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Development of a microbial process for methane generation from bituminous coal at thermophilic conditions

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ABSTRACT

There has been a growing interest in coal bed methane (CBM) both for energy production and reduction of greenhouse gases. CBM has been used as an alternative fossil resource and methane generation from coal reservoirs and is contributing in meeting clean energy demand. India has CBM generating potential but lacks in technology for in situ biogenic methane generation from its coal reservoirs. Therefore, to explore the possibility of enhancing biogenic methane production in coal seams particularly those present at greater depth, in this study, a thermophilic methanogenic consortium was enriched from samples collected from Banaskantha coal mines (depth of about 1200 m) of western India that had bottom-hole temperature of around 62 °C. Microbes were enriched with 1% (w/v) bituminous coal obtained from the same coal mines. Subsequently, effect of coal loading, temperature, pH and salinity were optimized for enhanced CBM generation for the selected consortium CBM 4. Maximum methane production of 22.9 mM/g of coal was observed by the thermophilic methanogenic consortium CBM 4 at temperature 60 °C, pH 7.5 and salinity 0.1% NaCl. This study suggested that the selected consortium isolated from Banaskantha coal mines is capable of utilizing high rank bituminous coal as a carbon–energy source at thermophilic condition. Thus, indicating a possibility of stimulating or augmenting this consortium in coal seams of similar temperature and to develop a microbial process for enhanced CBM generation.

1. Introduction

Coal is the major source of energy worldwide. It has also been playing a dominant role in the energy scenario of India. About 53% of the total nation's energy is supplied by coal in India and this percentage is expected to increase rapidly in the next few years (Baruah et al., 2013).

Although India has significant coal resources and accounts for the 3rd largest coal reserves in the world, majority of these are located in environmentally sensitive geographical areas or at a depth that renders its exploration and extraction economically unviable. Therefore, it becomes imperative to explore options for utilizing these coal resources to bridge the gap between demand and supply.

Coal bed methane (CBM) has emerged as clean non-conventional source of energy to supplement the rising demand of conventional hydrocarbons. The US coal industry has explored and utilized around 8.7 billion cubic meters of coal mine methane since the coal bed methane recovery started in the USA (EIA, 2006). Also, from the past few years, a huge impetus for the exploration of CBM has been given in developing countries including India, endowed with considerable coal reserves (Chakraborty et al., 2011).

The term CBM refers to a type of natural gas, composed majorly of methane, which is generally present in coal beds. CBM is produced by thermogenic, geological reactions and/or due to biological or biogenic activities (Taylor et al., 1998). Thermogenic methane is produced by the thermo-chemical devolatilization of coal, whereas biogenic methane production is the result of a series of biochemical reactions in which coal is converted into methane by a mixture of bacteria under anaerobic condition. In biogenic methane generation process, facultative bacterial strains first depolymerize complex organic compounds into intermediates, which can be later use by fermentative bacteria, generating substrates such as carbon dioxide (CO_2), hydrogen (H_2), and acetate for the methane production by methanogens (Fallgren et al., 2013). Biogenic methane is supposed to be consisting of around 40% of the total methane storage on earth. However, under favorable bio-geological conditions it is anticipated that there is an increased amount (65%) of methane produced in the reservoirs (Kotelnikova, 2002).

Although there is growing interest for enhancing biogenic coal bed methane generation, relatively less is known about the microbiology of coal beds. Microbial enhanced CBM, through bio-augmentation of selected microbes or by stimulation of indigenous microbes by adding nutrients, has the potential to produce methane from coal and also increases reservoir permeability via the microbial consumption of coal, waxes and paraffin (Scott, 1999).

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Enhancement of biogenic methane production from different ranks of coal in situ or ex situ has been explored by a number of investigators in recent years using a variety of nutrient combinations (Harris et al., 2008; Hendry and Midgley, 2014; Jones et al., 2008, 2010; Opara et al., 2012; Pfeiffer et al., 2010; Strapoc et al., 2008; Strąpoc et al., 2011).

All the previously reported studies on biogenic methane production from coal conducted in laboratory were at mesophilic condition. However, most of the unmineable coal seams/reserves that have CBM recovery potential are at a higher temperature range. Thus this study is focused towards biogenic methane production from coal at high temperature.

The current study aimed at developing a thermophilic methanogenic consortium from samples collected from high temperature coal seams with CBM potential. Also, in this study, optimization of parameters that may subsequently influence the in situ biogenic CBM production by selected thermophilic methanogenic consortium was taken into consideration. Further, the present study indicated potential of the isolated consortium for its possible application in enhanced coal bed methane recovery at high temperature bituminous coal reservoirs.

2. Materials and methods

2.1. Coal substrate and inoculum source

Four formation water samples were collected from Banaskantha coal mines situated in the northern part of Gujarat state (coordinates: 24° 10′ 23″ N, 72° 25′ 53″ E), western province of India (Fig. 1a). Coal mines were situated 220 km northwest from Ahmedabad in Gujarat. The climate is tropical with maximum and minimum temperatures of 42 °C and 14 °C respectively. The average annual rainfall is around 803.4 mm (31.63 in). The temperature of the drilling bores and the reservoir was around 62 °C with the depth of around 1200 m.

The formation water samples were collected from the well-head of the drilling bores into 100 ml anaerobic pre-sterilized serum bottles containing 1 ml of 2% Na_2S. Serum bottles were filled and sealed such that the samples are devoid of any air bubble. All the samples were transported at ambient temperature to the laboratory within 48 h, stored at 4 °C and processed immediately for activity measurements and microbial analysis.

The bituminous coal obtained from Banaskantha coal mines was used as a carbon source for this study (Fig. 1a). It belongs to CBM exploration acreage area lying in the Sanchor tectonic Block, covering an area of about 790 sq. km in Banaskantha district of North Gujarat. This sanchor block is surrounded in the north by the Serau fault and the Tharad fault in the south (Chakraborty et al., 2011; Sridhar et al., 1997). Geologically, this block lies at the junction of the Cambay basin to the south and Barmer Basin to the north as indicated in Fig. 1a. Coals are confined to the Middle Tharad Formation of Middle Eocene age (Trippi and Tewalt, 2011). The coals are overlain by shale sequence of Tharad formation, which is succeeded by Wav Formation of Oligocene sequence (Fig. 1b).

