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ORIGINAL PAPER

Biogenic methane production in formation waters from a large gas field in the North Sea

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Abstract Methanogenesis was investigated in formation waters from a North Sea oil rimmed gas accumulation containing biodegraded oil, which has not been subject to seawater injection. Activity and growth of hydrogenotrophic methanogens was measured but acetoclastic methanogenesis was not detected. Hydrogenotrophic methanogens showed activity between 40 and 80°C with a temperature optimum (ca. 70°C) consistent with in situ reservoir temperatures. They were also active over a broad salinity range, up to and consistent with the high salinity of the waters (90 g l^{-1}). These findings suggest the methanogens are indigenous to the reservoir. The conversion of H_2 and CO_2 to CH_4 in methanogenic enrichments was enhanced by the addition of inorganic nutrients and was correlated with cell growth. Addition of yeast extract also stimulated methanogenesis. Archaeal 16S rRNA gene sequences recovered from enrichment cultures were closely related to *Methanothermobacter* spp. which have been identified in other high-temperature petroleum reservoirs. It has recently been suggested that methanogenic oil degradation may be a major factor in the development of the world's heavy oils and represent a significant and ongoing process in conventional deposits. Although an oil-degrading methanogenic consortium was not enriched from these samples the presence and activity of communities of fermentative bacteria and methanogenic archaea was demonstrated. Stimulation of methanogenesis by addition of nutrients suggests that in situ methanogenic biodegradation of oil could be harnessed to enhance recovery of stranded energy assets from such petroleum systems.

Keywords (Extreme) thermophilic microorganisms and their enzymology · Thermophile ecology · Anaerobic bacteria · Archaea · Methanogens · Marine thermophiles and hyperthermophiles · Metabolism · Methanogenesis

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Introduction

Geochemical evidence suggests that a major end product of oil degradation in petroleum reservoirs is methane (Horstad and Larter 1997; Larter et al. 1999; Sweeny and Taylor 1999; Larter et al. 2005). Furthermore, methanogenic oil degradation may be a major factor in the development of the world's heavy oils and represent a significant and ongoing process in conventional deposits (Jones et al. 2008). Laboratory studies have demonstrated that methanogenic

degradation of hydrocarbons is feasible with stoichiometric conversion of pure alkanes and crude oil, to methane and CO_2. (Anderson and Lovley 2000; Siddique et al. 2006; Townsend et al. 2003; Zengler et al. 1999; Jones et al. 2008). It has also been proposed that in situ methanogenic biodegradation of oil could be harnessed to enhance recovery of stranded energy assets from petroleum reservoirs (Parkes 1999; Jones et al. 2008). This process would not only allow recovery of energy from residual oil as methane (typically $\gg$50% of oil remains in place after secondary production) but also improve the yield of oil by reducing the viscosity of the oil and re-pressurizing the reservoir.

The common occurrence of oil rimmed gas accumulations (ORGAs) has been postulated to result from extensive methanogenic biodegradation of reservoired oil (Larter and di Primio 2005). ORGAs represent reservoirs where the heavy oil and dry gas are in equilibrium (Larter and di Primio 2005). For instance, in the giant Troll field located 60 km off the west coast of Norway on the Horda platform, 74% of the accumulated petroleum is present as dry gas (93% mol% methane) and 26% is present as heavy oil (Horstad and Larter 1997). In this field it has been speculated that the heavy oil and dry gas are co-genetic and are to some extent formed from methanogenic biodegradation processes (Horstad and Larter 1997). Evidence for this comes from the presence of methane in the gas, which is isotopically light compared to thermogenic methane. Furthermore, the gas contains isotopically heavy CO_2, which is indicative of closed system reduction of CO_2 by hydrogen to methane (Larter et al. 1999; Jones et al. 2008). Since methanogenic biodegradation process may be occurring in many ORGAs today as well as other petroleum systems (Head et al. 2003) energy recovery from oilfields in the form of methane, based on accelerating natural methanogenic biodegradation, may offer a route to economic production of difficult-to-recover energy from oilfields. The purpose of this study was to determine the identity and activity of methanogens present within such a system and to experimentally determine the predominant pathway and potential rates of methane production.

