OPTIMIZATION OF BIO - GAS PRODUCTION

Thesis

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DOCTOR OF PHILOSOPHY

By

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CERTIFICATE

This is to certify that the thesis entitled "OPTIMIZATION OF BIO-GAS PRODUCTION" submitted by Datta Madamwar, ID No. 79PL21002, in partial fulfilment of the requirements of Ph.D. degree of the Institute, embodies original work done by him under my supervision.

Dated: 13th May 1985

(B.M. MITHAL)
Supervisor
Professor of Pharmacy

To

My beloved parents.

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C O N T E N T S

CHAPTER-I

INTRODUCTION

CHAPTER-I

INTRODUCTION

BIO-GAS: ENERGY RECOVERY FROM ANIMAL WASTES

Due to present world-wide shortage of energy, many alternative fuel sources are receiving attention. Among these, anaerobic digestion of waste matter resulting in production of bio-gas, a valuable source of energy, has attracted universal attention in the recent past.

Bio-gas is the term used to describe a mixture of gases which are produced when organic matter, such as animal wastes, is broken down or "digested" by bacteria in the absence of oxygen. This process is known as "anaerobic digestion". Bio-gas normally contains by volume 50-75 percent of methane (CH_4) and 25-50 percent of carbon-di-oxide (CO_2) with small amounts of other gases such as hydrogen sulphide, carbon monoxide, hydrogen, nitrogen etc. (1,2).

In an agricultural country like India, bio-gas can possibly be exploited to meet atleast the energy requirements of rural areas. The bio-gas production is neither capital intensive nor a highly technological process. As such it holds promise for third world

nations like India.

Bio-gas plants in city sewage treatment plants are called "sludge-digesters". Other names for bio-gas are "bihugas" in the Federal Republic of Germany, "gobar-gas" in India, "marsh-gas" in China and some other regions (3,4).

It was the discovery, some 200 years ago, that gases emitted from water-inundated marshlands were combustible which led to the theory and current practice of gas energy recovery from the decomposition of organic materials in a liquid but oxygen free environment. As early as in 1669, Shirley discovered marsh gas (5). On Novermber 14, 1776 Alessandro Votta (6) wrote a letter to a friend describing his unexpected discovery that "combustible air" was being formed continuously and in substantial quantities in all the lakes, ponds and streams in the vicinity of Como in northern Italy. The initial observation was made in Lake Verbano. Chemical knowledge of those days did not permit the characterization of Volta's inflammable gas. This was first accomplished in 1806 by William Henry (7), who showed that Volta's gas was apparently identical with the main constituent of synthetic illuminating

gas which was later called methane. In 1808, Humphry Davy (5) collected the gas from the decomposition of strawy cattle manure, and thus the bio-gas research started.

In the year 1868 Bechamp, a pupil of Louis Pasteur, clearly showed that methane is perhaps formed from simple carbon compounds by action of micro-organisms (8) and subsequently more adequate proof of the microbiological origin of methane was provided by Tappeiner in 1882 - 1884 (8). It was only a few years later, in 1896, that sewage gas was used for lighting a street in Exeter, England (5). After the second world war, especially in recent years, in view of the energy crisis, bio-gas technology which is low cost has been tapped for providing fuel for cooking, electricity and power for running farm machinery as well as high grade organic manures. Bio-gas technology not only helps in the utilization of animal and agricultural wastes but also aids in maintaining proper sanitation in the villages.

The present energy generating systems in developing countries depend largely on local resources such as wood, straw, or dung and whatever fossil fuel

supplies are locally available (Table 1). In most developing countries including India the economic base and the majority of the population are still rural, and machinery that requires energy (especially fossil fuel) is not heavily utilized. However, the lack of cheap and adequate energy often hampers rural development plan and retards improvement in the quality of rural life. Solving the problems of energy generation and distribution is central to implementation of plan for economic development, especially in rural areas. As imported fossil fuels become increasingly expensive, the urges of developing alternative fuel supplies from local materials are bound to grow.

An ideal energy source in rural setting would be one that is local in origin and can produce energy for heating, lighting, small scale electric power generation, and power for engines as needed. Moreover, where possible, it should also provide more energy than is now obtained from the same materials. Rural areas usually have large supplies of crop residues and animal wastes theoratically suitable for conversion into a usable source of energy. The process that appears to hold the greatest immediate potential for utilization of these materials as sources of fuel

is "anaerobic fermentation" (Bio-gas).

TABLE 1

Main energy sources that could possibly be provided to rural areas (9).

Energy source	Household			Agriculture/Industry		
	Cooking	Lighting	Heating	Power[1]	Transport	Heat energy[2]
Electricity	(X)	X		X		
Coke, coal	X					X
Kerosene	X	X	X	X		X
Diesel			X	X	X	X
Gas	X	X	X	X	X	X
Wood	X		X			X
Straw, vegetable wastes, crop residues	X		X			X
Dung	X		X			
Solar energy	(X)			(X)		X
Hydro				X		
Wind				X		
Alcohol				X	X	

1 Includes, for example, pump sets.
2 Includes steam
NOTE: (X) represents methodsthat are likely to be very expensive, be of limited application, or need further development.

In India the annual availability of cattle dung excreted by 178.9 million cattle and 57.9 million buffaloes is estimated to be about 970 million tonnes wet dung (10). The potential bio-gas production from the above cattle-dung would be about 65108 million m^3 per annum at an average production rate of 67.1 m^3 bio-gas per ton wet dung. The bulk of the cattle dung amounts to nearly 95% of the total animal and human excreta available in India. Hence, the production of bio-gas from cattle dung digestion assumes prime importance in the rural energy context.

In the Indian model of bio-gas plant, cow-dung slurry (cow-dung and water mixed in equal proportions) is placed in a well made of cement and lined with bricks. In the well, a drum is suspended upside down to create anaerobic condition for the decomposition of organic materials (figure 1) (5). On fermentation the methane gas is evolved and is taken out by pipes to the burners and used for cooking.

The gas produced during anaerobic digestion of natural material consists of a mixture of notably methane, CO_2, small amounts of other gases, (in particular H_2S and H_2). Methane is simplest and most abundant

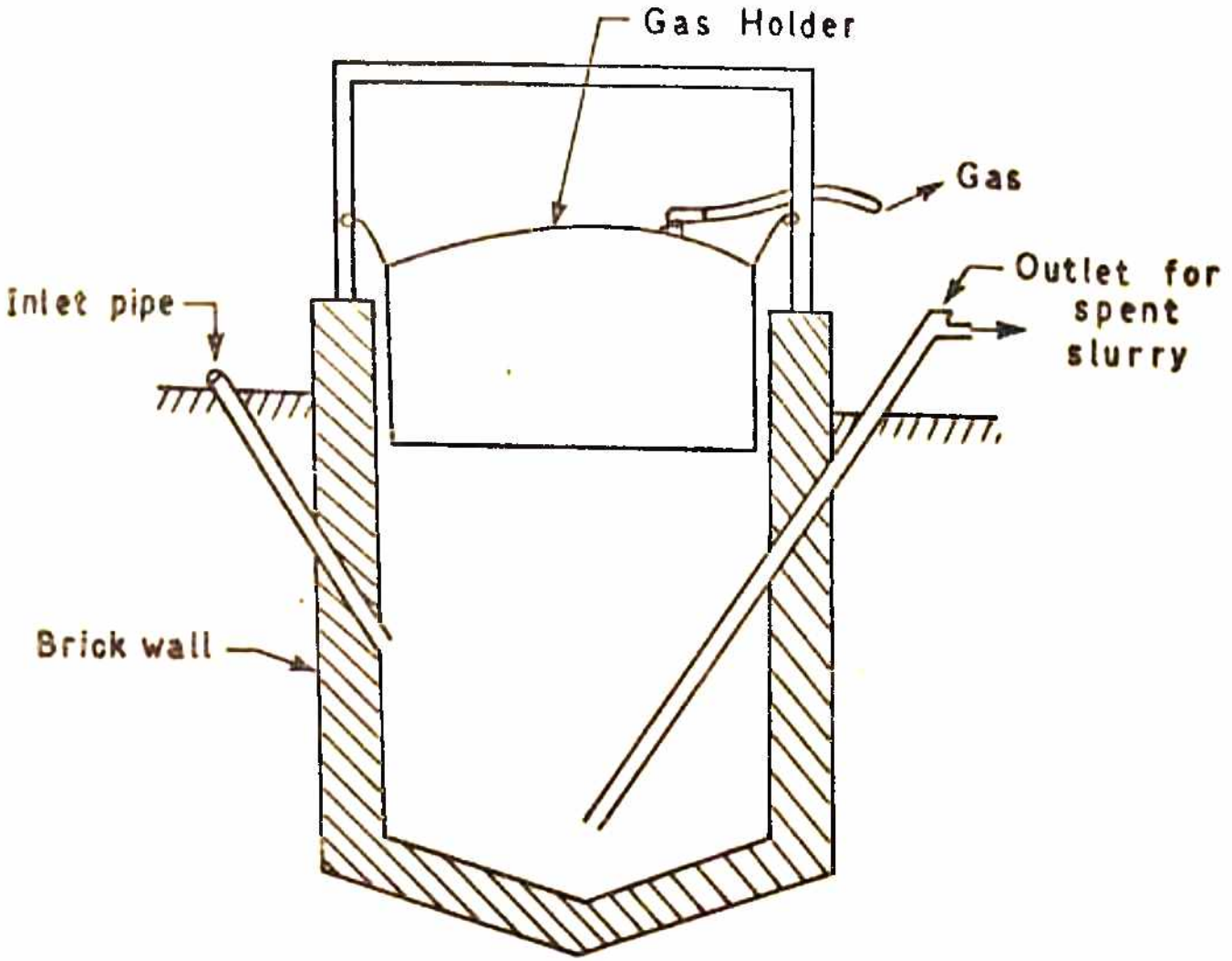

Fig. 1 BIO-GAS PLANT

hydrocarbon and the chief constituent of natural gas.

The production of bio-gas in anaerobic fermentation depends on the nature of organic materials. Cow-dung is one of the best source of bio-gas. Waste that accumulate at high animal population feeding operations consist primarily of fecal material. They also may include spilled feed, urinary deposits, and hair. Fecal wastes characteristics vary with animal diet, health, feed preparation procedures, retention time of the wastes before collection on pen floors, and the waste exposure (11-17) to wheather elements. These wastes when collected, have a content of 70 percent total solids of which 95 percent are volatile solids. Table 2 shows an analysis of typical animal waste (18-19).

TABLE 2

Analysis of typical cattle manure

	(Dry Matter Basis)	
	%	lb/ton
Proximate Analysis		
Crude protein	12.9	258
Fat	1.0	20
Fiber	33.1	662
Ash	5.2	104
Minerals		
Carbon	50.0	1,000
Nitrogen (Organic)	2.1	42
Phosphorus	0.4	8
Potassium	1.0	20
Calcium	1.0	20
Magnesium	0.4	8
Sodium	1.0	20
Sulfur	0.3	6
Iron	0.4	8
Amino Acids		
Alanine	0.47	9.4
Valine	0.27	5.4
Glycine	0.32	6.4
Isoleucine	0.20	4.0

Leucine	0.48	9.6
Proline	0.32	6.4
Threonine	0.26	5.2
Serine	0.25	5.0
Methionine	0.12	2.4
Hydroxyproline	0.03	0.6
Phenylalanine	0.27	5.4
Aspartic Acid	0.53	10.6
Glutamic Acid	0.82	16.4
Tyrosine	0.18	3.6
Lysine	0.18	3.6
Histidine	0.10	2.0
Arginine	0.15	3.0
Tryptophan	-	-
Cystine/2	0.05	1.0
Diaminopimelic Acid	-	-
Total Amino Acid	5.00	100.0
Amino Acid/Crude Protein	0.39	-

India took the lead in the development of bio-gas plants (locally known as gobar-gas plants). Nearly 70% of India's bio-gas plants, which now total more than 36000, were built during the fuel and fertilizer crisis of 1975-76. However, the Indian Council of Agricultural Research (ICAR) had begun anaerobic cow-dung fermentation as early as 1938-39 (7,20). In 1956, the government started setting up bio-gas plants on a large scale (5,7). In 1960's the "Khadi and Village Industries Commission" took keen interest in the setting up for bio-gas plants in villages. It provided financial assistance and free technical know how for this purpose. Currently, in India a number of agencies are involved in the development and propogation of bio-gas technology.

