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Microbial diversity and biogenic methane potential of a thermogenic-gas coal mine

Min Wei [a], Zhisheng Yu [a,*], Zheng Jiang [b], Hongxun Zhang [a]

[a] College of Resources and Environment, University of Chinese Academy of Sciences, 19A Yuquan Road, Beijing 100049, China
[b] Energy Group, Faculty of Engineering and the Environment, University of Southampton, High Field Campus, Southampton SO17 1BJ, UK

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ABSTRACT

The microbial communities and biogenic methane potential of a gas coal mine were investigated by cultivation-independent and cultivation-dependent approaches. Stable carbon isotopic analysis indicated that in situ methane in the coal mine was dominantly of a thermogenic origin. However, a high level of diversity of bacteria and methanogens that were present in the coal mine was revealed by 454 pyrosequencing, and included various fermentative bacteria in the phyla of *Actinobacteria*, *Bacteroidetes*, *Firmicutes*, and *Proteobacteria*, and acetotrophic, hydrogenotrophic, and methylotrophic methanogens. Methane was produced in enrichments of mine water samples supplemented with acetate under laboratory conditions. The microbial flora obtained from the enrichments could stimulate methane formation from coal samples. 16S rRNA gene clone library analysis indicated that the microbial community from coal cultivation samples supplemented with the enriched microbial consortium was dominated by the anaerobic fermentative Clostridiales and facultative acetoclastic *Methanosarcina*. This study suggests that the biogenic methane potential in the thermogenic-gas coal mine could be stimulated by the indigenous microorganisms.

1. Introduction

With constant industrial development, coal mining activity continues to increase in many countries. However, the coal recovery is low for the majority of coal mines. For example, it is about 30% for the majority of coal mines in China (Cui, 2006). Thus, the recovery and utilization of coal mines have been an issue in recent years. The large amount of coal in abandoned coal mines is a very attractive source for methane generation (Beckmann et al., 2011b). In fact, coal mine methane has been of great interest for both energy production and greenhouse gas reduction in recent years (Karacan et al., 2011). Additionally, it is a pressing concern for governments in terms of reducing coal mine gas explosions.

Methane emission from a coal seam can be generated from thermogenic, biogenic, or mixed pathways. Thermogenic methane is primarily generated through the thermal decomposition of mature organic matter. Biogenic methane includes primary and secondary biogenic gases, which is the result of anaerobic microbial degradation of the organic matter in coal at low temperatures (Ni et al., 2013). Mixed genesis methane refers to a mixture of methane genesis types with different geochemical characteristics from both biogenic and thermogenic origins.

There has been considerable interest in biogenic methane in recent years (Flores et al., 2008; Green et al., 2008; Papendick et al., 2011; Strąpoć et al., 2010), providing an opportunity for energy production via methane regeneration from coal mines. In general, biogenic methane is produced from acetate, hydrogen, or methyl-bearing substrates as precursors, which are respectively coupled with the acetotrophic, hydrogenotrophic, and methylotrophic methanogens. Acetotrophic and hydrogenotrophic methanogeneses are the predominant pathways identified in numerous microbial studies on biogenic methane formation of coal seams, such as abandoned coal mines in Germany (Beckmann et al., 2011b), the coal bed methane reservoir in the Powder River Basin (Green et al., 2008), and the Queensland coal seams in the Surat Basin (Papendick et al., 2011). Very few studies have focused on the methylotrophic methanogenesis with the exception of the Eastern Ordos Basin in China from our previous work (Guo et al., 2012) and a biogenic coal bed methane field in Alaska (Strąpoć et al., 2010).

The chemical composition of coal mine gas (C_1–C_3 gaseous alkanes), stable carbon and hydrogen isotope ratios of methane ($\delta^{13}C_{-CH4}$, $\delta^{13}C_{-CO2}$, and δD_{-CH4}), and isotopic fractionation between CO_2 and CH_4 ($\Delta^{13}C_{CO2-CH4}$) are routinely used to differentiate the various origins of coal mine methane (Conrad, 2005). Using the stable isotope method, the origins of methane from different coal seams are typically distinguished. It has been shown that biogenic methane reserves account for about 20% of gas reserves worldwide (Rice and Claypool, 1981). For example, the coal bed methane from the Powder River Basin (Flores et al., 2008) and eastern Australia (Faiz and

* Corresponding author. Tel./fax: +86 10 88256057.
E-mail address: yuzs@ucas.ac.cn (Z. Yu).

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Hendry, 2006) with the $\delta^{13}C_{-CH4}$ value < −55‰ and δD_{-CH4} < −300‰ suggested the biogenic origin. Mixed methane of both thermogenic and biogenic origin is also widely observed, e.g., coal mines in Ruhr Basin in Germany (Krüger et al., 2008) and coal seams in the San Juan Basin in the United States (Wawrik et al., 2012), and the Eastern Ordos Basin in China (Guo et al., 2012). However, the presence of thermogenic gas does not indicate whether or not there is a potential for biogenic methanogenesis in coal seams. Little is known about microbial communities and the biogenic potential in thermogenic gas coal mines (Kimura et al., 2010). Furthermore, the biogenic methane potential of thermogenic gas coal mines has never been evaluated.

In this study, coal and water samples were collected from a thermogenic-gas coal mine that was confirmed by the stable isotopic signatures. Molecular techniques combined with anaerobic cultivation were used to determine the potential for biogenic methane generation of the coal mine. We discuss the members of the microbial consortium that were mainly present in coal seams and whether biogenic methane could be generated from similar coal mines in the future.

