Microbial methane from carbon dioxide in coal beds

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Abstract

Microbial CH_4 chemistry and its formation in coal are summarised. The results of recent research on microbial CH_4 formation are reviewed from Australia, China, Germany, Japan, and the USA. Two fields of interest are considered in this report. Enhanced microbial CH_4 production is under investigation to improve the yield of CBM. A further development is to inject CO_2 in coal seams, using microbes to convert it to CH_4 for recovery.

There is reliable evidence for the production of CH_4 through recent microbial activity in coal beds. Microbial CH_4 may be enhanced artificially and there is an incentive to turn CBM, where appropriate, to a continuously renewing system. The introduction of large quantities of CO_2 from carbon capture systems could have a favourable effect on microbial CH_4 formation. However, there are uncertainties about the supply of H_2 which is essential to form CH_4. Field and laboratory studies are in progress to address gaps of knowledge in the geology, microbiology and engineering.

Acronyms and abbreviations

BGR	Federal Institute for Geosciences and Natural Resources, Germany
BMBF	Federal Ministry of Education and Research, Germany
CBM	coal bed methane
DMF	dimethylformamide
IEA	International Energy Agency
PRB	Powder River Basin
UQ	University of Queensland, Australia

Contents

1 Introduction

Coal mine methane (CH_4) has posed a danger in working coal mines and is a stronger greenhouse gas than CO_2, but is recognised as a valuable source of energy. Coal bed methane (CBM) from unmined coal production in the USA accounts for more than 70% of global CBM production, which exceeds 59 Gm^3. The remainder is produced in descending order of production by Australia, India, Canada, China, the UK, Colombia, Russia, Ukraine, and Austria (Flores, 2008). Power plants fired with coal mine CH_4 have been operating in Australia, China, France, Germany, Poland, Russia, the Ukraine, and the USA. The most favourable outlook for further power projects was identified for Australia, China, and Germany. Many countries in Eastern Europe, Africa and South America are interested in such projects but most would require international expertise and funding to ensure their success (Sloss, 2005, 2006).

Biological processing of coal was demonstrated in the early 1980s, with bacteria solubilising part of the organic phase of hard coal in Germany and wood-rot fungi solubilising lower rank coal in the USA. Early findings showed that low rank coals were more susceptible to biosolubilisation. Additionally, this susceptibility decreased progressively with increasing rank and increased with pre-oxidation (Catcheside and Ralph, 1999). In Germany, CH_4 from active and abandoned mining areas is being used increasingly for heat and power production, especially after the introduction of the 'renewable energy law' in 2000, which gives fiscal benefits to renewable energy sources. Hence there is interest in the discovery that part of the CH_4 has been produced recently by microbes (Krüger and others, 2008). Long-term (14 months) observations in the Ruhr Basin, Germany, showed that the carbon source for microbial CH_4 might be coal, but could also be other fossil or recent biomass. Further, between 38% and 90% of the recently sampled CH_4 was of microbial origin. The proof of living methanogenic microbes in mine water made it possible that at least a portion of this microbial CH_4 had been produced recently by CO_2 reduction (Thielemann and others, 2004a).

This raises the question as to whether CO_2 stored in coal beds, after capture from power plants, could be used to enhance microbial CH_4 production as a source of renewable CH_4. If so, this CO_2 sequestration would be a means of completely utilising the coal, recycling CO_2, and would complement underground coal gasification *(see the detailed review by* Couch, 2009). These processes would also increase the global resource of useable coal for gas production.

Enhanced microbial CH_4 production concerns firstly enhanced CBM production and secondly the effects of CO_2 sequestration on microbial activity. There are many gaps in the knowledge of various aspects of this topic. These are being addressed by ongoing and planned research in several countries. More information on technologies that can reduce greenhouse gas emissions derived from the use of fossil fuels is provided at www.ieagreen.org.uk by the IEA Greenhouse Gas R&D Programme, which is also an implementing agreement of the International Energy Agency (IEA).

This review focusses on biogenic CH_4 production from coal mines. There are also thermogenic sources of CH_4 which are not discussed here. It is necessary to distinguish between the organic setting which is mainly coal and the CH_4 associated with inorganic, sedimentary rocks. The formation of microbial CH_4 and the associated chemistry is summarised from results of recent studies in Australia, China, Germany, Japan, and the USA.

2 Methane production from coal beds

Biogenic CH_4 production, or methanogenesis can occur naturally in coal beds under specific geochemical conditions in the presence of methanogenic organisms. This CH_4 may contribute to undesirable CH_4 emissions or provide a useful energy source as CBM. The biogenic CH_4 production rate may be affected by coal rank, the availability of carbon (C), the presence of microbes, nutrient limitations, temperature and pressure, as well as coal surface area. In general, biogenic gas production occurs on the order of 100s to 1000s of years, but sometimes the rates are too low to be detected (Budwill, 2008a,b).

In addition to the 'background' production of microbial CH_4 in coal bed reservoirs, there is a great potential to enhance worldwide CH_4 reserves through the chemical conversion of CO_2 to CH_4, during CO_2 sequestration. This is very much still in the research stage (Massarotto, 2009).

2.1 Microbial methane chemistry

The biochemistry of methanogenesis has been detailed in earlier reports, for example, by Whiticar (1999) and Thauer (1998). The formation of microbial CH_4 is complex. Methane is produced from the reduction of CO_2 by over five reaction paths, involving eight enzymes, mostly at pH 6.8–7.2. The simplified reaction is:

$$4H_2(g) + CO_2(aq) \rightarrow CH_4(g) + 2H_2O\ (liq) \qquad 1$$

The reaction is isothermal with a release of energy up to -140 kJ/mol, at hydrogen (H_2) and CH_4 partial pressures of 1 bar (0.1 MPa) and for $CO_2(aq)$ and $H_2O(liq)$ of 1 mol/L. As H_2 is only present naturally in low concentrations, the energy release through this reaction is usually only 0 to -20 kJ/mol (Thielemann and others, 2004a,b).

The conversion of complex substrates such as coal to CH_4 requires fermentative and acetogenic bacteria in addition to methanogens. First fermentative bacteria hydrolyse and then ferment complex substrates to produce acetate, longer chain fatty acids, CO_2, H_2, NH^+_4, and HS^-. Acetogenic bacteria consume H_2 and CO_2 to produce more acetate. In addition, they can demethoxylate low-molecular weight, ligneous materials and ferment some hydroxylated aromatic compounds to produce acetate. Acetogens which produce H_2, convert fatty acids, alcohols and some aromatic and amino acid to the H_2, CO_2, and acetate needed by the methanogens. Low rank coals are especially enriched in lower molecular weight, leachable organic compounds which may be amenable to microbial degradation (Green and others, 2008).

The autotrophic pathway (equation 1) is distinct from heterotrophic metabolism. The autotrophic pathway depends on bacteria synthesising their own food by taking up inorganic compounds. Heterotrophic metabolism obtains ready made organic food from the environment, for example, the acetotrophic or methylotrophic pathway. The simplified overall equation for the acetotrophic pathway, which releases energy, is (Hoth and others, 2005):

$$CH_3COOH \rightarrow CH_4 + CO_2 \qquad 2$$

Fermentation reactions that produce H_2 are described by:

$$(CH_2O)n + nH_2O \rightarrow nCO_2 + 2nH_2 \qquad 3$$

The reduction of CO_2 would occur only with incomplete fermentation. The hydrogenic pathway of fermentation may stimulate the autotrophic methanogens. It may hinder acetate consuming methanogens. In anoxic marine sediments, acetogenic fermentation has been described by:

$$4H_2 + 2CO_2 \rightarrow CH_3COOH + 2H_2O \qquad 4$$

A combination of acetogenic fermentation (equation 4) and acetate consuming methanogenesis (equation 2) yields a second pathway of net CO_2 transformation to CH_4 (with the net equation 1). Biogenic coal bed CH_4 generation through transformation of CO_2 to CH_4 requires large reaction areas, long time scales and the avoidance of leakage to the atmosphere. All these demands can be met in the deep subsurface. Hence this favours the use of subsurface over surface technologies (Hoth and others, 2005).

The biogenesis of CH_4 is a process that concentrates H_2 in the final reaction products. The hydrocarbon substrates partially consumed by this process have varying ratios of H_2 to C. Coal has H_2:C ratios of less than one whereas oil has H_2:C ratios closer to two. Methane has a ratio of 4:1 and clearly represents the most H_2-enriched and reduced form of hydrocarbon. Hydrogen availability is the limiting factor in estimating the resource potential of a particular substrate (DeBruyn and others, 2004).

2.2 Microbial methane formation

Methane production from a coal substrate is strictly an anaerobic process. Aerobic degradation prior to anaerobic degradation may be helpful in improving coal bioavailability, but it is not required (Gilcrease, 2010). Anaerobic organisms work in a consortium (collection of different microbial species), providing methanogens with acetate or H_2 and CO_2 that then produce CH_4. The methanogen electron-transport chain and specific enzymes formed in the process use CO_2 as the final electron acceptor and reduce it to CH_4. Salts such as sulphates promote growth of sulphate reducers, that out-compete the methanogens for H_2 and acetate, inhibiting methanogen growth. Other inhibitors include oxygen (O_2), nitrates and sulphur. In simple terms, a methanogenic coal bed consortium works as a step-wise process by which some primary fermenting and hydrolytic microbes might break down coals directly to high molecular-weight, H_2-rich compounds. These can be consumed by other groups of microbe that convert these products to organic acids. Another pathway may involve oxidation of organic matter, hosted by coal, that partially or wholly solubilises the coal into humic acids. These organic acids can be utilised by another group of bacteria to produce the acetate or CO_2 and H_2 that the methanogens would need for metabolism. It has been demonstrated that low rank coals are particularly susceptible to these initial oxidation reactions. Alternatively, wood-degrading fungi have been shown to be capable of depolymerising coal humates into low molecular-weight organic compounds, like alkanes. These could also be consumed by the consortia, producing acetate, H_2 and CO_2 (Barker and Dallegge, 2006).

The conclusion from research on methanogenic consortia is that a sensitive chemical and biological balance appears to exist. This may explain why some coals generate CH_4 at present and other coals do not, although similar in bulk characteristics. For example, in nature, formation water associated with CBM appears to have a characteristic chemical composition with alkaline pH that contains sodium and bicarbonate, but typically lacks sulphate, calcium, and magnesium. This may be due to the reduction of sulphate by sulphate reducing bacteria and isolation of calcium and magnesium by chelating agents (Barker and Dallegge, 2006).

Temperature exerts a strong control on the rate of methanogenesis. Methanogenesis rates are lower when the ambient temperature is below 15ºC. The rates and overall activity generally increase at temperatures of about 100ºC. The optimal temperature for many methanogenic communities is about 20–40ºC (Barker and Dallegge, 2006). The temperature range for methanogenic activity is commonly reported as between 4–100ºC but thermophilic methanogens may generate CH_4 at temperatures >100ºC (Faiz and Hendry, 2006). Other environmental factors that limit microbial conversion of coal to CH_4 are: pH, salinity, nutrients, trace metals, and coal surface area/bioavailability (Gilcrease, 2008).

