**Biogenic methane production from coal: A review on recent research and development on
microbially enhanced coalbed methane (MECBM)**

Stephen Y. Park¹, Yanna Liang^{1,2*}

¹. Department of Civil and Environmental Engineering, Southern Illinois University, 1230
Lincoln Drive, Carbondale, IL 62901, USA.

2. Materials Technology Center, Southern Illinois University, 1230 Lincoln Drive, Carbondale,
IL 62901, USA.

* Corresponding author phone: 618-453-7808; fax: 618-453-3044

E-mail: liangy@siu.edu

Abstract

The biological conversion of coal to methane can be an efficient and environmentally friendly means of utilizing the current coal reserves, including those that are difficult to utilize with conventional methods. Understanding the details of microbial coal degradation leading up to methanogenesis is essential in order to establish new energy production techniques and industrial processes that are cost- and energy-effective. Various approaches, such as bioaugmentation, biostimulation and physical, chemical and biological pretreatment for the purpose of enhancing methane yield are reviewed. Specifically for biostimulation which holds great promise for the success of biogasification, detailed reviews are provided on elucidating the composition of a microbial community, developing suitable nutrient solutions, and the *in situ*

application of laboratory generated results. In addition, future research directions for maximizing methane production from coal are recommended.

Keywords: microbially enhanced coalbed methane (MECBM), bioaugmentation, biostimulation, methanogenesis, pretreatment, gas separation

1. Introduction

Coal is a widespread resource that has been used in many applications since the industrial revolution. Approximately 71.4% of the world's total fossil fuel reserves are in the form of coal [1]. Among the estimated 3.6 trillion ton of total coal reserves in the United States (US) that underlay 13% of its land mass [2], only approximately 236 billion ton (6.6%) are viable to mine with current acquisition technologies. Estimation by US Department of Energy is a little different. The mineable coals are less than 10% of a total reserve of 6 trillion ton [3]. In addition, approximately 58% of the US coal reserves are categorized as low-ranked lignite and subbituminous coals, which have considerably lower heating values and subsequent commercial value than their higher-ranked counterparts [4]. Moreover, the continued use of coal with conventional combustion technologies entails restrictions due to the release of toxic chemicals such as sulfoxides and nitroxides and greenhouse gas, CO₂ [5]. Thus, alternative methods of utilizing the existing coal reserves are currently being researched.

Currently, developing thermochemical methods make it possible to convert low rank coals into synthetic gas (syngas) – a mixture consisting primarily of methane (CH₄), carbon monoxide

(CO), hydrogen (H₂), carbon dioxide (CO₂), and water vapor (H₂O). These gasification procedures often require extreme processing conditions such as temperatures as high as 1,350°C and pressures ranging from 20 to 140 atm [1, 6]. The resulting syngas also requires extensive clean-up and purification steps for further use. Therefore, many thermochemical procedures for the conversion of low-ranked coals are deemed economically non-viable for commercialization.

It is possible to achieve gasification in more ambient conditions by means of microbial degradation, also coined as bio-gasification, as observed in biogenic coalbed methane (CBM). CBM is contributed by two processes, geological (i.e. thermogenic) and biological (i.e. methanogenic) [7]. Thermogenic CBM production is primarily controlled by coal rank and depth [8, 9]. In contrast, microbial CBM release is governed by numerous factors, such as salinity, microbe-accessible porosity and coal permeability [10]. Microbial production of CBM generally occurs at shallow depths, at temperatures less than 100°C and is of particular interest to current natural gas producers since: 1) it may represent a “renewable” resource and 2) the CBM recovery infrastructure is already in place [11]. Although it was found that coal could be degraded by microbial means since the early 20th century, it was not until a few decades ago when it was demonstrated with aerobic bacteria and fungi [12]. The biological conversion of coal to methane gas was mentioned in later studies [13].

As of now, more than 700 trillion cubic feet (TCF) of CBM exists in the US, with an estimated 100 TCF economically recoverable with existing technologies such as drilling wells and pumping out ground water. CBM currently accounts for approximately 10% of total natural gas production in the US [14]. The gas extraction process involves drilling wells and pumping out ground water so that the pressures in the coal seams decrease, subsequently allowing gas to escape. With the development of extraction technologies, the recoverable amount of CBM is

projected to increase. Much focus is put on the larger coal beds in the US, including the Powder River Basin [15-17] and the San Juan Basin – the most prolific methane-producing coal bed in North America, generating more than 0.93 TCF of natural gas annually [18]. Small-scale commercial CBM production in Australia began in 1996, with the extraction of mine methane from the Dawson Valley Field and the Moura coal mine in the Bowen Basin. Since then, production has rapidly increased, and in 2003 total cumulative CBM production reached about 40 petajoules (approximately 99 MMcf). Over 75% of produced CBM in Australia is derived from Permian coals of the Bowen Basin [19], but CBM is also commercially produced from the Sydney Basin [19]. As the largest consumer and producer of coal in the world, China has seen dramatically increasing CBM production over the years. Drilled CBM wells increased from 287 in 2004 to 4,657 in 2010, among which more than 3,700 were producing [20]. By 2010, the annual CBM production was 53 -70 billion cubic feet (BCT) [21]. By 2015, the annual production is expected to be 388 BCF [20].

