

## Accepted Manuscript

!-query id="q1" type="boolean" replies="Yes—No";ce:para; Your article is registered as a regular item and is being processed for inclusion in a regular issue of the journal. If this is NOT correct and your article belongs to a Special Issue/Collection please contact [s.murray@elsevier.com](mailto:s.murray@elsevier.com) immediately prior to returning your corrections.;ce:para;/query;-Biogenic methane production from Bowen Basin coal waste materials



!-query id="q2" type="boolean" replies="Yes—No";ce:para;The author names have been tagged as given names and surnames (surnames are highlighted in teal color). Please confirm if they have been identified correctly.;ce:para;/query;-Hang Zheng, Tianyu Chen, Victor Rudolph, Suzanne D. Golding

PII: S0166-5162(16)30197-5  
DOI: doi: [10.1016/j.coal.2016.09.006](https://doi.org/10.1016/j.coal.2016.09.006)  
Reference: COGEL 2718

To appear in: *International Journal of Coal Geology*

Received date: 13 May 2016  
Revised date: 4 September 2016  
Accepted date: 6 September 2016

Please cite this article as: Zheng, !-query id="q2" type="boolean" replies="Yes—No";ce:para;The author names have been tagged as given names and surnames (surnames are highlighted in teal color). Please confirm if they have been identified correctly.;ce:para;/query;-Hang, Chen, Tianyu, Rudolph, Victor, Golding, Suzanne D., !-query id="q1" type="boolean" replies="Yes—No";ce:para;Your article is registered as a regular item and is being processed for inclusion in a regular issue of the journal. If this is NOT correct and your article belongs to a Special Issue/Collection please contact [s.murray@elsevier.com](mailto:s.murray@elsevier.com) immediately prior to returning your corrections.;ce:para;/query;-Biogenic methane production from Bowen Basin coal waste materials, *International Journal of Coal Geology* (2016), doi: [10.1016/j.coal.2016.09.006](https://doi.org/10.1016/j.coal.2016.09.006)

This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

**Biogenic Methane Production from Bowen Basin Coal Waste Materials**Hang Zheng <sup>a\*</sup>, Tianyu Chen <sup>b</sup>, Victor Rudolph <sup>b\*</sup> and Suzanne D. Golding <sup>a\*</sup><sup>a</sup> School of Earth Sciences, <sup>b</sup> School of Chemical Engineering, The University of Queensland, Brisbane, QLD 4072, Australia**Abstract**

A microbial consortium derived from sewage sludge from the treatment of wastewater (Luggage Point Wastewater Treatment Plant, Brisbane, Australia) has been applied to Jameson Cell (J-cell) rejects ( $R_{o,max}=0.96\pm 0.008$ ) of a Bowen Basin coal preparation plant to assess the potential for biogenic methane production. A maximum methane yield of 26.20  $\mu\text{mol/g}$  J-cell rejects ( $0.64 \text{ m}^3 \text{ CH}_4/\text{ton}$ ) was observed, suggesting biogenic methane production from coal waste materials is a feasible process if yields can be improved. Molecular analysis performed on the microbial consortium showed similar microbial community compositions to those observed in natural coal bed environments. The study demonstrates that Australian coal waste materials can be used as a viable feedstock for biogenic methane production using microorganisms that are not native within the coal beds.

\* Corresponding Authors:

Hang Zheng. Ph: +61 3365 2130; Email Address: Jason.h.zheng@gmail.com

Victor Rudolph. Ph: +61 7 3365 4171; Email Address: v.rudolph@uq.edu.au

Suzanne Golding. Ph: +61 7 3365 1159; Email Address: s.golding1@uq.edu.au

## 1. Introduction

Natural gas is a premium energy source because of its ease of use, high energy content and low greenhouse gas intensity. An increasingly important source of gas is coal bed methane (CBM), which arises from both thermogenic and biological processes (Green et al., 2008; Moore, 2012; Strapoc et al., 2011). Thermogenic CBM is the result of coalification, which produces methane and other gases from chemical reactions occurring within the organic matter under elevated temperature and pressure as the coal bed matures (Clayton, 1998). Biogenic methane (biomethane) accumulation in coal beds proceeds through a series of stepwise biochemical reactions promoted by a consortium of microorganisms, which convert carbonaceous materials to methane under anaerobic conditions (Batstone and Jensen, 2011).

Using stable isotope ratio analysis, accumulation of biologically derived methane has been demonstrated in a number of basins worldwide, including the Powder River Basin (Green et al., 2008), Forest City Basin (McIntosh et al., 2008) and Gulf of Mexico (Warwick et al., 2008) in USA, Ruhr Basin in Germany (Krueger et al., 2008), Xinji Area in China (Tao et al., 2007), and Surat and Bowen basins in Australia (Ahmed and Smith, 2001; Hamilton et al., 2014; Kinnon et al., 2010; Li et al., 2008).

A number of laboratory scale studies have subsequently demonstrated the feasibility of using fresh coal as the main carbon-energy source for biomethane production by native coal bed microbial consortia (Ahmed and Smith, 2001; Opara et al., 2012; Papendick et al., 2011). Some studies have demonstrated that methane yields from coal can be improved by using chemical treatment (Huang et al., 2013).

This presents a potential opportunity to produce methane from waste coal, which is a by-product of coal preparation plants. Generally, 20 – 50 % of run-of-mine coal is rejected during coal beneficiation and is accumulated in reject piles (Groppo, 1991). Over five years

(2008-2013), the average coal production of Queensland, Australia alone was over 190 million metric tonnes (MT)(Geosurvey, 2014), with some 30 to 90 million MT of waste coal disposed of every year. Biogenically derived CBM fields generally have gas contents of 4-6 m<sup>3</sup>/ton (Moore, 2012), so there is the potential opportunity to produce 24 – 108 million m<sup>3</sup> of methane (assuming coal waste contains 20% coal materials).

An advantage of using waste coal as a substrate, besides the large and accessible stockpiles, is that it has already been crushed, providing a large surface area for microbial attack (Papendick et al., 2011). However, several challenges need to be addressed with a key one being the provision of a feasible consortium of micro-organisms that is capable of producing biomethane.

