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Unraveling the microbial interactions in coal organic fermentation for generation of methane — A classical to metagenomic approach

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ABSTRACT

Coal bed methane (CBM) obtained in the deep unmined coal reserves stands as a promising alternative source of energy for the diversified energy needs. Biogenic methane can consist of the significant portion of the gas from the coal beds as a result of microbial activity on the coal. The growing interest of enhancing the CBM production led to obtain the detailed information of the microbes and the development of a suitable microbial consortium capable of biotransforming coal to methane from the coal dust and the selectively enriched sump sediment water of the underground coal mines of the Jharia basin in the present study for the first time. The change of zeta potential from −40.6 to −8.3 mV indicated the probable modification of coal structure by the microbes. The collected subbituminous pure coal revealed a decrease in carbon content from 60.33% to 54.22% when inoculated with the enriched sump sediment water. The growth studies indicated significant microbial growth in the medium with C:N ratio (100:25), under anaerobic conditions. An increased methane production of 2.7 times was observed from the enriched sump sediment water as compared to coal dust inoculum. The prevalence of Firmicutes, Proteobacteria and the hydrogenotrophic methanogens was obtained in the coal dust and enriched sump sediment metagenomes via MG RAST server. The functional attributes obtained through SEED subsystem revealed the predominance of the monoaromatic and aliphatic compound degradation in both the metagenomes. This study aims to develop a microbial consortium through selective enrichment followed by the media amendment with the pre-digested organic nitrogen source for an enhanced coal biomethanation which can be applied to the unexplored and unmined coal seams of medium to low grade as an alternative form of energy.
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1. Introduction

With the declining of oil reserves expected in the first half of this century, coal reserve rises as a potential candidate for energy production on large scale (Parikh, 2006). The coal mining activities such as exploration, transportation, solid waste generation and emissions from the coal fired power-plant and other steps in energy generation from the coal, raised serious environmental concerns (Dai et al., 2012; Finkelman et al., 2002) that led to study associated to *in situ* coal derived cleaner form of energy, by producing biogenic methane. The deep coal seams of depths greater than 0.3–0.5 km represent the unmined coal resources that remain unexploited due to economic considerations but can be a rich source of methane (Valix and Thambimuthu, 2001). Utilization of unmined coal that is otherwise untapped, for biogenic methane production, could be advantageous.

Methane can act as a valuable alternative energy source that can be generated as long as the coal seam lasts, permitting countries to diversify the energy supply. The mined or abandoned coal seams are found to be good sources of methane gas formed either thermogenically during coalification or biogenically due to microbial activities in the coal (Faiz and Hendry, 2006; Thielemann et al., 2004). For the regeneration of biogenic coal bed methane (CBM), various laboratory based experiments as reported by Green et al. (2008) and Jones et al. (2008) as well as *in situ* studies revealed by Pfeiffer et al. (2010) focused on the involvement of particular microbial communities inhabiting the coal beds. Numerous reports on the presence of the microbial communities have been revealed in the coal beds of the USA (Barnhart et al., 2013; Gallagher et al., 2013; Green et al., 2008), Japan (Shimizu et al., 2007), China (Guo et al., 2012), India (Singh et al., 2012) and Australia (Papendick et al., 2011).

Several studies based on cloning and sequencing have suffered cloning bias and restricted throughput, leading to underrating and concealing of the realistic view of the biodiversity (Cheung et al., 2010). Therefore, more accurate information regarding the microbial communities of biogenic CBM production is required. The use of 454 pyrosequencing, a widely referred sequencing technique with a high read accuracy rate has overcome such methodological limitations and revealed more accurate microbial ecology data of a biogas reactor

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(Li et al., 2013), CBM reservoir (Guo et al., 2012) and effect of the coal oxidation on the microbial community structure (Gallagher et al., 2013). These reports supported the better understanding of the microbial diversity via pyrosequencing. Metagenomic approach explores the microbial diversity to determine the metabolic processes that are important for growth and survival in any environment (Dinsdale et al., 2008). The functional capabilities of the microbes in the environment are identified in terms of the abundance of the pathway associated to the biological processes (Sharon et al., 2011).

The bituminous and subbituminous ranks of coals are being produced from the Jharia basin of Jharkhand (Mazumder and Wolf, 2004) which is the largest producer of CBM in India. The presence of denitrifying bacteria in the formation water collected from Jharia coal bed basin was reported by Singh et al. (2012). The existence of the competent coal degrading bacteria deficient in electron acceptors for denitrification and acceleration of methanogenesis *in situ* by adding nitrite was concluded in the study (Singh et al., 2012). However, the sequential involvement of the microbes in the biogenic methanogenesis is yet to be elucidated.

This study deals with the analytical studies indicating coal as a bioprocess substrate followed by the determination of microbial growth at various coal concentrations and different C:N ratios; comparative gas production using coal dust and enriched sump sediment water as inocula; and detailed information on the likely sequential involvement of the microbial assemblages in the biotransformation of coal to methane from the coal dust and enriched sump sediment water inocula. Though a similar kind of work has been reported by Strąpoć et al. (2011) and Gallagher et al. (2013), to the best of our knowledge this is the first study at Jharia coal basin in India which aims to develop a microbial consortium through selective enrichment of the sump sediment water followed by the media amendment with the pre-digested organic nitrogen source for an enhanced coal biomethanation which can be applied to the unexplored and unmined coal seams of medium to low grade as an alternative form of energy.

