BIOGAS PRODUCTION FROM RICE HUSK BY ANAEROBIC DIGESTION

Thesis

Submitted to the Punjab Agricultural University
in partial fulfilment of the requirements
for the degree of
MASTER OF SCIENCE
IN
MICROBIOLOGY
(Minor : Biochemistry)

by
Amardeep Kaur
(L-93-BS-92-M)

Department of Microbiology
College of Basic Sciences and Humanities
PUNJAB AGRICULTURAL UNIVERSITY
LUDHIANA-141 004
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..... to my revered parents

and my loving husband

CERTIFICATE - I

This is to certify that the thesis entitled, **"Biogas production from rice husk by anaerobic digestion"**, submitted to the Punjab Agricultural University, Ludhiana in partial fulfilment of the requirements for the degree of Master of Science in the subject of Microbiology (Minor Subject: Biochemistry), is a bonafide research work carried out by **Amardeep Kaur (L-93-BS-92-M)**, under my supervision and that no part of this thesis has been submitted for any other degree.

The assistance and help received during the course of investigation have been fully acknowledged.

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CERTIFICATE - II

This is to certify that this thesis entitled, **"Biogas production from rice husk by anaerobic digestion"**, submitted by **Amardeep Kaur** (L-93-BS-92-M) to the Punjab Agricultural University, Ludhiana, in partial fulfilment of the requirements for the degree of Master of Science in the subject of Microbiology (Minor subject : Biochemistry), has been approved by the Student's Advisory Committee after an oral examination on the same, in collaboration with an External Examiner.

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ABSTRACT

Rice husk was used as feedstock for biogas production. It was pretreated by various methods viz. *Trichoderma reseii*, alkali, by soaking in cattle dung and water. The gas production rate with alkali and *T. reseii* pretreated rice husk was only 0.046 and 0.019 l^{-1} day^{-1} respectively. With 10%, 20%, 25% and 30% replacement of cattle dung with cattle dung pretreated rice husk, gas production rate was 0.130, 0.213, 0.167 and 0.137 l^{-1} day^{-1} respectively. It was 39% more with 20% replacement as compared to control, whereas 50% and 75% replacement of cattle dung with cattle dung pretreated rice husk produced negligible gas i.e. 0.0256 and 0.0028 l^{-1} day^{-1} respectively. With 10%, 25% and 30% replacement of cattle dung with water pretreated rice husk, 10% replacement yielded maximum gas i.e. 0.232 l^{-1} day^{-1} followed by 25% and 30% replacement i.e. 0.164 and 0.157 l^{-1} day^{-1} respectively. Comparison of batch with semi-continuous digester, both fed with 20% replacement of cattle dung with water pretreated rice husk, later yielded 36% more gas. Microflora count varied from $26x10^4$ - $90x10^6$ cfu ml^{-1} in all digesters.

Sahota

(Signature of Major Advisor)

Amardeep Kaur

(Signature of Student)

CONTENTS

Chapter I

INTRODUCTION

The problem of energy and associated agricultural inputs, shortages, environmental pollution and huge amount of agricultural wastes have created an urge among developing nations to process their wastes for energy. Processing of such energy and crop waste anaerobically is attractive since it holds the potential of producing renewable energy sources. Biogas production is an important aspect of anaerobic digestion. Biomethanation or anaerobic digestion is a complex microbiological process in which biomass is converted into a mixture of gases such as methane, carbon dioxide, hydrogen, hydrogen sulphide etc. by a consortium of bacteria. Most of agricultural - agroindustrial residues and animal wastes contain adequate quantities of nutrient (carbon and nitrogen) essential for the growth and metabolism of biogas producing bacteria. Few other advantages of biomethanation are reduction in dry matter, smell and pathogenic organisms and the better dewatering properties of the digested sludge.

In the last decenium anaerobic digestion has come into focus again. India being a tropical country with wide variety of crop residues, annual production of agro-industrial residues is 301.6 MT $year^{-1}$, rice husk and rice straw being 80.6 MT $year^{-1}$ (Garcha *et al.*, 1987). Potential of energy replacement in form of biogas derived from agro industrial residues is 718 MW x 10^{10} electricity or 6.618 log x 10^{10}

butane (Sahota, 1996). This tempted us to explore the possibility of biogas production from agricultural and agroindustrial residues.

Major factors affecting biogas production are feedstock composition, inoculum characteristics and biodegradability of substrate. All agroindustrial residues have certain natural organic compounds such as lignin which are refractory to decomposition under anaerobic conditions even at long retention times. So there is immediate need to explore physical, chemical and biological pretreatment techniques for improvement of biodegradability of wastes so that the same can be processed for biogas production when mixed with cattle dung.

For balanced digestion C:N ratio has to be 35-50 (Ghose and Das, 1982) whereas cattle dung alone has low C:N ratio which could be optimized by adding cellulosic waste such as straws, grasses, husk and other crop residues as substrate (Fraser, 1977). Agroindustrial or agricultural residues when mixed with cattle waste needs certain adaptation period and there can be increase or decrease in microflora. It is an important parameter to be studied as biogas production is directly related to the microflora present (Sahota, 1992).

To improve the existing biogas technology the present study is intended with isolation of microflora from anaerobic digester fed with cattle dung and rice husk in different proportions. Apart from checking the suitability of mixing cattle dung and rice husk in different proportions for maximum gas production our aim was

to study the most effective pretreatment method. Efforts were also made to study the effect of inoculum, substrate ratio and comparison between batch scale digestion and semi-continuous digestion.

Chapter II

REVIEW OF LITERATURE

2.1 ANAEROBIC DIGESTION

Anaerobic digestion is an effective fermentation of organic matter to biogas due to combined and co-ordinated metabolic activity of diverse anaerobic bacterial population.

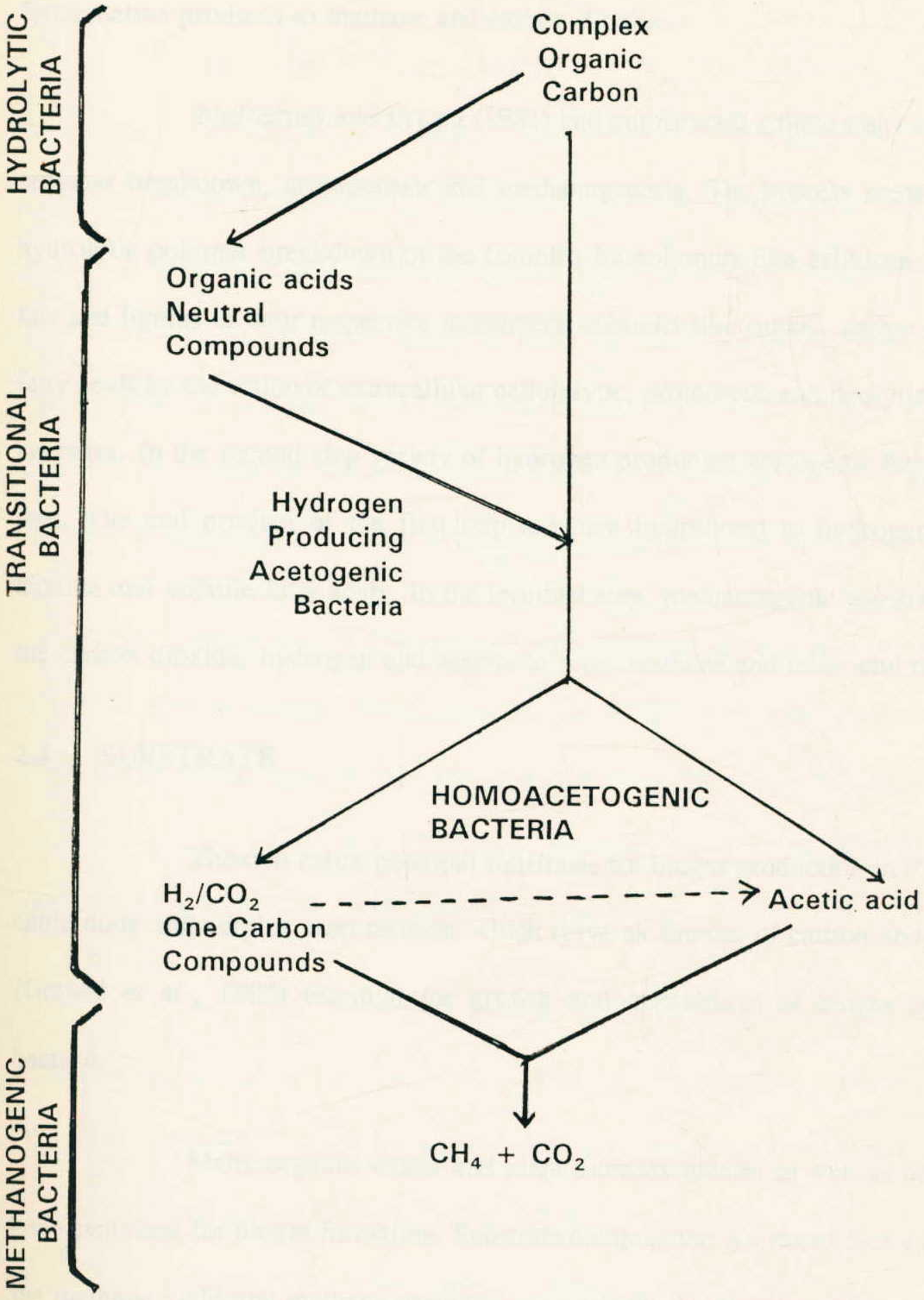
2.2 HISTORY

Biogas according to Tietzen (1975) was discovered by Shirley in 1667, Bechamp (1868) indicated for the first time that methane is formed by a microbial process, Tappeiner (1882) provided more adequate proof of the microbiological origin of methane. In China, in 1920's Luo Quorni constructed the earliest digester.

2.3 OVERVIEW OF ANAEROBIC DIGESTION

Kirsch and Skyes (1971) suggested involvement of two steps for conversion of organic matter to methane. First step is acidogenesis and the IInd is methanogenesis. Anaerobic digestion is carried out by two groups of physiologically diverse bacteria. In the first step acid forming bacteria hydrolysis and ferments

ANAEROBIC DIGESTION OF BIOMASS (CHYNOWETH, 1987)



Categories of Metabolically distinct bacteria in the methane fermentation

organic compound and in the second step methane forming bacteria converts their fermentative products to methane and carbon dioxide.

McInerney and Bryant (1981) had put forward a three step conversion-polymer breakdown, acetogenesis and methanogenesis. The process starts with the hydrolytic polymer breakdown of the complex biopolymers like cellulose, proteins, fats and lignins to their respective monomeric subunits like sugars, amino acids and fatty acids by the action of extracellular cellulolytic, proteolytic and lipolytic bacterial enzymes. In the second step variety of hydrogen producing acetogenic bacteria take part. The end product of the first step are then hydrolysed to hydrogen, carbon dioxide and volatile fatty acids. In the terminal step, methanogenic bacteria utilizes the carbon dioxide, hydrogen and acetate to form methane and other end products.

2.4 SUBSTRATE

The two major potential substrates for biogas production in Punjab are cattle dung and surplus crop residues which serve as sources of carbon and nitrogen (Grewal *et al.*, 1985) essential for growth and metabolism of biogas producing bacteria.

Many organic wastes and plant biomass species as well as blends has been evaluated for biogas formation. Substrate composition is a major factor affecting the methane yield and methane production rates from the digestion of biomass.

2.4.1 Cattle waste

Sorlini and Bonfanti (1989) concluded from their study that cattle manure could be efficiently utilized for production of methane gas. The gas production rate was approximately 110 ml g^{-1} of COD removed or 480 ml g^{-1} of TVS reduced in digester. This is due to the advantage of microflora present in the cattle waste which helps in better digestion and enhanced methanogenesis, because bioconversion of organic matter to methane is complex anaerobic fermentation involving the combined action of many micro-organisms with a wide variety of metabolic characteristics whereas in other substrates we had to add inoculum.

Jain *et al.* (1983) stated that batch digesters containing cattle waste can be started with or without inoculum since the cattle waste itself contains all the required microflora required for anaerobic digestion. Sahota (1992) reported total population (c.f.u. per ml) 8 x 10^{10} viable population, 3 x 10^{8} culturable, 4 x 10^{3} cellulolytic and 6.5 x 10^{4} methanogenic in cattle manure.

Hill (1983) reported that cattle waste was less concentrated as far as biological fermentation was concerned. Due to mechanization of agriculture, availability of cattle dung is becoming scarce and therefore, other sources rich in organic constituents may be exploited for this purpose. Another reason for exploiting other substrate is that there is low carbon to nitrogen ratio in animal wastes which is not optimum for gas production.

Since plenty of plant residues are available against the limited availability of animal dung so the use of former has to be exploited.

About 3/4th of the rural families in India do not possess the minimum requirement of five cattles for putting up a gobar gas plant. Thus their kitchen fuel need would continue to be met from fuel wood etc. India's forest resources are also declining at an alarming rate, barren lands cause soil erosion, silting up of rivers, lakes, dams etc resulting in flash floods etc. Hence only a locally available, cheap alternative fuel can help us and save our forests. Solution and need to have is to exploit crop residues for biogas production (Goswami, 1989).

