

Methanogenic Hydrocarbon Degradation: Evidence from Field and Laboratory Studies

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Key Words

Hydrocarbons · Coal · Oil · Fermentation · Methanogenesis · Methane production rate

Abstract

Microbial transformation of hydrocarbons to methane is an environmentally relevant process taking place in a wide variety of electron acceptor-depleted habitats, from oil reservoirs and coal deposits to contaminated groundwater and deep sediments. Methanogenic hydrocarbon degradation is considered to be a major process in reservoir degradation and one of the main processes responsible for the formation of heavy oil deposits and oil sands. In the absence of external electron acceptors such as oxygen, nitrate, sulfate or Fe(III), fermentation and methanogenesis become the dominant microbial metabolisms. The major end product under these conditions is methane, and the only electron acceptor necessary to sustain the intermediate steps in this process is CO₂, which is itself a net product of the overall reaction. We are summarizing the state of the art and recent advances in methanogenic hydrocarbon degradation research. Both the key microbial groups involved as well as metabolic pathways are described, and we discuss the novel insights into methanogenic hydrocarbon-degrading populations studied in

laboratory as well as environmental systems enabled by novel cultivation-based and molecular approaches. Their possible implications on energy resources, bioremediation of contaminated sites, deep-biosphere research, and consequences for atmospheric composition and ultimately climate change are also addressed.

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Introduction

Stable isotope studies carried out in the last two decades demonstrated that large amounts of biogenic methane are formed in oil and coal reservoirs, or contaminated aquifers [Horstad and Larter, 1997; Horstad et al., 1992; Larter, 2006; Thielemann et al., 2004; Weiner and Lovley, 1998]. Oil and coal, which are converted to methane by anaerobic microorganisms in the absence of oxygen (methanogenesis), are most likely the carbon sources for this methane. Microbial degradation of higher hydrocarbons to methane is independent from external electron acceptors besides CO₂, and demands only water as well as small amounts of nutrients and trace elements. Consequently, this methanogenesis provides a suitable model to explain oil biodegradation and gas formation in

reservoirs without oxidants, like oxygen, nitrate, manganese (IV), iron (III) or sulfate. Reservoir studies suggest that the microbial degradation rates of hydrocarbons to methane at temperatures between 40 and 70°C are in the order of 4–10 kg/m²/year at the oil-water contact area [Head et al., 2003]. The degrading biota appears to live predominantly at the interface between the oil and the water. Recently, methanogenic Archaea belonging to Methanosarcinales and Methanomicrobiales have been found in water droplets from water-in-oil emulsions in Pitch Lake, Trinidad and Tobago, the biggest asphalt deposit [Meckenstock et al., 2014], suggesting that microhabitats such as these droplets would be enough for in situ methanogenic biodegradation of hydrocarbons to occur.

Until the early 1980s, hydrocarbons were believed to be persistent under anoxic conditions [Atlas, 1981]. In the last few decades, it has been extensively proven that hydrocarbons are biodegradable under strictly anoxic conditions [Dworkin et al., 2006; Fuchs et al., 2011; Gieg et al., 2014; Gray et al., 2010; McInerney et al., 2009; Vogt et al., 2011; Widdel et al., 2010], and there is evidence of ongoing hydrocarbon degradation even in electron-acceptor-depleted environments, such as deep sediments, oil and coal reservoirs or contaminated aquifers, for example, where hydrocarbons represent a significant fraction of the organic matter. In these environments, methanogenesis is the only possible terminal biodegradation process.

Already in the early second half of the 20th century, Bokova [1953] suggested that oil biodegradation linked to methane production could be an important factor in the evolution of oil fields. This was consistent with previous ideas postulated by Kuznetsov [1950], who could isolate bacteria from oil samples but could not demonstrate methanogenic biodegradation of *n*-heptane. Later, Muller [1957] detected methanogenic conversion of longer *n*-alkanes from paraffinic oils. More recently, methanogenic hydrocarbon biodegradation has been identified as one of the main biodegradation processes in subsurface hydrocarbon-dominated environments, such as oil reservoirs [Jiménez et al., 2012; Jones et al., 2008; Milkov, 2010], coal deposits [Green et al., 2008; Krüger et al., 2008; Scott et al., 1994; Zhou et al., 2005] and shales [Krüger et al., 2014]. Biogenic methane represents a considerable amount of the natural gas resources [Rice and Claypool, 1981]. According to estimations by Milkov [2010], in the past, as much as $1,883 \times 10^9$ m³ of methane have potentially been generated by oil biodegradation in bitumen and biodegraded oil reservoirs worldwide through their geological history. Most of the so-produced methane most likely leaked into the atmosphere and ocean, affect-

ing the global carbon cycle and the planetary climate over geological time scales [Milkov, 2010].

Methanogenic hydrocarbon biodegradation has important biotechnological applications. Although it might be unwanted in oil reservoirs, because it decreases oil quality and value, the conversion of entrained oil to methane has been proposed as a method to enhance recovery of carbon in exhausted reservoirs [Gieg et al., 2008]. This transformation is of particular significance, as more than 50% of the original oil cannot be retrieved using conventional technologies [Youssef et al., 2009]. In addition, methanogenic hydrocarbon degradation can contribute to the natural attenuation of hydrocarbon spills in a variety of electron-acceptor-depleted environments, e.g. mineral oil or fuel-contaminated aquifers [Feisthauer et al., 2010, 2012; Gieg et al., 1999], when other electron acceptors such as Fe(III) and sulfate are depleted. Compared to other biodegradation processes, methanogenesis could be easier to sustain, precisely because it does not require any external electron acceptors.

In summary, in recent years, increasing evidence of the occurrence of in situ methanogenic hydrocarbon degradation has been published [Gieg et al., 2010; Gründger et al., 2015; Jiménez et al., 2012; Jones et al., 2008; Milkov, 2011], reflecting its environmental importance. In addition, laboratory experiments and the extensive use of molecular techniques have contributed to gain information about the methanogenic pathways, the conditions affecting methanogenesis and the microorganisms involved. The current review presents a comprehensive overview of the state of the art of methanogenic hydrocarbon degradation with respect to involved microorganisms, metabolic pathways and their environmental distribution and relevance.

Methanogenic Hydrocarbon Degradation

Methanogenic hydrocarbon biodegradation occurs in a series of steps and requires close syntrophic associations between fermentative bacteria and methanogenic Archaea [Zengler et al., 1999]. Syntrophic associations with in hydrocarbon-degrading cultures have been reviewed recently [Gieg et al., 2014; Sieber et al., 2012]. In this process, fermentative bacteria (e.g. some Clostridia and Proteobacteria) first transform hydrocarbons into smaller molecules such as short-chain fatty acids, alcohols or H₂. The involvement of *Smithella* and other related genera has gained general acceptance [Gray et al., 2011; Tan et al., 2014; Zengler et al., 1999]. Hydrocarbons first need to

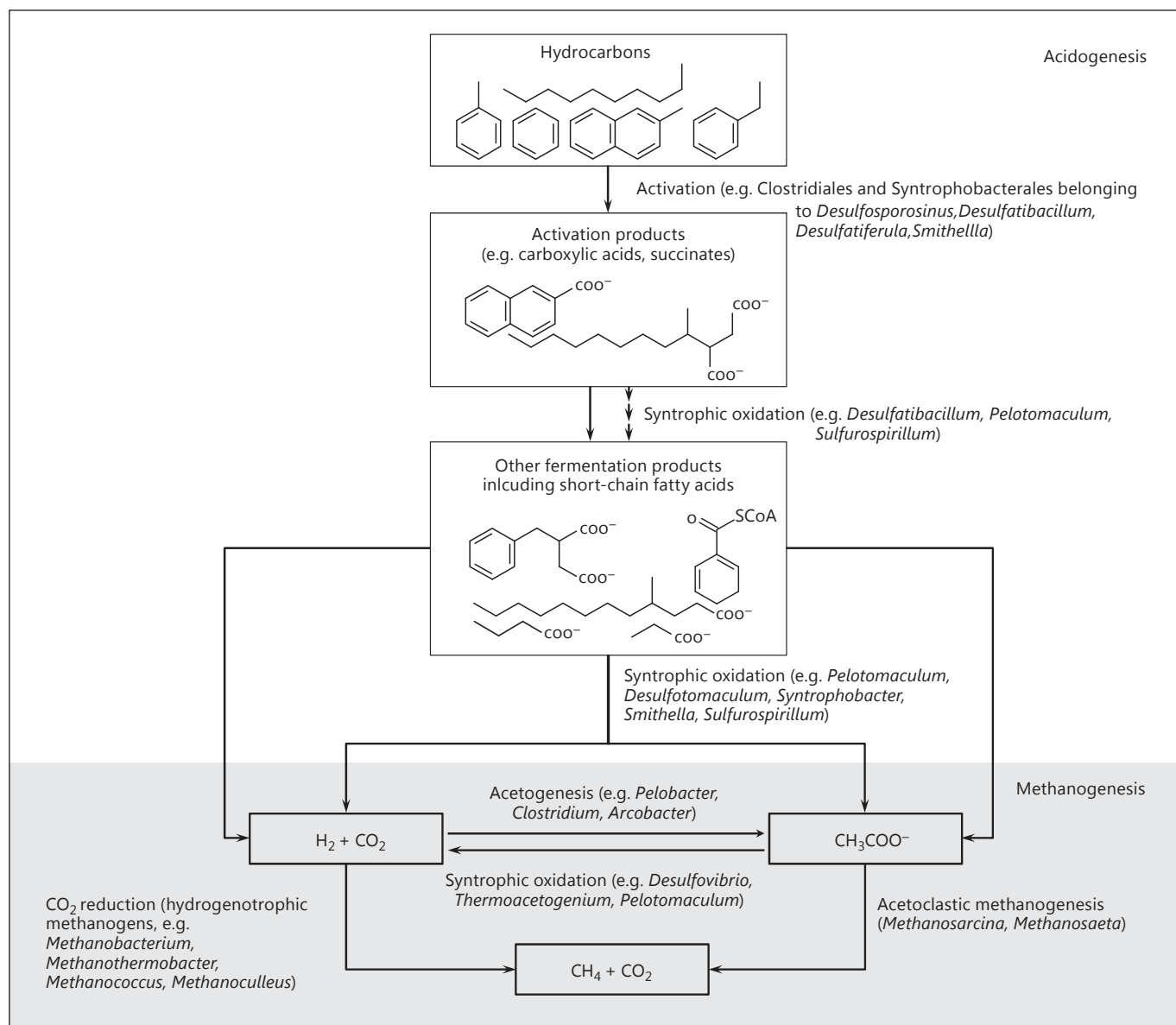


Fig. 1. Schematic diagram of microbial conversion of hydrocarbons to methane.

be activated (e.g. by addition of fumarate) to be further degraded [Heider, 2007; Tan et al., 2014]. Many of the involved reactions are endergonic and only become energetically feasible if the end products (formate, acetate or hydrogen) are kept at relatively low concentrations [McInerney et al., 2008]. According to calculations [Dolfing et al., 2008], the methanogenic transformation of alkanes is possible at hydrogen partial pressures lower than 4×10^{-5} atm. Different groups of methanogenic Archaea, mostly belonging to Methanomicrobia, use those prod-

ucts and transform them to methane and CO_2 through various pathways, mainly CO_2 reduction and acetoclastic methanogenesis (fig. 1).