Methods

Sampling and experimental setup

Samples of produced water from a North Sea oil rimmed gas accumulation were obtained from two production platforms (designated PX and PY respectively). This reservoir system has not yet been injected with seawater to enhance oil recovery; the in situ temperature of the formation waters is approximately 70°C. The samples, which comprised approximately equal volumes of water and oil, were collected in sterile Duran bottles (1 l). Sample bottles, which were completely filled and then immediately sealed with screw caps, were transported from the platforms at ambient temperature and were stored at 4°C until they were used to prepare methanogenic microcosms. Water samples were obtained from seven clusters produced from platform PX. The seven samples were mixed under nitrogen in equal quantities to produce a single PX composite sample. Water samples from six clusters produced from platform PY were also mixed to produce a single PY composite sample. Aliquots (100 ml) were taken for pH, inorganic anion and volatile fatty acid (VFA) analysis. Anions were measured using a Dionex ICS-1000 ion chromatograph (Dionex Ltd, Camberley, UK) equipped with an IonPac AS14 Anion-Exchange Column (Dionex) and using an isocratic carbonate (3.5 mM)/bicarbonate (1.0 mM) eluent. Volatile fatty acid (VFA) analysis was performed directly on aqueous samples using an ATI Unicam 610 gas chromatograph (ATI Unicam, Cambridge, UK). VFA were separated on a glass GC column (3 m $\times$ 0.46 mm ID) packed with 10% AT-1000 on Chromosorb WAW, 80/100 mesh (Alltech, Carnforth, UK) and quantified with a flame ionization detector and a Unicam 4815 integrator. The carrier gas was N_2 at a flow rate of 30 ml min^{-1} and the injector temperature was 120°C. The detector (120°C) and oven (140°C) temperatures were held constant throughout.

Microcosms and enrichment cultures were prepared in an anaerobic glove box (Coy, Milwaukie, USA). PX and PY composite samples (30 ml) were transferred into sterile 120 ml serum bottles and amended with Na_2S (1.5 mM, final concentration) to maintain reducing conditions. Microcosms were amended with different combinations of electron donors (80% H_2/20% CO_2, acetate or crude oil), inorganic nutrients and yeast extract (Table 1). Control microcosms containing 2-bromoethane sulfonate (BES, 10 mM final concentration) a specific inhibitor of methanogenesis, were prepared to determine the level of abiotic methane accumulation. All microcosms were prepared in triplicate, flushed with nitrogen to remove residual methane and closed with butyl rubber stoppers sealed with aluminium crimp tops. Microcosms were incubated at 60°C.

To determine the effect of temperature on methane production, sterile anaerobic growth medium (50 ml, Widdel and Bak 1992) was added to sterile 120-ml serum bottles and amended with Na_2S (1.5 mM, final concentration). All enrichment cultures were purged with nitrogen and closed with butyl rubber stoppers sealed with aluminium crimp tops. The headspace (70 ml) was flushed with 80% H_2/20% CO_2 by volume and the microcosms were inoculated with 1 ml from a PY formation water microcosm. All enrichment cultures were prepared in triplicate and incubated at 22, 40, 60, 70 and 80°C. An additional set of replicate cultures was prepared and

Table 1 Composition of initial methanogenic microcosms[c]

Electron donor	Inorganic nutrients[d]	Yeast extract[e]
Acetate (1 mM)[a]	+	+
Acetate (1 mM)[a]	+	−
H_2/CO_2[b,f]	+	+
H_2/CO_2[a,f]	+	−
H_2/CO_2[b,f]	−	−
Oil (100 mg)[a]	−	−
Oil (100 mg)[a]	+	−
No e^- donor[b]	+	+

[a] PX and PY formation water microcosms tested

[b] PY formation water microcosms tested

[c] All microcosm experiments were setup in triplicate and included triplicate BES inhibited controls

[d] The added inorganic nutrients comprised: trace elements ($FeSO_4$, H_3BO_4, $MnCl_2$, $CoCl_2$, $NiCl_2$, $CuCl_2$, $ZnSO_4$, Na_2MoO_4, Na_2SeO_3, Na_2WO_4), NH_4Cl (4.6 mM, final concentration), KH_2PO_4 (1.46 mM final concentration) and bicarbonate (1 mM, final concentration)

[e] 0.2% w/v yeast extract was added as a source of complex organic carbon including vitamins and amino acids

[f] Head space (90 ml) was purged with 80% H_2 20% CO_2 by volume

incubated at 60°C from which aliquots (0.5 ml) were removed periodically to conduct cell counts.