Anaerobic digestion of cattle dung and many other agro residues at initial total solids (TS) concentration varying from 16-25% in small, batch type, digesters under laboratory conditions has been reported to be satisfactory, for biogas production (Pathak *et al.*, 1985; Rajasekharan and Prasad Reddy, 1991; Shyam, 1991). The animal waste which have low carbon to nitrogen (C/N) ratio can be optimized by adding cellulosic waste such as grasses, straws, husk and other crop residues to increase methane production efficiency (Fraser, 1977).

Various substrates which can be used as feedstock for biogas production are discussed herewith.

2.4.2 Crop Residues

There is a great potential for producing biogas from crop residues as the yield of usable grain or other food staples is only about half the yield of total biomass under a wide range of conditions. Substantial quantities of unused stalks, straw, husk, bark and other crop residues are produced from a variety of crops which could be used for energy generation. The estimated production of agricultural residues in the form of crop residues and agroindustrial byproducts is 301.6 MT per year (Garcha *et al.*, 1987) and 80.6 MT of these agricultural residues is in the form of rice straw and rice husk.

Plant/crop residues have normally a high content of cellulose which has a combustion energy of 15 KJ g^{-1}, and if converted to methane, a preferred fuel, has a combustible energy of 50 KJ^{-1}, although the rate of bioconversion has been reported to be very slow (Khan *et al.*, 1983).

All crop residues are cellulosics in nature, but their individual characteristics vary over a wide range and hence selection of the residue with appropriate characteristic for a particular use or application is extremely important (Pathak *et al.*, 1986).

2.4.2.1 Rice Crop Residues

Rice husk and Rice straw

Anaerobic decomposition of various crop residues, leaves, grasses, berseem, oat, straw and hybrid napier etc. into organic acid and biogas at 55°C had

been studied (Acharya, 1958). It resulted in 40% reduction in total solids and 0.30 m^3 methane/kg of volatile solids.

Hills and Robert (1981) carried out digestion of dairy cow manure combined with rice hulls or rice straw in 4.4 ℓ laboratory digesters with working volume of 3.4 ℓ for maximum methane production per volume of anaerobic digester. The biogas comprised of 58.0% methane, 28.6% CO_2 and 14% other constituents like CO_2, O_2, N_2 and hydrogen etc. But Pathak *et al.* (1985) carried out the biogasification of cattle dung and rice straw mixture at different solid concentration. Nearly same amount of gas was produced per gm of solids converted when the solid concentration of the cattle dung slurry was 9.7%, 10.2% or 14.8%. A larger percentage of solids were converted when solid concentration was high. A mixture of cattle dung and chopped rice straw in the ratio of 1:2 (on dry weight basis) and containing 15.2% solids gave 97 ℓ gas per g of solids converted. The methane content was 58%.

Kalra and Panwar (1986) carried out anaerobic digestion of rice crop residues - husk and straw in 190 ℓ metallic digesters. One Kg of rice straw yielded about 220 ℓ of biogas under batch digestion. A mixture of rice straw and cattle dung 1:1 (dry wt basis) yielded 9.1% more gas than rice straw alone. Shyam and Sharma (1994) reported that gas yield was higher for substrates consisting of dung and agro residues than dung alone.

2.4.2.2 Fruit and vegetable waste

In India, about 18 MT of fruits and vegetables are produced and meager of 1% of the total produce is processed by the industry for the production of pulp, jam, jellies, chutney, squash and various other products and about 4 MT of solid wastes emanate annually which are largely wasted and also become a major source of pollution so Nand (1991) utilized fruit and vegetable processing waste for biogas production and yield was 0.3 m^3 $Kg\ TS^{-1}$.

Lubberding *et al.* (1988) carried out the anaerobic digestion of onion waste by mixed population of rumen microorganisms. Both peels and pulp were degraded with an efficiency of about 50-70% at retention time of only 60h. Onion peel yielded less amount of biogas i.e. 0.016 mmol g^{-1} VS digested as compared to onion pulp i.e. 0.65 mmol.

Apple pomace and vegetable waste (raddish leaves, cauliflower leaves and stalk and rotten cabbage) were subjected independently to anaerobic digestion on laboratory scale by Kalia *et al.* (1992). Each kilogram (dry weight) of apple pomace fed could generate 275 ℓ of biogas (59% CH_4), fresh vegetable waste generated 210 ℓ of biogas (72% CH_4) and rotten cabbage led to the generation of 320 ℓ of biogas (68% CH_4). The results shows that biomass can be easily exploited for biogas production.

Vishwanath *et al.* (1992) reported the effect of feeding different fruit and vegetable wastes, mango, pine apple, tomato, jackfruit, banana and orange in a

60 ℓ digester by cycling each waste every fifth day in order to operate the digester as and when their was a supply of feed. Digester performance in terms of biogas production was determined at different loading rates (LR) and at different hydraulic retention time (HRT) and the maximum biogas yield of 0.6 m^3 Kg VS^{-1} added was achieved at 20 day HRT and 40 KgTs m^3 day^{-1} LR. The hourly gas production was observed in the digester operated at 16 and 24 days HRT. The major yield (74.5%) of gas was produced within 12 h of feeding at a 16 day HRT whereas at a 24-day HRT 59.03% of the total gas could be obtained at this time.

Trujillo *et al.* (1993) reported that addition of tomato plant waste to the rabbit waste in proportion higher than 40% improves methane production.

2.4.2.3 Wheat Straw

Hassandar *et al.* (1989) evaluated the contribution of alkali treated plant residues as a supplement to cattle dung (1:1 w/w at 10% slurry level) for biogas production and quality. Addition of pretreated (1% NaOH for 7 days) lantana residue, wheat straw, apple leaf litter and peach leaf litter to cattle dung improved microbial digestibility and biodegradability during anaerobic fermentation at ambient temperature (28-31°C). Methane content being 63.6% in lantana slurry; 59.6% in apple leaf litter, 58% in wheat straw and 59.7% in peach leaf litter against 56.1% in cattle dung. The digestion efficiency in terms of biogas produced per gm of dry matter with pretreated plant residues was 341-372 ml g^{-1} and it was 31.42% higher than cattle dung.

2.4.2.4 Other plant/crop/residues

Nallathambi (1988) analysed digestibility of Gliricidia leaves for biogas production in 3 ℓ batch digesters at room temperature (32 ± 3°C). Results indicated a gas yield of 165-180 ml CH_4 g^{-1} VS added and 57-59% reduction of VS. An attempt has been made taking eucalyptus leaves for biogas production by Ali and Mathur (1990).

Dry anaerobic fermentation of cow dung-Subabul leaves mixture in multiple batched digesters was carried out at 20% solids concentration by Jain and Kumar (1990). The gas yields were 0.5 m^3 day^{-1} m^{3-1} digester volume.

2.4.2.5 Organic matter

NAS (1977) and Malik *et al.* (1989) reported that there are more than 30 types of wastes which have potential for methane generation (Table A)

2.5 RATE LIMITING STEPS

Factors affecting biogas yield from substrate are feedstock composition, inoculum characteristics and biodegradability of insoluble particles, variety of factors, including inherent characteristics of the molecule, structural blockage by components that are not readily degraded and toxic components.

Natural organic compound such as lignin shields the decomposition of organic components under anaerobic conditions. It is due to the lack of enzymes in anaerobic bacteria and an oxygen requirement by many enzymes to carry out the

(TABLE A)

Potential organic matter for methane generation

Crop wastes	Sugarcane trash, weeds, corn and related crop stubble, straw, spoiled fodder
Wastes of animal origin	Cattle shed wastes (dung, urine, litter), poultry litter, sheep and goat droppings, slaughter house wastes (blood and meat), fishery, leather, wool wastes
Wastes of human origin	Faeces, urine, refuse
By-products and wastes from agricultural based industries	Oil cakes, bagasse, rice bran, tobacco wastes and seeds, wastes from fruit and vegetable processing, press-mud from sugar factories, tea wastes, cotton dust from textile factories
Forest litter	Twigs, bark, branches, leaves
Waste from aquatic plants	Marine algae, sea weeds, water hyacinth

initial hydrolysis. Lignin forms complex with cellulose and thus shields its hydrolysis. A variety of physical, chemical and biological pretreatment techniques can be employed for enhancing biodegradability. Physical treatments were suggested by Dunlop *et al.* (1976), Millet *et al.* (1976), Jerger (1983) and Tsao and Chiang *et al.* (1983). These include size reduction which increase the number of exposed ends by cutting the long structures into many short ones.

Another is alkali pretreatment of cellulosics, which is the best known method of enhancement of biodigestion of cellulose. It results in rupture of lignin and an increase in surface area due to cellulose swelling and crystallization of cellulosics. Guggolz *et al.* (1971), Han and Callihan (1974) and Destroy *et al.* (1981) demonstrated the effectiveness of alkali pretreatment with an increased enzymatic digestibility. Ghosh *et al.* (1980) and Chynoweth *et al.* (1983) showed 10-15% increase in methane yield from water hyacinth in both semi-continuous and batch fed digesters after alkali pretreatment. Colleran *et al.* (1982) demonstrated that there is 50% increase in methane yield during digestion of wheat straw in two stage reactor system.

Hassandar *et al.* (1989) showed that treatment of biomass residue with 1% alkali solution at the rate of 250 g of NaOH Kg^{-1} TS for 7 days at ambient temperature resulted in increased production of biogas - 762 ml g^{-1} VS degraded with lantana crop residue as compared to cattle waste alone ie. 738 ml g^{-1} VS degraded.

Treatment of mango peel by composting for a period of 6 days

exhibited 8-fold increase in total gas production (Devi and Nand, 1989).Beg *et al.* (1986) successfully demonstrated that pretreatment of rice husk by *Pleurotus ostreatus* increased its biodegradability by decreasing its lignin and cellulose content by 40.9% and 19.1% respectively.

2.6 ENVIRONMENTAL FACTORS AFFECTING ANAEROBIC DIGESTION

Anaerobic digestion being a biological process is very sensitive to environmental factors like anaerobiosis, temperature, pH, changes in VFA, reduction in the organic matter, C:N ratio, micronutrients, the presence or absence of toxic materials in substrate and accumulation of inhibitory by-products or intermediates of the process.

2.6.1 Anaerobiosis

Methanogenesis being strict anaerobic microbial process, the major trophic groups and methanogens die in the presence of oxygen. Nyns (1986) designed a digester in such a way that the ecosystem is closed to atmospheric air so that the microflora is not exposed to oxygen tension.

2.6.2 Temperature

Temperature governs the process to a large extent since it controls the microbial growth rates in the digestion process. Sahota and Singh (1994) reported that maximum gas was produced in digesters maintained at 35-37°C. Low temperature, waste digestion is slower than mesophilic and thermophilic digestion.

2.6.3 pH

Methanogens are sensitive to even small changes in pH. During normal methane generation pH is maintained naturally in the range 6.5-8.0 by the bicarbonate buffer system. The optimal pH for methane production remained satisfactory between pH 6.6-7.6 (McCarty, 1974). The pH of the system depends on the rate at which the intermediates are fermented to methane. When the pH drops below 6.6, it adversely affects the activities of methane bacteria and hence methane generation, and the acid conditions of a pH 6.2 become toxic for them.

2.6.4 Reduction in organic matter

Volatile solids are commonly used as a measurement of organic matter in determining the loading and conversion efficiency during anaerobic digestion. Additional measurement of organic matter, such as, chemical oxygen demand; carbon in the feed, effluent and gas; and heating values of feed and effluent are often also utilized for estimation of organic composition (Speece, 1985).

2.6.5 Volatile fatty acids (VFA)

The most popular parameter for sludge digestion monitoring is the determination of volatile fatty acids (VFA) concentration. VFA accumulation reflected a kinetic uncoupling between acid producers and consumers and was typical in stress situation (Dague, 1968).

Singh *et al.* (1980) reported an optimum range of 2500-3500 ppm of VFA (in terms of acetic acid) to give maximum gas output and also to know the daily rate of utilization of acetate on the biogas digester fed on cattle waste as feedstock.

2.7 OPERATIONAL FACTORS

2.7.1 Composition of organic substrate

The major factor affecting the suitability and performance of biomass in anaerobic digestion is its composition. Chandler (1980) reported the linear relationship between VS reduction efficiency and lignin content to predict methane fermentation biodegradability.

2.7.2 Retention time- Organic loading rate

The rate at which cattle or organic waste is fed to the digester is referred to as volumetric loading rate. Loading rate is expressed in terms of either weight of volatile solids (TVS) added per unit volume of digester (Kg of TVS/m^3-d of digester volume). For operating high rate digester, the loading rate has to be 2-6 kg or TVS/m^3-d at an optimum detention time of 10-15 days and the concentration of organic solids (percentage of TVS) in the incoming waste has to be between 2 and 1 percent on a wet basis.

Lawrence (1971) as quoted by Jewell *et al.* (1976) reported the minimum SRT where the methane bacteria will not wash out as 7.5 days for 35°C, and 12 days for 28°C. Longer retention periods are desirable for complete

fermentation. Hobson *et al.* (1981) using 150 ℓ continuously stirred single-stage digester found that from 6 percent TS slurry gas production (70% methane) was 0.362 m^3 Kg TS^{-1} at 15 day detention time and 0.380 m^3 Kg^{-1} at a 20 day detention time at 35°C. Their experiment suggested an 18-20 day detention time for good digestion.

2.7.3 Degree of mixing

Mixing promotes improved digester performance by enhancing contact between organisms and substrates. But effect of mixing depend on digester size and configuration, feed composition and particle size, method of mixing, and a number of other factors.