A number of new designs have been published by Gobar Gas Research Station, Ajitmal. If one uses agricultural waste insulation and an external water jacket heated by a solar heater that delivers 1.5 liters of water per minute at 60°C, slurry retention time is reduced from 50 to 55 days to between 15 and 18 days, and gas production has also risen even in winter.

Nearly 27,000 small digesters have been installed in The Republic of Korea since 1969 through the efforts

of the Office of Rural Development (ORD). However, the cold winters and lack of cattle make Korea's experience with bio-gas quite different from India's. Most farmers do not operate the digesters between December and March, when temperatures are as low as -17°C, and gas production almost nil. The gas holders are covered with straw during these winter months. Vinyl covers were tried but were ineffective and furthermore the sophistication of heating the digester was not justified for the small plants (9). Operations is more favourable in the warmer South.

Fuel is not a major problem in the Philippines as firewood is plentiful. Consequently, interest in bio-gas stems from its pollution control and public health applications. Pigs and buffaloes provide most of the animal wastes, and despite some paychological inhibitions the National Housing Authority (NHA) is also promoting night-soil digestion, and one such digester is already operational. The major research activity is centered at the National Institute of Science and Technology (NIST), at the University of Philippines at Los Banos, and Maya Farms.

In countries like Thailand and Indonesia, not

much development of bio-gas technology has taken place since firewood is plentiful in most areas and animal wastes are not so plentiful (9).

In Japan, several institutions, including the National Institute of Animal Industry at Chiba, the Public Works Research Institute, the Fermentation Research Institute at Anage, M/S Hitachi Plant Construction, the Ministry of Agriculture, and the Agency of Industrial Science and Technology (MITI) have worked on anaerobic digestion of rural, urban, and industrial wastes for pollution control. They have adopted high temperature digester (in the thermophilic range) of some wastes (9).

In China, bio-gas is extensively used for cooking lighting, fertilizer, and for small internal combustion engines (23-24). As of September 1975, over 200000 family size digesters (10 m^3 capacity, generating about 5 m^3 of bio-gas per day) were operating in the province of Szechuan (25). The feed is a mixture of urine (30%), night soil (10%), and water(50%); vegetable matter is decomposed for 10 days before inclusion. Lime solution or grass ashes are added to maintain a pH 7-8.

Bio-gas systems are now receiving attention from

several international agencies following the crisis in the supply of energy and fertilizer. After its 1974 Colombo Declaration, the Economic and Social Commission for Asia and the Pacific (ESCAP) held bio-gas workshops (in New Delhi, August 1975, on Technology and Economics, and in Manila, October 1975,on Fermentation Technology), and began publishing newsletters (9).

Klass (26) presented a general review of the status of research and technology on the conversion of wastes and biomass to various forms of usable energy. The energy potential of biomass conversion to fuels was evaluated by many workers (27-34). Clausen et al. (35) reviewed various aspects of methane production from crop materials including sources of biomass in relation to crops and available land, kinetics of biomethanation, process description, and overall system economics. Chiranjivi (36) revised various metods of construction and operation. Most of these units were small scale, low-cost, and based on simple operating procedures at ambient temperatures.

APPLICATIONS

Williams (37) presented a general discussion of the application of small digester systems for use in remote rural areas of underdeveloped countries. As discussed earlier our firewood resources are depleting. Our hill sides are getting denuded and forests are disappearing. As biogas is more efficient than wood, consumption of firewood could be reduced considerably thus helping the forests to catch up in their regrowth through phased reforestation and felling programmes, which at present are fast losing ground.

It is a law of conservation of nature that whatever is taken from the land should be returned to it. At present only a small fraction of the animal wastes return to the land as compost manure, the rest of it is either burnt or is wasted, or washed away. As far as human wastes are concerned, almost all ot it is wasted. The net result is that not only is the soil deprived of essential manure, but it also loses its water retaining properties. This leads to erosion and the slow decay of good farm land. But putting human and animal wastes through the digester, after the gas is produced good humus rich manure can be

returned to the land, thus helping to restore the balance in nature.

The loss in quantity and quality of Cow-Dung Gas manure, is less when passed through the Cow-Dung Gas Plant than when composted. The manure obtained from Bio-Gas Plants contains about 1.5% Nitrogen (by dry weight) agaist 0.75% in ordinary farm compost.

Biogas digesters improve environmental health and reduce incidence of diseases and pollution as compared to openly handled manure. Animal and human wastes contain undesirable bacteria and parasite eggs. People living near the open storage pits may contact contagious diseases. The bio-gas digester conceals the animals and human excreta. It goes directly into the sealed pit. The fermentation process kills most disease germs, making surrounding environment much better.

Thus organic residues represent a potential energy source and possess following advantages (38-39).

* They are renewable.
* Their production and use represent a balanced

carbon cycle that does not result in elevation of ambient carbon dioxide levels.

* The conversion of organic waste residues reduces the environmental pollution.

Asian bio-gas systems are characterized by great diversity even though only a limited number have actually been built. Most of the systems are used for family cooking, although other uses are on the increase. Family size units are owned for the most part, by the well-to-do, as a host of reasons have made it difficult for poorer people to have bio-gas plants. Nonadoptation can be due to psychological and practical problems associated with the handling of various wastes and slurry, or simply due to lack of necessary resources (i.e. capital, input materials, land, time etc.). Bio-gas systems can succeed in areas where inputs have low opportunity costs, the alternative have high opportunity costs, and where plants can be operated with adequate efficiency.

Traditionally anaerobic digestion has been used as a method of treating municipal sewage (40-43) and as a method of producing methane gas from garbage(41, 42,44,45), food and industrial wastes (42,46,47),

manures and crop residues (41,44,48-55).

Although anaerobic digestion has long history of use, it has reputation for poor "process stability" and has never been universally accepted as an effective waste handling technique. Digesters are susceptible to malfunctioning due to shock of loading, temperature and variety of toxic substances. Malfunctioning manifests itself in terms of reduced gas production, reduced degradation of organic materials and increase in acidity. In addition, the rate of anaerobic digestion is relatively slow and the fermentation becomes slower with dips in environmental temperature.

BASIC PRINCIPLES OF BIOCONVERSIONS IN ANAEROBIC DIGESTION AND METHANOGENESIS

Anaerobic digestion involves many types of microorganisms and is a complex microbial fermentation. The process involves many different kinds of interacting microbial species, most of which do not directly produce CH_4 (56-61). Bacteria are the main biological agents involved in organic matter destruction and CH_4 production, but fermentative ciliate and flagellate protozoa and anaerobic fungi may also contribute in some ecosystems (56). The formation of methane by bacteria is common in nature. It occurs in those anaerobic environments in which there is a vigorous microbial fermentation of organic material. Such environments are sewage and organic waste digesters, aquatic sediments of lakes, estuaries, and marine systems, flooded soils, tundra, peat bogs, and marshes, where the main electron acceptor CO_2, is produced from degraded organic substrates (56,62,63).

Stages of the Fermentation

For simplification of the microbiology, chemistry and kinetics of the fermentation, several schemes has been

described that separate it into various stages involving different metabolic groups of bacteria (64-70). While it is emphasized that these bacterial groups cannot be separated when we talk about their metabolism since the efficient metabolism of group is dependent on the others (40, 71-75).

Figure 2 illustrate the scheme which best fits current information on methane fermentation (40,52,56, 76-82). The first stage involves species of fermentative bacteria which, as a group, hydrolyse lipids and the major polymers such as cellulose and protein and degrade the products of these to organic acids, alcohol, H_2 and CO_2. The second metabolic group, called the aceto-genic stage, produces acetate and H_2, and sometimes CO_2, from the organic acids and alcohols produced in the first stage. The third stage involves the methanogenic bacteria as such which utilize the products of the first two stages, CO_2, H_2, and acetate, in production of the final products, CH_4 and CO_2.

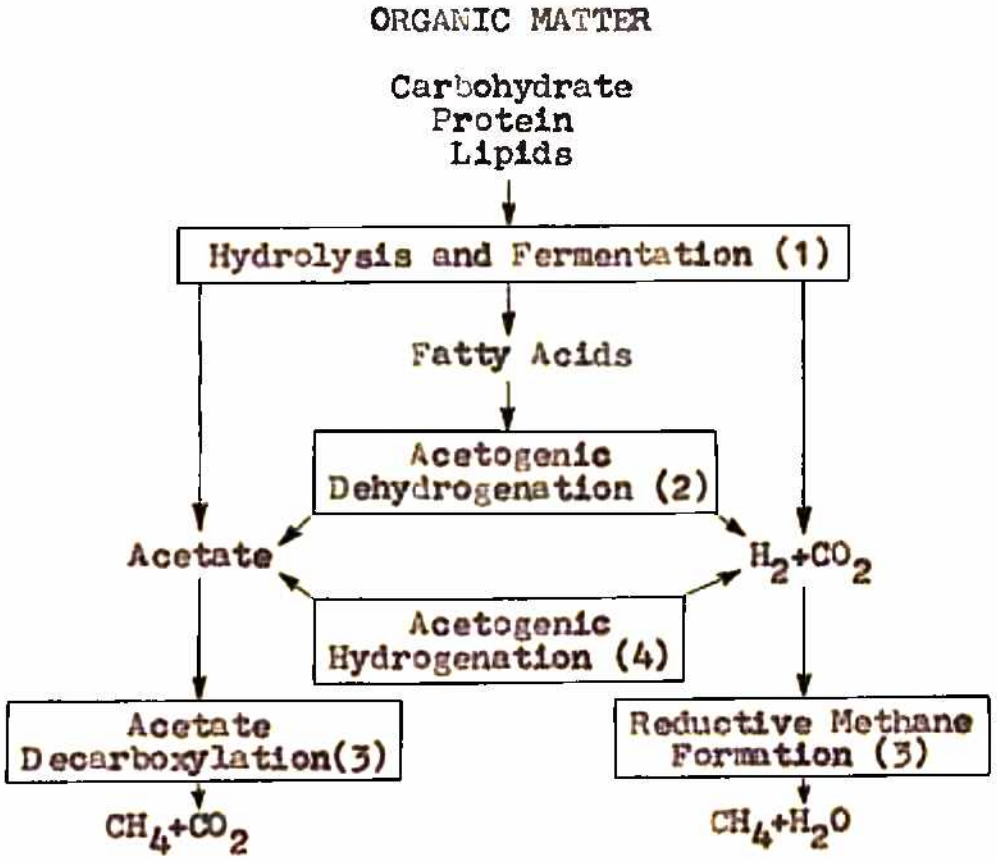

FIGURE 2. Three-stage scheme for the complete anaerobic degradation of organic matter showing the general pathways and the three major metabolic groups of bacteria: (1) fermentative bacteria; (2) obligate H_2- producing, i.e., proton-reducing, acetogenic bacteria; and (3) methanogenic bacteria. Acetate and sometimes other acids may be produced from H_2 and CO_2 by a fourth group of bacteria.

The Fermentative Bacteria: Little information is available concerning the more numerous fermentative bacteria active in anaerobic digesters. Results reviewed by Hobson et al. and others (40,83) indicate that most are strict anaerobes but some facultative anaerobes such as streptococci and Enterobacteriaceae are also involved. Bacteroides, chiefly Bacteroides ruminicola are very numerous and a large variety of other fermentative anaerobes including gram - negative curved motile rods, clostridia, bifidobacteria, and other gram-positive and gram-negative rods are found among the predominating flora (40, 84-86). Cellulolytic organisms are not very numerous but include both sporing and nonsporing gram-negative motile anaerobic rods which usually produce acetate, ethanol, H_2 and CO_2.

The Acetogenic Bacteria: The products of the first stage fermentation other than acetate, CO_2, and H_2, that is, mainly propionate and long-chained fatty acids and other organic acids are oxidised to acetate, but the kinds of acetogenic organisms involved and the electron sink product generated in the oxidation, presumably H_2, are largely unknown. Although much effort has been done, but organisms could not be isolated (56,77).

