STUDIES ON ACIDOGENIC PHASE DURING ANAEROBIC DIGESTION OF CATTLE DUNG WITH SUPPLEMENTATION

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1991

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GRANDPARENTS

STUDIES ON ACIDOGENIC PHASE DURING ANAEROBIC DIGESTION OF CATTLE DUNG WITH SUPPLEMENTATION

BY

RAJESH KUMAR

A thesis submitted to the National Dairy Research Institute (Deemed University), Karnal in partial fulfilment of the requirement for the degree of

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IN

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This is to certify that the dissertation entitled, **"STUDIES ON ACIDOGENIC PHASE DURING ANAEROBIC DIGESTION OF CATTLE DUNG WITH SUPPLEMENTATION"**, submitted by **Mr. RAJESH KUMAR** in partial fulfilment of the requirements for the award of the degree of **MASTER OF SCIENCE IN DAIRYING** in the discipline of **DAIRY MICROBIOLOGY** of the **NATIONAL DAIRY RESEARCH INSTITUTE (DEEMED UNIVERSITY), KARNAL (HARYANA), INDIA**, is a bonafide research work carried out by him under my supervision and guidance, and no part of the dissertation has been submitted for any other degree or diploma.

19/6/91

(KISHAN SINGH)
Major Advisor & Chairman (Guide)

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CHAPTER - 1

INTRODUCTION

1. INTRODUCTION

Several alternative fuel sources are gaining attention due to the present worldwide shortage of energy. Among these, one is anaerobic digestion in which methane gas is produced from variety of waste materials. It was a traditional method of sewage treatment but nowadays it has been applied for methane production from manures, garbage, food and industrial wastes, and crop residues. Due to successful application of anaerobic digestion in waste treatment, the system has gained recognition from food and fermentation industry as an alternative to purely conventional aerobic method. The benefits of this method are the production of energy and reduction in the level of population. Moreover, in contrast to high temperature and pressure required for thermochemical methanation, anaerobic methane production is carried out at near ambient temperature and pressure. This is very much relevant to a developing country like India where 60 % of foreign exchange earning is utilized for the import of commercial fuel sources.

During anaerobic digestion of organic matter, methane is produced as combustible component of biogas, mainly from a few terminal fermentation products such as organic acids, CO_2 and H_2. About 90-95 % of organic matter can be decomposed to biogas and rest is converted to bacterial cells.

Decomposition of organic materials involves activities of many anaerobic bacterial species. The process can be divided into different phases, which are: hydrolysis, acidification, hydrogenesis and methane formation. All phases are as a result of different interrelated species

of bacteria and the bacteria involved have been classified in five groups, viz., hydrolytic, fermentative, acetogenic, hydrogen producing and methanogenic. First three groups collectively lead to formation of organic acids and they constitute the acidogenic phase of anaerobic digestion. These bacteria can grow in presence of small amount of oxygen and create favourable conditions for next groups, which are obligate anaerobes and make use of organic acids by converting them into gaseous end products, i.e., CH_4 and CO_2 with minor quantities of nitrogen, hydrogen, CO and H_2S.

Since whole process of anaerobic digestion consists of different interdependent phases, it has become essential to have a thorough knowledge of every phase. This may help in overcoming many problems arising in way of maximising bioenergy production. Since organic acids are the main intermediates in biomethanation, the acidogenic phase of the process needs a considerable attention. The activity of bacteria in acidogenic phase is higher than methanogenic phase which leads to excessive acid production and impairing the methane production step. Study of acidogenic phase may help in coming across some of the important process variables that can regulate the whole process smoothly and help in maximising methane yield.

Cattle dung has been used as the main substrate for biogas production. But it has been found that gas producing potential is very less in cattle dung as compared to other agricultural and industrial wastes. This is because dung contains less biodegradable solids as compared to other wastes which are rich in such solids and hence lead to production of more gas. Many organic wastes like agricultural residues, garbage, forestry, and wastes from human and industrial sources have been tried as supplements to cattle dung in order to

enhance biomethane yield. In dairy industry, dairy effluent constitute major part of waste. This waste is rich in milk carbohydrates, lipids, proteins and inorganic compounds used in dairy plant. The effluent of dairy plant is usually not properly utilized and its disposal is still a problem. If, such wastes rich in biodegradable solids are used for biomethane recovery via anaerobic digestion, it will be beneficial for fuel recovery and pollution control.

In the present study, dairy effluent sludge has been used as a supplement to cattle dung for biogas recovery. Besides the microbiology of acidogenic phase anaerobic digestion, the extent of volatile fatty production, the biogas yield and the methane content have been studied. Main objectives of the study are:

1) To analyse different substrate slurries for various chemical and microbiological parameters.

2) To digest substrates anaerobically.

3) To analyse all the digested slurries for chemical and microbiological parameters at different time intervals.

CHAPTER - 2

REVIEW OF LITERATURE

2. REVIEW OF LITERATURE

2.1 HISTORICAL BACKGROUND

The fact that organic matter decomposing under the conditions where it is out of contact with air, will produce an inflammable gas, has been known for centuries, especially the marsh gas phenomenon. Occasional dancing flames of this gas (ignited perhaps by stray spark from nearby fire) visible at midnight have given rise to the legends of "Will-o-Wisp" or "fool's fire". Marsh gas was first identified by an Italian scientist Alessandro Volta in 1776. But chemical nature of this gas was not known. In 1806, William Henry found that Volta's gas was identical with main ingredient of synthetic illuminating gas, presently called as methane. Presence of methane in farmyard manure was noticed by Humphrey Davey in early 1800's. Bechamp (1868) showed that methane production was perhaps due to the microbial activity on simple carbon compounds. Tappenier in 1982-84 provided adequate proofs of microbial origin of methane (Sathianathan, 1975). After that a keen interest was taken on anaerobic digestion of organic wastes for methane production. Gas from 'skillfully designed' septic tank was used for street-lighting in Exeter (England) in 1895 (McCabe and Eckenfelder, 1957). After the first world war, several devices were developed and used for generating methane, a highly combustible gas.

The very first anaerobic digester of the world was setup in India as early as in 1900 (Sathianathan, 1977). But the main experimentation started in 1937 at Dadar, Bombay, where the gas produced was used to drive a lorry for disposing garbage (Sathianathan, 1977). Desai

(1939) conducted studies on anaerobic digestion of cattle dung and also got the credit of building the first anaerobic digester. The digester consisted two vessels, one having digesting dung and other holding fuel gas. A pipe through a gas cock allowed the gas to pass from digester to gas holder. During late 1940's, Kotwal and Borkar revealed that when urine was added to cattle dung, it fermented rapidly and resulted in more gas production per unit substrate.

2.2 SUBSTRATES FOR ANAEROBIC DIGESTION

2.2.1 CATTLE DUNG

Digestion of cattle dung slurries at 35°C was found to reach maximum at 15-20 or more days retention time (Zeeman et al., 1983; Summers et al., 1987). Efficient degradation of organic constituents was recorded in the slurries with total solid levels upto 13.5 % (Zeeman et al., 1983). During early stages of digestion, acid production was maximum with acetate being major constituent which was considered as key intermediate in anaerobic digestion (Singh et al., 1985). It was reported that the production of acids was controlled by rate of cellulose hydrolysis (Singh and Jain, 1986). Acid production led to decrease in pH of dung slurry from 7.5 to 6.75 within a day (Jain et al., 1983). Acid concentration was found to increase upon predigestion of cattle dung for 1 to 5 days, resulting in lowering of pH (Singh et al., 1983). Various levels of biogas production were reported under mesophetic conditions. The yields were 84.11 l from 500 g cattle dung (Bansal et al., 1977), 7.72-7.74 ft^3/kg wet dung (Neelakantan, 1987), 0.419 m^3/kg vs (Xavier and Nand, 1989) and 134.9 m^3 from 366 kg volatile solids (Vyas and Gupta, 1990). Considerable difference in gas yield (60.1 m^3 gas from 366 kgvs) under psychrotrophic conditions was reported (Vyas and Gupta, 1990).

Wohlt et al. (1990) reported 131-161 ml/g vs methane production under thermophilic conditions at 60°C. In comparison to the excreta of other animals, cattle dung was found to be a poor source of methane (Bansal et al., 1977; Hills, 1984; Neelakantan and Singh, 1991). Dung cakes have been reported to be a better substrate for anaerobic digestion (Chen et al., 1988).

2.2.2 OTHER SUBSTRATES

Screened dairy manure when subjected to anaerobic digestion (Lo et al., 1984a), maximum biodegradation was found at 3.6 days retention time which resulted in 27.6 % reduction in volatile solids at 30°C. It was reported that the higher biodegradation and gas production occurred using this waste in comparison to unscreened one (Lo et al., 1984b). Successful digestion of dairy manure was also carried out at 12°C (Lo and Liao, 1986), 20°C (Zeeman et al., 1983) and 14°C (Safley and Westerman, 1990). Biogas from cattle manure was found to be affected by temperature (Chayovan et al., 1988). Sheep and goat wastes were reported to produce more biogas than cow dung (Bansal et al., 1977; Jain et al., 1981; Neelakantan, 1987).

Poultry manure was successfully digested at 3.8, 6.0 and 8.1 % TS (Aubart and Fauchille, 1983) and at high NH_3-N concentration upto 7.5 g/l (Pechan et al., 1987). It has been found to be the best source of biogas in comparison to other animal excreta (Bansal et al., 1977; Hills, 1984; Neelakantan, 1987; Safley, 1989; Safley and Westerman, 1990).

Pig manure was subjected to mesophilic and thermophilic digestion, the optimum temperatures for digestion were found to be 25-30°C and 40-44°C, respectively (Hashimato, 1983). Critical solid

concentrations for methanogenesis were reported to be 3.5 % (Feilden, 1981). Startup time for pig manure was found between 40-60 days at 20-25°C (Zeeman et al., 1988). Effect of temperature was minimal at loading rate of 33.5 to 61.8 kg vs/m^3 and same was that of antibiotics as well as disinfectants at levels of their normal use (Hashimoto, 1984). Conway (1991) reported that methane production in animal gut was due to breakdown of organic matter by anaerobic bacteria.

Agricultural residues like various crop byproducts (Clausen et al., 1979), rice crop residue (Kalra and Panwar, 1986) and tomato solid wastes (Hill and Nicano, 1984; Koster, 1984) have been used as substrates. Plants such as Eupherbia tirucalli (Rajasekaran et al., 1989), aquatic weeds (Nipaney and Panholzer, 1987; Abassi et al., 1990) were tried. Spent straws from mushroom cultivation were found to yield more gas than that from untreated one (Bisaria et al., 1990).

Cheese factory wastes and whey were used as substrates for anaerobic digestion and their pH was found to decrease at shorter hydraulic retention times (Schindler, 1986). Three distinct phases with lactate, acetate and H_2/CO_2 as major intermediates were identified (Chartrain, 1986). Organic matter loading rate was found as an important factor in whey methanation (Lo and Liao, 1989). In case of dairy plant waste; waters, upto 60.2 % TS reduction during the digestion was recorded (Hills and Kayhanian, 1985).

Using wine distillery wastes, decrease in 40 % propionate and 11 % butyrate degradation were found when micro organisms were not alive. Rate of propionate degradation was found to be the rate limiting step (Segretian and Meletta, 1987).

In addition, other industrial wastes like fruit and vegetable wastes (Mahadevasami and Venkatraman, 1990), chocolate and biscuit

industry wastes (Ranade et al., 1989), textile industry wastes (Balasubramaniya et al., 1986) and paper mill wastes (Gizzen et al., 1990) were also used for methanation in anaerobic digester. It was reported that when the market wastes were digested at 5 % TS levels for 20 days which produced 30 l/kg TS/day gas (Ranade et al., 1987). Domestic wastes (Camp et al., 1987), municipal wastes (Brummeler and Koster, 1990; Chen et al., 1990), fructose (Duback et al., 1989), cellulose (Reig et al., 1989) and hydroquinone (Szewzyik and Schink, 1989) were reported as good substrates for anaerobic digestion.

It was reported that the biogas yield was higher (392 l/kg DM) in poultry litter followed by water-hyacinth (310 l/kg DM) and vegetable waste (273 l/kg DM), whereas cow dung and berseem hay produced the biogas least (113.55 and 113.6 l/kg DM, respectively) during the digestion of one month (Neelakantan and Singh, 1991).

2.2.3 SUPPLEMENTATION

Different combinations of the wastes have been tried in order to enhance the yield of methane. Cattle dung was supplemented with sheep waste (Jain et al., 1981), poultry waste, larval litter (Rajasekaran et al., 1986; Mallick et al., 1990). It was found that all the combinations increased biogas production in comparison to cattle dung alone. Combinations like cow dung with poultry litter (Rajasekaran et al., 1986) and a combination of cow dung, poultry litter, sugarcane bagasse (Mallick et al., 1990) were found to give the best results. Agricultural residues have been supplemented to the cattle dung such as rice straw, the mixture at 7.1, 10.2 and 14.8 % TS levels gave little

variation in CH_4 yield (Pathak et al., 1984). On mixing castor cake, maize cobs, mixed vegetable wastes and weeds, 75.4 % TS reduction was was reported (Lingaiah and Rajasekaran, 1986). Cow dung was also supplemented with Euphorbia tirucalli at 1:1 level (Rajasekaran et al., 1989), fresh and partially decomposed Ageratum (Kalia and Kanwar, 1990). A combination of partially decomposed Ageratum and cattle dung (3:2) resulted in 8 % increase in biogas production while digester failed to produce gas when fresh Ageratum was added (Kalia and Kanwar, 1990). Water-hyacinth when mixed at 3:7 level to cattle dung produced 0.64 l CH_4/l/day after 7-9 days at 7-9 % TS (Madamwar et al., 1990) while combination of water-hyacinth, algae, cattle dung and untreated rice husk in 1:1:1:0:9 ratio was found to be a good source of methane (Ghosh and Das, 1982). Supplementation of dung with Parthenium (Gunaseelan, 1987) and algal mixture of Oscillatoria, Chalybea, Euglena, Scenedemus, Spirulina and Merismopedia (Venkatraman and Kaushik, 1978) resulted in increase of biogas production. It was reported that the algal supplementation to goat dung increased the biogas production by 14.95 %, but no synergistic effect was observed (Neelakantan, 1987). About 15-30 % increase in biogas was reported when the cattle dung was mixed with vermiculite, charcoal and lignite and by 10-20 % with pebbles, glass marbles and plastic mesh (Geeta et al., 1986). Kumar et al. (1987) reported the stimulation in biogas production on charcoal addition to cattle dung at the rate of 5 % on dry matter basis. In addition to these, rock phosphate (Singh et al., 1980) and pressed mud (Singh et al., 1981) were supplemented to cattle dung. Pulp and paper mill effluent mixing with cattle dung resulted in 5.5-6.5 l/day gas production (Gupta and Awasthi, 1990).

Dairy cattle manure was supplemented with cellulose (Robbins et al., 1983) and crop residues (Hills, 1981). In latter case, C/N ratio of 25-32 was found to be the most favourable for biogas production. Industrial wastes such as winery waste (Lo and Liao, 1986) and cheese whey (Lo and Liao, 1989) were anaerobically digested in combination with cattle manure and resulted in an increase in biodegradation. Madamwar (1990) found that when cheese whey was combined with poultry waste, an improved digestion and higher methane production occurred.

Wong (1990) found that a ratio of 2:1 of pig manure and sewage sludge gave good volume of CH_4 and high reduction in organic load. Pig manure in combination with rabbit waste resulted in production of 215 l CH_4/kg VS (Aubart and Bully, 1984). Different combinations of pig manure with microcrystalline cellulose, hemicellulose, glucose and acetate were anaerobically digested (Ferrara et al., 1984) and it was found that combination with glucose gave maximum biogas production due to high rate of hydrolysis. Supplementation of swine manure with residues of wood pyrolysis (pyrolignitic acids) resulted in decrease in efficiency of anaerobic digestion (Andreoni et al., 1990).

2.3 STAGES OF ANAEROBIC DIGESTION

N.A.S. (1977) considered anaerobic digestion of complex polymeric organic fibers, to be a three-stage process. The stages were as polymeric breakdown, acid production and methane production. In first stage, carbohydrates, lipids and proteins were found to be hydrolysed to simpler compounds. Cellulolytic activity was found to be the most critical as the largest fraction of organic matter in most of the wastes was cellulose. Synergistic action of hydrolytic bacterial species was reported. Breakdown products of first phase become substrates for acid

producing bacteria, which constituted the stage of acid production. Acetic, propionic and lactic acids were found to be the major products. Acetic acid was observed as the single most important substrate for methane formation. In third-phase acetic acid, methanol, H_2 and CO_2 were reported to be converted into methane by methanogenic bacteria.

Calzadu et al. (1984) carried out anaerobic digestion of coffee pulp-juice in two phases. In first phase, acidogenesis occurred and was operated with 0.56 g VS/l/day. Methanogenesis occurred in second phase which was operated with 0.6 to 2.4 g VS/l/day. The system was reported to be better for biodegradation of coffee pulp juice. Bhadra et al. (1986) studied anaerobic digestion of native cellulosic wastes. Acidogenic phase was separated from methanogenic in two-stage system, which improved cellulose hydrolysis and process efficiency. Gupta and Awasthi (1990) also reported two-stages during anaerobic digestion of cow dung, pulp and paper mill effluent mixture. The stages were (I) acid formation and (II) methane production. The pH optimal for bacterial activity were 5.2 to 6.8 and 6.5 to 8.0 during the first and the second stages, respectively. Three stages during anaerobic digestion of dairy effluent namely, hydrolysis, fermentation and methanogenesis were reported (IDF, 1990).

2.4 EFFECT OF ENVIRONMENTAL FACTORS ON ANAEROBIC DIGESTION

2.4.1 TEMPERATURE

Venkatraman and Kaushik (1978) incorporated sundried algal mixture in cow dung at the rate of 3 %. Gas production at 30-31°C after 10 day digestion was found to be 1.64 times higher than that of the cow dung alone. The gas production was reduced to one half at 20-22°C and to one fourth at 16-18°C. Feiden (1981) reported maximum net energy production during anaerobic digestion at 25-30°C and 40-44°C.

Hashimato et al. (1981) studied gas production in temperature range of 30-65°C and found average gas production of 0.328 l/g VS fed. At 65°C, very less CH_4 was produced (0.118 l/g VS) as compared to that at 60°C (0.308 l/g VS). Varel et al. (1980) also reported similar results. Zeeman et al. (1983) investigated optimum temperature and retention time for mesophilic anaerobic digestion of dairy cow slurry. Digestion was performed at 30, 35 and 40°C for 10-30 days. Changing of temperature from 30 to 35°C had no significant effect.

Chen (1983) and Hashimato (1983) reported more energy production at 55°C than at 35°C. Bhadra et al. (1986) reported biogas productivity in single stage digestion ranging from 0.5-1.5 m^3/m^3/day at mesophilic (20-40°C) and 1.0-2.5 m^3/m^3/day at thermophilic temperature (40-60°C). Neelakantan (1987) reported 1.74, 5.74 and 7.72 ft^3 biogas per kg dry dung at winter, summer and 30°C, respectively. Nipaney and Panholzer (1987) studied the influence of temperature on biogas production from Pistia stratiotes in lab-scale batch digester. Gas yield ranged between 533, 707 l/kg VS and 2128 l/kg fresh weight of P. stratiotes after 30 day digestion at temperatures of 29.5, 33.0 and 37.5°C, respectively. It was reported that fluctuation in gas production depended upon pertubations in temperature of digester (Chayovan et al., 1988). Gollakota and Meher (1988) studied the effect of temperature on biogas production from Caster Cake. Experiments were carried out in five litre digester at 30°C and 37°C protected from light. Both rate and yield were higher at 37°C than at 30°C. Pain et al. (1988) carried out anaerobic digestion of cow dung slurry at 25 and 40°C. It was reported that gas values were approximately 30 % greater at 45°C than at 25°C. Zeeman et al. (1988) evaluated the procedures for fermentation of either pig or dairy cattle manure at low temperature of 5-20°C. Below 20°C, no methane formation initiated

without inoculum. Anaerobic digestion of caged layer manure was studied by Safley and Westerman (1990) at 14-23°C. Acceptable CH_4 yields were obtained at loading rates ranging between 0.15-0.57 kg VS/m^3/day.