The benefits of mixing are:

1. Helps in maintenance of uniform temperature throughout the digester.
2. Dispersion and dilution of potential inhibitors such as volatile acids, as in case of scum formation, there becomes a zone of local acid formation and concentration, which adversely affects the process.
3. Disintegration of coarse organic particles resulting in increased net surface area for increased microbial activity which will result in more degradation of the substrate.
4. Mixing also prevents the formation of fibrous mat at the surface of the fermenting material inside a digester in case of continuous systems.

2.8 NUTRITION AND GROWTH

The rate of activity of any class of microorganisms in the anaerobic digestion process is multiplicative function related to the concentration of all required nutrients. Absence of any one essential nutrient can stop the process as microbial regeneration time is a function of the concentration of nutrients present. The rate of substrate metabolism are also limited by nutrient limitation. Specific substrate utilization rate can be increased several fold when all required nutrients are in excess. Chynoweth (1981) reported that nitrogen deficient kelp resulted in digester imbalance presumably because of the selective inhibition of methanogenic bacteria.

Speece (1985) reported that the nutrients in decreasing order of importance are, nitrogen, sulfur, phosphorus, iron, cobalt, nickel, molybdenum, selenium, riboflavin and vitamin B_{12}. Methane fermentation will occur without all of the nutrients present but the rates will be reduced. All the nutrients of greater importance must be present before a lesser required nutrient will demonstrate stimulation. Ammonium appears to serve as the nitrogen source for all methanogens. Most methanogens utilize sulfide as a sulfur source but some can utilize cysteine (Mah *et al.*, 1976; Zehnder, 1978).

2.8.1 Nitrogen

The microbial population responsible for waste conversion and stabilization during anaerobic digestion require nitrogen, phosphorus and other elements for their optimal growth. A waste or substrate should consist of a proper

carbon: nitrogen: phosphorus ratio.

Nitrogen is required as a major nutrient. Excess of nitrogen, particularly in the form of ammonia, can inhibit the growth of the microbial population. Deficiency of nitrogen led to the non-formation of enzymes which are needed to utilize the carbon (Stafford *et al.*, 1980; Anderson *et al.*, 1982).

The carbon/nitrogen (C/N) ratio of the feedstock has been found to be useful parameter in evaluating digester performance and providing optimal nitrogen level. A C/N ratio of 50% was often cited as optimum (Ghose and Das, 1982).

2.8.2 Sulphur

The presence of sulphate may inhibit methanogenesis because the sulphate reducers have a lower half-saturation coefficient (kn) values that a methanogens for substrate such as hydrogen and acetate, the methanogens are dependent upon the production of sulfide for growth. According to (Bryant *et al.* 1971; Wolfe 1971 and Zehnder and Wuhrmann, 1977) methanogenic bacteria require fully reduced sulfur as sulfur source.

Khan and Tottier (1978) found that sulfur source of about 0.85 mM was essential for degradation of cellulose to methane. At 9 mM all inorganic sulfur compounds other than sulfate inhibited both cellulose degradation and methane formation. The inhibition increased in the following order:

Thiosulfate < sulfite < sulfide < H_2S

Sulfide was found to act as a sulfur source rather than a reducing agent (Wellinger and Wuhrmann, 1977).

2.8.3 Phosphate

Stafford *et al.* (1980) reported that phosphate was needed for both microbial growth and maintenance of an optimal pH and is also an important constituent of energy yielding system of bacterial population.

2.8.5 Trace elements

The complex chemical environment in an anaerobic digester makes it difficult to predict the equilibrium concentration of heavy metals available in solution for microbial nutrition. The metals may be chelated so strongly that bacteria are unable to utilize them. Bacteria produce substances called siderophores and enterochelins that capture and transport trace metals into the cell.

Diekert *et al.* (1980) earlier thought that nickel was not essential for growth of bacteria, but in last few years it became evident that nickel is an essential component of bacterial urease and in bacterial enzymes which convert hydrogen and carbon dioxide to acetate.

Iron, cobalt, molybdenum, selenium and tungsten have been shown to be stimulatory to methanogens (Jones and Stadtman, 1977).

2.8.5 Organic growth factors

Trace organic nutrient requirements for anaerobic digestion are not well

defined. Various trace organics stimulate methanogens. These are thiamine, vitamin B_{12}, riboflavin, hemoglobin, benzimidazole, proline and the complex organic sources, yeast extract, trypticase, milorganite etc.

Organic growth factors may be required for optimum activity of methanogens. These may include: Coenzyme M, Factor 420, acetate, 2-methyl butyric acid, vitamins, N-acetyl glucosamine, riboflavin, B_{12}.

2.9 TOXIC MATERIALS

Anaerobic digestion, being a complex process, its efficiency depends upon bacterial population, type of feed and environmental conditions. A sudden change in environmental, feeding conditions as incorporation of toxic material in digester lead to an unstable process and decreased efficiency of digester. The most common toxic materials which could reach the digester were many organic or inorganic material (Kugelman and Chin, 1971), heavy metals, antibiotics, phenols, chlorine compounds, detergents and sulfur compounds (Stafford *et al.*, 1980).

2.10 REACTOR DESIGN

Anaerobic digestion of biomass can be performed in variety of digesters promoting different HRT, SRT and MRT. These reactors are operated on different principles and their relative performance is affected by the biomass feedstock composition, since biomass feeds vary with regard to chemical composition, physical properties, so the reactor has to be designed according to the feedstock, economic viability and operation (Fannin and Biljetina, 1987).

In batch fed digesters the total volume is added to the reactor where it remain until digestion is complete. This is suited for biomass with high total solids. They are inexpensive, operation is easy and successful digestion is possible when fed with initial mixture of inoculum, organic substrates, nutrients and buffer.

In semi-continuous fed digester, daily feed stock is added and same amount is removed. This has advantage over batch fed as no feedback inhibition occurs and neither any toxic metabolites accumulates. Though the operation involves manual labour.

In Plug-flow reactors feed is added at the end and effluent is removed from the other. Mechanical mixing is not required as the biomass, when fed, passes through the reactor in plug like manner because the entry and exit is at the same time.

Other reactors are Up-flow solid reactor, Continuously stirred tank reactors, Upflow anaerobic sludge blanket reactors, Anaerobic filter reactors, Fluidized and Expanded bed reactors and Two-phase digesters.

Chapter III

MATERIALS AND METHODS

3.1 COLLECTION OF CATTLE WASTE

Cattle waste (without urine and bedding) which was not more than 24 hour old was collected from the Animal Farm of Punjab Agricultural University, Ludhiana. Cattle were routinely fed on concentrate, green fodder (berseem or millet) and dry fodder consisting of wheat straw.

3.2 SUBSTRATE

The feed stock, rice husk, used in this study was procurred from Rice sheller, village Barewal, Distt. Ludhiana and was stored as such in plastic bags for experimental work.

3.3 ANALYSIS OF CATTLE WASTE SLURRY AND RICE HUSK

Cattle waste slurry and rice husk were analysed for total solids, volatile solids and pH by procedure outlined in standard methods (APHA, 1985).

3.1 Determination of Total Solids

Clean silica crucible was heated at 100°C for 1 hour in hot air oven,

it was cooled in a desicator and weighed. Then weighed 1 g aliquot of the sample in the silica crucible. It was dried in hot air oven at 100°C to a constant weight overnight, cooled in a desicator and weighed again.

Calculation

Weight of empty silica crucible = A

Weight of crucible + sample = B

Weight of crucible + sample after drying at 100°C = C

$$\text{Moisture (\%)} = \frac{B - C \times 100}{B - A}$$

$$\text{Total solids (\%)} = \frac{C - A \times 100}{B - A}$$

The dried samples were retained for further estimation.

3.3.2 Total Volatile Solids

The dried samples obtained after determining total solids were then transferred into a muffle furnace and ignited the sample at 550±50°C for 1 hour, cooled in a desicator and weighed.

Calculations

Weight of empty silica crucible = A

Weight of crucible + sample = B

Weight of crucible + sample after drying at 100°C = C

Weight of crucible + sample after ignition at 550°C = D

$$\text{Total Volatile Solids (\%)} = \frac{C - D}{B - A} \times 100$$

3.3.3 Determination of pH

pH of the sample from various digesters was monitored every week using Systronics digital pH meter.

3.3.4 Determination of total volatile fatty acids

Total volatile fatty acids in the slurry sample were estimated by steam distillation method (Barnett and Reid, 1957).

Reagents

i) 5% oxalic acid

ii) 10% potassium oxalate

Procedure

The boiling flask of Markhan distillation apparatus was filled with distilled water and apparatus was steamed for 10 min. The condensate collected in the steam distillation compartment was emptied by punching the tube from the boiling flask. The water tap was opened to drain out the condensate. 1.0 ml of slurry sample and 2 ml of oxalic acid and potassium oxalate (1:1) mixture were added through the

inlet into the steam distillation compartment. It was ensured that the sample pipetted into the apparatus without touching the sides of the neck. With a received conical flask in position commenced the distillation, about 50 ml of distillate were collected and titrated against 0.05 N NaOH using phenolphathalein as an indicator. Before putting the next sample, the apparatus was thoroughly washed with distilled water.

Calculations

For calculation of total volatile fatty acid concentration, the following equivalent was used.

1 ml of 0.05 N NaOH = 0.05 mMol of acid

3.3.5 Reducing Sugars

Reducing sugars were determined by DNS method.

Reagents

i) DNS Reagent

ii) Potassium sodium tartrate

DNS Reagent

Dissolve 10 g of 3,5-dinitrosalicylic acid, 2.0 g of phenol and 0.5 g of sodium sulphate in 500 ml of 2% sodium hydroxide solution and then diluted to 1.0 L with distilled water. It was filtered and stored in a dark coloured bottle.

Potassium sodium tartrate

40 g of sodium potassium tartrate dissolved in distilled water and total volume was made 100 ml.

Procedure

One ml reaction mixture was mixed with 3 ml of DNS reagent. The mixture was heated for 10 min in a boiling water bath. In order to stabilize the colour, 1 ml of 40% sodium potassium tartrate was added to the mixture of reactants subsequent to developing of colour and prior to cooling to room temperature. The final volume in each tube was made to 7 ml with distilled water. The absorbance was measured at 575 nm using Spectronic.

3.4 INOCULUM PREPARATION

Inoculum was prepared by mixing 728 g fresh cattle waste with 91 g of digested cattle waste (slurry) from a cattle waste fed biogas plant. Then equal amount of water was added, mixed well, incubated for 3-4 days anaerobically at 37°C. This was used as inoculum.

3.4.1 Water displacement method for measuring gas

The slurries were kept in 5 l air-tight aspirator bottles with a working volume of 3.5 l. Gas was collected and measured by displacement of acidified salt saturated water solution in another aspirator bottle attached to the first with rubber tubing (Palanisamy, 1980).

3.5 MICROBIOLOGICAL ANALYSIS DURING ANAEROBIC DIGESTION

3.5.1 Isolation of cellulolytic microorganisms

3.5.1.1 Composition of medium and stock solution

The medium of Holdemann and Moore (1972) with slight modification was employed for isolation of cellulolytic microorganism from anaerobic digester

slurry. Concentration of salts used are given in $g\ell^{-1}$. Stock solution were made in distilled water and added in ml ℓ^{-1} medium.

Composition of media and stock solution are given below

Cellulolytic Medium	g/ℓ
Peptone	10.0
Yeast extract	10.0
Salt solution (SS II)	40.0
Rumen fluid	50.0
Resazurin stock solution (SS I)	1.0
Carboxy Methyl Cellulose	10.0
pH	7.0 ± 0.2

3.5.1.2 Rumen fluid

Two litres of digester fluid i.e. fermenting slurry of anaerobic digester was taken. The supernatant was decanted to one litre and 40 g yeast extract was added to it. Nitrogen was passed in the supernatant and it was incubated at 37°C overnight. Then centrifuged at 8000 rpm for 10 min in cold centrifuge. The supernatant was collected in rubber capped bottles, passed nitrogen and stored in the refrigerator.

3.5.1.3 Redox indicator (SS I)

Resazurin	0.10 g/10 ml

3.5.1.4 Stock solution (SS II)

	g/100 ml
K_2HPO_4	0.1
KH_2PO_4	0.1
$NaHCO_3$	0.2
NaCl	0.2
$CaCl_2$	0.02
$MgSO_4.7H_2O$	0.02

3.5.1.5 Preparation of stock solution

Stock solutions of substrates in requisite concentration were prepared in boiled and cooled distilled water under continuous gassing with oxygen free nitrogen. They were then distributed in anaerobic bottles maintaining anaerobic conditions. The bottles were sterilized by autoclaving at 121°C for 15 min.

3.5.1.6 Preparation of liquid medium

The ingredients were dissolved in boiled, distilled water and volume was made to one litre. The medium was filtered through absorbent cotton. It was cooled and under passage of oxygen free nitrogen, 1.0 ml of the resazurin stock solution (1 g l^{-1}) was added to the medium, pH of the medium was then adjusted to 7.0-7.2 by 1 N hydrochloric acid or 1% sodium bicarbonate solution.

PLATE I - EXPERIMENTAL SET UP OF BATCH DIGESTERS FED WITH CATTLE DUNG-PRETREATED RICE HUSK.

PLATE II - ROLL TUBE METHOD USED FOR ISOLATING ANAEROBIC MICRO-ORGANISMS FROM DIGESTERS.


