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Enhanced anaerobic degradation of Cumbu Napier hybrid grass through application of rumen microorganisms

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ABSTRACT

Anaerobic digestion of the cellulosic residues provide an attractive means of waste reduction and waste stabilization with simultaneous recovery of methane as an energy source. Anaerobic treatment of solid organic waste materials lack high degradation efficiencies because of low rate of hydrolysis due to the crystallinity of cellulose, the association of cellulose and hemicellulose with the lignin and the low cellulase activities present in digesters. However in nature several anaerobic microbial ecosystems are highly active in the conversion of lignocellulosic materials. Typical examples are the rumen of the ruminants. Therefore an enhancement of the cellulolytic activity in bio-digester is of utmost importance for an economically feasible anaerobic degradation process. Batch and continuous culture experiments with rumen microorganisms were carried out in order to study the anaerobic conversion of Cumbu Napier hybrid grass. In batch incubation maximum total fibre degradation of 54.4 per cent Cumbu Napier hybrid grass was obtained at 168 h of incubation time and methane production per gram of volatile solid digested in 7 days was 0.036 l. In semi-continuous experiments at loading rate of 15 g TS/l/d, at hydraulic retention times of 10-20 h, the total fibre degradation is reduced from 84.6 to 42.3 per cent at the end of retention period of 35 days. Average production of biogas per gram volatile solids digested per day varied between 0.13 to 0.36 l/day.

Keywords: Anaerobic digestion; Cumbu Napier hybrid grass; rumen; biogas

INTRODUCTION

Lignocellulosic biomass produced in conventional agriculture such as rice straw, corn stover, maize straw, wheat straw and Napier grass has a special interest because of the large quantities and inexpensive costs and is a potential resource for the production of biofuels like biogas. Cumbu Napier hybrid grass, a high yielding nutritious green fodder with soft stem and high leaf was selected for the study since it produced 400 tonnes/ha/yr as feedstock for biogas generation.

Anaerobic digestion of these cellulosic residues could provide an attractive means of waste reduction and waste stabilization with simultaneous recovery of methane as an energy source. However in contrast with the situation with

dissolved organic compounds present anaerobic treatments of solid organic waste materials lack high degradation efficiencies because of low rate of hydrolysis due to the crystallinity of cellulose, the association of cellulose and hemicellulose with the lignin and the low cellulase activities present in digesters. In nature several anaerobic microbial ecosystems are highly active in the conversion of lignocellulosic materials. Typical examples are the digestive tract of termites and the rumen of the ruminants. Therefore an enhancement of the cellulolytic activity in bio-digester is of utmost importance for an economically feasible anaerobic degradation process.

Conversion of cellulose into biogas proceeds by the action of various metabolically diverse populations of microorganisms comprising hydrolytic, fermentative, hydrogen-producing acetogenic and methanogenic species. A widespread natural cellulolytic system which operates with high efficiency is present in the rumen of ruminants. Several of the predominant bacteria and ciliates of this system are known to exhibit high levels of cellulase activity by which even crystalline cellulose can be hydrolyzed. The application of rumen microorganisms for an improved degradation of cellulosic residues and a simple semi-continuous cultivation system for rumen microorganisms can enhance the biogas production from the lignocellulosic wastes.

This paper reports on the anaerobic degradation of Cumbu Napier hybrid grass.

MATERIAL and METHODS

Substrate

Cumbu Napier hybrid grass CO (CN) 4 was collected from the Department of Forage Crops, Centre for Plant Breeding and Genetics, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu. The raw material was sundried for 7 days, shredded to pass through a 2-5 mm sieve mesh and stored at room temperature and its composition is shown in Table 1.

Table 1. Composition of Cumbu Napier hybrid grass

Component	Percentage
Neutral detergent fibre	84.6
Acid detergent fibre	31.8
Hemicellulose	9.5
Cellulose	52.8
Lignin	21.3

Culture and media

Rumen fluid collected from slaughtered cattle was used as a source of seed microorganisms. Firstly rumen solid digesta was taken from slaughtered cattle, squeezed and filtered using two layers of bandage cloth to collect rumen fluid and transferred to vials. These vials were purged with N_2 gas for 30 sec to ensure anaerobic conditions. The composition of artificial saliva is shown in Table 2.

