when subjected to higher temperature for acclimatisation. During the first shift down of digester temperature, the gas

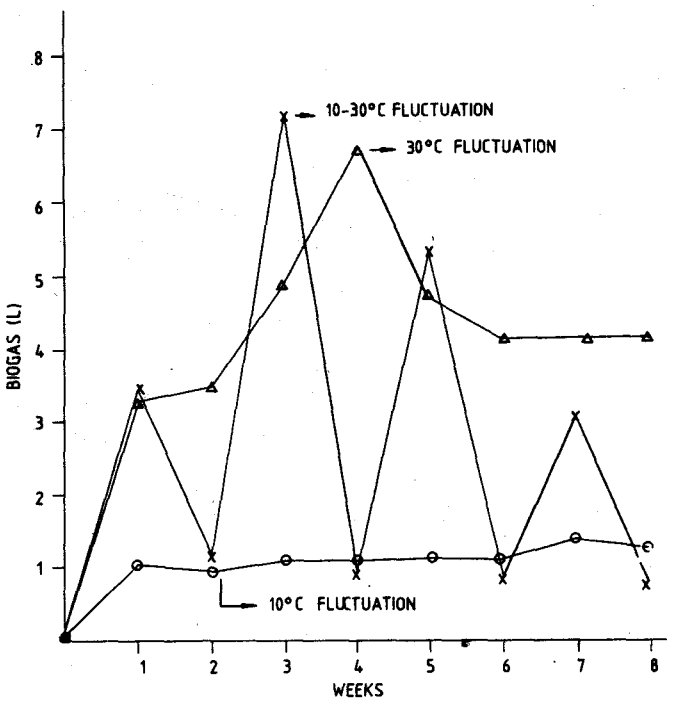

Figure 1. Effect of temperature on biogas production

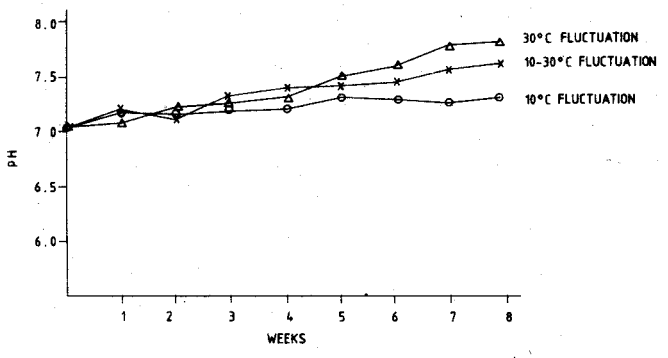

Figure 2. Effect of temperature on p*H* profile

production was same as the digester working at 10 °C. However, during subsequent weeks (after fifth week) the biogas production by digester was less than the control digesters maintained constantly at 10 °C and 30 °C. The results indicate that the efficiency of digester at 10 °C is not affected adversely during initial shocks of one week. However, subsequent temperature shocks gradually reduced the efficiency of consortium at low temperature.

Table 1 shows the CH_4 and H_2S content of digesting nightsoil. The CH_4 content was higher (62.0-68.4 per cent) and H_2S content was lower (10.0-12.0 per cent) at 10 °C in comparison to biogas produced at 30 °C, whereas the CH_4 and H_2S content from the digester temperature fluctuating between 10 °C and 30 °C was in between. The initial p*H* of the slurry was 7.06 which increased to 7.30 by the end of eighth week at 10 °C (Fig. 2). At 30 °C, the rise in p*H* was comparatively higher and slurry attained a p*H* of 7.84. The rise in p*H* was also observed in the digester subjected to temperature fluctuation. However, the values attained were in between the control digester values. Hashimoto[13], *et al.* also recorded the rise in p*H* with temperature. Nightsoil contains predominantly proteins and amino acids released due to protein hydrolysis which are further deaminated. The resulting NH_3 appears to be responsible for increse in p*H*[14] of the slurry.

The inoculum contained 3643 ppm VFA, of which acetic acid and propionic acid constituted 41 per cent and 52 per cent, respectively. At 10 °C, total VFA decreased till third week of digestion and increased afterwards, and attained the initial value by about seventh week. However, it was found that acetic acid accumulation was more (58 per cent) with concomitant decrease in propionic acid (19 per cent). At 30 °C, total VFA decreased as a function of time and by third week, it was reduced

