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REVIEW ARTICLE

Coal methanogenesis: a review of the need of complex microbial consortia and culture conditions for the effective bioconversion of coal into methane

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Abstract Microbial biodegradation of coal into low-molecular-weight compounds such as methane has been extensively researched in the last two decades because of the underlying environmental and industrial applications of this technique as compared to the chemical and physical methods of coal conversions. However, the irregular structure of coal and the need for complex microbial consortia under specific culture conditions do not make this biotransformation an ideal process for the development of anaerobic bioreactors. The most abundant species in a methanogenic culture are acetoclastic and hydrogenotrophic methanogens which utilize acetate and H_2+CO_2, respectively. Medium- to low-rank coals such as high-volatile bituminous, sub-bituminous and lignite are more promising in this bioconversion as compared to semi- and meta-anthracite coals. While covering the details of the ideal culture conditions, this review enlightens the need of research setups to explore the complex microbial consortia and culture conditions for maximum methane production through coal methanogenesis.

Keywords Bioconversion · Coal biodegradation · Methanogenic consortia · Microbial diversity · Methanogens

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Introduction

Coal represents more than 70% of all fossil fuels on this planet, while petroleum and natural gas represent only 17 and 2%, respectively (Sekhohola et al. 2013). However, this excessive and non-renewable source of energy should not be used abundantly due to the underlying environmental problems such as the emission of greenhouse gases and the resultant inorganic and residual pollution, which are more prominent concerns in the combustion of coal as compared to other fossil fuels (Shafiee and Topal 2009). The anthropogenic activities of today's mechanized era demand the utilization of non-renewable energy options for the economic and social stability of the modern society. Coalbed methane (CBM) is a non-conventional source of energy which is produced in deep and subsurface coal basins by the geological, thermogenic or microbial breakdown of complex coal polymers (Fakoussa and Hofrichter 1999; Harris et al. 2008). Coal methanogenesis or the biogasification of coal into methane has been extensively studied in recent years for its applications in the production of CBM.

Currently, more than 10% of natural gas production in the USA comprises CBM (EIA 2015). CBM production in the USA started in the 1980s and followed a linear increase from 1990 to 2006, with more than 20% of this gas being of microbial origin (EIA 2015). Coal can be converted into methane by a specific type of methanogenic consortia that are abundant in various anaerobic habitats, including coal seams and coal formation waters. The common substrates for methanogens are acetate, carbon dioxide, and other simple carbon compounds along with hydrogen.

Coal is a complex hydrophobic polymer consisting of a condensed polyaromatic structure which makes it a poor sub-

strate for methanogens (Speight 2012). However, there are various fungal and bacterial species that can actively degrade this complex polymer into simpler long-chain alkanes and single-ringed aromatic compounds, which are converted into volatile fatty acids upon further microbial action (Jones et al. 2010; Strąpoć et al. 2011; Haider et al. 2013). Such volatile fatty acids are ideal compounds for other types of species in the methanogenic culture, which transform long-chain fatty acids into short-chain carbon substrates for methanogens. Biogenic methane production has been an active process in many CBM wells across the world. The bioaugmentation and biostimulation techniques for the enhanced production of microbial methane have been recently developed to generate more CBM in coalfields. This review discusses the current understanding of coal methanogenesis and its relationship with the effects of coal rank, microbial diversity, field, and laboratory culture conditions along with the most probable pathway of biogenic methane production. In addition to the potential understanding of the overall concept of coal biogasification, the gaps in current knowledge have been discussed with a special focus on the biotreatment strategies for enhanced biogasification.

The effect of coal ranks on methanogenesis

There are many functional problems in the way of microbes to convert coal into fine biotechnological products (Fakoussa and Hofrichter 1999). Coal is a complex polyaromatic, hydrophobic and solid-state structure with large proportions of heterogeneous compounds. There are various parameters like coal structure, physicochemical properties, and quality to consider before assessing the effect of coal rank in biogenic methane production. Several studies have shown the effect of coal rank on the bioconversion of coal into methane in both field and laboratory frameworks (Faison and Crawford 1993; Faiz and Hendry 2006; Fallgren et al. 2013). Coal rank differs from coal type by the origin and history of coal formation (O'Keefe et al. 2013). Coal is characterized according to its economic significance, calorific value, and environmental effects, determining the industrial decisions about this fossil fuel. There are several ranks of coal according to the type and relative age of the dead biotic material, and they range from anthracite (the highest in rank) to lignite and peat (the lowest in rank). Coal rank is the degree of metamorphosis that leads to the reduction of oxygen, hydrogen, and moisture content while increasing the fixed carbon and heating value (Table 1) (ASTM 2015). Coal rank is important in order to understand the extent of structural susceptibility that can enhance microbial degradation of this complex polymer (Xiao et al. 2013).

Anthracite coals are the highest in rank and have the lowest numbers of cleavage sites and simple carbon compounds for microbial actions. The coalification process or sequential maturation of coal into higher ranks converts the simple carbon compounds into complex fixed carbon that cannot be used by the majority of microbes as the carbon substrate (Fig. 1). As has been mentioned by Murray (1996), the process of coalification for higher ranks involves the rearrangement of structural domains, which in turn results in the three-dimensional aggregation of aromatic carbon structures (Fig. 1). The degree of metamorphosis is determined by vitrinite reflectance (R_O), volatile matter content and gross calorific value (Table 1). It is a general assumption that anthracite coals cannot be degraded into liquid and gaseous compounds due to the difficulty in digestion of complex polyaromatic structures which are abundant in these ranks (Xiao et al. 2013). However, recent studies have shown the potential of anthracite coals for methanogenesis under special enrichment conditions, and with the help of several microbial consortia that can degrade complex aromatic compounds released from anthracite coals (Wei et al. 2014; Susilawati et al. 2015).

