Biogeochemistry of Microbial Coal-Bed Methane

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Abstract

Microbial methane accumulations have been discovered in multiple coal-bearing basins over the past two decades. Such discoveries were originally based on unique biogenic signatures in the stable isotopic composition of methane and carbon dioxide. Basins with microbial methane contain either low-maturity coals with predominantly microbial methane gas or uplifted coals containing older, thermogenic gas mixed with more recently produced microbial methane. Recent advances in genomics have allowed further evaluation of the source of microbial methane, through the use of high-throughput phylogenetic sequencing and fluorescent in situ hybridization, to describe the diversity and abundance of bacteria and methanogenic archaea in these subsurface formations. However, the anaerobic metabolism of the bacteria breaking coal down to methanogenic substrates, the likely rate-limiting step in biogenic gas production, is not fully understood. Coal molecules are more recalcitrant to biodegradation with increasing thermal maturity, and progress has been made in identifying some of the enzymes involved in the anaerobic degradation of these recalcitrant organic molecules using metagenomic studies and culture enrichments. In recent years, researchers have attempted lab and subsurface stimulation of the naturally slow process of methanogenic degradation of coal.

1. INTRODUCTION

Coal: a readily combustible rock containing >50 wt% and >70 vol% of carbonaceous material; is typically plant-derived organic matter at varying levels of chemical transformation, depending on thermal maturity

Coal rank: level of physical and chemical transformation of organic matter known as coalification; progresses from peat through lignite, subbituminous coal, bituminous coal, semianthracite, to anthracite and meta-anthracite

Methanogens: obligate anaerobic prokaryotic microorganisms that produce methane as a metabolic by-product. These archaea (not bacteria) reduce small molecules such as CO_2, acetate, and methanol to methane to gain energy

Coal-bed methane (CBM) production was initiated in the United States in the late 1980s and today accounts for approximately 10% of total production from all gas wells in this country (**Figure 1**). In 2009, the volume of produced CBM in the United States was 56×10^9 m^3 or $\sim 2 \times 10^{12}$ ft^3 (**http://www.eia.gov**). Early production of CBM focused on highly mature coals with thermogenic gas potential (e.g., Fruitland coals in the San Juan Basin). As CBM exploration progressed into less mature regions, methane was still found to be present in significant quantities, above what would be expected on the basis of thermogenic generation of gas. Even coals not mature enough to have begun generating thermogenic gas appeared to be methane rich (e.g., coals in the Powder River Basin). The universal abundance of methane in low-maturity coals and other organic-rich rocks, including shales and sandstones with organic debris, triggered geochemical studies, including gas isotopic analysis, to decipher the origin of the gas. Geochemistry repeatedly implied the microbial origin of gases in low-maturity rocks. Subsequently, some of the higher-maturity coals were discovered to contain mixtures of thermogenic hydrocarbons and microbial methane or secondary biogenic gas, e.g., the northern part of the San Juan Basin (Scott et al. 1994, Zhou et al. 2005). In multiple basins, regardless of coal rank, microbial methane generation was initiated or stimulated after the introduction of meteoric water into the system [e.g., Alberta (Bachu & Michael 2003) and Black Warrior (Pashin 2007)]. Furthermore, microbial methanogenesis is ongoing in multiple shallow coals [e.g., Powder River (Green et al. 2008)], dispersed organic matter–containing formations [e.g., Cook Inlet (Strąpoć et al. 2010a)], and shales [e.g., Michigan Basin (Martini et al. 2003)]. Global occurrences of microbial CBM are depicted in **Figure 1**.

During the past several years, geomicrobiological and phylogenetic studies of subsurface coal beds, shales, and sandstones with organic debris have described the active microbial communities [e.g., Hokkaido (Shimizu et al. 2007), Powder River Basin (Green et al. 2008), and the Ruhr Basin (Krüger et al. 2008)]. Several studies have demonstrated the ability of these communities to convert coal to methane (Shumkov et al. 1999, Harris et al. 2008, Orem et al. 2010). In general, these processes involve groups of fermentative bacteria that are syntrophically associated with methanogenic archaea. Recent, and mostly industry-based, efforts have targeted enhanced CBM subsurface generation via amending indigenous communities with nutrients and/or via inoculation with microbial consortia (Pfeiffer et al. 2010).

In this review, we describe how occurrences of microbial methane accumulations in coal beds are identified through the use of geochemical tools such as stable isotopes. Furthermore, geological and hydrogeological constraints of such accumulations are discussed. Detailed discussion about the microbial process of formation of methane from coal begins with a description of the origin and composition of coal as the substrate. Subsequently, we present current knowledge about and methods of analysis of microbial metabolic pathways and microbial communities involved in biodegradation of various moieties of coal's organic matter. Lastly, attempts of stimulation of enhanced microbial methane generation in coals are briefly described.

2. GEOCHEMISTRY AND GEOLOGICAL CONTROLS OF MICROBIAL COAL-BED METHANE

2.1. Geochemical Identification and Global Occurrence

Microbial methane generation from any coal, regardless of its burial history, requires suitable physicochemical subsurface conditions. Isotopically, microbially generated gas has distinct ^{13}C- and D-depleted signatures. These isotopic fingerprints were commonly utilized in gas origin

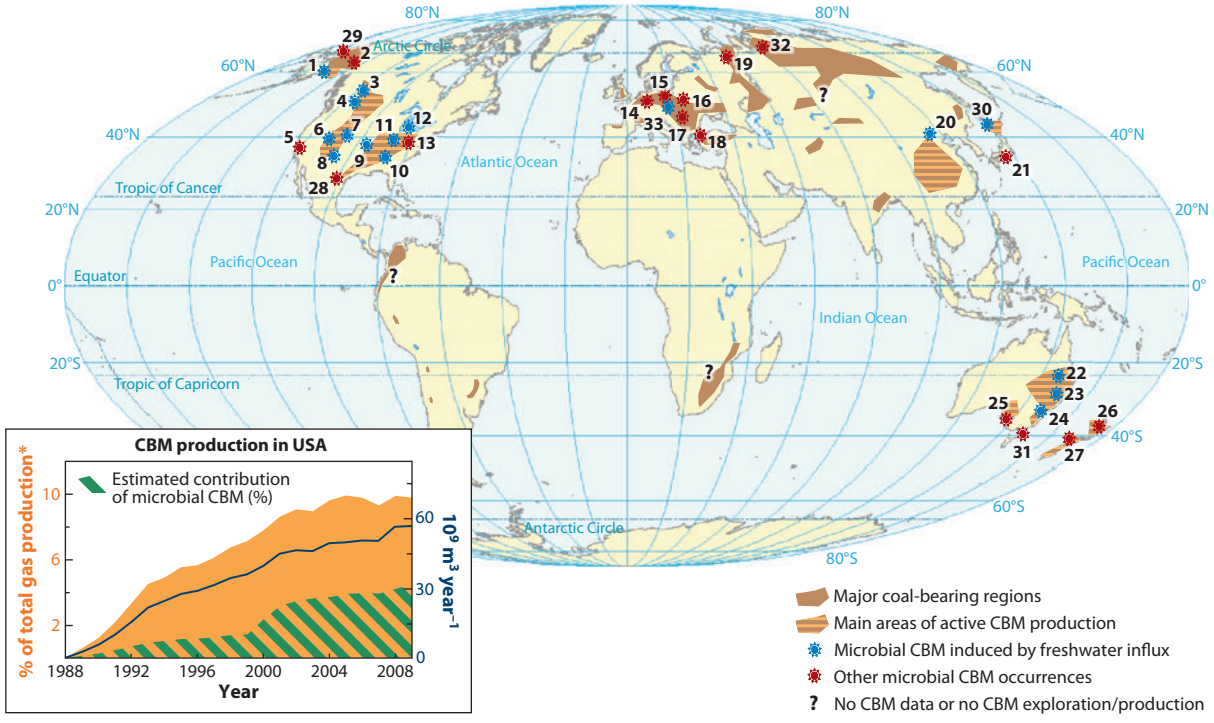


Figure 1

Global occurrences of microbial coal-bed methane (CBM). (*1*) Cook Inlet Basin, Alaska (Rice & Claypool 1981, Montgomery & Barker 2003, Strąpoć et al. 2010a). (*2*) Fort Yukon, Alaska (Harris et al. 2008). (*3*) Alberta Basin, Alberta (Bachu & Michael 2003). (*4*) Elk Valley, British Columbia (Aravena et al. 2003). (*5*) Maine Prairie, California (this review article). (*6*) Uinta Basin, Utah (Stark & Cook 2009, this review article). (*7*) Powder River Basin, Wyoming (Jin et al. 2007, Pfeiffer et al. 2010, Flores et al. 2008, Rice et al. 2008, Strąpoć et al. 2010a). (*8*) San Juan Basin, New Mexico and Colorado (Scott et al. 1994, Zhou et al. 2005, this review article). (*9*) Forest City Basin, Kansas (McIntosh et al. 2008). (*10*) Black Warrior Basin, Alabama (Pitman et al. 2003, Pashin 2007, Doerfert et al. 2009). (*11*) Illinois Basin, Indiana (Strąpoć et al. 2007, 2008). (*12*) Michigan Basin, Michigan (Martini et al. 2003). (*13*) Appalachian Basin, Pennsylvania (Volkwein 1995). (*14*) Ruhr Basin, Germany (Thielemann et al. 2004, Krüger et al. 2008). (*15*) Polish lignites (Strąpoć et al. 2010a). (*16*) Carpathian Foredeep (Kotarba 1998). (*17*) Pannonian Basin, Hungary (Veto et al. 2004). (*18*) Zonguldak Basin (Hösgormez et al. 2002). (*19*) Pechora Basin (Shumkov et al. 1999). (*20*) Xinji area, China (Tao et al. 2007). (*21*) Chiba Prefecture, Japan (Mochimaru et al. 2007). (*22*) Bowen Basin (Smith & Pallasser 1996, Ahmed & Smith 2001). (*23*) Surat Basin (Li et al. 2008). (*24*) Sydney Basin, Australia (Smith & Pallasser 1996, Faiz & Hendry 2006). (*25*) Port Phillip Basin, Australia (Li et al. 2008). (*26*) Waikato Basin, New Zealand (Butland & Moore 2008, Fry et al. 2009). (*27*) Greymouth, New Zealand (Butland & Moore, 2008). (*28*) Wilcox Group, Texas (Jones et al. 2008, Orem et al. 2010). (*29*) Nanushuk Formation, North Slope, Alaska (Jones et al. 2008). (*30*) Hokkaido, Japan (Shimizu et al. 2007). (*31*) Gippsland Basin, Australia (Midgley et al. 2010). *(32)* Northwestern Siberian plain (Yermakov 1970). *(33)* Upper Silesian Basin, Poland (Kotarba & Pluta 2009). Placement and shapes of the main coal basins on the map are approximate. Inset graph shows CBM production in the United States (data source: **http://www.eia.gov**). Asterisk indicates the percentage of gas produced from all gas-producing wells, and the green dashed area indicates the estimated contribution of microbial CBM to the total CBM produced (estimation based on isotopic compositions of produced methane). Presently the contribution of microbial methane to the total CBM produced is ~40% and largely owing to the significant production of purely microbial CBM in the Powder River Basin. Map modified from Open Univ. (2011).

classification diagrams to distinguish between thermogenic and biogenic gas accumulations (e.g., Schoell 1980, Rice & Claypool 1981, Whiticar 1999). Carbon and hydrogen isotopic values can also distinguish between major types of microbial methane generation pathways: CO_2 reduction from acetate fermentation and methane from methanol/methyl-utilizing processes. **Figure 2** presents an example of the spread of isotopic and compositional characteristics of gases collected

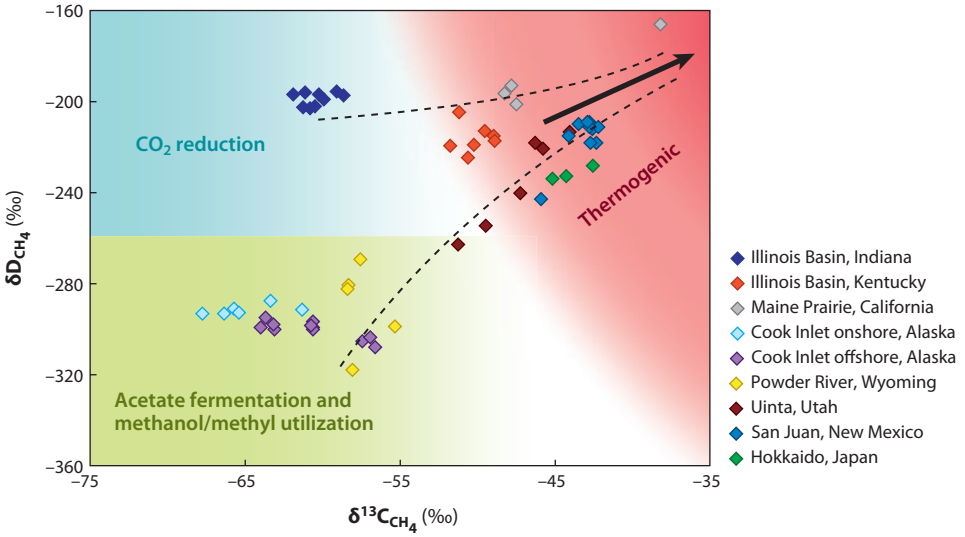


Figure 2

Carbon and hydrogen isotopic classification of methane showing differences between thermogenic gas and biogenic methane generated from different substrates. The arrow and red background color indicate increasing thermal maturity of thermogenic end-member methane. Dashed lines indicate examples of mixing lines. Data sources: Hokkaido, Shimizu et al. (2007); Illinois, Strąpoć et al. (2007); Cook Inlet onshore and Powder River, Strąpoć et al. (2010a); Maine Prairie, Cook Inlet offshore, Uinta, and San Juan, this review article.

from coal beds with microbial methane, thermogenic gas, and mixed-gas origins. In contrast to the classification of microbial gas, the classification of thermogenic gases relies on both molecular and stable isotopic patterns of methane, ethane, propane and butanes, which vary according to increasing thermal maturity (Berner & Faber 1988, Chung et al. 1988). Additionally, within a certain range of maturity, thermogenic gas from coal is volumetrically significant. Early thermogenic gas generation starts at ∼0.5% of vitrinite reflectance (R_o). Thermogenic gas production from coal reaches a transformation ratio (TR) value of 0.1 at ∼0.7% R_o. The major thermal cracking of coal to gaseous hydrocarbons occurs at R_o between 0.7% and 1.6%, reaching a TR of 0.8 (Waples & Marzi 1998). However, generation of the remaining 20% of gas continues well into the anthracite maturity range (2.5–5% R_o) (Schimmelmann et al. 2006).

The δD-δ^{13}C plot (**Figure 2**) indicates that the position of the thermogenic end member of the mixed gas depends on the coal's thermal maturity. Therefore, a mix of high-maturity thermogenic gas with a relatively small fraction of biogenic methane (e.g., that of San Juan) (**Figure 2**) can plot identically as pure low-maturity thermogenic gas (e.g., that of Illinois Basin, Kentucky). Microbial gas, however, is unique for its high dryness, owing to the lack of significant microbial generation of C_{2+} hydrocarbon gases (**Figure 3**). In addition to coal beds with exclusively thermogenic gas [e.g., in the southern part of the Illinois Basin (Strąpoć et al. 2007)] or biogenic gas [e.g., in the Powder River Basin (Flores et al. 2008)], mixed origin of CBM is even more common [e.g., in the San Juan Basin (Scott et al. 1994), Sydney Basin (Faiz & Hendry 2006), and Hokkaido (Shimizu et al. 2007)]. Isotopic mixing diagrams can be used to estimate the contribution of each end member (**Figure 3**). All the above-mentioned gas isotopic classification plots (**Figures 2** and **3**) should be used with caution and in tandem with or in the context of other available geological and geochemical data.