Well known case of acetogenic organisms are those involved in the degradation of alcohols such as ethanol. Methanobacillus omelianskii was thought to produce methane according to equation 1.

$$2\ CH_3\ CH_2\ OH + CO_2 \longrightarrow 2\ CH_3\ COOH + CH_4$$

$$\triangle G'_o = -31.7 \text{ kcal} \quad (1)$$

Bryant et al. (1969) (56) and others (57, 87-90) showed that the fermentation was actually carried out by a symergistic association of two species. One of these, the acetogenic S organism oxidises ethanol to acetate according to equation 2. The other organism was a methanogen which could not use ethanol but used H_2 according to equation 3.

$$CH_3\ CH_2\ OH + H_2O \longrightarrow CH_3\ COO^- + H^+ + 2H_2$$

$$\triangle G'_o = +1.5 \text{ kcal} \quad (2)$$

$$4H_2 + CO_2 \longrightarrow CH_4 + 2H_2O \quad \triangle G'_o = -33.2 \text{ kcal.} \quad (3)$$

However, first organism cannot effectively grow as a pure culture because the H_2 produced inhibits its growth. It can use no other system to excrete electrons. When combined as in the M. omelianskii culture, the two organisms grow very effectively in ethanol medium.

This study showed the necessity of the maintenance of a low partial pressure of H_2 via the methanogenic bacteria in order for oxidation of ethanol to occur.

The Methanogenic Bacteria: It is in the third stage of methane fermentation that real waste stabilization occurs. During this stage, the organic acids are converted by a special group of bacteria termed "methane formers" into the gaseous end products, CO_2 and CH_4. These bacteria are very strict anaerobes. They require lower oxidation reduction potential for growth than most anaerobic bacteria (about -300mv) and some species are rapidly killed by O_2 (40, 91-92). There are several different groups of methane formers, and the group is characterised by the ability to ferment a relatively limited number of organic compounds. They have quite different cell shapes and structures suggesting diverse philogenic origins (40, 89,90,92-99). For example, they include large sarcinae, coccus groups similar to micrococci, and streptococci, long cylindrical rods, long curved rods with tufts of flagella and short rods with a single polar flagellum (40,86,100). Yet, they all seems to have a some what similar and peculiar energy metabolism. All species except some such as Methanobacterium strain MoH and Methanobacterium thermoautotrophicum utilize H_2 and

CO_2 for growth as shown in equation 4.

$$4\ H_2 + CO_2 \longrightarrow CH_4 + 2H_2O \quad \Delta G'_o = -33.2 \text{ kcal} \qquad (4)$$

All of the marphologically diverse species so far studied contain Coenzyme M (40,101) and coenzyme 420 (72, 102-104), newly discovered coenzymes which have not yet been found elsewhere in nature. Coenzyme M has a methyl transfer function. Coenzyme 420 is an electron transport coenzyme which seems to serve in place of carriers such as ferredoxin in these bacteria. Methanogens also contains factor B, a heat stable cofactor with molecular weight of about 1000, required for the enzymatic formation of CH_4 from methyl coenzyme M (56, 105-107).

Table 3 shows the reviewed taxonomy of the methanogens based on analysis as proposed by Balch et al. (56,108).

TABLE 3

Proposed taxonomic scheme of Balch et al. based on comparative cataloging of the 16 S ribsomal RNA and substrates used for growth and methanogesis

Taxa	Type strain	Former designation	Substrates for growth and CH_4 production
Order I. Methanobacteriales (type order)			
Family I. Methanobacteriaceae			
Genus I. Methanobacterium (type genus)			
1. Methanobacterium formicicum (neotype species)	MF	Methanobacterium formicicum	H_2, formate
2. Methanobacterium bryantii	M.o.H.	Methanobacterium sp. strain M.o.H.	H_2
Methanobacterium bryantii strain M.o.H.G.		Methanobacterium sp. strain M.o.H.G.	H_2
3. Methanobacterium thermoautotrophicum	H	Methanobacterium thermoautotrophicum	H_2
Genus II. Methanobrevibacter			
1. Methanobrevibacter ruminantium (type species)	MI	Methanobacterium ruminantium strain MI	H_2, formate
2. Methanobrevibacter arboriphilus	DHI	Methanobacterium arbophilicum	H_2
Methanobrevibacter arboriphilus strain AZ		Methanobacterium sp. strain AZ	H_2
Methanobrevibacter arboriphilus strain DC		Methanobacterium strain DC	H_2, formate

3. Methanobrevibacter smithii

Order II. Methanococcales

Family I. Methanoccaceae

Genus I. Methanococcus

1. Methanococcus vannielii (neotype species)

2. Methanococcus voltae

Order III. Methanomicrobiales

Family I. Methanomicrobiaceae (type family)

Genus I. Methanomicrobium (type genus)

1. Methanomicrobium mobile(type species)

Genus II. Methanogenium

1. Methanogenium cariaci(type species)

2. Methanogenium marisnigri

Genus III. Methanospirilum

1. Methanospirillum hungatei

Family II. Methanosarcinacear

Genus II. Methanosarcina(type genus)

PS	Methanobacterium ruminantium strain PS	
SB	Methanococcus vannielii	H_2, formate
PS	Methanococcus sp. strain PS	H_2, formate
BP	Methanobacterium mobile	H_2, formate
JRI	Cariaco isolate JRI	H_2, formate
JRI	Black Sea isolate JRI	H_2, formate
JFI	Methanospirillum hungatii	H_2, formate

1. *Methanosarcina barkeri* (type species)

Methanosarcina barkeri strain 227

Methanosarcina barkeri strain W

MS	Methanosarcina barkeri	H_2, CH_3OH, CH_3NH_2, acetate
	Methanosarcina barkeri strain 227	H_2, CH_3OH, CH_3NH_2, acetate
	Methanosarcina barkeri	H_2, CH_3OH, CH_3NH_2, acetate

PROBLEM DELINEATED

Objective

The present project was started with the following objectives:

1. To devise techniques to enrich methane content in biogas so as to enhance its energy value.

2. To find ways and means of ensuring continuous production of gas even during low temperature conditions.

Approach

The following aspects of bio-gas production were planned to be studied.

Effect of manipulations of various parameters like temperature, loading rate, total solid content, pH, agitation, on the quantum of gas production and on the content of methane in the bio-gas of cattle-dung at laboratory scale.

In order to improve digestion process in terms of increase in total quantity of gas and also to enrich its methane content by keeping the metabolic processes of microbes at the optimum under varying thermal environments with the help of certain additives were studied, with

the ultimate objective of keeping bio-gas plants functional during low temperature climates.

This project was conducted in several phases and given in the several chapters (Chapter II to V) and lastly summary and conclusion has been given in the VI chapter.

CHAPTER-II

STANDARDIZATION OF EXPERIMENTAL CONDITIONS FOR ANAEROBIC TREATMENT OF CATTLE-DUNG

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STANDARDIZATION OF EXPERIMENTAL CONDITIONS FOR ANAEROBIC TREATMENT OF CATTLE-DUNG

INTRODUCTION

The anaerobic process is in many ways ideal for waste treatment. It has several significant advantages over other available method and is assured of increased usage in the future. However, inspite of the present significance and large future potential of this process, it has not generally enjoyed the favourable reputation it truly deserves. Digesters are also susceptible to malfunctioning due to shock of loading, temperature and pH. Under normal conditions, anaerobic waste treatment proceeds with a minimum control. However, if environmental conditions are changed, or if toxic materials are introduced the process may become unbalanced. An "unbalanced process" is defined as the one which is operating at less than normal efficiency.

Temperature, retention time, agitation, total solid content and pH are important factors responsible for unbalanced state (48,109-117). It is, therefore, desirable to find out optimum conditions interms of temperature,

retention time, agitation, total solid content and pH for operating digester with maximum efficiency.

It is also important to determine when a digester first becomes "unbalanced" so that control measures can be applied before it is too late. Several parameters must be watched for good control. Some of the parameter of importance are listed below:

* Total Gas Production
* Methane Percentage in Gas
* Volatile Acid Concentration
* pH
* Waste stabilization expressed as BOD and COD

The first indication about the efficiency of a digester is the total gas production. However, this parameter is useful as an indicator only when the daily feed is quite uniform and the daily gas production does not vary too widely from day to day under normal conditions.

Changes in the percentage of methane in the digester gas may sometimes indicate the onset of unbalanced conditions (118), as unbalanced treatment often results

in decreased methane production. This is accompanied by an increase in carbon dioxide percentage resulting in loss of energy value of bio-gas.

The most important parameter is the concentration of volatile acids. An increase in volatile acid concentration is frequently one of the first indicators of digester unbalance and indicates the onset of adverse conditions long before any of the other parameters are affected (92, 119).

Another indicator of digester unbalance is the decreasing pH, which usually results in a high volatile acids concentration. A significant drop in pH, however, does not normally occur until the digester is seriously affected.

Process stabilization can be judged by "Biochemical Oxygen Demand" (BOD) and "Chemical Oxygen Demand" (COD) indicate the extent of biodegradation (119,120).

Although none of the above parameters may be a sure sign of digester unbalance when used individually, together they give a good picture of digester operation efficiency (109).

Buhr and Andrews (121), Varel et al. (48) have reviewed the results of various studies on the effect of temperature and retention time (RT) on anaerobic fermentation. Although considerable work has been done on methanogenesis from beef cattle waste (121-125), sufficient data is not available tc adequately evaluate the overall potential of this process. Enough information is not available on the optimal temperature, and retention time. The quantity of bio-gas production from cattle-dung depends mainly on temperature of fermentation (126,127), and retention time (16,48). Besides these, many other factors, like agitation, total solid content, and pH affect the gas production. It was felt, therefore, necessary to find out optimum conditions interms of temperature, retention time, agitation, total solid content and pH for maximum gas production.

Fermentation of cattle-dung offers several advantages over other potential substrates. Adequate nutrients are available for the microbes to carry out the fermentation. Degradation of organic matter is usually between 30 and 65%. It is a means of pollution control, and the fermentator residue has a potential value as fertilizer (128,129). Cattle-dung is one of the major

raw materials available in the country. Therefore, cattle-dung was used in this study.

LABORATORY SCALE ANAEROBIC DIGESTER

Several bench scale anaerobic digesters were used (Figure 3). Each vessel consisted of 10 liter glass reaction bottle, having a working volume of 6 liters, and containing desired percentage of total solids, (Wet cattle-dung was collected, dried, powered and used). The digesters were agitated with magnetic stirrer. All digesters were maintained at desired temperature with ±1°C in a thermostat. Gas was collected and measured by displacement of acidified saturated salt solution (i.e. 200 g of Na_2SO_4 in 800 ml distilled water with 30 ml of conc. H_2SO_4) (130), making due corrections for atmospheric pressure and temperature. The digesters were fed on semicontinuous basis: Once per day with desired retention time. Prior to feeding equal quantity of sludge was withdrawn from the bottom of the digester. Substrate samples were routinely analysed for pH, volatile acids, BOD,and COD as per "standard procedures" (130) given below:

All chemicals used were of analytical grade.