2.4.2 pH

Acetate and fatty acids produced during anaerobic digestion tend to lower the pH of digester (Zeikus, 1977; Lo et al., 1986; Svendsen and Blackburn, 1986). Decrease of pH from 7.50 to 6.15 and 7.50 to 6.50 during the anaerobic digestion of cattle dung and cattle dung supplemented with pressed mud, respectively (Singh et al., 1981). Predigestion of organic matter for 1-5 days, decreased pH from 7.4 to 6.6 (Singh et al., 1983). It was reported that the pH decreased from 7.5 to 6.5 in one day (Jain et al., 1983) and 8.1 to 7.1 in 20 days (Balasubramaniya et al., 1986). Decrease of pH was found despite addition of rock phosphate as buffering agent (Singh et al., 1980).

Most of the microorganisms grow best under natural conditions, since other pH values may adversely affect mechanism by altering the chemical equilibrium of the enzymatic reaction or by actually destroying the enzymes. The methanogenic group of microorganisms is more sensitive to pH (Zeikus, 1980). The optimum pH of methanogenic group was reported to be 7.0 to 7.5 and activity had been found to decrease significantly at pH below 6.0 or above 8.0 (IDF, 1990). The optimum pH of acid forming bacteria has been found to be lower than that of methanogenic bacteria as the pH during the acidogenic phase was 3.2-3.8 (Calzadu et al., 1984), 5.2-6.8 (Gupta and Awasthi, 1990) and 5.5-6.5 (IDF, 1990).

The optimum range of pH for methane production has been reported between 7.0-7.2 although satisfactory gas production occurred between the pH range of 6.6-7.6 (NAS, 1977), whereas others reported the optimum

pH for gas production as 6.9-7.2 (Lo et al., 1984), 6.7-7.5 (Lingaiah and Rajasekaran, 1986), 7.0 (Gupta and Awasthi, 1990) and 7.2 ± 0.1 (Robbins et al., 1983). Acid conditions of pH 6.2 were reported toxic to methanogenic bacteria (NAS, 1977). No gas production has been found at pH 6.0 (Gupta and Awasthi, 1990) but growth of acidogenic bacteria was reported to continue with further drop in pH upto 4.5-5.0.

2.4.3 REDOX POTENTIAL (E_h)

Lo et al. (1986) found efficient mesophilic anaerobic digestion of screened manure at redox potential between -0.25 to -0.30 V. Svendsen and Blackburn (1986) studied the sequential phases in anaerobic digestion. They reported that during early stages E_h decreased and efficient CH_4 production occurred in E_h range between -163 to -220 mV. Kobayashi et al. (1989) studied anaerobic digestion of organic matter of dairy cattle manure. Redox potentials between -200 and -300 mV were found to favour better anaerobic digestion.

2.4.4 NUTRIENTS (CARBON AND NITROGEN)

Hills and Robberts (1981) carried out anaerobic digestion of dairy cattle manure with 2.14 % N and 40.9 % C. It was found that biogas production increased with an increase in C/N ratio. Singh et al. (1981) found reduction in carbon from 46.08 to 44.72 % in cattle waste and 14.38 to 40.95 % in mixture of cattle waste and pressed mud. It was reported that increase in nitrogen from 1.89 to 1.93 % and 1.81 to 2.07 % occurred in cattle waste and cattle waste - pressed mud mixture, respectively. But there was negligible difference of nitrogen before and after digestion of screened and unscreened manure (Lo et al., 1984). In another study, it was reported that nitrogen decreased from 1.71 to 0.20 % after 40 days digestion of the residue of textile processing

factory (Balasubramanya et al., 1986). Wong (1990) treated pig manure and sewage sludge, anaerobically at 37°C at 2:1, 1:1 and 1:2 ratio and found that the degradation of carbon was in order of 51.44 to 28.90, 54.64 to 28.26 and 66.76 to 28.58 %, respectively. The N/P ratio of 6.7 was reported optimum for anaerobic digestion (IDF, 1990).

2.4.5 DRY MATTER AND VOLATILE SOLIDS

Summers and Bousfield (1976) studied practical aspects of anaerobic digestion and reported 8.6, 32, 30 and 36 % reduction in total solids (TS) after 3, 5, 7 and 10 days, respectively. When poultry waste was subjected to anaerobic digestion at 3.8, 6.0 and 8.1 % TS levels, maximum biogas was produced at 3.8 % TS levels (Aubart and Fauchille, 1983). At higher digester loading (11.6 g TS/1 digester/day), the reduction in total solids and volatile solids (VS) was 30 and 30-40 %, respectively, when effluent was not recycled. At high rate of recycling (upto 100 %), about 30 % decrease in destruction of TS was reported (Chang et al., 1983). Robbins et al. (1983) carried out anaerobic digestion of cellulose dairy cattle manure mixture having 0-59 % VS at 37°C and found 40 % VS was reported to be the optimum for digestion of mixture. Predigestion of cattle dung was tried in batch and semi-continuous systems for 1-5 days. After 5 days, 12-21 and 8.31 % decrease in TS, while 11.14 and 9.93 % decrease in VS in the respective system was reported (Singh et al., 1983). Screened manure and water slurry, at 4.0 % TS and 2.8 % VS subjected to anaerobic digestion and found 27.6 % reduction in VS after 3.6 days retention time (Lo et al., 1984). Pathak et al. (1984) studied the effect of solid concentration on biogas production and noticed nearly the same amounts of gas produced per gram when TS of slurry were adjusted at 7.7, 10.2 and 14.8 %. Anaerobic digestion at different TS levels ranging from 2.25 to 18.0 % was studied

and it was found that the efficient digestion could be carried out upto 13.5 % TS. It was also reported that the gas production increased with increase in TS from 9.0 to 13.5 %. Reduction in TS (27.8 %) and VS (29.7 %) was found in slurry (4 % TS) after 20 days of anaerobic digestion (Singh et al., 1984). Degradation of TS to the extent of 46.3, 51.4 and 60.2 % was found when treated flushed dairy waste was subjected to anaerobic digestion (Hills and Kayhanian, 1985). About 45 % decrease in VS after 20 days were reported when willow dust treated with NaOH was subjected to anaerobic digestion, in batch digester (Balasubramaniya et al., 1986). It was observed that in biodegradation of cow dung and organic wastes mixed with oil cakes, there was reduction of 75.4 % TS and 71.8 % VS (Lingaiah and Rajasekaran, 1986). It was reported that there was variation in gas production with influent concentrations of 1, 2, 4, 6, 7 and 10 % TS and maximum biogas was produced at 4-6 % TS (Webb and Hawkes, 1985). Methanogenic fermentation of poultry manure at average concentration of TS between 11.3 and 14.1 % and 7.8-9.7 % VS under high NH_3-N concentration was studied. At these concentrations of solids, adverse effect of NH_3-N was observed (Pechan et al., 1987). The excreta from dairy and fattening cattle was digested in stirred tank anaerobic digester adjusting to 5-7 % TS in slurry, there was 32 % degradation after 20 days (Summers et al., 1987). When slurry having 13.4 % VS and 10.8 %, was digested anaerobically, it produced 0.10 m^3 biogas/kg VS in November and 0.20 m^3/kg VS in August months (Balsari, 1988). There was 60 % VS conversion rate in acidogenic phase of anaerobic fermentation, when organic refuse was used (Champ et al., 1989). Solid wastes from biscuit and chocolate industry were subjected to anaerobic digestion and 65 % degradation of VS was observed after 40 days (Ranade et al., 1989). There was reduction of 13-27 % TS and 23-44 % VS during anaerobic

digestion of cow dung and pulp and paper mill effluent at 30°C for 10-12 weeks (Gupta and Awasthi, 1990). A significant decrease in CH_4 production was noted when cattle dung slurry at 10 % VS levels was subjected to thermophilic digestion and reactor was reported having failed at VS concentration of 12 or 14 % (Wohlt et al., 1990).

2.4.6 C/N RATIO

The most suitable C/N ratio for anaerobic digestion was 30.0 as reported by NAS (1977). Singh et al. (1980) observed the effect of rock phosphate on biogas production from cattle waste and reported maximum reduction in C/N ratio in cow dung supplemented with rock phosphate at the rate of 0.5 %, while minimum reduction was found when supplementation was carried out at 1.0 % level and maximum gas was reported in earlier case. It was observed that the sheep waste having C/N ratio of 15.22 resulted in more cellulose degradation and gas production as compared to cow dung at 35.1 ratio (Jain et al., 1981). Ghosh and Das (1982) carried out at the anaerobic digestion of the mixture of cattle dung, rice husk, water-hyacinth and algae and it was reported that the maximum gas production occurred at C/N ratio of 29.5. It was also reported that maximum methane yield was observed at the C/N ratio in range of 25-32 (Hills and Robberts, 1981). Robbins et al. (1983) reported C/N ratio of 32.1 as optimum for anaerobic digestion of cellulose-dairy manure mixture. Lingaiah and Rajasekaran (1986) also found C/N ratio of 25-30 as optimum for biodegradation of cow dung and organic wastes along with oil cakes in anaerobic digester. Abassi et al. (1990) studied biogas potential of eight aquatic weeds. C/N ratio was different for all the weeds and no correlation of this parameter with biogas production was found.

2.4.7 VOLATILE FATTY ACIDS

Long chain fatty acids appeared at earlier stage were replaced by short chain fatty acids (C_8-C_{20}) after 8 days of anaerobic digestion. These acids disappeared after 20 days being hydrolysed to short chain groups of C_2-C_6 when farm wastes were subjected to anaerobic digestion (Hawkes et al., 1976). Singh et al. (1980) studied the effect of rock phosphate addition on biogas production from cattle waste. They found the amount of acids little above 3200 ppm after second week and after that reduction in amounts was recorded. Fischer et al. (1981) found acetate to be 93 % of total volatile fatty acids (TVFA) during anaerobic digestion of swine manure. These workers also reported that build up of propionate upset the digester. Volatile fatty acids (VFA) during anaerobic digestion of cattle and sheep wastes were found to be 390 and 430 mg/l, respectively. High build up of VFA, though lower than that in sheep waste was recorded when cattle waste was supplemented with sheep waste at 5 and 10 % rates (Jain et al., 1981). Singh et al. (1981) found maximum VFA build up in third week (6630 ppm) which was considerably decreased after eight weeks of anaerobic digestion of cattle dung. Propionate and butyrate accumulation was high. When pressed mud was incorporated to cattle dung, maximum TVFA was produced (5865 ppm) after second week, lower than that in case of cattle dung. Accumulation of propionate and butyrate was not significant.

It was noticed that during the anaerobic digestion of cellulose cattle manure mixture, 10 mM acetate was produced during first day which reached maximum upto 13 mM and then declined to 3 mM. Propionate was never found to be more than 3.0 mM (Robbins et al., 1983). TVFA of cattle waste slurry was found to increase from 194 to 246 mg/l after five day predigestion (Singh et al., 1983). Calzadu et al. (1984)

reported 90 % increase in acetic acid during acidogenic phase of anaerobic digestion of coffee pulp juice, while 39.8 % reduction in VFA was recorded in methanogenic phase. Cox (1983) found maximum (4360 mg/1) VFA during anaerobic digestion of sterile sewage sludge. In another study VFA was found to be below 0.5 kg m^{-3} in mesophilic digester (Hashimato, 1984). There was no VFA accumulation during the digestion of pig manure upto 25 days (Callander and Barford, 1984). Fischer et al. (1984) found that the VFA reached 525 mg/ml after one hour during anaerobic digestion of swine manure. A study on VFA in stored cattle slurry revealed that concentration in VFA was in order to acetic propionic butyric isovaleric isobutyric acid. The concentration of acids was found low at greater depth (Patni and Jui, 1985). The VFA was found never exceeding 150 mg/1 throughout anaerobic digestion of tomato processing wastes for 10-12 weeks (Sarda and Krishna, 1989). Acidogenic phase of anaerobic fermentation of domestic wastes was studied (Camp et al., 1989) and it was reported that specific rate of VFA production was 7.5 mM/g VS.

Dairy manure was digested anaerobically and the VFA was found in the range from 92.8 to 130.6 mg/1. Acetic acid was 92.5-94.37 % of TVFA (Sohal et al., 1990). It was noted that the level of acetic acid increased to the maximum, which caused imbalance in the activity of reactor due to higher acidogenesis (Goodwin et al., 1990). Summers and Bousfield (1976) found that 41.6, 81.0, 82.0 and 92.6 % reduction in VFA occurred after 3, 5, 7 and 10 days of anaerobic digestion. It was observed that 95 % VFA reduction occurred after three weeks of anaerobic digestion of piggery wastes (Poels et al., 1984). Lingaiah and Rajasekaran (1986) reported 55,85 to 79.03 % reduction in VFA during anaerobic biodegradation of cattle dung and organic wastes mixed with castor cake.

VFA like formic, acetic, propionic, butyric, valeric and caproic were the substrates for methanogenic bacteria (Taylor, 1975). In another study, it was found that when microbes were not active, propionic and butyric acid addition resulted in decrease of degradation rates of these acids to 40 and 11 %, respectively (Segretian and Moletta, 1987).

2.5 METHANE CONTENT

Summers and Bousfield (1976) reported presence of 70 % CH_4 in biogas. Singh et al. (1980) found that 62-66 % CH_4 is biogas produced from cattle waste and cattle waste-rock phosphate mixture. It was noted that 60-72 % CH_4 was present in biogas produced from cattle and sheep waste (Jain et al., 1981). Aubart and Fauchuille (1983) reported that 68.9 % CH_4 was present in gas produced from poultry manure digested at 37°C. CH_4 concentration of 55-60 % was obtained in biogas evolved after anaerobic digestion of beef cattle waste (Chang et al., 1983). When cattle waste was predigested in batch and semi-continuous systems, the biogas produced contained 68-75 % and 75-86 % methane (Singh et al., 1983). Mesophilic anaerobic digestion of screened and unscreened dairy manure resulted in maximum 63.8 % methane content (Lo et al., 1984). Fischer et al. (1984) found the average methane content of 52 ± 4 % during anaerobic digestion of swine manure at different influent solid concentrations. Webb and Hawkes (1985) carried out similar studies with poultry manure and found the maximum gas yield at 4-6 % TS levels with CH_4 content of 58.6 %.

Mahadevswami and Venkatraman (1986) studied bioconversion of poultry droppings and cow dung, biogas produced during anaerobic digestion contained 65 and 55-60 % CH_4, respectively. The anaerobic digestion of cattle manure and Parthenium mixture at 30 ± 1°C produced CH_4 ranging between 60-70 % (Gunaseelan, 1987). Nipaney and Panholzer

(1987) investigated Pistia stratiotes, an aquatic weed, as a substrate for biogas production which contained 58-68 % CH_4. Summers et al. (1987) reported average CH_4 content of 53 % after 20 or more day retention time in anaerobic digester. Devi and Nand (1989) pretreated mango peel for six days and reported eight-fold increase in gas production with an average of 58 % CH_4 content. There was 57 % CH_4 present in biogas produced from the solid wastes of biscut and chocolate industry during 40 days of anaerobic digestion (Ranade et al., 1989). Sarda and Krishna (1989) found 72 % CH_4 content in biogas during anaerobic digestion of tomato processing waste. Chen et al. (1990) reported 58 % CH_4 content during anaerobic digestion of municipal solid waste. Wong (1990) reported CH_4 content of 41-58 % during anaerobic treatment of pig manure and sewage sludge mixture. Lin (1990) noted 69 to 71 % CH_4 content in the gas produced from the anaerobic digestion of organic phosphate pesticide plant waste water. Kalia and Kanwar (1990) observed 62-77 and 50-60 % CH_4 content from Ageratum and cattle dung biodegradation, respectively. It was observed that biogas from sheep droppings and cattle dung digestion contained 70-75 and 56-60 % CH_4 content (Kanwar and Kalia, 1991).

2.6 MICROBIOLOGY OF ANAEROBIC DIGESTION

Cox (1983) studied non-methanogenic bacterial population in a high rate anaerobic digester. Most of the non-methanogenic bacteria were Gram-negative and possessed limited amylolytic activity. Total counts were between 10^8-10^9/ml in high rate digester as compared to 2 x 10^7/ml in controlled one. Total counts in anaerobic jar and globe were 8.7 x 10^7/ml and 2.93 x 10^8/ml, respectively, whereas amylolytic counts were 0.7 x 10^6/ml. The total bacterial counts were 7 x 10^7/ml in the slurry of pig manure digester (Fischer et al., 1984). There were

58.2 x 10^4/ml cellulolytic, 24.8 x 10^4/ml proteolytic and 151.3 x 10^3/ml acid forming bacteria in the slurry of cow dung and organic wastes mixed with oil cakes (Lingaiah, 1986). There were 49-86 x 10^6/g total bacteria, 37.5-109 x 10^3/g acid formers, 16-6.5 x 10^4/g cellulolytic, 24-14.5 x 10^3/g proteolytic and 8.0-2.0 x 10^3/g lipolytic bacteria at 37 and 28 ± 2°C in the slurry of cow dung digestion (Rajasekaran et al., 1986). Singh and Touro (1987) reported five groups of bacteria representing an anaerobic digestion namely, hydrolytic, fermentative, hydrogenogenic acetogenic, acetogenic and methanogenic. Neelakantan (1987) reported cellulolytic counts of dung slurry to be 25 x 10^5 cells/g and 15 x 10^5 cells/ml, when digested at 30°C and during winter season, respectively. Proteolytic, lipolytic and amylolytic counts were in the range of 5-30 x 10^5 cells/ml, 0.9-3.8 x 10^5 cells/ml and 8-42 x 10^5 cells/ml slurry, respectively. Bal et al. (1990) used different media for enumeration of anaerobically digested cattle dung. The acidogenic and methanogenic counts were 5 x 10^8 cfu/g and 12 x 10^9 cfu/g, respectively. Acetogens composed of proteolytic (22 x 10^8 cfu/g), lipolytic (3 x 10^8 cfu/g), amylolytic (23 x 10^8 cfu/g) and celulolytic (26 x 10^8 cfu/g) groups.

Nand (1991) enumerated methanogens and non-methanogens of digesters operated with rabbit-pellets and left over of experimental animal feeds, fruits and vegetable processing waste and cow dung. Considerably large number of cellulolysers, hemicellulolysers, pectolytes, proteolytes, lipolytes and other group of anaerobes (methanogens) were isolated and characterised taxonomically. Some of predominant were Clostridium cellobioparum, Ruminococcus albus (cellulose degrading), C. butyricum, C. sporopheroides, Streptococcus faecium, S. uberis (hemicellulose degrading), C. butyricum, C. baratti and

and S. avium (pectin hydrolyzing), C. sargotoforme, Butryvibrio spp., Eubacterium spp., Acetobacterium spp., Peptococcus spp. and Sarcina sp. (lipid hydrolyzing), Methanobacterium formicium, M. uliginosum, Methanococcus sp., Methanobrevibacter sp. and Methanosarcina sp. (methane producing). Rajasekaran and Srinivasan (1991) reported cellulolytic counts of 17.8 x 10^3/g and 24.5 x 10^3/g during anaerobic treatment of night soils and cow dung, respectively. Yeole and Ranade (1991) found more biogas from pig dung than cattle dung and concluded that chemical nature rather than natural microflora of pig manure was responsible for increase in gas production. Ramasamy et al. (1991) studied distribution in rumen and biogas digesters. They found that the total population of bacteria in range of 10^{11}/ml in rumen and 10^8-10^9/ml in biogas digester. Bacteroides and Clostridium dominated other micro-organisms in biogas digester. Population of acid formers and proteolytic bacteria were more in rumen than in digester. There was 194.58 x 10^3/g methanogenic bacteria in poultry droppings which increased to 237.08 x 10^3/g after digestion in slurry samples (Dhevagi et al., 1991). Neelakantan and Singh (1991) reported the increase in non-methanogenic bacteria after 30 days digestion. In cattle dung proteolytic bacteria were in higher count (36 x 10^5 cells/g), followed by amylolytic (28 x 10^5 cells/g), cellulolytic (18.4 x 10^5 cells/g) and lipolytic (4.4 x 10^5 cells/g) on 30th day. In case of pig dung proteolytic counts were 0.2-2.5 x 10^5 cfu/g and 0.01-0.05 x 10^5 cfu/g. Total anaerobic counts of berseem hay were 115 x 10^6 cfu/ml and that of water hyacinth combined vegetable market waste mixture were 140 x 10^6 cfu/ml after 30 days of digestion.

CHAPTER - 3

MATERIALS AND METHODS

3. MATERIALS AND METHODS

3.1 SUBSTRATE

3.1.1 CATTLE DUNG

Cattle dung was obtained from the Cattle Yard of National Dairy Research Institute (NDRI), Karnal.