2. Materials and methods

2.1. Study site and sampling

2.1.1. Geological context

The Jharia coal basin consists of 3000 m thick successions of lower Gondwana rocks of Permian age, lying between latitudes 23°37 N and 23°52 N and longitudes 86°05 E and 86°30 E in Jharkhand, India covering an area of about 456 km^2 (Fig. 1a). The sedimentary series overlies unconformably on the Archean gneissic basement, beginning with the glaciogenic sediments of the Talchir formation followed by fluvial and fluvio-lacustrine sediments successively of the Barakar, Barren measures and Raniganj formations (Fig. 1b) (Fox, 1931; Mehta and Murthy, 1957; Sengupta et al., 1979). The western part of the Jharia basin consists of predominantly underground mining area and presently comprises of four underground mines. The present study involves the sampling from the Moonidih coal block of western Jharia where mostly the coal seams are virgin and occur at a depth of ≥1000 m. Moonidih Block is occupied by rocks of Barakar formation, covers an area of 20.63 sq.km and is located in the central part of Jharia Coalfield. Coking coal in this block is available in the deeper level below Barren Measures. River Damodar flowing west to east further south of the block forms the main drainage system. Katri Nala flowing north to south west of the block joins Damodar River towards south west of the block. Moonidih project is the sole coal producing unit in the Moonidih coal block being the pioneer in deploying Powered Support Longwall (PSLW) technology mining in India since 1978. Moonidih mine is deep (present mining activities at a depth range of 450–650 m from the surface), and actively gassy with elevated strata temperature and occasional hot strata water (www.bccl.gov.in).

2.1.2. Sampling

Coal samples were collected on 18–19th April 2012 from 500 m below the earth surface and at an inclination of 3.5 km, along the coal

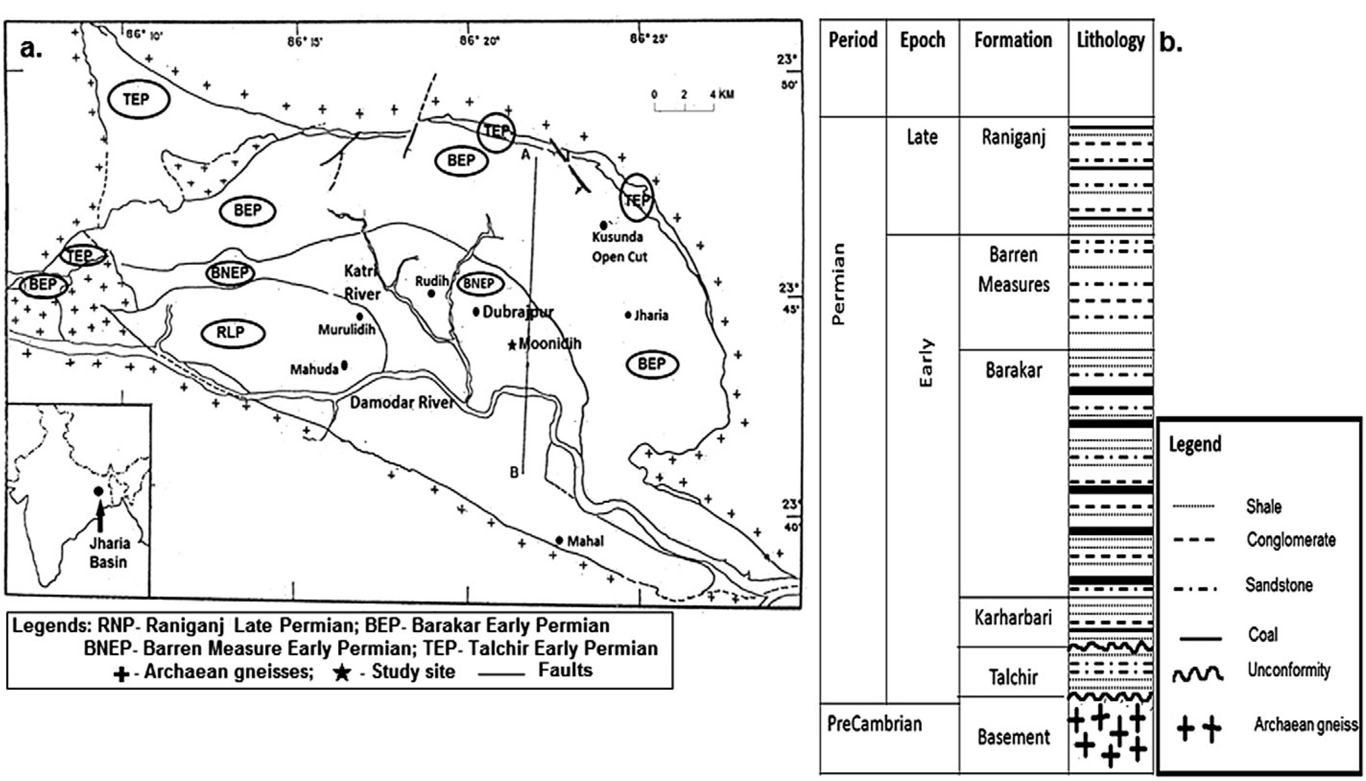

Fig. 1. a. Map of the Jharia coal bed basin showing the coal block under study located near the river Damodar. b. Litho-stratigraphy of the Jharia coal bed basin. Modified after Sengupta et al. (1979).

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