
CHARACTERIZATION OF WASTES FROM NATURAL RUBBER AND RUBBER WOOD PROCESSING INDUSTRIES AND THEIR UTILIZATION FOR BIOMETHANATION

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by

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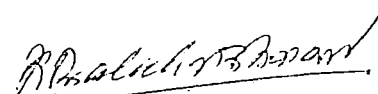
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C E R T I F I C A T E

This is to certify that this thesis is an authentic record of the research work carried out by Mr. Jacob Mathew, under my scientific supervision and guidance at the Rubber Research Institute of India, Kottayam, in partial fulfilment of the requirements for the degree of Doctor of Philosophy of the Cochin University of Science and Technology under the Faculty of Environmental Studies, and no part thereof has been presented for the award of any other degree, diploma, or associateship in any University.




Dr. K.P. Balakrishnan
(Supervising Guide)

D E C L A R A T I O N

I hereby declare that this thesis entitled, **Characterization of Wastes from Natural Rubber and Rubber Wood Processing Industries and Their Utilization for Biomethanation**, has not previously formed the basis of the award of any degree, diploma, associateship, fellowship or other similar titles for recognition.

Kottayam

September 1994



Jacob Mathew

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LIST OF ABBREVIATIONS

ADF	-	Acid detergent fibre
ADL	-	Acid detergent lignin
BOD	-	Biochemical oxygen demand
BS	-	Biodigested slurry
CD	-	Cowdung
COD	-	Chemical oxygen demand
CPE	-	Crumb processing effluent
CR	-	Crepe rubber
CRE	-	Crepe rubber effluent
CS	-	Crumb sludge
CW	-	Crumb waste
DO	-	Dissolved oxygen
DS	-	Dissolved solids
K	-	Potassium
LC	-	Latex concentrate
LCE	-	Latex concentrate effluent
N	-	Nitrogen
NDF	-	Neutral detergent fibre
P	-	Phosphorus
PSD	-	Predigested sawdust
RSS	-	Ribbed smoked sheets
SD	-	Sawdust
SPE	-	Sheet processing effluent
SS	-	Sewage sludge
TOC	-	Total organic carbon
TS	-	Total solids
TSR	-	Technically specified rubber
VFA	-	Volatile fatty acids
VS	-	Volatile solids

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CHAPTER 1

INTRODUCTION

INTRODUCTION

Environmental contamination is an inevitable consequence of human activity. But, in recent times, the activities of human beings became so complex and multidimensional that the quantity of by-products and unutilized resources have started mounting up at phenomenal rates, and in many instances, have exceeded the carrying capacity of the biosphere, resulting in pollution of air, water and land. The threat to the living systems, if not properly tackled, the doomsday is not very far. Unlike other industries, agro-based industries generate enormous solid and liquid wastes of carbonaceous and nitrogenous origin. Management of contamination by agroindustries, so as to mitigate or eliminate pollution problems, is rather complicated, but essential. Natural rubber industry is no exception in this regard. This calls for a detailed study of the whole programme in order to maintain developmental efforts and quality of environment.

Rubber tree (Hevea brasiliensis Muell. Arg.), is the most important commercial source of natural rubber and in

India, it is grown in an area of over five hundred thousand hectares with an annual production of 3.9 lakh tonnes during 1992-93. This is 5.3 and 6.8 per cent of the area under production and the total production of the world respectively. In India, 88.24 per cent of rubber growing area is in the state of Kerala. With the increase in area under this crop, considerable advancement in latex and rubber processing technology is also taking place. Various field management practices and processing of the raw materials are known to cause environmental damage. A wide range of fungicides, weedicides and insecticides used in crop protection and the chemicals used in rubber processing are known to cause an impact on environmental balance in rubber plantations [Yapa, 1984]. However, the most noticeable level of pollution in rubber plantation industry is caused by effluents from processing units containing sizeable amounts of non-rubber substances, besides unreacted processing chemicals. A glance at the geographical map of Kerala (Figure 1) shows the distribution of rubber processing units, which are located on river banks in view of easy availability of water for processing and for disposal of effluents. The number of factories under different types of processing, rubber production and their percentage are given in Appendix 2.

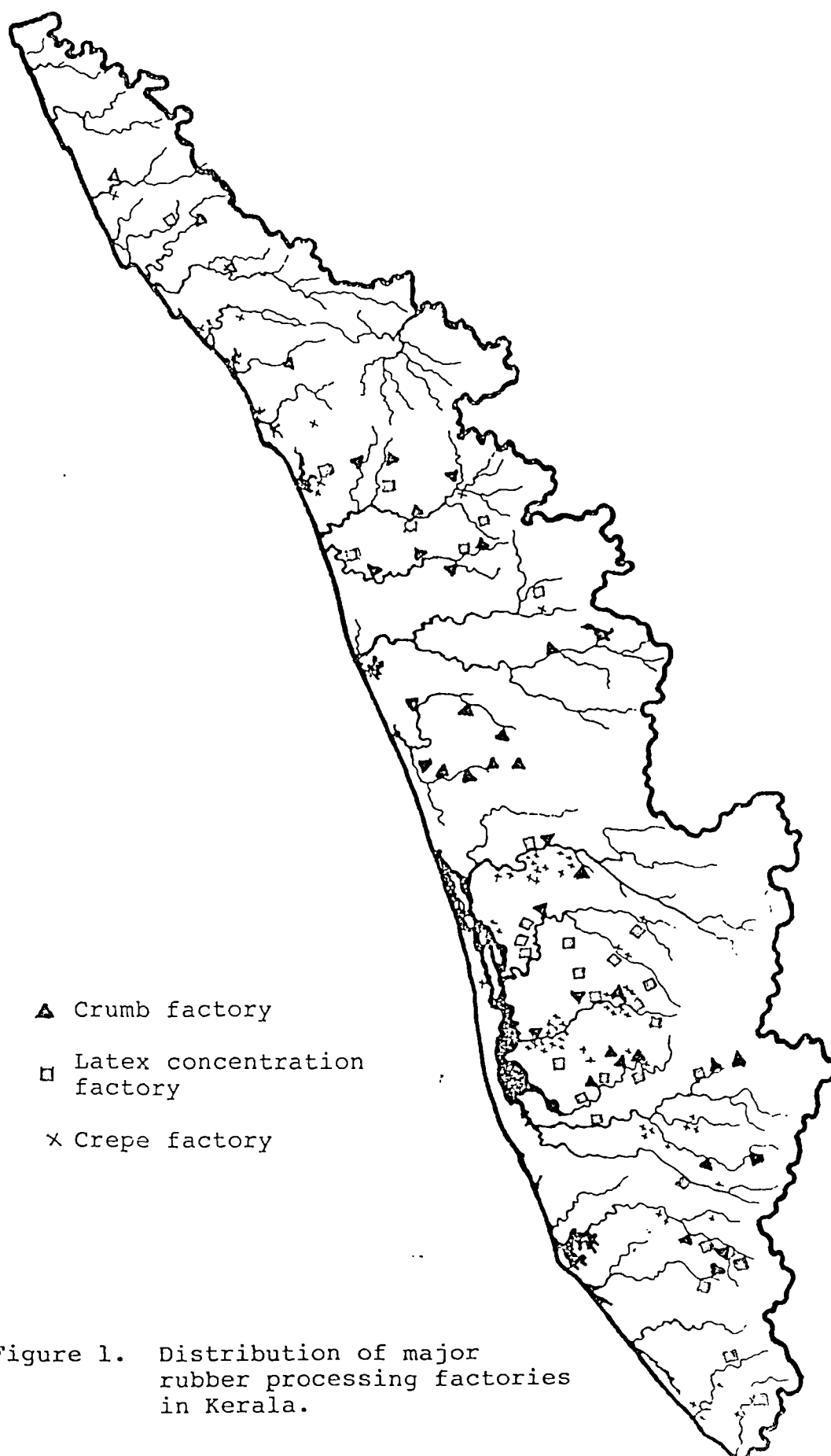


Figure 1. Distribution of major rubber processing factories in Kerala.

Natural rubber is obtained in the form of latex of which the rubber hydrocarbon constitutes 25-40 per cent. The non-rubber constituents, which include 2-2.5 per cent proteins, 1-1.5 per cent sugars, 1-2 per cent resins and 0.7-0.9 per cent ash, provide a substantial growth medium for microorganisms [Taysum, 1956]. Rubber hydrocarbon is processed in different forms like ribbed smoked sheets (RSS), various forms of crepe rubber (CR), technically specified rubber or crumb rubber (TSR) and latex concentrate (LC) [Kuriakose, 1992].

In small holdings and medium sized estates, processing of latex into RSS is very popular. Out of 3.9 lakh tonnes of the total rubber processed, 71.49 per cent is in the form of RSS [Rubber Board, 1993]. The processing capacity of such units varies from a few kilograms to a few tonnes and there are numerous such units. Effluent generated from such factories also range from a few litres to several thousand litres.

Crepe rubber is produced by 102 factories and the quantity of rubber processed in this form is 38,803 tonnes per annum, which form only 9.86 per cent of the total production. TSR covers 6.88 per cent of production through 31 factories. Latex concentrate factories numbering 62, process 11.75 per cent of the total rubber produced [Rubber Board, 1993]. The number of factories

and the quantity of rubber processed are increasing every year as seen in the Appendix 1.

The quantity of effluent generated per kg of processed rubber from various types of processing is given in the Appendix 2. Total estimated effluent generation from natural rubber processing in India during 1992-93 was 79,32,892 m³.

A large quantity of water is used for dilution of latex and for washing. During processing, inorganic and organic acids and ammonia in varying quantities are used. The wash water, unreacted chemicals, settleable solids in the form of bark shavings and sand particles constitute the effluent having high biochemical oxygen demand (BOD), chemical oxygen demand (COD), nitrogen etc.

Processing of rubber wood generates substantial amount of solid waste in the form of sawdust and bark. Saw dust has comparatively low combustion value and hence not widely used as domestic fuel [Tan and Stott, 1987].

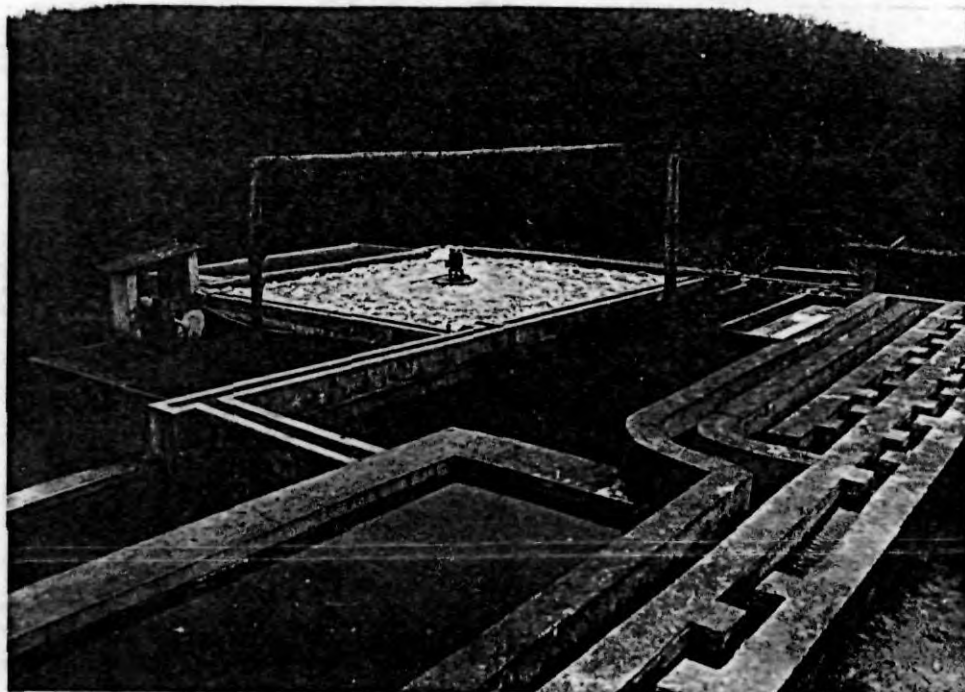
Recently, pollution from the rubber processing factories has become very serious due to the introduction of modern methods and centralized group processing practices. On an average, 25 l of effluent is generated from every kg of dry rubber processed.

It is mandatory for rubber processing factories to treat the effluent to attain the specifications stipulated by the Pollution Control Board (Plate 1a & b). The expenditure on the treatment systems in vogue is large and it adds to the cost of production of rubber. A successful effluent treatment system should be simple, economic and yielding returns. Microbial activities have successfully been employed for the treatment of waste to generate food and fuel.

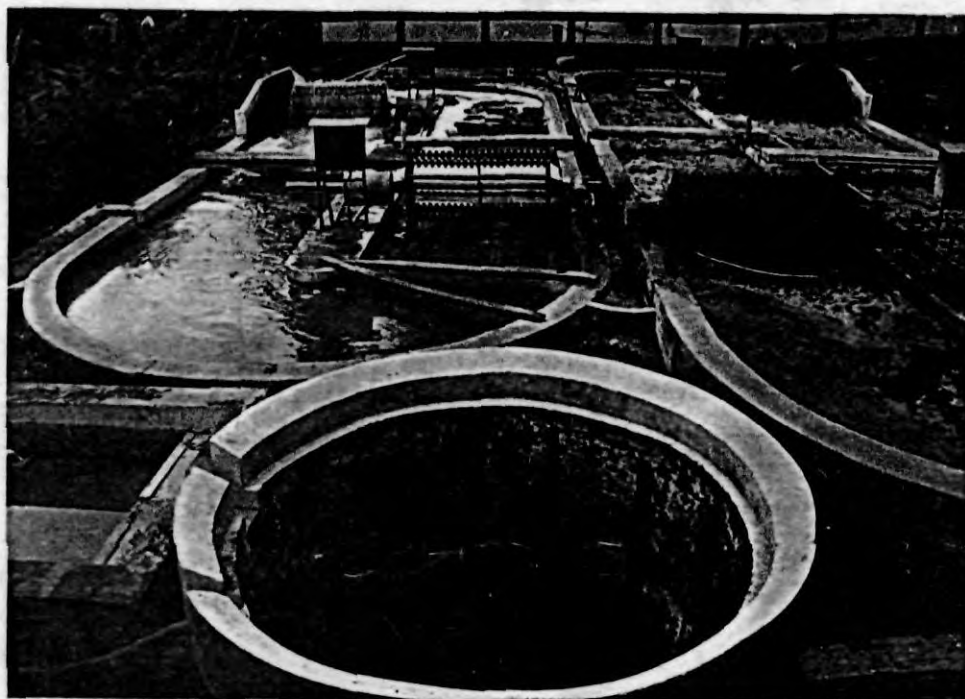
The world today is confronted with scarcity of fuel. Indiscriminate consumption of fossil fuels and denudation of forests contribute to energy crisis which necessitates exploration of the non-conventional sources of energy, that could be generated at low cost, by recycling and reuse of wastes. The anaerobic digestion of wastes by microorganisms is not only energy generating and environment friendly but also provides organic manure, rich in plant nutrients.

The possibility of utilizing liquid waste from rubber processing factories as well as solid waste like sawdust from rubber wood processing for generation of biogas is not fully explored. Rubber wood wastes being lignocellulosic can be depolymerized by microorganisms to monomers which serve as precursor to produce methane, alcohols and single cell proteins.

PLATE 1. EFFLUENT TREATMENT SYSTEMS



(a) Floating aerator



(b) Cage rotor

Since rubber cultivation is a continuous process involving the removal of old plants and replanting, rubber wood sawdust is available regularly.

In this study, an attempt is made to evaluate wastes from rubber and rubber wood processing as feed stock materials for biogas generation and to identify the optimum proportion of the liquid and solid wastes which ensures maximum gas production.

The experiments were designed and carried out with the following objectives.

1. To characterize and quantify the wastes from different types of rubber processing factories including rubber wood processing industries.
2. To evaluate and optimise the relative solid content for maximum biogas production.
3. To study the influence of different sources of microbial inoculum on the quantity of biogas production.
4. To study the effect of mixing of liquid waste with raw and predigested solid wastes on biogas production.
5. To evaluate the manurial values of the digested slurry.

CHAPTER 2

REVIEW OF LITERATURE

REVIEW OF LITERATURE

Water pollution due to sewage has gained sufficient attention in the past. Adequate information is available on its physicochemical and biological characteristics, impact on ecosystem and management. As a sequel to agricultural activities, being practised on an industrial basis, the quantity of wastes that reaches the aquatic environment has attained new dimensions leading to aquatic pollution. Effluents from natural rubber processing industries fall under this category. Extensive studies on rubber processing effluent were carried out in Malaysian rubber plantations.

However, in India information available on characterization, treatment and utilization of wastes from rubber based industries are scattered [Mathew et al., 1986]. The pertinent literature available on generation and characterization of wastes from rubber latex and wood processing and utilization of wastes for methanogenesis is reviewed.

Rubber is one of the important plantation crops in India. It occupies over five hundred thousand hectares of land producing annually about 3.9 lakh tonnes of natural

rubber [Rubber Board, 1993]. Thomas et al. (1980) reported that rubber harvested in the form of liquid from the bark of Hevea tree contained 30-40 per cent rubber hydrocarbon and varying quantities of non-rubber constituents like proteins, carbohydrates, lipids etc. The latex in situ is sterile but on tapping soon gets contaminated [Taysum, 1960]. The microorganisms decompose the organic compounds like proteins and carbohydrates, emitting unpleasant odour due to hydrogen sulphide. Consequently, BOD increases and causes environmental pollution [Ibrahim et al., 1974]. In addition to the organic compounds in latex, a sizeable quantity of inorganic and organic chemicals is used in the processing of latex. On an average 20 l of water per kg of rubber is used and the same is discharged as effluent loaded with native organic compounds of latex origin and other chemicals used for processing [Mathew et al., 1991a].

2.1. Characterization of wastes

All the four types of rubber processing factories generate effluents viz., sheet processing effluent (SPE) by ribbed smoked sheet (RSS) factories, crumb processing effluent (CPE) by technically specified rubber (TSR) factories, crepe rubber effluents (CRE) by crepe rubber (CR) factories and latex concentrate effluent (LCE) by latex concentrate (LC) factories. A study conducted by

Muthurajah et al. (1973) on the physical, chemical and biological properties of effluents from all the four types of rubber processing factories showed that they were pollutants. They have also reported that the effluent contains small amounts of rubber, varying quantities of proteins, sugars, lipids, carotenoids, inorganic and organic salts which increase the BOD, dissolved and suspended solids and nitrogen content of the receiving water bodies. Molesworth (1957), Muthurajah et al. (1973), Ponniah et al. (1975) found that effluents from LC and RSS factories were more polluting than CR and TSR factories. Mathew et al. (1986) also made similar observations. The LCE contained only 1/10 of the total microbial population compared to the CRE.

Attempts on effluent treatment was initiated in the sixties. Biological treatment by trickling filtration was tried initially and was reported to be efficient in reducing pollution [Molesworth, 1958, 1960]. In order to reduce the space required for the treatment and to increase the efficiency, improvements and modifications were made in the treatment systems from time to time [Chick, 1972, 1973; Muthurajah et al., 1973; John et al., 1974; Ponniah et al., 1976; Natta et al., 1977; Ibrahim et al., 1979; Hong, 1981; Ibrahim, 1983; Karim and Ibrahim, 1985]. However, the effluent treatment systems adopted at present demand more space and expenditure,

which add to the cost of production of natural rubber [Yeow and Yeop, 1983; Yapa, 1984].

2.2. Utilization of wastes

Taysum (1956) used effluents from rubber processing factories for culturing bacteria from latex. Mathew et al. (1987) reported that yeasts, Torulla utilis and Saccharomyces cerevisiae could be grown using sheet serum, a liquid waste from RSS processing factories. The effluents from natural rubber processing factories were also used for the production of ethanol by different groups of yeasts [Lau et al., 1989].

Diluted and partially treated effluents support the growth of unicellular algae like Chlorella sp. [Kulkarni et al., 1973; Kothandaraman and Nair, 1976; Mathew et al., 1987, 1991a].

Rubber processing factory effluents are reported to be used as a source of fertilizer and irrigation water for field crops [Tan et al., 1975; John et al., 1977; Dolmat et al., 1979; Lim and P'ng, 1984; Bachik et al., 1987; Wood and Lim, 1989; Karim et al., 1989]. Solid waste was also utilized for the cultivation of oyster mushrooms [Mathew et al., 1991b].

Rubber wood sawdust, the solid waste from rubber wood industry, contains different levels of cellulose and lignin[Tan and Stott, 1987]. Wahab (1986) and Kothandaraman et al. (1989, 1991) have successfully cultivated oyster mushrooms using the rubber wood sawdust.

Fang et al. (1985) and Pandey et al. (1990) made attempts to use the effluents from rubber processing factories for biogas production.

2.2.1. Methanogenesis

A variety of carbon compounds from simple sugars to complex polymerised cellulose is good substrate for biogas production. Manures from poultry, cattle, horse, urban refuse, human waste, sewage sludge, weeds and crop residues were used as raw materials for biogenesis of methane [Stafford et al., 1980a]. Energy crisis can partially be solved by the production of biogas from renewable feed stocks and agricultural wastes [Nagar, 1975; Munde, 1977; Daniel et al., 1990; Reichards et al., 1991].

The anaerobic digestion of piggery waste [Bousfield et al., 1974; Hobson et al., 1980; Hashimoto, 1983; Callander and Barford, 1984; Cullimore et al., 1985; Durand et al., 1988], poultry wastes [Bousfield et al., 1979; Hobson et al., 1980; Field et al., 1985,

Hunik et al., 1990], cattle waste [Hashimoto, 1981; Jain et al., 1983; Chen et al., 1988; Wohlt and Frobish, 1990] and agricultural wastes [Neelakantan et al., 1978; pfeffer, 1980; Hashimoto Gosh et al., 1985; Kalra and Panwar, 1986; Wong and Cheung, 1989] for biogas generation has been reported.

2.2.1.1. Agricultural wastes

The use of wastes from agriculture as alternate feed stock for biogas generation received more attention. Mishra (1954) discussed on the quantity and composition of gas produced by anaerobic decomposition of wastes like potato slices, maize seed, filter paper, sugarcane, bagasse, sawdust, groundnut shell, peptone, daincha and cattle dung.

Arokiaswamy (1978) also reported that anaerobic digestion of vegetable wastes, weeds, sheep and poultry waste generates biogas. Neelakantan et al. (1978) recorded higher yield of acid and biogas upon anaerobic digestion of plant materials like hybrid napier grass, berseem and paddy straw with and without the addition of 2.5 per cent nitrogen as ammonium sulphate.

Methanogenesis from crop residues was studied and suitable additions of other biodegradable material like cowdung to increase the biogas production was also studied

[Lingaiah and Rajasekharan, 1986]. Paddy straw was also used as a feed stock for biogas production [Neelakantan et al., 1978; Kalra and Panwar, 1986; and Hashimoto, 1989]. Methane from wheat straw and bajra [Chawla, 1973] and corn stover [Fujita et al., 1980] was reported. Leguminous plants also serve as a suitable substrate for biogas production. Gram pulses [Mishra, 1954] Leucaena leucocephala [Lalitha et al., 1984] and Glyricidia masculata [Gunaseelan, 1988] yielded different levels of biogas depending on their C:N ratio.

Biogas production from various weed biomass was also extensively studied. Mirabilis [Sharma and Panwar, 1985], lantana [Dar and Tandon, 1987], Euphorbia thirucalli [Rajasekharan et al., 1989], parthenium [Gunaseelan and Lashmanaperumalsamy, 1990], Eupatorium odoratum [Jagadeesh et al., 1990] and aquatic weeds like water hyacinth, salvinia, azola, nymphaea, hydrilla, utricularia [Abbasi et al., 1990; Mallik et al., 1990] produced biogas upon anaerobic digestion with suitable amelioration.

2.2.1.2. Agroindustry waste .

Refining the agricultural commodities in agroindustries to suit modern market requirements generates plenty of wastes. These wastes also can be used for biogas production [Hobson et al., 1981]. Knol et al.

(1978) reported that apple waste, carrots, asparagus, green peas, french beans, spinach and strawberries from a canning industry have been shown to produce biogas on anaerobic digestion. Kalia et al. (1992) observed methanogenesis from apple pomace and vegetable wastes like raddish leaves, cauliflower leaves and stalk of rotten cabbage. Sarada and Nand (1989) reported biogas generation from tomato processing waste. Wastes from mango processing factory generated 0.21 m^3 of biogas per kg of mango peals [Mahadevaswamy and Venkataraman, 1990].

Wood wastes of soft wood which are low in lignin content are good substrate for methanogenesis. Since the major components like, cellulose and hemicellulose are easily attacked by anaerobic cellulolytic organisms leading to the formation of simple carbohydrates, the substrate for methane production, much emphasis is given for these material in biogas production.

Wong and Chueng (1989) observed biogas generation from lignocellulosic wastes like cardboard, newspaper, sawdust and sugarcane waste, when fermented anaerobically with pigmanure. Willow dust, a solid cellulosic textile mill waste, with and without predigestion produced biogas when the solid liquid ratio was adjusted to 1:6 [Balasubramanya et al., 1981, 1986].

2.2.1.3. Pretreatment of the waste for methane production

The efficiency of the digestion process can be increased substantially by giving proper pretreatments which improve the biodegradability. Millet et al. (1975) 5) and Reig et al. (1989) stated that methane yields from digestion of lignocellulosics can be greatly enhanced by physico-chemical pretreatment that separates lignin from cellulose. A long adaptation period was required to obtain an active microbial population from untreated waste digesters that metabolized soluble chemical hydrolysis products of lignin [McCarty et al., 1976].

The degree of liquification could be considerably increased by pretreatment of solid wastes. Chemical treatment with acid or alkali for 1 h at pH 1.0 and 13.0 respectively increased the degree of liquification [Ilamurugu, 1985]. Enhanced liquification with alkali treatment favoured nearly 2-3 times more methanogenesis than that of untreated control [Pavlostathis and Gossett, 1985]. Haug et al., 1978 and Van Velsen et al. (1979) 9) reported that the thermal pretreatment of the feed materials during 1 h at 100°C under atmospheric pressure improved the degree of liquification by about 80 per cent.

Predigestion of substrate for biogas production is reported to enhance the methane content in the biogas.

singh et al. (1983) observed that methane in predigested cattle waste slurry increased from 68-75 per cent to 75-86 per cent. Kalia and Kanwar (1990) reported that predigested agroindustry waste when mixed with cowdung and fed to biogas plant, produced biogas with 62-77 per cent methane, as compared to 56-60 per cent methane from pure cattle dung.

Dudhbhate et al. (1984) studied the effect of pretreatment of cattle dung for augmentation of biogas production. The pretreatment of cattle dung slurry with Trichoderma reesi would enhance the cellulose degradation to 67 per cent as against 38 per cent in control with consequent rise in the biogas generation.

Vegetable substrates like radish leaves, cauliflower leaves and stalk of cabbage digested by bacteria yielded 210 l of biogas with 57 per cent methane [Kalia et al., 1992]. Oi et al. (1977, 1980) treated agrowastes with cellulolytic and hemicellulolytic enzymes and observed an increase in methane production as compared to that of untreated wastes.

2.2.1.4. Carbon nitrogen (C:N) ratio

De Renzo (1977) showed that digestion of paper pulp with sewage mixtures was feasible upto 9 per cent solids with a C:N ratio of 45:1. Other conditions being

favourable, a C:N ratio of 30:1 will permit digestion to proceed at an optimum rate.

Barnett et al. (1978) and Pyle (1978) stated that the optimum C:N ratio recommended for an anaerobic digester was 30:1. Addition of glucose or cellulose increased C:N ratio from 8 to 25 and resulted in methane production upto 60 to 70 per cent [Hills, 1979].

2.2.1.5. Inoculum

Seeding was recommended as a start up practice by Hobson and Shaw (1971) for quickening the digestion process. Addition of biodigested cowdung slurry at the rate of two per cent (v/v) to the cowdung slurry gave more gas production than cowdung alone [National Academy of Sciences, 1981]. Vanden Berg and Lentz (1981) observed that frequent inoculation of sludge as a starter during normal fermenter operation increased the gas production rate upto 10 to 15 per cent.

The use of spent slurry as inoculum at five per cent level reduced the retention time by four weeks and improved the gas production [Jain et al., 1982]. Lapp et al. (1984) reported that addition of actively digesting material from a municipal sewage sludge system improved the gas output. Hashimoto (1989) reported that methane production rate increased at a reducing rate up

to an inoculum substrate ratio of two, after which it remained constant. When straw was used as substrate for biogas production an inoculum concentration of 20 per cent v/v gave maximum methane [Hashimoto, 1989].

2.2.1.6. Feed concentration

Requirements for solid anaerobic fermentation as evidenced from the available literature varied from source to source. Early studies achieved successful digestion of sewage solids at 20 per cent and higher solid content [Keefer, 1947; Schulse, 1958]. The feasibility of efficient methane production from high solid organics was reviewed by Wong Chong (1975) and Hills (1980). It was shown that the rate and efficiency of conversion of mixture of straw and cow manure with initial solids at 25 per cent dry matter was surprisingly close to the decay rates in a 10 per cent solid mixture [Jewell et al., 1978].

Dilution of animal manure with 10.25 per cent solids concentration to obtain 7.9 per cent solids helped in obtaining maximum biogas output [Lapp et al., 1975]. Hobson et al. (1978) reported slow deterioration in digester performance at 14 per cent total solids (TS) and that at a TS per cent of 9.4, maximum gas production was achieved. When the per cent solids in the influent

slurry was increased, the gas production was reduced [Sathianathan, 1975]. Summers and Bousfield (1980), stated that 7 to 9 per cent of TS in the slurry suspension of piggery waste was optimum for rapid evolution of gas.

Mesophilic anaerobic digester produced higher methane yield per unit of volatile solids (VS) added and a higher solids degradation efficiency than the thermophilic digesters. Canel and Young (1980) noted that few bacteria function at high solid content. Ghose and Bhadra (1981) observed that 4 per cent TS in the influent slurry concentration was optimum for maximum methane production in swine fermenter. Hills and Roberts (1981) observed the optimal loading rates of 5.3 and 1.0 kg VS m³ for tomato and peach anaerobic digesters respectively. Wate et al. (1983) reported that 5.25 per cent TS and 4.00 per cent VS were ideal for maximum gas output from cattle dung. Using cowdung, Singh et al. (1980) noted that 13.5 per cent TS was optimum for maximum gas production. While Traore (1992) showed a 4 per cent suspension of dry material yielding 2.9 to 3.6 l of biogas litre⁻¹ day⁻¹. Gunaseelan (1988) noted 5 per cent was optimum for gas production.

2.2.1.7. Cellulose, hemicellulose and lignin degradation during anaerobic digestion

Carbohydrates (cellulose and hemicellulose) and lignin are the main digestible components of solid wastes.

Although these compounds except lignin are well digestible, they can be present in wastes in structural forms offering resistance to biodegradation. Lignin is virtually undegradable by anaerobic process and the cellulolytic enzymes can not penetrate the lignin complex because of steric hindrance. Khan (1977) reported that the cellulose was degraded to yield methane and carbon dioxide by mixed culture of organisms. The gas produced contained between 56 and 59 per cent methane with the total gas yield of 0.3 m³. Cultures of Acetovibrio cellulolyticus and Methanosarcina barkeri produced approximately 2.6 to 3.0 mol. of methane per mol. of cellulose degraded between third and eighth week of incubation [Khan, 1980]. Datta (1981) reported that an alkaline pretreatment helped in the effective fermentation of the soluble fraction of hemicellulose, cellulose and lignin. The decomposition rate for cellulose and hemicellulose was higher compared to the insignificant degradation of lignin [Jimenez et al., 1990].

The hemicellulose fraction was most easily degradable and degraded to the extent of 97.8 per cent in pig dung. The extent of cellulose degradation was increased by pretreatment of cowdung [Dudhbhate et al., 1984], or supplementation with sheep wastes at 5 to 10 per cent [Jain et al., 1981]. Laube and Martin (1981) studied the fermentation of cellulose by monoculture of

A. cellulolyticus and cocultures of A. cellulolyticus, M. barkeri, A. cellulolyticus and Desulfovibrio sp.. The monoculture produced acetate, hydrogen, carbon monoxide and ethyl alcohol. More acetate and less ethyl alcohol were formed by the cocultures than monocultures. These cultures hydrolysed 85 per cent of the cellulose present initially. The amount was increased to 90 per cent by increasing the population of M. barkeri in triculture.

2.2.1.8. Microbiology and biochemistry of methane production

Microorganisms serve as biocatalyst during methane generation. Ferrara et al. (1984) identified the role of microorganisms involved in the methane production.

Biogas production takes place primarily in two stages viz., acid forming and methane forming stages [Toerien and Hattingh, 1969; Kirsch and Sykes, 1971; Bryant, 1979; Svendsen and Blackburn, 1986]. In the first stage, acid forming bacteria convert organic materials like carbohydrates, lipids and proteins to formate, acetate, propionate, butyrate, ethanol, hydrogen and carbon dioxide [Hashimoto et al., 1980; Hill and Bolte, 1989]. Hansson (1981) proposed a scheme for methanogenesis from organic matter (Figure 2).

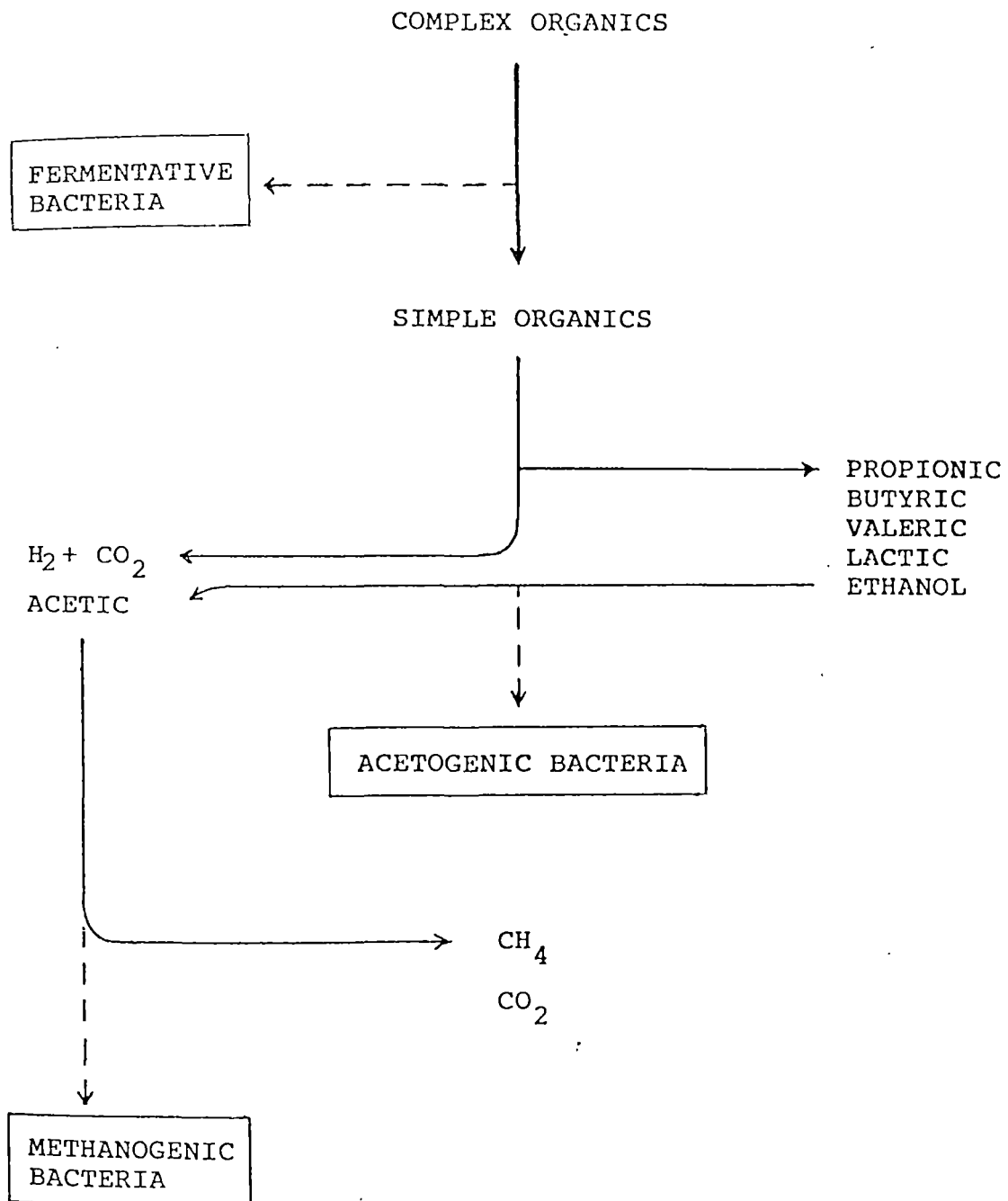


Figure 2. Main bacterial groups degrading organic compounds to CH₄ and CO₂ (Hansson, 1981).

Based on the substrates fermented and metabolic end products formed, four different trophic types of bacteria have been isolated from anaerobic digesters [Carrod and Wilke, 1978; Zeikus, 1980]. The four metabolic groups included were, 1) the hydrolytic bacteria 2) the hydrogen producing acetogenic bacteria 3) the homoacetogenic bacteria and, 4) the methanogenic bacteria.

During sludge digestion, it is reported that 70 per cent of methane is produced from methyl group of acetate [Hobson et al., 1974; Wolin, 1974; Mountfort and Agher, 1978]. Rapid conversion of formate to hydrogen and carbon dioxide by non-methanogens [Hungate, 1966] and importance of hydrogen as an intermediate rate limiting factor [Winfrey et al., 1977, Svendsen and Blackburn, 1986] have been reported. Propionate was thought to be a major intermediate in the rumen ecosystem [McCarty, 1964]. It was also reported that 15 per cent of the total methane production is derived from propionate [Kaspar and Wahrman, 1978; Svendsen and Blackburn, 1986].

The type of microbial population varied depending upon the substrates used. Rajasekharan (1980) studied the microbiological changes accompanying degradation of water hyacinth in an anaerobic digester. An increase in cellulolytic bacterial population was recorded due to water hyacinth incorporation. In the presence of

cellulosic wastes, Bacillus cereus, B. megaterium, Alcaligenes faecalis, Proteus vulgaris, Pseudomonas aeruginosa, P. riboflavina, P. reptilovoga and Leptospira biflexa have been isolated from anaerobic digesters [Toerien, 1967]. Starch wastes supported the growth of Micrococcus candidus, M. varians, M. ureae, B. cereus, B. megaterium and many species of Pseudomonas. Wastes rich in protein supported populations of Bacillus cereus, B. circulans, B. pulmilis, B. sphaerius, B. subtilis, M. varians, Escherichia coli and species of Pseudomonas. Based on temperature optima, Hobson et al. (1974) classified cellulolytic bacteria into two groups, viz., the mesophilic bacteria (35° to 40°C) and thermophilic bacteria (55° to 60°C). Synergistic action of these bacteria could lead to faster removal of cellulose than by pure cultures of either of these two.

