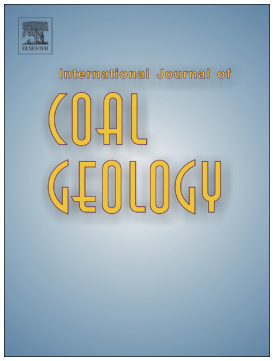

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Characterisation of Bioavailability of Surat Basin Walloon Coals for Biogenic Methane Production Using Environmental Microbial Consortia

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ABSTRACT

The study sets out to characterize the bioavailability of six Surat Basin Walloon coals from different stratigraphic layers in a single borehole to environmental methanogenic consortia. Factors that control bioavailability have also been investigated on grounds of coal petrographic composition and the organic composition of solvent-extractable matter. Finely crushed coal core samples were inoculated with digested sludge culture from domestic wastewater treatment in serum bottles kept anoxically before incubation at mesophilic temperature over 30 days for biomethane production. Degradation of coal compounds was demonstrated via GC-MS characterization of methanol and dichloromethane (DCM) extracts, in combination with aqueous volatile fatty acids and alcohols and TOC (total organic carbon) analysis on fresh and microbially-digested coal samples. The resulting methane yields ranged from 14 to 33 μmol/g, with an average of 21 μmol/g (0.515 m^3/tonne), comparable to those previously reported for subbituminous coals. Organic solvent-extractable materials that accounted for 3.8 to 12% of coal weight were generally dominated by aliphatic compounds, composed of mainly medium-long-chain *n*-alkanes, *n*-alcohols and esters. Aromatics were detected up to three fused rings, and are rich in dibenzofuran, alkyl benzene, diphenyls and alkyl PAH (polymeric aromatic hydrocarbon). The abundance of solvent-extractable matter was found to be positively associated with liptinite content, particularly suberinite, sporinite and liptodetrinite. Preservation of these compounds was thought to rely on vitrinite, such as telinite and collotelinite that are rich in micropores, serving as storage for the hydrocarbons. Environmental factors, such as microbe-carrying groundwater might compromise coal extractability by converting coal hydrocarbons to biogas. Bioavailability of coal was shown to be controlled by three factors: 1) Water solubility - Bioassay eliminated an average 98% of aqueous compounds (based on TOC), which were dominated by volatile fatty acids and alcohols, and to a lesser degree, medium-chain (primarily C_{10} to C_{20}) *n*-alcohols, esters and aliphatic amine; 2) Solvent extractability – 34.5% of solvent-extractable compounds were shown to be biodegraded (based on peak intensity in GC-MS), with methanol extracts being more bioavailable than DCM's; 3) Heterogeneous moieties, particularly aliphatic hydroxyl group, ester bond, ether bond and C-N bond in aliphatic amine - These functional groups present heteroatoms that can lower the activation energy of nearby bonds, making them vulnerable for microbial cleavage. Compound degradation in bioassays was shown to be clearly associated with methane yield, but only a small proportion degraded was converted to methane. Further improvement may be achieved via proper adaptation of the current microbial community.

Key words: unconventional natural gas; biogenic coal seam gas; bioavailability of coal; maceral compositions; solvent extraction; GC-MS characterization of solvent extract.

1. INTRODUCTION

1.1 THE EMERGING CONCEPT OF MICROBIALLY ENHANCED COALBED METHANE (MECOM) AS A FORM OF CLEAN FUEL

The demand for clean energy has increased rapidly since the late 1990s due to the concerns of pollution by burning traditional fossil fuels. As a typical form of clean energy (less soot pollution from burning), natural gas has attracted considerable interest, owing to its low carbon intensity compared to its high calorific value. Australia's consumption of natural gas has experienced steady growth in the first two decade of 21th century and is expected to account for 35% of total domestic energy consumption by 2035 (Ferguson et al., 2010). Australia also exports considerable amounts of liquefied natural gas (LNG). The source for much of this gas is from coal seams.

The demand is even higher internationally. The predicted export of Australian liquefied natural gas (LNG) in 2035 will see 19 fold growth with reference to that in 2000 (Ferguson et al., 2010), forming a potentially significant source of national revenue.

The formation of coal seam natural gas takes place via two processes: 1) thermogenesis, where hydrocarbons in coals are cracked by heat in the subsurface, producing methane and other gases; and 2) secondary biogenesis, where coals are gasified by methanogenic consortia via anaerobic digestion (e.g. Gao et al., 2014; Moore, 2012). The contribution of the latter to total coalbed methane (CBM) production has been proved to be significant (Draper & Boreham, 2006; Hamilton et al., 2014).. The Walloon Subgroup (Surat Basin, Queensland) coals investigated in this study produce almost entirely microbial CBM (Draper & Boreham, 2006; Hamilton et al., 2014). Secondary biogenesis presents the opportunity for making additional methane through microbial action. Research on microbial-enhanced coalbed methane (MECoM) production has been conducted in four general directions: 1) microbial stimulation involving in-situ nutrient amendment; 2) bioaugmentation through injection of enrichment culture; 3) increasing coal accessibility via physical fracturing; and 4) enhancement of bioavailability by biotic or abiotic pretreatments (Ritter et al., 2015). A substantial body of work has demonstrated the feasibility of the above methods (Fallgren et al., 2013a; Fallgren et al., 2013b; Furmann et al., 2013b; Green et al., 2008; Hang et al., 2017; Harris et al., 2008; Huang et al., 2013; Jones et al., 2010; Jones et al., 2008; Papendick et al., 2011; Ritter et al., 2015; Susilawati et al., 2013). Nevertheless, the field of MECoM research is still in the stage of exploration and a better fundamental understanding is necessary to assess the maximum methanogenic potential and possible improvements on the tiny conversion of coal to methane (normally below 1%. e.g. Furmann et al., 2013b; Jones et al., 2008; Green et al., 2008; Harris et al., 2008). There remains a critical piece lack of information concerning the bioavailability of coal, and reagarding what kinds of coals are suitable for enhanced methanogenesis.

1.2 CURRENT KNOWLEDGE ON COAL BIOAVAILABILITY

The understanding of coal bioavailability and its controlling factors, and in particular, the association between coal components and amenability to fermentation, to produce common methanogenic precursors such as acetate, is deficient. It may be expected that the thermal

maturity might be significant and it is widely accepted that lower rank coals are more biodegradable as a result of structural heterogeneity and lower degree of condensation (Fakoussa & Hofrichter, 1999; Orem & Finkelman, 2004; Rice & Claypool, 1981; Robbins et al., 2016; Scott, 1999). Robbins et al. (2016) reported a positive correlation between methane yield and contents of volatile fatty acids (VFAs), particularly acetate, which are richer in low rank coals. While it appears rank can influence biomethane production (e.g. Robbins et al., 2016), other studies cloud any clear relationship. For example, Jones et al. (2008) demonstrated significant variation in methane production from coals within the subbituminous rank and found final methane yields that were a magnitude smaller than those obtained by Gallagher et al. (2013) on coals of the same rank. Moreover, Fallgren et al. (2013a) found that bituminous coals produced more methane than lower rank lignites, whereas in a later study (Fallgren et al., 2013b), methane yields were reported to be two magnitudes higher from lignites than from other sources. This lack of consensus seems to preclude coal rank as the only determining factor for coal bioavailability.

A great deal of attention has also been given to maceral composition. Coal macerals are solid organic entities with unique features inherited from their plant material origin. Liptinite macerals have been claimed to have the highest methanogenic potential (Hunt, 1979; Isbister & Barik, 1993; Scott, 1999) as they have high hydrogen contents (generally >6% dry ash free, d.a.f.) and are rich in saturated moieties that are more easily biodegradable than aromatics (Head et al., 2003; Peters & Moldowan, 1993; Widdel et al., 2010). Nevertheless, Machnikowska et al. (2002) found liptinite concentrated in the biodegradation residue of a Polish subbituminous coal, which suggests that it is more refractory, and in a more recent study Furmann et al. (2013b) observed higher methane yields from vitrinite-rich coal extract as opposed to the coals with greater proportions of liptinite and inertinite. A possible explanation is that, for a given rank, the higher microporosity of vitrinite may provide enhanced surface accessibility and the higher abundance of heteroatoms in vitrinite may also offer advantageous conditions for microbial attack (Flores, 1998; Furmann et al., 2013b). The high heterogeneity of coal, and the confounding influences of many other factors (e.g. rank, depositional environment, exposure to ground water, microbial inocula used) makes it difficult to draw conclusions solely on the basis of coarsely classified composition like maceral groups.

A number of recently studies have sought to understand coal bioavailability in terms of organic chemistry to establish a direct linkage between compounds elimination and methane production. The organic part of coal is comprised of solid kerogen, infused with bitumen or solvent-extractable matter contained in the pores and cleats (Jones et al., 2013; Mastalerz & Glikson, 2000). The extractable materials are believed likely to be more degradable as result of smaller molecule size, higher mobility since they are not chemically bonded to the carbon skeleton of the coal, and because they contain many heteroatoms (Furmann et al., 2013a; Furmann et al., 2013b; Jones et al., 2010). They are, to varying degrees, leachable by water and are hence more accessible to aqueous microorganisms. Orem and Finkelman (2004) and Orem et al. (2007) classified the dissolved species in water produced from coal seams into the following groups: aromatic compounds, including polycyclic aromatic hydrocarbons (PAHs; mainly two to three ring entities), alkylbenzenes, substituted biphenyls, heterocyclic compounds, phenols and derivatives; and aliphatic compounds including long chain fatty acids, long chain *n*-alkanes and terpenoids. The coal-derived normal fatty acids have been

shown to be bioavailable by Jones et al. (2013) and Jones et al. (2010) since their experiments showed that fatty acids gradually accumulated in bioassays (using biomethane potential bottles containing coal, microbial consortia and liquid-phase nutrients) treated with 2-bromoethanesulfonate (BES, a methanogenic inhibitor), in contrast to the control culture with methane production. In addition, microbial inoculation of dichloromethane (DCM) extract of coal eliminated 14 to 91% of *n*-alkanes and 6 to 58% of aromatics (Furmann et al., 2013b). Longer-chain *n*-alkanes were more preferentially degraded, as were the aromatic compounds with fewer fused rings (Furmann et al., 2013b; Gao et al., 2013).

These findings set the tone for exploration of coal bioavailability using organic chemistry, but are limited by the number and diversity of samples and scope of individual studies, leaving conclusions that are hardly comprehensive. A notable issue in compound analysis is the focus on water samples, since these have low recovery of coal organics. The majority of coal extractable matter (e.g. *n*-alkanes, long-chain fatty acids) is hydrophobic and even if liberated by water, is prone to adsorptive loss on hydrophobic surfaces like the coal itself and the filter membrane during filtration (Jones et al., 2013). The resulting information on bioavailability could therefore be skewed and limited. Moreover, most bioavailability research in organic chemistry is rather narrow such that a clear relationship is yet to be established between the reported compounds and other coal characteristics such as petrographic composition. Elimination of compounds also needs to be closely associated with methane production. However, quantitative linkage between compounds degradation and methane yield has seldom been reported. Special caution also needs to be taken in regards to the presence of alternative electron acceptors such as sulphate or nitrate that could outcompete methanogenic pathways for carbon usage (Dar et al., 2008; Kristjansson & Schönheit, 1983; Oremland & Polcin, 1982; Sakthivel et al., 2012). In addition, most methane yields reported are specific to the composition of indigenous microbial consortia. However, spatial differences in coal and interburden character, in situ reservoir conditions, and hydrogeologic evolution between well sites could result in significant variation in microbial community structure and composition. This may have caused coals within and between field studies to be inconsistently degraded, making the effect of coal bioavailability difficult to interpret.

1.3 PURPOSE OF THIS STUDY

Experiments in this study seek to improve on the above aspects and explore the basic level of bioavailability of Surat Basin Walloon Subgroup coals in bioaugmented microcosms with the help of organic chemistry. The targeted compounds are those readily convertible to methane by exogenous microbial consortia without prolonged adaptation. The reason for using exogenous microbes is to provide an all-around and robust microbial community in contrast to indigenous consortia which might have limited species and population, thereby making the assessment of bioavailability more comprehensive and unbiased. For this purpose, we use anaerobic digester sludge from domestic waste water treatment plants as the inocula for coal bioassays in that it is non-selective, and contains a wide and robust spectrum of microbial species. Objectives of this study include the following: 1) Determine the biogenic methane yields from coal bioassays; 2) Characterise the composition of coal mobile compounds that are leachable by water or organic solvents; 3) Characterise the bioavailable compounds in coals and the association between biodegradation and methane production; and 4) Explore the relation of bioavailability and methane yield with petrographic characteristics and burial depth at a single well site.

2. SAMPLES AND METHOD

2.1 GEOLOGICAL SETTING AND COAL SAMPLING

The study well is a vertical CBM appraisal well drilled in the central north of the Surat Basin, Queensland (Fig. 1). Jurassic Walloon Subgroup coals in the Surat Basin host approximately two-thirds of Queensland's proven and probable (2P) CBM reserves (Towler et al., 2016). The Walloon Subgroup comprises the Upper and Lower Juandah Coal Measures and the Taroom Coal Measures, separated by the Tangalooma Sandstone (Fig. 2 (Hamilton et al., 2014). Walloon coals are hydrogen-rich (Khavari-Khorasani, 1987), containing a large proportion of perhydrous vitrinite, relatively high liptinite and low inertinite (Scott et al., 2007). The coals sit at the base of oil window (Tissot & Welte, 1984) and are rich in hydrocarbons that are degradable by indigenous microbial consortia (Boreham & Powell, 1991; Powell, 1993). Isotopic studies suggest that Walloon CBM in the Surat Basin is dominated by secondary biogenic methane generated through the CO_2 reduction pathway (Draper & Boreham, 2006; Hamilton et al., 2012; Hamilton et al., 2014). Being situated in a major recharge area for the Great Artesian Basin and surrounded by two aquifers, the Hutton Sandstone and the Springbok Sandstone, Walloon coals in the CBM production zone are subject to meteoric recharge that is interpreted to have introduced microorganisms and essential inorganic nutrients (Draper & Boreham, 2006; Baublys et al., 2015). These features are necessary requirements for in-situ coal biodegradation.

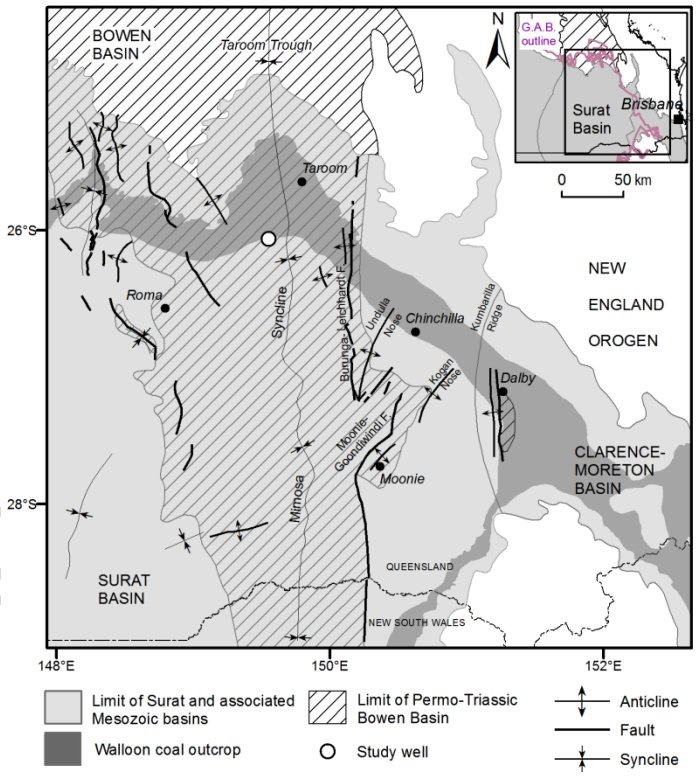


FIGURE 1: STUDY WELL LOCATION IN THE SURAT BASIN. BOWEN BASIN OUTLIER SOUTH OF DALBY TOWNSHIP BASED ON DAY ET AL. (2008); STRUCTURE MODIFIED FROM DAY ET AL. (2008) AND GEOLOGICAL SURVEY OF QUEENSLAND (2011). INSET: LOCATION OF THE STUDY AREA WITHIN EASTERN AUSTRALIA AND THE GREAT ARTESIAN BASIN (G.A.B.).

For this study, six Walloon Subgroup coal core samples were acquired after routine coal core canister desorption testing. In the field, fresh cores were sealed immediately on retrieval after

routine logging procedures. The sealed canisters were purged with helium for 60 s to remove air then sent to ALS Earth Data Pty Ltd, Stafford, Brisbane, where they were stored in a water bath at reservoir temperature throughout desorption (90 days). During this time, gas production was monitored twice a week until <1% of the total gas was produced within a week. Core samples for this study were collected within 30 min of commencing the 'offline' operation (after desorption is finished and samples taken out of the canister). Four of the 6 samples are from the Juandah Coal Measures at depths of 113.48 m (PEN9-003, where PEN standards for Penrhyn 9, the name of the sampling site), 201.34 m (PEN9-014), 264.10 m (PEN9-024), and 325.20 m (PEN9-029, near the upper boundary of Tangalooma Sandstone), and two are from the Taroom Coal Measures at depths of 441 m (PEN9-034, near the lower boundary of Tangalooma Sandstone) and 509.25 m (PEN9-043). Approximately 10 cm long whole coal core segments were vacuum-sealed in plastic bags and transported anoxically to the School of Chemical Engineering, The University Queensland (UQ), where they were transferred into glass containers and stored in an anaerobic chamber.

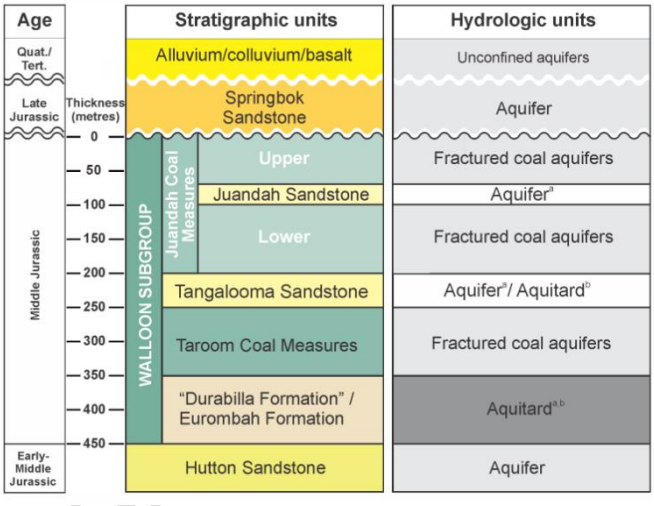


Figure 2: walloon subgroup stratigraphic sub-units, over- and underlying potable aquifers and hydrologic units relevant to this study. relative ages at left from mckellar (1998). [a] Worley parsons (2010), [b] lagendijk and ryan (2010).