Table 2. Composition of artificial saliva

Component	g/l
$NaHCO_3$	8.0
KH_2PO_4	1.0
K_2HPO_4	3.0
$CaCl_2.2H_2O$	0.03
NH_4Cl	0.18
$MgCl_2.6H_2O$	0.08

Batch incubation using rumen microorganism

In vitro degradation of Cumbu Napier hybrid grass was tested with 4.0 g of substrate, 10 ml of strained rumen fluid and 40 ml of fermentation buffer in 500 ml serum bottles closed with n-butyl rubber stoppers and aluminium caps. The bottles were subsequently flushed with N_2 gas for 30 seconds in order to create anaerobic conditions. Incubation was done at 39°C for 168 h in a shaking incubator. Samples for methane content determinations were taken at 0, 12, 24, 48, 72, 120 and 168 h after incubation. A one ml plastic syringe was used to draw 0.2 ml gas samples from the bottles. Two bottles were removed from the incubator at each of the above sampling times and stored at -20°C until fibre determination was carried out. Degradation was estimated by determining the loss in weight of neutral detergent fibre (NDF). Fermentations with only rumen fluid were included as controls. All fermentation experiments were repeated twice.

Semi-continuous experiment

Acidogenic phase: Acidogenesis from cellulose was performed in a 2.5 l volume continuous artificial rumen fermenter as described by Gijzen et al (1986). Inoculation and operation conditions of the fermenter device were slightly modified. Buffer solution according to Rufener et al (1963) was modified by the addition of NH_4C1 (1.5 g/l) as a nitrogen source.

Experiments were conducted by inoculating fermenter with 250 ml strained rumen fluid obtained from slaughtered cattle. After inoculation the fermenters were filled with buffer solution to a working volume of 1.5 l. Buffer input and filtered effluent flow rates were adjusted to 2.0 fermenter volumes per day (FV/day) for all experiments. No solids were withdrawn from the fermenter during the experimental period except for sample analysis. The substrate was added once daily except the first day when twice the normal daily amount was administered. The fermenter contents were mixed every 30 min for a period of 45 sec by means of a magnetic stirrer (125 rpm).

Methanogenic phase: The acidogenic reactor was placed in serial connection with an up-flow anaerobic sludge blanket (UASB) methane reactor with a total volume of 2.5 l. The methanogenic reactor consisted of a PVC (polyvinyl chloride) cylindrical pipe (900 mm internal diameter, 2800 mm height), a conical-shaped bottom (500 mm height), a settler and gas collection compartment. Biogas was collected by means of an inverted funnel construction in the gas collection compartment. The volume

of the gas collection compartment was 0.5 l. The reactor was started by the use of approximately 1.25 l settled granular sludge which was obtained from a fruit processing waste treatment UASB plant.

Liquid effluent from the acidogenic reactor containing dissolved organic matter was continuously fed to the methanogenic reactor at a rate of 1.5 l/day. At this flow rate the hydraulic retention times (HRT) in the sludge layer and methanogenic reactor were approximately 10 and 20 h respectively. To test the performance of a system with a closed fluid circuit the effluent of the methanogenic reactor was continuously fed to the acidogenic reactor at a rate of 0.9 l/day. In this case the buffer supply was disconnected. The reactors were thermostatically controlled at 39°C by using hot plate. The temperature of 39°C was derived from the in vivo situation of the rumen. A schematic representation of the two-phase process is shown in Fig 1.

Analytical procedures

Samples of 10 ml were drawn from the acidogenic reactor content and the methanogenic reactor effluent three times a week for determination of fibre and methane content of biogas. During the experiment performed at loading rate of 15 g dry wt/l/day of Cumbu Napier hybrid grass sampling was started one week after

Fig 1. **Semi-continuous experiment (a. Acidogenic reactor b. UASB-type methanogenic reactor Double stage peristaltic pump d. Magnetic shaker e. Gas collection flask f. Fermentation medium reservoir)**

inoculation after which a steady-state situation was reached. The samples were taken 4 hours after substrate addition.