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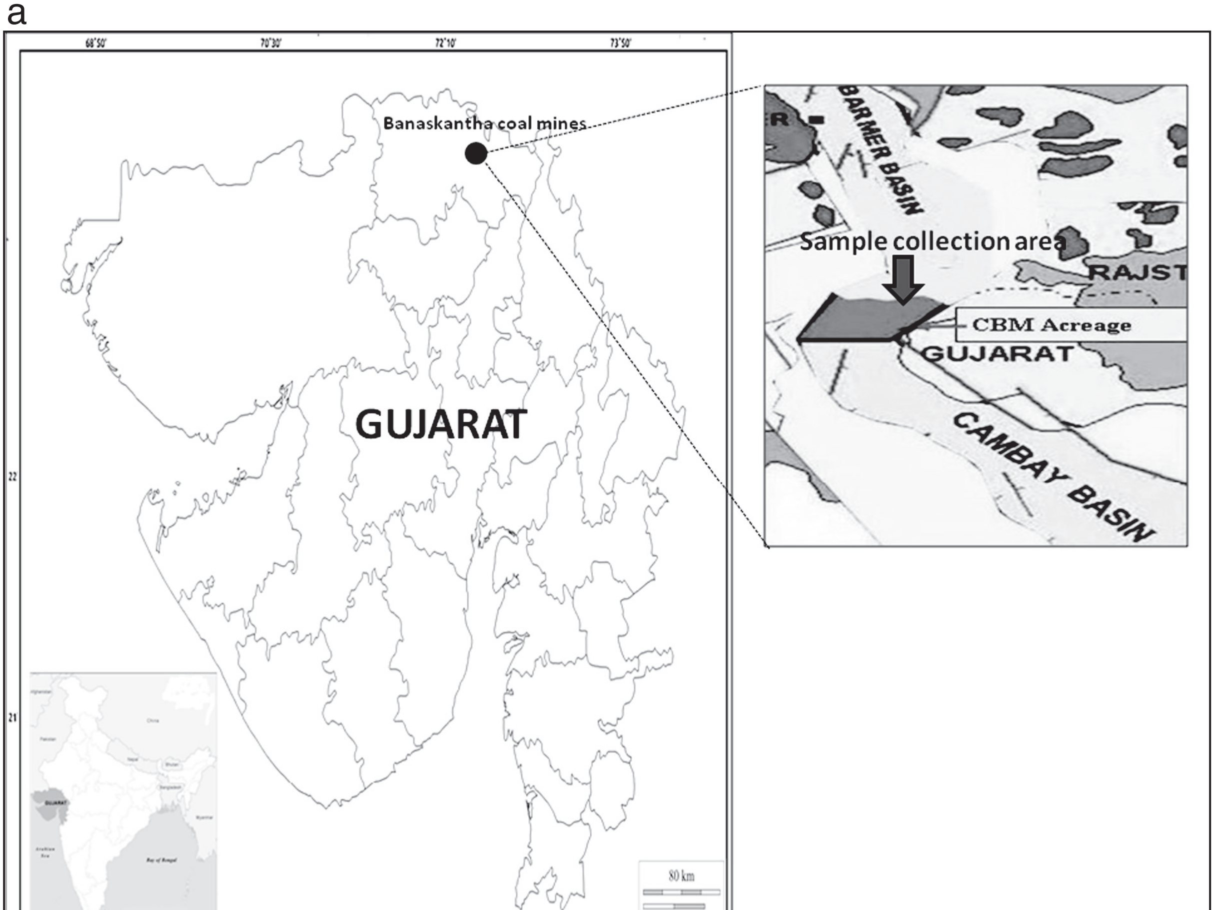

Fig. 1. a. Location of Banaskantha coal mines used for sample collection. b. Generalized and sub-surface litho-stratigraphy of the Banaskantha coal bed basin. Modified after Chakraborty et al. (2011). Modified after Singh et al. (1977); http://www.dghindia.org/Images/RajasthanBasin/Sanchor.jpg.