Groundwater flow along a coal bed aquifer flushes CH_4 and CO_2 downstream differentially because of their different solubilities and adsorption characteristics; CO_2 is about 20 times more soluble than CH_4 in water at low temperatures and has a stronger adsorption in most coals. This can lead to a separation between CO_2 and CH_4 along the flow path in the coal bed. The differential transport of coal bed gases by groundwater flow along coal bed aquifers can essentially separate CO_2 from CH_4, accumulating gas rich in CO_2 at downstream or near discharge zones if gas traps exist, and retaining gas with less CO_2 content in recharge regions. This may explain the CO_2 and CH_4 distribution in the San Juan and Powder River Basins in the USA. Active groundwater flow may facilitate the late-stage coal bed gas generation by supporting microbial activities in coal seams. This is due to nutrients, such as alcohols, organic acids, and phenols, carried in meteoric water along with gas-producing bacteria which flush microbial communities in coal seams and generate variable amounts of CO_2 and CH_4. In addition, O_2 carried in meteoric water may enhance microbial oxidation and decarboxylation reactions to produce CO_2 in coal seams close to recharge regions. The generated CO_2 may be further transported to a more reducing environment in coal seams, enriched in methanogens, where it is reduced to CH_4. These activities result in coal bed gas with distinct chemical and isotopic signatures (Cui and others, 2004).

In some unique basins that host coal bed CH_4 such as the Velenje lignite basin, Slovenia, CH_4 migrates faster than CO_2 and accumulates at the subsurface. This accumulation occurs within the water-saturated layers of the Velenje basin as a free gas phase. The CO_2 remains mostly adsorbed in the coal bed structure or preferentially dissolved in water (Kanduc and Pezdic, 2005).

Significant quantities of CO_2 are generated from coals during thermal maturation. Assuming that this CO_2 remains associated with a sourcing coal bed as uplift or erosion provide conditions conducive for microbial methanogenesis, the resulting quantities of CH_4 generated by complete CO_2 reduction can exceed the quantities of thermogenic CH_4 generated by factors of 2–5 (Kotarba and Lewan, 2004).

Isotopic fractionation of the carbon and hydrogen isotopic signatures of the CH_4 is one of the most useful diagnostic tools in the determination of the CH_4 source. It is used to distinguish CH_4 generated by microbial CO_2 reduction from thermogenic CH_4 formation. Biogenic CH_4 is generally isotopically light, with $\delta^{13}C$ values less than about -55‰. However, it is affected by other factors such as the isotopic composition of the original substrate, the temperature, partial pressure of H_2, methanogenic pathways and the species of methanogens involved. More definitive insights into the origin of these gases were obtained when $\delta^{13}C$ values were combined with H_2 isotope values of deuterium (δD) for CH_4 and gas dryness indices (*see* Faiz and Hendry, 2006).

Since both microbial CO_2 reduction and methyl fermentation (aceticlastic) reactions can occur during microbial activity in coals, both C and H_2 isotopes are necessary to distinguish between these two methanogenic pathways. Microbial CO_2 reduction derives H_2 from formation water, whereas microbial methyl fermentation uses H_2 primarily from methyl groups of organic matter and secondarily from formation water. Methane has a wide range of C and H_2 isotope ratios varying from -50‰ to -110‰ for $\delta^{13}C$ and from -150‰ to -400‰ for δD. Methane resulting from CO_2 reduction may be differentiated from that derived from microbial methyl fermentation by its isotopic characteristics. A summary of previous studies (Flores and others, 2008) showed the ranges:

Microbial CH_4	$\delta^{13}C$, ‰	δD,‰
CO_2 reduction	-55 to -110	-150 to -250
Methyl fermentation	-40 to -70	-250 to -400

The most comprehensive and fundamental work on this is considered to be by Whiticar (1999) and deals with C and H_2 isotope systematics of bacterial formation and oxidation of CH_4. Also, the biochemistry of methanogenesis is summarised by Thauer (1998).

Carbon and H_2 isotopic studies of CH_4 from gases accompanying bituminous coals and anthracites in coal basins of Germany, China, the former Soviet Union, The Netherlands, Australia and Poland revealed high variability of both $\delta^{13}C$ for CH_4 from -80‰ to -12‰ and δD for CH_4 of -333‰ to -117‰. Such high isotopic variations may result from various primary (generation) and secondary (migration) processes. For example, the Upper Silesian and Lublin coal basins in Poland contain coal bed gases including CH_4 and CO_2 which had been generated during thermogenic and probably microbial processes, followed by migration and mixing. After coalification the uplifted coal beds were subject to erosion, denudation and intensive degassing (Kotarba, 2001).

2.2.1 Sources of H_2

The supply of H_2 may be the most critical factor for the formation of CH_4 from CO_2 in coal reservoirs and therefore the methanogenic conversion of sequestered CO_2 (Gilcrease, 2009). This applies to both heterotrophic and autotrophic pathways (*see* Section 2.1). The production of H_2 gas by anaerobic bacteria is a widely known and well-documented phenomenon, occurring for example in coal samples in the presence of water (Faraj and others, 2004). Further information on microbial production and oxidation of CH_4 in the deep subsurface is provided by Kotelnikova (2002). Unfortunately, no information was found on H_2 supplies in coal seams but only on minerals in sandstones and basalts

Although H_2 concentrations in the deep subsurface far exceed that in surface aquatic environments, the H_2 content in subsurface fluids and the supply from juvenile geogas is small compared to the demand for autotrophic transformation of sequestered CO_2. The H_2 concentrations in the deep fluids represent only an intermediate state since H_2 is rapidly transformed. Hence the relatively low H_2 concentrations do not imply low H_2 supply rates. Earlier data cited showed that the H_2 content for Fe(III) zones varied in the range 0.2–0.8 nmol/L for SO_4 at terminal electron accepting processes from 1 to 4 nmol/L. By contrast, the H_2 content in methanogenic areas may even reach 5–30 nmol/L (*see studies reviewed by* Hoth and others, 2005).

In sandstones, iron rich clay minerals of cements are considered to be the most reactive minerals with respect to H_2 formation. This is because water reduction is commonly related to oxidation of mineral bound ferrous iron. The mineral cements from milled rock samples from the Westfal C sandstone formation at 2800 m depth were analysed to determine their composition and ability to generate H_2. Dissolved H_2 concentrations in the tests were 10–16 µmol/L, exceeding the reported environmental H_2 contents of, for example, sulphate reducing or methanogenic aquifers and of basaltic ground waters. The concentrations of H_2 formed are comparable to the calculated H_2 fluid concentrations of the mid-ocean ridge basalt (Kassahun and others, 2007b). Thus it has been shown that H_2 is generated on iron silicates. Further experiments within the RECOBIO research project (described in Chapter 4) emphasised the generation of H_2 on iron chlorites and its fast consumption, coupled to sulphate reduction (Hoth and others, 2008).

Anaerobic incubation tests of milled rock material from potential CO_2 sequestration sites in sandstone-hosted oil and gas fields in Germany revealed the generation of up to 500 nmol H_2 per gram rock sample. Dissolved H_2 concentrations of 20–450 µmol/L exceeded that of sulphate reducing or methanogenic aquifers at 1–30 nmol/L. The rock samples contained 1 wt% chlorite and up to 35 wt% of layer silicate. The results indicated microbial consumption of the H_2 for CO_2 transformation to CH_4 (Kassahun and others, 2007a). Organic matter on silicate mineral surfaces was also likely to be an important factor causing microbial colonisation and subsequent biofilm formation through the formation of molecular H_2 (Kassahun and others, 2008).

The deep subsurface methanogenesis of sequestered CO_2 was discussed by Koide and Yamazaki (2001) for Japanese sites. Sources of H_2 could include microbial decomposition of organic matter, geochemical water-rock interaction, and deep geothermal activities. They note that biogenic methanogenesis is active even in deep aquifers in basaltic lava layers, despite the fact that these lack organic matter. In deep basaltic aquifers, methanogens might produce CH_4 from CO_2 of inorganic origin and H_2 derived from reduction of thermal water by Fe(II) in basaltic glass. CO_2 injection into deep basaltic lava layers would make the aquifer environment similar to an ancient reducing environment where methanogens were dominant under conditions of high CO_2 pressure, high temperature, and low concentrations of organic matter. Artificial stimulation of the natural deep biogeochemical carbon cycle by CO_2 injection may facilitate greenhouse gas control with the added benefit of renewable CH_4 supplies. Perhaps in the future the biogeochemical carbon cycle in basaltic layers in the oceanic crust could provide a virtually limitless CO_2 sink and a source of CH_4 (Koide, 2001).

2.2.2 Studies in different countries

The formation of microbial CH_4 has been investigated recently in Australia, China, Germany, Japan, and the USA. The results are described in the following sections.

Australia

Commercial CH_4 production from coal has become a rapidly growing industry along the eastern seaboard. Two of the main basins where deep underground mining is widespread are the Sydney Basin in New South Wales and the Bowen Basin in Queensland. Extensive reservoir testing is being conducted for other eastern Australian basins such as the Surat, Genedah, Gloucester and Otway Basins (Faiz and Hendry, 2006).

The Bowen Basin, Queensland, is a major source of CBM from bituminous coal seams. Water and gas production have been variable across the field. Stable isotope analysis and accessory water quality tests were conducted on CBM production gas and water samples collected from two of the CBM producing coal seams. Time slice mapping over a four year period led to the conclusion that the domains of higher and lower gas production could be related to compartmentalisation of the reservoir due to tectonic activity that led to folding and faulting. The samples of water and gas were geochemically analysed to determine whether there were significant differences between tectonic regions of different production behaviour (*for geological, hydrological, and experimental details see* Kinnon and others, 2009).

The gas isotope analysis showed that production gases had both biogenic and thermogenic origins and that secondary biogenic gas generated through CO_2 reduction comprised a significant portion of the CBM produced from this field. It is generally accepted that CH_4 from Australian coal seam gases with $\delta^{13}C$ compositions less than -60‰ have biogenic origins and $\delta^{13}C$ compositions greater than -50‰ are thermogenic. Dry gases (those dominated by CH_4 rather than C_{2+} hydrocarbons) are characteristic of biogenesis. Waters associated with CBM are typically highly sodic. Tests showed that water sodicity increased with depth. The highest gas producing wells were distinguished by producing a mixture of biogenic and thermogenic CH_4, a well depth of between 200 m and 300 m, and more positive CO_2-CH_4 carbon isotopic fractionations between 60.8‰ and 62.9‰. Water recharge (washing) may introduce bacteria and increase biogenesis. Areas of recharge had less positive CO_2-CH_4 carbon isotopic fractionations ranging from 48.6‰ to 57.6‰; but the residual CH_4 is either enriched or depleted in ^{13}C depending upon whether aquifer flow rate is fast or slow respectively (Kinnon and others, 2009).