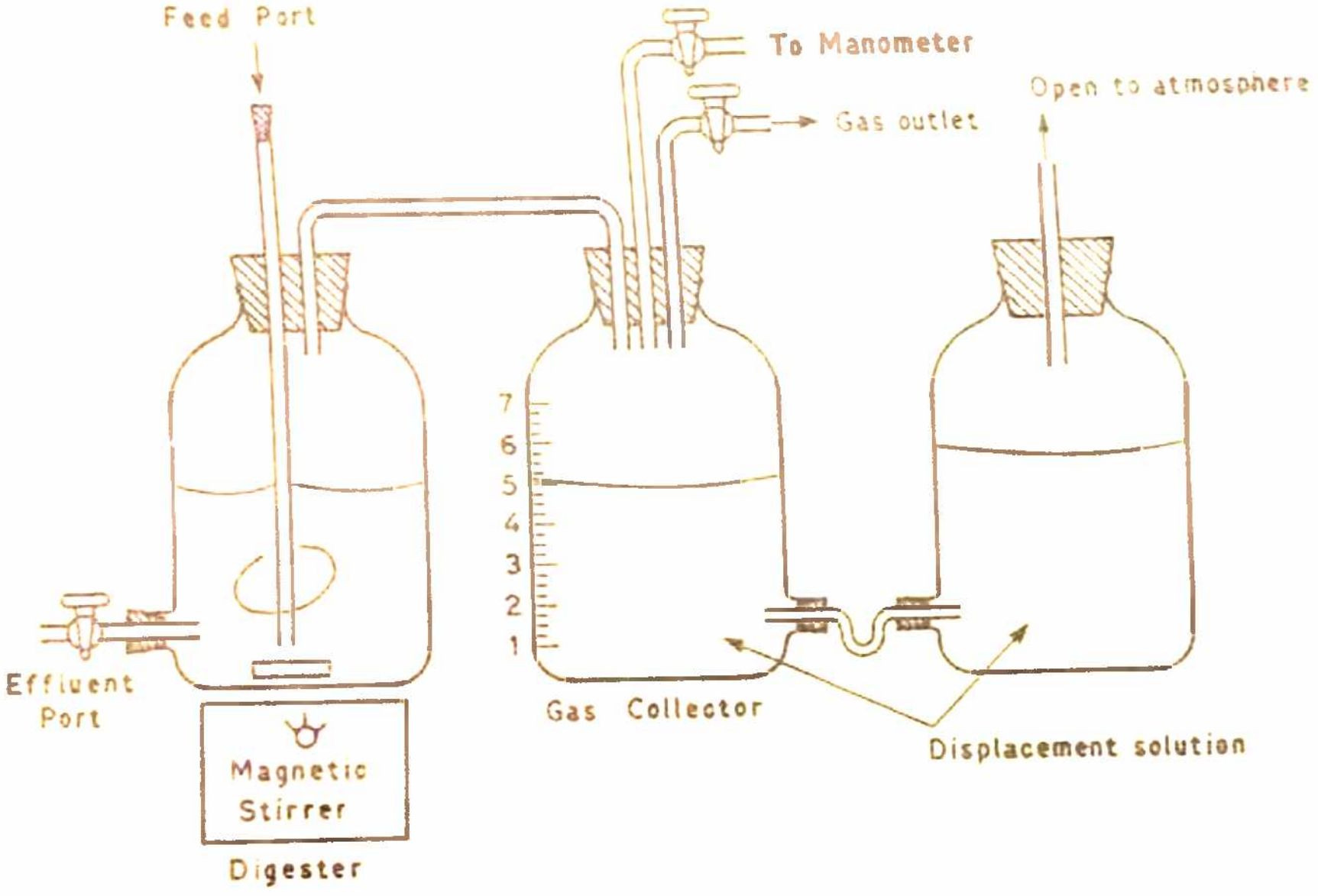

Fig.3 LABORATORY ANAEROBIC DIGESTER

Gas composition was analysed with "AIMIL Gas Liquid Chromatograph" with a stainless steel chromosorb 2 column, and a thermal conductivity detector. Helium served as the carrier gas for methane and carbon dioxide. Identification and percentage of methane and carbon dioxide were based on a comparision of RT and peak areas of unknows with those of standard amounts of the two gases.

pH was measured by Beckman pH meter.

Determination of Biochemical Oxygen Demand (BOD)

The BOD, by definition, is the quantity of oxygen required for the stabilization of oxidizable organic material present after 5 days incubation at 20°C. Complete stabilization, in most cases, would take a much longer time. The degree of oxidation occuring during a 5-day period depends on the type of microorganisms present in the seed and the type of nutrients.

Sufficiently aerated water saturated with Dissolved Oxygen (DO) was used for dilution water. 1ml each of phosphate buffer (pH 7.2), magnesium sulfate(22.5g $MgSO_4 . 7H_2O$ in a litre of distilled water), Calcium

chloride (27.5g of anhydrous $CaCl_2$ in 1 litre of water), and ferric chloride (0.25g of $FeCl_3 . 6H_2O$ in 1 litre of water) were added for each litre of water.

Initially, approximate BOD range was found out and samples were then diluted accordingly as per Table 4. Measured samples were added directly to BOD bottles (300ml capacity) and filled with just sufficient dilution water so that stopper was inserted without leaving air bubbles.

Modified Winkler method was used to determine DO after 15 min. and after 5 days.

For satisfactory BOD results, an adequate number of bacteria must be present. Seeding material was obtained from sewage disposal plant. 2ml of supernatant from settled sewage was used for every litre of dilution water. Supernatant was preserved by freezing.

For calculating BOD, the following formula was used.

$$\text{mg/litre BOD} = \frac{(D_1-D_2) - (B_1-B_2)f}{P}$$

where D_1 = DO of diluted sample 15 min. after preparation

TABLE-4

BOD range chart including appropriate dilutions and decimal fraction

BOD Range	Dilution Sample/300 ml	Decimal Fraction
0.01-6	300	-
4.5 -18	100	0.333
9.0 -35	50	0.133
18-70	25	0.083
45-180	10	0.033
90-360	5	0.017
225-900	2	0.007
450-1800	1	0.003
900-3600	0.5	0.0016
1125-4500	0.4	0.0013
1500-6000	0.3	0.001
2250-9000	0.2	0.0007
4500-18,000	0.1	0.0003

D_2 = Do of diluted sample after incubation

P = decimal fraction of sample used

B_1 = Do of diluted seed control 15 min. after preparation

B_2 = Do of diluted seed control after incubation

S = ratio of seed in sample seed in control

Dissolved Oxygen Test (DO): The sodium azide (Alsterberg) modification of Winkler method was used for DO determination, since this test is generally used to standardize an electronic oxygen monitor.

To a 300 ml sample, 2ml $MnSO_4$ solution (364g of $MnSO_4 . H_2O$ in 1 litre of water) was added, followed by addition of 2ml alkali-iodide reagent (500g NaOH and 135g NaI in 1 litre water + 40 ml of water containing 10g $NaIl_3$); below the surface in a stoppered flask and mixed by inverting. 2ml conc. H_2SO_4 was added by allowing it to run down the neck of the bottle. The content were mixed by gently inverting until dissolution of the floc was completed. 204ml of sample was removed and titrated with thiosulfate.

Following formula was used to calculate DO

$$\text{mg/litre DO} = \frac{\text{ml of } Na_2S_2O_3 \times \text{Normality of } Na_2S_2O_3 \times 8000}{\text{ml of original sample titrated}}$$

Determination of Chemical Oxygen Demand (COD)

The rapid method presently advocated by the National Canners Association was used for COD determination. The test measures the amount of oxygen consumed from a chemical oxidant such as potassium dichromate.

A standard COD determination, almost invariably, gives higher results than a 5 day BOD determination since the oxidation of organic material is more complete.

Added 1ml of mercuric sulfate solution (50g mercuric sulfate, 50ml of conc. H_2SO_4 in 1 litre of water) to each of two 500ml Erlenmeyer flask.

To 1 flask added 5ml of suitably diluted sample. To the second flask 5ml of distilled water was added.

20ml of dichromate oxidizing solution (2.5g potassium dichromate in a mixture of 500 ml each of conc. H_2SO_4 and 85% orthophosphoric acid) was added to each flask. Contents of the flasks were mixed with

gentle shaking and heated on steam bath at 92°C for 10 min. They were then cooled, and subsequently 150ml of distilled water was added. 10ml of potassium iodide solution (55g of KI in 200 ml of water) was added in each flask and contents litrated against sodium thiosulfate solution.

COD was calculated as below.

$$\text{ppp COD} = \frac{EN\ (A-B)}{D} \times 8{,}000$$

where A = ml thiosulfate used for blank

B = ml thiosulfate used for sample

N = Normality of thiosulfate

D = ml of sample used

E = dilution factor

Determination of Volatile Acids (Total organic acids)

Determination of volatile acids were done by "Column Partition Chromatographic Method" (Tentative).

The principle is as follows:

Chromatographic columns are capable of a dynamic partition or distribution of dissolved or dispersed substances between two immiscible phases, one of which

is moving past the other. Aqueous sample containing organic acids is adsorbed on a column of inert granular material and an appropriate organic solvent is passed through the column, the organic acids can thus be extracted from the aqueous sample. In the present work, silicic acid was used as the adsorbent column, acidified aqueous solution as the stationary phase, and normal butanol in chloroform as the mobile phase.

All of the short-chain 1- to 6- carbon organic or volatile acids were eluted with the solvent system used in the method and were reported collectively as the total organic acids. Total acids were measured by titration with standard base.

Determination of Total Solids

Measured volume of samples were poured into the weighed crucibles and heated to dryness on steam bath and further dried to constant weight in an oven at 103 - 105°C.

Calculations were done according to the formula:

$$\text{mg/litre total residue} = \frac{\text{mg/total residue} \times 1000}{\text{ml sample}}$$

EXPERIMENTAL DESIGN

Effect of Temperature and Retention Time

To study the effect of temperature and retention time, digesters were fed on semicontinuous basis: once per day with retention times of 3, 6, 8, 9, 12, 20 & 30 days for each temperature. The temperature ranged from 20°C to 60°C with 5°C increments between fermentors. Table 5 gives the experimental design for study of methane production from cattle-dung at various temperatures and RTs.

TABLE-5

Experimental design for study of methane production from cattle-dung at various temperature and RT.

Fermentor No.	Temp. (°C)	Loading rate (ml/digester/day) at given RT (days)						
		3	6	8	10	12	20	30
1	20	2000	1000	750	600	500	300	200
2	25	2000	↓	↓	↓	↓	↓	↓
3	30	2000	↓	↓	↓	↓	↓	
4	35	2000	↓	↓	↓	↓	↓	
5	40	2000	↓	↓				
6	45	2000	↓	↓				
7	50	2000	↓	↓				
8	55	2000	↓	↓				
9	60	2000	↓	↓				

Effect of Total Solid Content

To study this effect total solid content ranged from 5 to 15% with an increment of 2.5 percent between fermentors.

Effect of Agitation

The effect of agitation on gas production was studied in a 6 litre digester containing 7.5 percent of total solids. Agitation was done by a magnetic stirrer at 150 rpm. Stirring time varied from 2 to 10 hours per day.

Effect of Sodium Bicarbonate ($NaHCO_3$)

Similarly attempt was made to keep pH constant in the digester throughout the digestion by adding $NaHCO_3$ from 500mg per litre to 5000mg per litre of digester.

RESULTS AND DISCUSSION

Table 6 shows the gas production at different RT for various temperatures. Gradual increase in the gas production was found, when the temperature was increased from 20° to 35°C followed by decrease from 40° to 50°C and further increase from 50°C to 60°C at all RTs. A concurrent increase in methane content was observed with increase in total gas production.

Similarly, there was increase in gas production with increase in RT at least up to 12 days at all temperatures. However, methane percent was found to be more at short RT. Little is known about the maximum rates of gas production from animal wastes when lower temperatures are used. The present study shows a trend of increase in gas production with increase in RT, perticularly at low temperatures. Effect of temperature was most dramatic at 10-day RT. This indicates that the effect of temperature on the rate of gas production is more apparent at short than at long RT.

Process performance can also be judged by biochemical oxygen demand (BOD) and chemical oxygen demand (COD) which indicate the extent of biodegradation(20,87).

At long RTs, BOD and COD values were low indicating greater biodegradation as shown in Table 6.

The pH remained almost unaffected with variation in temperature as well as RT. In general, the pH was slightly lowered with increased RT at all temperatures. Process stability as evidenced by lower volatile acids consistently increased with increased RT. Maximum stability was achieved between 10 and 12 days RT in mesophilic temperature range.

These studies indicate that there is a little difference in the rates of gas production between 40° and 60° at R^T of 10 days or more. Though there is little advantage in fermentating waste between 50° and 60°C at short RT in terms of total gas production with improved methane content, this would be at the cost of more energy input in order to maintain the fermentor temperature.

Temperature in the range of 35° to 40°C was found to be most ideal between 10 and 12-day RT. In this range, not only increase in total gas production was observed, but also high methane content accompanied by enhanced rate of biodegradation was obtained.

