J. Mycopathol. Res. **40**(2) : 93–96 (2002)

Effect of inoculation of microwave irradiated methanogenic bacterium for improvement of biomethanation from jute caddis

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Biogas containing about 56% methane could be produced from jute waste and municipal solid waste by anaerobic fermentation using cow dung as inoculum. The start-up period for biogas generation was not reduced considerably by simply adding a methanogenic bacterium *Methanosarcina barkeri* DSM-804 in the digester. A distinct structural change of the jute fibre, as observed under Scanning Electron Microscope revealed the utilisation of jute caddis during fermentation for biogas production.

In the process of generation of biogas from digester some intermediate volatile fatty acids were detected, thus indicating the three-stepped process of methane generation from jute caddis, viz., acidogenesis, acetogenesis and methanogenesis as reported by earlier workers.

Inoculation of microwave irradiated *Methanosarcina barkeri* DSM-804 in the bio-digester increased the fermentative and methanogenic activity of the bacterium, resulting in higher efficiency and quicker generation of biogas.

Macro-and micronutrient analysis of the digester slurry showed that residual slurry after fermentation was rich in plant nutrients. So, this biogas residual slurry can serve as good quality manure for agriculture.

Key words : Biomethanation, microwave irradiation, bacterium, jute caddis

INTRODUCTION

The energy deficient age in which we live today demands that new and renewable sources of energy should be fully exploited. Generation of biogas through anaerobic digestion of organic matter is one such process, which has considerable potential to supplement energy supplies. However, in order to make biogas activity a real success in replacing non-renewable energy sources, efforts have to be made to enlarge the scope of anaerobic digestion by using different substrates. In the present study jute caddis and vegetable market wastes have been used as such substrates for biogas production.

Recently nonthermal effects of microwave on cell and tissue have been demonstrated and biointeraction of microwave is assumed to promote mutagenesis. Based on this, the present paper deals with the effect of microwave irradiation on methanogenic bacterium for biogas generation, using combination of jute waste and vegetable market wastes as substrates.

MATERIALS AND METHODS

Experiments were conducted in 10 litre glass aspirator bottles with 100 g jute caddis and 500 g vegetable market wastes for study of biogas generation in three sets of different experiments. In set 1, 1 kg cowdung was added as inoculum in the digester and diluted with 2 litre water.

The pH, redox potential (E_h), conductivity (C) and temperature (T) changes in the glass digesters were recorded at 0, 7, 10, 15 and 20 days from the digester slurry by the standard procedures.

To visualise the change in surface topology of the jute fibre when used as substrate for biogas generation, Scanning Electron Microscopy of the jute fibre from the bio-digesters before and after biogas production was done with the help of a Hitachi Scanning Electron Microscope model No. S-430.

The methane and carbon dioxide content of biogas in the digester (Set 1) was determined by Gas Chromatography. For this purpose, gas was collected from the digester and injected in a NUCON series 5700 model Gas Chromatograph equipped with a thermal conductivity detector and a Porapac Q glass column. Detector and column temperature was 50°C. Hydrogen served as the carrier gas at a flow rate of 20ml/min.

As intermediate product volatile fatty acids viz., formate, acetate, propionate and butyrate accumulated, in the process of formation of methane and carbon dioxide from the substrates - jute caddis and vegetable market wastes, were detected using a Gas Chromatograph equipped with a glass column packed with chromosorb by comparison with a standard. The column temperature was 180°C, while the injection port and Flame ionisation detector temperature was 200°C. Nitrogen was used as the carrier gas at a flow rate of 60ml/min.

Similar experiments, were set up in 10 litre glass aspirator bottles containing the same substrates but inoculated with a methanogenic bacterium *Methanosarcina barkeri* (DSM - 804) obtained from DSM, Germany and made available from the laboratory of Prof. A. R. Thakur, Dept. of Biophysics, Calcutta University in Set 2 while microwave irradiated (at a frequency of 26.5 GHz, 10 dbm power for 2 h) culture in Set 3. pH, redox potential (E_h), conductivity (C) and temperature (T) changes in the glass digesters were recorded as in Set 1.

Macro and micronutrient composition of the digester slurry was determined from airdried samples and expressed on oven dry-weight (105°C) basis. Total nitrogen, phosphorus and organic carbon contents were estimated following Jackson (1973). Other elements viz., Na, K, Ca, Zn and Mn were determined in a Perkin-Elmer AANALYST model atomic absorption spectrophotometer from ash of samples obtained by heating in a muffle furnace for six hours at 550°C using standard methods (Issac and Keber, 1971).

RESULTS AND DISCUSSION

The discussion deals with experimental data presented in Tables 1-4.

This reveals the fact that the jute caddis can be used as a substrate by the anaerobic bacteria in the biogas digesters, thus establishing the feasibility of utilising the lignocellulosic waste-jute caddis as a raw material for biogas production.

Table 1 : pH, redox potential (E_h), conductivity (C) and temperature (T) changes in diogas digesters until production of inflammable gas.

	Digester 1			
No. of days	pH	E_h (mV)	C (ms)	T (°C)
0	6.76	+30	8.8	32.2
7	5.13	-9.3	8.3	31.9
10	5.64	-93.9	8.0	29.3
15	6.51	-266.4	7.6	28.6
20	6.27	-325.0	7.2	31.3

Digester 1 contained
100 g jute caddis + 500 g vegetable market wastes + 1 kg fresh cowdung + 2 litre water

Table 2 : Analysis of biogas composition and volatile fatty acids in digesters.

	Volatile Fatty Acids in slurry (%)				Gas composition	
Sample	Formic acid	Acetic acid	Propionic acid	Butyric acid	CH_4 (%)	CO_2 (%)
A	3.2	52.5	Nil	25.8	56.3	43.7
B	Nil	17.9	Nil	42.3	25.5	74.5

A : 17th day of fermentation in digester
B : 27th day of fermentation in digester

Table 3 : Effect of inoculation of *Methanosarcina barkeri* (DSM-804) in biogas digester on pH, redox potential (E_h), conductivity (C) and temperature (T).

	Microwave irradiated				Microwave not irradiated			
No.	Digester 2				Digester 3			
of days	pH	E_h (mV)	C (ms)	T (°C)	pH	E_h (mV)	C (ms)	T (°C)
0	6.90	+30	8.4	30.2	6.90	+30	8.4	30.2
7	6.03	-186	7.6	31.1	5.73	-9	8.1	29.9
10	6.74	-233	7.4	30.3	6.04	-187	7.9	30.7
15	7.01	-327	7.1	30.6	6.52	-192	7.7	30.8
20	-	-	-	-	6.87	-255	7.5	31.3

Digester 2 : 100 g jute caddis + 500 g vegetable market wastes + microwave irradiated *Methanosarcina barkeri* (DSM - 804)
Digester 3 : 100 g jute caddis + 500 g vegetable market wastes + microwave not irradiated *Methanosarcina barkeri* (DSM - 804)

Table 4 : Analysis of slurry before (A) and after (B) biogas production.

	TS (%)	TVS (%)	Total organic C (%)	Total N (%)	Total P (%)	Total K (%)	Total Ca (%)	Total Mg (%)	Total Na (%)	Total Cu (%)	Total Zn (%)	C:N ratio
A	87.0	84.4	48.4	1.5	0.12	0.21	0.25	0.06	0.48	0.04	0.09	32.6
B	85.0	82.8	29.9	2.6	0.25	0.37	0.96	0.37	0.78	0.05	0.25	11.5

A : 17th day of fermentation in digester.
B : 27th day of fermentation in digester.

The untreated jute fibre surfaces appeared smooth and unbroken.

Results are presented in Table 1 until initiation of inflammable biogas production (i.e., 20 days) where the substrate, jute caddis and vegetable market wastes, was inoculated with cowdung. It is observed that pH of the substrate in biogas digester dropped to an acidic range from initial nautral pH and increased again to attain near neutral pH value before starting of generation of methane. During this time the E_h dropped steadily and conductivity also continued to decline slowly. It is noteworthy that E_h reached to a high degree of anaerobiosis before biomethanation activity started in full vigour. From the Table 2 it can be seen that methane gas to the extent of 56.3% was produced by using mixture of jute caddis and vegetable market wastes as substrate and cowdung as inoculum in the digester. This is supportive of an earlier work that indicated the feasibility of utilisation of jute caddis - a lignocellulosic waste for biogas production. The Table 2 also reveals that besides methane and carbon dioxide as the main gaseous end product, some intermediates i.e., volatile fatty acids (viz., formic acid, acetic acid and butyric acid) were also detected in the reactor medium. This indicates the multistep nature of anaerobic digestion of jute caddis and vegetable market wastes, and as such biomethanation is believed to occur in three main steps : acidogenesis, acetogenesis and methanogenesis.

The Table 3 shows the pH, E_h, conductivity and temperature data obtained upon inoculation of the methanogenic bacterial strain *Methanosarcina barkeri* (DSM 804), not irradiated and irradiated with microwave frequency 26.5 GHz and 10 dbm power for 2 hours in digesters 3 and 2 respectively. In the digester 2, biogas production occurred after 15 days of incubation when inoculated with *Methanosarcina barkeri* (DSM 804) irradiated with the microwave. Thus, from the study it can be said that the addition of the microwave irradiated methanogenic bacteria helped in the process of biomethanation by reducing the time requried for biogas production.

The Table 3 further reveals that simple addition of *Methanosarcina barkeri* (DSM 804) in digester 3 could not reduce biogas generation time. Thus, it can be deduced that microwave interaction with the methanogenic bacterial strain at 26.5 GHz frequency, 10 dbm power for two hours has increased its fermentative and methanogenic activity and eventually reduced the lag-period of growth resulting in higher efficiency and quicker generation of biogas.

This result thus supports the observation made by others that microwave irradiation is not only responsible for cell death due to heating but can also athermally affect the microbial system, resulting in altered metabolic behaviour.

Table 4 presents the data obtained from chemical analysis of slurry in digesters before and after biogas production. The residual slurry after biogas production was richer in plant nutrient like N, P, K, Ca, Mg, Na, Cu and Zn and thus can serve as good quality manure for agriculture. The lowering of carbon and nitrogen ratio in the residual slurry indicates good digestibility of the substrates (jute and vegetable market wastes) used for biomethanation.

ACKNOWLEDGEMENT

We acknowledge ICAR, New Delhi for granting the AP Cess project "Microwave induced biomethanation from jute waste and MSW composite" under which the research was conducted.

We are grateful to Director, NIRJAFT, Calcutta for providing the infrastructural and laboratory facilities for conducting the present research.

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(Accepted for publication May 6 2002)