As of 2011 in the US, microbial CBM accounts for 40% of the total CBM and is largely owing to the significant production of purely microbial CBM in the Powder River Basin [14]. Globally, methane yields in different basins [16, 22-27] have been reported to vary between 36-286 Standard Cubic Feet (SCF)/ton as detailed in Table 1. This large variation is a result of many factors. As reported, even in adjacent wells, methane productivity can vary significantly due to differences in burial depth, thickness of coal, gas content, porosity/permeability, effect of fracturing, structural setting and hydrogeological conditions [20]. In addition, source of methane plays an important role in the overall methane content. If thermogenic methane is present, then generally the overall methane yield (SCF/ton) is higher. It is commonly recognized that thermogenic methane is generated during the early stages of coalification [28]. As recovery of

CBM continues at a given location, methane release in coal beds has been reported to be decreasing *in situ*, thus calling for technologies to enhance microbial activity and thus recover more methane [29]. Furthermore, the United States Environmental Protection Agency reported that with the application of wastewater discharge requirement, ongoing CBM production sites are not economically viable [30]. Such analyses call for further research in increasing *in situ* feasibility as well as looking into alternative *ex situ* methods.

For the purpose of enhancing methane production from coal both *in situ* and *ex situ*, different approaches have been tested, such as: adding external microbial sources (bioaugmentation) [31-33] and supplementing chemicals and nutrients (biostimulation). Specifically for *in situ* application of Microbially Enhanced Coalbed Methane (MECBM), physical treatment, such as hydraulic fracturing of coal seams has been found to be effective, but only in short term [20]. As research and development on hydraulic fracturing for shale gas production have been intensively investigated, knowledge and successes from shale gas can certainly be applied to MECBM for making full use of coalbeds as methane reservoirs. Since excellent reviews [34-37] have been published on hydraulic fracturing, this topic is not reviewed here. Instead, this review focuses on two key concepts: bioaugmentation and biostimulation. Before these two approaches are discussed, a brief introduction of coal as a microbial substrate and microbial conversion of coal is provided. In addition, since CO₂ is released together with methane as a result of enhanced microbial activities, state-of-the-art methods for capturing CO₂ are also summarized.

2. Coal as a methanogenesis substrate

Compared to common organic materials that are consumed by anaerobic consortia, coal is not quite the ideal substrate due to its complex and recalcitrant structure. The following section overviews the different types of coal available for biogenic methane production, and possible

approaches of utilization. Low rank coals encompass lignite, leonardite and other subbituminous coals, which account for approximately 58% of the coal reserves in the United States. Compared to higher ranked coals, low rank coals have been buried for shorter periods of time and contain lower calorific value, but are easier to be degraded biologically. The relatively low heating capacity compared to higher ranked coals is triggering interest in the utilization of low rank coals as non-traditional fuels [4]. Laboratory studies using low rank coals have shown that substantial amounts of methane could be biologically produced [2, 14, 38, 39]. The main parent material of low rank coals is reported to be woody and lignocellulosic biomass, and thus the research of lignolytic and hydrolytic enzymes on the degradation of coal has been extensively studied [29].

Bituminous coals contain nuclei of aromatic and naphthenic rings coupled to one another by bridges of aliphatic chains, as well as a significant number of hydroxyl groups that structure the coal [40]. Higher ranked bituminous coals and anthracite are considered to be more recalcitrant than their lower-ranked counterparts. For example, in a laboratory environment, bituminous coal from a Utah mine was shown to have less biogenic methane production than North Dakota lignite [39]. Contrasting results have been reported as well, suggesting a positive correlation between coal rank and biogenic methane production [41]. A possible explanation of the contrasting result could be that bitumen has a greater potential than kerogen (or low rank coal) to be microbially converted into methane [42]. The microbial population and the initial metabolic steps of the coal degradation process are argued to have a greater impact on the overall performance than the coal rank [18]. Within the current infrastructure of high ranked coals being preferably utilized as conventional fuel sources, targeting coals of lower rank would appear to be relatively more feasible. The utilization would be favorable for regions with vast reserves of low

rank coals [43]. However, with growing focus on environmentally clean energy, making high ranked coals more available to microbial degradation may also be a growing topic of interest.

3. The process of bioconversion of coal

Compared to commonly available organic substrates such as carbohydrates, lipids, and proteins, coal is generally not a favorable microbial substrate. Approximately 85% of dry coal is comprised of organic material called macerals – which reflect the properties of the precursor biological material – while the remainder is typically in the form of aluminosilicate and sand pyrites (Lavana and Lal, 2011). Generally, coal is described as having an irregular, lignin-like polymer structure, with a limited fraction of biodegradable moieties, such as phenolics, carboxylic acids, alkanes, etc. [44]. Because of their recalcitrant structures, CBM formation through microbial processes requires the collective actions of microorganisms comprising three major metabolic groups: 1) hydrolytic and fermentative bacteria; 2) acetogenic bacteria, and 3) methanogenic archaea.

During the initial stage of bio-conversion, complex organic compounds in coals are decomposed by hydrolytic bacteria to simpler molecules such as: polyaromatic hydrocarbons (PAHs), monoaromatic carboxylic acids, ketones [45], single-ring aromatics, long-chain alkanes and long-chain fatty acids [38]. These molecules are then converted to short-chain volatile fatty acids, CO₂, H₂, and HS⁻ by fermentative anaerobes. Acetogenic bacteria, such as those belonging to the genus of *Clostridium*, can produce acetate from two pathways: H₂ producing from volatile fatty acids and CO₂ reducing by using H₂ as the electron donor. Based on analysis of microbial communities involved in coal bio-conversion, anaerobes belonging to phylum: *Spirochaetes*, *Bacteroidetes*, *Firmicutes*, *Proteobacteria*, *Betaproteobacteria*, and/or *Gammaproteobacteria*

have been identified [38, 45, 46]. These fermenters are noted for their ability to degrade polymers [45].