Hydrogenotrophic microorganisms, e.g. from the genera *Methanobacterium*, *Methanothermobacter*, *Methanocella* and *Methanococcus*, use H_2 as electron donor while reducing CO_2 to methane (equation 1). Interspecies electron transfer through H_2 or formate as electron carriers is a common process. Nevertheless, recent studies reported a direct interspecies electron transfer, in which no electron

Table 1. Direct and indirect geochemical evidence of biogenic methane in samples collected in field studies

Parameter	Examples	References
Hydrocarbon patterns (e.g. <i>n</i> -alkanes and aromatics) consistent with oil biodegradation, heavy oil	Dagang oil field, San Juan, Gippsland and Otway Basins	Aitken et al. 2013; Cai et al., 2015; Head et al., 2003; Jiménez et al., 2012; Jones et al., 2008; Milkov, 2011; Ross et al., 2010; Scott et al., 1994
Heavy $\delta^{13}\text{C}$ -CO ₂ (> +2‰), and dissolved organic carbon values (> +20‰), indicative of CO ₂ reduction to methane	Dagang oil field, San Juan, Gippsland, Otway and Western Siberian Basins, Forest City Basin	Jiménez et al., 2012; Jones et al., 2008; McIntosh et al., 2004, 2008; Milkov, 2010, 2011; Pallasser, 2000; Scott et al., 1994
Relationship between $\delta^{13}\text{C}$ -CH ₄ and δD -CH ₄	Forest City Basin, Illinois Basin, Antrim Shale, New Albany Shale	McIntosh et al., 2008; Schoell, 1980; Whiticar, 1999; Whiticar et al., 1986
Relationship between $\delta^{13}\text{C}$ -CH ₄ and $\delta^{13}\text{C}$ -CO ₂	Several basins	Milkov, 2011
Linear relationship of δD of CH ₄ and H ₂ O, and fractionation factors, indicative of use of water hydrogen by methanogens to produce methane	Illinois Basin	Martini et al., 1996; McIntosh et al., 2008; Schoell, 1980; Whiticar et al., 1986
Large separations between <i>n</i> -alkane homologs (up to +29‰)	Gippsland and Otway Basins	Pallasser, 2000
Dryness, low percentages of C ₂ ⁺ hydrocarbons (<2%)	Dagang oil field, Western Siberian Basin	Head et al., 2014; Jiménez et al., 2012; Milkov, 2010, 2011
Relationship between C ₁ /(C ₂ + C ₃) and $\delta^{13}\text{C}$ -CH ₄		
Low acetate concentrations (<1 mM)		
High alkalinity and dissolved organic carbon values (up to 70 mEq/kg)	San Juan Basin, Forest City Basin, Antrim Shale	Formolo et al., 2008; McIntosh et al., 2004, 2008
Ca/Mg ratios (<1.5)	Antrim Shale	McIntosh et al., 2004
Low concentrations of SO ₄ ²⁻ (<10 mM) and other electron acceptors	Dagang oil field, Forest City Basin, Antrim Shale	Jiménez et al., 2012; Martini et al., 1998; McIntosh et al., 2008

shuttle (formate or H₂) is required, between *Geobacter metallireducens* and members of Methanosarcinales, i.e. *Methanosaeta harundinacea* and *Methanosarcina barkeri* [Rotaru et al., 2014a, b]. Acetoclastic methanogens from the genera *Methanosarcina* and *Methanosaeta* utilize acetate as the terminal electron acceptor (equation 2). Acetate can also grow by the conversion of acetate to methane and HCO₃³⁻ (equation 3) [Dolfing, 2014; Hattori, 2008]. Finally, methylotrophic methanogens, e.g. *Methanospaera*, *Methanolobus* or *Methanosalsum*, use methylated compounds such as methanol or methylamines instead.



The occurrence of methanogenesis and the predominance of one or another of the methanogenic pathways in the environment may depend on a combination of factors such as temperature, CO₂ concentrations, salinity, pH, availability of electron acceptors and donors and nutrients, porosity and permeability, for example [Dolfing et

al., 2008; Kotsyurbenko et al., 2007; Mayumi et al., 2011; Milkov, 2011; Schlegel et al., 2011; Siegert et al., 2011; Waldron et al., 2007]. From the distribution of biodegraded oils worldwide, it seems that reservoirs buried to temperatures of more than 80°C are effectively sterilized with regard to hydrocarbon degraders [Head et al., 2003; Wilhelms et al., 2001]. However, in nutrient-rich environments, such as hydrothermal vents, methanogens can survive at more than 100°C [Takai et al., 2008]. In addition, temperature can select for different methanogenic communities [Blake et al., 2015].

Generally, it has been considered that SO₄²⁻ concentrations above 50 μM cause methanogens to be outcompeted by sulfate-reducing bacteria using the same substrates more efficiently, whereas lower concentrations could even enhance methanogenesis [Siegert et al., 2011].

Regardless of the prevailing pathway, the overall syntrophic reaction yields extremely low Gibbs free energy (around -10 kJ/mol or even less), even below of the predicted minimum increment of energy required for ATP synthesis (15 -20 kJ/mol) [McInerney et al., 2009, and the

references therein]. Consequently, methanogenic hydrocarbon-degrading microbial communities have typically extremely low growth rates.

Evidence of Microbial Conversion of Hydrocarbons to Methane in situ

Naturally occurring methane includes thermogenic and biogenic gas, which can be identified by distinct isotopic signatures of CH₄ ($\delta^{13}\text{C}$ and δD), CO₂ ($\delta^{13}\text{C}$) and H₂O (δD) [Whiticar, 1999]. Biogenic methane can also be detected using several other indirect geochemical indicators, including alkalinity, dissolved organic carbon, Ca/Mg ratios, gas dryness or concentrations of CO₂, acetate, SO₄²⁻ or O₂ (table 1) [McIntosh et al., 2008; Rice and Claypool, 1981]. Geochemists differentiate between primary microbial methane, formed by direct decomposition of sedimentary organic matter, and secondary microbial methane, formed during biodegradation of hydrocarbons [Milkov, 2010], either by CO₂ reduction or acetoclastic methanogenesis.

Geological and geochemical studies evidenced the wide distribution of secondary microbial gas in subsurface accumulations from on- and offshore sedimentary basins around the world, including oil reservoirs, coal deposits and shales [Etiope et al., 2009; Jiménez et al., 2012; Jones et al., 2008; Krüger et al., 2008; Martini et al., 2003; Milkov, 2011; Shimizu et al., 2007; Warwick et al., 2008; Zhou et al., 2005]. Production of biogenic methane has been detected in shallow systems such as New Albany and the Upper Devonian Antrim Shales in the Michigan Basin [Shurr and Ridgley, 2002]. Scott et al. [1994] calculated that around 15–30% of the coalbed gas in the San Juan Basin (USA) would derive from the biodegradation of wet-gas components, *n*-alkanes and other organic compounds at relatively low temperatures. Milkov [2010] estimated that a significant part of the shallow dry gas (>99% of methane) in the northern West Siberian Basin (which accounts for 17% of the world's conventional gas endowment) originated from the methanogenic biodegradation of petroleum. Oil legs from this basin were highly degraded and the isotopic signature of CO₂ suggested conversion of 40–70% oil-derived CO₂ to methane [Milkov, 2010].

Bacterial CO₂ reduction is coupled to a kinetic isotope effect, as the lighter carbon stable isotope (¹²C) is preferentially used [Whiticar, 1999]. This effect produces enrichment in the $\delta^{13}\text{C}$ of the remaining pool of CO₂ (with values >0), while the formed product CH₄ becomes lighter (with $\delta^{13}\text{C}\text{-CH}_4$ sometimes as negative as –110‰ vs.

Vienna Pee Dee Belemnite, VPDB) [Milkov, 2011]. 'Heavy' CO₂, enriched in ¹³C, typically found in strongly biodegraded reservoirs, thus indicates an extensive reduction of CO₂ to methane [Jiménez et al., 2012; Jones et al., 2008; Pallasser, 2000]. In contrast, the hydrogen isotopic discrimination for this pathway is low (δD between –170 and –250‰ relative to the Vienna Standard Mean Ocean Water, VSMOW) compared to other biological methanogenic pathways [Milkov, 2011]. Model studies [Morris et al., 2012] have shown that the concurrence of acetoclastic and hydrogenotrophic methanogenesis can lead to isotopically relatively heavy methane and low discrimination between CO₂ and CH₄, so CO₂ and CH₄ isotopic patterns should be interpreted with caution.

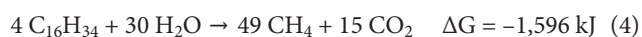
Combining several geochemical indexes allows identifying in situ methanogenic activities and distinct methanogenic pathways, and may be particularly useful when biogenic and thermogenic gases co-occur. For example, Martini et al. [1998] observed $\delta^{13}\text{C}\text{-CH}_4$ values consistent with a thermogenic or mixed gas (between –56 and –47‰) in the Antrim Shale. However, only the occurrence of microbial transformations could explain the unusually high $\delta^{13}\text{C}$ values of CO₂ coproduced with methane (+22‰) and dissolved inorganic carbon in formation waters (+28‰). In a recent study, the isotopic signatures of gases and fluids sampled from a water-flooded oil reservoir in Dagang, PR China, together with the discrimination between CH₄ and CO₂ (32–65‰) and the dryness C1/(C2+C3) and oil gas chromatographic mass spectrometry profiles exhibiting typical biodegradation patterns, i.e. lack of *n*-alkanes or changes in biomarker signatures, indicated an extensive biotransformation of oil to methane [Cai et al., 2015; Jiménez et al., 2012]. Biogenic gas, predominantly deriving from CO₂ reduction, was also predominant in a coal-associated sedimentary basin, as determined by the isotopic signatures ($\delta^{13}\text{C}$ and δD) of CO₂ and methane [Gründger et al., 2015]. Biogenic methane production has also been observed in hydrocarbon-contaminated aquifers after spills or leakage of hydrocarbon-containing waste [Feisthauer et al., 2012]. The hydrogen and carbon isotopic compositions were consistent with a predominance of acetoclastic methanogenesis [Feisthauer et al., 2010, 2012].

In vitro Studies

Despite the growing evidence of methanogenic hydrocarbon degradation in situ, until quite recently, microbial transformation of hydrocarbons under methanogen-

ic conditions had been hardly investigated, although the first reports of methane production by microbial cultures from oil field core materials were published more than 60 years ago [Bokova, 1953; Ekzercev, 1960]. These studies suggested that methane would be formed by the biodegradation of fatty acids present in oil to CO₂ and methane, and from the reduction of CO₂ by H₂. In the last decades, however, the topic has regained scientific attention and many studies attempting to explain the mechanistic aspects and to identify key microorganisms involved have been conducted. The conversion of hydrocarbons to methane has been extensively demonstrated in laboratory experiments (table 2) [Cai et al., 2015; Feisthauer et al., 2010; Gray et al., 2011; Gründger et al., 2015; Jiménez et al., 2012; Jones et al., 2008; Siegert et al., 2011; Tan et al., 2013; Wang et al., 2011; Zengler et al., 1999].

A famous experiment evidencing microbial transformation of *n*-alkanes to methane under strictly anoxic conditions is that of Zengler et al. [1999]. The authors incubated samples in sulfate-free mineral medium inoculated with anoxic ditch sediments spiked with 1.7 mmol *n*-hexadecane, and observed increasing methane formation (up to 4.6 mmol) linked to *n*-hexadecane consumption (0.059 mmol) in 810 days of incubation. The use of a ¹³C-labeled substrate confirmed that the CH₄ derived from *n*-hexadecane, mainly through acetoclastic methanogenesis. This proved that the complete mineralization of *n*-hexadecane to methane and CO₂ (which the authors proposed to take place in different steps leading to the net reaction described in equation 4) is thermodynamically feasible, even if the energy yield is lower than in other hydrocarbon-degrading reactions [Dolfing et al., 2008; Spormann and Widdel, 2000].