In most of the coal to biomethane literature, microbial consortia that are native to coal beds (i.e. formation water derived consortia) have been used to investigate biomethane potential (Fallgren et al., 2013b; Midgley et al., 2010) based on the idea that these native consortia are very well adapted to using coal as a substrate. Using native consortia for biomethane production also provides an easy basis for defending the process with respect to the local ecosystems, since nothing new is being introduced. It may be noted though, that these organisms generally come from the subsurface, i.e. a particular set of conditions with respect to pressure, temperature and available nutrients, and so they will be particularly adapted to these environmental conditions. When exposed to a different environment, it is likely that the consortia will either be inhibited (i.e. fail to maintain biomethane producing activities) or need a long time to re-adapt to the new environment.

We postulate that it is desirable to have a robust and diverse microbial consortium that is capable of maintaining stable biomethane production over a range of (potentially fluctuating) environmental conditions and coal constituents. One option is to use anaerobic consortia from

municipal wastewater treatment systems, since these are easily available in quantity, very diverse and employ similar biological processes as coal bed biomethane production. We accordingly report on the biomethane producing process from coal waste materials using wastewater sludge derived consortia, and identify the microbial community structure mediating this process.

## **2. Materials and Methods**

### **2.1. Waste coal substrate**

Waste coal used in this study was collected on site from the reject stream of the Jameson Flotation cell (J-cell) of a coal preparation plant in the Bowen Basin, Queensland, Australia. After collection, the J-cell rejects were sealed and transported to the laboratory where two identical 500 g ‘as received’ sister samples were extracted from the bulk sample for analysis and experiments in this study. The two identical samples were collected by pressing through the bulk samples through a sample splitter.

One 500 g sister sample of the J-cell rejects was used for proximate and ultimate analysis by ALS Laboratory Brisbane, Australia, following Australian Standards AS 1038.3 (Australia, 2000). Before analysis, the sample was air-dried and the particles that passed the 1mm sieve were selected for the proximate and ultimate analyses.

A portion (150 g) of the other 500 g ‘as received’ sister sample was analysed for moisture content by drying overnight at 105 °C. The dried sample was used for petrographic analysis using polished 20 g resin blocks examined using a Leica DM4500 P LED light microscope according to the Australian Standards (AS 2856.1-2000 and AS 2856.3-2000). Size analysis was carried out on a Malvern Mastersizer, preceded by 1 minute of sonication in Reverse

Osmosis (RO) water to disaggregate the particles. The rest of the 500 g 'as received' wet slurry sister sample was used as the substrate in the batch tests in this study.

## 2.2. Inoculum and culture adaptation

Effluent from a lab-scale anaerobic digester (37 °C) for treating sewage wastewater sludge (Luggage Point Wastewater Treatment Plant, Brisbane, Australia) was used as the inoculum. After collection, the inoculum was incubated at 37 °C for three days to deplete the residual methane then 1 mL was transferred to a number of 40 mL serum bottle (Sigma-Aldrich, Australia) containing 'as received' J-cell rejects and adapted Tanner medium, following the experimental set-up and operation procedures detailed below. The adapted Tanner medium used here contains minerals, trace metals and vitamin solutions with the concentrations as detailed previously by Papendick et al. (2011).

Methane production was observed after 60 days of adaptation and enrichment. Then 1 mL of mixed liquor from the best methane producing bottle was transferred to a new serum bottle containing fresh 'as received' J-cell rejects and Tanner medium. Four successive adaptation and enrichment batches were conducted before a stable culture was obtained for methane production. Contrast microscopy analysis showed that the cell concentration in this culture was approximately  $10^7$  cells/mL.

## 2.3. Experimental set-up and operation

### 2.3.1. Low inoculum: substrate ratio experiments

In each experiment set, nine serum bottles were used: three replicates, three positive controls and three negative controls. Each replicate contained 1 mL inoculum (the above mentioned

stable culture), 9 mL fresh Tanner medium, and 0.39 g 'as received' J-cell rejects (equating to 0.25 g dry weight). A positive control bottle containing the same material as the triplicates and 20 mM (final concentration) 2-bromoethanesulfonate (BES) measured any methane produced from processes other than methanogenesis. A negative control bottle containing 1 mL inoculum and 9 mL fresh Tanner medium provided a measure of the methane production from the inoculum in the absence of coal reject.

All the serum bottles were prepared and sealed with butyl-rubber stoppers (Rubber BV, Netherlands) and crimped with aluminium caps (Sigma-Aldrich, Australia) in an anaerobic chamber. The headspaces of the serum bottles were then vacuumed and flushed five times with pure N<sub>2</sub> (Coregas, Australia) and over pressured to 10 kPa(g) N<sub>2</sub> pressure, before incubation in a 37 °C non-shaking incubator (Thermoline Scientific, Australia).

### 2.3.2. High inoculum:substrate ratio experiments

High ratio experiments using 10 mL inoculum were conducted in parallel with the low ratio experiments to evaluate the effects of the initial inoculum to substrate ratio on the biomethane producing processes. Before transferring the inoculum into each bottle, the liquid phase of the 10 mL inoculum was separated by centrifuging (5 min, 4000 rpm) and discarded, and the residual sediment (microorganisms and spent J-cell rejects) was then added into each bottle. The purpose of centrifuging was to avoid bringing excess organic substrate into the bottles. The set-up of the triplicates, positive and negative controls followed the same protocols for the low ratio experiments.

#### 2.4. Gas and liquid analysis

Gas samples from the headspaces of the serum bottles were obtained through the septum using a 100  $\mu$ L syringe with pressure lock (Hamilton 81056). Methane concentrations in the gas samples were analysed using a Varian 3900 gas chromatograph (GC), equipped with a 30 m x 0.53 mm fused silica PLOT column (Rt<sup>®</sup>-Q-BOND Column, RESTEK, Australia) and a flame ionization detector (FID). Helium was used as the carrier gas at a flow rate of 25 mL/min. Calibrations using 1 % and 4 % methane standard gases (Coregas, Australia) were carried out each time before gas analysis. The net methane production from the triplicates was obtained by subtracting methane production of both the negative and positive controls.