To determine the effect of salinity on methanogens, enrichment cultures were prepared in triplicate with the same growth medium modified to provide a range of different NaCl concentrations (17, 257, 599, 1454, 1882 mM). All enrichment cultures were purged with nitrogen and closed with butyl rubber stoppers sealed with aluminium crimp tops. The headspace was flushed with 80% H_2/20% CO_2 by volume and the enrichment cultures were inoculated with 1 ml from a PY methanogenic enrichment culture obtained at 60°C. All the salinity experiments were incubated at 60°C.

Headspace gas analysis

Headspace gas samples (100 µl), which, on each occasion, were replaced with an equivalent volume of helium, were taken immediately after the microcosms or enrichment cultures were prepared and throughout the incubation period. Headspace gas samples were taken with a helium-flushed gas-tight syringe (SGE, Australia) and analysed using a gas chromatograph-mass spectrometer (GC–MS; Trio 1000 MS; Fisons, UK) fitted with a packed column (Pora Plot Q stationary phase, 25 m × 0.25 mm i.d.; Chrompack, The Netherlands). The carrier gas was helium, and the injector (250°C) and oven (35°C) were maintained at constant temperature. CH_4 was detected by single ion monitoring ($m/z = 15$) and quantified on the basis of peak area, using external standards of methane in a standard gas mix (0, 1, 0.1, 0.05, 0.025 vol % methane for low concentrations and 100, 80, 60, 40, 20, 10 vol % methane for high concentrations). Rates of methanogenesis in the replicated (x3) microcosm experiments and enrichment cultures were calculated from the linear increase in methane with time at the phase of most rapid methane production and were compared statistically by one-way analysis of variance (SPSS, Chicago, USA).

Microbial cell counts and molecular analysis of archaeal community structure

Liquid samples (0.5 ml) were removed from enrichment cultures using a nitrogen-flushed, sterile, 1 ml syringe. Samples were fixed by addition of 0.5 ml molecular grade filtered absolute ethanol (0.2 micron filtered) and stored at −20°C. Total cell counts were determined by epifluorescence microscopy (Olympus BX-60 microscope, Olympus UK, equipped with an oil emersion lens (×100) magnification) after staining with SYBR Gold. Briefly 50 µl of diluted (×100) SYBR-gold nucleic acid stain (Invitrogen, Paisley, UK), was added to fixed samples (1 ml) and incubated in the dark (room temperature, 30 min). For low cell numbers fixed samples were used neat; however, for higher cell numbers, samples were diluted to an appropriate concentration using 1x phosphate-buffered saline solution (PBS), After incubation, cells were vacuum filtered onto polycarbonate membrane filters (Isopore, 13 mm, 0.2 µm pore size, Millipore, Watford, UK), washed in PBS (1x) and covered with a cover slip on a standard microscope slide. Cells were viewed under oil immersion (100x) on an epifluorescence microscope (BX40, Olympus, London, UK), under a blue light filter. Images of 20 random fields of view were captured using a digital camera (Olympus E-400, Olympus UK) and cell counts were obtained using the Cell C image analysis software (Selinummi et al. 2005, http://www.cs.tut.fi/sgn/csb/cellc/). Specific growth rates were calculated from the slope of the exponential part of the growth curve plotted on a semi-log plot.

The methanogens from three replicate enrichment cultures, used to determine microbial growth, were analysed using ribosomal RNA based techniques. Cells from the enrichment cultures were filtered onto polycarbonate membranes (0.2 µm pore size) and DNA was extracted from the filters using a FastDNA Spin Kit (BIO 101 FastDNA Spin Kit (for soil), Q-Bio Gene, UK) 16S rRNA gene fragments (ca. 1 kb) were amplified from the DNA using the PCR, with primers Arch46 (Øvreås et al. 1997) and Arch1017 (Barns et al. 1994) selective for archaea. The PCR-amplified 16S rRNA gene fragments were purified using a Qiagen PCR clean up kit (Qiagen, Crawley, UK) and cloned using a TOPO TA Cloning® Kit (Invitrogen). All procedures were carried out according to the