The results of other studies which were carried out in the Gunndah and Bowen Basin, eastern Australia, show that the interaction of fluids with the coal seams along with the thermal degradation of organic matter has lead to formation of CO_2 and CH_4. The CO_2 generated from these processes has been incorporated into the Ca-Mg-Fe carbonates. The bulk of the CO_2 currently stored in the coal seams is of magmatic origin and variously associated with tectonism and major episodes of igneous activity. Biogenic and thermogenic alteration of organic matter, both produce CO_2 and CH_4 but the source of these products can be determined through geochemical analysis. Gas stable isotopes confirm generation of secondary biogenic CH_4 in CO_2-rich coal seams by reduction of CO_2. The relative proportions of different gases generated by thermal alteration depend on temperature and the type of organic matter. Significant CO_2 is produced through the thermal alteration of humic or coaly (type III kerogen) source rocks, whereas only minor CO_2 is generated from sapropelic (type I/IIkerogen) source rocks. The studies suggest that methanogenesis may provide an additional sequestration mechanism for CO_2 in coal seams. These coal seams with high CO_2 content are natural analogues of the processes likely to occur as a result of CO_2 injection and storage in coal systems (Golding and others, 2009a,b).

Li and others (2008) surveyed the microbial populations in some Australian CBM reservoirs (Surat, Sydney, and Port Phillip Basins). The results of polymerase chain reaction studies suggested that members of the domain *Archaea* (a unique group of microorganisms classified as archaeobacteria but genetically and metabolically different from all other known bacteria) were relatively rare in the coal samples and absent from the water samples. The dominant *archaeal* species belonged to the genus *Archaeologlobus*, an anaerobe with very weak CH_4 generating capacity. This result was unexpected as true methanogens had been detected in both the Sydney and Surat Basins. This suggested that there was potential to enhance, by artificial means, the biogenic production of CH_4 from these coal samples. Further coal and formation water samples collected from the CBM production coal basins in eastern Australia were being studied in detail to determine the mechanisms of secondary biogenic gas generation.

A review of microbial activity in Australian CBM reservoirs (*see* Fais and Hendry, 2006) summarises the processes involved in CBM formation. Geochemical data for gases and coal indicated extensive microbial activity, especially in coal seams shallower than about 600 m. Evidence suggested that CO_2 reduction was the main pathway of secondary biogenic CBM generation. There was no evidence for CBM formation from aceticlastic reactions, which differed from the results of studies of Powder River Basin (PRB) gases in the USA. The reason for the difference was not clear but the PRB coals are of lower rank and more permeable than the Australian coals. The variations in the water chemistry may also be another factor that could cause these differences.

China

The formation of secondary biological coal bed gas in the Xinji area, Anhui, China, was investigated by Tao and others (2007). The biogenic component of the coal bed gas was estimated at 60.1% to 68.5% of the total CH_4 generated. The coal bed gas was super-dry, which is the characteristic of secondary biogenic gas. By contrast, the thermogenic gas was wet from the thermal evolution of the coal. Intense tectonic uplift, faults and erosion in the studied area created favourable conditions for the infiltration of surface water, the abundance of microbes and hence the formation of secondary biogenic CH_4. The excess of biogenic over thermogenic CH_4 is attributed to the uplift of the coal beds or erosion of their overburdens with the presence of CO_2. The concentration of CO_2 in the coal bed gas samples from the Xinji area was only 0.51% to 1.93%, less than the 5% found in the Sydney and Bowen Basins in Australia.

Isotopic tests indicated that the CO_2 was of organic origin and derived from the coal bed. The content of N_2 in the coal bed gas was 2.86% to 42.4% and it had a strongly negative linear correlation with CH_4. This indicated that the N_2 was mainly from the atmosphere and was supported by the isotopic composition of N_2 and by helium data. As N_2 is a major component of the atmosphere, there is abundant N_2 dissolved in the surface water. Nitrogen can infiltrate into underground coal beds with the permeation of the surface water. The existence of atmospheric N_2 in the coal bed gas indicated an effective infiltration of surface water and favourable conditions in the coal bed for the bloom of microbes and formation of secondary biogenic gas, which can also be attributed to the rapid tectonic uplift and erosion in the area. The Xinji area coal bed gas was comprised of secondary biogenic gas, thermogenic gas and some atmospheric N_2. The proportion of secondary biogenic CH_4 was estimated at 60.1% to 68.5% and that of thermogenic CH_4 at 31.5% to 39.9% (Tao and others, 2007).

Germany

The formational history of coal seam gas in the Ruhr Basin was investigated using methods detailed by Thielemann and others (2004a,b). Isotope and gas wetness analyses indicated that the coal seam gas was a mixture of thermal and microbial CH_4. The latter was formed through CO_2 reduction and comprised 38–90% of the total coal seam and coal mine CH_4. The occurrence of coal seam gas varied over the three regions of the Ruhr Basin. In the centre, hard coal had been mined for 800 years, and intensively since 1850. This area showed a high proportion of microbial CH_4. In another area, coal had been mined only since 1900 and the CH_4 production showed only a small microbial signature. A third area had not been affected by mining. The microbial CH_4 from this area was <40%, similar to data from other regions, such as in Belgium, Turkey, and Australia. Further investigations were required to determine the timing of the CH_4 formation, although living CH_4 producing microbes were found in the mine water. Hence the CH_4 appeared to be at least partially of recent origin.

Krüger and others (2008) measured *in situ* isotopic signatures and CH_4 fluxes in a coal mine in the Ruhr Basin which had been abandoned and sealed in the 1960s. They collected samples for microbiological and phylogenetic investigations of the microbial populations in order to determine the presence and intensity of recent methanogenesis. The mine water sometimes contained mine timber or coal pieces. Gas emissions over different coal areas had a $\delta^{13}C\text{-}CH_4$ value of -39.8‰ and with mine timber -49.5‰. Control sites in the mine without coal or timber showed no CH_4 emissions. The potential H_2 substrate was not detected in any of the gas samples collected underground. Laboratory incubations showed a similar range of carbon isotopic signatures for coal and timber. Molecular techniques revealed that the archaeal community in enrichment cultures and unamended samples was dominated by members of the *Methanosarcinales*. The combined geochemical and microbiological investigations identify microbial methanogenesis as a recent source of CH_4 in abandoned coal mines.

The relative dominance of the hydrogenotrophic or acetoclastic pathway in coal and timber degradation was investigated. The addition of acetate to the incubations lead to a strong and relatively rapid stimulation of CH_4 production, while the effect of H_2 was less pronounced. The isotopic signature of the CH_4 produced was comparatively stable. Overall, this indicated that acetate might be a central intermediate in the degradation processes of timber and coal. Decomposition of natural organic compounds or hydrocarbons by anaerobic bacteria produces acetate and H_2 which are consumed by methanogenic *Archaea*. Hence the acetate and H_2 are maintained at very low concentrations. The results of the community species analysis confirmed that, contrary to previous observations, acetate was a central intermediate in recent mine gas formation. Since hard coal is effectively sterilised due to the high temperatures during its formation, the best explanation for the presence of diverse communities was a re-colonisation introduced by mining activities. Also, the transport of microbes through water in faults might possibly provide a further source of microbial life, as postulated for example in gold mines. Even after the end of mining activities, O_2 remains for a long time in the system. This residual O_2 probably initiates weathering of the coal and timber, thus facilitating a subsequent microbial degradation under anoxic conditions (Krüger and others, 2008).

A further study (*for details see* Beckmann and others, 2010) aimed to understand the microbial processes involved in mine gas formation in abandoned mines. It found that a complex community of procaryotes was involved in CH_4 formation, inhabiting distinct ecological niches in the sealed compartments of the coal mines. The results provided a basis for a deeper understanding of the underlying processes and timescales involved, and should help to determine more reliable estimates of global CH_4 inventories. The microbiological community analysis as well as the environmental conditions and the metabolites detected in the previous study (*see* Krüger and others, 2008) were consistent with the following scenario of CH_4 release:

- weathering of coal and timber is initiated by wood-degrading *fungi* and *bacteria* under a suboxic atmosphere;
- in the lower, O_2-depleted layers, f*ungi* and *bacteria* perform incomplete oxidation and release reduced substrates which can be channelled into methanogenesis;
- acetate appeared to be the main precursor of the biogenic CH_4 in the investigated coal mines.

Both the microbial community and the abundance of H_2 determine the extent of the CH_4 formation process. For example, the H_2 supply may result from the weathering of ferrous iron silicates in some aquifer systems. Different physiological types of microbes occur in the subsurface. The CO_2 reducing methanogens may compete with or benefit from acetate consuming methanogens, sulphate reducing and iron reducing bacteria, or fermenting organisms. The coupling between microbial metabolism and carbonate precipitation, which occurs in strongly reduced systems at higher CO_2 concentrations, is predominantly related to non-methylotrophic methanogenesis (Hoth and others, 2005).

Japan

The deep unmineable coal beds in Hokkaido, are both a potential CO_2 sink and untapped energy resource. Injected pure CO_2 behaves readily as a supercritical fluid around injection wells in deep coal seams. Supercritical CO_2-enhanced coal seam gas recovery and *in situ* production of microbial CH_4 could produce CH_4 for a closed-circuit power plant with zero CO_2 emissions (Koide, 2001; Koide and Kuniyasu, 2006; Koide and others, 2003).

In 2002, Japan launched a pilot project on CO_2 enhanced CBM production in Yubari, Hokkaido, in anticipation that CO_2 injection may stimulate microbial CH_4 generation by CO_2 reduction. One CO_2 injection well and one CBM production well have been installed and gas production has been monitored since 2004. The CO_2 injected has not been detected in the CBM production (CO_2 breakthrough). However, breakthrough of injected N_2 gas was observed. This is probably because N_2 migrates in coal seams faster than CO_2 and has lower adsorption to coal. The change in microbial community structures before and after N_2 injection as a substitute for CO_2 was compared (*for details see* Shimizu and others, 2007).

The isotopic ratios of the Yubari CBM implied an abiotic, thermogenic origin. However, the presence of methanogens in the coal seam was suggested by methanogenic enrichment cultures and gene sequences, most of which were closely related to methanogenic *Archaea* and acetogenic/H_2 generating bacteria in association with methanogens. This was consistent with the idea that most of the CBM was formed under high temperature and high pressure and that methanogenic and associated microbial populations developed with the decrease of *in situ* temperature due to the uplift of coal seams and/or increased flow of groundwater (Shimizu and others, 2007).