Study of enzymes produced and/or secreted by these hydrolytic and fermentative bacteria aforementioned revealed the presence of 151 bacterial protein families. The first group included 50 that are transporter and membrane proteins. These proteins are ATP-binding cassette (ABC) transporters responsible for transporting sugars, amino acids, phosphonate molecules, C-4 carboxylates (malate, succinate and fumarate) and binding to starch and collagen similar compounds. The second group was 43 proteins that are involved in cellular metabolism, such as: glutamate dehydrogenase which may be related to nitrogen deficiency. The third group consisted of 11 proteins that function in substrate utilization and conversion, for example: iron hydrogenase, glucokinase, rhamnulokinase, glycoside hydrolase and sulfite reductase. Presence of these proteins indicate that bacterial cells could utilize glucose, rhamnose, mixed sugars and sulfite for growth and for producing hydrogen. The fourth group comprised eight protein families that are responsive to oxidative stress, including rubrerythrin, superoxide dismutase and peroxidase. The fifth group included five protein families that are related to cell movement through flagella. The sixth group was 34 hypothetical proteins whose functions are not known at this point [47]. The presence of these proteins indicated that the bacterial cells use flagella to move to locations where substrates are more readily available. Once they are close to available substrates, they produce membrane proteins and transporters to uptake whatever they can in order to survive and grow on coal.

The last step in methane formation is by methanogenic archaea or methanogens. Methanogens have five orders: *Methanobacteriales*, *Methanomicrobiales*, *Methanosarcinales*, *Methanococcales*, and *Methanopyrales*. These microbes catalyze the terminal step in the

degradation of organic matter in anoxic environments where light and terminal electron acceptors other than CO₂ are limiting [48]. There are three main methanogenic pathways leading to biogenic CBM production in natural environments. Equation 1, 2 and 3 illustrate acetoclastic, hydrogenotrophic and methylotrophic reactions, respectively [25]. While the carbon substrate for the first two reactions is acetate and CO₂, the methylotrophic pathway covers a range of methyl-bearing C1 compounds, such as methanol, methylamine and methyl sulfide, etc.

The order of *Methanobacteriales* is generally hydrogenotrophic, using hydrogen to reduce CO₂ to CH₄. Some members of this order can use formate, CO, or secondary alcohols as electron donors for CO₂ reduction [49]. Most species within the order of *Methanomicrobiales* can consume formate and alcohols, but cannot use acetate and methylated C-1 compounds, such as methanol, methylamines and methyl sulfides [50]. The order of *Methanosarcinales* have the broadest range of substrates. Besides reducing CO₂ with H₂, dismutating methyl compounds, they can split acetate [48]. All of these three orders have diverse anaerobic habitats comprising marine and freshwater sediments, anaerobic digesters, and gastrointestinal tracts of animals. *Methanococcales*, however, have all been isolated from marine habitats and require salinity between 3.0 and 4.5 M for optimal growth [51]. In addition to hydrogen, formate can be used as electron donors. Acetate, C-1 compounds such as methanol and methylamines, and alcohols such as isopropanol and ethanol are not utilized as substrates for methanogenesis [51]. The last order of *Methanopyrales* is represented by only one species, *Methanopyrus kandleri* which is

hyperthermophilic and produces methane by CO₂ reduction with H₂. Genomic sequence analysis of *M. kandleri* suggests that it is closely related to *Methanobacteriales* and *Methanococcales* but possesses unusual features. It is the only methanogen known so far that catalyzes methanogenesis at temperatures higher than 100°C [52].

Different coal seams may have different methanogens present and different pathways resulting in methane formation. In formation water collected from the Illinois basin, approximately 90% of methanogens belonged to the order of *Methanobacteriales* which is hydrogenotrophic [46]. After being cultivated in the lab for several months, the originally minor order of *Methanomicrobiales* which is also mainly hydrogenotrophic became predominant. Grown on coal with a rich nutrient solution for 30 days, methanogens produced at least 98 protein families that were identified as statistically valid, among which 37 were tied specifically to methane production. These included: methyl-coenzyme M reductase, acetate kinase, phosphotransacetylase, acetyl-CoA decarbonylase synthase, formyl-methanofuran dehydrogenase, methylene tetrahydromethanopterin, methylene tetrahydromethanopterin (H₄MPT) reductase, methanol-5-hydroxybenzimidazolylcobamide methyltransferase, and formate dehydrogenase [47].

Acetoclastic methanogenesis is reported to be the main source of methane formation in abandoned coal mines in Germany [53]. This conclusion is based on ¹³C methane production from cultures where either ¹³C acetate or H₂ and ¹³CO₂ are used as the direct substrate. The dominant methanogen is *Methanosarcina* spp. in the order of *Methanosarcinales*. Although *Methanosarcina* spp. are known to use either hydrogen or acetate as discussed above, in the coal mined studied, acetoclastic pathway is the major one since acetate is more available than hydrogen and the archaeal cells are adapted to acetate already. Even with adequate supply of

hydrogen, this electron donor appears to be used by acetogens for producing acetate which is then converted to methane by the *Methanosarcinales* [54]. The acetoclastic pathway has also been observed in the Powder River Basin in US [16, 17].

In the Ordos basin in China, methanogens within the genus of *Methanolobus*, order of *Methanosarcinales* are predominant. As *Methanolobus*, an obligate methylotrophic methanogen, is a representative of the methyl-methanol utilizing methanogenic pathway in the subsurface, it is suggested that methyltrophic methanogenesis is the main pathway leading to biogenic methane formation in that basin [55]. This pathway has also been reported for methane release in northern Japan [56] and the Cook Inlet Basin in Alaska [57].