Liquid samples were collected from each bottle periodically, filtered immediately with 0.22  $\mu$ m membrane filter (Merck Millipore, Australia) and analysed for volatile fatty acids (VFAs) and alcohols, using an Agilent 7890A GC with a flame ionization detector (FID). Calibration was carried out using standard solutions made up of six VFAs and three alcohols (acetate, propionate, iso-butyrate, butyrate, iso-valerate, valerate, and hexanoate; ethanol, propanol and butanol) with concentrations of 20, 50, 100 and 500 mg/L.

#### 2.5. 16S rRNA gene sequencing of microbial community

For microbial community profiling, a 10 mL mixed liquid sample was taken at the end of the low ratio experiment for DNA extraction. Approximately 20 ng of the extracted DNA was used for amplifying the 16S rRNA gene through polymerase chain reaction by using universal primer set (926F and 1392R) under the conditions described by Vanwonterghem *et al.* (Vanwonterghem *et al.*, 2014). The amplicons were sequenced on the Roche 454 GS-FLX Titanium platform at the University of Queensland Australian Centre for Ecogenomics.



### 3. Results

#### 3.1. Substrate/J-cell rejects characterization

Petrographic analysis (Table 1) showed that coal contained in the J-cell rejects was medium rank coal with a maximum vitrinite reflectance ( $R_{o,max}$ ) of 0.96 %. The vitrinite group accounted for over 63 % of the total 500 maceral composition counts, while inertinite group, liptinite group and mineral content accounted for 25 %, 1.4 % and 10.4 %, respectively.

Proximate analysis showed that fixed carbon accounted for over 61 % of the J-cell rejects and ash content was 18.2 %, which is broadly consistent with the relatively high mineral content obtained from the petrographic analysis. Based on the ash free contents analysis, the J-cell rejects formula (dry ash free basis) can be empirically estimated as  $C_{3.9}H_{2.7}N_{0.3}S_{0.005}O$  according to the ultimate analysis. Size distribution analysis showed that 96.6 % of the J-cell rejects particles were in the range of 0.5 – 350  $\mu m$ , indicating most of the coal is in the form of fine particles.

**Table 1. Properties of Bowen Basin coal waste materials used as the primary carbon source. .**

	Yield (% weight)
Proximate Analysis (dry basis)	
Moisture	1.7
Volatile Matter	18.7
Ash	18.2
Fixed Carbon	61.4
Ultimate Analysis (dry ash free)	
Carbon	70.5
Hydrogen	3.96

Nitrogen	1.41
Sulfur	0.24
Oxygen	23.9
Petrographic Analysis vol.%	
Vitrinite	63.2
Liptinite	1.4
Inertinite	25
Mineral matter	10.4
Reflectance ( $R_{o,max}$ ) %	0.96±0.008

### 3.2. Culture adaptation

The original inoculum consortium (G1) produced very little methane from the J-cell rejects after 60 days of incubation ( $2.7 \pm 5.5 \mu\text{mol CH}_4/\text{g J-cell rejects}$ ). The consortium from the best biomethane producing bottle was used as inoculum to start the second generation (G2). Further successive adaptations were conducted and a stable biomethane yield of  $26.20 \pm 1.98 \mu\text{mol/g J-cell rejects}$  was observed after four generations. The reaction time also shortened from 60 days to less than 25 days. This adaptation process indicated that the environmental consortium contains microorganisms that are capable of converting J-cell rejects to biomethane, but the microorganisms were either in low abundance initially or needed time to shift their metabolic pathways for degrading the J-cell rejects.

### 3.3. Original methane and non-biological methane

Methane production from the positive controls with 20 mM BES reached the detectable level after 5 days of incubation ( $0.01 \pm 0.01 \mu\text{mol CH}_4/\text{g J-cell rejects}$ ) and remained the same for the rest of experimental period (Figure 1); this most likely arises from methane absorbed to the coal waste. Since this methane production was not the result of microbial activity, it was

subtracted from the biomethane production measured in the triplicates in both low and high inoculum:substrate ratio experiments.

### 3.4. Biomethane production from J-cell rejects

Yields of biomethane during the low- and high-ratio experiments are shown in Figure 1. Net biomethane production was observed starting from Day 3. The subsequent biomethane production profile showed the familiar S-curve, with a maximum cumulative biomethane yield of  $26.20 \pm 1.98 \mu\text{mol/g}$  J-cell rejects at 27 days in the low-ratio experiment and  $23.62 \pm 0.99 \mu\text{mol/g}$  J-cell rejects at 13 days in the high-ratio experiment. The corresponding maximum biomethane production rates were 5.54 and  $6.00 \mu\text{mol/g}$  J-cell rejects/day in the low- and high-ratio experiments, respectively. Increasing inoculum ratio could benefit the process start-up, but has no significant impact on the overall biomethane yield.