Table 7 gives data on the effect of total solid content on gas production. The total solid content in range of 7.5 to 10 percent was found to be most suitable which give better results in terms of both total gas production and methane content.

From table 8 it may be seen that there was no significant effect of agitation on methane production. However, occassional stirring improved the amount of total gas production. Continuous stirring allows scum formation on the fermentation mixture resulting in the decrease of gas production, whereas occassional stirring improved volatile acid concentration as well as showed increased rate of biodegradation.

pH is considered to be one of most important parameters in maintaining the efficiency of anaerobic fermention. Maximum efficiency in fermentation is obtainable near neutral pH (between 6 & 8). Generaly in the anaerobic process, high volatile acid concentration accounts for a decrease in pH value. $NaHCO_3$ is known as a buffering agent in presence of carbon dioxide. The major chemical system controlling pH is the carbon dioxide-bicarbonate system (109). In order to find out optimum concentration of $NaHCO_3$ for

maximum efficiency of digesters, study has been carried out and the data presented in table 9. There was increase in gas production with high methane content with increased dose of $NaHCO_3$ and concomitant low values of BOD, COD and volatile acids indicating better biodegradation and high process stability.

TABLE-6

Summary of effluent data during steady-state periods of digester operated at different RTs for various temperatures

Temp. (°C)	RT (days)	Total gas production l/day/digester	CH_4 (%)	BOD (mg/l)	COD (mg/l)	Volatile acids (mg/l)	pH
20°	3	1.85	56.5	14000	34000	2640	6.8
	6	2.1	54.0	14000	32800	2592	6.8
	8	2.15	54.0	13666	31800	2400	6.6
	10	2.4	53.0	13000	30200	2280	6.55
	12	2.65	51.5	12833	29600	2160	6.55
	20	2.7	51.0	12666	29200	2112	6.5
	30	2.5	51.0	12666	29000	2100	6.35

Contd....

Table-6 Contd....

25°	3	2.45	57.0
	6	3.2	570
	8	3.6	56.0
	10	3.95	56.5
	12	4.15	54.0
	20	4.30	52.5
	30	3.8	52.0
30°	3	2.95	57.0
	6	3.4	57.5
	8	3.9	56.5
	10	4.5	57.0
	12	4.7	57.5
	20	4.1	57.0
	30	4.0	54.5

13000	32800	2604	6.8
12166	31400	2542	6.8
11666	30000	2220	6.7
11166	29200	2100	6.65
11166	29000	2040	6.65
11000	28600	1968	6.6
11000	28400	1908	6.5

12166	31000	2340	6.8
11833	29600	2148	6.75
11666	28400	2028	6.8
11166	27200	2189	6.7
10833	27000	1806	6.7
10833	26800	1744	6.7
10833	26800	1734	6.7

Contd....

Table-6 Contd....

35°	3	3.5	58.0	11833	29600	2160	6.75
	6	4.15	58.0	11166	27800	1903	6.65
	8	4.6	58.0	10833	26800	1620	6.65
	10	5.1	58.0	10333	25600	1303	6.5
	12	5.2	57.5	10333	25200	1249	6.5
	20	4.75	56.0	10166	25000	1200	6.4
	30	4.45	56.0	10166	25000	1200	6.4
40°	3	3.7	58.0	12000	29400	2040	6.75
	6	4.2	58.5	11166	27800	1920	6.7
	8	4.75	58.0	10833	26400	1704	6.7
	10	5.15	58	10333	25400	1410	6.6
	12	5.15	57.0	10166	25200	1260	6.55
	20	4.8	54.5	10000	25000	1140	6.5
	30	4.35	54.0	10000	24800	1110	6.5

Contd....

Table-6 Contd....

45°	3	3.15	57.0	12166	29200	2400	6.8
	6	3.6	56.5	12000	28000	2016	6.75
	8	4.25	57.0	11666	26400	1800	6.75
	10	4.95	57.5	11166	26000	1608	6.7
	12	5.0	57.5	10833	25800	1404	6.7
	20	4.8	56.0	10333	25400	1296	6.6
	30	4.2	56.0	10333	25400	1236	6.6
50°	3	3.0	57.0	12833	29200	2412	6.8
	6	3.6	57.0	12166	28000	2004	6.7
	8	4.2	56.5	11833	26600	1758	6.65
	10	5.0	56.0	11000	25600	1518	6.65
	12	4.95	56.0	10833	25400	1422	6.65
	20	4.60	56.0	10666	25400	1362	6.60
	30	3.75	56.0	10666	25200	1260	6.6

Contd....

Table-6 Contd.....

55°	3	5.1	59.5	10833	25600	1506	6.7
	6	5.9	59.0	9166	24400	1260	6.7
	8	5.75	59.5	8883	24000	1176	6.7
	10	5.3	58.0	8333	23800	1116	6.5
	12	5.30	56.0	8333	23800	1104	6.55
	20	4.75	56.5	8166	23600	1080	6.5
	30	4.6	56.0	8166	23400	1032	6.5
60°	3	5.6	59.5	9666	24200	1236	6.75
	6	6.25	59.5	8333	23800	1128	6.75
	8	6.65	59.0	7666	23000	1050	6.70
	10	6.5	58.5	7166	22600	936	6.65
	12	6.4	58.0	7000	22600	924	6.65
	20	6.15	56.0	6333	22400	900	6.6
	30	5.9	56.0	6166	22200	900	6.5

TABLE-7

Effect of total solid content on gas production, digester maintained at 35 ± 1°C

S.No.	Total solid content (%)	Total gas production l/day/ digester	CH_4 (%)	BOD (mg/l)	COD (mg/l)	Volatile acids (mg/l)	pH
	5	4.1	55.0	12500	26800	15000	6.35
	7.5	5.1	58.0	10333	25600	1248	6.5
	10	5.0	59.0	10333	25800	1260	6.6
	12.5	4.9	57.5	11666	26000	1308	6.4
	15	4.6	56.0	12166	26400	1404	6.4

TABLE-8

Summary of effluent data during steady-state digester maintained at 35 ± 1°C to study the effect of agitation on gas production

	Period of agitation (Hrs/day)	Total gas production (l/day/digester)	CH_4 (%)	BOD (mg/l)	COD (mg/l)	Volatile acids (mg/l)	pH
Without agitation		4.6	57.0	12166	26200	1416	6.5
Continuous agitation	2	4.7	57.0	12000	26000	1404	6.4
	4	5.0	57.5	11666	25800	1344	6.45
	6	5.1	57.5	10333	25200	1248	6.4
	8	5.0	58.0	10333	25200	1250	6.60
	10	4.8	57.0	10833	26000	1308	6.60
	24	4.4	57.0	12500	26200	1416	6.5
Interrupted agitation	2	4.8	57.5	12000	25800	1344	6.7
	4	5.1	58.0	10333	25600	1250	6.5
	6	5.1	58.0	10333	25400	1248	6.5
	8	5.0	58.0	11666	25800	1308	6.45
	10	4.6	58.0	10833	26200	1416	6.4

TABLE-9

Effect of $NaHCO_3$ on gas production digester maintained at 35 ± 1°C

	Dose of $NaHCO_3$ (mg/l)	Total gas production (l/day/digester)	CH_4 (%)	BOD (mg/l)	COD (mg/l)	Volatile acids (mg/l)	pH
Control without $NaHCO_3$	0	5.1	58.0	10333	25600	1260	6.5
with $NaHCO_3$	500	5.1	58.0	10333	25600	1236	6.6
	750	5.35	58.0	10166	25000	1200	6.7
	1000	5.4	58.0	9833	24800	1176	6.75
	1500	5.45	58.0	9833	24600	1152	6.8
	2000	5.4	58.0	9833	24600	1152	6.8
	2500	5.4	56.5	9166	24600	1176	6.8
	3000	5.3	56.0	9833	24800	1176	7.1
	4000	5.35	56.0	10166	24800	1200	7.2
	5000	5.35	55.0	10166	25000	1212	7.3

CHAPTER-III

EFFECT OF SURFACTANTS ON ANAEROBIC DIGESTION OF CATTLE-DUNG

CHAPTER-III

EFFECT OF SURFACTANTS ON ANAEROBIC DIGESTION OF CATTLE-DUNG

INTRODUCTION

A surface active agent (or, more briefly, surfactant), when present at low concentration in a system, has the property of adsorbing onto the surfaces or interfaces of the system and of altering to a marked degree, the surface or interfacial free energies of those surfaces (or interfaces).

Surfactants are amongst the most versatile of the products of the chemical industry used in diverse forms in the motor oils as wetting, solubilizing and emulsifyingagents as detergents in laundry and homes, the drilling of muds for petroleum prospecting and as floatation agents used in benefication of ores (131-133).

Of late, surfactants have become subjects of intense investigation in the field of chemical kinetics and biochemistry because of the unusual properties of the polymeric forms (micelles) of these materials. They shows unusual catalysis of organic reactions (134)

and possess similarity to biological membranes and globular proteins. There are several reports which specify that surfactants improve enzyme production and enhances enzyme activity (131-135). In an investigation into the effect of solubilizers on the bacterial glutamic decarboxylase and glutaminase, Hughes(13) suggested that surfactants accelerated decarboxylation of glutamate and deamination of glutamine due to the removal of competitive inhibitors by the detergents. No study, however seems to have been made so far on the effect of surfactants on anaerobic digestion of cattle dung. In an effort to improve the gas production and its methane content, this study was taken up.

The following surfactants were studied.

1. Tween 20 (Polyoxyethylene sorbitan) monolaurate HLB -16.7
2. Tween 60 (Polyoxyethylene sorbitan) monostearate HLB -9.6
3. Tween 80 (Polyoxyethylene sorbitan) monooleate HLB -15.0
4. Span - 80(Sorbitan monooleate) HLB -4.3
5. Sodium lauryl sulfate HLB -40.0
6. Sodium oleate HLB -18.0
7. Soap-nut powder(Sapindus mukorossi fruits powder)

Surfactants were introduced in to the digesters with the feed sludge.

RESULTS AND DISCUSSION

Table 10 data and Figure 4 shows enhaced gas production with increasing amounts of tween 80, the optimum being at 100-150 µl per litre. The gas was also richer in methane content. Process stability as evidenced by lower content of voilatile acids (137,138) consistently increased with increased loads of tween 80, indicating that methane producing bacteria utilize acids at faster rate. Tween 80 gave lower values of BOD (Biochemical Oxygen Demand) and COD (Chemical Oxygen Demand) indicating greater biodegradation (19, 137,139) The presence of tween 80 in digester results in higher conversion efficiency.

Studied with other surfactants like tween 20, tween 60, span 20, sodium lauryl sulphate and sodium oleate also showed increased gas production with enriched methane content, indicating that surfactants in general, particularly non-ionic, enhance the conversion efficiency. Addition of soap-nut powder was found to be detrimental to the digester.

In conclusion, the addition of tween 80 results in exceptionally high performance stability and increase in rate of decomposition. These preliminary

evaluations indicate that tween 80, and tween 20 could be used to increase the potentiality of existing digesters. Though the exact mechanism cannot be given but from the analysis of the data, certain possibilities can be envisaged. Surfactants must be providing sites where substrate can accumulate, thereby providing highly localized substrate concentrations. These areas may be providing more favourable growth environment for bacteria-substrate systems.

TABLE-10

Summary of effluent data during steady-state periods

	Dose (µl/l or mg/l)	Total gas production (l/day/digester)	CH_4 (%)	BOD (mg/l)	COD (mg/l)	Volatile acids (mg/l)	pH
Control	-	5.1	58	10333	25600	1260	6.5
Tween 20	10	5.1	58	10333	25200	1248	6.5
	20	5.3	58	10000	24800	1200	6.5
	30	5.9	60	9833	24000	1176	6.7
	40	6.5	61	9166	22800	1128	6.8
	50	7.2	61	8333	21200	1058	6.8
	100	7.6	60	7833	18000	888	6.7
	200	7.7	61	6333	17200	840	6.7
	300	7.9	61	6166	16800	792	6.6

Contd....

Table-10 Contd....