Biogas Production From Buffalo Dung
Biogas Production From Alkali or NaOH Treated Rice Husk
Biogas Production From Buffalo Dung Treated Rice Husk
Biogas Production From Freesel Treated Rice Husk

The container in which the medium were dispensed were pregassed and desired volume of the medium was then transferred under continuous gassing. The containers were then closed securely using butyl rubber stoppers and aluminium seals. They were then autoclaved at 121°C for 15 minutes.

3.5.1.7 Preparation of solid medium

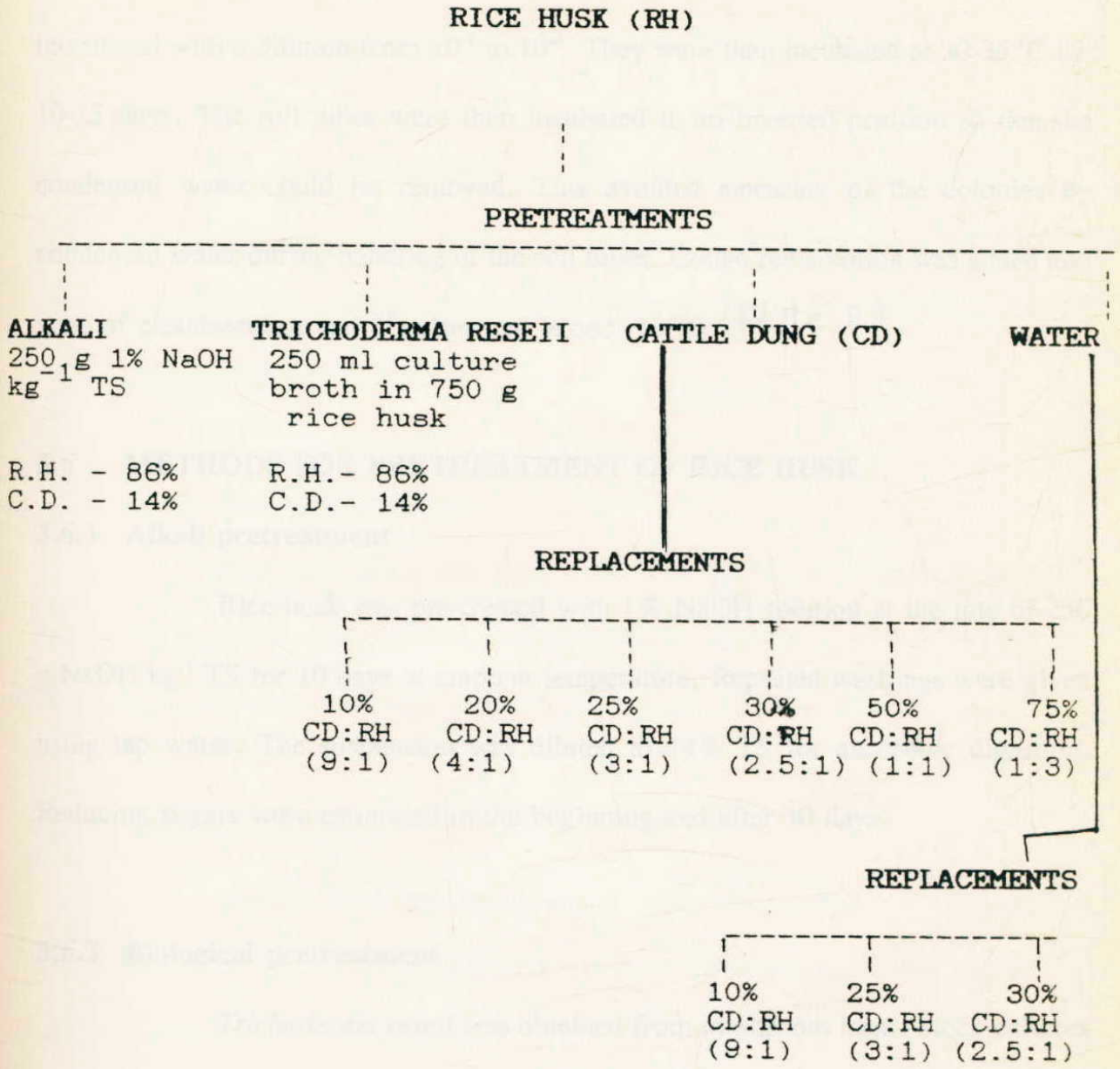
Liquid medium was cooled to 55°C. Agar in double strength was added to it maintaining the temperature at 55°C. Ten ml of molten medium was then dispensed in the roll tubes. The roll tubes with molten medium were then stoppered and autoclaved at 121°C for 15 min.

3.5.1.8 Collection of slurry sample

The sample was collected aseptically in sterile water blank in a way so that there was minimum exposure to oxygen.

3.5.1.9 Roll tube method for isolation of cellulolytic microorganisms

Six fold dilutions of the enrichment were done in the dilution medium. Roll tubes containing molten solid medium were kept at 50°C in a water bath. With the help of a sterile syringe and hypodermic needle 0.5 ml of the dilution was inoculated in one roll tube. Contents of the roll tube were mixed avoiding entrapment of gas bubbles. The tubes were then rolled under running tap water or on ice block



FLOW CHART OF VARIOUS PRETREATMENTS AND PERCENTAGES REPLACEMENTS OF CATTLE DUNG WITH PRETREATED RICE HUSK.

to have uniform layer of solid medium on the inner wall of the tube. Roll tubes were inoculated with a dilution from 10^{-4} to 10^{-6}. They were then incubated at 30-35°C for 10-15 days. The roll tubes were then incubated in an inverted position so that the condensed water could be removed. This avoided smearing of the colonies by condensed water during handling of the roll tubes. Congo red solution was added and zone of clearance observed (Teather and Wood, 1982). (Plate II)

3.6 METHODS FOR PRETREATMENT OF RICE HUSK

3.6.1 Alkali pretreatment

Rice husk was pre-treated with 1% NaOH solution at the rate of 250 g NaOH kg^{-1} TS for 10 days at ambient temperature. Repeated washings were given using tap water. The suspension was diluted to 14% TS for anaerobic digestion. Reducing sugars were estimated in the beginning and after 10 days.

3.6.2 Biological pretreatment

Trichoderma reseii was obtained from mushroom farm. Stock cultures were maintained on PDA (Potato dextrose agar) medium in test tube slants at 28°C.

Preparation of inoculum

Potato dextrose broth (containing 250 g potatoes boiled in water and filtered, 20 g glucose and total volume made to one litre) was used for the preparation of inoculum. The pH of the culture broth was adjusted to 5.0. The 250 ml capacity

conical flasks containing 100 ml PDA broth were inoculated with a few pieces of actively growing mycelium and incubated at 28 ± 1°C for 2 days.

Solid state fermentation

To carry out solid state fermentation 250 ml of PDA culture broth was added to each tray containing 750 gm wet rice husk. It was incubated at 28°C for 10 days. Aliquots of the contents of tray were employed for the estimation of reducing sugars initially and after 10 days.

3.6.3 Cattle dung pretreatment

Rice husk and cattle dung slurry (TS-7.5%) were mixed in an equal ratio (1:1) in a tray and fermented aerobically for 10 days at room temperature. Reducing sugars were estimated in the beginning and after 10 days.

3.6.4 Water pretreatment

To rice husk equal amount of water was added 1:1. The aerobic fermentation was carried out in a tray for 10 days. Reducing sugars were estimated in the beginning and after 10 days.

3.7 EFFECT OF ALKALI PRETREATMENT ON BIOGAS PRODUCTION RATE AND MICROFLORA

In digester alkali pretreated rice husk and water in ratio 1:1 along with 12% inoculum was added. Digester was allowed to run for 4 weeks at room

temperature. Reducing sugar content was estimated. TS and pH were analysed weekly. Microbial count was also taken at weekly intervals.(Plate I)

3.8 EFFECT OF BIOLOGICAL PRETREATMENT ON BIOGAS PRODUCTION

In digester *T. reseii* pretreated rice husk, water (1:1), 12% inoculum was placed. Digester was allowed to run for 4 weeks at room temperature. Reducing sugars, TS and pH and microbial count were analysed weekly.(Plate I)

3.9 EFFECT OF REPLACEMENT OF CATTLE DUNG WITH CATTLE DUNG PRETREATED RICE HUSK ON BIOGAS PRODUCTION

Four digesters were set up for carrying batch scale digestion at ambient temperature. Different ratios of cattle dung and rice husk were used. The rate of inoculum in all digesters was 4%. Water was added to give required initial total solid content. The parameters such as TS, VS, C, N and CH_4 content of biogas and pH values were analysed after weekly intervals. Microbial count was taken at weekly intervals.(Plate I)

3.9.1 Digester D_1 was control and contained cattle dung, water (1:1) and 4% inoculum. Initial Total solids were 7.4%.

3.9.2 Digester D_2 (25% Replacement) It contained rice husk and cattle dung in ratio 1:3. Initial Total solids were 10.5%.

3.9.3 Digester D_3 (50% Replacement) It contained rice husk and cattle dung in ratio of 1:1. Initial Total solids were 11%.

3.9.4 Digester D_4 (75% Replacement) It contained rice husk and cattle dung in ratio 3:1. Initial Total solids were 17%.

All digester were allowed to run for a period of 6 weeks at room temperature.

3.10 EFFECT OF DIFFERENT RATIOS OF HUSK AND CATTLE DUNG ON BIOGAS PRODUCTION

Further experiments were conducted varying the ratios of rice husk and cattle dung (low percentages replacement of cattle dung with rice husk). The rate of inoculum was 4% in all the digesters. Equal amount of water was added in all the digesters. Digestion was carried out for 6 weeks at ambient temperature. Digester slurry was analysed weekly for various parameters such as TS, VS, pH, VFA and microbial count.

3.10.1 Digester A (10% Replacement) It contained rice husk, cattle dung, in ratio of 1:9 and initial Total solids were 10.0%.

3.10.2 Digester B (20% Replacement) It contained rice husk and cattle dung, in ratio of 1:4 and initial Total solids were 10.0%.

3.10.3 Digester C (30% Replacement) was fed with rice husk and cattle dung, in ratio of 1:2.5. Initial Total solids were 10.0%.

3.11 EFFECT OF WATER PRETREATED RICE HUSK: CATTLE DUNG RATIO ON BIOGAS PRODUCTION

The water pretreated rice husk was used for this study. Different ratios of rice husk and cattle dung were fed in digesters for carrying batch scale digestion at ambient temperature. All digesters contained 4% inoculum and equal amount of water. Digester slurry was analysed for its TS, VS, pH and VFA content at weekly intervals. Microbial count was also taken at weekly intervals.

3.11.1 **Digester I** : Rice husk:cattle dung (1:9)

Digester II Rice husk:cattle dung (1:3)

Digester III Rice husk:cattle dung (1:2.5)

3.12 COMPARISON BETWEEN SEMI-CONTINUOUS AND BATCH DIGESTION

A biogas digester of 5 ℓ capacity containing cattle dung and rice husk in the ratio of 4:1 was operated semi-continuously by feeding once on alternative day with 40 ml of rice husk, cattle dung and inoculum slurry (40 ml of sample was withdrawn every time).

At the same time a batch digester was set up in the lab with cattle waste and rice husk in ratio 4:1. Retention time was 20 days in semi-continuous digester whereas batch digester was started immediately.

Slurry from semi-continuous digesters was analysed on alternative days for its TS, VS and VFA content, pH was also monitored on alternative days. Microbial count was taken on alternative days.

Slurry from the batch digester was analysed weekly and measured its TS, VS, and VFA content. The microbial count was also taken at weekly intervals. pH was also monitored every week.

Chapter IV

RESULTS AND DISCUSSION

The availability of agricultural residues being much greater as against the limited supply of animal dung, rice husk was exploited for the present study. Rice husk was pretreated by various methods and the most effective method which yielded maximum gas on anaerobic digestion was further employed to study the inoculum substrate ratio and then compared semi-continuous and batch-scale digestion. Rice husk did not need size reduction.

4.1 Effect of alkali (NaOH) and biological (*Trichoderma reseii)* pretreated rice husk on biogas production :

The experiment to evaluate biogas potential from alkali and biological pretreated rice husk was conducted from May to June and range of temperature was 36-42°C.

The effect of total solids (TS), pH and reducing sugars on biogas production rate was studied in two digesters fed with rice husk as feedstock which had been pretreated with alkali and biological method. The results are shown in Table 1 and Fig. 1.

