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Methanogenic potential of a thermophilic consortium enriched from coal mine

Meeta Lavania*, Simrita Cheema, Priyangshu Manab Sarma, Ramya Ganapathi, Banwari Lal*

The Energy and Resources Institute, Darbari Seth Block, India Habitat Center, Lodhi Road, New Delhi 110003, India

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ABSTRACT

India is rich in coal bed methane resources but still lacks in methane development technologies. To begin understanding this prospective source, in the present investigation, microbial methane production was examined from bituminous, subbituminous and lignite coals obtained from Jharia coal mines in India. Microbial populations were enriched over a period of 21 days on various methanogenic specific substrates including the three ranks of coal. Maximum methane production (49%) was obtained on *Methanosprillium* sp specific medium (sodium acetate and isopropanol) supplemented with subbituminous coal at 65 °C and pH 6.8. Phylogenetic analysis of 16S rRNA gene sequences from the selected CBM65 consortium revealed a syntrophic association between a hydrogenotrophic methanogen *Methanoculleus thermophiles* and fermentative bacteria *Comamonas* sp. This is the first report of methane production by a consortium enriched from Indian coal bed methane reservoirs at such high temperature. The results of this study shed light on the fact that Jharia coal mines are methanogenically active and offer a prospective source for coal bed methane extraction. It is also, considered as the richest source of methane in India further this data will help in determining the potential role of methane emitted from this site in influencing the global carbon cycle.

1. Introduction

Petroleum hydrocarbons are a major source of energy providing 50% of the energy used all over the world (Clark, 2007). However with the increasing demand and finite source of fossil fuel, there is an urgent need to look for an alternate source of hydrocarbons. Methane trapped in coal seams and its production from these coal mines has emerged as an important alternative. Ever since the coal bed methane recovery started in USA, the US coal industry has captured and used around 8.7 billion cubic meters of coal mine methane (EIA, 2006). Coal mine methane is a subset of coal bed methane (CBM) and refers to methane released due to coal mining activity, while CBM is trapped in coal seams and is often referred to as virgin CBM. Much of the world's coal resources are in bed of deep or otherwise unminable coal for which coal mining is uneconomic but many such coal beds might prove to be attractive for CBM recovery (Byrer and Guthrie, 1998). India is the third largest coal producer in the world and commercial production of CBM also has good prospects (Ojha et al., 2011).

Methane can be produced by either thermogenic formation occurring in subsurface carbon deposits or by biological or biogenic formation occurring usually at or near the earth surface (Taylor et al., 1998). Thermogenic gas is formed by the chemical devolatilization of coal that releases methane. On the other hand biogenic methane is the result of a series of biochemical reactions where coal is converted into methane by a complex group of bacteria in anoxic environments. Three functionally different trophic groups of bacteria are required for methanogenesis. These include (1) hydrolytic fermentative, (2) syntrophic acetogenic and (3) methanogenic bacteria (Boone, 1991). Hydrolytic fermentative and syntrophic acetogens hydrolyse complex polymers (cellulose, polysaccharide and protein) into monomers (fatty acids, sugars, amino acids, carbon dioxide, acetate and hydrogen). These monomers are finally utilized by methanogens to produce methane (Conrad, 1999).

The methanogens generate methane by several pathways (Boone, 1991). The universal mode of methane production is hydrogen - mediated reduction of carbon dioxide. Environmental factors (pH, salinity, temperature) and nutritional factors (inorganic

* Corresponding authors. Tel.: +91 11 24682100; fax: +91 11 24682144.
E-mail addresses: meetal@teri.res.in (M. Lavania), banwaril@teri.res.in (B. Lal).

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and organic) can affect methanogenesis (Boone, 1991). For example, in environments with slow rates of organic matter turnover, it is generally the hydrolysis of large polymers which restricts microbial activity in anoxic environments and low molecular weight intermediates do not accumulate appreciably and as a result methane production is limited (Ojha et al., 2011). Though the processes of biogenic methane production are poorly understood and highly complex processes they are pervasive in nature (William et al., 1998). It is believed that 20% of the natural gas in the earth is from methanogens of which 2/3 is by acetate fermentation and 1/3 by carbon dioxide reduction (Noble and Henk, 1998) there is strong evidence that it may be the predominant mechanism. Several studies have reported the degradation of coal by microbial cultures to produce methane. Taylor et al. (1998) investigated the microbial conversion of coal to methane using different anaerobic environments as sources for methanogenic consortia. Microflora present in water leached from coal mines were shown to generate methane (Thielemann et al., 2004). Furthermore, a methane-generating consortium extracted from coal was observed to grow on low-volatile bituminous coal as a sole carbon source (Thielemann et al., 2004). A microbial consortium was successfully applied to bioassay methane generating potential in 18 coal samples (Jones et al., 2008). Subbituminous coal from a nonproductive well in south Texas was stimulated to produce methane in microcosms when the native population was supplemented with nutrients (biostimulation) or when nutrients and a consortium of bacteria and methanogens enriched from wetland sediment were added (bioaugmentation) (Jones et al., 2010). In a recent study, microbial methane production from bituminous coal waste, lignite and bituminous coal materials under diverse conditions was shown (Opara et al., 2012).