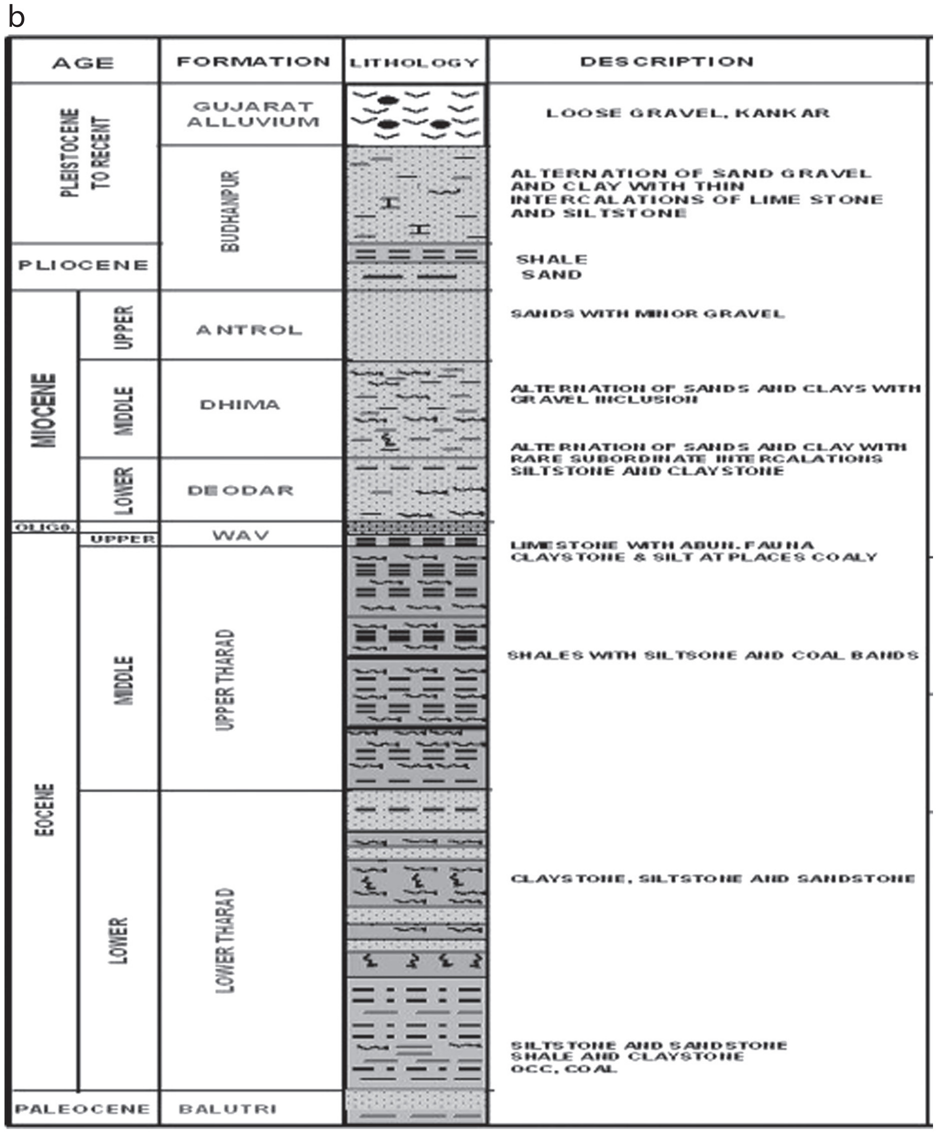

Fig. 1 (*continued*).

2.2. Physico-chemical analysis of formation water and coal

Physico-chemical analysis of formation water samples was done for hydrogen ion concentration (pH) 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. The presence of cation [calcium APHA 3500 (B)] and anion [chloride: APHA 4500, nitrate: IS 3025, phosphate: APHA 4500 (D) and sulfate APHA 4500 (E)] was also estimated in the formation water. The detailed analysis of coal in terms of ash, moisture, volatile matter and fixed carbon along with the specific carbon, hydrogen, nitrogen, sulfur and oxygen (CHNSO) profile was determined as per the guideline of ASTM standards.

2.3. Enrichment and screening of coal degrading thermophilic methanogenic consortium

An enrichment culture technique was used for the isolation of methanogens from the formation water samples. All the four formation water samples namely CBM 4, CBM 6, CBM 9 and CBM 12 were transferred to a nutrient medium containing bituminous coal as the carbon/energy source. Cultures were developed in modified MSP liquid medium containing (per liter) KH_2PO_4; 0.25 g, $MgCl_2.6H_2O$; 0.2 g, NaCl; 0.5 g, NH_4Cl; 0.4 g, $CaCl_2.2H_2O$; 0.025 g, yeast extract; 0.5 g, and $NaHCO_3$; 0.2 g (Lavania et al., 2014). The medium pH was adjusted to 7.00 ± 0.2. This medium was selected and modified as previously reported by some of the authors of this study (Lavania et al., 2014) for the methane production from coal. The bituminous coal collected from Banaskantha coal mines was pulverized (particles less than 500 μ) and then added in a serum bottle as a carbon source at a final concentration of 10 g/l. A volume of 100 μl of resazurin (10 g/l) was added as an oxygen indicator and the 1000 ml medium was then boiled for 10 min and cooled under a nitrogen purge to remove dissolved oxygen. Cysteine hydrochloride was then added at a final concentration of 0.5 g/l as a reducing agent to completely remove dissolved oxygen. A volume of 45 ml of medium was dispensed into 130 ml serum bottles flushed with O_2-free N_2. The serum bottles were sealed with butyl rubber stoppers and crimped with aluminum seals. Prior to inoculation, the sealed, pressurized tubes were sterilized in an autoclave at 121 °C for 15 min. At time zero, 45 ml of culture medium was inoculated with 5 ml of the culture using aseptic, strict anaerobic techniques and kept in an incubator at 60 °C for 20 days. Unless specified, all the experiments were performed in 130 ml anaerobic Wheaton serum bottles containing 45 ml of the liquid medium.

The incubated cultures were tested for methane production after 20 days by taking 0.5 ml of headspace gas samples from the anaerobic serum bottles using a gas-tight syringe. The expected headspace gases such as methane, hydrogen, carbon dioxide and nitrogen were quantified by gas chromatography as mentioned below in Section 2.6.

2.4. Effect of coal concentration on methane production by the methanogenic consortium

The effect of bituminous coal concentration on selected indigenous consortium CBM 4 was evaluated in terms of methane production at a range of concentration (0.5 g, 1 g, 1.5 g, 2 g, 2.5 g, 5 g and 10 g) in 130 ml anaerobic serum bottles containing 45 ml modified MSP medium. The 5 ml of freshly grown consortium CBM 4 was inoculated into above media bottles and kept at 60 °C for 20 days of incubation. Methane generated in the headspace of the experimental bottles was quantified by gas chromatography as mentioned in Section 2.6. Experiment was performed in duplicate and the data points are average of the duplicate ± standard deviation (less than 5% of average).

2.5. Effect of temperature, pH and salinity on methane production by the methanogenic consortium

The selected indigenous consortium CBM 4 was subjected to a range of temperature (37, 45, 55, 60, 65 and 70 °C), pH (6, 6.5, 7, 7.5 and 8) and salinity (0.05, 0.1, 0.2, 1, 2 and 4% NaCl) for determining the efficiency in terms of methane production. The pH was adjusted with 2 N HCl and 2 N KOH solutions. Experiments were performed in 130 ml anaerobic serum bottles containing 45 ml modified MSP medium with 0.5 g bituminous coal as a carbon source. Five milliliters of freshly grown consortium CBM 4 was inoculated and kept at 60 °C for 20 days of incubation. Headspace gases such as methane, carbon dioxide and hydrogen were observed by gas chromatography as per the method described in Section 2.6. In case of pH and salt concentration the incubations were done at 60 °C. All the experiments were performed in duplicate and the data points are average of the duplicate ± standard deviation (less than 5% of average).

2.6. Analytical method

The headspace gases (hydrogen, nitrogen, methane and carbondioxide) were quantified by a calibrated gas chromatograph (model GC-7890A, Agilent Ltd. USA) equipped with a molecular sieve packed stainless steel column (2 m × 2 mm id NUCON, INDIA) and a thermal conductivity detector (TCD). Argon was used as the carrier gas at a flow rate of 5 ml/min. The operating temperatures of the injection port, oven and the detector were 100, 50 and 150 °C respectively. In the present study, gas concentration values produced in the headspace of the media bottles were calculated as millimolar per gram of coal (mM/g coal).