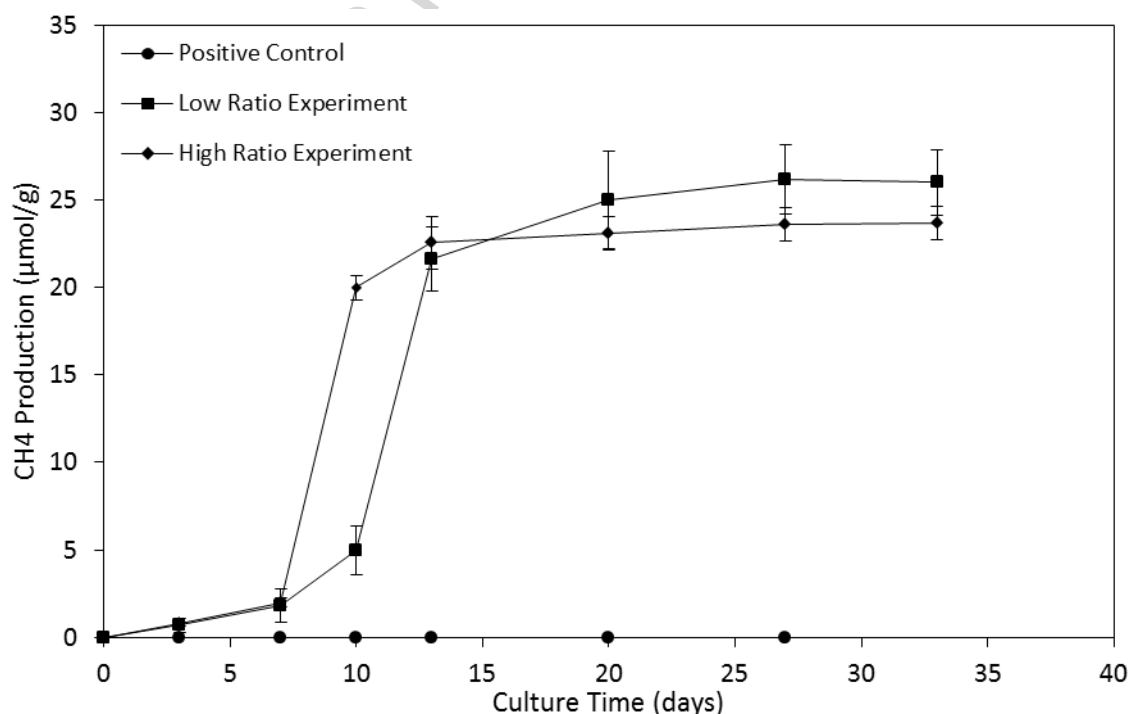


Figure 1. Biomethane productions of low and high inoculum:substrate ratios.

To capture the intermediate metabolic profiles of coal degradation and study the associated metabolic pathways, liquid samples were taken during and after the exponential biomethane production period. Samples of the low ratio experiment were then analysed by GC for VFAs of up to six carbons, and for ethanol, propanol and butanol. About 20 mg/L acetic acid was detected on Day 10 and less than 1 mg/L valeric acid was remaining on Day 33. No alcohol was detected in the degradation products. The dominance of acetate in the degradation products and its depletion at the end of the experiments suggest that acetate utilization was probably the main methanogenic pathway.

### 3.5. Microbial communities in the enriched culture

The distribution of microbial communities in the enriched culture was identified by pyrosequencing analysis (Figure 2). Over 8400 sequences were generated, which fell into 189 groups. Phylum *Proteobacteria* was the dominant bacteria (39.0 %), followed by the phyla *Spirochaetes* (23.2 %), *Bacteroidetes* (5.8 %) and *Firmicutes* (5.5 %). The genus *Methanosarcina* (8.8 %) and *Methanobacterium* (6.5 %) within the phylum *Euryarchaeota* were the dominant archaea in the adapted culture. Other microorganisms at less than 1 % individual abundance accounted for the remaining 13.4 % of the microbial population.

At genus level, *Dechloromonas* of the *Proteobacteria* lineages dominated the total community with an abundance of 26.0 %. *Treponema* that belongs to the phylum of *Spirochaetes* had an abundance of 17 %, which is followed by methanogens belong to the genus of *Methanosarcina* (8.8 %) and *Methanobacterium* (6.5 %).

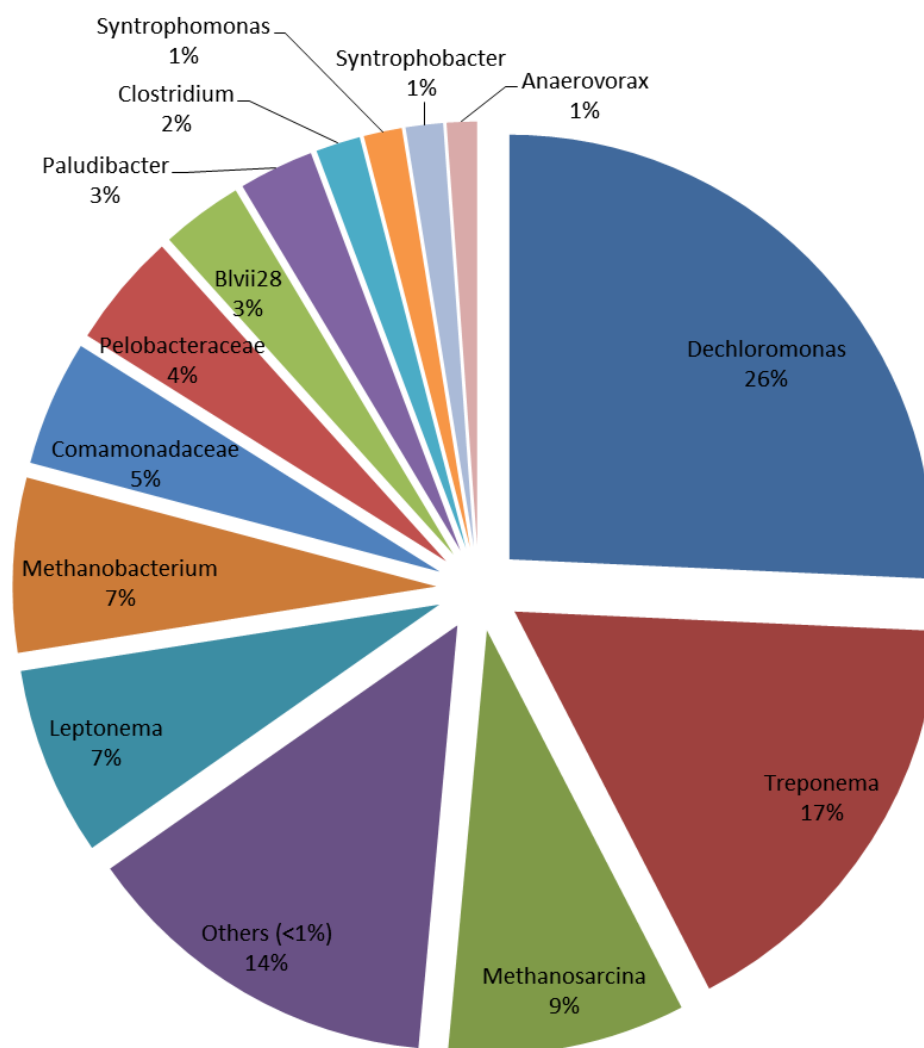


Figure 2. Microbial communities (genus level) identified by 16S rRNA gene pyrotag sequencing at the end of the batch experiments.