Generally speaking, for a given microbial community in coal bed formation water, the *in situ* bacterial community is more diverse than the archaeal kingdom. Regarding a community in the Illinois basin #6, the total bacterial species was 231 while the total species of archaea was 33. After enrichment, the bacterial species dropped to 185 and the archaea contained only nine species [46]. The same is true for a community in the Powder River Basin [58].

4. Bioaugmentation for coal bio-conversion to methane

As discussed above, producing methane from coal requires synergistic efforts from at least three groups of microorganisms. Thus, if bioaugmentation is considered, the augmented cells must be a community that is able to fulfill various coal conversion functions. Generally speaking, there are two ways to implement bioaugmentation. First, an indigenous microbial consortium taken from a coal seam can be enriched or concentrated in a laboratory environment. Once increased cell numbers are achieved, the community can be injected back to the coal seam with the anticipation that more cells will convert coal to methane faster. However, extreme care should be

taken to ensure that the laboratory environment (temperature, pressure, etc.) exactly mimics those in the field. Otherwise, the developed community will be largely different from those in the coal seam and may not function well under the field conditions. These cautions are not needed if the intended use of the developed community is for converting coal *ex situ*. In this case, conditions for generating methane from coal in a bioreactor can be used to cultivate the microbial community.

Second, communities that are external to coal seams can be developed and used for coal conversion either *in situ* or *ex situ*. For example, consortia obtained from termite guts [32, 33] and wetland sediment [38] have been studied for this purpose. The latter was used to augment a nonproductive coal mine and demonstrated faster methane release compared to those with only nutrient addition. For *in situ* application, if the indigenous microbial community is not active, then bioaugmentation can be a sound option. However, the ability for getting permits for injecting either enriched or engineered microbes to underground needs to be seriously taken into consideration. Again, for *ex situ* scenario, since the microbes will be contained in closed reactors, permitting should be less of a problem.

5. Biostimulation for coal bio-conversion to methane

Owing to the facts that: 1) indigenous microbes have been shown to convert coal to methane, 2) indigenous microbes in coal mines with no gas production history can be energized to produce methane from coal upon nutrient addition [59], and 3) microbes in mine water generated more methane than those from other microbial sources, such as cow dung, paddy field soil, termites [60], biostimulation has been studied extensively compared to bioaugmentation. Biostimulation works through meeting the microorganisms' needs in order to promote their growth and metabolic activities which include degrading coal to methane. Currently, most companies interested in MECBM have pursued biostimulation, such as Luca Technologies, Inc., Ciris

Energy and Next Fuel, Inc. [61]. To make biostimulation work, investigators should address at least four questions: 1) what are the microbes in a community? 2) what nutrients can bring the highest methane productivity? 3) can various pretreatment techniques increase coal bioavailability and indirectly improve methane yield? and 4) can the biostimulation strategy developed in laboratory environments be used *in situ*?

5.1. Understanding diversity of microbial communities

Since different coal seams may contain different microbial communities as a result of different temperature, pressure, geological location and source of microbes, the first step in biostimulation is to know what cells are present in a given location. In previous years, clone libraries of partial 16S ribosomal RNA genes obtained by polymerase chain reaction (PCR) of the community DNA have been used extensively to elucidate structure of a coal mine microbial community in Yubai, Japan [56], Australia [62], the Illinois basin [45], the Waikato coalfields in New Zealand [63], Alberta basin [64], and the Powder River Basin in US [65]. As expected, different bacterial and archaeal species have been identified at different places.

In light of the facts that: 1) conventional gene cloning and sequencing suffer from both cloning bias and limited throughput which lead to masking and underestimation of the actual diversity of a microbial community [66]; 2) cost for high throughput next-generation sequencing through either Roche's 454 pyrosequencing, Solexa, and Solid has decreased dramatically in recent years; and 3) the next generation sequencing technology is able to recover uncommon and exceedingly rare species, the number of publications on using state-of-the-art sequencing technology to understand composition of microbial communities in coal mines has been increasing. Coal mine communities in the Easter Ordos Basin in China [55], in the Jingmen-Dangyang basin in China [67], in the Illinois basin in US [46] and in the Powder River Basin

[15] are examples of this recent development (Table 3). Regarding microbial communities in the Illinois basin, through using 16S eubacterial specific primers, 27F and 519R, the percentage distribution of bacterial species within the formation water in the Illinois #6 basin was elucidated. Through using a primer pair dedicated for methane-producing methanogens, 86F and 448R, composition of archaea in the same community was understood. To understand the diversity of both bacteria and archaea in the whole community, the 16S rRNA gene V4 variable region PCR primers 515/806 [68] is found to be valuable. For example, for the original formation water collected from Illinois basin #6, it is revealed that bacterial and archaeal species accounts for 44% and 56% of the whole population, respectively [46]. Besides sequencing the 16S rRNA gene through next generation sequencing platforms, sequencing the metagenomes of a microbial community is increasingly becoming a reality these days due to affordable and reasonable costs. This approach seeks to target functional genes involved in relevant metabolic pathways and provides in-depth knowledge regarding a community's function. Metagenome sequencing has been reported for a microbial consortium capable of biotransforming coal to methane from the coal dust and the selectively enriched sump sediment water of the underground coal mines of the Jharia basin in India [69].

However, it needs to be noted that all PCR-based community characterizations (Sanger or next generation sequencing) are not without potential bias, such as different DNA extraction efficiencies and bias of primer selectivity [55]. To address these drawbacks, Fluorescence In Situ Hybridization (FISH), a 30-year old technique [70], may be a better approach to pursue. In this case, oligonucleotide probes hybridize to specific regions of 16S or 23S rDNA in fixed cells. The labeled cells can then be imaged using epifluorescence microscopy. The advantages of FISH include: 1) the availability of probes for a range of taxonomic specificity from domain to species;

2) the ability to generate a quantitative measurement of population structure of *in situ* microbial communities; 3) direct visualization of complex assemblages of bacteria and archaea; and 4) identification of actively growing microbial communities [14]. However, although FISH has been used broadly in the medical and environmental fields, its application to coal mine microbial communities has been limited to only one study [57] and certainly awaits further investigations.