2. Materials and methods

2.1. Study area and sample collection

Samples were collected from a coal mine located in Hubei, China (Fig. 1). The coal seam is Upper Triassic in age, belonging to the Jiuligang Formation in the Jingmen-Dangyang Basin. It is a sedimentary basin that evolved from a lake. The Jiuligang Formation is the coal-bearing strata characterized by a thin substrate of coal-bearing sediment and is composed of siltstone, sandy shale, mudstone, carbonaceous mudstone, and thin coal seams (Fig. 2). When compared to previous studies of coal mines in Hubei (Wei et al., 2013), the sampling mine in the present study is a representative gassy coal mine. The depth of the samples was 158 ± 5 m, and the average thickness of the coal seam was 0.8 m. The methane concentration in ventilation air varies from 0.3% to 1.7% over a year. High methane content seasons are spring and autumn. The gas samples were collected at three different newly exposed mining faces in the working area by injection into sealed 500 mL aluminum foil sampling bags. Coal samples were also collected from the three newly exposed mining faces at the same work site. For each coal sample, 4–5 kg of fresh working-face coal was obtained and immediately put in sterilized glass bottles. An uninterrupted drainage system was kept to drain mine water for the underground mine. Water samples were pumped from the drainage pipes without air and other contaminants. Three high-density polyethylene bottles of 10 L were filled with mine water with no headspace and tightly sealed with butyl rubber stoppers. The coal seam temperature was 33 ± 5 °C. Water temperature, pH, conductivity, and dissolved oxygen (DO) were measured with a portable detector (HQ 40d, Hach, Loveland, CO, USA). The in situ concentrations of CH_4 and CO_2 were measured with portable gas detectors GJC4/100 and GRG5H (China Coal Industrial Equipment Corp., Ltd., Beijing, China), respectively. The stable carbon isotopic compositions of CH_4 and CO_2 in coal mine gas were measured by Trace GC Ultra (Thermo Electron Corporation, Austin, TX, USA) and Thermo Quest Delta plus XL isotope ratio mass spectrometer (Thermo Finnigan, San Jose, CA, USA). Coal and water samples were kept on ice in the field and then transported to the lab. Coal properties were analyzed at the National Coal Quality Supervision and Inspection Center in China. Analysis of the chemical and physical properties of the water was carried out by Pony Testing International Group in Beijing, China.

2.2. Detection of biogenic methane potential of the coal mine

Coal samples were ground to coal powder and screened through a 100-mesh sieve (less than 150 μm) in an anaerobic glove box (Xingmiao YQX-11, Shanghai, China). Mine water samples (1 L) were filtered through sterile 0.22 μm pore-size membrane filters (Whatman Japan KK, Tokyo, Japan). Coal powder (0.3 g) and membranes containing the filtered microorganisms from water were respectively placed into 140 mL serum bottles containing 30 mL anaerobic medium. The anaerobic medium was prepared as previously described (Strapoc et al., 2008). Na_2S and cysteine were supplemented to maintain reducing conditions. To study the methanogenic pathway, available substrates were

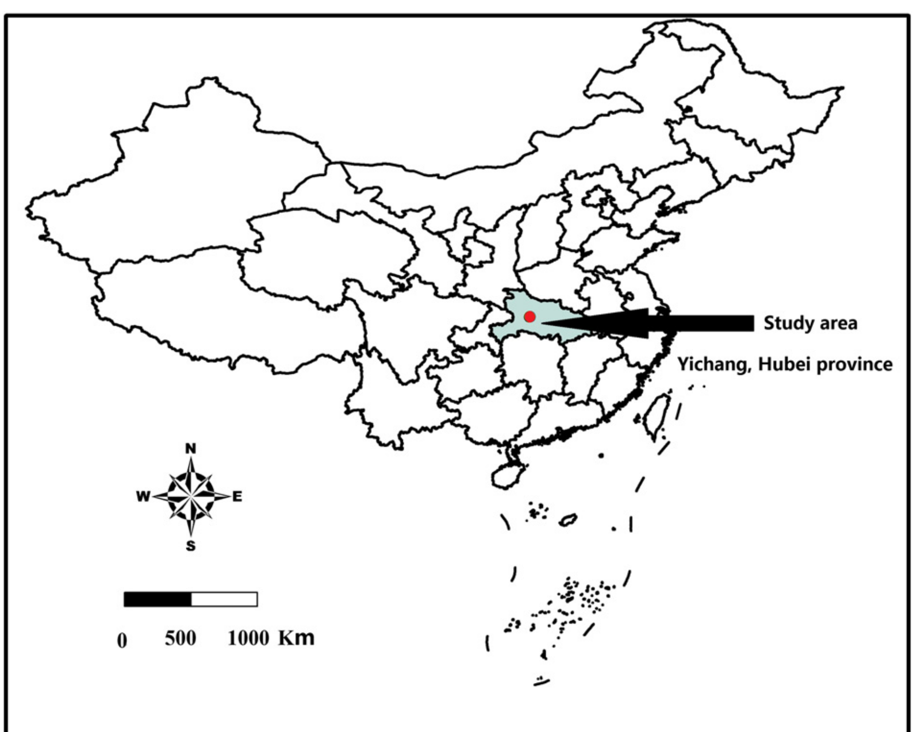

Fig. 1. Location of the thermogenic gas mine used for sample collection.