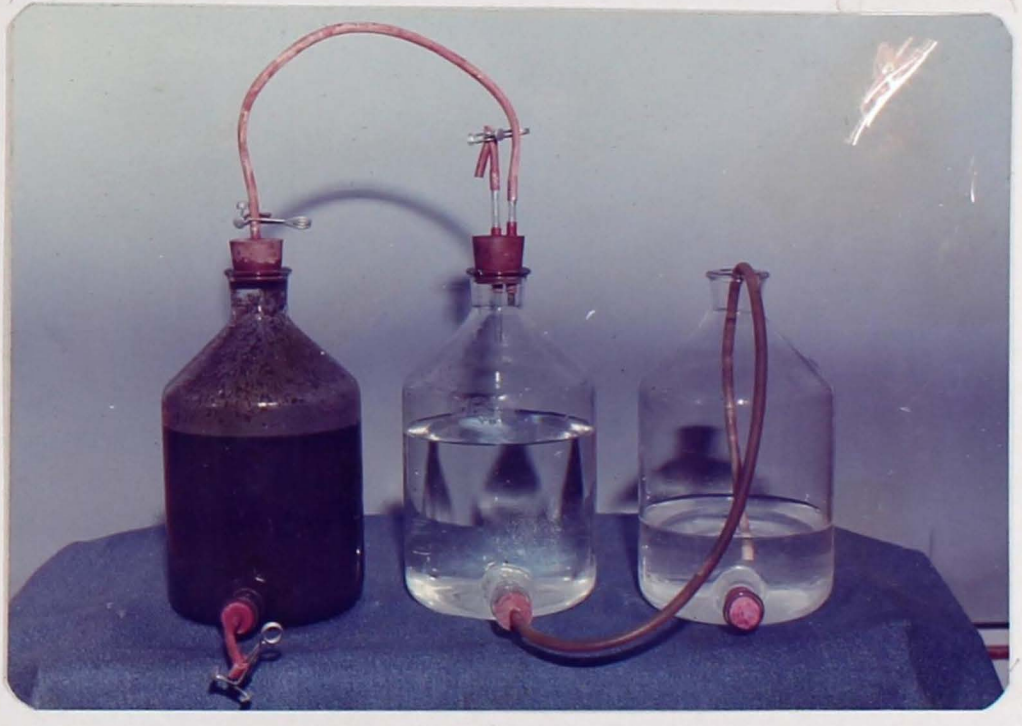
3.1.2 SUPPLEMENT

Dairy effluent sludge (dried) was obtained from Mother Dairy, New Delhi which was pulverised in a grinder.

3.2 ANAEROBIC DIGESTION ASSEMBLY

Anaerobic digestion assembly comprised of three Borosil Aspirator Bottles of 5 litre capacity. Each bottle was connected to the other with rubber tubings. The substrate slurry was charged in the first bottle, whereas the second bottle was filled with water. The third one was used for collection of water displaced by the gas produced. Bottom outlet of the third bottle was tightly closed with a rubber bung. Outlets of the other two bottles were connected through rubber tubing which served as sampling device in the first bottle and as outlet for the displaced water in the second bottle. Mouths of the first and the second bottles were connected through rubber tubing to serve as passage for gases. Rubber cork at the mouth of second bottle had an outlet for gases. Mouth of the third bottle was kept open and water outlet tube was placed in the bottle through it (Fig. 1).

Fig. 1. Assembly for the anaerobic digestion.



3.3 SUPPLEMENTATION

Cattle dung was supplemented with dairy effluent sludge at 1:1, 1:5 and 5:1 levels on dry matter basis. Cattle dung and dairy effluent sludge in 5:0 and 0:5 ratio were also taken as controls.

3.4 SUBSTRATE SLURRY COMPOSITION

Different substrates were mixed with water in order to get approximately 8-15 % total solids (TS). The samples were mixed thoroughly to obtain an uniform substrate slurry and the slurry samples were drawn out and analysed for different chemical and microbiological parameters.

3.5 CHARGING OF DIGESTER

The slurry was filled in preweighed aspirator bottles and re-weighed after loading. The weight of slurry was calculated by substracting weight of the empty bottle from that of the filled one.

3.6 ANAEROBIC DIGESTION

The charged assemblies in duplicate were subjected to anaerobic digestion for 20 days during the months of April and May. Gas production was measured daily from the water displaced by the gases. Samples of slurry were collected at intervals of five days and subsequently analysed for different chemical and microbiological parameters. Gas samples were also collected at the same intervals and analysed for CO_2 and CH_4 gases. Room temperature varied between 26.0-38.1°C with an average of 33°C.

3.7 SAMPLING

Samples of slurry and gas were collected after 5, 10, 15 and 20 days.

3.7.1 COLLECTION OF SLURRY SAMPLES

Slurry was vigorously shaken before sampling and negative pressure was created by connecting the water outlet tube to fresh

water tap. Water from tap pushed back the gas from the second bottle to the first bottle and slurry came out with pressure through the tube of the sampling device. Samples were collected in sample bottles and analysed for different chemical and microbiological parameters.

3.7.2 SAMPLING OF GAS

A 3-way valve was attached to a rubber bladder with the help of a latex tube and the second end of valve was attached to the gas outlet tube of aspirator bottle through latex tube (Fig. 2). Before opening the pinch cork of the gas outlet tube, the rubber tube connecting the two bottles was closed with another pinch cork. Negative pressure was generated similarly as in case of slurry sampling and the gas was collected in the rubber bladders (Fig. 3).

3.8 CHEMICAL ANALYSIS

3.8.1 TOTAL SOLIDS (TS)

Total solids in slurry were determined using Ohaus Moisture Determining Balance.

3.8.2 ASH CONTENT

Ash content was estimated by incineration at 600°C for 2-3 hours in a Muffle Furnace.

3.8.3 VOLATILE SOLIDS (VS)

Volatile solids were calculated as follows:

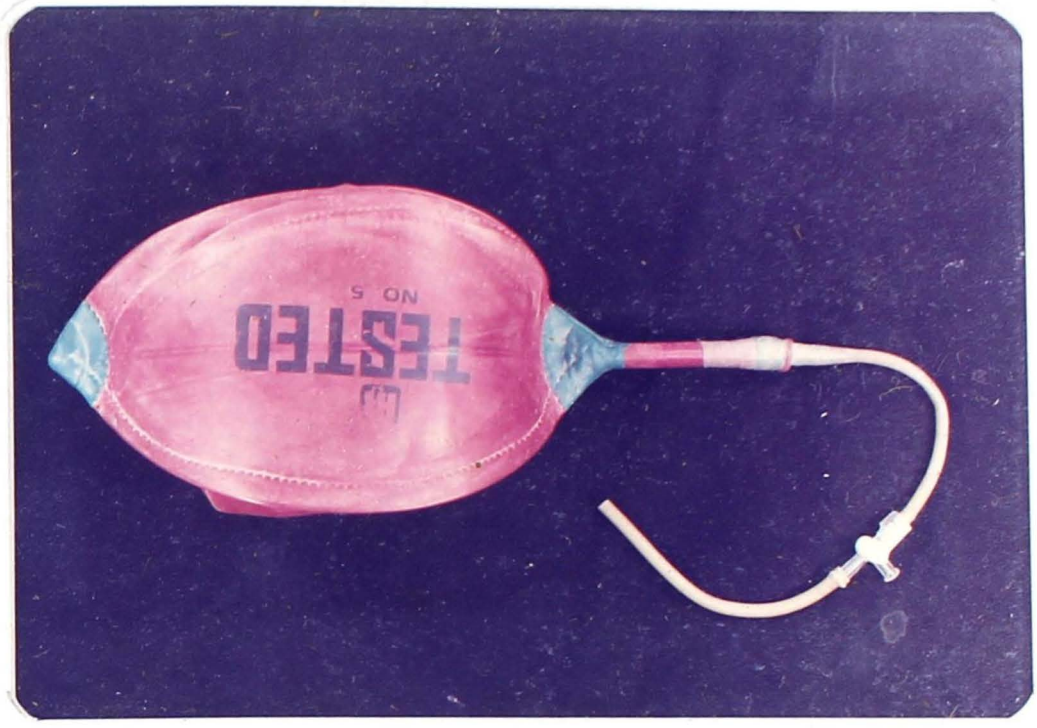
VS (%) = TS (%) - Ash content (%)

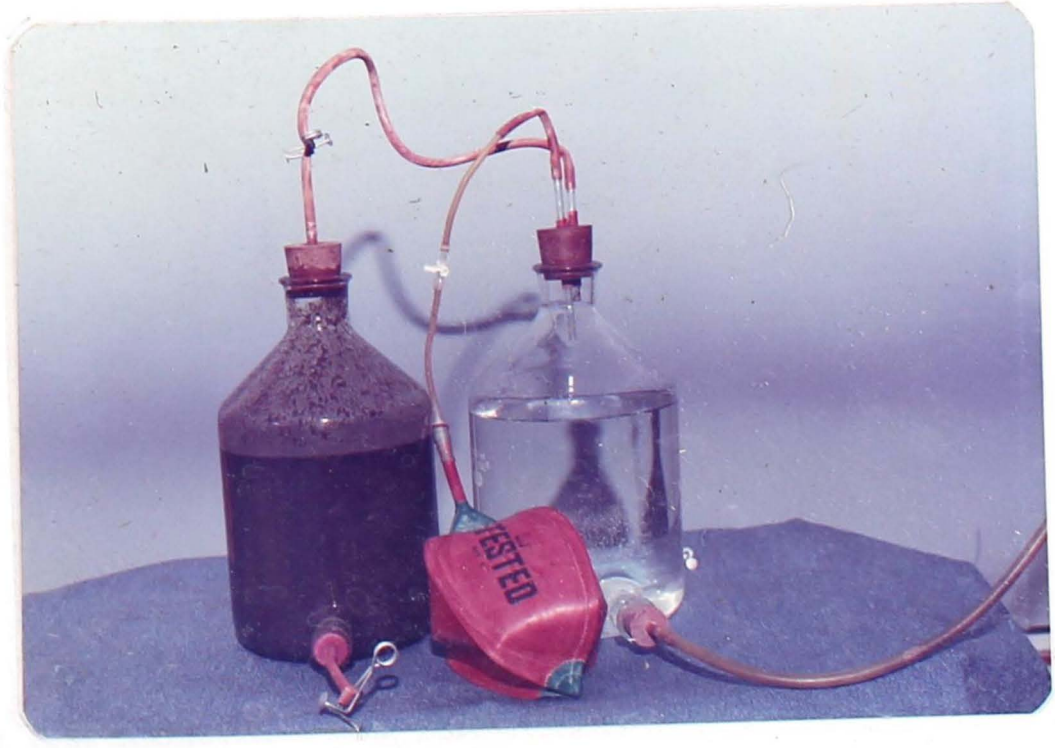
3.8.4 pH AND REDOX POTENTIAL (E_h)

pH and E_h of samples were measured with Digital pH Meter DPH 500.

Fig. 2. Gas sampling device.

Fig. 3. Gas sampling method.


TESTED
NO 5


TESTED

3.8.5 DISSOLVED OXYGEN (DO)

Dissolved oxygen of the sample was measured with an Oxygen Meter, YSI Model 54 A.

3.8.6 TOTAL VOLATILE FATTY ACIDS (TVFA)

TVFA content of slurry was determined by steam distillation of 1 ml filtered slurry on acidification followed by titeration with N/100 NaOH (Barnett and Reid, 1957), TVFA was expressed as milligrams acetic acid per litre slurry and calculated as:

$$\text{TVFA (mg acetic acid/l)} = \frac{A \times 1000 \times N \times (a-b)}{B}$$

Where,

A = Equivalent weight of acetic acid,

N = Normality of NaOH,

B = ml of sample taken,

a = Titer of sample, and

b = Titer of blank.

3.8.7 TOTAL NITROGEN

Total nitrogen was determined by micro-Kjeldahl method (AOAC, 1984).

3.8.8 ORGANIC CARBON

Total organic carbon was determined by a rapid titeration method (Walkley and Black, 1937). Ground (20 mesh) sample (0.1 g) of slurry was taken in 500 ml conical flask to which 20 ml of one normal $K_2Cr_2O_7$ and 20 ml conc. H_2SO_4 were added. Flask was shaken for one minute and kept for 30 minutes on asbestos pad. 200 ml of water, 10 ml H_3PO_4 (85 %) and 1 ml diphenylamine indicator were added. Then it was

titerated against one normal $FeSO_4.7H_2O$ till the colour flashed to green. Again 0.5 ml of normal $K_2Cr_2O_7$ was added to it and titerated with normal $FeSO_4.7H_2O$ dropwise till the blue colour turned to green. Volume of normal $FeSO_4.7H_2O$ used was recorded and organic carbon was calculated as follows:

$$\text{Amount of Carbon Oxidised} = \frac{(V_1 - V_2) \times 0.003 \times 100 \times C.F}{W}$$

Where,

V_1 = Volume of $K_2Cr_2O_7$ taken,

V_2 = Volume of $FeSO_4.7H_2O$ used,

W = Weight of sample taken, and

CF = Correction Factor (100/77).

3.8.9 C:N RATIO

C:N ratio of the samples were calculated after determining organic and total nitrogen as follows:

$$\text{C:N Ratio} = \frac{\text{Total organic carbon}}{\text{Total Nitrogen}}$$

3.8.10 VFA ANALYSIS

Strained slurry (4 ml) was mixed with 1 ml meta-phosphoric acid (25 % m-H_3PO_4 in 5 N H_2SO_4). After keeping overnight the contents were centrifuged at 4000 rpm for 20 min. Supernatant was analysed for individual VFA in gas chromatograph (Nucon 5000) equipped with flame ionization detector and stainless steel column packed with chromosorb 101. Injection block was maintained at 200°C for rapid vaporization of the sample. Column temperature of 200°C was used with a nitrogen carrier gas flow rate of 40 ml/min. Hydrogen flow rate to flame jet was 25 ml/min and air flow rate to detector chamber was 240 ml/min. The detector chamber temperature was maintained at 240°C (Erwin et al., 1961).

3.9 MICROBIOLOGICAL ANALYSIS

Samples were subjected to anaerobic bacterial counts using roll-tube technique (Hungate, 1969). Total anaerobic bacteria, cellulolytic, amylolytic, proteolytic, acid-formers and lipolytic bacterial counts were estimated in each sample.

3.9.1 DILUTION MEDIUM

The dilution medium was prepared with

Sodium bicarbonate	(0.5 %)
Sodium carbonate	(1.0 %) and
Resazurin	(0.0001 %).

It was boiled under CO_2 atmosphere and transferred to screw-capped test tubes in 10 ml quantities and autoclaved at 15 lb for 30 minutes. Serial dilutions of slurry samples were made in dilution medium and kept under CO_2 atmosphere.

3.9.2 MEDIUM FOR TOTAL ANAEROBIC BACTERIA

The chemical composition is as follows (Modified Hungate's Medium):

	(g/l)
K_2HPO_4	0.03
KH_2PO_4	0.02
$MgSO_4$	0.01
$CaCl_2$	0.01
$(NH_4)_2SO_4$	0.10
NaCl	0.10
Cystein HCl	0.20
$NaHCO_3$	0.50
Resazurin	0.0001
Glucose	0.50

Maltose	0.25
Cellobiose	0.25
Agar	20.0
Vitamin Solution*	1.0 ml
Trace Element Solution*	1.0 ml
pH	7.0

*Vitamin Solution (Laanbroek et al., 1985)

Biotin	10 mg
Nicotinic acid	100 mg
p-amino benzoic acid	50 mg
Thiamine	100 mg
Pantothenic acid	50 mg
Pyradoxamine	250 mg
Cobalamine	50 mg
Distilled water	1 litre

*Trace Element Solution (Laanbroek et al., 1985)

12.5 HCl	4.0 ml
$FeSO_4.4H_2O$	2000 mg
$ZnCl_2$	70 mg
$MnCl_2.4H_2O$	100 mg
$CoCl_2.2H_2O$	190 mg
$CuCl_2.2H_2O$	17 mg
$NiCl_2.6H_2O$	24 mg
$Na_2MoO_4.3H_2O$	36 mg
$Na_2WO_4.2H_2O$	39 mg
Distilled water	One litre

3.9.3 MEDIUM FOR CELLULOLYTIC BACTERIA

The chemical composition reported by Hungate (1957) is as follows:

	(g/l)
K_2HPO_4	0.03
KH_2PO_4	0.02
$MgSO_4$	0.01
$CaCl_2$	0.01
$(NH_4)_2SO_4$	0.10
Na_2CO_3	0.10
Cystein HCl	0.02
Resazurin	0.0001
Cellulose powder	1.0
Vitamin solution*	1.0 ml
Trace element solution*	1.0 ml
Agar	20.0
pH	7.0

* Composition of both the solutions is given in the medium for total anaerobic count.

3.9.4 MEDIUM FOR ACID FORMERS

The medium for enumeration of acid forming bacteria has the following composition (Chynoweth and Mah, 1977):

	(g/l)
NaCl	0.1
NH_4Cl	0.05
$MgCl_2$	0.005
$CaCl_2$	0.005
KH_2PO_4	0.005

$CoCl_2$	0.001
$(NH_4)_2MoO_4$	0.001
$NaHCO_3$	0.5
Glucose	0.5
Cystein HCl	0.05
$CaCO_3$	1.0
Agar	20.0
Na_2S	0.025
Bromothymal Blue	0.005

3.9.5 MEDIUM FOR PROTEOLYTIC BACTERIA

The composition is as follows (Abou Akkada and Blackburn, 1962):

Mineral(a) solution	15.0 ml
Mineral(b) solution	15.0 ml
Skim milk*	10.0 ml
Rumen fluid	10.0 ml
Glucose	0.1 g
Maltose	0.1 g
Cellobiose	0.1 g
Cystein HCl	0.05 g
Resazurin	0.0001 g
$NaHCO_3$	0.5 g
Agar	2.0 g
Distilled water	100 ml

Mineral(a) Solution:

K_2HPO_4	3.0 g/1 D.W.

Mineral(b) Solution:

	(g/1)
KH_2PO_4	3.0
$(NH_4)_2SO_4$	6.0
NaCl	6.0
$MgSO_4$	0.6
$CaCl_2$	0.6

* Skim milk was sterilized separately and added to the medium before pouring.

3.9.6 MEDIUM FOR LIPOLYTIC BACTERIA

The chemical composition reported by Hobson and Mann (1961) is as follows:

Mineral(a) solution*	15.0 ml
Mineral(b) solution*	15.0 ml
Clarified rumen fluid	40.0 ml
Cystein HCl	0.05 g
$NaHCO_3$	0.4 g
Resazurin	0.0001 g
Tributyrin	1.0 ml
Agar	2.0 g
Distilled water	100 ml

* Composition of Mineral(a) and Mineral(b) solution was similar to that in case of the medium for proteolytic bacteria.

3.9.7 MEDIUM FOR AMYLOLYTIC BACTERIA

The composition of the medium according to Kurihara et al. (1967) is as follows:

Casein enzyme hydrolysate	1.0 g
Bacto yeast extract	0.25 g

Mineral(a) solution	15.0 ml
Mineral(b) solution	15.0 ml
Strained rumen fluid	20.0 ml
Agar	2.0 g
Resazurin	0.001 g
Cystein HCl	0.05 g
$NaHCO_3$	0.4 g
Starch	0.5 g in 15 ml distilled water, and autoclaved at 5 lb in/30'
Distilled water	100 ml

All the media were sterilized at 15 lbs for 30 min and equilibrated with CO_2. Cotton plugs were replaced with sterilized rubber corks.

3.9.8 ROLL-TUBE TECHNIQUE

Medium upon sterilization was kept in water bath maintained at 45-50°C to retain it in liquid state. To the medium vitamin solution was added (if required) and flushed with CO_2 before pouring in tubes. One millilitre of sample from suitable dilution was transferred to a sterile roll-tube. About 5.0 ml of medium was added and spread uniformly along the sides of tubes using a spinner. After solidification of the medium, corks were removed from roll-tubes and the tubes were equilibrated with CO_2. Tubes were restoppered with sterile rubber corks and incubated in an anaerobic jar at 37°C for 4-7 days for total anaerobic, bacterial, lipolytic and acid forming bacterial counts; 2-3 days for proteolytic and amylolytic bacterial counts and 10-15 days for cellulolytic bacterial counts.

3.10 GAS ANALYSIS

Analysis of gas for CH_4 and CO_2 was carried out using Nucon Gas Chromatograph, which was equipped with thermal current detector (T.C.D.) and stainless steel column packed with Porapack-Q. Temperatures of injector, detector and oven were 40°C and current in detector was 200 mA. Gas flow rate in column was 60 ml/min.