manufacturer's instructions. White colonies containing inserts were selected at random and a small amount of cells from selected colonies was added to TE buffer (10 mM Tris–HCl, 1 mM EDTA, pH 7.5) and boiled for 3 min. 1 µl of this crude DNA preparation was used to PCR amplify insert DNA using primers pUCr (5′-CAGGAAACAGC-TATGAC-3′) and pUCf (5′-GTTTTCCCAGTCACGAC-3′). All sequencing was conducted using the DyeDeoxy chain termination method using a 3730 xl DNA analyzer (Applied Biosystems). Initially, 20 clones were partially sequenced (ca 500 bp) using the T3 sequencing primer T3 (5′-AATTAACCCTCACTAAAGGGA-3′). The partial sequences were all found to share greater than 99% sequence identity and longer 16S rRNA gene sequences (ca. 1000 bp.) were obtained from three of the clones using the primer T7 5′-GTAATACGACTCACTATAGGGC-3′. These three 16S rRNA sequences have been deposited in the GenBank database with accession numbers (FJ773215, FJ773216). Sequences were imported into the tree-building and database management program ARB and aligned (Ludwig et al. 2004). Sequences were inserted into the reference archaeal tree in ARB using the quick parsimony insertion tool (Ludwig et al. 2004) to identify the approximate affiliation of the sequences. A smaller neighbour joining distance tree was then constructed from 13 partial (≈910 bp) sequences from the genus *Methanothermobacter* and the genus *Methanobacter* using the method of Saitou and Nei (1987) with the Jukes and Cantor correction for multiple substitutions at a single site (Jukes and Cantor 1969). Bootstrap re-sampling was conducted with 100 replicates using the TREECON package (van De Peer and De Wachter 1994).

Results

Biogenic methane production potential in the ORGA formation water enrichments

Similar patterns of methanogenic activity were observed in both the PX and PY composite formation waters. In all experiments no methane production was observed in BES-inhibited controls indicating that there was no abiotic accumulation of methane from the partitioning of residual dissolved methane into the headspace. Methane production was observed in all of the H_2/CO_2 amended microcosms. The methane production observed in PY microcosms amended with H_2 and CO_2 (Fig. 1a) indicates the presence of hydrogenotrophic ($4H_2 + CO_2 \rightarrow CH_4 + 2H_2O$) methanogens in the formation waters. Addition of inorganic nutrients significantly increased methane production rates ($p < 0.001$, ANOVA) above those observed when only H_2 and CO_2 were provided (Fig. 1a). This indicated that the yield of methane was limited by the availability of inorganic nutrients in these oil field waters. The addition of yeast extract further increased methane production rates (Fig. 1a); however, methane exceeded the theoretical yield (8.6 mM CH_4 in head space) expected from the H_2 and CO_2 supplied. These findings suggest that yeast extract acted as a source of electron donor as well as a stimulant, a conclusion supported by the production of methane in microcosms to which only yeast extract was added (Fig. 1b). This also indicated that the formation waters must harbour fermentative bacteria that generate methanogenic substrates from the oxidation of organic compounds in the yeast extract.

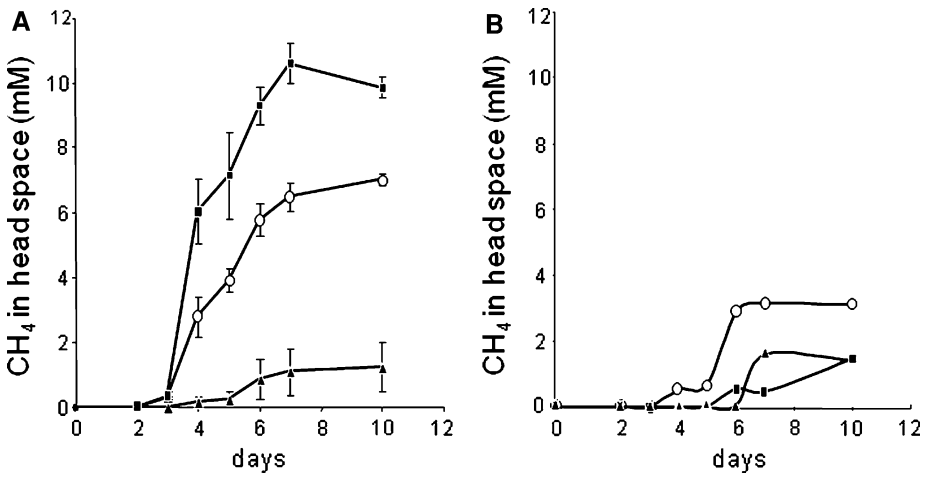

Fig. 1 **a** The influence of inorganic nutrients and yeast extract on methanogenesis from H_2/CO_2 in formation waters. *Triangles* (Formation waters + H_2/CO_2), *circles* (Formation waters + inorganic nutrients + H_2/CO_2), *Squares* (Formation waters + inorganic nutrients + yeast extract + H_2/CO_2). *Errors bars* denote 1x SE. **b** Methane generation from yeast extract in formation water. Different symbols (*triangles*, *circles* and *squares*) represent individual replicates of the same experiment (Formation waters + inorganic nutrients + yeast extract)