3. Results

3.1. Physico-chemical analysis of formation water and coal

Physico-chemical analysis of formation water is shown in Table 1. The formation water was found to be slightly alkaline with the presence of calcium, nitrate, sulfate, chloride and heavy metals including iron and zinc. Proximate analysis data showed that the moisture level in coal is very less (0.26%), and lower level of volatile matter (17.41%), along with 25.44% ash and 56.89% fixed carbon (Table 2). Also, the specific carbon, hydrogen, nitrogen, sulfur and oxygen (CHNSO) composition of the coal samples is tabulated in Table 2.

Table 1
Physico-chemical analysis of formation water of Banaskantha coal mines.

Test	Method	CBM#4	CBM#6	CBM#9	CBM#12
pH		7.55	7.46	7.23	7.32
Heavy metals					
Arsenics (mg/l)	EPA SW 846-7061A	ND	ND	ND	ND
Cadmium (mg/l)	EPA SW 846-7130	3.12	2.05	2.15	1.97
Chromium (mg/l)	EPA SW 846-7190	37.03	5.37	6.20	17.92
Copper (mg/l)	EPA SW 846-7210	47.14	2.55	8.68	32.58
Zinc (mg/l)	EPA SW 846-7950	180.96	22.38	17.08	26.43
Lead (mg/l)	EPA SW 846-7420	36.65	10.68	ND	23.25
Mercury (mg/l)	USEPA 846-7471A	ND	ND	ND	ND
Total iron (mg/l)	EPA SW 846-7380	6688.28	1318.97	1177.23	875.39
Other inorganics					
Calcium (mg/l)	APHA 3500 (B)	4.0	4.0	4.0	4.0
Chloride (mg/l)	APHA 4500	124.27	82.85	62.13	82.85
Nitrate (mg/l)	IS 3025	0.99	0.56	0.14	0.31
Phosphate (mg/l)	APHA 4500 (D)	ND	ND	ND	ND
Sulfate (mg/l)	APHA 4500 (E)	7.62	28.59	22.88	28.60

ND — not detected.

3.2. Enrichment and screening of indigenous coal degrading thermophilic methanogenic consortium

Four different thermophilic microbial consortia namely CBM4, CBM 6, CBM 9 and CBM 12 were enriched in anaerobically prepared modified MSP medium from the four individual formation water samples collected from Banaskantha coal mines. Growth of all the four consortia was monitored in terms of methane production in the headspace of the experimental bottles using bituminous coal at 60 °C. The consortium CBM 4 showed the highest methane production when compared with the other three enriched consortia (Fig. 2). After six enrichment cycles, 15.22 mM CH_4/g coal along with 11.98 mM CO_2/g coal was observed by the thermophilic methanogenic consortium CBM 4 (Fig. 2). Thus, this consortium designated as CBM 4 was selected for further studies as it showed maximum production of methane. The experimental control media bottles containing 0.5 g bituminous coal under similar condition showed CO_2 production of 2.24 mM/g coal along with H_2 production of 2.07 mM/g coal but no methane production at 60 °C after 20 days. The actively growing consortium CBM 4 was maintained at 60 °C by periodic transfer of 5 ml into 45 ml modified MSP medium bottle of 130 ml capacity containing 0.5 g bituminous coal after each 20 days of incubation for further studies.

3.3. Effect of coal concentration on methane production by the thermophilic methanogenic consortium

The effect of different coal concentrations on methane production by selected consortium CBM4 was evaluated. It was observed that with the increase in the coal concentration from 0.5 g to 2 g, there

Table 2
Analysis of coal sample.

Test	Method	Result
Proximate analysis		
Ash (%)	ASTM D3174-97	25.44
Moisture (%)	ASTM3173-87 (1996)	0.26
Volatile matter (%)	ASTMD3175-89A	17.41
Fixed carbon (%)	ASTMD3172-89	56.89
Ultimate analysis		
Carbon (%)	ASTM3178-89 (1997)	52.76
Hydrogen (%)	ASTM3178-89 (1997)	4.46
Nitrogen (%)	ASTM3179-89 (1997)	1.86
Sulfur (%)	ASTM3177-89 (1997)	0.53
Oxygen (%)	ASTM3176-89 (1997)	14.69

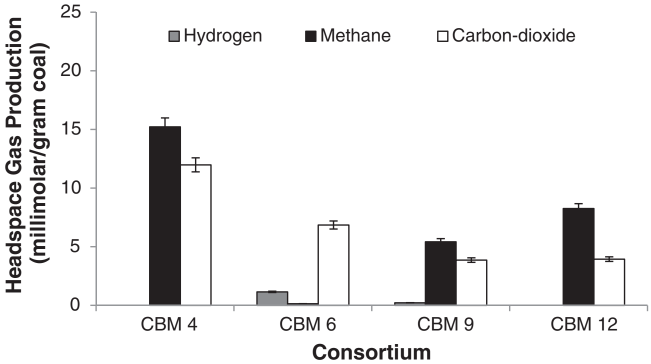

Fig. 2. Production of methane, carbon dioxide and hydrogen by microbial consortia (CBM 4, CBM 6, CBM 9 and CBM 12) isolated from Banaskantha coal mines.

was a concurrent increase in the methane production in the headspace of the experimental media bottles (Fig. 3a). However, increase in concentration of coal beyond 2 g indicated a decrease in methane production (Fig. 3a).

The maximum yield of methane per gram of coal by the consortium CBM 4 was also calculated. Maximum methane production of 8.23 mM, 8.44 mM, 9.19 mM, 9.52 mM, 8.57 mM, 7.8 mM and 4.6 mM was observed from coal concentration of 0.5 g, 1 g, 1.5 g, 2 g, 2.5 g, 5 g and 10 g respectively on the 20th day at 60 °C (Fig. 3a). This showed that maximum yield (mM/g) of methane per gram of coal (16.47 mM/g coal) along with CO_2 (11.53 mM/g coal) production was observed at 0.5 g coal concentration in the media at 60 °C (Fig. 3b).