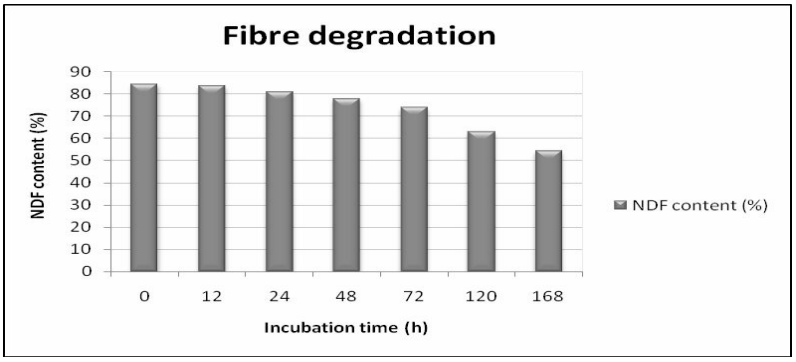
The amount of methane content produced in biogas was estimated in a Shimadzu GC-2014 gas chromatograph equipped with FID. The gas samples were introduced into the analyzer by filling the fixed loop (1.0 ml) on the sampling valve. Samples were injected into the column system by starting the analyzer which automatically activated the valve and back flush the samples according to the time programmed. The retention time of CH_4 was between 4 to 4.17 min.

Biogas production of both acidogenic and methanogenic reactors was measured daily by means of a gas measuring cylinder.

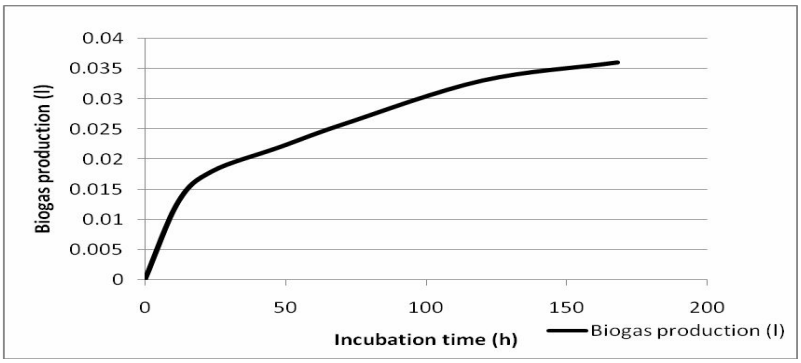
RESULTS and DISCUSSION

Batch incubations

The effect of incubation time on total fibre reduction and mean production of CH_4 is shown in Fig 2 A steady



(a)



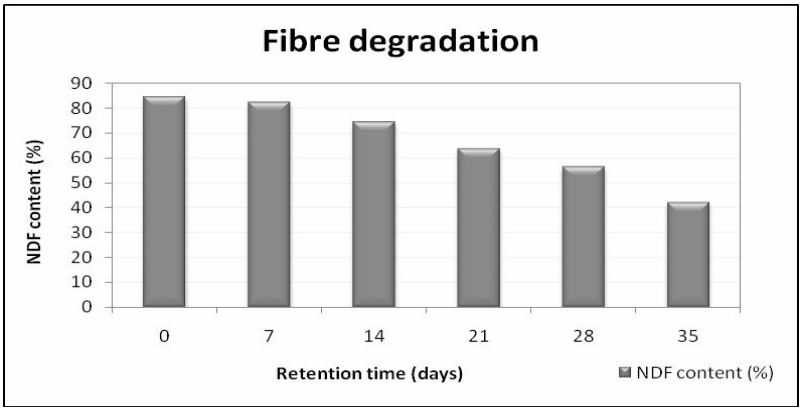
(b)

Fig 2. Total fibre reduction (a) and mean production of CH_4 (b) in batch incubation semi-continuous experiment