## 4. Discussion

### 4.1. Biomethane potential of coal waste materials

Biomethane yields from coal reported in the literature span a considerable range (0.24 - 320  $\mu\text{mol}$  methane/g coal, Table 2), although the reasons for this wide variability remain unexplained.

**Table 2. Biomethane yields and production rates observed from studies using various combinations of microorganisms and coals.**

Inoculum Sources	Coal type	Coal Sources	Incubating Conditions	CH <sub>4</sub> Yield (μmol/g)	References
Coal Bed Native Consortia	Lignite	Williston Basin, North Dakota, USA	22°C, Batch, in dark	0.24	(Fallgren et al., 2013a)
		Huolinguole, Inner Mongolia, China	23°C, Batch, in dark	240	(Fallgren et al., 2013b)
		Surat Basin, Queensland, Australia	23°C, Batch, in dark	320	(Fallgren et al., 2013b)
		Southern Sumatra island, Indonesia	23°C, Batch, in dark	330	(Fallgren et al., 2013b)
	Subbituminous	Powder River Basin, Wyoming, USA	22°C, Batch, in dark	0.38	(Fallgren et al., 2013b)
		Powder River Basin, Wyoming, USA	30°C, Batch	180	(Green et al., 2008)
		Powder River Basin, Wyoming, USA	30 °C, Batch, in dark	~205	(Gallagher et al., 2013)
		Surat Basin, Queensland, Australia	37°C, Batch	261	(Papendick et al., 2011)
	Bituminous	High Volatile, Pittsburgh No. 8, Pennsylvania (Freene County), USA	22°C, Batch, in dark	1.41	(Fallgren et al., 2013a)
		Low Volatile, Pocahontas No.3, Virginia (Buchanan Country), USA	22°C, Batch, in dark	2.47	(Fallgren et al., 2013a)
		Banaskantha coal mines, Gujarat state, India	60°C, Batch,	2290	(Rathi et al., 2015)
	Anthracite	Qingshui Basin, Zhaozhuang mining, China	30°C, Batch	8.99 mL/g (85 days)	(Xiao et al., 2013)
		Qingshui Basin, Sihe, China	30°C, Batch	4.5 mL/g (85 days)	(Xiao et al., 2013)
Environmental	Subbituminous	Upper Wyodak, Powder	22°C, Batch, in	23.2	(Jones et

Consortia	River Basin, Wyoming, USA	dark		al., 2008)
	Lower Wyodak, Powder River Basin, Wyoming, USA	22°C, Batch, in dark	8	(Jones et al., 2008)
	Pawnee, Powder River Basin, Wyoming, USA	22°C, Batch, in dark	9	(Jones et al., 2008)
	Wall, Powder River Basin, Wyoming, USA	22°C, Batch, in dark	1.7	(Jones et al., 2008)
	Big George, Wyoming, USA	22°C, Batch, in dark	2.2	(Jones et al., 2008)
	Monarch, Wyoming, USA	22°C, Batch, in dark	2.4	(Jones et al., 2008)
	Dietze 3, Wyoming, USA	22°C, Batch, in dark	3.3	(Jones et al., 2008)
Bituminous	North Slope Borough, Alaska, USA	22°C, Batch, in dark	3.3-5.1	(Jones et al., 2008)
Bituminous coal waste	Deer Creek, Utah, USA	23°C, Batch	16.05	(Opara et al., 2012)

The batch experiments conducted in this study consistently showed yields around 26  $\mu\text{mol CH}_4/\text{g}$  J-cell rejects, using the anaerobic sludge digestion derived consortium, comparable to the findings by Opara et al. (Opara et al., 2012) of 16  $\mu\text{mol CH}_4/\text{g}$  bituminous coal waste using cultures derived from various environments including waste coal, anaerobic digester and natural gas wells. Twenty six  $\mu\text{mol CH}_4/\text{g}$  rejects is equivalent to  $\sim 0.6 \text{ m}^3 \text{ CH}_4/\text{t}$ . Comparatively, the undisturbed methane contents in the coal seams in the Bowen Basin, Australia, which are exploited for coal seam gas, lie at depths of several hundreds of meters and typically contain 7-20  $\text{m}^3/\text{t}$  coal (Draper and Boreham, 2006).

Reject coal from preparation processes contain a number of additives (Osborne, 1988), including minerals (e.g. magnetite), frother and collector chemicals (e.g. Methyl-isobutyl-carbinol and diesel), and coagulants and flocculants. The process water in preparation plants

is typically recycled, so additives and dissolved salts build up in concentration. Some, like magnetite, may be beneficial to the biological processes while others such as frother and collector chemicals used in the J-Cell and coagulants and flocculants used in the dewatering are likely more toxic to microorganisms due to increased membrane fluidity and unspecific permeabilization of the membrane (Heipieper and Martínez, 2010). Yet others, e.g. diesel, are detrimental to cell membrane integrity (Heipieper and Martínez, 2010), but can also be substrate for some microorganisms (Boopathy, 2004; Das and Chandran, 2011). Quantifying the effects of the reagents used in preparation plants on biogenic methane production from coal waste materials is a focus of ongoing work.

#### **4.2. Microbial community of the adapted digester sludge**

16S rRNA pyrotag sequencing results showed that the digested sewage sludge derived consortium adapted to a mixture dominated by *Proteobacteria* (e.g. *Dechloromonas*), *Firmicutes*, *Spirochaetes* and *Methanomicrobia* (e.g. *Methanosarcina*), all of which are commonly found in native coal bed consortium derived cultures (Fry et al., 2009; Midgley et al., 2010; Penner et al., 2010; Ritter et al., 2015; Singh et al., 2012; Strapoc et al., 2011). These results and the prevalence of particular communities of micro-organisms provide information about the likely pathways involved in the breakdown of the coal.