Many of the research articles that have been published in the area of coal methanogenesis discuss this process with respect to the low-rank coals (Wise 1990; Faison and Crawford 1993; Faiz and Hendry 2006; Harris et al. 2008; Jones et al. 2008, 2010; Fallgren et al. 2013; Haider et al. 2013; Zhang et al. 2015). However, some exclusive efforts have also been made to understand the biogasification potential and biodegradation ability of anthracites. One example, in this regard, is the research setup designed by Xiao et al. (2013) to explore the biogasification potential of coal in Qinshui Basin, China. Qinshui Basin is rich in non-degradable coals, but Xiao et al. (2013) argue that the volatile matter acts as a controlling factor in the biomethane generation from different ranks of coals. Anthracite coals have 2–8% volatile matter content along with 98% fixed carbon (ASTM 2015). Generally, the volatile matter is the coal components that are liberated in the form of gases at high temperatures during the proximate analysis. Volatile matter also represents small percentages of trapped gases such as hydrogen, carbon dioxide, methane, and other low-molecular-weight hydrocarbons in coal macerals (Strąpoć et al. 2011). The presence of such compounds ensures high ignition rates and combustion potential (Speight 2012). In addition to the determination of spontaneous combustion, volatile matter is also important to the understanding of the potential of biogenic methane production from the respective coal ranks (Xiao et al. 2013).

It has been established that biogenic methane can commonly be produced in the coal cultures and in situ coal fields through acetate or carbon dioxide reduction pathways by several methanogenic communities (Zhang et al. 2015). Acetate and carbon dioxide often come from the initial biodegradation of complex organic compounds in low-rank coals which also have relatively more potential cleavage sites (Penner et al. 2010). However, with the presence of low-molecular-weight

Table 1 Extent of coalification and its effect on microbial degradation and methanogenesis

Coal type	Physicochemical characteristics				Coalbed methane analysis			Reference
	Vitrinite reflectance (%R_{oMax})	Volatile matter (%)	Fixed carbon (%)	Calorific value (Btu/lb)	Origin of CBM	Biodegradation potential	Reason behind degradation potential	
Anthracite								
Meta-anthracite	4.0–6.0	<2	>98	–	Metamict	Very low	Polyaromatic ring structure	Speight 2012
Anthracite	2.5–4.0	2–8	92–98	–	Thermogenic	Very low	Condensed and fused molecular structure	Strąpoć et al. 2011
Semi-anthracite	1.9–2.5	8–14	86–92	–	Thermogenic	Low	Yeast extract stimulation in laboratory incubations and presence of Actinobacteria for initial biodegradation	Xiao et al. 2013
Bituminous								
Low volatile bituminous	1.7–1.9	14–22	78–86	–	Thermogenic	Low	Condensed molecular structures	Fallgren et al. 2013
Medium volatile bituminous	1.2–1.7	22–31	69–78	–	Thermogenic	Low	No cleavage sites for microbial action	Sekhohola et al. 2013
High volatile A bituminous	0.9–1.2	>31[a]	<69[a]	>14,000	Thermogenic/biogenic	Medium to high	Volatile compounds provides initial substrate for methanogens	Volkwein et al. 1994
High volatile B bituminous	0.6–0.9	–	–	13,000–14,000	Thermogenic/biogenic	Medium to high	Same as above	Gupta and Birendra 2000
High volatile C bituminous	0.5–0.6	–	–	11,500–13,000	Thermogenic/biogenic	High	Abundance of cleavage sites for microbial action	Opara et al. 2012
Subbituminous								
Subbituminous A	<0.5	–	–	10,500–11,500	Thermogenic/biogenic	Medium to high	Presence of volatile compounds	Gonsalvesh et al. 2008
Subbituminous B	<0.5	–	–	9500–10,500	Biogenic	Medium to high	Presence of volatile compounds	Jones et al. 2010
Subbituminous C	<0.5	–	–	8300–9500	Biogenic	Medium to high	More cleavage sites for Proteobacteria	Jones et al. 2008
Lignite								
Lignite A	<0.5	–	–	6300–8300	Biogenic	High	Susceptible due to more hydrolytic sites	Haider et al. 2013
Lignite B	<0.5	–	–	<6300	Biogenic	High	Combined action of bacteria and methanogenic consortia	Harris et al. 2008

Anthracite and bituminous coals are characterized according to fixed carbon potential and other types are characterized according to calorific value (ASTM 2015; Murray 1996)

[a] According to the ASTM standard classification of coal ranks, more than 31% volatile matter in any coal sample categorizes this coal into a high volatile rank (Table 1; ASTM 2015). Similarly, the fixed carbon of such ranks is also categorized as indeterminate in the ASTM categorization table for the sake of simplification