5.2. Selection of nutrient solutions for enhancing methane production

Once the population of a microbial community is known, the next question is how to stimulate its activity, in particular, methane release. To achieve this purpose, a general approach is to evaluate methane yield in laboratory microcosms comprising ground coal samples, a microbial community collected from either the same location as the coal or other sources and a nutrient solution. These microcosms are cultivated under strictly anaerobic conditions and methane in headspace gas is monitored with time.

Different nutrient solutions with different names [71] [16] [72] [38] [39] [73] [49] have been tested by different investigators. Even though different medium recipes have different chemical compositions, generally, the nutrient solution is composed of at least six ingredients. The first one is major minerals, such as K, Na, Ca, Mg, nitrogen as NH_4^+ , P, and Cl. Tanner medium typically has SO_4^{2-} , while methanogenic medium excludes this anion to create a sulfate-free condition but normally include HCO_3^- which is a substrate for methanogens. These minerals supplied at concentrations of g/L are essential for cell function and activities. The second one is organic nitrogen source. Yeast extract or peptone or tryptone is generally added as a source of organic nitrogen and vitamins. In addition, yeast extract is suspected to have a chelating effect on some metal cations, which subsequently increases coal solubility and biodegradation [2]. These complex chemicals are often added at g/L level. The third ingredient is a vitamin solution which

may contain more than 10 vitamins and is included at less than mg/L concentrations. The fourth one is a trace mineral solution which provides elements of Mn, Co, Fe, Zn, W, Cu, Se, Mo, and B and is added at mg/L levels. Since a significant number of enzymes contain one or more heavy metal, these minerals cannot be eliminated from the solution. The fifth ingredient is reducing agent, such as Na₂S, cysteine-HCl, or mercaptoethanesulfonic acid. The latter is also called Coenzyme M which is an important substrate in later stages of methane formation. The reducing agents assist in creating and maintaining anaerobic environments. The last one is redox indicator, resazurin allows visual inspection of the anaerobic state of cell cultures.

Research in our own lab has shown that different medium can have a dramatically different effect on methane production [46]. When the Tanner medium was used at two concentrations: 20% and 50% of the final microcosm volume, no positive effect on methane yield was observed. A methanogenic medium [48], however, demonstrated increased methane production with increased medium concentration, from 20%, 50% to 100%. Thus, for a given coal sample and a microbial community, testing different nutrient solutions may be beneficial if the goal is to increase methane productivity.

Besides these standard medium recipes, some researchers have evaluated effects from the addition of formate, acetate or methanol on methane yield from coal. The addition of formate was hypothesized to induce the proliferation of methanogens, but it has been discussed that the dissimilatory metabolism of the substrate is a more probable cause [2]. Moreover, it should be noted that although the addition of supplemental nutrients as electron donors could stimulate microbial growth and increase the overall CH₄ production, it could also divert electrons away from methanogenesis, resulting in the accumulation of organic intermediates which could in turn limit biogenic CH₄ production. The supplementation of a solvent has also been investigated. The

effect of solvent addition, so far, is mixed with contradictory results. In one study, where three solvents were tested, methanol, pyridine, and N,N-dimethylformamide (DMF), only DMF at 0.25 vol.% produced 346% more methane than the no-solvent control cultures. However, the researchers were unable to conclude whether the enhanced methane production in the presence of DMF was due to enhanced coal utilization or direct degradation of DMF [16]. In another study, where ethanol served as the solvent, enhanced methane production was observed only when ethanol was added in the amount of 5 or 10 mg to 10 g coal from Power River Basin [74]. Other investigators have added surfactants to cultures to enhance coal bioconversion. Like organic solvents, surfactants may also enhance the aqueous solubility of coal but have negative impacts as well. One obvious concern is that they are toxic to microbial cells at concentrations above certain levels. Among three non-ionic surfactants (Zonyl FSN, Triton X-100, and Brij 35) studied, only the first one at 50% of its critical micelle concentration (CMC) produced 93% and 57% more methane than no-surfactant controls and cultures with 100% CMC Zonyl FSN [25].

5.3. Pretreatment for increasing bioavailability of coal

In addition to use of surfactant and solvent for enhancing coal solubility, pretreatment of the initial coal matrix can be performed to mitigate the recalcitrance of coal and increase the availability of usable substrates. As the rate-limiting step of biogenic CBM is typically the solubilization step, various methods including chemical and biological pretreatment can be employed. In terms of chemical treatment, the use of an inexpensive Lewis base, such as ammonium, to increase the pH, neutralize the carboxylic acid functional groups, and subsequently enhance the solubility of coal was suggested [4, 75]. Strong reagents, such as nitric acid, sodium hydroxide, potassium permanganate and catalyzed hydrogen peroxide, have been tested on enhancing bioavailability of subbituminous coal. Although the first two chemicals

solubilized up to 14% of coal carbon, results from a bioassay using *Pseudomonas putida* F1 indicated that coal pretreatment with permanganate at high concentration led to the largest fraction of bioavailable solubilized constituents [76]. When permanganate concentration was 0.1 mol/L, 5.4% of coal was solubilized into the aqueous phase in 21 days. The resulting liquid phase was converted to methane by a microbial community when a nutrient solution was supplemented [77].