Thermal maturity: a measure of degree of organic matter transformation in response to geothermal conditions

Vitrinite reflectance (R_o): a measure of thermal maturity of organic matter in rocks; a percentage of the incident light reflected from a polished surface of maceral vitrinite measured with a reflected light microscope using an oil immersion objective

Additionally, carbon isotopic differences between CH_4 and CO_2 ($\Delta^{13}C_{CO_2-CH_4}$) can be helpful in deciphering gas origin (**Figure 3*a***). Thermogenic processes are characterized by low $\Delta^{13}C_{CO_2-CH_4}$ owing to high temperatures. Low-temperature microbial enzymatic processes lead to ^{13}C enrichment in residual CO_2 (Conrad 2005). In coals with a mixture of thermogenic and biogenic gases, $\Delta^{13}C_{CO_2-CH_4}$ can be more suitable than the absolute value of $\delta^{13}C$ for discriminating gas origin (Smith & Pallasser 1996, Strąpoć et al. 2007).

In spite of the great utility of these isotopic methods, they cannot account for all three methanogenic pathways. Specifically, methane samples derived from acetate fermentation plot together with methane samples derived from methanol, methylamine, or methylsulfide utilization. The isotopic signatures of these two pathways are similar because the methane hydrogen and carbon atoms are derived from methyl groups that are either disproportionated from acetate or cleaved from methanol, methylamines, or methylsulfides. In contrast, the CO_2 reduction pathway results in slightly lower $\delta^{13}C_{CH_4}$ values and a distinct value corresponding to the δD of formation water, which is typically deuterium enriched compared with carbon-bound hydrogen (Smith et al. 1992, Conrad 2005). Other substrates that can be utilized by methanogens include formate, carbon monoxide, and other simple alcohols (e.g., ethanol, isopropanol, and butanol), but their associated isotopic fractionations are not well-known. Nonetheless, in addition to coals with dominantly acetate- or CO_2-utilizing methanogens, recent studies not only have shown the presence of obligate methyl/methanol-utilizing methanogens (Shimizu et al. 2007, Doerfert et al. 2009, Mochimaru et al. 2009, Toledo et al. 2010) but have even suggested that such methanogens have a leading role in methane production (Strąpoć et al. 2010a).

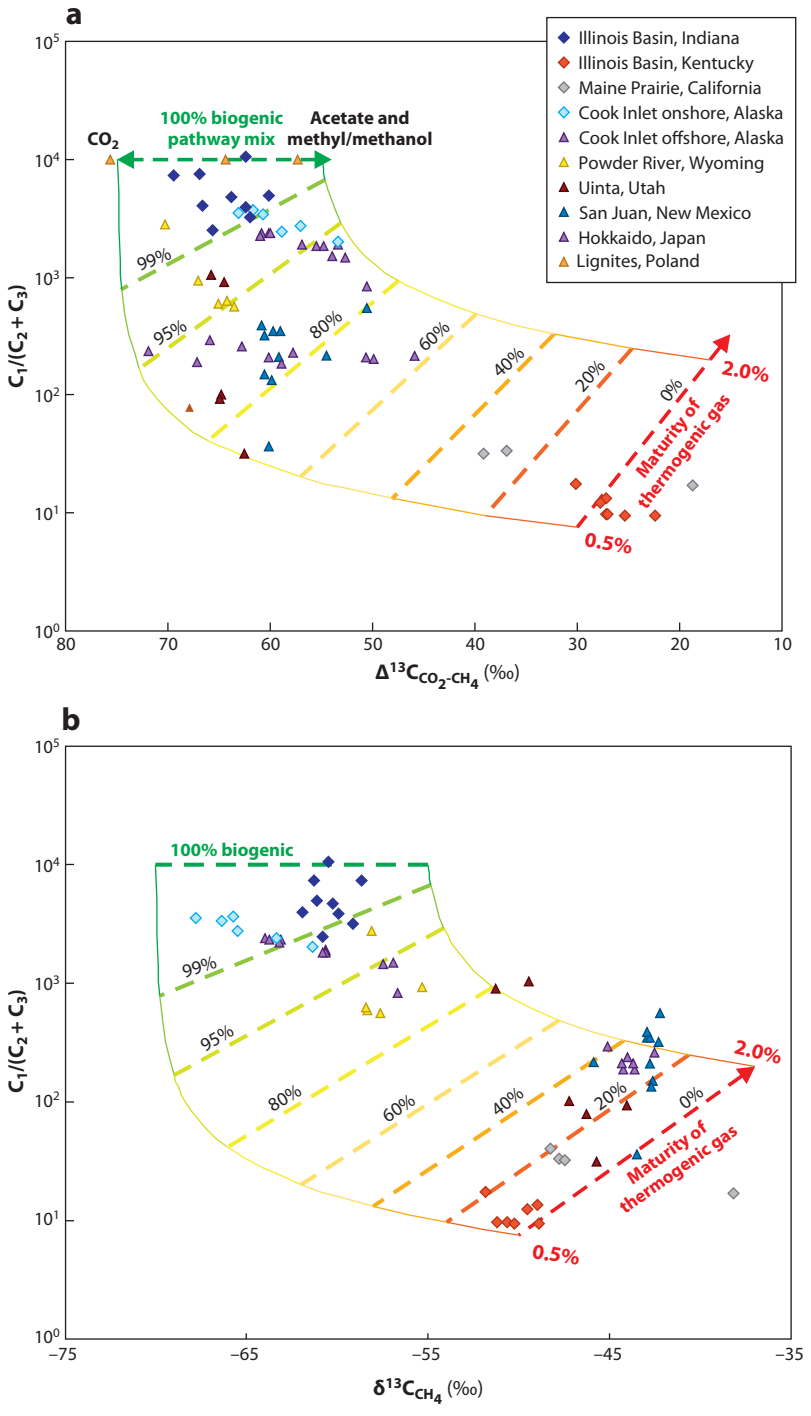
Transformation ratio (TR): a level of conversion of organic matter into hydrocarbons during thermal maturation. Expressed as a fraction (values from 0 to 1); a value of 1 designates complete conversion

Isotopic fractionation: the enrichment of one isotope relative to another in a chemical or physical process such as bond breaking

2.2. Impact of Burial History and Hydrogeology on Microbial Coal-Bed Methane Generation

Numerous CBM basins containing microbial methane experienced microbial reinoculation during their postburial geological history. Exceptions could be basins that were never buried deeply enough to become temperature sterilized or basins that were never uplifted sufficiently after coalification. The most likely source of microbial inoculation is an influx of waters in contact with shallower environments containing microbial communities. Numerous examples include Forest City Basin (McIntosh et al. 2008); Sydney Basin (Ahmed & Smith 2001, Faiz & Hendry 2006); Bowen Basin (Ahmed & Smith 2001); Surat Basin (Li et al. 2008); Alberta Basin (Bachu & Michael 2003); Elk Valley in British Columbia (Aravena et al. 2003); Uinta Basin (this review article); Cook Inlet (Montgomery & Barker 2003, Strąpoć et al. 2010a); Upper Silesian Basin in Poland (Kotarba & Pluta 2009); the Xinji area in China (Tao et al. 2007); and Hokkaido, Japan (Shimizu et al. 2007).

In the Powder River Basin (Flores et al. 2008), the map of water δD shows the impact of the Pleistocene water in the recharge areas of the basin. In the Black Warrior Basin (Pitman et al. 2003, Pashin 2007), two events of uplift, microbial inoculation and methane generation, were recorded by the precipitation of ^{13}C-enriched calcite. In the San Juan Basin, uplift- and topography-driven recharge of meteoric water from the mountains inoculated coals and stimulated biomethane generation in the north and northwest parts of the basin (Scott et al. 1994, Ayers 2002, Zhou et al. 2005, this study). Additionally, artesian overpressure in the northern part of the basin allows for a larger gas capacity of the Fruitland coals. Similar artesian inflow has been observed in the western part of the Uinta Basin (Buzzards Bench and Drunkards Wash) along with its association with enhanced microbial methane along the freshwater conduits (Stark & Cook 2009). The formation water in the Ferron Member–containing coals was estimated to be ~30 ka using ^{14}C. Alternative isotopic methods for the dating of older groundwaters are ^{36}Cl (half-life, 301 ka) and ^{129}I (half-life, 15.7 Ma) (for a detailed description, see Snyder et al. 2002 and Lu et al. 2008).



In the Illinois Basin, the same types of coal from two parts of the basin with significantly different burial histories contain diametrically different types of gases. The southern part of the basin (western Kentucky) experienced deeper burial associated with an abandoned rift system and the potential impact of hydrothermal fluids. The resulting graben was never erosionally uplifted as much as the rest of the basin. As a result, this part of the basin generated exclusively thermogenic gas, whereas shallow and immature coals in the eastern flanks of the basin generated almost pure biogenic methane (**Figure 4**) (Strąpoć et al. 2008). Similar phenomena were documented for the occurrences of biogenic shale gas in which low salinity of diluted basinal brines coincided with isotopic signatures of microbial methane [Antrim Shale in the Michigan Basin (Martini et al. 2003), New Albany Shale (Strąpoć et al. 2010b)]. For global occurrences of microbial methane in coals, see **Figure 1**.

3. COAL COMPOSITION AND MATURITY: FROM PLANTS TO A MOLECULE

Organic carbon, the dominant component of coals, is the primary source of coal-bed gas through its abiotic and biotic breakdown. Therefore, below we briefly review the composition of the organic coal fraction that depends on the makeup of the original plant material, the conditions of organic matter decomposition within a peat mire, and postdepositional chemical transformations.

3.1. Peat-Forming Plant Communities

Peat, a precursor of coal, originates predominantly from plant communities on peat mires. The first indications of plants inhabiting the land came from Middle Ordovician to Late Silurian age strata, but they became widespread only in the Early Devonian (Traverse 2008, Taylor et al. 2009, and references therein). The formation of significant peat deposits required massive expansion and diversification of plant communities, and although coal deposits are known from the Middle Devonian from the Kuznetsk Basin in Russia (Gorsky 1964), only in the Early Carboniferous did large coal deposits form (**Figure 5**). In the Lower Carboniferous, representatives of most of the plant groups that would form the luxuriant vegetation of the upcoming coal age were present. The Upper Carboniferous (Pennsylvanian) featured further development of this vegetation, and during that period most of North America and Europe were covered by peat-forming mires (the Euro-American Coal Province). Located within 10° of the paleoequator, the paleomires were dominated by lower vascular plants such as lycopods, ferns, and calamites (Phillips et al. 1985, Winston 1989, Eble & Grady 1993, Willard 1993). The gymnosperm *Cordaites* appeared during that period and increasingly became a contributor to peats.

Permian peat-forming plants were restricted mainly to the Gondwana supercontinent. Therefore, Permian coals are found on several continents, including Africa, Antarctica, Australia, China, and South America (**Figure 5**), and are known as the Gondwana Coal Province. Permian floras

Figure 3

Gas origin classification and mixing diagrams. Dashed lines represent fractions of biogenic gas in mixture. Thermogenic gas is represented by a range of values corresponding to thermal maturity (expressed as vitrinite reflectance, R_o), and biogenic gas is represented by a range of values related to methanogenic pathways (CO_2 reduction versus methylotrophic and acetotrophic pathways). (*a*) Gas dryness and isotopic fractionation between CO_2 and CH_4 ($\Delta^{13}C_{CO_2-CH_4}$). (*b*) Gas dryness and $\delta^{13}C$ of methane. Data sources: Poland, Strąpoć et al. (2010a); Hokkaido, Shimizu et al. (2007); Illinois, Strąpoć et al. (2007); Cook Inlet onshore and Powder River, Strąpoć et al. (2010a); Maine Prairie, Cook Inlet offshore, Uinta, and San Juan, this review article.

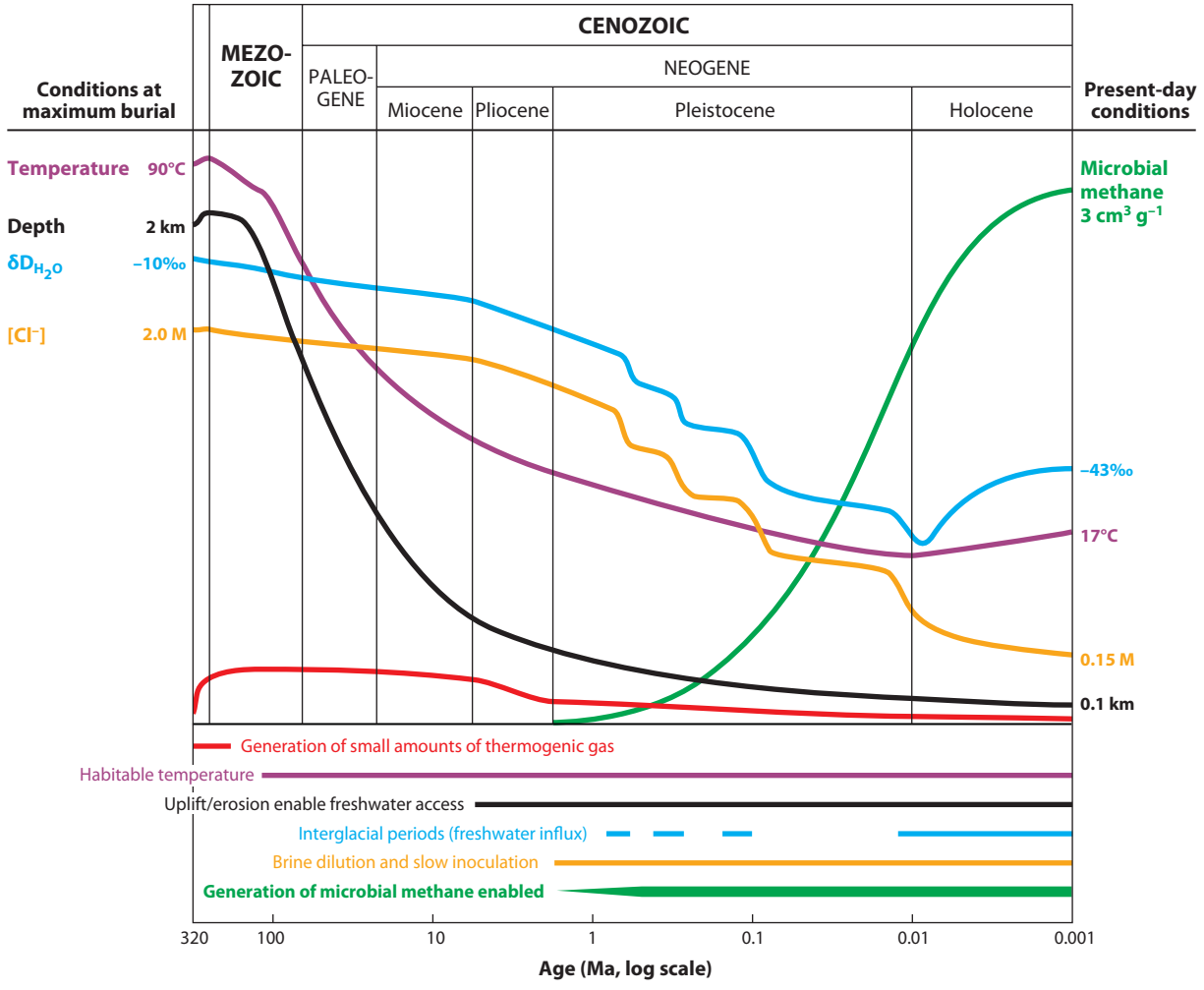


Figure 4

Conceptual diagram depicting uplift and freshwater influx diluting basinal brine and inoculating coals with microbes, yielding recent microbial methane generation. The example shown is from the eastern Illinois Basin. After erosional uplift exposed coals to shallow depths (of less than 300 m) ~5 Ma, slow influx of freshwater was initiated and was followed by a sequence of intense floods by inter- and postglacial melt waters characterized by low δD values. Influx waters not only introduced the microbes to coals but also created habitable conditions for mesophiles by diluting the original Pennsylvanian basinal brines (which originally had a 2 M chloride concentration).