Among these bacterial groups, members affiliated with *Dechloromonas* (within the phylum of *Proteobacteria*) were most abundant in the enriched culture and are able, with electron acceptors such as nitrate, Fe (III) and sulfate, to degrade anaerobically aromatic hydrocarbons (Chakraborty et al., 2005; Coates et al., 2001; Fry et al., 2009; Grbicgalic and Vogel, 1987; Weiner and Lovley, 1998) such as benzene, a likely product of coal depolymerisation.



The second most abundant bacterial group is *Treponema*, which have been identified in anaerobic reactors from various studies (Leven et al., 2007; Schrank et al., 1999; Zhang et al., 2009) and in rumen microbial communities (Stanton and Canaleparola, 1980; Tajima et al., 1999). They have the ability to mediate complex organic fermentation and acetogenesis processes under mesophilic conditions (Iida et al., 2000; Kudo et al., 1987) providing substrates for subsequent biomethane production. The coal depolymerisation process releases complex organics, including aromatic and aliphatic molecules, which are known to be anaerobically degraded to CH<sub>4</sub> and CO<sub>2</sub> by methanogenic mixed cultures sourced from sewage sludge and petroleum-contaminated aquifers (Grbicgalic and Vogel, 1987; Weiner and Lovley, 1998), with acetate and propionate acting as precursors intermediate products (Weiner and Lovley, 1998) for methanogenesis. Combining the observation of complex organics degrading bacteria in the microbial community and the detection of acetate as one of the main intermediate metabolic products, it may be deduced that *Dechloromonas* and *Treponema* in the microbial community, as observed here, have depolymerised the coal and degraded the solubilized organics to intermediates such as acetate, which was then consumed by methanogens to produce methane (Orem et al., 2010).

The archaeal community identified in the enriched culture mainly consisted of *Methanosarcina* (8.8 %) and *Methanobacterium* (6.5 %). *Methanosarcina* is a versatile genus of acetate-utilizing methanogens, which is capable not only of cleaving acetate, but also oxidizing acetate, providing H<sub>2</sub> and CO<sub>2</sub> to be further converted to methane by *Methanosarcina* itself and/or other hydrogenotrophic methanogens. The shift between acetoclastic and acetate oxidation can be influenced by temperature, organic acid concentration and ammonia concentration (Karakashev et al., 2006; Schnurer et al., 1996; Zinder and Koch, 1984). Generally, acetate oxidation is favoured at temperatures between

50 °C and 65 °C and high ammonia concentrations (Karakashev et al., 2006; Kudo et al., 1987; Zinder and Koch, 1984); however, these conditions are not applicable to the current study. This suggests that *Methanosarcina* more likely followed acetoclastic methanogenesis. In contrast, *Methanobacterium* is an obligatory hydrogen-utilizing methanogen, and its dominance indicates that some bacteria (e.g. *Firmicutes*) were mediating processes to produce H<sub>2</sub> that was subsequently consumed by *Methanobacterium*. The fact that no H<sub>2</sub> was detected and acetate consumption was observed in this case may be the result of these metabolic processes.

The microbial community structure may be interpreted as follows: fermentation of hydrolysed substrates by members of *Proteobacteria*, *Firmicutes* and *Spirochaetes* produces methanogenic substrates, which are then consumed by the hydrogenotrophic and acetoclastic methanogens (e.g. *Methanosarcina* and *Methanosarcina*) to produce methane. The microbial ecosystem is very similar to those detected in other coal-to-methane studies using native microbes, supporting the overall conclusion that the key coal-to-methane functional groups can be sourced from non-native environment.

#### 4.3. Vitality of the adapted digester sludge

Biomethane can be produced from Australian coal waste materials using digested sludge derived microbial consortium. While previous studies using native formation water derived cultures (**Error! Reference source not found.**) reported long incubation times (at least 30 days and up to 85 days (Xiao et al., 2013)) to achieve the maximum biomethane yield, sludge derived cultures provided comparable methane in less than 25 days. We surmise that critical micro-organisms are scarcely present in formation waters, e.g. those critical for the initial coal depolymerisation that may be more likely sessile, and take some time to re-establish. By

contrast these are more abundant in the sewage sludge, thereby reducing the incubation period.

It remains unclear, however, what factors influence coal bioavailability.

## 6. Conclusion

- Australian medium rank coal waste materials (J-cell rejects) provide a substrate suitable for biogenic degradation to methane.
- Sewage sludge bio-solids contain a sufficient diversity that, after a modest period of adaption, they can degrade coal as well as the consortia obtained in-situ from coal bed waters. This would be convenient for coal processing since sludge is readily and abundantly available.
- After adaption, the environmental consortium had very similar communities as the in-situ derived cultures.
- Bowen Basin waste coal can be partially digested to biomethane, providing (in this study) a yield of  $\sim 0.6\text{m}^3/\text{ton}$  reject. This production amount is unlikely to be viable and needs to be substantially improved, e.g. by an order of magnitude, to become commercially attractive.

## 7. Acknowledgements

The authors would like to thank Vale Australia and the Queensland Government for providing financial support through the Research Partnerships Program, Dr Paul Evans for undertaking the pyrosequencing and cell count analysis, Professor Joan Esterle for collecting J-cell rejects samples and Dr Sandra Rodrigues and Dr Rita Susilawati for undertaking the petrographic analysis for this study.