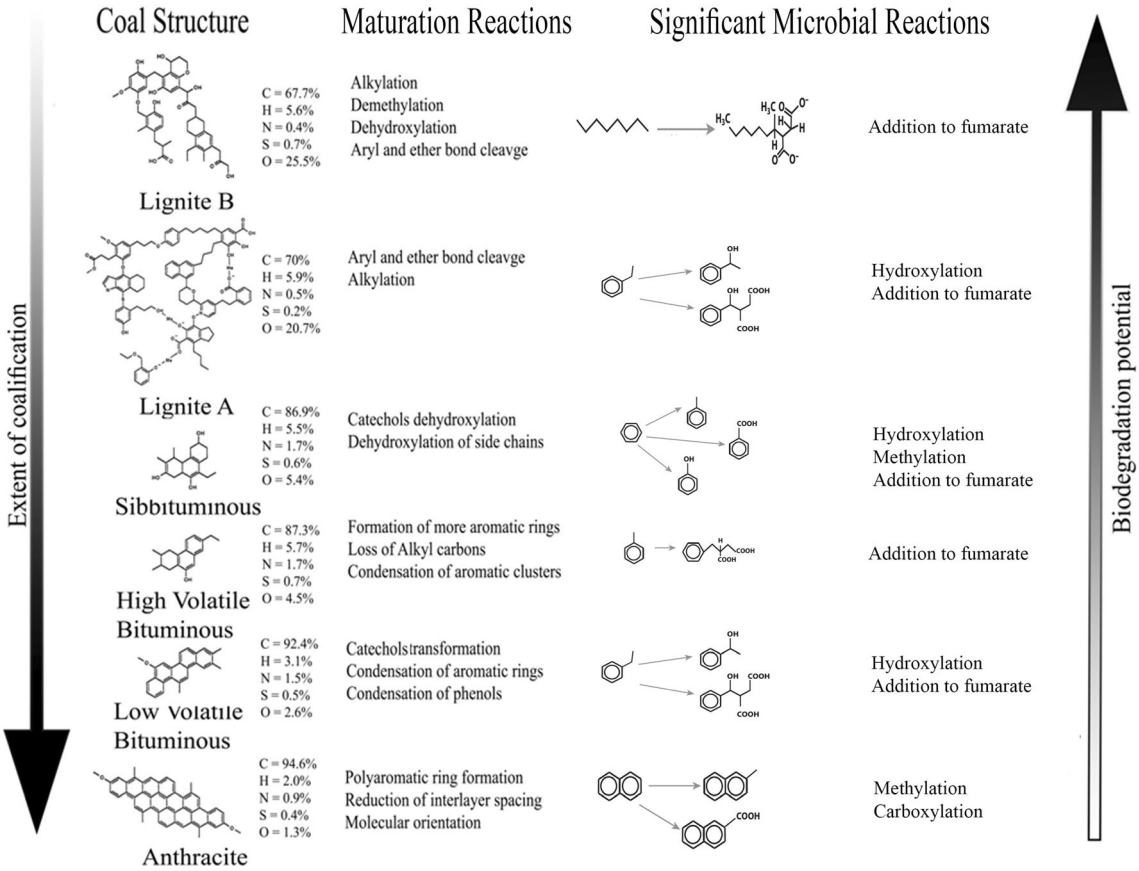

Fig. 1 Structural representation of coal ranks and chemical reactions in coalification along with the significant microbial reactions that could contribute to the biodegradation of coal into methane (coal structures adapted from Fakoussa and Hofrichter 1999 and Strąpoć et al. 2011)

hydrocarbons and gaseous compounds, the methanogenic communities can initialize the substrate utilization in high-rank coals without the help of coal-degrading bacteria and fungi (Sekhohola et al. 2013). Coal ranks such as high-volatile bituminous and sub-bituminous have the greatest potential for microbial methane generation among all other types because of the abundance of volatile matter entities in these ranks (Table 1). According to the ASTM standard classification of coal ranks, more than 31% volatile matter in any coal sample categorizes this coal into high volatile ranks (Table 1; ASTM 2015). Similarly, fixed carbon of such ranks is also categorized as indeterminate in the ASTM categorization table for the sake of simplification. Both volatile matter and fixed carbon percentages are usually determined to check the coal quality for combustion, and a high amount of volatile matter is not good for the environment whilea low amount of fixed carbon creates issues for the economic purposes. These properties make coals with high volatile matter and low fixed carbon less desirable in coal combustion. On the other hand, the same properties of coal can positively affect the biodegradation potential of complex compounds into low-molecular-weight compounds for final methane generation (Crawford 1992; Faison and Crawford 1993; Fallgren et al. 2013).

Ideal coalfield sites and sources for coal methanogenesis

Most of the coalbed methane in the anthracite basins has a thermogenic nature. The methane is produced due to the degradation of coal because of high temperatures and pressure in these basins over long periods of times. Some examples are the North China Plain, the Qinshui Basin, the South Wales Coal Basin, and the Ruhr Basin (Murray 1996; Thielemann et al. 2004; Xiao et al. 2013). On the other hand, biogenic methane production requires highly volatile bituminous and other low-rank coals (Sekhohola et al. 2013). Based on the organic composition and other geochemical characteristics, coal methanogenesis can be expected to occur anywhere with ideal environmental conditions. Several studies have identified the high rates of coalbed methane production in the abandoned coal mines that are the potential sources of pollution. It has also been estimated that more than 7% of global greenhouse methane comes from abandoned coal mines due to thermogenic and biogenic gasification (Denman et al. 2007). However, biogenic methane production has prominently been associated with low-rank coals (Crawford 1992). Beckmann et al. (2011) investigated coal samples and mine timber from

several abandoned coal mines in Germany, which were closed in the 1960s after excessive mining, and have concluded that favourable conditions for the methanogenic consortia can be found in the abandoned coal mines because of appropriate H_2 conditions. The results of this study are interesting to note because they indicate the potential of coal wastes as an active source of biogenic methane.