3.4. Effect of temperature, pH and salinity on methane production by the methanogenic consortium

For effective implementation of biogenic CBM in coal reservoirs an attempt was made to study the role of the physiological factors that may possibly affect the in situ biogenic methane production.

Thermophilic methanogenic consortium CBM4 showed an increasing trend of CH_4 and CO_2 production as the temperature increased from 37 °C to 60 °C. However, at 65 °C, a sharp decline in CH_4 production with the increase in CO_2 production was observed (Fig. 4a). Maximum production of methane (17.99 mM/g coal) along with carbon dioxide (17.07 mM/g coal) was observed at 60 °C (Fig. 4a).

Methane production was tested in a pH range of 6.0 to 8.0. The CH_4 production along with CO_2 increased as pH value increased from 6.0 to 7.5 (Fig. 4b). The CH_4 production was maximum (19.39 mM/g coal) along with 13.56 mM/g coal of carbon dioxide at pH 7.5 (Fig. 4b). However, at pH 8.0, decrease in CH_4 (17.44 mM/g coal) and CO_2 (11.52 mM/g coal) production was observed (Fig. 4b). Therefore, it was concluded that the optimum pH value for maximum production of methane by selected thermophilic consortium CBM 4 was at pH 7.0–7.5 at 60 °C.

High salt concentration (NaCl) had detrimental effect on methane production by the indigenous consortium CBM 4 as shown in (Fig. 4c). Methane production was comparatively low (0.22 mM CH_4/g coal) at 4% NaCl along with 4.44 mM CO_2/g coal and 3.21 mM H_2/g coal. Maximum CH_4 (19.84 mM/g coal) along with CO_2 production of 13.74 mM/g coal was observed at salinity 0.1%(w/v) NaCl concentration i.e. close to the formation water salinity from which the consortium was isolated (Fig. 4c). Beyond 0.1% NaCl concentration, a decrease in trend of methane production was observed (Fig. 4c).

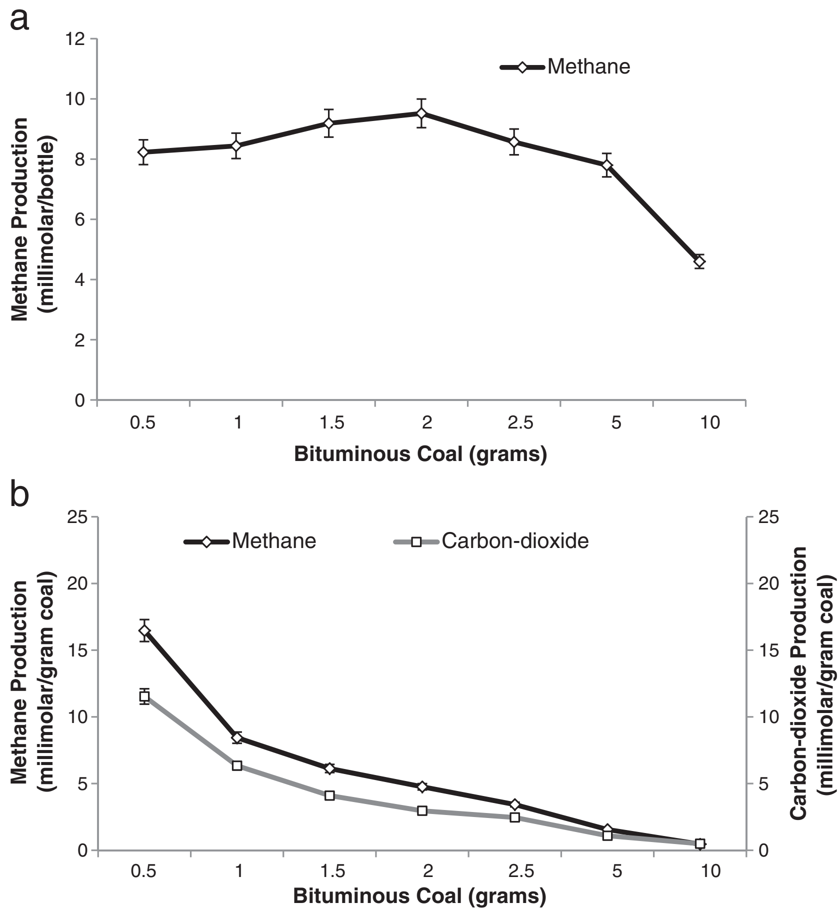

Fig. 3. a. Effect of coal concentration on methane production in headspace of media bottle by the thermophilic methanogenic consortium CBM4 in MSP medium. b. Effect of coal concentration on methane and carbon-dioxide yield per gram of coal by the thermophilic methanogenic consortium CBM4 in MSP medium.