2.2 COMPOSITIONAL AND PETROGRAPHIC CHARACTERISATION

For compositional and petrographic analysis, the outer layer of each coal core sample was chiselled off and the samples crushed and ground to the size range used in the experiments (300 – 500 µm). This particular size range is thought to provide sufficient surface area for colonization and mass transfer, while keeping the ash content low. 10 g of each powdered sample was sent to the ALS Pty Ltd. Coal Division for proximate and ultimate analysis, following Australian Standards AS 1038.3 (for proximate composition, Standards Australia, 2000), AS 1038.6.4 (for carbon, hydrogen and nitrogen, Standards Australia, 2005)) and AS 1038.6.3.3 (for total sulfur, Standards Australia, 1997).

Petrographic analysis was carried out at the School of Earth Sciences, UQ. Samples were prepared by passing the bulk powder through a splitter box that generates two homogeneous

subsets with compositions representative of the size range. The same operation was repeated for one of the two subsets until a suitable amount powder was obtained (roughly 10 g). The powdered samples were then mounted in epoxy resin and polished according to the recommendation in the ISO 7404-2 (2009) standard for petrographic analyses of coal under reflected white light. The random vitrinite reflectance (R_r%) and maceral composition were determined according to ISO 7404-5 (2009) and ISO 7404-3 (2009), respectively. Samples were analysed using a Leica DM6000 M microscope in air, with fluorescent light used to assist in the identification of macerals, particularly liptinite group macerals. Maceral analysis was performed using the International Committee for Organic Petrology (ICCP) classification and nomenclature (ICCP, 1998; ICCP, 2001; Sýkorová et al., 2005; Taylor et al., 1998).

2.3 SETUP OF BIOASSAYS

Coal bioassays were set up anoxically in an anaerobic chamber inflated with nitrogen. Adapted Tanner media (Tanner, 2007) was prepared for microbial culturing, providing essential sources of non-carbon nutrients (minerals, trace metals, vitamins, $NaHCO_3$ buffer, and $Na_2S{\cdot}9H_2O$ as anti-oxidant). The modified recipe eliminated the use of TES buffer that contains oxidised sulphur, a potential inhibitor of methanogenesis (Dar et al., 2008; Kristjansson & Schönheit, 1983). For each coal bioassay, 0.25 g coal powder (size range 300 – 500 μm) was added to a 37 mL biomethane potential bottle (BMP bottle) together with 9 mL growth media. The bottle was sealed with a butyl rubber stopper and crimped with an aluminium cap to keep it gas-tight. The headspace was vacuumed and refilled with nitrogen to a slight over pressure to prevent intrusion of air. The coal-media mixture was then autoclaved at 120 ^{o}C for 20 minutes before inoculation. Anaerobic digester sludge from domestic wastewater treatment plants (sourced from Luggage Point Wastewater Treatment Plant, Brisbane, Australia, provided by the Advanced Water Management Centre, within UQ) was used as the inocula after a period of pre-incubation to exhaust the native carbon. The autoclaved bottles were inoculated with 1 mL of the above sludge culture using sterile 3 mL disposable syringes and 21 gauge needles before being incubated at 37 ^{o}C in dark.

Bioassays were set up in quadruplicate for each coal sample along with quadruplicate negative controls containing only media and inocula, and triplicate desorption controls with only media and coal. The presence of control cultures allows determination of net microbial production of methane from bioassays.

2.4 COAL EXTRACTION

Solvent extraction of coal powders (300 – 500 μm) took place in a two-step Tecator Soxtec system HT2 1045 (shown in Fig. 3), a method adapted from Soxhlet with improved efficiency. Extraction starts with a boiling stage, where coal powder is contained by a paper thimble (only permeable to solvent) and submerged in solvent held by an aluminium cup. A clamp-on hot plate attaches the cup air-tightly to the extraction chamber and provides heat for boiling. The rising vapour is condensed by cooling water in the condenser and returns to the thimble through a bridging glass tunnel. In the second stage of rinsing, the thimble is lifted away from the cup, suspended in the extraction chamber. Solvent refluxes from the condenser and washes down the free compounds on the coal surface, improving the recovery of extracts in the cup. Membrado Giner et al. (1996) showed that a combination of 1 minute boiling and 30 minutes rinsing produced the equivalent yield to 24 hours of Soxhlet extraction.

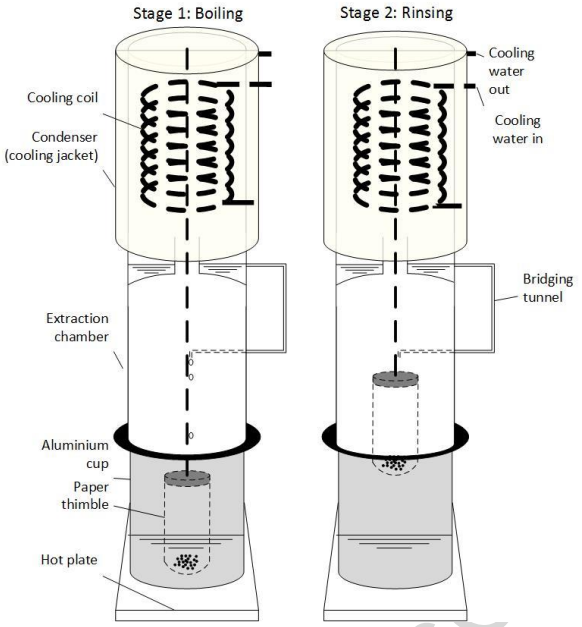


FIGURE 3: SCHEMATIC DIAGRAM OF SOXTEC SYSTEM FOR COAL EXTRACTION. DRAWING ADAPTED FROM FOSS SOXTEC™ SYSTEM, TECATOR™ LINE.

In this study, fresh coal samples were sequentially extracted with three solvents with increasing hydrophobicity: water, HPLC grade methanol and AR dichloromethane. The purpose is to maximise the recovery of compounds with different natures: hydrophilic, amphipathic and hydrophobic, as well as to study the effect of hydrophobicity on compound bioavailability. A parallel set of extractions was performed with only the organic solvents (i.e. methanol and then DCM). The difference in the organic extracts of the two sets would define the water-soluble compounds. Digested samples from bioassays, as well as the negative controls, were also extracted with methanol and DCM. The resulting extracts were compared to those of fresh coals to give information on coal bioavailability. Figures. 4 and 5 illustrate

the solvent extraction procedure and method for interpretation of the outcomes.

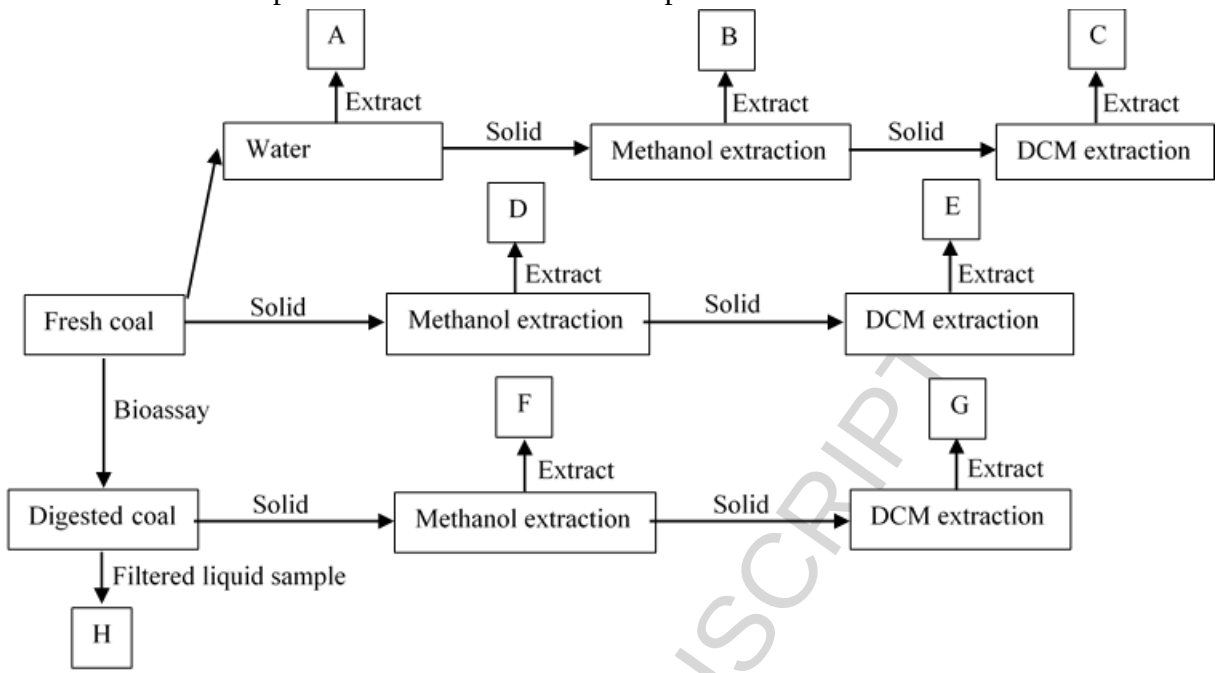


FIGURE 4: SCHEMATIC DIAGRAM OF SOLVENT EXTRACTION AND PRODUCT. BLOCKS WITH ALPHABETS LETTERS REPRESENT EXTRACTION PRODUCTS. NEGATIVE CONTROLS (NC1, NC2, NC3, AND NC4) WERE SET UP ACCORDINGLY TO ESTABLISH THE BASELINE FOR ANALYSIS.

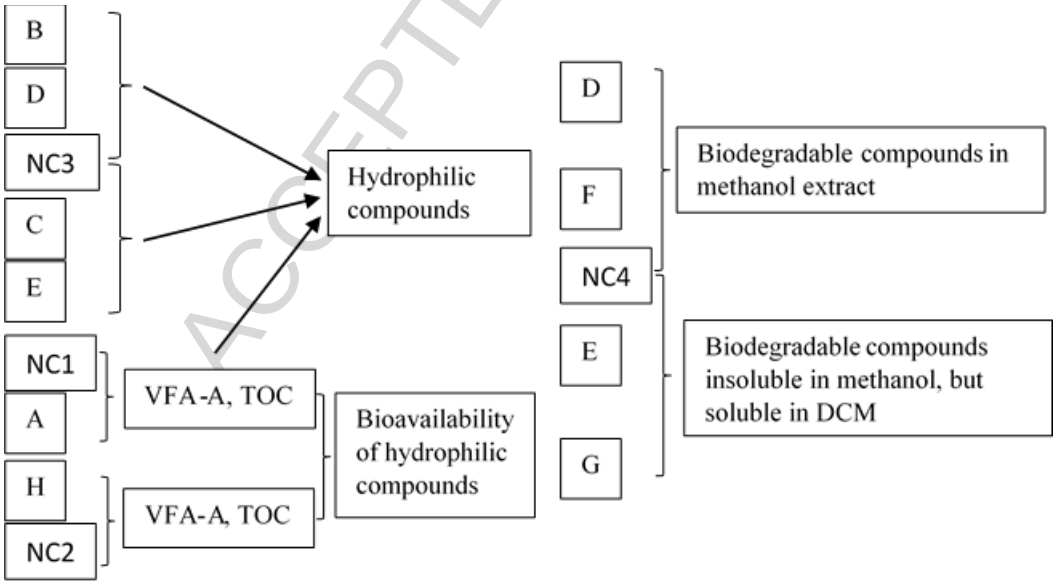


FIGURE 5: SCHEMATIC DIAGRAM FOR INTERPRETATION OF SOLVENT EXTRACTION EXPERIMENT. VFA-A – VOLATILE FATTY ACIDS AND ALCOHOLS, TOC – TOTAL ORGANIC CARBON IN WATER SOLUTION.

During sequential extraction, solid residues from the previous round were dried overnight at 37°C before being extracted with the next solvent. The digested coal samples were prepared by combining each quadruplicate of coal bioassays (to add up to 1 g for each coal) and centrifuging at 3750 rpm for 5 minutes. The bottom pellet was dried at 37 °C overnight before extraction.

All extracts were transferred to glass tubes and concentrated to 1 mL at room temperature under a gentle stream of nitrogen. Methanol extracts were further evaporated to 0.5 mL and refilled with DCM to 1 mL to enhance recovery of hydrophobic compounds. The concentrates were then sealed and sent for GC-MS analysis. Tables 1 and 2 summarise the operation parameters and extraction yields.

TABLE 1: PARAMETERS FOR SOLVENT EXTRACTION.

Parameters	Stage	Value
Extraction time (min)	Boiling	60
	Rinsing	60
Coal loading (g/extract)		1
Volume of solvent (mL)		30

TABLE 2: SUMMARY OF SOLVENT EXTRACTION YIELD.

Samples	PEN9-003	PEN9-014	PEN9-024	PEN9-029	PEN9-034	PEN9-043
Fresh coal (g)	1	1	1	1	1	1
Water extractable (g)	0.0011	0.0007	0.0009	0.0014	0.0005	0.0013
Methanol extractable (g)	0.0807	0.0470	0.0465	0.0365	0.0282	0.0849
DCM extractable (g)	0.0385	0.0300	0.0206	0.0205	0.0094	0.0293
Total extractable (%)	12.0	7.77	6.80	5.84	3.81	11.6

2.5 VOLATILE FATTY ACID AND ALCOHOLS AND TOTAL ORGANIC CARBON ANALYSIS

Volatile short-chain fatty acids and alcohols (referred to VFA-As hereafter) are potential in-situ coal fermentation substrates that reside in the coal matrix (Hang et al., 2017). Upon dissolution in water, they are readily degradable by microbial consortia. To quantify VFA-As in coal, 1 mL samples of the water extracts were filtered by Millex GP (33 µm) micro-filter after being cooled down to room temperature in the sealed extraction chamber maintained air-tight (to prevent loss of volatile compounds in the vapour phase).

To prepare for analysis, 0.4 mL of the filtered extract was transferred to a glass vial containing 0.32 mL Milli Q water and 0.08 mL 10% formic acid solution. The original concentration was diluted by two times to reduce the concentration to within the detection range (<100 ppm). The mixture was then sent to the analytical laboratory of the Advanced Water Management Centre (within the University of Queensland) for analysis, using an Agilent 7890A GC with a flame ionization detector (FID).

The total dissolved organic carbon (TOC) in the same water extract of coal was also measured. 1 mL of each filtered extract was added to 7 mL of Milli Q water and sealed in a glass tube with a Parafilm. The samples were sent to the same laboratory for TOC analysis.

For both VFA-As and TOC analysis, a blank containing only Milli Q water was tested together with the samples to establish the baseline.

2.6 GC-MS ANALYIS OF COAL EXTRACTS

The concentrated organic extracts were analysed by GC-MS for structural identification and quantification. A Shimadzu GCMS-QP2010 equipped with a CTC PAL autosampler and a Restex Rxi-5MS 30m × 0.25 mmID × 1.0 μm d.f. (film thickness) column was used for analysis. 1 μL of sample was injected in splitless mode at an injector temperature of 250 ^{o}C, and carried by helium gas at 1.34 mL/min through the column. The column temperature was programmed as 1) initially at 80 ^{o}C, hold for 4.7 minutes; and 2) increase to 300 ^{o}C at 12 ^{o}C/min and hold for 15 min. Mass scan started at time 4.5 minutes (solvent delay), running in full scan mode, covering the m/z (mass/charge) range of 35 to 800 D. The ion source was operated at 200 ^{o}C with an interface temperature of 250 ^{o}C.

All data were recorded and processed through LabSolutions GCMSsolution Version 4.20 (Shimadzu Corporation). Compound identification combined an initial automatic similarity match against the internal mass spectral databases, and a further manual verification of each individual peak and comparison against the NIST MS Search 2.0 database. Only those with match qualities greater than 60% were reported. Diagnostic fragments were also employed to assist the interpretation of *n*-alkanes (m/z = 57, 85) (Brassell et al., 1980), acyclic isoprenoids (m/z = 57, 183) (Petrov et al., 1990), and PAHs (individual $M^{+\bullet}$) (Brassell et al., 1980). In addition, pure 1-heptadecanol (Sigma-Aldrich, 98%) and hexadecanoic acid, methyl ester (Sigma-Aldrich, >99%, capillary GC) were used as qualitative standards to confirm the identification of aliphatic esters and alcohols, which have not been frequently reported in coal organic extracts. The two selected compounds are the most abundant of each kind. A commercial standard of *n*-alkanes of C_{10} to C_{30} was also employed to set up a retention time index for *n*-alkanes. Solvent blanks were run in parallel to account for impurities in the background. Phthalates (m/z = 149), a common plasticiser contaminant (plastic bags were used for transport of coals to the lab), were found in all samples, and were disregarded in data analysis.

Concentrations of identified compounds were approximated by areas under peaks (intensity units, on absolute scale). Error of measurement was found to be generally within 10% by analysing a single sample (methanol extract of fresh PEN9-003 coal) three times. Peak areas were then normalized with reference to the sum of those in the methanol extract of the fresh PEN9-003 coal. This enabled study of the relative abundances of different compounds within a sample, and changes in compound concentration in microbial-digested coals.

2.7 MEASUREMENT OF METHANE CONCENTRATION

A Varian 3900 gas chromatograph equipped with an FID detector and an RT-Q-BOND column was used to measure the methane concentration in bioassay. The set temperatures for the injector, column and detector were 105 ^{o}C, 50 ^{o}C and 200 ^{o}C, respectively. Injected samples were carried by a constant flow of 4 mL/min of helium gas to the detector.

For each injection, a 100 μL gas sample was drawn from the headspace of the microcosm, using an aseptic 100 μL syringe equipped with stainless steel needle and a shut-off valve. The sample was then injected in a splitless mode. Calibration was performed using 1% and 15% methane standard gas (balanced with CO_2) before and after each set of measurements to

ensure accuracy of results. Methane concentration was monitored roughly twice a week to keep track of production.

3. RESULTS

3.1 COMPOSITIONAL AND PETROGRAPHIC CHARACTERISTICS OF COALS

Coal property data are presented in Table 3 and 4. All samples are rich in volatile matter that accounts for an average 41.9% of total mass. Volatile matter (dry basis) decreases, increases and decreases with depth and peaks near the Tangalooma Sandstone (PEN9-029 and PEN9-034). Juandah coals in general have higher ash contents, with a peak in PEN9-024. The proportion of ash is significantly lower in the Taroom Coal Measures, roughly 1/3 that of the Juandah coals. Hydrogen contents are >6% in all samples (dry ash free basis, d.a.f.), confirming the perhydrous nature of Walloon coals. As such, the vitrinite in this study can be regarded as perhydrous vitrinite according to the Seyler's coal chart (see Fig. 6 in Lowry, 1963). The H/C molar ratio ranges from 0.93 (in PEN9-043) to 1.47 (in PEN9-024) with an average of 1.07. These reasonably high values could imply a lesser degree of structural condensation that is consistent with the low rank and high volatile matter contents. As the most abundant heteroatom, oxygen accounts for 10.0% to 13.8 % of total mass on a dry-ash-free basis. Oxygen atoms occur in coal via heterogeneous linkages such as ether, ester, carbonyl and hydroxyl groups, which present good potential for microbial attack (Fakoussa & Hofrichter, 1999). PEN9-003 is richest in oxygen, followed by PEN9-024. Across all samples, nitrogen and sulfur were detected in lower concentrations with average contents of 1.36% and 0.445% respectively. PEN9-024 and PEN9-043 contain the highest amount of nitrogen while PEN9-014 is richest in sulfur.

