From the results it is clear that 49·9% of the total proteins of the blood has been represented as haemocyanin in the active snails and the remaining percentage as proteins other than haemocyanin (low molecular weight proteins[19]). The low molecular weight proteins decreased as the haemocyanin content decreased upon starvation, showing an inverse relationship. Such inverse relationship between haemocyanin and proteins other than haemocyanin has been demonstrated in the blood of *Maia* and *Carcinus*[15 16]. The fact that decrease in the low molecular weight proteins is associated with an increase in the concentration of haemocyanin in the blood of the aestivated snails suggests the utilization of these proteins in the synthesis of haemocyanin. However, the experimental evidence for the synthesis of haemocyanin in *Pila globosa* is awaited. It may, therefore, be concluded that the hepatopancreas in the snail, *Pila globosa*, perhaps, serves as a storage organ for copper and also acts as a copper donor, at times of need, for the synthesis of haemocyanin.

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ANAEROBIC FERMENTATION OF PLANT MATERIALS INTO ACIDS AND BIOGAS

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ABSTRACT

Plant materials, *viz.*, berseem, hybrid napier, oat, dhub grass, paddy straw with and without 2·5% nitrogen as ammonium sulphate were anaerobically fermented at 5% solids concentration at 35° C for 20 days. The hybrid napier and berseem yielded the highest plant acid recovery of 30·5% and 32·0% of their dry matter when the plant materials were digested for 10 or 15 days, filtered, charged with water again and digested upto 20 days. However, with other plantt materials, the plant acid recovery ranged from 21·5% to 31·8%. The pH changes during digestion were also observed. The biogas yield from berseem plant acids, in the methanogenic phase digester, ranged from 0·46 to 0·78 litre/g plant acids added as a single charging whereas with triple charging a maximum biogas yield of 0·85 litre/g plant acid was obtained. The biogas comprised of 58% methane, 28% carbon dioxide and 11% of other constituents like carbon monoxide, oxygen, nitrogen, hydrogen, etc.

INTRODUCTION

UTILIZATION of agricultural and animal wastes as an energy source has received wide attention recently. Wolf and Keenan[9] suggested a two stage digestion of dog food. Batty[4] and Sarma[8] emphasized that energy crops could be converted into methane through anaerobic fermentation with reference to conversion of solar energy into fuel gas. Hence, in the present study, the possibility of anaerobically digesting in a first stage digester, plant residues like berseem, oat, hybrid napier, dhub grass and paddy straw with and without nitrogen supplementation, to yield maximum volatile fatty acids was attempted, and the plant acids so produced were fed into a second stage methanogenic fermenter for the production of biogas.

Materials and Methods

The plant materials, *viz.*, berseem, hybrid napier, oat, dhub grass and paddy straw were obtained from N.D.R.I., Karnal. The digested cattle dung slurry and digested rumen fluid were obtained from the gobar gas plant and fistulated buffalo from the experimental farm respectively. The plant materials were analysed for dry matter, ash content, organic matter, carbon and nitrogen (Piper[7]).

A slurry containing 5% solids was prepared by mixing finely chopped plant residues, *viz.*, berseem, oat, hybrid napier, dhub grass, paddy straw and paddy straw with 2·5% nitrogen as ammonium sulphate. The samples were charged with 2·5 litres water in Haffkin's fermentation flasks and 1% starter inoculum of rumen fluid containing rumen bacteria and cattle manure digested slurry containing anaerobic bacteria was added to each flask. The flasks were stoppered with one pored rubber bunks fitted with bent capillary tube, other end which dipped in water in a beaker to prevent entry of air into the flask (Manocha[5]). The flasks were incubated at 35° C and the contents of the flasks were shaken daily. In one series of experiments, the flasks were incubated for 20 days and in other series, the plant extract was filtered on the 5th day, 10th day and 15th day of digestion and flasks were charged again with the same volume of water to obtain 5% solids and incubated again up to 20 days. In the four series of experiments, the total acids obtained comprised of 5 + 15 days, 10 + 10 days, 15 + 5 days and 20 days fermented plant extract. The above samples were analysed for the pH using an Elico LI–10 pH meter, and volatile fatty acid by steam distillation (Barnett and Reid[3]). Composite samples were kept in deep-freezer until the second methanogenic digestion was carried out.