USA

The Powder River and San Juan Basins, USA, are two of the most productive CBM reserves in the world. Geochemical and isotopic indicators establish that the gas in these basins contains microbial CH_4. The microbial biodegradation of the coal beds is a significant component of the total gas resources in the San Juan Basin and the sole source for the shallow coals of the PRB. Localised hydrological conditions and subsurface geology are likely to play important roles in controlling the extent of biodegradation and of methanogenesis. Biodegradation of hydrocarbons coupled with methanogenesis may develop regardless of the organic matter source across a range of inherited thermal maturities in the coal. The highest degrees of biodegradation were confined to particular coal seams, suggesting that zones of biodegradation were ultimately controlled by stratigraphic variation in the subsurface. These variations included fractures and cleat systems in coals, allowing localised groundwater flow through particular strata, and stimulating microbial biodegradation of hydrocarbons (Formolo and others, 2008).

The biodegradation signatures preserved in the coals did not specifically follow observed trends in petroleum, crude oil, or in shale-hosted gas reservoirs. They showed elevated biodegradation of aromatic compounds prior to complete or extensive removal of more aliphatic compounds in both thermally mature and thermally immature coal seams. More research is necessary to link the signatures of organic matter biodegradation to active microbial methanogenesis (Formolo and others, 2008).

Flores and others (2008) investigated the composition and isotopes of gases and co-produced water from samples collected basin wide from varied CBM coal reservoirs in the PRB, Montana and Wyoming. They linked the analytical data to the basin wide coal geology and stratigraphy. Their conclusions, relating to recent CH_4 formation, were that:

- gas generated from CO_2 reduction accumulated and was dominant in the centre of the basin;
- thermogenic gas migrated into the deep, central part of the basin and mixed with microbial gas, forming gas with a transitional isotopic signature;
- groundwater recharge events continued to the present;
- groundwater recharge events, also previously interpreted to have played an important role in the generation of microbial gas from CO_2 reduction along the eastern basin margin, played an important role in the generation and accumulation of younger gas derived from methyl fermentation in the northwestern part of the basin;
- several generations of methanogenesis produced younger gases from methyl fermentation along basin margins that overprinted older gas derived from mixed CO_2 reduction and methyl fermentation.

Geological factors play an important role in the origin of the coal bed gases. These include the direction of groundwater recharge, depth of burial, thermal and maturation history, lateral and vertical continuity of stratigraphic units, degree of faulting and fracturing, coalification processes, and the natural burning of the coal beds. All these factors influence the extent, methanogenesis, gas composition, gas generation, accumulation, and preservation of these resources. Thus, ongoing and future microbiological, biogeochemical, and hydrological studies must correlate their data and results to the coal geology, structural geology, and stratigraphy of the basin in order to be meaningful (Flores and others, 2008).

The effect of coal beds of differing thermal maturity on the origin of coal bed gases was investigated by Strąpoć and others (2007) in the southeastern Illinois Basin, Indiana, USA. The gradient of thermal maturity in the Illinois Basin gives rise to biogenic and/or thermogenic coal bed gases depending on coal properties and geologic setting. The gas generation pathways in Illinois Basin coals at lower maturity in Indiana were compared with those with higher maturity in western Kentucky. Biogenic CH_4 from CO_2 reduction was prevalent in the less mature Indiana coals, whereas more mature coals from western Kentucky produced predominantly thermogenic hydrocarbons through cracking of coal organic matter. The two different types of coal bed gas were distinct in gas and isotopic composition. The fast fluid exchange with oxygenated surface waters in Indiana coal beds makes it unlikely that hydrocarbon gases have been accumulating since the deposition of Pennsylvanian coal swamps. Instead, the presence of viable methanogens in coal waters with abundant CBM, suggests that biogenic CH_4 in Indiana coal beds is continuously forming as a quasi-renewable form of fossil energy, although the rate of *in situ* CH_4 generation remains unknown.

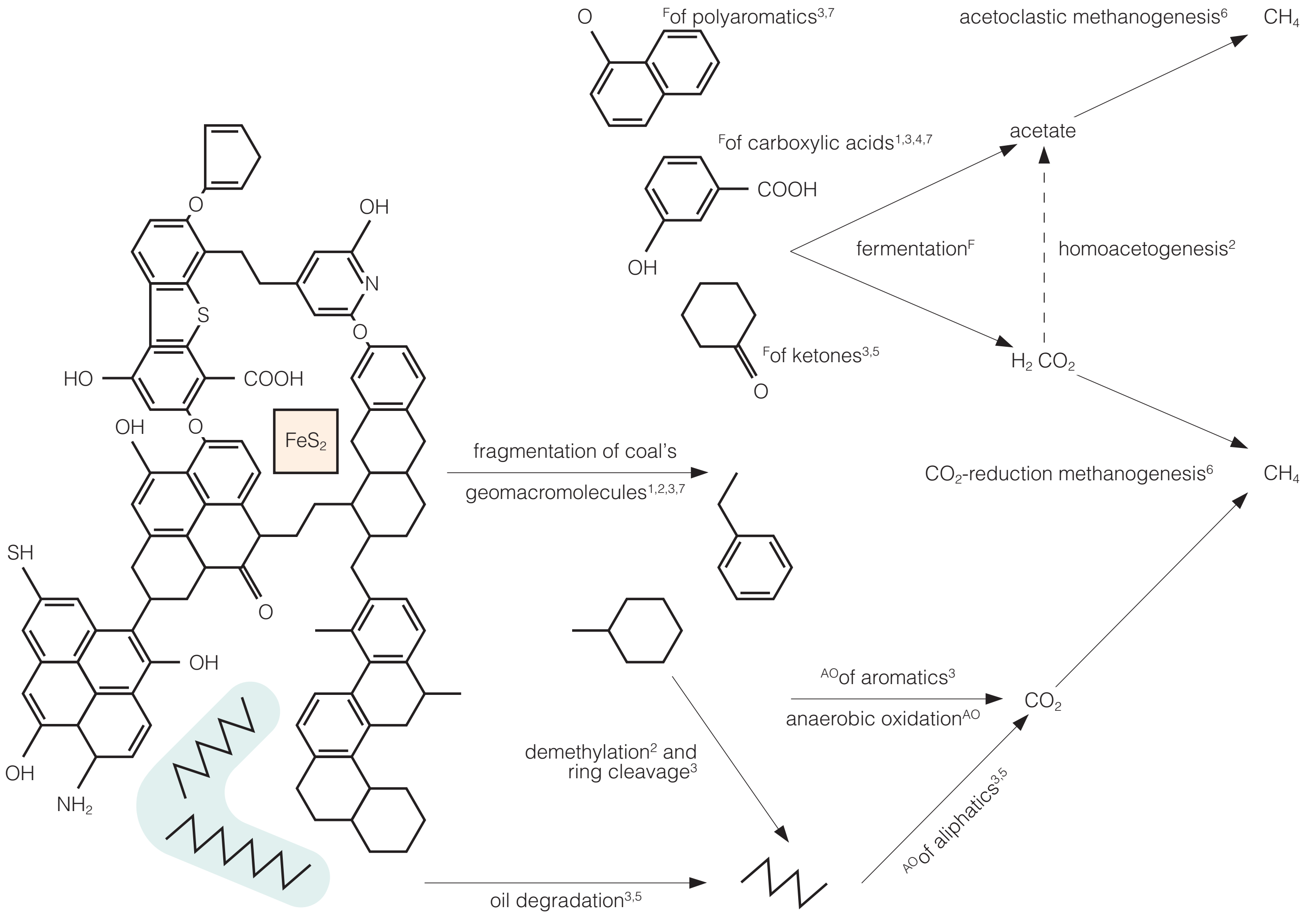
The isotopic results of tests on the coal bed gas from Indiana indicated microbial methanogenesis by CO_2 reduction. A plausible scenario for decomposition of coal organic matter by microbial consortia leading to methanogenesis through CO_2 reduction could be that (Strąpoć and others, 2007):

- methanogens require elemental H_2 and CO_2 which can be produced by other microbes;
- H_2 and CO_2 are probably provided by cooperating species that decompose organic matter; such as fermenting anaerobes which generate CO_2, acetogens producing H_2, proton reducers and others.

Predominant macerals of Indiana coals, and especially vitrinite, are suitable substrates to support microbial generation of H_2 and CO_2 by fermenters and acetogens, thus providing the raw materials for methanogenesis as a terminal step of organic matter decomposition in coal. Alternative supplies of H_2 could result from thermal maturation of underlying marine shales, rich in organic matter, or may be generated by deep metamorphic processes. If that were the case, methanogenic *Archaea* could generate CH_4 in the subsurface independently from bacterial consortia decomposing organic molecules to CO_2 and H_2 (Strąpoć and others, 2007).

Molecular and geochemical studies were performed on microbial communities and CBM throughout the CBM formations in the eastern Illinois Basin. The results suggest that organic matter is biodegraded to simple molecules, such as H_2 and CO_2 which fuel methanogenesis, principally by *Methanocorpusculum*, generating large CBM reserves. The rate limiting step of coal biodegradation is the initial fragmentation of the macromolecular, polycyclic, lignin-derived macromolecules which tend to be relatively resistant to degradation. Lignin degradation can be achieved by extracellular enzymes used by fungi and some microbes. It has also been shown that up to 40% of the weight of some coals can be dissolved using extracted microbial enzymes. Enrichments have been developed capable of anaerobic degradation of methylated and ethylated aromatic compounds or even polycyclic aromatic hydrocarbons. In the study of the eastern Illinois Basin, enrichments from coal waters showed high rates of CH_4 generation and the presence of abundant methanogens. Anoxia, low salinity, and moderate temperatures (about 17ºC here) are common characteristics of all described *Methanocorpusculum* niches. However, the species is tolerant of temperatures as low as 1ºC, although it grows primarily in the range 25–35ºC. Proposed mechanisms for stepwise biodegradation of organic matter in coal are shown in Figure 1. The data from the study suggested that the contribution of acetoclastic methanogenesis to bulk CH_4 generated was minor (Strąpoć and others, 2008).

The presence of minerals in coal cleats may have great significance for CH_4 generation and extraction from coal beds. This is because mineral fillings affect fluid flow and permeability, and the isotopic composition of authigenic (secondary) calcite is diagnostic for the onset of microbial methanogenesis relative to calcite mineralisation. Although coal bed gas CO_2 in the Illinois Basin, Indiana, features strong ^{13}C-enrichment in the presence of microbial CO_2 reduction, the available $\delta^{13}C_{calcite}$ values are not in thermodynamic equilibrium with modern coal bed CO_2. Relatively negative $\delta^{18}O_{calcite}$ values indicate that calcites in Indiana coal cleats crystallised at a time when CO_2 in coal beds was far more ^{13}C-depleted than it is today. Apparently microbial fractionation of CO_2 through CO_2 reductive methanogenesis



^{1}spirochaeta, ^{2}sporomusa, ^{3}cytophaga, ^{4}acidoaminococcus, ^{5}flavobacterium, ^{6}methanocorpusculum, ^{7}rhodobacter(?)