There is a need to gather more data about methane generation from coal bed cultures as the information so far is limited. In the Jharia coal field (India) which is considered to be the most prospective area, the methane content is estimated to be between 7.3 and 23.8 m^3 per ton of coal within the depth range of 150–1200 m (Ojha et al., 2011). Analysis indicates every 100 m increase in depth is associated with a 1.3 m^3 increase in methane content (M2M 2005). Yet efficient methane production is limited here largely due to lack in detailed reservoir characterizations. The presence of hydrogenotrophic *Methanobacterium* sp. was found in Jharia coal fields (Singh et al., 2012). However, the study has not provided any information on the biogenic methane production. In the present study we have used a laboratory based approach to characterize the indigenous microbes in these coal seams and assess their potential for stimulating methanogenesis across different range of coals (lignite, subbituminous and bituminous). Unlike many other studies, this research focuses exclusively on enhancing methane production at high temperature (65 °C) using *in situ* coal, conditions typically encountered in CBM operations. This will help in overcoming the differences that arise in optimizing *in situ* methanogenesis.

2. Materials and methods

2.1. Site description

Jharia coal mines are the most important storehouse of prime coke coal in India containing 23 underground and nine open cast mines. The coal field is situated about 260 km northwest of Calcutta in the heart of the Damodar River Valley (70.30 °E; 22.25 °N) covering about 280 km^2 and produces bituminous, subbituminous and lignite coal suitable for coke. This region has a typical desert climate. The average temperature during summer is 50 °C, while in winter it is 5 °C with medium rainfall. The coal samples were collected in December 2009 and used in the experiments after sterilization. The temperature of the coal seams was 60–70 °C with 0.5% salinity of formation water.

2.2. Sample collection and processing

The coal (lignite, subbituminous and bituminous) and formation water samples from Jharia coal mines were procured through the Oil and Natural Gas Corporation Ltd (part owner) stored and shipped under argon atmospheric conditions. The concentration of chloroform-extractable organic matter was determined via Soxhlet extraction. Briefly, coal (30 g) was extracted using 300 mL chloroform over 24–48 h. The volume of the resulting extract was decreased by rotary evaporation and subsequently processed by standard geochemical procedures to separate the bitumen into asphaltene and maltene fractions. The mass of extracted maltene was determined by reducing a known volume to dryness under a stream of nitrogen in a tared vial and weighing the resulting residue. Dissolved Organic Carbon (DOC) concentration was determined by mixing coal (50 g) and deionized water (100 mL) and allowing extraction for 96 h at 25 °C. The resulting extract was filtered (0.45 μm) and analyzed for DOC by the wet-oxidation method.

Physio-chemical analysis of formation water was done for hydrogen ion concentration (pH) and electrical conductivity according to the American Petroleum Institute (API) standards. The presences of heavy metals such as arsenic, cadmium, chromium, copper, lead and mercury were also estimated as per the standard methods (EPA SW 846-7061A; EPA SW 846-7130; EPA SW 846-7190; EPA SW 846-7210; EPA SW 846-7420; USEPA 846-7471A respectively).

APHA method) with their detection limits being 0.01, 0.01, 0.01, 0.02, 0.01 and 0.0005 mgL^{-1} respectively. The presence of cation [calcium (APHA 3500 (B)] and anion [chloride: APHA 4500, nitrate: IS 3025, phosphate: APHA 4500 (D) and sulphate APHA 4500 (E)] were also estimated in the formation water.

2.3. Inoculum source

The depth of Jharia coal seams was ~645 m and estimated pressure at the bottom of the well was 6.4 kg m^{-1}. The collection device consisted of a 10 cm diameter, 1 m long polyvinyl chloride pipe that was sealed at the bottom and capped with an open steel mesh at the top. Both pipe and mesh enclosure were sterilized with a bleach solution at the field site and then lowered to the bottom of the well on a wire line. Samples (1000 mL) were collected in 1000 mL of anaerobic sterilized serum bottles and analyzed on site for pH and conductivity. Sample bottles were then transported to laboratory for further analysis.

2.4. Establishment of methanogenic culture

An enrichment culture technique was used for isolation and characterization of methanogens from the formation water sample. Three methanogenic specific media was used as reported (Imachi et al., 2000; Waldron et al., 2007; Cheng et al., 2008). For our study we have abbreviated them as MSP for *Methanosprillium* sp., MSA for *Methanosarcina* sp. and MBA for *Methanobacter* sp. This annotation has been referred in all the sections. The MSP medium contained (per liter of demineralized water): KH_2PO_4, 0.5 g; $MgSO.7H_2O$, 0.4 g; NaCl, 0.4 g; NH_4Cl, 0.4 g; $CaCl_2.2H_2O$, 0.05 g; $FeSO_4.7H_2O$, 0.002 g; yeast extract, 1 g; sodium acetate, 1 g; sodium formate, 2 g; $NaHCO_3$, 4 g; resazurin, 0.001 g; L-cysteine HCl, 0.5 g; $Na_2S.9H_2O$, 0.5 g; isopropanol, 7.5 ml at pH 6.5. The composition of MSA medium (per liter of demineralized water) was: K_2HPO_4,