Table 1a : Biodegradation of TS and changes in pH pattern during gas production in digesters fed with alkali pretreated and *T. reseii* pretreated rice husk

Weeks	A			B		
	TS (%)	pH	*Gas production (L)	TS (%)	pH	*Gas production (L)
0	14.0	7.5	_-	14	7.5	-
1	9.8	7.9	0.780	12.3	7.2	0.500
2	8.7	7.8	1.800	9.4	7.0	0.750
3	8.1	7.7	1.100	8.8	6.5	0.320
4	7.5	8.2	0.800	8.3	6.5	0.100

A = Alkali Pre-treatment

B = Biological Pre-treatment

* in terms of litres of water displaced

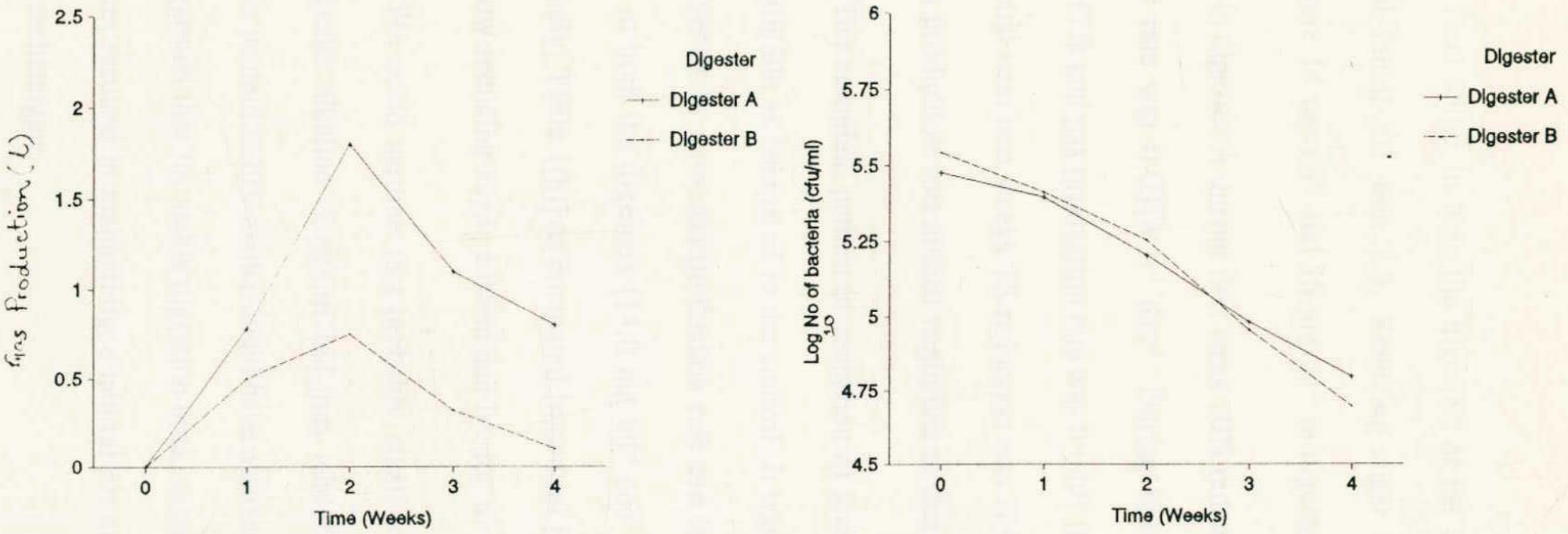


Fig 1. Effect of incubation time on microbial count and gas production from digesters fed with alkali and biologically pretreated rice husk

Total solids in both the digesters at the beginning of the experiment were 14% and initial pH was 7.5. Reducing sugar initially were nil but after pretreatment were 14 mg ml^{-1} and 16 mg ml^{-1} in digesters A and B respectively.

In digester A during first week 30% reduction of TS was recorded and gas production rate was 0.0318 $l l^{-1}$ day^{-1}. During next two weeks the total solid reduction was 17% and gas production rate was 0.0591 $l l^{-1}$ day^{-1}. In digester B during the first week and next two weeks TS reduction was 12% and 28% respectively and consequent gas production was almost negligible at that time.

The complete pattern of reduction of total solids in digester A and B was almost negligible as compared to the control. It was 75% less in digester A and 90% less in digester B. Low gas production rate can be attributed to low reducing sugars content in both the digesters [14.0 mg ml^{-1} and 16.0 mg ml^{-1} in digesters A and B respectively, Table 1(b)] as compared to control [35 mg ml^{-1} reducing sugars, Table 2(b)]. Low reducing sugar content can be due to incomplete pretreatment.

We could assume that probably alkali was not washed off properly making the digester alkaline in nature and thus inhibiting methanogenesis. Higher content of crude protein in dry matter could have affected the stable digestion. Laura (1971) had suggested that for stable digestion urea, calcium carbonate and manure if added in digester resulted in maintaining a neutral pH and favourable environmental is created for methanogens.

Table 1b : Relative performance of alkali and *T. reesii* pretreated rice husk fed anaerobic digester

Parameter	Digester	
	A	B
Digester working volume (l)	3.5	3.5
Duration of experiment (days)	28	28
Retention time (days)	10	10
Reducing sugar content after 10 days (mg ml^{-1})	14	16
Performance :-		
TS % reduction	46%	40%
Cumulative gas production (l)	4.480	1.670
Gas production rate (l l^{-1} day^{-1})	0.046	0.019

Anaerobic digestion of cattle waste is a slow process, mainly because it is usually poor in biodegradable organics. On the other hand vegetative matter is highly degradable and may create the problem of digester failure due to the fast development of organic acids (Hobson *et al.*, 1981). Hence rice husk residues were co-digested with cattle waste to increase biogas productivity, without the problem of digester failure.

Sun *et al.* (1987) reported that the use of crop manure mixture increases the rate and extent of plant biomass degradation and thereby reduces the retention time as compared to crop waste alone.

4.2 Effect of replacement of cattle dung with cattle dung pretreated rice husk on biogas production :

From the previous experiment it is learnt that co-digestion can prevent digester failure. So the approach of two stage digestion, aerobic and anaerobic fermentation were carried out in this experiment. This permitted complete digestion of lignocellulosic complexes at faster rates and accomplished a reduction in the level of crystallinity and disruption of such lignocellulosic structures.

To study the effect of total solids (TS), volatile solids (VS), pH and volatile fatty acids (VFA) on gas production in digesters fed with different concentrations of cattle dung and cattle dung pretreated rice husk the experiments were carried out. The results are shown in Table 2 and Fig. 2 & 3. The reducing

sugar content after pretreatment in digester D_1, D_2, D_3 and D_4 were 35, 60, 22 and 20 mg ml^{-1} respectively.

The initial total solid content of control was 7.4% in digester D_1, and it varied as 10.5%, 11% and 17% in digesters D_2, D_3 and D_4 respectively. The peak value in biogas production rate was in D_2 digester (0.1670 $l l^{-1}$ day^{-1}) indicating that there was maximum degradability and utilization of feedstock materials. Gas production rate was 0.0213$l l^{-1}$ day^{-1} in D_3 digester and was almost negligible 0.00238 $l l^{-1}$ day^{-1} in D_4 digester. The low biogas yield from rice husk could also be due to high lignin content in D_3 and D_4 digester as 50% and 75% replacement was there. The efficiency of utilization of raw material depended on the overall digestibility of the substrate, which is primarily a function of the microflora in cattle dung and lignin content in rice husk. Sun *et al.* (1987) have reported that higher initial total solids boost total biogas yield. But low gas production could be due to low inoculum rate 4% (46 x 10^6 cfu ml^{-1}) whereas they have used 30%. Secondly it was supplemented with pig manure which has 30-50% easily digestible dry matter. It also has high nitrogen content and this non-ammonia nitrogen is converted to ammonia during fermentation modifying the pH value.

In D_1 digester (Fig. 2) degradation of total solids during first week and next two weeks were 12% and 23% respectively and consequent gas production was 0.1020 $l l^{-1}$ day^{-1} and 0.1079 $l l^{-1}$ day^{-1} respectively. In D_2 digester degradation of TS was 29% and 36% during first 3 weeks and consequent gas production was 0.1979

Table 2a : Comparative changes in TS, VS, pH and VFA during gas production in digesters fed with different concentrations of pretreated[1] rice husk and cattle dung

Weeks	D_1 Control					D_2					D_3					D_4				
	TS %	VS %	pH	[3]VFA	[2]Gas production	TS %	VS %	pH	[3]VFA	[2]Gas production	TS %	VS %	pH	[3]VFA	[2]GAs Production	TS %	VS %	pH	[3]VFA	[2]Gas production
0	7.4	5.4	7.5	12.5	-	10.5	6.0	7.5	16.0	-	11	6.1	7.5	17.5	-	17	11	7.0	12.5	0.150
1	6.5	5.0	6.0	72.5	2.500	7.4	5.7	6.5	62.5	4.850	9.5	5.2	6.8	22.5	0.310	13	8.8	7.0	17.5	0.100
2	5.1	4.5	5.5	87.5	2.790	5.3	4.9	6.2	72.5	4.010	8.0	4.9	6.5	27.5	0.230	9.2	7.2	6.5	20.0	0.100
3	5.0	3.3	5.5	77.5	2.500	4.7	3.0	6.0	82.5	4.650	6.2	3.5	6.2	22.5	0.700	7.6	5.8	6.2	17.5	-
4	4.2	3.1	6.0	57.5	4.250	4.5	2.8	6.7	62.5	3.370	5.0	3.0	6.5	17.5	0.950	5.4	4.9	6.8	12.5	-
5	3.9	2.5	8.5	22.5	3.370	4.4	1.9	7.9	47.5	3.730	3.3	2.5	7.8	10.0	0.900	4.2	4.1	8.0	10.0	-
6	3.5	1.5	9.0	20.0	2.900	4.3	1.6	8.0	37.5	3.950										

1 Rice husk pretreated by soaking in slurry
2 in terms of litres of water displaced
3 in terms of mMol of acid per 1000 ml
D_1 Control containing only cattle dung
D_2 25% replacement with rice husk
D_3 50% replacement with rice husk
D_4 75% replacement with rice husk

Table 2b : Digester performance of pretreated rice husk and cattle dung in varying concentrations

Parameter	Digester			
	D_1	D_2	D_3	D_4
Digester working volume (ℓ)	3.5	3.5	3.5	3.5
Duration of experiment (days)	42	42	35	35
Retention time (days)	10	10	10	10
Reducing sugar content after 10 days (mg ml^{-1})	35	60	22	20
Performance				
TS reduction %	52.7	59.0	70.0	75.0
VS reduction %	72.2	75.3	65.5	62.7
Cumulative gas production (ℓ)	18.310	24.560	3.140	0.350
Gas production rate ($\ell\ell^{-1}$ day^{-1})	0.1245	0.1670	0.0256	0.0028

ll^{-1} day^{-1} and 0.1767 ll^{-1} day^{-1}. In digester D_3 and D_4 TS reduced by 13-23% during first week and 34%-41% during next two weeks and consequent gas production was 0.0126 ll^{-1} day^{-1}, 0.0061 ll^{-1} day^{-1} and 0.0189 ll^{-1} day^{-1}, 0.0040 ll^{-1} day^{-1} respectively. Various organic constituents were also degraded to a greater extent in the plant-cattle waste mixture than in cattle waste alone. The major part of fermentation was complete in five weeks. Our results are in agreement with Hassandar *et al.* (1989) and Shawarby *et al.* (1984) where batch digesters were fed with alkali treated crop residue and cattle waste, 17-31% biodegradation and maximum 57% methane were observed. In D_1 digester maximum gas was produced during 4th week and degradation of TS after 4th week remained almost constant. The highest gas production rate was in the Ist IInd and IIIrd week and it remained practically same after IVth week (Table 2a). The maximum degradation of TS was 55% till third week.

The total solids degradation pattern was 52.7%, 59%, 70% and 75% (Table 2b) in digesters D_1, D_2, D_3 and D_4 respectively. In D_3 digester i.e. with 50% replacement TS degraded by 70% and gas production rate was 0.0213 ll^{-1} day^{-1} while in D_4 digester with 75% replacement TS degraded by 75% and still gas production was negligible. Higher total solids degradation should have attributed to higher biomethanation but on contrary gas production was negligible. The reason for failure here could be low nitrogen content and organic overloading in D_3 and D_4 digesters. Whereas, in D_2 digester, with 25% replacement, C:N ratio was optimum to carry out

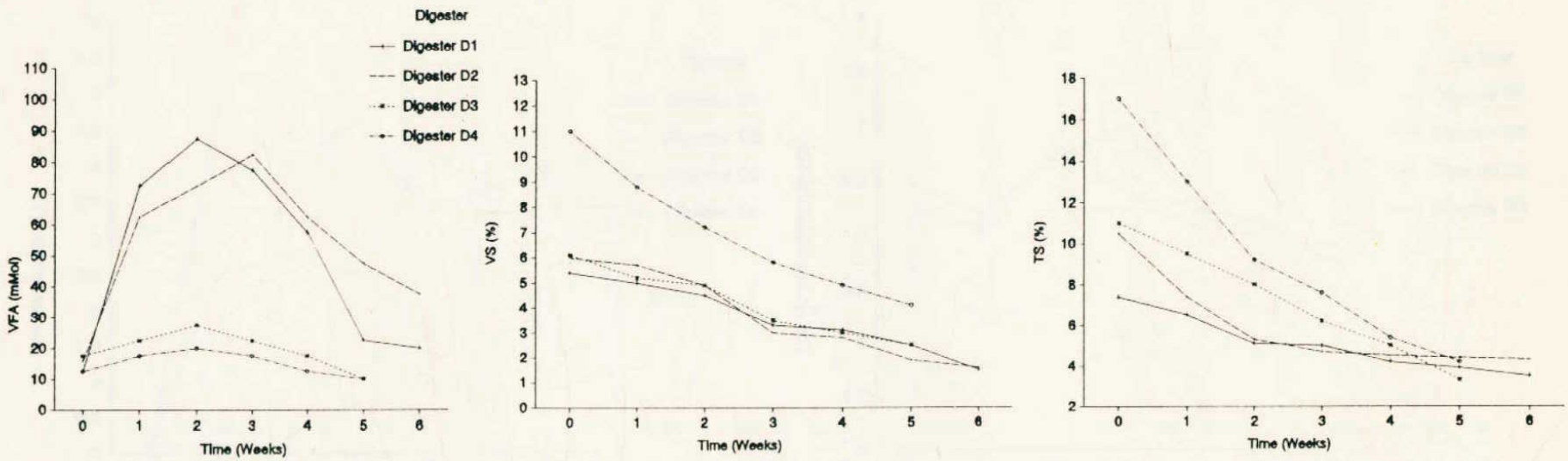


Fig 2. Changes in total solids, volatile solids, and volatile fatty acids in anaerobic digesters fed with varying concentrations of cattle dung and cattle dung pretreated rice husk