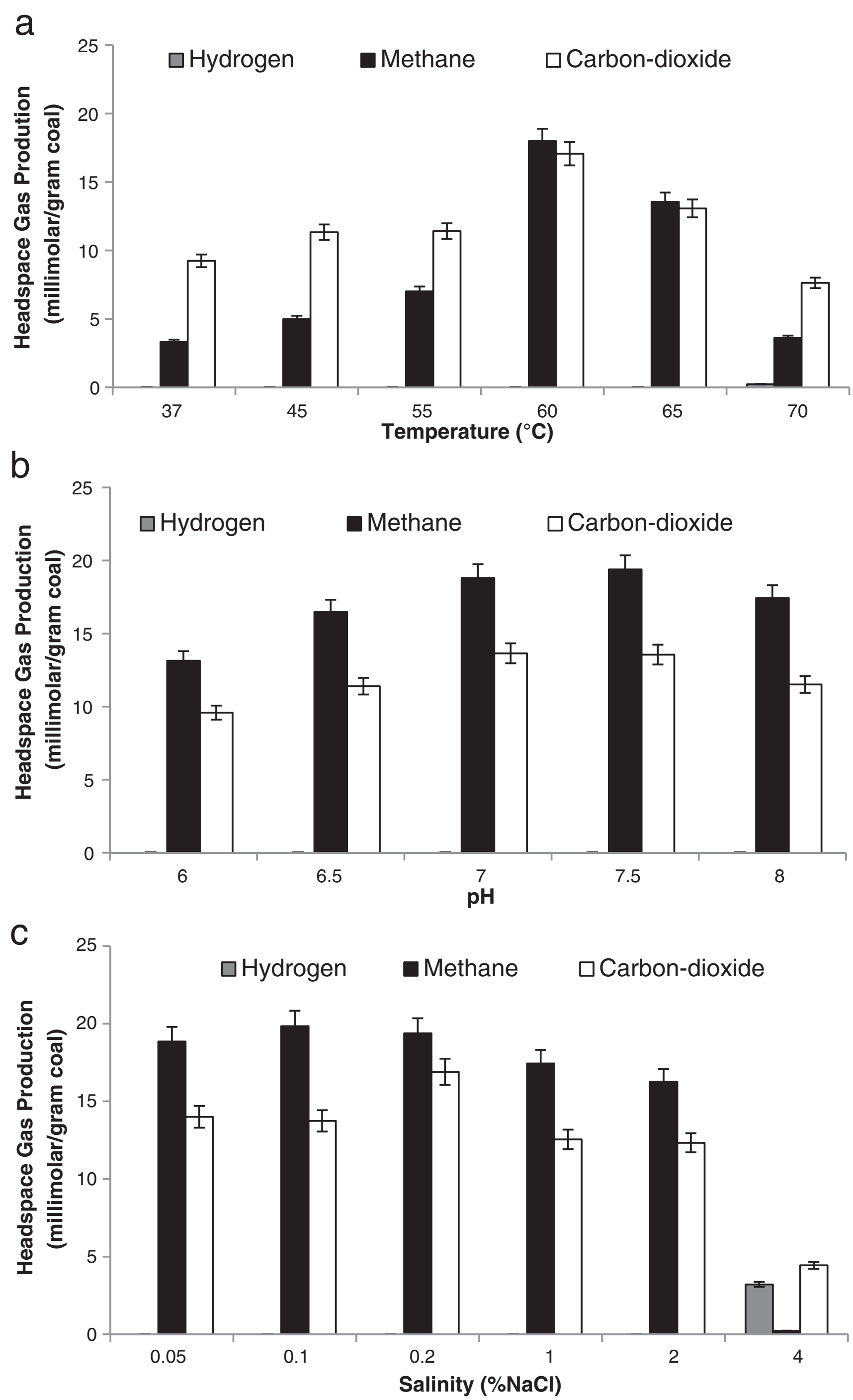

Fig. 4. Effect of environmental parameters on production of methane, carbon dioxide and hydrogen by thermophilic methanogenic consortium CBM4 in MSP medium with nitrogen as headspace. Data recorded after 20 days of incubation. a. At different temperatures (37 °C, 45 °C, 55 °C, 60 °C, 65 °C, 70 °C). b. At various pH values (6, 6.5, 7, 7.5, 8). c. At various salt concentrations (0.05, 0.1, 0.2, 1, 2, 4%).

3.5. Biogenic methane production by the selected methanogenic consortium CBM 4 at optimum conditions

Methane production by consortium CBM 4 was carried out with the optimized parameters of 0.5 g bituminous coal in 45 ml anaerobic modified MSP medium of pH 7.5 and salinity of 0.1% (w/v) NaCl concentration at temperature 60 °C. Methane and carbon-dioxide production was periodically analyzed after every 5 days interval for the period of 30 days. Maximum accumulated methane observed was 22.9 mM/g coal on the 20th day (Fig. 5). As indicated in Fig. 5, increase in methane production was only up to 20 days; thereafter the methane production decreased. Hence, optimum retention time was considered to be 20 days in this study. The experimental control media bottles containing 0.5 g bituminous coal under above similar condition showed no CH_4, 2.38 mM CO_2 along with 0.53 mM of H_2 at 60 °C after 20 days.

4. Discussion

CBM is envisaged to play a major role in meeting the energy needs in countries that have significant coal reserves. Thus breakthroughs in

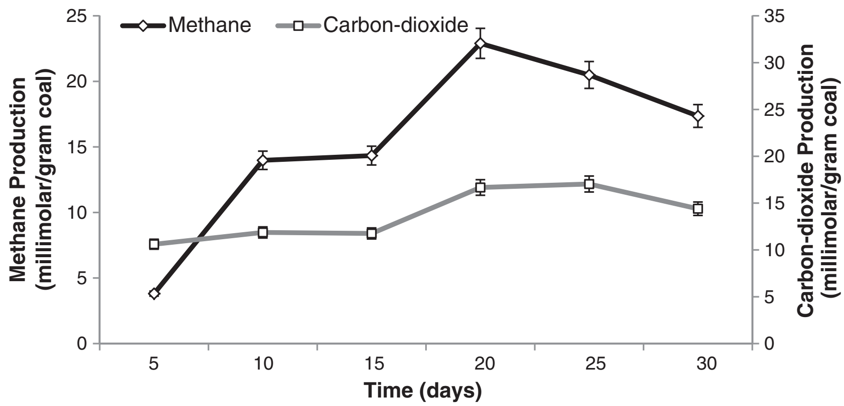

Fig. 5. Biogenic methane and carbon-dioxide production at optimum conditions.

technologies for enhancing in situ methane generation would be of interest for this non-conventional source of energy from coal reservoirs. The aim of the present study was to develop indigenous thermophilic anaerobic methanogenic consortium from coal reservoir with the potential to generate methane from coal, augmentation of which can enhance coal bed methane recovery of the reservoir.

In the present study, microbes from the formation water samples of Banaskantha coal reserves were used as a source for biogenic methane production. The enriched and selected consortium CBM 4 showed the highest methane production (15.22 mM/g coal) at 60 °C in modified MSP medium when bituminous coal was used as a carbon source (Fig. 2).

There are reports indicating formation of biogenic methane in the range of 0.1–0.25 mM/g coal at mesophilic conditions after an incubation period of more than a month (Gupta and Gupta, 2014; Strapoc et al., 2008). However, considering the applicability of this technology at high temperature range, there are very few reports on the biogenic methane potential of thermophilic coal reserves (Kimura et al., 2010; Wei et al., 2014). In a previous report by the authors of this study (Lavania et al., 2014), a bacterial consortium enriched from Jharia coal mines showed 49% methane production after 21 days at 65 °C with 1% (w/v) of sub-bituminous coal when supplemented with sodium acetate and sodium formate as carbon source. In the current study, consortium CBM 4 enriched from Banaskantha coal mines showed 15.22 mM of methane after 20 days at 60 °C when provided with 1% (w/v) of bituminous coal as the carbon–energy source. However, in the current study, yeast extract was added in the medium considering its importance cited in previous studies (Gonzalez et al., 2003; Gray et al., 2009; Kobayashi et al., 2012). Also, in many laboratory studies, methanogens isolated from coal reservoirs were enriched in a basal medium consisting of yeast extract along with coal as a substrate (Green et al., 2008; Harris et al., 2008; Lavania et al., 2014; Papendick et al., 2011; Strąpoc et al., 2011). Gilcrease and Shurr (2007) had emphasized the necessity of yeast extract in the context of coal dependent methanogenesis for successful in situ application of microbial enhanced CBM. Also, several companies, including Luca Technologies, Inc., and Ciris Energy, whose primary objective was to stimulate microbial enhanced CBM production in CBM wells through adding nutrients have commercially used yeast extract in their amendment mixture as a multinutrient (Ritter et al., 2015). Though, there are differences in the media used to investigate methane production in this study and the oligotrophic, higher-pressure in situ environment. However, Mahaffey (2012) stated that the most productive nutrient mixtures were those that were developed in the laboratory and further used for field implementation.

It is established by previous studies that the bacterial diversity present in the formation water varies from reservoir to reservoir depending on the physiological and geological parameters of coal reserves (Green et al., 2008; Jones et al., 2010; Strapoc et al., 2008; Susilawati et al., 2014). Different coal reservoirs have variable environmental condition such as temperature, pH, salinity and coal loading. Due to this reason, microbial community composition, function, and metabolic pathways are often distinct to a coal basin, and may even vary by location within a basin (Barnhart et al., 2013; Strąpoc et al., 2011). Thus, for a successful implementation of the technology, it becomes imperative to develop consortia specific to reservoir conditions. Accordingly, the primary aim of this study was to develop a suitable microbial system for Banaskantha coal reservoirs for enhanced CBM recovery.

It is possible that there may be a change in indigenous microbial community before and after enrichments. However, according to the recent review by Ritter et al. (2015), the measurements of microbial populations from the pilot study of Luca Technologies indicated that they were successful in maintaining desirable microbial community in situ in response to the nutrient amendment. Thus, stimulation or augmentation along with nutrient medium, as also developed in this study, can help in the growth of enriched microbes inside the coal seams, countering competition.

The rank of coal increases with the depth (Hamilton et al., 2014). The bituminous coal (a higher rank coal) formation occurs at elevated temperature and pressure, which is generally present at greater depth inside the reservoirs. Thus, this bituminous coal present in the lower depths of the reservoir is not easily minable. Therefore, the second aim of the current study was to enrich and optimize a thermophilic methanogenic consortium for enhanced methane production by utilizing bituminous coal as a substrate.

For the successful in situ implementation of the process, suitability of the reservoir conditions for the selected microbial system must be understood as the environmental conditions prevalent might affect rates of methane generation (Ritter et al., 2015). Head et al., 2014 also reported that the factors such as temperature, pH, salinity and available organic nutrients could affect methanogenesis. Therefore, studying the effect of these parameters on selected methanogenic consortium can help in in situ stimulation or augmentation of this consortium in the CBM field for microbial enhanced CBM generation. Though, it is difficult to control or modify the reservoir parameters such as temperature, pH, salinity and coal loading during in situ microbial stimulation or augmentation in different coal reservoirs. However, the approach of controlling these parameters by injecting water or specific nutrient can be done. As reported previously, injection of water or nutrient promotes enhanced methanogenesis by either transporting microorganisms into organic-rich reservoirs, providing moisture necessary for microbial activity, decreasing salinity, removing waste products and transporting in nutrients necessary for microbial growth (Barnhart et al., 2013; Ritter et al., 2015; Schlegel et al., 2011; Strapoc et al., 2008). Although much work has not been done in this regard, a recent review article by Ritter et al., 2015, reported that ExxonMobil (company) proposes in situ stimulation commercially, by controlling chemistry, salinity, temperature and pressure, with the possibility of adding nutrients to enhance production.

Coal concentration is one of the most important factors for the enhanced biogenic CBM generation. The bioavailability of coal to the microbes for methanogenesis depends majorly on the coal concentration (Green et al., 2008). Therefore, in this study, effect of different coal concentrations on methane production by consortium CBM 4 was evaluated. It was found that consortium CBM 4 showed enhanced methane production with the increase in coal concentration at 60 °C (Fig. 3a). However, beyond 2 g (corresponds to 4% w/v) of coal concentration in medium, decrease in the biogenic coal to methane conversion was observed. Previously, Papendick et al. (2011) stated that coal was the yield-limiting nutrient and the coal was not inhibitory or toxic to the methanogenic consortia at the highest concentration of 4% (w/v) at 37 °C. Similarly, it was found that the bituminous coal used in the present study was the yield limiting nutrient and was not inhibitory or toxic to consortium CBM 4 up to 4% (w/v) of coal concentration at thermophilic (60 °C) conditions. However, consortium CBM 4 showed maximum yield (mM/g) of methane (16.47 mM/g coal) at 0.5 g bituminous coal concentration (Fig. 3b).

Temperature has significant effect upon microbial growth and coal solubility and thereby affecting CBM production (Hamilton et al., 2014). Optimum temperature range for biogenic gas production for methanogens taken from formation water has been reported between 26 and 55 °C (Cheng et al., 2011; Nakamura et al., 2013). However, in the present study, maximum methane concentration was observed at 60 °C by the indigenous thermophilic methanogenic consortium CBM 4 (Fig. 4a). This data suggests significant application of the consortium CBM 4 for enhanced biogenic CBM generation at high temperature, which is usually observed in coal mines with greater depth. This temperature also correlates to the reservoir temperature from where the formation water sample was collected. Increase in aqueous solubility of coal substrates with temperature is one of the major reasons other than the enhanced cell metabolism and growth kinetics for higher methanogenesis rate (Green et al., 2008). This in turn increases the rate and extent of substrate mass transfer from the coal solids. Green et al. (2008), stated that if dissolution represents the rate-limiting step in methane production from coal solids, increased solubility leads to enhanced methanogenesis.