generally resemble Late Pennsylvanian paleofloras, but notable differences include the increased contribution of coniferous plants and the expansion of the distinctive genus of conifers, *Glossopteris* (Traverse 2008, Taylor et al. 2009). The Early Mesozoic (Triassic and Jurassic) peat-forming communities of North America were dominated by cycads and cycadeoids, and therefore this period is referred to as the age of the cycads. Triassic and Jurassic coals are found on several other continents as well, notably in countries such as Australia and China. Late Cretaceous and Early Tertiary coal floras represent a fundamental change in mire composition; flowering plants (angiosperms) appear

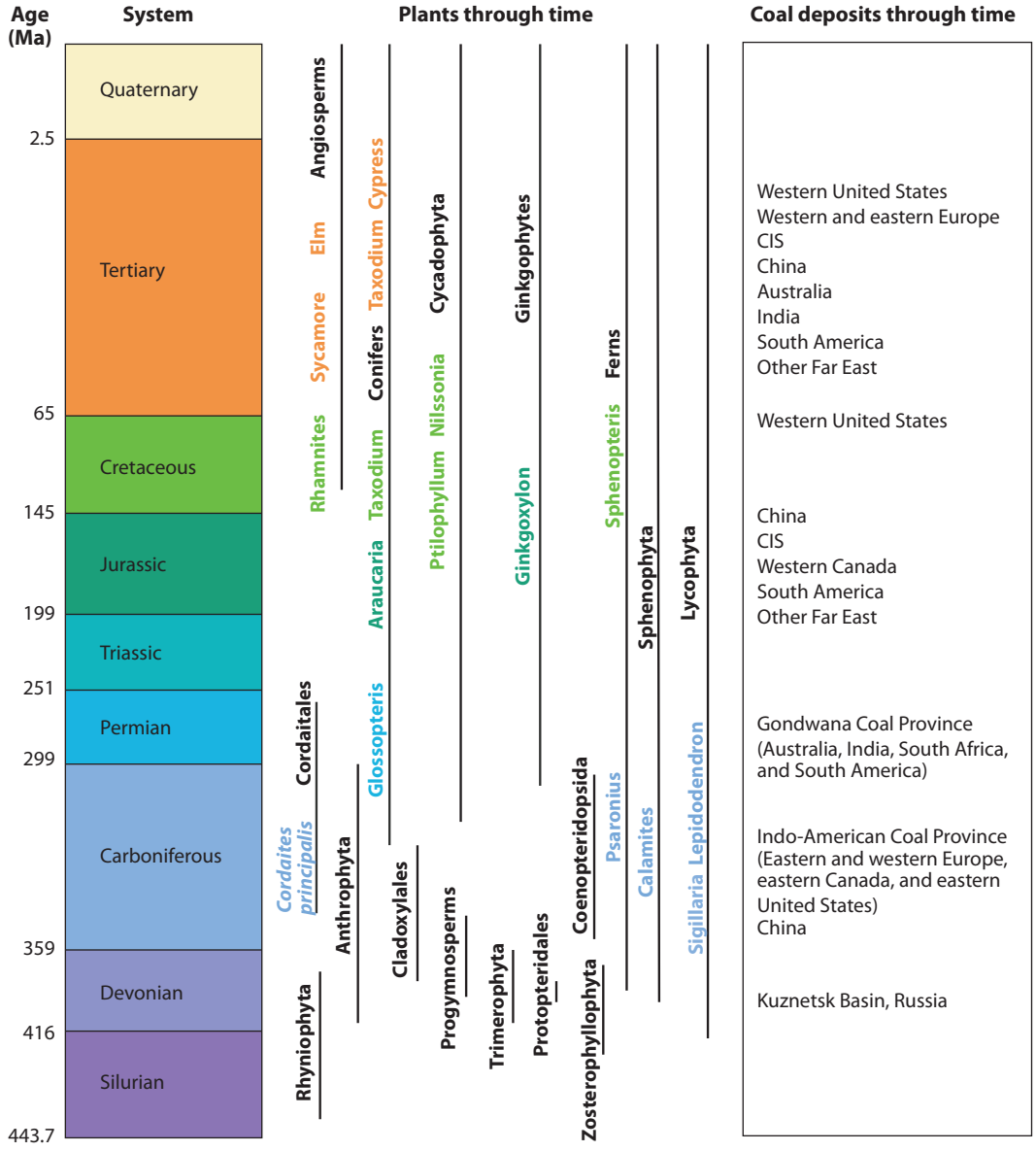


Figure 5

Plant distribution and coal deposits through time. Plant groups are given in black, whereas major representatives of plants that contributed to coal formation of a given time period are shown in the same color as the corresponding time period. Compiled on the basis of Traverse (2008), Taylor et al. (2009), and references therein. CIS, Commonwealth of Independent States.

for the first time in the geological record (Crane 1987). This change resulted in an emergence of modern flora. Mesozoic coals in North America are restricted essentially to the Atlantic coastal plain and are associated with foreland basins that were formed during the opening of the present-day Atlantic Ocean (Leckie et al. 2004). Tertiary coals show a dominant contribution from conifers and angiosperms (e.g., Bechtel et al. 2002, Drobniak & Mastalerz 2006). Cross & Phillips (1990)

provide an excellent review of coal-forming plants from the Devonian to the Paleogene in North America.

Macerals: microscopically recognizable organic entities in coal, analogous to minerals in rocks. Classification of macerals depends on what part of the plants or other organisms are detected and what thermochemical transformations have occurred

Recent peat-forming floras reflect a continuation and evolution of Late Cretaceous/Early Tertiary mire floras. In North America, mire plant communities continue to diversify and specialize. For example, the Florida Everglades and Okeefenokee swamps are dominated by cypress, grasses, various hardwoods, and a saline-tolerant flora (Cohen & Spackman 1977), whereas in Minnesota bogs and fens, in addition to sphagnum, spruce, tamarack, willow, and birch, are principal contributors. Moss, sedges, and grasses dominate the groundcover (e.g., Siegel et al. 2001). In contrast, equator-raised (ombrogenous) mires (in Indonesia and elsewhere) are dominated by large angiosperm trees, whereas planar (topogenous) peats consist of brackish-water mangroves and nipa palms and freshwater, herbaceous, aquatic plants (Anderson 1964).

Apart from evolutionary changes in flora throughout geological history, the character of peat-forming communities can vary significantly within each time period, depending on the combination of many factors, primarily climate, tectonics, and paleotopography. These factors influence the geometry of mire, hydrology, water source, and the availability of nutrients and consequently the character of the peat and, to a large extent, the resultant coal (Cecil et al. 1985, Esterle et al. 1989, Moore 1989, Calder 1993, Eble & Grady 1993). Ombrotrophic, rain-fed, domed mires are considered to be the principal precursors of good-quality, large, low-sulfur, low-ash coal seams (Cecil et al. 1985, Neuzil et al. 1993).

3.2. Coal Macerals

The original plant composition and contribution of different plant parts result in the different coal composition expressed by coal macerals. Macerals are microscopically recognizable organic entities in coal. They are distinguished microscopically by the color, shape, morphology, and degree of preservation of the cell structure, reflectance level, and intensity of fluorescence. Macerals were introduced by analogy to minerals in rocks (Stopes 1935). There is, however, a major difference between the concept of minerals and that of macerals. In contrast to minerals that have a homogeneous composition and a fixed internal structure, macerals consist of a mixture of organic compounds that undergo both physical and chemical changes during coalification. Macerals are classified into three groups: the vitrinite (huminite in low-rank coals) group, the inertinite group, and the liptinite group. Each maceral group is subdivided into maceral subgroups and macerals (for a detailed classification and examples of photomicrographs of coal macerals, see **http://igs.indiana.edu/coal/atlas/index.cfm**). Taylor et al. (1998) provide details of the origin and characteristics of coal macerals.

The vitrinite group represents woody plant material (e.g., stems, trunks, roots, and branches) that is derived from the lignin and cellulose of plant tissues (Taylor et al. 1998). Subdivision into macerals within this group depends on the degree of preservation of cell structure. The liptinite group includes components that are chemically more resistant to physical and chemical degradation than are macerals of the vitrinite group, and it includes, for example, pollen, spores, cuticles, waxes, and resins. Liptinite macerals are enriched in hydrogen, owing to a greater amount of aliphatic components compared with the other two maceral groups. The inertinite maceral group originates from the same material as do the vitrinite group and the liptinite group but has a higher degree of aromatization and condensation. Inertinite macerals have a greater carbon content than vitrinite and liptinite group macerals at the same rank because they were carbonized, oxidized, or subjected to chemical or bacterial attacks prior to coalification, usually in the peat stage (Taylor et al. 1998, Scott & Glasspool 2007).

As a consequence of originating from different plants and different plant parts, maceral groups have different chemical properties, as reflected by their elemental and molecular composition. The range of differences changes with coalification level (coal rank). For example, at high volatile bituminous rank, significant differences between maceral groups exist in elemental composition, and in carbon and oxygen content in particular, exemplified in **Figure 6** for the Lower Block Coal from Indiana. For this coal, vitrinite presents the lowest carbon content (78.6%), followed by sporinite (84.7%) and fusinite (90.7%). Oxygen follows the opposite trend. Elemental changes are accompanied by changes in functional group abundances. The aliphatic nature of sporinite is documented by the prominent peaks in the aliphatic stretching region of 2,800–3,000 cm^{-1} that dominate the spectrum, in contrast to their much smaller absorbance in vitrinite and even less significant absorbance in fusinite as a result of the more aromatic character of this maceral. These chemical differences between coal macerals, so distinct at high volatile C bituminous and lower ranks, progressively decrease at higher ranks as coal structure becomes more aromatic. As demonstrated by electron microprobe studies, vitrinite and sporinite properties converge at the maturation level corresponding to a reflectance of 1.25% and 88.5% carbon, and semifusinite joins the coalification path of vitrinite and sporinite at 89.5% carbon, which corresponds to R_o values of 1.8–2.0% (Mastalerz & Bustin 1993).

3.3. Evolution of Coal Chemistry with Rank

The process of coalification encompasses physical and chemical changes that begin shortly after deposition and burial and that continue during thermal maturation. **Figure 7** shows the progression in optical characteristics and elemental composition of coal samples selected to represent selected coal ranks from lignite to anthracite. Petrographically, liptinite macerals are present up to high volatile A bituminous rank and are absent from medium volatile bituminous coal of reflectance 1.28% or higher. At the anthracite rank, coal is uniform, and distinguishing vitrinite from inertinite is impossible. With regard to elemental chemistry, an increase in carbon content and a decrease in oxygen content are the most prominent characteristics (**Figures 7** and **8**). The loss of oxygen is particularly pronounced at the subbituminous C/B rank. However, individual oxygen-containing functional groups have their unique maturation paths. The most typical path is an early increase of abundance in the early stages of coalification followed by diminishing to disappearance (Drobniak & Mastalerz 2006, Petersen et al. 2008). A hydrogen content of ~5.0% persists through the ranks, including medium volatile bituminous, and decreases in low volatile bituminous coal and anthracite. Nitrogen content in all samples is less than 2% and does not show a consistent relationship with rank (**Figure 7**).

On a molecular level, the first processes of wood transformation are cellulose elimination and lignin transformation (Hatcher et al. 1981, Russell & Barron 1984, Drobniak & Mastalerz 2006). Lignin transformation begins with the cleavage of aryl ether bonds, including hydroxyl and methoxyl groups, and the cleavage of β-O-4 aryl ethers (Hatcher & Clifford 1997). These reactions produce a phenolic OH, resulting in the formation of catechol-like structures. This, in turn, leads to the alkylation of aromatic rings (Botto 1987). Another important reaction in the early stages of coalification is the cleavage of aryl-O bonds in lignin, specifically methoxyl groups attached to the aromatic rings through a demethylation process (Hatcher et al. 1988, Stout et al. 1988). Basically, the structural composition of subbituminous coal is that of the lignin precursor that lost its methoxyl groups through demethylation and dehydroxylation and lost its side-chain hydroxyls.

Further evolution from subbituminous coal to bituminous coal involves the transformation of the catechol-like structure to phenols (Hatcher & Clifford 1997) that undergo condensation to aryl

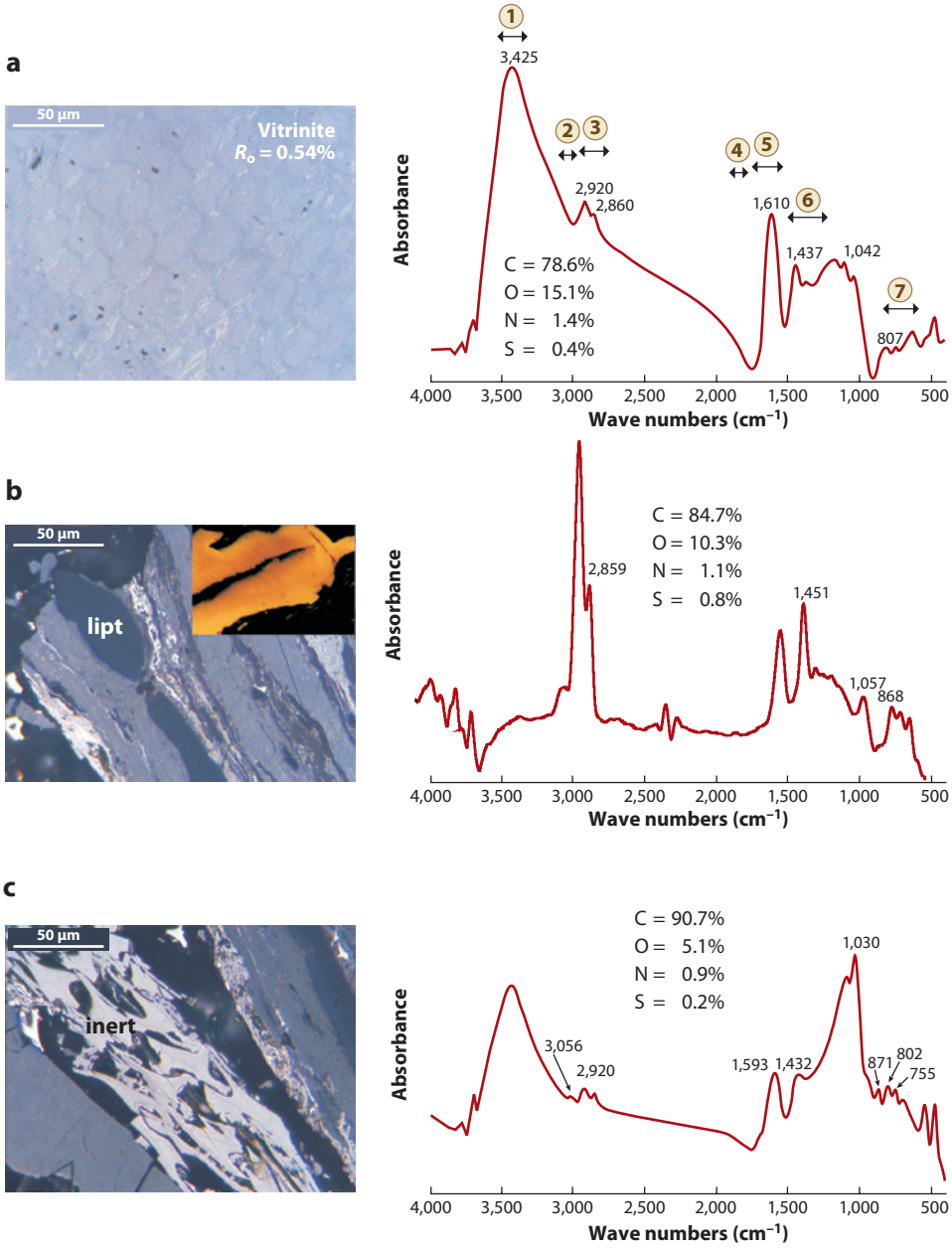


Figure 6

Photomicrographs of high volatile carbon bituminous coal from Indiana [with a vitrinite reflectance (R_o) of 0.54%] using reflected light, oil immersion. (*a*) Vitrinite with a well-preserved cell structure. (*b*) Liptinite (lipt) represented by sporinite. The inset shows a large megaspore in fluorescent light from the same coal. (*c*) Inertinite (inert) represented by fusinite. Next to the photomicrographs are Fourier transform infrared spectra of the vitrinite, sporinite, and fusinite, respectively. Functional group regions: (*1*) hydroxyl, (*2*) aromatic stretching, (*3*) aliphatic stretching, (*4*) oxygenated groups, (*5*) aromatic carbon, (*6*) aliphatic bending, and (*7*) aromatic out of plane. The elemental compositions of these macerals, obtained with an electron microprobe, are given with the spectra.