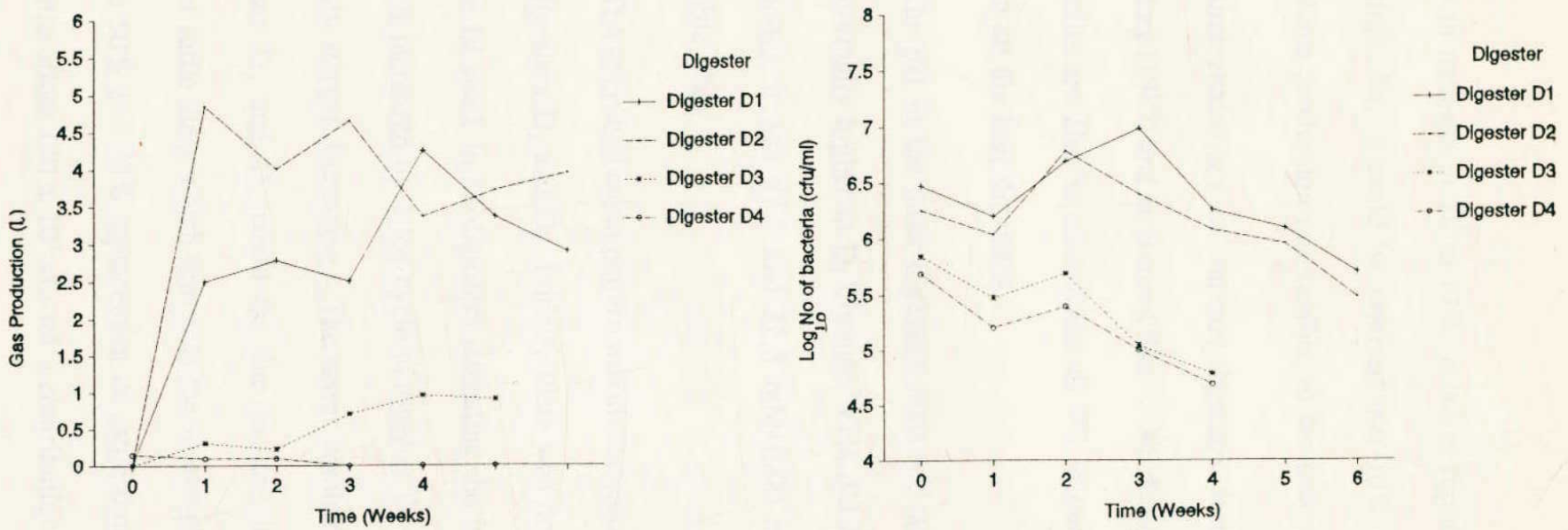


Fig 3. Microbial count and gas production from digesters fed with different concentration of pretreated rice husk and cattle dung

fermentation. With increase in TS to 17%, it led to significantly lesser reduction in total volatile solids. So, it could be inferred that 10% initial TS appeared to be optimum as methane production rate reached to the maximum.

Biodegradability of various digesters feeds were studied in batch digester by Stuckey (1983) and he showed that VS destruction varied from 11.1%-84.9%. Our results are also in accordance as VS in our studies also varied from 62.7%-75.3% in all the four digesters.

The pH in the three digesters remained within the range of 6.0-8.0 while it was abnormally higher in D_1 digester. VFA did not accumulate much in all the digesters though it was 87.5 and 82.5 mM/1000 ml in digesters D_1 and D_2 respectively (Table 2a).

The microbial count pattern was almost same in all the digesters though it was less in digesters D_3 and D_4. Initially there was decrease in count by 1-2 log cycle during the Ist week in all digesters indicating the lag phase (Fig. 3) and after acclimatization it increased by 1 log cycle in digester D_1 and D_2 after 2nd week and then it gradually started decreasing. The lesser number of bacteria (28×10^3 cfu ml^{-1}) in digester D_3 and D_4 could be due to low inoculum content and less concentration of cattle dung which serves as the source of inoculum in digesters . In digesters where 50% and 75% replacement of cattle dung with rice husk served as the feedstock microflora (40×10^3 cfu ml^{-1}) contribution for biogas production was very less. The other possible reasons for inefficient digestion could be improper C:N

ratio in the digesters. Overloading and higher initial total solid content could have hindered the thorough mixing of content. So we can say that the total biogas yield and biogas production rate could have decreased due to higher rate of replacement of cattle dung with rice husk.

4.3 Effect of low percentages replacement of cattle dung with pretreated rice husk on biogas production :

Digester performance of three batch digesters are summarized herewith. Table 3 and Fig. 4 gives the extent of conversion of total solids (TS), volatile solids (VS), and volatile fatty acid in digesters fed with different concentrations of pretreated rice husk and cattle dung.

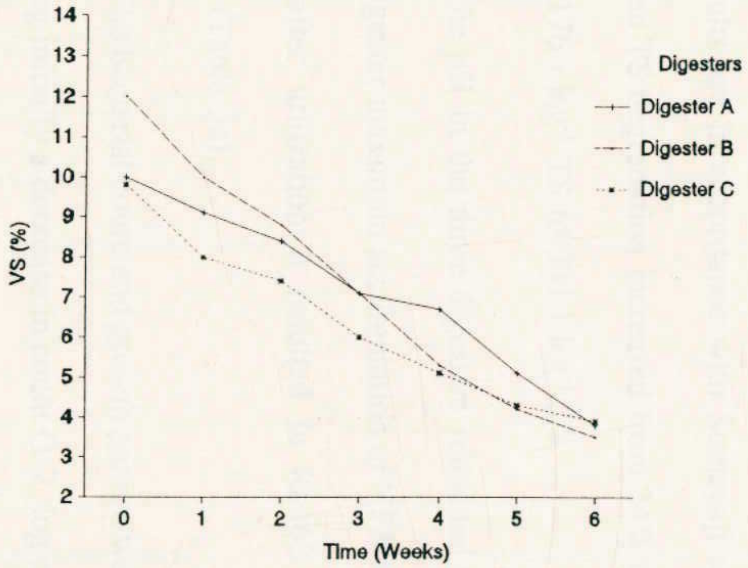
The initial TS content was 10% in all the three digesters A (10% replacement), B (20% replacement) and C (30% replacement) (Table 3a).

The reduction of TS in digesters A, B and C varied from 16%, 22% and 11% and consequent gas production rate was 0.079, 0.122 and 0.075 $l l^{-1}$ day^{-1} respectively during the 1st week suggesting that there is a direct relationship between TS turnover and gas production when C:N ratio is optimum and no organic overloading is there. In the next 2 weeks TS reduction was 17%, 24% and 21% respectively in digesters A, B and C and consequent gas production rate was 0.140 $l l^{-1}$ day^{-1}, 0.274 $l l^{-1}$ day^{-1} and 0.163 $l l^{-1}$ day^{-1} respectively. During further next 2 weeks there was 7.2%, 33% and 25% reduction in TS in digester A, B and C and gas

Table 3a : Relative changes in TS, VS, pH and VFA pattern on gas production in digesters fed with different concentrations of pretreated[1] rice husk and cattle dung

Weeks	A					B					C				
	TS %	VS %	pH	[2]VFA	[3]Gas production	TS %	VS %	pH	[2]VFA	[3]Gas production	TS %	VS %	pH	[2]VFA	[3]GAs Production
0	10	10	7.2	10.0	-	10	12.0	7.4	12.5	-	10	9.8	7.7	15.0	-
1	8.4	9.1	7.2	17.5	1.950	7.8	10.0	7.2	57.5	3.000	8.9	8.0	7.5	22.5	1.850
2	7.6	8.4	7.0	42.5	2.770	6.7	8.8	7.0	62.5	7.250	7.5	7.4	7.2	27.5	3.780
3	6.9	7.1	6.5	57.5	4.130	5.9	7.1	6.5	87.5	6.200	7.0	6.0	6.5	42.5	4.250
4	6.6	6.7	6.3	42.5	4.000	5.8	5.3	6.8	72.5	5.630	6.2	5.1	6.9	37.5	3.100
5	6.4	5.1	7.4	37.5	3.950	3.9	4.2	7.8	57.5	3.950	5.2	4.3	7.2	17.5	3.900
6	4.1	3.8	8.0	22.5	2.310	3.1	3.5	8.1	22.5	2.250	4.1	3.9	8.0	10.0	3.370

1 Rice husk pretreated by soaking in cattle dung slurry
2 in terms of mMol of acid per 1000 ml
3 in terms of litres of water displaced
A 10% replacement of cattle dung with pretreated rice husk
B 20% replacement of cattle dung with pretreated rice husk
C 30% replacement of cattle dung with pretreated rice husk



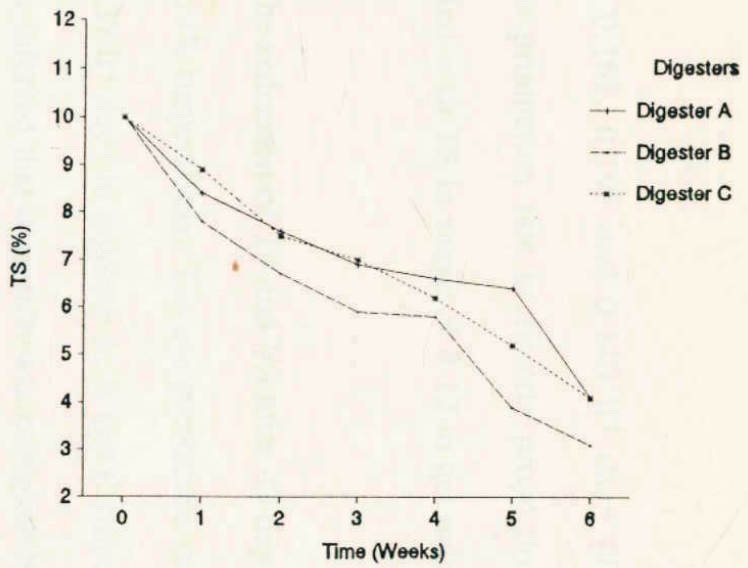


Fig 4. Changes in total solids and volatile solids in digesters fed with different concentrations of pretreated rice husk and cattle dung

production was 0.162, 0.195 and 0.140 ll^{-1} day^{-1} (Table 3a). Our observations suggests that gas production rate is directly proportional to TS degraded. So the digester should fed with TS in range of 8-12 to get maximum gas production by TS turnover.

The reduction of TS and VS after 42 days in digester A, B and C was 59%, 67% and 59% respectively. The gas production rate was 0.130 ll^{-1} day^{-1}, 0.213 ll^{-1} day^{-1} and 0.137 ll^{-1} day^{-1} in digester A, B and C respectively (Table 3b & Fig. 5). From this it was inferred that digestion was complete by 7th week.

The peak gas production rate 0.213 ll^{-1} day^{-1} in digester B is attributed mainly to a higher percentage of degradable solids in cattle dung rice husk mixture (20%). Our results are in accordance with Somayaji and Khanna (1994) who also showed that when TS degradation increased from 24% to 51% the daily biogas yield increased from 176 l kg^{-1} TS to 331 l kg^{-1} TS.

The pH in the three digesters remained within the range of 6.3-8.1. In balanced B digester maximum accumulation of VFA during the 3rd week was 87.5 mM and was after utilization it resulted in (0.163 ll^{-1} day^{-1}) increased biogas production rate (Table 3a).

