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Biogenic methane potential for Surat Basin, Queensland coal seams

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ABSTRACT

Produced water samples collected from Surat Basin coal seams in eastern Queensland, Australia were shown to contain viable microbial consortia with the ability to convert native Walloon coal into methane. Methane generation rates of up to 1.0 m^3/t coal/day (30 scf/ton/day) were observed, with overall yields up to 6.5 m^3/t coal (210 scf/ton); in comparison, total methane reserves for the Surat Basin are typically 4 to 8 m^3/t. This is the first direct evidence of real-time biogenic coal-to-methane potential for an Australian coal seam sample. Six of the eight Surat Basin water samples tested positive for biomethane production when H_2–CO_2 was provided as the sole methanogenic substrate; five produced methane from methanol, but acetoclastic methanogenesis was not observed. An active H_2–CO_2 pathway could have implications for the conversion of sequestered CO_2, if an *in-situ* source of reduced hydrogen is present in excess. In a simple experiment, biomethane production from a Walloon coal was not enhanced when the bicarbonate concentration in the medium was doubled. It is anticipated that the low surface area/solubility of coals may limit their bioavailability; coal-to-methane rates and final yields increased in proportion to the coal particle surface area. Similarly, when a Zonyl FSN surfactant was added to improve coal bioavailability, the initial methane production rate increased by 240%, and the final methane yield increased by 180% in comparison to a no-surfactant control. Overall, these results suggest an opportunity to enhance Surat Basin coalbed methane reserves via *in-situ* stimulation of indigenous microbial consortia.

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1. Introduction

1.1. CBM production in the Surat Basin

Over 64% of the proven and probable '2P' coalbed methane (CBM) reserves in Australia are in the Surat Basin, Queensland (Geoscience Australia, 2010). The Surat Basin CBM industry has experienced significant growth over the past decade; the vast extent and greater permeability of shallow, low-rank Walloon coals makes them an attractive target for low-cost and potentially sustainable CBM development. The Queensland Government's implementation in 2007 of a 'Smart Energy Policy' provided further impetus for exploration, by mandating that gas-fired generation contributes 15% of the state's power supply by 2010 (Queensland Government, 2011). Walloon CBM contributed approximately 93PJ to Queensland's energy supply in 2009–2010, over 43% of the state's annual total CBM production, and there are plans to expand current production for liquefied natural gas (LNG) export (Queensland Government, 2011).

1.2. Geologic context

The Walloon Coal Measures are laterally extensive across the Surat and Clarence-Moreton Basins in southern Queensland and northern New South Wales (NSW) (Fig. 1). Most CBM production occurs in the north and east of the Surat Basin in Queensland, generally at depths of 200–600 m below surface. Historically, up to 30 named seams were recognized in the Surat Basin (Scott et al., 2004); however, correlation proved difficult due to the discontinuity, variable thickness, and complex cross-sectional geometry of individual coal seams or plies (Fielding, 1993; Jones and Patrick, 1981). Consequently, there is now a tendency towards correlation of coal-rich intervals and their combination with overbank facies, commonly referred to as 'seam groups'. In our study area, the Walloon Coal Measures have been promoted to the Middle Jurassic Walloon Subgroup of the Injune Creek Group (Jones and Patrick, 1981; Scott et al., 2004). Coals are concentrated in the lower Taroom Coal Measures, and the upper Juandah Coal Measures (Fig. 2). Coal rank ranges from subbituminous to high-volatile bituminous (Beeston et al., 1997; Salehy, 1986; Scott et al., 2007).

Surat Basin methane is produced from multiple seam groups across the Taroom and/or the Juandah Coal Measures. For the Dalby and Roma fields sampled in this study, the top of the Walloon Subgroup is less than 100 m and 100–400 m below ground level,

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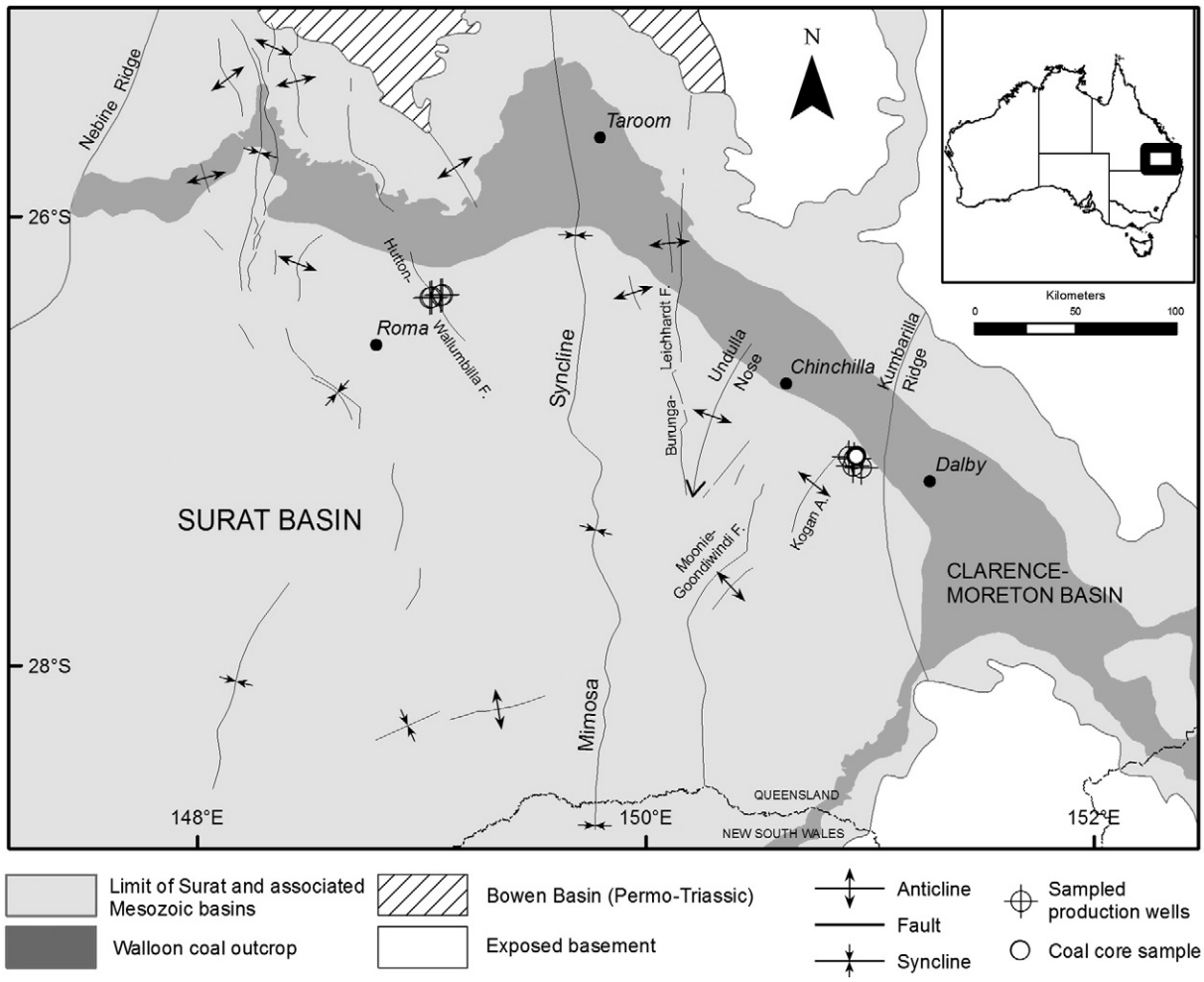

Fig. 1. Map showing location of Surat and Clarence-Moreton Basins, and subcrop/outcrop of the Walloon Coal Measures/Walloon Subgroup (dark grey). The study wells are located near the towns of Roma and Dalby.