ethers or dibenzofuran-like structures. Aromatic ring condensation is indicated by an increased abundance of naphthalenes and fluorenes. Possible ring closure and aromatization of the alkyl side chains result in increased aromaticity. With progressive coalification, phenolic structures are lost in favor of benzene-like structures. These pyrolytically produced benzene-like structures, with their associated aliphatic side-chain carbons, may condense and form the polycyclic aromatic ring systems present in higher-rank coals (e.g., Fakoussa & Hofrichter 1999).

Semianthracite and anthracite are characterized by highly aromatic structures. At the onset of the semianthracite rank, a sudden molecular orientation takes place, and small aromatic stacks aggregate into clusters (Oberlin et al. 1980, Rouzand et al. 1991). Interlayer spacing gradually decreases to the anthracitic rank and further to graphite (Wilks et al. 1993).

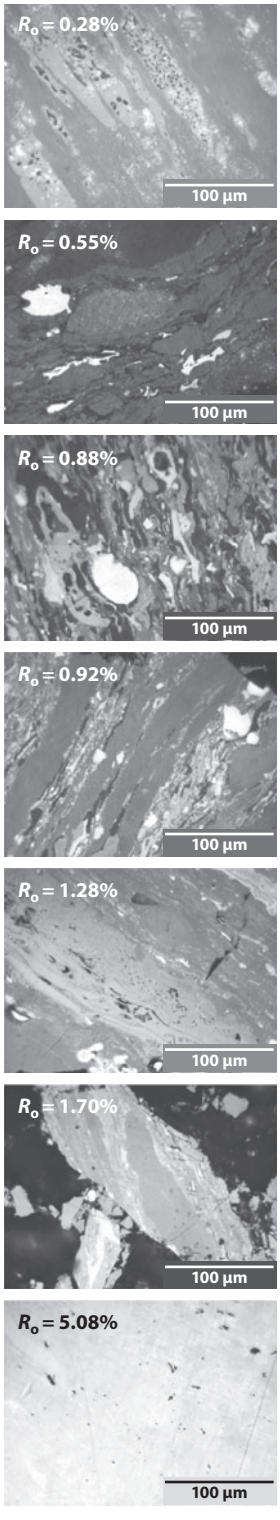
On the basis of chemical and structural data, the generation of chemical models of coal has been attempted for more than 60 years, beginning with Fuchs & Sandhoff (1942). Although the models differ, reflecting the specifics of the chemical data used, the coal is viewed simply as a macromolecule consisting of condensed aromatic rings connected by ether, alkoxy, and sulfur bridges and having hydroxyl groups, methoxyl groups, and carboxyl groups attached to the aromatic nuclei. Reviews of molecular modeling of coal structure can be found in van Krevelen (1993) and in Vandenbroucke & Largeau (2007).

From a microbial CBM perspective, increasing coal maturity increases the level of recalcitrance to biomethanation. For example, Jones et al. (2008) observed microbial methane generation from subbituminous coals (in Texas, Alaska, and the Powder River Basin), but not from higher-maturity coals from Pennsylvania. In our study, we observe a direct negative correlation between coal maturity and maximum methanogenic rates in the laboratory incubations of microbial consortia with coals over a range of R_o values (**Figure 9**). There appears to be a maximum methanogenesis rate that can be obtained from a coal at a given maturity. A similar pattern of increased methanogenesis rates with lower-maturity substrates was observed when artificially matured plant material, forming so-called biocoal, was subjected to biodegradation by a microbial community derived from a subsurface coal deposit (**Figure 10**). Again, an increased level of recalcitrance [higher Btu (British thermal unit)] of the substrate dictated a logarithmic decrease in methanogenesis rate. In addition to coal maturity, maceral composition of coal may potentially influence the biodegradation rate. For example, macerals richer in heteroatoms (i.e., O, S, N) can be expected to be more prone to microbial degradation (see Section 4, below).

4. STEPWISE MICROBIAL DEGRADATION OF COAL TO METHANE

4.1. Methods for Detecting and Identifying Metabolites

Elucidating the biological pathways of coal-derived methane requires the detection and identification of metabolic intermediates. Field metabolomics has been used to document the in situ degradation of different classes of compounds. Metabolite profiling provides information that not only is compound specific but also may be indicative of described biological pathways (Gieg & Suflita 2005). Groundwater samples are collected, immediately acidified, and stored at 4°C until they are solvent extracted and analyzed by gas chromatography–mass spectrometry. Samples are often derivatized using compounds such as BSTFA [(*N*,*O*-bis(trimethylsilyl)trifluoroacetamide] or trimethylchlorosilane to enhance the volatility of the compounds during gas chromatography. Alternatively, liquid chromatography–electrospray ionization tandem mass spectrometry is employed due to its high sensitivity and specificity. For both methods, putative metabolites are identified using authentic standards and/or previously published mass spectra. Several field investigations have used these methods to detect in situ metabolites indicative of microbial degradation



of BTEX (benzene, toluene, ethylbenzene, and xylene) compounds (Beller 2000, Elshahed et al. 2001, Gieg & Suflita 2002, McKelvie et al. 2005, Beller et al. 2008, Parisi et al. 2009, Gieg et al. 2010); polycyclic aromatic hydrocarbons (PAHs) (Gieg & Suflita 2002, Ohlenbusch et al. 2002, Phelps et al. 2002, Griebler et al. 2004, Safinowski et al. 2006, Parisi et al. 2009, Gieg et al. 2010); and alicyclic, alkane, and alkene hydrocarbons (Gieg & Suflita 2002, Rios-Hernandez et al. 2003, Parisi et al. 2009, Gieg et al. 2010).

4.2. Putative Metabolic Pathways Involved in Biodegradation of Coal to Methane

Coal chemical and structural characteristics depend on the rank of coal (see Sections 3.2 and 3.3), and the organic fraction of coal consists of a complex mixture of aromatic and aliphatic hydrocarbons as well as nitrogen-, sulfur-, and oxygen-containing heterocyclic compounds (NSOs) (e.g., Kabe et al. 2004; see also **Figure 11** for generic coal structure). Due to the hydrophobicity, heterogeneity, and recalcitrance of partially aromatic and lignin-derived macromolecules, the degradation of coal under anoxic conditions requires a community of microorganisms with a range of metabolic strategies. Under oxic conditions, the initial step of coal biodegradation is likely catalyzed by biosolubilization and extracellular enzymatic depolymerization (for reviews, see Catcheside & Ralph 1999 and Fakoussa & Hofrichter 1999). To date, extracellular processes of anaerobic microbial communities are not well-known. However, under methanogenic conditions, the paradigm for microbial conversion of complex organic matter to methane involves the primary fermentation of polymers and monomers to fatty acids, organic acids (e.g., lactate, succinate, acetate), alcohols (e.g., methanol), and hydrogen and carbon dioxide. Degradation subsequently follows via secondary fermenting bacteria (syntrophs); homoacetogenic bacteria; and acetoclastic, methylotrophic, and hydrogenotrophic methanogens (for review, see Schink 2006). The same investigators have hypothesized that this metabolic model is applicable to the bioconversion of coal to methane as well. In addition to the above fermentation products, coal matrices are also a source of other substrates such as methylamines, methylsulfides, ethanol, and carbon monoxide. The requisite methanogenic pathways can differ among basins, fields, and wells and can depend on the physicochemical properties of the microenvironment (Strąpoć et al. 2010a).

Although the mechanisms of coal activation under anoxic conditions are not well understood, several studies have investigated the modes by which anaerobes activate aromatic and aliphatic hydrocarbons, which are coal components (**Figure 11**). These pathways include the addition to fumarate, hydroxylation, C1 addition/carboxylation, and methylation (Abu Laban et al. 2009, 2010; Boll & Heider 2010; Tierney & Young 2010; Widdel & Grundmann 2010; Widdel & Musat 2010). Investigation of these pathways has also led to the discovery of the enzymes that activate hydrocarbons under anoxic conditions. Examples of such enzymes include benzylsuccinate synthase (Leuthner et al. 1998, Widdel & Musat 2010), naphthyl-2-methyl-succinate synthase (Annweiler et al. 2000, Selesi et al. 2010), alkylsuccinate synthase (Callaghan et al. 2008, Grundmann et al. 2008), ethylbenzene dehydrogenase (Johnson et al. 2001, Kniemeyer & Heider 2001), and anaerobic benzene carboxylase (Abu Laban et al. 2010). Some of these pathways are not well understood and have been subject to ongoing debate. Progress in understanding the genetics and

Figure 7

Dominant coal transformation reactions with increasing maturity, elemental changes, and photomicrographs of different rank coals. R_o denotes vitrinite reflectance. Abbreviations: vol., volatile; bit., bituminous. Coal transformation reactions are compiled from Hatcher & Clifford (1997), Drobniak & Mastalerz (2006), and Cao et al. (2011).

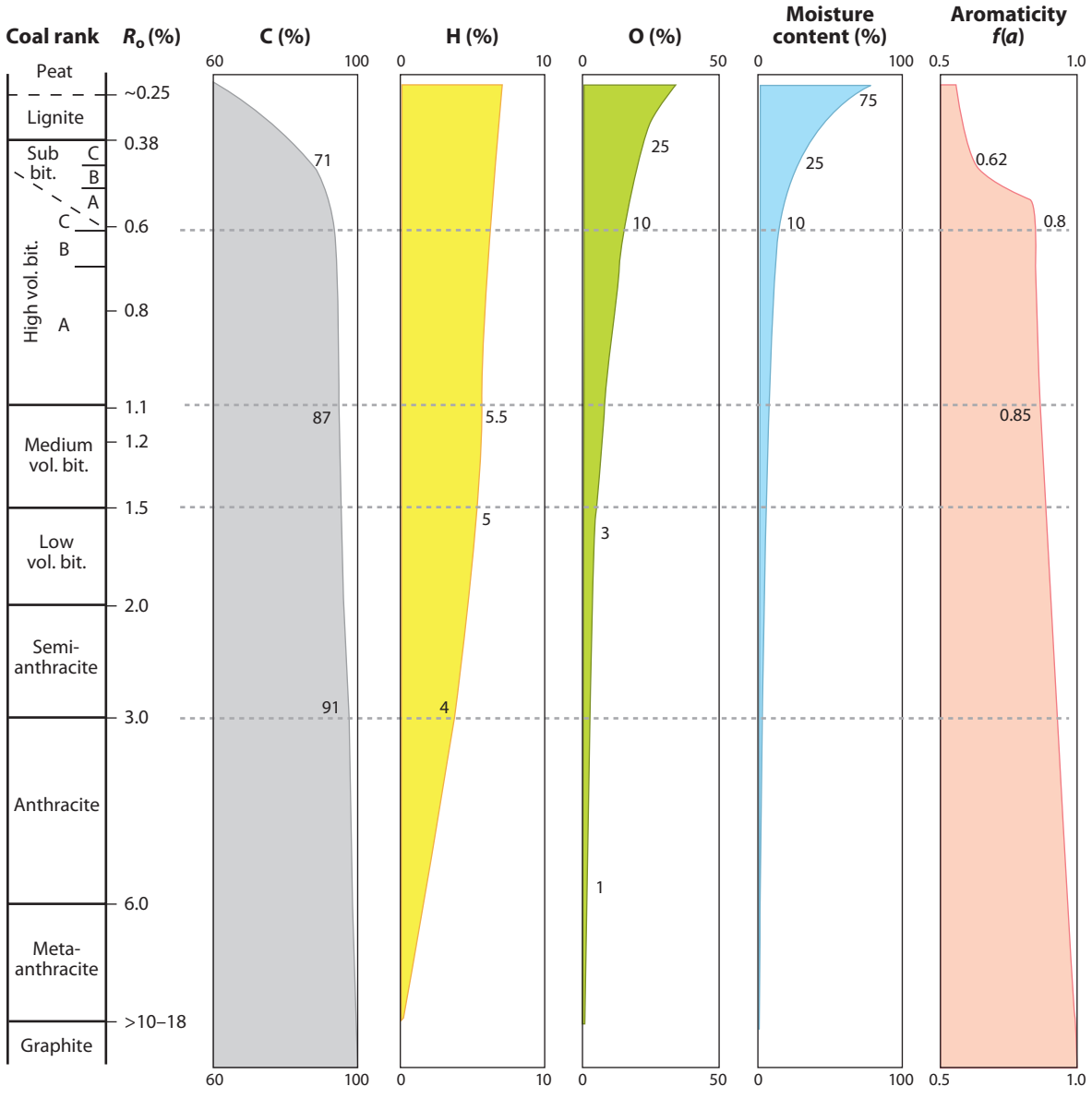


Figure 8

Changes in selected chemical properties of coal throughout coalification (coal ranks). R_o denotes vitrinite reflectance. Abbreviations: vol., volatile; bit., bituminous. Modified from Stach et al. (1982).

biochemistry of anaerobic degradation of coal monomers such as alkanes, alkylbenzenes, toluene, and PAHs (e.g., naphthalenes and phenanthrenes) may facilitate future investigations targeting the processes by which microbes attack the aromatic core- and heteroatom-containing fragments of coal. A priori knowledge of metabolic pathways facilitates metabolite profiling and chemical analysis of field samples and can provide insight into the processes of coal bioconversion. Although most studies to date have focused on coal-bed inorganic geochemistry (e.g., gas and redox