Proteolytic enzyme activity in digester liquor was measured by Agardy et al. (1963). Proteolytic activity varied with the type of waste digested and with the associated gram positive organisms [Toerien, 1967].

High level of methane production under specific nutritive and ecological conditions was reported in the following genera; Methanobacterium, Methanobacillus, Methanosarcina and Methanococcus [Zajic, 1971]. Mah et al. (1977) and Svendsen and Blackburn (1986) reported that

methanogenic bacteria obtained energy from the oxidation of hydrogen coupled with reduction of carbon dioxide to methane.

The role of methanogenic, anaerobic and facultative anaerobic bacteria in relation to methane generation from hemicellulose and cellulose was studied [Andrew, 1979; Tsao, 1987; Jerger et al., 1982]. Rajasekharan and Nagarajan (1979) reported a positive correlation to the biogenesis of methane and methanogenic bacterial population. The associative action of various groups of organisms in the production of biogas was studied by Godbole et al. (1981). Inoculation of sterilized cattle dung with cellulolytic and noncellulolytic either singly or in combination with methanogenic organisms resulted in the production of more biogas.

2.2.1.9. Volatile fatty acids (VFA)

Volatile fatty acids are the intermediates in the anaerobic digestion process. The adverse effect of VFA at high concentrations is attributed either to the toxicity of the VFA themselves or to the decrease in pH brought about by the VFA content [Van Velsen and Lattinga, 1980]. Henderson (1973) and Prins et al. (1972) have reported that long chain fatty acids formed from lipids can be inhibitory or stimulatory to methanogenic and fermentative

bacteria, depending on acid concentration. Wiegant and Zeeman (1986) reported that increased concentration of propionate inhibits the methanogenesis from acetate.

The VFA generated viz., acetic, propionic and butyric acids which are substrates for methanogenesis might, at higher concentration, prove to be inhibitory [Hill and Bolte, 1989]. McCarty and McKinney (1961) found that acetic acid in high concentrations did not inhibit methanogenesis. Hobson and Shaw (1976) also observed that the growth of M. formicium was not inhibited by concentrations upto $10,000 \text{ mg}^{-1}$ of acetic or equivalent butyric acid. The undissociated VFA are inhibitory since the bacterial cell wall is far more permeable to undissociated molecules than their ionised state. VFA content was found to increase and pH reduces upon reduction in the hydraulic retention time of the fermentor [Ranade et al., 1989].

Van Velson and Lettinga (1980) stated that in the digestion of pig manure, no adverse effects of the VFA have been observed even at 500 mg^{-1} acetic acid concentrations. Wilkie et al. (1986) reported a reduced VFA concentration accompanied by augmented methane due to the addition of N, P and micronutrients. Singh et al. (1980) stated that an acetate level of 2500 to 3000 ppm was found to be optimum for maximum production of biogas.

2.2.1.10. pH

Methane producing organisms grow at pH values between 5.9 and 7.7 and the optimum being 6.1 to 6.9 [Paynter and Hungate, 1968]. On the other hand, Singh (1974) reported that the best pH for rapid fermentation was between 6.8 and 8.0.

Conversion of acetic acid to methane was found to be optimum at the pH of 6.5 to 7.1 at a temperature of 40-45°C [Vanden Berg et al., 1976]. The pH below 6.2 was toxic to methanogenic bacteria. The change in pH during the digestion of piggery waste, poultry litter, cattle and goat dung ranged from 6.3 to 7.9 [Bansal et al., 1977]. The biogas production was low when the pH was less than 6.0 and more than 8.0 and the optimum pH range for maximum gas production was 6.4 to 7.2 [Ranade et al., 1980]. They also reported that optimum pH for methanogenesis ranges from 7.1 to 7.5. Traore (1992) observed that a pH of 7.5 was optimum for fast methanogenesis. However, a pH from 6.5 to 7.4 was found to be optimum for gas production and pH above 7.8 had inhibitory effect [Visser et al., 1993].

2.2.1.11. Gas composition

During the conversion of carbohydrates to carbon dioxide and methane, equal volumes of each gas are

produced. However, all the carbon dioxide enter into reactions with water and hydroxide concentrations. The carbon dioxide content of the gas output increased with increased temperatures [Zajic, 1971]. The methane content of the gas produced at 60°C ranged 55-61 per cent as compared to 54-70 per cent at 35°C [Pfeffer, 1974]. The anaerobic digestion of pig manure at 35°C yielded methane upto 72 per cent and carbon dioxide upto 28 per cent [Anglo et al., 1978]. Barnett et al. (1978) reported that anaerobic digestion of cowdung, chicken manure, pig manure, farm wastes, sewage sludge and elephant grass produced gas that contained 65, 60, 65 to 70, 60 to 70, 68 and 60 per cent methane respectively.

Neelakantan et al. (1978) reported that anaerobic digestion of plant materials, yielded upto 58 per cent methane. On the other hand, Sharma et al. (1987a) reported 69 per cent of methane from Mirabilis leaf as against 62 per cent from cattle dung. Traore (1992) also recorded methane content of 56-59 per cent from the Calotropis. Nipanay and Panholzer (1987) recorded 58-68 per cent methane from weeds like Pistia sp. and Parthenium upon anaerobic decomposition. But Abbasi and Nipanay (1991) obtained a low methane output of 58-68 per cent from Pistia sp.

Anaerobic digestion of willow dust yielded biogas with 60 per cent methane, 40 per cent carbon dioxide and traces of other gases. The gas produced from cattle and sheep wastes digestion varied between 60 and 72 per cent [Jain et al., 1981]. Oba and Honda (1981) reported methane content of 60 to 84 per cent during anaerobic fermentation of agricultural and cattle wastes. Murugesan (1982) reported that biogas produced from the poultry droppings and rumen fluid incorporated treatments contained about 55 to 63 per cent of methane during the third to seventh week of digestion. Methane content from the biodigestion of agricultural residues was 70 per cent, as reported by Lalitha et al. (1984). Sarada and Nand (1989) reported 72 per cent methane in the biogas produced from tomato processing wastes.

Predigestion of substrate for biogas production is reported to enhance the methane content in the biogas. Singh et al. (1983) observed that methane in predigested cattle waste slurry increased from 68-75 per cent to 75-86 per cent. Kalia and Kanwar (1990) reported that predigested agroindustry waste when mixed with cowdung and fed to biogas plant produced biogas with 62-77 per cent methane, when compared to 56-60 per cent methane from cattle dung.

2.2.2. Manurial values of the biodigested slurry

Generally, solids that are neither broken down nor converted into methane, settle down in the digester. Depending on the raw materials used and the conditions of digestion, the sludge that settles down contains many elements essential to plant life viz., nitrogen, phosphorous, potassium and other micronutrients. It can be used (1) as manure directly after diluting with water (2) as dried manure (3) as a starter inoculum for compost and (4) as a manure by impregnating it with urea and super phosphate [Sathianathan, 1975; Balasubramanya et al., 1986].

Laura and Idani (1972) reported that the spent slurry and sun dried slurry were superior to farm yard manure and compost. The anaerobic digestion of cowdung produced better quality of manure rich in plant nutrients [Singh, 1974; Singh and Miglani, 1977]. Biswas (1977) reported that the residual slurry could be utilized as a manure in various ways.

Effect of application of biodigested slurry as manure on crop growth depended upon the crop to which it was applied. Application of slurry to replace half of the nitrogenous fertilizer in vegetable crops, gave better yield than complete replacement of synthetic fertilizers with it. Total replacement of the nitrogenous fertilizer

with slurry influenced maximum yield in fodder crops [Dahiya and Vasudevan, 1986]. Biodigested slurry is found to increase the growth of aquatic weeds like lemna [Balasubramanya and Kasthuribai, 1992) and phytoplankton [Sehgal et al., 1991].

CHAPTER 3

MATERIALS AND METHODS

MATERIALS AND METHODS

3.1. Characterization of wastes

3.1.1. Identification of rubber processing factories

Four different types of technologies are in vogue for primary processing of natural rubber. Effluents from five factories in each type were studied. Information on the factory site, production capacity and source of water was collected to assess the effluent load and its impact on the ecosystem.

3.1.2. Collection and preservation of the effluent samples

Sheet rubber processing effluent (SPE), Crumb rubber processing effluent (CPE), Crepe rubber effluent (CRE) and Latex concentrate effluent (LCE), from different rubber processing factories of Kerala and Tamil Nadu were collected for physicochemical and microbiological examinations. Samples were collected every month during the dry months. Three samples were taken from each factory.

Colour, odour and pH of the samples were recorded at the time of collection. The samples for physicochemical

examinations were collected in 2.5 l polyethylene containers. Sterilized glass bottles (200 ml) were used for collecting samples for microbiological examinations, avoiding contamination and these samples were used within 24 h after collection.

Standard methods for the examination of water and waste water [American Public Health Association, 1975] were followed in the collection, preservation and analysis of the samples wherever possible.

3.2. Analysis of samples

3.2.1. Physicochemical properties

3.2.1.1. Colour and odour

Colour of the effluent samples was compared visually and the odour by sniffing.

3.2.1.2. Moisture content

Moisture content of the different waste materials used in this study was estimated by the method described by Johnson and Ulrich (1960).

Ten grams of the sample was dried in the hot air oven at 105°C for 12 to 16 h. The loss in weight was expressed as moisture percentage on oven dry basis.

3.2.1.3. Total solids (TS)

A clean evaporating dish was heated at $550 \pm 50^{\circ}\text{C}$ for 1 h in a muffle furnace, cooled, weighed and stored in a desiccator. Hundred ml of the sample was transferred to the dish and evaporated to dryness in a steam bath. The evaporated sample was dried for 1 h at $104 \pm 1^{\circ}\text{C}$. Then the dish was cooled in the desiccator and weighed. The weight of the TS content was determined as,

$$\text{Total solids, mg l}^{-1} = \frac{(A-B) \times 1000}{V}$$

where

A = weight of sample with dish

B = weight of the dish

V = volume of sample in ml.

3.2.1.4. Volatile solids (VS)

The dish after the determination of TS was transferred to a muffle furnace and the sample ignited at 600°C for 1/2 h. This was then cooled to room temperature in a desiccator and the final weight was recorded. The VS content was calculated as,

$$\text{Total volatile solids, mg l}^{-1} = \frac{(A-C) \times 1000}{V}$$

where

A = Weight of sample with dish before ignition

C = Weight of sample with dish after ignition

V = Volume of sample in ml.

3.2.1.5. Suspended solids

A filter disc was placed in a Gooch crucible and washed with three changes each of 20 ml distilled water, vacuum suction was used to remove all traces of water.

Hundred ml of a well mixed sample was filtered through the dried and weighed crucible under vacuum, the crucible assembly dried at $104 \pm 1^{\circ}\text{C}$ for 1 h in an oven, cooled in a desiccator and weighed. The filtrate was preserved for the determination of dissolved solids. The weight of suspended solids was calculated as,

$$\text{Suspended solids, mg l}^{-1} = \frac{(A-B) \times 1000}{V}$$

where

A = Weight of crucible assembly with residue

B = Weight of crucible assembly

V = Volume of sample in ml.

3.2.1.6. Dissolved solids (DS)

The filtrate was evaporated in a tarred porcelain dish, preheated to $550^{\circ}\text{C} \pm 50^{\circ}\text{C}$ and cooled. The dish was heated at 105°C for about 1 h, cooled and weighed to determine the DS as,

$$\text{Dissolved solids, mg l}^{-1} = \frac{(A-B) \times 1000}{V}$$

where

A = Weight of dried residue with dish

B = Weight of dish

V = Volume of filtrate used in ml.

3.2.1.7. pH

The pH of the liquid samples was determined using a pH meter at the time of collection. For solid samples, 5 g of powdered residue was resuspended and mixed with 25 ml of distilled water and pH measured.

3.2.1.8. Dissolved oxygen (DO)

The DO in the effluent was determined by modified Winkler's method [American Public Health Association, 1975].

The effluent sample was taken in a BOD bottle and 1 ml each of manganous sulphate and alkaline iodide solution added, stoppered and the contents were well mixed. When the precipitate settled down, the stopper was removed and 2 ml of concentrated sulphuric acid was added, stoppered and mixed by gentle inversion of the bottle until the precipitate dissolved completely. Then 203 ml of the solution was taken and titrated against 0.025 N sodium thiosulphate solution, using starch as indicator. The end point was indicated by the disappearance of blue colour.

3.2.1.9. Biochemical oxygen demand (BOD)

Dilution water was prepared by adding 1 ml each of phosphate buffer, calcium chloride, magnesium sulphate and ferric chloride solutions to 1 l of distilled water and saturating it with air for 24 h. One per cent solution of known volume of the sample was pipetted into BOD bottles which were filled with dilution water and stoppered avoiding entrapment of air bubbles. The DO in the effluent sample was determined previously. The diluted sample and a blank dilution water were incubated for 5 days at 20°C. After 5 days the DO in the incubated sample and blank was determined and the BOD was calculated,

$$\text{BOD (mg l}^{-1}\text{)} = \frac{(\text{DO}_0 - \text{DO}_5 - \text{BC}) \times 100}{\text{per cent sample}}$$

where

DO_0 = Initial dissolved oxygen content

DO_5 = Dissolved oxygen content after incubation for 5 days.

BC = Blank correction.

3.2.1.10. Chemical oxygen demand (COD)

In a round bottomed flask, 20 ml of the sample was taken and to this 0.4 g mercuric sulphate, 10 ml potassium dichromate solution and 30 ml silver sulphate-sulphuric acid solution were added. The contents of the flask were refluxed for 2 h, cooled and washed down the condenser with about 25 ml of distilled water. Then the contents of the flask were transferred to a 500 ml conical flask, washing out the reflux flask 4 to 5 times. Then the mixture was diluted to 140 ml and the excess dichromate was titrated against ferrous ammonium sulphate solution using ferroin indicator, the end point being given by the change of colour from bluish green to reddish brown. A blank was run in the same manner.

$$\text{COD (mg l}^{-1}\text{)} = \frac{a - b \times N \times 8000}{V}$$

where

- a = Blank titre value
- b = Sample titre value
- N = Normality of ferrous ammonium sulphate
- V = Volume of sample taken (ml).

3.2.1.11. Total nitrogen

Hundred ml of the sample was taken in a Kjeldahl flask. Ten ml concentrated sulphuric acid and 1 ml copper sulphate solution were added to this and boiled under a hood until the solution became clear and was then allowed to cool.

The contents of the flask were transferred to a distillation flask and diluted to about 300 ml. This solution was made alkaline with sodium hydroxide using phenolphthaline indicator. The distillate was collected in 50 ml boric acid solution in a conical flask.

Two hundred ml of the distillate was collected and 0.5 ml mixed indicator solution added. This was titrated against 0.02 N sulphuric acid. End point was the colour change from pale green to lavender. A blank was also run simultaneously.

$$\text{Total nitrogen} = \frac{(A-B) \times 280}{V}$$

where

A = ml 0.02 NH_2SO_4 required for sample

B = ml 0.02 NH_2SO_4 required for blank

V = Volume of sample

3.2.1.12. Ammonia nitrogen

Hundred ml of the sample was taken, pH was adjusted to 10.5 by adding 1 ml of zinc sulphate solution and 0.5 ml sodium hydroxide. After settling the supernatant was filtered through Whatman No.42 filter paper. Aliquot of sample of 2.0, 4.0, 6.0, 8.0, 10.0, 12.0 and 15.0 ml were taken and each diluted to 50 ml. One drop of EDTA was added and mixed well. Two ml Nessler's reagent was added and the volume made up to 100 ml, mixed well and after 10 min, percentage transmission was read at 410 nm in a Spectronic 20 colorimeter. A blank was also run. A calibration curve using suitable aliquots of standard solution was prepared.

3.2.1.13. Phosphate

Fifty ml of the filtered clear sample was taken in a conical flask. Two ml ammonium molybdate and then 0.5 ml of stannous chloride were added and mixed well. A blank using distilled water was also prepared in the same way.

The colour developed was measured at 690 nm. on the spectrophotometer. The concentration of phosphate was arrived at with the help of a standard curve.

3.2.1.14. Sulphate

Two hundred ml of the sample was taken in a beaker, the pH adjusted to 4.5 with HCl using methyl red indicator and additional 2 ml HCl was added. This was boiled for one minute and 10 ml hot barium chloride solution was added slowly with constant stirring. The beaker was kept in a water bath to digest the precipitate at 80^o-90^oC for 2 h. The content of the beaker was filtered through a Whatman No.42 filter paper. The precipitate was washed with hot water. The filter paper with precipitate was placed in a previously weighed silica crucible and dried. The dried paper was ignited at 800^oC for 1 h, cooled in a desiccator and weighed.

$$\text{Sulphate as (SO}_4\text{) mg l}^{-1} = \frac{W \times 411.5}{V}$$

where

W = Weight of BaSO₄

V = Volume of sample.

3.2.1.15. Total organic carbon (TOC)

TOC in the various samples was determined by the wet digestion method of Walkley and Black as described by piper (1950).

3.2.1.16. Cellulose, hemicellulose and lignin

The cellulose content in the sample was determined by the method of Updegraff (1969).

Five ml of acetic nitric acid reagent was added to 0.1 g of the sample taken in a test tube and mixed thoroughly. The tube was placed in a water bath (100°C for 20 min). Then the content was centrifuged (5000 g for 20 min) and the supernatant discarded. The residue was washed with distilled water and then dissolved in sulphuric acid (67 per cent). One ml of this solution was then diluted to 100 ml. Total sugar in the hydrolysate was estimated using anthrone reagent. Oven dried pure cellulose assayed in a similar manner served as standard.

The hemicellulose content was estimated by the method developed by Goering and Van Socst (1975).

3.2.1.17. Neutral detergent fibre (NDF)

One g of the sample was transferred to a refluxing flask and 100 ml of cold neutral detergent solution was

added. To this, 2 ml of decahydronaphthalene and 0.5 g of sodium sulphate were added. It was heated to boiling and refluxed for 60 m. The contents were filtered through sintered glass filter by suction and washed with hot water. Finally, two washings were given with acetone and the residue transferred to a crucible, dried at 100°C for 8 h, weighed and expressed as NDF (w/w).

3.2.1.18. Acid detergent lignin (ADL)

ADL was determined by the method developed by Goering and Van Soest (1975). For the estimation, the acid detergent fibre (ADF) was determined using 1 g of the sample with 100 ml of cold acid detergent solution. Refluxing was done as detailed above. The hemicellulose content was calculated as the difference between NDF and ADF, $\text{hemicellulose} = \text{NDF} - \text{ADF}$.

The dried ADF was treated with 72 per cent sulphuric acid for 3 h, filtered, washed free of acid, dried at 100°C and weighed. The residue was then ignited in a muffle furnace at 550°C for 3 h, cooled and weighed. The ADL content was calculated by the loss of weight upon ignition.

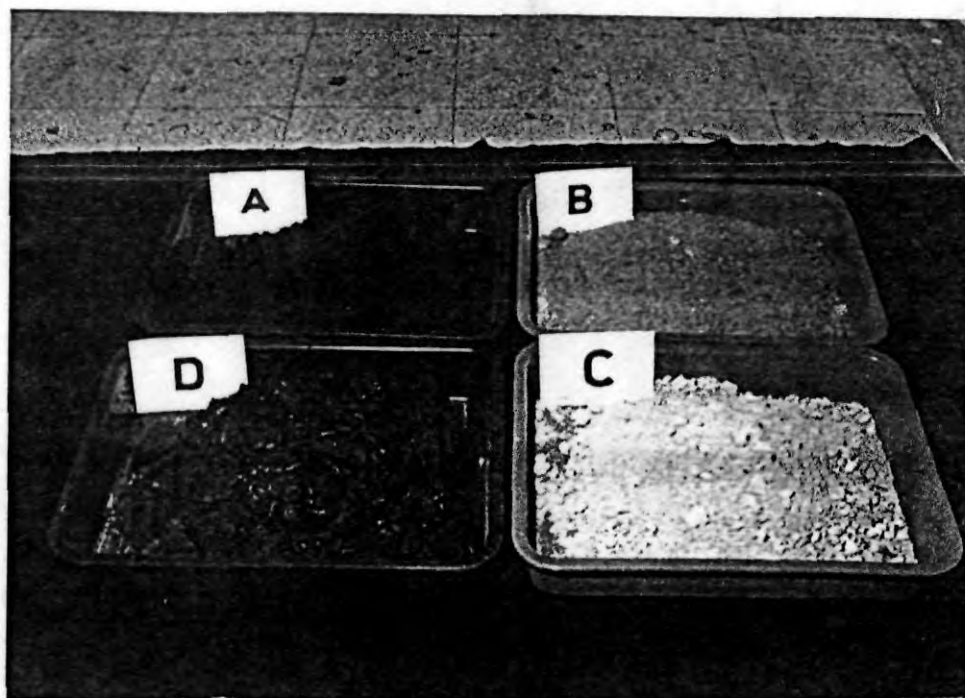
3.3. Studies on biogas production

In the present study, the following organic wastes viz., solid wastes from crumb rubber processing factory; crumb waste (CW), rubber wood sawdust (SD), predigested sawdust by mushroom composting (PSD) (Plate 2), crumb processing effluent (CPE), latex concentrate effluent (LCE) and sheet processing effluent (SPE) were used as feed stock for generating biogas. Cowdung (CD) was used as control. Biodigested slurry (BS) from the biogas plant, sewage sludge (SS) from the sewer and crumb sludge (CS) from the effluent collection tank of crumb rubber factory served as sources of inoculum.

3.3.1. Collection of organic wastes for biogas generation

Crumb waste (CW) and CPE were collected from the common rubber trap of the effluent treatment plant (Plate 3a & b). LCE and SPE were collected from latex concentrate and ribbed smoked sheet processing factories respectively. Rubber wood sawdust was obtained from the saw mills in and around Kottayam (Plate 4a & b) and predigested sawdust after oyster mushroom (Pleurotus florida) cultivation on rubber wood sawdust from the Rubber Research Institute of India (RRII) experiment station (Plate 5a & b). Fresh cowdung was obtained from a cattle yard near RRII experiment station.

PLATE 2. SUBSTRATES FOR BIOGAS PRODUCTION



- A. Crumb waste
- B. Rubber wood sawdust
- C. Predigested sawdust
- D. Cowdung

BS was collected from a gober gas plant, CS from the effluent collection tank of the crumb rubber processing factory near RRII experiment station and SS from a sewer at Kottayam.

3.3.2. Estimation of volatile fatty acids (VFA)

The VFA estimation was carried out by distillation method [American Public Health Association, 1975].

One hundred ml of centrifuged sample was taken in a 500 ml distillation flask and 100 ml distilled water was added to this. To avoid the bumping of the solution, 4 to 5 glass beads were added. Five ml sulphuric acid was added and mixed well to avoid settling of acid. The contents were distilled and the distillate (50 ml) collected in a 250 ml conical flask and titrated with 0.1 N NaOH using phenolphthalein as indicator. The end point was noted after the development of the pink colour which persisted on standing for a short time.

$$\text{VFA as acetic acid (mg l}^{-1}\text{)} = \frac{\text{Vol. of NaOH (ml)} \times 6000}{\text{Vol. of sample (ml)} \times 0.7}$$

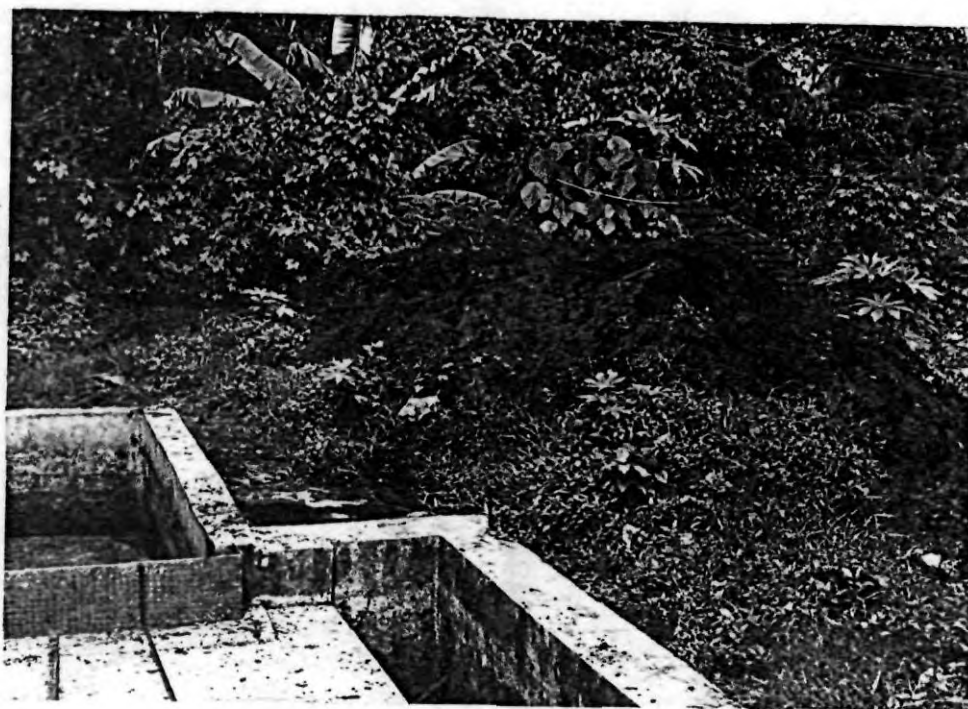
3.3.3. Analysis of enzymes

Analysis of cellulase and β -glucosidase was done at initial, middle and final stages of digestion.

PLATE 3. SOLID WASTE FROM CRUMB FACTORY

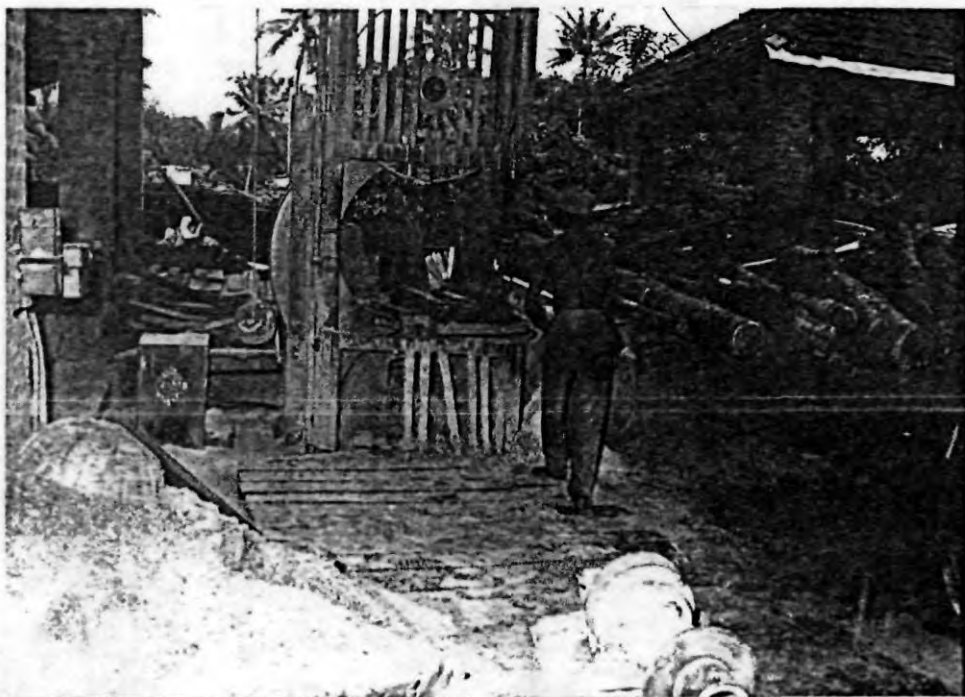


(a) Field coagulum with bark materials



(b) Solid waste

PLATE 4. SOLID WASTE GENERATION IN SAWMILL



(a) Rubber wood sawing



(b) Heap of sawdust

3.3.3.1. Cellulase

The reducing sugar produced by cellulase on carboxymethyl cellulose was measured using Nelson's reagent [Nelson, 1944] colorimetrically as per the method of Pancholy and Rice (1973).

3.3.3.2. β -glucosidase

The β -glucosidase activity was assayed by measuring the amount of glucose released from the substrate (Salicin) as per the method described by Olah and Sherwood (1973).

3.4. Microbiological properties

The different groups of microorganisms viz., total bacteria, yeast, acid forming bacteria, methanogenic bacteria, cellulolytic bacteria, proteolytic bacteria and lipolytic bacteria were enumerated using the appropriate media given in the Appendix 3.

The standard serial dilution plate technique of Pramer and Schmidt (1965) was employed for the enumeration of microbiological population.

3.5. Estimation of methane content

Methane content in the biogas mixture was estimated using a gas chromatograph (Shimadzu, Model No.GC9F) fitted

with FID and poropack Q column. Nitrogen was used as carrier gas. The temperatures of oven, injector and detector were maintained at 90°C, 110°C and 125°C respectively. 0.5 ml of gas was injected each time and the area of peak was measured by CR3A integrator. The volume of methane was calculated from the standard methane in nitrogen gas.

3.6. Manurial values

The total nitrogen content of the samples was estimated by Micro-Kjeldahl method described by Humphries (1956). Phosphorous and potassium were estimated by the method described by Jackson (1962).

3.7. Statistical analysis

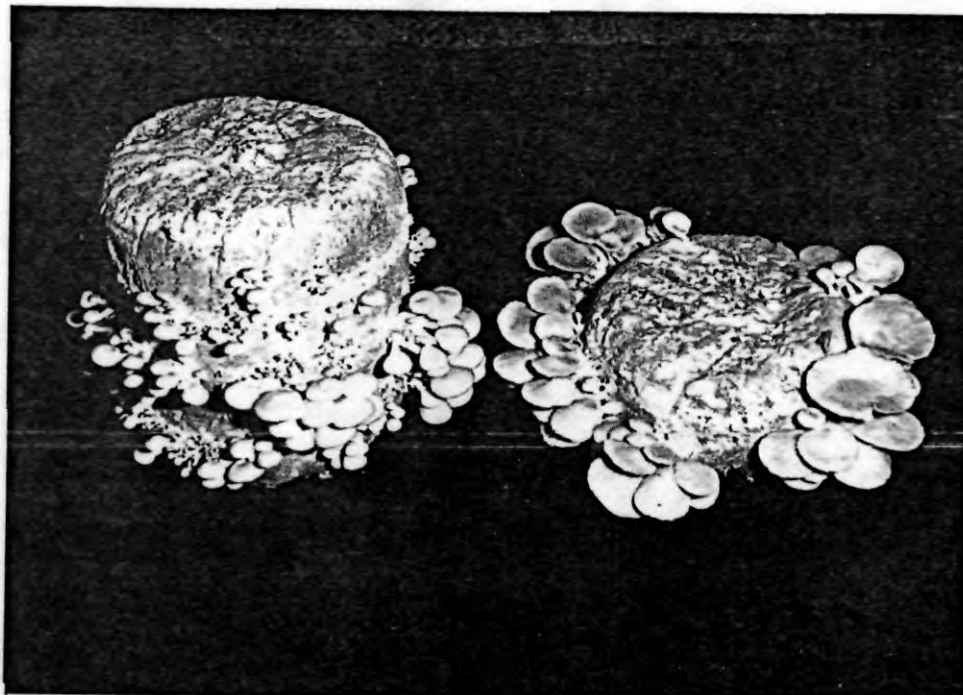
The data collected were statistically analysed using completely randomised factorial design outlined by Steel and Torrie (1960).

3.8. Details of experiments

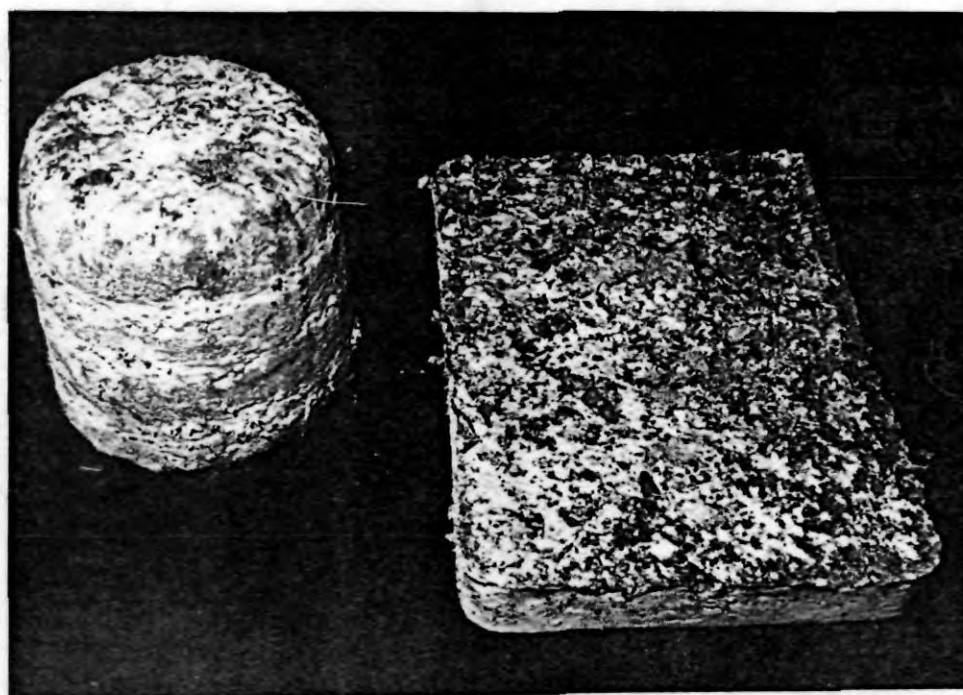
3.8.1. Effect of solid concentrations on gas generation

Solid waste from crumb rubber processing, rubber wood sawdust and PSD from mushroom composting were used as the substrates for methane production.

ATE 5. PREDIGESTION OF SAWDUST WITH Pleurotus florida



(a) Mushroom on sawdust



(b) Predigested sawdust

since cowdung is the conventional substrate for biogas generation, it was included as one of the treatments for comparison. Various substrates were mixed with water to bring the TS content to 5, 10 and 15 per cent. Initial carbon and nitrogen contents of the wastes were determined as detailed earlier. The C:N ratio of each slurry (mixture) was adjusted to 30 using urea solution [Singh, 1971], as this is reported to be optimum condition for normal gas generation [Singh, 1974; Barnett et al., 1978 and Fujita et al., 1980]. The following 12 treatments were preferred in the present investigation.

T ₁	Crumb waste	- 5 per cent
T ₂	Crumb waste	- 10 per cent
T ₃	Crumb waste	- 15 per cent
T ₄	Sawdust	- 5 per cent
T ₅	Sawdust	- 10 per cent
T ₆	Sawdust	- 15 per cent
T ₇	Predigested sawdust	- 5 per cent
T ₈	Predigested sawdust	- 10 per cent
T ₉	Predigested sawdust	- 15 per cent
T ₁₀	Cowdung	- 5 per cent
T ₁₁	Cowdung	- 10 per cent
T ₁₂	Cowdung	- 15 per cent

The slurry (2 l each) prepared as above was carbonated and then poured into 2.5 l amber coloured bottles and sealed airtight with a single holded rubber

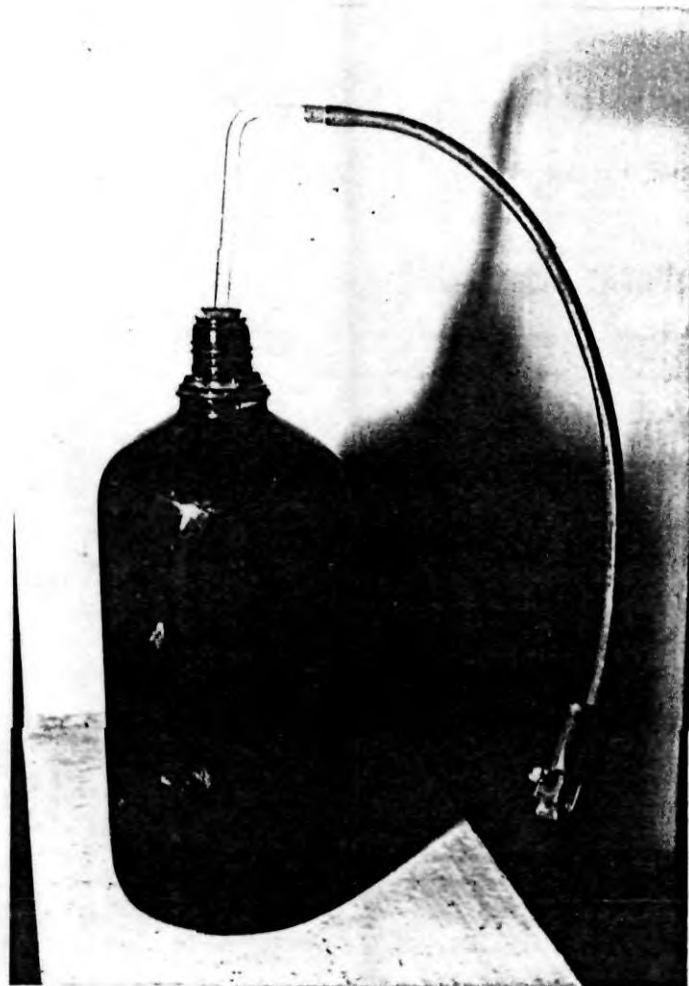
cork fitted with glass tubing for gas collection (Plate 6). BS collected from an active biogas plant was used as inoculum at the rate of 50 g per bottle. Three replications were maintained in each treatment. The experiment was conducted at room temperature ($27 \pm 2^{\circ}\text{C}$) for 10 weeks.

3.8.2. Effect of inocula on gas generation

Biodigested slurry, crumb sludge and sewage sludge were used as inocula. Substrates for biogas production using CW, sawdust, PSD and cowdung were prepared at 10 per cent level and their C:N ratio adjusted to 30:1. Fifty grams each of the inocula were mixed with the prepared substrate. The treatments were as under.