TABLE 3: PROXIMATE AND ELEMENTAL COMPOSITION OF COAL SAMPLES.

Coal Measure	**Juandah**				**Taroom**	
Coal	**PEN9-003**	**PEN9-014**	**PEN9-024**	**PEN9-029**	**PEN9-034**	**PEN9-043**
Proximate composition (dry basis)						
Moisture %	8.3	8.8	7.1	4.9	5.9	6.1
Ash%	18.7	17.9	34.6	12.9	5.1	6.4
Volatile matter %	40.3	39	30.5	49.7	48.7	43.2
Fixed carbon %	32.7	34.3	27.8	32.5	40.3	44.3
Elemental composition (dry ash free basis)						
Carbon %	78.2	78.8	78	79	81.6	80.5
Hydrogen %	6.33	6.41	6.52	6.59	6.72	6.25
Nitrogen %	1.17	1.37	1.55	1.37	1.27	1.55
Sulfur %	0.47	0.59	0.4	0.47	0.38	0.36
Oxygen %*	13.8	12.8	13.5	12.6	10.0	11.0

* Oxygen in elemental composition is calculated by subtracting the proportion of other elements measured from 100%.

The vitrinite reflectance range is relatively narrow (R_r 0.45-0.59%, subbituminous to high volatile bituminous C). There is a clear trend of increasing reflectance with depth, consistent with a down-hole increase in rank.

Fig. 6 displays the vertical variability in maceral composition. Vitrinite contents (vol.%) range from 32.4% to 60.8% (a.r.), liptinite contents from 12.4% to 49.2%, and inertinite contents in all samples are low (max. 2.4%). PEN9-014 and PEN9-043 have the highest vitrinite contents, and PEN9-034 has the highest proportion of liptinite. Overall, maceral group compositions are extremely variable across samples of different depth.

At submaceral level, telovitrinite group macerals (telinite and mainly collotelinite) dominate the vitrinite group in PEN9-043 (31.8% of total maceral composition), PEN9-014 (30.6%) and PEN9-029 (25.0%). Detrovitrinite (vitrodetrinite and collodetrinite) is richest in PEN9-014 (22.2%), followed by PEN9-003 (18.4%), PEN9-043 (18.4%), PEN9-029 (17.8%), PEN9-034 (17.4%) and PEN9-024 (16.6%). Gelovitrinite (corpogelinite) peaks in PEN9-034 (21.0%), followed by PEN9-003 (16.6%), PEN9-029 (11.4%), PEN9-043 (8.4%), PEN9-014 (8.0%) and PEN9-024 (3.8%). The only inertinite group maceral observed in the samples was semifusinite, which is highest in PEN9-003 (2.4%). The liptinite group is dominated by suberinite with lesser sporinite, liptodetrinite, cutinite, resinite and exsudatinite. PEN9-034 has the highest suberinite content (37.4%) and a high sporinite content (6.8%). PEN9-014 is richest in cutinite (3.8%), resinite (2.4%) and exsudatinite (3.4%), while PEN9-029 has the highest amount of liptodetrinite (4.4%). No systematic relationship is observed between the pattern of submaceral distribution and coal depth.

TABLE 4: PETROGRAPHIC CHARACTERISTICS OF COAL SAMPLES FROM JUANDAH AND TAROOM COAL MEASURES ON AN AS RECEIVED BASIS (VOL. % A.R. = VOLUME PERCENTAGE ON AN AS RECEIVED BASIS).

Coal Measure		**Juandah**				**Taroom**	
Coals		**PEN9-003**	**PEN9-014**	**PEN9-024**	**PEN9-029**	**PEN9-034**	**PEN9-043**
Maceral group	**Individual maceral**	**(Vol. % a.r.)***					
Vitrinite		**48.8**	**60.8**	**32.4**	**54.2**	**46.8**	**58.6**
	Telinite	0.2	1.4	0.4	1.0	0.4	0.4
	Collotelinite	13.6	29.2	11.6	24.0	8.0	31.4
	Vitrodetrinite	1.8	0.8	2.8	1.4	0.0	1.0
	Collodetrinite	16.6	21.4	13.8	16.4	17.4	17.4
	Corpogelinite	16.6	8.0	3.8	11.4	21.0	8.4
Inertinite	Semifusinite	**2.4**	**0.8**	**0.2**	**0.4**	**0.6**	**1.2**
Liptinite		**32.7**	**24.6**	**12.4**	**29.2**	**49.2**	**24.8**
	Cutinite	0.4	3.8	1.8	0.6	1.2	0.4
	Sporinite	7.0	4.0	3.4	4.6	6.8	4.8
	Suberinite	20.6	10.0	5.8	18.6	37.4	16.6
	Resinite	2.0	2.4	0.8	0.6	0.8	1.0
	Exsudatinite	0.4	3.4	0.0	0.4	0.0	0.0
	Liptodetrinite	2.3	1.0	0.6	4.4	3.0	2.0
Mineral matter		**16.2**	**13.8**	**55.0**	**16.2**	**3.4**	**15.4**
Vitrinite reflectance (R_r %)		**0.45**	**0.46**	**0.49**	**0.49**	**0.54**	**0.59**

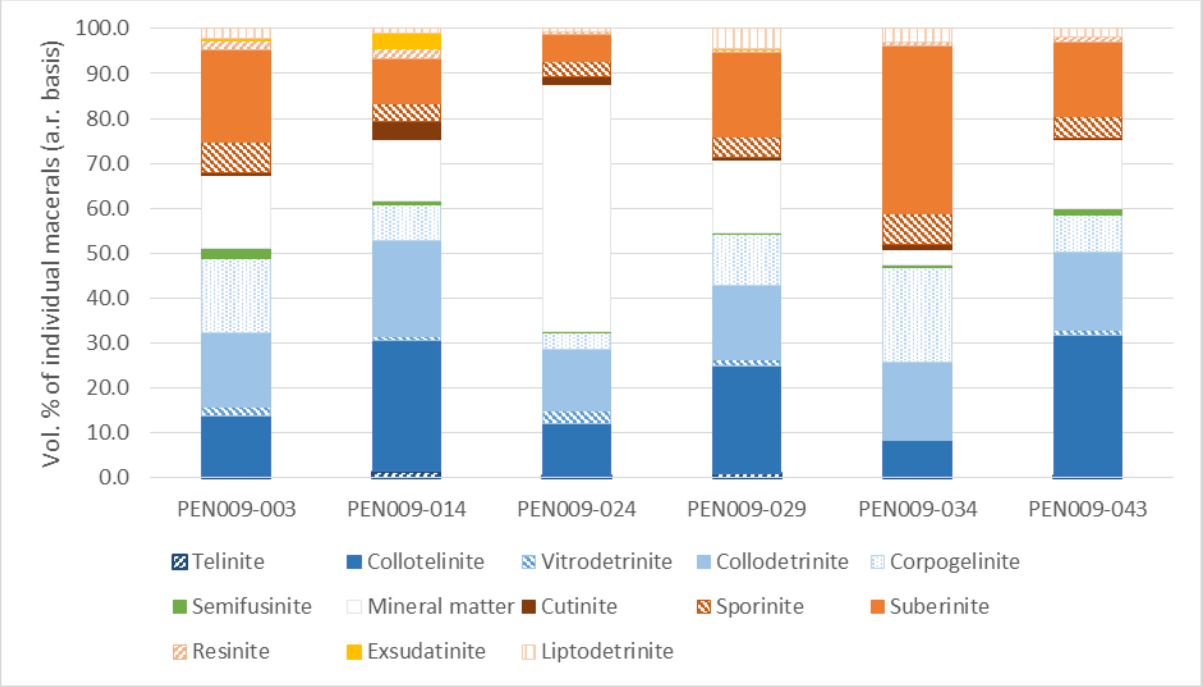


FIGURE 6: MACERAL COMPOSITIONS OF COALS ON AN AS RECEIVED BASIS. PEN9-003, PEN9-014 AND PEN9-029 ARE FROM JUANDAH COAL MEASURES WHILE PEN9-034 AND PEN9-043 ARE FROM TAROOM COAL MEASURES. BLUE = VITRINITES, GREEN = INERTINITES, RED (ALSO YELLOW) = LIPTINITES, AND WHITE = MINERAL MATTER. THE DETAILED COMPOSITION IS GIVEN IN TABLE 4.

3.2 METHANE PRODUCTION FROM BIOASSAYS

Figure 7(A) shows the net microbial methane production from bioassays (total methane less methane from inocula and desorption). The values represent final yields after 30 days of incubation, when the production plateau was established. Methane yield ranges from 14 to 33 µmol/g with an average of 21 µmol/g (0.515 m^3/tonne). This figure falls within the scale previously reported for subbituminous coal (Harris et al., 2008; Jones et al., 2008; Penner et al., 2010), but is much less than the average gas content of Walloon subgroup coals (5.36 m^3/tonne d.a.f of primarily biogenic methane) (Hamilton et al., 2012). Multiple reasons may account for this (discussed in more detail later in Section 4.3), most prominently associated with the limitations of laboratory bioassays. Methane production started almost immediately after inoculation and finished within 30 days (Fig. 7 (B)). The relatively fast kinetics implies the presence of readily degradable compounds (e.g. VFA-As).

A comparison of methane yields between different samples reveals the relationship of coal bioavailability with depth and stratigraphy. Methane yield is found to decrease at first and then increase with depth in the Juandah Coal Measures until the upper bound of Tangalooma Sandstone. It then decreases near the lower bound of Tangalooma Sandstone in Taroom Coal Measures, and increases again within the Taroom Coal Measures. The highest production takes place in the sample (PEN9-029) just above the Tangalooma Sandstone, while the lowest occurs in the sample (PEN9-034) just below the Tangalooma Sandstone. This pattern is generally consistent with the dominant downhole gas content trend in Walloon CSG wells (positively parabolic) (Hamilton et al., 2012).

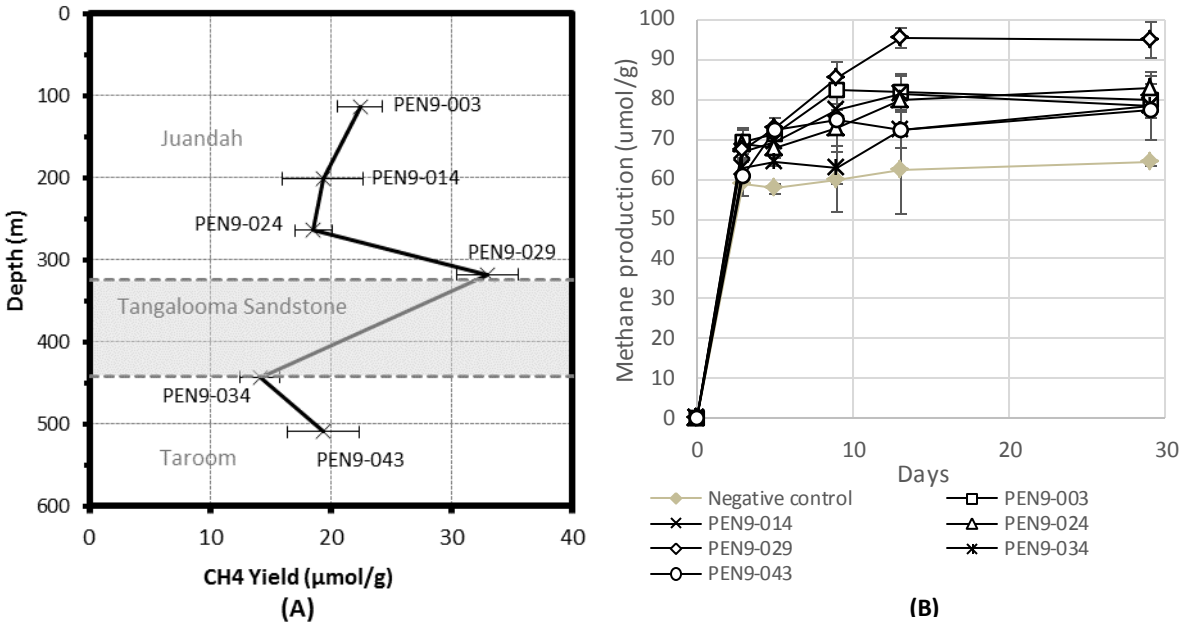


FIGURE 7: (A) METHANE YIELDS FROM BIOASSAYS ACROSS SAMPLES OF DIFFERENT STRATIGRAPHIC LAYERS. THE VALUES PLOTTED ARE THE FINAL NET YIELDS ACHIEVED AT DAY 30, WITH BASELINE METHANE FROM THE NEGATIVE CONTROL (CONTAINS NO COAL) BEING DEDUCTED. (B) METHANE PRODUCTION CURVE; ERROR BARS SHOW ± ONE STANDARD DEVIATION FROM THE MEAN.

3.3 VFA-AS AND TOC

The concentrations of volatile fatty acids and alcohols (VFA-As), as well as total soluble organic carbon in the aqueous solution of the six coals (see Fig. 4, fraction A) are given in Table 5. Ethanol and acetic acid are the only two VFA-As that were detected at significant concentrations. Juandah coal extracts are in general, richer in VFA-As, with PEN9-029 being the highest. Proportions of VFA-As in the total water-soluble organic carbon (TOC) were calculated to be (in order) 18.6%, 39.7%, 35.1%, 45.8%, 39.6%, and 15.7% for the six samples, respectively. This suggests the release of other organic compounds upon extraction of coals with water. The concentration of water-soluble TOC is trivial with respect to the total mass of coal (< 1‰). The proportion of it in the total extractable matter is also small (< 1.6%). These are consistent with the extraction yield (in Table 2), underlining the highly hydrophobic nature of coal compounds.

Variation of VFA contents with coal was compared to that of methane yields, and is illustrated in Fig. 8. The two variables correlate favourably with the exception of PEN9-003 and PEN9-043 coals, which have other significant carbon substrates (see Section 3.4). The theoretical maximum methane achievable from VFA-As was also calculated, assuming 100% conversion of VFA-As through methanogenic pathway. The proportions of the calculated maximum with respect to the observed methane yield are 40.1%, 73.1%, 73.4%, 96.2%, 65.5%, and 46% for the six samples (Table 5), respectively. This implies a seemingly important contribution of VFA-As to coal bioavailability, especially in PEN9-29 coal, despite the low concentrations.

Both ethanol and acetic acids were found to be completely eliminated in the bioassays (see Fig. 4, fraction H). The total organic carbon content was reduced by an average of 98% in the bioassay residues. This suggests the water soluble coal compounds are highly bioavailable.

TABLE 5: VOLATILE FATTY ACIDS AND ALCOHOLS IN WATER ELUENTS (SEE FIG. 4, FRACTION A) OF PEN 9 COAL SAMPLES

Samples	PEN9-003	PEN9-014	PEN9-024	PEN9-029	PEN9-034	PEN9-043
Ethanol (mg/g)	0.183	0.332	0.274	0.875	0.210	0.235
Acetic acid (mg/g)	0.165	0.199	0.282	0.193	0.146	0.0752
TOC in fresh (mg/g)	0.749	0.536	0.634	0.932	0.359	0.791
TOC digested (mg/g)	0.0149	0.01	0.023	0.0164	0.0113	0.0123
Max. contribution of VFA-As to CH_4 (%)	40.1	73.1	73.4	96.2	65.5	46.0

* TOC in fresh = TOC in fresh coals; TOC in digested = TOC in microbially-digested coals.
* Max. contribution of VFA-As to CH_4 = the percentage of maximum yield of methane producible from acetic acid and ethanol assuming 100% conversion through methanogenic pathway in the actual methane yield observed.

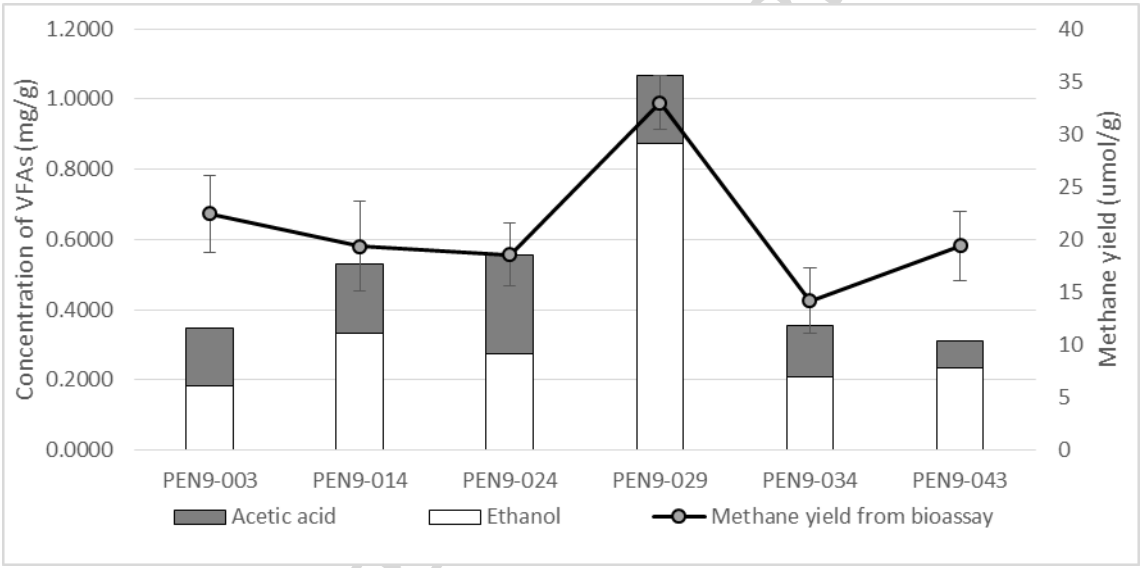


FIGURE 8: DISTRIBUTION OF VFA-AS IN WATER ELUENTS OF COALS (COLUMNS) AND COMPARISON TO METHANE YIELDS (LINE). VARIATION OF VFA-AS CONTENTS FOLLOWS A GENERALLY CONSISTENT PATTERN WITH THAT OF METHANE YIELD, SUGGESTING A LIKELY SIGNIFICANT CONTRIBUTION OF VFA-AS TO METHANE PRODUCTION.

