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Fatty Acids Profile during Anaerobic Digestion of Night Soil– Effect of Temperature, Calcium Carbonate and Selectively-enriched Inoculum

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ABSTRACT

Anaerobic biodegradation of night soil was carried out at 5-30 °C with 1.8-10.62 per cent volatile solids (VS). Biogas production increased with the temperature and VS up to 6.2 per cent. Further increase in VS caused higher volatile fatty acids (VFA) accumulation resulting in decreased gas production. Acetate and propionate accounted for 62-83 per cent of total VFA. Butyrate to isobutyrate ratio increased with VS. Calcium Carbonate promoted VS degradation, biogas production and VFA degradation. The increased methanogenic and decreased sulphate-reducing bacteria caused proportional changes in CH_4 and H_2S gases. Enrichment with H_2 oxidising methanogenic consortium is beneficial by enhancing VFA utilisation by two to three fold.

1. INTRODUCTION

Anaerobic digestion of solid organic wastes into biogas is of current interest as it reduces the volume of the waste in addition to the generation of valuable renewable energy in the form of methane. Civil, industrial and agricultural wastes have been successfully treated by anaerobic digestion. However, failure of several reactors has been due to low *p*H and volatile fatty acids (VFA) accumulation resulting in digester souring. Although these VFA are the source of substrate for methanogenic bacteria; at high concentrations they inhibit the methanogens[1,2]. This problem is further aggravated at low temperatures, which reduce the methanogenic activity[3].

Studies have been made earlier on anaerobic digestion with sewage sludge, cowdung, industrial and animal wastes at low temperatures[4,5]. However, investigations on night soil (NS) have been scarce. Night soil is degraded in nature by soil bacteria. At low temperatures, i.e. 5-20 °C, the activity of these bacteria is retarded resulting in the accumulation of these wastes. The problem in India is of special significance in areas, such as Leh and Siachen glacier where the army has been deployed due to strategic conditions. These areas are not only facing the problem of organic pollution but also have the risk of epidemics (diarrhoea, cholera, jaundice, etc.) arising due to the contamination of water with NS. The disposal of these wastes by incineration is not practicable as NS contains large amount of moisture. The degradation of NS by anaerobic digestion offers a good alternative.

The present investigation was undertaken to study the effect of temperature and volatile solid (VS) concentrations on the biogas production and VFA accumulation. An attempt is also made to determine the effect of $CaCO_3$ and selectively-enriched bacterial consortium on the degradation of the accumulated VFA.

2. MATERIALS & METHODS

2.1 Inoculum Preparation

Slurry was collected from local digesters running on

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cowdung and was gradually adapted to NS in 25 l digesters with semi-continuous feeding. The temperature was gradually decreased from 35 to 20 °C in six months. The inoculum was taken from the optimally running digesters.

2.2 Experimental Setup

All the experiments were carried out in serum vials of 120 ml capacity containing 70 ml of slurry. The NS was diluted and mixed with inoculum (1 : 1 ratio) to give 1.8, 3.27, 4.74, 6.2, 7.67 and 10.62 per cent VS concentration. The head space of the vials was flushed off with O_2-free N_2 gas. The vials were then sealed and incubated at 5, 10, 15, 20, and 30 °C without shaking, for 15 days. To study the effect of $CaCO_3$, sterile solution was added in increments to maintain the *p*H at 7.2. Anaerobic degassed sterile distilled water was added in control to maintain the slurry volume constant. The serum vials for each experiment were used in duplicate.

2.3 Enrichment of Acetogens & Methanogens

One millilitre of the slurry was collected from the biogas digesters running at 20 °C and inoculated into 20 ml of medium taken in 60 ml of serum vials[6]. For methanogens, the head space of the vials was filled with H_2 and CO_2 in 80:20 proportion and 100 g ml^{-1} vancomycin was added to the medium to prevent eubacterial growth. For acetogens, different VFA (propionic, butyric, isobutyric, isovaleric, and valeric) were added separately at 0.4 per cent (v/v) concentration to the Balch medium and the head space was filled with N_2 gas. After attaining good growth, subcultures were made once in every 20 days into the fresh medium[6]. After subculturing the acetogenic and methanogenic consortium five times, the medium was centrifuged at 8,000 rpm for 30 min and the cells collected were suspended in sterile anaerobic water (obtained by flushing with O_2-free N_2 gas) to give an optical-density (OD) of 0.8 at 520 nm (10^5 cells/ml). One millilitre of this culture was inoculated into the serum vials (along with the inoculum) to determine the effect of these cultures on VFA degradation. Chemicals were procured from Hi-media Laboratories Pvt Ltd and Sigma Chemical Co.

2.4 Estimations

Total gas production was measured by plunger displacement of an airtight syringe. Methane and hydrogen sulphide [gas chromatography (GC) with thermal conductivity detector (TCD), Porapak Q column] and VFA [(GC with flame ionisation detector (FID), FFAP column)] were determined by gas chromatograph. Volatile solids were estimated according to the standard method[7]. Analysis was done on two samples.

3. RESULTS

3.1 Effect of Temperature & VS Concentration on Biogas Production & VFA Accumulation

The results of experiments to find the effects of temperature and VS concentration on cumulative biogas production are shown in Table 1. The gas production at all VS concentrations increased with temperature. Also increase in VS concentration continuously increased the gas production at 5 °C; however, at 10-30 °C the gas production increased only up to 6.2 per cent. Further increase in VS concentration decreased the gas production. Analysis of variance indicates significant differences in biogas production at various temperatures and volatile solid concentrations.

Table 1. Effect of temperature and volatile solids on biogas production

Temperatu (°C)	Biogas production, ml (%)						
Volatile solids	1.8	3.27	4.74	6.2	7.67	10.6	LSD*
5	10	16	38	30	47	59	2
10	48	155	190	181	170	78	4
15	71	187	212	235	195	114	6
20	162	260	371	388	286	205	11
30	275	442	496	520	394	207	3
LSD*	4	19	11	21	16	7	

*Least significant difference

The *p*H of the digested slurry ranged from 7.4 (at low VS) to 6.4 (at high VS).

Figure 1 shows the effect of temperature on the accumulation of VFAs at different VS concentrations. Acetate and propionate accounted for 62-83 per cent of the total VFA accumulated. In general, the VFA concentration increased directly with VS concentration and inversely with temperature. Interestingly, as VS concentration increased, per cent isobutyrate decreased with a concurrent increase in per cent butyrate. However, the concentration of isovalerate (per cent) remained more or less the same although the valerate (per cent) content increased with substrate concentration.

3.2 Effect of $CaCO_3$ on Biogas Production & VFA Degradation

The VS degradation ranged from 12.5 to 34.1 per cent in control and was higher in diluted slurries. Calcium Carbonate promoted VS degradation at all temperatures. Increase in temperature caused increase in biodegradation (Table 2). The biogas production at different times in slurry with 6.2 per cent VS is shown in Table 3. There was about one and half to three times increase in biogas production with $CaCO_3$ as compared to control. At 20-30 °C the maximum biogas was detected around the fourth day and later biogas production declined up to 14th day. At 10 °C the biogas production did not show any definite trend and was

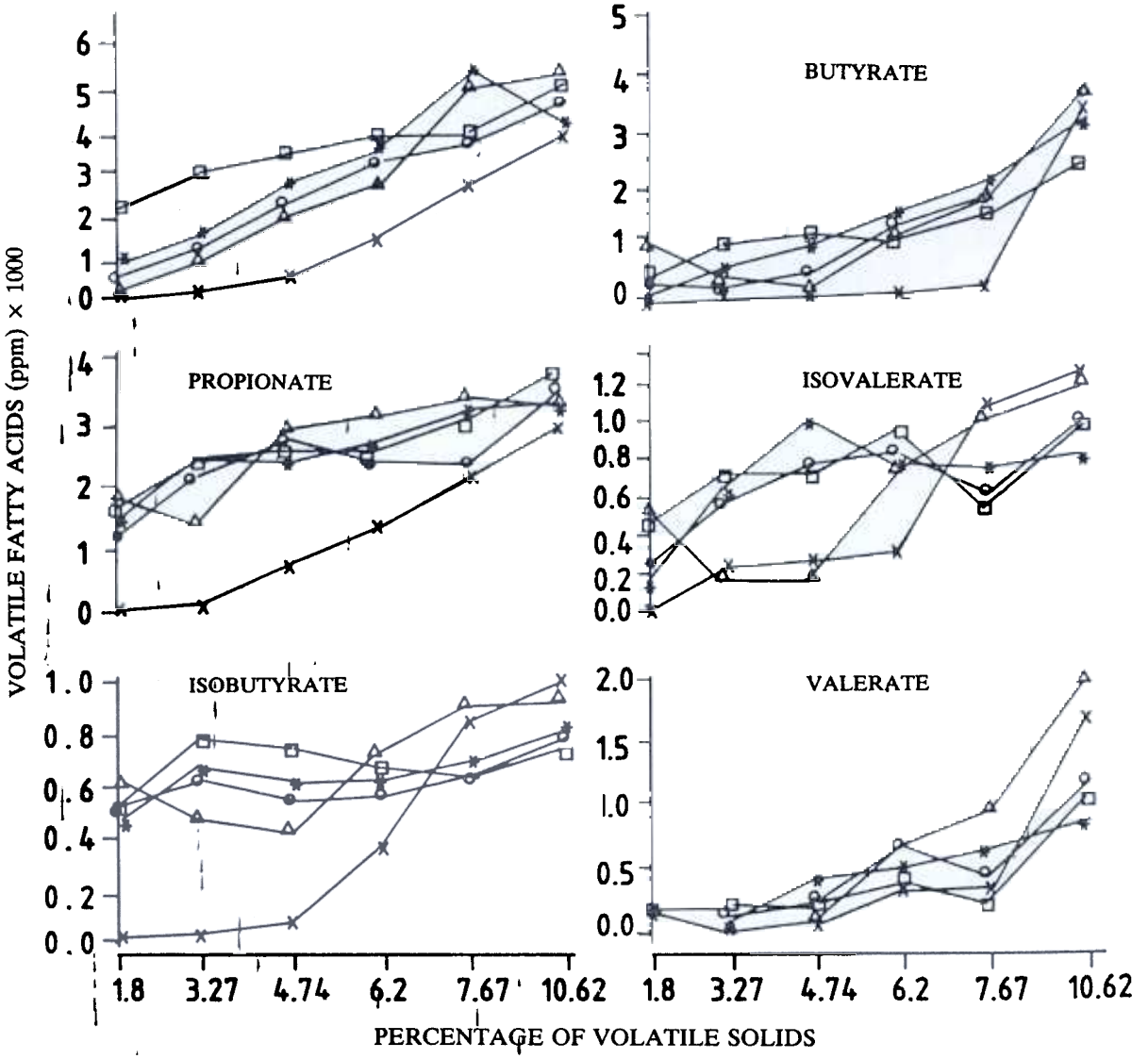

Figure 1. Effect of temperature on the VFA profile at different VS concentrations. Symbols : □, 5 °C; ∗, 10 °C; O, 15 °C; △, 20 °C; ×, 30 °C.

Table 2. Effect of $CaCO_3$ on VS degradation at different temperatures (Incubation time: 13 days)

Temperature (°C)	Per cent VS	Per cent degraded (dry wt)	
		Control (LSD)	$CaCO_3$ (LSD)
10	1.8	21.4 ± 1.6	33.9 ± 2.4
	6.2	16.8 ± 0.8	25.8 ± 1.8
	10.6	12.5 ± 1.3	20.6 ± 0.4
20	1.8	31.6 ± 2.3	48.1 ± 4.3
	6.2	24.3 ± 0.6	35.4 ± 2.8
	10.6	19.2 ± 1.1	27.5 ± 0.9
30	1.8	34.1 ± 3.2	50.0 ± 0.8
	6.2	25.3 ± 0.7	37.0 ± 0.6
	10.6	20.8 ± 1.2	29.3 ± 0.2

Table 3. Biogas production in the presence and absence of $CaCO_3$ at 6.2 per cent VS

Days	10 °C		20 °C		30 °C	
	Control	$CaCO_3$	Control	$CaCO_3$	Control	$CaCO_3$
2	13.0	10.0	36.8	40.0	40.6	74.0
4	11.5	15.7	52.0	68.7	70.0	105.6
6	7.9	14.4	36.5	42.2	42.2	80.9
8	11.9	23.2	25.1	38.1	26.9	71.3
10	11.0	14.3	18.8	31.7	15.5	78.2
12	9.4	29.6	9.5	31.5	8.4	56.2
14	5.6	15.4	4.4	25.3	4.0	52.1
Total	70.3	122.6	183.1	277.6	207.6	518.3
SD	±6.8	±7.3	±14.1	±7.8	±11.7	±16.3

produced almost at the same rate. The *p*H of the slurry after digestion varied from 6.8 to 7.2 in the presence of $CaCO_3$ and 6.4 to 6.6 in control.

The H_2S content decreased from 23 to 14 per cent at 10 °C, 27 to 18 per cent at 20 °C and 14 to 10 per cent at 30 °C in the presence of $CaCO_3$. The sulphate reducing bacterial (SRB) count reduced from 1.4×10^5 to 0.9×10^5 in the presence of $CaCO_3$ at 20 °C. Similar response was observed at other temperatures, whereas, the amount and per cent of CH_4 content increased with

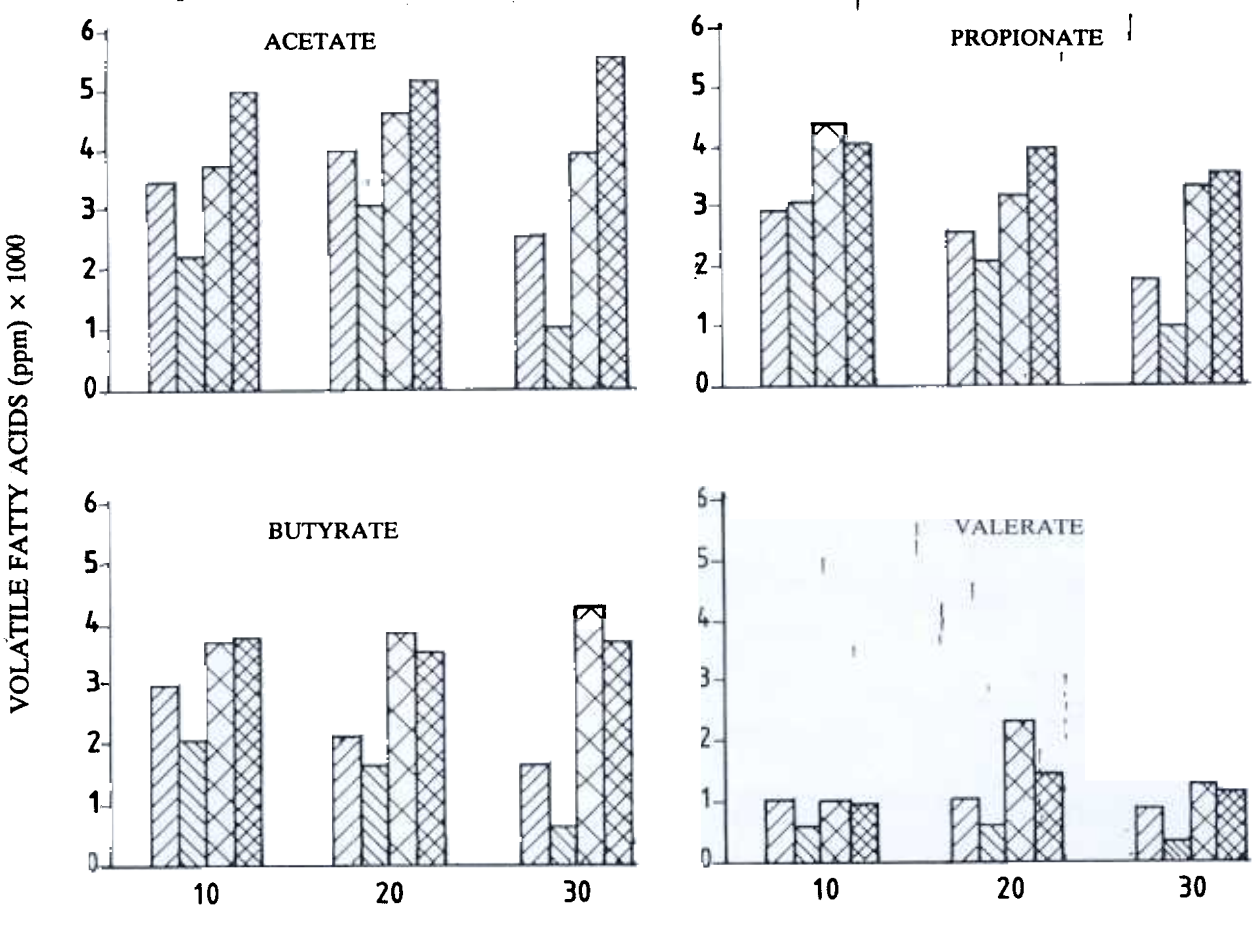

Figure 2. Effect of $CaCO_3$ on the degradation of VFA at different temperatures. Symbols (VS): ▨, 6.2 % Control; ▧, 6.2 % $CaCO_3$; ▩, 10.6 % Control; ■, 10.6 % $CaCO_3$.

temperature and $CaCO_3$ had a beneficial effect. It increased the methane content from 50 to 55 per cent at 10 °C, 52 to 60 per cent at 20 °C and 55 to 63 per cent at 30 °C. The methanogenic count enhanced from 1.4×10^5 to 2.2×10^5 (cells/ml) in the presence of $CaCO_3$ at 20 °C.

The effect of $CaCO_3$ on the accumulation of VFA at different temperatures with different VS concentrations is shown in Fig. 2. There is no detectable difference in levels of VFA in both control and in the presence of $CaCO_3$ at 1.8 per cent VS. However, $CaCO_3$ showed a mixed response at 6.2 per cent VS. It stimulated the utilisation of acetate, propionate, and butyrate at all temperatures, but it is more pronounced at 30 °C. At higher VS concentrations, there is no appreciable effect. Calcium Carbonate promoted valerate utilisation at all temperatures irrespective of substrate concentration (i.e both 6.2 and 10.62 per cent VS). However, there is no appreciable utilisation of isobutyrate irrespective of temperature and VS concentration (data not shown).

3.3 Effect of Selectively-Enriched Inoculum on VFA Degradation

The effect of selectively-enriched acetogenic (obligate proton reducing bacteria) and H_2 oxidising methanogenic consortium on VFA degradation is shown in Fig. 3. Addition of only acetogens along with inoculum has no effect on VFA utilisation, and their concentrations remained more or less the same as in control (only inoculum added). However, there is a considerable decrease (2-3 times) in VFA levels in the presence of H_2 oxidising methanogenic consortium with an increase in methane content from 53 to 59 per cent.

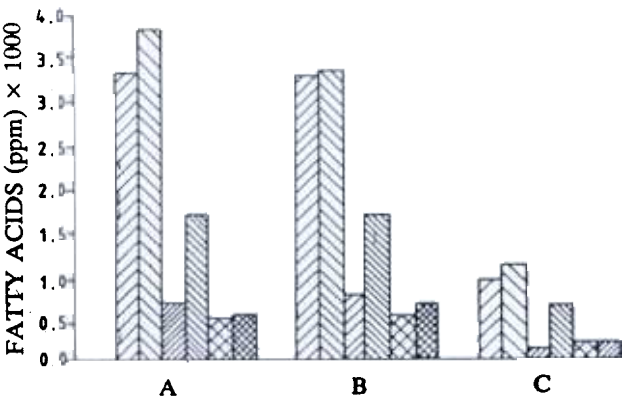

Figure 3. Effect of selective acetogenic and methanogenic consortium on VFA degradation. Symbols : ▨, acetate; ▧, propionate; ■, isobutyrate; ■, butyrate; ▩, isovalerate; ▩, valerate. A: only inoculum ; B: Inoculum + acetogens ; C: Inoculum + methanogens.

4. DISCUSSION

The increased production of biogas with higher VS at higher temperatures is due to the increased hydrolysis of biopolymers and high methanogenic activity (Table 1). This is supported by higher per cent VS degradation, lower amounts of VFA and increased methane content. The decrease in gas production at higher than 6.2 per cent VS concentration is due to the accumulation of high amounts of VFA. Earlier, Kennedy and Vandenberg[4] reported that increase in loading caused accumulation of VFA with concurrent fall in methane content in anaerobic fixed film reactors. The higher amounts of VFA at lower temperatures (<20 °C), fall in agreement with the earlier report by Zeeman, *et al*[5] and may be due to the decrease in the methanogenic activity which is rate-limiting at lower temperatures[3,5]. The toxic effect of VFA appears to be due to the fall in *p*H which ranged from 7.4 to 6.3. Further, several investigators[8,9] have observed the propionate inhibition of methanogens under unbalanced anaerobic conditions accompanied by an increased H_2 pressure. Recently, Barredo and Evison[10] have reported that 20 mm propionate inhibited methanogenic activity appreciably which is more pronounced at higher *p*H, i.e. 8. In this study, there is comparatively higher propionate accumulation which might have resulted in decreased methane content. The toxic effects of other fatty acids on anaerobic digestion are not well documented.

The initial peak of biogas production on fourth day at 20-30 °C may be due to the degradation of fatty acids which are already present in NS. The increased gas production in the presence of $CaCO_3$ is due to the increased degradation of VS (Table 2). The higher *p*H (6.8-7.2) of digested slurry in the presence of $CaCO_3$ compared to control (6.4-6.6) shows that $CaCO_3$ maintains the *p*H of fermenting slurry in desirable range for biodegradation. Earlier Torre and Stephanopoulos[11] have also reported that maintaining *p*H by *NaOH* or bicarbonate had a beneficial effect on biogas production. The decrease in H_2S content in the presence of $CaCO_3$ was due to a decrease in sulphate-reducing bacteria count from 1.4×10^5 to 0.9×10^5 cells/ml. The

increase in methane content was due to the increase in methanogens from 1.4×10^5 to 2.2×10^5 cells/ml. Probably $CaCO_3$ by inhibiting sulphate-reducing bacteria promoted methanogens allowing more availability of substrate, i.e. acetate. However, the reasons for inhibition of sulphate-reducing bacteria by $CaCO_3$ are not understood.

The lesser amounts of VFA at 30 °C may be due to the increased methanogenic activity which removes H_2, thereby favouring the utilisation of the higher fatty acids by the obligate proton-reducing bacteria (Fig. 2). This is confirmed by increased VFA degradation (2 to 3 fold) by enrichment with H_2 oxidising methanogens (Fig. 3). Earlier reports revealed enhanced growth of *Syntrophomonas wolfei* and *Syntrophobacter wolini* (which are the major fatty acids degrading bacteria in the digesters) in the presence of H_2 oxidising sulphidogen/methanogen[12,13]. This is due to the removal of H_2 by sulphidogens/methanogens thereby allowing the conversion of higher chain fatty acids. From the present studies it is concluded that low temperature caused increased VFA accumulation which is more pronounced at higher VS concentrations. Buffering with suitable chemicals will be helpful for anaerobic digestion of NS at low temperatures. Further retention of methanogens by immobilisation or cell recycle may have a great potential in future for better performance of the digester.

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