respectively. An average Dalby CBM well contains 20–30 m of net coal, while an average Roma well contains ~15 m; individual coal plies can be up to 3–4 m thick (Draper and Boreham, 2006). Coal permeability is variable; values up to 200 mD have been recorded in the Roma field, while measured permeabilities in the eastern Surat range from <1 to >500 mD (Scott et al., 2004). Coal permeabilities in one well near the Dalby sampling sites ranged from 0.07 to 42.38 mD. Walloon methane is mainly biogenic in origin, and was generated primarily via the CO_2 reduction pathway (Draper and Boreham, 2006). The carbon isotope compositions for methane span a narrow range from $\delta^{13}C$ −57.3‰ to −54.2‰ (n = 9). The corresponding hydrogen isotope compositions for methane also fall within a narrow range (δD −215.5‰ to −203.3‰) (Draper and Boreham, 2006). It is generally accepted that thermogenic methane has a carbon isotope composition higher than −50‰ and biogenic methane has a carbon isotope composition lower than −60‰, due to the preferential consumption of ^{12}C by methanogens. Intermediate compositions may be produced by mixing of biogenic and thermogenic gases or secondary processes such as water-flushing and thermal cracking of bitumen (e.g., Boreham et al., 1998, 2001; Faiz and Hendry, 2006; Flores et al., 2008; Golding et al., 1999; Kinnon et al., 2010; Smith and Pallasser, 2001; Whiticar, 1996, 1999). The minor C_{2+} components from Walloon coals have carbon isotope compositions ranging from −43.9 to −24.5‰. These values fall within the narrow isotopic range of natural gases from the Bowen and Surat Basins, which were sourced from Permian coals (Draper and Boreham, 2006). The intermediate Walloon methane compositions could reflect mixing with migrated Permian thermogenic gases, or alternatively, early stage thermogenic gas generation from the Walloon coals themselves (Draper and Boreham, 2006). In this context, it is

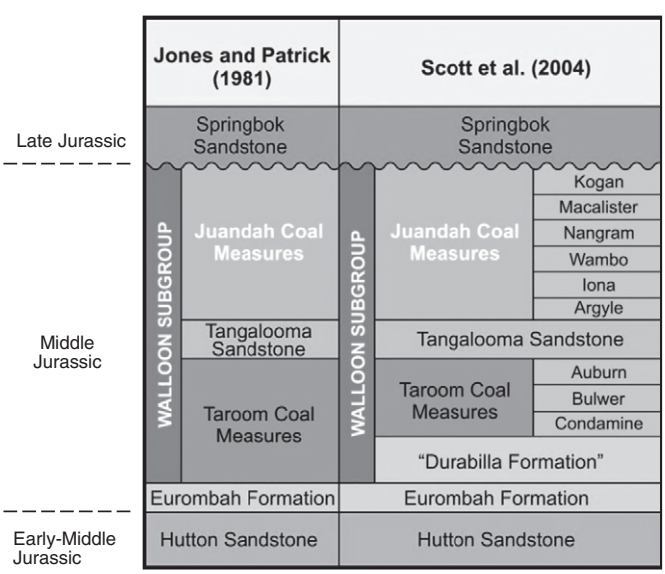

Fig. 2. Litho-stratigraphy of the Walloon Subgroup.

possible that thermogenic gas migrated from a reflectance high (0.70%; Beeston et al., 1997) in the central-south of the Surat Basin in Queensland.

1.3. Evidence of viable methanogenic consortia in coal seams

Microbially enhanced coalbed methane (MECoM) is defined as the introduction of microbial nutrients and/or microbial consortia into coal beds, with the goal of stimulating the *in-situ* biological conversion of coal to methane (Scott, 1999). For the conversion of complex organic substrates (such as coal) to methane, fermentative and acetogenic bacteria are required in addition to the methanogens (McInerney and Bryant, 1981); this collection of different species in syntrophism is referred to as a microbial consortium. The introduction of a new microbial consortium may not be necessary, as indigenous microbial populations may already be present in many situations (Faison, 1992). Specifically, methanogen DNA has been recovered from US coal seams in the Gulf Coast (Jones et al., 2010), Illinois (Strąpoć et al., 2008), and Powder River (Klein et al., 2008) Basins, as well as from coal seams in Canada (Penner et al., 2010) and Japan (Shimizu et al., 2007). Culture tests on coal/water samples from various sites demonstrate not just the presence of viable methanogens (McIntosh et al., 2008; Shimizu et al., 2007; Strąpoć et al., 2008), but also complete microbial consortia capable of converting native coal to methane given the proper environment and nutrient supplements (Green et al., 2008; Harris et al., 2008; Jones et al., 2010; Penner et al., 2010). There is limited evidence for viable methanogenic consortia in Australian coal seams to date; methanogens were detected in a Gippsland Basin sample, but the consortium did not demonstrate coal-to-methane activity when tested with a non-native coal (Midgley et al., 2010). Conversely, Li et al. (2008) found no genuine methanogens in coal or water samples taken from the Sydney, Surat and Port Phillip Basins. Overall, the observed rates (up to 0.5 m^3/t coal/day or 16 scf/ton/day) and yields (up to 4.2 m^3/t or 133 scf/ton) for some laboratory coal-to-methane cultures (using native coals and consortia) are quite promising, and suggest this could lead to an economical process for generating new methane *in-situ*. A summary of basic MECoM concepts, along with an overview of the research and development needed for commercialization, is provided elsewhere (Gilcrease and Shurr, 2007; Scott, 1999; Strąpoć et al., 2011).

1.4. Coal bioavailability may limit methanogenesis rates/yields

When dealing with complex solid substrates such as coal, physicochemical barriers (such as a phase boundary) between the substrate and the microorganisms can limit the rate and/or extent of biodegradation; this is defined as limited *bioavailability* (Volkering et al., 1998). Chemically, coal is characterized by an irregular, lignin-like polymer structure, with a limited fraction of biodegradable moieties (phenolics, carboxylic acids, alkanes, etc.) (Faison, 1992); others have proposed coal-to-methane biodegradation pathways that highlight coal structures most susceptible to microbial attack (Jones et al., 2010; Strąpoć et al., 2008; Strąpoć et al., 2011). Levels of dissolved organics in coal seam waters are quite low (Orem et al., 2007), and much of the internal coal surface area is not accessible to microorganisms (Faison, 1992; Scott, 1999). Fragmentation of the coal geopolymer into soluble, biodegradable intermediates may require hydrolytic exoenzymes located on or near the microbial surface (Faison, 1992; Jones et al., 2010; Strąpoć et al., 2008). As such, the physical separation of aqueous microorganisms and internal coal surfaces may represent significant mass transfer limitations and lead to limited coal bioavailability. For the bioconversion of Wyodak coal to methane, methanogenesis rates increased with coal particle surface area, suggesting the overall rate is limited by the coal dissolution rate (Green et al., 2008). Jones et al. (2008) report that only 0.25% of the total carbon in a Texas coal sample was bioavailable; for a Wyodak coal culture, biomethane production did not resume after the addition/replacement of nutrients/microorganisms. In a follow on study (Jones et al., 2010), they report that the methanogenesis plateau coincided with the depletion of organic precursors, and argue that without modification, the remaining coal was not bioavailable. The concept that coal bioavailability may limit methanogenesis rates/yields has practical implications regarding the nature and efficacy of potential field stimulation treatments (Gilcrease and Shurr, 2007; Green et al., 2008).

1.5. Motivation for CO_2 injection and possible outcomes

There is keen interest in using coal seams for CO_2 sequestration, as coal has a high capacity for CO_2 adsorption, and CO_2 can displace methane as a means of secondary methane recovery (Tsotsis et al., 2004; White et al., 2005). Some have speculated that if the H_2–CO_2 methanogenic pathway is active, the injected CO_2 could serve as a methanogenic substrate, resulting in a net increase in *in-situ* biogenic methane production (Budwill et al., 2003; Stricker et al., 2008). There is isotopic evidence that CO_2 from magmatic and hydrothermal sources was emplaced into coal seams in the eastern Australian coal basins and served as a biomethane substrate (Draper and Boreham, 2006; Faiz et al., 2007; Golding et al., 2011); that said, it is not known whether a new addition of CO_2 will result in a stoichiometric increase in biomethane generation. While the hydrogen atoms incorporated into methane during biological CO_2 reduction are derived from the formation water (Whiticar et al., 1986), a reduced form of hydrogen (H_2) is still needed to drive the reaction, as shown in Eq. (1) (Table 1). As such, the addition of CO_2 will not stimulate additional biomethane production unless CO_2 is the limiting reagent, and H_2 is present in excess. When inorganic H_2 is present from magmatic or hydrothermal sources, biomethane production from CO_2 can occur in the absence of other reduced organic matter (Shock, 1990; Winfrey, 1984); however, when the sole source of H_2 is the anaerobic fermentation of organic matter, CO_2 is produced in excess, and H_2 is the limiting methanogenic substrate, as illustrated by the overall fermentation of glucose to methane in Eq. (2) (Table 1). The H:C ratio for glucose is 2:1, and there is a net production of 3 mol CO_2 per mole of glucose; in comparison, the H:C ratio for a subituminous rank coal is approximately 1:1. Even if biomethane production is primarily from select coal substrates such as alkanes or long chain fatty acids, such compounds have the same H:C ratio as glucose. As such, it seems unlikely that H_2 would be present in excess if the sole source of H_2 for methanogenesis is the microbial fermentation of coal substrates. Unless there are significant H_2 fluxes to/within a coal seam, it is not reasonable to expect that injected CO_2 will lead to a stoichiometric increase in biomethane generation (Klein et al., 2008).