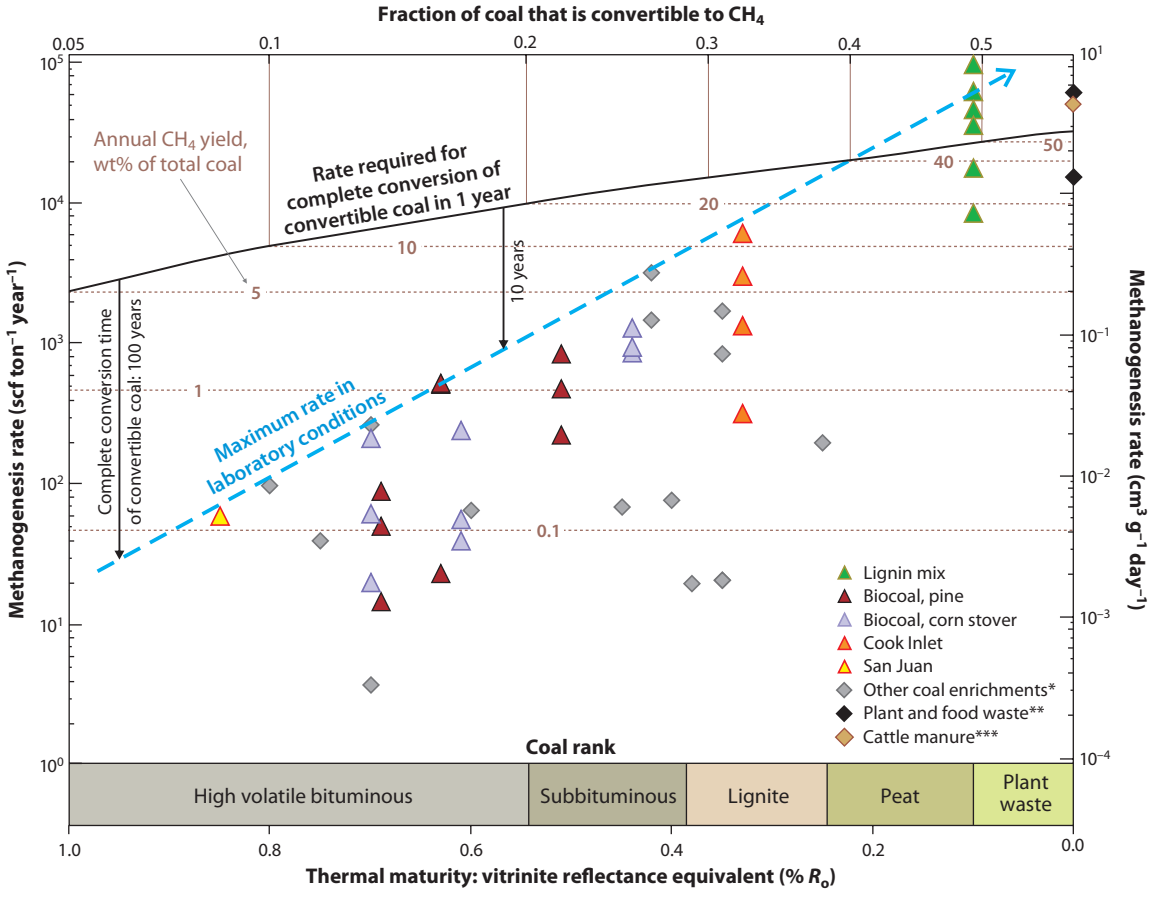


Figure 9

Rates of methanogenesis from coals of varying maturities. In general, the higher the maturity is, the lower the rate of conversion to methane can be expected. All data plot under the hypothetical maximum-rate blue line. The fraction of convertible coal decreases with increasing maturity. The black solid line represents the rate required to turn the entire convertible fraction of coal to methane in one year, and the black arrows show how many years are required to do so at a particular maturity, given laboratory conditions with the provided nutrients, vitamins, and trace metals. Triangles denote data from this study. Diamonds denote data from the following studies: single asterisk, Volkwein (1995), Shumkov et al. (1999), Menger et al. (2000), Green et al. (2008), Harris et al. (2008), Jones et al. (2008), and Krüger et al. (2008); double asterisk, Akunna et al. (2007); triple asterisk, Budiyono et al. (2010). Abbreviation: scf, standard cubic feet.

chemistry, pH, alkalinity, and trace elements), a few recent studies have aimed to characterize the organic composition of produced waters from coal beds.

4.2.1. Aliphatics. Several studies have shown the presence of longer-chain alkanes, isoprenoids, and compounds containing long aliphatic chains in coal formation waters or within the extractable organic matter (EOM) of coal. Coal extracts can yield a wide range of saturated hydrocarbons (Formolo et al. 2008). Orem et al. (2010) observed significant concentrations of C_{22}-C_{36} *n*-alkanes and hexadecanoic acid, which were released during incubations of the Wilcox Coal from Texas. Monomethylalkanes and alkylcyclohexanes (**Figure 12**), which are typical of microbial mats and coals (Dong et al. 1993, Kenig 2000, Orem et al. 2007), have also been detected in some produced fluids from CBM fields (Orem et al. 2007). Extracts also contained biomarkers such as hopanes

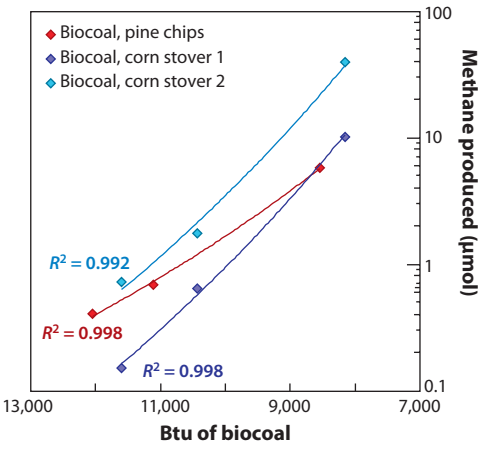


Figure 10

Methane production rate from biocoal (artificially matured pine chips and corn stover) using a coal-derived microbial community. Note the good correlation between the logarithmic increase of rate and the decreasing biocoal Btus (British thermal units). Trend lines are power type.

and terpanoids (**Figure 12**; Orem et al. 2007, Formolo et al. 2008). Biodegradation of aliphatic and cyclic hydrocarbons can be a source of metabolites such as fatty acids in coal waters (Orem et al. 2007, Wawrik et al. 2010). Typical products of *n*-alkane biodegradation via addition to fumarate are methylalkylsuccinates, which have been observed in the San Juan Basin (Wawrik et al. 2010). These intermediates can be further oxidized to methanogenic substrates. Studies have demonstrated the presence of low-molecular-weight, water-soluble organics from low-maturity coals (of less than 0.6% R_o) (e.g., acetate, formate, and oxalate) (Vieth et al. 2008, Glombitza et al. 2009). In these studies, the dominant extractable compounds and their concentrations were dependent on coal maturity and the organofacies of the source material. The detection of these compounds in coal samples suggests that a small fraction of shallow, low-maturity coal may serve as a potential feedstock source for microorganisms (Vieth et al. 2008, Glombitza et al. 2009). Conversely, other studies have indicated the potential toxicity or the ability to inhibit methanogenesis of some intermediates, such as fatty acids (e.g., Jones et al. 2010, this study). The inhibition of methanogenesis may be associated with the lowering of pH via the accumulation of organic acid intermediates.

4.2.2. Aromatics. Aromatic compounds are also commonly found in coal formation waters, coal EOM, and methanogenic coal incubations. Several studies have resulted in the detection of PAHs and their functional derivatives (e.g., phthalic acid), benzene derivatives (e.g., benzoic acid), phenols, biphenyls, and aromatic amines (Orem et al. 2007, Ulrich & Bower 2008, Orem et al. 2010). A more recent study combined enrichment studies and molecular methods (see Section 5) with metabolite profiling to analyze formation waters collected from high-maturity coal (~1% R_o) from the San Juan Basin. The most prevalent metabolites were those consistent with microbial hydroxylation or carboxylation of the aromatic core of coal (e.g., phenol and benzoate). Although not as prevalent, metabolites indicative of the addition of alkanes and alkenes to fumarate were also detected, as mentioned above (Wawrik et al. 2010). Similarly, studies of extracts from low-maturity (Fort Union from Powder River Basin) and higher-maturity (Fruitland from San Juan Basin) coals by Formolo et al. (2008) have suggested that the preferential specialization in biodegradation of aromatics over aliphatics may be universal in subsurface coal ecosystems.

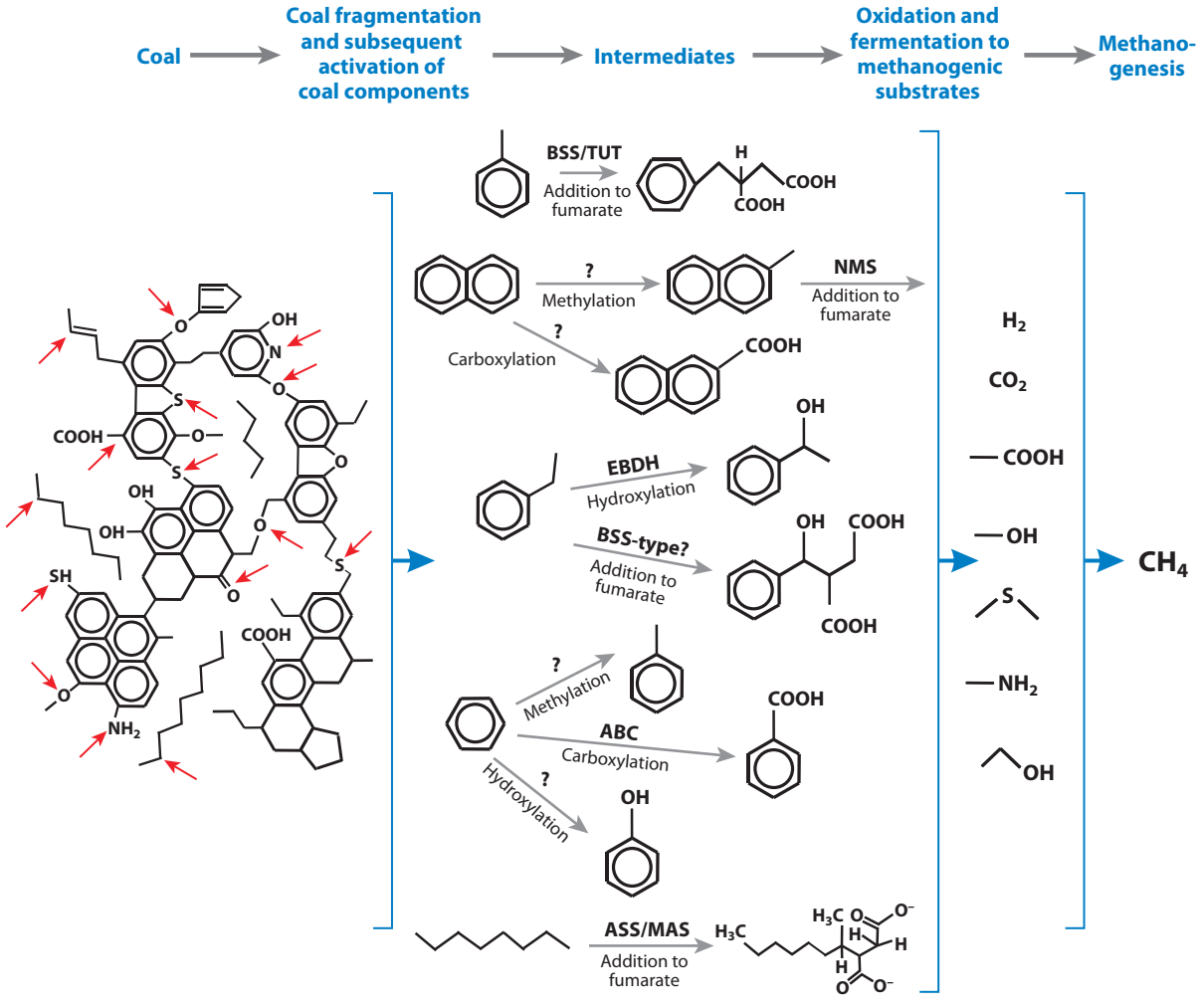


Figure 11

Schematic of microbial decomposition of coal to a variety of hydrocarbon intermediates and methanogenic substrates. Red arrows designate potential activation and cleavage sites. Anaerobic activation mechanisms for some aliphatic and aromatic hydrocarbons are shown. Enzyme abbreviations: ABC, anaerobic benzene carboxylase; ASS, alkylsuccinate synthase; BSS, benzylsuccinate synthase; EBDH, ethylbenzene dehydrogenase; MAS, (1-methylalkyl)succinate synthase; NMS, naphthyl-2-methyl-succinate synthase; TUT, toluene-utilizing enzyme (analogous to BSS). Question marks indicate that the requisite enzyme is unknown.

However, biomethanation of mature and more aromatic cluster–dominated coals is likely to be slower than that of less mature coals with open chemical structures similar to those of lignin protoplasts.

4.2.3. Heteroatoms. In addition to strictly aromatic and aliphatic moieties, lignin-derived coal macromolecular structures provide a multitude of heteroatom bonds and linkages as potential target activation sites for biodegradation. The potential importance of heteroatom stimulation of coal depolymerization was highlighted by the detection of aromatic NSOs in produced water samples from CBM wells in the Powder River Basin (Orem et al. 2007). The NSO-containing coal

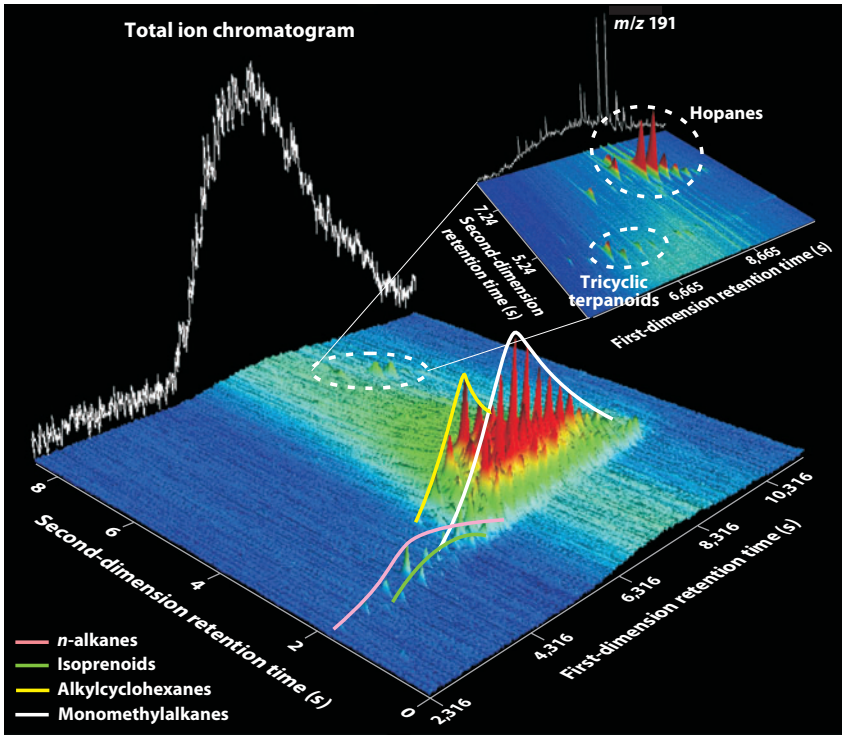


Figure 12

Two-dimensional gas chromatography–mass spectrometry (GC-GC/MS) analysis of a urea-adducted dichloromethane (DCM) extract of 100 liters of coal formation water from the eastern Illinois Basin. The detected compounds include a homologous series of monomethylalkanes and alkylcyclohexanes. A small amount of *n*-alkanes and isoprenoids can be observed. The inset shows hopanes and tricyclic terpanoids using representative fragment ion with mass-to-charge ratio (*m*/*z*) 191.

moieties may be initially targeted at specific sites, including functional groups (hydroxy, methoxy, carboxylic, and methyl groups) and linkages between cyclic hydrocarbons or aromatic clusters (e.g., ether linkages and intracyclic heteroatoms). As an example, demethoxylation of aromatics is a viable step in biodegradation as shown by stable isotopic probing (Liu & Suflita 1993). Consequently, heteroatom-rich macerals, from liptinite (the richest) to vitrinite to inertinite (the least rich), are more prone to microbial degradation (**Figure 8**). Functional groups attached to carbon atoms in the kerogen have lower bond dissociation energies than do aliphatic or aromatic C-C bonds. For example, bond enthalpies of functional groups attached to a phenyl are 118 kcal mol^{-1} for other phenyl groups, 103.5 kcal mol^{-1} for methyl groups, 101 kcal mol^{-1} for methoxy groups, 99.3 kcal mol^{-1} for formyl groups, and 98.8 kcal mol^{-1} for acetyl groups (Blanksby & Ellison 2003). Additionally, bonds between carbon and heteroatoms (e.g., ether or thioether bridges) are more reactive than C-C bonds (Sheremata 2008), as indicated by the bond dissociation energies: 365 kJ mol^{-1} for C-C, 344 kJ mol^{-1} for C-O, 342 kJ mol^{-1} for C-N, and 307 kJ mol^{-1} for C-S (Savage 2000). Thermodynamically attractive heteroatom bonds may also serve as activation sites for the biodegradation of coal, similar to what occurs in the thermal decomposition of asphaltenes (Sheremata 2008), which are arguably chemical analogs to lignin.