3.11 STATISTICAL ANALYSIS

Data were analysed in randomised block design (Steel and Torrie, 1981). Analysis of variance (ANOVA) was carried out. Dunken's multiple range test (DMRT) was utilized to differentiate the treatments as well as replicates from each other, as the effects were found significant by analysis of variance (Steel and Torrie, 1981).

CHAPTER - 4

RESULTS AND DISCUSSION

4. RESULTS AND DISCUSSION

4.1 TOTAL SOLIDS

Total solids in different substrate combinations ranged from 7.2 to 13.25 % (Table 1). Decrease in total solids of all the substrates was noticed during the digestion period. Cattle dung supplemented with dairy effluent sludge in the ratio of 1:1, showed maximum degradation (23.1 %), whereas other combinations 0:1 (dairy effluent sludge only) and 1:5 gave 22.2 % and 18.5 %, respectively, while very less degradation was found during the digestion of cattle dung alone (9.6 %) and cattle dung - dairy effluent sludge combination at 5:1 ratio (9.3 %). Higher degradation of total solids in the slurries with higher levels of dairy effluent sludge might be due to the presence of easily degradable solids in the waste. Decrease in total solids might occur due to their degradation by anaerobic microflora and subsequent conversion into gaseous end products. Gupta and Awasthi (1990) found 13-27 % decrease in total solids when cattle dung was supplemented with pulp and paper mill effluent. Similar degradation rates (12.21 - 27.80 %) were reported by Singh et al. (1983, 1984).

4.2 VOLATILE SOLIDS

Dairy effluent sludge was poor in volatile solids (4.15 %), whereas cattle dung was rich (7.48 %) which accounted 57.63 and 80.55 % on dry matter basis, respectively (Table 2). Volatile solids decreased with the progress of anaerobic digestion. Maximum (33.2 %) degradation was found in 1:1 combination followed by dairy effluent sludge alone

Table 1. Total solid content (%) during the anaerobic digestion of cattle dung and dairy effluent sludge.

Period (Days)	Total solids (%)				
	Cattle dung : Dairy effluent sludge				
	1:1	5:1	1:0	0:1	1:5
0	10.60	9.68	9.28	7.20	13.25
5	10.25	9.49	9.05	6.90	12.20
10	9.88	9.15	8.86	6.70	11.90
15	8.65	8.92	8.71	6.20	11.40
20	8.15	8.78	8.39	5.60	10.80

Table 2. Volatile solids (%) during the anaerobic digestion of cattle dung and dairy effluent sludge.

Period (days)	Volatile solids (%)				
	Cattle dung : Dairy effluent sludge				
	1:1	5:1	1:0	0:1	1:5
0	7.34	7.32	7.48	4.15	8.23
5	7.05	6.80	7.23	3.89	7.41
10	6.53	6.40	7.06	3.54	7.17
15	5.48	6.21	6.90	3.21	6.80
20	4.90	6.07	6.62	2.82	5.91

(32.05 %) and 1:5 combination (28.62 %). Cattle dung digestion resulted with minimum loss (11.5 %), while in 5:1 combination, it was 17.1 %. Higher degradation of volatile solids in dairy effluent sludge might be due to easily available organic matter for biodegradation. Biodegradability of milk solids is more because of the fact that the polymeric compounds are lesser in amount in this case as compared to the cattle dung. In latter case, hemicellulose and lignin like polymeric compounds require more time for their hydrolysis. The combination of 1:1 was found optimum for better biodegradability. After 20 days of anaerobic digestion, 27.6 % reduction of volatile solids in screened manure (Lo et al., 1984), 25.6 % in animal excreta (Singh et al., 1984), 32.0 % in excreta of dairy and fattening animals (Summers et al., 1987) and 23.44 % in cattle dung supplemented with pulp and paper mill effluent (Gupta and Awasthi, 1990) have been reported.

4.3 pH

The pH values of all the substrate decreased during the earlier periods of digestion and later on, increased (Fig. 4). Maximum decrease (5.29 %) in pH was found in cattle dung effluent mixture in ratio of 5:1 followed by cattle dung (5.41 %) and 1:1 supplementation (5.63 %). The pH in case of dairy effluent sludge and 1:5 supplementation showed comparatively less decrease and final values were found higher than the original value. Decrease in pH occurred due to the acid production during the digestion. Acid accumulation due to the lower rate of methanogenesis caused decrease in pH to a great extent. When the utilization of acid produced started the pH values increased. Decrease in pH values were observed during the anaerobic digestion of cattle dung and cattle dung supplemented with charcoal from 7.5 to 6.15 and from 7.50 to 6.50,

Fig. 4. pH changes during the anaerobic digestion of cattle dung and dairy effluent sludge

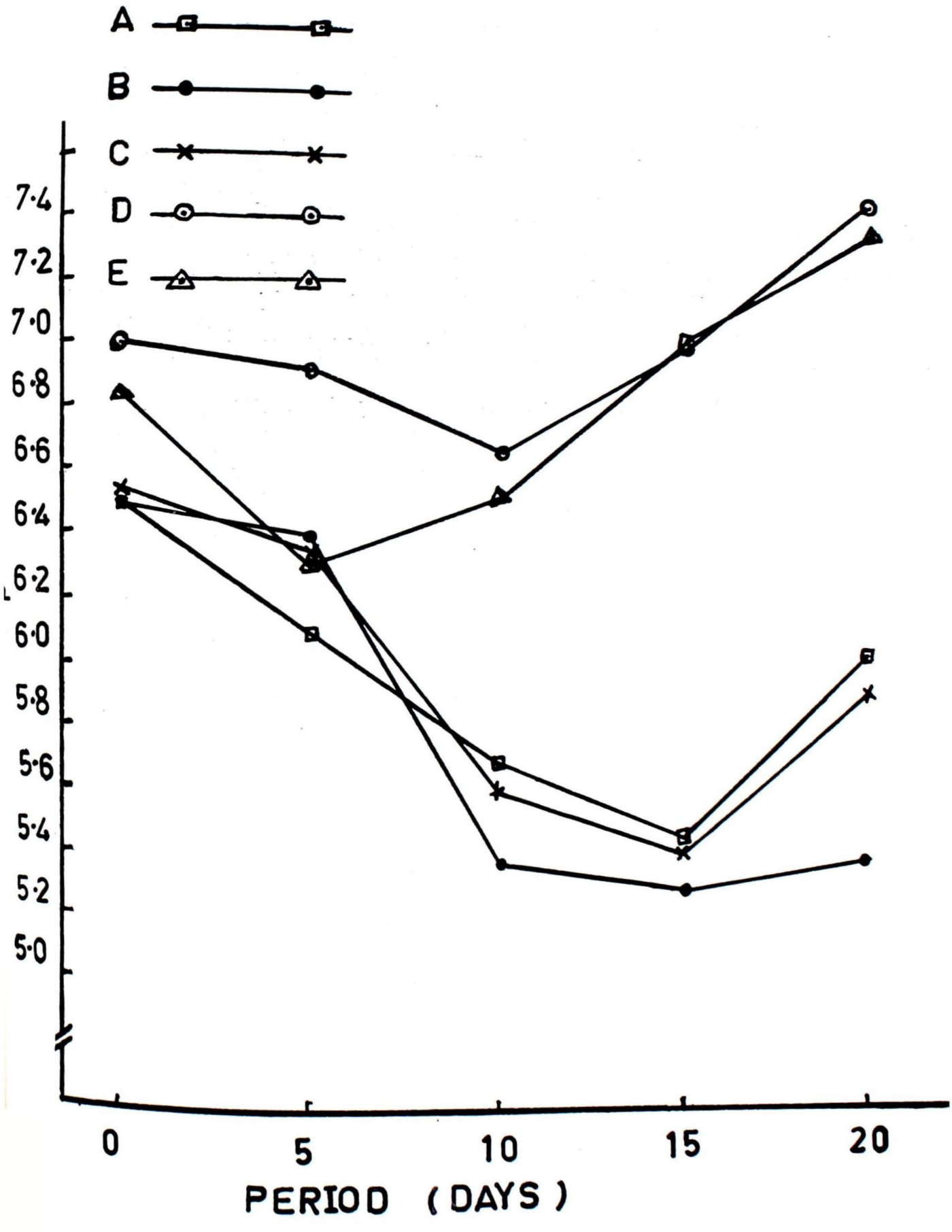
A ---- 1:1

B ---- 5:1

C ---- 1:0

D ---- 0:1

E ---- 1:5


A
B
C
D
E
7·4
7·2
7·0
6·8
6·6
6·4
6·2
6·0
5·8
5·6
5·4
5·2
5·0
0
5
10
15
20
PERIOD (DAYS)

respectively (Singh et al., 1981). After 20 days, digestion of willow dust and textile mill waste, decrease in pH from 8.1 to 7.1 was observed (Balasubramaniya et al., 1986).

4.4 E_h

Figure 5 shows the E_h changes during the anaerobic digestion of cattle dung and dairy effluent sludge. Redox potential (E_h) of substrate slurries changed during the anaerobic digestion in opposite the order of pH values. The E_h values reached highest at +106 mV in 5:1 supplementation ratio followed by +99 mV in cattle dung and +86 mV in 1:1 ratio. Dairy effluent sludge (0:1) and 1:5 supplementation ratios showed lower E_h values (50 mV) and the final values were -10 and -7 mV, respectively. When organic matter is degraded to gaseous end product, oxidation number of carbon changes. The highest (+4) oxidation number of carbon is found in CO_2 while lowest (-4) in CH_2. CO_2 and CH_4 produced by oxidation and reduction processes, respectively. Increase in redox potential indicates the oxidation of organic wastes during the digestion period. This was supported by production of CO_2 in higher amounts than methane (Fig. 19). When CH_4 production increased or exceeded CO_2 production, decrease in E_h values was observed. Negative values of E_h coincided with higher methane production (Fig.19). The favourable values of E_h for the efficient CH_4 production were reported as -250 to -300 mV (Lo et al., 1986), -163 to -220 mV (Svendsen and Blackburn, 1986) and -200 to -300 mV (Kobayashi et al., 1990).

4.5 DISSOLVED OXYGEN

Fig. 6 depicts the amount of dissolved oxygen which decreased during the anaerobic digestion. Initially, the values were in the range of 0.2-0.3 mg/l which decreased at slow rate and whole of oxygen

Fig. 5. E_h (mV) changes during the anaerobic digestion of cattle dung and dairy effluent sludge

A ---- 1:1

B ---- 5:1

C ---- 1:0

D ---- 0:1

E ---- 1:5

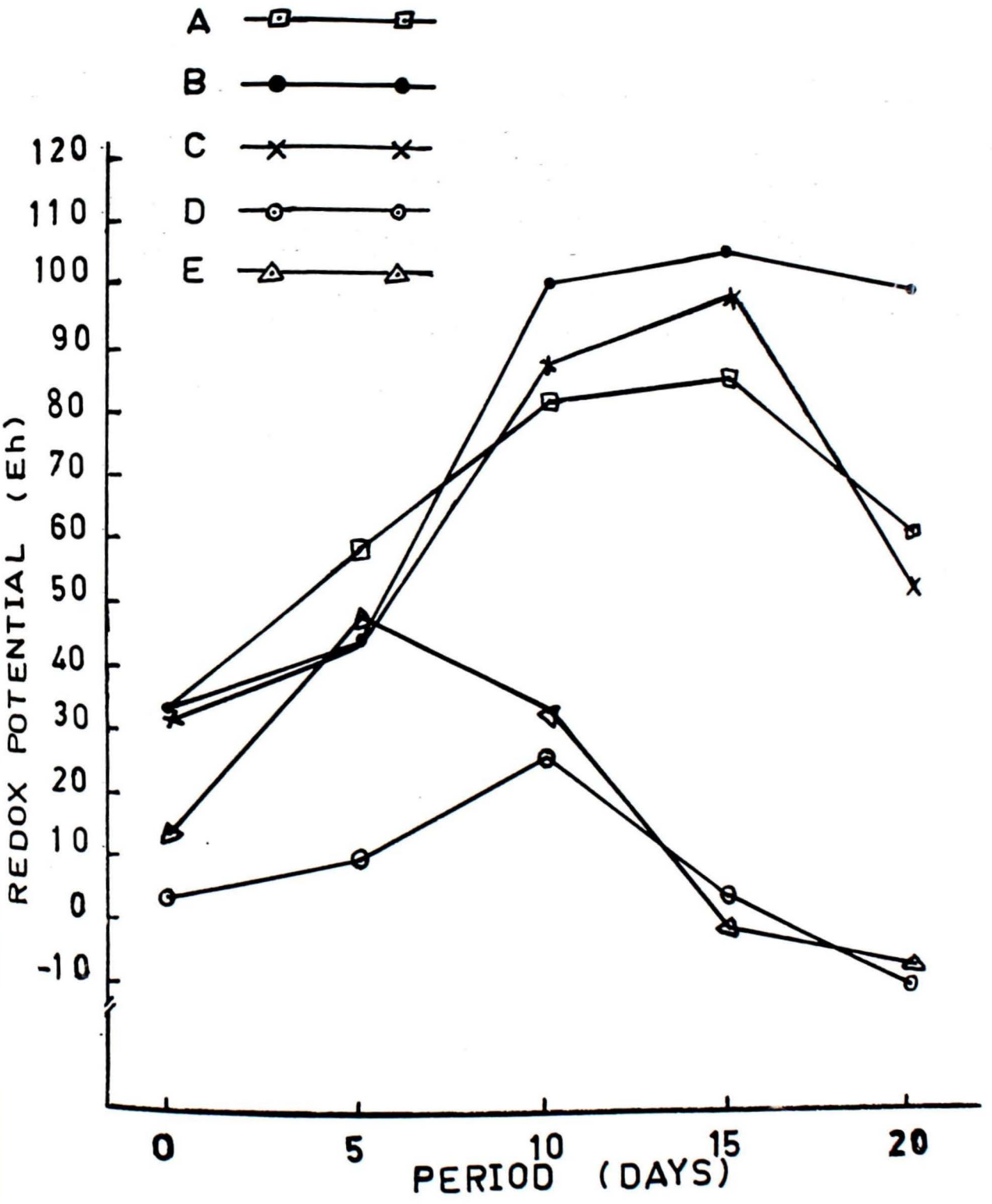

A
B
C
D
E
120
110
100
90
80
70
60
50
40
30
20
10
0
-10
REDOX POTENTIAL (Eh)
0
5
10
15
20
PERIOD (DAYS)

Fig. 6. Dissolved oxygen (mg/l) during the anaerobic digestion of cattle dung and dairy effluent sludge

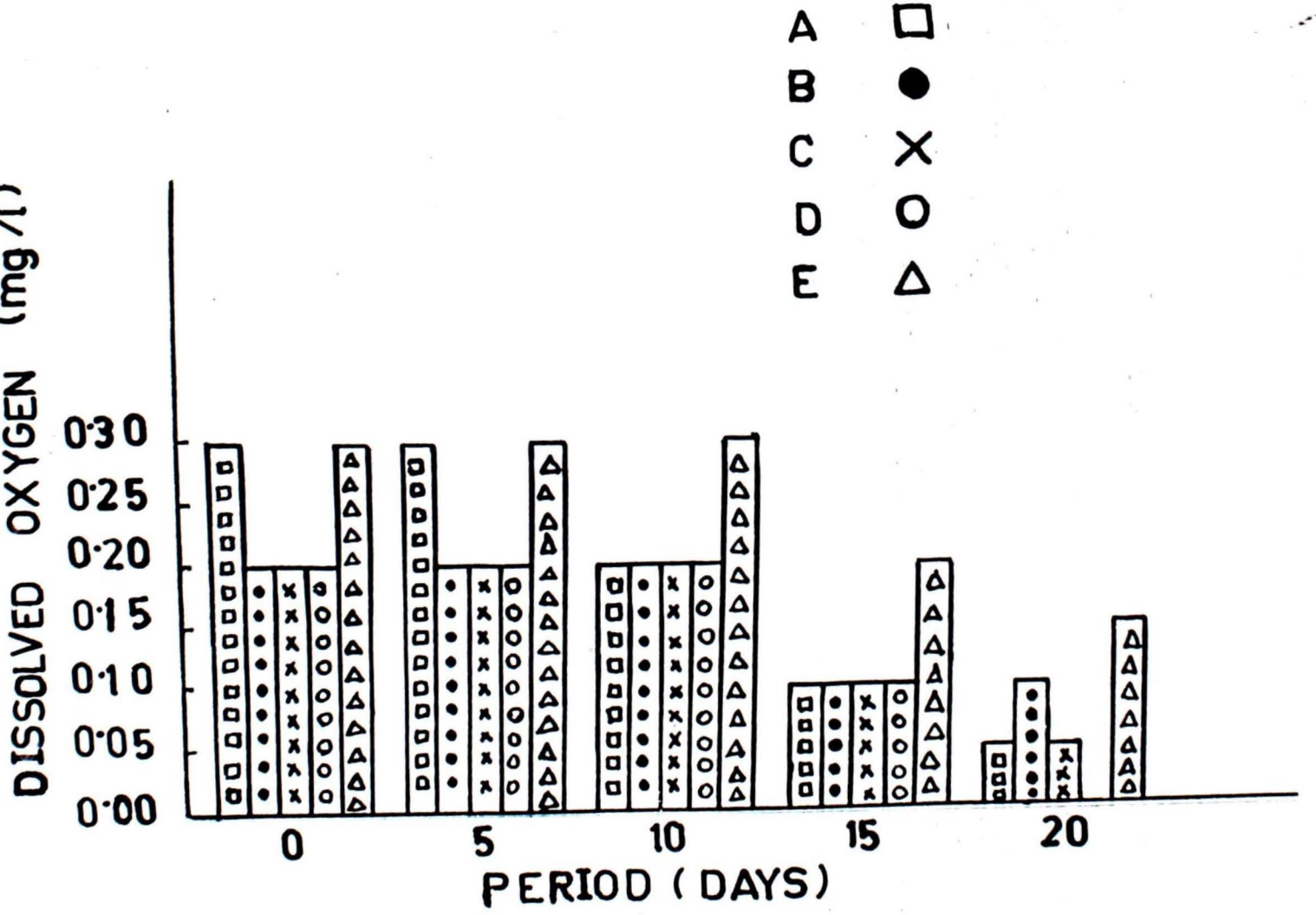
A ---- 1:1

B ---- 5:1

C ---- 1:0

D ---- 0:1

E ---- 1:5


A
B
C
D
E
DISSOLVED OXYGEN (mg/l)
0·30
0·25
0·20
0·15
0·10
0·05
0·00
0
5
10
15
20
PERIOD (DAYS)

disappeared after 20 days in the slurry of dairy effluent sludge while other slurries showed the final concentration of oxygen in the range of 0.05 to 0.15. Dissolved oxygen decreased due to its utilization by the facultative anaerobic bacteria and sharp decrease might account for rapid takeup of O_2 by the bacteria present in the slurries.

4.6 TOTAL NITROGEN

Table 3 represents the changes in nitrogen content during the anaerobic digestion of cattle dung and dairy effluent sludge. The decrease in nitrogen was found after the digestion of substrate slurries except in case of supplementation ratio of 1:5. Nitrogen contents of dairy effluent sludge were higher than that of cattle dung. Rapid decrease in nitrogen contents was found after 15 days except in case of 1:5 mixture of cattle dung and dairy effluent sludge. NAS (1977) reported that upto 18 % original nitrogen could be lost during anaerobic digestion. Usually nitrogen loss is more when NH_3-H content is higher. Decrease in total nitrogen from 1.71 to 0.20 % after 40 days of digestion was reported by Balasubramaniya et al. (1986), while increase in nitrogen content from 1.58 to 3.18, 1.59 to 4.08 and 1.58 to 5.96 % during the anaerobic digestion of pig manure and sewage sludge in ratio of 2:1, 1:1 and 1:2, respectively, was observed by Wong (1990).

4.7 ORGANIC CARBON

Cow dung was found to contain the highest amount of organic carbon, while dairy effluent sludge contained the least (Table 4). Very less carbon was degraded in cattle dung (2 %), while reduction was higher in combinations containing higher amount of dairy effluent sludge. Combinations of 1:1, 0:1 and 1:5 showed 13.2, 12.8 and 12.4 % degradation of organic carbon content. Higher reduction of organic carbon content

Table 3. Nitrogen content (%) during the anaerobic digestion of cattle dung and dairy effluent sludge (on DM basis).