1.6. Goals of this study

The goals of this study were as follows: (1) demonstrate that viable methanogenic consortia are present in Surat Basin coalbed methane seams, and are capable of using native Walloon coal as the sole carbon-energy substrate; (2) determine which methanogenic pathway(s) are active; (3) determine if the bioavailability of Walloon coal can be enhanced, leading to increased methanogenesis rates/yields; and, (4) determine if added carbon dioxide results in a net increase in the amount of biomethane generated.

Table 1
Methanogenic reactions (Zinder, 1984, 1993).

Stoichiometry	$\Delta G^{\circ\prime}$ (kJ/mol CH_4)	
$4\,H_2 + HCO_3^- + H^+ \rightarrow CH_4 + 3\,H_2O$	−136	Eq. (1)
$C_6H_{12}O_6 + 3\,H_2O \rightarrow 3\,CH_4 + 3\,HCO_3^- + 3\,H^+$	−135	Eq. (2)
$4\,CH_3OH \rightarrow 3\,CH_4 + HCO_3^- + H_2O + H^+$	−105	Eq. (3)
$CH_3COO^- + H_2O \rightarrow CH_4 + HCO_3^-$	−31	Eq. (4)

2. Materials and methods

2.1. Sample collection for inocula

Produced water samples were collected from four wells near Roma, Queensland on January 7, 2009, and a second set were collected from four wells near Dalby, Queensland on January 8, 2009 (Fig. 1). Due to the multi-seam completion approach, the majority of samples were mixed waters from both the Juandah and the Taroom Coal Measures. The Dalby and Roma water samples were sourced from 97 to 459 m and 124 to 682 m below ground level, respectively. Water samples were collected in one quart mason jars (Ball) containing 0.2 g $Na_2S{\cdot}9H_2O$ solids as a reductant; the jars were purged with nitrogen and autoclaved at 121 °C for 15 min prior to use. A horizontal 2-in. ball valve was opened directly off the wellhead, and a non-sterile 1 m length of PVC tubing was used to direct the produced water flow to the sample jar in a quiescent manner; both the ball valve and the PVC tubing were flushed thoroughly with produced water prior to sampling. Sample jars were filled and sealed such that no air bubbles were collected with the sample. Jars were shipped overnight to our laboratory in Rapid City, South Dakota, where they were stored inside an anaerobic chamber at room temperature, and remained sealed except for brief periods needed for inocula transfers. Temperature, pH, and conductivity of the produced water were measured at the well site using a Fisher Scientific Accumet multimeter (model 13636AP85). Water samples were also collected for inorganic chemical analysis by ALS Laboratory Group, Environmental Division Brisbane (Stafford, QLD, Australia).

2.2. Coal substrate

For cultures containing coal, subbituminous Walloon coal was used as the primary carbon source. This coal was obtained on January 8, 2009 as a core from a completed Dalby well (Fig. 1); it had been stored outdoors in a core box under a tarp cover since 2003. The core subsection came from a depth of 308 m, which corresponds to the Taroom Coal Measures. Drilling mud and the immediate core surface were pared away with a razor blade, and the freshly exposed coal surface was surveyed using SEM-EDS. The dominant mineral present was kaolinite, with lesser calcite, montmorillonite, anatase, mixed layer illite/smectite, quartz, dolomite, ankerite, siderite, pyrite and rhodochrosite. Trace Ce–Nd–La-monazites, base metal sulfides, and barite were also present. No evidence of weathering alteration of sulfide or carbonate minerals was observed. The pared core was then crushed inside an anaerobic chamber using a mortar and pestle. Proximate, ultimate, and petrographic analyses (Table 2) were run on a 70 g sister sample of the same core by Energy Resources Consulting Pty Ltd. The crushed coal was sieved into size fractions inside the anaerobic chamber, and then stored in gas tight bottles. Polished resin blocks were constructed from subsamples of this crushed coal; the observed pyrite was highly reflective and not pitted. SEM-EDS of the polished blocks found no evidence of pyrite and carbonate alteration or gypsum formation, again indicating the coal had been fairly well preserved. Unless noted otherwise, a 300–600 μm particle size fraction of coal was used in the culture experiments. Laser diffraction analysis with a Malvern Mastersizer 2000 provided particle size distribution within each fraction using a general purpose analysis model of irregular shaped particles.

2.3. Medium

An enrichment culture technique was used for detecting and characterizing methanogenic consortia present in coal seam gas formation waters (Green et al., 2008). Formation water samples or an enrichment culture were transferred to a nutrient medium containing coal or standard methanogenic substrates (acetate, methanol, or H_2–CO_2 headspace) as the sole carbon-energy source. The basal medium was adapted from a recipe by Tanner (2002) for culturing bacteria and fungi. Defined minerals, trace metals, and vitamin solutions were combined with distilled water in 100 mL batches, and their final concentrations were as reported previously (Green et al., 2008). N-[Tris(hydroxymethyl)methyl]-2-aminoethanesulfonic acid (TES) (2 g/L) was added as a pH buffer; the possible effect of this organic buffer on coal solubility was not tested. For the initial methanogen screening experiment, sodium acetate trihydrate was added to a final concentration of 0.73 mM; when screening methanogenic substrates, it was added at 36.7 mM. For the abiotic controls, the methanogen inhibitor 2-bromoethanesulfonic acid (BESA) was added to a final concentration of 10 mM. For the surfactant experiments, either Brij 35, Triton X-100, or Zonyl FSN were added at 50% or 100% of the critical micelle concentration (the CMC equals 0.09 mM, 0.014 vol.%, and 0.3 vol.%, respectively). After adjusting the medium pH (to 2.7 with HCl for coal cultures, or 6.5 with sodium hydroxide for no-coal cultures), sodium bicarbonate (2.0 g/L) was added. 20 μL of resazurin (1.0 g/L) was added as an oxygen indicator (resazurin has a pink color at redox potentials above about −150 mV), and the 100 mL solution was then boiled for ~60 s and cooled under a nitrogen purge to remove dissolved oxygen. Once cool, the solution was transferred to an anaerobic chamber (Coy 7150–000) where 0.5 mL of fresh 40 g/L $Na_2S{\cdot}9\ H_2O$ reducing solution was added; a bed of activated carbon was maintained inside the chamber to prevent sulfide poisoning of the palladium catalyst used to maintain an oxygen free atmosphere (Balch and Wolfe, 1976). For the methanol screening experiment, methanol was now added to a final concentration of 4 g/L. The medium was then dispensed inside the chamber in 10 mL aliquots to Balch tubes (Bellco 2048–00150) containing 0.25 ± 0.005 g (unless noted otherwise) Walloon coal, sealed with butyl rubber stoppers (Bellco 2048–11800), and then removed from the chamber. Outside the chamber, a gassing manifold with needle tips (Balch and Wolfe, 1976) was used to vacuum and fill the headspace with 125 kPa nitrogen for three cycles (final nitrogen headspace pressure was set at 125 kPa). For the initial methanogen screening experiment, the 15 mL headspace was pressurized to 160 kPa with 80% H_2–20% CO_2; for the H_2–CO_2 screening, it was pressurized to 300 kPa. Prior to inoculation, the sealed, pressurized tubes were sterilized in an autoclave at 121 °C for 15 min. Final medium pH after autoclaving was 7.6.

Table 2
Properties of Walloon coal sample used as the primary carbon source.

	Yield %
Proximate analysis (air dried) %	
Moisture	3.6
Volatile matter	37.9
Ash	24.4
Fixed carbon	34.1
Total sulfur %	0.35
Ultimate analysis (dry ash free) %	
Carbon	75.9
Hydrogen	6.46
Nitrogen	1.27
Sulfur	0.49
Oxygen	15.9
Petrographic analysis vol.%	
Vitrinite	63.9
Liptinite	21.0
Inertinite	0.4
Mineral matter	14.7
Reflectance ($R_{o,\ max}$) %	0.48 ± 0.046

2.4. Growth conditions

At time zero, 10 mL of culture medium was inoculated with 1 mL of raw well water or 1 mL of the maintenance culture using aseptic,

strict anaerobic techniques (as described by Hungate (1969) and modified by Balch and Wolfe (1976)). Culture tubes were placed horizontally in an incubator shaker at 37 °C and agitated at 50 rpm to enhance coal-liquid mass transfer rates. Cultures were removed from the shaker only for short sampling periods. Cell concentrations were not quantified, as the presence of coal solids would have interfered with such measurements.