T ₁	Crumb waste	+ biodigested slurry
T ₂	Crumb waste	+ sewage sludge
T ₃	Crumb waste	+ crumb sludge
T ₄	Sawdust	+ biodigested slurry
T ₅	Sawdust	+ sewage sludge
T ₆	Sawdust	+ crumb sludge
T ₇	Predigested sawdust	+ biodigested slurry
T ₈	Predigested sawdust	+ sewage sludge
T ₉	Predigested sawdust	+ crumb sludge
T ₁₀	Cowdung	+ biodigested slurry
T ₁₁	Cowdung	+ sewage sludge
T ₁₂	Cowdung	+ crumb sludge

PLATE 6. BATCH DIGESTION



As detailed for the previous experiment, the slurry (2 l each) was prepared by mixing with water and taken in 2.5 l amber coloured bottles and sealed air tight. In each treatment three replications were maintained. The bottles were kept at room temperature ($27 \pm 2^{\circ}\text{C}$) for 10 weeks.

3.8.3. Effect of effluents on gas generation

Solid wastes like CW, sawdust, PSD and cowdung were mixed with CPE, SPE and LCE at 10 per cent substrate and C:N ratio was adjusted to 30:1. Fifty g of BS was added as inoculum. The details of the treatments are given below.

T ₁	Crumb waste	+ Sheet processing effluent
T ₂	Crumb waste	+ Crumb processing effluent
T ₃	Crumb waste	+ Latex concentrate effluent
T ₄	Sawdust	+ Sheet processing effluent
T ₅	Sawdust	+ Crumb processing effluent
T ₆	Sawdust	+ Latex concentrate effluent
T ₇	Predigested sawdust	+ Sheet processing effluent
T ₈	Predigested sawdust	+ Crumb processing effluent
T ₉	Predigested sawdust	+ Latex concentrate effluent
T ₁₀	Cowdung	+ Sheet processing effluent
T ₁₁	Cowdung	+ Crumb processing effluent
T ₁₂	Cowdung	+ Latex concentrate effluent

The slurry (2 l each) was prepared as above and the experiment was carried out as outlined in experiment 1.

In all the experiments in addition to measurement of gas generated daily by water displacement [Mishra, 1954], the other parameters like moisture content, TS, VS, pH, cellulose, hemicellulose, lignin, VFA and cellulolytic enzyme activity were determined at initial (0'day), middle (35th day) and final (70th day) stages of digestion process. Samples were drawn simultaneously for the microbiological assay of cellulolytic, proteolytic, lipolytic, acid forming and methanogenic bacteria. Samples of BS from all the treatments were also analysed for their nitrogen, phosphorus and potassium contents at the end of the experiment.

CHAPTER 4

EXPERIMENTAL RESULTS

EXPERIMENTAL RESULTS

4.1. Physicochemical and microbiological properties of liquid wastes from rubber processing factories

4.1.1. Sheet processing effluent (SPE)

Effluent from RSS processing factories, (SPE) was milky white and turbid due to uncoagulated rubber hydrocarbon and other organic compounds (Plate 7a & b). The level of dissolved solids (DS) was more than the suspended solid. Volatile solids (VS) content was very high (1845 mg l^{-1}) when compared to suspended solids (Table 1). The pH was less than that of influent water. Biochemical oxygen demand (BOD) was 1415 mg l^{-1} while chemical oxygen demand (COD) was 3260 mg l^{-1} (Table 2). The total nitrogen phosphates and chlorides in SPE were in the order of 200, 10 and 15.5 mg l^{-1} respectively. This effluent harboured a large population of saprophytic microorganisms. The population of yeast was high, while the population of E. coli and Streptococcus sp. was very low (Table 3).

4.1.2. Latex concentrate effluent (LCE)

The LCE was milky white and turbid, smelling ammonia (Plate 8a & b, 9a & b). The total, dissolved, suspended

Table 1. Physical properties of effluents

Properties	Effluents			
	SPE	LCE	CPE	CRE
Colour	Milky white	Milky white	Brownish	Brownish
Odour	Acidic	Ammonia	Foul	Foul
Total solids	4200	8800	1670	1400
Dissolved solids	3450	6880	620	480
Suspended solids	750	1920	1050	925
Volatile solids	1845	3815	365	352

All values except colour and odour are mg l^{-1}

PLATE 7. EFFLUENT GENERATION IN SHEET FACTORY



(a) Sheet making



(b) Effluent discharge

and volatile solids were in the order of 8800, 6880, 1920 and 3815 mg l^{-1} which were higher than that of other three effluents (Table 1).

The pH of LCE was very low (3.8). BOD and COD were 3,500 mg l^{-1} and 10,500 mg l^{-1} respectively. It contained 1,860 mg l^{-1} of total nitrogen, 1825 mg l^{-1} of ammonia nitrogen, 200 mg l^{-1} of phosphate and 4340 mg l^{-1} sulphate (Table 2). Unlike other effluents, the LCE contained high quantity of ammonia nitrogen.

The population of different groups of microorganisms is given in Table 3. It contains 8×10^4 total bacteria, 12×10^3 yeasts, 28.8×10^4 proteolytic bacteria, 20.8×10^4 acid producing bacteria and 9.6×10^4 lipolytic bacteria. The population of coliforms, E. coli and Streptococci were 4500, 250 and 350 per ml of effluent respectively (Table 3). The bacterial population in general was less in this effluent.

4.1.3. Crumb processing effluent (CPE) and crepe rubber processing effluent (CRE)

The processing methods for crumb and crepe rubbers are almost identical. The effluents from these, CPE and CRE are brownish and emit foul odour (Plate 10a & b, 11a & b). In both cases, TS were more followed by suspended solids, DS and VS (Table 1).

Table 2. Chemical properties of effluents

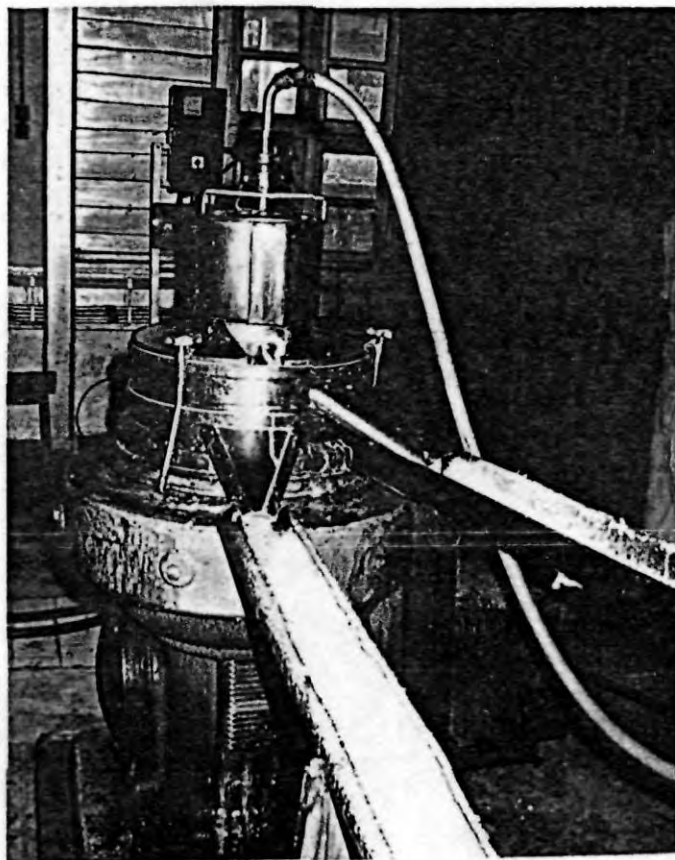
Properties	Effluents			
	SPE	LCE	CPE	CRE
pH	4.6	3.8	6.2	6.0
BOD	1415.0	3500.0	310.0	340.0
COD	3260.0	10,500.0	1200.0	1350.0
Total nitrogen	200.0	1860.0	125.0	140.0
Ammonia nitrogen	-	1825.0	-	-
Phosphates	10.0	200.0	10.5	12.5
Sulphates	-	4340.0	1186.0	950.0
Chlorides	15.5	28.0	8.5	14.5

All values except pH are mg l^{-1}

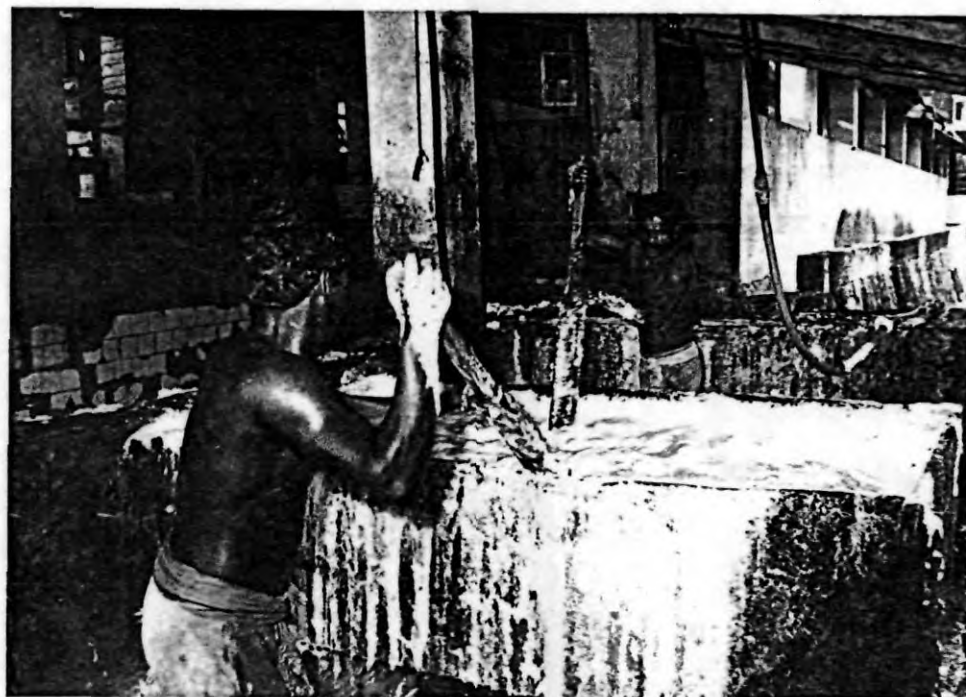
Table 3. Microflora of the effluents

Organisms(ml^{-1})	Effluents			
	SPE	LCE	CPE	CRE
Total bacteria ($\times 10^4$)	12.2	8.0	16.0	24.0
Yeasts ($\times 10^3$)	125.0	12.0	35.2	26.5
Cellulolytic bacteria ($\times 10^2$)	-	-	8.2	9.8
Proteolytic bacteria ($\times 10^4$)	30.6	28.8	28.5	31.2
Acid producing bacteria ($\times 10^4$)	25.3	20.8	24.6	26.8
Lipolytic bacteria ($\times 10^4$)	12.8	9.6	13.8	13.2
Coliform	13,400.0	4,500.0	11,500.0	15,600.0
<u>E. coli</u>	250.0	250.0	18,650.0	21,450.0
Streptococci	5,500.0	350.0	22,300.0	25,450.0

PLATE 8. EFFLUENT GENERATION IN LATEX CONCENTRATION FACTORY



(a) Latex centrifugation

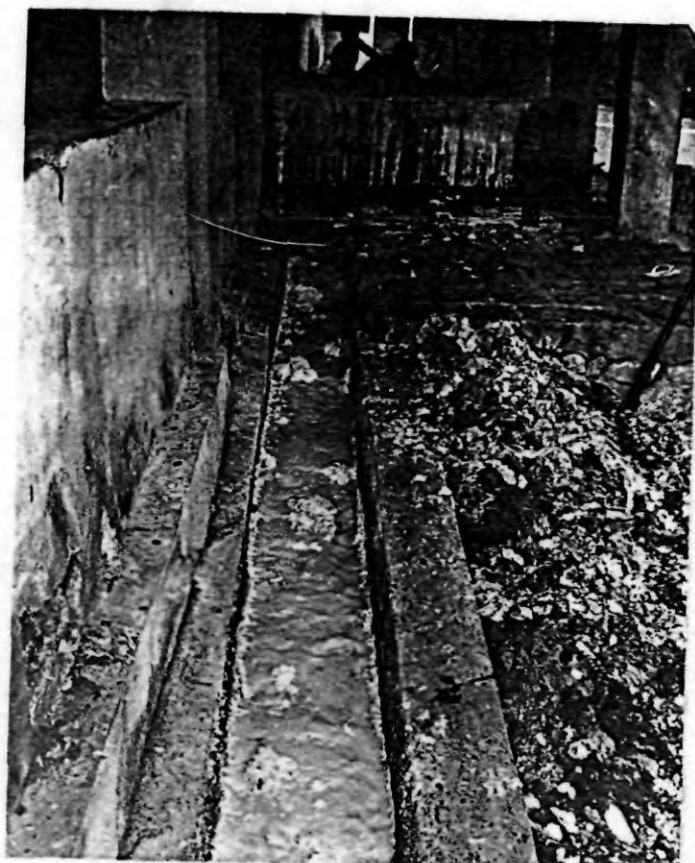


(b) Skim coagulation

PLATE 9. EFFLUENT FROM LATEX CONCENTRATION FACTORY



(a) Washing of latex containers



(b) Effluent discharge

All the four types of solids were less in these effluents. Between CPE and CRE, the solids were more in the former. Levels of BOD and COD were also less, while pH was more in the CPE and CRE (Table 2).

The microbial population except that of yeasts was more in CRE than CPE (Table 3). The population of proteolytic, acid producing and lipolytic bacteria in CPE and CRE were almost equal to that of SPE. Other groups of bacteria were more in CPE and CRE.

4.2. Physicochemical properties of solid wastes

The moisture content was more in crumb. waste (CW) followed by predigested sawdust (PSD) and raw sawdust. But the moisture content of cowdung was more than the above wastes. VS of sawdust was more than the CW and PSD (Table 4), and was comparable with cowdung. Organic carbon of all the three solid wastes was the same and it was less in cowdung.

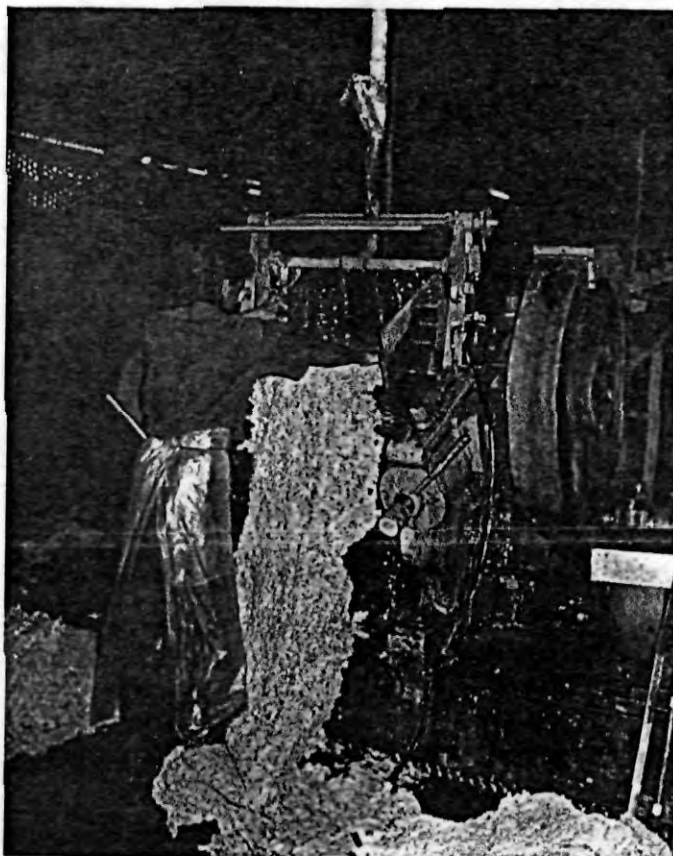
Total nitrogen, phosphorus and potassium contents were less in sawdust when compared to the other two solid wastes and cowdung (Table 4).

Cellulose content in sawdust was more than the CW and PSD which are almost equal. However, cellulose in cowdung was lower than the other solid wastes. Hemicellulose was also more in sawdust followed by CW, PSD and cowdung.

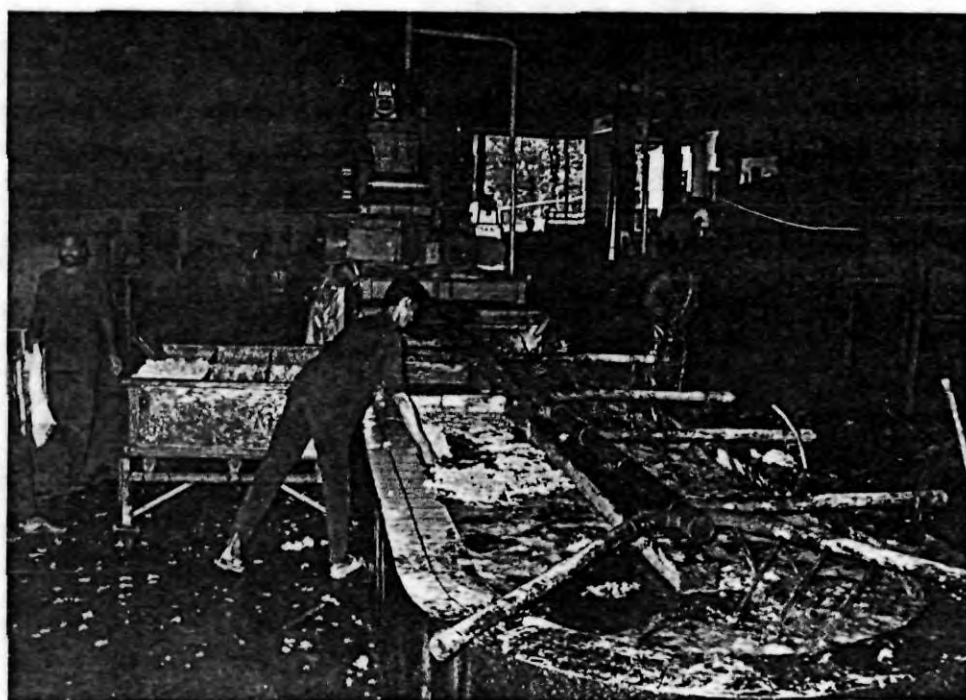
Table 4. Physicochemical properties of solid wastes (%)

Wastes	Moisture	Total solids	Volatile solids	Total organic carbon	Nitrogen	Phosphorous	Potassium	Cellulose	Hemicellulose	Lignin	C/N ratio
Crumb waste	70.28	29.72	13.28	46.34	1.42	0.86	0.78	38.42	40.36	8.95	33:1
Sawdust	32.28	67.72	16.48	52.20	0.58	0.21	0.38	44.28	48.15	24.25	90:1
Predigested sawdust	65.58	31.42	12.43	48.27	1.20	0.68	0.74	38.56	32.48	12.56	40:1
Cowdung	82.08	19.92	16.23	26.38	1.25	0.75	0.59	21.05	20.15	9.12	20:1

PLATE 10. EFFLUENT GENERATION IN CRUMB FACTORY



(a) Rolling and washing

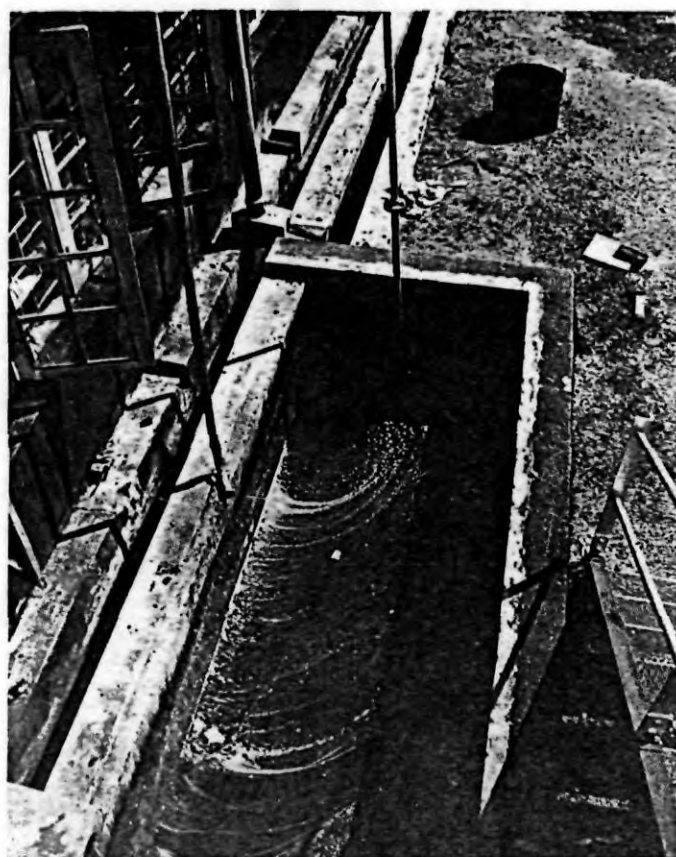


(b) Final washing

PLATE 11. EFFLUENT GENERATION IN CREPE FACTORY



(a) Coagulum washing
and rolling



(b) Effluent discharge

Like cellulose and hemicellulose, lignin content was more in sawdust (Table 4). Lignin content in PSD was next to sawdust followed by cowdung and CW. C:N ratio was maximum in sawdust (90:1) followed by PSD (40:1) and CW (33:1). The least C:N ratio was recorded in cowdung (20:1) (Table 4).

4.3. Experiment 1. Effect of solid concentrations on biogas generation

Crumb waste, sawdust, PSD and cowdung at 5, 10 and 15 per cent levels were studied for different parameters in relation to the quantity of biogas produced by anaerobic digestion.

4.3.1. Total solids (TS) destruction

Percentage destruction of TS in general was maximum at 10 per cent level while further increase in the concentration did not show any positive correlation (Table 5). Among the different substrates, PSD showed maximum TS destruction while it was not significant in other substrates (Figure 3).

4.3.2. Volatile solids (VS) destruction

Destruction of VS was higher than those of TS destruction in all the four substrates (Table 5).

Table 5. Total and volatile solids destruction at different solid concentrations (%)

Substrate	Total solids					Volatile solids				
	Solid concentrations (%)					Solid concentrations (%)				
	5	10	15	Mean		5	10	15	Mean	
CW	23.55	24.47	22.40	23.42		31.59	33.08	33.42	32.69	
SD	22.03	23.32	22.03	22.46		41.59	42.65	42.65	42.60	
PSD	24.05	29.60	27.40	27.01		52.84	57.19	54.07	54.70	
CD	22.07	24.51	23.30	23.29		32.25	36.47	34.58	34.43	
Mean	22.92	25.47	23.74			39.56	42.58	41.18		
CD for substrate (P = 0.05) - 2.43 CD for concentration (P = 0.05) - 2.10 CD for interaction (P = 0.05) - 4.21										
CD for substrate (P = 0.05) - 2.02 CD for concentration (P = 0.05) - 1.75 CD for interaction (P = 0.05) - 3.5										

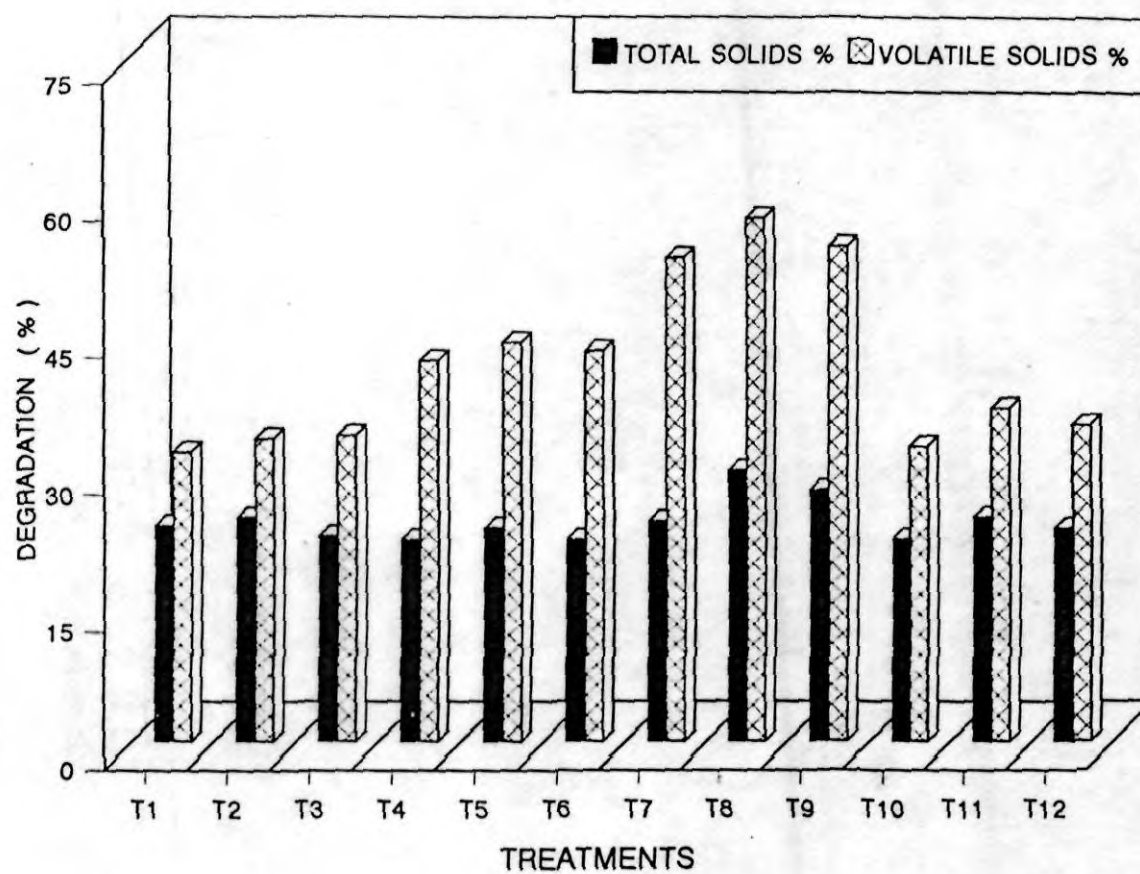


Figure 3. Degradation of total and volatile solids at different solid concentrations.

The highest percentage of destruction of VS was recorded in PSD followed by sawdust, cowdung and CW. The rate of VS destruction was maximum at 10 per cent level and thereafter the percentage of destruction declined (Figure 3). PSD showed maximum destruction of VS.

4.3.3. Cellulose, hemicellulose and lignin degradation in relation to gas generation

4.3.3.1. Cellulose

The loss of cellulose during biodigestion of the substrates CW, PSD and cowdung was uniform, but sawdust recorded the least level of cellulose degradation (Table 6). Increase in the level of substrates upto 10 per cent augmented the degradation of cellulose in all the substrates except sawdust. The highest level of cellulose degradation was recorded in 10 per cent concentration of CW followed by PSD (Figure 4).

4.3.3.2. Hemicellulose

The hemicellulose degradation was more in CW, followed by PSD, sawdust and cowdung irrespective of their concentration (Table 6). Among different levels of substrates, 15 per cent TS showed maximum hemicellulose degradation.

Table 6. Degradation of cellulose, hemicellulose and lignin at different solid concentrations (%)

Substrate	Cellulose				Hemicellulose				Lignin			
	5	10	15	Mean	5	10	15	Mean	5	10	15	Mean
CW	23.43	24.38	24.07	23.96	47.94	46.02	48.57	47.51	16.48	18.63	12.15	15.75
SD	16.55	14.52	15.53	15.53	35.93	38.51	37.76	37.40	12.79	13.00	12.88	12.89
PSD	23.26	24.07	23.87	23.73	41.65	43.67	45.05	43.45	2.86	2.07	1.12	2.01
CD	20.07	22.09	23.54	21.90	34.96	30.74	35.85	33.85	3.43	3.68	3.81	3.64
Mean	20.82	21.26	21.75		40.12	39.73	41.80		8.89	9.34	7.49	
CD for substrate	(P = 0.05)	-	2.53		CD (P = 0.05)	substrate	-	4.22	CD (P = 0.05)	substrate	-	1.28
CD for concentration	(P = 0.05)	-	2.19		CD (P = 0.05)	concentration	-	2.98	CD (P = 0.05)	concentration	-	1.11
CD for interaction	(P = 0.05)	-	4.39		CD (P = 0.05)	interaction	-	5.96	CD (P = 0.05)	interaction	-	2.22

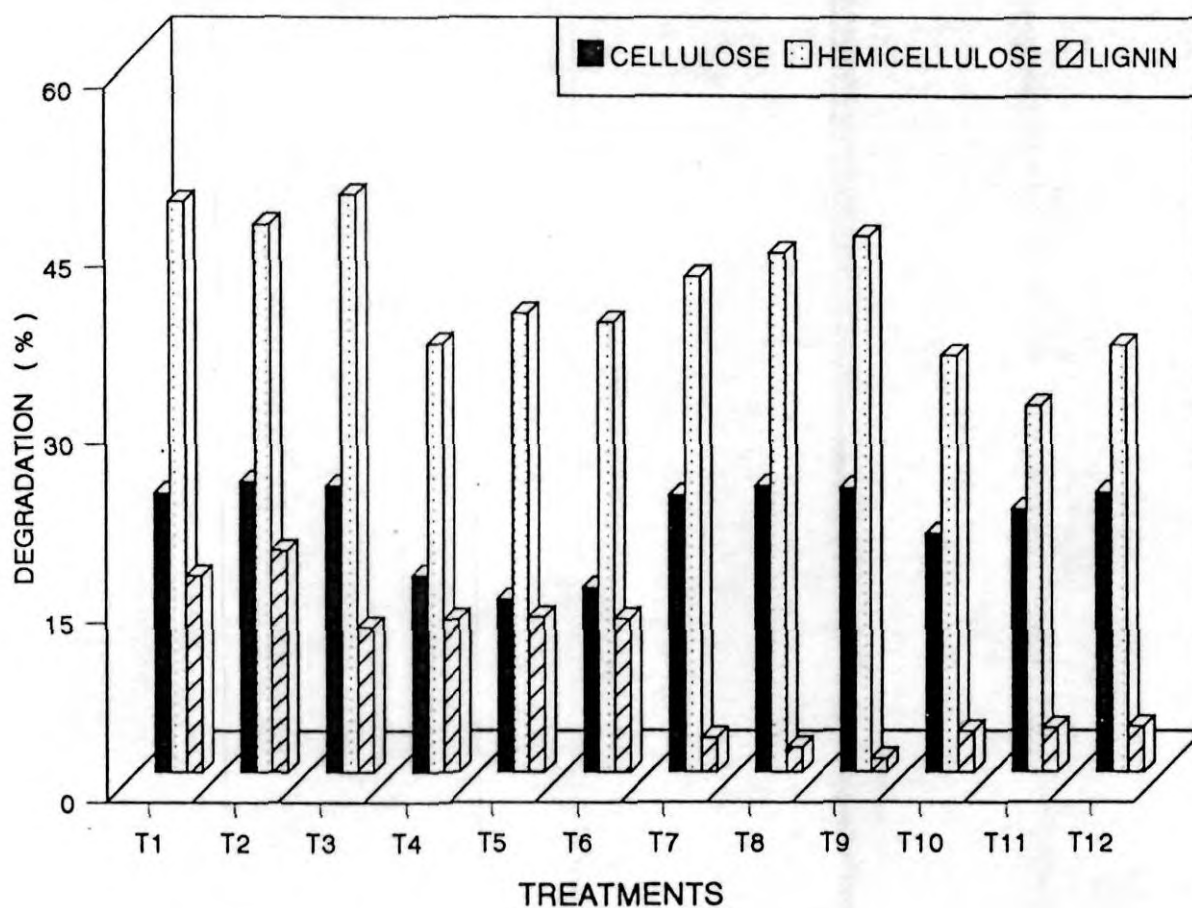


Figure 4. Degradation of cellulose, hemicellulose and lignin at different solid concentrations.

4.3.3.3. Lignin

Lignin degradation was more in CW followed by sawdust, cowdung and PSD (Table 6). Lignin degradation at 10 per cent substrate concentration was significantly higher than 15 per cent level, but was almost equal to 5 per cent substrate concentration (Figure 4).

4.3.4. Volatile fatty acids (VFA) content

Predigested sawdust showed maximum concentration of VFA during the initial and middle phases (Figure 5), whereas in the final stage CW registered the highest level. Increase in percentage of substrate showed a significant increase in fatty acids concentration at all the stages for all the feed stocks.

Fatty acid level in all the substrates irrespective of concentration increased upto the middle stage and then reduced significantly. This reduction was much pronounced in the PSD.

4.3.5. pH

Anaerobic digestion of wastes at different concentration resulted in decrease in pH at the middle stage (Table 7). However, in the final stage it was around neutral in all the treatments.

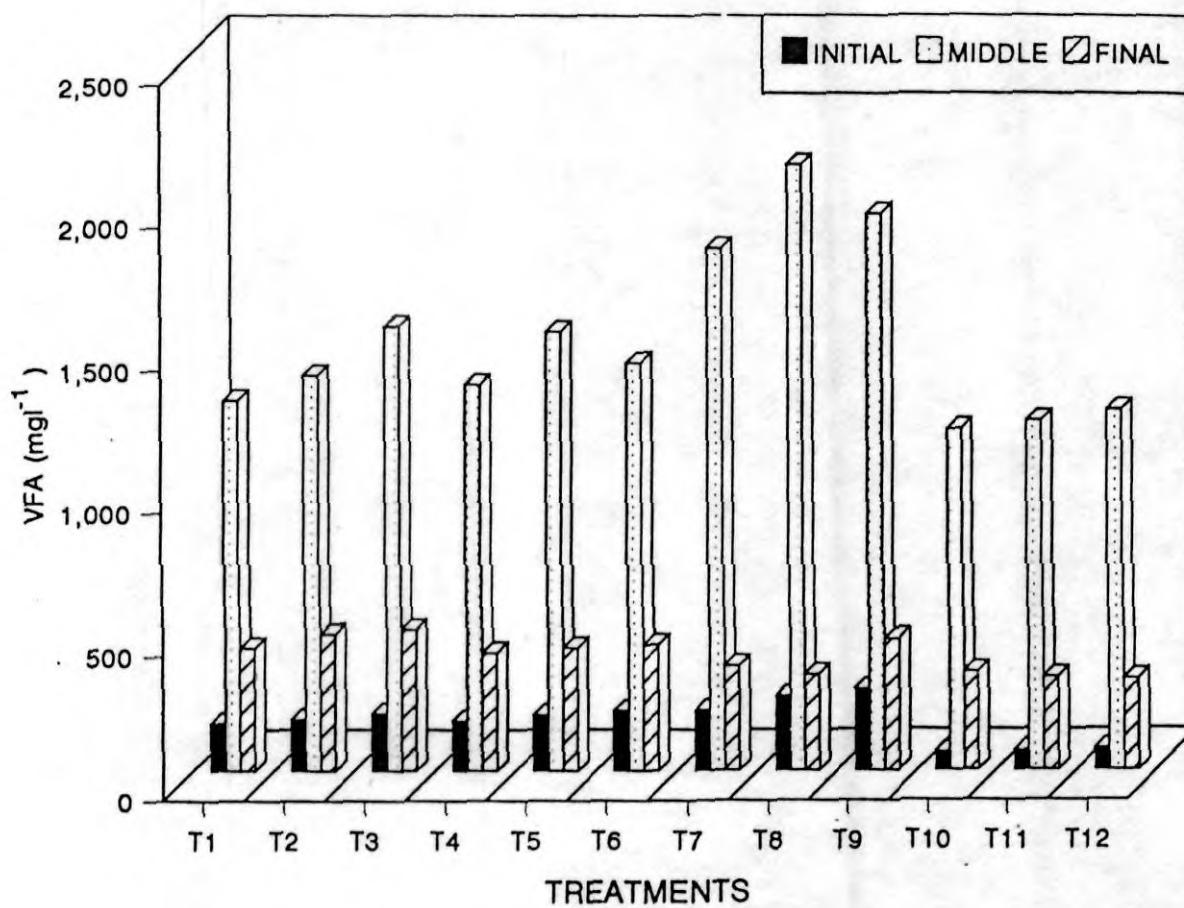


Figure 5. Volatile fatty acids at different solid concentrations.

Table 7. Changes in pH at different solid concentrations

Treatments	Initial	Middle	Final
T ₁	7.1	6.8	7.2
T ₂	7.0	6.8	7.0
T ₃	7.1	6.6	6.9
T ₄	7.0	6.7	6.9
T ₅	6.9	6.6	6.8
T ₆	7.0	6.7	7.0
T ₇	7.1	6.4	7.2
T ₈	7.0	6.2	7.2
T ₉	7.0	6.3	7.3
T ₁₀	7.1	6.8	7.4
T ₁₁	7.0	6.8	7.1
T ₁₂	7.1	6.7	7.2

PSD recorded lower pH than the other treatments. Among the three concentrations the drop in pH was more at 10 per cent level in PSD at the middle stage.

4.3.6. Enzyme activity

4.3.6.1. Cellulase

The cellulase activity in all the substrates tested increased upto middle stage and then decreased (Figure 6). Significant increase in cellulase activity was also recorded with the increase in the substrate concentration during the start of the experiment. However, in the middle and final stages cellulase activity increased upto 10 per cent concentration of substrate and thereafter reduced. In the initial stage PSD showed maximum cellulase activity followed by cowdung, CW and sawdust. However, in the middle stage this enzyme activity was more in sawdust and PSD than the CW and cowdung. In the final stage the highest cellulase activity was found in sawdust followed by cowdung, PSD and CW.

4.3.6.2. β -Glucosidase

The activity of β -glucosidase was greatly influenced by the substrates, their concentrations and the stages of biogas production (Figure 7).