3.4 GC-MS CHARACTERISATION OF COAL SOLVENT-EXTRACTABLE COMPOUNDS

Figure 9 shows the example GC-MS total ion current (TIC) chromatograms a) for an *n*-alkane standard mixture, as well as a hexadecanoic acid, methyl ester and 1-heptadecanol standard; b) for PEN9-003 and c) for PEN9-043 methanol extracts extracted directly from the fresh coals (Fig. 4, fraction D), after extraction with water (Fig. 4, fraction B) and after microbial digestion (Fig.4, fraction F). Example peaks are labelled on the graphs (Fig. 9). Clear discrepancy can be observed between chromatograms of water extracted (Fig. 4 fraction B) and microbially-digested (Fig. 4, fraction F), indicating elimination of hydrophobic compounds in bioassays. Notable difference can also be observed between those of fresh and water extracted coals, suggesting some dissolution of organic-solvent-extractable compounds in water. Distribution of organic compounds in organic solvent extracts of the six samples is summarised in Fig. 10.

Water extraction produced a small yield for all coals, accounting for an average 1.3% of the mass of total extractable matter (as seen in Table 2). Apart from the VFA-As shown in Fig. 8, *n*-alcohols, aliphatic esters and aliphatic amines were also found to solubilize in water to a significant extent. This was demonstrated by a decrease in peak intensities of the relevant compounds in the methanol extract of water-extracted coals (annotated in Fig. 9). In contrast, methanol extraction returned the highest compound recovery that accounts for an average 67.7% of total extraction yield (as shown in Table 2). This implies a possible wide spread of polar functional groups in coal extractable matter, but may also be due to an advantage in being the earlier solvent in the sequential extraction process, with access to a wider spectrum of extractable compounds. The remaining 31% of extractable material was captured in the subsequent DCM wash step. The majority of compounds detected are found in both methanol and DCM extracts. The former generally demonstrates a higher content of heteroatoms. Among the six samples, the shallowest and the deepest coals PEN9-003 and PEN9-043 contain the highest proportion of solvent-extractable materials: 12% and 11.6% respectively (see Table 2). Extractability of coal decreased with depth in the first five samples, and had its minimum just below the lower boundary of Tangalooma Sandstone (3.8% in PEN9-034). The extraction yields compare favourably to GC-MS results. An average 67.3% (based on peak intensity, i.e. area under peak) of detected compounds are shown to be derived from methanol extracts (see Fig. 10b).

Taking a closer look at the composition of organic extracts (combined methanol and DCM), aliphatic compounds are dominant in all coals with proportions from the shallowest to deepest coals of 69%, 59%, 69.5%, 70%, 60.8%, and 81.9%, respectively (see Fig. 10c). This is consistent with the perhydrous nature of Walloon coals (Scott et al., 2007). Normal alkanes, normal alcohols and aliphatic esters form three major groups of aliphatic compounds that are collectively responsible for 88.9%, 84.7%, 87.5%, 86.2%, and 90% of aliphatic peaks in each sample (Fig. 10 D, E). Others like cyclic and acyclic isoprenoids, ethers, cyclic aliphatic ketones and aliphatic amine occurred in much lower concentrations. *n*-Alkanes ($C_{17\text{-}29}$) are, in all cases, the most prevalent group of compounds. Abundance of *n*-alkanes peaks in PEN9-043, and decreases moderately through samples PEN9-014, PEN9-003, PEN9-024, PEN9-029 and PEN9-034. The proportion of *n*-alkanes ranges from 27.6% in PEN9-003 to 51.7% in PEN9-029 with an average of 42.5%. The distribution of *n*-alkanes follows a unimodal pattern, with maximum at C_{27}. Longer-chain alkanes (C_{23}- C_{29}) occurred at higher concentrations than the shorter chain compounds ($C_{17} - C_{23}$). *n*-Pentacosane (C_{25}) and *n*-heptacosane(C_{27}) are the two most abundant compounds of the group. Odd-carbon-numbered-chain homologues are again found to be dominant. The carbon preference index (CPI, Marzi, et al., 1993) calculated for each coal is given in Table 6. Among the six samples, PEN9-003 and 043 coals are distinguished by a wealth of long chain normal alcohols (C_{10} to C_{20}) that make up 20.8% and 22.7% of the total peak areas in the two samples, respectively. This is significantly higher than the average of 2.23% in the other coals. The alcohols display a dominance of odd homologues, with emphasis on 1-tridecanol (C_{13}) and 1-pentadecanol (C_{15}). The same two samples are also the richest in fatty esters, which account for 13% and 10.8% of total peak areas. The average proportion of the esters is 10.6% with moderate difference among samples. The fatty esters identified in this experimental study are composed primarily of mid-long chain fatty acids ($C_{14} - C_{24}$), associated with methyl or ethyl alcohols with the exception of propanoic acid 2-methyl-, 2-ethyl-3-hydroxy hexyl ester and 2-propenoic acid tridecyl ester. Hexadecanoic acid methyl ester, octadecanoic acid methyl

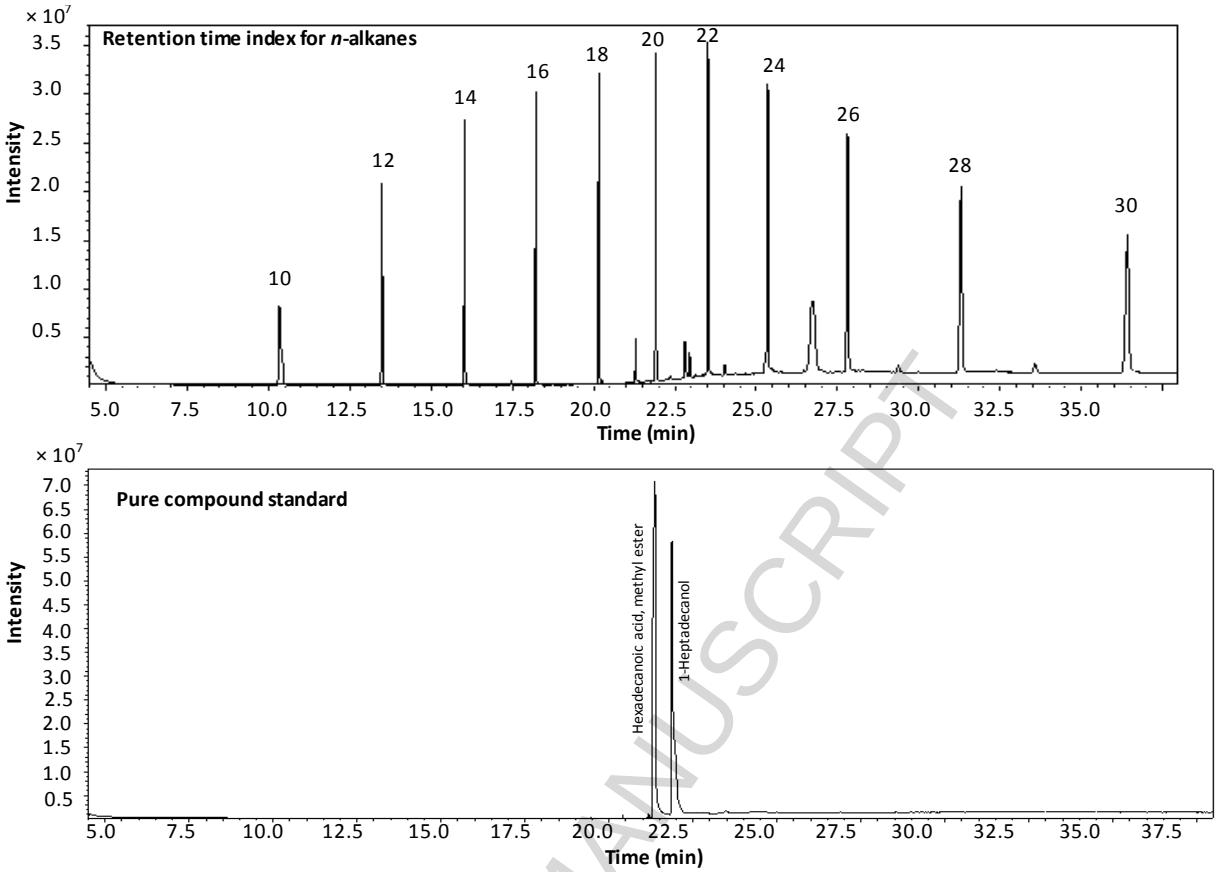
ester, and tetracosanoic acid methyl ester, were identified as the most abundant saturated esters. Others include pentadecanoic acid methyl ester, octadecanoic acid 1-methylethyl ester, heptadecanoic acid methyl ester, and propanoic acid 2-methyl-, 2-ethyl-3-hydroxyhexyl ester in decreasing order of abundance. Among the unsaturated esters, 9-octadecenoic acid methyl ester, (E)- occurred at the highest concentrations. Also present are hexanedioic acid bis (2-ethyl hexyl) ester, ethyl 9-tetradecenoate, 2-propanoic acid tridecyl ester, ethyl oleate, ethyl 9-hexadecenoate, and 9-hexadecenoic acid methyl ester, (Z)-, beginning with the richest. The saturated esters demonstrate a slight dominance over the unsaturated by a ratio of 1.25 based on peak area.

The distribution of aromatic compounds appears to be more diverse and scattered (Fig. 10 D, E). Compound groups including dibenzofuran, alkyl benzene, alkyl PAH, and acetyl diphenyl were detected in significant quantities. The average proportions of these groups are 7.25%, 6.04%, 6.35% and 4.59%, respectively. The less abundant classes are phenolic ester, acetyl-alkyl tetralin, phenyl cyclic aliphatics, alkyl octahydrophenanthrene, alkyl biphenyl, dehydroabietylamine, biphenyl amine, alkyl octahydrophenanthrenol, alkyl benzonaphthyridinone, alkyl indene, and phenyl-pyridinyl amide, with proportions typically below 2% of total extractable mass. The alkyl benzenes are composed of a benzene ring with primarily single substitution of $C_{12\text{-}14}$ (predominantly C_{13}) branched alkanes. Occurrence of multiple substitution (1,2,3,6-) is infrequent and was only identified in the PEN9-029 extract at low concentration. The detected PAHs contain exclusively alkyl naphthalenes and phenanthrenes with dominance of the former. The concentration of alkyl-PAH decreases and then increases with burial depth, inflecting around the Tangalooma Sandstone. 4,4'-Diacetyldiphenylmethane and dibenzofuran are the only significant compounds of their groups (group acetyl diphenyl dibenzofuran), yet the richest individuals of the aromatic kind. The two shallowest coals PEN9-003 and PEN9-014 contain the highest quantity of aromatic compounds (based on total areas of aromatic peaks), and PEN9-014 in particular, has the highest proportion.

TABLE 6: GEOCHEMICAL INDICES FOR SAMPLE EXTRACTS.

Samples	**PEN9-003**	**PEN9-014**	**PEN9-024**	**PEN9-029**	**PEN9-034**	**PEN9-043**
CPI in *n*-alkanes	1.4	1.64	1.33	1.27	1.12	1.59

* CPI = carbon preference index, calculated using equation: $\mathrm{CPI} = \frac{\sum(C_{17-27})_{odd} + \sum(C_{19-29})_{odd}}{2\times(\sum C_{18-28})_{even}}$, modified from Marzi, et al., (1993).



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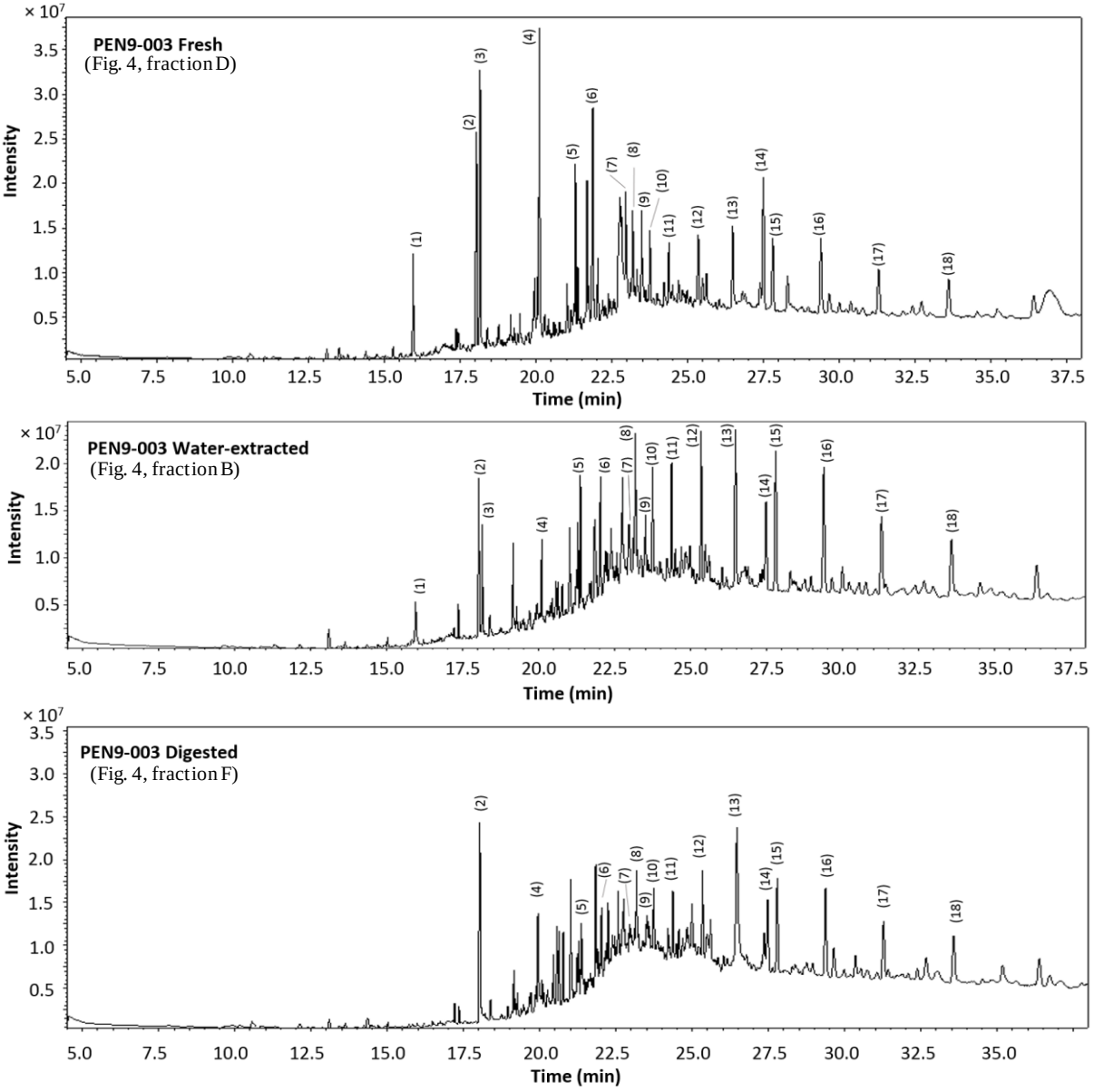

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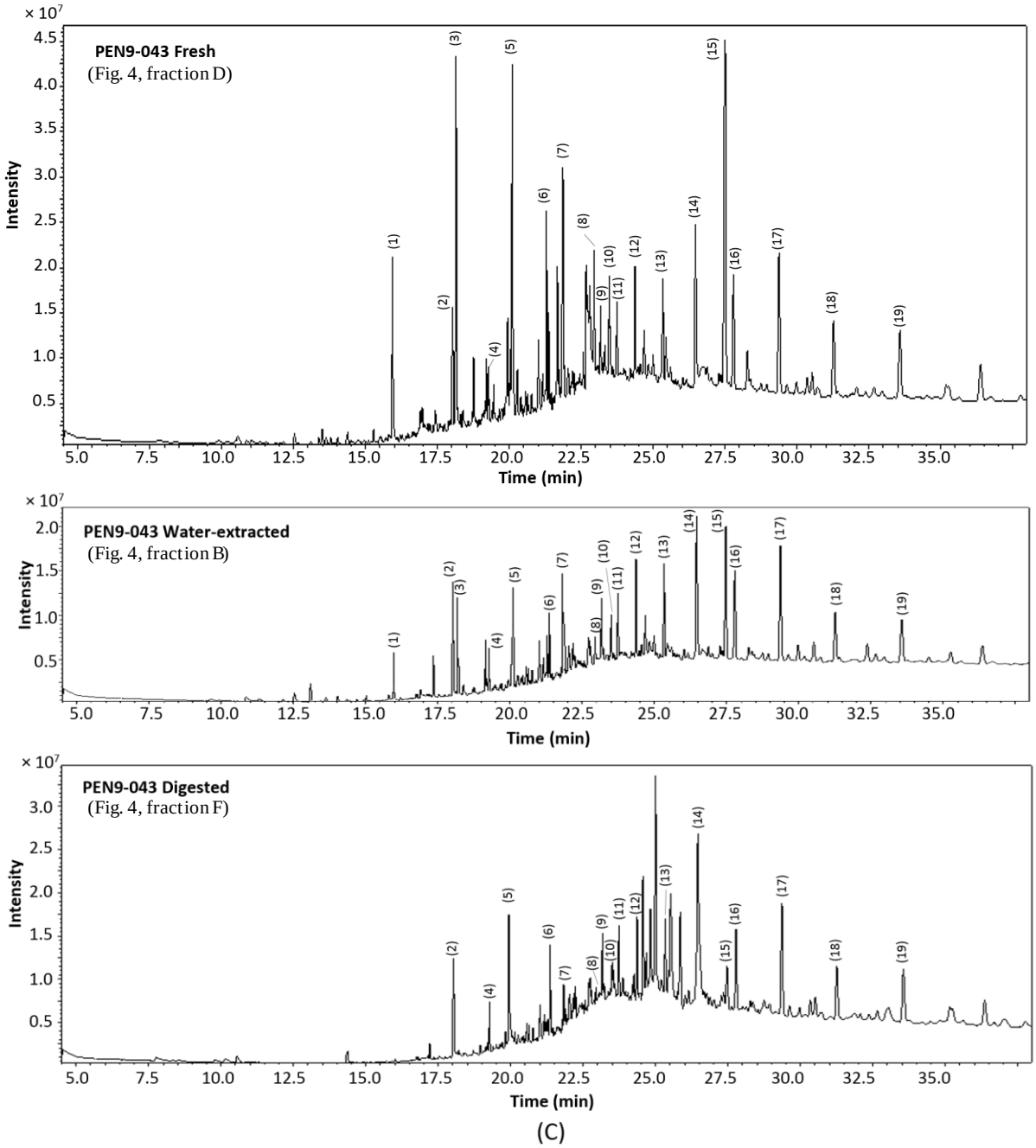
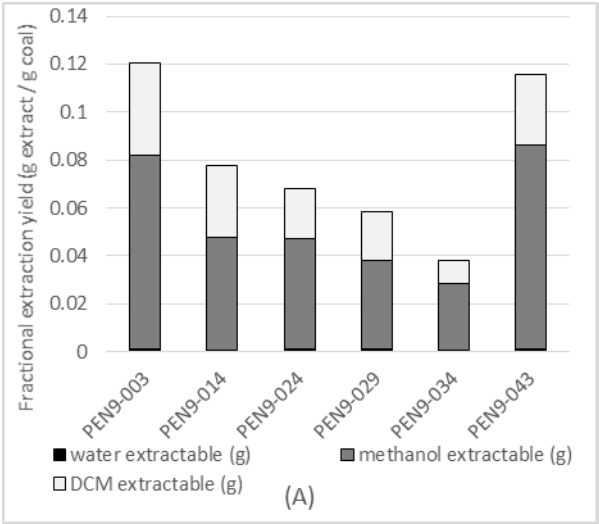
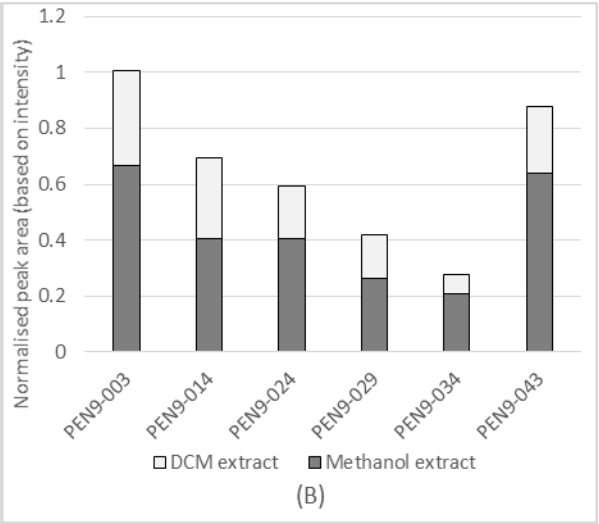