Microcosms treated with acetate did not generate methane unless yeast extract (which was shown to act as an electron donor (Fig. 1b)) was also added. The absence of methane production in microcosms amended with acetate indicated that acetoclastic methanogenesis ($CH_3COOH \rightarrow CH_4 + CO_2$) or syntrophic acetate oxidiation ($CH_3COOH + 2H_2O \rightarrow 4H_2 + 2CO_2$) coupled to hydrogenotrophic methanogenesis does not occur in these formation waters or alternatively these organisms had simply not survived sampling, storage and preparation of the microcosms. Acetate was detected at concentrations of 0.409 mM (PX) and 0.079 mM (PY) in the composite water samples. These sub-millimolar values and the absence of any other VFA are consistent with the findings of a previous survey of North Sea formation waters (Barth and Riis 1992). Oil treated microcosms incubated for over 24 months did not yield any detectable methane production.

The effect of temperature on hydrogenotrophic methanogenesis in the ORGA formation water enrichments

Samples from experiments used to measure methanogenesis directly in the PY formation waters (see above) were used to inoculate enrichment cultures containing anaerobic mineral salts medium. These enrichment cultures, incubated at a range of temperatures, indicated that PY hydrogenotrophic methanogens had a temperature optimum between 60 and 70°C (Fig. 2) with complete conversion of the added H_2/CO_2 in less than 100 h for the 60 and 70°C experiments and within 300 h for the 40°C experiments. Even at 80°C low rates of methane production were measured. In contrast no methanogenic activity was detected at 22°C after 450 h of incubation.

The effect of salinity on hydrogenotrophic methanogenesis in formation water enrichments

Measured chloride concentrations in the PX and PY composite samples (1520 and 1445 mM, respectively) correspond to 88 and 84 g l^{-1} of NaCl which is nearly three times that found in sea water ($\approx$35 g l^{-1} of NaCl). Despite these high salt concentrations significant methane production occurred in formation water amended with H_2 and CO_2 (Fig. 1). No significant difference ($p = 0.302$, ANOVA) was observed in the rates of methane production in the initial PY formation water microcosms and in subsequent enrichment subcultures incubated at a range of lower salinities (1–85 g l^{-1} of NaCl). No methane production was detected at 110 g l^{-1} of NaCl.

Growth characteristics of methanogenic enrichments

Cell counts in the original formation water samples were low (10^3–10^4 cells ml^{-1}); however, methanogenic enrichment cultures indicated that methanogenesis was coupled to rapid growth (Fig. 3). A comparison of cell counts and methane production indicated cell growth that occurred following a 5-h lag phase. The mean specific growth rate calculated from the exponential phase of growth was 0.14 ± 0.017 h^{-1} corresponding to a doubling time of 2.15 ± 0.24 h. Addition of fresh H_2 and CO_2 at 120 h resulted in rapid production of methane. The cell counts over this period remained relatively constant. However, this is because the amount of substrate added was only sufficient to yield an increase in cell numbers from ca. 10^4 cells/ml to ca. 10^7 cells/ml. This increase in numbers would not be detectable against the background cell count of 10^7 cells/ml at 120 h.

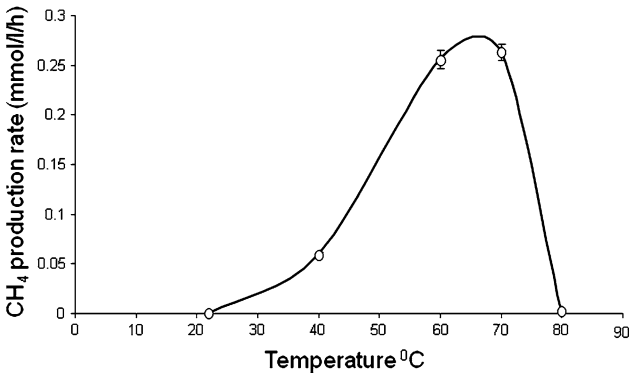

Fig. 2 The influence of temperature on rates of hydrogenotrophic methanogenesis in enrichment cultures. *Errors bars* denote 1x SE