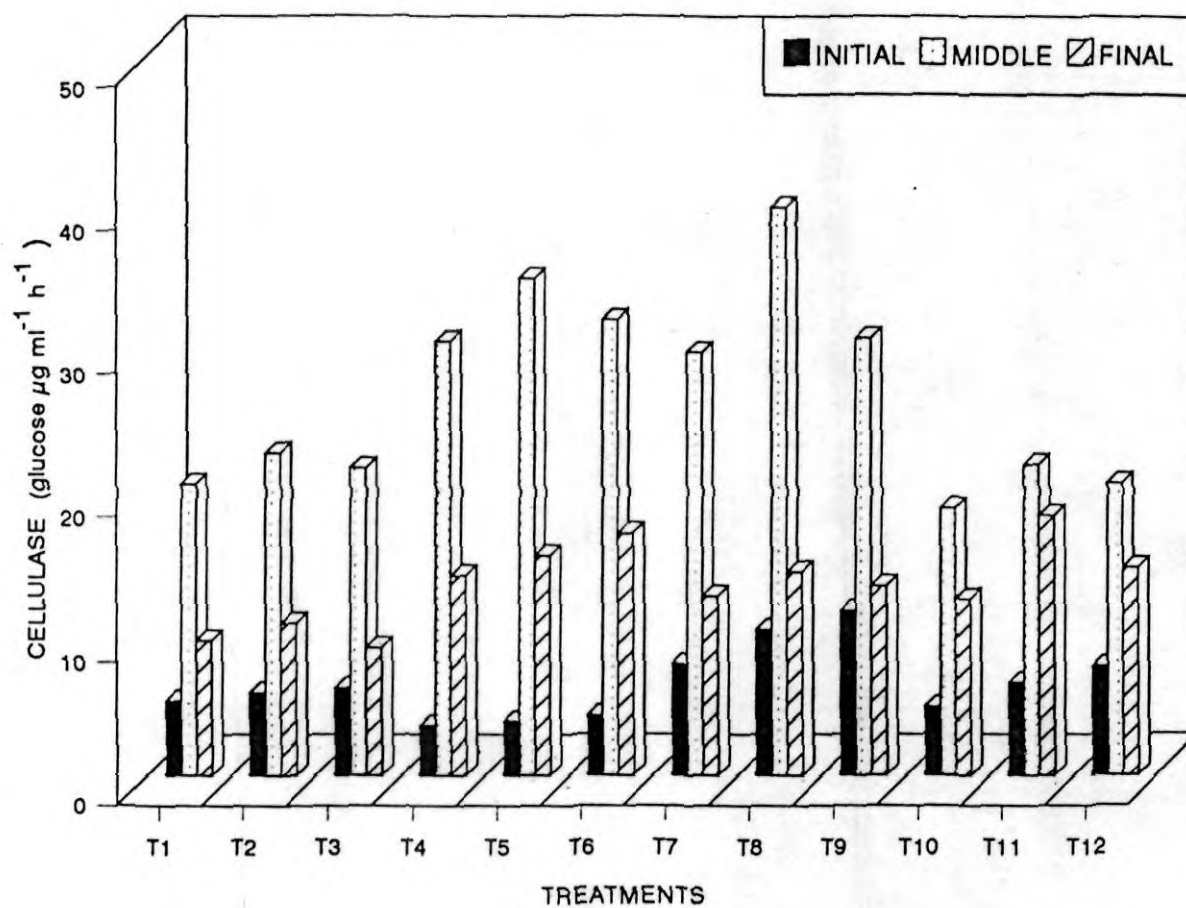


Figure 6. Cellulase activity at different solid concentrations.

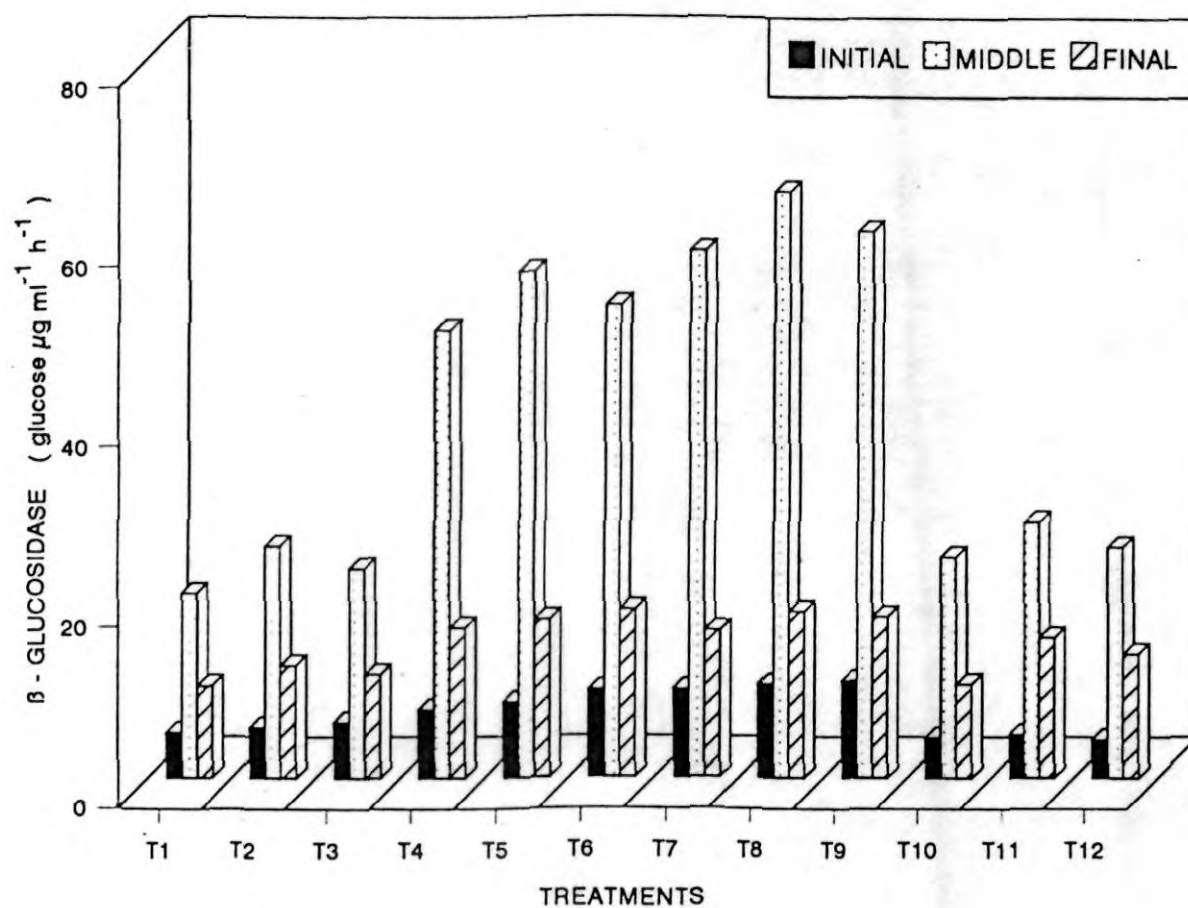


Figure 7. β -Glucosidase activity at different solid concentrations.

β -Glucosidase activity increased marginally with the increase in concentration of the substrate in the initial stage. But in the middle and final stages it increased upto 10 per cent substrate level and thereafter reduced. In general, the activity of this enzyme increased upto middle stage and thereafter decreased. PSD caused enhanced β -glucosidase activity during the initial and middle stage in all concentrations. In the middle stage, next to PSD, higher enzyme activity was noticed in sawdust. CW and cowdung caused lower activity than the former two substrates and this trend was maintained till the end of the experiment.

4.3.7. Enumeration of microbial population

4.3.7.1. Cellulolytic bacteria

Neither the substrate nor its concentration influence the population of cellulolytic bacteria in the initial stage (Table 8). During the middle stage, PSD showed the highest cellulolytic bacterial population followed by sawdust. Cellulolytic bacterial population was more at 10 per cent concentration in all the substrates (Table 8). Higher substrate concentration did not influence the population. A similar trend was also noticed in the final stage. At the final stage also the cellulolytic bacterial population was more in PSD.

Table 8. Population of cellulolytic bacteria at different solid concentrations ($\times 10^4 \text{ g}^{-1}$)

Substrate	Initial				Middle				Final			
	-----				-----				-----			
	5	10	15	Mean	5	10	15	Mean	5	10	15	Mean
Solid concentrations (%)												
CW	15.20	16.50	16.80	16.16	29.30	30.50	31.20	30.33	8.20	9.80	10.20	9.40
SD	15.40	18.60	19.10	17.70	38.30	42.40	43.10	41.26	10.20	11.30	11.80	11.10
PSD	17.60	20.40	18.50	18.83	56.80	65.60	58.30	60.23	35.00	39.50	38.30	37.60
CD	17.80	19.20	20.80	19.26	32.70	36.20	35.40	34.76	10.50	11.50	10.90	10.96
Mean	16.50	18.67	18.80		39.27	43.67	42.00		15.97	18.02	17.80	
CD for substrate	(P = 0.05) - 2.94				CD (P = 0.05) substrate - 2.67				CD (P = 0.05) substrate - 2.09			
CD for concentration	(P = 0.05) - 2.55				CD (P = 0.05) concentration - 2.31				CD (P = 0.05) concentration - 1.81			
CD for interaction	(P = 0.05) - 5.10				CD (P = 0.05) interaction - 4.62				CD (P = 0.05) interaction - 3.63			

The population of cellulolytic bacteria increased with reaction time upto middle stage and thereafter decreased in all the four substrates, irrespective of their concentration.

4.3.7.2. Acid forming bacteria

Initial population of acid forming bacteria was more in CW and cowdung than in sawdust and PSD substrates. But in the middle and final stages it was maximum in PSD (Table 9). It increased with the increase in the concentration of the substrate from 5 to 10 per cent, but not further. Like cellulolytic bacteria, acid forming bacteria also increased progressively with biomethanation upto middle stage and later on, it decreased.

4.3.7.3. Lipolytic bacteria

Lipolytic bacterial count was high in all the substrates studied. The population was significantly higher in sawdust than in the other substrates at the initial stage (Table 10). Their population increased with concentration of substrates. At 15 per cent substrate concentration, the population was significantly higher than that at 5 per cent level.

The population was the highest in the middle stage at 10 per cent substrate with maximum in PSD and cowdung.

Table 9. Population of acid forming bacteria at different solid concentrations ($\times 10^4 \text{ g}^{-1}$)

Substrate	Initial				Middle				Final			
	5	10	15	Mean	5	10	15	Mean	5	10	15	Mean
	Solid concentrations (%)											
CW	20.60	21.80	22.10	21.50	30.20	32.80	33.10	32.03	20.26	21.50	20.80	20.85
SD	10.60	11.20	11.80	11.20	33.50	35.90	36.80	35.40	21.60	24.20	23.80	23.20
PSD	10.50	14.60	12.80	12.63	48.40	56.60	50.40	51.80	30.70	40.60	32.30	34.53
CD	19.60	23.50	24.50	22.53	30.10	33.40	31.70	31.73	16.50	18.60	19.40	18.16
Mean	15.32	17.77	17.80		35.55	39.67	38.00		22.26	26.22	24.07	
CD for substrate	(P = 0.05) - 1.86				CD (P = 0.05) substrate - 2.74				CD (P = 0.05) substrate - 2.16			
CD for concentration	(P = 0.05) - 1.61				CD (P = 0.05) concentration - 2.37				CD (P = 0.05) concentration - 1.87			
CD for interaction	(P = 0.05) - 3.23				CD (P = 0.05) interaction - 4.75				CD (P = 0.05) interaction - 3.74			

Table 10. Population of lipolytic bacteria at different solid concentrations ($\times 10^3 \text{ g}^{-1}$)

Substrate	Initial				Middle				Final			
	-----				-----				-----			
					Solid concentrations (%)							
	5	10	15	Mean	5	10	15	Mean	5	10	15	Mean
CW	10.60	11.20	11.80	11.20	13.90	15.60	16.80	15.43	3.10	2.80	3.60	3.16
SD	12.30	14.20	15.10	13.86	16.20	18.70	20.00	18.30	3.60	4.60	5.20	4.46
PSD	9.80	11.20	12.50	11.16	18.50	21.80	22.50	20.93	4.20	3.80	4.60	4.20
CD	9.30	10.30	11.60	10.40	18.50	20.60	21.30	20.13	11.50	15.40	13.40	13.43
Mean	10.50	11.72	12.75		16.77	19.17	20.15		5.60	6.65	6.70	
CD for substrate	(P = 0.05) - 1.74				CD (P = 0.05) substrate - 2.15				CD (P = 0.05) substrate - 0.63			
CD for concentration	(P = 0.05) - 1.50				CD (P = 0.05) concentration - 1.86				CD (P = 0.05) concentration - 0.55			
CD for interaction	(P = 0.05) - 3.01				CD (P = 0.05) interaction - 3.73				CD (P = 0.05) interaction - 1.10			

In the final stage, cowdung registered the highest population of lipolytic bacteria than the other three substrates. Though the increase in the substrate concentration from 5 to 10 per cent augmented the population, further increase had no significant effect.

4.3.7.4. Proteolytic bacteria

Cowdung and PSD showed maximum population followed by fresh sawdust and CW in the initial stage. With regard to the concentration of substrate 10 per cent and 15 per cent showed significantly higher population of proteolytic bacteria than 5 per cent level (Table 11).

In the middle stage of biometanation, the population of proteolytic bacteria was more when compared to the initial stage. PSD registered the highest population followed by sawdust, cowdung and CW. Both 10 per cent and 15 per cent concentration harboured comparatively higher population.

At the end of the experiment the population was maximum in cowdung followed by sawdust, CW and PSD. The proteolytic bacteria increased upto 10 per cent level in the final stage.

The proteolytic bacteria showed a slight increase due to the progress in the fermentation only upto the middle stage and then decreased to initial level except in PSD which showed a sharp decline.

Table 11. Population of proteolytic bacteria at different solid concentrations ($\times 10^4 \text{ g}^{-1}$)

Substrate	Initial				Middle				Final			
	5	10	15	Mean	5	10	15	Mean	5	10	15	Mean
Solid concentrations (%)												
CW	25.93	29.30	26.80	27.34	35.20	39.30	38.40	37.63	23.20	27.60	28.20	26.33
SD	29.30	31.40	31.80	30.83	43.60	45.20	46.30	45.03	26.50	29.30	30.20	28.66
PSD	30.50	36.20	37.20	34.63	47.50	50.40	51.60	49.83	18.60	19.40	20.50	19.50
CD	32.30	35.20	35.80	34.43	36.40	42.40	41.60	40.13	33.20	35.50	36.30	35.00
Mean	29.50	33.02	32.90		40.67	44.32	44.47		25.37	27.95	28.80	
CD (P = 0.05) substrate												
CD (P = 0.05) concentration												
CD (P = 0.05) interaction												
CD for substrate	(P = 0.05)	-	2.31		CD (P = 0.05) substrate	-	1.89		CD (P = 0.05) substrate	-	1.87	
CD for concentration	(P = 0.05)	-	2.00		CD (P = 0.05) concentration	-	1.64		CD (P = 0.05) concentration	-	1.62	
CD for interaction	(P = 0.05)	-	4.01		CD (P = 0.05) interaction	-	3.28		CD (P = 0.05) interaction	-	3.24	

4.3.7.5. Methanogenic bacteria

Methanogenic bacterial population in the beginning of the experiment depended on the nature of the substrates used. CW registered a high count of methanogenic bacterial population followed by PSD, sawdust and cowdung (Table 12). Concentration of the substrates did not show any appreciable difference in methanogenic bacterial population.

The population of this bacteria increased steadily with the progress of the digestion upto middle stage in all the treatments. In general, both 10 and 15 per cent concentration of substrate showed almost same methanogens which was significantly higher than at 5 per cent level of substrates. There was no change in the population of methanogenic bacteria in the final stage except for a slight reduction in all the treatments.

4.3.8. Biogas production

Both the substrates and their concentrations greatly influenced the biogas production. The highest level of biogas production was recorded in PSD followed by sawdust, cowdung and CW (Figure 8). Biogas production increased with the increase in the concentration of the substrates upto 10 per cent beyond which there was a reduction (Figure 9). In general, the gas production was high during the fourth to sixth week of biodigestion (Figure 10).

Table 12. Population of methanogenic bacteria at different solid concentrations ($\times 10^4 \text{ g}^{-1}$)

Substrate	Initial				Middle				Final			
	5	10	15	Mean	5	10	15	Mean	5	10	15	Mean
Solid concentrations (%)												
CW	16.80	18.40	19.50	18.23	43.20	46.70	47.20	45.70	38.20	40.20	39.30	39.23
SD	9.30	9.80	10.30	9.80	55.40	60.30	55.60	57.10	49.30	52.60	50.30	50.73
PSD	10.30	11.40	11.60	11.10	96.20	120.30	115.90	110.80	90.40	106.30	101.60	99.43
CD	9.10	9.60	9.80	9.50	30.40	32.30	33.80	32.16	20.60	22.40	23.80	22.26
Mean	11.37	12.30	12.80		56.30	64.90	63.12		49.62	55.37	53.75	
CD for substrate	(P = 0.05) - 1.20				CD (P = 0.05) substrate	- 2.46				CD (P = 0.05) substrate	- 2.50	
CD for concentration	(P = 0.05) - 1.04				CD (P = 0.05) concentration	- 2.13				CD (P = 0.05) concentration	- 2.16	
CD for interaction	(P = 0.05) - 2.08				CD (P = 0.05) interaction	- 4.26				CD (P = 0.05) interaction	- 4.33	

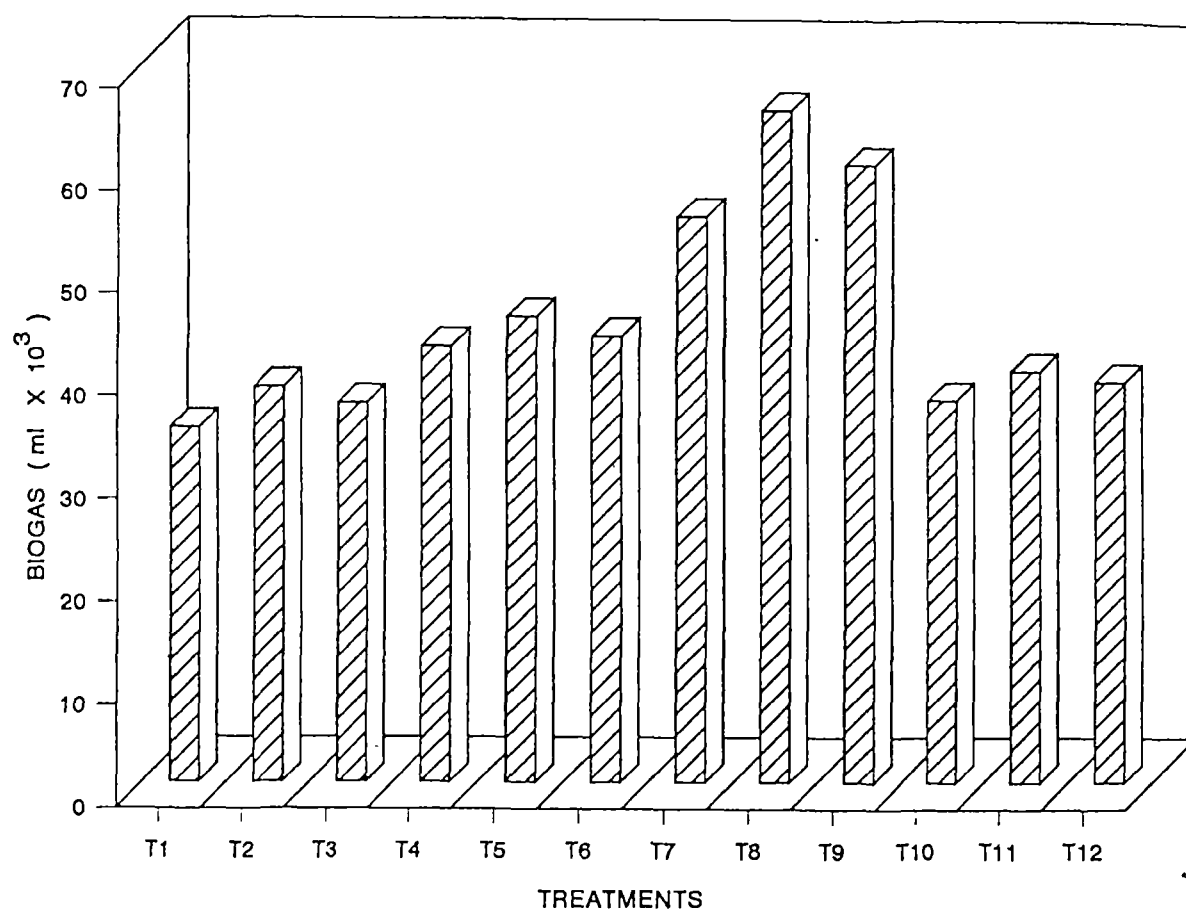


Figure 8. Biogas production in treatments with different solid concentrations.

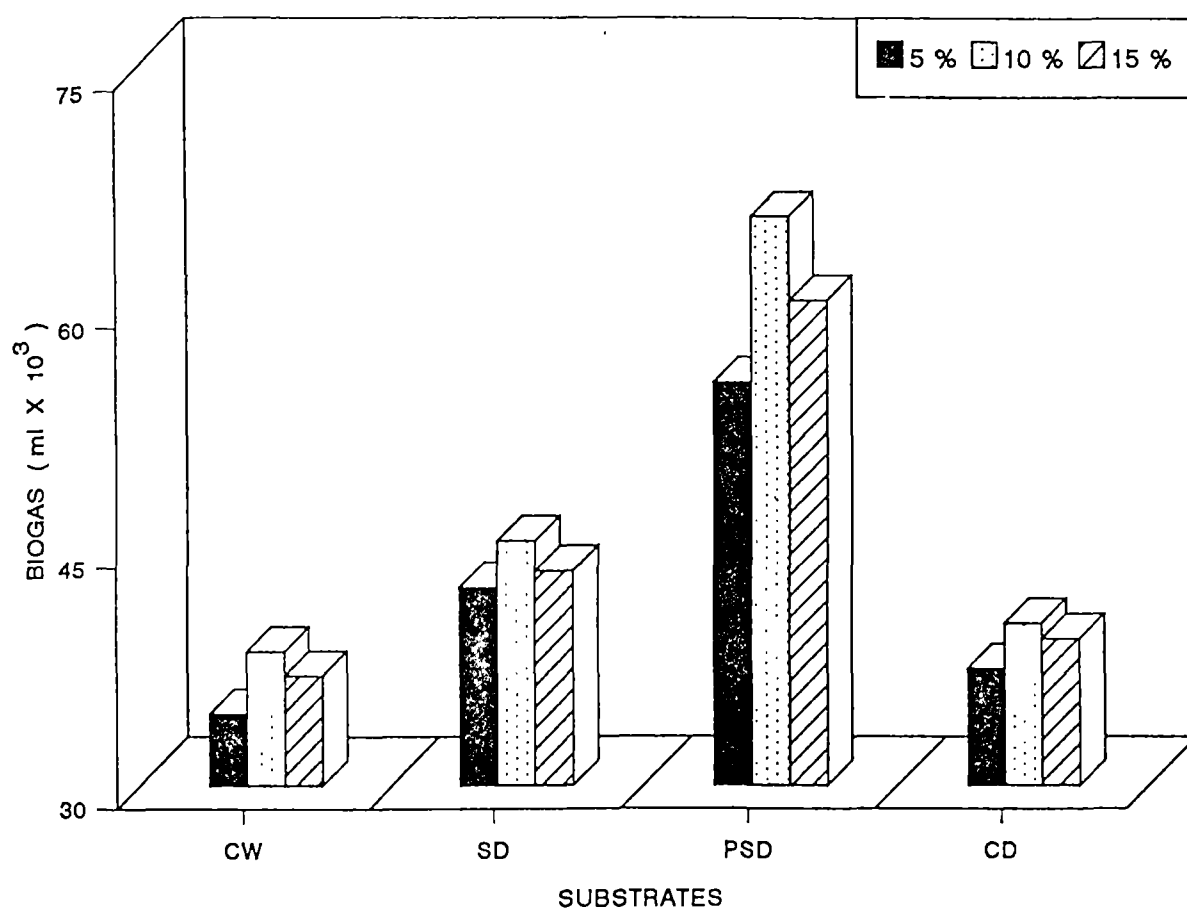


Figure 9. Biogas production in substrates with different solid concentrations.

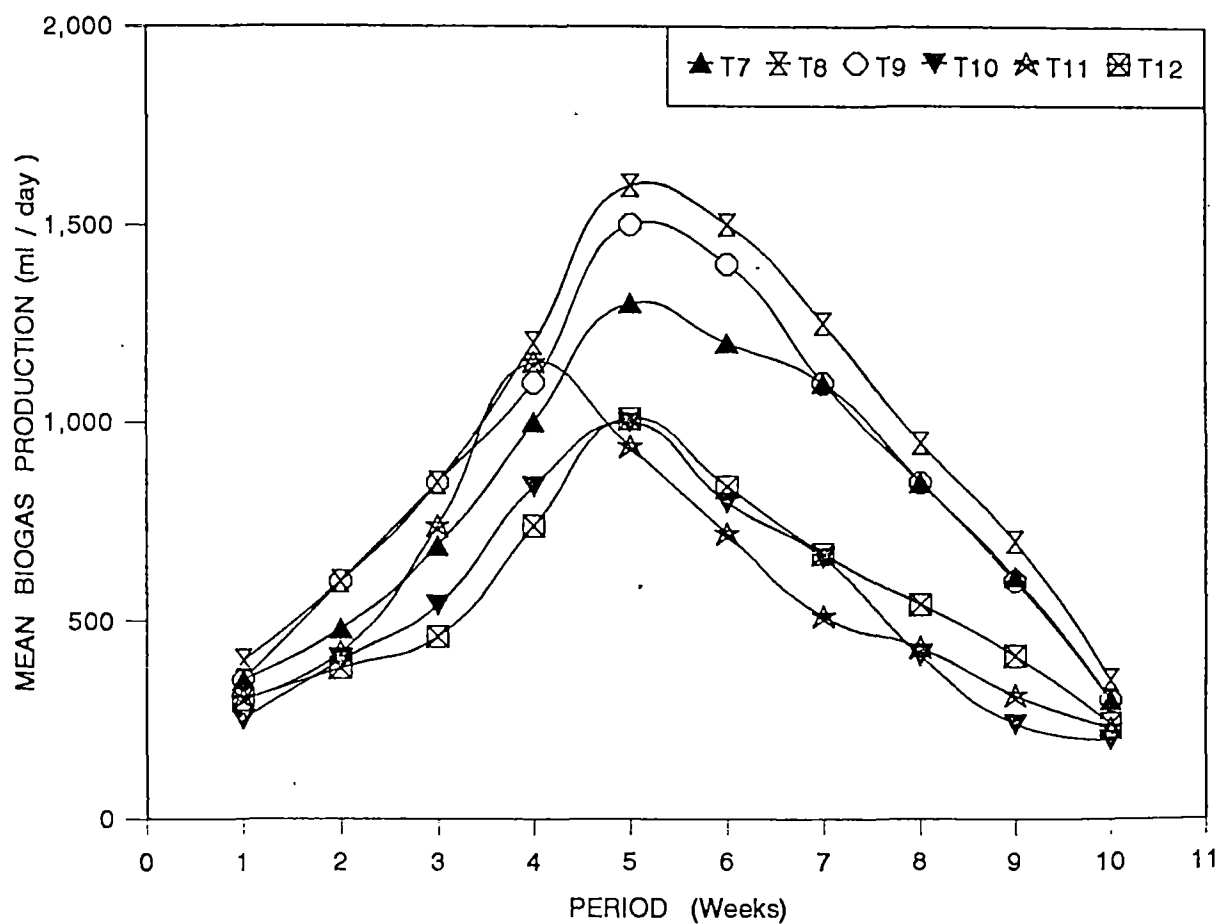
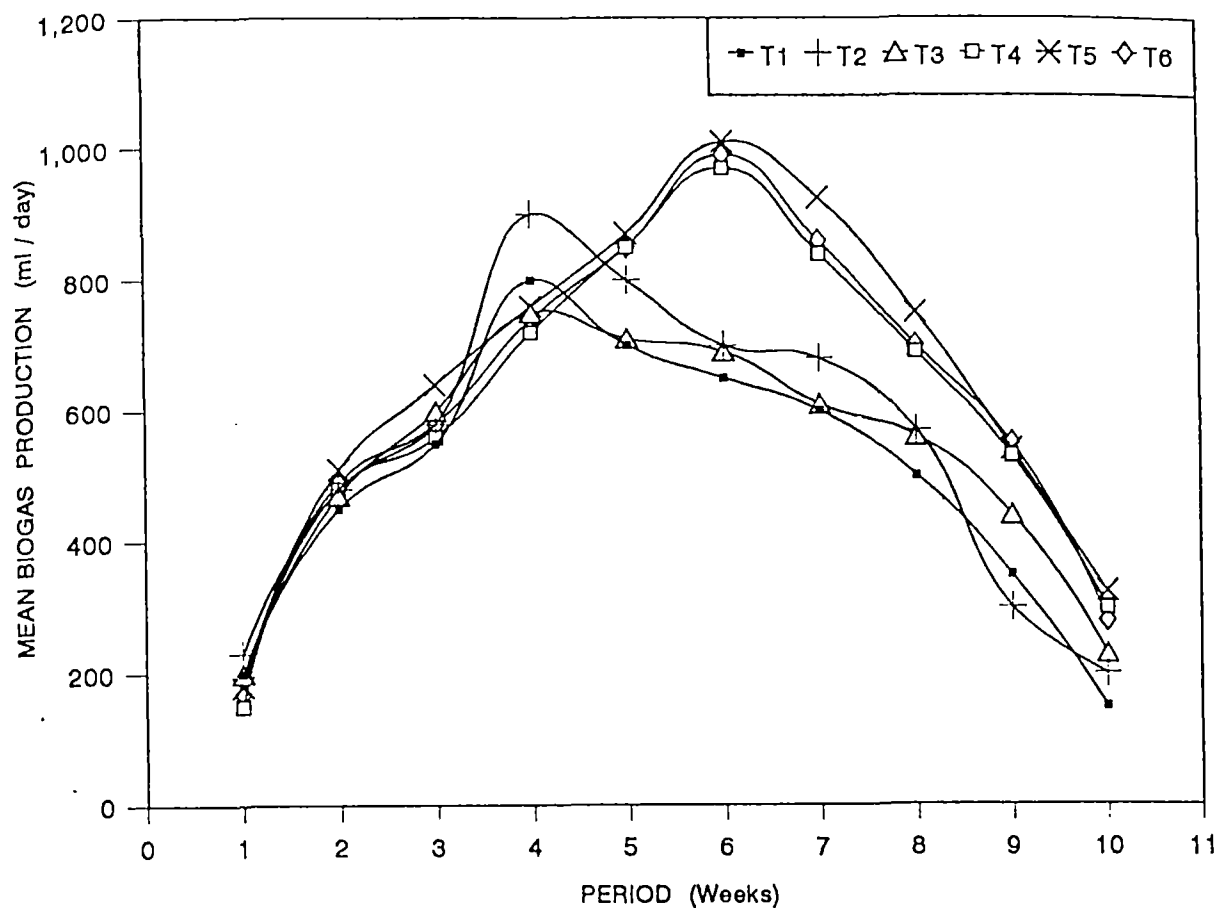


Figure 10. Influence of solid concentrations on gas output.

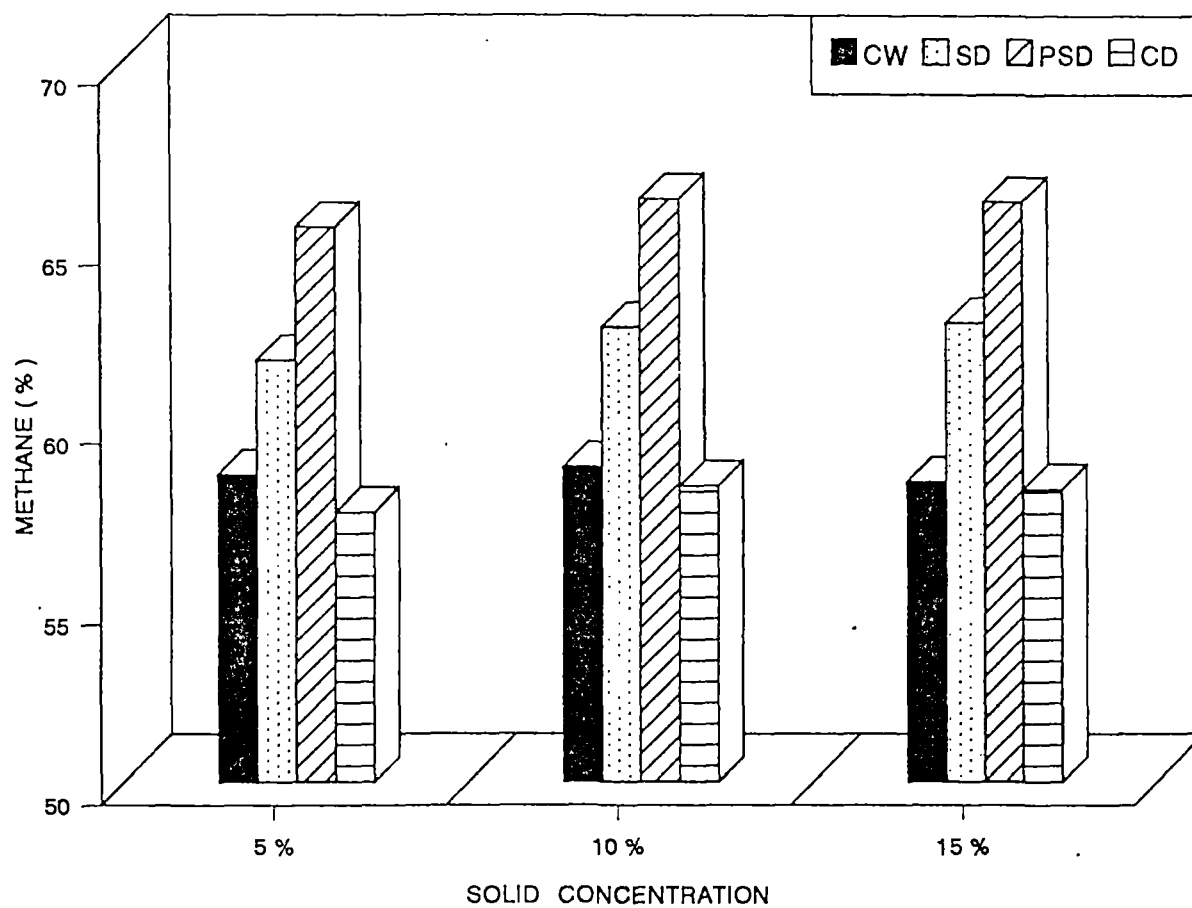


Figure 11. Methane content in the biogas at different solid concentrations.

Methane content of the biogas produced in PSD was maximum followed by undigested sawdust (Figure 11). Low levels of methane percentage was recorded in CW and cowdung. In both sawdust and PSD the percentage of methane increased with the increase in the TS concentration upto 10 per cent but further increase had no effect.

4.4. Experiment 2. Effect of inocula on biogas generation

Crumb waste, sawdust and PSD and cowdung were mixed with biodigested slurry (BS), sewage sludge (SS) and crumb sludge (CS) separately and changes in various parameters were studied in relation to methane production.

4.4.1. Microbiological properties of inocula

Biodigested slurry contained more of cellulolytic, acid forming and methanogenic bacteria followed by SS and CS (Table 13). However, both proteolytic and lipolytic bacteria were more in SS. CS registered lowest values of the anaerobic bacteria.

4.4.2. Total solids destruction

The rate of destruction of TS was more in PSD followed by sawdust (Table 14). The lowest rate of destruction of TS was in CW and cowdung.

Table 13. Microbiological properties of different inocula

Organism (g^{-1})	Biodigested slurry	Crumb sludge	Sewage sludge
Cellulolytic bacteria ($\times 10^4$)	42.3	23.6	34.4
Acid forming bacteria ($\times 10^4$)	51.8	29.2	30.2
Lipolytic bacteria ($\times 10^3$)	18.4	15.8	28.7
Proteolytic bacteria ($\times 10^4$)	19.8	5.7	30.2
Methanogenic bacteria ($\times 10^4$)	58.5	38.8	42.4

Table 14. Total and volatile solids destruction with different inocula (%)

Substrate	Total solids				Volatile solids			
	-----				-----			
	Inocula							
	BS	SS	CS	Mean	BS	SS	CS	Mean
CW	21.11	22.41	23.12	22.21	31.67	33.28	31.03	31.99
SD	26.62	24.17	22.27	24.35	39.98	34.97	28.97	34.64
PSD	27.72	25.32	23.98	25.67	48.78	45.10	35.52	43.13
CD	23.98	22.78	21.03	22.59	36.85	35.47	29.21	33.84
Mean	24.85	23.67	22.60		39.32	37.20	31.18	
CD for substrate	(P = 0.05) -			1.11	CD for substrate		(P = 0.05) -	1.20
CD for inoculum	(P = 0.05) -			0.96	CD for inoculum		(P = 0.05) -	1.04
CD for interaction	(P = 0.05) -			1.93	CD for interaction		(P = 0.05) -	2.08

BS in general recorded maximum destruction of TS and the other inocula were in the order of SS and CS (Figure 12).

All the three inocula registered high total solid destruction in PSD and sawdust than in the other two substrates. Cowdung inoculated with BS showed more TS destruction than CW. SS did not cause much change in these two substrates. On the other hand the TS destruction was more in CW inoculated with CS.

4.4.3. Volatile solids destruction

The rate of destruction of VS was in the order of PSD, sawdust, cowdung and CW (Table 14). The influence of inoculation on VS destruction was more in the case of BS followed by SS and CS. PSD inoculated with BS caused maximum destruction of VS. SS was next to BS with regard to VS destruction. The lowest rate of destruction of PSD was noted when CS was used as inoculum. There was a significant difference in VS destruction among four substrates when BS was used as inoculum (Figure 12).