A subsequent study by Feisthauer et al. [2010] determined the stable carbon and hydrogen isotopic signatures of methane, CO₂ and water during microbial formation of methane from *n*-hexadecane. The narrow ranges for the carbon and hydrogen isotopic discrimination between substrate and methane suggested a co-occurrence of acetoclastic and CO₂-reducing methanogenesis.

After the pioneering study by Zengler et al. [1999], biodegradation of *n*-hexadecane under methanogenic conditions has been repeatedly documented [Anderson and Lovley, 2000; Jiménez et al., 2012; Siegert et al., 2011]. In addition, several other alkanes have also proven to be converted to methane and CO₂. This includes short- to medium-length compounds [Siddique et al., 2006, 2011] or long ones, like *n*-C₂₈ or *n*-C₃₂ (tables 2, 3). Siddique et

al. [2006] reported methanogenic biodegradation of short-chain *n*-alkanes by enrichment cultures from oil sands tailings taking place in an unusual sequence (C₁₀ > C₈ > C₇ > C₆). The authors postulate that this might be caused by an increase in the octanol/water partition coefficient (*K_{ow}*) with increasing molecular weight. Another explanation might be a sort of selective transport across cell membranes of the *n*-alkane-degrading microorganisms as proposed by Kim et al. [2002]. Although gaseous *n*-alkanes can also be oxidized under anoxic conditions [see Musat et al., this volume, pp 211–226], their transformation under methanogenic conditions has not been shown yet.

Linear alkanes are the most readily degraded hydrocarbons. This would explain why *n*-alkane-rich light crude oil provides higher methanogenic yields as compared to other complex hydrocarbon substrates (table 3). Enrichment cultures from the Dagang oil reservoir were able to degrade all medium- and long-chained *n*-alkanes (*n*-C₁₀ to *n*-C₃₆) from crude oil in less than 200 days, producing methane at a rate of 76 ± 6 μmol/day/g oil added [Cai et al., 2015], paralleling previous observations [Gieg et al., 2008; Jones et al., 2008]. The presence of abundant aliphatic constituents in kerogen-rich Posidonia and Alum shale samples (reflected by high hydrogen index values) determined its potential as methanogenic substrate for methanogenic hydrocarbon-degrading enrichment cultures [Krüger et al., 2014]. In this case, methane production (ranging from 1 to 10³ nmol/day/g TOC) was influenced by the quality of the TOC and inversely correlated to the thermal maturity of the organic matter.

Recently, methanogenic biodegradation of C₇ and C₈ iso-alkanes (methylhexanes and heptanes) was demonstrated [Abu Laban et al., 2014]. The biodegradation was found to be isomer specific, consistent with some previous in situ observations [Jiménez et al., 2012], and some of the compounds could only be degraded (probably co-metabolically) when in iso-alkane mixtures. The presence of putative succinylated iso-alkane metabolites suggested fumarate addition as activation mechanism.

Some aromatic hydrocarbons, such as benzene, toluene, *o*-xylene, ethylbenzene or 2-methylnaphthalene, have also proven to be biodegradable under methanogenic conditions [Abu Laban et al., 2015; Edwards and Grbić-Galić, 1994; Feisthauer et al., 2010; Fowler et al., 2012, 2014; Jiménez et al., 2012; Siegert et al., 2011; Sun et al., 2014]. Siegert et al. [2011] observed methane production (up to 58.1 nmol CH₄/ml/day) in enrichment cultures of hydrocarbon-contaminated harbor sediments growing with ethylbenzene. However, no methane was produced when

Table 2. Overview of methanogenic hydrocarbon-degrading consortia, growing on coal, oil and single hydrocarbons

Substrate	Origin of the inoculum, description of the habitat	Incubation temperature (if available), remarks	Identified taxa (if available)	References ^a
Light oil, heavy oil, <i>n</i> -alkanes (<i>n</i> -C ₁₂ – <i>n</i> -C ₁₈)	Plussee, Germany; Eutrophic lake with stable anoxic hypolimnion; sample depth: 28 m	28° C	–	Eller et al., 2005
Light oil, heavy oil, <i>n</i> -alkanes (<i>n</i> -C ₁₂ – <i>n</i> -C ₁₈)	Eckernförde Bay, Germany; Baltic Sea brackish water; sample depth: 28 m	28° C	–	Feisthauer et al., 2010; Siegert et al., 2011
<i>n</i> -hexadecane	Bremen, Germany; freshwater ditch		<i>Syntrophus</i> , <i>Methanosaeta</i> , <i>Methanoculleus</i> , <i>Methanospirillum</i>	Zengler et al., 1999
Light oil, heavy oil, shales, coal, timber, <i>n</i> -alkanes (<i>n</i> -C ₁₀ – <i>n</i> -C ₃₂)	Kuhgraben, Bremen, Germany; another freshwater ditch; sample depth: 2 m; in situ temperature during sampling: 25° C	28° C, active enrichment on <i>n</i> -C ₃₂	<i>Syntrophus</i> , <i>Methanoculleus</i> , <i>Methanospirillum</i>	Feisthauer et al., 2010; Krüger et al., 2014; Siegert et al., 2011
Light oil, heavy oil, <i>n</i> -hexadecane, BTEX	Brazil; sample from intertidal sediments of brackish water mangroves	28° C	–	–
<i>n</i> -hexadecane, ethylbenzene	Zeebrugge Harbor, Belgium; heavy metals and oil-contaminated sediments. Sample depth: 3 m	28° C	–	Siegert et al., 2011
<i>n</i> -hexadecane, BTEX	Weissandt-Gölsau, Germany; gas samples from groundwater aquifer contaminated with crude oil	28° C	–	Feisthauer et al., 2010
Coal, <i>n</i> -hexadecane, BTEX	Ruhr Basin, Germany; groundwater and coal-bearing sediments from an open-pit brown coal mine	30° C	<i>Desulfomonile</i> , <i>Smithella</i> , <i>Desulforhopalus</i> , <i>Desulfatiferula</i> , <i>Pseudomonas</i> , <i>Acetobacterium</i> , <i>Nocardiodaceae</i> , <i>Anaerolineaceae</i> , <i>Deferribacteres</i>	Gründger et al., 2015
Coal, timber	Ruhr Basin, Germany; timber and coal from a coal mine closed since 1960s; sample depth: 800 m	30° C	<i>Clostridium</i> , <i>Desulfovibrio</i> , <i>Pelobacter</i> , <i>Burkholderia</i> , <i>Geobacteraceae</i> , <i>Hydrogenophaga</i> , <i>Methanosarcina</i>	Beckmann et al., 2011; Krüger et al., 2008
Light oil, <i>n</i> -hexadecane, toluene	Arctic sediments from the Baltic Sea	4° C	–	Algora et al., 2013, 2015
<i>n</i> -hexadecane	Lusi Mud Vulcano, Sidoarjo village, Northern Java, Indonesia; mud samples from the framing dike of the crater	30° C, very active	–	Mazzini et al., 2007, 2012
<i>n</i> -hexadecane	Paclele Mici volcano, Romania; mud volcano field with naturally occurring oil seepage	30° C	–	Alain et al., 2006; Feisthauer et al., 2010
Light oil, heavy oil, <i>n</i> -hexadecane, BTEX, 2-methylnaphthalene	Dagang oil field, PR China; well-head samples from wells from a water-flooded medium- to high-temperature (30–80° C) reservoir	30° C	<i>Pseudomonas</i> , <i>Smithella</i> , <i>Syntrophorhabdus</i> , <i>Syntrophobacter</i> , <i>Desulfobulbus</i> , <i>Methanosaeta</i> , <i>Methanoculleus</i> , <i>Methanofollis</i> , <i>Thermoplasmata</i>	Cai et al., 2015; Jiménez et al., 2012, 2015
Oil, <i>n</i> -hexadecane, <i>n</i> -hexadecanoic acid, <i>n</i> -octadecanoic acid	South Platte alluvial aquifer in Weld County near Denver, Colo., USA; gas condensate-contaminated groundwater sediments	21° C	<i>Clostridium</i> , <i>Desulfobulbus</i> , <i>Desulfatibacillum</i> , <i>Desulfotomaculum</i> , <i>Desulfovibrio</i> , <i>Syntrophus</i> , <i>Methanoculleus</i> , <i>Methanospirillum</i> , <i>Methanosaeta</i> , <i>Methanosarcina</i>	Gieg et al., 2008; Morris et al., 2012; Townsend et al., 2003
2-Methylnaphthalene, 2,6-dimethylnaphthalene	South Platte alluvial aquifer in Weld County; gas condensate-contaminated groundwater sediments	21–23° C	<i>Clostridium</i> , <i>Desulfobulbus</i> , <i>Desulfovibrio</i> , <i>Methanoculleus</i> , <i>Methanosaeta</i> , <i>Methanolinea</i>	Berdugo-Clavijo et al., 2012; Gieg et al., 2008; Townsend et al., 2003
Toluene	South Platte alluvial aquifer in Weld County; gas condensate-contaminated groundwater sediments	21° C	<i>Desulfosporosinus</i> , <i>Syntrophaceae</i> , <i>Desulfovibrionales</i> , <i>Chloroflexi</i> , <i>Spirochaetes</i> , <i>Methanoculleus</i> , <i>Methanolinea</i> , <i>Methanosaeta</i>	Fowler et al., 2012, 2014; Gieg et al., 1999
Oil	Medicine Hat, Alta., Canada; Glauconitic C low-temperature (30° C) oil field subjected to nitrate injection for souring control	33° C	<i>Smithella</i> , <i>Pseudomonas</i> , <i>Methanosaeta</i> , <i>Methanoculleus</i> , <i>Methanobacterium</i>	Berdugo-Clavijo and Gieg, 2014

Table 2 (continued)

Substrate	Origin of the inoculum, description of the habitat	Incubation temperature (if available), remarks	Identified taxa (if available)	References ^a
<i>n</i> -alkanes (<i>n</i> -C ₁₅ – <i>n</i> -C ₂₀)	Shengli oilfield, PR China; well head samples from a production well at 70° C 2,058 m in depth	37 and 55° C	<i>Firmicutes</i> , <i>Thermodesulfobiaceae</i> , <i>Thermotogaceae</i> , <i>Nitrospiraceae</i> , <i>Dictyoglomaceae</i> , <i>Archaeoglobales</i>	Mbadinga et al., 2012; Wang et al., 2011; Zhou et al., 2012
Benzene, toluene	Ferulic-acid-degrading sewage sludge	–	–	Grbić-Galić and Vogel, 1987; Grbić-Galić and Young, 1985
Benzene	Tsuchiura, Japan; lotus field soil	25° C	<i>Clostridium</i> , <i>Methanoculleus</i> , <i>Methanoregula</i> , <i>Methanosaeta</i> , <i>Thermoplasmata</i>	Sakai et al., 2009
Benzene	Forested sandy alluvium area in Glen Falls, N.Y., USA; coal tar waste-contaminated sediments	–	<i>Pseudomonas</i> , <i>Pelomonas</i> , <i>Delftia</i>	Liou et al., 2008
Oil	Newcastle, UK; Tyne River sediments	–	<i>Smithella</i> , <i>Marinobacter</i> , <i>Thauera</i> , <i>Methanocalculus</i> , <i>Methanogenium</i> , <i>Methanomicrobiaceae</i>	Gray et al., 2011; Jones et al., 2008
<i>n</i> -alkanes (<i>n</i> C ₁₄ – <i>n</i> C ₁₈), BTEX, naphtha	Mildred Lake Settling Basin, Alta., Canada; oil sands tailings ponds	20° C	<i>Syntrophus</i> , <i>Desulfuromonas</i> , <i>Desulfobacterales</i> , <i>Methanosaeta</i> , <i>Methanoculleus</i>	Siddique et al., 2006, 2011
<i>n</i> -alkanes (<i>n</i> C ₆ – <i>n</i> C ₁₀), 2-methyl-pentane, 2-methylcyclopentane	Mildred Lake Settling Basin; Oil sands tailings ponds	28° C	<i>Peptococcaceae</i> , <i>Anaerolineaceae</i> , <i>Desulfobacteraceae</i> , <i>Smithella</i> , <i>Syntrophus</i> , <i>Methanosaeta</i> , <i>Methanoculleus</i>	Abu Laban et al., 2014; Siddique et al., 2006; Tan et al., 2013, 2015
Benzene, naphthalene, phenanthrene	Baltimore Harbor, Md., USA; Harbor sediments	30° C	<i>Aquificae</i> , <i>Bacteroidetes</i> , <i>Thermotogae</i> , <i>Clostridia</i> , <i>Pseudomonas</i> , <i>Methanosarcina</i> , <i>Methanoculleus</i> , <i>Methanococcus</i>	Chang et al., 2005a, b
Phenanthrene, anthracene	Landfield leachate contaminated sediments	20° C	<i>Methylibium</i> , <i>Legionella</i> , <i>Rhizobiales</i>	Zhang et al., 2012a, b
<i>n</i> -alkanes (C ₂₈ –C ₅₀)	San Diego Bay, Calif., USA; bay sediment	Long-chained <i>n</i> -alkanes	<i>Smithella</i> , <i>Methanoculleus</i> , <i>Methanosaeta</i>	Marks et al., 2015

^a Referring to the sampling site, the enrichment culture or both.