The bacterial count and growth pattern was similar in all the 3 digesters (Fig. 5), showing initially a decrease in count (1-2 log cycle) indicating the lag phase of bacteria and after acclimatization the count increased during the IInd week by 1

Table 3b : Digester performance of water pretreated rice husk fed anaerobic digester

Parameter	Digester		
	A	B	C
Digester working volume (ℓ)	3.5	3.5	3.5
Duration of experiment (days)	42	42	42
Retention time (days)	10	10	10
Reducing sugar content after 10 days (mg ml^{-1})	45	65	45
Performance			
TS reduction %	59	67	59
VS reduction %	62	70.8	60.2
Cumulative gas production (ℓ)	19.130	31.350	20.250
Gas production rate (ℓ $ℓ^{-1}$ day^{-1})	0.1300	0.2130	0.1377

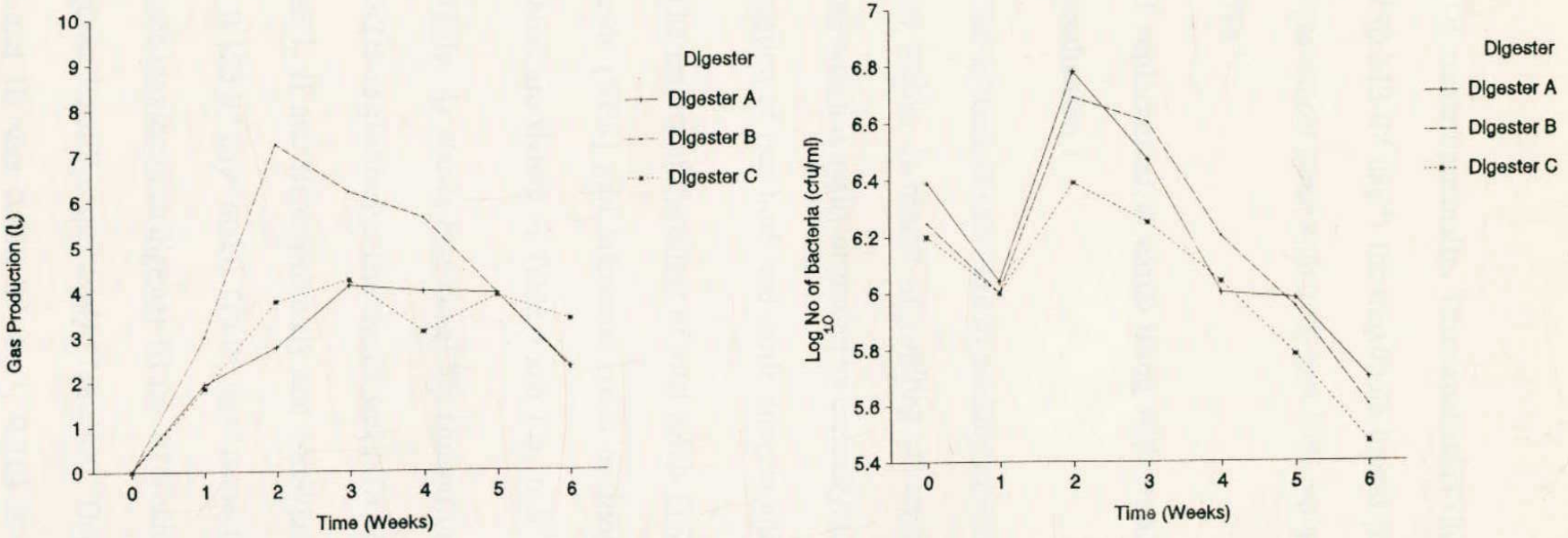


Fig 5. Microbial count and gas production from digesters fed with cattle dung pretreated rice husk and cattle dung

log cycles then it decreased gradually. This experiment showed that 20% replacement is recommended (0.213 l^{-1} day^{-1}) for maximum biogas production rate whereas gas production rate was almost same with 10% and 30% replacement i.e. 0.130 l^{-1} day^{-1} and 0.1377 l^{-1} day^{-1}.

4.4 Effect of replacement of cattle dung with water pretreated rice husk on biogas production :

Pretreatment of rice husk by soaking in water was found to be simplest and economically viable. It results in swelling of crystalline lignin structure and exposing cellulose which is easily degraded by cellulolytic microflora present in cattle dung, on co-digestion of rice husk and cattle dung in anaerobic digester.

The results of the effect of total solids (TS), volatile solids (VS), pH, volatile fatty acids (VFA) and microbial count on biogas production from water pretreated rice husk are shown in Table 4 and Fig. 6 & 7.

Table 4a shows that initial TS content in digester I, II and III were 9.2, 9.8 and 10.2% respectively. During Ist week TS degraded by 5.4%, 19% and 9.8% in digester I, II and III respectively and consequent gas production rate was 0.232 l^{-1} day^{-1}, 0.123 l^{-1} day^{-1} and 0.175 l^{-1} day^{-1} respectively. Total reduction of TS after 49 days were maximum in digester III i.e. 61% whereas TS reduced by 53.2% and 57% in digester I and II respectively (Table 4b). The total gas production rate in digesters I, II and III was 0.232 l^{-1} day^{-1}, 0.164 l^{-1} day^{-1} and 0.157 l^{-1} day^{-1}

Table 4a : Relative changes in TS, VS, pH and VFA on gas production in digesters fed with different concentrations of water pretreated rice husk and cattle dung

Weeks	I					II					III				
	TS %	VS %	pH	[1]VFA	[2]Gas production	TS %	VS %	pH	[1]VFA	[2]Gas production	TS %	VS %	pH	[1]VFA	[2]Gas Production
0	9.2	8.5	7.0	12.5	-	9.8	11	7.2	22.5	-	10.2	8.7	7.0	7.5	-
1	8.7	7.6	7.0	37.5	5.700	7.9	8.40	7.1	42.5	3.020	9.2	7.9	6.9	12.5	5.330
2	7.2	6.4	7.0	42.5	7.250	7.6	7.1	7.0	42.5	3.900	8.1	7.0	6.9	17.5	6.700
3	6.7	5.9	6.6	72.5	7.300	7.2	5.8	6.5	42.5	5.650	6.3	5.9	7.1	37.5	4.250
4	6.3	4.1	7.4	62.5	6.600	6.9	4.7	6.4	62.5	4.000	6.0	4.2	7.3	42.5	3.750
5	6.1	3.4	7.8	42.5	5.330	7.1	3.5	7.1	57.5	5.550	5.7	4.0	7.7	37.5	2.850
6	5.9	2.8	8.0	37.5	5.150	5.8	2.1	7.5	22.5	4.420	4.2	3.1	8.0	22.5	2.100
7	4.3	1.3	8.2	22.5	2.500	4.2	1.7	8.7	17.5	1.750	3.9	2.4	8.2	12.5	2.000

1 in terms of mMol of acid per 1000 ml

2 in terms of litres of water displaced

I 10% replacement of cattle dung with water pretreated rice husk

II 25% replacement of cattle dung with water pretreated rice husk

III 30% replacement of cattle dung with water pretreated rice husk

Table 4b : Digester performance of water pretreated rice husk and cattle dung fed batch digester

Parameter	Digester		
	I	II	III
Digester working volume (ℓ)	3.5	3.5	3.5
Duration of experiment (days)	49	49	49
Retention time (days)	10	10	10
Reducing sugar content after 10 days (mg ml^{-1})	85	70	52
Performance			
TS % reduction	53.2	57	61
VS reduction %	84.7	84.5	72.4
Cumulative gas production (ℓ)	39.830	28.290	26.980
Gas production rate($\ell\ \ell^{-1}$ day^{-1})	0.232	0.164	0.157

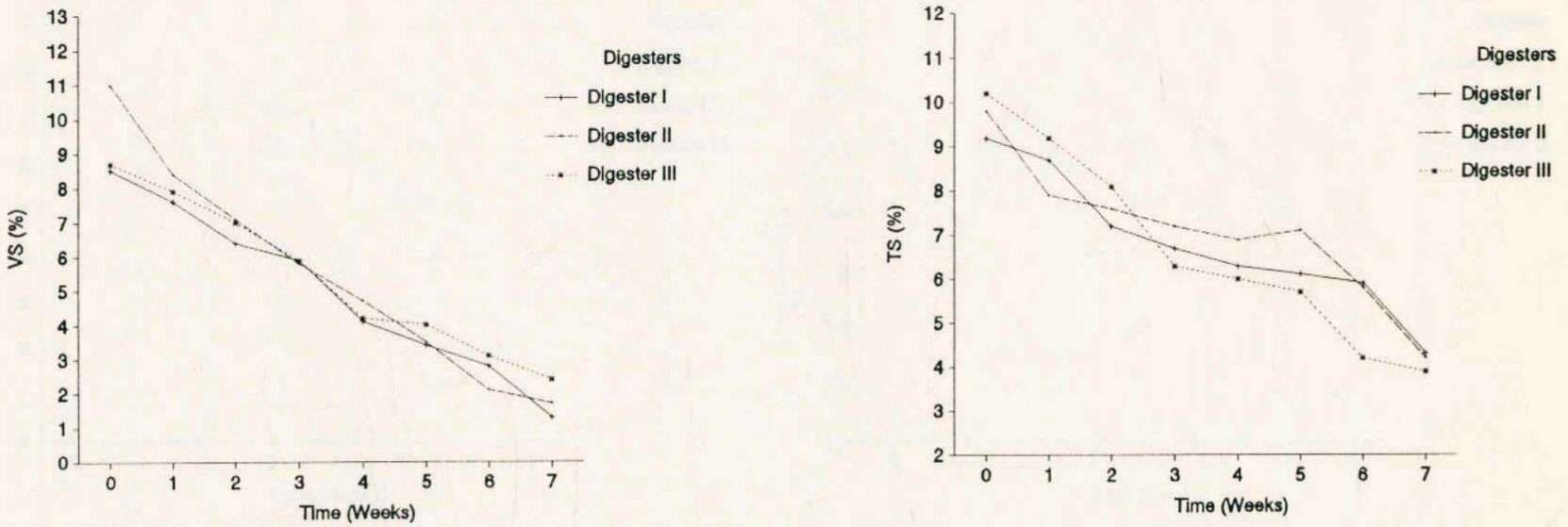


Fig 6. Changes in total solids and volatile solids in digesters fed with water pretreated rice husk and cattle dung

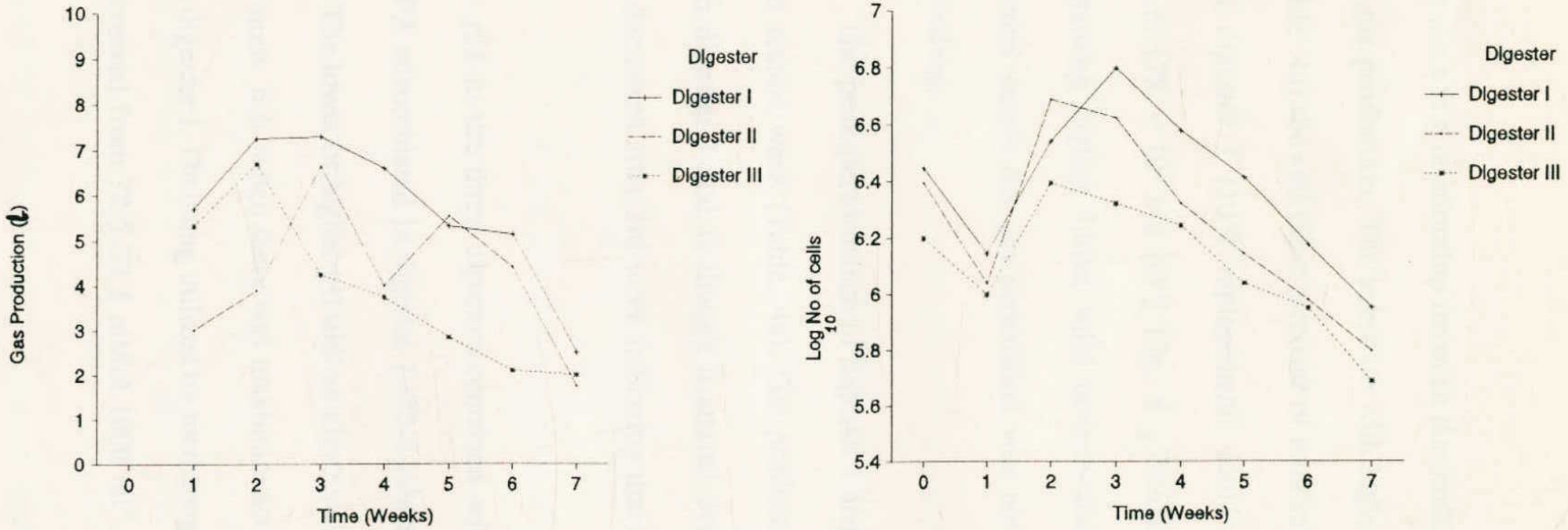


Fig 7. Effect of incubation time on microbial count and gas production from digesters fed with different concentrations of pretreated rice husk and cattle dung

indicating that there is a relationship between the biodegradability of solid content of substrate and gas production. The substrate with highest initial total solids (digester III - , Table 4a) showed rapid turnover of total solids but the highest rate of gas production in digester I (10% replacement) could be attributed to the greater microbial count (78 x 10^5 cfu ml^{-1}) Fig. 4 , Pathak *et al.* (1985) observed that substrate containing highest initial solid concentration (15.2%) gave the highest reduction of total solids but gas production was not the highest, the reason was organic overloading.

The peak performance of digester I and II was in third week and for digester III in second week (Table 4a). Gas production was practically same after third week in digester I and II though it started decreasing but in digester III it continuously decreased after 3rd week indicating that biodegradability was complete after that.

pH in the three digesters remained within the range of 6.4-8.7 and maximum VFA accumulated in digester I (72.5 mMol/100 ml) during the third and fourth week. The lower or higher pH did not effect stable digestion in these digesters. pH was minimum 6.6 when there was maximum accumulation of VFA (72.5 mM 1000 ml^{-1}) in digester I. On being utilized by methanogens pH increased from 6.6-8.2 and VFA decreased from 72.5-22.5 mMol 1000 ml^{-1}.

The bacterial growth pattern is shown in Fig 7. Initially the count was almost same being highest in digester I (34 x 10^5 cfu ml^{-1}). During Ist week the count decreased by 1 log cycle in digester II and III (89 x 10^4 cfu ml^{-1}) but remained almost same in digester I and it increased (98 x 10^5 cfu ml^{-1}) during the IInd and IIIrd week and then a gradual decrease was observed indicating the death phase which is mainly due to feed back inhibition, nutrient depletion or accumulation of toxic metabolites.