4.4.4. Cellulose, hemicellulose and lignin degradation in relation to gas generation

4.4.4.1. Cellulose

The destruction of cellulose inoculated with three different inocula was in the descending order of PSD, CW,

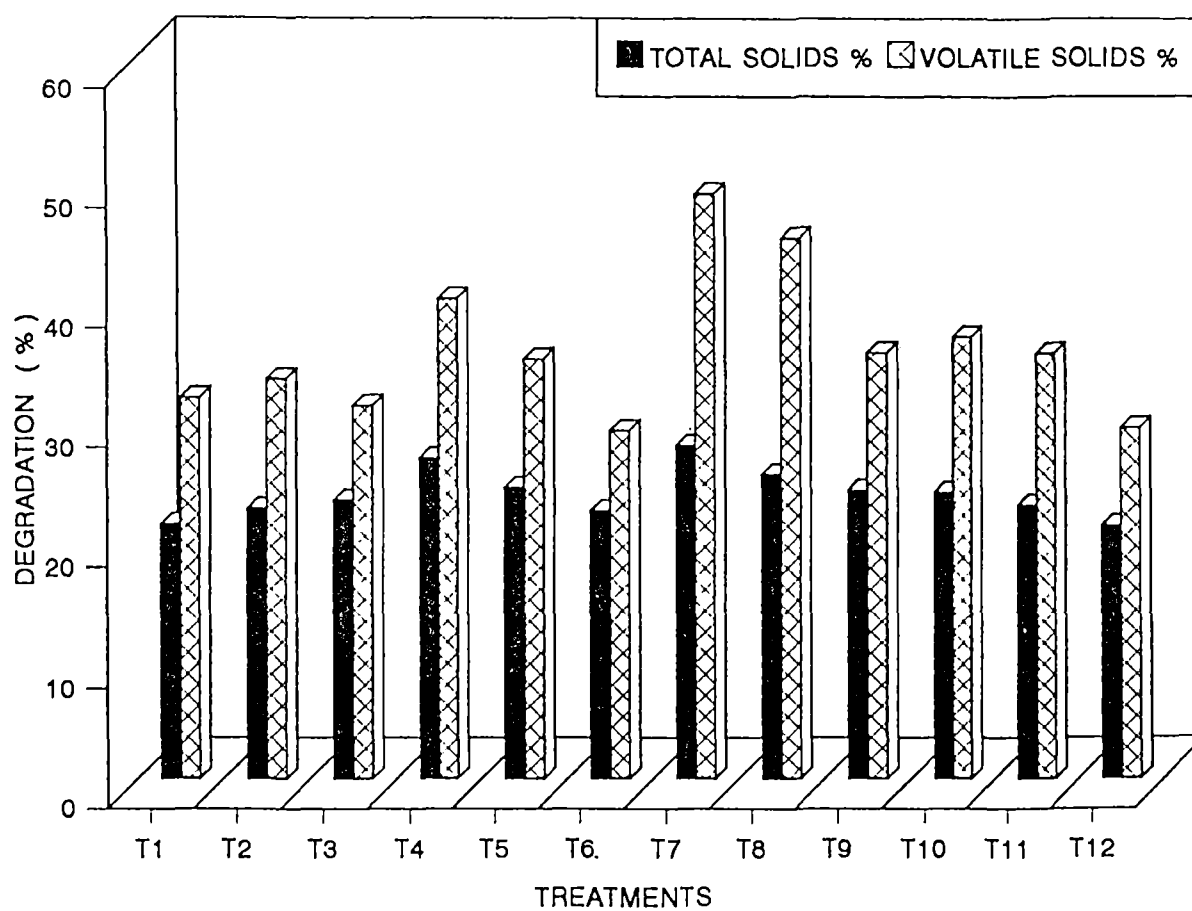


Figure 12. Degradation of total and volatile solids with different inocula.

sawdust and cowdung (Table 15). The three inocula did not show much variation in cellulose degraded. When individual substrates were considered, higher cellulose destruction was found in PSD (Figure 13). However, the highest percentage of destruction was in CW inoculated with CS.

4.4.4.2. Hemicellulose

Hemicellulose degradation upon biomethanation was the highest in PSD followed by both cowdung and sawdust treatments (Table 15). CW registered the lowest level of hemicellulose degradation. BS and SS inocula degraded more hemicellulose than CS. Maximum degradation of hemicellulose was noticed when SS was added to PSD (Figure 13).

4.4.4.3. Lignin

The lignin destruction in general was the highest in CW followed by cowdung, sawdust and PSD (Table 15). The effect of inocula on degradation of lignin was in the descending order of CS, SS, and BS. Maximum lignin degradation was in the combination of CS and CW and the minimum was in BS and PSD combination. When individual substrates were taken into consideration, combinations involving CS showed good lignin degradation followed by SS (Figure 13).

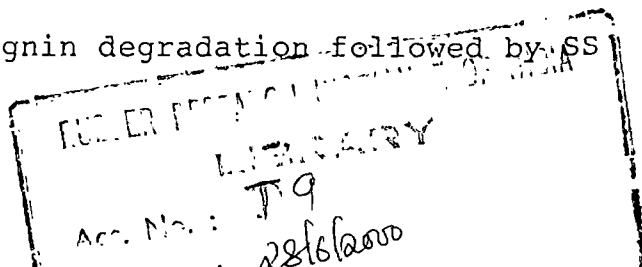


Table 15. Degradation of cellulose, hemicellulose and lignin with different inocula (%)

Substrate	Cellulose				Hemicellulose				Lignin			
	-----				-----				-----			
	Inocula				Inocula				Inocula			
	BS	SS	CS	Mean	BS	SS	CS	Mean	BS	SS	CS	Mean
CW	20.65	21.90	27.65	23.40	26.10	25.55	24.52	25.39	11.48	18.16	19.87	16.50
SD	22.12	22.32	20.03	21.49	30.03	28.92	26.77	28.57	10.97	12.39	13.93	12.43
PSD	25.52	25.25	25.94	25.57	40.79	42.56	39.28	40.87	4.75	8.27	10.46	7.82
CD	21.11	20.06	20.21	20.46	29.54	28.28	28.95	28.92	12.40	14.59	16.19	14.39
Mean	22.35	22.38	23.45		31.61	31.32	29.88		9.90	13.35	15.11	
CD for substrate	(P = 0.05)	(P = 0.05)	(P = 0.05)	0.94	CD (P = 0.05)	CD (P = 0.05)	CD (P = 0.05)	0.89	CD (P = 0.05)	CD (P = 0.05)	CD (P = 0.05)	0.81
CD for inoculum	(P = 0.05)	(P = 0.05)	(P = 0.05)	0.81	CD (P = 0.05)	CD (P = 0.05)	CD (P = 0.05)	0.77	CD (P = 0.05)	CD (P = 0.05)	CD (P = 0.05)	0.70
CD for interaction	(P = 0.05)	(P = 0.05)	(P = 0.05)	1.63	CD (P = 0.05)	CD (P = 0.05)	CD (P = 0.05)	1.54	CD (P = 0.05)	CD (P = 0.05)	CD (P = 0.05)	1.41

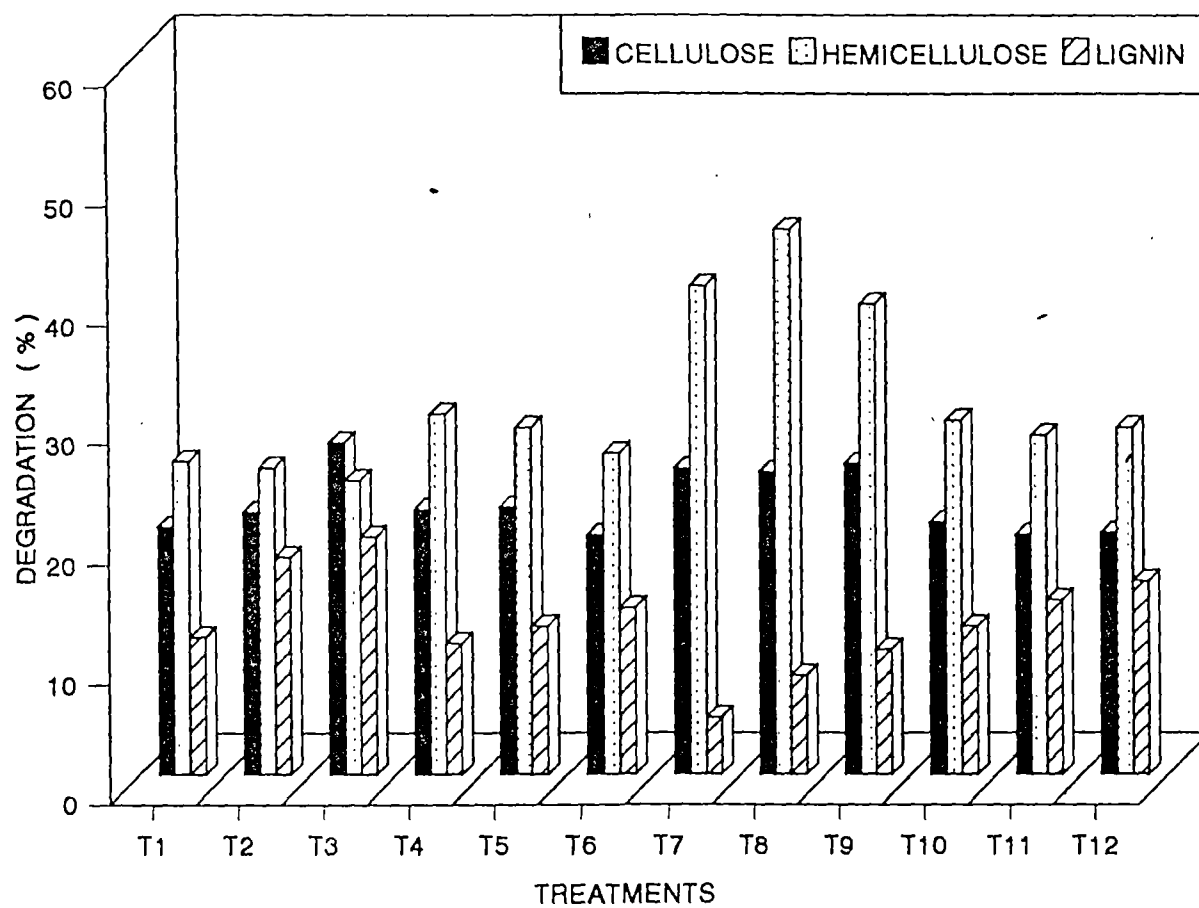


Figure 13. Degradation of cellulose, hemicellulose and lignin with different inocula.

4.4.5. Volatile fatty acid content

In the initial stage, VFA was present in all the treatments. Irrespective of the inocula, VFA was more in PSD followed by sawdust, CW and cowdung. Addition of SS to four different substrates caused a higher level of VFA. The treatment involving SS and PSD registered the highest VFA in the initial stage. In the middle stage BS in combination with different substrates registered high VFA. However, at the end of the experiment CW with different inocula registered higher levels of VFA followed by sawdust, PSD and cowdung. Among the three inocula, BS with the different substrates contained more VFA and in others it was low (Figure 14).

There was a sharp increase in VFA in all the treatments upto middle stage but it decreased at the end of the experiment. These changes were more pronounced for PSD.

4.4.6. pH

The pH of digestion mixture decreased in the middle stage in all the treatments and during the final stage again it rose to a level which was more than the initial. In the middle stage the pH ranged from 6.2 to 6.8 (Table 16). The maximum reduction in pH was in PSD. PSD and BS combination recorded the lowest pH and produced maximum gas.

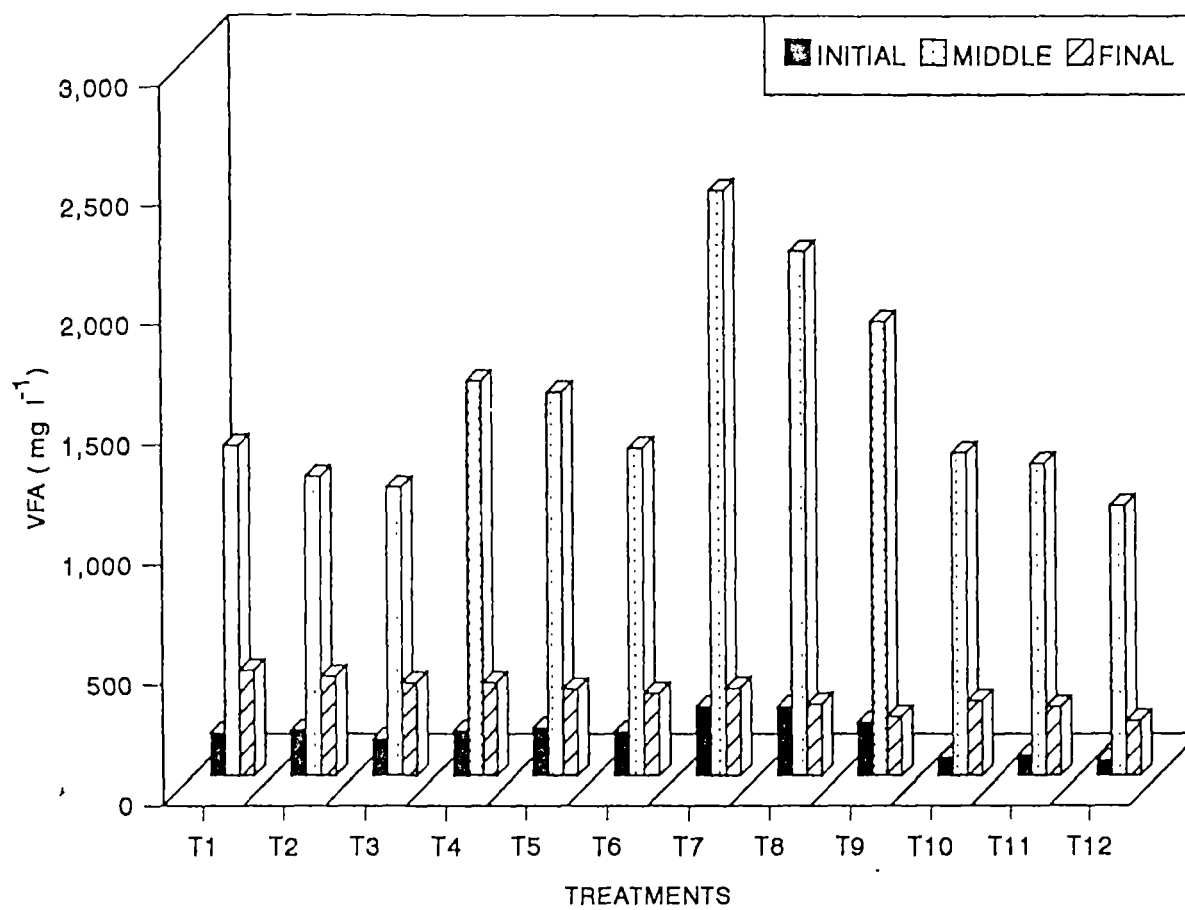


Figure 14. Volatile fatty acids with different inocula.

Table 16. Changes in pH with different inocula

Treatments	Initial	Middle	Final
T ₁	7.0	6.7	7.1
T ₂	7.0	6.8	7.4
T ₃	7.1	6.8	7.2
T ₄	6.9	6.5	7.1
T ₅	7.0	6.6	7.0
T ₆	7.1	6.8	6.9
T ₇	7.1	6.2	7.3
T ₈	7.0	6.4	7.2
T ₉	7.0	6.5	7.3
T ₁₀	7.1	6.8	7.2
T ₁₁	7.0	6.8	7.3
T ₁₂	7.1	6.8	7.1

4.4.7. Enzymatic activity

4.4.7.1. Cellulase

Significant difference in cellulase was noticed in different substrates, highest activity being associated with PSD followed by cowdung, CW and sawdust at the initial stage (Figure 15). Among the inocula, BS had more enzyme activity than CS and SS. However, when a combination of individual substrates and inocula was considered the difference in cellulase activity was not much pronounced. Irrespective of the inoculum, PSD showed more cellulase activity.

Significant variation was observed in the activity of cellulase in the four different substrates at the middle stage of the biogenesis of methane. PSD showed maximum enzyme activity and in other substrates, it was in the order of sawdust, cowdung and CW. Addition of BS resulted in enhanced cellulase activity than SS and CS at this stage. All the three inocula significantly favoured maximum cellulase in PSD followed by sawdust. In the final stage the difference in enzyme activity was comparatively less.

All the substrate in combination with BS, SS, and CS individually showed enhanced enzyme activity at the middle stage and was subsequently reduced.

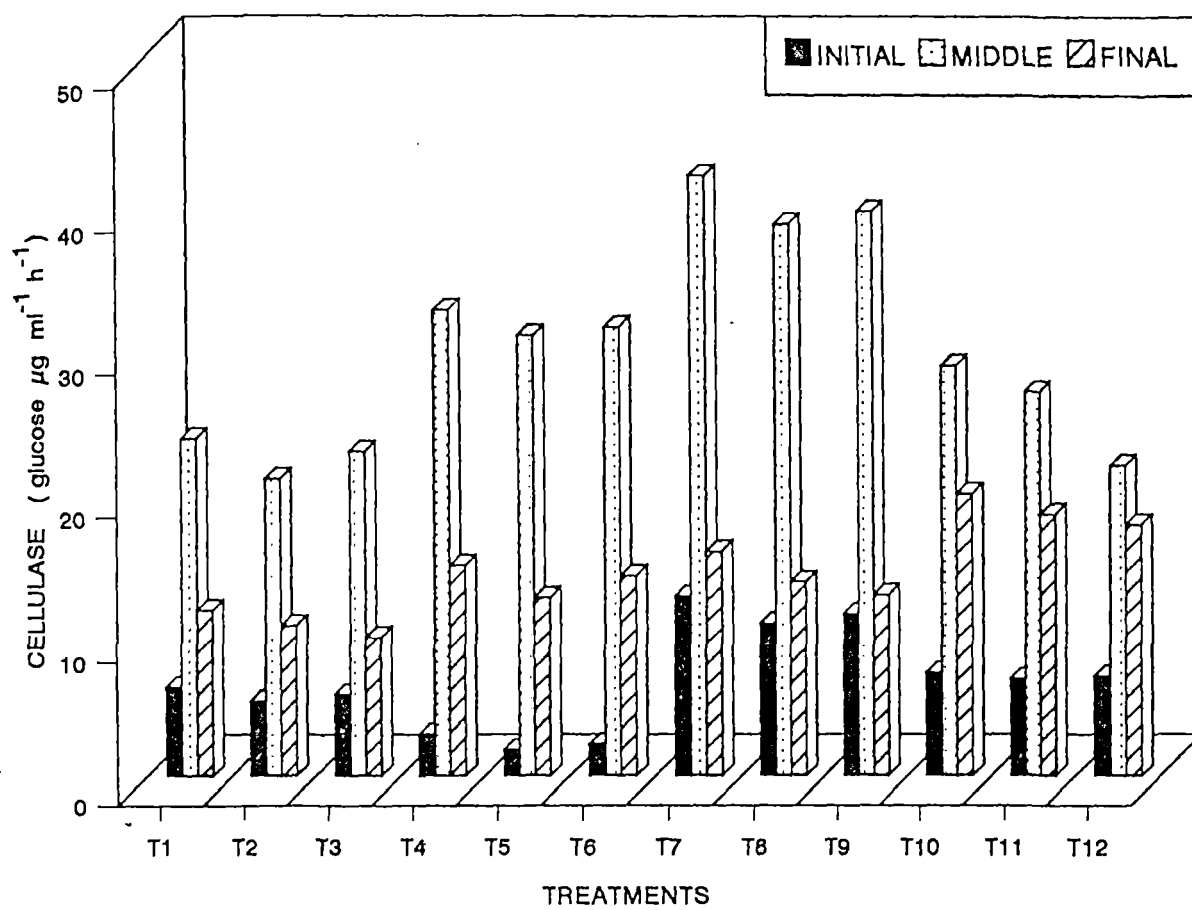


Figure 15. Cellulase activity with different inocula.

4.4.7.2. β -Glucosidase

In general, β -glucosidase activity was higher than cellulase activity. In the initial stage β -glucosidase was maximum in PSD in combination with different inocula (Figure 16). BS inoculation in all the substrates increased enzyme activity. The combination of PSD and BS was superior to other combinations. The β -glucosidase activity in the middle stage also was very high in the treatments involving PSD. Saw dust treatments were next to PSD in the activity of β -glucosidase. Incorporation of BS registered more of β -glucosidase activity with all the four substrates. In the final stage also PSD with different inocula registered more β -glucosidase activity. β -Glucosidase activity increased upto middle stage and then dropped in all the substrates-inocula combinations.

4.4.8. Enumeration of microbial population

4.4.8.1. Cellulolytic bacteria

Initially PSD registered maximum cellulolytic bacteria followed by cowdung, CW and sawdust (Table 17). Addition of BS to four substrates favoured the population of bacteria followed by CS and SS.

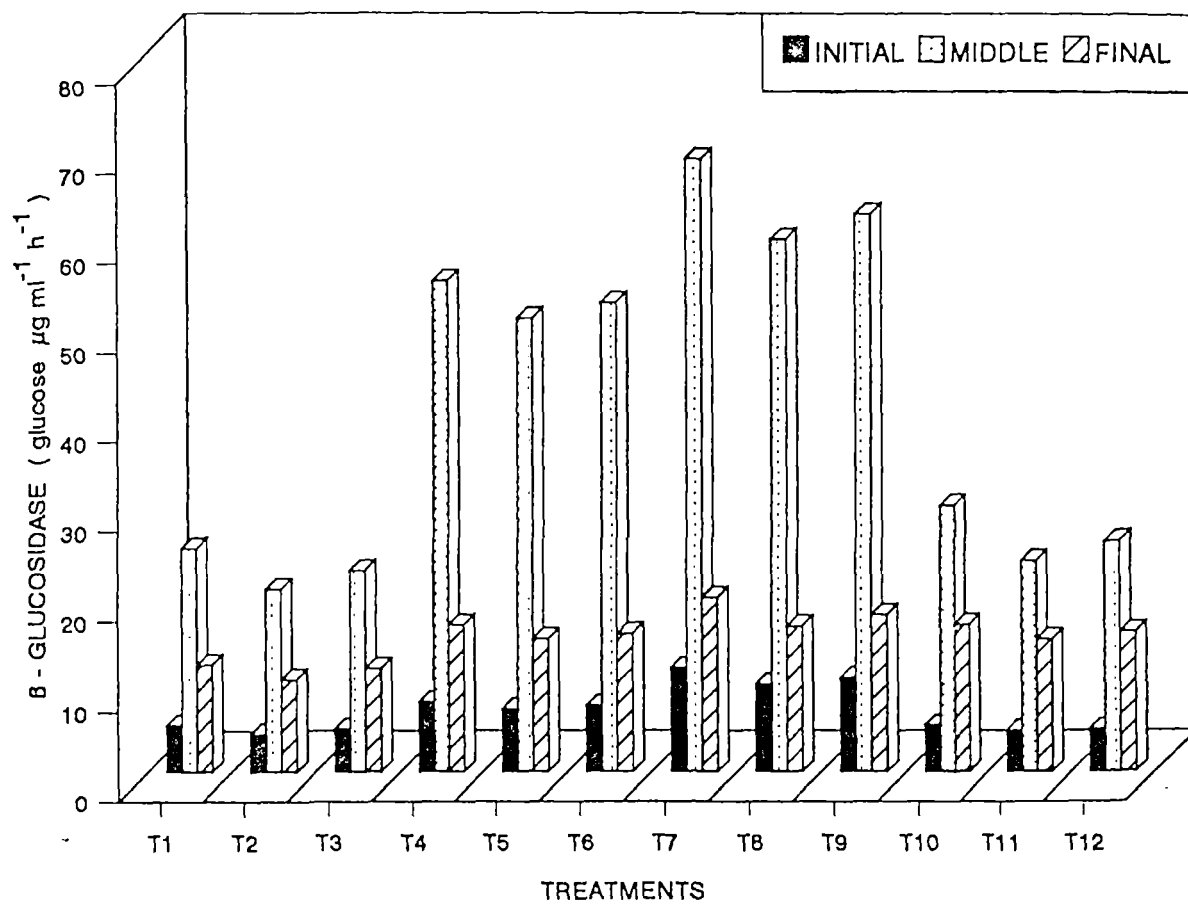


Figure 16. β -Glucosidase activity with different inocula.

Table 17. Population of cellulolytic bacteria with different inocula ($\times 10^4 \text{ g}^{-1}$)

Substrate	Initial				Middle				Final			
	-----				-----				-----			
					Inocula							
	BS	SS	CS	Mean	BS	SS	CS	Mean	BS	SS	CS	Mean
CW	17.50	15.70	16.60	16.60	32.40	30.20	31.80	31.46	10.50	8.70	9.80	9.66
SD	14.20	12.30	13.20	13.23	43.50	38.60	40.60	40.90	12.40	11.30	12.80	12.16
PSD	21.70	19.50	20.30	20.50	66.90	55.30	58.60	60.26	38.80	27.60	30.40	32.26
CD	20.50	18.40	19.50	19.46	37.60	32.50	34.80	34.96	12.80	11.60	12.50	12.30
Mean	18.47	16.47	17.40		45.10	39.15	41.45		18.62	14.80	16.37	
CD for substrate	(P = 0.05)	(P = 0.05)	(P = 0.05)	0.57	CD (P = 0.05)	CD (P = 0.05)	CD (P = 0.05)	1.77	CD (P = 0.05)	CD (P = 0.05)	CD (P = 0.05)	1.76
CD for inoculum	(P = 0.05)	(P = 0.05)	(P = 0.05)	0.49	CD (P = 0.05)	CD (P = 0.05)	CD (P = 0.05)	1.53	CD (P = 0.05)	CD (P = 0.05)	CD (P = 0.05)	1.52
CD for interaction	(P = 0.05)	(P = 0.05)	(P = 0.05)	0.99	CD (P = 0.05)	CD (P = 0.05)	CD (P = 0.05)	3.07	CD (P = 0.05)	CD (P = 0.05)	CD (P = 0.05)	3.05

In the middle stage of the experiment also, PSD recorded higher count of cellulolytic bacteria. This was followed by sawdust, cowdung and CW respectively. Addition of BS showed higher cellulolytic bacterial count in the middle stage as well. The best combination for the activity of cellulolytic bacteria in the middle stage onwards was PSD and BS.

On the whole, the cellulolytic bacterial population increased upto the middle stage and then reduced in all the substrate-inocula combinations.

4.4.8.2. Acid forming bacteria

In the initial stage of biogas production different inocula did not influence acid forming bacterial population, while the substrates did. Acid forming bacteria were maximum in cowdung followed by CW, PSD and sawdust (Table 18). But in the middle stage PSD showed more population than sawdust and cowdung. Unlike initial stage there was marked difference in the population of acid forming bacteria among the inocula in the middle stage.

In the final stage also, PSD contained more acid forming bacteria than other treatments. The treatment combinations with BS harboured more acid producing bacteria.

Table 18. Population of acid forming bacteria with different inocula ($\times 10^4 \text{ g}^{-1}$)

Substrate	Initial				Middle				Final			
	-----				-----				-----			
	BS	SS	CS	Mean	BS	SS	CS	Mean	BS	SS	CS	Mean
Inocula												
CW	22.50	21.80	19.50	21.26	32.40	30.30	29.40	30.70	22.60	20.30	18.40	20.43
SD	12.50	13.80	11.60	12.63	36.90	35.40	28.90	33.73	26.26	23.80	24.30	24.78
PSD	15.50	14.90	12.30	14.23	57.40	56.50	48.20	54.03	39.80	38.30	31.80	36.63
CD	24.60	23.20	28.80	25.53	34.80	32.90	30.80	32.83	19.40	18.50	17.30	18.40
Mean	18.77	18.42	18.05		40.37	38.77	34.32		27.01	25.22	22.95	
CD for substrate	(P = 0.05)	-	0.44	CD (P = 0.05)	substrate	-	1.82	CD (P = 0.05)	substrate	-	2.03	
CD for inoculum	(P = 0.05)	-	0.38	CD (P = 0.05)	inoculum	-	1.58	CD (P = 0.05)	inoculum	-	1.75	
CD for interaction	(P = 0.05)	-	0.77	CD (P = 0.05)	interaction	-	3.16	CD (P = 0.05)	interaction	-	3.51	

Acid producing bacterial population showed a gradual increase upto middle stage and then declined in all the treatments.

4.4.8.3. Lipolytic bacteria

In the initial stage, sawdust inoculated with all the three inocula recorded maximum lipolytic bacteria. Among the inocula, SS with the different substrates contained more number of lipolytic bacteria initially. The highest population was observed in the treatment consisting of sawdust and SS. In the middle stage, PSD and cowdung harboured higher number of lipolytic bacteria (Table 19). SS continued to favour lipolytic bacterial population. SS and PSD combination resulted in maximum population of lipolytic bacteria. In the final stage the lipolytic bacteria was more in cowdung than in other treatments irrespective of the inoculum.

The population of lipolytic bacteria increased with the progress of fermentation upto middle stage and then reduced considerably.

4.4.8.4 Proteolytic bacteria

During the initial and middle stages, the proteolytic bacteria were more in PSD than in the other substrates. Among the three inocula, SS in combination with all the substrates harboured more of these bacteria.

Table 19. Population of lipolytic bacteria with different inocula ($\times 10^3 \text{ g}^{-1}$)

Substrate	Initial				Middle				Final			
	-----				-----				-----			
	Inocula				Inocula				Inocula			
	BS	SS	CS	Mean	BS	SS	CS	Mean	BS	SS	CS	Mean
CW	12.20	13.50	11.80	12.50	16.30	18.50	17.20	17.33	3.40	4.50	4.10	4.0
SD	15.20	16.80	14.90	15.63	19.10	21.50	20.53	20.37	5.20	6.30	5.80	5.76
PSD	10.60	12.50	9.50	10.86	22.50	25.30	19.60	22.46	4.20	5.60	4.80	4.86
CD	11.60	12.60	10.30	11.50	21.80	22.30	21.50	21.86	16.20	17.50	15.40	16.36
Mean	12.40	13.85	11.62		19.92	21.90	19.70		7.25	8.47	7.52	
CD for substrate	(P = 0.05)	(P = 0.05)	(P = 0.05)	0.62	CD (P = 0.05)	CD (P = 0.05)	CD (P = 0.05)	1.88	CD (P = 0.05)	CD (P = 0.05)	CD (P = 0.05)	1.34
CD for inoculum	(P = 0.05)	(P = 0.05)	(P = 0.05)	0.53	CD (P = 0.05)	CD (P = 0.05)	CD (P = 0.05)	1.63	CD (P = 0.05)	CD (P = 0.05)	CD (P = 0.05)	1.16
CD for interaction	(P = 0.05)	(P = 0.05)	(P = 0.05)	1.07	CD (P = 0.05)	CD (P = 0.05)	CD (P = 0.05)	3.27	CD (P = 0.05)	CD (P = 0.05)	CD (P = 0.05)	2.33

However, at the end of the experiment, cowdung showed higher population (Table 20). At the middle and end of the experiments, the inocula did not influence proteolytic bacterial population.

The population of proteolytic bacteria showed an increase due to fermentation upto the middle stage and then reduced in all the treatments.

4.4.8.5. Methanogenic bacteria

The population of methanogenic bacteria in the initial stage was significantly higher in the PSD followed by CW. Among the inocula, BS supported a higher population of methanogenic bacteria (Table 21).

In the middle stage also PSD registered very high population of methanogenic bacteria, while sawdust treatment showed only half the population. The lowest count of methanogens was noted in cowdung in the middle stage. Among the three inocula, BS mixed with all the four substrates registered higher population followed by SS and CS.

At the end of the experiment the population of methanogenic bacteria in all the treatments was less when compared to the middle stage. Among different inocula, BS retained a higher population of methanogenic bacteria followed by SS and CS till the end of the experiment.

Table 20. Population of proteolytic bacteria with different inocula ($\times 10^4 \text{ g}^{-1}$)

Substrate	Initial				Middle				Final			
	-----				-----				-----			
					Inocula							
	BS	SS	CS	Mean	BS	SS	CS	Mean	BS	SS	CS	Mean
CW	30.50	31.80	30.80	31.03	40.80	42.50	41.30	41.53	28.90	29.20	30.10	29.40
SD	32.30	33.90	31.80	32.66	45.0	47.40	48.20	46.86	30.80	31.30	29.90	30.66
PSD	37.40	38.90	36.80	37.70	51.80	56.20	50.50	52.83	20.20	23.80	21.90	21.96
CD	36.40	37.90	35.80	36.70	43.60	44.80	41.30	43.23	36.80	37.20	35.20	36.40
Mean	34.15	35.62	33.80		45.30	47.72	45.32		29.17	30.37	29.27	
CD for substrate	(P = 0.05)	-	0.61		CD (P = 0.05)	substrate	-	2.22	CD (P = 0.05)	substrate	-	2.09
CD for inoculum	(P = 0.05)	-	0.53		CD (P = 0.05)	inoculum	-	1.92	CD (P = 0.05)	inoculum	-	1.81
CD for interaction	(P = 0.05)	-	1.06		CD (P = 0.05)	interaction	-	3.85	CD (P = 0.05)	interaction	-	3.62

Table 21. Population of methanogenic bacteria with different inocula ($\times 10^4 \text{ g}^{-1}$)

Substrate	Initial				Middle				Final			
	-----				-----				-----			
					Inocula							
	BS	SS	CS	Mean	BS	SS	CS	Mean	BS	SS	CS	Mean
CW	17.50	15.30	16.20	16.33	45.20	40.60	42.60	42.80	39.60	35.50	37.40	37.50
SD	10.40	9.50	9.80	9.90	61.50	58.60	55.70	58.60	53.80	49.30	45.60	49.56
PSD	22.60	18.50	19.20	20.10	130.40	118.60	100.50	116.50	120.80	110.50	95.30	108.86
CD	10.20	9.60	9.20	9.66	33.80	30.60	28.50	30.96	23.80	22.50	20.60	22.30
Mean	15.17	13.22	13.60		67.72	62.10	56.82		59.50	54.45	49.72	
CD for substrate	(P = 0.05) - 0.67				CD (P = 0.05) substrate				CD (P = 0.05) substrate			
CD for inoculum	(P = 0.05) - 0.58				CD (P = 0.05) inoculum				CD (P = 0.05) inoculum			
CD for interaction	(P = 0.05) - 1.16				CD (P = 0.05) interaction				CD (P = 0.05) interaction			

There was a sharp increase in the methanogenic bacterial population upon the addition of inoculum upto the middle stage and thereafter it reduced in all the treatments.

4.4.9. Biogas production

Biogas production was maximum in PSD followed by sawdust, cowdung and CW (Figure 17). Among the three inocula, biogas production was more in BS followed by SS and CS. The most suitable combination of substrate and inoculum was PSD and BS (Figure 18). The lowest level of biogas production was recorded in a combination of CW and CS. The gas production was high during fourth and sixth week of biodigestion (Figure 19).

All the three inocula with PSD favoured increase in the percentage of methane, when compared to the other substrates (Figure 20). Among the three inocula, BS was better, followed by SS and CS. Percentage of methane in sawdust treatments was more than that recorded in CW and cowdung.

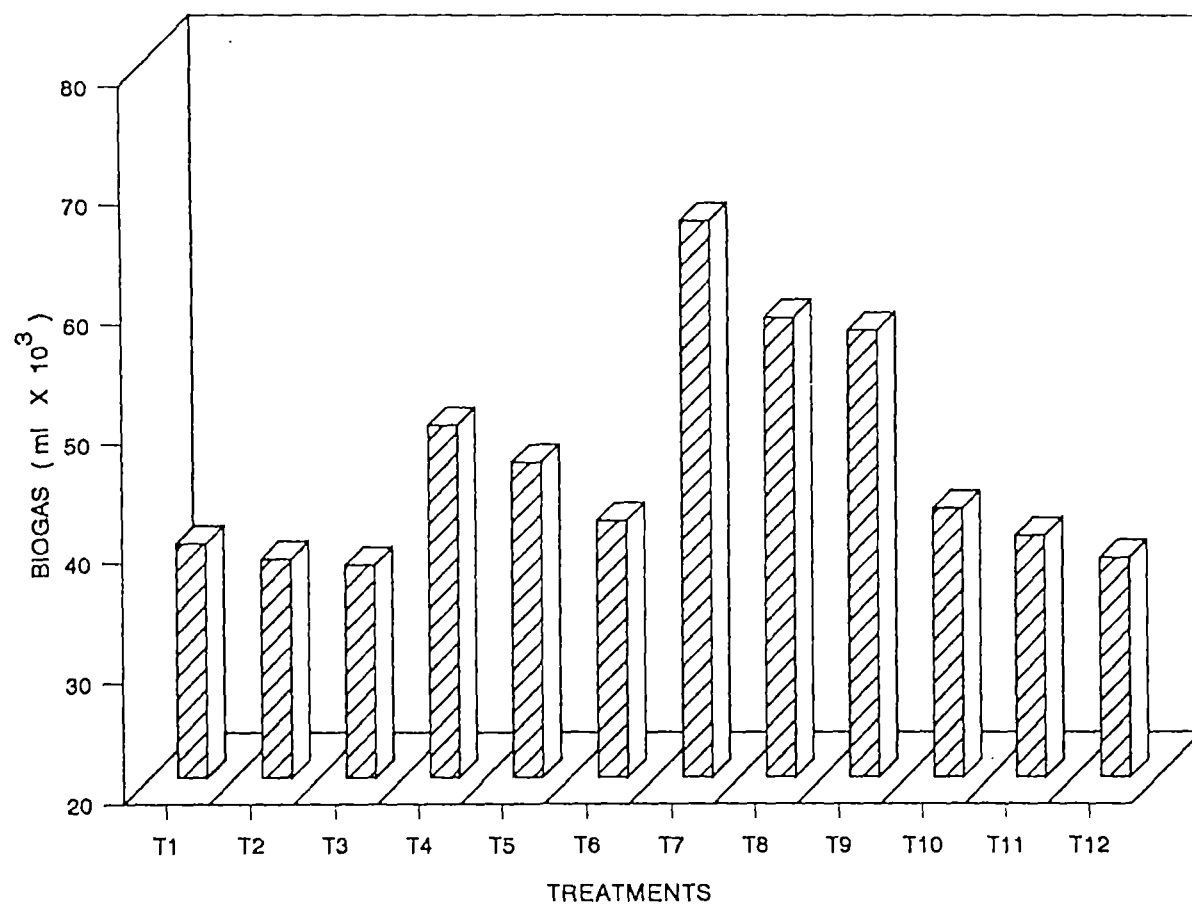


Figure 17. Biogas production in treatments with different inocula.

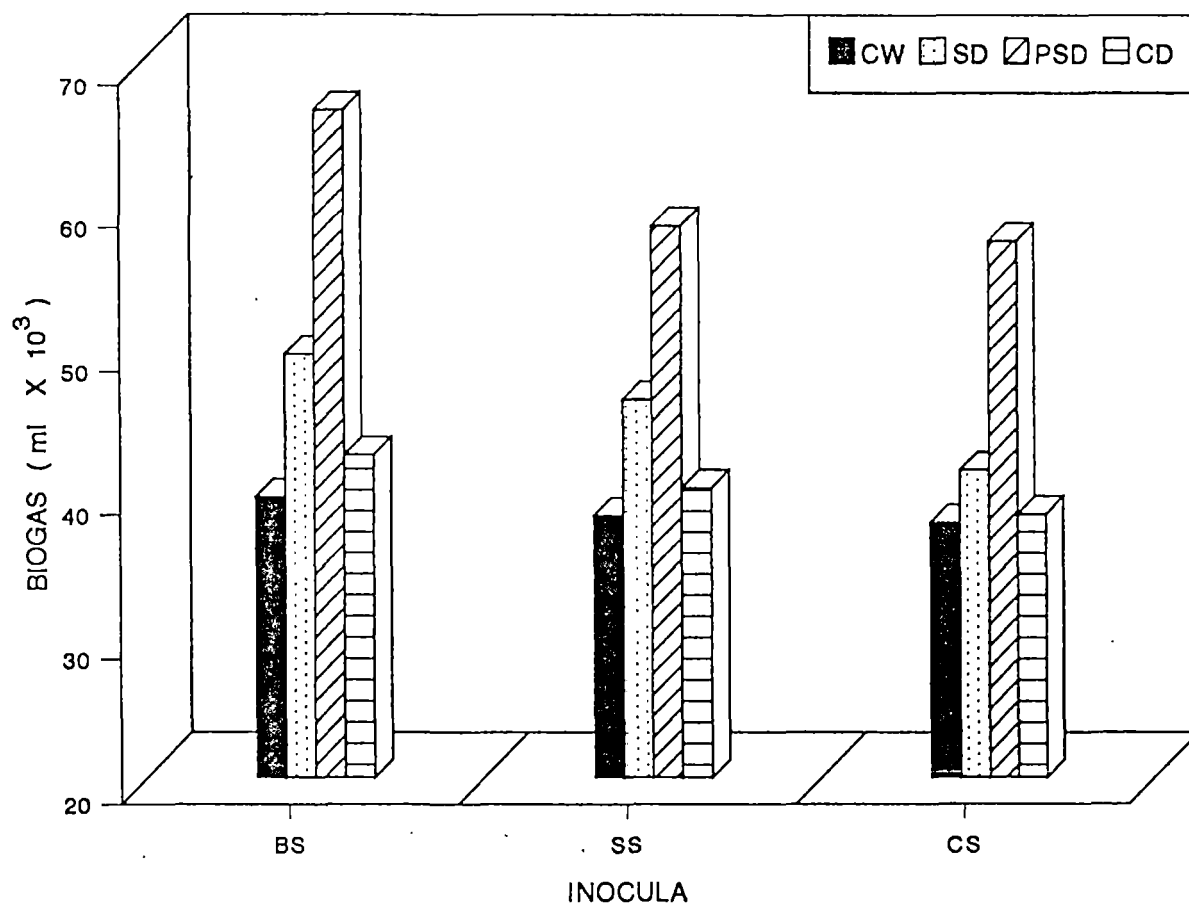


Figure 18. Biogas production with various inocula and substrates.