Period (days)	Nitrogen (%)				
	Cattle dung : Dairy effluent sludge				
	1:1	5:1	1:0	0:1	5:1
0	3.57	2.66	2.03	4.69	3.05
5	3.68	2.65	2.08	5.25	3.12
10	3.72	2.35	1.92	3.85	3.78
15	3.71	2.31	1.82	3.64	3.36
20	2.31	1.82	1.68	2.94	3.29

Table 4. Organic carbon (%) during the anaerobic digestion of cattle dung and dairy effluent sludge (on DM basis).

Period (days)	Organic carbon (%)				
	Cattle dung : Dairy effluent sludge				
	1:1	5:1	1:0	0:1	1:5
0	40.27	43.95	46.83	33.51	36.31
5	40.01	41.66	46.57	32.78	35.31
10	38.45	40.69	46.33	30.74	35.01
15	36.80	40.47	46.03	29.57	34.67
20	34.96	40.19	45.89	29.23	31.80

may be due to the availability of simpler organic compounds in milk solids which are easily degraded, whereas carbon is present in the form of complex compounds like hemicellulose and lignin in dung and thus is degraded slowly. Reduction in carbon from 46.08 to 44.72 and 44.38 to 40.95 % was reported during the anaerobic digestion of cattle dung and cattle dung - pressed mud mixture (Singh et al., 1981). Similarly, decrease from 54.44 to 28.9, 54.64 to 28.26 and 66.76 to 28.58 % was recorded in carbon contents of pig manure - sewage sludge mixtures at 2:1, 1:1 and 1:2 ratios, respectively during the anaerobic fermentation (Wong, 1990).

4.8 C/N RATIO

It was observed that the C/N ratio was different in all the samples of slurries (Fig. 7). The minimum (7.15) C/N ratio was found in dairy effluent sludge and maximum (23.07) was in cattle dung. The C/N ratio decreased during the early stages of digestion. An increase in the C/N ratio was recorded during later stages of the fermentation. In the slurry of 1:1 mixture, the C/N ratio first decreased by 12.1 % and then increased (34.1 %). The slurry of 5:1 proportion showed 33.7 % increase in C/N ratio after that it decreased (4.8 %) during the later stages of fermentation. Cattle dung slurry showed an increase of 18.42 % and then decreased (2.9 %), whereas dairy effluent sludge recorded 39 % increase in C/N ratio and then decreased (12.7 %). The slurry with 1:5 supplementation showed a decrease of 18.7 % in C/N ratio. In this study, maximum methane production was observed at the range of 9.64 to 15.13 C/N ratio. NAS (1977) reported that C/N ratio of substrate should not fall beyond the range of 30:1 to 50:1 for the efficient anaerobic digestion. The change in optimum C/N ratio resulted in the change of

Fig. 7. C/N ratios during the anaerobic digestion of cattle dung and dairy effluent sludge

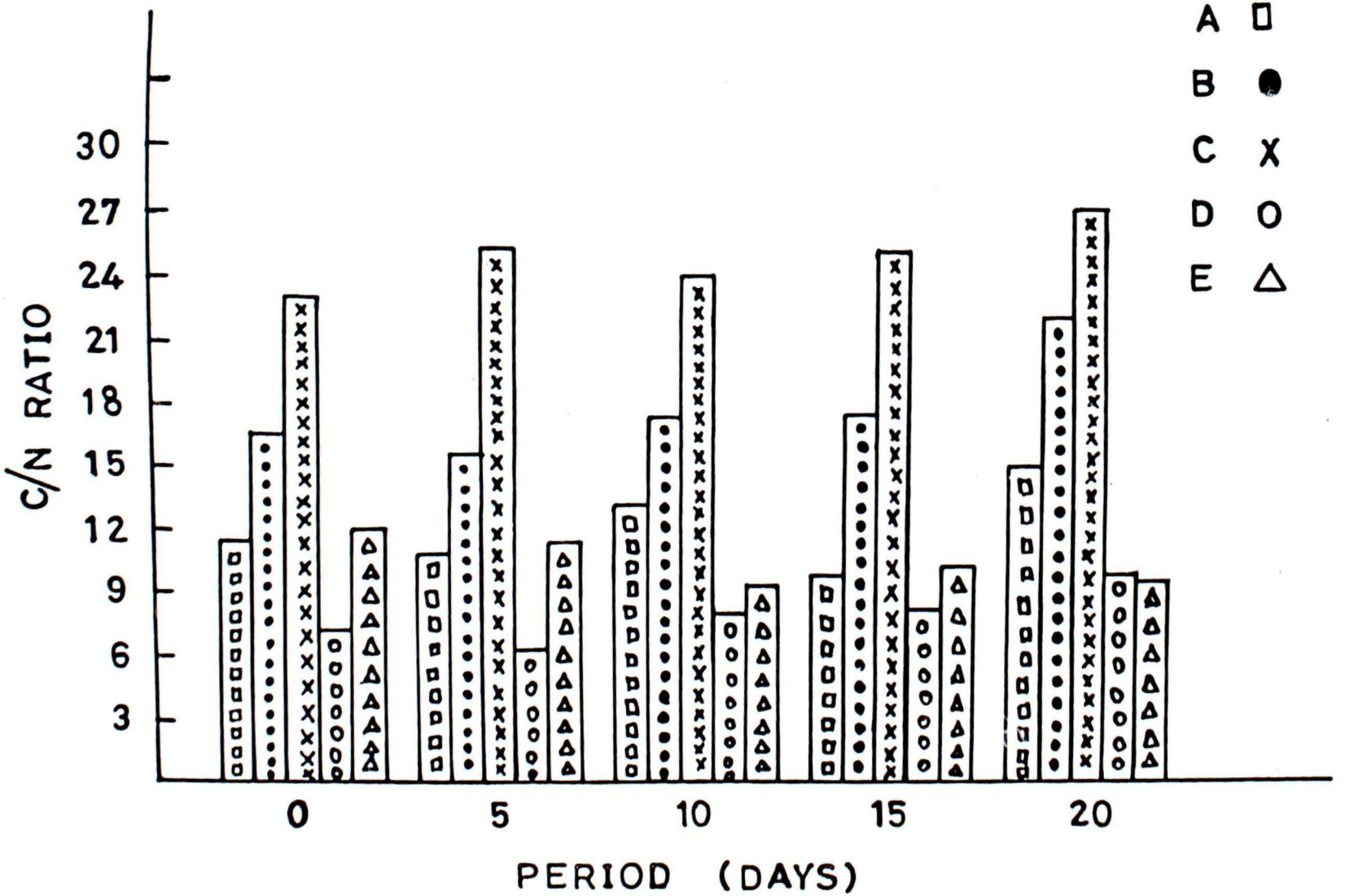
A ---- 1:1

B ---- 5:1

C ---- 1:0

D ---- 0:1

E ---- 1:5


A
B
C
D
E
C/N RATIO
30
27
24
21
18
15
12
9
6
3
0
5
10
15
20
PERIOD (DAYS)

temperature of fermentation and it was noted that the most suitable C/N ratio was 30. Jain et al. (1981) also found more degradation of organic solids, mainly cellulose at the C/N ratio of 15.22. The optimum C/N ratios for anaerobic digestion were also reported as 25-32 (Hills and Robberts, 1981), 29.5 (Ghosh and Das, 1982) and 25-30 (Lingaiah and Rajasekaran, 1986). In the present study, the degradation of nitrogen content of slurry samples may result in increase of C/N ratio.

4.9 VOLATILE FATTY ACIDS (VFA)

Figure 8 shows the total volatile fatty acids (TVFA) of the slurry samples during the anaerobic digestion of cattle dung and the dairy effluent sludge. Increase in TVFA was recorded in all the cases, which continued to increase even after 20 days in the ratio of 1:1 and 5:1. Maximum TVFA (8190 mg acetic acid/l) was noticed in 5:1 ratio which was followed by 1:1 ratio (7680 mg acetic acid/l). These values indicated the excessive acidogenesis and acid accumulation. Methane production in such cases was very less upto 15 days. Rest of the combinations recorded increase and then decrease in TVFA concentrations. In case of cow dung (1:0), acid accumulation upto the level of 7790 mg acetic acid/l occurred in 15 days which decreased considerably to 4500 mg acetic acid/l after 20 days. Values of TVFA in dairy effluent sludge (0:1) and 1:5 combinations were comparatively lower than other combinations. Decrease in TVFA concentrations in the substrates started after the 5th and 10th day. Cattle dung, dairy effluent sludge and 1:5 combination showed that acidogenic stage lasted for 15, 10 and 5 days, respectively, after that methanogenesis occurred which decreased the concentration of TVFA. Singh et al. (1981) found maximum VFA (6630 ppm) build up in the third week and second week (5869 ppm) during the anaerobic digestion of cattle dung and the cattle dung - pressed mud mixture, respectively. Maximum VFA

Fig. 8. Changes in total volatile fatty acids (mg acetic acid/l) during the anaerobic digestion of cattle dung and dairy effluent sludge

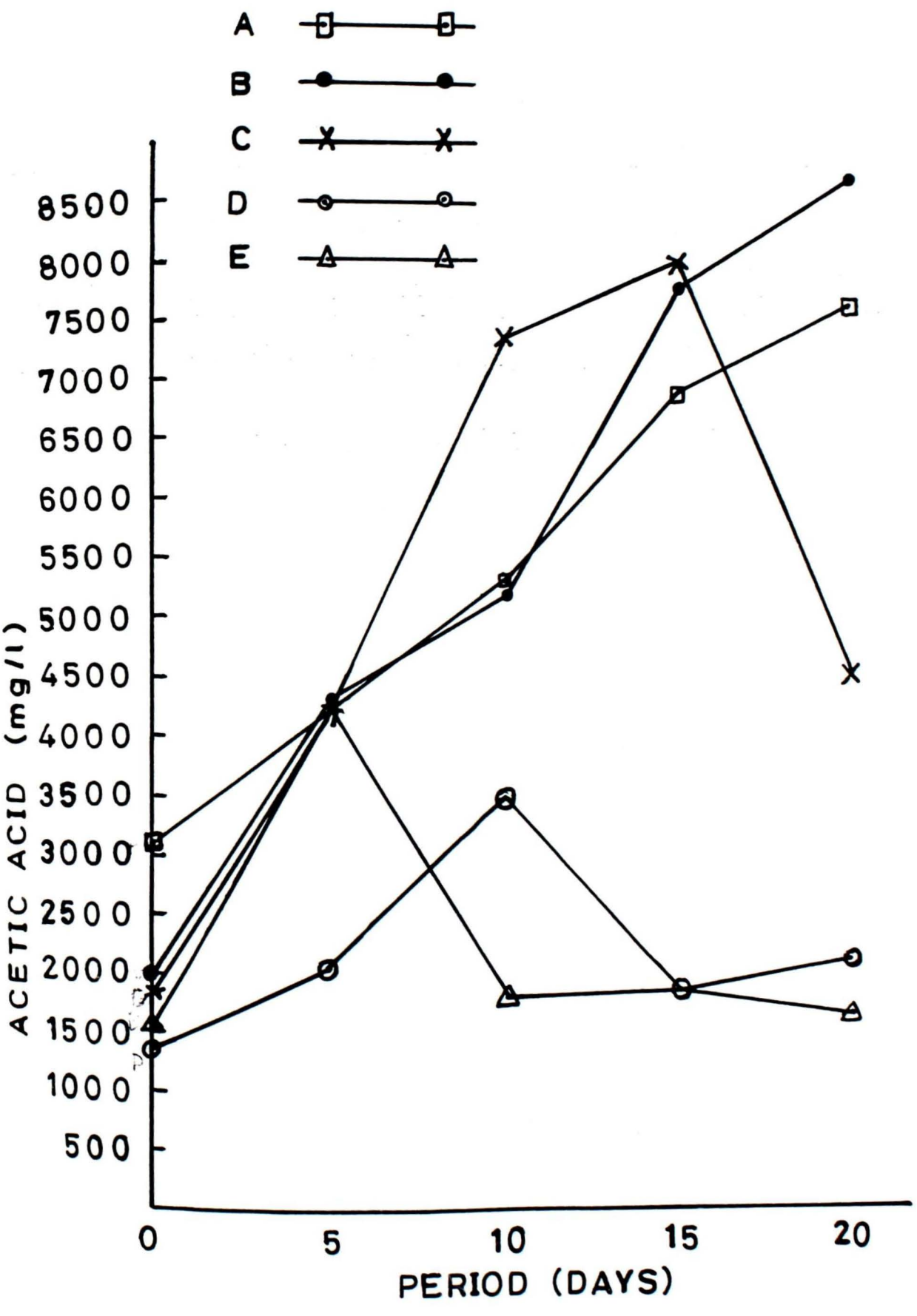
A ---- 1:1

B ---- 5:1

C ---- 1:0

D ---- 0:1

E ---- 1:5


A
B
C
D
E
8500
8000
7500
7000
6500
6000
5500
5000
4500
4000
3500
3000
2500
2000
1500
1000
500
ACETIC ACID (mg/l)
0
5
10
15
20
PERIOD (DAYS)

(4360 mg/l) were produced in sewage sludge (Cox, 1983), 0.5 kg m^{-3} (Hashimato, 1984) and 150 mg/l (Sarda and Krishna, 1989). Present study revealed that the production of acids occurred when cow dung was supplemented with an equivalent and one-fifth proportions of dairy effluent sludge.

The fractions of VFA are given in Figures 9, 10 and Table 5. Acetic acid constituted the major part of the TVFA. Its values in 1:1 combination, ranged from 42.5 and 82.7 mM/l. After 15 days, a considerable decrease in acetate production was recorded. In 5:1 combination, fluctuation in acetate concentration was recorded. Acetate concentration increased upto 15 days in cattle dung (1:0) then decreased rapidly after 20 days. Dairy effluent sludge (0:1) showed the presence of acetic acid as single representative of VFA at 0 day which increased later on. In 1:5 combination, decrease in acetate commenced after 10 days of fermentation. The proportions of individual fatty acids were found to vary with time as well as the nature of substrate (Fig. 9). In the initial stages, propionic acid was more than butyric acid and the change in proportions was recorded in later stages. Butyric acid was found to be totally degraded after 15 days and 20 days in dairy effluent sludge (0:1) and 1:5 combination, respectively, while total degradation of propionic acid occurred after 15 days in 1:5 combination. But build up in this acid was found after 20 days digestion in 1:5 combination (12.8 mM/l), dairy effluent sludge (16.5 mM/l) and 1:1 combination (24.4 mM/l). Butyrate concentration was found to increase after 20 day digestion of 1:1, 5:1 and 1:0 (cow dung) slurries. Concentrations of VFA were found in order of acetic propionic butyric valeric isobutyric isovaleric (Patni and Jui, 1985). Similar trend in VFA was seen in this study, here acetic propionic butyric acid were recorded.

Table 5. The fractions of VFA during the anaerobic digestion of cattle dung and dairy effluent sludge.

Period (days)	Concentration of VFA (mM/l)				
	Cattle dung : Dairy effluent sludge				
	1:1	5:1	1:0	0:1	1:5
	ACETIC ACID				
0	42.5	22.9	28.4	21.6	20.9
5	39.8	56.6	32.5	20.6	56.9
10	75.2	73.8	71.6	36.6	27.5
15	82.7	53.4	74.4	23.9	20.0
20	63.8	79.7	50.6	14.1	18.4
	PROPIONIC ACID				
0	12.3	5.8	6.9	-	5.6
5	9.6	8.4	7.8	5.6	9.1
10	15.0	11.3	11.3	7.2	8.1
15	11.5	13.8	14.7	5.3	-
20	24.4	14.1	9.7	16.5	12.8
	BUTYRIC ACID				
0	5.4	4.4	6.9	-	2.2
5	11.5	6.9	5.3	3.8	5.3
10	12.3	18.8	15.6	5.3	3.8
15	16.9	16.6	15.0	-	0.9
20	21.6	16.6	13.4	-	-

Fig. 9. VFA fractions (%) during the anaerobic digestion of cattle dung and dairy effluent sludge

A ---- 1:1

B ---- 5:1

C ---- 1:0

D ---- 0:1

E ---- 1:5

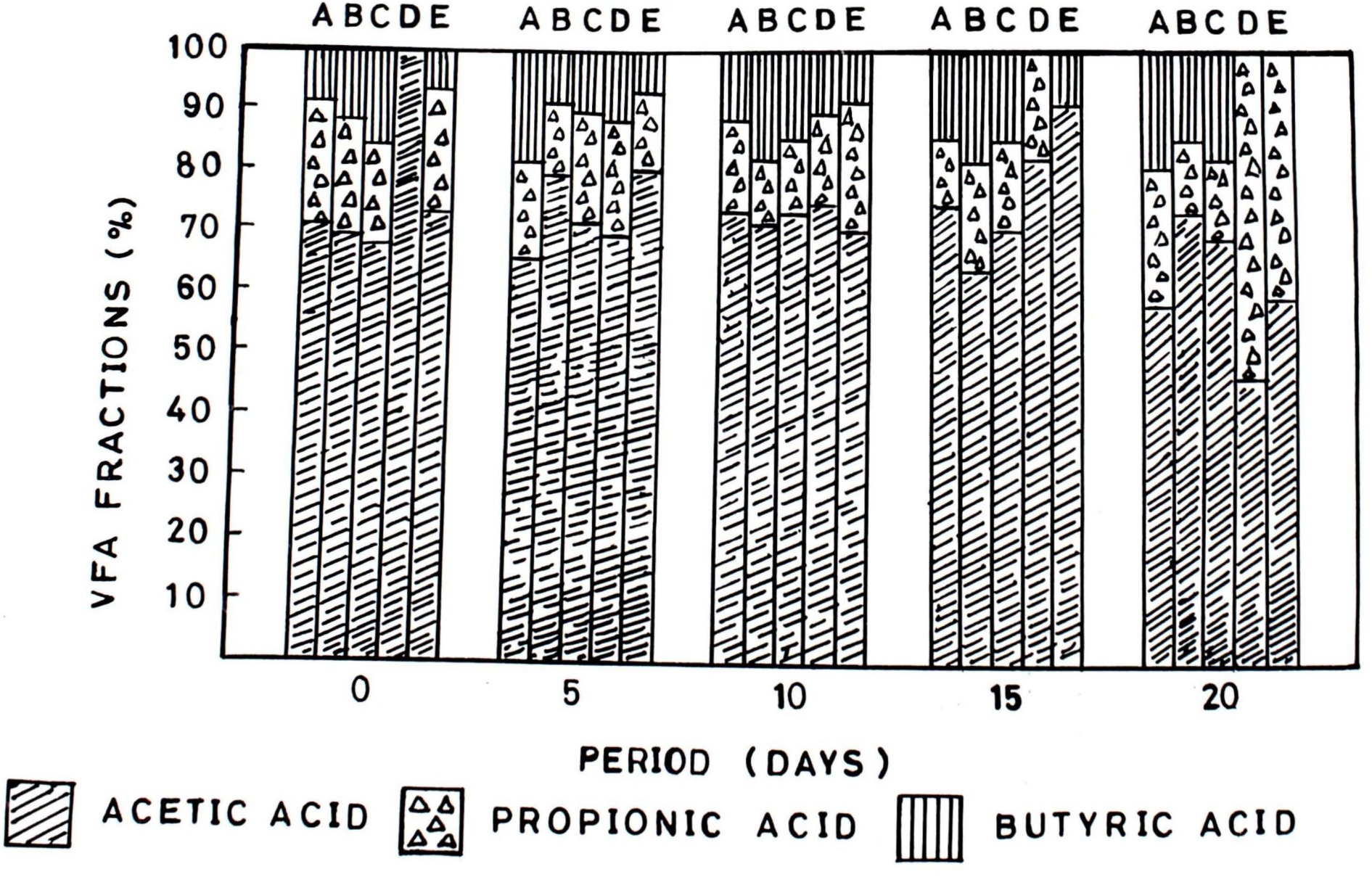

A B C D E
A B C D E
A B C D E
A B C D E
A B C D E
100
90
80
70
60
50
40
30
20
10
VFA FRACTIONS (%)
0
5
10
15
20
PERIOD (DAYS)
ACETIC ACID
PROPIONIC ACID
BUTYRIC ACID

Table 10. (a) Chromatographic pattern of VFA during the anaerobic digestion of cattle dung and dairy effluent sludge