Figure 1 Proposed mechanisms for biodegradation of organic matter in coal (Strąpoć and others, 2008) did not occur at the time of calcite crystallisation, possibly owing to prior sterilisation of coal during deep burial and heating. This suggests that inoculation of uplifted coals with methanogenic microbial consortia and the onset of microbial methanogenesis were relatively late events. This further supports the work of Strąpoć and others (2007), suggesting the previously heat-sterilised sedimentary environment had not yet been inoculated with methanogenic microbial consortia and that microbial methanogenesis may be a relatively recent development. Inoculation with CO_2-reducing methanogens must have occurred when microbes were migrating with fluids from cooler strata above the coal seams. Prior, discontinuous mineralisation along cleats may have preserved the cleat framework, kept pathways open for fluid migration, and thus facilitated access of methanogenic bacteria into coals. On the other hand, cleat mineralisation could also inhibit the migration of fluids, including that of coal bed gases by sealing the faults (Solano-Acosta and others, 2008).

Laboratory experiments on subbituminous, humic coals from Alaska resulted in additional, CH_4-rich gas generation after adding nutrients to the coal cuttings and canister water and culturing the microbial consortia under anaerobic conditions. The canister water contained common, fluorescent, rod-like microbes comparable to *Methanobacterium* species and rod, cocci and spherical forms of microbes attached to the coal surface. These microbes apparently represented at least a portion of the microbial consortia needed to depolymerise coal, as well as to generate the observed secondary CH_4 emission (Barker and Dallegge, 2006).

3 Enhanced microbial methane production

The benefits of enhancing biogenic CH_4 production rates are to reverse declining production in older wells, improve it in coal seams with low gas content and possibly increase permeabilities. A further development is to inject CO_2 for storage in coal seams, using microbes to convert it to CH_4 for enhanced recovery. Field trials are necessary to demonstrate the technology (Budwill, 2008a,b).

3.1 Enhanced CBM production

Investigations aimed at enhancing CBM reserves, but not yet recycling CO_2, are also pertinent to the subject of this review. Extensive laboratory-based studies have confirmed that the introduction of a suitable nutrient stimulates the growth and activity of indigenous methanogens, leading to the real-time generation of new CH_4 from coal. Research is therefore in progress to develop a technology which would involve the injection of nutrients into deep, unmineable coal beds (*see* Figure 2) or other unconventional reservoirs for clean energy generation (Budwill, 2008a).

The nutrient needs to be introduced into the target reservoir seam to ensure that it comes into contact with the native population of bacteria and methanogens as much as possible in order to achieve a successful field demonstration of the methanogenesis technology. However, microbial activity typically concentrates close to the nutrient injection point. This produces a limited amount of CH_4 from easily solubilised and metabolised geological matrix components. Microbial activity is also limited to areas where water is retained within the reservoir pore-fracture network. These conditions may mean that a large portion of available reservoir surface may not be utilised within the formation because it is beyond the zone of nutrient and microbial influence. This limits new CH_4 generation (Budwill, 2008a).

A patent entitled ‘Biogenic fuel gas generation in geologic hydrocarbon deposits’ (US 12/136,728) by Luca Technologies Inc (LUCA, 2009a,b) covers methods of cultivating CH_4 production from geologic deposits such as coal, oil or shale by:

- collecting a methanogenic bacterial consortium from water extracted from an underground hydrocarbon formation, under anaerobic conditions;
- using the anaerobic microbial concentrate to inoculate another geologic hydrocarbon deposit; and
- harvesting the CH_4 that the consortium produces over time from anerobic water within the deposit or from the well head space.

An analogous patent, covering the same aspects of microbially enhanced CH_4 production methods has been issued in New Zealand (Patent No. 5638868). Initial success with these methods in cultivating CH_4 production under field conditions in the Wyoming coal beds suggests this technology offers the potential for long-term, large-scale sustainable energy generation (LUCA, 2009b).

Traditional extraction techniques for CBM production entail pumping groundwater out of the coal beds. A damaging and unforeseen consequence of this pumping is that it can allow

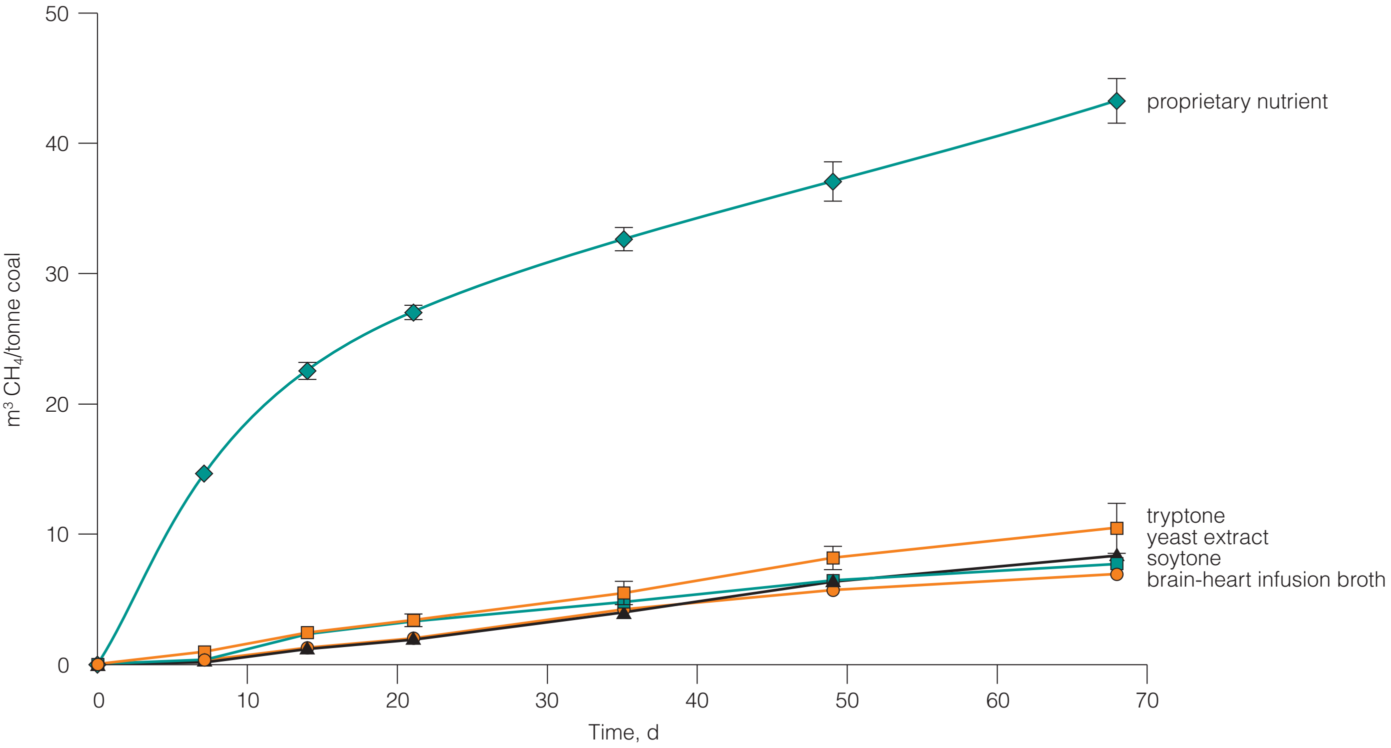


Figure 2 Enhanced CH_4 production in coal-based methanogenic cultures grown with crushed coal and amended with nutrients (Budwill, 2008a)

the influx of air, containing abundant free O_2 which is toxic to the CH_4 producing microorganisms. Removal of water also damages the microorganisms. LUCA has established a library of anaerobic core samples from known and suspected geobioreactors for study and experimentation, using genomics and modern biotechnology. Developing a better understanding of the biology and ecology of these organisms should hopefully lead to the ability to create functional geobioreactors from currently barren or non-CH_4 producing hydrocarbon reservoirs. It is believed by LUCA that well managed, functional geobioreactors not only have the potential to meet the need for CH_4 but that it may also be possible to engineer CH_4-producing organisms genetically to produce free H_2 instead as their final product (LUCA, 2009a).

The low rank, high permeability, and high water content of PRB coal suggest that seam conditions are still favourable for CH_4 biogenesis. A fundamental understanding of the processes of biogenesis could lead to enhanced CH_4 production (Green and others, 2008).

Earlier experiments with coal cores from the PRB of northeastern Wyoming were described by LUCA (2004). Biogenic CH_4 production could be increased rapidly and substantially by the addition of specific nutrients and other amendments in laboratory experiments. In addition, laboratory results suggested that formation water had an as yet unidentified role in stimulating biogenesis – perhaps acting as a conduit of nutrients to entrenched microbes within the coal. A better understanding of how this microbial community interacts with its environment and how its well-being could be managed through prudent production practices was considered paramount in efforts to produce clean energy from the methanogenic bioreactor. Laboratory evidence for recent biogenic CH_4 formation in oil is described by DeBruyn and others (2004).

The patented Arctech Process converts coal into CH_4 and humic substances. Natural microorganisms are adapted to digest coal under anaerobic conditions, resulting in a mixture of CH_4 and humic substances. The microbes have been engineered from the digestive tract of termites. The humic-rich carbon by-product may be used to improve soil fertility, replenish water, and neutralise munitions, converting them into organic fertiliser. A wholly owned subsidiary, Humaxx, has been formed by Arctech, Inc to market clean CH_4 and humic-rich products. Further information is available at www.arctech.com and www.humaxx.com (Chopra, 2009).

Bioconversion is accomplished by a three-step process, using appropriate nutrient components (Humaxx, 2009):

1. microbes convert the coal into volatile organic liquids, by a hydrolytic and fermentation process;
2. the liquids, along with gases produced, are contacted with CH_4 producing microbes (methanogens) that hydrogenate the acetate and CO_2 into CH_4;
3. the CH_4 is separated and unconverted residual coal residue is converted into humic acid for formulating agricultural and environmental products.

Coal-culturing experiments reported by Barker and Dallegge (2006) indicated that it may be possible to use simple nutrients, such as yeast extract, to stimulate the indigenous methanogenic consortia as an alternative to injecting alien methanogen cultures. They calculated that if they could achieve a one percent conversion of PRB coals by nutrient stimulation, they could increase the recoverable CBM by around 13–30%.

Studies have shown that the overall biodegradation rates for organic solids can be limited by the solid-liquid mass transfer rate when the microbial concentration is high and/or the solid surface area is low. Hence the rate and quantity of CH_4 generated from coal may depend on the exposed surface area, and the insolubility and impermeability of coal represent major constraints. Although the diameter of coal pores ranges from 0.04 to 30 μm many of these pores are too small to permit microbial entry (<0.2 μm). Thus microbial access is mostly limited to the cleat surfaces of the coal. Experiments were carried out to demonstrate the presence of viable methanogenic consortia in PRB CBM seams, capable of using coal as the primary substrate; determine which intermediates are utilised by the methanogens; characterise different members of the microbial consortium; evaluate the effects of temperature, pH, and particle size on biomethane production from coal; and determine if the system is mass transfer limited and, if so, evaluate solvents for enhancing these rates *(for details see* Green and others, 2008).