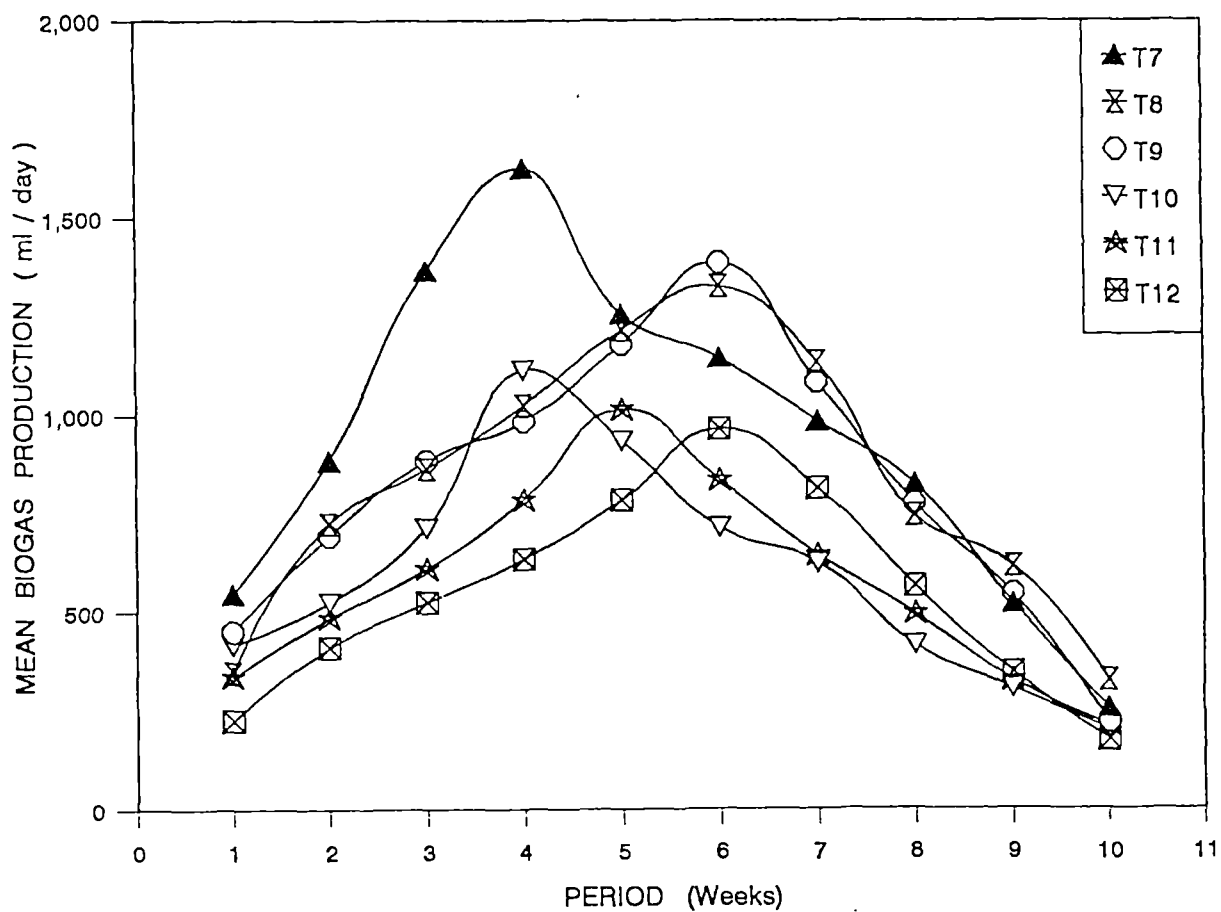
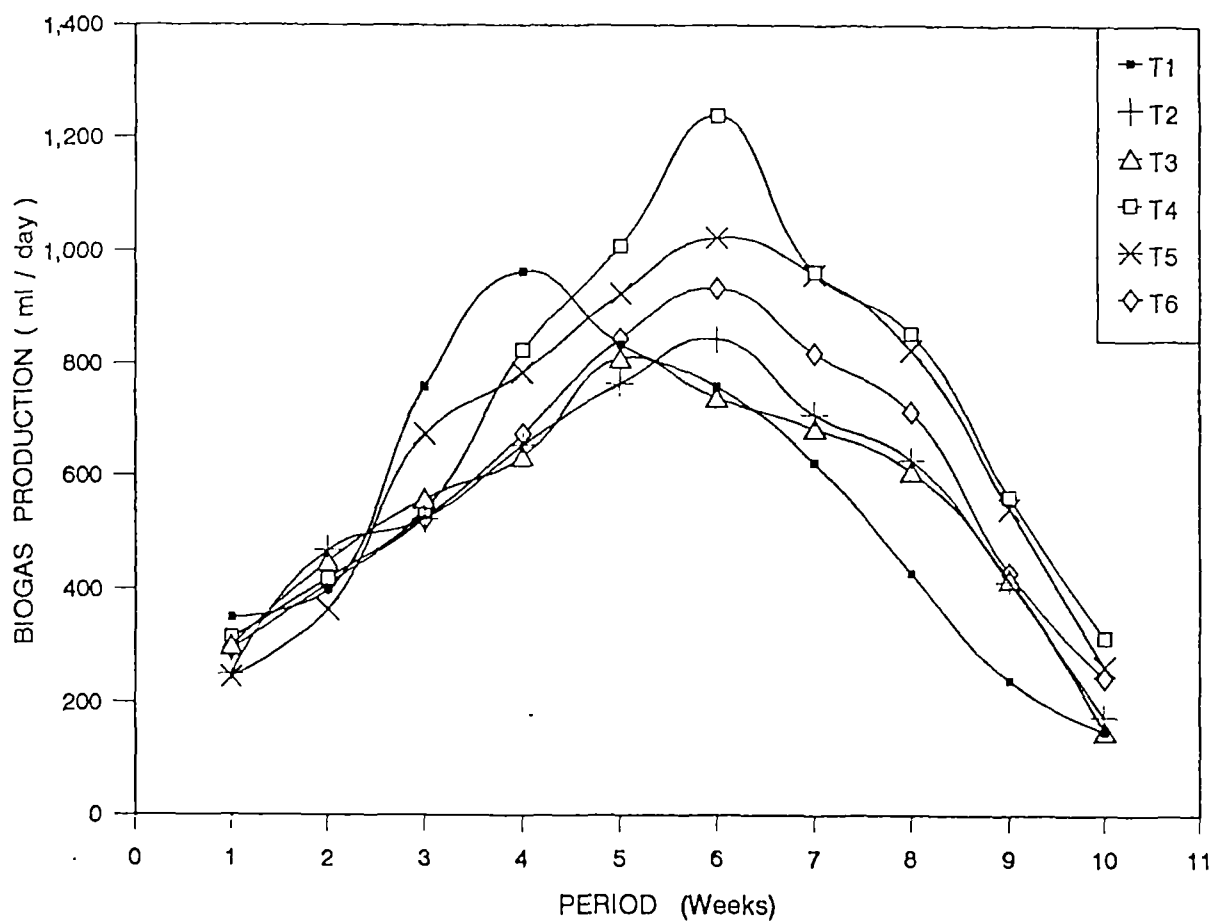


Figure 19. Influence of inocula on gas output.

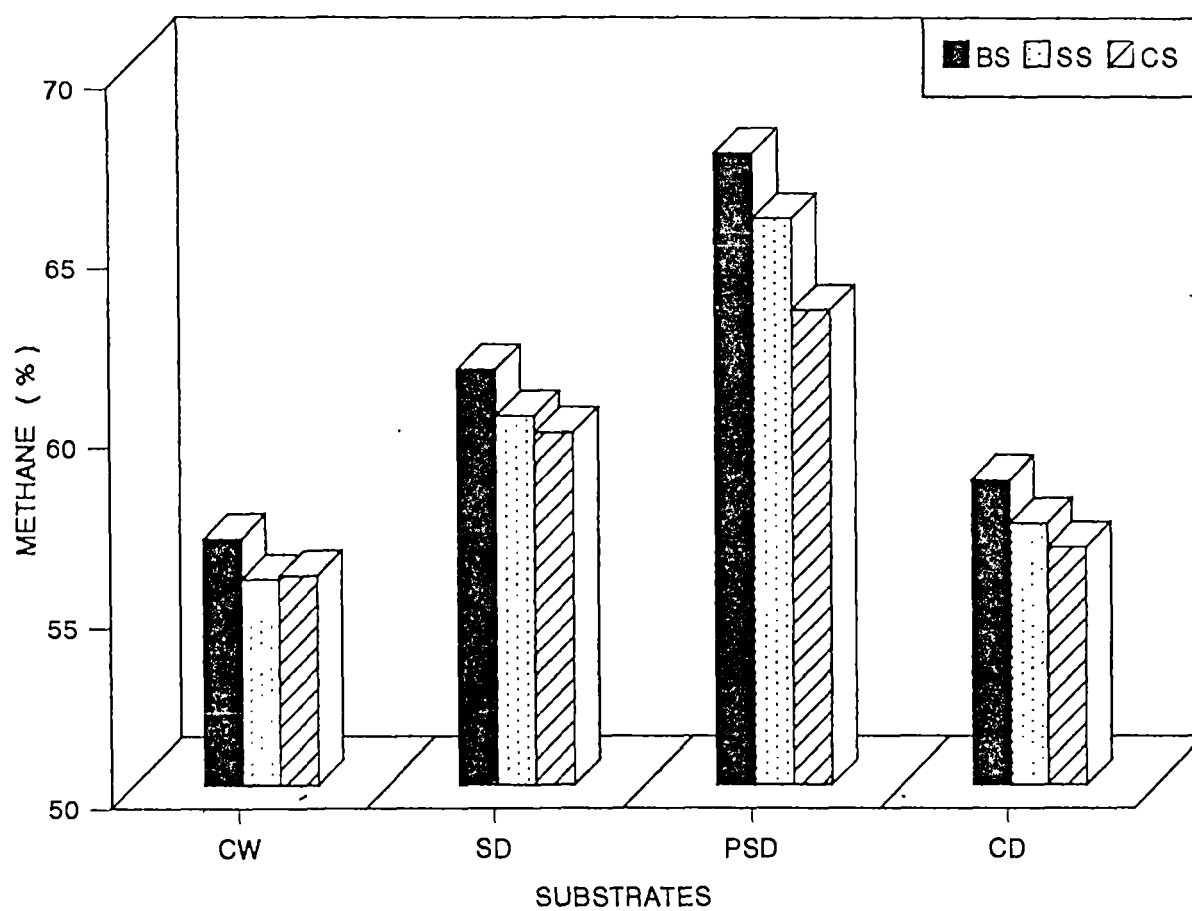


Figure 20. Methane content in the biogas with different inocula.

4.5. Experiment 3. Effect of effluents on biogas generation

The effect of combination of CW, sawdust, PSD and cowdung with SPE, CPE and LCE was investigated for changes in various parameters in relation to biogas production.

4.5.1. Total solids destruction

Methanogenesis resulted in the destruction of TS and significant difference was recorded among various substrates and diluents (Table 22). All the four substrates with SPE caused higher percentage of TS destruction while LCE caused the least change. PSD with SPE and CPE showed higher percentage of TS destruction (Figure 21).

4.5.2. Volatile solids destruction

Volatile solid destruction was generally more in PSD with all the three diluents (Table 22). However, maximum destruction of VS was noted in PSD and SPE combination (Figure 21).

4.5.3. Cellulose, hemicellulose and lignin degradation in relation to gas generation

4.5.3.1. Cellulose

Considerable degradation of cellulose was recorded upon biogenesis of methane (Table 23).

Table 22. Total and volatile solid destruction with different diluents (%)

Substrate	Total solids				Volatile solids			
	Diluents							
	SPE	CPE	LCE	Mean	SPE	CPE	LCE	Mean
CW	27.53	26.04	20.48	24.68	31.31	29.63	30.14	30.36
SD	29.54	27.85	24.52	27.30	33.85	31.28	32.68	32.60
PSD	31.03	30.18	28.56	29.92	53.52	48.65	31.83	44.66
CD	29.41	28.75	23.24	27.13	32.60	31.89	31.33	31.94
Mean	29.37	28.20	24.20		37.82	35.36	31.49	
CD for substrate	(P = 0.05)	-	0.81	CD for substrate	(P = 0.05)	-	1.50	
CD for diluent	(P = 0.05)	-	0.70	CD for diluent	(P = 0.05)	-	1.29	
CD for interaction	(P = 0.05)	-	1.40	CD for interaction	(P = 0.05)	-	2.59	

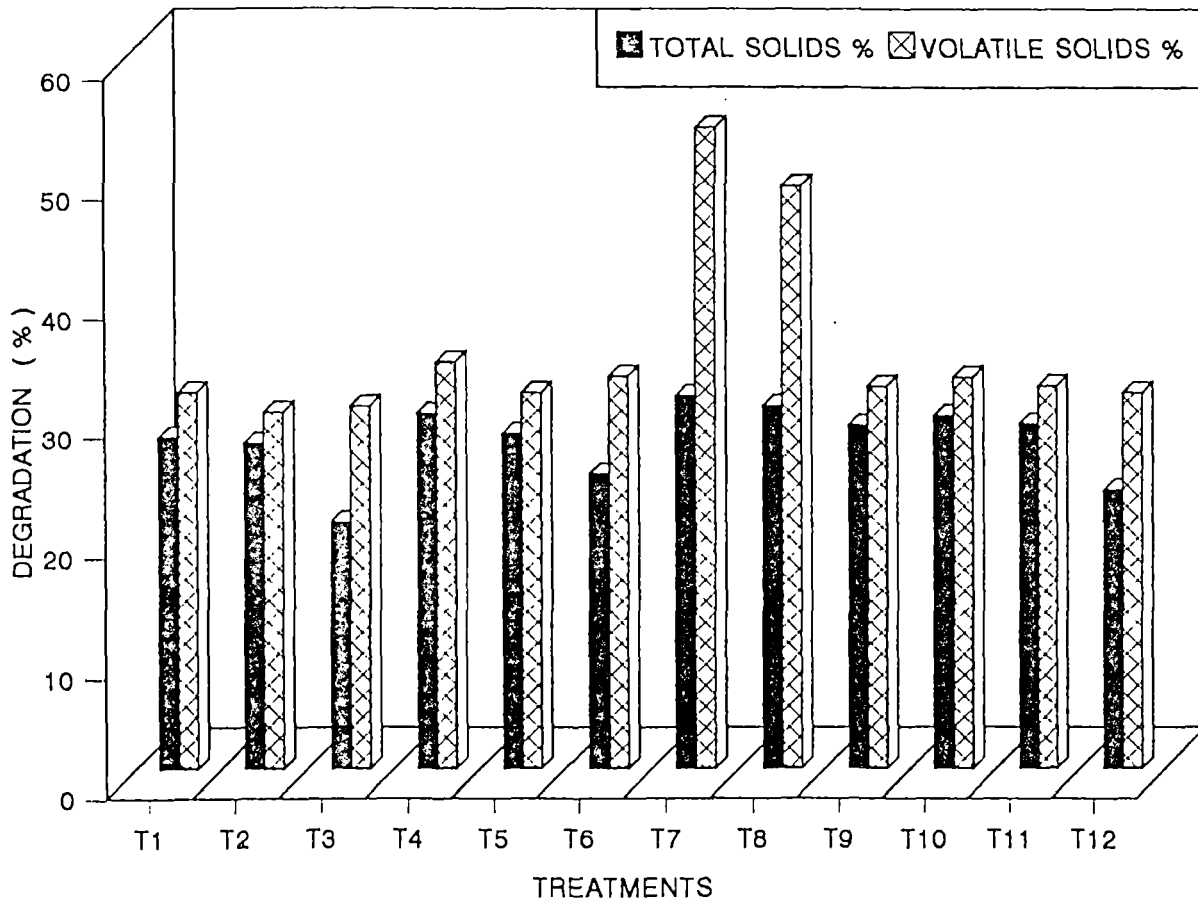


Figure 21. Degradation of total and volatile solids with different diluents.

PSD caused high level of cellulose degradation, while SPE with PSD favoured maximum cellulose degradation, followed by CPE and LCE respectively (Figure 22).

4.5.3.2. Hemicellulose

The degradation pattern of hemicellulose was same as that of cellulose (Table 23). PSD was superior to other substrate while SPE registered more degradation when the diluents were compared (Figure 22).

4.5.3.3. Lignin

The degradation of lignin was considerably lower in all the treatments (Table 23). Neither the substrates nor the diluents showed any significant influence on lignin degradation (Figure 22).

4.5.4. Volatile fatty acid content

VFA was more in the treatments of PSD with SPE, CPE and LCE followed by sawdust (Figure 23). Cowdung treatment showed least VFA. Irrespective of treatments there was a steep rise in VFA upto the middle stage which reduced afterwards. The increase in VFA was more in treatments of PSD with SPE followed by the same substrate with CPE.

Table 23. Degradation of cellulose, hemicellulose and lignin with different diluents (%)

Substrate	Cellulose				Hemicellulose				Lignin			
	-----				-----				-----			
	SPE	CPE	LCE	Mean	SPE	CPE	LCE	Mean	SPE	CPE	LCE	Mean
Diluents												
CW	20.0	18.41	18.33	18.91	34.87	35.45	31.26	33.86	5.70	5.06	4.10	4.95
SD	23.41	22.18	21.61	22.40	40.29	39.79	35.35	38.47	5.79	6.51	5.30	5.86
PSD	26.74	24.85	22.64	24.74	45.17	42.56	39.36	42.36	5.79	5.40	5.12	5.43
CD	20.37	19.83	18.31	19.50	36.26	34.85	31.95	34.35	4.88	4.84	3.91	4.54
Mean	22.63	21.31	20.22		39.14	38.16	34.48		5.54	5.45	4.60	
CD for substrate	(P = 0.05)	-	1.45	CD (P = 0.05)	substrate	-	1.68	CD (P = 0.05)	substrate	-	1.32	
CD for diluent	(P = 0.05)	-	1.25	CD (P = 0.05)	diluent	-	1.46	CD (P = 0.05)	diluent	-	1.14	
CD for interaction	(P = 0.05)	-	2.51	CD (P = 0.05)	interaction	-	2.92	CD (P = 0.05)	interaction	-	2.28	

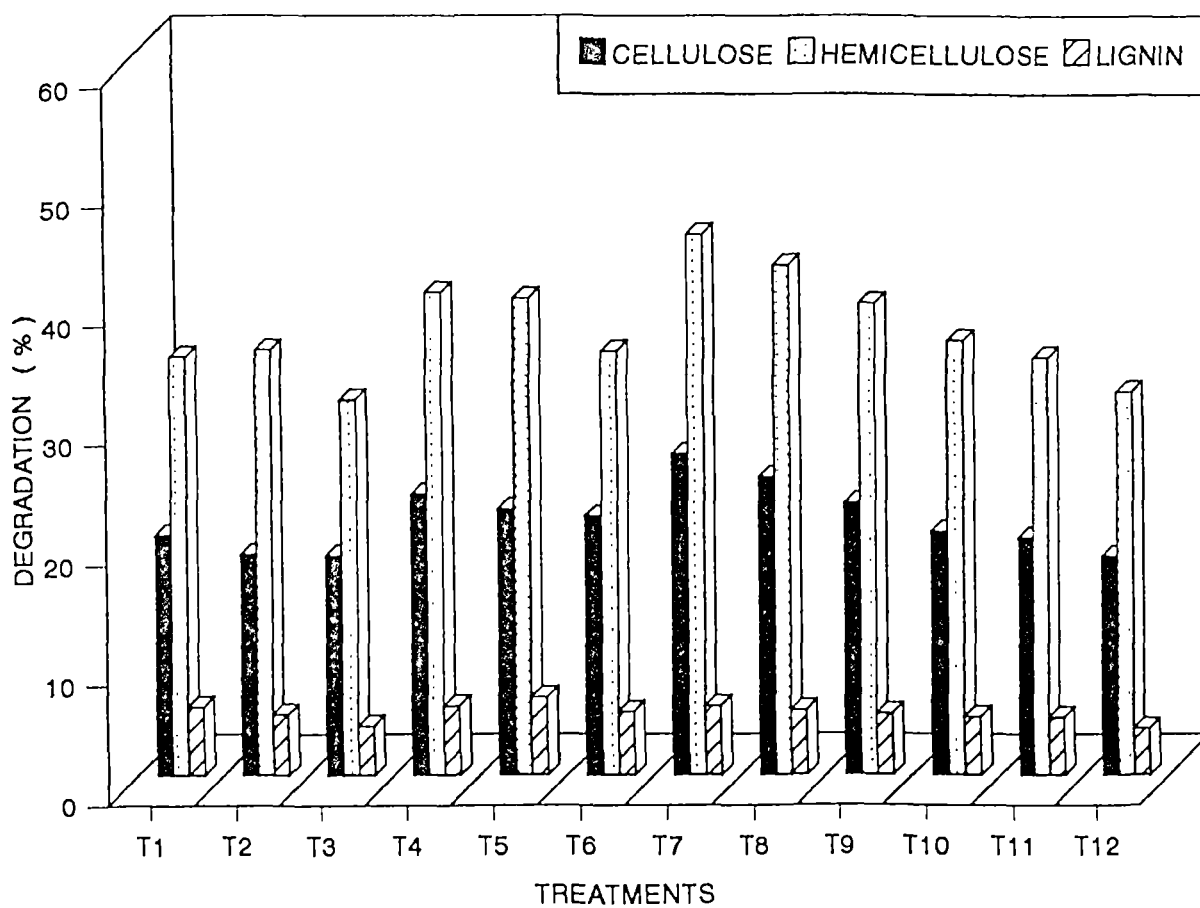


Figure 22. Degradation of cellulose, hemicellulose and lignin with different diluents.

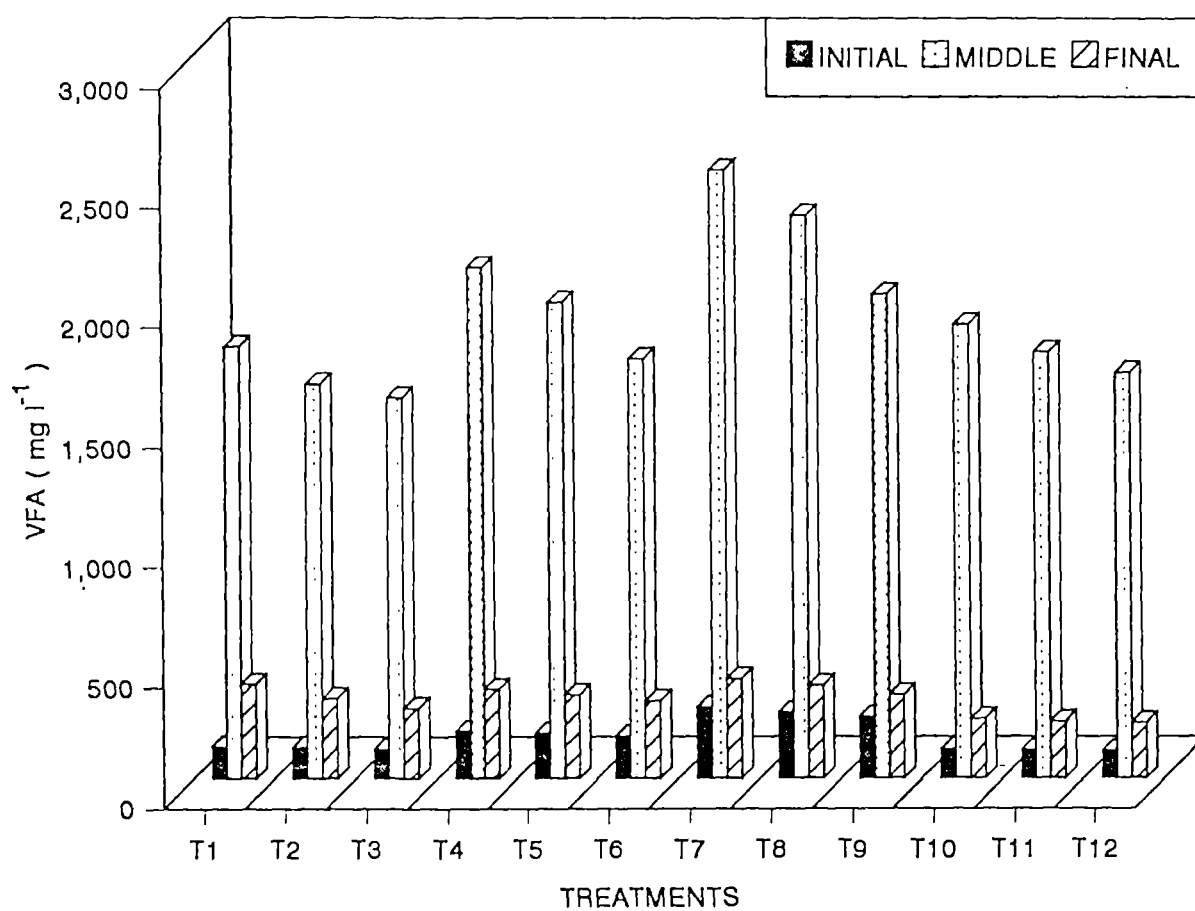


Figure 23. Volatile fatty acids with different diluents.

4.5.5. pH

The pH of the digestion mixture dropped during the middle stage and again showed higher values at the end of the experiment, in all the treatments. The drop in pH was more in PSD incorporated treatments (Table 24). The lowest pH in the middle stage was in the treatment of PSD diluted with SPE.

4.5.6. Enzymatic activity

4.5.6.1. Cellulase

The activity of cellulase showed wide variations with the treatments during all the three stages of methanogenesis. In the initial stage, cellulase activity was not pronounced. However, CW and PSD showed maximum cellulase activity (Figure 24). When the diluents were considered, CPE registered maximum cellulase activity. PSD with CPE registered the highest cellulase activity. The cellulase activity increased with the progress in fermentation upto middle stage and thereafter reduced. In the middle stage PSD had maximum cellulase activity, when substrates were taken for comparison. In the case of diluents sheet processing effluent showed more cellulase activity than the other two diluents. PSD with SPE showed the highest cellulase activity in the middle stage.

Table 24. Changes in pH with different inocula

Treatments	Initial	Middle	Final
T ₁	6.9	6.8	6.9
T ₂	6.9	6.8	6.8
T ₃	7.0	6.8	6.9
T ₄	6.8	6.4	6.9
T ₅	6.8	6.6	7.0
T ₆	7.0	6.8	6.9
T ₇	7.0	6.2	6.9
T ₈	6.8	6.3	6.8
T ₉	6.8	6.5	6.7
T ₁₀	7.0	6.7	7.0
T ₁₁	7.0	6.8	7.1
T ₁₂	6.8	6.7	6.9

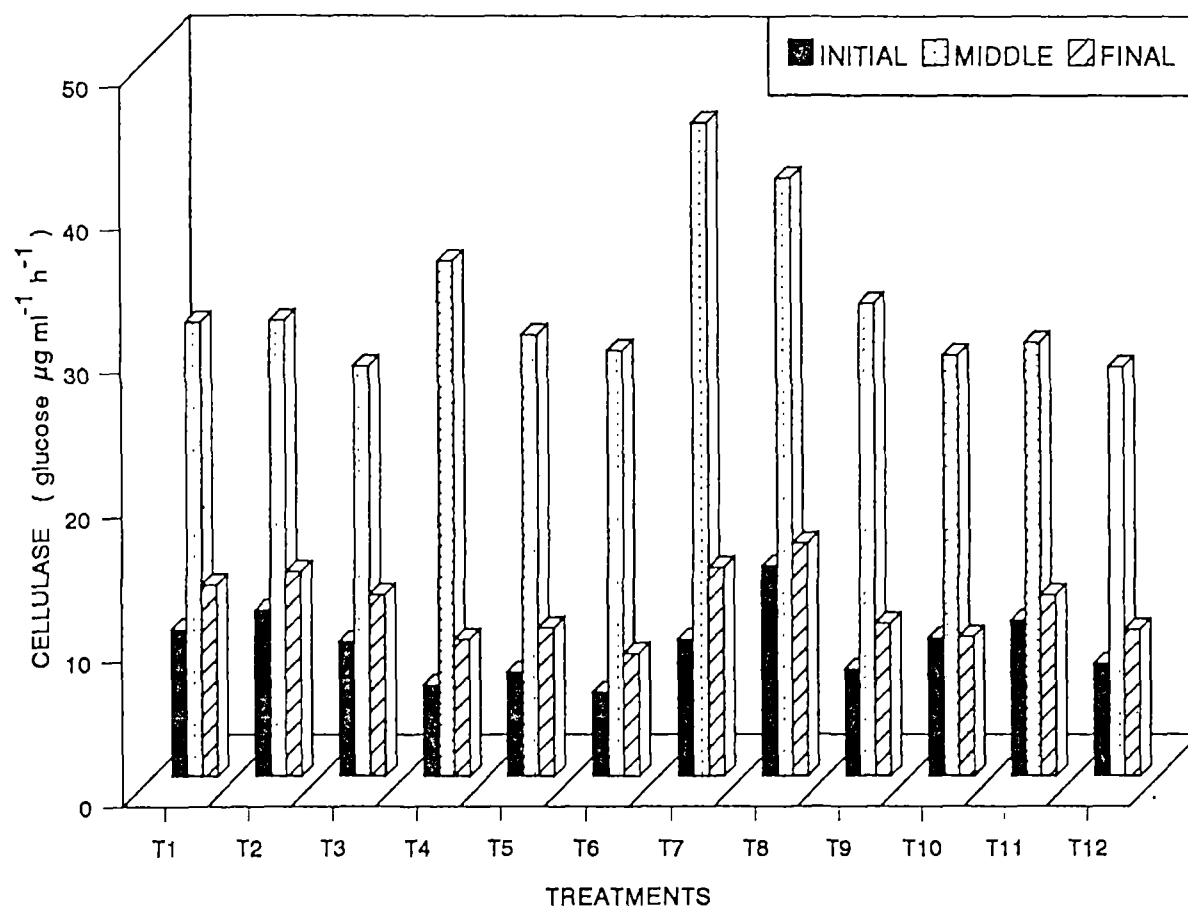


Figure 24. Cellulase activity with different diluents.

The activity of this enzyme was not showing a definite trend with regard to either substrate or diluent in the final stage.

4.5.6.2. β -Glucosidase

The activity of β -glucosidase was noted in all the treatments throughout the experiment. PSD mixed with different diluents showed more β -glucosidase activity in the initial stage. Among the diluents, CPE with various substrates showed higher levels of β -glucosidase (Figure 25).

Irrespective of treatments the β -glucosidase increased upto middle stage and then reduced. In the middle stage the treatments involving PSD showed higher enzyme activity followed by sawdust. In the case of diluents, SPE showed more enzyme followed by CPE and LCE. On the whole, PSD with SPE showed the highest β -glucosidase activity. In the final stage though there was not much variation in β -glucosidase activity, except PSD which registered more enzyme activity.

4.5.7. Enumeration of microbial population

4.5.7.1. Cellulolytic bacteria

The population of cellulolytic bacteria was more in PSD and cowdung (Table 25) in the initial stage.

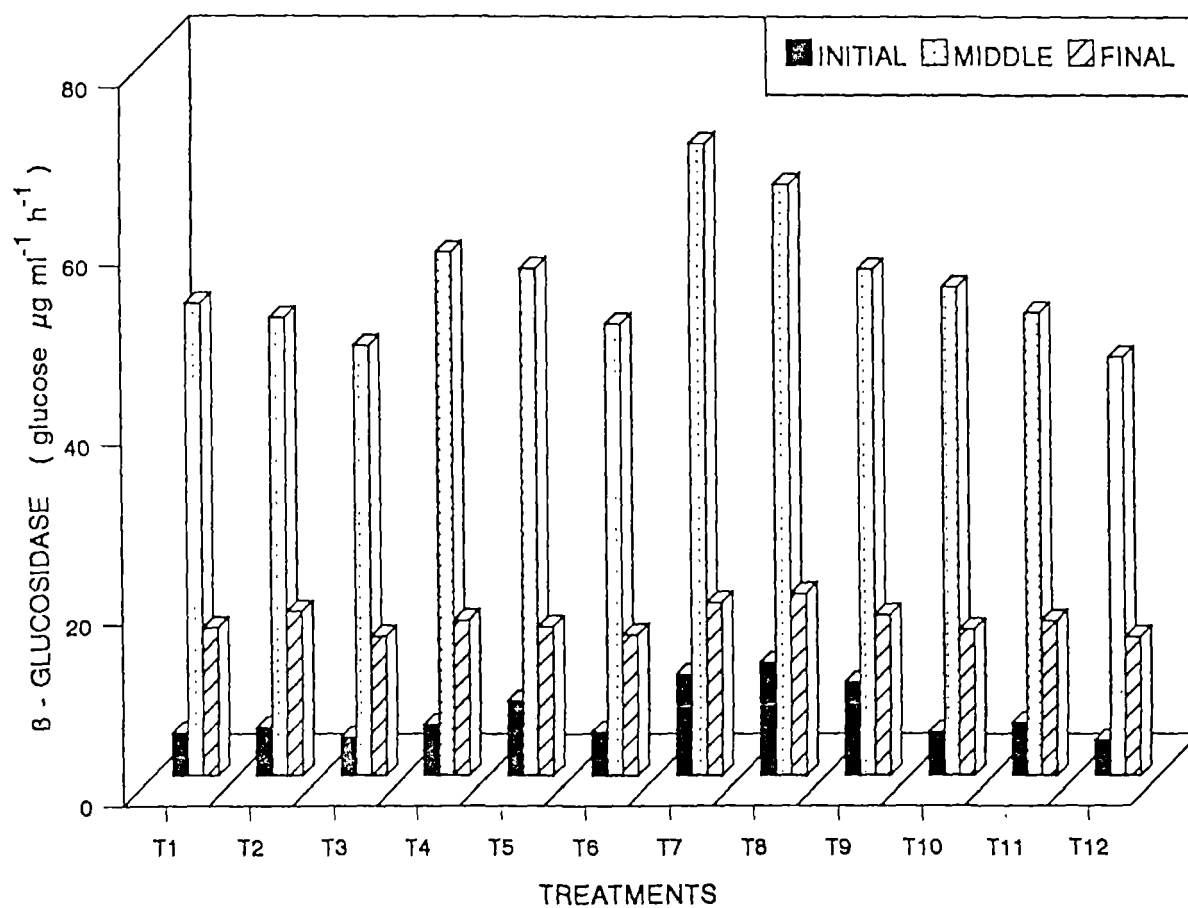


Figure 25. β -Glucosidase activity with different diluents.

Table 25. Population of cellulolytic bacteria with different diluents ($\times 10^4 \text{ g}^{-1}$)

Substrate	Initial					Middle					Final				
	-----					-----					-----				
	Diluents					Diluents					Diluents				
	SPE	CPE	LCE	Mean		SPE	CPE	LCE	Mean		SPE	CPE	LCE	Mean	
CW	14.60	16.80	15.30	15.56		38.50	36.40	32.30	35.73		10.50	9.50	9.80	9.93	
SD	12.30	14.70	12.80	13.26		47.20	45.60	37.40	43.40		11.20	12.90	10.80	11.63	
PSD	19.20	20.50	19.80	19.83		52.50	50.20	45.60	49.76		29.30	31.60	28.30	29.73	
CD	18.40	21.80	19.20	19.80		39.30	36.40	36.70	37.46		27.60	36.70	28.40	30.90	
Mean	16.12	18.45	16.77			44.37	42.15	38.25			19.65	22.67	19.32		
CD for substrate	(P = 0.05)	-	0.98	CD (P = 0.05)	substrate	-	1.65	CD (P = 0.05)	substrate	-	0.94				
CD for diluent	(P = 0.05)	-	0.85	CD (P = 0.05)	diluent	-	1.42	CD (P = 0.05)	diluent	-	0.81				
CD for interaction	(P = 0.05)	-	1.70	CD (P = 0.05)	interaction	-	2.85	CD (P = 0.05)	interaction	-	1.63				

Among the diluents, CPE registered more population of cellulolytic bacteria during the initial stage. The population of this group of bacteria increased upto the middle stage and then decreased. In the middle stage also the PSD harboured higher cellulolytic bacteria followed by sawdust, cowdung and CW. With regard to diluents the cellulolytic bacterial count was more in SPE followed by CPE and LCE incorporated treatments. The highest population of cellulolytic bacteria was observed in PSD diluted with SPE.

In the final stage also PSD and cowdung contained more of cellulolytic bacteria.

4.5.7.2. Acid forming bacteria

In the initial stage, the population of acid forming bacteria was more in the treatments containing cowdung and different diluents. Regarding diluents, LCE with all the four substrates harboured more acid forming bacteria followed by SPE and CPE (Table 26). Acid forming bacteria increased upto middle stage and then reduced in all the treatments. PSD with all the diluents showed higher population of acid forming bacteria in the middle stage followed by sawdust, cowdung and CW. SPE and CPE also contained more acid forming bacteria in the middle stage.