2.5. Maintenance culture

Raw formation water stored at room temperature under anaerobic conditions was used as the inoculum for all but one of the experiments. Over time, coal-to-methane yields for experiments inoculated with raw well water decreased (presumably due to limited shelf life of critical consortium members), so the coal particle size experiment was inoculated from a maintenance culture. The medium composition was identical to that used in the *Coal as sole carbon and energy source* experiments (Section 3.4); 10 mL of ten month old formation water was used to inoculate 100 mL of medium containing 1.6 g Walloon coal (particles less than 140 mesh) as the sole carbon-energy source. This culture was incubated under a 125 kPa nitrogen atmosphere at 37 °C and 50 rpm for four months, and was then used to inoculate the particle size experiment.

2.6. Gas analysis

Culture tube headspace samples were drawn using aseptic, anaerobic technique with a 100 μL gas syringe (Hamilton 81056) equipped with a shut-off valve (Hamilton 35083) and a sterile 23 gauge needle (BD 305193). Methane analysis was performed using a Hewlett-Packard 6890 gas chromatograph, equipped with a Chrompack Carboplot P7 25-m × 0.53-mm fused silica capillary column (HP 19095P-CO2) and a thermal conductivity detector using helium (5 mL/min) as the carrier gas. Calibration standards consisting of 4% or 10% methane (Matheson Tri-Gas GMT10404TC, GMT10320TC, respectively) were injected at atmospheric pressure to generate the calibration plot. Further details have been previously reported (Green et al., 2008).

2.7. Determination of theoretical methane yields

For experiments using direct methanogenic substrates (H_2–CO_2, methanol, or acetate), theoretical methane yields were calculated using the stoichiometry given in Eqs. (1), (3), and (4) (Table 1), respectively.

3. Results

3.1. Water analysis

Table 3 summarizes the raw water properties for the wells in this study; all yielded sodium bicarbonate-chloride type waters typical of Surat Basin aquifers in the near-surface recharge areas (Quarantotto, 1989), and the Walloon Subgroup in general (Draper and Boreham, 2006). Fig. 3 is a corresponding Schoeller plot of the water quality data that have been grouped according to study field; production waters from both fields are characterized by low calcium, magnesium and sulfate concentrations. Following a review of water quality for the six main CBM basins in the USA, Van Voast (2003) suggested that formation waters associated with thermogenic/biogenic CBM have a distinctive geochemical signature: very low concentrations of sulfate (less than 500 mg/L or 10 meq/L), low concentrations of calcium and magnesium, and high concentrations of sodium, bicarbonate, and sometimes chloride. Our study wells are consistent with this CBM water signature, which was also observed for production waters from several other Queensland CBM projects (Draper and Boreham, 2006; Kinnon et al., 2010), including the Walloon Subgroup (Draper and Boreham, 2006). The production waters of the Dalby field are distinguished by higher total dissolved solids than the Roma field, manifest largely in higher concentrations of sodium and chloride (Fig. 3).

Table 3
Raw well water analyses.

	Roma waters				Dalby waters			
	CC2	CC4	H4	H7	D43	D45	K12	K37
Temperature (°C)	36.6	34.7	38	38.1	35.2	32.4	30.8	32.7
pH	8.48	8.54	8.24	8.35	8.34	8.35	7.84	8.28
Conductivity (mS)	2.98	3.23	3.73	3.37	5.94	7.25	11.24	8.36
Carbonate alkalinity (mg/L)[a]	39	39	12	17	43	19	<1	<1
Bicarbonate alkalinity (mg/L)[a]	829	711	719	943	1550	1070	330	597
Sulfate (mg/L)	4	<1	<1	<1	<1	<1	<1	<1
Chloride (mg/L)	426	632	728	555	1050	1730	3580	2460
Calcium (mg/L)	4	3	4	3	5	7	28	14
Magnesium (mg/L)	<1	<1	<1	<1	2	4	11	5
Sodium (mg/L)	691	747	797	780	1430	1620	2460	1950
Potassium (mg/L)	4	3	4	5	5	9	10	7

[a] as $CaCO_3$.

3.2. Methanogen screening

The methanogen screening experiment was designed to culture-test eight Surat Basin CBM formation waters for the presence of viable methanogens. Each water sample was inoculated into nutrient medium amended with the two most common methanogenic substrates, acetate and H_2–CO_2. No methane was detected in the headspace at 80 h, whereas all samples tested positive at 250 h with varying methane concentrations (Fig. 4). Theoretical yields for H_2–CO_2 and acetate (195 and 7.3 μmol CH_4, respectively, based on Eqs. (1) and (4)) are displayed by shaded areas in the background. Although substrate-free controls were not run for this experiment, later experiments showed maximum methane levels for no-substrate controls of 3 μmol/tube (Table 4). The experimental values shown in Fig. 1 may not represent final methane yields, as no measurements were taken after 250 h. Nonetheless, all water samples tested positive for viable methanogens when provided with acetate and H_2–CO_2 substrates.

3.3. Screening methanogenic substrates

Although a complete methanogenic consortium can metabolize a broad range of complex organic molecules, methanogens in pure culture have a narrow range of simpler substrates. This experiment was designed to determine which methanogenic pathways were active by

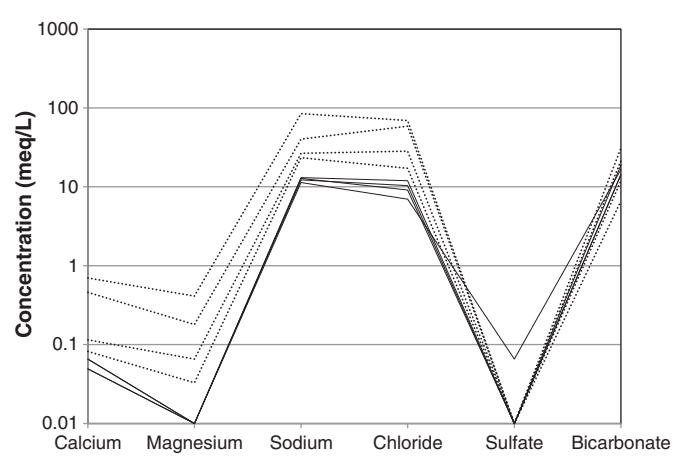

Fig. 3. Schoeller plot of water quality data from the Roma (solid lines) and Dalby (dotted lines) study fields.

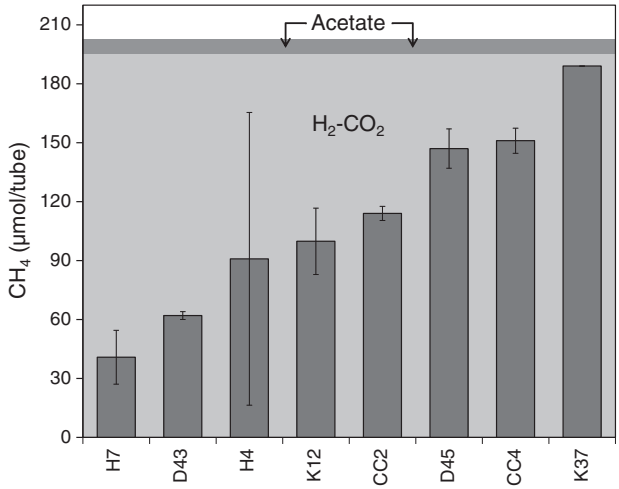

Fig. 4. Methane production from acetate/H_2–CO_2 after 250 h. Cultures were inoculated with raw well water 20 days after field sampling. Growth medium contained 0.73 mM sodium acetate and 160 kPa H_2–CO_2 in 15 mL headspace, for which dark grey and light grey background areas, respectively give theoretical yields (Eqs. (1), (4)). Error bars represent one standard deviation for duplicate cultures.

culturing the samples on either acetate, methanol, or H_2–CO_2 as the sole carbon-energy substrate. Nutrient medium with either 5 g/L sodium acetate trihydrate, 4 g/L methanol, or 300 kPa H_2–CO_2 as the sole carbon-energy source was inoculated with formation water and monitored over 500 hours. From the growth curves (not shown), methane production occurred between 100 and 350 h for the H_2–CO_2 cultures, lagged up to 300 h and continued through 500 h for the methanol cultures, and was not observed for the acetate cultures. To determine final yields for the methanol cultures, methane levels were measured again after six months. The results of all eight samples are displayed in Table 4. Formation waters used to inoculate this experiment had been in storage for eight months; at this point, CC2 and K37 samples appear to have lost all methanogenic activity. Methane production levels with acetate were similar to the no-substrate control and were assumed inactive. Of the remaining six water samples, all tested positive for active hydrogenotrophy, and all but K12 tested positive for methylotrophy.