Well-bore water samples from the PRB tested positive for the presence of living microbial communities, capable of generating CH_4 from the coal under laboratory conditions. Methanogens, acetogens and fermentative species were identified. The results for the effects of temperature, pH, particle size and enhancing solvents are shown in Figure 3. Increasing the incubation temperature from 22ºC to 38ºC, increased CH_4 production by 300%. Lowering the pH of the culture medium from 7.4 to 6.4, increased the rate of methanogenesis by 680%. Increasing the coal particle surface area by 890% (through smaller particle size), increased CH_4 production rates by over 200%. Microbial CH_4 production in coal slurries was also enhanced by the addition of the solvent N,N-dimethylformamide (DMF). These results suggest an opportunity to enhance CBM reserves by stimulating the activity of existing methanogenic consortia *in situ*. In particular, reservoir treatments that enhance coal solubility, dissolution rates and/or surface area may be beneficial (Gilcrease, 2008; Green and others, 2008).

3.2 CO_2 sequestration

The CO_2 sequestered in reducing, deep environments may serve as electron acceptor and carbon source for microbial pathways. Methane formation represents a long-term transformation to an energy source, while autotrophic sulphate reduction is coupled to the problem of acid gas generation (H_2S). The main question is the long term biogeochemical transformation which was studied at a gas field, Schneeren, in Germany. This is connected to Upper Carboniferous, compacted and hence low permeable, coal-bearing sandstones. Sulphate in the formation waters sustains an active community of sulphate reducing organisms. Sulphate reduction is the main process due to high sulphate

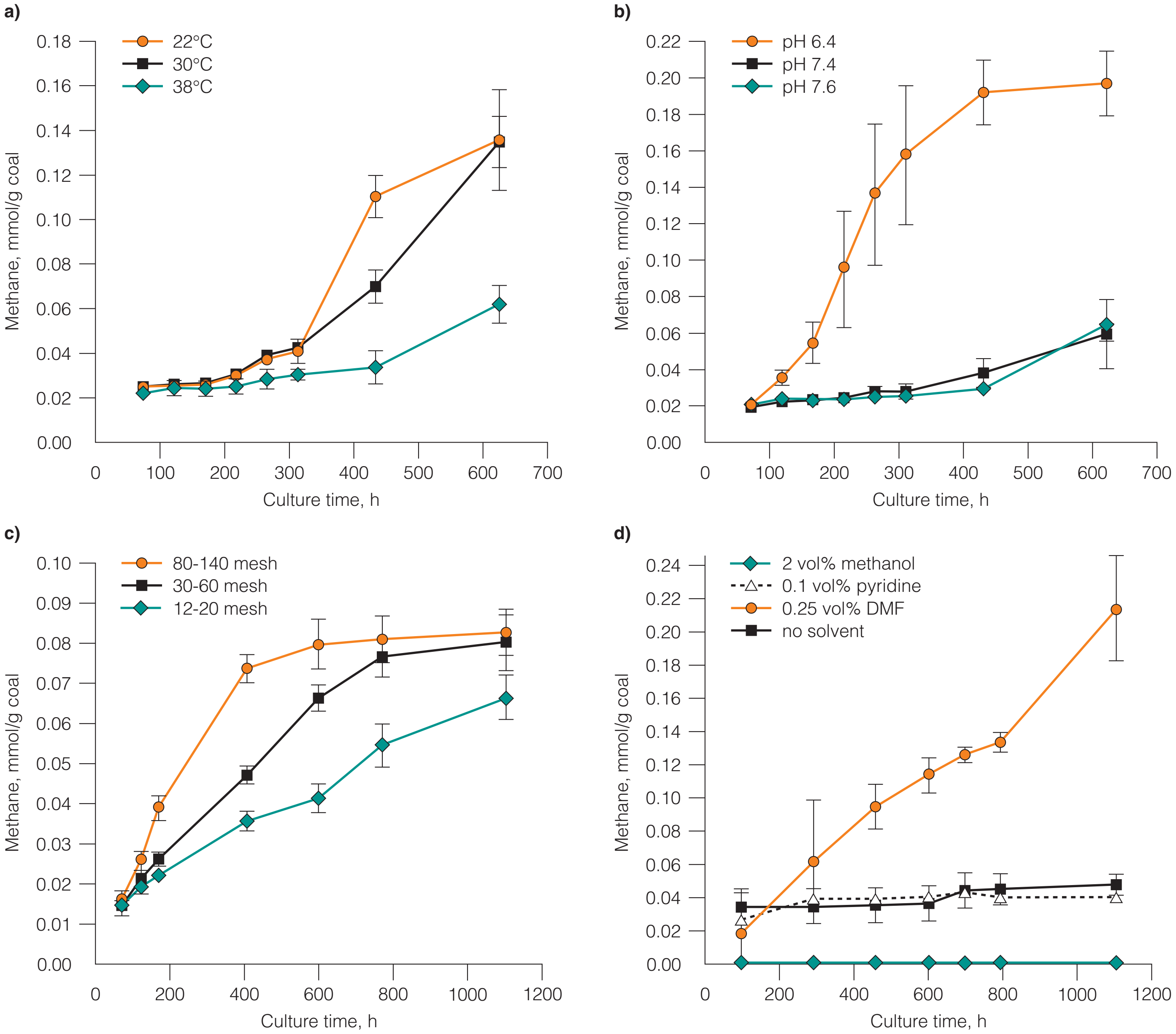


Figure 3 **Effect of temperature, pH, particle size, and enhancing solvents on microbial CH_4 production** (Green and others, 2008)

concentrations in the formation waters. After depletion of the sulphate, CH_4 formation can start. Generally the processes are linked to carbonate formation through a shift within the pH values and the supply of cations. These carbonate phases can result in an important increase of the sequestration capacity (Hoth and others, 2007a,b).

There is insufficient knowledge of the impact of sequestrated CO_2 on the deep biosphere. The occurrence of microorganisms in gas field fluids was therefore studied by Ehinger and others (2009) to obtain more fundamental knowledge of the microbial processes in potential CO_2 sequestration sites. Active microbial cells were absent in gas field fluids with high salinity (239 g/L Cl^-) and low pH (4.9) but present in those with lower salinity and higher pH values. A consortium of fermenting and sulphate-reducing bacteria together with the methanogenic *Archaea* were involved in the complex degradation processes in the gas reservoir fluids. Only two methanogenic genera dominated, *Methanlobus* and *Methanoculleus*, in the Schneeren gas field. This combination had also been reported for a deep coal seam aquifer at Yubari in northern Japan (Shimizu and others, 2007).

The abundance of various sulphate reducing, fermenting and methanogenic microorganisms, coexisting in the deep saline, sulphate-rich gas field fluids, indicate that the gas reservoir Schneeren harbours microbial activity despite the extreme physicochemical conditions. Variability in the composition of the methanogenic community shows their adaptation to altered environmental conditions and the dynamic within these methanogenic processes. Sequestration of CO_2 in such deep gas reservoirs will change physical and chemical parameters. For example, a cooling effect is predicted in the storage formation due to the expansion and evaporation of the CO_2 after injection. These cooling effects can be significant in fissures and cavities if CO_2 is migrating upwards. Cooling may decrease microbial activity (*see* Section 3.1) although small temperature changes could enhance microbial activity in some regions of the reservoir. There is some evidence for release of minerals and dissolved metals, as well as organic material which would affect microbial activity. It is not currently possible to predict the effect of the injection of large amounts of CO_2 on the biogeochemistry in the subsurface because investigations are at an early stage (Ehinger and others, 2009).

Natural CO_2 occurrences have three principal sources: mantle or mantle-derived igneous rocks, metamorphism or dissolution of marine carbonate-bearing sedimentary rocks, and modification of organic material. Coal seams containing high CO_2 contents occur in many coal basins worldwide, for example, in the Gunnedah and Bowen Basin, eastern Australia (*see* Section 2.2). High purity CO_2 deposits (>90%) occur in several fields in the US (Colorado), Hungary (Pannonian Basin), France (Massif Central) and Turkey (West and East Anatolia). Also, in central Italy, CO_2 dissolved in cold spring waters is derived largely from deep, mantle related sources. Nearly pure high concentrations of CO_2 occur in Permian gas fields of Germany, connected to Late Tertiary volcanism. These basins provide natural analogues of the processes likely to occur as a result of CO_2 injection and storage in coal systems (Golding and others, 2008, 2009a,b).

The CO_2 is stored predominantly as adsorbed molecules on micropore surfaces (adsorption trapping). These allow higher densities and greater volumes at shallower depths than in sandstone and carbonate reservoirs where CO_2 is stored initially as a free phase (structural/stratigraphic trapping). In the long term, CO_2 dissolves in formation water and reacts with minerals in the host formation (solution/ionic trapping) and may be precipitated as carbonate minerals (mineral trapping). Natural analogue studies in the Bowen and Gunnedah Basins in Australia indicate that magmatic CO_2 has been stored in coal and sandstone formations since the Mesozoic through a combination of adsorption and mineral carbonation reactions. Gas stable isotopes confirm generation of secondary biogenic CH_4 in CO_2-rich coal seams by reduction of CO_2, suggesting that methanogenesis may provide an additional sequestration mechanism for CO_2 in coal seams (*see* Section 2.2). These observations compare well with those seen previously in the Sydney Basin (Golding and others, 2008, 2009a,b).

The mineralogy and geochemistry of high CO_2 coal seams in the Bowen and Gunnedah Basins were compared with adjacent low CO_2 coal seams of the same formation. Experimental details are described by Golding and others (2009b). The study aimed to determine the impact of high CO_2 contents on the coals and the mechanisms that kept the CO_2 naturally sequestered. Hydrogen isotope compositions of CH_4 were used to distinguish between microbial CO_2 reduction and methyl fermentation. The CH_4 δD values ranged from -213‰ to -223‰ in the low CO_2 hole and -214‰ to -221‰ in the high CO_2 hole. The more negative $\delta^{13}C$ values of CH_4 from the high CO_2 hole (mainly <-60‰) relative to the low CO_2 hole (mainly >-60‰) suggested that CH_4 in the high CO_2 environment formed by microbial reduction of CO_2, when considered with the δD values. This may indicate that CH_4 generated by coalification has been displaced by CO_2 of inorganic origin with the current CH_4 in place largely the result of subsequent microbial CO_2 reduction.

The experiments carried out at the Yubari site, northern Japan (*see* Section 2.2) used N_2 injection at one well and subsequent production of N_2 at the other as a substitute for CO_2. This affected the genetic diversity of the methanogenic community, as well as the pH and Eh in the groundwater of the production well. This evidence suggested that N_2 injection into the coal seam might affect the cycling of organic matter by methanogens *in situ*. Similarly, the planned large-scale CO_2 injection (and breakthrough) would affect groundwater properties and methanogenic community structure. The degree and extent of the possible influence of CO_2 sequestration should be assessed and the recoverability of geochemical and microbial properties after CO_2 sequestration should also be estimated (Shimizu and others, 2007).