## 8. References

- Ahmed, M., Smith, J.W., 2001. Biogenic methane generation in the degradation of eastern Australian Permian coals. *Organic Geochemistry* 32, 809-816.
- Council of Australia Standard, 2000. Coal and coke - Analysis and testing - Proximate analysis of higher rank coal, AS 1038.3
- Batstone, D.J., Jensen, P.D., 2011. Anaerobic Digestion, in: Wilderer, P. (Ed.), *Treatise on water science*. Elsevier, p. 2012.
- Boopathy, R., 2004. Anaerobic biodegradation of no. 2 diesel fuel in soil: a soil column study. *Bioresource Technology* 94, 143-151.
- Chakraborty, R., O'Connor, S.M., Chan, E., Coates, J.D., 2005. Anaerobic degradation of benzene, toluene, ethylbenzene, and xylene compounds by *Dechloromonas* strain RCB. *Applied and Environmental Microbiology* 71, 8649-8655.
- Clayton, J.L., 1998. Geochemistry of coalbed gas – A review. *International Journal of Coal Geology* 35, 159-173.
- Coates, J.D., Chakraborty, R., Lack, J.G., O'Connor, S.M., Cole, K.A., Bender, K.S., Achenbach, L.A., 2001. Anaerobic benzene oxidation coupled to nitrate reduction in pure culture by two strains of *Dechloromonas*. *Nature* 411, 1039-1043.
- Das, N., Chandran, P., 2011. Microbial degradation of petroleum hydrocarbon contaminants: an overview. *Biotechnology research international* 2011, 941810-941810.
- Draper, J.J., Boreham, C.J., 2006. Geological controls on exploitable coal seam gas distribution in queensland. *APPEA Journal* 46, 343-366.
- Fallgren, P.H., Jin, S., Zeng, C., Ren, Z., Lu, A., Colberg, P.J.S., 2013a. Comparison of coal rank for enhanced biogenic natural gas production. *International Journal of Coal Geology* 115, 92-96.
- Fallgren, P.H., Zeng, C.P., Ren, Z.Y., Lu, A.H., Ren, S.J., Jin, S., 2013b. Feasibility of microbial production of new natural gas from non-gas-producing lignite. *International Journal of Coal Geology* 115, 79-84.
- Fry, J.C., Horsfield, B., Sykes, R., Cragg, B.A., Heywood, C., Kim, G.T., Mangelsdorf, K., Mildenhall, D.C., Rinna, J., Vieth, A., Zink, K.-G., Sass, H., Weightman, A.J., Parkes, R.J., 2009. Prokaryotic populations and activities in an Interbedded coal deposit, including a previously deeplyburied section (1.6-2.3 km) above 150 Ma basement rock. *Geomicrobiology Journal* 26, 163-178.
- Gallagher, L.K., Glossner, A.W., Landkamer, L.L., Figueroa, L.A., Mandernack, K.W., Munakata-Marr, J., 2013. The effect of coal oxidation on methane production and microbial community structure in Powder River Basin coal. *International Journal of Coal Geology* 115, 71-78.
- Geosurvey, Q., 2014. Queensland coal industry 5 year summary, in: Mines, D.o.N.R.a. (Ed.).
- Grbicgalic, D., Vogel, T.M., 1987. Transformation of toluene and benzene by mixed methanogenic cultures. *Applied and Environmental Microbiology* 53, 254-260.
- Green, M.S., Flanagan, K.C., Gilcrease, P.C., 2008. Characterization of a methanogenic consortium enriched from a coalbed methane well in the Powder River Basin, USA. *International Journal of Coal Geology* 76, 34-45.
- Grosso, J.a.W.-G., B, 1991. Plant Waste and Environmental Considerations, in: III, L.J. (Ed.), *Coal Preparation*. Society for Mining, Metallurgy, and Exploration, INC Littleton, Colorado, pp. 785-850.
- Hamilton, S.K., Golding, S.D., Baublys, K.A., Esterle, J.S., 2014. Stable isotopic and molecular composition of desorbed coal seam gases from the Walloon Subgroup, eastern Surat Basin, Australia. *International Journal of Coal Geology* 122, 21-36.
- Heipieper, H.J., Martínez, P.M., 2010. Toxicity of Hydrocarbons to Microorganisms, in: Timmis, K. (Ed.), *Handbook of Hydrocarbon and Lipid Microbiology*. Springer Berlin Heidelberg, pp. 1563-1573.
- Huang, Z., Urynowicz, M.A., Colberg, P.J.S., 2013. Stimulation of biogenic methane generation in coal samples following chemical treatment with potassium permanganate. *Fuel* 111, 813-819.

- Iida, T., Ohkuma, M., Ohtoko, K., Kudo, T., 2000. Symbiotic spirochetes in the termite hindgut: phylogenetic identification of ectosymbiotic spirochetes of oxymonad protists. *Fems Microbiology Ecology* 34, 17-26.
- Jones, E.J.P., Voytek, M.A., Warwick, P.D., Corum, M.D., Cohn, A., Bunnell, J.E., Clark, A.C., Orem, W.H., 2008. Bioassay for estimating the biogenic methane-generating potential of coal samples. *International Journal of Coal Geology* 76, 138-150.
- Karakashev, D., Batstone, D.J., Trably, E., Angelidaki, I., 2006. Acetate oxidation is the dominant methanogenic pathway from acetate in the absence of Methanosaetaceae. *Applied and Environmental Microbiology* 72, 5138-5141.
- Kinnon, E.C.P., Golding, S.D., Boreham, C.J., Baublys, K.A., Esterle, J.S., 2010. Stable isotope and water quality analysis of coal bed methane production waters and gases from the Bowen Basin, Australia. *International Journal of Coal Geology* 82, 219-231.
- Krueger, M., Beckmann, S., Engelen, B., Thielemann, T., Cramer, B., Schippers, A., Cypionka, H., 2008. Microbial methane formation from hard coal and timber in an abandoned coal mine. *Geomicrobiology Journal* 25, 315-321.
- Kudo, H., Cheng, K.J., Costerton, J.W., 1987. Interactions between treponema-bryantii and cellulolytic bacteria in the invitro degradation of straw cellulose. *Canadian Journal of Microbiology* 33, 244-248.
- Leven, L., Eriksson, A.R.B., Schnurer, A., 2007. Effect of process temperature on bacterial and archaeal communities in two methanogenic bioreactors treating organic household waste. *Fems Microbiology Ecology* 59, 683-693.
- Li, D., Hendry, P., Faiz, M., 2008. A survey of the microbial populations in some Australian coalbed methane reservoirs. *International Journal of Coal Geology* 76, 14-24.
- McIntosh, J., Martini, A., Petsch, S., Huang, R., Nuesslein, K., 2008. Biogeochemistry of the Forest City Basin coalbed methane play. *International Journal of Coal Geology* 76, 111-118.
- Midgley, D.J., Hendry, P., Pinetown, K.L., Fuentes, D., Gong, S., Mitchell, D.L., Faiz, M., 2010. Characterisation of a microbial community associated with a deep, coal seam methane reservoir in the Gippsland Basin, Australia. *International Journal of Coal Geology* 82, 232-239.
- Moore, T.A., 2012. Coalbed methane: A review. *International Journal of Coal Geology* 101, 36-81.
- Opara, A., Adams, D.J., Free, M.L., McLennan, J., Hamilton, J., 2012. Microbial production of methane and carbon dioxide from lignite, bituminous coal, and coal waste materials. *International Journal of Coal Geology* 96-97, 1-8.
- Orem, W.H., Voytek, M.A., Jones, E.J., Lerch, H.E., Bates, A.L., Corum, M.D., Warwick, P.D., Clark, A.C., 2010. Organic intermediates in the anaerobic biodegradation of coal to methane under laboratory conditions. *Organic Geochemistry* 41, 997-1000.
- Osborne, D.G., 1988. Coal preparation technology. Graham & Trotman, London; Boston.
- Papendick, S.L., Downs, K.R., Vo, K.D., Hamilton, S.K., Dawson, G.K.W., Golding, S.D., Gilcrease, P.C., 2011. Biogenic methane potential for Surat Basin, Queensland coal seams. *International Journal of Coal Geology* 88, 123-134.
- Penner, T.J., Foght, J.M., Budwill, K., 2010. Microbial diversity of western Canadian subsurface coal beds and methanogenic coal enrichment cultures. *International Journal of Coal Geology* 82, 81-93.
- Rathi, R., Priya, A., Vohra, M., Lavania, M., Lal, B., Sarma, P.M., 2015. Development of a microbial process for methane generation from bituminous coal at thermophilic conditions. *International Journal of Coal Geology* 147, 25-34.
- Ritter, D., Vinson, D., Barnhart, E., Akob, D.M., Fields, M.W., Cunningham, A.B., Orem, W., McIntosh, J.C., 2015. Enhanced microbial coalbed methane generation: A review of research, commercial activity, and remaining challenges. *International Journal of Coal Geology* 146, 28-41.
- Schnurer, A., Schink, B., Svensson, B.H., 1996. *Clostridium ultunense* sp nov, a mesophilic bacterium oxidizing acetate in syntrophic association with a hydrogenotrophic methanogenic bacterium. *International Journal of Systematic Bacteriology* 46, 1145-1152.