Table 26. Population of acid forming bacteria with different diluents ($\times 10^4 \text{ g}^{-1}$)

Substrate	Initial				Middle				Final			
	-----				-----				-----			
	SPE	CPE	LCE	Mean	SPE	CPE	LCE	Mean	SPE	CPE	LCE	Mean
Diluents												
CW	23.80	21.30	24.20	23.10	31.70	36.20	26.30	31.40	18.80	17.90	18.50	18.40
SD	23.40	22.60	24.30	23.43	33.70	31.30	28.80	31.26	18.20	19.50	21.60	19.76
PSD	22.40	20.20	25.30	22.63	42.70	38.40	32.60	37.90	20.66	18.30	20.70	19.88
CD	26.40	24.70	27.30	26.13	30.20	29.30	28.60	29.36	19.60	18.70	20.70	19.66
Mean	24.00	22.20	25.27		34.58	33.80	29.08		19.31	18.60	20.37	
CD for substrate	(P = 0.05)	-	0.91	CD (P = 0.05)	substrate	-	1.44	CD (P = 0.05)	substrate	-	1.29	-
CD for diluent	(P = 0.05)	-	0.79	CD (P = 0.05)	diluent	-	1.24	CD (P = 0.05)	diluent	-	1.12	-
CD for interaction	(P = 0.05)	-	1.58	CD (P = 0.05)	interaction	-	2.49	CD (P = 0.05)	interaction	-	2.24	-

Among all the treatments the treatments involving PSD and SPE harboured the highest population of acid forming bacteria in the middle stage. At the end of the experiment there was not appreciable difference in acid producing bacterial population among the various treatments.

4.5.7.3. Lipolytic bacteria

Considerable variation was noticed in the population of lipolytic bacteria with various treatments in the initial, middle and final stages of the methanogenesis. Both PSD and cowdung contained higher population in the initial stage. Regarding diluents, LCE harboured more lipolytic bacteria than the other two treatments. As the fermentation progressed, the population of lipolytic bacteria increased upto middle stage and then reduced (Table 27).

In the middle stage, both SPE and CPE contained more lipolytic bacterial population than LCE. Among the substrates, CW and PSD, contained more population of this bacteria. When combinations are considered the SPE with PSD carried more lipolytic bacteria. During the final stage the difference in various treatments was not significant.

Table 27. Population of lipolytic bacteria with different diluents ($\times 10^3 \text{ g}^{-1}$)

Substrate	Initial				Middle				Final			
	-----				-----				-----			
	Diluents				Diluents				Diluents			
	SPE	CPE	LCE	Mean	SPE	CPE	LCE	Mean	SPE	CPE	LCE	Mean
CW	11.50	10.80	12.80	11.70	24.20	23.40	21.40	23.00	16.50	17.10	16.70	16.76
SD	9.80	9.50	10.30	9.86	22.60	21.20	20.10	21.30	15.70	14.80	16.00	15.50
PSD	13.10	12.60	14.20	13.30	25.20	24.30	20.40	23.30	16.30	15.80	17.60	16.56
CD	12.60	11.20	13.40	12.40	22.30	20.10	18.50	20.30	16.80	15.60	17.40	16.60
Mean	11.75	11.02	12.67		23.57	22.25	20.10		16.32	15.82	16.92	
CD for substrate	(P = 0.05)	-	0.90		CD (P = 0.05)	substrate	-	1.42	CD (P = 0.05)	substrate	-	1.17
CD for diluent	(P = 0.05)	-	0.78		CD (P = 0.05)	diluent	-	1.23	CD (P = 0.05)	diluent	-	1.01
CD for interaction	(P = 0.05)	-	1.57		CD (P = 0.05)	interaction	-	2.46	CD (P = 0.05)	interaction	-	2.03

4.5.7.4. Proteolytic bacteria

In the initial stage of the experiment the population of proteolytic bacteria greatly depended on the substrate and diluent. PSD showed maximum population followed by cowdung (Table 28). With regard to diluents, LCE upon incorporation with different solids was having more lipolytic bacteria followed by SPE and CPE.

The proteolytic bacterial population in all the treatments increased upto middle stage and then reduced. In the middle stage, bacterial count was higher in PSD followed by cowdung, sawdust and CW. The trend in bacterial counts noted in different diluents in the initial stage was maintained throughout the experiment. The maximum population of proteolytic bacteria was in the sawdust-SPE combination in the middle stage.

4.5.7.5. Methanogenic bacteria

The population of methanogenic bacteria was greatly influenced by the type of substrates as well as the diluents used. Maximum population was recorded in PSD followed by sawdust, cowdung and CW. Among the diluents both SPE and CPE contained higher population of methanogenic bacteria (Table 29). PSD in combination with SPE and CPE registered higher population of methanogens. This trend was maintained throughout the experiment.

Table 28. Population of proteolytic bacteria with different diluents ($\times 10^4 \text{ g}^{-1}$)

Substrate	Initial			Middle			Final		
	-----			-----			-----		
				Diluents					
	SPE	CPE	LCE	Mean	SPE	CPE	LCE	Mean	
CW	28.40	27.30	30.70	28.80	40.30	38.40	42.60	40.43	20.20 19.20 29.30 22.90
SD	25.30	24.60	26.20	25.36	56.40	45.70	42.40	48.16	21.20 20.80 23.50 21.83
PSD	37.50	35.20	40.60	37.76	55.30	54.40	52.40	54.03	20.40 19.50 23.50 21.13
CD	35.40	33.20	38.40	35.66	48.70	46.20	56.70	50.53	26.20 24.30 23.20 24.56
Mean	31.65	30.07	33.97		50.17	46.17	48.52		22.00 20.95 24.87
CD for substrate	(P = 0.05)	-	0.96		CD (P = 0.05)	substrate	-	1.22	CD (P = 0.05) substrate - 1.64
CD for diluent	(P = 0.05)	-	0.83		CD (P = 0.05)	diluent	-	1.05	CD (P = 0.05) diluent - 1.42
CD for interaction	(P = 0.05)	-	1.67		CD (P = 0.05)	interaction	-	2.11	CD (P = 0.05) interaction - 2.84

Table 29. Population of methanogenic bacteria with different diluents ($\times 10^4 \text{ g}^{-1}$)

Substrate	Initial				Middle				Final			
	-----				-----				-----			
					Diluents							
	SPE	CPE	LCE	Mean	SPE	CPE	LCE	Mean	SPE	CPE	LCE	Mean
CW	9.80	10.40	9.20	9.80	82.60	75.70	72.40	76.90	62.80	65.60	50.50	59.63
SD	18.50	20.30	18.20	19.00	108.30	95.80	90.40	98.16	92.50	86.20	82.50	87.06
PSD	23.80	24.30	19.20	22.43	136.30	112.50	95.10	114.63	118.20	96.50	85.20	99.96
CD	11.30	12.50	11.00	11.60	100.50	98.30	92.10	96.96	65.80	62.50	59.70	62.66
Mean	15.85	16.87	14.40		106.92	95.57	87.50		84.82	77.70	69.47	
CD for substrate	(P = 0.05)	-	0.93	CD (P = 0.05)	substrate	-	1.35	CD (P = 0.05)	substrate	-	1.66	-
CD for diluent	(P = 0.05)	-	0.80	CD (P = 0.05)	inoculum	-	1.17	CD (P = 0.05)	diluent	-	1.44	-
CD for interaction	(P = 0.05)	-	1.61	CD (P = 0.05)	interaction	-	2.34	CD (P = 0.05)	interaction	-	2.88	-

The population of methanogens increased upto middle stage and then decreased. PSD with SPE contained comparatively more population of this bacteria than other combinations in the middle and final stage. Considerable number of methanogenic bacteria was maintained in all the treatments upto the end of the experiment.

4.5.8. Biogas production

Predigested sawdust recorded maximum biogas followed by sawdust (Figure 26). Both CW and cowdung produced the same amount of biogas. Among the diluents, SPE showed more yield of biogas followed by CPE and LCE. PSD with SPE registered maximum biogas followed by the same substrate with CPE and LCE (Figure 27). The gas production was at peak during third to seventh week of digestion (Figure 28).

In general, PSD generated biogas with higher methane content followed by sawdust (Figure 29). Both CW and cowdung were inferior to sawdust with and without pretreatment. Among the three diluents, SPE caused more methane generation followed by CPE and LCE. The highest methane content was recorded when PSD was mixed with SPE.

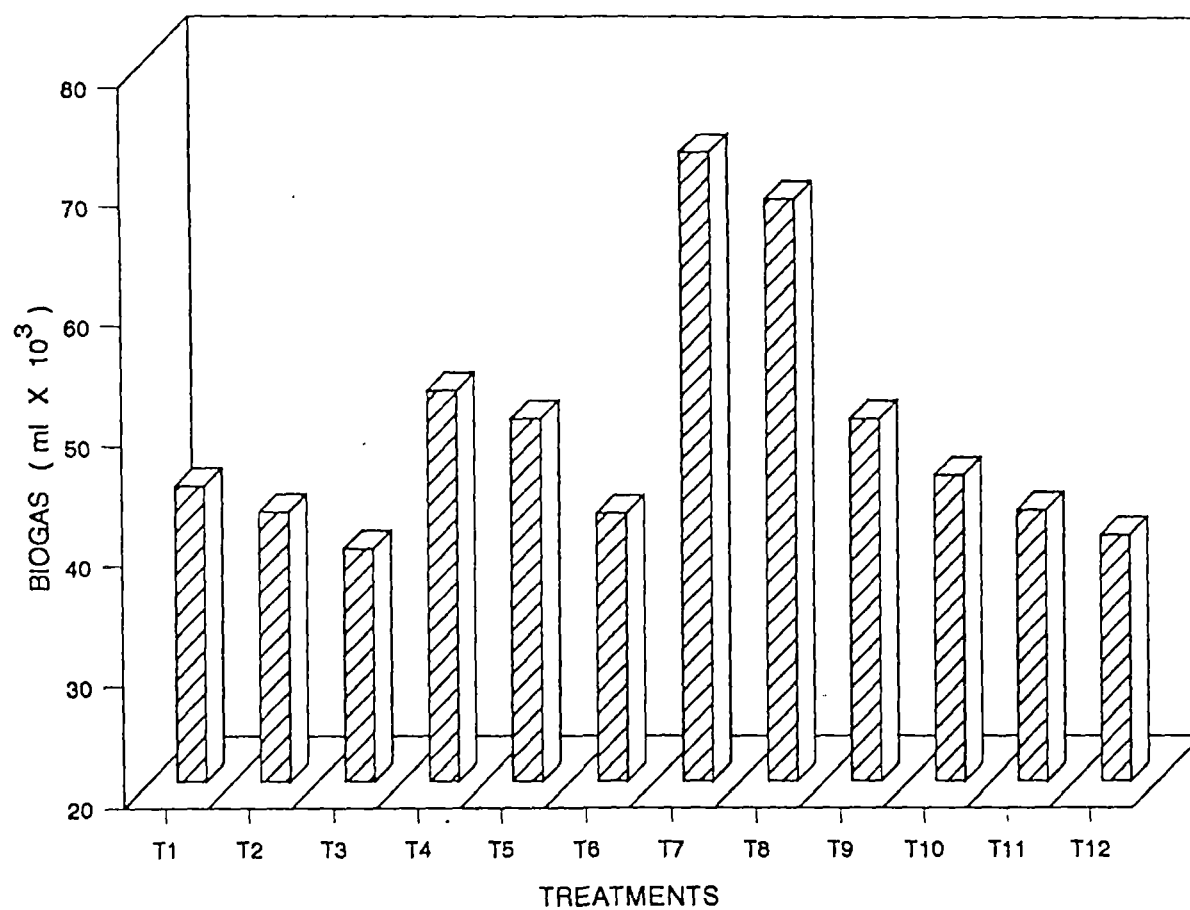


Figure 26. Biogas production in treatments with different diluents.

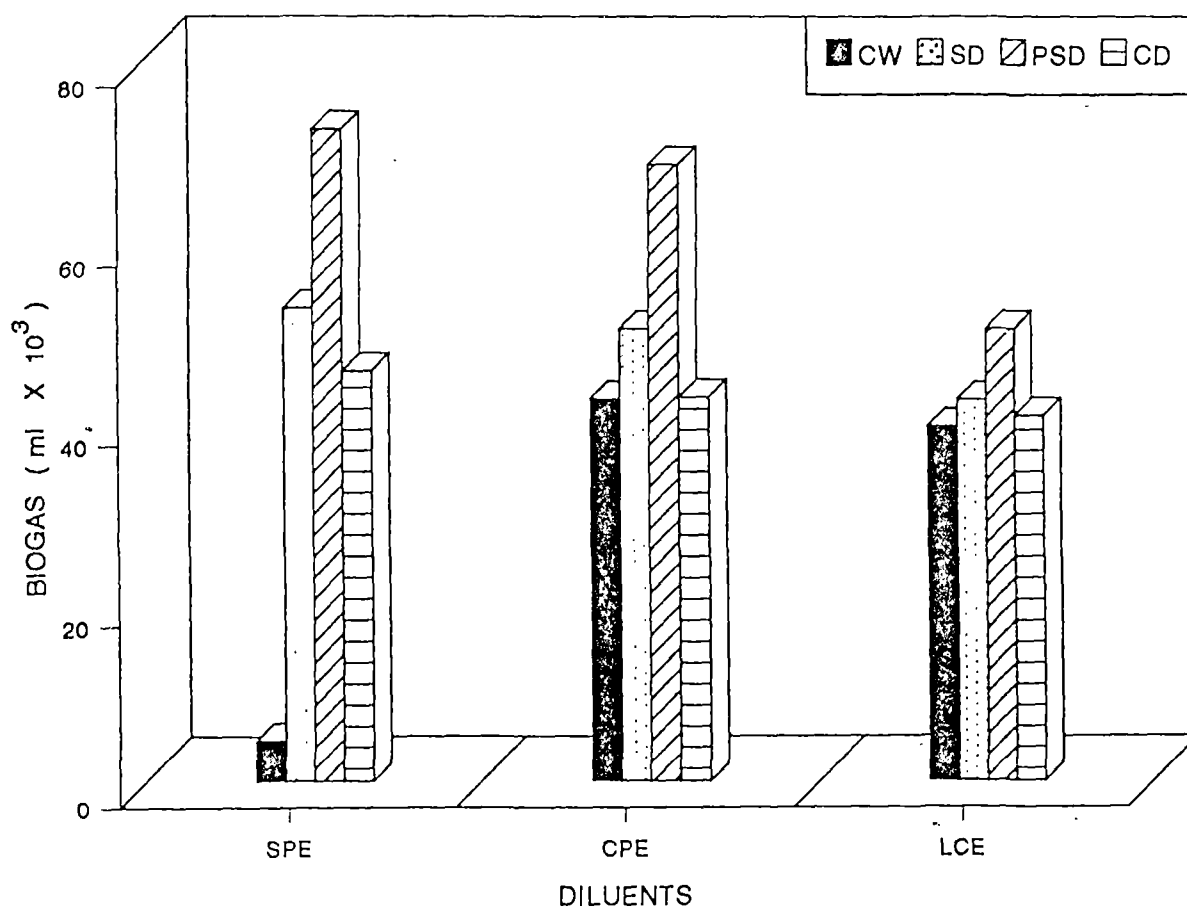


Figure 27. Biogas production with various diluents and substrates.

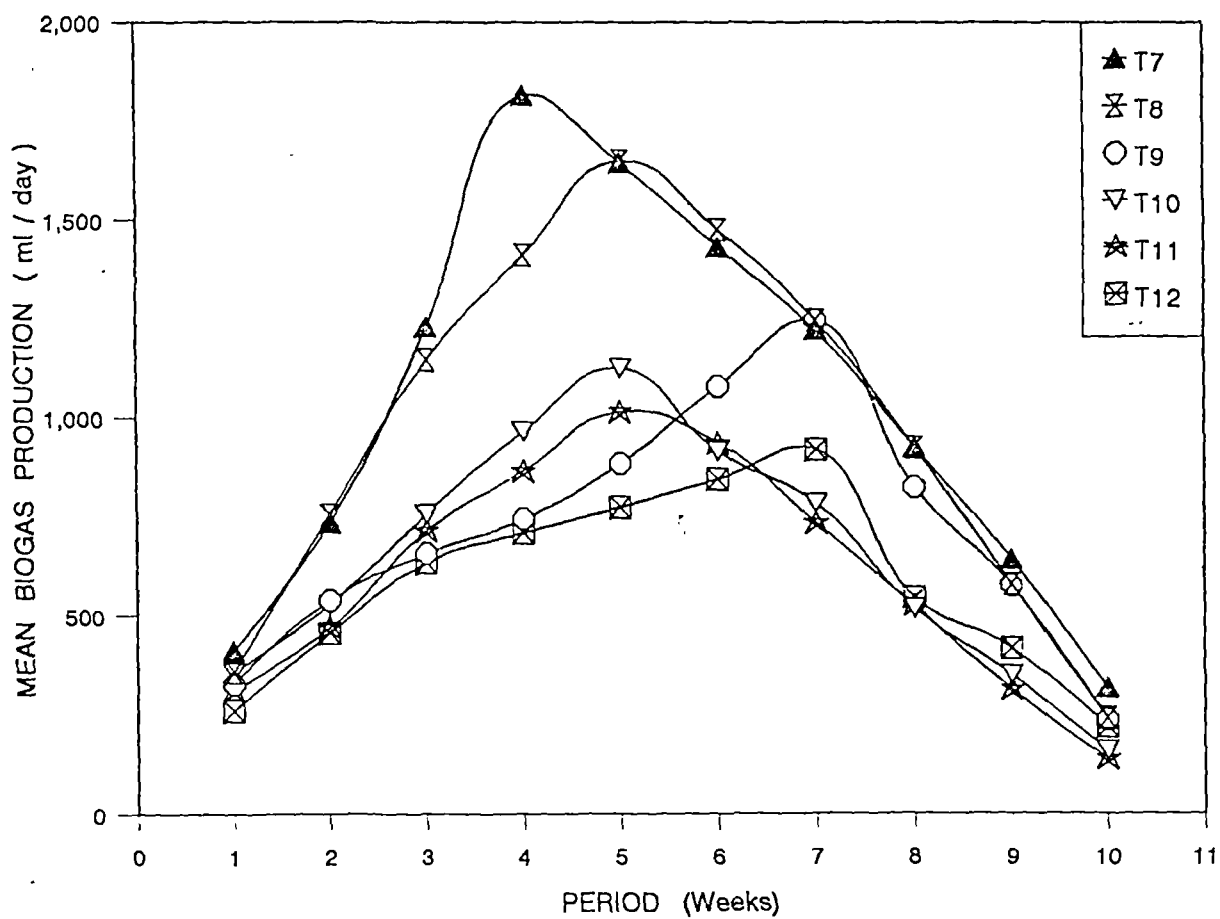
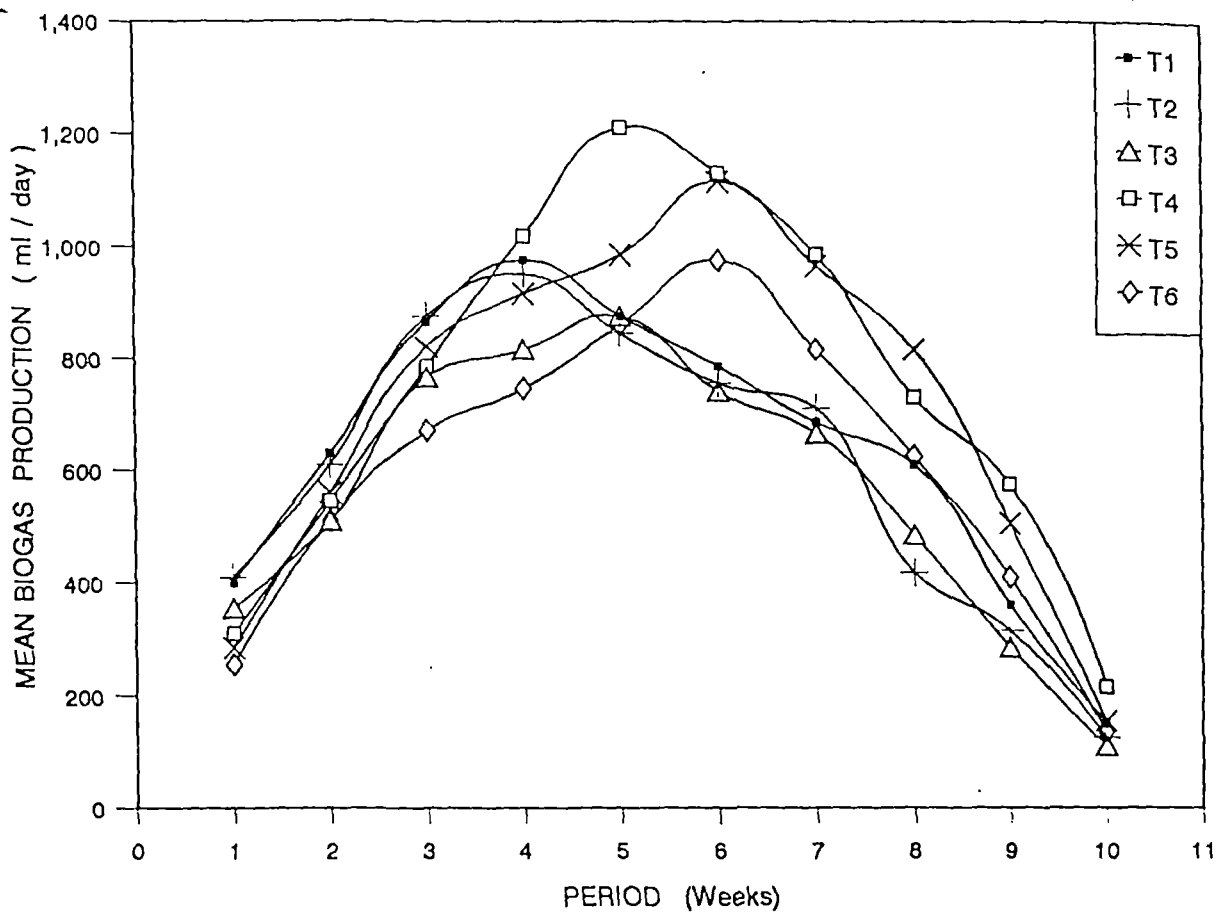


Figure 28. Influence of diluents on gas output.

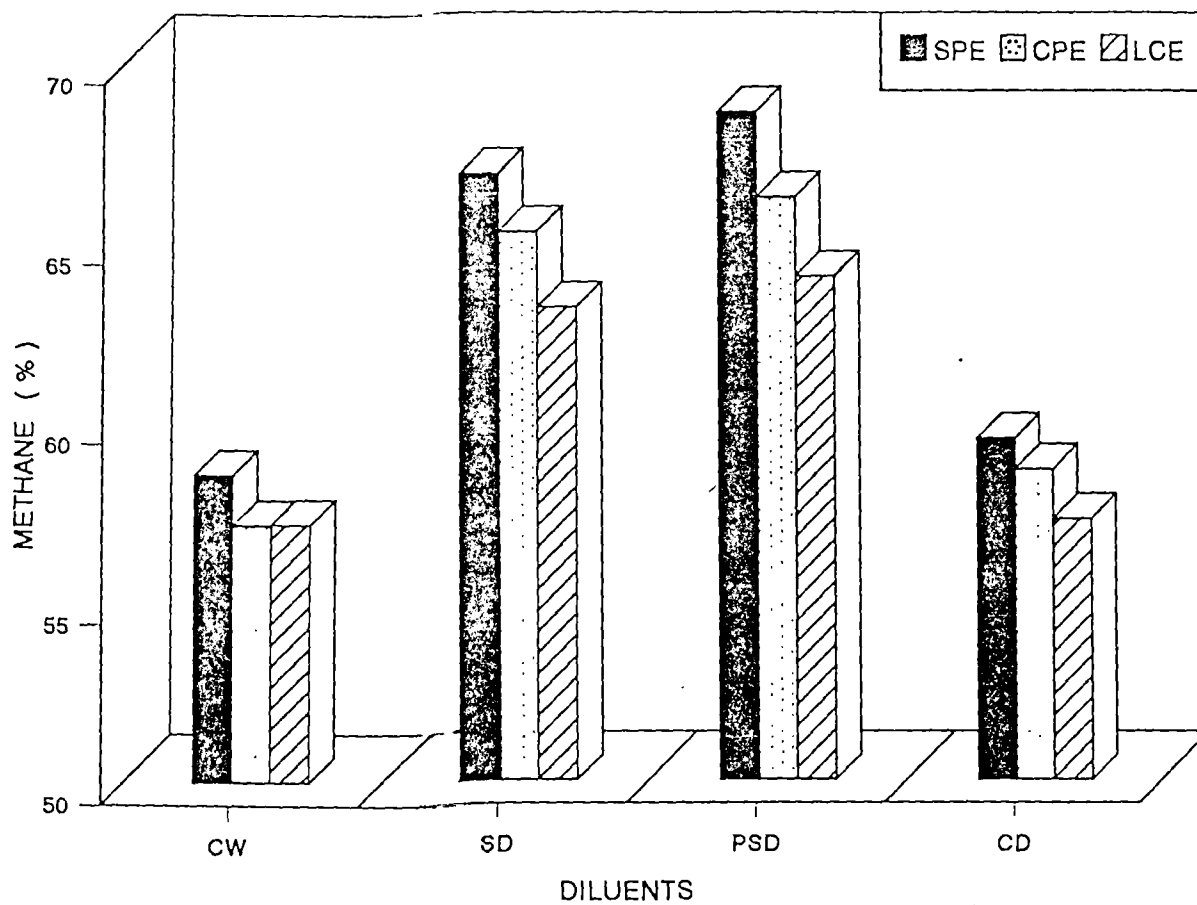


Figure 29. Methane content in the biogas with different diluents.

Table 30. Manurial value of biodigested slurry (%)

Treatments	N	P	K
T ₁	1.98	0.92	0.78
T ₂	1.60	0.58	0.42
T ₃	1.86	0.78	0.62
T ₄	1.82	0.80	0.68
T ₅	1.26	0.58	0.45
T ₆	1.43	0.62	0.52
T ₇	2.26	0.92	0.78
T ₈	1.62	0.66	0.52
T ₉	1.92	0.82	0.65
T ₁₀	1.95	0.90	0.76
T ₁₁	1.62	0.60	0.55
T ₁₂	1.80	0.70	0.62

4.5.9. Manurial values of biodigested slurry

The biodigested slurry obtained at the end of the experiment contained the major plant nutrients viz., nitrogen, phosphorous and potassium. The nutrient value of the PSD slurry was more than the other three substrates. The spent liquor of PSD diluted with SPE contained 2.26 per cent N, 0.92 per cent P and 0.78 per cent K (Table 30). The sawdust diluted with the different effluents recorded low manurial values.

CHAPTER 5

DISCUSSION

DISCUSSION

Agro-based industries are one of the largest contributors to industrial pollution and amongst this, the natural rubber processing industries play a significant role in polluting water bodies in Kerala state. Out of five hundred thousand hectares of rubber plantation in India, Kerala state alone has 75.3 per cent. The area under rubber is on the increase (Appendix 1). By the year AD 2000, the demand for natural rubber is estimated to be 6.8 lakh tonnes which should be met either by extending the area or increasing the productivity. The increase in production would correspondingly lead to the increase in the number and size of processing factories and consequently lead to generation of larger quantum of effluent. The survey on the rubber processing factories suggests that there are large number of RSS factories, besides 78 crepe, 31 crumb and 62 latex concentrate factories, generating 7932892 m³ effluent, while producing a total of 3.9 lakh tonnes of rubber during 1992-93 (Appendix 2).

5.1. Physicochemical and microbiological properties of liquid wastes from rubber processing factories

Proteins, sugars, lipids, carotenoids, inorganic and organic solids of latex, various chemicals added for

processing and water used for processing constitute the effluent from natural rubber processing factories [Ponniah et al., 1975]. These nutrient rich effluents containing fairly large amount of dissolved organic and inorganic solids, besides suspended solid particles support a large number of general and indicative bacteria [John et al., 1974; Dolmat et al., 1979]. The fast growth of microorganisms causes depletion of the oxygen content in the effluent and receiving water bodies.

The present study revealed that the latex concentrate effluent (LCE) contained more suspended solids, dissolved solids, total solids (TS) and volatile solids (VS) which resulted in substantial growth of microorganisms resulting in high BOD and COD. Similar observations were made by Muthurajah et al. (1973); Sethu et al. (1977); Dolmat (1978) and Mathew et al. (1986, 1988). They have confirmed that dissolved solids play a significant role in the salinity of the effluent. The high BOD and COD values of the LCE indicate that the TS in the effluents are mainly organic, especially fine particles of rubber hydrocarbon, with high oxygen requirements for their oxidation. Muthurajah et al. (1973) attributed the enhanced BOD and COD in LCE to the high concentration of proteins, sugars, lipids, inorganic and organic salts in addition to high ammonia nitrogen.

The LCE is highly acidic. The addition of sulphuric acid for recovery of rubber by its coagulation from the skim portion of latex resulted in the reduction of pH of the effluent [Rubber Research Institute of Malaysia, 1974]. Sulphuric acid also contributes to the sulphate content of the effluent. Phosphate content in the effluent is also high due to the addition of diammonium phosphate in the latex. Highly acidic effluent loaded with salts adversely affect plant growth and causes corrosion of structures in river [Yapa, 1984].

The LCE contains large amount of total and ammoniacal nitrogen. The addition of substantial quantity of ammonia in the preservation of latex contributes to this. Ammonia is toxic to fishes and also encourages algal bloom. So the discharge of such effluents to water-ways is discouraged [Middleton, 1977].

It is found that the population of different groups of microorganisms in the LCE was less when compared to that in the effluent from other processes. Pelczar et al. (1993) recorded low counts of bacteria in nutrient rich medium and which he attributed to low pH.

Pollution by sheet processing effluent (SPE) was less than LCE, but was more than other effluents. Even though the effluents from large scale RSS factories adversely affect the environment, small units spread out in the

rubber tract do not cause noticeable impact on the quality of the environment. The pollution by large units, however, should be treated on par with that of latex concentrate factories.

During RSS processing, addition of formic or acetic acid causes reduction in pH of the effluent. This effluent causes very high levels of BOD and COD, indicating that the TS are mainly of organics with high oxygen requirements for their oxidation. SPE contained all the different groups of bacteria in large number. The yeast population in the effluent was very high compared to other effluents. Enhanced microbial activity including yeasts in SPE has already been reported [Mathew et al., 1987]. The optimum pH required by yeasts for its growth is 4 to 5 and obviously the RSS effluent favoured its proliferation [Rubber Research Institute of Malaysia, 1974].

Though the pollution load in crumb processing effluent (CPE) and crepe rubber effluent (CRE) was comparatively less, the environmental impact would be much higher due to the discharges of high volume. TSR and CR processing factories generate 26.3 and 24.5 l of effluent respectively per kg of processed rubber, which ultimately reach and pollute water bodies [John et al., 1974].

Effluent from agroindustries is reported to contain considerable quantities of suspended and dissolved solids as well as organic acids, which are good substrates for biomethanation [Knol et al., 1978; Balasubramanya et al., 1986; Wong and Cheung 1989; Mahadevaswamy and Venkataraman, 1990 and Kalia et al., 1992]. The present study revealed that the wastes from natural rubber and rubber wood processing contain high levels of valuable substrates for biogenesis of methane.

5.2. Physicochemical properties of solid wastes

The quantity of TS, VS, carbon, nitrogen, phosphorous, potassium, cellulose, hemicellulose and lignin was high in the solid wastes from rubber based industries. The C:N ratio of optimum range in these solid wastes suggests their usefulness as a raw material for methane production [Fujita et al., 1980]. Crumb waste (CW) is mainly composed of bark remnants rich in cellulose and such sludge from crumb factories with C:N ratio 33:1 was ideal for biogas production as reported by Singh (1974), Fujita et al. (1980), Hashimoto (1981), Pathak et al. (1985) and Abbasi et al. (1990). Low nitrogen content in sawdust is overcome by addition of urea or bio-degradation of lignocellulosic materials by cellulolytic fungi. The presence of organic and inorganic nutrients at optimum level makes sawdust biodigestible,

even though the nitrogen level is poor. The degradation by cellulolytic fungi brought down the C:N ratio from 90:1 to 40:1 which is within the minimum required limit for biomethanation [De Renzo, 1977].

5.3. Experiment 1. Effect of solid concentration on biogas generation

Biogas production from a given substrate is influenced by its nature and composition, available nutrients, pH, temperature, inoculum and diluents used. The result of the present study using solid wastes and sawdust clearly indicated that biogas could be produced from the solid wastes of rubber processing factories and from sawdust or predigested sawdust (PSD).

Solid wastes like CW, sawdust and PSD contain mainly cellulose, hemicellulose, lignin and minerals like nitrogen, phosphorus and potassium [Tan and Stott, 1987]. The C:N ratio in solid substrates other than sawdust is optimum for biogas production.

Biogas production from organic wastes is the result of a series of reactions brought out by a consortium of bacteria [Bryant, 1976, 1979] under anaerobic conditions, at the expense of mainly lignocellulosic wastes. This process is biphasic involving hydrolysis and acidogenesis by a group of bacteria (fermentative) followed by

methanogenesis by another group. The organic acids used for coagulation of latex [Thomas et al., 1980], have a positive influence on biogas production from solid and liquid wastes of rubber processing factories by entering in the first phase. Singh et al. (1985a) observed that biogas production demanded an improvement in the amount of acetate formation or addition. The enhanced biogas production in the wastes of rubber processing factories is obviously due to the extraneous organic acids as well as from the acidogenesis under anaerobic condition.

Solid portion in the biodigestion mixture is the main source of biogas production. Optimum substrate concentration for maximum biogas production depends on many factors like content of carbon, nitrogen, phosphates etc. Depending on these factors, a substrate concentration varying from 7.9 to 20.0 per cent is recommended [Keefer, 1947; Lapp et al., 1975]. Therefore, the effect of the concentrations of solid waste from rubber and rubber wood processing on various parameters which contribute to biomethanation was studied in this experiment.

5.3.1. Total and volatile solids destruction

Total solid and VS in the digestion mixture are the major factors for the biogas production. The rate of

degradation of TS content of the slurry at 5, 10 and 15 per cent levels of the substrates varied significantly throughout the experiment. It decreased gradually upto the final stage in all the treatments and the extent of reduction varied with the wastes employed. This experiment revealed that the degradation was maximum in PSD at 10 per cent solid concentration. The reduction in the volume of TS coupled with enhanced activity of bacteria and cellulolytic enzymes confirm the role of microorganism in the degradation of organic matter during biomethanation [Kirsch and Sykes, 1971; Barnett et al., 1978; Bousfield et al., 1979; Hills and Roberts, 1981]. Singh et al. (1980, 1982) who emphasised the need for optimum substrate concentration for normal biogas production as observed in the present study.

Depending upon the nature and quantity of raw materials, the solid content varied and this influenced the degradation brought out by microbial activity and gas output. The low rate of degradation of TS in 5 and 15 per cent might be due to non availability of sufficient nutrients and saturation of end products of ligno-cellulosic portion respectively.

The enhanced TS destruction in PSD was due to the action of lignocellulolytic fungi and release of simple sugars [Joseph et al., 1991]. Break down of cellulose and

lignin by the mushroom fungi as well as the enzymes produced by anaerobic bacteria has accelerated degradation. Millet et al. (1975) observed that methane yield from lignocellulose could be enhanced by physicochemical treatment that separates lignin from cellulose.

The percentage of TS reduction was 29.6 in the PSD at 10 per cent solid concentration. Higher the TS destruction more is the biogas generated. Summers and Bousfield (1980) reported that maximum gas yield was obtained with reduction of 36 per cent of TS.

Volatile solids, being easily degradable, positively influence the production of methane. Predigestion of sawdust by P. florida led to maximum VS degradation during methanogenesis. Selective proliferation of microorganisms that could best metabolise the organic fractions in the various wastes incorporated treatment at optimum per cent of substrate could ensure enhanced biogas production. Solid wastes from crumb rubber processing factories as well as cowdung were partially degraded and hence the lesser VS degradation.

Destruction percentage of VS varied from 31.59 to 57.19 and it contributed greatly to biomethanation. Aller (1979) and Pathe et al. (1982) recorded maximum gas yield at 40-64 per cent destruction of volatile solids. In the

present study significant positive correlation was observed between total and volatile solid destruction and gas production. The highest gas production (65585 ml) was recorded in the treatment which showed maximum destruction of TS and VS (29.60 and 57.19 per cent respectively). Similar phenomenon was observed in biogas production using cowdung [Schellenbach, 1980]. Hashimoto and Robinson (1982) obtained higher methane yield when the percentage reduction of TS and VS of straw and manure mixture were 39.81 and 41.40 respectively.

5.3.2. Cellulose, hemicellulose and lignin degradation in relation to biogas generation

The solid wastes of rubber processing factories consist of mainly cellulose and lignin in considerable quantity as they originated from the trunk of rubber trees. The physicochemical properties of lignocellulosic wastes determine the rate of degradation of these compounds as well as biogas production [Sushilkumar and Biswas, 1982]. Both cellulose and hemicellulose as such are highly susceptible to the action of microorganisms. But in nature they exist in a complex with lignin and show varying degree of microbial attack. Therefore, liberation of cellulose and hemicellulose from the lignocellulose moiety either by chemical or enzymatic reactions is reported to have profound influence on the

quality of biogas produced as well as the methane content [Jimenez et al., 1990].

The percentage destruction of cellulose was more in CW, PSD and cowdung. But the lowest rate of cellulose degradation was recorded when sawdust was used as substrate. Even though these values were not statistically significant, the PSD registered the highest percentage of cellulose degradation as well as more biogas production with higher methane content. Winter and Cooney (1980), Jain et al. (1981), Hills (1982) and Hashimoto (1983, 1984) suggested that cellulose degradation is a prime factor in biogas production. The low level of cellulose degradation in sawdust might be due to the ligneous structure shielding the cellulose materials from enzymatic hydrolysis [Han et al., 1975; Badger et al., 1979; Robbins et al., 1979].

The lignocellulolytic fungi belonging to the genera, Pleurotus are capable of degrading lignin and releasing cellulosic components for further microbial attack [Theradimani and Marimuthu, 1991]. The higher cellulose degradation in sawdust predigested with P. florida in the present study confirms the above findings. There is a direct relationship between cellulose decomposition and biogas production. The enhanced gas production at lesser cellulose degradation observed in the case of sawdust,

however, might be due to operation of other mechanisms of biogas generation as outlined by Hansson (1981).