The second-methanogenic digester was started in one litre flask, with digester cattle dung slurry, filtered through muslin cloth into which the berseem plant acids were charged at the rate of 1·0, 1·2, 1·5, 2·0, 2·25 and 2·5 g/litre and the biogas yield was noted for a period of ten days during digestion at 35° C. The biogas formed displaced water from the adjacent one litre flask into a measuring cylinder (Manocha[5]). The effect of three split charging of 1·2 g berseem plant acids @ 0·4 g on 0 hr., 3rd day and 6th day on the total biogas yield was also studied. The biogas samples were analysed for their constituents, *viz.*, CH_4, CO_2, CO, H_2, O_2, N_2, etc. (A.P.H.A.[2]).

Results and Discussion

(*a*) *Composition of Plant Materials*

The chemical composition of plant materials, *viz.*, berseem, hybrid napier, oat, dhub grass and paddy straw is given in Table I. The dry matter content of the above plant materials was 19·0, 16·4, 27·0, 49·6 and 96·0% respectively. The organic matter content (dry matter basis) ranged from 81·6 to

Table I

Chemical composition of plant materials

Sl. No.	Plant material	Moisture content	Organic matter (Dry matter basis)	Carbon	Nitrogen	C : N ratio
1	Berseem	81·0	89·2	51·74	3·192	16·2 : 1
2	Hybrid napier	83 6	89·4	51·86	2·618	19·8 : 1
3	Oat	73·0	89·4	51·86	1·302	39·8 : 1
4	Dhub grass	50·4	87·0	50·46	1·211	41·7 : 1
5	Paddy straw	4·0	81·6	47·33	0·433	109·3 : 1

89·4%. C : N ratio of berseem and hybrid napier was 16·2 : 1 and 19·8 : 1, whereas that of oat and dhub grass was 39·8 : 1 and 41·7 : 1 respectively. Paddy straw had a wide carbon nitrogen ratio of 109·3 : 1.

(*b*) *Plant Acids Fermentation*

Figure 1 presents the concentration of volatile fatty acids formed at 5 days interval upto 20 days of digestion of the plant material. It was observed that hybrid napier and berseem plant acids had higher concentration of volatile fatty acids as compared to oat, dhub grass, paddy straw and paddy straw with nitrogen during 20 days digestion.

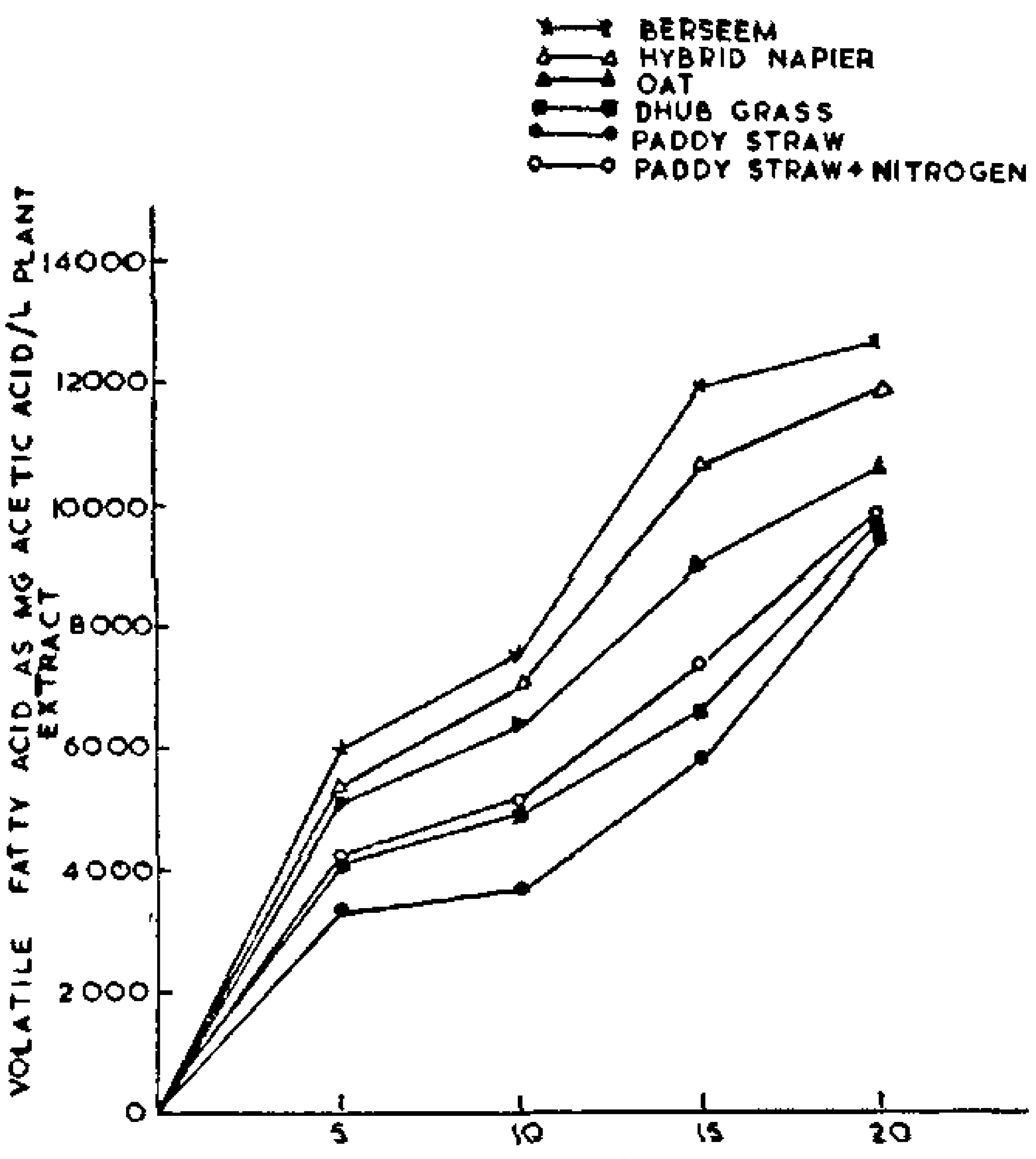

FIG 1 VOLATILE FATTY ACID CONCENTRATION DURING ANAEROBIC DIGESTION OF PLANT MATERIALS

The berseem plant acids concentration was 5,940 and 12,720 mg as acetic acid per litre on the 5th and 20th day of digestion respectively, whereas hybrid napier plant acids concentration was 5,340 mg and 11,820 mg as acetic acid per litre on the 5th and 20th day of digestion respectively. Addition of nitrogen to paddy straw increased the plant acid concentration during anaerbic digestion.

It was observed that the rate of volatile fatty acid formation from plant residues decreased after 5th to 10th days digestion. This might be due to low pH which might develop in the plant extract during digestion or might be due to end product inhibition.

Figure 2 shows the changes in pH of the digesting slurry during 20 days digestion. The original buffering capacity as N lactic acid/100 gm dry matter of plant materials is given as follows : Oat—48·1 ml ; Hybrid Napier—36·6 ml ; Berseem—57·9 ml ; Dhub grass—64·5 ml ; Paddy straw—44 ml.

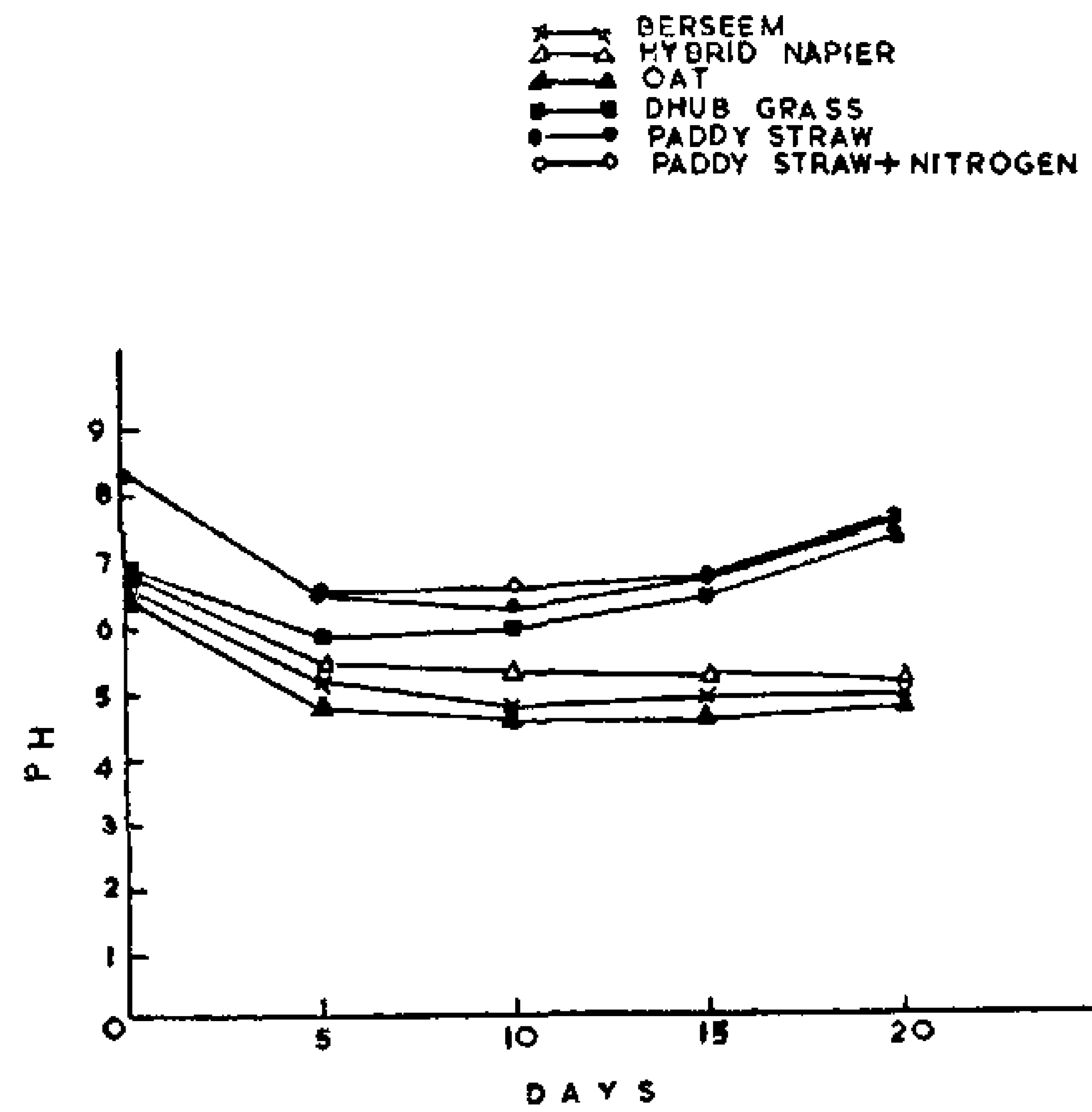

FIG 2 PH CHANGES DURING ANAEROBIC DIGESTION OF PLANT MATERIALS

The initial pH of paddy straw was 8·1 which decreased to 6·3 to 6·5 during 10th and 15th day of digestion and increased to 7·3 on the 20th day. The initial pH of dhub grass was 6·5 which decreased to 5·8 from 5th to 10th day and increased to 7·3 on the 20th day. The initial pH of other plant materials like oat, hybrid napier and berseem ranged from 6·2 to 6·5 which decreased to 5·2 to 5·3 on the 5th day and further decreased to 4·4 to 4·8 upto 20th day.

It was observed that the pH of the plant extract decreased at a faster rate on the 5th and 10th day of digestion due to higher rate of plant acids formation. The resultant pH changes are due to interaction of the buffering capacity of plant tissues and the type of the acids formed during different periods of digestion. Sarma[8] reported that the alfalfa plant acids were found to be primarily made of C_2, C_3, i-C_4, C_4, i-C_5 and C_5 acids and an unidentified relatively long chain fatty acid.

Table II presents the effect of dilutions at different charging time on the rate of plant acids (mg) formed from 1 g dry matter of plant material and the extent of dry matter loss,

TABLE II

Production of volatile fatty acids and dry matter loss during anaerobic digestion of plant materials

Sl. No.	Plant material	VFA as mg/g dry matter				% dry matter loss				Ratio of VFA/dry matter loss			
		charging time in days				charging time in days				charging time in days			
		5+15	10+10	15+5	20	5+15	10+10	15+5	20	5+15	10+10	15+5	20
1.	Berseem	256·8	319·2	320·4	254·4	73·7	76·3	77·4	72·6	0·347	0·418	0·414	0·350
2.	Hybrid napier	235·2	261·6	304·8	236·4	49·4	53·6	59·8	38·4	0·476	0·489	0·509	0·616
3.	Oat	256·8	273·6	318·0	213·6	53·3	55·6	55·6	53·7	0·482	0·492	0·572	0·398
4.	Dhub grass	133·2	247·2	242·4	195·6	47·2	52·4	53·2	46·8	0·282	0·472	0·456	0·418
5.	Paddy straw	187·2	204·0	214·8	171·6	23·4	34·0	37·0	30·0	0·800	0·600	0·581	0·572
6.	Paddy straw + nitrogen	202·8	204·0	234·0	198·0	23·0	40·5	41·0	44·0	0·882	0·504	0·572	0·450

It was observed that when the plant materials were digested for 15 + 5 days double charging fermentation, maximum plant acid recovery was obtained ranging from 21·48 to 32·04% in the various plant materials, whereas, with 20 days continuous digestion the plant acid recovery was 17·16 to 25·44% only.

In the case of berseem, the volatile fatty acid formation during 15 + 5 days digestion from 1 g dry matter was 320·4 mg with 77·4% dry matter loss. The highest plant acid recovery from 1 g dry matter with respect to other plant materials, *viz.*, hybrid napier, oat, dhub grass, paddy straw and paddy straw *plus* nitrogen was found to be 304·8, 318·0, 242·4, 214·8 and 234·0 mg as acetic acid. The corresponding dry matter loss to obtain the above quantity of plant acid recovery was found to be 59·8, 55·6, 53·2, 37·0 and 41·0%. The plant acid recovery per g dry matter loss with 15 + 5 days digestion in respect of berseem, worked out to be 0·414 g whereas with other plant materials, it ranged from 0·456 to 0·581 g.

Even though, the C : N ratio of oats and dhub grass was closely similar and ranged between 39·8 and 41·7 : 1, the plant acid recovery was more with oats than dhub grass. The berseem and hybrid napier had a C : N ratio of 16·2 : 1 and 19·8 : 1 and the plant acid recovery ranged from 320·4 mg and 304·8 mg/g dry matter. It was found that, the plant acid recovery from oats with 15 + 5 days fermentation was 318·0 mg/g dry matter and was found to be more than that of hybrid napier plant acid recovery, although the oat material had a wider C : N ratio of 39·8 : 1. The plant acid recovery with paddy straw with a C : N ratio of 109·3 : 1 was 214·8 mg/g dry matter, whereas with the addition of 2·5% nitrogen, the C : N ratio narrowed down to 16·13 : 1 and the plant acid recovery increased to 234·0 mg/g dry matter. The berseem plant material and the nitrogen supplemented paddy straw had a similar C : N ratio, but the plant acid recovery with berseem plant was much higher than the latter. During anaerobic digestion, the plant acid recovery may depend on the percentage composition of the organic fraction of the plant biomass and its biodegradability, even though nitrogen factor appears to be partly limiting in some cases. However, the supply of available nitrogen is relatively insignificant in anaerobic digestion with paddy straw (Acharya[1]).