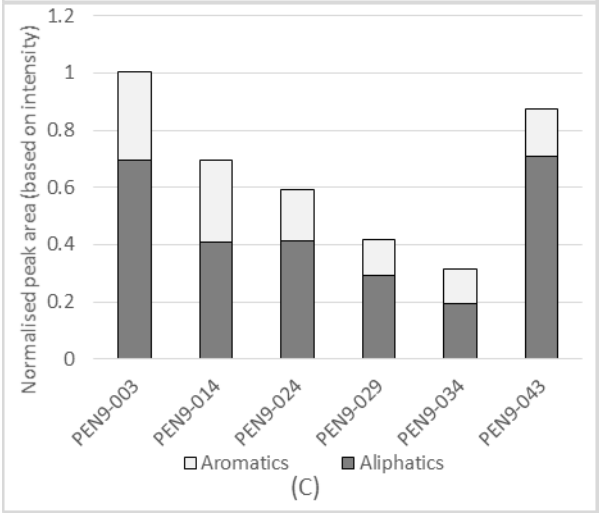
(C)

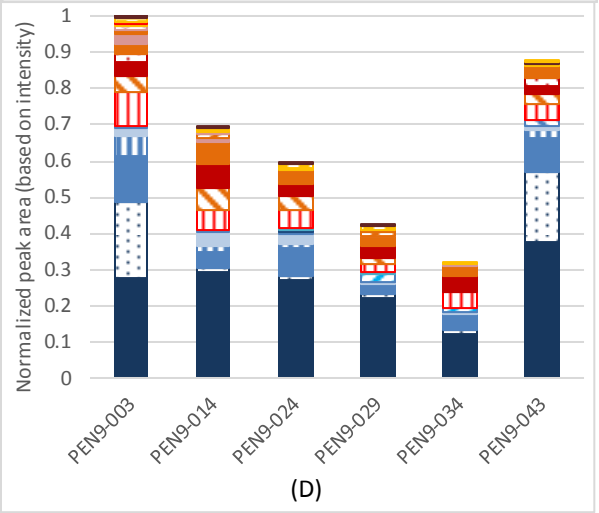
FIGURE 9: EXAMPLES OF GC-MS TOTAL ION CURRENT CHROMATOGRAMS. (A) RETENTION TIME INDEX FOR *N*-ALKANES FROM C_{10} TO C_{30} , AND PURE COMPOUND STANDARDS OF HEXADECANOIC ACID, METHYL ESTER AND 1HEPTADECANOL; (B) METHANOL EXTRACTS OF PEN9-003 FRESH COAL, WATER EXTRACTED COAL AND MICROBIALLY-DIGESTED COAL FROM BIOASSAY. THE LABELLED COMPOUNDS ARE 1) 1-DECANOL, 2) DIBENZOFURAN, 3) 1-TRIDECANOL, 4) 1-PENTADECANOL, 5) HEXADECANOIC ACID, METHYL ESTER, 6) 1-HEPTADECANOL, 7) METHYL STEARATE , 8) NAPHTHALENE, 7-BUTYL, 1-HEXYL, 9) 1-EICOSANOL, 10) 4,4'-DIACETYLDIPHENYLMETHANE, 11) *N*-TRICOSANE, 12) *N*-TETRACOSANE, 13) *N*-PENTACOSANE, 14) BIS(2-ETHYLHEXYL) PHTHALATE (CONTAMINANT), 15) *N*-HEXACOSANE, 16) *N*-HEPTACOSANE, 17) *N*-OCTACOSANE, AND 18) *N*-NONACOSANE. CLEAR DISCREPANCY HAS BEEN OBSERVED BETWEEN THE CHROMATOGRAMS OF METHANOL EXTRACTS FROM FRESH AND WATER-EXTRACTED COALS, INDICATING COAL ORGANICS HAVE A DEGREE OF WATER-SOLUBILITY. (C) METHANOL EXTRACTS OF PEN9-043 FRESH COAL, WATER EXTRACTED COAL AND MICROBIALLY-DIGESTED COAL FROM BIOASSAY. THE LABELLED COMPOUNDS ARE 1) 1-DECANOL, 2) DIBENZOFURAN, 3) 1-TRIDECANOL, 4) *N*-HEPTADECANE, 5) PRISTANE, 6) 1-PENTADECANOL, 7) HEXADECANOIC ACID, METHYL ESTER, 8) 1-HEPTADECANOL, 9) METHYL STEARATE, 10) NAPHTHALENE, 7-BUTYL, 1-HEXYL, 11) 1-EICOSANOL, 12) 4,4'-DIACETYLDIPHENYLMETHANE, 13) *N*-TRICOSANE, 14)

N-TETRACOSANE, 15) *N*-PENTACOSANE, 16) BIS(2-ETHYLHEXYL) PHTHALATE (CONTAMINANT), 17) *N*-HEXACOSANE, 18) *N*-HEPTACOSANE, 19) *N*-OCTACOSANE, 20) *N*-NONACOSANE. CLEAR DISCREPANCY HAS BEEN OBSERVED BETWEEN THE CHROMATOGRAM OF METHANOL EXTRACTS FROM THE MICROBIALLY DEGRADED COAL AND THOSE FROM FRESH AND WATER-EXTRACTED COALS, INDICATING THAT COAL ORGANICS WERE DEGRADED IN BIOASSAYS.









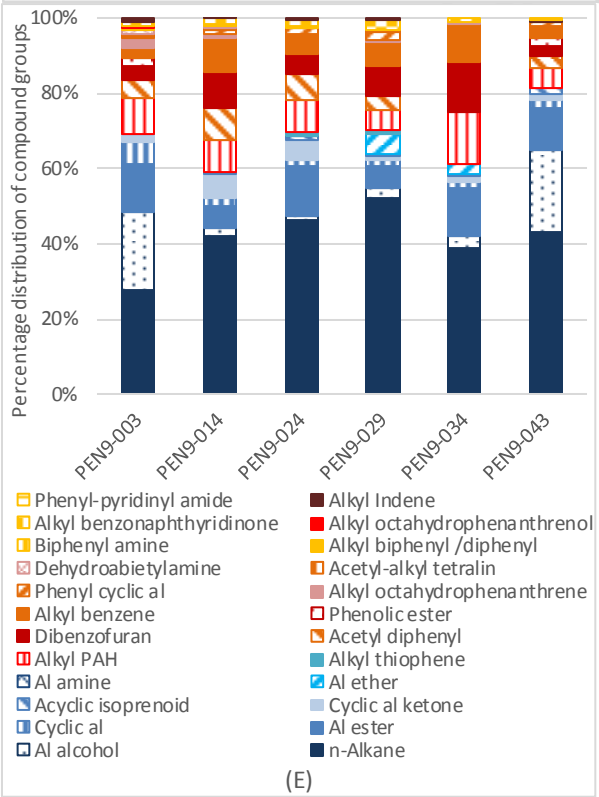


FIGURE 10: CHARACTERISATION OF COAL EXTRACTS USING GC-MS. (A) YIELD OF SEQUENTIAL EXTRACTION WITH THREE SOLVENTS BASED ON WEIGHT (DRY BASIS) – WATER, METHANOL AND DCM. METHANOL EXTRACT CONTAINS THE MOST EXTRACTABLE MATTER, FOLLOWED BY DCM AND WATER. (B) EXTRACTABILITY OF FRESH COAL SAMPLES WITH METHANOL AND DCM BASED ON GC-MS PEAK INTENSITY. (C) RELATIVE ABUNDANCE OF ALIPHATIC AND AROMATIC COMPOUNDS IN COAL EXTRACTABLE MATTERS BASED ON GC-MS PEAK INTENSITY. ALIPHATICS OCCUR BY A HIGHER PROPORTION THAN AROMATICS IN ALL SAMPLES. (D) CHARACTERISATION AND QUANTIFICATION OF COMPOUND GROUPS IN COAL EXTRACTABLE MATTER. DATA COMBINES METHANOL EXTRACT AND DCM EXTRACT. BLUE- ALIPHATIC COMPOUNDS, RED/YELLOW = AROMATIC COMPOUNDS. (E) PERCENTAGE DISTRIBUTION OF COMPOUND GROUPS IN COAL EXTRACTABLE MATTER BASED ON GC-MS PEAK INTENSITY. QUANTIFICATION OF COMPOUNDS WAS DONE BY MEASURING THE AREA UNDER PEAKS. SUMMED PEAK AREAS OF PEN9-003 EXTRACT (BOTH METHANOL AND DCM) WAS USED AS THE NORM TO GIVE NORMALIZED PEAK AREAS IN GRAPHS B, C AND D. * NOTE: AL = ALIPHATIC, AR = AROMATIC. DIPHENYL IS COMMONLY EQUIVALENT TO BIPHENYL, BUT HERE WE USE DIPHENYL TO REFER TO COMPOUNDS THAT HAVE AT LEAST ONE ADDITIONAL CARBON LINKING THE TWO PHENYL GROUPS.

3.5 BIOAVAILABILITY OF COAL EXTRACTS

Differences between the solvent-extractable compounds of fresh (Fig. 4, fraction D & E) and microbially-digested coals (Fig. 4, fraction F & G) reveal the bioavailability of the coal samples. Table 7 summarises the percentage elimination of different portions of coal extracts. The two most extractable samples PEN9-003 and PEN9-043 were also the most degradable. An overall 46% and 45.6% (based on peak area) of extracted materials were eliminated in bioassays grown on the two coals, in contrast to the average 28.8% observed for the remaining samples. The high elimination rates are attributable to the aliphatic components in the extracts. Biodegradation had broken down 56.6% and 49.9% of the aliphatic compounds in the extracts of the two coals. This is significantly higher than the average of 33.3% for the remaining samples. In comparison, aromatic compounds are less bioavailable and showed very similar levels of degradation in all samples, with an average 20% of elimination. In terms of solvents, methanol extracts have appeared to be more degradable than DCM extracts. This may be in part, due to the presence of more polar moieties in methanol extract but could also be due to the fact that methanol was the first solvent so that a wider variety of compounds were encountered than were those in the subsequent DCM.

TABLE 7: A GENERAL SUMMARY OF COMPOUNDS ELIMINATION IN COAL EXTRACTS. ELIMINATION IS GIVEN BY THE QUOTIENT OF LOSS IN GC-MS INTENSITY AFTER BIODEGRADATION AND THE INTENSITY IN EXTRACTS OF RAW COALS MULTIPLIED BY 100%.

Samples	PEN9-003	PEN9-014	PEN9-024	PEN9-029	PEN9-034	PEN9-043
			Elimination %			
Total extract	46	24.4	27.7	28.6	34.5	45.6
Methanol extract	59.9	27.9	31.2	29.2	34.7	56
DCM extract	18.2	19.5	20.5	26.6	33.5	16.1
Aliphatics	56.6	27.6	32.6	33.3	39.8	49.9
Aromatics	22.2	19.9	15.9	17.8	18.2	25.9

Taking a closer look into the compound groups, the importance of individual groups to the overall bioavailability of coal is summarised in Fig. 11. Aliphatic compounds contribute significantly to bioavailability in all samples. The proportions of biodegraded aliphatic groups to the total elimination of extractable compounds (peak area lost upon microbial digestion) in bioassays are 85%, 68%, 84%, 81%, 78% and 91% for the six coals respectively.

The three major aliphatic groups: *n*-alkanes, aliphatic alcohols, and aliphatic esters are of paramount significance. Collectively they are responsible for 60% (in PEN9-014) to 89% (in PEN9-043) of total compounds degraded (Fig. 11 B). Aliphatic alcohols, in particular, contributed to the bioavailability of the PEN9-003 and PEN9-043 coals. This single group accounts for over 40% of total elimination that distinguishes the two samples from the others in both amount and extent of biodegradation (Fig. 11 A). Aliphatic ester is the second contributing group in the two samples, responsible for 25% and 21% of total elimination. This is followed by *n*-alkanes with 13% and 18%. Among the other 4 samples, *n*-alkanes are the top contributors to coal bioavailability, accounting for an average 34.5% of biodegradation. This is followed by aliphatic esters: 16% (PEN9-029) to 32% (PEN-034), and alcohols: 5% to 10%. Aliphatic ether forms another significant group in the PEN9-029 and PEN9-034 coals, bringing about 12% and 6% of total biodegradation, respectively. In general, bioavailability of coals is less dependent on aromatic compounds, and even for PEN9-014 in which it is most important, they provide only 32% of total compound elimination. The contribution is much less in the other samples with the average being 16%. Dibenzofuran is one of a few prominent aromatic groups that has a significant impact on coal bioavailability, accounting for 14% in PEN9-014, 9% in PEN9-034, 6% in PEN9-029, and less significantly 2% and 1% in PEN9-003 and PEN9-043. A similar trend was observed with alkyl benzene, providing 7% of compound elimination in PEN9-014 extracts, followed by 6% in PEN9-034, 4% in PEN9-024 and PEN9-029, and 2% and 1% in PEN9-043 and PEN9-003, respectively. For other groups: 5% of total elimination comes from phenolic ester in PEN9-003 and PEN9-043; average 3.7% for acetyl diphenyl in PEN9-014, PEN9-024 and PEN9-029; and average 3.7% for alkyl PAH in PEN9-014, PEN9-024 and PEN9-034.

Figure 12 summarises the bioavailability of individual compound groups based on averages for the six samples. Aliphatic compounds of *n*-alcohol, ester, ether, and amine, and aromatics including phenolic ester and dehyroabietylamine demonstrated top degradability with more than 50% being eliminated in bioassays. Aliphatic alcohols were almost completely degraded in all samples (insignificant peaks remain in the extracts of digested samples). The low TOC (Table 5) in bioassay residues precludes the possibility of significant compound loss due to water leaching. Aliphatic and phenolic esters were also substantially degraded by over 80%. This is followed by aliphatic amine (77%), ether (61%) and dehyroabietylamine (52%). Nevertheless, the contribution of the last three groups to the overall bioavailabilty of coal is minimal, as a result of their low concentrations. On the contrary, the highest concentration aliphatic group, *n*-alkane, and aromatic group, dibenzofuan turned out to be just moderatedly degraded (on average 20.9% and 25.5%, respectively, Fig. 12). This limits the overall bioavailability of the coal extracts. Compounds with heteratoms are in general, more degradable in bioassays (average 45.2% eliminated) than those of simply hydrocarbons (average 14.1% eliminated). This is consistent with the general belief that heterogeneous linkages are more vulnerable to microbial cleavage (Fakoussa & Hofrichter, 1999; Furmann et al., 2013a). The error bars show reasonably small variation, suggesting similar extent of degradation of individual compounds across different samples.

The total compound elimination of each sample is plotted in the same graph with methane yields minus the amount producible from VFA-As in Fig. 13. The two curves demonstrate matching trends. A futher regression analysis reveals a strong correlation between the two

sets of data with an R^2 value of 0.901 (Fig. 13 B). This indicates a likely tight association between biogenic methane production and the observed compound elimination in bioassays.

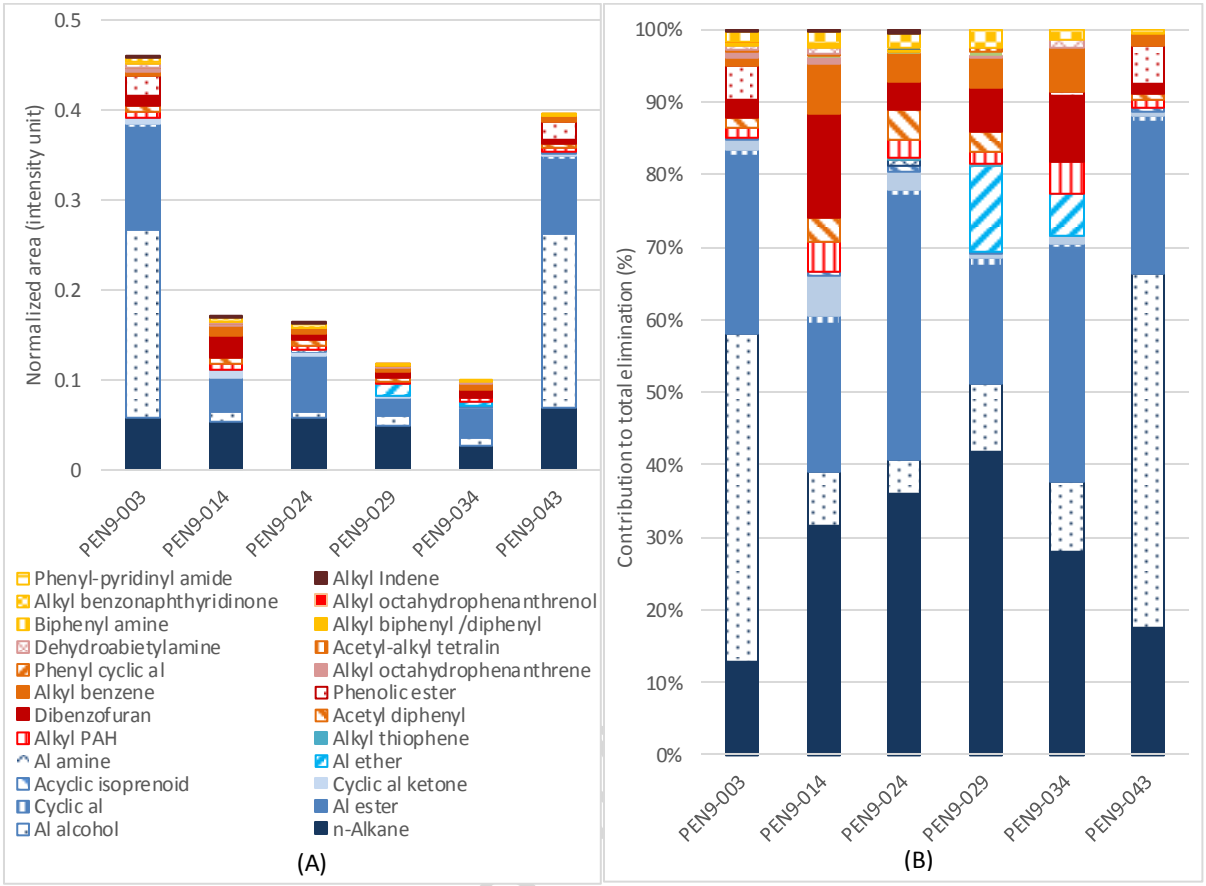


FIGURE 11: QUANTIFICATION OF BIODEGRADATION EXTENT IN COMPOUNDS GROUPS OF COAL ORGANIC EXTRACTS. (A) QUANTIFICATION OF COMPOUND LOSSES DUE TO BIODEGRADATION. THE VALUES SHOW THE DIFFERENCE BETWEEN THE DETECTED GC-MS PEAK AREAS OF FRESH AND DIGESTED SAMPLES, NORMALIZED WITH RESPECT TO THE TOTAL PEAK AREA OF FRESH PEN9-003 COAL EXTRACT. DATA COMBINES METHANOL EXTRACT AND DCM EXTRACT. (B) PERCENTAGE CONTRIBUTION OF COMPOUNDS GROUPS TO OVERALL BIOAVAILABILITY OF INDIVIDUAL SAMPLES.