Hemicellulose destruction was more in CW and PSD. Increasing the concentration of slurry upto 10 per cent level correspondingly raised the hemicellulose degradation which is directly proportional to the quantum of biogas produced. Among the different substrates, the maximum biogas production was noticed in the treatment of PSD at 10 per cent concentration. Similar observations were made by many workers [Datta, 1981; Jain et al., 1981; Guo and Mah, 1981] who stressed the importance of degradation of hemicellulose and cellulose in predigested substrates. In the case of PSD, biogas production is directly related to the quantum of hemicellulose degradation. Datta (1981) and Pathe et al. (1982) have reported similar findings. Iannotti et al. (1979) reported that hemicellulose was the single largest compound destroyed (65 per cent) in anaerobic digestion. This was observed to be true for swine waste digestion [Hobson and Shaw, 1971], water hyacinth and bermuda grass digestion [Gosh et al., 1985], suggesting that under mesophilic digestion the hemicellulose is more subjected to microbial enzyme activity than the lignocellulosic fraction.

Lignin is comparatively resistant to the attack of microorganisms [Guo and Mah, 1981] and it also prevent the

degradation of cellulose and hemicellulose and thereby influence the biogas generation. In the present study lignin destruction in all the treatments was less when compared to that of cellulose and hemicellulose. Boruff and Buswell (1934) and Iannotti et al. (1979) reported that the lignin itself is very resistant to the degradation by anaerobic fermentation. The lesser lignin content of PSD, when compared to sawdust, and the percentage of lignin degradation indicated that a portion of lignin was degraded by lignocellulolytic fungi and the left over lignin was comparatively resistant to the enzymatic degradation by anaerobic bacteria. In the anaerobic system, higher biogas production is often associated with low lignin degradation [Datta, 1981; Pathe et al., 1982] as observed in the present study.

5.3.3. Volatile fatty acids

Volatile fatty acids, the precursor for methanogenesis, determine the extent of gas production. When rubber processing waste and cowdung were subjected to methanogenesis, the VFA content in all the treatments increased steadily upto middle stage. However, the level of VFA declined at the final stage. The optimum VFA for maximum biogas production varies with different substrates. Hobson and Shaw (1976) observed that the

growth of M. formicium was not inhibited by concentration upto 10000 mg l^{-1} of acetic or butyric acids. Van Velson and Lettinga (1980) reported that VFA as high as 5000 mg l^{-1} as acetic acid did not inhibit biogas production. In the present study, upto $2118.26 \text{ mg l}^{-1}$ of VFA was recorded which led to enhanced gas output. Singh et al. (1980) stated that an acetate level of 2500-3000 ppm was found to be optimum for maximum production of biogas with 84-87 per cent methane from cattle waste. Both high and low substrate concentrations limit the process of hydrolysis and VFA generation and thereby influence the biogas production.

5.3.4. pH

Action of anaerobic bacteria on organic compounds leads to the production of organic acids which lower the pH values [Jain et al., 1981]. In the present experiment enhanced activity of acid producing bacteria and accumulation of VFA upto the middle stage were observed. The low pH in the middle stage is obviously due to the production of organic acids, the precursors of methane. Methanogenic bacteria utilize the organic acids as energy source and produce methane. This inturn reduce acid concentration and increase the pH. Anaerobic digestion proceeds most optimally between pH 6.6 and 7.6 [McCarty, 1964] and the process stability largely resulted due to

chemical equilibria established between the three primary buffers of VFA, bicarbonate and ammonia [Pohland, 1968]. Either the shift in the buffer action or reduction in organic acids or both is responsible for ascending trend in pH values at the end of the experiment as observed by Albertson (1961). In PSD at 10 per cent level the lowest pH was recorded which indicate that the higher methanogenesis observed was at the expense of organic acids. Such enhanced production of biogas associated with reduction in pH was recorded by Ghose and Das (1982) and Lapp et al. (1982).

5.3.5. Microbiology and biochemistry of biogas production

Methanogenesis involves a series of microbial activity that convert solid and liquid substrates into gaseous form. Cellulolytic bacteria are responsible for the break down of cellulosic compounds to simple sugars and play a major role in the initiation of biogenesis of methane. The population of cellulolytic bacteria was same in all the treatments during the onset of the experiment, later it increased upto the middle stage and reduced thereafter. The PSD (10%), in general, showed higher counts of cellulolytic bacteria. The population of cellulolytic bacteria in digestion mixture during the middle and final stage was directly proportional to the volume of gas

produced. A similar trend in the cellulolytic bacterial population was reported in the biodigestion of animal waste [National Academy of Sciences, 1981].

The activity of cellulase and β -glucosidase also increased upto middle stage and thereafter subsided. Both the enzymes increased with the increase in the substrate concentration.

More gas was generated from PSD which harboured more cellulolytic bacteria and enzymes. While studying the microbial ecology during anaerobic fermentation of cellulosic material, Chhonkar (1981a, 1981b) and Singh et al. (1982) noted such phenomenon and stressed the importance of cellulolytic bacteria and their enzymes in the initiation of methanogenesis.

The cellulose content was more in all the three concentrations of sawdust. But they recorded lesser population of cellulolytic bacteria than PSD. Materials with high lignin content are normally poor substrate for cellulolytic bacteria [Kalra and Panwar, 1986]. However, there was not much difference in the cellulase content of sawdust and PSD. It appears that lignin in the lignocellulose matrix affects only the growth of cellulolytic bacteria, but not the cellulase activity. The β -glucosidase activity in sawdust was lesser than that in PSD. Presumably it is due to selective inhibition

of this fraction of cellulase by free lignin or its break down products, the phenolic compounds [Chahal, 1982].

The exact structure of the lignin-cellulose complex is still not clearly understood. There appears to be very few covalent bonds between lignin and cellulose. There are, however, ester bonds between the uronic acids of hemicellulose and the phenol groups of lignin. Lignin appears to surround cellulose fibre as a three dimensional net. It may be this net that inhibits enzymatic degradation of the cellulose fraction of the complex [Bellamy, 1974]. Based on the material employed, cellulolytic bacterial population varies and this influences biogenesis of methane [Hobson and Shaw, 1974]. The lesser gas production in CW and cowdung can be attributed to the low levels of cellulose, cellulase and cellulolytic bacteria contained in these.

The major pathway of biogas production is through volatile organic acids like propionic, butyric, valeric and lactic [Hansson, 1981]. The acid producing bacteria, metabolizes the simple sugars and proteins to VFA. In the present study considerable variation was noticed in the acid producing bacterial population with the highest in PSD. Differences in the inherent chemical constitution of the substrates cause variation in acid forming bacteria [Hobson, 1982]. The organic acids in the

digestion mixture are intermediary products of fermentation [Toerien et al., 1967] and methanogenesis. In all the experiments, the treatment which recorded higher counts of acid producing bacteria also recorded higher VFA concentration and gas output. Deshpande et al. (1979) reported that increased acid producing bacterial activity led to increased gas generation. The acid producing bacteria are capable of tolerating very high volatile acid concentration and functioning effectively. VFA content during middle stage of methanogenesis was more in 10 per cent substrate concentration in all treatments except cowdung. It indicates that 10 per cent substrate concentration is ideal for optimum VFA production. Fast depletion of VFA during the final stage could be attributed to their utilization for methanogenesis.

Significant variation in the proteolytic bacterial population was observed in the slurry of different treatments. The difference in the population is due to the variations in the composition of waste materials employed [National Academy of Sciences, 1981]. The proteolytic bacteria, while depolymerising the proteinaceous compounds to meet the nitrogen demand cause fermentation in the anaerobic digester. Higher proteolytic bacterial population was recorded in the

middle stage especially at higher substrate concentration and thereafter the population decreased gradually. Much variations were encountered by Hobson (1973) in the proteolytic bacterial population with changes in the quality and quantity of input materials used, initial level of starter inoculum etc. as observed in the present study.

Plant materials contain varying quantities of lipids and they also undergo degradation and contribute to biogas production [Singh and Tauro, 1987]. In the present study, all the treatments contained lipolytic bacteria and their population increased upto the middle stage and thereafter, reduced except in cowdung. The lipolytic bacterial population increased with the increase in the concentration of the substrates. The variation in population of this group of bacteria in the different treatments might have depended on lipid content of feed stocks employed, as recorded by Novak and Carlson (1969). The long chain fatty acids formed from lipids may be inhibitory or stimulatory to methanogenic and fermentative bacteria, depending on acid concentration [Prins et al., 1972; Henderson, 1973].

Lipolytic bacteria were abundant in PSD treatment. These bacteria are capable of fermenting glycerol ester [Hobson, 1973]. It is probable that the fatty acids

liberated during lipolysis by various lipolytic organisms might have been oxidized via, β -oxidation to acetic acid, carbon dioxide and methane. The variation in lipolytic bacterial population in cowdung may be due to difference in the effect of long chain fatty acids formed from lipids, which inhibit anaerobic digestion [McCarty, 1964].

The final stage of biogas production is effected by an array of methanogenic bacteria. Obviously the rate of methanogenesis is proportional to their population. The population of methanogenic bacteria varied in different substrates and concentrations. Among the treatments the methanogenic population was maximum in the middle stage and thereafter declined. The rate of increase of the methanogens was more in the treatments involving PSD and this substrate at 10 per cent level registered the highest population throughout the experiment. Higher level of gas production is attributable to the relatively higher cell densities indicating the significant role of methanogenic bacteria. Significant positive correlation between methanogenic bacterial population and biogas production as reported by Young and McCarty (1969) is evidenced in the present investigation.

5.3.6. Biogas production

The end product of methanogenesis is a mixture of methane, carbon dioxide, carbon monoxide and traces of hydrogen sulphide. The factors which influence the microbes and their enzyme activities determine quantitative and qualitative nature of biogas. In the present study, biogas production was more in PSD. PSD as well as other substrates recorded more biogas at 10 per cent substrate concentration. The same concentration favourably influenced degradation of solids and production of acids. Considering all the factors of methanogenesis discussed elsewhere, 10 per cent substrate concentration is found to be ideal for biogas production from the solid wastes from rubber based industries. The present study also stresses the need for predigestion of rubber wood sawdust, a lignocellulosic waste, with white rot saprophytic fungi like Pleurotus sp.. Lignin adversely affect biodegradation [Bellamy, 1974; Hills, 1979]. According to Robins et al. (1979) approximately 44 per cent of fermentable material is shielded by lignin. More biogas production in PSD is mainly due to delignification by P. florida before using it as substrate for biogas production.

Biogas production attained a maximum level during the fourth and sixth week in treatments involving sawdust at

different levels while in CW and cowdung the peak gas production was earlier. CW and cowdung are subjected to the action of anaerobic bacteria before feeding the digester and hence the early biogas production. The delayed gas production in sawdust treatments implies that such substances offer resistance to microbial degradation. Delay in attaining peak level of biogas production in PSD clearly indicates that it is due to slow build up of microbes, capable of degrading cellulose, hemicellulose, etc. [Khan, 1977]. The early production of biogas at 10 per cent substrate concentration indicate the presence of easily degradable carbohydrates. At 15 per cent, the end product might have suppressed the enzyme activity.

Methane content in the biogas is an important factor that determines the combustion value. In the present study PSD at 10 per cent level recorded a higher level of methane indicating its superiority over the biogas produced in other substrates. This finding is comparable with the observations of Kalia (1990) and Singh et al. (1983) who proved higher methane generation in predigested agricultural wastes. Such an increase in methane content could also be attributed to the presence of optimum level of organic acids [Hansson, 1981]:

5.4. Experiment 2. Effect of inocula on biogas generation

The quality and quantity of inoculum in the slurry of biogas plant are the factors that determine the biogas production. The type of microorganisms present in the inoculum has profound influence on biodegradation, acidogenesis and methanogenesis. The volume of the inoculum needed is based on the number of different groups of anaerobic bacteria present. In the present study, biodigested slurry (BS), sewage sludge (SS) and crumb sludge (CS) upon inoculation with CW, sawdust, PSD and cowdung showed marked variations in different parameters including the biogas production.

5.4.1. Total and volatile solids destruction

Biodigested slurry with PSD, and sawdust showed more destruction of TS as well as VS. BS is loaded with optimum proportion of different groups of bacteria and therefore triggered the degradation of both TS and VS. An inoculum has to undergo acclimatization and stabilization in the new environment [Toerien, 1967] and once acclimatized to a particular substrate the process of methanogenesis is accelerated. In the present study, BS from the biogas plant seems to contain all the different groups of microorganisms involved in methanogenesis.

However, the BS did not show any favourable effect when CW was used as the substrate. The CW was collected from effluent tank in which TS and VS are already subjected to the action of different groups of microorganisms.

Predigested sawdust inoculated with BS produced more biogas than other combinations. Corresponding to the degree of biogas production there was an increase in destruction of TS and VS, due to the presence of suitable bacteria in the inoculum. The usefulness of BS as a source of microbial inoculum has been recognised by many workers. Wato et al. (1983) found that well digested cattle dung from a biogas plant was a good inoculum for initiating microbial communities in the anaerobic digester. Hashimoto (1984) obtained higher gas yield when digested slurry from biogas plant was used as inoculum. Ghose and Das (1982) and Hills (1982) reported that addition of 10 per cent inoculum from anaerobic digester favoured maximum growth of microbial population in the new digester.

5.4.2. Cellulose, hemicellulose and lignin degradation in relation to biogas generation

The solid portion of substrates in the BS comprised mainly of partially digested cellulose, hemicellulose and lignin. Hence, the inoculum is expected to act as a starter for microbial action and biogas production.

In the present study, maximum degradation of cellulose and hemicellulose was recorded in PSD treatment. Interestingly the biogas production is inversely related to lignin degradation. The decrease in lignin degradation observed in this study could be mainly due to the accumulation of phenolic compounds [Theradimani and Marimuthu, 1991] during anaerobic degradation of sawdust by P. florida.

5.4.3. Volatile fatty acids

The VFA content was more in treatments involving BS. Simple sugars are the precursors for production of VFA mediated by acid producing bacteria. The cumulative activity of cellulolytic and acid producing bacteria is responsible for higher VFA content in this study as suggested by Winter and Cooney (1980) and Jain et al. (1981).

Higher VFA was recorded in the middle stage in all the treatments. This is due to the activity of acid forming bacteria, the population of which was also more in the middle stage of biogas production. In the final stage, the methanogenic bacteria were more dominant and converted VFA to methane which led to the low VFA level at the end of the experiment. Singh et al. (1980) also observed such phenomena in cattle dung fermentation.

The inocula (BS) and the substrate (PSD), both contributed for higher biogas production. Cowdung and CW were only partially digested and lower substrate availability, especially of cellulose is likely to be responsible for low levels of VFA and biogas production.

5.4.4. pH

In the initial stage of the experiment, the pH was in the neutral range (6.8 to 7.2), gradually decreased in the middle stage (6.4-6.8) and again rose to neutral range in the final stage of digestion, irrespective of the treatments. The increase in the acidity during the middle stage is due to the action of acidogenic bacteria which produced VFA and later when methanogenic bacteria became active, the pH increased. Similar findings were also reported by Ghose and Das (1982), Lapp et al. (1982); Hashimoto (1982b) and Hashimoto and Robinson (1982). They attributed the pH drop to the acid spectrum due to the acid produced by acid forming bacteria during the first few weeks of fermentation.

5.4.5. Microbiology and biochemistry of biogas production

Among the three inocula, the BS showed enhanced activity of cellulolytic bacteria, acid producing bacteria and methanogenic bacteria throughout the experiment.

This was followed by SS and CS. The activity of these bacteria was directly proportional to biogas generated. Maximum biogas (66, 420 ml) was produced in PSD inoculated with BS. Ferry et al. (1974) Bryant (1976) and Laube and Martin (1981) have also made similar observations on bacterial degradation and suggested that the enhanced action was due to the qualitative and quantitative nature of microbes present in the inocula.

Methane production is mediated by various groups of microorganisms on solid and liquid wastes. Cellulose, the polymerized glucose is depolymerized in the first stage of biogas production. In the case of PSD the first stage i.e., cellulose degradation was partially carried out by P. florida. Lignin and phenols which retard the microbial activity were also partially degraded, facilitating the increased gas production.

The proteolytic and lipolytic bacterial population were more in treatments with SS during initial and middle stages of fermentation. Compared to BS and CS, SS is rich in these two groups of bacteria as sewage is the domestic waste loaded with plenty of proteinaceous and fat materials [Lapp et al., 1984], thus improving the gas output in biogas plants.

Both cellulase and β -glucosidase activity in all the substrates were more when BS was used as inoculum.

BS consists of actively fermenting substrates and microbes and thereby containing more enzymes. This in combination with PSD, resulted in higher biogas output due to the enhanced cellulolytic and other enzyme activity.

5.4.6. Biogas production

In general, biogas production was more in all the substrates inoculated with BS. Among the substrates, PSD showed more gas production. The higher level of biogas with more methane content in BS treatment is due to favourable microbial consortium which hastened the degradation of organic compounds. BS, therefore, is considered to be a better source of microbial inoculum [Wate et al., 1983].

The enhanced biogas and more percentage of methane production in PSD is attributed to the quantitative and qualitative bacterial population coupled with the availability of preconditioned substrates in PSD and reduction in the lignin content.

Predigested sawdust with BS showed peak level of gas production at fourth week. In this treatment, the prior degradation of substrate makes carbohydrates easily available and also depletes the lignin content. Besides, the microbes in BS were adopted to the substrate and this resulted in the advancement of methanogenesis.

5.5. Experiment 3. Effect of diluents on biogas generation

Substrate is one of the important factors influencing biogas production in nature. The substrate consists not only the solid materials added, but also the diluents used for adjusting the concentration of substrate in the digestion mixture. Diluents other than water contain different organic and inorganic compounds as well as some inert materials, which influence biogas production [Singh et al., 1985b]. Corresponding to the change in biogas production, changes in physicochemical properties of the slurry, microorganisms and their enzyme activities take place.

5.5.1. Total and volatile solids destruction

The digestion mixture consists of both TS and VS and their rate of degradation is directly proportional to the biogas production [Sax et al., 1980]. In the present study, gas production is accompanied by significant changes in different solids. The destruction of TS ranged from 20.48 to 31.03 depending on the substrates and diluents used. The predigestion under aerobic condition has led to development of selective organisms suited to the environment causing higher total solid destruction. Similar results were recorded by Kirsch and Sykes (1971) and Barnett et al. (1978).

The destruction of TS and VS followed the same trend, although the latter showed a higher range (29.63 to 53.62). A positive correlation was observed between the VS destruction and the total gas production and this is in conformity with the findings of Varel et al. (1980) and Pathe et al. (1982).

5.5.2. Cellulose, hemicellulose and lignin degradation in relation to biogas generation

Like TS, their major constituents i.e., cellulose and hemicellulose also showed considerable variation in degradation depending on the diluents used. Highest percentage of degradation of these two components was recorded in PSD diluted with SPE followed by the same substrate with CPE. The enhanced gas output is mainly due to the biodegradation of the solid substances [Datta, 1981] under the influence of the diluents containing proteins, vitamins and other growth promoting substances [Thomas et al., 1980]. The role of proteins and vitamins on the growth of microbes is well established [Singh and Tauro, 1987]. PSD with SPE were the best combination in terms of biogas production. The hemicellulose destruction was high when compared to that of cellulose and lignin. Such differential degradation was reported by Datta (1981), Pathe et al. (1982) and Gosh et al. (1985) who

suggested that the five carbon sugars, the building blocks of hemicellulose, are susceptible to the attack by bacteria under anaerobic condition.

The chemical composition of lignin as well as phenols produced from lignin led to reduced microbial activity and biogas production. However, the rate of destruction of lignin was low ranging from 3.91 in cowdung with LCE to 5.79 in PSD with SPE. The strong matrix of lignin with cellulose substrate appears to resist, though not prevent biodegradation of lignin. Hills and Roberts (1981) in their experiments with dairy manure and field crop residues observed a reduced level of lignin.

5.5.3. Volatile fatty acids

Volatile fatty acids, the intermediary substrate leading to methanogenesis and the biogas production is directly proportional to its concentration [McCarty and McKinney, 1961]. The treatment which involve SPE recorded maximum VFA as well as biogas. SPE contains formic and acetic acids which were added for coagulation of latex, besides organic acids produced by the microorganisms. Readily available organic acids led to more biogas production.

5.5.4. pH

Various biochemical reactions as well as their end products influence the pH which play an important role in biogas production. McCarty (1964) reported that methane production proceeds very well as long as the pH was maintained between 6.6 and 7.6. The optimum pH range appears to be between 6.1 to 6.6 in the middle stage, when rubber factory wastes are used as substrates. When the fermentative bacteria continue to produce acids, the pH drops to 4.5 to 5.0 and the digester becomes 'struck' or 'pickled'. The fatty acid itself or due to its effect in decreasing the pH leads to toxic effects [Van Velson and Lettinga, 1980]. The enzyme activity was also comparatively less at low pH leading to reduced gas generation [Andrews, 1968]. Growing mushroom fungi in sawdust produce cellulase which release simple sugars capable of yielding more acid, resulted in low pH. House (1978) reported more acid production when reducing sugars are readily made available.

5.5.5. Microbiology and biochemistry of biogas production

An increase in cellulolytic bacterial count upto the middle stage of fermentation was noted in the present experiment by depolymerizing cellulose into simple sugars

and thereby promote biogas production [National Academy of Sciences, 1981]. The variation in cellulolytic bacterial activity observed in different substrates and diluents combinations is due to the differences in their compositions.

The acid producing bacterial population was more in SPE with different substrates in the middle stage and the maximum being in PSD. The increased acid forming bacterial population in PSD with SPE is possibly due to the lesser lignin content and increase in the end products of the hydrolytic cleavage that formed the initial substrate for the acid producing bacteria [Bryant, 1976]. SPE contains considerable number of acid forming bacteria [Taysum, 1960; John et al., 1975] which also contributed to this higher population when added to substrates that contained the precursors like sugars. Deshpande et al. (1979) attributed increased acid producing activity for higher gas generation as noticed in the present investigation.

Protein is one of the sources of nitrogen in the digestion mixture for biogas production. In the present study no relation could be observed between the population of proteolytic bacteria and biogas production. Hobson (1973) reported that the variation in proteolytic bacterial population might be due to changes in the type

and amount of input material and the starter inoculum added. The mushroom compost is rich in protein [Zadrazil, 1978] and the predigestion of sawdust using mushroom fungi contributed to a high population of proteolytic bacteria. SPE contain considerable quantity of proteins [Thomas et al., 1980] and caused the augmentation in the proteolytic bacteria in treatment involving this diluent.

The population of lipolytic bacteria in various treatments did not show significant variation with respect to the substrates or diluents. In general, there was an increase of these bacteria upto middle stage and subsequent reduction. Availability of lipids in the digestion mixture might have influenced the lipolytic bacterial population as well as their activity.

The methanogenic bacterial population varied significantly in wastes in combination with different diluents in the middle stage. The highest population of methanogens in the middle stage was recorded in the PSD diluted with SPE which also registered maximum gas. A positive correlation was observed between gas production and methanogenic bacterial population [Young and McCarty, 1969; Lawrence, 1971 and Rajasekaran and Nagarajan, 1979].

5.5.6. Biogas production

The quantity and quality of biogas, especially, methane content are given much consideration in biogas technology. The lower biogas yield in CPE and LCE when compared to SPE was due to their low pH which has negative effect on both methanogenic bacteria and the enzyme activity [Vanden Berg et al., 1976]. Low pH also makes the bacteria unable to use the acids quickly rendering the digestion process ineffective as suggested by Singh (1974). The presence of sulphate, the natural electron acceptor, is one of the factors that adversely affect the biogas production [Bryant, 1976; Athanassopoulos, 1989]. In LCE, high levels of sulphate are present and cause reduction in biogas production. The results of this study were in conformity with the findings of Bansal et al. (1977) who reported the optimum pH range of 6.4 to 7.2. Ammonia acts as a strong inhibitor against the formation of methane from hydrogen and carbon dioxide [Hashimoto, 1986; Heinsichs et al., 1990; Wohlt et al., 1990]. The low levels of biogas production in the present study might also be due to the high ammonia content in LCE.

The favourable role of organic acids on methanogenesis is well established [McCarty and McKinney, 1961; Singh et al., 1983]. Acetic acid used to coagulate latex in the sheet rubber processing served as substrate for methane generation.

The advancement in attaining peak level of biogas is an important factor when the efficiency of biodigestion is taken into consideration. PSD with SPE recorded peak level of biogas in the fourth week of biodigestion, which is probably due to the cumulative effect of various groups of microorganisms in the substrate, diluents and inocula. The organic acid contained in the SPE also might have favoured the early gas production.

Sheet processing effluent as diluent with different organic wastes resulted in an increase in biogas production. This diluent contains organic acids as well as fine particles of rubber hydrocarbon from latex. The latex yielding plant materials are reported to be more gas producers owing to the rubber hydrocarbon content and easily available nutrients [Rajasekharan et al., 1989 and Traore, 1992]. SPE rich in these components resulted in more biogas with higher percentage of methane. The present study clearly shows that SPE is a better source for biogas production.

5.5.7. Manurial values of biodigested slurry

In the biogenesis of methane the anaerobic biodegradation of organic and inorganic nutrients leads to drastic changes in substrate/reaction mixture. At the end of the fermentation, the slurry contains N, P and K which could serve as manure for higher plants. The NPK contents

were more in PSD diluted with SPE, indicating the higher manurial value. The slurry from the treatment which favoured more gas production also showed higher manurial value. Mishra (1954), Nagar (1975) and Krishnappa et al. (1979) in their comparative studies reported superiority of the BS and farm yard manure in their nitrogen over compost. The increase in nitrogen of CW and PSD incorporated treatments as well as cowdung diluted with different effluents is due to the higher initial nitrogen content of the substrates in addition to the build up of the microbial biomass that converts ammoniacal nitrogen to nitrates in the anaerobic environment. Eckholm (1976) and Singhal (1977) also reported more nitrogen content in the BS.

The phosphorous content ranges from 0.58 to 0.92 per cent in the BS. During methanogenesis, phosphates play a major role in energy cycle [National Academy of Sciences, 1981]. Unlike nitrogenous and carbonaceous compounds, phosphates and potassium are not lost due to microbial metabolism. Hence, significant variation in phosphate content in the slurry might be due to differences in their sources.

Compared to nitrogen and phosphorous, the potassium content was less in all the treatments. However, among different sources potassium was more in SPE.

The results of the present study revealed the magnitude of pollution by various rubber processing factories as well as rubber wood saw mills.

Energy bills of factories are escalating at an alarming rate. Biogas production from the liquid and solid wastes would help in reducing the expenditure on energy, besides controlling pollution. When compared to cowdung, the conventional substrate for biogas generation, solid and liquid wastes from rubber based industries yielded more biogas. Rubber hydrocarbon is present in rubber wood as well as in the wastes of rubber processing factories. Latex yielding plants are reported to be good substrates for biogas production [Traore, 1992]. The enhanced level of biogas production from the wastes from rubber based industries might be due to the presence of nutrients and rubber hydrocarbon in them. Substitution of electrical energy, at least partially by renewable energy from biogas generated from these wastes would definitely contribute to the reduction in the cost of processing of rubber.

CHAPTER 6

SUMMARY

SUMMARY

The liquid and solid wastes from natural rubber based industries were characterized and their use for the production of biogas investigated with a view to conserve conventional energy, and to mitigate environmental degradation. The following investigations were carried out to identify, assess and overcome the problems.

1. Characterization and quantification of the wastes
2. Utilization of wastes from rubber processing and rubber wood industries.

The physicochemical and bacteriological analysis showed that rubber factory effluents contain large quantities of solids, both organic and inorganic, creating a high oxygen demand. Bacterial population was higher in crepe rubber effluent (CRE), followed by crumb processing effluent (CPE), sheet processing effluent (SPE) and latex concentrate effluent (LCE). The solid wastes from rubber processing varied depending on the physicochemical properties. Since the major components of the solid wastes are lignocellulosic in nature and the liquid wastes

contain nutrients, both the wastes were used for generation of biogas. The effects of different levels of solid content, sources of inoculum and incorporation of liquid wastes with solid wastes, on biogas production were investigated. The changes in different solids, cellulose, hemicellulose, lignin, cellulolytic, acid producing, proteolytic, lipolytic and methanogenic bacteria and hydrolytic enzymes liberated, at initial, middle and final stages of 10 weeks incubation time, were monitored. The possibility of the use of spent slurry as organic manure is discussed.

Digestion mixture with crumb waste (CW), sawdust, predigested sawdust (PSD) and cowdung was prepared at 5, 10 and 15 per cent concentration of solids and used for anaerobic digestion. The amount of gas evolved and the changes undergone in the digestion mixture were studied. Maximum degradation of TS, VS, cellulose and hemicellulose was observed in PSD at 10 per cent level. The VFA content was also more in this treatment. At 10 per cent level of PSD, the activity of cellulolytic, acid producing, proteolytic, lipolytic and methanogenic bacteria were more in the middle stage of methanogenesis. Corresponding to the population of various bacteria there was a change in the activity of cellulase and β -glucosidase. Enhanced activity of different groups of bacteria resulted in higher production of biogas rich in methane content.

Favourable changes leading biogas generation and quantum of biogas generated in sawdust was next to PSD. Both sawdust and PSD were superior to CW and cowdung for biogas production under the influence of microbial activity and biochemical changes.

Biodigested slurry (BS) with different substrates was identified based on the parameters investigated, as very ideal for biogas production. Among various substrates PSD with BS led to maximum biogas production. The degradation of TS, VS, cellulose and hemicellulose was higher in the treatment involving PSD and BS. Population of cellulolytic, acid producing, proteolytic, lipolytic and methanogenic bacteria as well as the activity of enzymes- cellulase and β -glucosidase, and VFA were more on PSD inoculated with BS. Activity of cellulolytic enzymes and microbial population generally increased upto middle stage and then decreased with time. The percentage of methane in the biogas was also more in the PSD and BS treatments.

Among the liquid wastes from rubber processing used as diluents in combination with PSD, SPE promoted more biogas production with high methane content in the gas. The factors that favour methane production like TS, VS, cellulose and hemicellulose degradation were favoured in this treatment which led to higher methane biogenesis.

The population of bacteria and the quantity of enzymes that cause degradation of cellulose, the major component of TS and VS were more, especially during the middle stage in PSD and SPE combination. Similarly the population of proteolytic, lipolytic, acid producing and methanogenic bacteria were also more in PSD and SPE.

The spent liquor contained major plant nutrients i.e., N, P and K. PSD with SPE contained 2.26, 0.92 and 0.78 per cent of N, P and K respectively, indicating its usefulness as an organic manure for plants.

The results of the present investigation open a new avenue with significant dimensions in the conservation of conventional energy. The substantial amount of high quality biogas generated from natural rubber and rubber wood industry waste would be a covetable alternative source of energy. Simultaneously this process also abates the environmental degradation. The results further highlight ways and means to use agricultural wastes as alternative sources of energy.

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APPENDIX

APPENDIX 1

Distribution of rubber processing factories and the quantum of rubber processed

Types of rubber processed	1975		1980		1985		1993	
	No. of factories	Quantity processed (MT)	No. of factories	Quantity processed (MT)	No. of factories	Quantity processed (MT)	No. of factories	Quantity processed (MT)
RSS	*	63,750	*	1,04,890	*	1,30,090	*	2,81,335
TSR (Crumb)	5	1,659	9	2,416	11	4,981	31	27,107
Crepe	78	20,685	130	32,594	102	32,959	79	38,803
Latex concentrate	17	7,050	19	13,200	35	18,420	62	46,245

* Numerous

APPENDIX 2

Quantity of rubber processed and the effluent generated (1992-'93)

Type of rubber processing	No. of factories	Quantity of rubber processed (MT)	Total production (%)	Effluent generation rate (litre/kg)	Estimated quantity of effluent ³ generated (m ³)
RSS	*	2,81,335	71.49	19.35	54,43,832
TSR (Crumb)	31	27,107	6.88	26.30	7,12,914
Crepe	79	38,803	9.86	24.50	9,50,673
Latex concentrate	62	46,245	11.75	17.85	8,25,473
Total		3,93,490	100		79,32,892

* Numerous

APPENDIX 3

Microbiological properties of the slurry

The different types of anaerobic microorganisms viz., cellulolytic, proteolytic, lipolytic, methanogenic and acid forming bacteria were enumerated at three different stages from the slurry specimens of all batch type experiments, using respective media, favouring their growth.

1. Enumeration of cellulolytic bacteria

The medium devised by Hungate (1957) was used for the enumeration of anaerobic cellulolytic bacterial population by the roll tube method.

Media used

Potassium dihydrogen phosphate	-	0.02 g
Dipotassium hydrogen phosphate	-	0.03 g
Magnesium sulphate	-	0.01 g
Calcium chloride	-	0.01 g
Sodium chloride	-	0.10 g
Ammonium sulphate	-	0.10 g
Cysteine hydrochloride	-	0.02 g
Sodium bicarbonate	-	0.50 g

2. Enumeration of anaerobic proteolytic bacteria

The enumeration of proteolytic bacteria by the roll tube method was carried out by using the methods of Blackburn and Hobson (1962) and by Abou Akkada and Blackburn (1963).

Media used

Glucose	-	100.00 mg
Minerals (a)	-	15.00 ml
Minerals (b)	-	15.00 ml
Cystein hydrochloride	-	0.05 g
Casein	-	0.50 g
Rumen fluid (centrifuged)	-	10.00 ml
Tryptose	-	0.30 g
Agar	-	2.00 g
Resazurin	-	0.0001 g
Sodium bicarbonate	-	0.50 g
Distilled water	-	100.00 ml

Minerals (a)

Dipotassium hydrogen phosphate	-	3.00 g
Distilled water	-	1000 ml

Resazurin	- 0.0001 g
Rumen fluid (not clarified)	- 30.00 ml
Cellulose powder (swollen cellulose)	- 1.00 g
Agar	- 2.00 g
Distilled water	- 100 ml

The medium was prepared and 10 ml of the medium was dispensed to washed vials. The mouth of the vials were sealed with rubber stopper and crimped with aluminium caps to make them air tight with the help of a crimper. Then the bottles were sterilized at 15 lb pressure for 20 min. Serial dilutions of the samples were made upto 10^{-4} under $N_2:CO_2$ (70:30) gas phase. After sufficient cooling of the medium one ml of the well mixed dilution was injected in the vials containing medium by using sterile hypodermic syringe. The contents of the vials were rolled immediately in such a way that agar medium was distributed uniformly in the form of a thin layer on the inner surface of the vials. The gassing was done by using gassing manifold [Batch and Wolfe, 1976] with 70:30 proportion of $N_2:CO_2$. These vials were incubated for 3 to 4 weeks and the cellulolytic bacteria were identified by zones of clearing around the colonies.

Minerals (b)

Potassium dihydrogen phosphate	-	3.0 g
Ammonium sulphate	-	6.0 g
Sodium chloride	-	6.0 g
Magnesium sulphate	-	0.6 g
Calcium chloride	-	0.6 g
Distilled water	-	1000 ml

The procedures for plugging of rubber corks, aluminium caps sterilization, inoculation and gassing of $N_2:CO_2$ mixture were done in the same way as described earlier for cellulolytic bacterial enumeration. The proteolytic bacterial colonies were enumerated after incubation upto three weeks.

3. Enumeration of anaerobic lipolytic bacteria

The lipolytic bacterial population were enumerated by the roll tube method by using the medium developed by Hobson and Mann (1961) and the composition of the medium was as follows:

Minerals (a)	-	15.00 ml
Minerals (b)	-	15.00 ml
Rumen fluid	-	40.00 ml
Cysteine hydrochloride	-	0.05 g

Sodium bicarbonate	-	0.40 g
Resazurin	-	0.0001 g
Linseed oil	-	1.00 ml
Agar	-	2.00 g
Distilled water	-	100 ml

Mineral solutions 'a' and 'b' were prepared as described under proteolytic bacterial population enumeration. The medium was shaken thoroughly to disperse the oil before dispensing. The 10^{-3} serial dilution was used for lipolytic bacteria. The roll tube method was followed as described earlier. The lipolytic bacterial colonies that developed after incubation for three weeks were enumerated.

4. Enumeration of methanogenic bacteria

For the enumeration of methanogenic bacteria, Smith and Hungate (1958) medium with the following composition was used in roll tubes.

Media used

Dipotassium hydrogen phosphate	-	0.10 g
Potassium dihydrogen phosphate	-	0.10 g
Sodium chloride	-	0.20 g
Ammonium chloride	-	0.10 g

Magnesium sulphate	-	0.01 g
Calcium chloride	-	0.01 g
Resazurin	-	0.0001 g
Sodium bicarbonate	-	0.60 g
Rumen fluid (centrifuged)	-	30.00 ml
Agar	-	2.00 g
Sodium pyruvate	-	0.10 g
Sodium dithionate	-	0.003 g
Distilled water	-	100 ml

The above said medium was prepared in a 500 ml flask. After sterilization of the flasks at 15 lb pressure for 30 minutes, CO₂ gas was passed into the flasks immediately to maintain the CO₂ gas atmospheric inside the flasks. The medium was then kept in a water bath at 40°C to 50°C, under CO₂ bubbling. The serial dilutions upto 10⁻⁴ was made under CO₂ gas phase. Five to ten ml of the medium was pipetted out into a sterilized test tube by continuously passing the CO₂ gas into the test tube. One ml of the 10⁻⁴ dilution of the sample was pipetted out into the test tube and immediately stoppered tightly with a sterile rubber stopper so that the gas will not escape from the tube. Then the test tubes were rolled on a wet cloth. The tubes were incubated for two to three weeks. The methanogenic bacteria were identified by clear cream coloured colonies.

5. Enumeration of acid forming bacteria

The acid forming bacteria was estimated as per the method described by Chynoweth and Mah (1977).

Media used

	(per cent w/v)
Sodium chloride	- 0.10
Ammonium chloride	- 0.05
Magnesium chloride	- 0.005
Calcium chloride	- 0.005
Potassium dihydrogen phosphate	- 0.005
Cobalt chloride	- 0.001
Ammonium molybdate	- 0.001
Sodium bicarbonate	- 0.50
Glucose	- 0.50
Cysteine hydrochloride	- 0.05
Sodium sulphide	- 0.025
Bromothymol blue	- 0.005
Precipitated calcium carbonate	- 1.00
pH	- 7.00

6. Enumeration of general bacteria

Nutrient glucose agar medium was used for the enumeration of general bacteria.

Media used

Peptone	-	5 g
Glucose	-	5 g
NaCl	-	5 g
Beef extract	-	3 g
Agar	-	15 g
Water	-	1000 ml
pH	-	6.8

7. Enumeration of yeasts

The yeast population was enumerated using the modified Martin's Rose Bengal streptomycin agar medium.

Composition

Oxoid malt extract agar	-	4%
K_2HPO_4	-	1 g
$MgSO_4 \cdot 7H_2O$	-	0.5 g
Rose Bengal	-	0.0033 %
Streptomycin	-	75 g/ml
Water	-	1000 ml
pH	-	5.5