Another active location that has been discovered recently for coal methanogenesis is coal-bearing sediments. Gründger et al.(2015) reported the high rates of biogenic methane production in the Cenozoic sediments. These sediments consist of high amounts of organic material derived from nearby coal mines. The major source of the coal-derived organic material and coal particles in these sediments is fluvial deposits. Nearby rivers and underground streams brought the methanogens and nutrients together for the biological reactions (Gründger et al. 2015). Waldron et al. (2007) investigated the biogenic methane production in the sedimentary rocks of northern Michigan coal fields and reported the diversified substrate preferences by different methanogenic consortia acting in these sedimentary rocks. Such coal-bearing sediments can be used to study the ideal conditions of methanogenesis because of the relative rates of this microbial process on these sites.

Ideal culture conditions for ex situ coal methanogenesis

Various research setups have been designed in recent years to discover the ideal culture conditions for maximum gas production from coal by different types of methanogenic consortia. Some of these studies have been exclusively conducted in laboratory settings while others have also been designed to check the ideal conditions of CBM production in coal mines. Methanogenic consortia identified in coal basins are anaerobic in most cases but also show flexibility in this culture condition (Crawford 1992). It has been estimated that less than 0.3 V of oxidation/reduction potential in the cultures can actively degrade the coal polymers into methane (Games et al. 1978; Xiao et al. 2013). However, it has also been accepted that the methanogenic consortia in various coal cultures can also be aerotolerant to some extent (Opara et al. 2012; Zhang et al. 2015). A major goal in the development of a methanogenic culture having coal as the sole carbon substrate should be to acquire strictly anaerobic conditions that can be correlated with these conventional microbiological studies (Karakashev et al. 2006; Jones et al. 2008, 2010; Ohtomo et al. 2013).

The second condition for obtaining an ideal methanogenic culture for coal biodegradation is the optimum temperature that ranges from mesophilic to thermophilic for these microbial species (Chen et al. 2008). Mesophilic methanogens have been isolated from various anaerobic bioreactors designed for complex polymer degradation such as organic wastes, wastewater sludge and low-rank coals (Pohland and Bloodgood 1963; Huser et al. 1982; Zinder et al. 1984; Speight 2012). In addition to the mesophilic conditions (30–40 °C), methanogenesis has also been detected at very low temperatures (e.g. 4 °C), as the methanogenic activity is common in anoxic rice field soils at this temperature (Kotsyurbenko et al. 1996; Peng et al. 2008). While these sources of methanogenesis are very different from coal biodegradation into methane, they can give important hints about the optimum temperature ranges for methanogenic consortia which all produce methane despite having different types of origins and environments. The analysis of different origins and environments of methanogens is necessary to define a set of culture conditions that can be applied in the laboratory settings of coal methanogenesis. After reviewing the optimum temperature preferences of methanogens, it can be proposed that the coal biodegradation experiments should be performed at temperatures between 30 and 60 °C (Speight 2012).

Nutrient amendment and adjustment to the optimum pH are two other factors that can be used to enhance microbial methane production in the respective cultures (Jones et al. 2010; Sekhohola et al. 2013). Methanogens can digest various kinds of simple substrates such as CO_2, acetate or other single carbon compounds. The influx of simple carbon compounds like acetate salts can increase the rate of initial breakdown and the final output of methane generation (Jones et al. 2010). Minimal salt medium is one of the most common media used for methanogenic studies, and it has NH_4Cl, K_2HPO_4, and $MgCl_2$ in different concentrations (Rhee et al. 1997; Lambo et al. 2009). Additionally, Jones et al. (2010) used $NaHCO_3$, NH_4Cl, NaH_2PO_4 and KCl as the nutrient media for the cultivation of methanogens. The use of acetate salts such CH_3COONa in the bioreactors enhances the ability of methanogens to thrive in the low-nutrient conditions when the acetate and H_2+CO_2 have not yet been produced by the initial breakdown of the organic polymers by bacterial and fungal species (Orem et al. 2010). Usually, the initial pH of the medium is set to be around 7, but can be changed considerably according to the active microbial species in the reactor and the type of inoculums (Speight 2012).

The most common habitats for methanogens are anoxic field soils (Peng et al. 2008), cattle manure (Borja et al. 1996), wastewater sludge (Steinberg and Regan 2008), marine sediments (Barnes and Goldberg 1976), and coal formation waters (Singh et al. 2012) along with many others. All the habitats mentioned above contain complex carbon compounds which hint toward the ability of methanogens to degrade such complex compounds. However, all such methanogenic species come from a diverse range of microbial consortia in which specific bacterial communities provide the intermediate products for the final methanogenic pathway (Sekhohola et al. 2013). Such habitats can be used as

inoculum material in the methanogenic bioreactors (Jones et al. 2008). The anoxic conditions should be present for the collection and handling of such inoculum material. Ex situ studies, or more specifically t laboratory setups, have revealed the effect of the addition of such material as the potential source of methanogenic consortia (Jones et al. 2010). Therefore, such complex microbial sources can provide ideal types of consortia in ex situ bioreactor development.

By summarizing all the above-mentioned culture conditions in which coal methanogens would survive and produce maximum methane, it can be seen that an ideal methanogenic culture would have a high anoxic environment, medium to high temperature, and neutral pH. Additionally, the amendment of minimal salt media can help in the initial survival of methanogens in the consortia. The enrichment of methanogens from the above-mentioned source can, in addition, provide positive results for overall coal biodegradation into methane.