Table 1. Effect of temperature on CH_4 and H_2S production

Duration (weeks)	CH_4 production (%)			H_2S production (%)		
	A	B	C	A	B	C
1	62.37 (± 3.2)	60.70 (± 2.8)	61.10 (± 3.0)	10.00 (± 0.80)	11.00 (± 0.80)	10.80 (± 0.84)
2	62.50 (± 3.4)	60.50 (± 2.8)	62.00 (± 3.2)	11.10 (± 0.82)	12.90 (± 0.85)	11.30 (± 0.83)
3	62.00 (± 3.4)	60.50 (± 2.9)	61.60 (± 3.1)	10.90 (± 0.82)	13.50 (± 0.85)	12.20 (± 0.84)
4	64.00 (± 2.6)	60.00 (± 2.8)	62.50 (± 3.2)	11.40 (± 0.82	14.30 (± 0.85)	12.40 (± 0.83)
5	66.00 (± 2.7)	59.30 (± 3.1)	62.40 (± 3.2)	12.10 (± 0.83)	14.80 (± 0.84)	13.60 (± 0.83)
6	68.20 (± 2.6)	58.20 (± 3.0)	60.30 (± 3.0)	12.00 (± 0.83)	14.50 (± 0.84)	13.90 (± 0.84)

A – Digestion of nightsoil at 10 °C, B – Digestion of nightsoil at 30 °C, and C – Digestion of nightsoil from 10 °C and 30 °C
Values are arithmatic mean ± standard deviation of five readings.

Table 2. Amount of total VFA produced during anaerobic degradation of nightsoil at different temperatures

Duration (weeks)	Production of VFA (ppm) at					
	10 °C		30 °C		10 °C & 30 °C	
1	(1892)	4056	(1014)	1400	(831)	1282
2	(1150)	1550	(730)	1129	(710)	1108
3	(1033)	1434	(545)	972	(430)	736
4	(1524)	2466	(550)	956	(825)	1050
5	(1846)	2980	(520)	824	(440)	623
6	(2023)	3075	(490)	836	(370)	779
7	(2360)	3780	(510)	902	(329)	783

* Values in parenthesis indicate acetate level (ppm).
Initial VFA–3643 ppm (1520)
Data indicate arithmatic mean of four values obtained from four independent experiments.

to one-fourth of initial value (Table 3). The presence of lesser amount of VFA at higher temperature is in contrast to the results reported earlier by Hashimoto[13], *et al.* They observed that increase in VFA level was proportional to rise in temperature. Similar trend was observed for the digester working at fluctuating temperature. Further, the change of digester temperature from 30 °C to 10 °C caused slight increase in total VFA during each cycle. Moreover, it was observed that total VFA level in the digester was relatively less than the other two digesters functioning at constant temperatures of 10 °C and 30 °C, respectively. It might be due to the deleterious effect of fluctuating temperature on acidogenic bacteria.

Examination of digester slurry for enumeration of methanogens and SRB at the end of the experiment revealed the highest counts of methanogens and the lowest counts of SRB at 10 °C. Hydrogenotrophic methanogens were the lowest in the digester fluctuating between 10 °C to 30 °C, while the acetate utilising methanogenic counts were present in least number at 30 °C. These results are in agreement with the CH_4 and H_2S content of biogas (Table 3). The data also corroborate that the repeated heating and cooling reduces the counts of hydrogenotrophic methanogens. The higher amounts of VFA at 10 °C can be attributed to the lower counts of SRB as they utilise VFA[15]. The observation under fluorescence microscope showed the dominance of *Sarcina* (50 per cent) followed by rod-shaped methanogens (40 per cent) in the slurry digested at 10 °C. In contrast, the digester slurry functioning at 30 °C had only 10 per cent Sarcina with rod-shaped bacteria amounting to 80 per cent. The slurry from fluctuating digester also had lower number of *Sarcina* and the prominent methanogens (85 per cent) were of *Cocci* type.

Table 3. Effect of temperature on methanogens and sulphate reducing bacteria

Temperature (°C)	Methanogens		Sulphate reducing bacteria
	H_2+CO_2	Acetate	
Initial	44×10^5	25×10^5	24×10^3
10	12×10^6	52×10^5	54×10^3
30	98×10^5	43×10^5	87×10^3
10 & 30	25×10^5	17×10^5	77×10^3

The study concludes that heat shock at 30 °C to low temperature adapted slurry showed hardly any negative effect during the first two cycles of fluctuation. However, repeated exposure of the digester to 30 °C elicits marginal deleterious effect on the anaerobic consortium. Further, the effect is mainly ascribed to the reducing number of acidogens and hydrogenotrophic methanogens.

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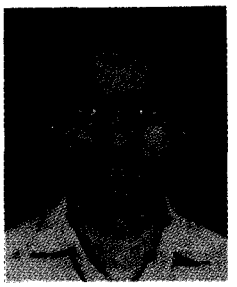

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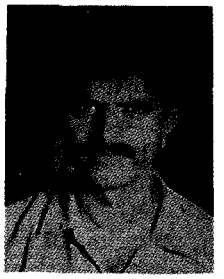

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