3.4. Coal as sole carbon and energy source

If *in-situ* bioconversion of coal to methane is occurring in real time, viable microbial consortia collected from coal seam formation waters should also convert native coal into methane *in vitro*. In this experiment, nutrient medium amended with 0.25 g Walloon coal as the sole carbon and energy source was inoculated with five month old formation water to test the coal-to-methane activity of the entire microbial consortium. Since the coal was autoclaved with the medium, this experiment tested microbial consortia present in the formation water rather than in the coal substrate. Methane production was monitored until methanogenesis ceased around 15 days; final yields for the samples collected near Roma (H4, H7, CC2, CC4) ranged from 154 ± 3 to 261 ± 66 µmol CH_4/g coal (3.64 to 6.17 m^3/t coal), and maximum rates ranged from 12 to 48 µmol CH_4/g coal/day (0.28 to 1.1 m^3/t coal/day). The Dalby area water samples (D43, D45, K12, K37) yielded significantly less methane at an average of 15 µmol CH_4/g coal (0.35 m^3/t coal). These results indicate that the microbial consortia needed for real-time *in-situ* coal methanogenesis are indeed present in these Surat Basin coal seam waters.

In Fig. 5, the final methane yields (per gram of coal substrate) are plotted as a function of the produced water chlorinity. The highest methane yields were observed for the four Roma area wells, where the formation water chlorinity values were all less than 20 mM. This suggests that formations with lower salinities may provide a more favorable environment for consortia with the ability to convert coal to methane. While a subsequent D43 experiment yielded close to 60 µmol CH_4/g coal under equivalent conditions (see Section 3.6), it was inoculated with a D43 *enrichment* culture that had already adapted to the lower chloride concentration of our culture medium (2.3 mM). Clearly, the culture medium matches Roma well salinities more closely than the higher salinity Dalby wells. An inverse trend between methane yield and chloride concentration was not observed in the initial methanogen screening experiment (Fig. 4); K37 and D45 were among the top three producing cultures when grown on H_2–CO_2 plus acetate.

3.5. Coal quantity effect

In the initial cultures with Walloon coal as the sole carbon-energy substrate, methane production stopped after 15 days. To determine whether the cultures were nutrient limited (excess bioavailable coal) or coal limited (excess Tanner medium), H7 and CC2 formation waters were inoculated into 10 mL nutrient medium amended with 0.1, 0.2, or 0.4 g coal. If coal is the yield-limiting substrate, then the total methane formed should increase in proportion to the amount of coal added. For the H7 cultures, Fig. 6.A shows both methanogenesis rates and final methane yields increasing with the total weight of coal added; the maximum methane produced was 26.9 µmol CH_4 from 0.4 g coal, which was 110% and 350% greater than the 0.2 and

Table 4
Final methane yields for substrate screening experiment. Yields reported as µmol CH_4 produced per tube with % theoretical yield in parentheses. Theoretical yield is based on 367, 936 and 367 µmol from H_2–CO_2, methanol, and acetate, respectively, as substrate and Eqs. (1), (3), and (4). Uncertainty represents one standard deviation for triplicate cultures.

		CH_4 produced (µmol/tube)			
		H_2–CO_2	MeOH	Acetate	No-substrate
Formation water	H4	242 ± 50 (65%)	7.8 ± 0.8 (1%) *	0.3 ± 0.1 (~0%)	2.8 ± 4.5
	H7	306 ± 65 (83%)	33 ± 17 (4%)	2.9 ± 0.2 (1%)	2.6 ± 0.9
	CC2	0.1 ± 0.1 (~0%)	n.d.	0.5 ± 0.8 (~0%)	0.2 ± 0.2
	CC4	255 ± 69 (69%)	264 ± 73 (28%)*	1.8 ± 0.1 (1%)	0.95 ± 0.04
	D43	265 ± 22 (72%)	151 ± 114 (16%)	1.2 ± 0.3 (~0%)	2.2 ± 0.3
	D45	18 ± 16 (5%)	261 ± 28 (28%)*	2.1 ± 0.4 (1%)	1.5 ± 0.2
	K12	48 ± 70 (13%)	n.d.	n.d.	n.d.
	K37	n.d.	n.d.	n.d	1.8 ± 0.9

* Measured at 6 months. n.d. = not detected.

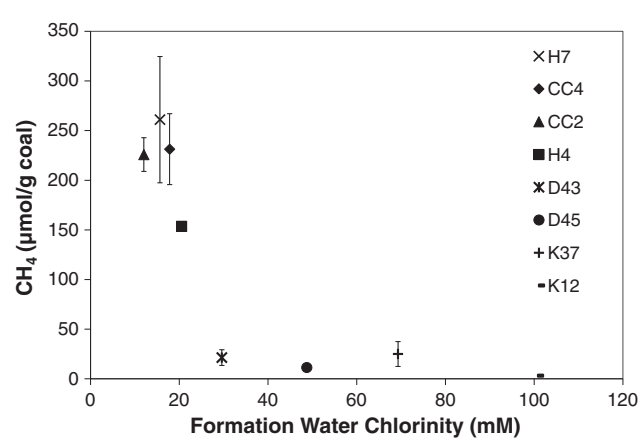

Fig. 5. Methane yields from Walloon coal after 15 days, plotted against source well chloride concentration. Chlorinity of the culture medium was 2.3 mM. Error bars represent one standard deviation for triplicate cultures. Yield values shown are corrected for methane observed in no-coal control cultures. No methane was detected in uninoculated controls.

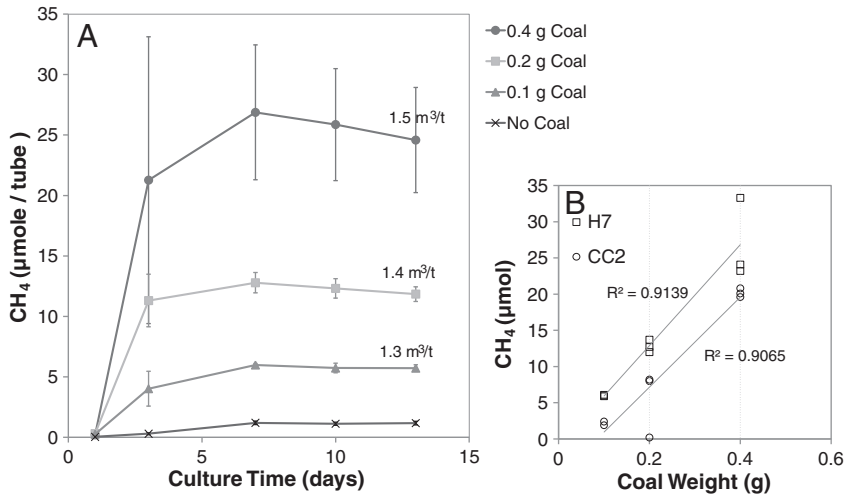

Fig. 6. Methane production as a function of total Walloon coal (0.1, 0.2, or 0.4 g) added as the sole carbon-energy source to 10 mL growth medium. (A) H7 methane production curves with final yield values also shown in m^3 per t coal. Error bars represent one standard deviation for triplicate cultures. (B) Final methane yields for H7 and CC2 cultures (no-coal control levels subtracted) as a function of coal quantity.

0.1 g coal cultures, respectively. The BESA-inhibited controls produced no detectable methane, and the no-substrate controls produced negligible methane levels. Fig. 6.B shows the final methane yields were directly proportional to the amount of coal added for both H7 and CC2 cultures; this indicates that coal was the yield-limiting nutrient in these experiments, and that coal was not inhibitory/toxic to the microbial consortia at the highest coal loading of 0.4 g. The observed increase in methanogenesis rates with coal surface area (proportional to the amount of coal added) is consistent with a process where coal dissolution is the rate-limiting step.

3.6. Coal particle size effect

While coal-to-methane rates and yields increased in proportion to the total coal added (Fig. 6.B), this may have been the effect of increased coal particle surface area. The internal pore structure of coal is mostly too small to harbor microbes, and intraparticle pore diffusion is a very slow process; as such, only surface substrates may be readily bioavailable. If the coal surface is the sole provider of organic substrates, an increase in the total particle surface area (decreasing the particle size while keeping the total coal weight constant) should increase coal-to-methane rates and yields. In this particle size experiment, a D43 maintenance culture was used to inoculate medium amended with 0.25 g Walloon coal of three particle size fractions (<300 µm, 300–600 µm, and 600–850 µm). Laser diffraction was used to measure the size distribution within each fraction, from which external surface areas of 106, 20, and 12 cm^2 were calculated (assuming perfect spheres with a 1.3 g/cm^3 coal density (Mutton, 2003)). The methane production curves are displayed in Fig. 7, which show increasing methanogenesis rates and yields with decreasing particle size. The inlaid plot to Fig. 7 clearly shows the increase in final methane yield with coal surface area (R^2 for best fit line = 0.904). These results indicate that surface mass transfer is the rate-limiting step, and suggest that only surface substrates are bioavailable during the limited culture period.

3.7. Surfactant effect

Surfactants have been shown to enhance or inhibit the biodegradation of insoluble organics; results vary depending on the organism, the substrate, and the type and concentration of surfactant used (Mihelcic et al., 1993). The goal of this experiment was to determine if the addition of surfactants at or below the critical micelle concentration (CMC) would enhance the microbial conversion of Walloon coal to methane. The three non-ionic surfactants tested separately in this experiment were Zonyl FSN, Triton X-100, and Brij 35; Zonyl FSN and Triton X-100 both were shown to be non-toxic to *E. coli* cultures at concentrations up to twice the CMC (Talari, 2004). Brij 35 at concentrations up to 10 g/L was non-toxic to *Pseudomonas* strains used in the biodegradation of naphthalene and phenanthrene (Volkering et al., 1995). Each surfactant was tested at 50% and 100% of the CMC. To determine whether the surfactants served as methanogenic substrates, a no-coal control was run for each surfactant containing one CMC of the surfactant in 10 mL of medium (surfactant no-coal). Seven month old H7 formation water was used as the inoculum, and the greatest enhancement in methane production from coal was observed with Zonyl FSN at one-half the CMC (Fig. 8). Cultures with 50% CMC Zonyl FSN produced 93% and 57% more methane than no-surfactant controls and 100% CMC Zonyl FSN cultures, respectively. Zonyl FSN was not used directly as a methanogenic substrate, as methane production for the surfactant no-coal culture was identical to the no-carbon control (no coal or Zonyl FSN). Brij 35 and Triton X-100 produced methane yields similar to the no-surfactant control at both 50% and 100% of the CMC. These results

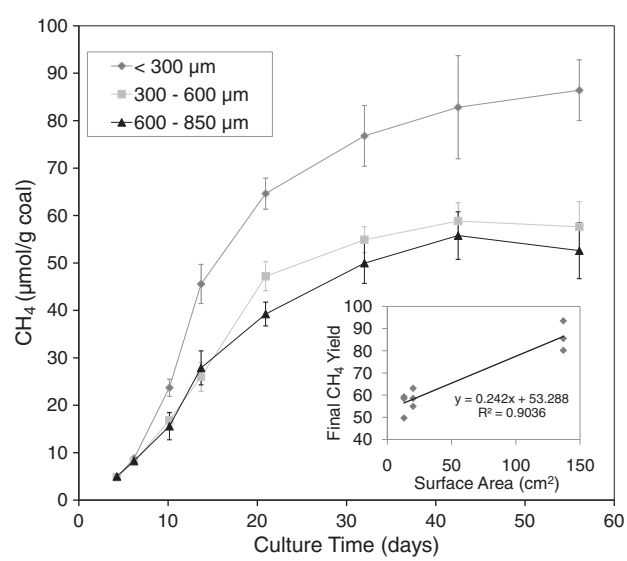

Fig. 7. Methane production curves for the particle size experiment. Error bars represent one standard deviation for triplicate cultures. Inset figure displays final methane yield (µmol CH_4/g coal) as a function of coal particle external surface area.

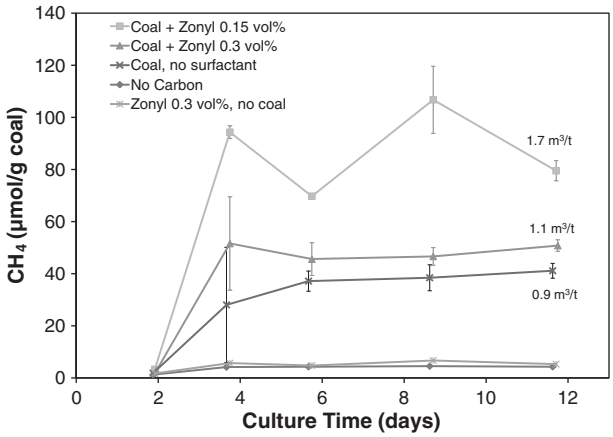

Fig. 8. Methane production from 0.25 g Walloon coal in the presence of Zonyl FSN surfactant at 50% and 100% of the CMC. Seven month old H7 formation water was used as the inoculum. Error bars represent one standard deviation for triplicate cultures.

indicate that it may be possible to enhance biomethane rates/yields from Walloon coal by adding the proper surfactant at the right concentration.

3.8. Effect of excess bicarbonate on methane production

This experiment explored whether the addition of supplemental CO_2 would enhance the production of microbial methane from Walloon coal. If excess H_2 is available from the fermentation of coal substrates (methane production is CO_2 limited), then additional CO_2 could allow for the production of additional methane. To test this, CO_2 in the form of sodium bicarbonate was added at 2 g/L and 4 g/L in nutrient medium amended with 0.25 g coal. For comparison, the amount of bicarbonate in the H7 formation water is equivalent to 1.58 g/L sodium bicarbonate. To eliminate any effects of added CO_2 on pH, sulfuric acid and sodium hydroxide were added to the normal and excess bicarbonate media, respectively, such that the initial pH was 7.6 for all cultures. Displayed in Fig. 9, both the maximum methane production rate (0–7 days) and the final yield decreased (by 28% and 13%, respectively) when the bicarbonate concentration was doubled. Less than 0.1 µmol methane was observed in the BESA controls. For the no-coal controls, the final methane yield was only 17% of the "2 g/L $NaHCO_3$-Coal" culture, and the effect of added bicarbonate was negligible. These results support the hypothesis that in the anaerobic biodegradation of coal to methane, CO_2 is not the limiting methanogenic substrate.

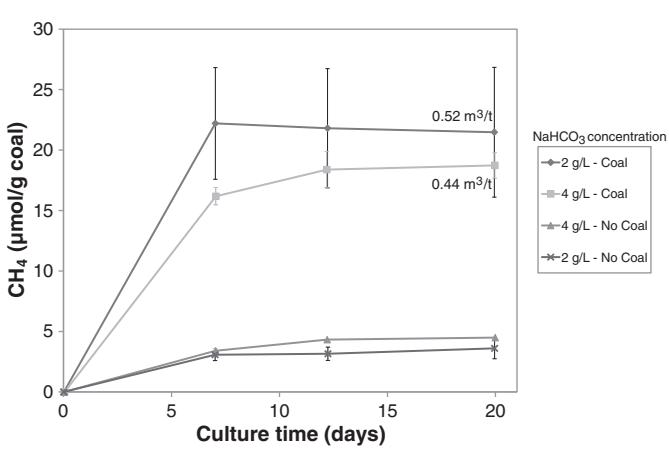

Fig. 9. Methane production from Walloon coal as a function of sodium bicarbonate concentration (2 vs. 4 g/L). Media was inoculated with H7 well water. Error bars represent one standard deviation for triplicate cultures.

4. Discussion

4.1. Geochemical interpretation

Compared to other macerals, hydrogen-rich liptinitic macerals generally have higher biogasification rates (Scott, 1999). Walloon coals are low rank ($R_{o,\ max}$ 0.35–0.70%; Beeston et al., 1997; Scott et al., 2007) and hydrogen-rich (Khorasani, 1987) due to abundant perhydrous vitrinite (average 76.8%) and a relatively high liptinite content (average 19.5%) (Scott et al., 2007), making them a good candidate for MECoM. The Walloon coal used in our culture experiments has a somewhat higher liptinite content (24.6% on a mineral matter free basis); because of its higher H/C ratio, it has the potential to yield more new biomethane than an average Walloon coal. The range of vitrinite reflectance values corresponds to the early stages of thermogenic hydrocarbon generation; the coals are at the base of the oil window (0.5–1.3% R_o) (Tissot and Welte, 1978) and rich in liquid hydrocarbons to serve as potential feedstock for indigenous microbial consortia. In higher rank coals these liquid hydrocarbons would have been cracked to produce methane and higher hydrocarbon gases, thus reducing the fraction of biodegradable moieties.

Coalbed water in the Surat Basin including the Roma and Dalby CBM fields is typically alkaline with a pH range of 8 to 9 that generally increases with depth (Hodgkinson et al., 2009). The production waters from the Roma and Dalby fields in the current study are brackish with low calcium and magnesium concentrations, and high sodium, bicarbonate and chloride concentrations (Table 3). As noted previously, these waters have the same proportions of major and minor cations and anions as CBM production waters from the Bowen Basin and USA (Draper and Boreham, 2006; Kinnon et al., 2010; Van Voast, 2003). Coal measures in the Surat Basin (and the majority of coal basins producing CBM) act as fractured regional aquifers confined by relatively impermeable units; as such, the chemistry of the CBM production waters mainly reflects the chemical and biological processes occurring within the coal seam environment over time.