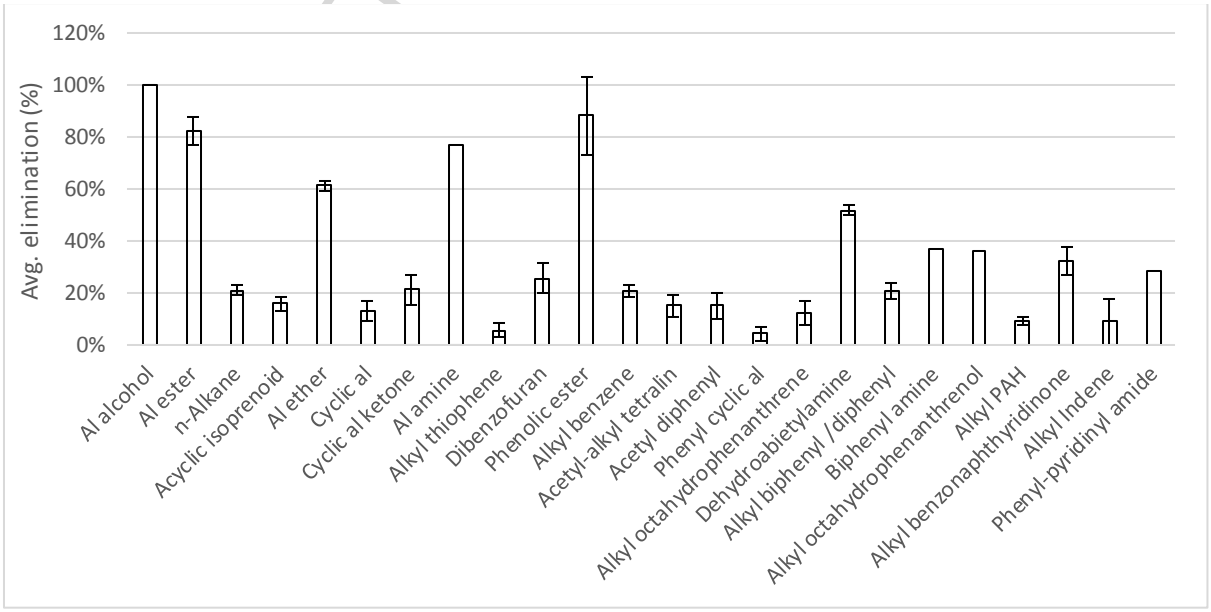


FIGURE 12: BIODEGRADABILITY OF INDIVIDUAL COMPOUND GROUPS. VALUES SHOW THE PERCENTAGE OF LOSS OF PARTICULAR COMPOUND GROUPS IN BIOASSAYS (BASED ON GC-MS PEAK INTENSITY) BASED ON AVERAGE OF THE SIX COALS. ERROR BARS REPRESENT A STANDARD DEVIATION AWAY FROM THE MEAN VALUES. GROUPS ALIPHATIC ALCOHOLS, ALIPHATIC ESTERS, PHENOLIC ESTERS, ALIPHATIC AMINES, AND ALIPHATIC ETHERS HAVE DEMONSTRATED HIGHEST BIODEGRADABILITY WITH AVERAGE ELIMINATION GREATER THAN 60%.

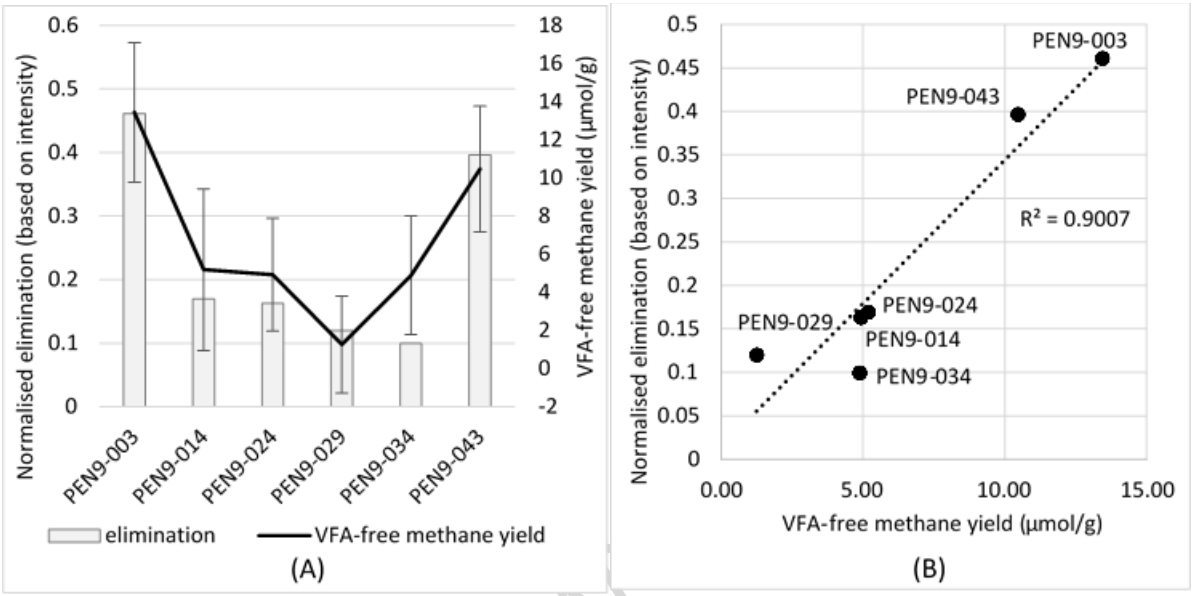


FIGURE 13: (A) COMPARISON BETWEEN VFA-FREE METHANE YIELD AND BIO-ELIMINATION OF COAL EXTRACTABLE MATTERS. VFA-FREE METHANE YIELD WAS CALCULATED BY SUBTRACTING THE OBSERVED METHANE BY THE MAXIMUM AMOUNT OF METHANE PRODUCIBLE FROM VFA-AS ASSUMING 100% CONVERSION. NORMALIZED ELIMINATION WAS CALCULATED BY DIVIDING THE DIFFERENCE BETWEEN THE SUMMED PEAK AREAS OF FRESH AND BIOASSAY RESIDUE BY THAT OF THE FRESH PEN9-003 COAL. (B) LINEAR REGRESSION SHOWING THE RELATION BETWEEN COMPOUND ELIMINATION AND VFA-FREE METHANE YIELD, WITH AN R^2 VALUE.

4. DISCUSSION

4.1 RELATION OF COAL BIOAVAILABILITY WITH PETROGRAPHIC CHARACTERISTICS AND BURIAL DEPTH

A linkage between coal petrographic characteristics with bioavailability or extractability of coal is always of interest yet controversial. In this study, neither methane production nor extraction yield correlates strongly with maceral composition at first glance. However, if PEN9-029 and PEN9-034 are disregarded, the total peak intensity (as measured in GC-MS) in organic extracts (combining methanol and DCM extracts, Fig.4 fraction D + E) of the remaining four coals is found to be positively associated with contents of liptinite (R^2=0.82, as shown in Fig. 14 A). This is consistent with the general view that liptinite macerals are oil-prone due to the perhydrous nature of source materials such as spores, cutin, suberin, resins, waxes, balsams, latex, fats, and oils (Levine, 1993; Ratanasthien et al., 1999; Saxby & Shibaoka, 1986; Taylor et al., 1998; Wilkins & George, 2002). The same linear relationships are observed at submaceral level, specifically suberinite, sporinite and liptodetrinite from the liptinite group (suberinite is shown in Fig. 14). The R^2 values for suberinite is rounded to 1.0,

suggesting a strong correlation. Suberinite, in particular, originates from bark and roots (Taylor et al., 1998) and is known to generate substantial amounts of C_{12+} waxy normal hydrocarbons at reflectance below 0.6% (Khavari-Khorasani & Michelsen, 1991). Sporinite has its origin from the outer cell walls of spore and pollen that consists of mainly sporopollenin, an oxidative polymer of carotenoids (Brooks & Shaw, 1978; Powell et al., 1991; Taylor et al., 1998). This allows it to generate lower molecular weight compounds of branched and cyclic or aromatic nature upon thermal cracking (Mukhopadhyay & Hatcher, 1993; Powell et al., 1991). Liptodetrinite is of finely detrital nature, originating from fragments and degradation remains of sporinite, cutinite, resinite, alginate and suberinite (Mukhopadhyay & Hatcher, 1993; Taylor et al., 1998). Molecular hydrocarbons within liptodetrinite are said to be non-waxy and low-molecular-weight, similar to those in sporinite (Powell et al., 1991; Wilkins & George, 2002). The fact that suberinite is present in dominant quantity and that it correlates tightly with contents of extractable matter (with R^2 value of almost 1 in Fig. 14 B) seems to support it as a major control for solvent extractability in the studied samples. This is also consistent with the dominance of waxy compounds in the extracts, a characteristic of suberinite hydrocarbons. Sporinite and liptodetrinite, as the second and third most abundant liptinite macerals, are thought to have significant contribution to the formation of non-waxy compounds. However, the fact that the contents of the two macerals fail to associate with concentration of cyclics and aromatics in the solvent extract (shown in Fig. 10) seems to preclude them as the only coal fractions that produce these compounds. Resinite, for example, is capable of generating terpenoids, which are readily convertible to aromatics upon maturation (Mukhopadhyay & Hatcher, 1993). The relatively rich resinite contents in PEN9-014 and PEN9-003 (shown in Table 4) match favourably with the high abundance of aromatics detected in the solvent extracts of the two samples (shown in Fig. 10). Perhydrous vitrinites of the telovitrinite (telinite and collotelinite) and detrovitrinite (vitrodetrinite and collodetrinite) groups have also demonstrated the potential to generate oil (Wilkins & George, 2002 and the references therein) with non-waxy characteristics (Powell et al., 1991). Vitrinites in this study are identified as perhydrous as per discussion in Section 3.1.

The anomalous behaviour of PEN9-029 and PEN9-034 coals (the outliers in Fig. 14) in relation to the linear regression analysis in Fig. 14 is not to be overlooked. The two samples are rich in oil-prone macerals, yet produced the least solvent extraction yield. In particular, PEN9-029 is distinguished by a wealth of VFAs and alcohols, whose contents are 2.5 times the average of the other 5 samples (Fig.8). This is in addition to the regional trend of generally higher biogenic gas contents at the stratigraphic level where these 2 coals were sampled (i.e. near the Tangalooma Sandstone; Hamilton et al., 2012), suggesting a likely realtime in-situ fermentation that gives rise to VFA-As and eventually biogas at the expense of coal extractable matter, which is shown to be bioavailable in this study. This hypothesis may not readily explain the discordance of PEN9-034 that contains little VFA-As. It is however, noteworthy that in contrast to the rich fraction of liptinite, especially suberinite in the PEN9-034 coal, the contents of telinite and collotelinite are remarkably low (the lowest of the six samples). These particular macerals are known for their well-preserved plant cell structure that offers porosity for oil storage (Mukhopadhyay & Hatcher, 1993). Lack of telinite and collotelinite in PEN9-034 coal may indicate the inability to store hydrocarbons, consequently causing migration of oils that have been generated. It is noted that PEN9-003 coal with the highest extraction yield also has relatively low telinite and collotelinite contents.

However, this may be compensated by the likely presence of macropores, which can be produced by tectonic uplift (PEN9-003 is the shallowest) and occur in much lower abundance in deeper coals (such as PEN9-034; Littke & Leythaeuser, 1993). It is therefore hypothesized that production of hydrocarbons in the samples, especially waxy compounds is largely dependent on liptinite, whereas their preservation requires vitrinite and the absence of strong microbial activity capable of converting them to biogas.

In addition to extraction yield, depositional environment and exposure to underground microorganisms could also directly affect coal bioavailability in laboratory bioassay. Stratigraphic control on laboratory coal biomethane yield was not only observed in this study (in Fig. 7A) but also samples (such as those in Jones et al., 2008) from other closely associated locations. Since different coal seams vary in permeability and fracture development, the access of coal to microorganisms introduced via meteoric recharge could also be different. These microbes function as in-situ fermenters (Hamilton et al., 2015; Jones et al., 2008). They might then produce intermediates such as acetate (Robbins et al., 2016). These compounds may themselves be precursors for methanogenesis or be easily converted into appropriate precursors which are readily bioavailable and, if not fully consumed in the coal seams, will contribute to methane yield in bioassays. In contrast, the higher hydrocarbons could have been processed via other pathways in laboratory bioassays, producing other end products such as CO_2, in the presence of alternative electron acceptor such as sulfate and ferric ion that could be present in sludge culture. A likely example is the PEN9-029 (lower Juandah) coal that has both the highest VFA-As concentration (Fig. 8) and biomethane yield (both from coal seam and laboratory, Fig. 7), a phenomenon that cannot be explained solely by coal properties such as proximate, ultimate and petrographic composition, or rank. Importantly, lower Juandah coals are more permeable owing to higher vitrain contents and better cleating, which may have enhanced microbe ingress via groundwater flow (Hamilton et al., 2015; Ryan et al., 2012),. On the other hand, an active microbial consortium in the coal seam might also be expected to deplete the local bioavailable fractions, decreasing the biomethane potential of the coal. This leads towards the hypothesis that while coal composition could be a primary factor for bioavailability (supported by extractability in the previous paragraph and further discussion in Sections 4.2 and 4.3), post-depositional modification of coal by in-situ microorganisms is capable of exerting a secondary effect that could potentially increase methane yield in bioassays.

It should however be noted that the coal samples characterised in this study are specific to the size range of 300 – 500 μm, which may not fully represent the composition of the in situ coal seam. This is because different types of macerals vary in strength and brittleness and might segregate disproportionally across size fractions upon crushing, causing bias to the above interpretations. Moreover, macerals in difference size fractions may vary in accessibility not only due to the change in total surface area, but also because some macerals are physically entrapped in others (e.g. corpogelinite occurs mostly as cell infillings in suberinte, Mukhopadhyay & Hatcher, 1993) and would only be exposed when they are crushed. The enlargement in surface area after crushing would also magnify the rate and yield of methane production as opposed to those in the field (Papendick et al., 2011). We thereby recommend the implications drawn in this study to be mainly linked to the compositional features themselves rather than being extrapolated across the Surat Basin Walloon CSG play. That

said, the sensible relationship observed between field gas contents and laboratory results warrants future investigation.

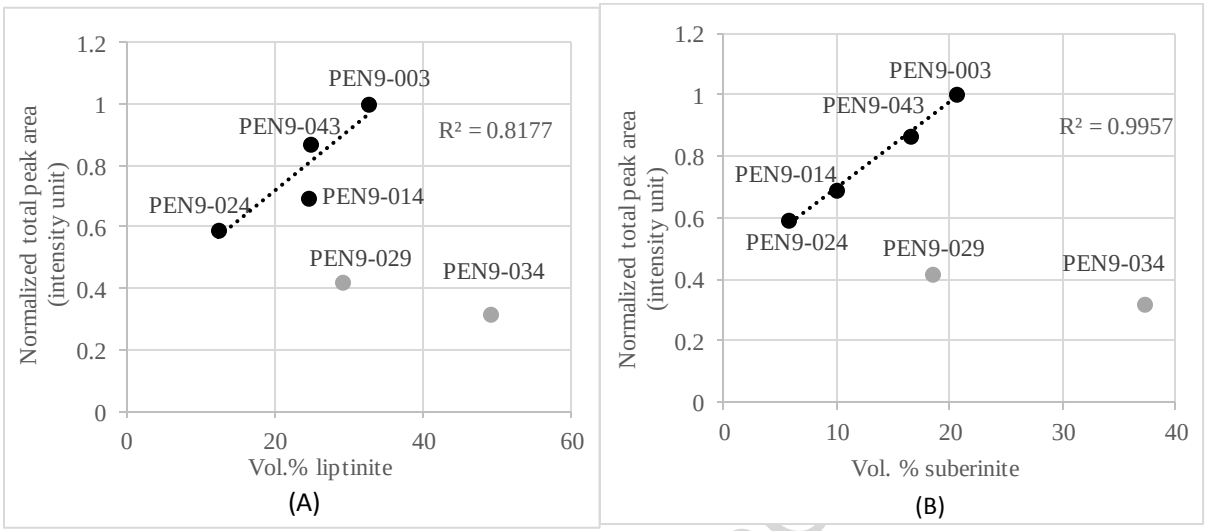


FIGURE 14: LINEAR REGRESSION BETWEEN TOTAL PEAK INTENSITY OF ORGANIC-SOLVENT-EXTRACTABLE MATTER AND CONTENTS OF MACERALS (A) LIPTINITE GROUP; (B) SUBERINITE IN FOUR OF THE SIX SAMPLES. PEN9-029 AND PEN9-034 WERE TREATED AS OUTLIERS. STRONG CORRELATIONS SUGGEST THAT THE ABUNDANCE OF LIPTINITE, PARTICULARLY SUBERINITE, IS CRUCIAL FOR GENERATION OF SOLVENT-EXTRACTABLE MATTER IN THE SAMPLES. NEVERTHELESS, THE TWO OUTLIERS INDICATES THERE ARE OTHER FACTORS (E.G. GELIFIED VITRINITE, EXPOSURE TO GROUNDWATER AS DISCUSSED IN SECTION 4.1) THAT CAN AFFECT PRESERVATION OF THE EXTRACTABLE MATTER.

4.2 ORIGIN AND JUSTIFICATION OF THE IDENTIFIED COMPOUNDS IN COAL EXTRACT

4.2.1 *N*-ALKANES

Compound characterisation using a combination of solvent extraction and GC-MS has long been used to examine coal composition. In this study, particular focus has been applied to three aliphatic groups: *n*-alkanes, *n*-alcohols and aliphatic esters since these dominate the extractable compounds detected and greatly contribute to degradability of coals. Being a major biomarker group, the presence of *n*-alkanes in coal organic extracts has been extensively reported (Fabiańska et al., 2013; Furmann et al., 2013a; Gao et al., 2013; Romero-Sarmiento et al., 2011), spanning a wide spectrum of C_{13} to C_{30} that encompass the range detected in this experiment.

All of the six PEN9 coal extracts were enriched in long-chain homologues of *n*-alkanes from C_{23} to C_{29}. A dominance of odd-carbon-numbered homologues was evident with CPI values greater than unity (Table 6). These signify an origin of epicuticular leaf waxes from terrigenous plants, a typical feature of coals containing cutinite (e.g. the studied samples) (Brooks et al., 1969; Didyk et al., 1978; Eglinton & Hamilton, 1967; Reddy et al., 2000). The bulk quantity of *n*-alkanes could also be derived from decomposition of suberinite as discussed above (Khavari-Khorasani & Michelsen, 1991).

4.2.2 *N*-ALCOHOLS

Compared to alkanes, the presence of *n*-alcohols in coal extracts is less frequently documented, presumably as it is not a typical coal biomarker. Like *n*-alkanes, the occurrence of *n*-alcohols was also demonstrated in the precursor epicuticular leaf waxes of higher plants (Dzou et al., 1995; Eglinton & Hamilton, 1967; Mudge et al., 2009). Bacterial biomass represents another possible direct input for mainly shorter chain homologues ($C_{14} - C_{18}$) (Mudge et al., 2009; Parkes & Taylor, 1983). *n*-Alcohols of bacterial origin are characterised by a dominance of odd-carbon-numbered chains (Parkes & Taylor, 1983), which is consistent with the profile observed in this study (as described in Section 3.4). This supports a likely significant contribution from bacteria during coalification. The fact that odd-carbon-numbered chains also dominate the *n*-alkanes, and that these are microbially convertible to *n*-alcohols via hydroxylation in a manner where the number of carbon is preserved (Ishige et al., 2003; Soltani et al., 2004), suggests that formation from plant waxes during coalification could also be a significant pathway. Given the high abundance of normal alcohols that were found (especially in PEN9-003 and PEN9-043), co-occurrence of the two mechanisms is likely and is reasonable as they both involve microorganisms. Direct evidence of *n*-alcohols in coal was presented by Glombitza et al. (2016) in a series of New Zealand coals with maturity up to R_0 0.52%. Such information is, however, limited in the current field of research.

The large difference in alcohol content between PEN9-003 and PEN9-043 and the rest of the samples is also noteworthy. This might be attributed, in part, to the elevated content of waxy liptinite (Fig. 14), which formation involves participation of bacterial biomass (Mukhopadhyay & Hatcher, 1993; Taylor et al., 1998). However, the liptinite difference is insufficient to explain fully the large discrepancy in alcohol content. Post-depositional microbial modification may again be crucial to the preservation of the hydrocarbons. Samples PEN9-003 and PEN9-043 that are richest in *n*-alcohols might have encountered little microbial activity post-uplift, whereas those with low alcohol content might have undergone strong microbial alteration that exhausted the compounds in exchange for biogas (e.g. PEN9-029 which is likely to have more groundwater exposure, see Section 4.1).

4.2.3 FATTY ESTERS

Fatty esters detected in this experimental study occurred in the saturated and unsaturated form, both with and without side chains. In most cases, fatty acids constitute the backbones of the esters, accompanied by single or double carbon unit alcohols. The presence of fatty esters (either as esters or free fatty acids) in coal extractable matter has been reported by a number of studies (Glombitza et al., 2009b; Oldenburg et al., 2000; White et al., 1979; Zink et al., 2008). As with the alkanes, saturated normal fatty acids or esters are also a constituent of leaf wax of higher plants (Dzou et al., 1995; Eglinton & Hamilton, 1967; Mudge et al., 2009), with predominance in even-carbon-numbered members (Eglinton & Hamilton, 1967; Glombitza et al., 2009b), a feature that agrees with the observations of this study. Waxy esters can also be produced from *n*-alkanes and 1-alkanols by microorganisms (Ishige et al., 2003; Ishige et al., 2002), a reaction that could happen during diagenesis together with formation of *n*-alcohols. Bacterial cell mass is an alternative important resource for fatty acids with shorter chain length ($< C_{20}$) (Glombitza et al., 2009b; Oldenburg et al., 2000; White et al., 1979; Zink et al., 2008). In particular, C_{16} and C_{18} fatty acids, in both saturated

(palmitic acid and stearic acid respectively) and unsaturated forms are the main components in the phospholipids of bacteria cell membranes (White et al., 1979; Zink et al., 2008). Methyl esters of palmitic acid (C_{16}) and stearic acid (C_{18}) were also identified in extracts of bacterial biomass, for example, *Arthrobacter* sp. and *P. aeruginosa* (Botvinko et al., 2014). Extracellular methyl palmitate, methyl stearate and methyl oleate were also found in methylotrophic bacteria as growth factors and adaptogens (Botvinko et al., 2014). The fact that the saturated normal fatty acids in this study are dominated by C_{16} and C_{18} homologues and that they occurred exclusively in the form of methyl esters seem to again imply a significant input from bacteria biomass during coalification.

We caution, however, that the use of methanol as a solvent could trigger esterification with free fatty acids during extraction. That said, the methyl esters detected could have actually been fatty acids, which are abundant in both terrestrial plants and microorganisms (Eglinton & Hamilton, 1967; White et al., 1979). Distribution of n-fatty acids in coals has been reported to follow a bimodal pattern with the first maximum at C_{16} and C_{18} and second around C_{24} and C_{26} in New Zealand coals (Glombitza et al., 2009b). This corresponds with our finding that C_{16}, C_{18} and C_{24} are the most prevalent.

Research has also reported the occurrence of other methyl and ethyl esters of unsaturated and branched fatty acids, detected in this study. In particular, hexanedioic acid bis(2-ethylhexyl) ester was isolated as a secondary metabolite in the terrestrial *Streptimyces* (Elleuch et al., 2010); tetradecanoid acid (myristic acid) was found in leaf epicuticular waxes (Eglinton & Hamilton, 1967); and branched fatty acids have been associated with bacterial origin (Johns et al., 1977).

Although the six PEN9 samples show less variation in the content of aliphatic esters, the concentrations are still notably higher in the two coals (PEN9-003 and PEN9-043) that are rich in *n*-alcohols. This could support the hypothesis made in the previous section, suggesting a combined effect of source material (and therefore maceral composition) and post-depositional modification on the contents of the compounds.

4.2.4 AROMATIC COMPOUNDS

Occurrence of aromatic compounds in organic extracts of subbituminous coals has also been reported globally (Fabiańska et al., 2013; Furmann et al., 2013a; Furmann et al., 2013b; Gao et al., 2013; Romero-Sarmiento et al., 2011). Liptinite macerals, such as resinite, sporinite and liptodetrinite that originate from materials rich in cyclics and aromatics (eg. resin, spores and pollent, and degradation remains of resinite and sporinite; Taylor et al., 1998) are capable of generating molecular aromatic compounds upon maturation (Mukhopadhyay & Hatcher, 1993; Taylor et al., 1998). Perhydrous vitrinite is another source of extractable aromatics (Powell, 1993). Vitrinite macerals are composed predominantly of aromatic structures mainly derived from the lignin of woody plants (Hatcher & Clifford, 1997; Hatcher et al., 1992; Strąpoć et al., 2011). Coalification of lignin involves dihydroxylation, demethylation (cleavage of aryl-O bond) and cleavage of β-O-4 aryl esters to form phenol-like and catechol-like structures (Hatcher & Clifford, 1997; Hatcher et al., 1988; Stout et al., 1988). Further maturation transforms the phenols to benzofuran and diarylethers, which are then pyrolyzed to benzene-like structures (Botto, 1987; Hatcher & Clifford, 1997). Dibenzofuran and alkylbenzenes occur at this stage within subbituminous to high-volatile bituminous coal ranks (Hatcher & Clifford, 1997; Hatcher et al., 1992). The resulting alkyl side chains of benzene

rings may be further condensed to polymeric aromatic ring systems upon heating (Fakoussa & Hofrichter, 1999; Hatcher & Clifford, 1997). The detection of a significant amount of dibenzofuran and alkyl benzenes, and to a lesser extent, phenolic esters, in in the extracts of the six PEN9 coals is consistent with the above description. The presence of dibenzofuran was also evidenced by other studies on extracts of subbituminous and bituminous coals (Fabiańska et al., 2013; Romero-Sarmiento et al., 2011). Polyaromatic hydrocarbons in this experiment occur exclusively as naphthalene and phenanthrene derivatives. This suggests a relatively moderate thermal alteration, which is reasonable for low ranks coals. The predominance of these compounds has been supported by other studies on coals of similar ranks (Fabiańska et al., 2013; Gao et al., 2013; Romero-Sarmiento et al., 2011). Occurrence of 4,4'-Diacetyldiphenylmethane (group acetyl diphenyl), another significant compound in this study, was also reported in produced water from the Powder River Basin, northeastern Wyoming (Orem et al., 2007), providing support for the identification. The abundance of aromatic compounds in the six coal extracts first decreases and then increases with burial depth, inflecting around the Tangalooma Sandstone (Fig. 10C). This matches favourably with the general downhole gas trend of Walloon coal CSG wells (maximizes near the Tangalooma Sandstone, Hamilton et al., 2012), and may again indicate a significant impact of ongoing in-situ microbial activities on the preservation of extractable compounds (i.e. aromatics might have also been converted to biogas by indigenous microbes).

4.2.5 VFA-As

The presence of acetic acid and ethanol in the water extract of coals is noteworthy and some justification of their occurrence seems warranted. Although detected in low quantities, they would be significant sources of methane yield if being fully converted by methanogens (as discussed in Section 3.3).

Since coal is an excellent adsorbent for small organic molecules, any laboratory method to characterise coal bioavailability should avoid potential environmental contaminants. With that in mind, we use 10% benzalkonium chloride instead of ethanol for general laboratory aseptic practice. Silica gel desiccant in the anaerobic chamber is regularly regenerated to prevent accumulation of VFA-As that might come off opened culture bottles during inoculation. Crushed coal samples are stored anaerobically in glass bottles with metal caps and rubber seals so that exposure to the environment is minimized. Crushing of coal cores was carried out using consistent procedures within 48 hours, in the sequence of well number (i.e PEN9-003 being the first, and PEN9-043 being the last). The fact that VFA content varies notably among samples and that it peaks in the fourth sample (PEN9-029, with intermediate contents of porous vitrinite as shown in Table 4) demonstrates an intrinsic source of the compounds instead of environmental. This is further supported by the similar trends between the concentration of VFA-As (shown in Fig. 8, ethanol and acetate are precursors to biogenic methane) and the dominant downhole gas content trend in Walloon CSG wells generally (Hamilton et al., 2012).

The question remains, however, as to how the ethanol and acetic acid are generated and preserved in the coal samples. Although not frequently reported, acetic acid was found in the formation water across multiple wells in the Powder River Basin associated with active acetoclastic methanogenesis (Ulrich & Bower, 2008). It is identified as a key fermentative product of coal and an immediate precursor for methane production (Moore, 2012; Papendick et al., 2011; Strąpoć et al., 2011). Even though most of the acetate formed in-situ may be

expected to be consumed by methanogens, partial preservation is still possible. For example, in an environment with convective flow of groundwater, acetate molecules might have been washed away from biofilm and adsorbed to the surfaces of micropores (< 2nm, Clarkson & Marc Bustin, 1996)) that have high affinity for acetate but are inaccessible to methanogens. Occurrence of acetate in laboratory aqueous solution of coal was also reported by Robbins et al. (2016). In contrast, observation of ethanol in coal is unusual and needs explanation. We propose the potential pathways for ethanol formation in coal seams:

1) Conversion from coal gas – Ethanol can be produced from CO_2, CO and H_2 in coal seam gas by the microbial strain Clostridium ljungdahlii along with production of equimolar acetate under anaerobic conditions (Elmore, 1990; Phillips et al., 1993). If the depletion rate of acetate by methanogens exceeds that of ethanol, accumulation of ethanol might occur. Examination of the indigenous microbial community is required to support this hypothesis.
2) Fermentation from coal source materials – The typical fermentation product of both coal waxes and aromatics is acetyl-CoA, formed via β-oxidation (Cravo-Laureau et al., 2005; Harwood et al., 1998; Porter & Young, 2014). Direct formation of ethanol from higher hydrocarbons has not been reported yet. Cellulosic materials, a common structural component of precursor plants, seem to be the only plausible substrate for ethanol and acetic acid fermentation. Although microbial elimination of cellulose is believed to be thorough during diagenesis, a small portion of cellulose might still have been preserved and became available to microbes upon recharge of groundwater in modern times.
3) Hydrolysis of ester bond – Ester bonds on coal surface may undergo either microbial hydrolysis (Fakoussa & Hofrichter, 1999; Hofrichter & Fakoussa, 2001; Glombitza et al., 2009a) or abiotic cleavage via equilibrium reactions with pore water (Glombitza et al., 2009a) to give free acids and alcohols. As such acetic acid or ethanol may be formed upon hydrolysis of an acetic or ethyl ester bond sticking out of the coal matrix.
4) Groundwater carrying plant debris – Meteoric water carrying plant debris may enter the coal bearing aquifers (e.g. through natural recharge or even abandoned gas wells). This can provide cellulosic materials for ethanol and acetic acid fermentation.

Due to the possible presence of coal seam microorganisms in PEN9-029 (as previously discussed), the relatively high VFA concentrations in the sample may be considered at least plausible.

4.3 BIOAVAILABILITY OF SOLVENT-EXTRACTABLE COMPOUNDS

The above results have demonstrated the bioavailability of the PEN9 Walloon coals to environmental microbial consortia. The fact that methane production in all bioassays finished within 1 month suggests that even without prolonged adaptation, a certain portion of coal still exhibits bioavailability to environmental cultures. Moreover, the primary phase of methane production takes place within the first 13 days of the 1 month incubation period. Such fast kinetics indicates the readily degradable nature of the bioavailable compounds to conversion via methanogenic pathways. The quick onset of methane production and significant baseline methane from negative control (with only inocula, no coal) also indicate some carry-over of carbon source from the inocula digested sludge. This could potentially benefit the cultures by not only boosting the initial microbial population, which was diluted 10 times in new assays upon inoculation, but by also providing energy for initiating degradation of more recalcitrant compounds.

4.3.1 ASSOCIATION BETWEEN COMPOUND ELIMINATION AND METHANE GENERATION

Methane generation from bioassays has been previously shown to correlate with compound elimination in coal extractable matter. However, it is still unclear how much of the eliminated compounds were finally converted to methane. A more explicit examination using mass balance is given to elucidate the maximum possible methane production assuming all eliminated compounds are metabolised through methanogenic pathways. A sample calculation based on the major groups of PEN9-003 extract is shown in Table 8.

TABLE 8: SAMPLE MASS BALANCE BETWEEN COMPOUND ELIMINATION AND METHANE PRODUCTION FOR PEN9-003

Compound groups	Reaction (formula based on weighted average)	Mass of compound (mg/g)	Avg. elimination (%)	Theoretical max. CH_4 (mmol/g)
n-Alcohol	$CH_3(CH_2)_{15.8}CH_2OH + 7.9H_2O \rightarrow 13.35CH_4 + 4.45CO_2$	24.9	100	1.24
Aliphatic ester	$C_{17.9}H_{34.4}O_2 + 8.4H_2O \rightarrow 12.8CH_4 + 5.1CO_2$	15.5	87.7	0.619
n-Alkane	$CH_3(CH_2)_{23.5}CH_3 + 12.3H_2O \rightarrow 19.4CH_4 + 6.1CO_2$	33.1	21.5	0.385
VFA-As	$CH_3CH_2OH + H_2O \rightarrow 1.5\ CH_4 + 0.5\ CO_2$	0.183	100	0.00597
	$CH_3COOH \rightarrow CH_4 + CO_2$	0.165	100	0.00275

* Mass of compounds is approximated by multiplying the fraction of compounds based on GC-MS peak intensity to solvent extraction yield in Table 2. The average formula of compounds is based on GC-MS intensity-weighted average. The theoretical maximum CH_4 yield is calculated by assuming complete conversion of compounds via methanogenic pathway.

The first three groups (in Table 8) that account for 83% of total compound elimination in the organic extract of PEN9-003 (as shown in Fig. 11) are used to approximate the theoretical methane yield from degradation of extractable compounds. Together with VFA-As, they are capable of producing a maximum of 2.25 mmol of methane/g coal (55 m^3/tonne). This is significantly higher than the average gas content reported in the field (5.36 m^3/tone d.a.f. of primarily biogenic methane) (Hamilton et al., 2012). The actual maximum generation could be even higher, taking into account the remaining 17% of compound elimination. However, the observed methane yield from bioassay turned out to be just 1% of the calculated value. This suggests the presence of powerful alternative sinks for the degraded carbons. The fact that the other 5 samples show analogous scales of methane production and compound elimination to those in PEN9-003 coal suggests that the low conversion to methane is common to all samples. We hereby speculate on a couple of factors that could explain this observation.

1) The great microbial diversity in the inocula digested sludge, once thought to be an advantage for compound degradation, may actually limit the amount of carbon available to methanogens. Different microbial species may process hydrocarbons in various ways that might not always lead to formation of methanogenic precursors. Carbon dioxide is a common end product of hydrocarbon metabolism in the presence of other electron acceptors such as sulphate, nitrate and ferric ion (Harwood et al., 1998; Holliger &

Zehnder, 1996; Porter & Young, 2014), which could occur in domestic wastewater where the inocula was sourced.

2) In addition, methane production needs to compete with biomass growth for carbon usage. In the initial stage of bioassay, the low microbial population, diluted 10 times upon inoculation, and high carbon concentrations could favour microbe growth with the result that a great proportion of carbon substrates are likely to be incorporated into biomass. At the later stages of incubation, where bioavailable carbon became limiting, microorganisms would be compelled to degrade biomass to fulfil their energy demand. The fast growing bacteria strive harder for substrates, and might have outcompeted the Archaea methanogens. This could result in methanogens being decomposed and consumed by other bacteria as a carbon source, reducing the overall methane yield.