1 = Acetic acid A ---- 1:1

2 = Propionic acid B ---- 5:1

3 = Butyric acid

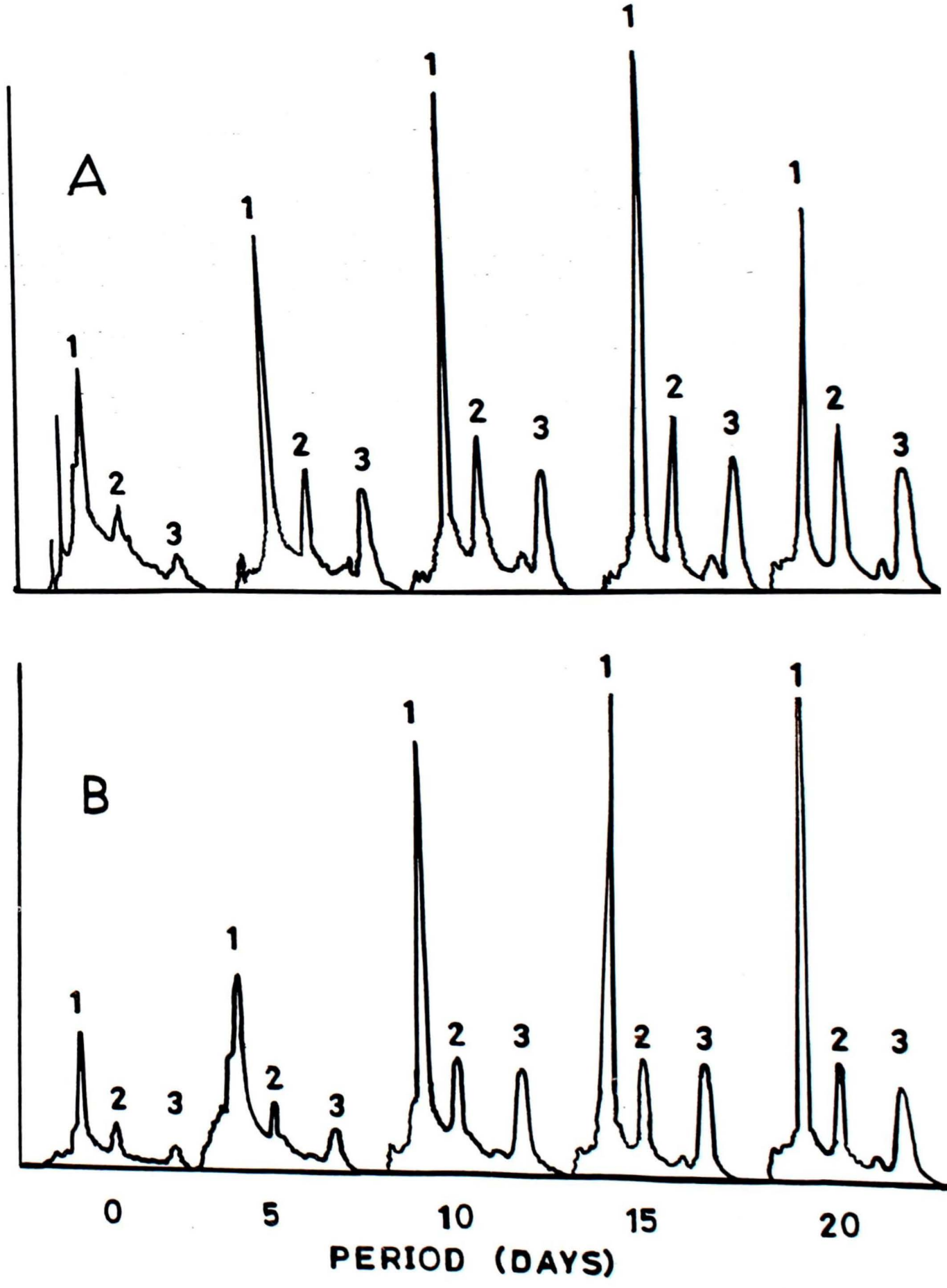
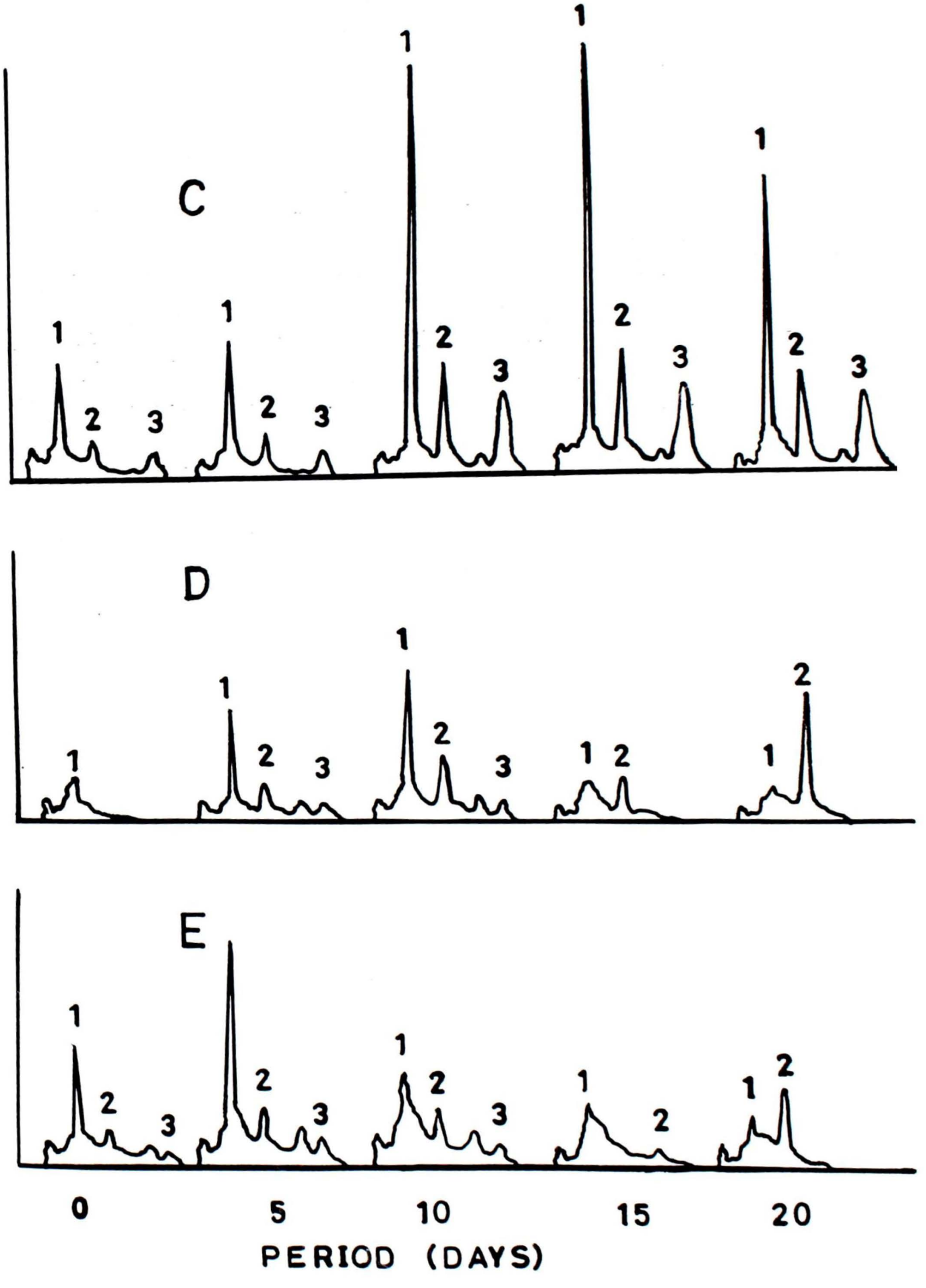

A
B
1
2
3
0
5
10
15
20
PERIOD (DAYS)

Fig. 10. (b) Chromatographic pattern of VFA during the anaerobic digestion of cattle dung and dairy effluent sludge

1 = Acetic acid C ---- 1:0

2 = Propionic acid D ---- 0:1

3 = Butyric acid E ---- 1:5


C
D
E
0
5
10
15
20
PERIOD (DAYS)

Singh et al. (1981) found high propionic and butyric acid accumulations during anaerobic digestion of cattle dung. The accumulations were not significant when pressed mud was added to the cattle dung.

Acetic acid was found to constitute 92.5 to 94.37 % of TVFA (Sohal et al., 1990). In undigested slurries, the range of acetic acid was 67.3 to 100 % of TVFA in the present study. Acetate was found to be the key of intermediates during the methane production and the maximum methane is produced from this acid (NAS, 1977). In the present study, methane content increased alongwith decrease in acetic acid concentration. Accumulation of propionic acid resulted in decrease in volumeric biogas production from dairy effluent sludge (0:1) and 1:5 combination. More biogas was produced from 1:1 combination, proportionate accumulation of propionate was lesser in this case. Fischer et al. (1981) also noted that the propionic acid production upset the digester.

4.10 MICROBIOLOGY OF ANAEROBIC DIGESTION

Various groups of non-methanogenic bacteria were enumerated using Astell roll-tube technique (Fig. 11).

4.10.1 TOTAL ANAEROBIC BACTERIAL COUNT

An increase in total anaerobic count was observed after 20 days of digestion (Table 6). Maximum population was observed in slurry of dairy effluent sludge after 15 days of fermentation. The initial counts in this slurry were very less at the time of loading. The increase in counts might be maximum due to the availability of easily degradable solids in dairy effluent sludge. When dairy effluent sludge was supplemented (5 parts) to cattle dung (1 part), the total anaerobic counts were found minimum. Higher counts after 20 days of the period revealed that conditions in digester were suitable for the growth of anaerobic

Fig. 11. Enumeration of different groups of anaerobic bacteria using roll tube technique

A ---- Acid forming bacteria

B ---- Proteolytic bacteria

C ---- Amylolytic bacteria

D ---- Lipolytic bacteria

E ---- Cellulolytic bacteria

F ---- Total anaerobic bacteria

A B C D E F

Table 6. Anaerobic bacterial counts during the anaerobic digestion of cattle dung and dairy effluent sludge.

Period (days)	Total anaerobic bacterial count (x 10^6 cfu/ml)				
	Cattle dung : Dairy effluent sludge				
	1:1	5:1	1:0	0:1	5:1
0	350	640	350	100	200
5	450	500	420	400	250
10	380	850	510	440	180
15	580	330	700	1390	110
20	950	510	920	860	340

bacteria. Cox (1983) found that total anaerobic counts were between 10^8-10^9/ml in high rate digester while 2 x 10^7/ml in controlled one. It was reported that the total anaerobic counts in slurries of pig manure were 7 x 10^7/ml (Fischer et al., 1984), 49-86 x 10^6/g were in cow dung (Rajasekaran et al., 1986) and 10^8-10^9/ml in biogas digester (Ramasamy et al., 1991). Neelakantan and Singh (1991) reported the presence of 115 x 10^6 cfu/ml and 140 x 10^6 cfu/ml of anaerobic bacteria in berseem hay and water hyacinth combined vegetable market waste mixture, respectively. Morphological study revealed that Gram positive rods dominated the population of these bacteria (Fig. 12).

4.10.2 CELLULOLYTIC BACTERIA

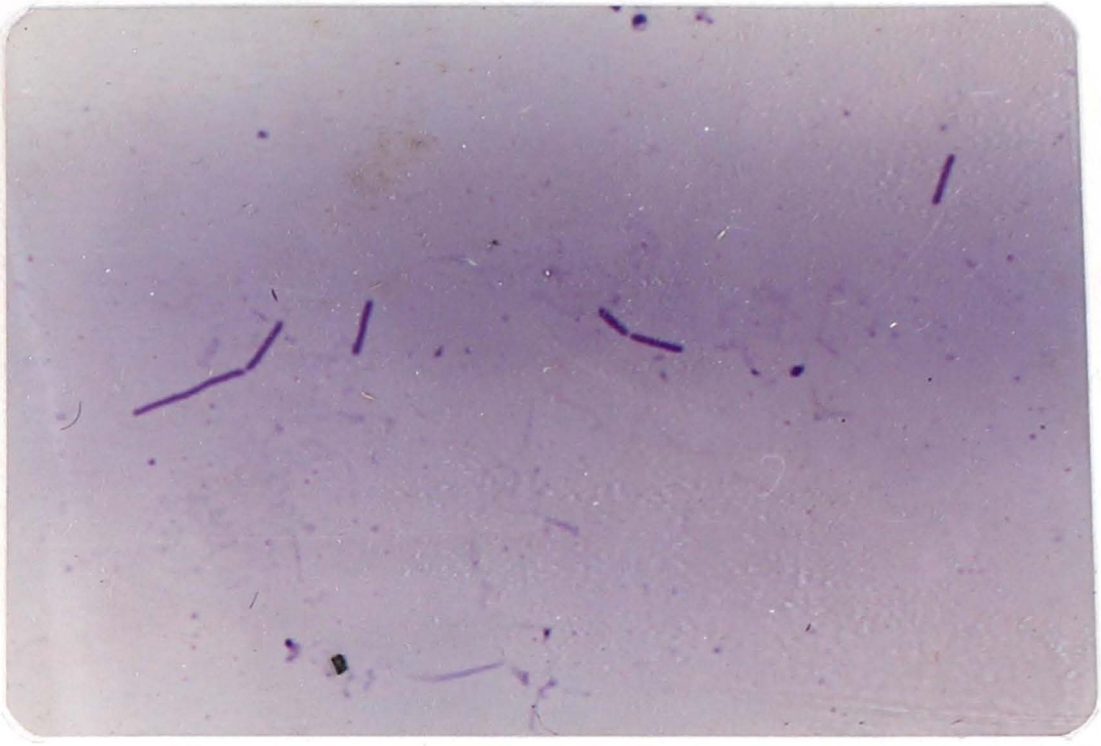
Table 7 shows the cellulolytic bacterial counts during the anaerobic digestion of various supplementation combinations of cattle dung and dairy effluent sludge. Cow dung (1:0) showed the highest number of cellulolytic bacteria at the time of charging the digester. During the anaerobic digestion, the counts decreased gradually, this might be due to limited cellulolytic activity in the slurry. Other combinations showed an increase in the cellulolytic bacterial growth. The population was higher in these samples on the 20th day. The maximum counts were recorded in dairy effluent sludge (0:1) and 1:1 combination. The maximum cellulolytic population after 20 days of digestion was 118 x 10^6 cfu/ml. The maximum cellulolytic activity occurred in slurries of 1:1 and 0:1 combinations. Bal et al. (1990) reported 26 x 10^8 cfu/g cellulolytic bacteria in cattle dung. Cellulolytic counts were reported to be 15 - 25 x 10^5 cells/g (Neelakantan, 1987) and 18.4 x 10^5 cells/g cattle dung slurries (Neelakantan and Singh, 1991). Gram positive rods were predominant among the population of cellulolytic bacteria in the present study (Fig. 13).

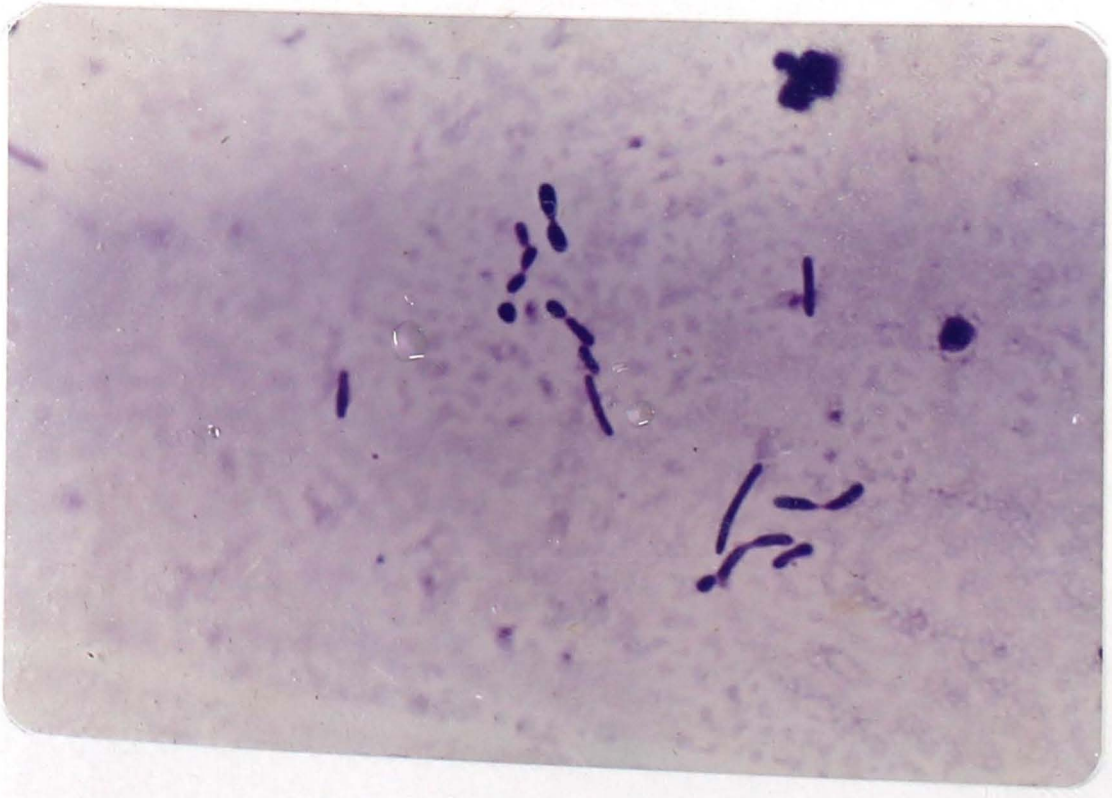
Table 7. Cellulolytic bacteria counts during the anaerobic digestion of cattle dung and dairy effluent sludge.

Period (days)	Cellulolytic counts (x 10^6 cfu/ml)				
	Cattle dung : Dairy effluent sludge				
	1:1	5:1	1:0	0:1	1:5
0	8	35	84	2	10
5	65	30	52	8	15
10	56	40	35	42	14
15	72	73	30	53	30
20	118	103	22	67	42

Fig. 12. Morphological characteristics of total anaerobic bacteria.

Fig. 13. Morphological characteristics of cellulolytic bacteria.





4.10.3 AMYLOLYTIC BACTERIA

Table 8 depicts the amylolytic bacterial counts during the digestion of cattle dung and dairy effluent sludge. The population of amylolytic bacteria was lesser than that of cellulolytic bacteria. In all the slurry samples except dairy effluent sludge, the amylolytic count decreased after the 15th day, whereas in slurry of dairy effluent sludge, the amylolytic counts showed graded increase. Maximum (72 x 10^6 cfu/ml) population was recorded in 1:1 combination, whereas 1:5 showed relatively lower counts. The reason for decrease in count after 15 days might be due to the complete degradation of starch during the digestion of slurries. Cattle dung slurry was reported to contain an amylolytic counts of 0.7 x 10^6/ml (Fischer et al., 1984), 23 x 10^8 cfu/g (Bal et al., 1990) and 28 x 10^5 cells/g (Neelakantan and Singh, 1991). Morphological studies of this group showed that the most of the bacteria were Gram positive and spores, rods and cocci were main constituents of the population (Fig. 14).

4.10.4 PROTEOLYTIC BACTERIA

The population of proteolytic bacteria increased after 20 days of digestion (Table 9). Maximum (88 x 10^6 cfu/ml) population was recorded in the case of 1:1 combination. The combination 1:5 showed a trend of maximum increase of population during the fermentation. The higher rates of population increase were observed in slurries containing dairy effluent sludge. The increase in population might be due to the higher amount of crude protein content present in these slurries. The maximum increase in population in 1:5 slurry indicated the maximum proteolytic activity. The proteolytic bacterial counts of 5-30 x 10^5 cells/g (Neelakantan, 1987), 22 x 10^8 cfu/g (Bal et al., 1990) and

Table 8. Amylolytic bacterial counts during the anaerobic digestion of cattle dung and dairy effluent sludge.

Period (days)	Amylolytic bacterial counts (x 10^6 cfu/ml)				
	Cattle dung : Dairy effluent sludge				
	1:1	5:1	1:0	0:1	1:5
0	6	15	25	8	23
5	40	30	52	20	22
10	70	65	58	27	12
15	72	53	52	25	18
20	56	49	28	38	17

Table 9. Proteolytic bacterial counts during the anaerobic digestion of cattle dung and dairy effluent sludge.