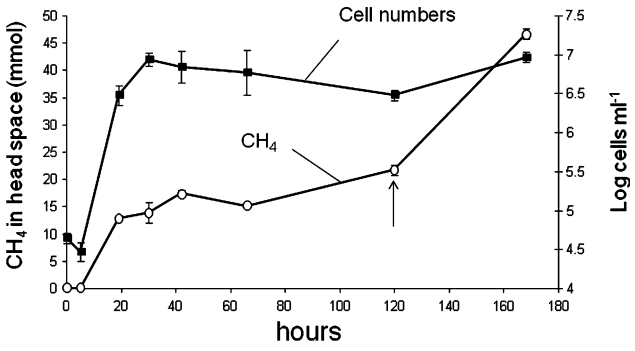

Fig. 3 The relationship between methane production and cell growth in methanogenic enrichment cultures over gassed with H_2/CO_2 and incubated at 60°C. Squares (log cells ml^{-1}), circles (methane produced (mmol)). The *arrow* indicates the second addition of H_2/CO_2. *Errors bars* denote 1x SE

Analysis of archaeal 16S rRNA gene sequences

Comparative sequence analysis demonstrated that two of the representative sequences from the enrichment subculture clone library were identical. The other sequence differed by only a single base over 957 bp. These two sequences shared >99% sequence identity with a number of cultured and uncultured *Methanothermobacter* spp. (Fig. 4).

Discussion

Phylogeny, activity and growth characteristics of ORGA derived methanogens

Past studies of high-temperature petroleum reservoirs have shown that there is a prevalence of hydrogen utilizing methanogens in such environments (Magot et al. 2000; Nazina et al. 2006). More specifically, both culture and culture-independent studies have identified *Methanothermobacter* spp. as common constituents of archaeal oil field communities (e.g. Magot et al. 2000, Orphan et al. 2000). In this study, 16S rRNA gene sequences recovered from formation water enrichments were found to be most closely related to *M. thermoautotrohpicus*, of which ΔH is the type strain (Wasserfallen et al. 2000). *M. thermoautotrohpicus* species show temperature-related growth and activity similar to that observed in this study and this group has a wide environmental distribution, present in thermophilic anaerobic digesters, hot springs and high-temperature oil and gas fields (Nazina et al. 2006; Mochimaru et al. 2007; Li et al. 2007). The sequences recovered from the North Sea ORGA in this study were most closely related (≥99.9% similarity) to a *Methanothermobacter* sp. NAK2-a2 identified in a natural gas field in Japan (Mochimaru et al. 2007).

Previous reports of methanogens and their activity in North Sea oil formation waters have differed in their