Typically, coalbed waters evolve down-gradient through the processes of microbial sulfate reduction, bicarbonate enrichment and calcium and magnesium depletion (Van Voast, 2003). In recharge areas, weathering and oxidation of pyrite and marcasite in the coal results in high-sulfate waters as does dissolution of gypsum. With increasing depth and decreasing oxygen concentration, sulfate-reducing bacteria convert dissolved sulfate and organics to sulfide (which is re-precipitated) and bicarbonate, respectively. The attendant increase in bicarbonate concentration may lead to the precipitation of calcite and dolomite that will reduce the concentration of calcium and magnesium in the coalbed waters (Van Voast, 2003). Ion exchange with clays may also result in elevated sodium concentrations and low calcium and magnesium concentrations (Van Voast, 2003). In this context, the wells with slightly higher calcium and magnesium concentrations are associated with the Dalby field, where the shallow depths could have limited the extent of microbial sulfate reduction; that said, no sulfate was detected in the Dalby wells, and the higher calcium and magnesium levels more likely reflect a change in geographical location and/or aquifer lithology.

4.2. Methanogen and methanogenic substrate screening

Using a culture screening method, this study demonstrates the presence of viable methanogens in all of the Surat Basin production wells tested (both Roma and Dalby sites). This is in contrast to a previous 16S rDNA analysis of Surat Basin formation water (Talinga seam), where both raw formation water and yeast extract enrichment cultures tested negative for methanogens (Li et al., 2008); in this case, methanogen levels may have been below detection limits, and yeast extract was not a sufficient enrichment for these methanogens. Most methanogens have the ability to utilize H_2–CO_2 (Thauer et al., 1993), and acetate still

plays a major role in the natural production of methane (Winfrey, 1984). Since an active H_2–CO_2 pathway was later demonstrated for all but two of the wells sampled, enrichments with H_2–CO_2 plus acetate may provide a broader and more sensitive technique (compared to yeast extract enrichment) for methanogen detection in the Surat Basin.

Culture screening with specific methanogenic substrates suggests that hydrogenotrophy and/or methylotrophy are the active pathways for any real time *in-situ* methanogenesis; historically, hydrogenotrophy was the dominant biological pathway for Surat Basin coal seam methane generation (Draper and Boreham, 2006). It seems unusual that no acetoclastic methanogenesis was observed (Table 4), as this pathway is common in freshwater environments, and has been observed in other coal seams (Green et al., 2008; Ulrich and Bower, 2008; Whiticar et al., 1986); that said, it is possible that acetoclastic methanogens were present in the original water samples, but were lost during the eight months of storage. Both the H_2–CO_2 and methanol methanogenesis pathways were active for wells H4, H7, CC4, D43, and D45; this indicates the presence of multiple methanogenic species or a single species with the ability to utilize both substrates (e.g. *Methanosarcina frisia*). Most methylotrophic methanogens have an optimum chloride concentration greater than 200 mM (Keltjens and Vogels, 1993), so it is unusual that we also observed methylotrophy for our lower chlorinity wells (as low as 16 mM Cl^- for H7).

4.3. Coal as sole carbon and energy source

These results indicate that complete microbial consortia with the ability to convert native Walloon coal into methane are indeed present in waters produced from Surat Basin coal seams. Given the proper nutrients and environment, these consortia could be stimulated to produce new natural gas *in-situ* in real time. While methanogens were also detected in a Gippsland Basin, Australia sample, no coal-to-methane activity was observed in laboratory culture; the consortium may not have been adapted to the non-native coal that was used (Midgley et al., 2010). The maximum coal-to-methane yield observed in our study was for the H7 culture (Fig. 5), which produced 261 μmol/g coal (6.2 m^3/t or 200 scf/ton). Walloon Subgroup coals have a 3.5 m^3/t mean gas content (Scott et al., 2007), meaning our laboratory conditions yielded 177% of the existing coal seam methane content in 25 days. If our maximum culture rate of 1.0 m^3/t/day (30 scf/ton/day) could be extrapolated to the field, the existing methane content would be replenished in less than four days. While our Surat consortia were cultured under ideal laboratory conditions (i.e., by crushing the coal and providing a full suite of growth factors), realizing just a fraction of these coal-to-methane rates/yields *in-situ* would have a major impact on field reserves and longevity.

In another study of 18 different subbituminous coal samples, only two tested positive for endemic coal-to-methane consortia (Jones et al., 2008). That said, the presence of native coal-to-methane consortia has been demonstrated in similar lab culture studies for the Powder River Basin in Wyoming (Green et al., 2008; Jones et al., 2008), the Gulf Coast Basin in Texas (Jones et al., 2008, 2010), the Fernie Basin in British Columbia (Penner et al., 2010), and for coal seams near Fort Yukon, Alaska (Harris et al., 2008). In each study, coal or formation water was used to inoculate a mineral medium containing coal as the sole or primary carbon-energy source, and biological methane production from coal was demonstrated. Estimated methane production rates and final yields are summarized in Table 5, which also includes two bioaugmentation studies with Texas coals; our H7 culture rates and yields are quite robust in comparison to other coal-derived consortia. While laboratory rates and yields for the termite consortium were an order of magnitude greater, this could be due in part to the smaller coal particle size used; also, it is not known if this foreign consortium could compete in a coal seam bioaugmentation scenario (Gilcrease and Shurr, 2007; Vogel, 1996). In addition to coal particle size, there are a host of possible explanations why one consortium outperforms another; activity is potentially affected by sampling methods, culture conditions, coal bioavailability, and selectivity of the *in-situ* coal seam environment. Nonetheless, the rates and yields for our Surat consortia look quite promising, and warrant further study.

Table 5
Coal-to-methane rates and yields for various studies.

Inoculum source	Coal source	Coal size (mm)	Avg. rate (μmol/g coal/day)	Yield (μmol/g coal)	Source
Coal-derived consortia					
Surat, Queensland	Walloon (Queensland)	0.30–0.60	10	260	Current study
Powder River, Wyoming	Wyodak (Wyoming)	0.25–0.60	7.6	180	Green et al. (2008)
Wilcox, Texas	Wilcox (Texas)	2–10	2.1	60	Jones et al. (2010)
British Columbia	Obed Mine (Alberta)	Finely ground	0.5	42	Penner et al. (2010)
Ft. Yukon, Alaska	Ft. Yukon (Alaska)	Crushed	0.8	14	Harris et al. (2008)
Bioaugmentation consortia					
Wetland sediment enrichment	Wilcox (Texas)	2–10	3	80	Jones et al. (2010)
Wood-eating termite	Texas lignite	0.045	650	7200	Harding et al. (1993)

Looking at our coal-to-methane yields across the eight wells tested, there appears to be an inverse correlation between coal methanogenesis and formation water salinity (Fig. 5); conversely, there was no correlation between salinity and methanogenesis from H_2–CO_2 plus acetate substrates (Fig. 4). This suggests that the complete consortium (needed to convert coal to methane) may be more sensitive to higher chloride concentrations than the subset of H_2–CO_2/acetate utilizing methanogens; similarly, Liu and Boone (1991) found that lignocellulose-to-methane subcultures were more NaCl sensitive than subcultures producing methane from H_2–CO_2 or acetate. While higher salinity is known to inhibit many methanogens (Waldron et al., 2007), the chlorine levels measured for the Dalby wells (30 to 101 mM) are well below the maximum reported chloride concentration of 2140 mM for hydrogenotrophic methanogenesis (Ollivier et al., 1998). Antrim Shale formation waters from a broad spectrum of natural chloride concentrations (8.4 to 3490 mM) were screened for methanogenic activity *in vitro* from 20 to 2500 mM Cl^- (Waldron et al., 2007); for a given sample, methanogenesis activity was maximized when cultured in a medium close to the native chloride concentration. Because our nutrient medium only contained 2.3 mM Cl^-, our coal-to-methane screening may have selected for consortia adapted to this low chloride concentration; therefore, we cannot conclude if the Dalby wells have less active coal-to-methane consortia, or our medium salinity was unsuitable for stimulating these consortia. It should be noted that all the higher salinity wells were associated with the Dalby site; as such, there may be other unknown differences between the Roma and Dalby sites that resulted in the range of activities shown in Fig. 5.