Tween 60	10	5.1	58	10333	25600	1260	6.5
	50	5.1	58	10333	24000	1260	6.55
	100	5.3	59	10166	22200	1200	6.55
	200	6.0	59	8333	21200	1176	6.5
	300	6.1	59	7833	20000	1104	6.7
	500	6.0	59	7500	19200	1080	6.65
Tween 80	1	5.3	59	10166	24800	1176	6.7
	10	5.8	61	9833	24000	1128	6.7
	20	6.4	62	8333	22800	1080	6.9
	30	6.8	64	6333	21200	936	7.1
	50	7.9	65	5166	20000	864	7.2
	100	8.9	65	4500	17200	792	7.2
	125	9.3	66	4333	15200	720	7.1
	150	9.4	65	4166	13200	708	7.0
	200	9.6	65	3833	13000	696	7.0

Contd....

Table-10 contd....

Span 80	10	5.1	58	10333	25600	1266	6.5
	20	5.1	58	10166	25200	1236	6.5
	50	5.3	58	9833	24800	1200	6.5
	100	5.4	60	9166	24620	1176	6.7
	150	5.7	61	9166	24000	1176	6.7
	200	5.6	61	8333	21200	1152	6.6
	300	5.7	61	8333	20800	1152	6.7
Soap-nut powder	10	5.1	58	10333	25600	1260	6.5
	50	5.1	58	10500	25600	1260	6.5
	100	5.1	56	10833	26000	1308	6.6
	200	5.0	57	11666	26800	1344	6.45
	500	4.8	58	12000	27000	1404	6.4
	1000	4.7	58	12166	27200	1416	6.3
	2000	4.3	58	12500	28000	1416	6.35

Contd....

Table-10 Contd....

Sodium lauryl sulphate	10	5.1	58
	20	5.1	58
	30	5.4	61
	50	5.8	62
	100	6.4	63
	150	6.7	63
	200	6.5	62
	300	6.5	63
Sodium oleate	10	5.1	58
	50	5.1	57
	100	5.3	57
	200	5.4	56
	300	5.5	57
	500	5.5	57

10333	25200	1260	6.5
10166	24620	1260	6.7
9833	24000	1236	6.7
9166	23200	1176	6.8
8333	22800	1104	7.1
7500	21200	1080	7.2
7500	20000	984	7.1
7333	19200	936	7.1

10333	25400	1248	6.5
9833	25000	1224	6.5
9166	24600	1200	6.6
8333	24000	1176	6.45
8000	22800	1152	6.3
8000	22400	1104	6.35

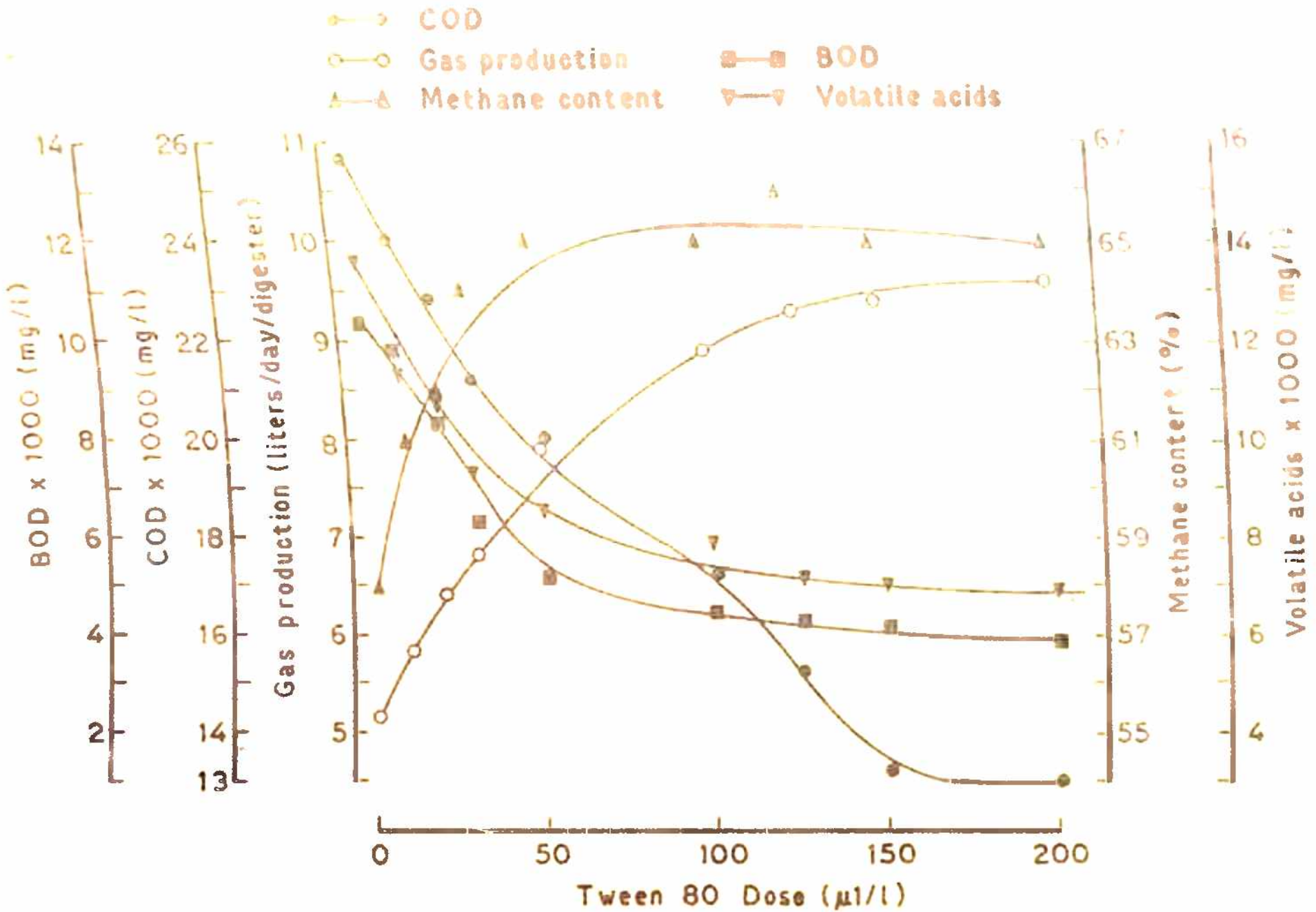

Fig. 4 ANAEROBIC DIGESTION PROFILE OF CATTLE-DUNG IN PRESENCE OF TWEEN 80 AT 35 ± 1°C

CHAPTER-IV

ADSORBENTS IN ANAEROBIC DIGESTION OF CATTLE-DUNG

CHAPTER-IV

ADSORBENTS IN ANAEROBIC DIGESTION OF CATTLE-DUNG

GENERAL

In an effort to improve the digestion process, the effect of various doses of different adsorbents like powdered activated charcoal, powdered ordinary charcoal, bentonite, kaolin and saw dust have been evaluated in a bench scale digesters. Recently it has been shown that addition of powdered activated charcoal stabilizes process performance of anaerobic digesters of sewage sludge (43,137). No study, however, seems to have been made so far on the effect of activated charcoal and other adsorbents on fermentation of cattle-dung. Therefore it was felt necessary to study this aspect with the ultimate aim of improving gas production in biogas fermentation.

ADSORBENTS

Adsorbents are chemically inert powders which have the ability to adsorb gases, toxins, and bacteria.

Whenever the surface of a solid or a liquid is allowed to come into contact with gas or the vapour of a liquid, there is usually increased concentration of the gas or the vapour molecules at the surface, regardless of gas or the surface. This phenomenon of surface concentration is known as "adsorption"(140,141).

Some of the adsorbents like activated carbon are used as a contact catalyst in various reactions of isomerization, polymerization, oxidation and halogenation. They also find use as a carrier for other catalysts, perhaps to a greater extent than generally realized(142). Some times they show catalytic properties by indirect action e.g. certain reactions are accelerated by activated carbon as a result of the adsorption of inhibitors as in recovery of iodine from iodides present in the petroleum salt brines. Nitrous acid is employed to oxidize the iodides to iodine - a reaction that may be retarded by the presence of inhibitors in the brine. Treatment of the brine with an activated carbon removes the inhibitors and enables the oxidation reaction to proceed (142).

Carbon may accelerate biological activity by

adsorbing poisons, or may retard it by adsorbing nutrients necessary for the growth of microorganisms. Amati (143) noted that carbon retarded the fermentation of sugars, but several other workers have reported an acceleration of biological activity (144-146). Lampe (147), working with molasses, reported that 0.1% of activated carbon greatly accelerated the fermentation.

In this study following adsorbents were used.

1. Powdered Activated Charcoal

The residue from the destructive distillation of various organic materials, treated to increase its adsorptive power. Procured from BDH.

2. Powdered Ordinary Charcoal

Obtained by burning wood and powdering.

3. Kaolin

A native hydrated aluminium silicate, powdered, and freed from gritty particles by elutriation. Procured from BDH.

4. Bentonite

Bentonite,hydrated aluminum silicate free from grit obtained from BDH.

5. Saw-dust

Obtained from local saw mill.

Several bench scale anaerobic digesters were used (Figure 3). Adsorbents (120 mesh) were incorporated with the feed sludge.

In one set all digesters were maintained at 35 ± 1°C in a thermostat and in other set, all digesters were exposed to ambient temperature.

RESULTS AND DISCUSSION

Table 11 (Figure 5) shows enhanced gas production with increasing amount of powdered activated charcoal (PAC), the optimum being 3,000 to 4,000mg per litre. The gas produced was also richer in methane content. Process stability as evidenced by lower volatile acid (137,138), consistently increased with increased levels of PAC. Activated charcoal gave lower values of BOD and COD indicating greater biodegradation (19,139).

Similar trend was observed in case of digester operated at ambient temperature (Figure 6). The average of the data recorded from July to November when the

ambient temperature varied from 40° to 15°C are given in Table 12. Table 11 contains the data of controlled temperature (35 ± 1°C). This interesting observation which was found in second set of experiments clearly indicates that powdered activated charcoal also serves the function of shock absorbent so that gas production is not significantly affected with variation in temperature.

Studies with other adsorbents like powdered ordinary charcoal (POC), bentonite, and kaolin which were done only with digesters operated at controlled temperature of 35 ± 1°C, also showed increased gas production with higher methane content. However, improvement was not as good as with PAC. Addition of saw dust was found to be detrimental to the digester.

It is concluded from this study that addition of powdered activated charcoal results in exceptionally high performance stability and increase in rate of decomposition. Even powdered ordinary charcoal was found to be effective. But its effect was less than activated charcoal may be because of lesser surface area. Thus PAC and POC could be used to increase the potentiality of existing digesters. Analysis of the

data shows that carbon provide sites for the anaerobic reaction to occur. Some observation has been made by other workers (43, 137). It appears that carbon enhances the methane forming step of the digestion process.

TABLE-11

Summary of effluent data during steady-state periods of digesters maintained at 35 ± 1°C in presence of adsorbents

	Dose (mg/l)	Total gas production (l/day/digester)	CH_4 (%)	BOD (mg/l)	COD (mg/l)	Volatile acids (mg/l)	pH
Control		5.1	58	10333	25600	1260	6.5
Powdered activated charcoal	100	5.2	58	10000	24000	1200	6.5
	500	5.5	60	9666	21200	1140	6.8
	1000	6.6	63	7833	17600	1050	7.1
	2000	7.0	65	6166	16800	750	7.0
	3000	7.8	68	4333	15200	720	7.1
	4000	8.0	67	3333	13200	690	6.9
	5000	7.6	67	3000	13000	720	6.9
	6000	7.0	67	3000	12000	690	7.0
	7000	6.9	66	2666	12000	600	7.0

Contd....

Table-11 Contd....

Powdered ordinary charcoal	100	5.1	58	10333	25200	1248	6.5
	500	5.2	58	10000	24400	1200	6.5
	1000	5.3	58	9666	24000	1200	6.5
	2000	5.7	60	9000	23600	1140	6.6
	3000	5.9	61	7833	20000	1092	6.8
	4000	6.5	61	6166	17200	1068	6.8
	5000	6.8	63	6166	16000	948	6.7
	6000	6.8	63	5996	15600	900	6.8
	7000	6.5	63	5500	15200	900	6.8
Bentonite	100	5.1	58	10000	25600	1248	6.5
	500	5.7	58	10000	25200	1200	6.7
	1000	6.0	61	9666	24400	1176	6.7
	2000	6.0	61	8666	22400	1080	6.75
	3000	6.0	61	8000	18800	1056	6.8
	4000	5.9	61	7000	17600	984	6.7
	5000	5.8	62	6666	17200	984	6.8

Contd....

Table-11 Contd....

Kaolin	100	5.1	58	10000	25200	1248	6.7
	500	5.5	59	9666	25200	1248	6.5
	1000	5.7	60	9666	24400	1248	6.5
	2000	5.7	59	9000	23200	1176	6.7
	3000	5.7	60	8000	21600	1176	6.7
	4000	5.7	60	7666	20000	1128	6.6
	5000	5.8	60	7000	19200	1056	6.6
Saw dust	100	5.1	57	10333	25600	1248	6.8
	500	5.0	57	10000	25600	1248	6.8
	1000	5.0	58	11000	26400	1296	6.4
	2000	4.9	58	11333	27200	1320	6.4
	3000	4.9	58	11333	27200	1560	6.2
	4000	4.8	58	11333	27600	1680	6.2
	5000	4.7	58	11666	28000	1704	6.1

TABLE-12

Summary of effluent data during steady-state periods of digesters at ambient temperature in presence of powdered activated charcoal (The average of the data recorded from July to November when ambient temperature varied from 40°-15°C)

	Dose (mg/l)	Total gas production (l/day/digester	CH_4 (%)	BOD (mg/l)	COD (mg/l)	Volatile acids (mg/l)	pH
Control	0	4.0	55	13000	31000	2640	6.5
Powdered activated charcoal	100	4.0	55	12666	30000	2592	6.5
	500	4.4	55	11666	28800	2520	6.5
	1000	5.0	58	11000	26800	2400	6.5
	2000	5.7	62	10000	24200	2016	6.8
	3000	6.2	62	8000	22000	1680	6.7
	4000	6.2	62	6333	21000	1584	6.8
	5000	6.2	62	5666	20800	1500	6.8
	6000	5.5	62	5000	20000	1308	6.8

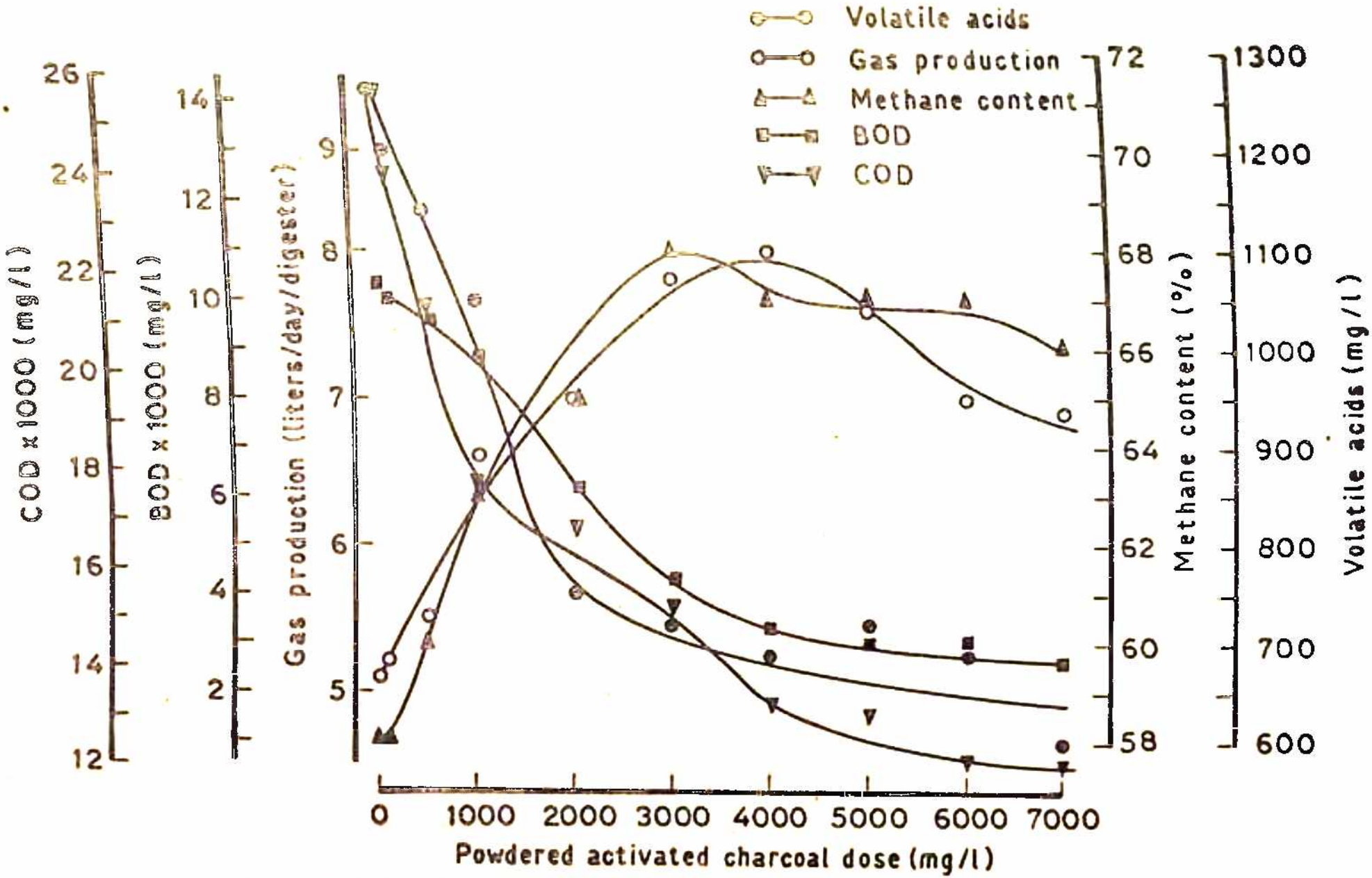

Fig. 5 ANAEROBIC DIGESTION PROFILE IN PRESENCE OF POWDERED ACTIVATED CHARCOAL MAINTAINED AT 35±1°C

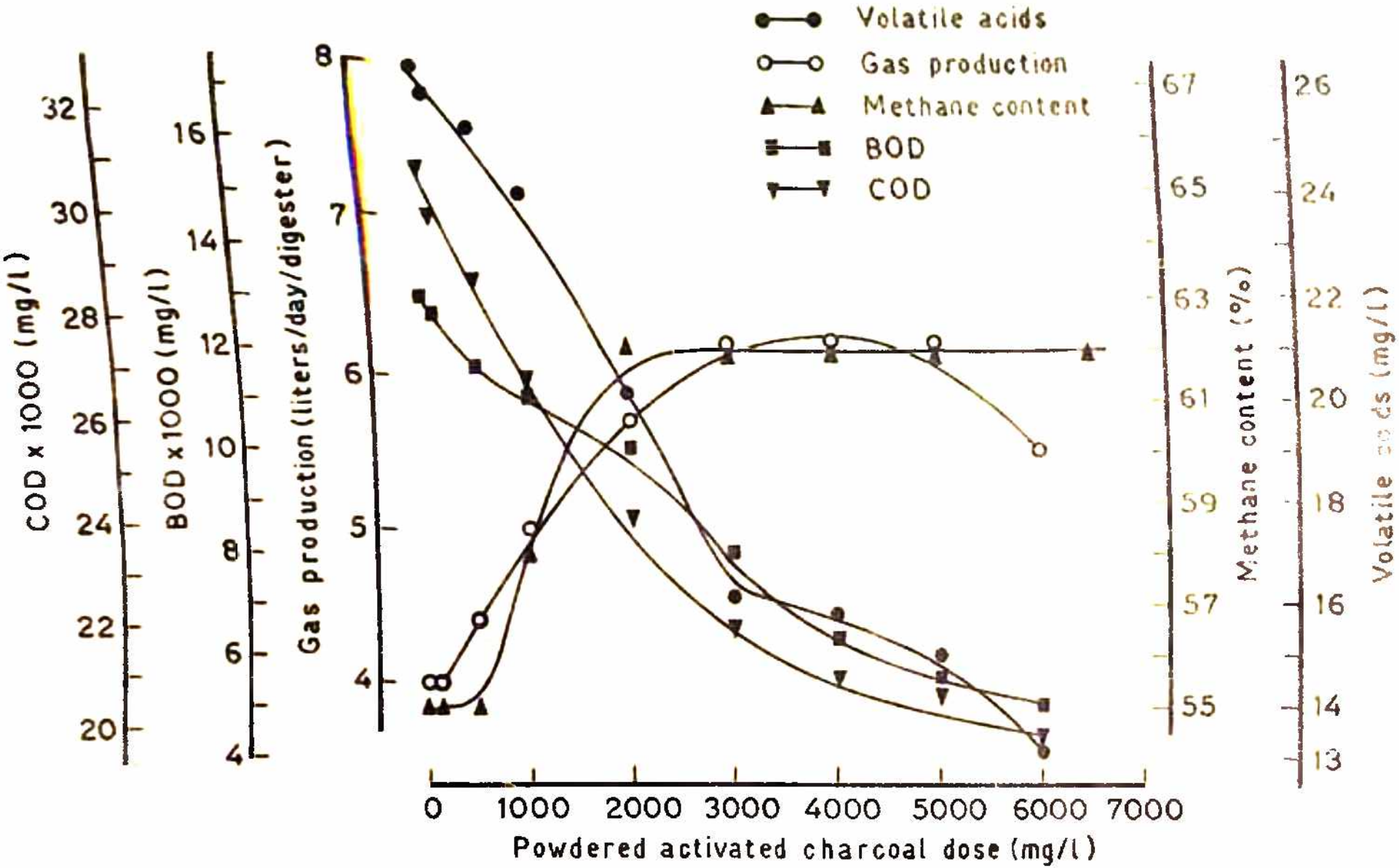

Fig. 6 ANAEROBIC DIGESTION PROFILE OF CATTLE-DUNG IN PRESENCE OF POWDERED ACTIVATED CHARCOAL MAINTAINED AT AMBIENT TEMPERATURE

CHAPTER-V

ADDITIVES IN ANAEROBIC DIGESTION OF CATTLE-DUNG

CHAPTER-V

ADDITIVES IN ANAEROBIC DIGESTION OF CATTLE-DUNG

GENERAL

Addition of powdered activated charcoal results in increase of total gas production with high methane content (Chapter IV). Based on review of literature, (43), it is evident that carbon is responsible for improved digestion. The surface of the activated carbon provides adsorption sites where substrate can accumulate, thereby providing high localized substrate concentration. These areas of adsorption provide a more favourable growth environment for bacterial substrate systems (45). No study, however, seems to have been made so far on the effect of other additives like pectin on anaerobic digestion of cattle-dung.

Since pectin is also used, as one of the adsorbent in many cases (132), it appeared desirable to study the effect of pectin on anaerobic digestion of cattle-dung with the ultimate aim of improving the production of

gas with increased methane content. The impact of pectin on volatile acid, pH, and process stability has also been examined. This chapter present the results of two sets of the experiments involving pectin addition to bench-scale anaerobic digesters. One set of experiments was carried out at controlled temperature of 35 ± 1°C while the other at ambient temperature from 40° to 15°C.

Similarly effects of addition of sugar cane bagasse on gas production, methane content, pH, volatile acids and biodegradability has been studied.

Pectin

The cellular tissues of citrus fruits, apples, and many other fruits contain pectin, which is an mucilagenous carbohydrate. It is soluble in about 20 parts of water, yielding a viscous, apalescent colloidal solution which is slightly acidic in reaction. Pectin is insoluble in alcohol. It is considered to be made up of chain molecules, consisting largely of polygalacturonic acids which are partially in the form of methyl esters. In this study two forms of pectin have been used. Commercial pectin obtained from BDH and a crude form was prepared in the laboratory from fruits of Citrus medica plant.

Sugar Cane Bagasse

A huge amount of cellulose goes waste either in intermediate or terminal stages of utilization or processing. In India the total quantity of agricultural by products or wastes which are cellulosic in nature accounts for nearly 50-100 million tons per year, 50% of which is cellulose (148). Sugar cane bagasse one of the major cellulose waste accounts for 5.3 million tons/annum (148).

MATERIALS AND METHODS

In one set all digesters were maintained, at 35 ± 1°C in a thermostat and in other set, all digesters were exposed to ambient temperature - ambient temperature varied from 40° to 15°C that is from August to November. In case of crude pectin as well as for sugar cane bagasse all digesters were maintained at 35 ± 1°C.

Pectin and sugar cane bagasse were incorporated with feed sludge.

RESULTS AND DISCUSSION

A trend of enhanced gas production with increased

amount of commercial pectin is evident from Figure 7. Maximum enhancement (of over 150 percent) was achieved with addition of 10 g/l pectin. (Figure 7, Table 13). In addition to increasing total gas production, pectin was responsible for higher methane content in digester gas. As shown in Figure 7, as much as 65 percent methane was present in the total gas. Similar trend was also observed even in case of digester operated at ambient temperatures. The average of the data recorded from August to November when the ambient temperature varied from 40° to 15°C are given in Table 14. Table 13 contains the data of controlled temperature (35 ± 1°C).

Process stability as evidenced by lower volatile acids (137,138) consistently increased with increased levels of pectin (Table 13). Average acid concentration ranged from 1260 mg/l in the digester with no pectin to 660 mg/l in the highest dosed digester. Effect of pectin on volatile acid concentration was found to be more prominent in digester at ambient temperature, whereas volatile acid concentration was reduced to less than half. It indicated that the volatile acids were consumed at a faster rate than that in the controlled experiments where no pectin was used.

In both sets of experiments pH was found to be more or less constant that is near the neutral region.

Process performance can also be judged by biochemical oxygen demand (BOD) and chemical oxygen demand (COD) values which indicate extent of biodegradation (19,137,139). Pectin in both experiments gave low values of BOD and COD indicating greater biodegradation as shown in Table 13 and 14. Even the addition of crude form of pectin obtained by using dried, powdered rind along with some inner part of Citrus medica fruits resulted in increased methane production and greater process stability.

In the present study it was found that pectin is not utilised to appreciable extent in the anaerobic digestion process. On the contrary its quantity remained was found to be only slightly less than the loaded amount Therefore it seems that pectin acts in a way similar to powdered activated carbon. Pectin may be providing adsorption sites where substrate can accumulate, thereby providing high localized substrate concentration. These areas of adsorption provide a more favourable growth environment for bacteria substrate system.

Another intersting observation was made in the second set of experiments where digesters were operated

at ambient temperature varying from 40° to 15°C. As shown in Table 14, addition of pectin showed considerable increase in the total gas production with high methane content. This clearly indicates that pectin also serve the function of shock absorbent so that gas production is not significantly affected with variation in temperature.

Studies with sugar cane bagasse (Table 13), done only at controlled temperature of 35 ± 1°C, showed increased gas production but not increase in methane content. Improvement in general was not found to be as good as pectin.

This study indicates that addition of pectin and sugar cane bagasse results in exceptionally high performance stability and increase in rate of decomposition.

TABLE-13

Summary of effluent data during steady-state periods of digester maintained at 35 ± 1°C

	Dose (mg/l)	Total gas production (l/day/digester)	CH_4 (%)	BOD (mg/l)	COD (mg/l)	Volatile acids (mg/l)	pH
Control		5.1	58	10333	25600	1248	6.5
Commercial pectin	100	5.1	59.0	10333	24000	1200	6.5
	500	6.4	60.5	9566	21200	1056	6.7
	1000	7.2	62.0	7833	17600	744	6.75
	2000	8.5	63.5	6166	15200	696	6.7
	4000	10.1	64.5	4333	14000	696	6.6
	6000	12.5	65.0	3333	13000	672	6.65
	8000	12.7	65.0	3000	12200	660	6.7
	10000	12.8	65.0	3000	11800	660	6.7

Contd....

Table-13 Contd....

Crude pectin	1000	5.9	58	10333	25000	1248	6.7
	2000	6.7	60	10000	24400	1200	6.75
	5000	8.8	61.5	8000	24000	1116	6.7
	8000	10.2	62.5	6333	21200	1056	6.4
	10000	11.0	63.0	5000	19400	960	6.6
	12000	11.3	63.0	4333	17000	744	6.5
	15000	11.5	63.0	4000	16600	720	6.5
Sugar cane bagasse	2000	6.0	58	9833	21800	1200	6.5
	5000	7.6	58	7833	21000	1128	6.8
	10000	8.7	58	7500	18000	984	6.8
	12000	9.2	58	5166	17200	792	6.8
	15000	9.4	58	5166	17000	750	7.0
	17000	9.0	56	6333	17200	840	6.9
	20000	8.9	56	7180	18000	888	6.9

TABLE-14

Summary of effluent data during steady-state periods of digester at ambient temperature. (The average of the data recorded from Aug. to Nov. when the ambient temperature varied from 40°C to 15°C)

	Dose (mg/l)	Total gas production (l/day/digester)	CH_4 (%)	BOD (mg/l)	COD (mg/l)	Volatile acids (mg/l)	pH
Control		3.2	53	13000	31000	2640	6.5
Commercial pectin	100	3.2	53	12666	30000	2592	6.5
	500	3.4	55	11666	28800	2400	6.4
	1000	4.3	56.5	11000	26400	2208	6.45
	2000	5.0	58.0	10000	24200	2016	6.55
	4000	5.6	60.5	8000	22400	1608	6.7
	6000	5.8	61.0	6333	20000	1584	6.75
	8000	6.2	62.0	5666	19200	1500	6.6
	10000	6.4	62.5	5000	18800	1306	6.65
	15000	6.5	62.0	5333	18600	1296	6.7

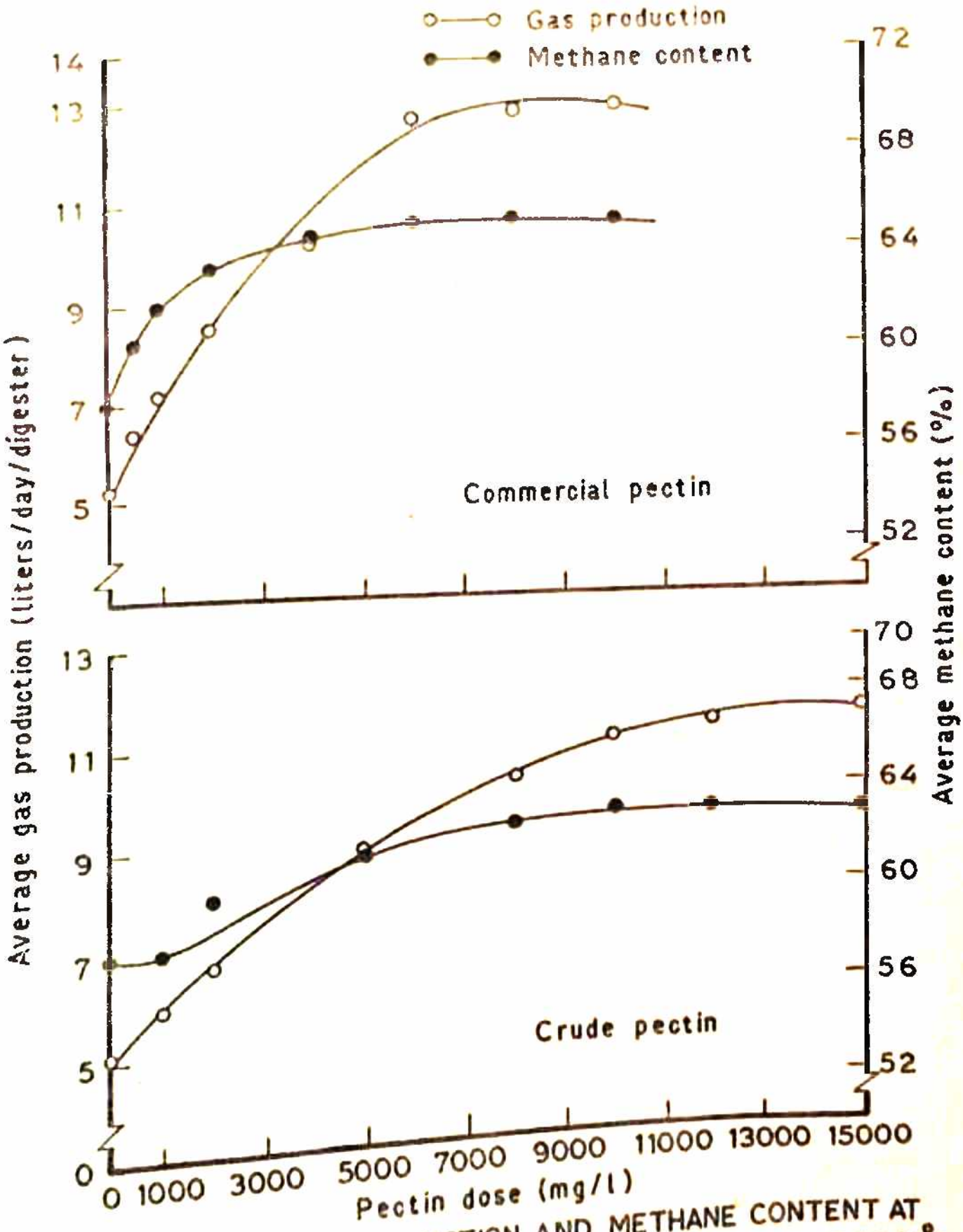

Fig.7 AVERAGE DAILY GAS PRODUCTION AND METHANE CONTENT AT VARIOUS DOSES OF PECTIN IN DIGESTER MAINTAINED AT 35±1°C

CHAPTER-IV

SUMMARY AND CONCLUSIONS

CHAPTER-VI

SUMMARY AND CONCLUSIONS

Anaerobic digestion of waste matter resulting in production of bio-gas which is a valuable source of energy has received much attention recently. In an effort to improve, the anaerobic digestion process, effect of manipulations of various parameters like temperature, retention time, total solid content, agitation, and pH has been studied on the quantum of gas production and on the content of methane in the bio-gas of cattle-dung at laboratory scale. With respect to these parameters experimental conditions has been standardized. It has been found that temperatures of the order of 35 ± 1°C with 10-12 days retention time gives maximum efficiency. Interrupted agitation of about 10 hours improved the amount of gas production with increased rate of biodegradation.

To increase the potentiality of existing digesters (in terms of increase in total quantity of gas and enrichment of its methane content), by keeping the metabolic processes of microbes at the optimum under varying thermal environments with the help of certain additives have been studied. Substances such

as powdered activated charcoal, pectin, and tween 80 showed exceptionally enhanced gas production with enriched methane content. They also gave lower content of volatile acids indicating high process stability. It is evident from this study that, in presence of additives like powdered activated charcoal, pectin , and tween 80, volatile acids were consumed at a faster rate than that in the control.

It has been documented that protein and carbohydrate fermentating bacteria grow rapidly, and the substrates are rapidly degraded into fatty acids even under unbalanced state (109). However, the fermentation of fatty acids does not occur until the retention time or other factors are favourable for the growth of fatty acid fermentating bacteria (73,74). Therefore the rate limiting step in methane fermentation often involves the degradation of fatty acids which is related to the efficiency of H_2 utilization by methanogenic bacteria. From the present study, it certainly appears that, these additives enhance the methane forming step of the digestion process. They also increased the rate of biodegradation by reducing the values of BOD and COD.

Powdered activated charcoal and pectin served the

function of shock absorbents so that gas production does not get significantly affected with variation in temperature.

Analysis of the data shows that powdered activated charcoal and pectin may be providing adsorption sites where substrate can accumulate therby providing high localized substrate concentration. These areas of adsorption provide a more favourable growth environment for bacteria-substrate system.

Tween 80 may be improving digestion process either by providing more favourable environment for microbes to grow or removing inhibitors from the environment through its unusual property of polymeric forms (micelles) (133).

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