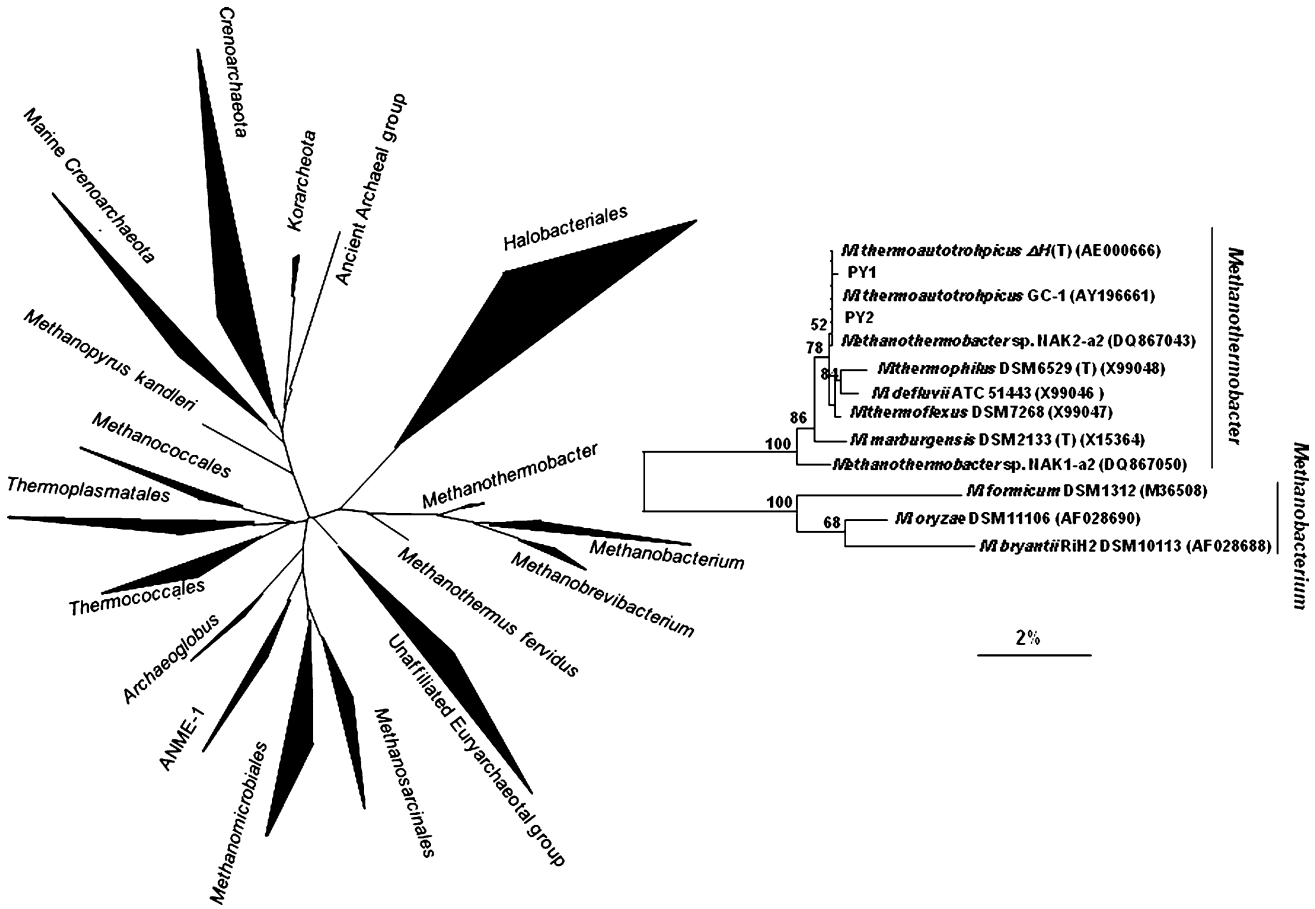

Fig. 4 Phylogenetic distance trees based on the comparative analysis of partial (1000 bp) *Archaeal* 16S rRNA sequences recovered from the enrichment cultures. The larger tree represents the deeper branching lineages within the archaeal domain constructed using the quick parsimony method of the ARB software package (Ludwig et al. 2004). The smaller neighbour joining tree was constructed from 11 partial 16S rRNA sequences from *Methanothermobacter* and *Methanobacteria* spp and sequences from this study (PY1 and PY2). Genbank accession numbers for the sequences are provided in parenthesis. The *scale bar* denotes 2% sequence divergence and the values at the nodes indicate the percentage of bootstrap trees that contained the cluster to the right of the node. Bootstrap values less than 50 are not shown

findings. For instance, while a hydrogenotrophic methanogen *Methanothermococcus thermolithotrophicus* was isolated from the Statfjord field in the Norwegian sector of the North Sea (Nilsen and Torsvik 1996), no methanogenic activity or growth could be detected in the Ninian field, approximately 100 miles east of Shetland (Mueller and Nielson 1996). The presence and absence of methanogens in these two fields are likely linked to their production history since both these North Sea oil reservoirs have undergone extensive sea water injection to restore reservoir pressure for secondary oil recovery. Seawater injection can have two major effects: 1. the introduction of sulphate and the presence of organic carbon in formation waters may drive high rates of sulphate reduction and suppress methanogenesis (an explanation invoked for the absence of methanogens in the Ninian production waters, Mueller and Nielson 1996); 2. the injection of sea water lowers reservoir temperature which may inhibit the growth of the indigenous high-temperature-adapted organisms. The original temperature of the Statfjord reservoir was 80°C prior to injection, so it was suggested that the *Methanothermoccocus* sp. isolated in enrichments (temperature range of 17–62°C and optimum growth at 60°C) was not necessarily indigenous (Nilsen and Torsvik 1996).

