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Microbiology of petroleum reservoirs

Michel Magot[1],*, Bernard Ollivier[2] & Bharat K.C. Patel[3]
[1]*SANOFI Recherche, Centre de Labège, BP 132, F31676 Labège, France*
[2]*Laboratoire ORSTOM de Microbiologie des Anaérobies, Université de Provence, CESB-ESIL, Case 925, 163 Avenue de Luminy, F13288 Marseille Cedex 9, France*
[3]*Faculty of Science and Technology, Griffith University, Nathan, Brisbane, Australia 4111*
*(*Author for correspondence)*

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Abstract

Although the importance of bacterial activities in oil reservoirs was recognized a long time ago, our knowledge of the nature and diversity of bacteria growing in these ecosystems is still poor, and their metabolic activities *in situ* largely ignored. This paper reviews our current knowledge about these bacteria and emphasises the importance of the petrochemical and geochemical characteristics in understanding their presence in such environments.

Microbiology of oil reservoirs: concerns and questions

Since the beginning of commercial oil production, almost 140 years ago, petroleum engineers have faced problems caused by micro-organisms. Sulphate-reducing bacteria (SRB) were rapidly recognised as responsible for the production of H_2S, within reservoirs or top facilities, which reduced oil quality, corroded steel material, and threatened workers' health due to its high toxicity (Cord-Ruwich et al. 1987). The first extensive microbiological study describing the widespread presence of SRB in oil-producing wells was published in 1926 (Bastin 1926). In his paper, Bastin addressed what remained for a long time an enigma: were these bacteria indigenous, or were they introduced into the reservoirs by waters descending from the surface, or by oil production operations? Until recently, it was considered that the deep subsurface was sterile, and that bacteria isolated from such environments could only be of exogenous origin. Our perception of this has changed recently with the unexpected discovery of large and diverse populations of microbes possessing a range of different metabolic activities in subterranean aquifers (Amy & Haldeman 1997). During the last decade, a body of convergent observations has also shown that indigenous microorganisms inhabit aquatic as well as oil-bearing deep subsurface environments. This review focuses on indigenous as well as exogenous bacteria in subterranean oil fields.

Physico-chemical characteristics of oil reservoirs

The possibility for living organisms to survive or thrive in oil field environments depends on the physical characteristics and chemical composition of the ecosystem. Temperature is the main limiting factor for microbial growth in oil reservoirs. Since temperature increases with depth at a mean rate of 3°C per 100 m (but regional geothermal gradients may be significantly different), deep oil reservoirs which attain an *in situ* temperature exceeding 130–150 °C cannot sustain bacterial growth. This temperature range is considered the highest theoretical limit for growth due to the thermal instability of biological molecules (Stetter et al. 1993a). Different types of data suggest that the presence of indigenous bacteria in oil fields could be limited to a threshold temperature between 80 and 90°C. Philippi (1977) noted that *in situ* oil biodegradation was never observed in reservoirs whose temperature exceeded 82°C. Analysis of a set of 87 water samples collected from North American oil reservoirs showed that fatty acid concentrations were maximum at a temperature of 80°C in the reservoir (Fisher 1987; Barth 1991). This indicated that maximum biodegradation occurs below 80°C, and that thermal decarboxylation occurs above this temperature. In a microbiological study, hyperthermophilic bacteria could not be isolated from 100 oil field water samples whose reservoir temperatures were higher than 82°C (Bernard et al. 1992; Magot, unpubl.). Hyperthermophilic micro-organisms growing at temperatures as high as 103°C have been isolated from some reservoirs, but the authors suggested that they represented exogenous bacteria resulting from seawater injections (Stetter et al. 1993b; Grassia et al. 1996).

Salinity and pH of formation waters can also limit bacterial activity. The salinity ranges from almost fresh to salt-saturated water, and pH generally from 5 to 8. However, the pH measured at atmospheric pressure does not necessarily reflect the actual in situ pH, as it is influenced by dissolution of gasses under high pressure. The *in situ* pH is usually in the range 3–7. This physical characteristic has to be taken into consideration when designing culture media, or interpreting the potential indigenous nature of bacteria recovered from deep subsurface samples.

The pressure within oil reservoirs (up to 500 atm) is not considered to preclude the development of bacteria in situ although it can influence their physiological or metabolic properties.

The availability of electron donors and acceptors governs the type of bacterial metabolic activities within oil field environments. The oil industry undertakes routine chemical analyses of oil fields hence a wide body of data are available; nevertheless critical data (e.g., nitrogen and phosphorus availability) necessary for understanding microbial metabolism is not routinely performed thereby thwarting our ability to understand microbial processes *in situ.* Since oil fields are deep subterranean environments and are generally isolated from the surface waters, their redox potentials are very low and some electron acceptors are most generally absent: in particular oxygen, nitrate and ferric iron. Stratal waters generally contain sulphate at various concentrations and carbonate, factors which have led to the assumption that the major metabolic processes occurring in such ecosystems are sulphate reduction, methanogenesis, acetogenesis and fermentation.

The potential electron donors include CO_2, H_2 of geochemical or bacterial origin, and numerous organic molecules. Organic acids are present, but not in all oil reservoirs, and concentrations higher than 20 mM have been recorded (Barth 1991; Barth & Riis 1992). Acetate is the most abundant, but benzoate, butyrate, formate, and propionate are also commonly detected. More complex organic acids known under the generic name of 'naphtenic acids' are also present in many crudes, at concentrations up to 100 mM. Among the thousands of organic molecules found in crude oil, several chemical families have been shown to be degradable under anaerobic conditions: the degradation of *n*-alkanes, homocyclic aromatic compounds commonly referred to as BTEX, polycyclic aromatic compounds, nitrogen and sulphur heterocyclics (Grbic-Galic 1990; Aeckersberg et al. 1991; Ruetter et al. 1994; Krumholz et al. 1996) has been reported, but samples from which these bacteria were isolated were collected from top facilities and may represent exogenous organisms. Connan et al. (1996) showed that *in vitro* anaerobic biodegradation of crude oil by an indigenous bacterial community reproduced biodegradation effects which have been recorded under natural conditions. Resins and asphaltenes are important fractions of crude oil whose composition has been partly elucidated. These fractions are composed of thousands of complex hydrocarbons which can contain heteroatoms of nitrogen, sulphur and oxygen. Although generally ignored, they are an abundant potential source of electron donors for anaerobic metabolism. Their presence could explain the observation that diverse groups of strict anaerobes can grow with crude oil as a sole carbon and energy source without any modification of the alkanes or light aromatic compounds (Magot, unpubl.).

The availability of nitrogen sources in oil fields has not been extensively studied. If nitrogen gas is present, then it could be assimilated by nitrogen-fixing micro-organisms. Nitrogen is also available in nitrogen heterocyclic compounds. The availability of phosphorus in oil fields has not been studied.

Sample recovery: influence on the interpretation of microbiological data

Problems associated with oil reservoir sample recovery have been recently reviewed by McInerney & Sublette (1997). For economical reasons, wellhead sampling is the only way of collecting samples from petroleum reservoirs and hence sources of contamination are numerous and maximum care has to be taken to interpret data from microbiological analyses. A different and potentially important source of contamination of oil reservoirs is waterflooding, i.e., reservoir repressurization by water injection into the oil-bearing stratum. Whatever the source of water injection (seawater, river water, or even formation water), exogenous bacteria can penetrate the formation under study and be recovered at producing wells. Even in non-waterflooded reservoirs, which are considered the best models to study indigenous bacteria, drilling, well equipment operations, and damaged (leaking) tubings or casings, may be other sources of contamination. The carbon steel of the well-tubing itself can create a specific ecosystem since cathodically produced hydrogen can be used as an electron donor; Magot et al. (1993) described the localised development of SRB in well tubings, resulting in the so-called 'well souring' phenomenon.

When the *in situ* temperature or salinity of the oil field under study is high, a careful examination of the physiological properties of recovered bacteria can be of great help in understanding their possible indigenous nature. On the other hand, it is often very difficult to discriminate contaminants from the indigenous flora for low salinity–low temperature oil fields. In this case, the presence of aerobic bacteria is generally an indicator of contamination from any of the above-listed sources.

Microorganisms from oil reservoirs

A wide variety of bacteria have been isolated from, or have been detected in oil field samples by molecular techniques. Aerobic, facultatively anaerobic (Adkins et al. 1992; Nazina et al. 1993; Zvyagintseva et al. 1995; Voordouw et al. 1996; Telang et al. 1997), and microaerophilic microorganisms belonging to the genera *Campylobacter, Oceanospirillum* and *Thiomicrospira* (Voordouw et al. 1996; Telang et al. 1997) have been detected in oil field samples. However, their *in situ* activities have never been reported as significant. The lack of details on sample recovery, and possible entry of surface water resulting from water injection or local geological characteristics, often make the interpretation of the presence of aerobic bacteria in such anaerobic ecosystems quite difficult. The use of the term 'indigenous' for microorganisms that were known to have been introduced into the reservoir by waterflooding is also often confusing (Matz et al. 1992).

Available data suggest that only strict anaerobes can be considered as truly indigenous to oil reservoirs. This consideration is strengthened by the physiological characteristics of some of the isolated species that, when reported, were shown to be closely adapted to the *in situ* conditions (e.g., Bernard et al. 1992). The presence of closely related bacteria in remote oil fields (Grassia et al. 1996; Magot 1996) also supports the existence of a widespread anaerobic biosphere in oil reservoirs. Nevertheless, our current knowledge of the microbial ecology of oil fields is still largely insufficient for us to confidently exclude oxygen-respiring bacteria as exogenous contaminants of oil fields.

Based on the above backdrop, we summarise below our current knowledge on the presence of strict anaerobes in oil reservoirs.

Sulphate-reducing bacteria

Because of their detrimental effects, SRB have been the most commonly studied bacterial group from oil field waters. A distinction is generally made between mesophilic SRB, which are merely involved in corrosion processes of top facilities, and thermophilic SRB, mainly responsible for *in situ* reservoir souring.

Mesophilic SRB

The most frequently isolated mesophilic SRB from oil field production waters belong to the genus *Desulfovibrio* (Table 1). *Desulfovibrio longus* and *D. vietnamensis* are considered halotolerant, whereas *D. gabonensis* is seen as a moderate halophile requiring 5–6% NaCl for optimal growth. The three species are incomplete oxidizers and use a limited range of substrates which include hydrogen, lactate, and pyruvate. In an extensive study of SRB from oil fields, 21 strains isolated from 15 production waters by Tardy-Jacquenod et al. (1996a) were identified as *Desulfovibrio* species based on 16S rRNA gene sequence analysis (Magot, unpubl.). Some of these strains were identified as representatives of recently described species that have so far been isolated from oil reservoir waters only, or were published as new species: *D. longus* (Magot et al. 1992), *D. gabonensis* (Tardy-Jacquenod et al. 1996b) or *D. vietnamensis* (Nga et al. 1996). Other strains were identified as representatives of species isolated from ecosystems not related to petroleum reservoirs and these included *D. desulfuricans, D. oxyclinae* (Krekeler et al. 1997) and *D. longreachii*

Table 1. Mesophilic and thermophilic sulphate-reducing bacteria isolated from oil fields

Species	Salinity (%)		Temperature (°C)		Complete oxidizer	References
	Range	Optimum	Range	Optimum		
Archaeoglobus fulgidus	0.02–3	2	60–85	76	+	Beeder et al. (1994)
Desulfacinum infernum	0–5	1	40–65	60	+	Rees et al. (1995)
Desulfobacter vibrioformis	1–5	ND	5–38	33	+	Lien and Bieder (1997)
Desulfobacterium cetonicum	Up to 5	1	20–37	30–35	+	Galushko & Rozanova (1991)
Desulfomicrobium apsheronum	0–8	ND	4–40	25–30	–	Rozanova et al. (1988)
'Desulfotomaculum halophilum'[a]	1–14	4–6	30–40	35	–	Tardy-Jacquenod et al. (1998)
Desulfotomaculum kuznetsovii	0–3	0	50–85	60–65	+	Nazina et al. (1988)
Desulfotomaculum nigrificans	Up to 4	1	40–70	60	–	Nazina & Rozanova (1978)
Desulfotomaculum thermocisternum	Up to 5	0,3–1,2	41–75	62	–	Nilsen et al. (1996b)
Desulfovibrio gabonensis	1–17	5–6	15–40	30	–	Tardy-Jacquenod et al. (1996b)
Desulfovibrio longus	0–8	2	10–40	35	–	Magot et al. (1992)
Desulfovibrio vietnamensis	0–10	5	12–45	37	–	Nga et al. (1996)
Thermodesulfobacterium mobile	ND	ND	45–85	65	–	Rozanova & Pivovarova (1988)
Thermodesulforhabdus norvegicus	0–5.6	1.6	44–74	60	+	Beeder et al. (1995)

[a] Taxonomic validation is pending.

(Redburn & Patel 1994). The remaining strains represented as yet undescribed new species which are phylogenetically related to known taxa. This example also illustrates how difficult it is to assess the indigenous nature of mesophilic SRB in oil production waters. The indigenous characteristic can nevertheless sometimes be clearly excluded, as in the case of *D. longus* (Magot et al. 1992), and the new *Desulfotomaculum* species *D. halophilum* (Tardy-Jacquenod et al. 1998) which are both mesophiles but were isolated from high-temperature reservoir wellhead samples.

Desulfomicrobium apsheronum (Rozanova et al. 1988), a halotolerant SRB isolated from stratal waters in the Apsheron peninsula, tolerates up to 8% NaCl for growth and oxidizes lactate to acetate incompletely. It grows autotrophically and hence can be easily distinguished from the three *Desulfovibrio* species described above. Members of the genus *Desulfobacter* have been identified by oligonucleotide probes in oil field environments (Telang et al. 1997), and *Desulfobacter vibrioformis* has been isolated recently from a water–oil separation system (Lien & Beeder 1997). It is a moderate halophilic microorganism that shows optimal growth between 1 and 5% NaCl. Acetate is the only carbon and energy source used in dissimilatory sulphate reduction. *Desulfobacterium cetonicum*, which was isolated from a flooded oil stratum, displays the unusual ability to oxidize ketones (Galushko & Rozanova 1991).

In situ studies using molecular techniques have recently been used to study SRB microflora. Voordouw et al. (1992) used reverse sample genome probing on 56 samples taken from seven oil fields and determined the presence of two distinct communities of SRB. Ten out of the 34 species detected were unique to fresh water and 18 to the saline oil field environments while only six microorganisms were cultured from both communities. Cord-Ruwisch et al. (1987) also indicated that salt had a strong selective effect on SRB growth. 16S rRNA gene studies from several different oil fields by Voordouw et al. (1996) indicated that all the Gram-negative SRB detected belonged either to the family Desulfobacteriaceae or the family Desulfovibrionaceae.

Thermophilic SRBs

It is has been well documented that thermophilic *Desulfotomaculum* species are present in oil field environments (Rozanova & Nazina 1979; Rosnes et al. 1991; Christensen et al. 1992; Nilsen et al. 1996b). *Desulfotomaculum kuznetsovii* which was initially isolated from an underground thermal mineral water ecosystem (Nazina et al. 1988) was subsequently recovered from two wellhead samples taken from non-waterflooded oil fields in the Paris Basin (Magot, unpubl.). *Desulfotomaculum thermocisternum*, which grows optimally at 62°C, was isolated below the sea floor of the Norwegian sector of the North Sea. It incompletely oxidizes a wide range of substrates including lactate, ethanol, butanol, and carboxylic acids (C3 to C10 and C14 to C17) in the presence of sulphate (Nilsen et al. 1996b). An incomplete lactate and alcohol oxidizer with a growth temperature range between 40 and 70°C has been recovered from an oil sample originating from Western Siberia and named as a new subspecies of *Desulfotomaculum nigrificans*, *D. nigrificans* subsp. *salinus* (Nazina & Rozanova 1978). Although members of the genus *Desulfotomaculum* were not detected using enrichment cultures, their presence in the Gullfaks oil field in the North Sea was shown using genus specific fluorescent antibodies produced against these microorganisms (Nilsen et al. 1996a).

Desulfacinum infernum, *Thermodesulforhabdus norvegicus* and *Thermodesulfobacterium mobile* are other thermophilic SRB isolated from North Sea oil reservoirs. In contrast to the *Desulfotomaculum* species, *D. infernum* (Rees et al. 1995) and *T. norvegicus* (Beeder et al. 1995) are complete oxidizers. *D. infernum* is a Gram-negative organism that is a member of the delta subdivision of the class *Proteobacteria*. It uses acetate, butyrate, palmitate, and alcohols and grows optimally at 60°C. *T. norvegicus* is also a member the delta subdivision of *Proteobacteria* and like *D. infernum* oxidises acetate, butyrate and palmitate. *Thermodesulfobacterium mobile* (previously named as *Desulfovibrio thermophilus* (Rozanova & Khudyakova 1974; Rozanova & Pivovarova 1988)) and *T. commune* (Zeikus et al. 1983) are the most thermophilic sulphate-reducing microorganisms. They have an upper temperature limit for growth of 82°C and are members of the third deepest phylogenetic branch of the domain *Bacteria*. *T. mobile* was isolated from a North Sea oil reservoir (Christensen et al. 1992) and *T. commune* from a continental oil reservoir located in the East Paris Basin (L'Haridon et al. 1995). Members of both species are rod-shaped and utilize H_2, formate, lactate and pyruvate as electron donors for sulphate reduction. Strains of both species have also been isolated from other non-waterflooded reservoirs in the Paris Basin (Magot, unpubl.).

In the domain *Archaea*, sulphate-reducing bacteria are only represented by hyperthermophilic members of the genus *Archaeoglobus*. *Archaeoglobus fulgidus* was isolated from hot oil field waters in the Norwegian sector of the North Sea (Beeder et al. 1994). *A. fulgidus*, '*A. lithotrophicus*' and *A. profundus* have also been found in reservoir fluid from the Thistle platform in the East Shetland Basin of the North Sea (Stetter et al. 1993b). As DNA/DNA hybridization studies have revealed genomic similarities between these species and those isolated from submarine hot vents, it has been speculated that their presence most probably resulted from seawater entering the reservoir or perhaps from other anthropogenic contaminations. However, other more recent reports on the microbiology of a continental oil reservoir and a marine oil reservoir have suggested that such hyperthermophiles are indigenous to oil fields and may be widespread deep in the crust of the earth (L'Haridon et al. 1995). In contrast to *A. fulgidus* Z, the type species, *A. fulgidus* strain 7324, which was isolated from a North Sea oil reservoir, has a lower optimum growth temperature (76°C) and cannot grow autotrophically on H_2+CO_2. It grows on lactate and pyruvate as carbon and energy sources. Growth on valerate has only been observed when hydrogen is present (Beeder et al. 1994).

It is clear from what has been said above that contaminating and/or indigenous sulphate-reducing bacteria represent an important microbial community of petroleum reservoirs which thrive over a wide range of salinities and temperatures and are perhaps ubiquitous to the oil ecosystem. Interestingly, H_2, CO_2, acetate, propionate, butyrate, and other short-chain organic acids, from C5 to C7, are often found in oil field waters with acetate concentration being the highest. Molecular hydrogen is also found and can be produced from either geothermal reactions or from thermophilic microbial fermentations. Because of the availability of organic compounds (C2 to C7 organic acids), hydrogen and CO_2 in the oil–water interface, growth of hydrogenotrophic SRB requiring acetate as carbon source could most probably occur. Beeder et al. (1994) have suggested that hydrogen is important for the growth of *Archaeoglobus fulgidus* strain 7324 on valerate in oil field water. In addition, many of the thermophilic SRB described above are known to perform dissimilatory reduction of sulphate in the presence of acetate and/or butyrate. The possibility that SRB can also use different components of crude oil *in situ* has been discussed above.

Methanogenic bacteria

Methanogenesis has only recently been reported from deep oil-bearing strata (Belyaev & Ivanov 1983; Ivanov et al. 1983; Connan et al. 1996). Isolation of methanogens has been successful from slightly to highly saline oil well waters at mesophilic temperatures (Table 2). Thermophilic, but not hyperthermophilic, strains have also been isolated from hot oil

Table 2. Mesophilic and thermophilic methanogens isolated from oil reservoirs

Species	Optimum	Temperature (°C)		Substrates used			References
	NaCl (%)	Range	Optimum	Methylamines	Acetate	Hydrogen	
Methanobacterium bryantii	0–2	25–40	37	–	–	+	Davydova-Charakhch'yan et al. (1992a)
Methanobacterium ivanovii	0.09	10–55	45	–	–	+	Belyaev et al. (1983); Borzenkov et al. (1997)
Methanobacterium thermoaggregans	2–4	40–70	60	–	–	+	Ng et al. (1989)
Methanobacterium thermoalcaliphilum	0–2	30–80	65	–	–	+	Davydova-Charakhch'yan et al. (1992a)
Methanobacterium thermoautrophicum	0-30	40–70	60	–	–	+	Ollivier & Magot, unpubl.
'*Methanocalculus halotolerans*'	5	25–45	38	–	–	+	Ollivier et al (1998)
Methanococcus thermolithotrophicus	1.4–2.4	17–62	60	–	–	+	Nilsen & Torsvik (1996)
Methanohalophilus euhalobius	6	10–50	28–37	+	–	–	Obraztsova et al. (1988)
'*Methanoplanus petrolearius*'	1–3	28–43	37	–	–	+	Ollivier et al. (1997)
Methanosarcina mazei	0.1–2	10–50	37	+	+	–	Obraztsova et al. (1987); Borzenkov et al. (1997)
Methanosarcina siciliae	2.4–3.6	20–50	40	+	–	–	Ni & Boone (1991)

reservoirs. It is generally regarded that the combination of both high temperature and high salinity in oil fields drastically reduce microbial populations, including those of methanogens. Thermophilic moderately halophilic anaerobes have not yet been reported from oil reservoirs or from any other deep subsurface environments, but it is likely that anaerobes with such traits are present since a new bacterium, *Halothermothrix oreni*, which fits this physiology precisely, has been isolated from a terrestrial hypersaline ecosystem (Cayol et al. 1994).

Borzenkov et al. (1997) demonstrated that methanogenesis decreased with an increase in salinity in a study of the Bondyuzhskoe oil field in Tatarstan. In addition, the concentration of methylamine, a methanogenic substrate, increased with the mineralization of stratal waters suggesting that methylotrophic methanogens are also present in subterrestrial saline ecosystems, a situation which is analogous to that reported for terrestrial saline and hypersaline environments (Ollivier et al. 1994). The methylotrophic *Methanococcoides euhalobius* (renamed *Methanohalophilus euhalobius*) has been isolated from an exploratory well stratal sample which had a salinity of 140 g/l (Obraztsova et al. 1988; Davidova et al. 1997). *Methanosarcina siciliae* HI350, isolated from an oil well at High Island in the Gulf of Mexico (Ni & Boone 1991; Ni et al. 1994), is a slightly halophilic methanogen which uses methanol, methylamines and dimethyl sulphide as substrates. Another moderately halophilic methanogen, which also uses methanol and trimethylamine, has been isolated from an Alsacian oil field (France) containing about 90 g/l NaCl (Ollivier et al. 1998). In addition to the coccoid methanogens cited above, the presence of a methylotrophic rod-shaped bacterium, strain GF 283, growing optimally in the presence of 15% NaCl, has been reported from the Bondyuzhskoe oil field (Borzenkov et al. 1997). However, no further taxonomical description of this strain is available.

Despite evidence of methanogenesis from acetate enrichment cultures initiated from oil field waters, under mesophilic and thermophilic conditions (Belyaev et al. 1983; Nilsen & Torsvik 1996), only one acetoclastic methanogen, *Methanosarcina mazei* strain 47, has been isolated (Obraztsova et al. 1987). It used acetate, methanol and methylamines, but not H_2+CO_2 for growth. Experiments conducted in North Sea oil reservoirs indicated that methane was produced in enrichments cultures with acetate as the substrate at 60, 80 and 92°C (Nilsen & Torsvik 1996). However, no pure acetoclastic methanogenic culture could be recovered from such enrichments. A similar result was observed by Davidova-Charakhch'yan et al. (1992a) from acetate enriched media inoculated with waters from high-temperature formations in the Mykhpai field (Western Siberia). In both cases, the authors suggested that acetate could have been degraded in-directly by a syntrophic coculture rather than directly by acetoclastic methanogens. The presence of acetate oxidizers acting syntrophically with hydrogenotrophic methanogenic or sulphate-reducing bacteria can be expected in oil field waters.

Hydrogenotrophic mesophilic or thermophilic methanogens are common inhabitants of slightly saline stratal waters. They are generally rod-shaped bacteria with the exception of *Methanococcus thermolithotrophicus*, isolated from a North Sea oil field reservoir (Nilsen & Torsvik 1996), '*Methanoplanus petrolearius*' (Ollivier et al. 1997) and '*Methanocalculus halotolerans*' (Ollivier et al. 1998), which produce irregularly disc-shaped cells. Rod-shaped methanogens found in oil reservoir fluids include *Methanobacterium thermoautrophicum* (Ivanov et al. 1983), *M. bryantii* (Davidova-Charakhch'yan et al. 1992a), *M. ivanovii* (Belyaev et al. 1983), and phenotypic variants of *Methanobacterium thermoaggregans* (Ng et al. 1989) and *M. thermoalcaliphilum* (Davidova-Charakhch'yan et al. 1992a). All these micro-organisms share a common feature in the use of hydrogen, which is most probably their primary source of energy in oil fields.

The isolation of a novel halotolerant member of *Methanobacterium thermoaggregans* from the high temperature and moderately saline San Miguelito oil field provides evidence that virgin oil reservoirs can contain an *in situ* methanogenic population (Ng et al. 1989). The hydrogenotroph, *Methanocalculus halotolerans* grows in a salinity range of 0–12% NaCl, which is the highest salinity reported to date for any hydrogenotrophic methanogen (Ollivier et al. 1998). This is interesting as the highest NaCl concentration supporting hydrogen oxidation via methanogenesis in terrestrial ecosystems has been reported to be only 9% NaCl (Oremland & King 1989; Ollivier et al. 1994). This suggests that *M. halotolerans* is most probably indigenous to the subterranean oil field environment. *Methanococcus euhalobius* showed a strict dependence on calcium for growth, a feature not found in any other methanogen. This physiological trait was attributed to the development of this methanogen in stratal brines with a high calcium content from where it originated and therefore is an indigenous characteristic of this micro-organism adapted to its environment (Borzenkov et al. 1997).

As indicated above, biologically or abiotically generated hydrogen may be an important energy source for methanogens in oil field environments. In addition, acetate, which has been found to accumulate to concentrations of up to 20 mM in North Sea oil reservoirs (Barth & Riis 1992) may be an important source of energy for these microorganisms which could be used directly or by indirect syntrophic associations. The specific presence of methylotrophic methanogens in saline and hypersaline oil field waters indicate the possible presence of methylated compounds, especially methylamines, in oil field waters. It has been suggested that these compounds originate from the breakdown of osmoregulatory amines in terrestrial hypersaline environments (Ollivier et al. 1994), and this suggestion can also be extended to the subsurface saline oil field environment.

Fermentative bacteria

Mesophilic, thermophilic, and hyperthermophilic fermentative bacteria also constitute an important microbial community of the oil field environment (Table 3).

Mesophilic fermentative bacteria

Although halophilic anaerobes have been previously isolated from oil–water injection filters (Rengpipat et al. 1988), the first characterization of an haloanaerobe from oil brines was reported by Gevertz et al. (1991). The isolate grew optimally at 2.5% but did not grow above 10% NaCl. A further five moderate halophiles isolated from the hypersaline brines of the Southeast Vassar Vertz Sand Unit (Bhupathiraju et al. 1991, 1993) produced acids, solvents, and gases from carbohydrates. These are interesting metabolites for potential microbial enhancement of oil recovery (MEOR).

Characterized haloanaerobes isolated from oil field brines mainly belong to the genus *Haloanaerobium*, order *Haloanaerobiales*, and these include *H. acetoethylicum* (formerly named *Halobacteroides acetoethylicus*) (Rengpipat et al. 1988; Patel et al. 1995; Rainey et al. 1995), '*H. congolense*' (Ravot et al. 1997) and *H. salsugo* (Bhupathiraju et al. 1994). The three species are heterotrophic moderate halophiles which grow optimally with 10% NaCl. '*Haloanaerobium congolense*' differs from *H. acetoethylicum* and *H. salsugo* in the range of substrates used and in production of acetate and not ethanol from carbohydrate fermentation.

A free-living moderately halophilic spirochete, '*Spirochaeta smaragdinae*', was isolated from an African offshore oil field in the Congo (Magot et al.

Table 3. Characterized fermentative bacteria isolated from oil reservoirs

Species	NaCl (%)		Temperature		Reduction of sulfur compounds		References
	Range	Optimum	Range	Optimum	S°	$S_2O_3^{-}$	
Acetoanaerobium romashkovii	ND	ND	30–60	37	ND	ND	Davydova-Charakhch'yan et al. (1992b)
Anaerobaculum thermoterrenum	0–2	1	28–60	55	+	+	Rees et al. (1997)
Dethiosulfovibrio peptidovorans	1–10	3	20–45	42	+	+	Magot et al. (1997b)
Geotoga petraea	0.5–10	3	30–55	50	+	ND	Davey et al. (1993)
Geotoga subterranea	0.5–10	4	30–60	45	+	ND	Davey et al. (1993)
Haloanaerobium acetoethylicum	6–20	10	15–45	34	ND	ND	Rengipat et al. (1988)
Haloanaerobium congolense	4–24	10	20–45	42	+	+	Ravot et al. (1997)
Haloanaerobium salsugo	6–24	9	22–51	40	ND	ND	Bhupathiraju et al. (1994)
Petrotoga miotherma	0.5–10	3	35–65	55	+	ND	Davey et al. (1993)
Spirochaeta smaragdinae	1–10	5	20–40	37	+	+	Magot et al. (1997a)
Thermoanaerobacter brockii	0–4.5	ND	37–75	55–60	+	+	Cayol et al. (1995)
Thermotoga elfii	0–2.4	1.2	50–72	66	–	+	Ravot et al. (1995a)
Thermotoga hypogea	0–1.5	0–0.2	56–90	70–75	–	+	Fardeau et al. (1997)
Thermotoga subterranea	0–2.4	1–2	50–75	70	–	+	Jeanthon et al. (1995)

1997a). It is the only member of this genus which has been isolated from the deep subsurface. From the same oil field, a dominant, slightly halophilic vibrioid, designated *Dethiosulfovibrio peptidovorans*, was isolated (Magot et al. 1997b). Its physiological and phylogenetic traits are unique amongst oil field fermentative isolates as it used protein extracts such as peptones and amino acids, but was unable to ferment sugars. Acetate, isobutyrate, isovalerate, and 2-methylbutyrate were the only organic acids detected from peptone fermentation. The bacterium was also found to actively corrode iron when reducing thiosulphate to sulphide.

Cloning and sequencing of PCR-amplified 16S rRNA genes of oil field microbial communities revealed the presence of a limited number of anaerobes belonging to the genera *Clostridium*, *Eubacterium* and *Synergistes* (Voordouw et al. 1996). Isolation and phenotypic characteristics of these strains have not yet been reported.

Davydova-Charakhch'yan et al. (1992b) isolated a new acetogenic anaerobe, *Acetoanaerobium romashkovii*, from formation waters of oil fields in Tataria and western Siberia. This bacterium grows on H_2+CO_2 as sole carbon and energy sources, and also uses different C1 compounds, amino-acids and sugars.

Thermophilic fermentative bacteria

Thermophilic fermentative bacteria have been isolated with a much greater frequency than the mesophilic fermenters; this is a reflection of the high temperature characteristics of most oil fields. Thermophilic anaerobes, in general, have also been the most widely studied, mainly because of the industrial potential of their thermostable enzymes. An increased interest has been kindled because of the isolation of hyperthermophilic microorganisms with growth temperatures of 103°C from hot oil field reservoirs (Stetter et al. 1993b). These authors provided evidence of the presence of *Thermotoga* strains in oil fields and, soon after, isolation of *Thermotoga elfii* (Ravot et al. 1995a), *Thermotoga subterranea* (Jeanthon et al. 1995), and *Thermotoga hypogea* (Fardeau et al. 1997) from such ecosystems was reported. Interestingly, in contrast to the previously described species of the genus *Thermotoga*, none of the new oil field isolates reduced elemental sulphur to sulphide, but instead reduced thiosulphate to sulphide. This latter reductive process was thereafter shown to be a feature shared by other members of the order *Thermotogales* (Ravot et al. 1995b). Though *T. elfii* and *T. subterranea* were found to be inhibited by elemental sulphur, sulphur reducing *Thermotoga*-like isolates have been reported to exist in oil field waters. This suggests that both sulphur- and thiosulphate-reducing members of the order *Ther-*

motogales inhabit oil field environments (Ravot et al. 1995b). On the basis on their salinity range for growth, *T. elfii, T. hypogea* and *T. subterranea* were proposed to represent a new ecological group within the genus *Thermotoga* originating from the subsurface and adapted to levels of salinity intermediate between those of marine and terrestrial species. All of these species produced acetate as the major volatile fatty acid and L-alanine from glucose metabolism. The production of L-alanine from sugar fermentation by members of the order *Thermotogales* has been interpreted as a possible remnant ancestral metabolism (Ravot et al. 1996).

Davey et al. (1993) were first to report the presence of micro-organisms belonging to the genera *Geotoga* and *Petrotoga* of the order *Thermotogales*, from Oklahoma and Texas petroleum reservoirs, and three new species, namely *Geotoga petraea, G. subterranea* and *Petrotoga miotherma* were characterized. Members of all three species are moderately thermophilic heterotrophs which grow over a broad range of salt concentrations (0.5–10%).

Studying various hot oil reservoirs of the world, Grassia et al. (1996) provided evidence of the importance of microorganisms that were morphologically and physiologically related to other members of the order *Thermotogales*, namely *Fervidobacterium, Geotoga, Petrotoga, Thermotoga*, and *Thermosipho* strains. However, physiological characterization of *Fervidobacterium* or *Thermosipho* isolates from oil field environments has not been reported to date.

Members of the family Thermoanaerobiaceae, which include the genera *Thermoanaerobacter* and *Thermoanaerobacterium*, have been recovered frequently from hot and slightly saline reservoirs (Grassia et al. 1996). Members of the genus *Thermoanaerobacter* reduce thiosulphate to sulphide whereas those of the genus *Thermoanaerobacterium* reduce thiosulphate to elemental sulphur. Formal characterization of *Thermoanaerobacterium* isolates from oil fields has not been reported. A strain from a French hot continental oil reservoir was found to be identical to *Thermoanaerobacter thermohydrosulfuricus* (L'Haridon et al. 1995). Several other strains isolated from French and African oil fields have been recognized as a new subspecies of *Thermoanaerobacter brockii, T. brockii* subsp. *lactiethylicus* (Cayol et al. 1995). Beside carbohydrates, the latter used hydrogen as electron donor in the presence of thiosulphate (Fardeau et al. 1993). In addition, thiosulphate reduction significantly improved peptide and amino acid consumption (Faudon et al. 1995).

Anaerobaculum thermoterrenum was isolated from production fluid from the Redwash oil field in Utah (Rees et al. 1997). It grew optimally at 55°C not only on sugars, but also on organic acids such as citrate, fumarate and tartrate. Both sulphur and thiosulphate were used as electron acceptors.

Recent work has provided evidence on the occurrence of hyperthermophilic fermentative *Archaea* in hot oil terrestrial and marine reservoirs (Stetter et al. 1993b; L'Haridon et al. 1995). Based on DNA–DNA hybridization studies, *Thermococcus celer, T. littoralis* and a new species of the genus *Pyrococcus*, '*P. lithotrophicus*', were identified. The latter was shown to grow at temperatures up to 102°C, whereas *Thermococcus* isolates grew at an incubation temperature of 85°C. For members of all these species, growth is generally observed on peptides or yeast extract and they all share the property of reducing elemental sulphur to sulphide (Stetter et al. 1993b).

The intriguing ability to reduce thiosulphate to sulphide is shared by a large number of fermentative bacteria from the petroleum reservoirs cited above. The involvement of thiosulphate-, non-sulphate-reducing bacteria in some specific cases of microbial corrosion of top facilities has also been demonstrated (Crolet & Magot 1996; Magot et al. 1997b). However, the presence of thiosulphate in oil reservoirs has not been adequately investigated, and further studies will need to be done in order to understand the significance, if any, of microbial thiosulphate metabolism *in situ*.

Iron-reducing bacteria

Mesophilic iron-reducing bacteria have been detected in oil field fluids and identified as *Shewanella putrefaciens* (formerly *Alteromonas putrefaciens*) (Semple & Westlake 1987; Nazina et al. 1995). This bacterium can also reduce elemental sulphur, sulphite and thiosulphate into sulphide (Moser & Nealson 1996), and uses H_2 or formate as electron donor with iron oxyhydroxide as an electron acceptor (Nealson & Saffarini 1994). The first thermophilic Mn- and Fe-reducing bacterium, *Deferribacter thermophilus*, has recently been isolated from production waters of the waterflooded Beatrice oil field in the North Sea (Greene et al. 1997). In the presence of iron, manganese, and nitrate as electron acceptors, it can use yeast extract, peptone, Casamino acids, tryptone, hydrogen and numerous organic acids including acetate, lactate and valerate as energy sources. Although these results indicate that the oil field environment could be a novel niche in the terrestrial subsurface for isolating new iron- and manganese-reducing bacteria, it is difficult to predict whether this type of metabolism can occur *in situ* as the presence of ferric iron or manganese oxides in oil fields has not been investigated and is an analogous situation to that described for thiosulphate (see above).

Conclusions

The main paradox of petroleum microbiology is probably that, although microbiological studies of oil reservoirs waters started early in this century, it is still considered to be in its infancy. A glance at the bibliography cited in this review indicates that the detailed characterisation of oil field bacteria was undertaken not much more than a decade ago. As a consequence, very little is known about the microbial diversity in these ecosystems, and almost nothing about the in situ activities of these communities.

To develop our knowledge of these very specific ecosystems, an interdisciplinary approach, between specialists involved in petroleum exploration and production (geologists, geochemists, chemists, reservoir engineers) and microbiologists is needed. It is obvious that such collaboration will greatly improve our knowledge of the deep subsurface living world and hence that of the entire biosphere. Progress in understanding microbial metabolisms occurring in the deep underground will also greatly benefit the oil industry, a few examples can be briefly mentioned.

The economical impact of reservoir souring (biological *in situ* production of H_2S) in oil production is considerable. Its mechanism is only partially understood: SRB are obviously involved, and waterflooding is thought to bring otherwise limiting nutrients into the reservoir. Microbiological studies to determine the presence or absence of indigenous SRB, and the quantification of the available and limiting nutrients prior to water injection, could help in the design of prevention measures against field souring.

In situ microbial enhanced oil recovery, i.e., the use of bacterial activities to extract more oil from oil fields, is an old idea about which a general consensus has not been reached in the oil industry. Although hundreds of field trials have been done (for a review, see Premuzic & Woodhead 1993), including a number of successful ones, it is still not clear whether indigenous bacteria may be more useful than injected ones. It is still poorly understood what type of metabolism has to be stimulated to achieve the best efficiency, or even if the positive results obtained in the past can be attributed to bacterial activity. In close collaboration with the petroleum industry, a MEOR field trial should include chemical, geochemical, geological and microbial studies. The data obtained would make a rational choice of the bacterial and metabolic targets during the experiment possible, and allow the design of laboratory experiments and the construction of numerical models. Collection of the largest set of parameters before and after the start of the MEOR process, including microbiological data, should also contribute to a better understanding of the mechanisms involved.

Despite the fact that numerous observations strengthened the hypothesis of indigenous microbial life within oil field waters, we currently do not know if these microbial communities are restricted (and therefore specific) in the subterrestrial environment to the oil field ecosystem. If this were the case, it would be a major breakthrough into the most strategic activity in the oil industry, i.e., oil exploration. To recognize the hypothetical tracer character of specific bacteria or bacterial communities, microbiological investigations have to be extended to a very large set of oil reservoirs of different characteristics (salinity and temperature), and to be compared to that of other deep subsurface environments from which oil is absent. The use of the most recent molecular techniques applied to microbial ecology (e.g., nucleic acid probes, gene amplification, cloning and sequencing of 16S rRNA), which have too rarely been used so far, would help to constitute such an extremely important set of data.

Note added to proof

Several descriptions of bacteria from oil reservoirs, which improve our knowledge of the biodiversity of these ecosystems, have been published after the preparation of this review. They are briefly summarized below.

Desulfobulbus rhabdoformis, a sulphate-reducing, propionate-oxidizing bacterium, has been isolated from a water–oil separation system on a North Sea oil platform (Lien et al. 1998a). Evidence of anaerobic oxidation of *o*-xylene and *m*-xylene by novel sulphate-reducing bacteria isolated from enrichment cultures originating from the water phase of North Sea oil tank has been reported by Harms et al. (1999). Lien et al. (1998b) isolated *Petrotoga mobilis*, a thermophilic sulphur- and thiosulphate-reducing heterotroph, from a North Sea oil reservoir water sample. Milekhina et al. (1998) reported that aerobic *Rhodococcus* species are the main representatives of hydrocarbon-oxidizing microbial communities of waterflooded oil fields in Tatarstan. It can reasonably be hypothesized that these bacteria have been introduced in the reservoir by water injection, as well as *Marinobacter aquaeolei*, a halophilic aerobe, able to grow anaerobically in the presence of nitrate and isolated from a Vietnamese oil-producing well (Huu et al. 1999). This strain has been reported to oxidize hydrocarbons aerobically. Indirect evidence for the presence of a halophilic aerobic *Archaea* has been reported from an upper Devonian oil field in Tatarstan, but evidence for its ability to grow under in situ conditions needs further investigation (Zvyagintseva et al. 1998).

Leu et al. (1998) investigated four thermophilic enrichment cultures from three different oil fields samples using 16S rDNA sequence analysis. They found sulphate-reducing *Desulfotomaculum*-related sequences in all samples, and *Clostridium*-, *Desulfomicrobium*- and *Thermoanaerobacter*-related sequences from a sandstone core.

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References

Adkins JP, Cornell LA & Tanner RS (1992) Microbial composition of carbonate petroleum reservoir fluids. Geomicrob. J. 10: 87–97.

Aeckersberg F, Bak F & Widdel F (1991) Anaerobic oxidation of saturated hydrocarbons to CO_2 by a new type of sulfate-reducing bacterium. Arch. Microbiol. 156: 5–14.

Amy PS & Haldeman DL (1997) Denizens of the deep. In: Amy PS & Haldeman DL (Ed) The Microbiology of the Terrestrial Deep Subsurface (pp 1–3). CRC Lewis, New York.

Barth T (1991) Organic acids and inorganic ions in waters from petroleum reservoirs, Norwegian continental shelf: a multivariate statistical analysis and comparison with american reservoir formation waters. Appl. Geochem. 6: 1–15.

Barth T & Riis M (1992) Interactions between organic acid anions in formation waters and reservoir mineral phases. Org. Geochem. 19: 455–482.

Bastin ES (1926) The problem of the natural reduction of sulphates. Bull. Am. Assoc. Petrol. Geol. 10: 1270–1299.

Beeder J, Nilsen RK, Rosnes JT, Torsvik T & Lien T (1994) *Archaeglobus fulgidus* isolated from hot North Sea oil field water. Appl. Environ. Microbiol. 60: 1227–1231.

Beeder J, Torsvik T & Lien T (1995) *Thermodesulforhabdus norvegicus* gen. nov., sp. nov., a novel thermophilic sulfate-reducing bacterium from oil field water. Arch. Microbiol. 164: 331–336.

Belyaev SS & Ivanov MV (1983) Bacterial methanogenesis in underground waters. Ecol. Bull. 35: 273–280.

Belyaev SS, Wolkin R, Kenealy WR, De Niro MJ, Epstein S & Zeikus JG (1983) Methanogenic bacteria from the Bondyuzhskoe oil field: general characterization and analysis of stable-carbon isotopic fractionation. Appl. Environ. Microbiol. 45: 691–697.

Bernard FP, Connan J & Magot M (1992) Indigenous microrganisms in connate water of many oil fields: a new tool in exploration and production techniques. In: SPE 24811. Proceedings of the Society of Petroleum Engineers, vol. II (pp 467–475). Society of Petroleum Engineers, Inc., Richardson, TX.

Bhupatiraju VK, Sharma PK, McInerney MJ, Knapp RM, Fowler K & Jenkins W (1991) Isolation and characterization of novel halophilic anaerobic bacteria from oil field brines. Dev. Petrol. Sci. 31: 131–143.

Bhupathiraju VK, McInerney MJ & Knapp RM (1993) Pretest studies for a microbially enhanced oil recovery field pilot in a hypersaline oil reservoir. Geomicrobiol. J. 11: 19–34.

Bhupatiraju VK, Oren A, Sharma PK, Tanner RS, Woese CR & McInerney MJ (1994) *Haloanaerobium salsugo* sp. nov., a moderately halophilic, anaerobic bacterium from a subterranean brine. Int. J. Syst. Bacteriol. 44: 565–572.

Borzenkov IA, Belyaev SS, Miller YM, Davidova IA & Ivanov MV (1997) Methanogenesis in the highly mineralized stratal waters of the Bondyuzhskoe oil field. Microbiology (Engl. Tr.) 66: 104–110.

Cayol JL, Ollivier B, Patel BKC, Prensier G, Guezennec J & Garcia JL (1994) Isolation and characterization of *Halothermothrix orenii* gen. nov. sp. nov., a halophilic, thermophilic, fermentative, strictly anaerobic bacterium. Int. J. Syst. Bacteriol. 44: 534–540.

Cayol JL, Ollivier B, Patel BKC, Ravot G, Magot M, Ageron E, Grimont PAD & Garcia JL (1995) Description of *Thermoanaerobacter brockii* subsp. *lactiethylicus* subsp. nov., isolated from a deep subsurface French oil well, a proposal to reclassify *Thermoanaerobacter finnii* as *Thermoanaerobacter brockii* subsp. *finnii* comb. nov., and emended description of *Thermoanaerobacter brockii*. Int. J. Syst. Bacteriol. 45: 783–789.

Christensen B, Torsvik T & Lien T (1992) Immunomagnetically captured thermophilic sulfate-reducing bacteria from North Sea oil field waters. Appl. Environ. Microbiol. 58: 1244–1248.

Connan J, Lacrampe-Couloume G & Magot M (1996) Origin of gases in reservoirs. In: Dolenc A (Ed) Proceedings of the 1995 International Gas Research Conference. Government Institutes Inc., Rockville, MD.

Cord-Ruwich R, Kleinitz W & Widdel F (1987) Sulphate-reducing bacteria and their activities in oil production. J. Petrol. Technol. 1: 97–106.

Crolet JL & Magot M (1996) Non-SRB sulfidogenic bacteria in oilfield production facilities. Mat. Perf., March 1996: 60–64.

Davey ME, Wood WA, Key R, Nakamura K & Stahl DA (1993) Isolation of three species of *Geotoga* and *Petrotoga*: two new genera, representing a new lineage in the bacterial line. System. Appl. Microbiol. 16: 191–200.

Davidova IA, Harmsen HJM, Stams AJM, Belyaev SS & Zehnder AJB (1997) Taxonomic description of *Methanococcoides euhalobius* and its transfer to *Methanohalophilus* genus. Antonie van Leeuwenhoek 71: 313–318.

Davydova-Charakhch'yan IA, Kuznetsova VG, Mityushina LL & Belyaev SS (1992a) Methane-forming bacilli from oil fields of Tataria and western Siberia. Microbiology (Engl. Tr.) 61: 299–305.

Davydova-Charakhch'yan IA, Mileeva AN, Mityushina LL & Belyaev SS (1992b) Acetogenic bacteria from oil fields of Tataria and western Siberia. Microbiology (Engl. Tr.) 61: 306–315.

Fardeau ML, Cayol JL, Magot M & Ollivier B (1993) H_2 oxidation in the presence of thiosulfate by a *Thermoanaerobacter* strain isolated from an oil-producing well. FEMS Microbiol. Lett. 113: 327–332.

Fardeau ML, Ollivier B, Patel BKC, Magot M, Thomas P, Rimbault A, Rocchiccioli F & Garcia JL (1997) *Thermotoga hypogea* sp. nov., a xylanolytic, thermophilic bacterium from an oil-producing well. Int. J. Syst. Bacteriol. 47: 1013–1019.

Faudon C, Fardeau ML, Heim J, Patel BKC, Magot M & Ollivier B (1995) Peptide and amino acid oxidation in the presence of thiosulfate by members of the genus *Thermoanaerobacter*. Curr. Microbiol. 31: 152–157.

Fisher JBF (1987) Distribution and occurrence of aliphatic acid anions in deep subsurface waters. Geochim. Cosmochim. Acta 51: 2459–2468.

Galushko AS & Rozanova EP (1991) *Desulfobacterium cetonicum*. sp. nov: a sulfate-reducing bacterium which oxidizes fatty acids and ketones. Microbiology (Engl. Tr.) 60: 102–107.

Gevertz D, Paterek JR, Davey ME & Wood WA (1991) Isolation and characterization of anaerobic halophilic bacteria from oil reservoir brines. Dev. Petrol. Sci. Ser. 31: 115–129.

Grassia GS, McLean KM, Glénat P, Bauld J & Sheehy AJ (1996) A systematic survey for thermophilic fermentative bacteria and archaea in high temperature petroleum reservoirs. FEMS Microbiol. