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Effect of microwave on cell biology of *Methanosarcina barkeri*

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Microwaves are part of electromagnetic spectrum and are considered to be that radiation ranging from frequency of 300 million cycles per second (300 MHz) to 300 billion cycles per second (300 GHz), which corresponds to a wavelength range of 1 metre down to 1 milli-metre. This non-ionizing electromagnetic radiation is absorbed at molecular level and manifests as changes in vibrational energy of the milecules or heat. Of late non-thermal effect of specific microwave has been dicumented. There is evidence that microwave cause different biological effects depending upon field strength, frequencies, wave forms, modulation and duration of exposure.Various studies has been conducted to evakuate the effect of microwave irradiation on eubacteria, cyanobacteria, moulds, yeasts etc. but no study has been done for such effect on archaebacteria. In this study we have taken an attempt to evaluate the bio-effects on such a methanogenic archaebacteria viz. Methanosarcina barkeri DSM-804 obtained from Deutsche Samnlung Von Mikroorganismen Und Zellkulturon Gmbh,Germany. It is reported that this bacteria are able to induce biomethanation due to irradiation of microwave frequencies ranging from 13.5 to 36.5 GHz. among which 31.5 GHz frequency was the most effective one.

In this study an attempt has been taken to understand the changes in cell biology due to irradiation of microwave at 31.5 GHz frequency for two hours at 10 dbm power on pure broth culture of Methanosarcina barkeri DSM-804. The cell count and cell diameter showed 24.32% &56.71% increase and 10.76% &21.50% increase from 7th day right from visible bubble production (CH_4) to significant declining stage of gas bubble production due to microwave irradiation on pure bacterial culture of *Methanosarcina barkeri* DSM-804 respectively. The irradiated broth culture also showed high fluorescence activity seen under a fluorescence microscope. This may be cause of higher biological activity related to biomethanation.

Key words : Microwave effect, cell biology; *Methanosarcina barkevi*

INTRODUCTION

Biomethanation, which is popularly known as generation of biogas, is a complex and multistage process of organic matter decomposition in anaerobic environment to end product methane. Biomethanation from lignocellulosic organic material takes place by anaerobic babteria through concerted action of fermentation, acetogenesis and finally methanogenesis (Nagamani and Ramasamy, 1999). So, efficiency of biomethanation depends on the final step i.e. methanogensis by methanogenic bacteria although a consortium of cellulolytic and acetogenic bacteria is the prerequisite.

Scientists have focused attention on the bio-effects of different electromagnetic frequencies. Microwave forms a part of the electromagnetic spectrum and in recent reports various athermal effects of selected frequencies of microwave on cell and tissue responses have been documented (Banik *et al.*, 2003, Rai *et al.*, 1994)

This piece of work is dedicated to a study of irradiation of two specific frequencies of (28 GHz and 31.5 GHz) microwave on test microorganism an archaebacteria *Methanosarcina barkeri* (DSM-80) on its cell morphology and biomethanation property.

MATERIALS AND METHODS

Methanosarcina barkeri (DSM-804), a methanogenic bacterial strain obtained from Deutsche Sam nlung Von Microorganismen Und Zelkulturon Gmbh, Germany, was made avaibale from the laboratory of Prof. A.R. Thakur, Department of biophysics, University of Calcutta was used as the test organism. The culture strain was prepared anaerobically under 1 atmosphere of 80 % nitrogen and 20% carbon dioxide. The methanogenic bacterial culture was grown in medium 120 for 4 days before irradiatio of microwave. Microwave frequencies of 28.0 GHz and 31.5 GHz were irradiated for two hours continiously from a microwave generating source placed at a height of 2 meter. The cultures were then incubated at 30°C for 3 days before inoculation in biogas digesters.

Three sets of experiments were conducted in 10 litre glass aspirator bottles with a mixture of 100/g jute caddis, 500/g vegetable market waste and 1kg fresh cowdung. The mixture was grinded and then diluted with 2 litre tap water. Set 1 served as control where microwave unirradiated *Methanosarcina barkeri* (DSM-804) culture was inoculated. In Set 2 and Set3 *Methanosarcina barkeri* irradiated with microwave frequencies of 28.0 GHz and 31.5 GHz respectively were inoculated. pH, redox potential (E_h) and conductivity (C) changes in these above biogas digesters were determined at 7, 10, 15 and 20 days from digester slurry in all three sets by standard procedures.

Methane and carbon dioxide gas generate in the head space of biogas digesters was determined Plant with the help of Nucon made (Model No. 5700) gas chromatograph equipped with a thermal conductivity detecter and a porapack Q glass column. Detector and column temperature was kept at 50°C. Hydrogen served as the carrior gas at a flow rate of 20 ml/min.

Cell morphology of *Methanosarcina barkeri* (DSM-804) was done from pure cultures of the bacteria grown in broth medium for 20 days at 35°C under normal condition and after irradiation with 28.0 and 31.5 GHz microwave. Direct count of cells was studied with the help of a heamocytometer and cell dimention was measured by an ocular micrometer under a microscope. Fluorescence activity of the bacteria was studied under a fluorescence microscope starting from gas bubble initiation to gas bubble termination stage.