Microbial diversity in methanogenic consortia

Methanogenic consortia can have many kinds of microbes including fungi, bacteria, and archaea. The consortia act in a syntrophic manner to give the desired product which can be different for different types of consortia, substrate, and the environment. However, sometimes, the active production of one chemical or product by one type of organisms inhibits the growth and productivity of another type of microbial species. One example, in this regard, is the activity of sulfate-reducing bacteria such as *Desulfotomaculum* spp. which hinders the activity of hydrogenotrophic methanogens due to the utilization of H_2 (Detmers et al. 2001). On the other hand, the overall methanogenic pathway consists of many biodegradation stages of complex organic compounds like polyaromatic hydrocarbons (PAHs) into simple intermediate compounds such as acetate which would then be utilized by methanogens and sulfate-reducing bacteria simultaneously (Sekhohola et al. 2013). Despite the advent of modern molecular and metagenomic techniques to identify unculturable microbial species, the determination of the exact numbers and types of microbial species in such a consortium is still a very difficult task. However, the efforts have been made and have contributed to a large number of research articles containing information about methanogenic species (Fig. 2).

One of the most prominent environmental drivers that determine the community structure in a methanogenic consortium is the origin of the coal organic matter available to these microbial communities. The origins of coal organic matter are the formation water, hard coal and laboratory coal samples which can be in powdered form or pretreated with different chemicals and/or fungal species (Haider et al. 2013). Some of the studies that have been conducted in the past ten years for the determination of microbial diversity in coal methanogenic cultures were based on these environmental drivers and can produce very different results. For example, the study conducted by Guo et al. (2012) and Gründger et al. (2015) were specifically designed to determine the microbial diversity in the formation waters and hard coal subsurface in coal mines. Both research articles report different methanogenic archaea but similar fermenting bacteria. Guo et al. (2012) describe the abundance of *Methanolobus*, while Gründger et al. (2015) report *Methanosarcinales* and *Methanomicrobiales* as the methane-producing communities. Both studies indicate the presence of proteobacteria as the fermenting and/or syntrophic bacteria.

After the analysis of the research articles published in recent years, it can be said that the most common methanogenic genera found in the laboratory cultures, coal mines, and formation waters are *Methanosarcinales*, *Methanobacteriales*, and *Methanomicrobiales* (Meslé et al. 2013). There are seven exclusive genera that have been specifically associated with methane generation from three different pathways. Among them, *Methanosarcina* is the most common, having versatile substrate preferences including acetate, H_2+CO_2, and C_1 compounds such as methanol, dimethyl sulfide, and trimethylamines (Jones et al. 2010; Gründger et al. 2015). On the other hand, *Methanocalculus*, *Methanoculleus*, *Methanobacterium*, and *Methanothermobacter* are all hydrogenotrophic, while *Methanosaeta* is acetoclastic (Meslé et al. 2013). More than ten bacterial phyla have commonly been identified in most of the coal degradation studies. The dominate examples include proteobacteria (Guo et al. 2012), actinobacteria (Deng and Fong 2011), firmicutes and bacteroidetes (Meslé et al. 2013). The most common bacterial genera that act both as fermenters and syntrophic associates are proteobacteria (Table 2). Various fungal species such as *Coriolus versicolor*, *Streptomyces badius*, P*hanerochaete chrysosporium*, *Heterobasidion annosum*, *Coriolus hirsusutus*, and *Neosartorya fischeri* have been identified in the bioconversion of coal into useful products (Ghani et al. 2015). Fungal biotreatment of coal has been a common research interest as compared to the research trends in coal methanogenesis and other related coal biodegradation concepts. While all these fungal species have been found to degrade coal into useful products aerobically, the application of *Penicillium chrysogenum* has been studied as the pretreatment agent for coal methanogenesis (Haider et al. 2013). The potential of this fungal species to degrade coal into the substrates for methanogens can be used to elaborate the significance of fungal species in methanogenesis.

While the original environment of the coal organic matter can play an important role in the determination of the microbial diversity of a coal methanogenic culture, the biogeographic origins of the coal organic matter can also be used to estimate the general community structure of this type of