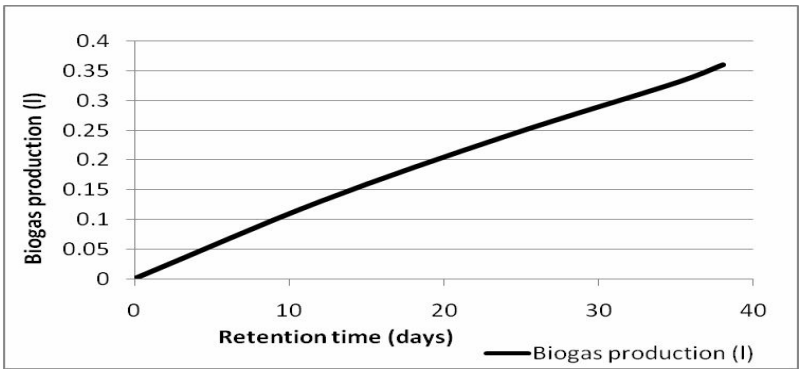
degradation of total fibre was observed throughout the incubation period. At the end of the incubation (168 h) total fibre fractions from Cumbu Napier grass were degraded by 52 per cent.

The effect of retention time on total fibre reduction and mean production of CH_4 is shown in Fig 3. A steady degradation of total fibre was observed throughout the retention time. At the end of the retention time (35 days) the total fibre fractions from Cumbu Napier grass were degraded by 43 per cent. The methane content varied from 55 to 62 per cent and this could be due to the action of rumen microorganism as shown in Fig 4.

The suitability of a substrate to anaerobic conversion into biogas depends primarily on its composition and on the digestion system employed. The study



(a)



(b)

Fig 3. Total fibre reduction (a) and mean production of CH_4 (b) from Cumbu Napier grass

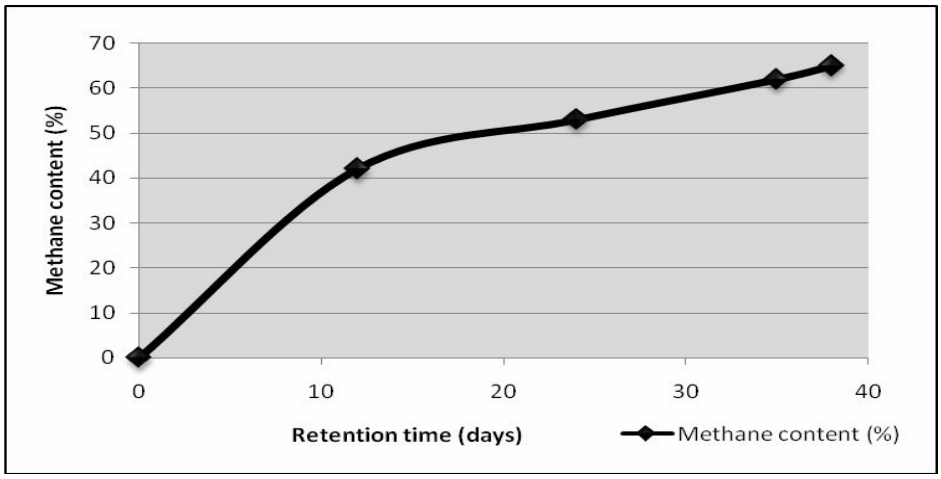


Fig 4. Methane content from Cumbu Napier grass in semi-continuous experiment

showed that Cumbu Napier hybrid grass can be mainly converted into VFAs by about 60 per cent in less than 3 days by employing rumen microorganisms. Since VFAs are precursors of methane, they can be converted by coupling a high-rate methanogenic reactor to the rumen reactor.

In the artificial rumen reactor which is a semi-continuous culture system, the rumen microorganisms appear to be more efficient than batch culture incubations in terms of fibre degradation and yields of the fermentation products. This was probably due to more favourable conditions prevailing in the semi-continuous culture system. Continuous removal of fermentation products resulted in a stable pH and hence a suitable environment for fast growth and high enzyme activity was established.

ACKNOWLEGMENTS

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