With regard to biological treatment, fungal strains have been studied intensively in the 1980s and 1990s. Wood-rotting fungi such as *Trametes versicolor* and *Poria monticola* have been found on or in the vicinity of lignite deposits and coal mine timber, suggesting that the enzymes or extracellular metabolites from fungal metabolism may contribute in the solubilization of coal [78]. Some macromolecules in low rank coals are bonded together by polyvalent exchangeable cation bridges between negatively charged groups on the coal macromolecules. Breaking the ionic linkages result in polar coal macromolecules, which are relatively soluble in water. Oxalate ions are able to chelate these cation bridges. It was found that fungal cultures *Phanerochaete chrysosporium* and *T. versicolor* are capable of solubilizing lignite by secreting oxalate ions [4]. Most importantly, fungi strains possess several mechanisms for coal degradation/liquefaction: oxidative enzymes (peroxidases, laccases), hydrolytic enzymes (esterases), alkaline metabolites and natural chelators. Readers interested in this aspect are recommended to consult a review paper by Fakoussa and Hofrichter [29]. As a result of fungal metabolism on coal, a series of aliphatics, single ring aromatics and polyaromatic hydrocarbons are released, which could in turn be converted into methane [43]. However, since coal-degrading fungal strains are aerobic, this solubilization approach may only be applied ex situ. In situ application of this fungal pretreatment approach would necessitate seeding the coal seams with desired fungi and

providing air or oxygen for their metabolism. Once supplied air or oxygen is consumed, the coal seam would become anaerobic and methanogenesis may take place thereafter. The practicality of this aerobic/anaerobic cycle, however, will be highly dependent on the site condition and whether a permit to inject fungal strains can be obtained.

5.4. *In situ* application of biostimulation

Besides adding nutrient solutions and use of chemical/biological pretreatment for improving methane yield from coal, different cultivation conditions have been tested. These include, temperature, with or without shaking during cultivation, coal particle size, and coal loading. As a result of all of these different conditions, plus different kinds of coals and microbial communities studied, it is almost impossible to compare results reported from different investigations (Table 2). However, as demonstrated by one study using Indian hard coal, parameters, such as coal loading, pH, temperature and particle size of coal need to be optimized for a given microbial community in order to achieve maximum methane production [60]. In this study, a coal loading of 1:10, pH of 7.0-7.5, temperature of 35°C, and particle size between 25 and 60 µm are identified as the best conditions for obtaining a methane yield of 169 SCF/ton with a methane content of 92.6%.

Although methane yield among different basins cannot be compared, the common recognition from all published studies on biostimulation for coal bio-conversion is that nutrient limitations restrict microbial activities *in situ* and addition of nutrient solutions enhances methane productivity in all coal basins tested so far. Certainly, this conclusion is encouraging for bio-gasification of coal to methane, but, all of the studies summarized in Table 2 do not simulate *in situ* conditions. First, except one study by Wawrik et al. [18], which employed a flow through column test, all others conducted their experiments using serum bottles where ground coal,

microbes and nutrients were capped and sealed. This approach is adequate for investigating methane production from coal *ex situ*. However, since powdered coals were used and *in situ* temperature and pressure were not considered, the attained results are difficult to apply in the field. Second, although Wawrik's work did consider pore pressure in the column test, mechanical conditions of vertical and lateral stresses were not taken into consideration. In addition, instead of using chunks of coals, this study used powdered coal. Thus, to date, no bio-gasification studies replicating truly *in situ* conditions have been reported in scientific journals. This, however, does not exclude investigations that have been performed by commercial companies who have been active in the CBM field. Several companies, such as Luca Technologies, Inc., Next Fuel, Inc., and Ciris Energy, have conducted field trials on MECBM [61].

In addition to the lack of tests conducted under *in situ* conditions and the uncertainty related to transferring laboratory studies to the field, cost of biostimulation either *ex situ* or *in situ* has not been addressed so far in the literature. The majority of studies on biostimulation of coal to methane start with a standard medium as described above. Different carbon sources, surfactants and solvents are all additives to the chosen standard medium. A closer look of each medium recipe reveals that: 1) each medium is highly complex, which may include more than 20 or 30 chemicals; and 2) if the standard medium even without additives is used in large scale either *ex situ* or *in situ*, the cost is not going to be low. Thus, it is necessary to simplify the standard medium recipe and only use the components that are critical to methane yield. By doing so, cost-effective nutrient solutions can be developed for specific microbial communities and coal mines.

6. Separation of methane from biogas released from CBM wells

Bioaugmentation and biostimulation can indeed yield higher methane productivity in SCF/ton or m³/ton. This has been confirmed by field trials conducted by companies, such as Luca

Technologies, Inc., Ciris Energy, Next Fuel, Inc. etc. In the case of Luca Technologies, Inc., as of 2010, the reported gas production from the nutrient-amended sites over 2 years were in total $8.2 \times 10^8 \text{ ft}^3$ greater than the untreated areas [79]. In 2012, wells that were successfully treated were reported to produce $4.5 \times 10^7 \text{ ft}^3$ more gas per treated well, or approximately 50% of their original peak productivity over a 4-year period [80, 81]. Regarding Ciris Energy, as of 2012, gas recovery from treated wells were reported to be 8-20 times the untreated gas production [82]. Detailed analysis in terms of commercial field trials is provided by Ritter [61] and is not reviewed here.

