Elucidation mechanism of organic acids production from organic matter (grass) using digested and partially digested cattle feed

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Abstract The process of anaerobic digestion is highly influenced by the environmental and operational factors like organic acids concentration and the reactor volume occupied by the feed material. The optimum level of organic acids is commonly assumed to be in the range between 2,500 and 3,500 mg/l for the anaerobic digestion process. It was observed that the production of total organic acids during hydrolysis of grass using cattle dung slurry (CDS) as the inoculum reached up to 4,850 mg/l in 6 days, while on the other hand it reached 5,700 mg/l within 4 days when rumen content was used as inoculum. The organic acids production continued to the 30th day in the case of rumen content, while in the case of CDS it stopped within 10 days because of pH drop. As compared to CDS the anaerobic digestion of grass with rumen content showed better degradation and biogas production with nearly 80% of methane and up to 80 and 95% reduction in chemical oxygen demand and organic acids respectively.

Keywords Anaerobic digestion; cattle dung; hydrolysis; lignocellulosic waste; methanogenesis; rumen

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Introduction

Waste utilization, rather than its treatment, emphasizes shifting the process from reducing the potential for pollution to synthesis of useful products, like gases and chemicals. Biomass amenability to conversion depends largely on the characteristics of the biomass, the substrate and the process requirements for the conservation technology under consideration. Stabilization of biological wastes via anaerobic degradation leads to the production of useful energy rich fuel gases. Anaerobic digestion is a multiple stage process among which hydrolysis is the main step during which the complex insoluble substrate macromolecules are hydrolyzed into simpler and more soluble intermediates by putrefying bacteria. During hydrolysis microorganisms produce extracellular enzymes, for example cellulase, amylase, protease and lipase which degrade the cell wall of lignocellulosic waste (Yang *et al*., 2001), and addition of these enzymes in an anaerobic reactor enhances the degradation of bio-waste (Sonakya *et al*., 2001a). The organic acids produced during the hydrolysis stage serve as a substrate for methanogens which results in methane production. The optimum level of organic acids is considered to be in the range between 2,500 and 3,500 mg/l for the anaerobic digestion process (Sonakya *et al*., 2001b).

Lignocellulosic materials are hard to degrade through anaerobic decomposition (Kalia *et al*., 2000). But in certain natural microbial systems, in the rumen for instance, it is known to proceed at high rates. The rumen is a highly complex ecosystem where microorganisms live in a symbiotic relationship that facilitates fiber digestion. Simulation of the rumen system could improve the efficacy of the anaerobic digestion of plant material as the rumen harbors bacteria, fungi, and protozoa which produce fiber-degrading enzymes (Lee *et al*., 2000; Russell and Rychlik, 2001). It has also been reported that rumen microorganisms were helpful in improving the low efficiency of acidogenic fermentation (Shin *et al*., 2001).

This study focused on the production of organic acids from grass as a feed. The source of hydrolytic bacteria (inoculum) was from digested cattle feed (i.e. cattle dung) and partially digested cattle feed (PDCF) in the rumen of slaughtered cattle.

Materials and methods

Feed material

The grass samples were collected from the open landscape. Then it was cut into small pieces (1–2 cm in length) with the help of a grass cutter. The fresh sample was characterised for total solids (TS), total organic carbon (TOC), chemical oxygen demand (COD) etc. The fresh cattle dung (CD) was collected from the dairy, and the rumen content from the rumen of cattle collected from the slaughter house.

Hydrolysis of grass

The preliminary experiments of hydrolysis of grass were done in 500 ml plastic bottles under semi-anaerobic conditions at 2% slurry on the basis of total solids. The experiments were run till the organic acids production stopped. One milliliter sample was collected at an interval of 24 hours for organic acids estimation.

Methanogenesis (reactor set up)

For this experiment the reactors were made up of glass (6 l volume) with a lid having 4 openings which were fitted with plastic pipes. The first two openings were for inlet and outlet of feed which were connected with a two-way pump, and the next two were for gas outlet and for pH adjustment. In the first reactor (first stage) the chopped grass and fresh rumen were mixed and incubated at 37°C for hydrolysis. The gas counter was attached to the methane reactor (second stage) which continuously monitored the biogas production during methanogenesis (Figure 1).