It was observed that the low pH and resultant fatty acid concentration might interfere in the plant acid production. Hence, the dilution effect due to subsequent chargings of water after filtration on 5th, 10th and 15th days has resulted in higher recovery of plant acids. This might be due to the inhibitory action that has been neutralised by subsequent charging, which otherwise would have interfered with bacterial action on substrate during conversion into plant acids.

(c) Biogas Production from Plant Acids

Table III presents the biogas yield from berseem plant acids during 10 days methanogenic digestion at 35° C. The total biogas yield with plant acid charging at the rate of 1·0, 1·2, 1·5, 2·0, 2·25 and

TABLE III

The yield of biogas (litres) with single and triple charging rates of berseem plant acids (g as acetic acid)

Sl. No. Observation	Single charging						Triple charging
	1·0	1·2	1·5	2·0	2·25	2·5	1·2
1. Biogas yield (l)	0·680	0·936	1·120	1·119	1·214	1·146	1·025
2. Biogas (l)/g plant acid	0·680	0·780	0·746	0·559	0·544	0·458	0·854

2·5 g/litre cattle manure slurry was 0·680, 0·936, 1·120, 1·119, 1·214 and 1·146 litres. The biogas yield per g plant acid worked out to be 0·680, 0·780, 0·746, 0·559, 0·544 and 0·458 respectively for the above charging rates. The berseem plant acid charging at the rate of 1·2 g yielded the highest biogas recovery. When 1·2 g plant acid was charged in three instalments at the rate of 0·4 g on the 0 hr, 3rd day and 6th day of methanogenic digestion at 35° C, the biogas yield during 10 days digestion was 1·025 litres. Hence, with triple charging of 1·2 g of same plant acid the biogas yield increased from 0·780 to 0·854 litres as compared to single charging. The biogas samples comprised of 58·0% methane, 28·0% carbon dioxide, 1·0% carbon monoxide, 6·0% oxygen, and 7·0% of other gases like hydrogen and nitrogen. Sarma[8] obtained a biogas yield of 0·85 to 1·07 litres/g of lucerne plant acids added which comprised of 50 to 52% methane.

With the highest berseem plant acid recovery during 15 + 5 days digestion and a biogas yield of 0·854 litre/g acid, the biogas that could be obtained/kg dry matter of berseem plant worked out to be 9·66 cu.ft. Assuming the above biogas yield from other plant acids also, the biogas yield that could be obtained from hybrid napier, oat, dhub grass, paddy straw and paddy straw *plus* 2·5% nitrogen worked out to be 9·19, 9·59, 7·46, 6·48 and 8·06 cu.ft./kg dry matter of the plant materials respectively. Sarma[8] obtained a biogas recovery of 10·45 cu.ft./kg dry matter of alfalfa hay in two stage digestion with NaOH addition in the first stage digester for higher plant acid recovery. Pfeffer and Khan[6] reported that heat and caustic pre-treatment could yield 20% increase in biogas production from fermentation of organic municipal refuse. It is concluded that crop residues could be efficiently converted into methane by two stage anaerobic bacterial fermentation in the context of conversion of solar energy into fuel gas.

TABLE IV

Chemical composition of biogas

Sl. No.	Constituents	Per cent
1.	CH_4	58·0
2.	CO_2	28·0
3.	CO	1·0
4.	O_2	6·0
5.	N_2, H_2 etc.	7·0

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