The maximum gas production rate in digester I could be attributed to the highest microbial count (26 x 10^6 cfu ml^{-1}) due to presence of higher percentage of inoculum in form of cattle dung and low TS content being fed with only 10% rice husk.

4.5 Comparison between semi-continuous and batch digester performance

The results of analysis of slurry before and after digestion are given in Table 5 and Fig 8 & 9. The chemical composition of the samples were the same as the substrates were prepared from one lot of fresh cattle dung and rice husk soaked in water. The C:N ratio was 30-35. The characteristics of the digested slurry were distinctly different from those of the fresh slurry.

It was observed that pH of the slurry in semi-continuous digester varied slightly and ranged from 6.9-7.5. On the other hand in batch digester pH of the fermenting slurry, initial being 7.5, dropped to 6.5 during the 3rd week. There was accumulation of VFA (42.5 mM) upto 3 weeks in batch digester, whereas it did not

Table 5a : Comparative changes in total solids (TS), Volatile solids (VS), pH and Volatile fatty acids (VFA) and gas production during batch scale and semi-continuous digestion fed with pretreated rice husk and cattle dung

Days	Semi-continuous (A)					Batch-scale (B)				
	TS %	VS %	pH	[1]VFA	[2]Gas production	TS %	VS %	pH	[1]VFA	[2]Gas production
18th Oct	6.50	7.80	7.5	22.5	-	7.5	8.40	7.4	22.5	-
20th "	6.10	7.15	7.5	17.5	1.800	-	-	-	-	-
22nd "	5.40	7.00	7.4	17.5	1.580	-	-	-	-	-
24th "	6.20	6.80	7.2	15.5	2.550	-	-	-	-	-
26th "	5.90	6.55	7.2	15.5	2.250	6.9	7.1	7.2	42.5	3.020
28th "	5.50	6.35	7.1	15.5	1.850	-	-	-	-	-
30th "	4.85	6.10	7.0	15.5	2.150	-	-	-	-	-
1st Nov	4.70	5.90	6.9	15.5	2.500	-	-	-	-	-
3rd "	4.55	5.25	6.9	10.0	1.950	5.8	5.9	7.0	42.5	3.900
5th "	3.75	4.90	7.0	10.0	0.950	-	-	-	-	-
7th "	3.25	4.85	7.1	10.0	1.100	-	-	-	-	-
9th "	3.00	4.30	7.1	10.0	1.000	4.9	5.2	6.5	30.5	5.650

1 in terms of mMol of acid per 1000 ml
2 in terms of litres of water displaced

Table 5b : Digester performance of batch and semi-continuous digester fed with pretreated rice husk

Parameter	Digester	
	A	B
Digester working volume (ℓ)	3.5	3.5
Duration of experiment (days)	21	21
Retention time (days)	20	20
Reducing sugar content after 10 days (mg ml^{-1})	4	6
Performance		
TS % reduction	53.8	34.6
VS reduction %	44.8	38
Cumulative gas production (ℓ)	19.680	12.370
Gas production rate($\ell\ \ell^{-1}$ day^{-1})	0.2677	0.171

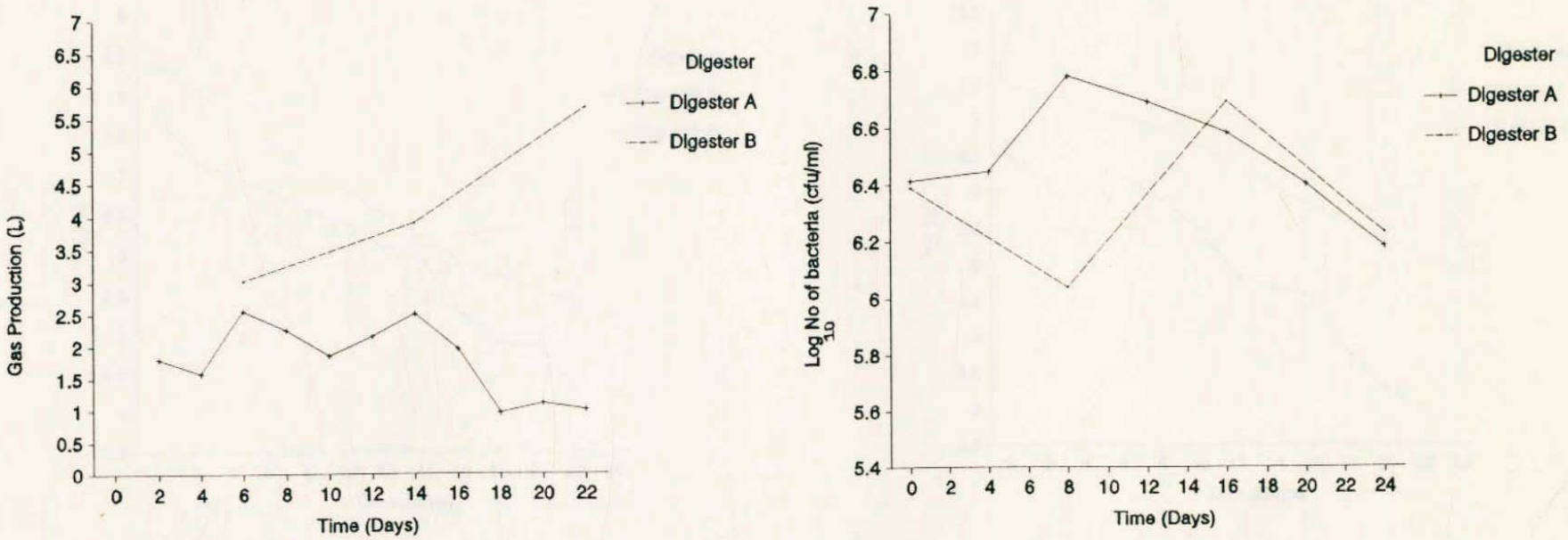
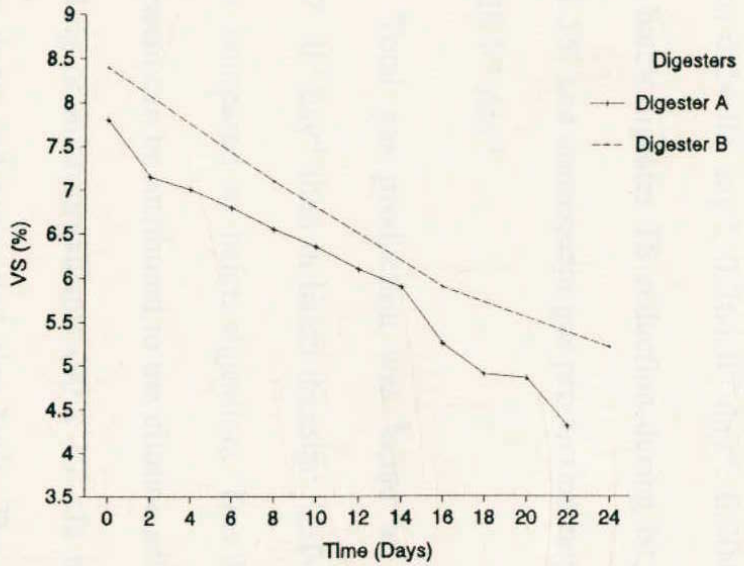


Fig 8. Relationship between bacterial count and biogas production in semi-continuous and batch digesters



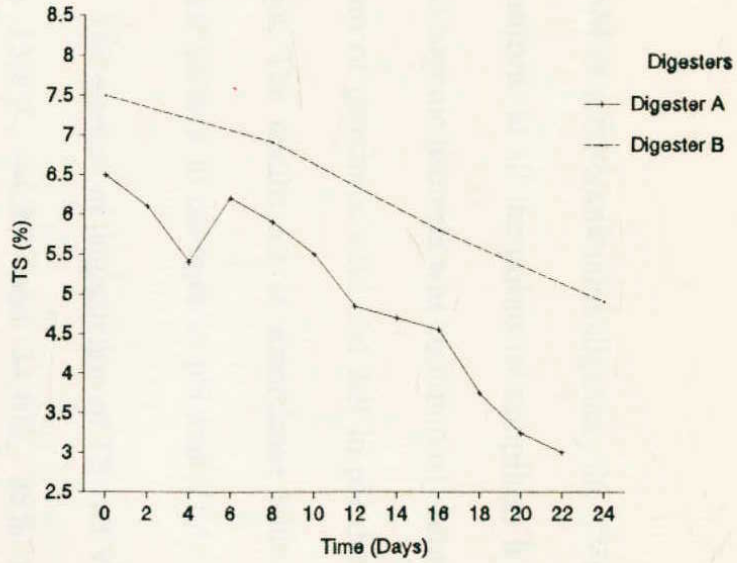


Fig 9. Comparison between total solids and volatile solids in semi continuous and batch digesters

exceed 22.5 mM in semi-continuous digester, the TVFA in semi-continuous digester were almost uniform at all the points of sampling, it might be due to that hydrogen produced by acetogenic bacteria was continuously removed by methanogens and there was no build up of propionic acid and fall in pH due to addition of fresh slurry on alternative days. The results are in accordance with Jain *et al.* (1983), where they observed similar pattern in changes in pH and VFA.

The content of degradation of TS and VS in semi-continuous and batch digester were 53.8%, 44.8% and 34.6%, 38% respectively similar were the observations of Shawarby *et al.* (1982). In the semi-continuous digester TS reduction during the Ist, IInd and IIIrd week was 9.2%, 20% and 36% and consequent gas production was 0.24 l^{-1} day^{-1}, 0.264 l^{-1} day^{-1}, 0.204 l^{-1} day^{-1} respectively. On the other hand in batch digester TS reduction during Ist, IInd and IIIrd week was 8%, 15.9% and 15.5% and consequent gas production rate was 0.125 l^{-1} day^{-1}, 0.159 l^{-1} day^{-1} and 0.228 l^{-1} day^{-1}.

Total gas production was found to be higher in semi-continuous digester 0.267 l^{-1} day^{-1} than in batch digester i.e 0.171 l^{-1} day^{-1} which was almost 36% more as compared to batch digestion. This higher efficiency in the semi-continuous system can be attributed to the dilution effect of any toxic material and to the daily feeding of microorganisms (90 x 10^5 cfu ml^{-1}) and nutrient in digester in form of cattle dung and pretreated rice husk. The main problems with anaerobic digestion of crop residues in semi-continuous digester were observed to be feeding

and output, floating of fresh material in slurry and the consistency and mixing of the slurry. Also it is time consuming and retention time is needed before the digester is operated because there is lag period before the methane is produced. Therefore batch digestion was adopted in all the experiments. In batch digestion sampling is not difficult, no initial inoculum is required and no retention time is needed but disadvantage in batch digestion is that in the initial stages of digestion the metabolic pathways lead to the production of propionic, acetic and butyric acids due to non-removal of H_2 from the system by methanogens. With this there is accumulation of VFA and fall in pH which inhibits the multiplication of methanogenic bacteria (Bryant, 1979) and sometimes leads to digester failure and also one of the reasons for less gas production.

Chapter V

SUMMARY AND CONCLUSIONS

The problem of energy and associated agricultural inputs, shortages, environmental pollution and huge amount of agricultural wastes tempted us to explore one of agricultural byproduct i.e. rice husk for biogas production. Rice husk has to be pretreated because of lignin (17.20%) cellulose (44.10%) association. Suitability of mixing dung and rice husk for maximum gas production was studied. The comparative study between a semi-continuous operated and batch digester fed with pretreated rice husk was also made.

Rice husk was pretreated with alkali and *Trichoderma reseii* but gas production was negligible i.e. 0.046 $\ell\ell^{-1}$ day^{-1} and 0.019 $\ell\ell^{-1}$ day^{-1} respectively and as compared to control it was 63% and 84% less.

Rice husk was pretreated by soaking in cattle dung slurry for 10 days. Replacement of 25% cattle dung with rice husk resulted in more biogas production i.e. 0.1670 $\ell\ell^{-1}$ day^{-1} as compared to control 0.1245 $\ell\ell^{-1}$ day^{-1}. 50% and 75% replacement with rice husk produced negligible gas i.e. 0.0256 $\ell\ell^{-1}$ day^{-1} and 0.0028 $\ell\ell^{-1}$ day^{-1} respectively.

With 10%, 20% and 30% replacement of cattle dung with rice husk, gas production rate was 0.130 $\ell\ell^{-1}$ day^{-1}, 0.213 $\ell\ell^{-1}$ day^{-1} and 0.1377 $\ell\ell^{-1}$ day^{-1} respectively.

On pretreatment of rice husk by soaking in water 10% replacement yielded maximum gas 0.232 $\ell\ell^{-1}$ day^{-1} followed by 25% and 30% replacement i.e. 0.164 $\ell\ell^{-1}$ day^{-1} and 0.157 $\ell\ell^{-1}$ day^{-1} respectively.

On comparison of semi-continuously operated digester with batch-scale digester the former fed with 25% replacement of cattle dung with rice husk yielded maximum gas as compared to all the other experiments i.e. 0.2677 $\ell\ell^{-1}$ day^{-1} whereas batch digester with similar replacement yielded 0.171 $\ell\ell^{-1}$ day^{-1}.

To conclude, maximum gas production 0.2677 $\ell\ell^{-1}$ day^{-1} was in semi-continuously operated digester fed with 25% replacement of cattle dung with water pretreated rice husk followed by the batch digester 0.232 $\ell\ell^{-1}$ day^{-1} fed with 10% replacement of cattle dung with water pretreated rice husk.

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