Nevertheless, small molecules such as VFA-As are themselves easily convertible to methanogenic precursors (e.g. acetate), which generates ATP upon conversion to methane (Boone, 2000; Zeikus, 1977). This makes methanogenesis from VFA-As a more energetically favourable process than anabolic reaction (to form cell mass) that requires input of energy. The ATP generated can also help overcome the energy barrier in initiating degradation of higher hydrocarbons. This suggests a likely high conversion of VFA-As to methane, in contrast to the generally low conversion of other higher hydrocarbons. This hypothesis is consistent with the matching trend between VFA-A concentrations and the observed methane yields (as shown in Fig. 8). On the other hand, the above results may imply an appreciable hidden biomethane potential in the studied coals that could be unravelled when a better water chemistry and a relevant robust microbial community are given.

4.3.2 BIOAVAILABILITY AND COMPOUND CHARACTERISTICS

GC-MS analysis of solvent extracts on fresh and bioassay residue has demonstrated elimination of compounds through microbial digestion. Aliphatic compounds are, on average, more substantially degraded than aromatics. This is attributable to the high stability of aromatic ring structure as a result of delocalization of π electrons. In addition, methanol extracts displayed higher degradability than DCM extracts. This is due both to the fact that methanol has high affinity for polar functional groups, which are likely to serve as initiating sites for microbial attack (Furmann et al., 2013b; Hofrichter & Fakoussa, 2001; Strąpoć et al., 2011), and that the use of methanol as the first solvent in sequential extraction allows more biodegradable compounds to be sequestrated than in the subsequent DCM. Groups with the highest degradability (i.e. *n*-alcohol, esters and aliphatic amine), shown in Fig. 12, are predominantly aliphatic and concentrated in the methanol extracts, supporting the statement above. The fact that both aliphatic and aromatic compounds were significantly but incompletely degraded suggests concurrent utilisation of the two types during microbial degradation. This is consistent with the finding in the Furmann et al. (2013b) study.

To determine the bioavailability of coal compounds, aqueous solubility is commonly the first indicator to examine. Microbial digestion managed to eliminate an average 98% of soluble organic carbons in bioassays as per TOC results in Table 5. This implies a positive correlation between degradability and compound hydrophilicity. The latter is a characteristic of small polar molecules such as VFA-As, which are well known for their high bioavailability (Liu et al., 2013; Robbins et al., 2016). Also dissolved in water are larger compounds including *n*-alcohols, esters and aliphatic amines. These three groups presented

the highest degradability in organic extracts (as shown in Fig. 12), further justifying the above relationship. High aqueous solubility of compounds could also ease the mass transfer constraints, accelerating the methanogenic process.

Solvent extractability is the second factor to examine for coal bioavailability. Fig. 15 depicts the relationship of compound elimination with total extractable matters based on peak intensities (area under peaks). A strong positive correlation was observed with a high linear regression R^2 value of 0.875. This suggests that the bioavailability of coal is to a large degree related to solvent-extractable materials, thus, the bitumen fraction in contrast to the complex bound organic matrix. However, the precision of using extractability as a determining factor is limited due to the variance in compound distribution and variation in bioavailability of individual compounds.

A more refined evaluation can be made on the grounds of structural characteristics of extractable materials, a third and advanced factor to assess coal bioavailability. Compounds that are highly degradable in this study are distinguished by functional moieties of aliphatic hydroxyl groups, ester bond, carbon-nitrogen bond in aliphatic amine and ether bond. The heteroatom could form an activation site with low bond dissociation energy, either within the hetero-linkage (e.g. ester bond) or on neighbouring C-C bonds (e.g. hydroxyl group), facilitating microbial cleavage (Oyeyemi et al., 2015; Oyeyemi et al., 2014a; Oyeyemi et al., 2014b). Moreover, the presence of heteroatoms could also add polarity to compounds, enhancing the accessibility in aqueous solution.

Strict hydrocarbons (with only C and H) in this study are in contrast, much less degraded by the environmental digested sludge. A longer adaptation time or introduction of relevant microbial species would be necessary for them to be efficiently metabolised. On the other hand, these hydrocarbons conceive huge potential for additional methane production (Aitken et al., 2013; Foght, 2008; Furmann et al., 2013b; Gao et al., 2013; Harwood et al., 1998; Holliger & Zehnder, 1996; So et al., 2003) if the microbial community is robust and well-adapted.

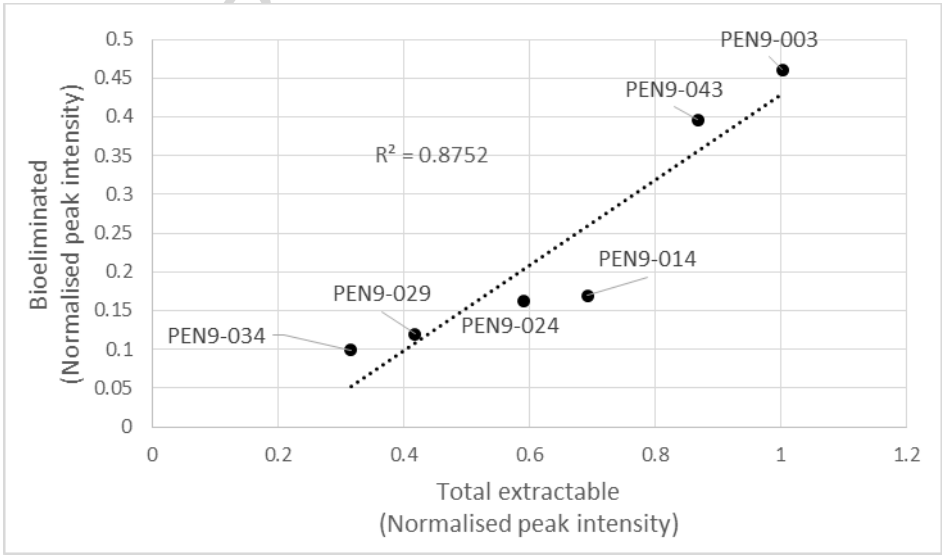


FIGURE 15: RELATIONSHIP BETWEEN QUANTITY OF EXTRACTABLE COMPOUNDS AND BIOELIMINATED COMPOUNDS. VALUES REPRESENT TOTAL PEAK INTENSITIES NORMALIZED WITH RESPECT TO THAT OF THE FRESH PEN9-003 SOLVENT EXTRACT.

5. CONCLUSIONS

1) This study has confirmed the basic bioavailability of the 6 Penrhyn 9 Walloon coals to environmental methanogenic consortia. The average methane yield obtained from 30 days incubation was 21 μmol/g (0.515 m^3/tonne). The trend by which methane production varied with burial depth is generally consistent with that of gas content in the study area. All samples were subbituminous to high volatile bituminous rank with relatively high vitrinite contents and elevated liptinite and hydrogen contents.
2) Extraction of coal with organic solvent recovered 3.8% - 12% of coal compounds based on weight. In general, aliphatic compounds were found to be more abundant than aromatics. *n*-Alkanes that occurred at the highest concentration in all samples, are thought to originate from plant wax during coalification. *n*-Alcohols and aliphatic esters that are concentrated in PEN9-003 and PEN9-043 coals, are thought to reflect an input of bacterial biomass. Aromatic compounds were detected up to three-fused rings. Distribution of aromatics is more diverse, including major groups of dibenzofuran, alkyl benzene, alkyl PAH, and acetyl diphenyl.
3) Content of solvent-extractable matter in coal is mainly affected by the following factors:
    a. *Liptinite content*. Suberinite, the dominant type of liptinite, is thought to contribute to the wax contents, whereas sporinite, liptodetrinite, as well as perhydrous vitrinite, contribute to the formation of cyclic and aromatic compounds.
    b. *Vitrinite*. Vitrinite macerals such as telinite and collotelinite are gelified and have well-preserved plant cell structures with microporosity that might be essential for storage of solvent-extractable hydrocarbons.
    c. *Post-uplift microbial modification* could exert a secondary effect on coal extractability. Preservation of extractable matter is thought to be compromised by microorganisms in meteoric recharge which can convert coal organics to biogas.

    Based on these findings, it was hypothesized that the content of extractable hydrocarbons in this study is dependent both on liptinite for generation and vitrinite for storage, in the absence of vigorous microbial activity capable of converting hydrocarbons to biogas.
4) On the grounds of organic chemistry, the basic bioavailability of coal to environmental cultures can be assessed by three factors hierarchically:
    a) *Water solubility*. VFA-As are the major compounds in water extracts of coal and can be responsible for a predominant proportion of methane being produced (40% to 96%). Also dissolved, but to less extent, are *n*-alcohols, esters and aliphatic amine. Water-soluble matter collectively showed an average 98% of elimination in bioassay.
    b) *Organic solvent extractability*. Bioavailability of organic extract was found, to a large degree, to be proportional to solvent extraction yield. The average compound elimination in organic solvent extract is 34.5%.
    c) *Aliphatic components with hydroxyl and amine group as well as ester and ether bonds*. These functional groups are characteristics of extractable compounds that exhibited the highest bioavailability in bioassay. The heteroatom linkages create sites with low bond dissociation energy that are amenable to microbial cleavage.
5) The proportion of biodegraded hydrocarbons being processed through methanogenic pathways is low. A large part of the carbon source may have been incorporated into biomass at an early stage to support cell growth and reproduction. This could also be a result of the unadapted microbial community in digested sludge, which might have

processed the carbons through other pathways. On the other hand, the low conversion implies a promising potential for improvement on methane yield, given that a more robust and relevant community is present.

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SUPPLEMENTARY DATA

TABLE 9: GC-MS DETECTED SOLVENT-EXTRACTABLE COMPOUNDS AND THEIR DEGRADABILITY (PRESENTED AS FRACTIONS: LOSS/ORIGINAL AMOUNT)

Compound class	Compound	Formula	Avg. elim.	Stdv.	Soluble solvents
n-Alcohols	1-Decanol	C10H22O	1	0	W, M

	1-Undecanol	C11H24O	1	0	W, M
	1-Dodecanol	C12H36O	1	0	W, M
	1-Tridecanol	C13H28O	1	0	W, M
	1-Pentadecanol	C15H33O	1	0	W, M
	1-Heptadecanol	C17H36O	1	0	W, M
	1-Nonadecanol	C19H40O	1	0	W, M
	1-Eicosanol	C20H42O	1	0	W, M
Al esters	Propenoic aicd,2-methyl-,2-ethyl-3-hydroxy hexyl ester	C12H24O3	0.869	0.096	W, M
	Methyltetradecanoate	C15H30O2	0.817	0.131	W, M
	2-Propanoic acid, tridecyl ester	C16H30O2	0.556	0.129	W, M, D
	Ethyl9-tetradecenoate	C16H30O2	0.986	0.010	W, M
	Pentadecanoic acid, methyl ester	C16H32O2	0.809	0.050	W, M
	2-Ethylhexyl 2-ethylhexanoate	C16H32O2	0.970	N.A.	W, M
	Hexadecanoid acid, methyl ester	C17H34O2	0.905	0.052	W, M, D
	9-Hexadecenoic acid, methyl ester,(Z)-	C17H32O2	0.667	0.187	W, M
	Heptadecanoic acid, methyl ester	C18H36O2	0.220	N.A.	W, M
	Ethyl9-hexadecenoate	C18H34O2	0.838	0.042	W, M
	Hexadecanoic acid, 14-methyl, methyl ester	C18H36O2	0.182	N.A.	W, M
	9-Octadecenoic acid, methyl ester, (E)-	C19H36O2	0.876	0.108	W, M, D
	Octadecanoic acid, methyl ester	C19H38O2	0.976	0.045	W, M
	Ethyl(Z)-octadec-9-enoate	C20H38O2	0.982	0.000	W, M
	Octadecanoic acid, 1-methylethyl ester	C21H42O2	0.993	N.A.	W, M
	Hexanedioic acid, bis(2-ethylhexyl) ester	C22H42O4	0.441	0.030	W, M
	Tetracosanoic acid, methyl ester	C25H50O2	0.639	0.026	W, M
***n*-Alkanes**	*n*-Heptadecane	C17H36	0.262	0.056	M, D
	n-Eicosane	C20H42	0.303	0.077	M, D
	n-Heneicosane	C21H44	0.237	0.085	M, D
	n-Docosane	C22H46	0.308	0.041	M, D
	n-Tricosane	C23H48	0.291	0.055	M, D
	n-Tetracosane	C24H50	0.275	0.022	M, D
	n-Pentacosane	C25H52	0.040	0.097	M, D
	n-Hexacosane	C26H54	0.295	0.073	M, D
	n-Heptacosane	C27H56	0.295	0.041	M, D
	n-Octacosane	C28H58	0.086	0.033	M, D
	n-Nonacosane	C29H60	0.133	0.048	M, D
Branched alkanes	Pristane	C19H40	0.165	0.026	M, D
	Phytane	C20H42	0.163	0.014	M, D
Al ether	Oxirane, [(dedecyloxy)methyl]-	C15H30O2	0.718	0.126	M, D
	Oxirane, heptadecyl-	C19H38O	0.554	N.A.	D
	Oxirane, [(hexadecyloxy)methyl]-	C19H38O2	0.336	N.A.	D
Cyclic al	18-Norabietane	C19H34	0.147	0.063	D
	Phenanthrene,7-ethenyl-1,2,3,4,4a,4b,5,6,7,8,8a,9-dodecahydro-1,1,4b,7-tetramethyl-,[4aS-(4a,alpha.,4b.beta.,7.alpha.,8a.alpha)]-	C20H32	0.230	N.A.	D
	17.alfa.21.beta.-28,30-Bisnorhopane	C28H48	0.122	0.052	M, D
	Muurolane-B	C15H28	0.123	N.A.	D
Cyclic al ketone	4a,trans-4b,cis-8a,trans-10a-Perhydro-cis-2,4b,8,8-tetramethyl-trans-2,10a-ethanophenanthren-12-one	C20H32O	0.220	N.A.	D

	5.alpha.,17.alpha.-Pregnan-12-one	C21H34O	0.218	0.072	M, D
Al amine	1-Dodecanamine,N,N-dimethyl-	C14H31N	0.770	N.A.	W, M
Alkyl thiophene	Thiophene,2,5-bis(1,1,3,3-tetramethylbutyl)-	C20H36S	0.057	0.025	M, D
Dibenzofuran	Dibenzofuran	C12H8O	0.255	0.058	M, D
Phenolic ester	Benzenepropanoic acid,3,5-bis(1,1-dimethylethyl)-4-hydrox	C18H28O3	0.883	0.148	W, M
Alkyl benzene	1-(3-Methylbutyl)-2,3,6-trimethylbenzene	C14H22	0.086	N.A.	M, D
	Benzene, (1-methylundecyl)-	C18H30	0.324	0.067	M, D
	Benzene, (1-pentyloctyl)-	C19H32	0.302	0.312	M, D
	Benzene, (1-butylnonyl)-	C19H32	0.142	0.043	M, D
	Benzene, (1-propyldecyl)-	C19H32	0.075	0.048	M, D
	Benzene, (1-methyldodecyl)-	C19H32	0.206	0.042	M, D
	benzene, (1-methyltridecyl)-	C20H34	0.260	0.019	M, D
Acetyl-alkyl tetralin	7-Acetyl-6-ethyl-1,1,4,4-tetramethyltetralin	C18H26O	0.151	0.042	M, D
Acetyl diphenyl	4,4'-Diacetyldiphenylmethane	C17H16O2	0.152	0.048	M, D
Phenyl cyclical	1,5,6,7-Tetramethyl-3-phenylbicyclo[3.2.1]hepta-2,6-diene	C17H20	0.044	0.027	M, D
Phenyl octahydrophenanthrene	4b,8-Dimethyl-2-isopropylphenanthrene, 4b,5,6,7,8,81,9,10-octahydro-	C19H28	0.194	0.003	M, D
	1-Methyl-10,18-bisnorabieta-8,11,13-triene	C19H28	0.176	0.043	D
	7-Isopropyl-1,1,4a-trimethyl-1,2,3,4,4a,9,10,10a-octahydrophenanthrene	C20H30	0.064	0.022	D
Alkyl biphenyl / diphenyl	4,4'-Diisopropylbiphenyl	C18H22	0.136	0.031	M, D
	1,1'-Biphenyl,3-methyl-	C13H12	0.225	0.030	M, D
	1,1'-Biphenyl,2,2',5,5'-tetramethyl-	C16H18	0.146	N.A.	M
	Benzene,1,1'ethylidenebis[3,4-dimethyl]-	C18H22	0.073	N.A.	M
	Benzene,1,1'-ethylidenebis[4-ethyl]-	C18H22	0.098	N.A.	M
Dehydroabietylamine	Dehydroabietylamine	C20H31N	0.519	0.023	M, D
Biphenyl amine	[1,1'-Biphenyl]-4-amine	C12H11N	0.368	0.054	D
Alkyl octahydrophenanthrenol	3-Phenanthrenol,4b,5,6,7,8,8a,9,10-octahydro-4b,8,8-trimethyl-,(4bS-trans)-	C17H24O	0.361	N.A.	D
Alkyl PAH	Naphthalene, 7-butyl-1-hexyl-	C20H28	0.084	0.049	M, D
	Naphthalene,1,6-dimethyl-4-(1-methylethyl)-	C15H18	0.168	N.A.	M, D
	10,18-Bisnorabieta-5,7,9(10,11,13-pentaene	C18H22	0.103	0.078	M, D
	1,4,5,8-Tetramethylnaphthalene	C14H16	0.155	0.029	M, D
	Retene	C18H18	0.056	0.040	M, D
	8-Isopropyl-1,3-dimethylphenanthrene	C19H20	0.145	N.A.	D
Alkyl benzonaphthyridinone	10H-Benzo[b][1,8]naphthyridin-5-one,7-ethyl-2,4-dimethyl-	C16H16N2O	0.326	0.053	M, D
Alkyl Indene	Cyclopent[a]indene,3,8-dihydro-1,2,3,3,8,8-hexamethyl-	C18H22	0.063	0.072	M, D
Phenyl-pyridinyl amide	2-Pyridinepropanamide, N-phenyl-	C14H14N2O	0.288	N.A.	M

* Elimination is based on the average of the six coal samples. Elimination is calculated by: $(\text{Intensity}_{\text{fresh coal}} - \text{Intensity}_{\text{digested coal}})/ \text{Intensity}_{\text{fresh coal}}$. Soluble solvents represents the type of solvent (W = water, M = methanol, and D = DCM), with which extraction had reduced the peak intensity (area under peaks) of a particular compound by more than 5% (i.e. at least 5% of compound is dissolved in the solvent after extraction).

Highlights

- Bioavailability of coal is controlled by three factors: water solubility, solvent extractability, and abundance of heterogeneous functional groups.
- Generation of extractable matter in coal is largely dependent on vitrinite, particularly suberinite
- Preservation of extractable matter relies on vitrinite but can be compromised by microbial activities in groundwater.