In contrast, the methanogens enriched in this study from the oil rimmed gas accumulation (which has not undergone injection and does not contain significant sulfate <0.1 mM) show an optimum temperature which is consistent with the in situ reservoir temperature (ca.70°C; Fig. 2). Furthermore, the halotolerence of the hydrogenotrophic methanogens is also indicative of their adaptation to in situ conditions, which encompasses salinities considerably greater than seawater. The very broad salinity range, over which the methanogens were active, is similar to other halotolerent hydrogenotrophic methanogens isolated from hydrocarbon reservoirs although the salt tolerance is somewhat lower than the maximum reported (125 g l^{-1} NaCl; Ollivier et al. 1998). It is also noteworthy that methanogenesis occurred in freshly collected waters almost immediately on the provision of hydrogen and CO_2 (Fig. 1a) suggesting that a large proportion of microbial cells present prior to enrichment were methanogens well adapted to in situ conditions.

The doubling times of the enriched methanogens calculated from growth experiments (Fig. 3) are similar to those reported for the closely related *M. thermoautotrophicus* strain ΔH grown in batch cultures (de Poorter et al. 2007); however, since cell numbers in the formation water prior to enrichment were relatively low compared to the enrichments, in situ growth and activity is likely restricted by low rates of hydrogen production in the deep subsurface environment from which the formation waters were derived. There are a number of possible abiotic sources of hydrogen in the subsurface. Amongst others it may be produced from the hydrolysis of silicate minerals (Charlou et al. 2002); catagenesis of organic matter (Hunt 1979) or the radiolysis of water by radioactive isotopes (Apps and van de Kamp 1993). However, anaerobic degradation of hydrocarbons may drive methanogenesis in many petroleum reservoirs, including those found in the North Sea, and recent evidence suggests that crude oil degradation via methanogenesis principally proceeds through hydrogenotrophic methanogenesis (Jones et al. 2008).

An additional product of anaerobic hydrocarbon degradation is acetate (Zengler et al. 1999). As such, in petroleum reservoirs undergoing methanogenic biodegradation, acetate-stimulated methanogenesis might be expected to occur. In a previous survey of North Sea reservoirs (Barth and Riis 1992), it was found that high levels of oil degradation were closely correlated with low concentrations of acetate (0.01–3.3 mM) in the Gullfaks, Troll, Heidrun Nord and Snorre fields. These low acetate concentrations (also a feature of the PX and PY formation waters) were interpreted as evidence of a biological sink for acetate in these environments. Given this likely sink for acetate in the PX and PY formation waters it was surprising that acetate did not stimulate methanogenesis in the enrichments either by acetoclastic methanogenesis,or alternatively by syntrophic acetate oxidation coupled to hydrogenotrophic methanogenesis (this is a process thought to occur in many petroleum systems (Nazina et al. 2006; Nilsen and Torsvik 1996; Davydova-charakhch'yan et al. 1992)). With regard to acetoclastic methanogenesis, crude oil (Warren et al. 2004) or high salinity (Waldron et al. 2007) are known to have inhibitory effects on this process. However, the lack of activity may be due to the sensitivity of acetotrophic organisms to sampling, storage and enrichment.

Methanogenic degradation of oil in a North Sea ORGA

We have not detected methane production in our enrichments provided with oil as an electron donor; however, to date no microbial consortium capable of degrading hydrocarbons under high-temperature conditions has been isolated from any petroleum reservoir; this is a major challenge necessitating rigorous and innovative sampling and culture techniques (Jeanthon et al. 2005). Although methanogens appear to be relatively robust, often tolerating considerable exposure to oxygen (Gray et al. 2002), the sensitivity of syntrophs which provide methanogenic substrates in methanogenic co-cultures, is well documented (e.g. Hatamoto et al. 2007) and can partly be ascribed to very slow growth rates and the susceptibility of syntrophs to inhibition by accumulation of hydrogen.

Despite the difficulties of isolating methanogenic hydrocarbon-degrading consortia from petroleum reservoirs, anaerobic degradation of hydrocarbons (specifically alkanes) coupled to methanogenesis has been demonstrated in the laboratory at low temperature and pressures (Zengler et al. 1999; Jones et al. 2008; Townsend et al. 2003; Anderson and Lovley 2000). These laboratory experiments were inoculated with near-surface sediments, and bacteria related to the genus *Syntrophus* have been inferred to be responsible for the initial oxidation of hexadecane and oil to hydrogen and acetate before conversion to methane. A wide diversity of fermentative bacteria have, however, been identified in petroleum reservoirs (e.g. Orphan et al. 2000; Nazina et al. 2006). This is of interest, because the production of methane in the microcosms supplied with yeast extract indicated the presence of a microbial consortium capable of syntrophically degrading organic carbon to methane.

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