Table 3. Methane production rates in methanogenic enrichment cultures originating from freshwater ditch sediments supplemented with different hydrocarbon substrates

Substrate added	CH ₄ production rate, nmol CH ₄ /g TOC/day
Light oil	360–420
Heavy oil	170–250
Lignite	110–150
Hard coal	50–60
Shale (Posidonia)	140–190
PAH (2-methylnaphthalene)	20–50
BTEX (ethylbenzene, toluene)	30–70
Paraffin (<i>n</i> -C ₃₂)	40–70
<i>n</i> -alkanes (C ₁₂ –C ₁₈)	350–560

naphthalene was the only substrate added (similar results were later obtained by Berdugo-Clavijo et al. [2012]). The authors concluded that methanogens may not directly participate in the degradation of naphthalene, as previous studies suggested toxicity of this compound for methanogenic microbiota [Sharak Genthner et al., 1997].

However, alkylated naphthalenes can be biodegraded under methanogenic conditions. The conversion of 2-methylnaphthalene to methane by enrichment cultures from Dagang was confirmed in stable isotope tracer experiments by production of ¹³C-labeled CO₂ and methane from ¹³C-labeled 2-methylnaphthalene [Jiménez et al., 2012]. Berdugo-Clavijo et al. [2012] reported significant methane production (up to 400 μmol) in crude-oil-degrading enrichment cultures amended with 2-methylnaphthalene or 2,6-dimethylnaphthalene and identified putative metabolites (2-naphthoic acid and 6-methyl-

2-naphthoic acid) indicating the biodegradation of these substrates.

Methanogenic biodegradation of higher polycyclic aromatic hydrocarbons (PAHs) seems to be energetically feasible as well [Dolfing et al., 2009], and it is likely occurring in situ in highly biodegraded oil reservoirs. However, to date, very few studies have reported biodegradation of tricyclic PAH (phenanthrene or anthracene) under methanogenic conditions [Chang et al., 2005a; Zhang et al., 2012a, b], and the degradation of tetra- or pentacyclic PAHs remains to be demonstrated. Similarly, methanogenic biodegradation of heterocyclic hydrocarbons (like carbazoles) can probably take place in situ, as patterns consistent with biodegradation have been observed [Huang et al., 2003], but there is a general lack of knowledge of the associated mechanisms.

The use of coal as methanogenic substrate has also been demonstrated in laboratory experiments [Harris et al., 2008; Krüger et al., 2008; Orem et al., 2010], although coal becomes more recalcitrant to degradation with increasing thermal maturity [Strapoć et al., 2011]. Coal contains low-molecular-weight components like hydrocarbons and naphthenic acids, which might partly act as carbon substrates for methanogenesis. Holowenko et al. [2001] observed an increase in methane production with the addition of simple surrogate naphthenic acids (3-cyclohexylpropanoic acid or 4-cyclohexylbutanoic acid) to microcosms that contained a Base Mine Lake fine tailing sample, which the authors attributed to the biodegradation of the lateral chain. However, higher naphthenic acids inhibited the degradation process under these conditions.

In another study, crushed core material from a reservoir was applied to provide a solid surface for the microbial community and nutrients, and enhance their access to sedimentary organic matter and crude oil [Gieg et al., 2008, 2010]. Solid particles (sand, lava or amberlite) were also required to sustain the degrading capacity of a sulfidogenic benzene-degrading consortium [Herrmann et al., 2008], as physiologically active microorganisms tended to grow attached to the surfaces. Solid surfaces may improve substrate availability and biofilm formation, enhancing the biodegradation of hydrocarbons. Nevertheless, microbial consortia can also be successfully shifted to oil-free and solid matrix-free culture media for experiments with labeled and nonlabeled substrates such as hydrocarbons and carboxylic acids [Morris et al., 2012].

Methanogenesis does not require the presence of external electron acceptors, but low concentrations of sulfate and Fe(III) could support methane formation. For

example ferrihydrite triggers growth of *Methanosarcina*-related methanogens [Siegert et al., 2011]. However, methanogenesis may be negatively affected by sulfate and nitrate at concentrations of more than 5 and 1 mM, respectively [Siegert et al., 2011].

Microbial Ecology and Molecular Biology of Methanogenic Hydrocarbon Degradation

Hydrocarbon degradation by methanogenic microbial communities has been extensively proven in microcosm studies (see previous section). However, cultivation approaches may result in a biased representation of active or highly abundant microorganisms [Amann et al., 1995] as the biogeochemical conditions in laboratory microcosms usually differ from in situ conditions. Generally, community members in laboratory enrichment cultures and in natural habitats are identified by molecular techniques. The extensive use of metagenomics, metatranscriptomics and metaproteomics aims at circumventing isolation and cultivation biases and provides information about abundant noncultivated taxa of which the functions are often completely unknown [Rappé and Giovannoni, 2003]. These tools can also be used to identify key genes related to the function of methanogenic hydrocarbon-degrading communities, from the peripheral hydrocarbon degradation catabolism, such as *assA* or *bssA*, to the methane-generating pathway, e.g. *mcrA* [Callaghan et al., 2010; Kuntze et al., 2008; Steinberg and Regan, 2009; Tan et al., 2015; von Netzer et al., 2013]. Further information about functional genes for aromatic hydrocarbon degradation, such as those involved in fumarate addition, is summarized by von Netzer et al. [this volume, pp 180–194].

The analysis of concentrations of the methyl-coenzyme M reductase F430 prosthetic group and its isotopic signatures allows detecting and quantifying methanogenic microorganisms in environmental samples, and simultaneously provides an idea about their substrates [Kaneko et al., 2014; Takano et al., 2013].

Molecular studies have allowed the detection of a variety of microorganisms from oil reservoirs and other hydrocarbon-impacted environments, including aerobes, microaerophilic taxa, fermenters, sulfate reducers and methanogens [An et al., 2013b; Magot et al., 2000], many of which have never been cultivated. The composition of these hydrocarbon-degrading microbial communities seems to be determined by the availability of electron acceptors [Head et al., 2014]. Many of the phylotypes iden-

tified in methanogenic hydrocarbon-degrading microbial communities from a variety of habitats are similar. Firmicutes and Proteobacteria (particularly Gamma-, Delta- and Epsilonproteobacteria), mostly, but also Bacteroidetes and Spirochaetes are frequently found in coalbeds, oil reservoirs and other hydrocarbon-bearing systems [Gray et al., 2010; Strapoć et al., 2011].

According to An et al. [2013a], peripheral degradation pathways (hydroxylation, carboxylation and fumarate addition) would be performed by Proteobacteria, Firmicutes and Actinobacteria. Among the Deltaproteobacteria, *Smithella*, *Syntrophus* and other related genera belonging to the Syntrophaceae are often observed in methanogenic microbial communities from hydrocarbon-bearing systems [Johnson et al., 2015; Ramos-Padrón et al., 2011; Shimizu et al., 2007; Siddique et al., 2011] and are frequently enriched in methanogenic cultures (table 2). These organisms seem to be involved in the methanogenic degradation of *n*-alkanes [Zengler et al. 1999; Gray et al. 2011]. Gründger et al. [2015] reported an enrichment of Syntrophaceae (affiliated to the genera *Smithella* and *Desulfomonile*) in methanogenic *n*-hexadecane-degrading cultures from coal-bearing sediments. Moreover, Gray et al. [2011] observed a predominance of *Smithella* in oil-degrading methanogenic enrichment cultures and its exponential growth in parallel to alkane degradation and methane accumulation, whereas *Mari-nobacter*, a known hydrocarbon degrader, did not participate in the biodegradation. In addition, alkylsuccinate genes (*assA*) closely related to those of *Smithella* spp. were found to be expressed in a methanogenic *n*-octacosane-degrading (*n*-C₂₈) enrichment culture [Marks et al., 2015].

Several sulfate-reducing hydrocarbon-degrading Deltaproteobacteria, also belonging to the Syntrophobacterales, e.g. *Desulfoglaeba alkanexedens* [Davidova et al., 2006], or Desulfobacterales, e.g. *Desulfatibacillum* spp. [Callaghan et al., 2012; Cravo-Laureau et al., 2005], *Desulfobacula toluolica* [Wöhlbrand et al., 2013], *Desulfotignum toluenicum* [Ommedal and Torsvik, 2007] or *Desulfatiferula olefinivorans* [Cravo-Laureau et al., 2007], have been isolated and described. Some studies have shown the ability of sulfate reducers to grow syntrophically by fermentation when sulfate concentrations are too low. A coculture of the *n*-hexadecane-degrading *Desulfatibacillum alkenivorans* with a hydrogenotrophic methanogen yielded methane [Callaghan et al., 2012]. Also *Desulfatiferula* has been identified in methanogenic enrichment cultures [Gründger et al., 2015]. Sulfate reducers in relative high abundance were detected by quantitative PCR of the *dsrA*

genes in the Dagang oil field, which is a low-sulfate environment [Jiménez et al., 2012].

Gammaproteobacteria have also been frequently detected in hydrocarbon-rich environments [Gray et al., 2010]. Particularly, *Pseudomonas* has been found to be abundant in coal-bearing sediments [Gründger et al., 2015; Penner et al., 2010] and in production waters from different oil reservoirs [Cai et al., 2015; Nazina et al., 2006; Ren et al., 2011]. This genus has conventionally been considered aerobic or facultative anaerobic using nitrate as electron acceptor. However, Berdugo-Clavijo and Gieg [2014] observed an enrichment of *Pseudomonas* in a methanogenic oil-degrading enrichment culture, suggesting that this genus may have to grow syntrophically. In addition, molecular analyses (i.e. 454-pyrosequencing and cloning) confirmed that syntrophic (*Smithella* and related taxa) together with other hydrocarbon-degrading (e.g. *Pseudomonas* and *Thauera*) bacteria were among the most represented bacterial phylotypes in methanogenic oil- and 2-methylnaphthalene-degrading enrichment cultures from the Dagang oil field [Jiménez et al., 2015].