Analyses

Organic acids estimation was done by GC (Carlo Erba, HRGC 5300 Mega series) using DB-FFAP column (30m in length) and helium as a carrier gas. TS, OTS, OTR etc., were analysed by the standard procedures given in DIN 38414. The gas analysis was done by an Infra-Red Gas Analyser (Geotech company, UK).

Results and discussion

Organic acids production

In the present study two sets of preliminary experiments were conducted for the optimisation of hydrolysis of grass. The properties of grass are listed in Table 1. In the first set of experiments cattle dung was used as a source of hydrolytic bacteria. On the other hand, in the second set of experiments, rumen content was used as a source of hydrolytic bacteria. These experiments were conducted in duplicate and the results are presented on the basis of the mean of the two sets of experiments.

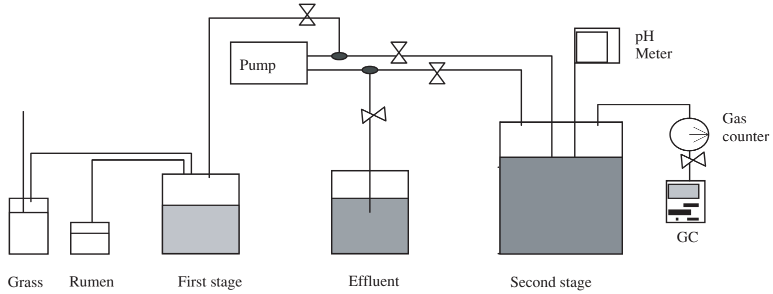

Figure 1 Small scale two stage anaerobic digester used for degradation of grass

Table 1 Properties of fresh grass used as a feed stock

COD g/kg	1,132.0
TS g/kg	400.0
TOC %	46.14
TR g/kg	0.24
OTS %	91.20
Hemicellulose %	14.00
Cellulose %	28.30
Lignin %	5.40
Ash %	0.46

In the first set of experiments the total organic acids production varied from 2,250 to 4,850 mg/l during the incubation period of 10 days. During this period the acetic acid production remained constant until the 6th day but on the 8th day it increased to 2,500 mg/l. After that it decreased and on the 10th day it stopped completely (Figure 2). The production of propionic acid was much higher than butyric and valeric acid, although some variations were also observed during the process. On the 8th day the production of total organic acids increased due to an increase in butyric acid production. Inanc *et al*. (1999) reported that the higher production of propionic acid has a worse effect on the anaerobic digestion process than other acids produced during hydrolysis.

In the second set of experiments the total organic acids production varied from 3,500 to 7,250 mg/l during the incubation period of 30 days. Initially it increased constantly but after the 2nd day it increased more rapidly and on the 4th day it increased up to 5,700 mg/l. The next peak up to 7,250 mg/l of total organic acids was observed on the 20th day (Figure 3). After that the acid concentration started declining and production ceased on the 30th day. On average, butyric acid production was higher than propionic and valeric acid, which is beneficial for the process of methanogenesis.

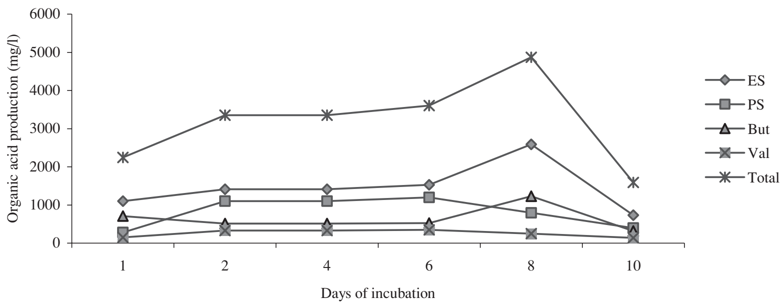

Figure 2 Production of organic acids from the reactor inoculated with cattle dung slurry

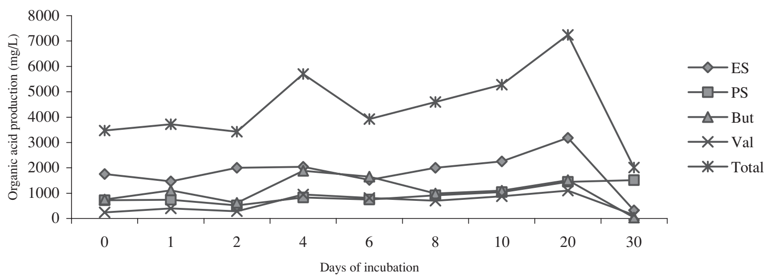

Figure 3 Production of organic acids in the reactor inoculated with partially digested cattle feed

