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Investigation of methanogen population structure in biogas reactor by molecular characterization of methyl-coenzyme M reductase A (*mcrA*) genes

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Abstract

The methanogen community in biogas reactor running on cattle dung was investigated in two different seasons; summer (April, 36 °C) and winter (December, 24 °C), in the year 2004 by a culture-independent approach. Community structure was determined by phylogenetic analyses of 343 and 278 *mcrA* clones belonging to summer and winter month libraries, respectively. In summer month's library, 41.7% clones were affiliated to *Methanomicrobiales*, 30% to *Methanosarcinales*, 19% to *Methanobacteriales*, 5% to *Methanococcales* and a total of 4.3% clones belonged to unclassified euryarchaeotal lineages. In winter month's library, *Methanomicrobiales* encompassed 98.6% clones, and *Methanobacteriales* included 1.4% of total clone diversity. Biogas plant performance data collected during the winter month indicated significant reduction in daily biogas produced as compared to summer month because of lowering in ambient temperature and associated shift in microbial community. Results from this molecular study showed the existence of highly diverse and complex methanogens communities present in biogas plant.
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1. Introduction

Biogas technology plays a very significant role in rural areas of developing countries where various cellulosic biomasses are available in plenty. The estimated population of cattle in India is about 250 million, and if one third of the dung produced can be used for biogas production, then more than 12 million biogas plants can be operated yearly (Kashyap et al., 2003; Yadvika et al., 2004). Around 3 million biogas plants are set up in India (Khoiyangbam et al., 2004). Floating-dome (Khadi and Village Industries Commission [KVIC] type) and fixed-dome design (Janata type) with capacity of 3–20 m^3 are the most popular models in India (Khoiyangbam et al., 2004). The process of biogas production requires complex interaction of several varieties of bacteria that must be in equilibrium in order for the biogas plant to perform efficiently. Among various factors that affects biogas production, e.g., pH, temperature, hydraulic retention time (HRT), C/N ratio, etc., temperature is the most critical parameter for successful production of biogas. It affects not only the carbon and electron flow but also determines the composition of methanogenic community (Chin et al., 1999; Fey and Conrad, 2000). The optimum temperature for the mesophilic biogas production process is 35 °C and therefore a biogas plant must be maintained between

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30 and 35 °C for most favorable functioning (Mital, 1996; Yadvika et al., 2004). Most of the biogas plants in rural India are operated at ambient temperature without any temperature control, therefore variation in ambient temperature affects daily gas yield. During winter season, the reduction in biogas production has been observed and it poses a serious problem to the practical application of this technology. Kalia and Singh (1996) found that biogas production in May–July was 1700 l/day which reduced to around 99 l/d in January–February in hilly regions of India. Khoiyangbam et al. (2004) quantitatively estimated the methane flux in fixed-dome biogas plants in hilly (16–25 °C) and plain regions (21–33 °C) of northern India. They reported a strong effect of temperature on methane production, and a drastic fall was observed during winter. The failure of many biogas plants to operate reliably and with a sustained performance has underlined the need for more basic information on the microbiological aspects of biogas process. The low growth rate of methanogenic bacteria can make the biogas production system highly sensitive to environmental changes and perturbation in one trophic level may affect the entire community. Such imbalances are reflected by reduced efficiency of the biogas plant and may lead to process failure or at least require long recovery periods. To help in understanding such imbalances and controlling biogas plant performance more efficiently, in-depth analysis of microbial community structures are required. Few studies have been carried out to investigate the effect of temperature shift on the microbial community structure in thermophilc biogas reactors treating cattle manure (Ahring et al., 2001; Nielsen et al., 2004). However, to the best of our knowledge no culture-independent survey have been carried out for the characterization of methanogen diversity present in cattle dung fed biogas plants operating under ambient temperature which are popular in India.

Methanogens play a pivotal role in the production of biogas and convert H_2/CO_2, acetate, formate or methanol to methane (Ferry, 1993). Methanogens can be studied specifically using a characteristic "functional" marker gene *mcrA* coding α-subunit of methyl-coenzyme M reductase (MCR), the key enzyme of methanogenesis. Unlike other enzymes in methanogenic metabolism, MCR appears to be unique to methanogens and is involved in the final stage of methanogenesis causing reduction of methyl group attached to coenzyme M (Friedrich, 2005; Lueders et al., 2001). The presence of MCR enzyme defines a cell as a methanogen so *mcrA* sequences might be used for methanogen phylogenies (Luton et al., 2002). The phylogeny of methanogens based on *mcrA* closely resembles the 16S rDNA therefore *mcrA* was used as a suitable target for PCR-based detection in many molecular ecological studies such as landfill (Luton et al., 2002), termite gut (Ohkuma et al., 1995), and cattle rumen (Tatsuoka et al., 2004).

A biogas plant is a closed, comparatively homogenous and stable ecosystem. Since there is scarce information about this ecosystem, a molecular inventory will be the first approach to expand our understanding of the methanogenic communities of the biogas plants. The aim of this study was to examine the methanogenic community structure of smooth functioning cattle dung fed biogas plant in two different seasons by constructing *mcrA* clone libraries.

2. Methods

2.1. Design and operation of biogas reactor

Cattle dung was obtained daily from a specific dairy in Pune, India to avoid variation in physical and chemical characteristics of dung according to the diet of cattle. These characteristics are likely to affect the species composition and relative abundance of the groups of bacteria that play an important role in producing biogas from the slurry (Meynell, 1976), and will therefore have an effect on the quantity and quality of gas produced. Total solid (TS) estimation was carried out for each batch of dung and adjusted to have final concentration ~8.5% in slurry by adding tap water for daily addition in biogas plant. Biogas reactor used in the present study was of 25 l capacity, KVIC type, non-stirred and floating-dome model. The biogas plant was running over a period of 1 year at ambient temperature in single-stage, semi-continuous mode and achieved steady-state conditions with respect to cattle dung digestion. Biogas plant was charged daily with 830 ml of cattle dung slurry for 30 days HRT which is used generally for cattle dung digestion in India.

2.2. Sampling and analysis

Pune city experiences the summer season from April to June and the rainy season begins from early July and goes up to November. The winter season starts from December and lasts up to March. During year 2004, maximum mean ambient temperature (36 °C) was found during April and minimum (24 °C) in December. Daily minimum and maximum temperatures were also recorded. The ambient and slurry temperature of biogas plant was measured by using an ordinary mercury thermometer, at the time of sampling. Effluent slurry samples were collected from the outlet of biogas plant during the month of April and December, 2004 on every 1st, 10th and 30th day, and brought immediately to laboratory on ice. Parameters such as TS, volatile solids (VS), and pH of influent and effluent slurry were estimated as per standard methods (APHA, 1992). Biogas produced was measured daily using a wet gas-flow meter (Toshniwal, India) and analyzed on gas chromatograph (Chemito 3800, Toshniwal) for CH_4 and CO_2 content using the method described by Savant et al. (2002). Volatile fatty acids (VFA) content in the slurry was estimated on gas chromatograph using the method described by Dighe et al. (1998). All measurements were done in triplicate and the averages were taken as the representative values.

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