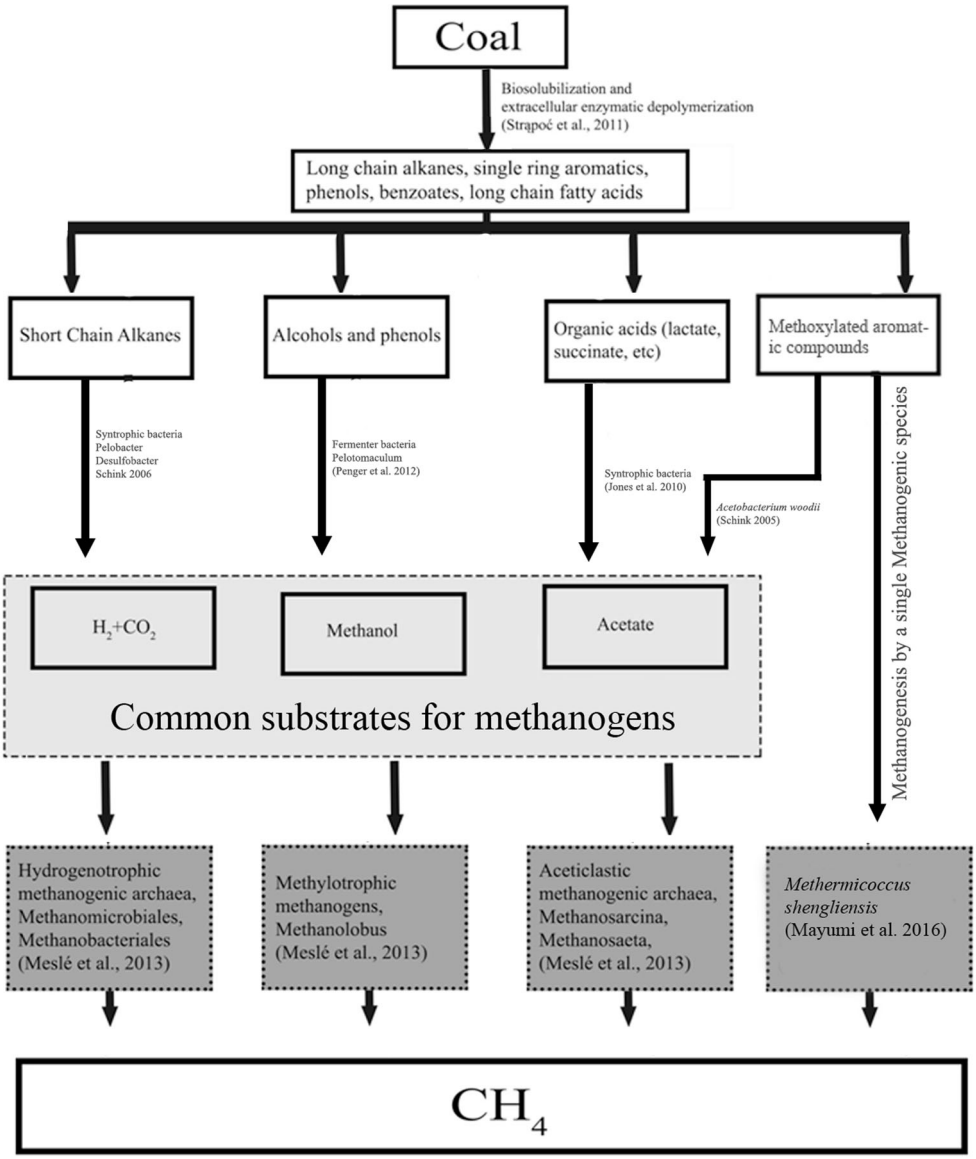

Fig. 2 Simplified pathways in microbial methane production and the active microbial species in methane production at different stages

culture. For example, Beckmann et al. (2011) and Gründger et al. (2015) report the nature of microbial consortia in the hard coal samples and formation water of coal mines in Germany. On the other hand, Guo et al. (2012) report the microbial diversity of coal methanogenic culture of formation waters in China. The enrichment experiments conducted by Harris et al. (2008), Jones et al. (2010), Opara et al. (2012), and Ohtomo et al. (2013) report the efficiency of different types of *Methanosarcina* spp. in the conversion of coal organic matter extracted from the coal mines of the USA. All these studies hint toward some common methanogenic pathways and genera in the efficient conversion of coal into methane.

Aside from the environmental drivers and biogeographic trends that can generate methane in situ or in laboratory bioreactors, coal-degrading methanogenic consortia can also be differentiated according to the nature of the metabolic reactions that they use for their growth in the mixed cultures. As has been categorized in Table 2, many of the subtypes of proteobacteria act as fermenters in the biodegradation of coal polymers. The fermenter bacteria are a special type of microbial species in the methanogenic consortia which break down the complex polymers in coal. Such polymers are degraded into organic acids, medium- and short-chain fatty acids, C_1 compounds, acetate and H_2+CO_2 (Strąpoć et al. 2011). The fermentation reactions occur in the form of fragmentation, and the resultant activation provides intermediate products for another type of bacterial species which are known as syntrophic communities in the methanogenic consortia. Some examples of such metabolic reactions are methylation, carboxylation, and hydroxylation along with the addition of fumarate. All

Table 2 Microbial diversity according to the type of substrates and products in the coal cultures

Major microbial community	Common examples	Substrate in coal cultures	Metabolic intermediates and products	References
Fermenter Bacteria	Actinobacteria, bacteroidetes, firmicutes, α-proteobacteria, β-proteobacteria, and γ-proteobacteria	Polyaromatic rings, Long aliphatic hydrocarbon chains and organic acids	Single ringed aromatic compounds, shorter aliphatic chains and fatty acids	Fallgren et al. 2013; Meslé et al. 2013; Xiao et al. 2013
Syntrophic communities	δ-proteobacteria, and ε-proteobacteri	Water-soluble compounds, Organic acids, fatty acids, Sulphates, Nitrates, and Alkanes	Acetate, Short chain aliphatic compounds, benzene, H_2+CO_2 and Single carbon compounds	Fakoussa and Hofrichter 1999; Jones et al. 2008; Zhang et al. 2015
Methanogenic communities	*Methanosarcinales*, *Methanomicrobiales*, *Methanobacteriales*	Acetate, H_2+CO_2, single carbon compounds	Methane	Fakoussa and Hofrichter 1999; Gupta and Birendra 2000; Harris et al. 2008; Strąpoć et al. 2011; Wang et al. 2015

such reactions provide the final products that would then be utilized by the methanogens. In this regard, it can be said that the fermenter bacteria are the most common types of bacteria in coal cultures (Table 2). The syntrophic communities provide favourable metabolic conditions and products for the growth of methanogens. One example of syntrophic associations is the metabolism of *Methanobacillus omelianskii* for keeping the low partial pressure of hydrogen in the bioreactors (Barker 1939). On the other hand, methanogens in the bioreactors can be subdivided into acetate reducers, CO_2 reducers, and the methylotrophs (Fig. 2). All of these methanogenic species play a crucial role in the bioconversion of coal into methane.