Higher methane yield (SCF/ton) as a result of biostimulation and/or bioaugmentation certainly is encouraging, but none of these companies disclosed the composition of biogas released from CBM wells and discussed the possibility that stimulated microbial activities may also bring higher CO_2 release. Similar to anaerobic digesters converting agricultural feedstocks, methane content in the headspace gas generally does not exceed 75%, although some biogenic CBM can carry CH_4 contents that are up to par with commercial natural gas with a methane content of 90-95% [16, 24, 83]. Gas as a product of methanogenesis may contain significant amounts of impurities including CO_2 and hydrogen sulfide (H_2S). The upgrading of biogas is key in order to increase the heating value of the gas and fulfill requirements of various infrastructures that utilize it. Carbon dioxide found in CBM ranges from 0 to 99%, and thus the purification of biogas may be necessary [84]. One of the most common methods of removing acid gases such as CO_2 and H_2S is absorption, which is possible through the difference in binding forces of polar chemicals, such as CO_2 or H_2S , and non-polar chemicals such as CH_4 . Media that can be used to separate the polar chemicals from CH_4 can consist of water [85] or organic solvents such as

polyethylene glycol [86], mono ethanol amine or dimethyl ethanol amine [87] and can exist as columns or counter-flows.

At elevated pressures, gas impurities can be separated via pressure swing adsorption which utilizes molecular sieves consisting of activated carbon, silicagel, alumina or zeolite. The molecular sieves can be regenerated through desorption at reduced pressures. At moderate temperatures, CBM with a CH₄ content of 55.2% (v/v) could be enriched up to 98% (v/v) via vacuum pressure swing adsorption, where CO₂ is removed with solid potassium hydroxide under high pressures reaching 300 kPa [88]. The pressure swing adsorption method is not only capable of removing CO₂, but also O₂ and N₂ from CH₄ depending on different mesh sizes or gas pressures which determine adsorption selectivity. — Gas adsorption can also be employed for *in situ* CBM recovery, taking advantage of the characteristics of medium to high rank coal of adsorbing twice as much CO₂ by volume as methane [84].

The difference in permeability of the various gases in CBM can be used to separate the gases in membrane systems. Gas permeation is an economic alternative to absorption and adsorption for the treatment of CBM. A wide array of materials has been explored for the development of gas separation membranes. Non-polymeric membranes consisting of materials such as alumina, zeolites, and carbon have high chemical and thermal stability and are generally considered to have better separation properties when compared to their polymer counterparts, but are restricted by their high costs and poor mechanical properties [89]. For example, triamine-grafted pore-expanded mesoporous silica was reported to display excellent separation between CO₂ and CH₄ [90]. Various organic polymers including polysulfone, cellulose acetate, and polyimide have been considered for industrial scale applications.

Cryogenic separation of CH₄ and CO₂ has also been considered, since CH₄ has a boiling point of -160°C whereas CO₂ has a boiling point of -78°C. Carbon dioxide can be separated from biogas as a liquid through cooling at elevated pressures. This method can be advantageous as CO₂ in liquid form can act as a solvent for any other existing impurities. Although the high pressures and intensive cooling can be energy intensive, cryogenic separation can be of value when the final product is methane in liquid form, which has benefits regarding energy density and transportability [91].

7. Concluding remarks

Although recent studies have shown that the conversion of coal to CH₄ through the metabolism of anaerobic consortia is possible and would open numerous advantages to the current coal energy infrastructure, MECBM development is still in its infancy [92]. Future research is urgently needed to develop cost-effective techniques for enhancing methane yield from coal both *in situ* and *ex situ*. Specifically, the scientific community involved in coal bio-conversion are recommended to consider the following. It needs to be noted that these future research recommendations are general and do not differentiate between *in situ* and *ex situ* conditions or processes.

- 1) Understand the bio-conversion process better. Similar to anaerobic digestion of agricultural feedstocks, the anaerobic fermentation process employing a community of microbes has been poorly understood. Deeper and thorough knowledge of this process may enable controlling or engineering the pathways to achieve desired outcomes.
- 2) Develop a suite of techniques for maximizing methane yield. As discussed above, microbial communities are capable of converting coal to methane. To increase the rate,

however, physical/chemical/biological pretreatment may be necessary and beneficial for the overall process.

- 3) Identify cost-effective nutrient solutions for a given coal bed and microbial consortium.

The nutrient solution needs to be simple to make, as inexpensive as possible and result in the highest methane yield as possible.

- 4) Identify optimal conditions for achieving the highest methane productivity. Parameters, such as: coal loading, pH, temperature, coal particle size, shaking, test duration, solvents, surfactants and external carbon sources need to be evaluated at a case-by-case scenario.

- 5) Determine suitable ways for nutrient delivery. This can be accomplished through fracking or other means. In addition, the time interval for delivering nutrients needs to be known for a given location.

- 6) Conduct economic analysis of the whole process to determine whether MECBM is cost-effective. MECBM, at commercial scales, will be affected by market price of natural gas and other commodities which are difficult to predict. Thus, economic scenarios at different prices levels for related commodities need to be considered.

- 7) Determine impact to the underground environment if nutrients are injected to the subsurface. Specifically, effect on drinking water quality needs to be evaluated if the coal mine is close to source of drinking water.

- 8) Evaluate properties of coal after bio-conversion. These properties will determine how the residual coal will be utilized after certain period of biogasification.

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Table caption:

Table 1: Methane yield from different basins.

Table 2: Reported *ex situ* methane production from coal in simulated CBM environments.

Table 3: Dominant bacterial and archaeal genera identified in different samples as a result of next generation DNA sequencing.

561

562 Figure 1: Proposed pathway for biogasifying coal to methane. This pathway is based on coal
563 intermediates identified in references [38, 45] and proteins identified in reference [47].