RESULTS AND DISCUSSION

Table 1 represents the changes of pH, redox potential (E_h) and conductivity (C) in biogas digesters fed with a combination of jute caddis, vegetable market waste and cattle dung as substrate and microwave non irradiated and irradiated at 28.0 GHz and 31.5 GHz *Methanosarcina barkeri* (DSM)-804 as inoculum. The trend of changes of all three parameters were the same in all three digesters. pH dropped at initial stage and then rises to near neutral value whereas the E_h values continued to reduce and reached to a very negative value. Conductivity values also initially dropped and then continued to increase till initiation of biogas production. This clearly indicate that during active biomethanation near neutral pH a very low redox potential was necessary. It was also evident from the study that in sets inoculated with microwave irradiated *Methanosarcina barkeri* (DSM-804) the ideal condition was established quicker in comparison to inoculation of non-irradiated ones.

Table 1 : Change in pH, Rodox potential (E_h) and Conductivity (C) in Biogas Digesters

	Digester – 1			Digester – 2			Digester – 3		
No. of day	pH	Eh	C	pH	Eh	C	pH	Eh	C
0	6.9	+30	8.43	6.9	+30	5.73	6.9	+30	5.73
7	6.85	–245	3.8	6.81	–322	4.59	6.76	–340	4.66
10	6.97	–280	4.56	6.96	–330	4.9	6.88	–352	5.24
15	7.1	–323	5.72	7.13	–343	5.4	7.0	–344	6.28
20	7.17	–350	6.2	7.25	–365	5.88	7.2	–350	6.5

Digester 1 : 100g jute caddis + 500g MSW + 1Kg fresh cowdung + 2 litre water + Microwave not irradiated *Methanosarcina barkeri* (DSM-804).

Digester 2 : 100g jute caddis + 500g MSW+ 1 Kg fresh cowdung + 2 litre water + Microwave irradiated (at 28.0GHz) *Methanosarcina barkeri* (DSM-804.)

Digester 3 : 100g jute caddis + 500g MSW + 1Kg fresh cowdung + 2 litre water + microwave irradiated (at 31.5 GHz) *Methanosarcina barker* (DSM-804).

From Table 2 it is evident that methane content in biogas both at initiation and termination stage was

higher in digester sets treated with microwave irradiated *Methanosarcina barkeri* in comparison to microwave unirradiated ones. Frequency of microwave was also responsible in controlling effective than 28.0 GHz microwave on biomethanation by *Methanosercina barkeri* DSM-804.

Table 2 : Analysis of biogas composition in biogas digesters

	Digester – 1		Digester – 2		Digester – 3	
No. of day	CH_4	CO_2	CH_4	CO_2	CH_4	CO_2
18	35.4	64.6	50.5	49.5	54.4	45.6
28	39.1	50.9	41.4	58.6	46.2	53.8

18 days : Initiation stage of biogas production
28 days : Termination stage of biogas production

Table 3 showed the changes in morphology of *Methanosarcina barkeri* DSM-804 upon microwave irradiation at a frequency of 31.5 GHz. The cell count and cell dimension increased from the initiation of gas bubble on 7th day and attend a maximum at 9th day and then continued to decline and reached a minimum value at the point of termination of gas bubble i.e., on 11th day. From comparative studies between microwave un-irradiated and irradiated *Methanosarcina barkeri* (DSM-804), it was noticed that the cell count, in haemocytometer, increased to a maximum of 56.71 % on 10th day, while the cell size increased to a maximum of 21.5 % on the same day. So, from this study it is understood that due to irradiation of mucrowave on *Methanosarcina barkeri* (DSM-804) not only the cells grew faster (Banik *et al.*, 2002) but also cell number and cell size increased which may be the cause of increased biomethanation activity by *Methanosarcina barkeri* DSM-804.

Table 3 : Change in cell morphology of *Methanosarcina barker* (DSM-804) upon microwave irradiation

Number of Days	Microwave not irradiated culture		Microwave irradiated culture		Increase in Number (%)	Increase in size (%)
	No. of cells/cc	Size (μ)	No. of cells/cc	Size (μ)		
7*	22.4×10^6	2.32	29.6×10^6	2.6	24.32	10.76
8	28.8×10^6	2.92	59.2×10^6	3.64	51.35	19.78
9	56.8×10^6	3.1	113.6×10^6	3.84	50	19.27
10	46.4×10^6	2.92	107.2×10^6	3.72	56.71	21.50
11	44.0×10^6	2.52	96.0×10^6	2.80	54.16	10.0

* Microscopic study was done from gas bubble initiation to gas bubble termination stage.

From Table 4 it is evident that fluorescence activity of *Methanosarcina barkeri* DSM-04 was durectly related to biomethanation. Fluorescence activity was noticed in cultures from the day of initiation of methane gas in the culture bottles. It was also observed that biomethanation as well as fluorescence activity was noticed in microwave irradiated cultures on 7th day while the same started on 9th day in culture not irradiated with microwave.

Table 4 : Fluorescence activity of *Methanosarcina barker* (DSM-804) due to irradiation microwave

No. of days	Microwave not irradiated culture	Microwave irradiated culture
7	–	+
8	–	+
9	+	+
10	+	+
11	+	+

* Microscopic study was done at gas bubble initiation to gas bubble termination stage.
+ Fluorescence activity seen.
– Fluorescence activity not seen.

So, from the present study it is evident that microwave power has ability to induce biomethanation activity in *Methanosarcina barkeri.* Further study is on progress.

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