Energy substrates for methanogenic consortia

It must be noted that a methanogenic consortium has all three types of functional metabolic communities, including fermenting, syntrophic and methanogenic species. Various studies on biogenic methane production have revealed that there is no single common pathway used by all the identified consortia in coal bioreactors (Fig. 2). There can be as many pathways as the microbial species playing their part in the final output. Sometimes, methane production becomes impossible due to the unfavourable culture conditions such as the excessive formation of H_2S (Detmers et al. 2001) or H_2 (Barker 1939). On the other hand, the anoxic conditions in the culture media have also been shown to play a diverse role in various types of consortia. Before the confirmation of aerotolerance by several methanogenic consortia (Opara et al. 2012; Xiao et al. 2013), it was considered that most of the methanogenic species are strictly anaerobic (Gijzen 2002; Orem et al. 2010). However, the presence of oxygen in limited amounts has recently been correlated with enhanced methane production rates. All such conditions make the methanogenic pathways extremely complex to be understood as a simple set of metabolic reactions.

There are three most common substrates in the biogenic methane production besides methoxylated aromatic compounds which have recently been discovered to be degraded by some methanogenic species (Fig. 2). The common substrates are acetate, H_2+CO_2, and C_1 compounds (e.g. methanol). Depending on these common types of substrates, the methanogenic species can be considered as acetoclastic, hydrogenotrophic and methylotrophic (Penger et al. 2012; Meslé et al. 2013). These distinctions can enhance the understanding of the biochemical reactions that have various C_1 compounds and hydrogen as their end products (Table 2). However, the exceptions to these common biochemical trends are the CO_2 reduction, O-demethylation, and acetyl–coenzyme A metabolism of different compounds of methoxy-benzoates (Mayumi et al. 2016). These methoxylated aromatic compounds are substrates for *Methermicoccus shengliensis* (Mayumi et al. 2016).

The common substrates that have been described above are produced by the fermenter and syntrophic bacteria before and along with the metabolic growth of the methanogens (Ulrich and Bower 2008). The fermenter bacteria utilize short and medium fatty acids, proteins, amino acids, and dNTPs along with many other complex organic compounds in their metabolic reactions (Meslé et al. 2013). However, none of these compounds are readily present in coal and have to be produced by the hydrolytic reactions through extracellular enzymatic digestion by fungal species. The ideal types for coal biodegradation are highly volatile bituminous, highly volatile sub-bituminous and lignite (Table 1). These types of coal have the cleavage sites in their complex polymer structure which can be attacked by bacterial and fungal exoenzymes (Haider et al. 2013).

After the initial degradation, the coal-derived products (e.g. long-chain alkanes, fatty acids, phenolic compounds and

single-ringed aromatic compounds) are obtained which can then be utilized by fermenter bacteria (Fig. 2). The single-ringed aromatic compounds such as methoxy-benzoate, trimethoxy-benzoate, trimethoxy-cinnamate, methoxy-phenol, and trimethoxy-benzylalcohol can also be used by *Methanomethylovorans hollandica* or *Methermicoccus shengliensis* to produce methane (Mayumi et al. 2016). Some of the common metabolic reactions after fermentation are acinetobacter respiration and fermentation (Jones et al. 2010). This stage generates various kinds of volatile fatty acids that can now be utilized by H_2-producing acetogenic bacteria (Beckmann et al. 2011).

The final stage of fermentation is the production of the ideal substrates for methanogens. However, the interplay between different methanogenic substrates is also a common process at this stage. For example, the syntrophic acetate-oxidizing bacteria convert acetate into H_2+CO_2, which is a common product for hydrogenotrophic methanogens (Karakashev et al. 2006). On the other hand, H_2+CO_2 can also be converted back into the acetate by homoacetogenic bacteria (Ohtomo et al. 2013). Furthermore, acetate can also be produced from methoxy-benzoate, trimethoxy-benzoate, and trimethoxy-cinnamate through the reaction of *Acetobacterium woodii* (Schink 2006). Overall, these interchanges of the substrates can collectively hinder or enhance the activity of methanogens depending on the culture conditions provided by the syntrophic communities.

Bioprocessing of coal by methanogens

The bioprocessing of coal is one of the most widely accepted applications in the area of coal biotechnology (Klein 1999). However, little is known about the effect of coal characteristics before and after biotreatment in methanogenic bioreactors. The basic goal behind most of the research, in this regard, is to understand the microbial reactions for better gas production. The bioprocessing of coal to enhance the quality of coal while decreasing the emission of greenhouse gases has been explored only for bacterial and fungal species (Klein 1999; Gonsalvesh et al. 2008). There is a need to develop research protocols addressing the effect of methanogenesis on the quality of coal, because it can be assumed that the biotreatment increases the overall calorific values while decreasing the percentage organic sulfur which can be important for subsequent coal combustion.

The possibility of improved coal quality after methanogenic biotreatment can be explained with the help of individual biochemical pathways and intermediate products that can enhance the overall coal quality. For this initial assumption, various physicochemical parameters such as moisture content, volatile matter, and the ratio of total to fixed carbon play an important role. It has been found that volatile fatty acids are the key intermediate products in biogenic methane production from coal (Jones et al. 2010). The same type of volatile fatty acids along with gases like hydrogen, carbon dioxide, and methane constitutes the volatile matter content of coal (Speight 2012). Volatile matter is an important factor in describing the overall rank and quality of coal because it helps in the spontaneous combustion of this industrial fuel. Similar assumptions have also been presented for fixed carbon and calorific value of coal species. After reviewing the bioprocessing studies that have revealed the potential of several microbial treatments for the enhanced quality of coal, the coal samples after methanogenic treatment can also be assessed to check their potential as cleaner fuels with high calorific values. However, few research setups have been designed to check the effect of methanogenesis on the quality of coal samples for subsequent combustion.

Research trends in coal methanogenesis

The hints about the microbial methane production in different habitats can be traced back to 1776 when methane gas was first discovered by Alessandro Volta (Gijzen 2002). The discovery of various methanogens from time to time enables biological scientists to look back into the classification systems of prokaryotic and eukaryotic organisms while emphasizing the organisms that can thrive in extreme conditions. On the other hand, the role and presence of fungi and bacteria in coal degradation was discovered before the 1960, and the microbial conversion of coal into other compounds was identified in the early 1980s (Fakoussa 1981). The development of anaerobic bioreactors for the digestion and treatment of wastewater and solid wastes was carried out in the 1970s and 1980s, respectively (Gijzen 2002).

The biodegradation of coal into useful compounds has been a widely visited topic since the discovery of fungal species like *Polyporus versicolor* and *Poria monticola* that can actively degrade the condensed aromatic polymers in coal (Cohen and Gabriele 1982). Biotechnological research on the bioconversion of coal into useful products including methane was one of the main trends in the discovery of fungal biodegradation of coal. Various research setups focused on the in situ bioconversion potential of coal along with the bioprocessing of this fossil fuel for cleaner energy production (Ehrlich and Brierley 1990; Faison and Crawford 1993; Gonsalvesh et al. 2008). Some specific examples include microbiological desulfurization, elimination of inorganic impurities that generate ash, and increasing the calorific value. The microbiological desulfurization of coal is one of the most widely applicable options with regard to the applications of microbial species in coal methanogenesis (Dugan and Apel 1978).

In the past decade, a great amount of attention has been given to in situ biogenic methane production along with small and large bioreactor development for this bioconversion. There are many examples of research setups exploring in situ methanogenic populations in the coalfields across the world (Faiz and Hendry 2006; Butland and Moore 2008; Flores et al. 2008; Beckmann et al. 2011; Agyarko and Mansoori 2013; Fallgren et al. 2013). There are two types of studies in this regard: the first type deals with the determination of the types and extent of methanogenic consortia in the coal basins by metagenomic analysis and other molecular techniques, and the second type deals with the culturing of such microbial species in the laboratory reactors for checking the potential of coal methanogenesis in the development of ideal culture conditions and bioreactors.

In the past few years, the effect of various nutrient amendments in coal methanogenesis has been checked to discover the ideal culture parameters for methane production (Jones et al. 2010). One example is the research setup designed by Ünal et al. (2015) to explore the effect of trace elements such as iron, nickel, cobalt, zinc and manganese. Such trace elements are present in the form of inorganic impurities, and their oxides are the main components present in the ash produced by coal combustion. These trace elements are required by microbial species as co-factors and can be effectively utilized to achieve maximum gas production rates (Harris et al. 2008). Wang et al. (2015) designed a research setup to check the effect of various substrates in coal biogasification. A similar type of research setup was also designed by Jones et al. (2010). All of these projects highlight the promising results for the development of bioaugmentation and biostimulation strategies for coal methanogenesis.

The current trends in the research suggest the importance of coal rank and microbial community structures in the maximum methane production from coal. While these research setups have been exclusively designed to check the methanogenic potential of low-quality coal, there is a need to develop research setups that focus on the assessment of medium-quality coal ranks that are high in volatile matter. Additionally, the next generation sequencing analysis must be applied to identify and quantify microbial communities in methanogenic cultures in a more comprehensive manner.

Conclusion

Coal methanogenesis is a complex set of microbial processes that convert this hard blackish rock into a cleaner energy source. Coalbed methane has been extensively studied for exploring the potential of this non-conventional source of energy in the near future. Coal activation under anoxic conditions has not yet been fully understood and there are a number of critical knowledge gaps in the understanding of the microbial reactions that can break down complex aliphatic and aromatic hydrocarbons in coal components. Recent studies have revealed the key role of medium- and low-quality coal types in the enhanced bioconversion into methane. However, the generalized assumption that low-rank lignites are more prone to microbial degradation under anoxic conditions has recently been seriously challenged , indicating the potential of medium to highly volatile bituminous coal in maximum gas conversion as compared to other types of coal. This new finding could produce significant insights into the field of coal methanogenesis as it would lead to the understanding of metabolic reactions and intermediate determinants in biogenic methane production from coal. The microbial diversity, culture conditions and ex situ bioreactor development for coal methanogenesis have been extensively studied during the last three decades due to the potential applications of this technology. The metabolic reactions and intermediate products that govern the overall microbial populations and gas production rates have not yet been understood but some biochemical pathways like initial fermentation reactions and methanogenic reduction of acetates and carbon dioxide have been found to be common in the light of the current literature review. Currently, the focus of researchers should be to determine the efficient coal ranks and microbial species in the maximum bioconversion of coal into methane.

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