5. MICROBIAL ASSOCIATIONS INVOLVED IN BIODEGRADATION OF COAL TO METHANE

5.1. Methods of Exploration

Characterization of metabolic processes in a complex microbial community requires an integrative approach that combines physiological and molecular biology experiments with analytical chemistry. At the present time, the key challenges include elucidating microbial coal activation, identifying the requisite microorganisms, and understanding the system's physiological constraints. Methods frequently employed in environmental studies include 16S rRNA phylogenetic surveys; functional gene detection in community DNA, including full-genome shotgun analysis (metagenomics); fluorescence in situ hybridization (FISH)-targeted analysis of communities and syntrophic associations; metabolite profiling to implicate specific biodegradation pathways (metabolomics); MS-MS (tandem mass spectrometry) analysis of expressed proteins (proteomics); mRNA detection to assay for actively transcribed genes (transcriptomics); detection of high-molecular-weight intermediates and metabolites [dissolved organic matter (DOM)] by FTMS (Fourier transform mass spectrometry) (for review of methods of DOM analysis, see Mopper et al. 2007); and NanoSIMS (secondary ion mass spectrometry) isotopic scanning analysis of individual microbial cells or microbial associations (e.g., Behrens et al. 2008). **Figure 13** shows an overview of these commonly used techniques, several of which are described in detail in the next section.

The majority of coal microbiological studies focus on the formation water owing to rare access to fresh, intact coal cores and their limited volume (compared with large volumes of CBM-coproduced wastewater that is readily available), which can cause difficulties in successful cultivation. Hence, there is potential bias toward planktonic microbes, as opposed to sessile microbes, which adhere to coal surfaces. However, CBM-coproduced water typically contains a large quantity of coal fines with potentially satisfying amounts of sessile microbe populations (**Figure 14**). Nonetheless, several recent studies successfully analyzed microbial phylogeny from freshly retrieved solid coal core materials from the Powder River Basin (Klein et al. 2008) and Alberta Basin (Penner et al. 2010).

5.2. Enrichment Studies

Investigating the biological activity associated with methane formation from coal presents considerable challenges. The coal-bed environment imposes strict physiological limitations in the absence of bioavailable electron acceptors, such as oxygen, sulfate, nitrate, or iron. In fact, methanogenesis cannot proceed until all such alternative electron acceptors are exhausted. Enrichment studies must therefore be conducted under strictly anaerobic conditions, taking into account the physiological requirements of methanogenic consortia (for a review on anaerobic cultivation techniques, see Wiegel et al. 2006). As a consequence, only a limited number of enrichment studies seeking to demonstrate the presence of methanogenic archaea and the direct bioconversion of coal to methane have been conducted (Shumkov et al. 1999, Thielemann et al. 2004, Green et al. 2008, Harris et al. 2008, Krüger et al. 2008, Penner et al. 2010, Ünsal et al. 2010, Wawrik et al. 2010). These limited studies do, however, indicate that the requisite methanogenic archaea can be found in coal-bed formation water. For example, live populations of methanogenic archaea were reported for coal-mine water collected in the Ruhr River Basin when incubated in the presence of hydrogen and fatty acids (Thielemann et al. 2004). Similarly, both acetoclastic and hydrogenotrophic methanogens were detected in enrichments using mine timber and hard coal (Krüger et al. 2008).

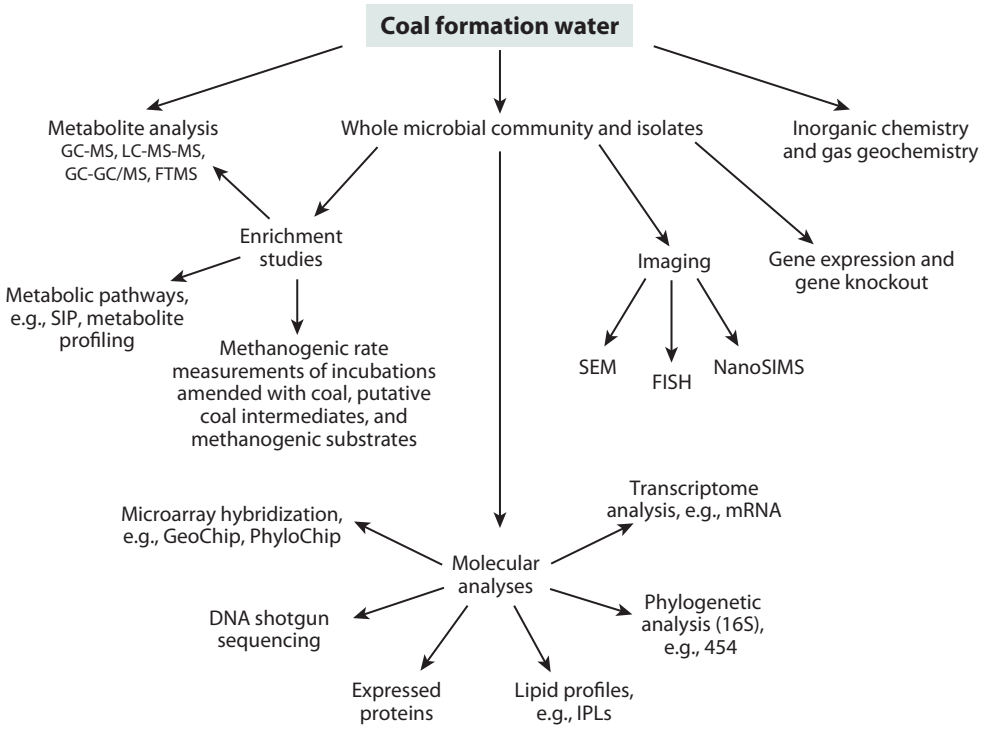


Figure 13

Methods for the analysis of microbial communities and metabolic pathways in coal formations. FISH, fluorescence in situ hybridization; FTMS, Fourier transform mass spectrometry; GC-GC/MS, two-dimensional gas chromatography–mass spectrometry; GC-MS, gas chromatography–mass spectrometry; IPL, intact polar lipid; LC-MS-MS, liquid chromatography–tandem mass spectrometry; MS-MS, tandem mass spectrometry; NanoSIMS, secondary ion mass spectrometry; SEM, scanning electron microscopy; SIP, stable isotope probing.

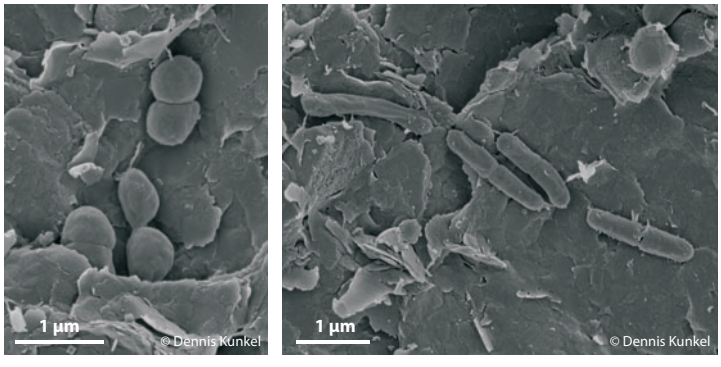


Figure 14

Scanning electron microscopy images of methanogenic incubations with coal (Cook Inlet, Alaska). Microbial cells are closely associated with coal surfaces, pores, and fractures. Copyright © 2010 Dennis Kunkel.

The addition of hydrogen and carbon dioxide to communities obtained from the Powder River Basin led to high rates of methanogenesis in enrichment bottles, whereas the addition of acetate apparently did not stimulate methane production (Harris et al. 2008). The same study reports methane production from low-rank coals collected from Fort Yukon, Alaska, after amendment with inorganic nutrients. Green et al. (2008) report incubations of Wyodak coal with well-bore water from the Fort Union Formation in the Powder River Basin. Temperature- and pH-dependent methane production was observed, and methanogenesis was significantly enhanced by reducing the particle size (increasing the surface area) of amended coals. Greater methane production was similarly reported with increased cation flux and pH in a two-stage coal bioreactor inoculated with a methanogenic consortium isolated from coal (Shumkov et al. 1999). A more recent enrichment study on Powder River coal microbial communities demonstrated that amendments with trace elements, including iron, nickel, cobalt, and molybdenum, enhanced methane production from coal (Ünsal et al. 2010). Furthermore, the maturity of coals also appears to play an important role. As mentioned in Section 3.3, enrichments of coals of varying maturity indicated that methane production rates are greatest in coals of lower maturity (**Figures 9** and **10**).

16S rRNA gene: encodes the prokaryotic 16S rRNA subunit, a component of the ribosome (which functions in protein synthesis); is orthologous to the eukaryotic 18S rRNA gene

5.3. Molecular Analysis of Methanogenic Communities in Coals

Enrichment studies have demonstrated that produced waters from coal beds contain the requisite microbial consortia capable of methanogenesis and that these consortia are able to utilize some coal components (see Section 4.1). However, neither the physiological and biochemical basis of coal methanogenesis nor the identity of relevant microorganisms can be understood from enrichment studies alone. Molecular biology techniques can be combined for a more comprehensive survey of microbial community composition and metabolism (**Figure 13**). Molecular techniques are particularly revealing of potentially important microbial community members because a large majority of microbes in the environment cannot be grown under commonly used culture conditions (Zengler et al. 2002). The most widely accepted approach for determining microbial community profiles is the sequence analysis of partial 16S ribosomal RNA genes obtained by polymerase chain reaction amplification of community DNA, and several phylogenetic studies utilizing this approach on coal-inhabiting microbial communities have recently been reported. One of the first studies conducted on a coal seam near Yubari, Japan (Shimizu et al. 2007), reported a clone library dominated by archaeal sequences most closely related to the hydrogenotrophic and methylotrophic archaeal genera *Methanoculleus* and *Methanolobus*, respectively. The detected bacteria included genera most closely related to *Acetobacterium* and *Syntrophus*, as well as a range of other proteobacterial and firmicute lineages. A similar study on coal beds located in eastern Australia reported α-Proteobacterial lineages and Firmicutes (mainly Clostridiales) (Li et al. 2008). Archaeal 16S rRNA gene sequences were affiliated with the *Sulfophobococcus*, *Archaeoglobus*, and *Thermococcus* lineages, which are not generally thought to include methanogenic members. Conversely, methanogenic archaea belonging to the genus *Methanocorpusculum* were detected in coal-bed water collected from the Illinois Basin (Strąpoć et al. 2008). Bacterial 16S rRNA gene sequences belonged to α-Proteobacteria, Firmicutes, Clostridia, and Spirochaetes. A more in-depth study of coals collected from the Waikato coalfields in New Zealand reported similar patterns, with communities composed mainly of an array of Proteobacteria, Firmicutes, Bacteroidetes, and methanogenic archaea (Fry et al. 2009). An abundance of bacterial (α-, β-, γ-Proteobacteria) and archaeal (*Methanosarcina* and Methanobacteriales) lineages were detected in Alberta Basin formation water, whereas the core material yielded only bacterial 16S rRNA gene sequences (Penner et al. 2010). In a study of the Powder River Basin, some archaea (*Methanobacterium* and *Methanothermococcus*) were successfully detected in the coal core material, but a greater diversity of

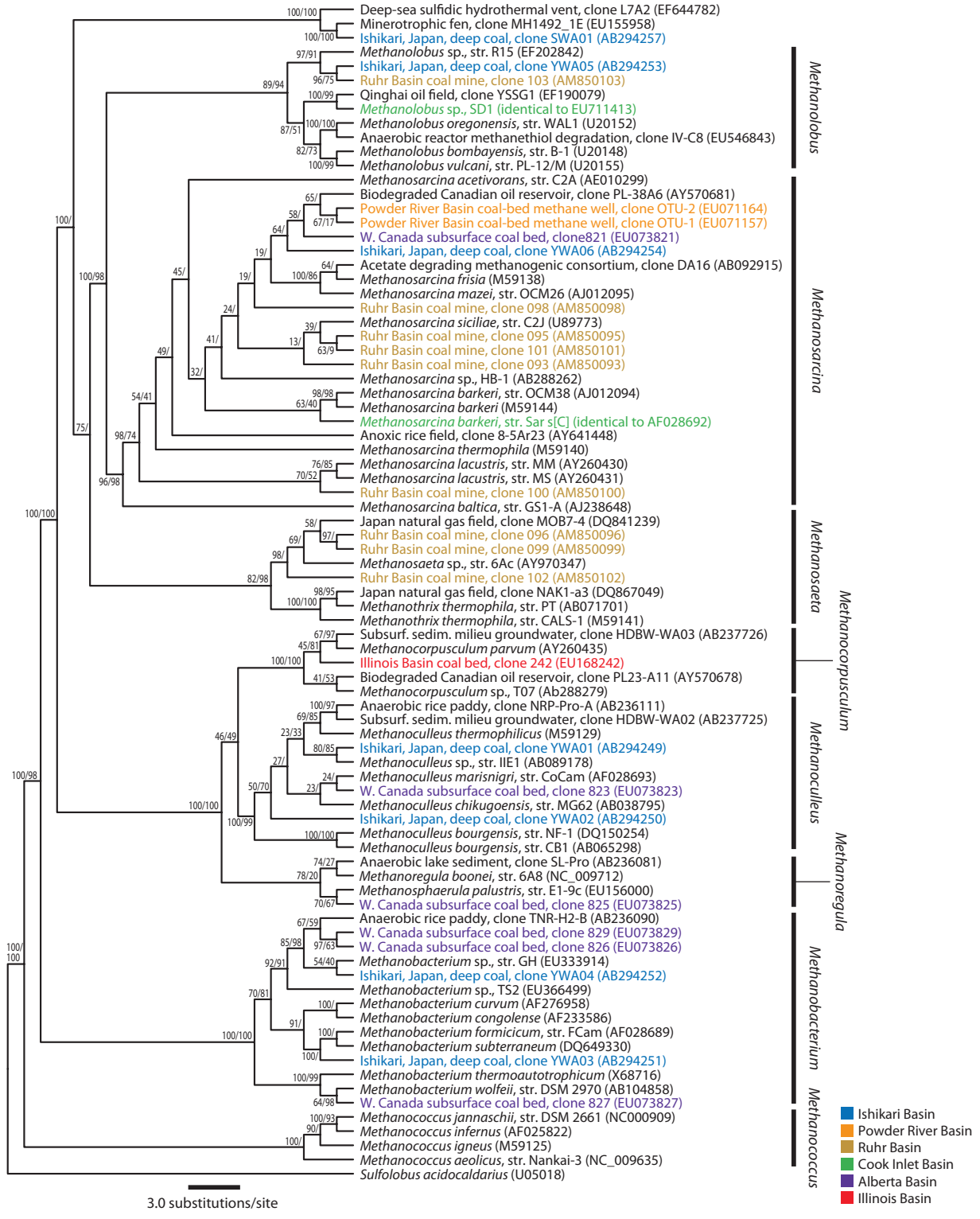
methanogens was found in the formation water samples from the same well (Klein et al. 2008). A compilation of publicly released bacterial and archaeal sequences found in coal is shown in **Figure 15**. Archaeal populations of coal beds include a wide variety of methanogens representing the entire portfolio of methanogenic pathways. The most common lineages include *Methanosarcina*, *Methanolobus*, *Methanobacteria*, *Methanocorpusculum*, *Methanosaeta*, *Methanococci*, *Methanoculleus*, *and Methanoregula*. Dominant bacterial phyla include Firmicutes, Spirochetes, Bacteroidetes, and all subgroups of Proteobacteria.