4 Ongoing and planned research

Research is in progress and planned in several countries to address critical gaps in our current knowledge of the effects of CO_2 injection on microbial CH_4 formation. These include:

- potential fracturing of the coal seams;
- dissolution of constituents in coal and their bioavailability;
- destruction of permeable networks in the coal structure;
- potential increase in H_2 availability to counteract the H_2 limiting factor in coal;
- H_2 from water.

Australia

A research project is in progress at the University of Queensland (UQ) on underground conversion of CO_2 to CH_4, using coal as a substrate. The scope is (Massarotto, 2009):

1. active promotion of microbial CH_4 technology as a research project to local CBM companies;
2. collection and analysis of formation waters from coal fields, which has confirmed the presence of methanogenic bacteria;
3. the coal and water samples were provided to Dr Patrick Gilcrease, South Dakota School of Mines, USA, and he has confirmed good quantities of biogenic CH_4 generation in preliminary tests. This collaboration may extend to a one-year sabbatical at UQ in 2010;

Details of a project using coals as CH_4 bioreactors, initiated with Gilcrease, are summarised by Golding (2009). The gas to supply the proposed Gladstone liquefied natural gas facilities will require an enormous area to be drained of water and then CH_4 – an area that will need to be continually expanded as sections of the coal measures are exhausted of their gas inventory. Preliminary research results suggest that CH_4 can be regenerated *in situ* within exhausted areas. This essentially creates a sustainable supply of coal seam CH_4 and permits the reuse of wells and associated infrastructure. Moreover the regeneration occurs in a remarkably short period of time, within weeks in a controlled laboratory environment. It uses microorganisms which occur naturally and are already present within the coal seams. The aim is to expand the preliminary results and develop them to methods suitable for field development. A successful outcome would significantly increase coal seam CH_4 production and reserves, and reduce the cost of their recovery.

The approach proposed for this project is to confirm and characterise the processes involved in microbial CH_4 generation in the Walloon coal measures in the Surat Basin, Queensland, as a basis to understand and develop the concepts, preceding work on other coal types, ranks and measures (not part of this project). Field studies and laboratory experiments will address the following geology, microbiology, and engineering gaps (Golding, 2009):

- evaluate the distribution of microbial CH_4 resources, the role of geology and formation water chemistry in microbial CH_4 generation, and the geochemical signatures of associated gas and formation waters;
- define the main biogeochemical processes active in the Walloon coal measures and the relative importance of the acetate fermentation and CO_2-reduction pathways in generating *in situ* microbial CH_4;
- undertake coal seam and formation water metagenomics in conjunction with sequencing of isolates to improve understanding of biogeochemical processes and ecosystem functioning in medium-depth coal seams;
- determine the reaction rates, coal to CH_4 conversion factors and yields, and the nutrient and environmental requirements and limits (including temperature and pressure), of living microbial consortia from the coal seams to elucidate the potential for microbially enhanced coal seam gas; and
- evaluate potential physical and chemical reservoir treatments to stimulate the *in situ* activity of methanogenic consortia in coal seams, determine their effect on CO_2 permeability/injectivity and CH_4 permeability/productivity at wells, and on the overall fluid transport process in the reservoir.

Canada

The Alberta Research Council (now part of Alberta Innovateas – Technology Futures)has been investigating and developing technology to enhance biogenic CH_4 production, or methanogenesis, in deep unmineable coal beds and other unconventional reservoirs for clean energy generation. Further research is planned to (Budwill, 2008a):

- enhance nutrient delivery and dispersion;
- maximise microbe-coal interaction;
- utilise robust geochemical monitoring tools to monitor the process.

A continuous flow-through column that can simulate reservoir conditions of elevated pressure and groundwater movement will be used to conduct the experiments involving nutrient introduction. The end result will be a methodology for *in situ* methanogenesis which will be transferable to different geological conditions and realise the full potential of CH_4 production (Budwill, 2008a).

It is important to understand the microbial ecology and methanogenesis processes so that *in situ* conditions can be modified to enhance microbial CH_4 production to economically viable amounts. Proposed research therefore includes (Budwill, 2008a, 2010):

- identification of the native microbial species in unconventional reservoirs using DNA sequencing techniques;
- linking the assemblage of microbial species with the reservoir geochemistry;
- participation in a metagenomics project that will look at community structure and function of coal beds and other energy-related environments such as oil sands and oil reservoirs (*see* www.hydrocarbonmetagenomics.com).

The outcomes of this research will allow for the prospecting of unconventional reservoirs for the stimulation of methanogenesis. This may be in the form of ensuring that

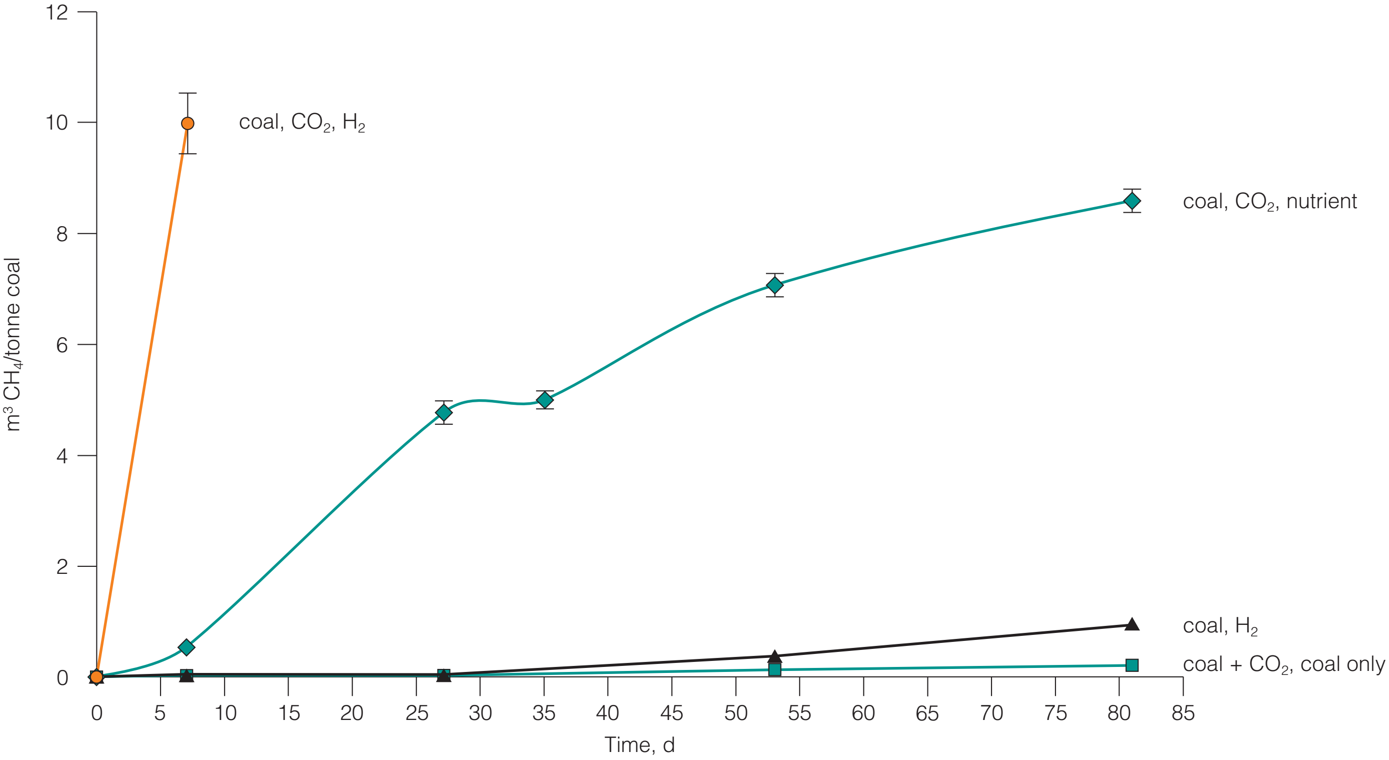


Figure 4 The importance of H_2 in methanogenesis (Budwill, 2008b)

active methanogenic communities are present in the target coal seam; or identifying the dominant bacterial and/or *Archaeal* species and customising nutrient amendments to stimulate growth and metabolic activity of the species. The research will also allow for the development of strategies for *in situ* monitoring, without which analysis of the complex interactions between changes in nutrient availability, naturally occurring microbial communities, and geological matrix properties cannot be quantified during a field trial, much less understood (Budwill, 2008a).

The injection of CO_2 for storage requires H_2 for enhanced production of CH_4 (*see* Figure 4). Hence coal containing CO_2 and H_2 produced CH_4 more quickly than coal with CO_2 and nutrients (Budwill, 2008b).

Future research is required to (Budwill, 2008b):

- determine the optimum nutrient injection procedure to provide greater nutrient distribution and to maximise the CH_4 production potential;
- assess water saturation characteristics;
- understand the effect of coal surface chemistry on methanogenic activity, including which components are being degraded and limits to biofilms;
- model the geochemistry of biogenic coal bed environments;
- determine the microbial ecology of coal bed environments.

Germany

The Federal Ministry of Education and Research (BMBF) has been funding the RECOBIO project which is linked to a national R&D programme called GEOTECHNOLOGIEN (*see* http://www.geotechnologien.de). Research within RECOBIO 1 was carried out from 2005 to 2008 by the Technical University of Freiberg, Dresden Groundwater Research Centre and the GEOS Ingenieurgesellschaft mbH, Freiberg. The recycling of sequestered CO_2 by microbial transformation in the deep subsurface was studied. Sub-projects included a laboratory investigation of the biogeochemical CO_2 transformation with molecular-genetic methods and the impact of mineral reactions on the microbial and biogeochemical transformation of sequestered CO_2. The long-term biogeochemical reactions are important to interpret the resulting pressure reductions in CO_2 which might otherwise be interpreted as leakage in the storage reservoir (Ehinger, 2009; Hoth, 2009; Hoth and others, 2005).

The research is continuing within RECOBIO 2 which focuses on the biogeochemical transformation of injected CO_2 in the deep subsurface, including induced carbonate phase formation and the importance of accompanied impurities in the stored CO_2. The Technical University of Freiberg and the Federal Institute for Geosciences and Natural Resources (BGR) are investigating the effects of elevated CO_2 partial pressures on the efficiency of CH_4 production in different coal and gas reservoirs (GEOTECHNOLOGIEN, 2009; Hoth, 2009; Krüger, 2009).

The influence of microbial processes, as well as fluid/CO_2 interactions and reservoir topology, determine the storage efficiencies and long-term safety for the sequestration of CO_2 in underground reservoirs. The consequence of changes in these parameters under elevated CO_2 concentrations are being studied at the BGR within the frame of the EU-Network of Excellence CO_2GeoNet (Krüger, ND).