Members of Epsilonproteobacteria might be involved in the fermentation of hydrocarbons as well. Acetotrophic and acetogenic *Arcobacter* species, together with fermenters belonging to *Sulfurospirillum*, have often been detected in oil reservoirs or coal deposits, such as coal-bearing sediments from the Ruhr area [Gründger et al., 2015], the Waikato Basin in New Zealand [Fry et al., 2009] or the Pelican Lake oil reservoir in Alberta, Canada [Grabowski et al., 2005a, b], among many others [Gray et al., 2010; Head et al., 2014; Hubert et al., 2012].

Firmicutes belonging to Clostridiales have been detected in several oil reservoirs [Mochimaru et al., 2007], tailing ponds [Ramos-Padrón et al., 2011] and methanogenic enrichment cultures (table 2), and could take part in the fermentation of hydrocarbons. Among them, *Pelotomaculum* species are considered strict syntrophs, as they can only oxidize propionate (an intermediate of hydrocarbon fermentation) when cocultured with a H₂ scavenger. In addition, several *Pelotomaculum*-related phylotypes have been identified as syntrophic benzene degraders [for an overview, see Vogt et al., 2011], so this substrate might also be a niche for Clostridiales.

Archaeal communities are often less abundant than bacteria [Jiménez et al., 2012; Orphan et al., 2000] and usually less diverse, with just a few predominant operational taxonomic units [Schlegel et al., 2011]. Orphan et al. [2000] reported only a minor presence of archaeal clones in 16S rRNA gene clone libraries from production water from a high-temperature oil field in California.

High temperature favors CO₂ reduction versus acetoclastic methanogenesis [Dolfing et al., 2008]. Whereas acetoclastic methanogens have been found in low-temperature reservoirs [Pham et al., 2009], a variety of thermophilic hydrogenotrophic methanogens, like *Methermicoccus shengliensis* [Cheng et al., 2007] or *Methanobacterium thermoaggregans* [Nazina et al., 1995], have been isolated from hydrocarbon-related high-temperature systems. In a recent study, changes in the incubation temperature of Arctic sediments resulted in distinct methanogenic community structures [Blake et al., 2015]. Moreover, the addition of H₂/CO₂, acetate or methanol did not affect methanogenic rates or the microbial structure at 5°C, but favored hydrogenotrophic methanogenesis at 30°C [Blake et al., 2015].

However, as already stated, there are other influencing factors, like salinity, pH or substrate concentrations, so actually, hydrogenotrophic and acetotrophic methanogens may coexist. For example, Zengler et al. [1999] detected *Methanosaeta* species together with *Methanospirillum* and *Methanoculleus* in *n*-hexadecane-degrading enrichment cultures. Similar results were obtained by Berdugo-Clavijo et al. [2012]. An et al. [2013a] identified genes for both hydrogenotrophic and acetotrophic methanogenesis in oil sands tailing ponds. In fact, co-occurrence of both H₂/formate (e.g. *Methanobacterium*, *Methanococcus*, *Methanospirillum*, *Methanococcus*, *Methanoculleus* and *Methanoregula*) and acetate-utilizing (*Methanosarcina* and *Methanosaeta*) methanogens has been reported in several other hydrocarbon-bearing systems such as the Gippsland Basin [Midgley et al., 2010], the Illinois Basin [Strapoć et al., 2008], the Ishikari coal field [Shimizu et al., 2007] and coalbeds from Alberta [Penner et al., 2010].

Stable isotope probing (SIP) techniques can provide a link between biodegradation processes and specific microbial taxa and can help determine the main methanogenic pathway. They are based on the incorporation of isotopically labelled substrates, such as ¹³C-hydrocarbons, to cellular biomarkers or biomolecules (lipids, nucleic acids and proteins) and can be used to identify active microorganisms without any prior knowledge of their identity [Radajewski et al., 2000]. The use of SIP for analyzing anaerobic hydrocarbon degradation is summarized by Vogt et al. [this volume, pp 195–210]. Based on DNA-SIP analysis, Beckmann et al. [2011] found predominance of acetoclastic methanogenesis in liquid cultures of hard coal and timber growing with either ¹³C-acetate or H₂/¹³CO₂. In H₂/¹³CO₂-amended cultures, the substrates were mainly used by acetogens related to *Pelobacter acetylenicus* and *Clostridium* species. Active me-

thanogens utilized acetate instead of the thermodynamically more favorable hydrogen, which could reflect the adaptation of the microbial community to the low H₂ concentrations in coal mines.

In a recent study, Morris et al. [2012] investigated the carbon flow in a model methanogenic community capable of hydrocarbon degradation by using a combination of stable isotope fractionation, protein-based SIP and metaproteomics. Labeling experiments with ¹³C substrates showed that the proteins of the acetoclastic and hydrogenotrophic methanogens were equally labeled, suggesting acetoclastic and hydrogenotrophic methanogenesis contributed similarly to substrate consumption and thus methanogenesis in this model consortium, indicating complex interactions within the methanogenic and bacterial community.

Future Research Directions

There is a current considerable interest in methanogenic hydrocarbon biodegradation and its application in energy recovery and bioremediation. The combined activity of fermentative and methanogenic microorganisms during conversion of crude oil into methane and CO₂ in the absence of sulfate is widespread in subsurface petroleum reservoirs. This combination of fermentation and methanogenic processes has been proposed as a means to enhance energy recovery from stranded energy assets (i.e. reservoirs where over 70% of the resource can be left in place due to extraction limitations) by stimulating microbial activity. Assuming that light crude oils consist of ~10–15% of *n*-alkanes or BTEX, the microbial degradation of this fraction would convert 50% of these into methane, so approximately additional 5–10% of the total oil mass could be recovered. Thus, the induced conversion of oil or coal into methane thereby increases the production lifetime of these reservoirs.

Furthermore, methanogenic hydrocarbon degradation processes might in the future also be of interest for the bioremediation of contaminated aquifers and other deeper geological systems [Feisthauer et al., 2010], since the respective microorganisms seem to be ubiquitously distributed. Also, no expensive nutrients would be required and no harmful by-products, like H₂S produced under sulfate-reducing conditions, are to be expected. From a biochemical perspective, these unique syntrophic degradation processes hold great promise to detect and further develop novel enzymatic reactions for biotechnological applications.

During the last years, the availability of new sampling techniques, together with the development of more sensitive single-cell approaches and new-generation sequencing methods, has enabled a deeper knowledge of the extent, function and importance in deep, often methanogenic subsurface environments [Edwards et al., 2012]. In addition, new methodological progress in the cultivation of microorganisms at high pressures and temperatures, resembling in situ conditions of the deep biosphere [Frerichs et al., 2014; Imachi et al., 2011] together with the development of single-cell techniques has allowed the growth of ‘unculturable’, sometimes also methanogenic, microorganisms in the laboratory. In addition, advances in molecular biology have made it possible to identify biodegradation pathways and the microorganisms involved. SIP techniques or the analysis of metabolites and degradation products by high-resolution mass spectrometry (such as HPLC-MS/MS: High Performance Liquid Chromatography coupled to tandem Mass Spectrometry, FT-ICR-MS: Fourier Transform Ion Cyclotron Resonance Mass Spectrometer/Spectrometry, HPLC-ESI-QTOF: High Performance Liquid Chromatography coupled to Electrospray Ionization Quadrupole Time of Flight Mass Spectrometry) can provide a direct link between the metabolic processes taking place and the microorganisms mainly involved [Jehlich

et al., 2008, 2010; Kaneko et al., 2014; Lenhart et al., 2014; Lueders et al., 2004; Schmidt et al., 2014].

The elucidation of methanogenic hydrocarbon-degrading microbial communities and their degradation pathways has thus made great progress during the past decade, but it is still in its infancy. Biochemistry and physiology of anoxic hydrocarbon degradation in reservoirs and contaminated aquifers are not thoroughly understood, and many questions remain open, e.g. the biodegradation of higher-molecular-weight PAHs or gaseous hydrocarbons, the role of microbial community members of which many have unknown function, the elemental cycling and energy fluxes within the microbial communities, the interactions between environmental factors (pressure, availability of nutrients and trace metals) and regulation of methanogenic communities.

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References

- Abu Laban N, Dao A, Foght J: DNA stable-isotope probing of oil sands tailings pond enrichment cultures reveals different key players for toluene degradation under methanogenic and sulfidogenic conditions. *FEMS Microbiol Ecol* 2015;91:fiv039.
- Abu Laban N, Dao A, Semple K, Foght J: Biodegradation of C7 and C8 iso-alkanes under methanogenic conditions. *Environ Microbiol* 2014, DOI: 10.1111/1462-2920.12643.
- Aitken CM, Jones DM, Maguire MJ, Gray ND, Sherry A, Bowler BFJ, Ditchfield AK, Larter SR, Head IM: Evidence that crude oil alkane activation proceeds by different mechanisms under sulphate-reducing and methanogenic conditions. *Geochim Cosmochim Acta* 2013; 109:162–174.
- Alain K, Holler T, Musat F, Elvert M, Treude T, Krüger M: Microbiological investigation of methane- and hydrocarbon-discharging mud volcanoes in the Carpathian mountains, Romania. *Environ Microbiol* 2006;8:574–590.
- Algora C, Gründger F, Adrian L, Damm V, Richnow H-H, Krüger M: Geochemistry and microbial populations in sediments of the northern Baffin Bay, Arctic. *Geomicrobiol J* 2013; 30:690–705.
- Algora C, Vasileiadis S, Wasmund K, Trevisan M, Krüger M, Puglisi E, Adrian L: Manganese and iron as structuring parameters of microbial communities in Arctic marine sediments from the Baffin Bay. *FEMS Microbiol Ecol* 2015;91:fiv056.
- Amann RI, Ludwig W, Schleifer KH: Phylogenetic identification and in situ detection of individual microbial cells without cultivation. *Microbiol Rev* 1995;59:143–169.
- An D, Brown D, Chatterjee I, Dong X, Ramos-Adrón E, Wilson S, Bordenave S, Caffrey SM, Gieg LM, Sensen CW, Voordouw G: Microbial community and potential functional gene diversity involved in anaerobic hydrocarbon degradation and methanogenesis in an oil sands tailings pond. *Genome* 2013a;56:612–618.
- An D, Caffrey SM, Soh J, Agrawal A, Brown D, Budwill K, Dong X, Dunfield PF, Foght J, Gieg LM, Hallam SJ, Hanson NW, He Z, Jack TR, Klassen J, Konwar KM, Kuatsjah E, Li C, Larter S, Leopatra V, Nesbø CL, Oldenburg T, Pagé AP, Ramos-Adrón E, Rochman FF, Saidi-Mehrabad A, Sensen CW, Sipahimalani P, Song YC, Wilson S, Wolbring G, Wong M-L, Voordouw G: Metagenomics of hydrocarbon resource environments indicates aerobic taxa and genes to be unexpectedly common. *Environ Sci Technol* 2013b;47:10708–10717.
- Anderson RT, Lovley DR: Biogeochemistry: hexadecane decay by methanogenesis. *Nature* 2000;404:722–723.
- Atlas RM: Microbial degradation of petroleum hydrocarbons – an environmental perspective. *Microbiol Rev* 1981;45:180–209.
- Beckmann S, Lueders T, Krüger M, von Netzer F, Engelen B, Cypionka H: Acetogens and acetoclastic Methanosarcinales govern methane formation in abandoned coal mines. *Appl Environ Microbiol* 2011;77:3749–3756.
- Berdugo-Clavijo C, Dong X, Soh J, Sensen CW, Gieg LM: Methanogenic biodegradation of two-ringed polycyclic aromatic hydrocarbons. *FEMS Microbiol Ecol* 2012;81:124–133.
- Berdugo-Clavijo C, Gieg LM: Conversion of crude oil to methane by a microbial consortium enriched from oil reservoir production waters. *Front Microbiol* 2014;5:197.
- Blake LI, Tveit A, Øvreås L, Head IM, Gray ND: Response of methanogens in Arctic sediments to temperature and methanogenic substrate availability. *PLoS One* 2015;10:e0129733.

- Bokova EN: Formation of methane during microbial degradation of oil. *Polevaya Promyslovaya Geochim* 1953;2:25–27.
- Cai M, Jiménez N, Krüger M, Guo H, Jun Y, Straaten N, Richnow HH: Potential for aerobic and methanogenic oil biodegradation in a water flooded oil field (Dagang oil field). *Fuel* 2015;141:143–153.
- Callaghan AV, Davidova IA, Savage-Ashlock K, Parisi VA, Gieg LM, Suflita JM, Kukor JJ, Wawrik B: Diversity of benzyl- and alkylsuccinate synthase genes in hydrocarbon-impacted environments and enrichment cultures. *Environ Sci Technol* 2010;44:7287–7294.
- Callaghan AV, Morris BEL, Pereira IAC, McInerney MJ, Austin RN, Groves JT, Kukor JJ, Suflita JM, Young LY, Zylstra GJ, Wawrik B: The genome sequence of *Desulfatibacillum alkenivorans* ak-01: a blueprint for anaerobic alkane oxidation. *Environ Microbiol* 2012;14:101–113.
- Chang W, Um Y, Hoffman B, Holoman TRP: Molecular characterization of polycyclic aromatic hydrocarbon (PAH)-degrading methanogenic communities. *Biotechnol Prog* 2005a;21:682–688.
- Chang W, Um Y, Holoman TRP: Molecular characterization of anaerobic microbial communities from benzene-degrading sediments under methanogenic conditions. *Biotechnol Prog* 2005b;21:1789–1794.
- Cheng L, Qiu T-L, Yin X-B, Wu X-L, Hu G-Q, Deng Y, Zhang H: *Methermicoccus shengliensis* gen. nov., sp. nov., a thermophilic, methylotrophic methanogen isolated from oil-production water, and proposal of *Methermicocaceae* fam. nov. *Int J Syst Evol Microbiol* 2007;57:2964–2969.
- Cravo-Laureau C, Grossi V, Raphel D, Matheron R, Hirschler-Réa: Anaerobic *n*-alkane metabolism by a sulphate-reducing bacterium, *Desulfatibacillum aliphaticivorans* strain cv2803t. *Appl Environ Microbiol* 2005;71:3458–3467.
- Cravo-Laureau C, Latat C, Joulain C, Matheron R, Hirschler-Réa: *Desulfatiferula olefinivorans* gen. nov., sp. nov., a long-chain *n*-alkene-degrading, sulphate-reducing bacterium. *Int J Syst Evol Microbiol* 2007;57:2699–2702.
- Davidova IA, Duncan KE, Choi OK, Suflita JM: *Desulfoglaeba alkanexedens* gen. nov., sp. nov., an *n*-alkane-degrading, sulphate-reducing bacterium. *Int J Syst Evol Microbiol* 2006;56:2737–2742.
- Dolfing J: Thermodynamic constraints on syntrophic acetate oxidation. *Appl Environ Microbiol* 2014;80:1539–1541.
- Dolfing J, Larter SR, Head IM: Thermodynamic constraints on methanogenic crude oil biodegradation. *ISME J* 2008;2:442–452.
- Dolfing J, Xu A, Gray ND, Larter SR, Head IM: The thermodynamic landscape of methanogenic PAH degradation. *Microb Biotechnol* 2009;2:566–574.
- Dworkin M, Falkow S, Rosenberg E, Schleifer K-H, Stackebrandt E, Widdel F, Boetius A, Rabus R: Anaerobic biodegradation of hydrocarbons including methane; in Dworkin M, Falkow S, Rosenberg E, Schleifer K-H, Stackebrandt E (eds): *The Prokaryotes*. New York, Springer, 2006, pp 1028–1049.
- Edwards EA, Grbić-Galić D: Anaerobic degradation of toluene and *o*-xylene by a methanogenic consortium. *Appl Environ Microbiol* 1994;60:313–322.
- Edwards KJ, Becker K, Colwell F: The deep, dark energy biosphere: intraterrestrial life on earth. *Annu Rev Earth Planet Sci* 2012;40:551–568.
- Ekzerceva VA: Formation of methane by microorganisms in oil fields. *Geokhimiya* 1960;1:362–370.
- Eller G, Känel L, Krüger M: Co-occurrence of aerobic and anaerobic methane oxidation in the water column of lake Plussee. *Appl Environ Microbiol* 2005;71:8925–8928.
- Etiopie G, Feyzullayev A, Milkov AV, Waseda A, Mizobe K, Sun CH: Evidence of subsurface anaerobic biodegradation of hydrocarbons and potential secondary methanogenesis in terrestrial mud volcanoes. *Mar Petrol Geol* 2009;26:1692–1703.
- Feisthauer S, Seidel M, Bombach P, Traube S, Knöllner K, Wange M, Fachmann S, Richnow HH: Characterization of the relationship between microbial degradation processes at a hydrocarbon contaminated site using isotopic methods. *J Contam Hydrol* 2012;133:17–29.
- Feisthauer S, Siegert M, Seidel M, Richnow HH, Zengler K, Gründger F, Krüger M: Isotopic fingerprinting of methane and CO₂ formation from aliphatic and aromatic hydrocarbons. *Org Geochem* 2010;41:482–490.
- Formolo M, Martini A, Petsch S: Biodegradation of sedimentary organic matter associated with coalbed methane in the Powder River and San Juan Basins, USA. *Int J Coal Geol* 2008;76:86–97.
- Fowler SJ, Dong X, Sensen CW, Suflita JM, Gieg LM: Methanogenic toluene metabolism: community structure and intermediates. *Environ Microbiol* 2012;14:754–764.
- Fowler SJ, Gutierrez-Zamora ML, Manefield M, Gieg LM: Identification of toluene degraders in a methanogenic enrichment culture. *FEMS Microbiol Ecol* 2014;89:625–636.
- Frerichs J, Rakoczy J, Ostertag-Henning C, Krüger M: Viability and adaptation potential of indigenous microorganisms from natural gas field fluids in high pressure incubations with supercritical CO₂. *Environ Sci Technol* 2014;48:1306–1314.
- Fry JC, Horsfield B, Sykes R, Cragg BA, Heywood C, Kim GT, Mangelsdorf K, Mildenhall DC, Rinna J, Vieth A, Zink K-G, Sass H, Weightman AJ, Parkes RJ: Prokaryotic populations and activities in an interbedded coal deposit, including a previously deeply buried section (1.6–2.3 km) above 150 Ma basement rock. *Geomicrobiol J* 2009;26:163–178.
- Fuchs G, Boll M, Heider J: Microbial degradation of aromatic compounds – from one strategy to four. *Nat Rev Microbiol* 2011;9:803–816.
- Gieg LM, Davidova IA, Duncan KE, Suflita JM: Methanogenesis, sulphate reduction and crude oil biodegradation in hot Alaskan oil fields. *Environ Microbiol* 2010;12:3074–3086.
- Gieg LM, Duncan KE, Suflita JM: Bioenergy production via microbial conversion of residual oil to natural gas. *Appl Environ Microbiol* 2008;74:3022–3029.
- Gieg LM, Fowler SJ, Berdugo-Clavijo C: Syntrophic biodegradation of hydrocarbon contaminants. *Curr Opin Biotechnol* 2014;27:21–29.
- Gieg LM, Kolhatkar RV, McInerney MJ, Tanner RS, Harris SH, Sublette KL, Suflita JM: Intrinsic bioremediation of petroleum hydrocarbons in a gas condensate-contaminated aquifer. *Environ Sci Technol* 1999;33:2550–2560.
- Grabowski As, Blanchet D, Jeanthon C: Characterization of long-chain fatty-acid-degrading syntrophic associations from a biodegraded oil reservoir. *Res Microbiol* 2005a;156:814–821.
- Grabowski As, Nercessian O, Fayolle F, Blanchet D, Jeanthon C: Microbial diversity in production waters of a low-temperature biodegraded oil reservoir. *FEMS Microbiol Ecol* 2005b;54:427–443.
- Gray ND, Sherry A, Grant RJ, Rowan AK, Hubert CRJ, Callbeck CM, Aitken CM, Jones DM, Adams JJ, Larter SR, Head IM: The quantitative significance of syntrophaceae and syntrophic partnerships in methanogenic degradation of crude oil alkanes. *Environ Microbiol* 2011;13:2957–2975.
- Gray ND, Sherry A, Hubert C, Dolfing J, Head IM: Methanogenic degradation of petroleum hydrocarbons in subsurface environments: remediation, heavy oil formation, and energy recovery. *Adv Appl Microbiol* 2010;72:137–161.
- Grbić-Galić D, Vogel TM: Transformation of toluene and benzene by mixed methanogenic cultures. *Appl Environ Microbiol* 1987;53:254–260.
- Grbić-Galić D, Young LY: Methane fermentation of ferulate and benzoate: anaerobic degradation pathways. *Appl Environ Microbiol* 1985;50:292–297.
- Green MS, Flanagan KC, Gilcrease PC: Characterization of a methanogenic consortium enriched from a coalbed methane well in the Powder River Basin, USA. *Int J Coal Geol* 2008;76:34–45.
- Gründger F, Jiménez N, Thielemann T, Straaten N, Lüders T, Richnow HH, Krüger M: Microbial methane formation in deep aquifers of a coal-bearing sedimentary basin, Germany. *Front Microbiol* 2015;6:200.
- Harris SH, Smith RL, Barker CE: Microbial and chemical factors influencing methane production in laboratory incubations of low-rank subsurface coals. *Int J Coal Geol* 2008;76:46–51.

- Hattori S: Syntrophic acetate-oxidizing microbes in methanogenic environments. *Microbes Environ* 2008;23:118–127.
- Head IM, Gray ND, Larter SR: Life in the slow lane; biogeochemistry of biodegraded petroleum containing reservoirs and implications for energy recovery and carbon management. *Front Microbiol* 2014;5:566.
- Head IM, Jones DM, Larter SR: Biological activity in the deep subsurface and the origin of heavy oil. *Nature* 2003;426:344–352.
- Heider J: Adding handles to unhandy substrates: anaerobic hydrocarbon activation mechanisms. *Curr Opin Chem Biol* 2007;11:188–194.
- Herrmann S, Kleinstüber S, Neu TR, Richnow HH, Vogt C: Enrichment of anaerobic benzene-degrading microorganisms by in situ microcosms. *FEMS Microbiol Ecol* 2008;63:94–106.
- Holowenko FM, Mackinnon MD, Fedorak PM: Naphthenic acids and surrogate naphthenic acids in methanogenic microcosms. *Water Res* 2001;35:2595–2606.
- Horstad I, Larter SR: Petroleum migration, alteration, and remigration within Troll field, Norwegian North Sea. *AAPG Bull* 1997;81:222–248.
- Horstad I, Larter SR, Mills N: A quantitative model of biological petroleum degradation within the Brent Group reservoir in the Gullfaks field, Norwegian North Sea. *Org Geochem* 1992;19:107–117.
- Huang H, Bowler BFJ, Zhang Z, Oldenburg TBP, Larter SR: Influence of biodegradation on carbazole and benzocarbazole distributions in oil columns from the Liaohe Basin, NE China. *Org Geochem* 2003;34:951–969.
- Hubert CRJ, Oldenburg TBP, Fustic M, Gray ND, Larter SR, Penn K, Rowan AK, Seshadri R, Sherry A, Swainsbury R, Voordouw G, Voordouw JK, Head IM: Massive dominance of Epsilonproteobacteria in formation waters from a Canadian oil sands reservoir containing severely biodegraded oil. *Environ Microbiol* 2012;14:387–404.
- Imachi H, Aoi K, Tasumi E, Saito Y, Yamanaka Y, Saito Y, Yamaguchi T, Tomaru H, Takeuchi R, Morono Y, Inagaki F, Takai K: Cultivation of methanogenic community from subsurface sediments using a continuous-flow bioreactor. *ISME J* 2011;5:1913–1925.
- Jehlich N, Fetzter I, Seifert J, Mattow J, Vogt C, Harms H, Thiede B, Richnow H-H, von Bergen M, Schmidt F: Decimal place slope, a fast and precise method for quantifying ¹³C incorporation levels for detecting the metabolic activity of microbial species. *Mol Cell Proteomics* 2010;9:1221–1227.
- Jehlich N, Schmidt F, von Bergen M, Richnow H-H, Vogt C: Protein-based stable isotope probing (protein-sip) reveals active species within anoxic mixed cultures. *ISME J* 2008;2:1122–1133.
- Jiménez N, Cai M, Straaten N, Yao J, Richnow HH, Krüger M: Microbial diversity in methanogenic hydrocarbon-degrading enrichment cultures isolated from a water-flooded oil reservoir (Dagang oil field, China). *International Symposium on Applied Microbiology and Molecular Biology in Oil Systems*. Stavanger, 2015.
- Jiménez N, Morris BEL, Cai M, Gründger F, Yao J, Richnow HH, Krüger M: Evidence for in situ methanogenic oil degradation in the Dagang oil field. *Org Geochem* 2012;52:44–54.
- Johnson JM, Wawrik B, Isom C, Boling WB, Callaghan AV: Interrogation of Chesapeake Bay sediment microbial communities for intrinsic alkane-utilizing potential under anaerobic conditions. *FEMS Microbiol Ecol* 2015;91:1–14.
- Jones DM, Head IM, Gray ND, Adams JJ, Rowan AK, Aitken CM, Bennett B, Huang H, Brown A, Bowler BFJ, Oldenburg T, Erdmann M, Larter SR: Crude-oil biodegradation via methanogenesis in subsurface petroleum reservoirs. *Nature* 2008;451:176–180.
- Kaneko M, Takano Y, Chikaraishi Y, Ogawa NO, Asakawa S, Watanabe T, Shima S, Krüger M, Matsushita M, Kimura H, Ohkouchi N: Quantitative analysis of coenzyme F430 in environmental samples: a new diagnostic tool for methanogenesis and anaerobic methane oxidation. *Anal Chem* 2014;86:3633–3638.
- Kim IS, Foght JM, Gray MR: Selective transport and accumulation of alkanes by *Rhodococcus erythropolis* S+14He. *Biotechnol Bioeng* 2002;80:650–659.
- Kotsyurbenko OR, Friedrich MW, Simankova MV, Nozhevnikova AN, Golyshin PN, Timmis KN, Conrad R: Shift from acetoclastic to H₂-dependent methanogenesis in a west Siberian peat bog at low pH values and isolation of an acidophilic Methanobacterium strain. *Appl Environ Microbiol* 2007;73:2344–2348.
- Krüger M, Beckmann S, Engelen B, Thielemann T, Cramer B, Schippers A, Cypionka H: Microbial methane formation from hard coal and timber in an abandoned coal mine. *Geomicrobiol J* 2008;25:315–321.
- Krüger M, van Berk W, Arning ET, Jiménez N, Schovsbo NH, Straaten N, Schulz H-M: The biogenic methane potential of European gas shale analogues: results from incubation experiments and thermodynamic modelling. *Int J Coal Geol* 2014;136:59–74.
- Kuntze K, Shinoda Y, Moutakki H, McInerney MJ, Vogt C, Richnow H-H, Boll M: 6-Oxocyclohex-1-ene-1-carbonyl-coenzyme A hydrolases from obligately anaerobic bacteria: characterization and identification of its gene as a functional marker for aromatic compounds degrading anaerobes. *Environ Microbiol* 2008;10:1547–1556.
- Kuznetsov SI: Possibilities of production of methane in oil fields of Saratov and Buguruslan (in Russian). *Mikrobiologiya* 1950;19:193–202.
- Larter S: The controls on the composition of biodegraded oils in the deep subsurface. Part II. Geological controls on subsurface biodegradation fluxes and constraints on reservoir fluid property prediction. *AAPG Bull* 2006;90:921–938.
- Lenhart TR, Duncan KE, Beech IB, Sunner JA, Smith W, Bonifay V, Biri B, Suflita JM: Identification and characterization of microbial biofilm communities associated with corroded oil pipeline surfaces. *Biofouling* 2014;30:823–835.
- Liou JSC, DeRito CM, Madsen EL: Field-based and laboratory stable isotope probing surveys of the identities of both aerobic and anaerobic benzene-metabolizing microorganisms in freshwater sediment. *Environ Microbiol* 2008;10:1964–1977.
- Lueders T, Manefield M, Friedrich MW: Enhanced sensitivity of DNA- and rRNA-based stable isotope probing by fractionation and quantitative analysis of isopycnic centrifugation gradients. *Environ Microbiol* 2004;6:73–78.
- Magot M, Ollivier B, Patel BKC: Microbiology of petroleum reservoirs. *Antonie van Leeuwenhoek* 2000;77:103–116.
- Marks C, Wawrik B, Callaghan AV, Pruit S, Duncan KE, Davidova IA, Suflita JM: Metagenome and transcript analyses of a methanogenic consortium utilizing *n*-octacosane. *International Symposium on Applied Microbiology and Molecular Biology in Oil Systems*, Stavanger, 2015.
- Martini AM, Budai JM, Walter LM, Schoell M: Microbial generation of economic accumulations of methane within a shallow organic-rich shale. *Nature* 1996;383:155–158.
- Martini AM, Walter LM, Budai JM, Ku TCW, Kaiser CJ, Schoell M: Genetic and temporal relations between formation waters and biogenic methane: Upper Devonian Antrim Shale, Michigan Basin, USA. *Geochim Cosmochim Acta* 1998;62:1699–1720.
- Martini AM, Walter LM, Ku TCW, Budai JM, McIntosh JC, Schoell M: Microbial production and modification of gases in sedimentary basins: a geochemical case study from a Devonian shale gas play, Michigan Basin. *AAPG Bull* 2003;87:1355–1375.
- Mayumi D, Mochimaru H, Yoshioka H, Sakata S, Maeda H, Miyagawa Y, Ikarashi M, Takeuchi M, Kamagata Y: Evidence for syntrophic acetate oxidation coupled to hydrogenotrophic methanogenesis in the high-temperature petroleum reservoir of Yabase oil field (Japan). *Environ Microbiol* 2011;13:1995–2006.
- Mazzini A, Etiope G, Svensen H: A new hydrothermal scenario for the 2006 Lusi eruption, Indonesia. *Insights from gas geochemistry*. *Earth Planet Sci Lett* 2012;317–318:305–318.
- Mazzini A, Svensen H, Akhmanov GG, Aloisi G, Planke S, Malthe-Sørensen A, Istadi B: Triggering and dynamic evolution of the Lusi mud volcano, Indonesia. *Earth Planet Sci Lett* 2007;261:375–388.
- Mbadinga S, Li K-P, Zhou L, Wang L-Y, Yang S-Z, Liu J-F, Gu J-D, Mu B-Z: Analysis of alkane-dependent methanogenic community derived from production water of a high-temperature petroleum reservoir. *App Microbiol Biotechnol* 2012;96:531–542.

- McInerney MJ, Sieber JR, Gunsalus RP: Syntrophy in anaerobic global carbon cycles. *Curr Opin Biotechnol* 2009;20:623–632.
- McInerney MJ, Struchtemeyer CG, Sieber J, Mouttaki H, Stams AJM, Schink B, Rohlin L, Gunsalus RP: Physiology, ecology, phylogeny, and genomics of microorganisms capable of syntrophic metabolism. *Ann NY Acad Sci* 2008;1125:58–72.
- McIntosh J, Martini A, Petsch S, Huang R, Nüsslein K: Biogeochemistry of the forest city basin coalbed methane play. *Curr Opin Biotechnol* 2008;76:111–118.
- McIntosh JC, Walter LM, Martini AM: Extensive microbial modification of formation water geochemistry: case study from a midcontinent sedimentary basin, United States. *Geol Soc Am Bull* 2004;116:743–759.
- Meckenstock RU, von Netzer F, Stumpp C, Lueders T, Himmelberg AM, Hertkorn N, Schmitt-Kopplin P, Harir M, Hosein R, Haque S, Schulze-Makuch D: Water droplets in oil are microhabitats for microbial life. *Science* 2014;345:673–676.
- Midgley DJ, Hendry P, Pinetown KL, Fuentes D, Gong S, Mitchell DL, Faiz M: Characterisation of a microbial community associated with a deep, coal seam methane reservoir in the Gippsland basin, Australia. *Int J Coal Geol* 2010;82:232–239.
- Milkov AV: Methanogenic biodegradation of petroleum in the West Siberian basin (Russia): significance for formation of giant Cenomanian gas pools. *AAPG Bull* 2010;94:1485–1541.
- Milkov AV: Worldwide distribution and significance of secondary microbial methane formed during petroleum biodegradation in conventional reservoirs. *Org Geochem* 2011;42:184–207.
- Mochimaru H, Yoshioka H, Tamaki H, Nakamura K, Kaneko N, Sakata S, Imachi H, Sekiguchi Y, Uchiyama H, Kamagata Y: Microbial diversity and methanogenic potential in a high temperature natural gas field in Japan. *Extremophiles* 2007;11:453–461.
- Morris BEL, Herbst F-A, Bastida F, Seifert J, von Bergen M, Richnow H-H, Suflita JM: Microbial interactions during residual oil and n-fatty acid metabolism by a methanogenic consortium. *Environ Microbiol Reports* 2012;4:297–306.
- Muller FM: On methane fermentation of higher alkanes. *Antonie van Leeuwenhoek* 1957;23:369–384.
- Nazina T, Shestakova N, Grigor'yan A, Mikhailova E, Tourova T, Poltarau A, Feng C, Ni F, Belyaev S: Phylogenetic diversity and activity of anaerobic microorganisms of high-temperature horizons of the Dagang oil field (P. R. China). *Microbiology* 2006;75:55–65.
- Nazina TN, Ivanova AE, Borzenkov IA, Belyaev SS, Ivanov MV: Occurrence and geochemical activity of microorganisms in high-temperature, water-flooded oil fields of Kazakhstan and Western Siberia. *Geomicrobiol J* 1995;13:181–192.
- Ommedal H, Torsvik T: *Desulfotignum toluenicum* sp. nov., a novel toluene-degrading, sulphate-reducing bacterium isolated from an oil-reservoir model column. *Int J Syst Evol Microbiol* 2007;57:2865–2869.
- Orem WH, Voytek MA, Jones EJ, Lerch HE, Bates AL, Corum MD, Warwick PD, Clark AC: Organic intermediates in the anaerobic biodegradation of coal to methane under laboratory conditions. *Org Geochem* 2010;41:997–1000.
- Orphan VJ, Taylor LT, Hafenbradl D, Delong EF: Culture-dependent and culture-independent characterization of microbial assemblages associated with high-temperature petroleum reservoirs. *Appl Environ Microbiol* 2000;66:700–711.
- Pallasser RJ: Recognising biodegradation in gas/oil accumulations through the $\delta^{13}\text{C}$ compositions of gas components. *Org Geochem* 2000;31:1363–1373.
- Penner TJ, Foght JM, Budwill K: Microbial diversity of Western Canadian subsurface coal beds and methanogenic coal enrichment cultures. *Int J Coal Geol* 2010;82:81–93.
- Pham VD, Hnatow LL, Zhang S, Fallon RD, Jackson SC, Tomb J-F, DeLong EF, Keeler SJ: Characterizing microbial diversity in production water from an Alaskan mesothermic petroleum reservoir with two independent molecular methods. *Environ Microbiol* 2009;11:167–187.
- Radajewski S, Ineson P, Parekh NR, Murrell JC: Stable-isotope probing as a tool in microbial ecology. *Nature* 2000;403:646–649.
- Ramos-Padrón E, Bordenave S, Lin S, Bhaskar IM, Dong X, Sensen CW, Fournier J, Voordouw G, Gieg LM: Carbon and sulfur cycling by microbial communities in a gypsum-treated oil sands tailings pond. *Environ Sci Technol* 2011;45:439–446.
- Rappé MS, Giovannoni SJ: The uncultured microbial majority. *Annu Rev Microbiol* 2003;57:369–394.
- Ren H-Y, Zhang X-J, Song Z-Y, Rupert W, Gao G-J, Guo S-X, Zhao L-P: Comparison of microbial community compositions of injection and production well samples in a long-term water-flooded petroleum reservoir. *PLoS One* 2011;6:e23258.
- Rice DD, Claypool GE: Generation, accumulation, and resource potential of biogenic gas. *AAPG Bull* 1981;65:5–25.
- Ross AS, Farrimond P, Erdmann M, Larter SR: Geochemical compositional gradients in a mixed oil reservoir indicative of ongoing biodegradation. *Org Geochem* 2010;41:307–320.
- Rotaru A-E, Shrestha PM, Liu F, Markovaite B, Chen S, Nevin KP, Lovley DR: Direct interspecies electron transfer between *Geobacter metallireducens* and *Methanosarcina barkeri*. *Appl Environ Microbiol* 2014a;80:4599–4605.
- Rotaru A-E, Shrestha PM, Liu F, Shrestha M, Shrestha D, Embree M, Zengler K, Wardman C, Nevin KP, Lovley DR: A new model for electron flow during anaerobic digestion: direct interspecies electron transfer to Methanosarcina for the reduction of carbon dioxide to methane. *Energ Environ Sci* 2014b;7:408–415.
- Sakai N, Kurisu F, Yagi O, Nakajima F, Yamamoto K: Identification of putative benzene-degrading bacteria in methanogenic enrichment cultures. *J Biosci Bioeng* 2009;108:501–507.
- Schlegel ME, McIntosh JC, Bates BL, Kirk MF, Martini AM: Comparison of fluid geochemistry and microbiology of multiple organic-rich reservoirs in the Illinois Basin, USA: Evidence for controls on methanogenesis and microbial transport. *Geochim Cosmochim Acta* 2011;75:1903–1919.
- Schmidt F, Koch BP, Witt M, Hinrichs K-U: Extending the analytical window for water-soluble organic matter in sediments by aqueous Soxhlet extraction. *Geochim Cosmochim Acta* 2014;141:83–96.
- Schoell M: The hydrogen and carbon isotopic composition of methane from natural gases of various origins. *Geochim Cosmochim Acta* 1980;44:649–661.
- Scott AR, Kaiser WR, Ayers WB: Thermogenic and secondary biogenic gases, San Juan Basin, Colorado and New Mexico – implications for coalbed gas producibility. *AAPG Bull* 1994;78:1186–1209.
- Sharak Genthner BR, Townsend GT, Lantz SE, Mueller JG: Persistence of polycyclic aromatic hydrocarbon components of creosote under anaerobic enrichment conditions. *Arch Environ Contam Toxicol* 1997;32:99–105.
- Shimizu S, Akiyama M, Naganuma T, Fujioka M, Nako M, Ishijima Y: Molecular characterization of microbial communities in deep coal seam groundwater of Northern Japan. *Geobiology* 2007;5:423–433.
- Shurr GW, Ridgley JL: Unconventional shallow biogenic gas systems. *AAPG Bull* 2002;86:1939–1969.
- Siddique T, Fedorak PM, Foght JM: Biodegradation of short-chain n-alkanes in oil sands tailings under methanogenic conditions. *Environ Sci Technol* 2006;40:5459–5464.
- Siddique T, Penner T, Semple K, Foght JM: Anaerobic biodegradation of longer-chain n-alkanes coupled to methane production in oil sands tailings. *Environ Sci Technol* 2011;45:5892–5899.
- Sieber JR, McInerney MJ, Gunsalus RP: Genomic insights into syntrophy: the paradigm for anaerobic metabolic cooperation. *Annu Rev Microbiol* 2012;66:429–452.
- Siegert M, Cichocka D, Herrmann S, Gründger F, Feisthauer S, Richnow H-H, Springael D, Krüger M: Accelerated methanogenesis from aliphatic and aromatic hydrocarbons under iron- and sulphate-reducing conditions. *FEMS Microbiol Lett* 2011;315:6–16.
- Spormann A, Widdel F: Metabolism of alkylbenzenes, alkanes, and other hydrocarbons in anaerobic bacteria. *Biodegradation* 2000;11:85–105.

- Steinberg LM, Regan JM: *mcrA*-targeted real-time quantitative PCR method to examine methanogen communities. *Appl Environ Microbiol* 2009;75:4435–4442.
- Strapoć D, Mastalerz M, Dawson K, Macalady J, Callaghan AV, Wawrik B, Turich C, Ashby M: Biogeochemistry of microbial coal-bed methane. *Annu Rev Earth Planet Sci* 2011;39:617–656.
- Strapoć D, Mastalerz M, Schimmelmann A, Drobnik A, Hedges S: Variability of geochemical properties in a microbially dominated coalbed gas system from the eastern margin of the Illinois Basin, USA. *Int J Coal Geol* 2008;76:98–110.
- Sun W, Sun X, Cupples AM: Identification of *Desulfosporosinus* as toluene-assimilating microorganisms from a methanogenic consortium. *Int Biodeter Biodegrad* 2014;88:13–19.
- Takai K, Nakamura K, Toki T, Tsunogai U, Miyazaki M, Miyazaki J, Hirayama H, Nakagawa S, Nunoura T, Horikoshi K: Cell proliferation at 122°C and isotopically heavy CH₄ production by a hyperthermophilic methanogen under high-pressure cultivation. *Proc Natl Acad Sci USA* 2008;105:10949–10954.
- Takano Y, Kaneko M, Kahnt J, Imachi H, Shima S, Ohkouchi N: Detection of coenzyme F430 in deep sea sediments: a key molecule for biological methanogenesis. *Org Geochem* 2013;58:137–140.
- Tan B, Dong X, Sensen CW, Foght J: Metagenomic analysis of an anaerobic alkane-degrading microbial culture: potential hydrocarbon-activating pathways and inferred roles of community members. *Genome* 2013;56:599–611.
- Tan B, Nesbo C, Foght J: Re-analysis of omics data indicates *Smithella* may degrade alkanes by addition to fumarate under methanogenic conditions. *ISME J* 2014;8:2353–2356.
- Tan B, Semple K, Foght J: Anaerobic alkane biodegradation by cultures enriched from oil sands tailings ponds involves multiple species capable of fumarate addition. *FEMS Microbiol Ecol* 2015;91:fiv042.
- Thielemann T, Cramer B, Schippers A: Coalbed methane in the Ruhr Basin, Germany: a renewable energy resource? *Org Geochem* 2004;35:1537–1549.
- Townsend GT, Prince RC, Suflita JM: Anaerobic oxidation of crude oil hydrocarbons by the resident microorganisms of a contaminated anoxic aquifer. *Environ Sci Technol* 2003;37:5213–5218.
- Vogt C, Kleinstaub S, Richnow HH: Anaerobic benzene degradation by bacteria. *Microb Biotechnol* 2011;4:710–724.
- von Netzer F, Pilloni G, Kleindienst S, Krüger M, Knittel K, Gründger F, Lueders T: Enhanced gene detection assays for fumarate-adding enzymes allow uncovering of anaerobic hydrocarbon degraders in terrestrial and marine systems. *Appl Environ Microbiol* 2013;79:543–552.
- Waldron PJ, Petsch ST, Martini AM, Nüsslein K: Salinity constraints on subsurface archaeal diversity and methanogenesis in sedimentary rock rich in organic matter. *Appl Environ Microbiol* 2007;73:4171–4179.
- Wang L-Y, Gao C-X, Mbadinga SM, Zhou L, Liu J-F, Gu J-D, Mu B-Z: Characterization of an alkane-degrading methanogenic enrichment culture from production water of an oil reservoir after 274 days of incubation. *Int Biodeter Biodegrad* 2011;65:444–450.
- Warwick PD, Breland FC Jr, Hackley PC: Biogenic origin of coalbed gas in the northern Gulf of Mexico coastal plain, USA. *Int J Coal Geol* 2008;76:119–137.
- Weiner JM, Lovley DR: Rapid benzene degradation in methanogenic sediments from a petroleum-contaminated aquifer. *Appl Environ Microbiol* 1998;64:1937–1939.
- Whiticar MJ: Carbon and hydrogen isotope systematics of bacterial formation and oxidation of methane. *Chem Geol* 1999;161:291–314.
- Whiticar MJ, Faber E, Schoell M: Biogenic methane formation in marine and freshwater environments: CO₂ reduction vs acetate fermentation-isotope evidence. *Geochim Cosmochim Acta* 1986;50:693–709.
- Widdel F, Knittel K, Galushko A: Anaerobic hydrocarbon-degrading microorganisms: an overview; in Timmis K (ed): *Handbook of Hydrocarbon and Lipid Microbiology*. Berlin, Springer, 2010, pp 1997–2021.
- Wilhelms A, Larter SR, Head I, Farrimond P, di Primio R, Zwach C: Biodegradation of oil in uplifted basins prevented by deep-burial sterilization. *Nature* 2001;411:1034–1037.
- Wöhlbrand L, Jacob JH, Kube M, Musmann M, Jarling R, Beck A, Amann R, Wilkes H, Reinhardt R, Rabus R: Complete genome, catabolic sub-proteomes and key-metabolites of *Desulfobacula toluolica* tol2, a marine, aromatic compound-degrading, sulphate-reducing bacterium. *Environ Microbiol* 2013;15:1334–1355.
- Youssef N, Elshahed MS, McInerney MJ: Chapter 6 microbial processes in oil fields: culprits, problems, and opportunities; in Allen I, Laskin SS, Geoffrey MG (eds): *Advances in Applied Microbiology*. Amsterdam, Academic Press, 2009, vol 66, pp 141–251.
- Zengler K, Richnow HH, Rossello-Mora R, Michaelis W, Widdel F: Methane formation from long-chain alkanes by anaerobic microorganisms. *Nature* 1999;401:266–269.
- Zhang S, Wang Q, Xie S: Stable isotope probing identifies anthracene degraders under methanogenic conditions. *Biodegradation* 2012a;23:221–230.
- Zhang SY, Wang QF, Xie SG: Molecular characterization of phenanthrene-degrading methanogenic communities in leachate-contaminated aquifer sediment. *Int J Environ Sci Technol* 2012b;9:705–712.
- Zhou L, Li K-P, Mbadinga S, Yang S-Z, Gu J-D, Mu B-Z: Analyses of *n*-alkanes degrading community dynamics of a high-temperature methanogenic consortium enriched from production water of a petroleum reservoir by a combination of molecular techniques. *Eco-toxicology* 2012;21:1680–1691.
- Zhou Z, Ballentine CJ, Kipfer R, Schoell M, Thibodeaux S: Noble gas tracing of groundwater/coalbed methane interaction in the San Juan basin, USA. *Geochim Cosmochim Acta* 2005;69:5413–5428.