4.4. Effect of coal bioavailability on methanogenesis rates and yields

Since microorganisms are inherently aquatic, and coal represents a separate solid phase, the dissolution (mass transfer) of coal substrates into aqueous solution governs *bioavailability* in our system, and is likely required before coal biodegradation can occur (Green et al., 2008). Alternatively, biodegradation of a solid substrate like coal may require direct attachment of the primary degrading organisms to the coal surface (Mihelcic et al., 1993; Wick et al., 2002). If dissolution/primary degradation represents the rate-limiting step in the coal-to-methane pathway, then overall methanogenesis rates will be proportional to the exposed

coal surface area. In both the coal amount (Fig. 6) and coal particle size (Fig. 7) experiments, initial coal methanogenesis rates increased in proportion to the external coal surface area. This was also observed in an analogous culture study with a Powder River Basin consortium growing on different size fractions of Wyodak coal (Green et al., 2008). Given that microbial access to surfaces within a coal seam will be limited (Faison, 1992; Scott, 1999), field strategies for enhancing *in-situ* methanogenesis rates (above and beyond the introduction of supplemental non-carbon nutrients) may need to target mass transfer within the coal seam, as discussed elsewhere (Gilcrease and Shurr, 2007; Green et al., 2008).

It is likely that only a fraction of coal organic molecules have chemical structures that are amenable to microbial metabolism (defined as readily *biodegradable* in this context) (Faison, 1992); long chain fatty acids, alkanes, single-ring aromatics, small polyaromatics, and ketones may be primary intermediates in the bioconversion of coal to methane (Orem et al., 2010; Strąpoć et al., 2008). While the micropores in coal range from 0.04 to 30 μm in diameter, many of these pores are too small for microbial entry (Faison, 1992); in this case, organics within a coal particle will not come in contact with the degrading consortium. Since intraparticle pore diffusion of organics is a very slow process (Geerdink et al., 1996), only surface organics will be bioavailable for the relatively short timescales of our experiments. If only a small fraction of those coal surface molecules are biodegradable, then we would expect coal-to-methane yields to be proportional to the external surface area of the coal substrate. For both the coal amount (Fig. 6) and coal particle size (Fig. 7) experiments, final coal-to-methane yields were indeed proportional to the total external coal surface area. The maximum fraction of coal converted to methane was 0.44 wt.% (H7, Fig. 5), confirming that a significant fraction was not utilized; this implies that unless coal is physically, chemically, or biologically altered, only a finite amount of methane can be readily derived from coal. By enhancing coal bioavailability, there is an opportunity to enhance both methanogenesis rates and yields, and extend the methane production period. For example, hydraulic fracturing could be used to produce new coal seam cracks, creating additional surface area for microbial attack. Separately, the observed increase in final methane yield with coal amount (Fig. 6B) is consistent with the idea that coal organics (not the supplemental nutrients) were the yield limiting substrate in these cultures. To avoid the cost of excess nutrients, field treatments should operate at a nutrient to coal ratio where the nutrient supplements are yield limiting.

A more realistic estimate for field *in-situ* methane production potential would be to extrapolate based on the ratio of estimated *in-situ* coal surface area to laboratory coal particle surface area (Scott, 1999). Given that the Surat Basin contains medium ash coals with an approximate specific gravity of 1.4, the estimated surface area of vitrain layer-confined cleats plus master cleats is 203 m^2/t coal (Table 6). For our 300–600 μm Walloon coal substrate, the calculated surface area was 20 cm^2/0.25 g or 8000 m^2/t. With this surface area ratio, our maximum laboratory culture rate of 1.0 m^3/t/day (30 scf/ton/day) would correspond to an *in-situ* rate of 0.025 m^3/t coal/day (0.81 scf/ton/day). At this rate it would take 140 days to replenish the coal seam gas content (3.5 m^3 of methane per metric ton of coal), which is still significant from a production standpoint. On the other hand, if we scale up our best laboratory coal-to-methane yield (6.2 m^3/t) based on this same surface area ratio, the estimated *in-situ* yield is only 0.16 m^3/t (5.0 scf/ton). As such, there is a clear need for techniques that will enhance *in-situ* coal-to-methane yields, as discussed earlier.

4.5. Surfactant screening

Enhanced methane production rates and yields with the addition of a non-ionic surfactant (Zonyl FSN) may be further evidence that this process is limited by the bioavailability of Walloon coal. In a related study, the addition of 0.25 vol.% N,N-dimethylformamide (DMF) solvent to a Powder River Basin culture enhanced the methane yield from a subbituminous Wyodak coal by 350% (Green et al., 2008).

Table 6
Hypothetical total cleat surface area in coals of 1.4 g/cm^3 density, per metric ton of coal.

	Vitrain layer confined cleats[a]	Master cleats[a]
Corresponding volume of coal block if it weighs 1 metric ton (m^3)	0.714	0.714
Length of one side of a cube of this weight (mm)	714	714
Hypothetical average cleat height, which equals layer height for vitrain layer-confined cleats[b] (mm)	4	70
Number of cleats in a given horizontal interval (assuming directly proportional relationship between cleat height and spacing[b], and equal presence in face and butt cleat orientation)	357	20.4
Number of vitrain layers if the coal is interbanded, comprised of 50% vitrain layers of hypothetical height 4 mm	89.25	N/A
Number of cleats in the entire coal block	31,862	208
Approx. surface area of one cleat's walls = 2 × length × height (m^2)	5.71×10^{-3}	0.1
Approx. surface area of all hypothetical cleats of a given class (m^2)	182	20.8
Total surface area of vitrain layer-confined cleats and master cleats (m^2) per metric ton of coal	203	

[a] Terminology of Dawson and Esterle (2010).
[b] From Dawson and Esterle (2010).

Both solvents and surfactants enhance the aqueous solubility of organics, which increases the concentration driving force for dissolution (mass transfer) of coal substrates into the aqueous medium; however, surfactant micelles present at or above the CMC can trap substrates and actually reduce their bioavailability in some cases (Grimberg et al., 1996; Mihelcic et al., 1993). This may explain why the effect of Zonyl FSN on coal methanogenesis rates and yields was greater at 50% CMC than at 100% CMC (Fig. 8). Surfactants can make the coal surface more hydrophilic, which may increase its bioavailability (Isbister and Barik, 1993); they have been reported to enhance cell adherence in some instances, (Volkering et al., 1998) and prevent cell attachment in others (Mihelcic et al., 1993). If extracellular enzymes are involved in the coal solubilization process, surfactants may also prevent enzyme sorption and subsequent denaturation on the coal surface (Faison, 1992). Because of their amphipathic structure, surfactants can associate with the analogous phospholipid structure of cell membranes; this can enhance cell permeability and substrate transport at lower concentrations (Leon et al., 1998; Mihelcic et al., 1993), but can result in inhibition/cell lysis at higher concentrations (Volkering et al., 1998). In summary, the effect of surfactants on biodegradation tends to be system specific, but our results with Zonyl FSN indicate they can have a net positive effect on the bioconversion of coal to methane. Needless to say, the cost of any field surfactant treatment must be justified in terms of the value of any additional methane generated.

4.6. Effect of added bicarbonate on methane production

Our experimental results would seem to support the hypothesis that in the anaerobic biodegradation of coal to methane, CO_2 is not the limiting methanogenic substrate; while some of this added CO_2 may get reduced to methane, the net methane production from the system remains unchanged. While it is possible that our base level of bicarbonate (which was similar to natural levels) was already providing CO_2 in excess, our results are consistent with a separate culture study where coal-to-methane rates and yields were the same for a bicarbonate vs. a no-bicarbonate medium (Jones et al., 2008). The concept of an H_2-limited system would appear to be consistent with recent commercial patent disclosures for stimulating new biomethane production from coal, which are based on the addition of hydrogen gas or hydrogen-rich substrates (Downey, 2010; Pfeiffer et al., 2010). Stable isotope analysis can confuse this issue of H_2-limitation;

while the hydrogen atoms on biomethane may be derived from formation water (Whiticar et al., 1986), a reduced form of hydrogen (generally H_2) is still needed to drive the CO_2 reduction reaction from a thermodynamic standpoint (Madigan et al., 2003; Whiticar et al., 1986). Unless an external source of reduced hydrogen is available within a coal seam (e.g., inorganic hydrogen from nearby volcanic activities (Baross et al., 1982; Deuser et al., 1973)), CO_2 addition will not directly increase the net biomethane produced. That said, the injection of CO_2 will change the pH, water content, water chemistry, and porosity of the coal seam (Massarotto et al., 2010), which could have significant indirect effects on *in-situ* biomethane production. These effects could be positive or negative; biomethane production from Wyodak coals was stimulated at a lower pH (Green et al., 2008), but reduced permeability and water content would be detrimental to microbial consortia at some point.

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