Previous studies showed biogenic methane generation in the range of 0.1–0.25 mM per gram coal by using coal as a substrate at mesophilic conditions (Green et al., 2008; Gupta and Gupta, 2014; Papendick et al., 2011). However, generation of high amount of biogenic methane from high rank (bituminous) coal at thermophilic temperature range is reported first time in this study. There are many coal reservoirs in India like Banaskantha and Jharia coal seams, which are high temperature bituminous coal reserves. The above results, indicates possible application of CBM 4 consortium for biogenic CBM generation at high temperature ranged coal reservoirs.

Another important parameter in regulating the anaerobic process is pH, as a change in pH of the environment can result in loss of biological activity. A neutral pH range of 6.6 to 7.8 is conventionally preferable for methane generation by methanogens (Lay et al., 1997; Ward et al., 2008). Hao et al. (2012) also stated that the pH as low as 5.5 would extend the lag-phase of methane production from acetate and change the dominant pathway of methanogenesis. Similarly, Gao et al., 2010 mentioned the adverse effect of higher pH (more than 8.0) on methanogenesis. Recently, Gupta and Gupta (2014) showed that the maximum biogenic methane production from coal was observed at pH range of 7.0–7.5. Concurrently, in this study the methane production gradually decreased below pH 7.0, while methane production from coal was highest at pH 7.5. Thus, indicating the optimum pH value for the selected methanogenic consortium CBM 4 (Fig. 4b).

Changes in the salinity also affect growth and methanogenic activity (Head et al., 2014). As reported earlier, many methanogens isolated from high temperature sites such as oil/gas or coal reservoirs (Lavania et al., 2014; Zhou et al., 2013) have salinity range (0.05–0.1%NaCl) similar to as mentioned in this study. Papendick et al. (2011) had also suggested that formation water with lower salinity favors the growth of methanogens. Similarly, in the present study, maximum methane production by consortium CBM 4 occurred at lower salinity (0.1%NaCl) i.e. close to the collected formation water salinity (Fig. 4c). This confirms the fact that the microbes isolated from the Banaskantha formation water favor low salinity for the methane generation from coal.

Thus, after considering the optimized parameters (60 °C, 0.1% salinity with pH 7.0 and 0.5 g bituminous coal), methane production by the consortium CBM 4 was increased from 15.22 mM/g coal to 22.9 mM/g coal (Fig. 5). This indicated 33.53% increase in the methane production by consortium CBM 4 when operated at the optimum conditions. Optimum condition in laboratory may not coincide with the in situ environment. Compared to the laboratory condition, generally, in situ environment is devoid of moisture, nutrients, trace elements and consists of high pressure. However, according to recent review by Ritter et al. (2015), there are successful applications of laboratory based research implemented in field. Several studies have been reported on determining the optimal reservoir conditions for methane production by indigenous microbial consortium at laboratory scale (Green et al., 2008; Gupta and Gupta, 2014; Papendick et al., 2011).

In the earlier reports the methane production by coal degrading methanogenic consortia at mesophilic conditions was around 0.042–7.2 mM/g coal, whereas in the present study 22.9 mM CH_4/g coal was achieved at temperature 60 °C from bituminous coal. Table 3 depicts a comparison between the present study and the reported work from literature. The rate and yield of CBM 4 consortium in the present study are very promising and can be taken up subsequently for future studies in field.

There can be possible explanations for the enhanced performance of consortium CBM 4 as indicated in Table 3. According to Fallgren et al.

Table 3
Methane production rate and yields from coal in variable studies [a].

Inoculums	Temperature (°C)	Coal source	Coal Size (μm)	Avg. rate (mM/g coal/day)	Yield (mM/g coal)	Source
Bio-stimulation consortia						
Consortium CBM 4, Banaskantha, India	60 °C	Banaskantha (India)	<500	1.145	22.9	Current study
Jitpur coal mine, India	35 °C	Jitpur (India)	15–60	0.005	0.21	Gupta and Gupta (2014)
Sumatra island, Indonesia	23 °C	Sumatra island (Indonesia)	<1000	0.004	0.33	Fallgren et al. (2013)
Surat, Queensland	37 °C	Walloon (Queensland)	300–600	0.01	0.26	Papendick et al. (2011)
Powder river, Wyoming	30 °C	Wyodak (Wyoming)	250–600	0.007	0.18	Green et al. (2008)
Wilcox, Texas	22 °C	Wilcox (Texas)	2000–10,000	0.002	0.06	Jones et al. (2010)
British Columbia	30 °C	Obed Mine	Finely ground	0.0005	0.042	Penner et al. (2010)
Bio-augmentation consortia						
Wetland sediment enrichment	22 °C	Wilcox (Texas)	2000–10,000	0.003	0.08	Jones et al. (2010)
Wood-eating termite	Not provided	Texas lignite	45	0.6	7.2	Harding et al. (1993)

[a] This table was modified from Papendick et al. (2011).

(2013), methanogenic activity of the consortium can be affected by sampling methods, culture conditions, coal bioavailability and selectivity of the in situ coal seam environment. High methane yield obtained in the present study may be due to the aqueous solubility and bioavailability of coal as a substrate at high temperature. Also, there might be possibilities of the high rate of biodegradation of coal into intermediate compounds, which were further converted into methane by methanogens of the consortium. Craig Venter had previously reported that the bacteria from underground coal seams have unique enzymes, which can break down coal and convert it into methane. However, to understand this biodegradation of coal and metabolic diversity, microbes present in the consortia need to be identified. Therefore, in particular, microbial diversity in the coal degrading consortium CBM 4 and its metabolic pathways from coal to methane will be subsequently taken into consideration for further studies.

The insights of microbial activity towards the enhanced generation of CBM, highlight the need for additional studies to prove the feasibility of this approach. Taking this fact into consideration in the present study, we undertook controlled laboratory experiments to identify the influential factors of in situ biogenic methane production from bituminous coal at high temperature. The present work encompasses the development of thermophilic methanogenic consortium and the environmental conditions that led to increase in the methane production rate from bituminous coal, such that these can be targeted to subsequent large-scale feasibility studies.

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