- Schrank, K., Choi, B.K., Grund, S., Moter, A., Heuner, K., Nattermann, H., Gobel, U.B., 1999. *Treponema brennaborensis* sp. nov., a novel spirochaete isolated from a dairy cow suffering from digital dermatitis. *International Journal of Systematic Bacteriology* 49, 43-50.
- Singh, D.N., Kumar, A., Sarbhai, M.P., Tripathi, A.K., 2012. Cultivation-independent analysis of archaeal and bacterial communities of the formation water in an Indian coal bed to enhance biotransformation of coal into methane. *Applied Microbiology and Biotechnology* 93, 1337-1350.
- Stanton, T.B., Canaleparola, E., 1980. *Treponema bryantii* sp. nov., a rumen spirochete that interacts with cellulolytic bacteria. *Archives of Microbiology* 127, 145-156.
- Strapoc, D., Mastalerz, M., Dawson, K., Macalady, J., Callaghan, A.V., Wawrik, B., Turich, C., Ashby, M., 2011. Biogeochemistry of Microbial Coal-Bed Methane, in: Jeanloz, R., Freeman, K.H. (Eds.), *Annual Review of Earth and Planetary Sciences*, Vol 39, pp. 617-656.
- Tajima, K., Aminov, R.I., Nagamine, T., Ogata, K., Nakamura, M., Matsui, H., Benno, Y., 1999. Rumen bacterial diversity as determined by sequence analysis of 16S rDNA libraries. *Fems Microbiology Ecology* 29, 159-169.
- Tao, M., Shi, B., Li, J., Wang, W., Li, X., Gao, B., 2007. Secondary biological coalbed gas in the Xinji area, Anhui province, China: Evidence from the geochemical features and secondary changes. *International Journal of Coal Geology* 71, 358-370.
- Vanwonterghem, I., Jensen, P.D., Dennis, P.G., Hugenholtz, P., Rabaey, K., Tyson, G.W., 2014. Deterministic processes guide long-term synchronised population dynamics in replicate anaerobic digesters. *Isme Journal* 8, 2015-2028.
- Warwick, P.D., Breland, F.C., Jr., Hackley, P.C., 2008. Biogenic origin of coalbed gas in the northern Gulf of Mexico Coastal Plain, USA. *International Journal of Coal Geology* 76, 119-137.
- Weiner, J.M., Lovley, D.R., 1998. Rapid benzene degradation in methanogenic sediments from a petroleum-contaminated aquifer. *Applied and Environmental Microbiology* 64, 1937-1939.
- Xiao, D., Peng, S.P., Wang, B.Y., Yan, X.X., 2013. Anthracite bio-degradation by methanogenic consortia in Qinshui basin. *International Journal of Coal Geology* 116, 46-52.
- Zhang, H., Banaszak, J.E., Parameswaran, P., Alder, J., Krajmalnik-Brown, R., Rittmann, B.E., 2009. Focused-Pulsed sludge pre-treatment increases the bacterial diversity and relative abundance of acetoclastic methanogens in a full-scale anaerobic digester. *Water Research* 43, 4517-4526.
- Zinder, S.H., Koch, M., 1984. Non-aceticlastic methanogenesis from acetate- acetate oxidation by a thermophilic syntrophic coculture. *Archives of Microbiology* 138, 263-272.

## Highlights

- Australian medium rank coal waste materials (J-cell rejects) provide a substrate suitable for biogenic degradation to methane.
- Sewage sludge bio-solids contain a sufficient diversity that, after a modest period of adaption, they can degrade coal as well as the consortia obtained in-situ from coal bed waters. This would be convenient for coal processing since sludge is readily and abundantly available.
- Bowen Basin waste coal can be partially digested to biomethane, providing (in this study) a yield of  $\sim 0.6\text{m}^3/\text{ton}$  reject. This production amount is unlikely to be viable and needs to be substantially improved, e.g. by an order of magnitude, to become commercially attractive.