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566 8. References

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Table 1: Methane yield from different basins

Location	Coal rank	CH ₄ yield (ft ³ /t)	CH ₄ content (%)	Gas origin	Reference
Illinois Basin, USA	High volatile C to A bituminous	67–106	> 90	Thermogenic and biogenic	[22]
Springfield, Indiana, USA	High volatile bituminous	71-106	NA	> 99% biogenic	[23]
Seelyville, Indiana, USA	High volatile bituminous	78-162			
South-central Wyoming, USA	Subbituminous – high volatile bituminous	115–263	97	Thermogenic and biogenic	[24]
Powder River Basin, USA	Subbituminous	57–78	97	Biogenic	[16]
San Juan Basin, USA	Subbituminous – low volatile bituminous	36-222	NA	Thermogenic and biogenic	[27]
Surat Basin, Queensland, Australia	Subbituminous – high volatile bituminous	141–282	NA	Thermogenic and biogenic	[25]
Ruhr Basin, Germany	NA	NA	1–95	Thermogenic and biogenic	[26]

NA – Data not available

Table 2. Reported *ex situ* methane production from coal in simulated CBM environments.

Coal source	Coal rank	Source of microbial community	Methane production rate (ft ³ /t/d)	Temperature (°C)	Shaking (rpm)	Test duration (d)	Particle size (µm)	Methane yield (ft ³ /t)	Coal loading (g/L)	Reference
Illinois basin, USA	High volatile B bituminous	Formation water	5.6	28	No	20	< 420	111	200	[46]
Utah, USA	Bituminous		73.1 ^a	23	No	30	< 74	2193	667	
	Lignite	Waste coal	40.2 ^a	23	No	30	< 74	1206	667	[39]
	Coal waste		43.8 ^a	23	No	30	< 74	1314	667	
Pocahontas No. 3, USA	Low volatile bituminous	Formation water	2.1 ^b	22	No	60	NA	126	50	[59]
Pittsburgh No. 8, USA	High volatile bituminous	Formation water	1.2 ^b	22	No	60		72	50	
Powder River Basin, USA	Subbituminous	Well water	2.7	22	100	26	250-600	47	25	[16]
Powder River Basin, USA	Lignite and subbituminous	NA	0.01-0.03	NA	No	365	Crushed	4.5-12	667	[2]
Alaska, USA	Lignite and subbituminous	NA	0.07-0.15	NA	No	60	Crushed	4.2 – 9.1	800	
Texas, USA	Subbituminous	Wetland sediment	0.5-0.7	22	No	105	NA	55.0 – 80.0	156	[73]
Texas, USA	Subbituminous	Wetland sediment	0.8	22	No	70	2000-10000	56	117	[38]
San Juan Basin, USA	NA	Formation water	0.33 ^c	31	No	243	Ground	79	NA	[18]

Wallon, Australia	Subbituminou s	Formation water	30	37	50		300-600	210	25	[25]
Jitpur, India	Hard coal	Mine water	2.7	35	No	63	25-60	169	100	[60]
German fat coals	Hard coal	NA	0.04-0.12	35-36	No	180	NA	7.2-21.6	NA	[53]

a: with a 50% nutrient solution

b: calculated based on $\mu\text{mol CH}_4/\text{g coal-day}$

c: calculated from the highest methane production rate of $0.37 \mu\text{mol CH}_4/\text{g coal-day}$

Table 3: Dominant bacterial and archaeal genera identified in different samples as a result of next generation DNA sequencing.

Site location	Sample type	Dominant bacterial genera	Percentage (%)	Dominant archaeal genera	Percentage (%)	Reference
Eastern Ordos Basin, China	Water	<i>Arcobacter</i>	60.4	<i>Methanolobus</i>	81.2	[55]
		<i>Azonexus</i>	32.6	<i>Methanosarcina</i>	18.6	
		<i>Bacteroidetes</i>	7.2			

Jingmen Dangyang Basin, China	Coal	<i>Brevundimonas</i>	17.2	<i>Methanolobus</i>	99.8	[67]
		<i>Hydrogenophaga</i>	23.5			
		<i>Acinetobacter</i>	19.9			
		<i>Bacteroidetes</i>				
	Rock	<i>Brevundimonas</i>	11.6	<i>Methanolobus</i>	99.9	
		<i>Acinetobacter</i>	23.1			
		<i>Bacteroidetes</i>	7.2			
		<i>Hydrogenophaga</i>	21.0			
	Water	<i>Methylosoma</i>	4.8	<i>Halobacteria</i>	74.7	
		<i>Candidatus</i>	8.1			
	Coal	<i>Ferribacterium</i>	5.6	<i>Methanobrevibacter</i>	14.8	
		<i>Pseudomonas</i>	16.5	<i>Methanosaeta</i>	39.2	
		<i>Rheinheimera</i>	14.6	Halobacteria	5.1	
		<i>Meniscus</i>	11.1			
Illinois Basin, United States	Water	<i>Meniscus</i>	11.0	<i>Methanobacteria</i>	85.6	[46]
		<i>Syntrophus</i>	10.0	<i>Methanococcus</i>	5.8	
		<i>Ruminococcus</i>	8.1			
		<i>Dasania</i>	4.7			
		<i>Candidatus</i>	4.4			
		<i>Crocinitomix</i>	3.6			
		<i>Desulfofustis</i>	3.5			
		<i>Thermovirga</i>	3.5			
		<i>Sulfurimonas</i>	3.4			
		<i>Desulphuromusa</i>	3.4			
		<i>Maritimimonas</i>	3.3			
		Powder River Basin, United States	Coal slurry	<i>Clostridium</i>	87.0	
<i>Spirochaetes</i>	2.7			<i>Methanobacteria</i>	NA [*]	
<i>Pelobacter</i>	NA [*]			<i>Methanocorpusculum</i>	NA [*]	
<i>Pseudomonas</i>	NA [*]					

*NA: Not Available.

Figure