Period (days)	Proteolytic bacterial counts (x 10^6 cfu/ml)				
	Cattle dung : Dairy effluent sludge				
	1:1	5:1	1:0	0:1	1:5
0	43	58	53	44	15
5	47	51	63	45	11
10	85	64	67	55	30
15	79	65	62	62	41
20	88	60	72	80	50

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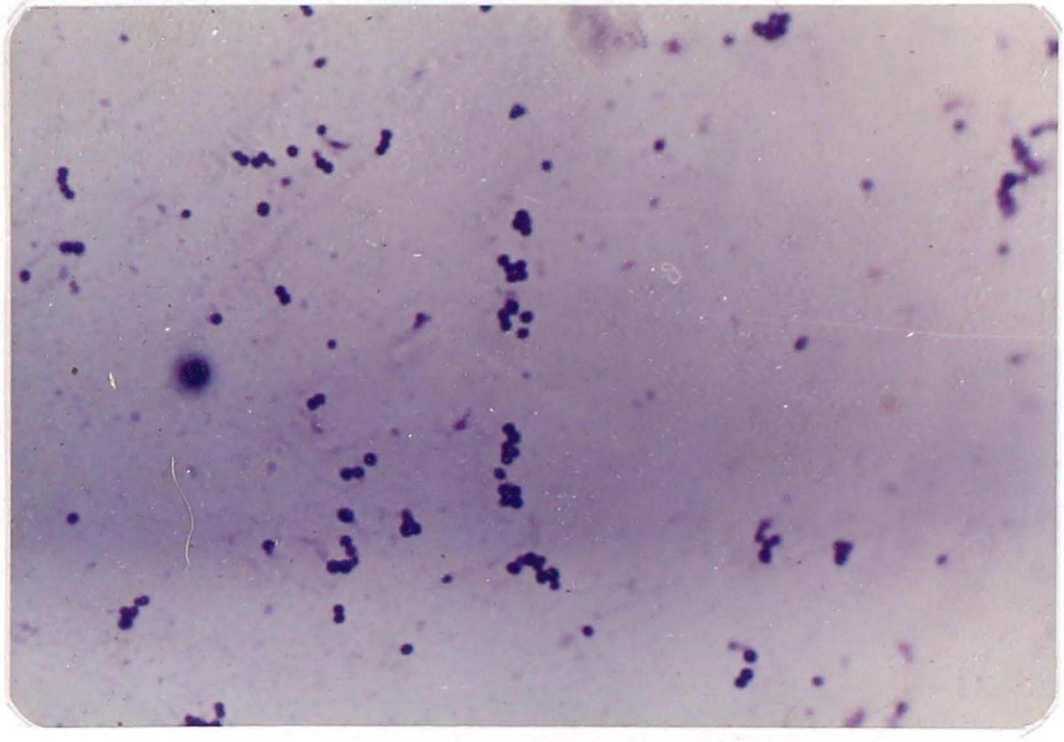
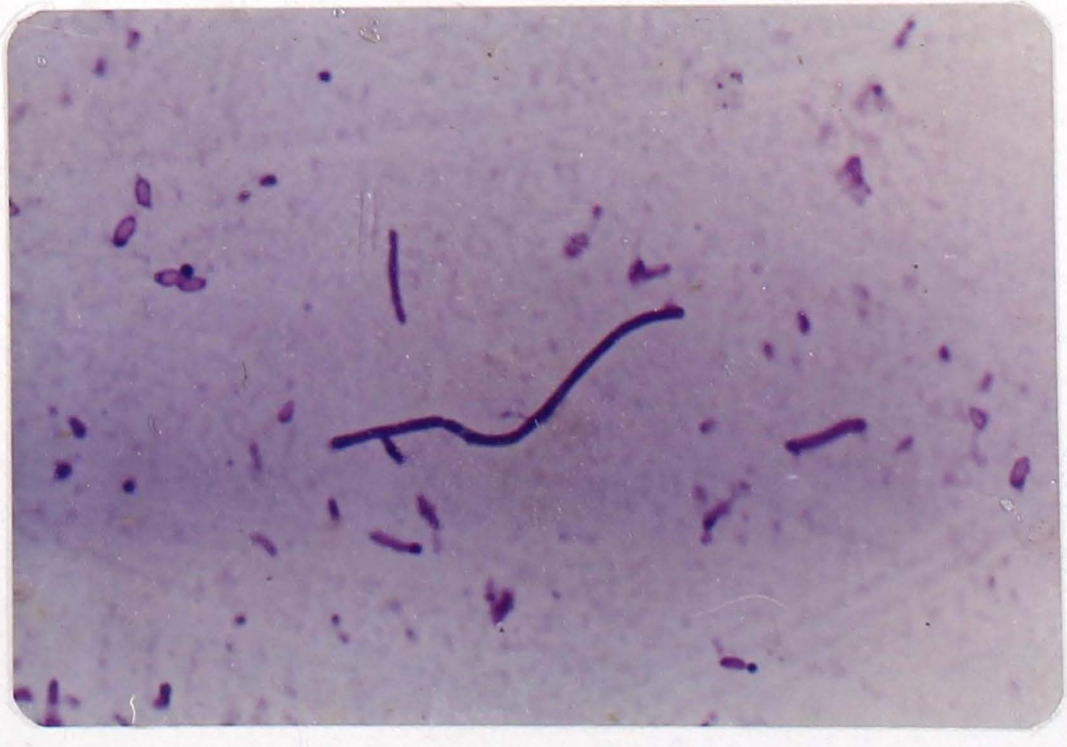
Fig. 14. Morphological characteristics of amylolytic bacteria.

Fig. 15. Morphological characteristics of proteolytic bacteria.





0.2-2.5 x 10^5 cfu/g (Neelakantan and Singh, 1991) were reported in the slurries of cattle dung. The microscopic examination of bacteria showed the predominance of Gram positive club-shaped rods. Many spore forming rods were also encountered (Fig. 15).

4.10.5 LIPOLYTIC BACTERIA

Table 10 shows the population of lipolytic bacteria during the anaerobic digestion of cattle dung and dairy effluent sludge. The combination of 1:1 showed a decrease in counts after 20 days, whereas all the other combinations showed an increasing trend. Maximum (46 x 10^6 cfu/ml) population was observed in the case of 5:1 combination. Increase in the lipolytic counts might be due to the appropriate lipid degradation by these bacteria. The lipolytic bacteria were predominantly Gram positive. Morphologically, cocci constituted the major part of the bacterial population. The cocci were mostly present singly, in pairs, chains and groups of four (Fig. 16). In the cattle dung, the lipolytic bacteria were 3 x 10^8 cfu/g (Bal et al., 1990). Counts of 0.9-3.8 x 10^5 cells/g (Neelakantan, 1987) and 4.4 x 10^5 cells/g (Neelakantan and Singh, 1991) of lipolytic bacteria in cow dung slurries have been reported.

4.10.6 ACID FORMING BACTERIA

Table 11 depicts the acid forming bacteria during the digestion of cattle dung and dairy effluent sludge. The population of these bacteria was very less as compared to other hydrolytic bacteria in the slurries. The maximum population of this group was 5.9 x 10^6 cfu/ml in the cattle dung slurry. Rajasekaran et al. (1986) reported 37.5-109 x 10^3/g of acid formers in cow dung slurry undergoing digestion. Morphologically, these bacteria were predominantly rod shaped (Fig. 17). Gram positive bacteria were present in all the fields during the microscopic examination.

Table 10. Lipolytic bacterial counts during the anaerobic digestion of cattle dung and dairy effluent sludge.

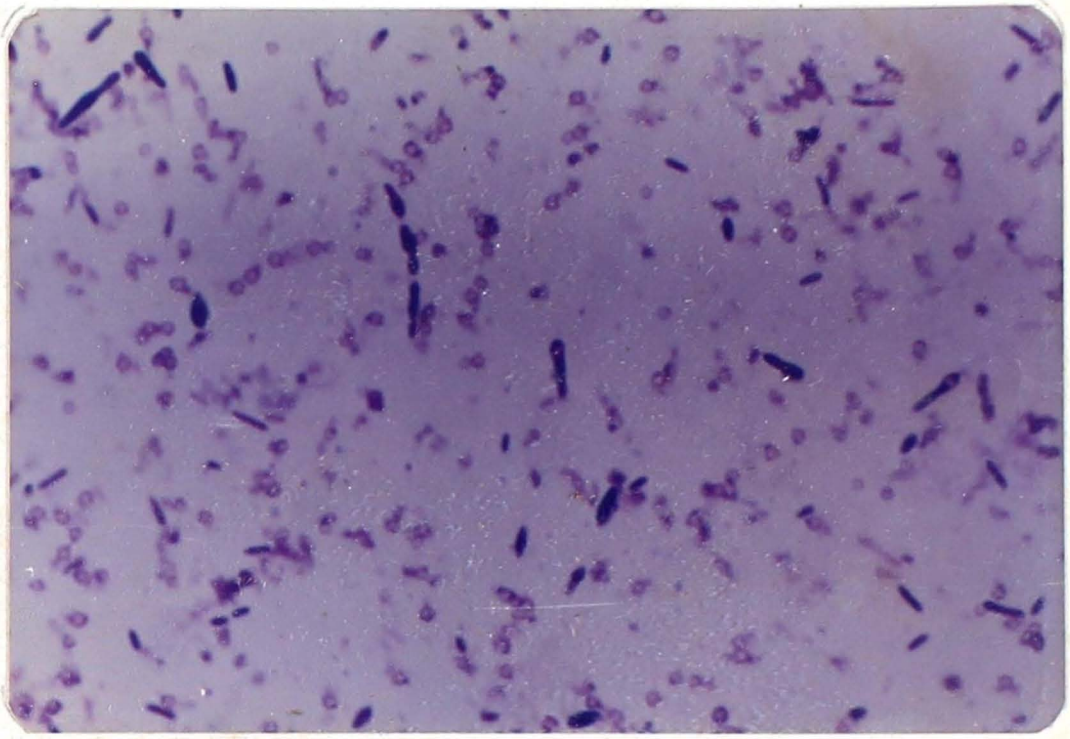
Period (days)	Lipolytic bacterial counts (x 10^6 cfu/ml)				
	Cattle dung : Dairy effluent sludge				
	1:1	5:1	1:0	0:1	1:5
0	59	9	17	2	3
5	38	14	15	9	13
10	25	24	31	11	17
15	30	40	30	10	18
20	31	46	40	14	35

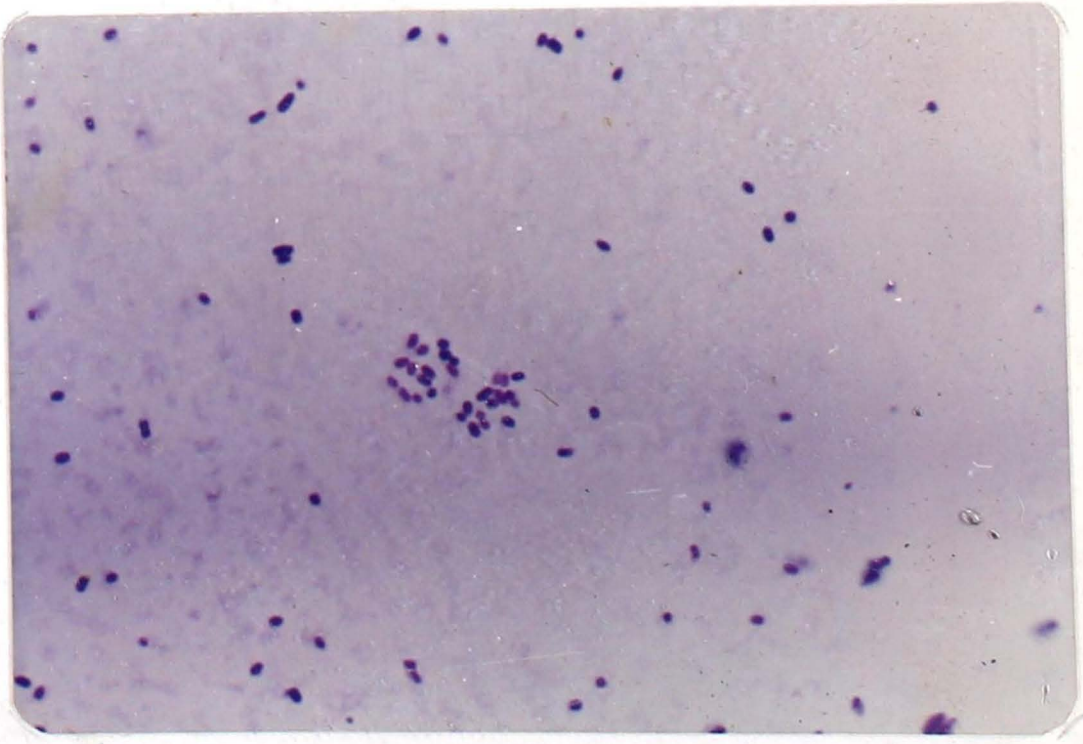
Table 11. Acid producing bacteria during the anaerobic digestion of cattle dung and dairy effluent sludge.

Period (days)	Acid forming bacterial counts (x 10^6 cfu/ml)				
	Cattle dung : Dairy effluent sludge				
	1:1	5:1	1:0	0:1	1:5
0	0.7	0.3	4.0	3.0	0.5
5	3.1	2.5	5.2	3.8	1.8
10	5.5	5.8	5.9	3.4	2.1
15	2.8	2.9	4.1	3.0	1.2
20	2.3	2.4	3.0	1.0	3.0

Fig. 16. Morphological characteristics of lipolytic bacteria.

Fig. 17. Morphological characteristics of acid forming bacteria.





4.11 BIOGAS PRODUCTION

It was observed that the biogas production in all the combinations was low during the early fermentation periods (Fig. 18) which might be due to the inoculum (rumen fluid) that contain less methanogenic bacterial population. Maximum (142.68 ml/g VS) biogas was produced with dairy effluent sludge (0:1) followed by 1:1 combination (82.76 ml/g VS), 1:5 combination (80.2 ml/g VS) and cattle dung (43.95 ml/g VS). The combination of 5:1 produced the least (32.25 ml/g VS) biogas. Maximum gas in dairy effluent sludge might be due to the early onset of methanogenesis in the digester and the presence of easily degradable solids. Biogas production from cattle dung was reported 7.72-7.74 ft^3/kg wet dung (Neelakantan, 1987) and 0.419 m^3/kg VS (Xavier and Nand, 1989). Biogas yield of 5.5-6.5 1/day was found when cattle dung supplemented with pulp and paper mill effluent (Gupta and Awasthi, 1990), 392 1/kg DM from poultry litter, 310 1/kg DM from water hyacinth and 273 1/kg DM from market vegetable waste (Neelakantan and Singh, 1991).

4.12 METHANE CONTENT OF BIOGAS

There was very less methane production in earlier stages of digestion but increased gradually (Fig. 19). In combination of 1:5 and 1:0 (dairy effluent sludge), the methane production started increasing after 10 days and reached maximum after 20 day of digestion. In other cases, methane production was very less upto the 15th day of the digestion and biogas contained higher concentration of CO_2. After 20 days, There was a sudden increase in methane content. In 1:1 slurry, maximum increase in CH_4 was found after 20 days. Overall, maximum (48.95 %) concentration of CH_4 was found in the case of dairy effluent sludge followed by the combination of 1:1 (44.7 %) and 1:5 (43.96 %). The

Fig. 18. Biogas production (ml) during the anaerobic digestion of cattle dung and dairy effluent sludge

A ---- 1:1

B ---- 5:1

C ---- 1:0

D ---- 0:1

E ---- 1:5

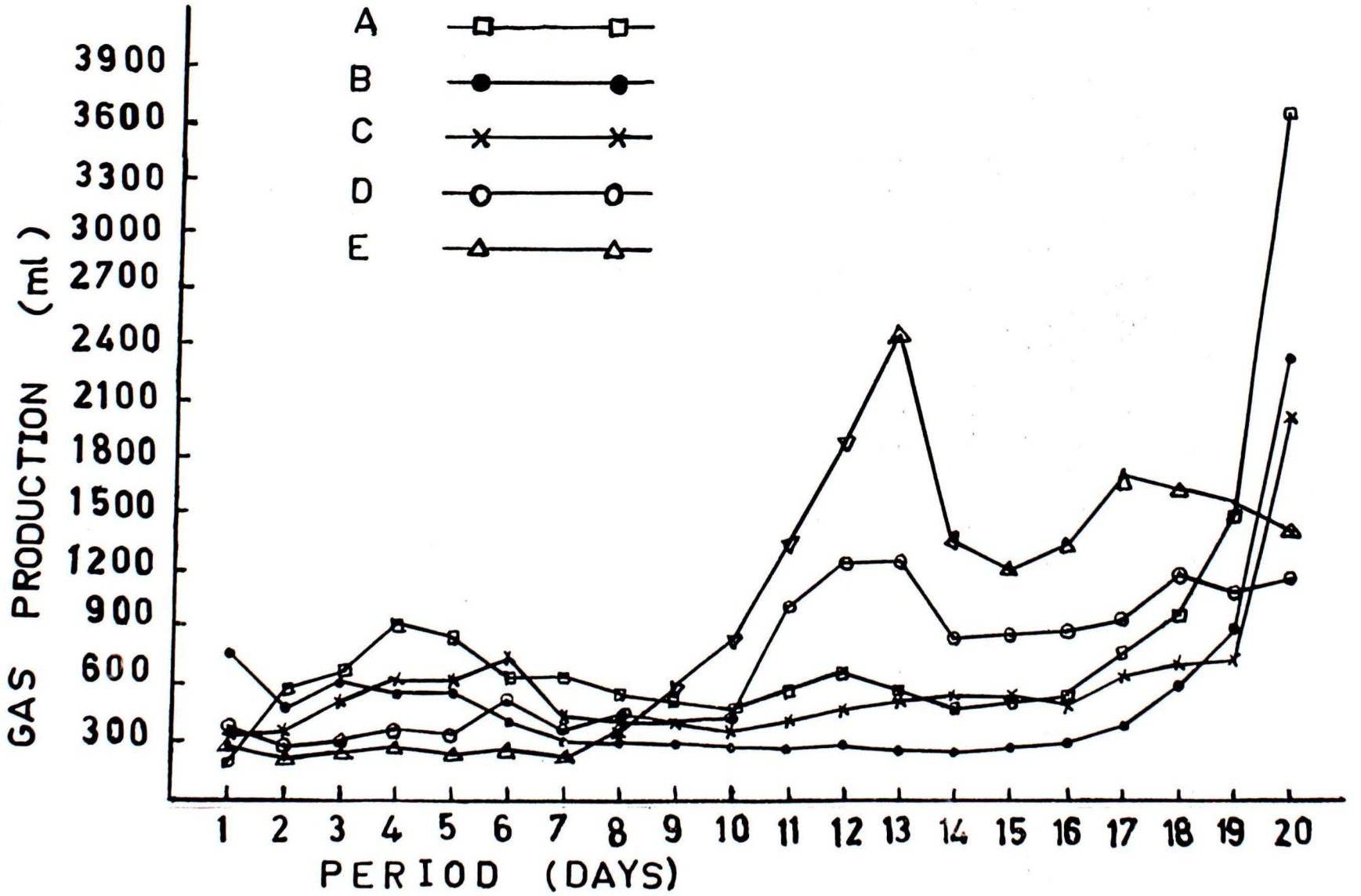

A
B
C
D
E
3900
3600
3300
3000
2700
2400
2100
1800
1500
1200
900
600
300
GAS PRODUCTION (ml)
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20
PERIOD (DAYS)

Fig. 19. Biogas constituents (%) during the anaerobic digestion of cattle dung and dairy effluent sludge