The biochemical and genetic basis for coal degradation remains unclear from 16S rRNA gene–based studies. Metagenomic approaches are therefore typically employed in microbial ecology studies to target functional genes involved in relevant metabolic pathways. Microarrays, such as the GeoChip (He et al. 2010), allow for rapid and cost-effective profiling of samples. For example, using the GeoChip, researchers demonstrated that genes involved in anaerobic alkane, toluene, and ethylbenzene degradation are present in San Juan Basin formation water (Wawrik et al. 2010). The drawback of microarray approaches is that they can be used only to survey for targets that are highly similar to already known gene sequences. Much of the functional gene diversity of microbes in the environment remains undescribed. More traditional cloning and end-sequencing approaches can be used to obtain genetic profiles from microbes for which no a priori sequence data are available, and a great number of aerobic and anaerobic metabolic genes were detected in San Juan Basin production water in this manner (Toledo et al. 2010). Nevertheless, survey methods that are based on sampling are susceptible to the typically skewed abundance class distribution of microbial communities (**Figure 16*a***). This distribution is the result of the small number of abundant species and the large number of rare species. Sampling-based surveys are therefore burdened by the continued reisolation of abundant sequences at the expense of identifying rare species. This phenomenon puts a premium on the ability to perform DNA sequencing faster and cheaper. Both array and cloning technologies, however, are being rapidly replaced with metagenomic analysis that employs next-generation (NextGen) sequencing technologies. This is facilitated by the entrance of several companies into the marketplace that have developed novel and highly cost-effective sequencing approaches. The current leaders in this area include Roche's 454 pyrosequencing technology [Roche Applied Science (Margulies et al. 2005)], Solexa [Illumina (Bennett 2004)], and Solid [Applied Biosystems (Shendure et al. 2005)]. The 454 platform is currently the most widely adopted method due to its high fidelity and greater read lengths compared with those of other NextGen technologies. This platform has enabled deeper, more comprehensive microbial surveys to be performed (**Figure 16*b***). Panels *c* and *d* of **Figure 16** show examples of reproducibility of 16S rRNA gene surveys generated by 454 analyses of organic matter–associated environmental samples. To provide an integrated view of microbial

Supplemental Material

Figure 15

Phylogenetic trees of 16S rRNA gene sequences of methanogens associated with coal-bed methane from six basins: Ishikari Basin (*blue*), Powder River Basin (*orange*), Ruhr Basin (*brown*), Cook Inlet Basin (*green*), Alberta Basin (*purple*), Illinois Basin (*red*). See **Supplemental Figure 1** for data on non-Proteobacteria bacterial groups; α-, δ-, and ε-Proteobacteria; and γ- and β-Proteobacteria (follow the Supplemental Materials link from the Annual Reviews home page at **http://www.annualreviews.org**). The neighbor-joining dendrogram was generated in PAUP* v.4b10. Bootstrap values from neighbor joining and maximum parsimony (in that order) are shown for each node. Sequences were aligned using the NAST aligner at **http://greengenes.lbl.gov** (DeSantis et al. 2006). NAST-aligned sequences were imported into ARB, a graphical software package with database tools for analyzing DNA sequences (Ludwig et al. 2004). Phylogenetic analyses were performed with distance and maximum-parsimony methods using PAUP* v.4b10 (Swofford 2000). Neighbor-joining trees (neighbor-joining search, jukes-cantor distance, 2,000 bootstrap replicates) were compared against maximum-parsimony consensus trees (heuristic search, 2,000 bootstrap replicates). Abbreviations: str., strain; subsurf. sedim., subsurface sediment.



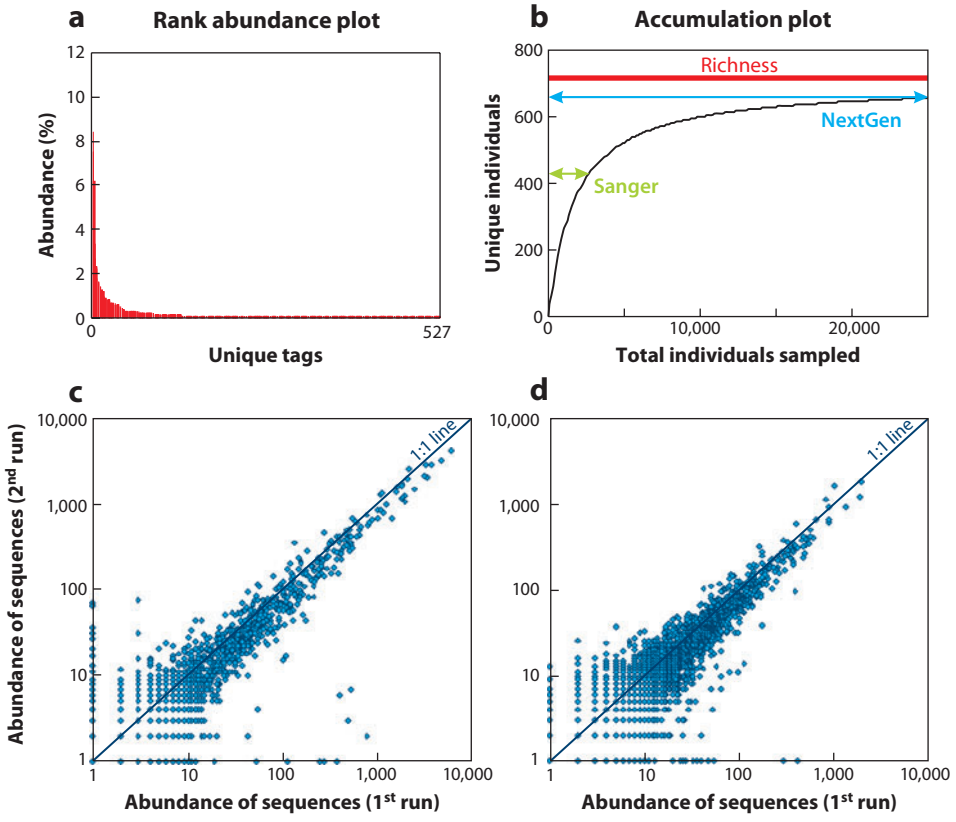


Figure 16

(*a*) Rank abundance plot of a 16S rRNA gene–based survey of a microbial community. This survey comprised ~5,000 ribosomal sequence tags, of which 527 tags were unique and were derived from distinct species. (*b*) Theoretical accumulation plot of a sampling-based survey of a microbial community. The thick red horizontal bar represents the total number (richness) of species present. The horizontal arrows indicate the typical level of coverage achieved with traditional Sanger sequencing versus next-generation (NextGen) technologies. (*c*) Reproducibility of 16S rRNA gene surveys created with the 454 pyrosequencing technology; each point represents a unique 16S rRNA gene plotted as the number of times detected in two replicate sequencing runs. (*d*) The same analysis as in panel *c*, but of a different environmental sample. (*a*,*b*) Large onshore petroleum seep. (*c*,*d*) Shallow subsurface environmental samples.

community metabolism in coal beds, future projects are therefore likely to combine intensive sequencing and bioinformatics efforts with enrichment studies, metabolite profiling, microscopy, and gene expression analysis (mRNA and proteomics).

5.4. Fluorescence In Situ Hybridization

FISH is a culture-independent technique for the identification of bacteria and archaea in mixed communities. Oligonucleotide probes hybridize to specific regions of 16S or 23S rRNA in fixed cells. The labeled cells can then be imaged using epifluorescence microscopy. Advantages of this technique include the visualization of complex assemblages, the identification of the actively growing microbial community, and the ability to design probes with a range of taxonomic specificity from domain to species. A relatively recent modification to this method, magneto-FISH, allows

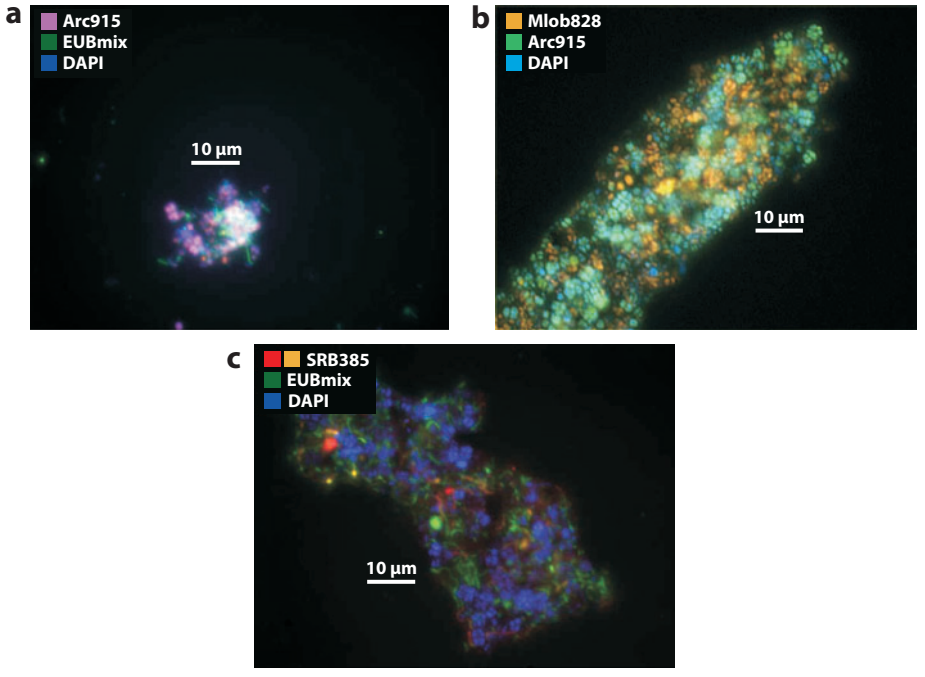


Figure 17

Epifluorescence micrographs depicting major bacterial and archaeal lineages observed in production water samples from the Cook Inlet Basin (Dawson et al. 2010). Probe specificities: EUBmix, all bacteria; Arc915, most archaea; Mlob828, genus *Methanolobus*; SRB385, some Firmicutes and ∂-Proteobacteria; DAPI, general DNA stain. Samples were fixed in 1% (w/v) paraformaldehyde, washed with 1× phosphate-buffered saline (PBS), and stored in a 1:1 PBS/ethanol solution at −20°C. FISH experiments were carried out as described in Hugenholtz et al. (2001). (*a*) Bacterial cells (*green*) associated with archaeal cells (*red*). (*b*) *Methanolobus* cells (*orange*) among other archaeal cells (*green*) and bacterial cells (*blue*). (*c*) Bacterial cells (*green*) including Firmicutes (*orange/red*) intertwined with archaeal cells (*blue*).

for further isolation of target microorganisms and their physically associated partners (Pernthaler et al. 2008).

Several previous studies have identified bacteria and methanogenic archaea associated with the breakdown of coal and the production of methane using both culturing and 16S rRNA cloning (Shimizu et al. 2007, Green et al. 2008, Krüger et al. 2008, Li et al. 2008, Strąpoć et al. 2008, Fry et al. 2009, Dawson et al. 2010, Midgley et al. 2010). These studies provided a first glimpse of the environmental microbes associated with coal beds but did not reflect the relative population sizes of the active bacterial and archaeal populations. FISH can be used to generate a quantitative measure of the population structure of in situ coal-bed communities, as well as to visualize associations between bacterial and archaeal cells such as syntrophic relationships. Information about spatial relationships and community structure derived from FISH provide important clues about the pathways and associated microbial taxa responsible for coal fragmentation, hydrocarbon fermentation, and methanogenesis.

Examples of epifluoresence micrographs depicting close spatial associations of bacteria and archaea from the Cook Inlet Basin are shown in **Figure 17**. Short filamentous and rod-shaped bacterial cells intertwine with *Methanosarcina* and *Methanolobus* cocci, forming large clusters of cells. Based upon hybridization to FISH probes, the rod-shaped cells are a mixture of *Bacteriodales*

and *Acetobacterium*, whereas the filamentous cells are a mixture of Firmicutes and other unidentified lineages. Previous studies of methanogenic environments show a similar co-occurrence of methanogens and acetogens (Kotelnikova & Pedersen 1998, Struchtemeyer et al. 2005) or methanogens and sulfate-reducing bacteria (Moser et al. 2005). Investigation of a deep coal seam (Shimizu et al. 2007) revealed clones of the genera *Methanolobus*, *Acetobacterium*, and *Syntrophus*. Thus, FISH provides a visual and quantitative picture of bacterial-archaeal associations and points to the metabolic potential of anaerobes at a studied site.