Japan

The research and development programme on ‘underground microbial CO_2 sequestration and CH_4 factory’ (*see* Section 2.2) was scheduled for the period 2002-04 (Koide and others, 2003). Shimizu and others (2007) note that gas production at the Yubari site has been monitored since 2004. No further information has been found on any subsequent research plans.

USA

Collaboration is in progress between the University of Queensland, Australia, and the South Dakota School of Mines, USA (*see above and* Massarotto, 2009). Gilcrease (2009) speculates that when coal is used as a source of C and H_2 for microbial CH_4, the system is H_2 limited rather than CO_2 limited, and that adding CO_2 does not enhance CH_4 yields. He is currently in the process of evaluating this hypothesis using thermodynamics, stoichiometry, and laboratory experiments. However, if a geological source of reduced hydrogen (other than coal) is available at a site, then the addition of CO_2 could stimulate additional methane production. No further information on research plans for this topic has been obtained from US researchers contacted.

5 Conclusions

There is reliable evidence for the production of CBM through recent microbial activity in coal beds. However, the conditions to initiate, support, and enhance this production are not fully understood. Nevertheless, microbial CH_4 may be enhanced artificially and techniques have been patented in the USA and New Zealand. The usual CBM production methods of removing water prevent further microbial CH_4 formation. There is an incentive to turn CBM, where appropriate, to a continuously renewing system by changing the production methods and by stimulating production.

The introduction of large quantities of CO_2 from carbon capture systems could have a favourable effect on microbial CH_4 formation. However, there are uncertainties about the supply of H_2 which is essential to form CH_4. Research is in progress to determine the effect of CO_2 storage on microbial CH_4 formation. The coal seams in Australia with high CO_2 content, and containing recent microbial CH_4, provide natural analogues of the processes likely to occur as a result of CO_2 injection and storage in coal systems.

Methane production from coal beds

The microbial formation of CH_4 in coal seams is widely accepted. There is great potential for increasing CH_4 reserves through the chemical conversion of CO_2 to CH_4, by enhanced methanogenesis. However, this is still in the research stage. The formation of microbial CH_4 from complex substrates such as coal requires several steps. First fermentative bacteria hydrolyse and then ferment complex substrates to produce acetate, longer chain fatty acids, CO_2, H_2, NH^+_4, and HS^-. Acetogenic bacteria consume H_2 and CO_2 to produce more acetate. In addition, they can demethoxylate low-molecular weight, ligneous materials and ferment some hydroxylated aromatic compounds to produce acetate. Acetogens which produce H_2, convert fatty acids, alcohols and some aromatic and amino acid to the H_2, CO_2, and acetate needed by the methanogens to produce CH_4. In terrestrial, coal-bearing basins, the transformation of CO_2 to CH_4 requires large reaction areas, appropriate microbial communities and formation water chemistry, long time-scales, and a reservoir that restricts the escape of CH_4 to the atmosphere. These demands can be met in the deep subsurface. The biogenesis of CH_4 is a process that concentrates H_2 in the resulting hydrocarbon products. Coal has H_2:C ratios of less than one whereas oil has H_2:C ratios closer to two. Methane has a ratio of 4:1 and represents the most H_2-enriched form of hydrocarbon molecule. Hydrogen availability is the limiting factor in estimating CH_4 resource potential.

Formation water associated with CBM in nature appears to have a characteristic chemical composition. It has alkaline pH, containing sodium and bicarbonate, but typically lacks sulphate, calcium, and magnesium. The temperature range for methanogenic activity is 4–100ºC. The optimal temperature for many methanogens is between 20ºC and 40ºC. Thermophilic methanogens may generate CH_4 at temperatures >100ºC. Other environmental factors that limit microbial conversion of coal to CH_4 are: pH, salinity, nutrients, trace metals, and coal surface area/bioavailability

Isotopic fractionation and gas wetness or dryness are commonly used to distinguish CH_4 generated by CO_2 reduction from thermogenic CH_4 sources. Biogenic CH_4 is generally isotopically light, with $\delta^{13}C$ values less than about -55‰. However, it is affected by other factors such as the isotopic composition of the original substrate, temperature, partial pressure of H_2, methanogenic pathways, and the species of methanogens involved. The origin of these gases may be determined in conjunction with H_2 isotope values of δD for CH_4 and gas dryness indices. This differentiates between microbial CO_2 reduction, deriving H_2 from formation water, and microbial methyl fermentation using H_2 primarily from methyl groups of organic matter and secondarily from formation water.

Hydrogen formation from water reduction is commonly related to oxidation of mineral bound ferrous iron and may be a process occurring in several environments. This process has been observed in reactions involving cements of sandstones in potential CO_2 sequestration sites in Germany. It has also been shown that organic matter on silicate mineral surfaces is likely to favour microbial colonisation through the formation of molecular H_2. The deep basaltic aquifers in Japan lack organic matter but show active biogenic methane formation. The H_2 may be derived from reduction of water by iron in basaltic glass.

Secondary biogenic CH_4 formed through CO_2 reduction comprises a significant portion of the CBM produced in eastern Australian coal basins. There is no evidence for CBM formation through the acetate pathway. The coal seams with high CO_2 content are natural analogues of the processes likely to occur as a result of CO_2 injection and storage in coal systems. Interaction of fluids with the coal seams and thermal degradation has lead to formation of CO_2 and CH_4 with the CO_2 incorporated into the Ca-Mg-Fe carbonates in long-term storage. Evidence suggests that there is potential for artificially enhancing the biogenic production of CH_4 from CO_2 injection into coals.

The recent microbial CH_4 formation in the Powder River and San Juan basins in the USA did show a minor contribution from the acetate pathway for CH_4 formation although the CO_2 pathway dominated (*see* Figure 1). Differences from the Australian results may be due to the lower rank and greater permeability of the US coals, and possibly variations in water chemistry. In the US microbial bio-degradation of the coal (providing H_2 and CO_2) was stimulated by localised groundwater flow through fractures and cleat systems.

Isotopic tests of coal bed gas in the Xinji area, China, indicated that the CO_2 was derived from organic matter in the coal but there was less CO_2 than in the Australian coal basins. Uplift of the coal beds or erosion of their overburdens with CO_2 created favourable conditions for infiltration of surface water and microbes for CH_4 formation. In Hokkaido, Japan, isotopic ratios of the CBM suggested that it had mostly formed under high temperature and pressure but that methanogenic and associated microbial populations proliferated at lower temperatures following the uplift of coal seams and/or increased flow of groundwater.

The occurrence of recently formed coal seam gas varies over three regions of the Ruhr Basin in Germany. It is considered that this variability may be partly due to differences in the mining activities. The microbial contribution of CH_4 seems to be more pronounced at sites of active and especially abandoned coal mining than at unmined places. An abandoned coal mine where the mine water contained both mine timber and pieces of coal showed evidence for CH_4 production through both H_2 and acetate. The best explanation was that residual O_2 after mining activities initiated weathering of the coal and timber, facilitating a subsequent microbial degradation under anoxic conditions. The CO_2 reducing methanogens may compete with or benefit from acetate consuming methanogens, sulphate reducing and iron reducing bacteria, or fermentation processes. The processes of CH_4 formation and other microbial processes can generally be linked to carbonate formation which can substantially increase the CO_2 sequestration capacity.

Enhanced microbial methane production

New CH_4 may be generated from coal by introducing a suitable nutrient such as yeast extract to stimulate indigenous microbial activity. Research is in progress in Canada to inject nutrients into deep, unmineable coal beds to stimulate CH_4 formation (*see* Figure 2). There are limitations caused by microbial activity concentrating close to the nutrient injection point, and large areas of available reservoir surface may be unavailable due to lack of water.

Enhanced CBM production processes have been patented in the USA, by Luca Technologies Inc and Arctech Inc and in New Zealand. Successful CH_4 production under field conditions in the Wyoming coal beds indicates that the Luca technology offers the potential for long term, large scale sustainable energy generation. Laboratory studies suggest that formation water has an as yet unidentified role in stimulating biogenesis – perhaps acting as a conduit of nutrients to microbes in the coal. However, traditional extraction techniques for CBM production entail pumping groundwater out of the coal beds. The removal of water damages the CH_4 producing microorganisms and can allow the influx of air which is toxic to them. LUCA considers that well managed, geobioreactors have the potential not only to meet the need for CH_4 but that it may also be possible to engineer CH_4 producing organisms genetically to produce free H_2 instead as their final product. The Arctech Process converts coal into CH_4 and humic substances, using microorganisms. In addition to the CH_4, the humic substances may be used to improve soil fertility, replenish water, and neutralise munitions, converting them into organic fertiliser.

The rate and quantity of CH_4 generated from coal may depend on the exposed surface area. The insolubility and impermeability of coal are major constraints. Many coal pores are too small to permit microbial entry (<0.2 µm). Access to microbes is mostly limited to the cleat surfaces of the coal. Experiments have investigated the most favourable conditions for methanogenic consortia in PRB coal seams producing CBM to determine if the system is limited by mass transfer and to evaluate solvents to enhance these rates (*see* Figure 3). Increased temperature, lower pH, increased coal particle surface area (smaller particle size), as well as adding a solvent, increased CH_4 production.

Injection of CO_2 for storage in coal seams and using microbes to convert it to CH_4 for recovery is under investigation. The effects of CO_2 sequestration on the deep biosphere are not well known. It is not currently possible to predict the effect of injecting large amounts of CO_2 on the biogeochemistry in the subsurface because investigations are at an early stage. Sequestration of CO_2 in deep gas reservoirs, for example in Germany, will change physical and chemical parameters. A cooling effect is predicted in the storage formation due to the expansion and evaporation of the CO_2 after injection. Microbial activity is affected by small temperature changes, release of minerals and dissolved metals as well as organic compounds. The Japanese tests with N_2 injection as a substitute for CO_2 suggested that large-scale CO_2 injection would affect groundwater properties and methanogenic community structure.

Natural analogues of the processes likely to occur as a result of CO_2 injection and storage in coal basins are provided by coal seams containing high CO_2 contents in Australia, France, Hungary, Turkey, and the USA. These environments provide the natural framework to study, test, and implement both CO_2 sequestration methods and CH_4 formation. They will therefore continue to be the focus of future research.

Research plans

Preliminary research results in Australia suggest that CH_4 can be regenerated within exhausted CBM production areas using naturally occurring microorganisms which are present within the coal seams. The aim is to expand the results and to develop methods suitable for field deployment. Field studies and laboratory experiments will address gaps of knowledge in the geology, microbiology and engineering associated with coal bed CH_4 reserves. Nutrient introduction to enhance microbial CH_4 production to economically viable amounts is planned in Canada. The recycling of sequestered CO_2 by microbial transformation in the deep subsurface is under investigation in Germany. This will include induced carbonate phase formation and the importance of impurities in the stored CO_2 as well as the effects of elevated CO_2 partial pressure on the efficiency of CH_4 production. Storage efficiencies and long-term safety for sequestration of CO_2 in underground reservoirs are being studied. No further information has been found on current and planned research in Japan and the USA.

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