Methane production

During the hydrolysis stage the grass fibres were separated by filtering the feed through a sieve (1.4 mm pore size), and the liquid was fed into the second reactor for methanogenesis (Figure 3). The two stage reactor was set up because some advantages over a single stage reactor were anticipated. Firstly, in a single stage reactor hydrolytic bacteria produce organic acids which may cause the pH to drop. This is inhibitory for methanogenesis. Secondly, methanogenic bacteria are far more sensitive to accumulation of organic acids (Veeken and Hamelers, 1999) which may cause sickening of the digester. In a two stage reactor the pH in the hydrolytic reactor (first stage) is normally low (5.5 to 6.5) and in the second stage it can be maintained higher (nearly 7.0 to 7.2) by the flow rate of the influent from the first reactor. The digestion of bio-waste is in delicate balance between the rate of hydrolysis and the rate of methanogenesis because methanogenic bacteria are far more sensitive to accumulation of organic acids, and the corresponding pH drop, than acidogenic and fermentative bacteria (Veeken and Hamelers, 1999). So hydrolyzed slurry was used to keep optimum organic acids concentration and low hydraulic retention time (HRT). The experiment was run at an HRT of 4 days at a feeding rate of 2.5 l every alternate day with one day hydrolyzed slurry and the total biogas production was observed over a digestion period of 6 weeks (nearly 10 cycles). Nearly 100 l of biogas was produced at the rate of 2.3 l/day. The CH_4 percentage was 60% up to two cycles of feed, and later gradually increased up to 80%, and the CO_2 percentage decreased and became stable between 15 to 20% as shown in Figure 4. The observed biogas production varied between 15 to 20 l/week. During the whole process pH was maintained at regular intervals, or when required, by adding 2N NaOH solution. The two stage anaerobic digestion reactor was also inoculated with cattle dung but because of low production of organic acids no substantial results were obtained (data not shown). The performance of the anaerobic reactor with respect to organic acids degradation was also good and led to 98% reduction in organic acids and 85% reduction in COD after 6 cycles (data not shown).

Conclusions

Bacterial populations were inhibited when the propionic acid concentration was more than 1,000 mg/l, while they were able to tolerate acetic and butyric acid concentrations up to 10,000 mg/l. The higher concentration of propionic acid also inhibits the degradation of complex organic matter (Inanc *et al*., 1999). An early termination of the hydrolysis of grass was observed when cattle dung was used as the inoculum. This could be attributed to the fact that propionic acid concentration was increased on the second day. The termination point of the hydrolysis of grass with rumen as the inoculum was achieved late because the propionic acid concentration increased continuously and crossed the optimum level on the 20th day, where it was 1,450 mg/l.

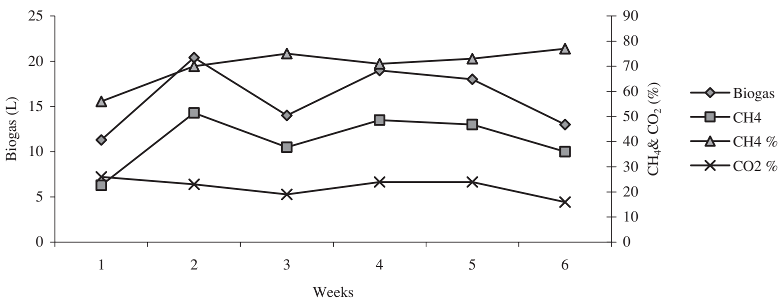

Figure 4 Biogas production in degradation of grass in two stage anaerobic reactor

On the basis of these conclusions, hydrolysis of grass was carried out using rumen content (partially digested cattle feed) as a source of inoculum, because after 24 hours the organic acids concentration reached the optimal range for methanogenesis and for keeping low HRT. On the other hand, with cattle dung, the optimum level of organic acids was achieved in 4 to 6 days, for better anaerobic digestion. This is probably because of the higher concentration of hydrolytic bacteria in the rumen than in cattle dung. The anaerobic digestion of grass in two stage systems works better with rumen content as inoculum than with cattle dung. The performance of the anaerobic reactor, on the basis of organic acids degradation was also better. Up to 98% reduction in organic acids was observed and 85% reduction in COD after 6 cycles.

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