A ---- 1:1

B ---- 5:1

C ---- 1:0

D ---- 0:1

E ---- 1:5

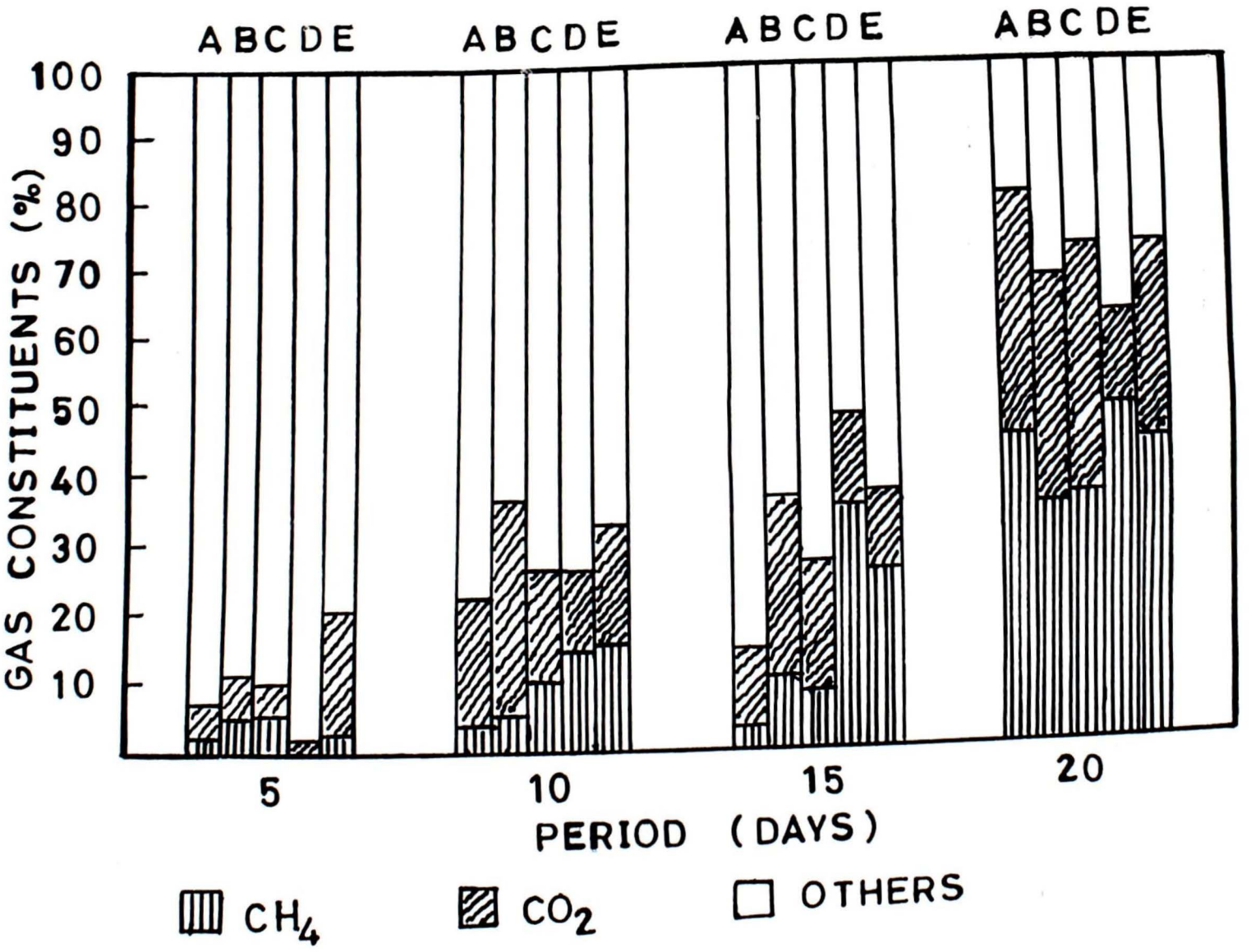

A B C D E
A B C D E
A B C D E
A B C D E
100
90
80
70
60
50
40
30
20
10
GAS CONSTITUENTS (%)
5
10
15
20
PERIOD (DAYS)
CH4
CO2
OTHERS

Fig. 20. Chromatographic pattern of constituents of biogas produced after a 20 days of anaerobic digestion of cattle dung and dairy effluent sludge

M ---- CH_4

C ---- CO_2

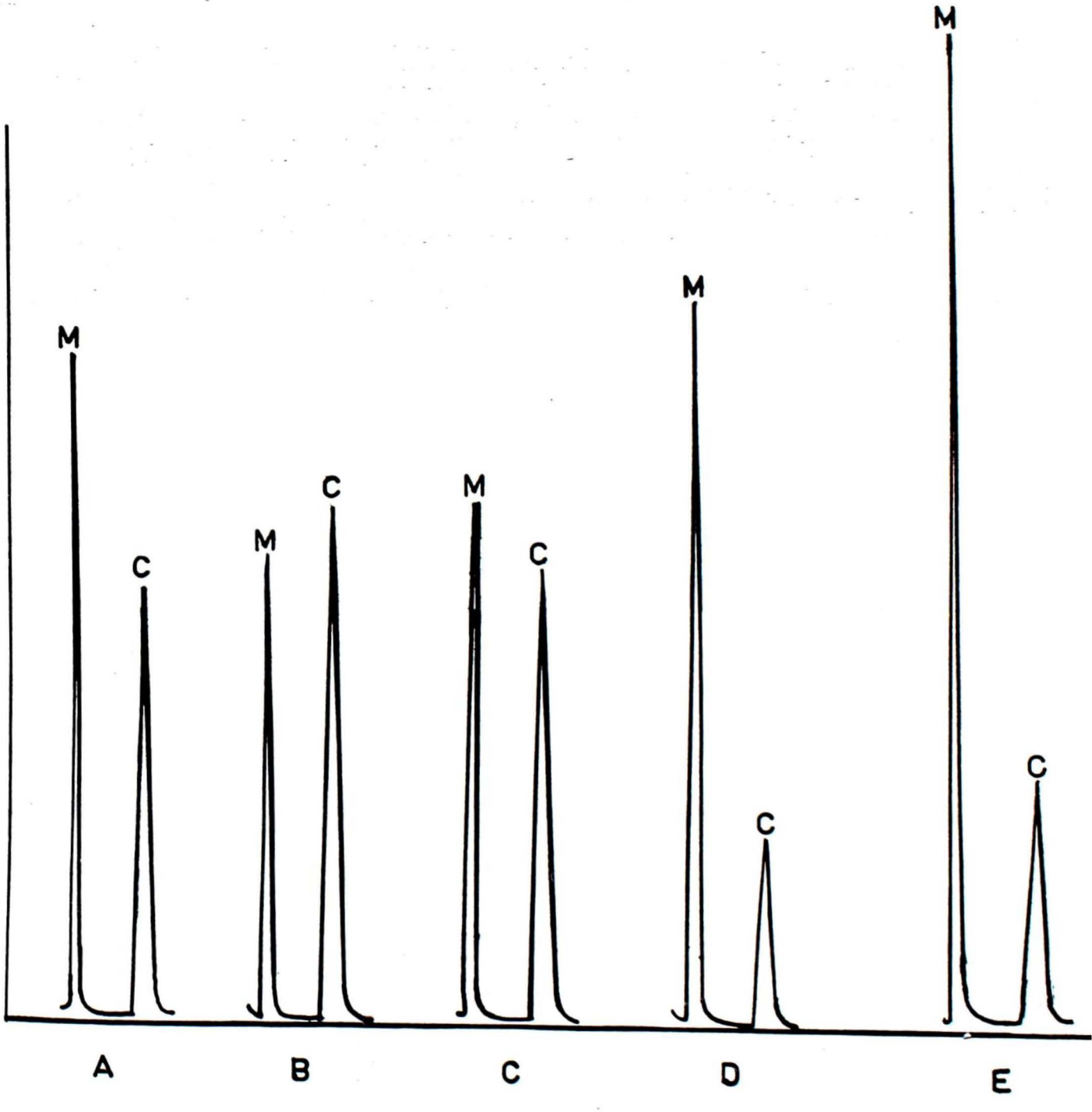
A ---- 1:1

B ---- 5:1

C ---- 1:0

D ---- 0:1

E ---- 1:5


M
C
M
C
M
C
M
C
M
C
A
B
C
D
E

combinations of 1:0 and 5:1 produced 36.2 % and 35.2 % CH_4. Gas chromatographic pattern of CH_4 and CO_2 in biogas after 20 days is shown in Figure 20. High concentration of methane in dairy effluent sludge might be due to the higher degradation of volatile solids (Table 2), negative redox potential (Fig. 5) and high acetate degradation (Table 4). Methane content increased after 15 days in 1:1, 1:0 and 5:1 combinations, indicating that the methanogenesis started after two weeks in these slurries. Methane contents reported during the anaerobic digestion of cattle dung were 70 % (Summers and Bousfield, 1976), 62-66 % (Singh et al., 1980), 60 % (Jain et al., 1981), 55-60 % (Mahadevswami and Venkatraman, 1986) and 56-60 % (Kanwar and Kalia, 1991). Gunaseelan (1987) found 60-70 % methane during anaerobic digestion of cattle manure and Parthenium mixture. Methane content was lower in the present study. This was probably because of two reasons, one, the inoculum was rumen fluid which contained lesser numbers of methanogenic bacteria. Secondly, methanogenesis commenced after 15 days and digester was operated under acidogenic phase.

4.13 STATISTICAL ANALYSIS

Analysis of variance revealed that all the treatments had significantly different effects on all the parameters except dissolved oxygen (Table 12). The effect on propionic acid was found significant at 5 % level, whereas other parameters were significant at 1 % level. Effect of periods of digestion was insignificant on dissolved oxygen, acetic and butyric acids, whereas significantly different on total solids, propionic acid, carbon and C/N ratio at 1 % level and on pH, E_h, nitrogen, acetic acid and volatile fatty acids at 5 % level. The result showed that all the

Table 12. Analysis of variance for different parameters.

Source	d.f.	TS		VS		pH		E_h		D.O.		N	
		M.S.S.	F-Value	M.S.S.	F-Value	M.S.S.	F-Value	M.S.S.	F-Value	M.S.S.	F-Value	M.S.S.	F-Value
1	2	3	4	5	6	7	8	9	10	11	12	13	14
Supplementations	4	18.42*	154.44*	11.05	107.24*	1.41	11.68*	4962.14	11.08*	0.018	1.397+	3.78	20.05**
Digestion periods	4	2.14	17.9*	2.075	11.68*	0.39	3.23**	1411.14	3.15**	0.014	1.079+	0.67	3.53**
Error	16	0.12		0.107		0.12		447.89		0.013		0.19	

Contd....

Contd....(Table 12)

Source	d.f.	C		C/N		VFA		Acetic Acid		Propionic Acid		Butyric Acid	
		M.S.S.	F-Value	M.S.S.	F-Value	M.S.S.	F-Value	M.S.S.	F-Value	M.S.S.	F-Value	M.S.S.	F-Value
1	2	15	16	17	18	19	20	21	22	23	24	25	26
Supplementations	4	173.14	214.91*	225.03	125.72*	154325.19	6.54*	1466.45	6.54*	48.70	3.72**	164.29	9.97*
Digestion periods	4	10.79	13.39*	9.77	5.46*	87987.99	3.73**	629.48	3.73**	62.57	4.79*	47.76	2.89+
Error	16	0.81		1.79		23585.43		253.89		13.08		16.48	

* Significant at 1 % level of significance.

** Significant at 5 % level of significance.

\+ Non-significant.

parameters were affected differently by all the combinations except dissolved oxygen.

Comparative effects of different treatments are evendent from Table 13 showing mean differences of different treatments. Cattle dung (T_3) and dairy effluent sludge (T_4) were found to be significantly different from each other in terms of the effect on various parameters, while cow dung and 5:1 combination (T_2) were found somewhat similar to each other except their effect on volatile fatty acids, carbon and C/N ratio. Nitrogen, carbon and C/N ratio were found not to be affected significantly by cow dung and 1:1 combination (T_1). Cow dung and 1:5 combination (T_5) were different from each other except their effect on volatile solids, acetic and propionic acids. Among all the combinations, 1:1 and 5:1 were the most closely related, 5:1 and 1:5 combinations had the same difference as that between cow dung and 1:5 combination, whereas 1:1 and 1:5 were close to each other in terms of the effect on volatile solids, nitrogen, acetic acid and C/N ratio. The effect of all the combinations was different on carbon, whereas the effect on acetic acid was similar except that of dairy effluent sludge.

Table 14 depicts the comparative differences in effects of digestion periods on various parameters. The effect of digestion periods was similar on dissolved oxygen, acetic and butyric acids. The effect of twenty day period was significant. Ten days digestion period was found not to affect nitrogen, propionic acid and C/N ratio, whereas five days digestion affected E_h, volatile fatty acids and carbon content only. During five and ten days of the digestion, only volatile fatty acids and E_h were affected differently. E_h, VFA and C/N ratio were significantly affected during the period of the ten and fifteen days. During the last five days of the digestion, E_h, nitrogen and volatile fatty acids were

Table 13. Mean differences of different treatments (supplementations) for various parameters using Dunken's Multiple Range Test (DMRT).

Parameters	Treatments				
	T_1	T_2	T_3	T_4	T_5
TS*	9.51^{a}	9.20^{a}	8.86^{a}	6.52^{b}	11.91^{c}
VS*	6.26^{a}	6.56^{a}	7.06^{ac}	3.52^{b}	7.11^{ac}
pH*	5.99^{a}	5.77^{a}	6.01^{a}	6.96^{b}	6.78^{b}
E_h*	66.60^{a}	77.20^{a}	63.20^{a}	6.80^{b}	17.40^{b}
D.O.	0.28^{a}	0.16^{a}	0.24^{a}	0.14^{a}	0.24^{a}
N*	3.40^{ad}	2.36^{b}	1.91^{b}	4.07^{cd}	3.32^{a}
C*	38.10^{a}	41.39^{b}	46.33^{c}	31.17^{d}	34.62^{e}
C/N*	11.51^{a}	17.83^{b}	24.44^{c}	7.89^{de}	10.31^{ae}
VFA*	5432^{ab}	5490^{a}	5134^{b}	2168^{c}	2158^{c}
Acetic Acid*	60.80^{a}	57.28^{a}	51.50^{ac}	23.36^{bcd}	28.74^{ad}
Propionic Acid**	14.56^{a}	10.68^{ac}	10.08^{ac}	6.92^{bc}	7.12^{bc}
Butyric acid*	13.54^{a}	12.66^{a}	11.24^{a}	1.82^{b}	2.44^{b}

Similar superscripts mean no significant difference.

* Significant at 1 % level of significance.

** Significant at 5 % level of significance.

Table 14. Mean differences of different digestion periods for various parameters using DMRT.

Parameters	Digestion period				
	R_1	R_2	R_3	R_4	R_5
TS*	10.00^{a}	9.58^{ad}	9.30^{bd}	8.78^{be}	8.34^{ce}
VS*	6.91^{a}	6.48^{ad}	6.14^{bd}	5.72^{be}	5.26^{ce}
pH**	6.67^{a}	6.39^{ac}	5.97^{be}	6.08^{bc}	6.40^{ac}
E_h**	23.60^{a}	41.00^{b}	66.00^{c}	58.80^{d}	39.80^{b}
D.O.	0.24^{a}	0.24^{a}	0.22^{a}	0.12^{a}	0.24^{a}
N**	3.20^{a}	3.36^{a}	3.12^{ac}	2.97^{ac}	2.41^{bc}
C*	40.17^{a}	39.27^{bcd}	38.24^{be}	37.51^{ade}	36.41^{c}
C/N*	13.82^{a}	13.31^{a}	13.81^{a}	14.23^{ac}	16.83^{bc}
VFA*	1894^{a}	3796^{b}	4614^{c}	5242^{d}	4818^{c}
Acetic acid	27.26^{a}	41.28^{a}	56.94^{a}	50.88^{a}	45.32^{a}
Propionic acid*	6.12^{a}	8.10^{a}	10.58^{ac}	9.06^{ac}	15.50^{bc}
Butyric acid	13.80^{a}	6.56^{a}	11.16^{a}	9.88^{a}	10.32^{a}

Similar superscripts mean no significant difference.

* Significant at 1 % level of significance.

** Significant at 5 % level of significance.

significantly changed. After the first ten days, the changes in values of the parameters were significantly different except that of nitrogen, propionic acid and C/N ratio. After next ten days, i.e., from 10th - 20th day, changes in parameters except pH, VFA, propionic acid and carbon content. Therefore, it could be concluded that the significant changes in parameters were more during 10 days intervals as compared to 5 days. The effect of periods of digestion was minimum on propionic acid and nitrogen while E_h and volatile fatty acids were found to be affected significantly.

CHAPTER - 5

SUMMARY AND CONCLUSION

5. SUMMARY AND CONCLUSION

Anaerobic digestion of organic materials, is a three phase process comprising of hydrolysis, fermentation and methanogenesis. This process is carried out by different groups of bacteria resulting in the final production of combustible biogas.

In the present study, the acidogenic phase of the anaerobic digestion of cattle dung with supplementation of dairy effluent sludge was investigated. Different proportions of cattle dung and dairy effluent sludge with rumen liquor inocula were subjected to anaerobic digestion and the relevant chemical and bacteriological analysis including VFA and methane production were carried out at different time intervals.

Total solids and volatile solids were found to decrease with the progress of digestion. Maximum degradation of total solids (23.1 %) and volatile solids (33.2 %) were found in case of cattle dung and dairy effluent sludge mixture of 1:1 ratio. Digestion of cattle dung alone resulted in very less degradation. The pH decreased in all the slurry samples due to the acidogenesis. The decrease was more pronounced in cattle dung and 5:1 combination. The ratio of 1:5 and dairy effluent sludge alone showed comparatively slight decline in pH and final pH in these cases was higher than the initial values.

The redox potential of the slurries was found to indicate oxidised conditions except in 1:5 and 0:1 combinations, where reduced conditions were observed after 20 days of the digestion. Initially, dissolved oxygen was very less which further decreased very slowly. Oxygen was completely removed during the digestion of dairy effluent sludge.

Carbon contents decreased gradually with the advancement of the digestion. Slurries containing higher amounts of dairy effluent sludge (0:1, 1:5 and 1:1) showed relatively higher carbon utilization. Nitrogen contents were maximum (4.69 %) in dairy effluent sludge and the minimum (2.03 %) was in the cattle dung. C/N ratio varied between 7.15 and 23.07. The ratios in all slurries increased after 20 days except for 1:5 combination which was decreased. Final C/N ratio was in the range of 9.67 to 27.32.

The VFA varied with supplementation combinations. Accumulation of acids, representing higher rate of acidogenesis than methanogenesis, was found in 1:1, 5:1 and 1:0 combinations. Ratios of 0:1 and 1:5 showed no acid accumulation. Maximum VFA was found in 5:1 combination (8190 mg acetic acid/l), followed by cattle dung (7790 mg acetic acid/l). Acetic acid was the main volatile fatty acid which constituted 67.3-100 % of TVFA initially which was decreased to 46.08-72.19 % of TVFA after 20 days. Maximum acetic acid was produced in 5:1 combination (79.7 mM/l). Butyric and propionic acids were present in nearly equal proportions. Butyric acid was completely used up after 20th day in 0:1 and 1:5 combinations. Propionic acid was found to increase at the end of the digestion period.

Total anaerobic counts were in the range of 10^8-10^{10} cfu/ml. The cellulolytic, amylolytic, proteolytic and lipolytic bacteria were present in the range of 10^6-10^7 cfu/ml. The acid formers were less in number (10^5-10^6 cfu/ml) as compared to other groups.

Biogas production was very low in acidogenic stage. After 10 days of digestion, higher yields were recorded in 0:1 and 1:5, whereas an increase was noticed in 1:0, 1:1 and 5:1 combinations after the

18 days of digestion. Maximum methane content (48.95 %) was recorded in the biogas produced from the dairy effluent sludge, whereas in cattle dung digestion, it was minimum (36·32 %). Maximum biogas (142.68 mg/g VS) production was observed in dairy effluent sludge, whereas the minimum (32.25 ml/g) was in 5:1 combination.

Statistically, all the supplements had significantly different effect on the various chemical parameters except dissolved oxygen. Dairy effluent sludge (0:1) and cow dung (1:0) were found to be significantly different in term of the effect on various parameters. The ratio of 5:1 and 1:1 were similar to each other, whereas 1:5 combination was different. The digestion periods did not affected the acetic and butyric acids. Intervals of 10 days were found to have significantly more effect on the parameters as compared to 5 day intervals.

CONCLUSION

The present study revealed that the total solids, volatile solids and carbon decreased during the anaerobic digestion of cattle dung supplemented with dairy effluent sludge. The pH showed decreasing trends in early stage of fermentation which increased later on. The redox potential during the acidogenic phase represented oxidised conditions. The C/N ratio was found to increase with the progress of time and higher methane yields were obtained at lower C/N ratios. The volatile fatty acids increased during the early periods of digestion. Acetic acid was the main the VFA producted. Propionic acid accumulation occurred after 20 days of the digestion. Anaerobic bacterial population increased during digestion. Acid formers were found to be less in number than the other hydrolytic and fermentative bacteria. Methane content of biogas increased with the progress of digestion. Cattle dung when

supplemented with dairy effluent sludge showed higher gas production. Cattle dung alone and supplementation ratios of 1:1 and 5:1 were found to result in high acid accumulation. Acidogenic phase in these slurries lasted for two weeks and lower methane yields were recorded. In 1:5 combination, the acidogenesis lasted only for one week and reducing conditions were found after 20 days period. Dairy effluent sludge served as a good substrate for anaerobic digestion and the decrease in pH values was not so pronounced as in cattle dung. It could be concluded from this work that dairy effluent sludge would be a good source for methane recovery and could be used alone or the combination with cattle dung in digester.

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