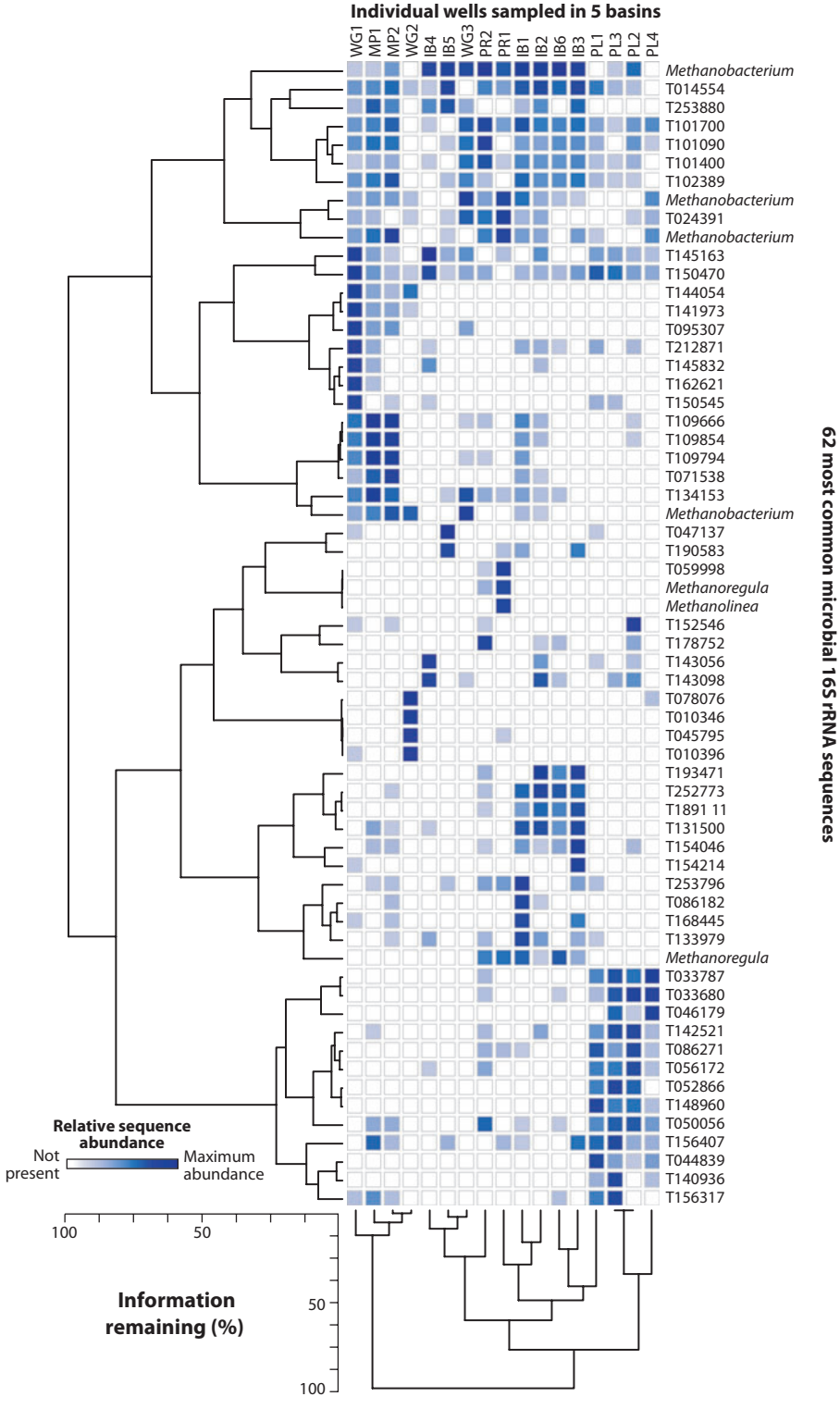
5.5. Geostatistical Methods of Exploring Microbial Assemblages

Microbial assemblages can be also explored by statistical methods. Examples include various types of clustering using microbial population data. **Figure 18** shows a 2D cluster analysis (PC-ORD, MjM software; McCune & Grace 2002) of samples from several coal basins and microbial 454 sequence counts. Particular samples, as well as specific microbial individuals, group into clusters, which may reflect their ecological or metabolic associations. For instance, bacterial sequence groupings in a cluster dominated by one type of methanogen reflect the potential association with hydrocarbon-degrading bacteria, which produce the substrates for methanogens (e.g., carbon dioxide/hydrogen-utilizing *Methanobacterium*).

Ordination is another multivariate statistical method for determining the relationship between microbial communities and geochemistry and for extracting dominant patterns in complex data sets (Legendre & Legendre 1998). Nonmetric multidimensional scaling (NMDS) is a robust, unconstrained, nonparametric ordination method that plots similar objects close together in ordination space (Minchin 1987, Legendre & Legendre 1998). NMDS also provides a good method for overlaying environmental data. We used metaMDS in R (The R Project for Statistical Computing, **http://www.r-project.org**) and the vegan package, based on Kruskal's MDS (Oksanen 2011), using Wisconsin double standardization, where wells were standardized by maxima and then by species by total (**Figure 19**). The calculated stress is 10; stresses less than 10 are considered to yield reliable results (McCune & Grace 2002). The function envfit (vegan package) was used to overlay environmental factors and to determine the relationship between sequence data and geochemistry (temperature, pH, total dissolved solids, sulfate concentration, and gas carbon isotopic parameters, $\delta^{13}C_{CH_4}$ and $\Delta^{13}C_{CO_2-CH_4}$). Ordination of the Polish lignite and Maine Prairie microbial sequence counts form distinct clusters, whereas the Illinois Basin and one of the Powder River samples form a third dominant cluster. The environmental fit analysis shows the associations between geochemical parameters and the NMDS axes. For instance, temperature is the most significant parameter and is strongly correlated with both NMDS axes, with a p value of < 0.001 (based on 1,000 permutations of the environmental fit overlay). The other significant parameters are $\Delta^{13}C_{CO_2-CH_4}$, $\delta^{13}C_{CH_4}$, and TDS. The $\Delta^{13}C_{CO_2-CH_4}$ increase in the main Illinois Basin cluster (with a strong negative correlation with NMDS axis 1) is related to the prevalence of CO_2 reduction methanogenesis, with a typically high carbon isotopic fractionation between CO_2 and CH_4.

Figure 18

2D cluster of example data set from several coal basins: PL, Polish lignites; PR, Powder River; IB, Illinois Basin; MP, Maine Prairie, California; WG, West Grimes, California. Shades of blue depict abundances of individual sequences: The darker the blue, the greater the abundance. Clustering of wells from the same basins is evident, as is clustering of bacterial groups associated with certain types of methanogens. All sequences of methanogens are labeled with genus name. Phylogenetic affiliation of bacterial sequences is not shown; sequences are labeled with generic numbers starting with the letter T. Some microbial clusters appear to be universally present in most samples (*upper part of the diagram*), whereas some clusters tend to be basin specific (e.g., *bottom right*).



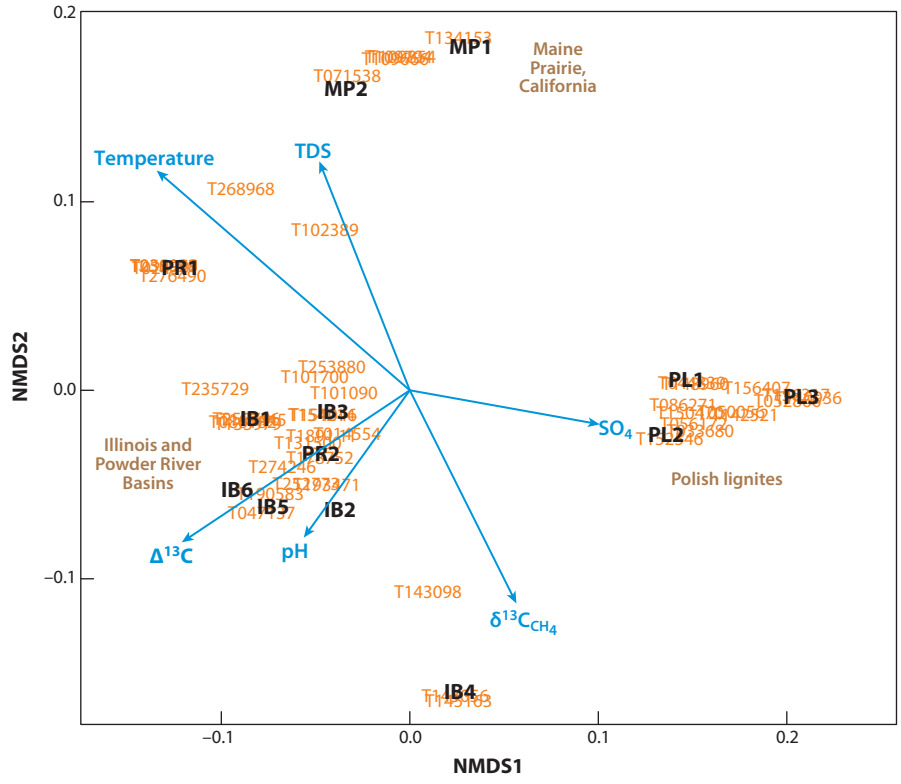


Figure 19

Nonmetric multidimensional scaling (NMDS) of microbial sequence counts, with an environmental overlay of geochemical parameters. The sample (*black*) and sequence (*orange*) identifiers are the same as in **Figure 18**, except that archaeal sequences here are also coded with generic numbers starting with the letter T. There are three distinct clusters: Polish lignites; Maine Prairie, California; and the Illinois Basin together with Powder River samples. The most significant environmental factors, which are correlated to the ordination axes, are temperature (p value <0.001), separating the cool Polish lignites from the hotter Illinois Basin; $\Delta^{13}C$ (p value = 0.02), distinctly higher in the Illinois Basin, which is dominated by CO_2 reduction methanogenesis (high associated carbon fractionation); and total dissolved solids (TDS) (p value = 0.05), highest in Maine Prairie.

6. STIMULATION OF MICROBIAL METHANE GENERATION FROM COAL

Multiple researchers have performed methanogenic incubations with coal as a sole carbon source and without hydrogen addition (e.g., Shumkov et al. 1999, Menger et al. 2000, Pfeiffer et al. 2010, Green et al. 2008, Harris et al. 2008, Jones et al. 2008, Krüger et al. 2008, Orem et al. 2010; see also **Figure 8** for a summary of published rates obtained from incubations of coal at different maturity ranks). Slow conversion rates often require multimonth or even multiyear incubations to obtain accurate coal-to-methane yields. In other words, in addition to finding out how quickly methane can be generated from coal, we need to ascertain what fraction of coal can be ultimately converted to methane by microbes. The yield will likely also depend on coal maturity. The conversion rates could be potentially improved by the addition of stimulating nutrients, but the ultimate yields might not be significantly improved. Several authors proposed chemical stimulation of methanogenesis from coal (Pfeiffer et al. 2010, Green et al. 2008, Krüger et al. 2008, Toledo et al.

2010). Tested nutrient additions included ammonia, phosphate, yeast extract, tryptone, milk, agar, trace metals, and vitamins (e.g., Jin et al. 2007, Pfeiffer et al. 2010). However, the addition of carbon sources other than coal may cause overestimation of the coal-degrading capability of the consortia. Nutrient additions typically applied in laboratory studies are similar to the enrichment media used for the targeted cultivation of specific microbes (for a compilation of growth media and access to numerous archaeal and bacterial isolates, see **http://www.dsmz.de/microorganisms**).

In addition to chemical stimulation of microbial conversion of coal to methane, some authors have suggested and experimented with the addition of selected microbial consortia (e.g., Jin et al. 2007). Examples of consortia used for methanogenic inoculation with coal in laboratory settings include a cultivated consortium indigenous to studied coal (Pfeiffer et al. 2010), a consortium obtained from termite guts (Srivastava & Walia 1998, Menger et al. 2000), and a consortium obtained from an abandoned coal mine used as sewage disposal [Appalachian Basin (Volkwein 1995)].

Several researchers have proposed subsurface enhancement of microbial methane (Scott et al. 1994, Volkwein 1995, Scott 1999, Menger et al. 2000, Budwill 2003, Faiz et al. 2003, Scott & Guyer 2004, Thielemann et al. 2004, Jin et al. 2007). For example, a patent by Menger et al. (2000) suggested digestion of lignite in an underground chamber using termite microflora composed of acid formers and methanogens. Jin et al. (2007) suggested fracturing the reservoir for better simultaneous nutrient delivery and enhanced surface area of coal. The only description of a multiwell field trial was presented in a patent application by Pfeiffer et al. (2010) (see also **http://www.lucatechnologies.com**). In situ microbially enhanced CBM stimulation performed in the Powder River Basin showed an increase in methane production after nutrient treatment (e.g., phosphate) compared with the expected production decline curve. The addition of microbes preconcentrated from the same formations seemed to stimulate gas production from CBM wells as well.

SUMMARY POINTS

1. Extensive review of multiple coal basins suggests that almost any relatively shallow coal bed at present-day temperatures of less than 80°C can contain methanogenic microbial communities capable of generating secondary microbial methane in addition to often preexisting thermogenic gas. Unsterilized, low-maturity coals (lignite and subbituminous coals, as found in, e.g., Powder River and Cook Inlet) are less recalcitrant than higher-maturity coals. Therefore, low-maturity coal cannot be overlooked in coal-bed methane (CBM) exploration. Coals at intermediate maturity (high volatile bituminous) can be accessible and fertile environments as well. These bituminous coals typically have well-developed cleat and fracture systems (e.g., in the Illinois Basin) and therefore are more permeable than lignites. Consequently, larger surface areas are exposed to microbial attack, whereas the organic matter is still molecularly labile enough for microbial attack. However, in these burial-sterilized coals, reinoculation with microbes is likely required. Therefore, more microbial methane can be expected in the flanks of a basin and along the reinoculation paths (e.g., faults, areas with extensive groundwater recharge). Additionally, uplift and long exposure to relatively shallow depths can compensate for slow subsurface methanogenesis rates over geological time. Burial history, in tandem with hydrogeological regimes, can either promote (e.g., northern part of San Juan Basin) or exclude (e.g., southeast Illinois Basin) parts of a basin from microbial methane generation. Coals higher in rank than high volatile bituminous typically are not as favorable for microbial gas generation because of their recalcitrant character, although microbial generation may still occur locally.

2. Recent developments in genetic and other microbiological methods enabled deep microbial surveys and detailed metabolic studies that shed light on the deep subsurface ecosystems. Through the use of a combination of molecular and culturing techniques, significant progress has also been made in deciphering the machinery of microbial processing of coal-derived intermediates as well as in gaining an understanding of specific syntrophic associations between bacteria and methanogens. The least-understood processes in coal-to-methane transformation are the initial steps of anaerobic coal degradation.
3. Field-scale stimulation of microbial methane production requires knowledge of the geological history of the basin, the microbial populations present in the subsurface, and the geochemistry of the formation water. Stimulation techniques may include nutrient and microbe additions to enhance rate-limiting steps of coal biodegradation. Present-day generation of microbially enhanced CBM as a large natural gas resource remains to be evaluated in terms of rates, yields, and economic feasibility. Recent laboratory results and initiation of large-scale field trials may provide answers in upcoming years.

FUTURE ISSUES

Although much progress toward understanding microbial gas systems has been made in the past decade, many questions remain to be answered before this resource can be adequately explored and subsequently utilized.

1. What fraction of coal can be ultimately converted to methane by microbes? To what extent does maceral composition of coal influence microbial gas generation, and what makes a maceral more microbial methane prone? Is it possible, with analogy to thermogenic gas, to define a coal of a specific rank to be the best candidate for microbial gas generation? Or could a combination of rank and maceral composition perhaps be such a predictor? What role, if any, does mineral matter in coal play in microbial gas generation?
2. The initial steps of coal degradation need to be elucidated. Future developments in transcriptomics (mRNA) and proteomics (produced proteins), as well as the analyses of high-molecular-weight intermediates and extracellular enzymes, could shed light on the rate-limiting initial steps in coal biodegradation steps as well as on the entire cascade of reactions from coal to methane.
3. How can we better apply our laboratory discoveries to aid field-scale microbial methane stimulation? What are the limiting factors: access to large volumes or surfaces of kerogen, accommodation space, delivery of nutrients, and/or inherently slow rates of subsurface metabolism? Are the stimulated subsurface coal-to-methane rates using nutrients and specialized microbes high enough to convert vast global coal reserves into long-lived, microbially regenerating gas resources?

DISCLOSURE STATEMENT

C.T. is a ConocoPhillips stockholder; M.A. has significant stock holdings in Taxon Biosciences, Inc., which conducts activities in this field.

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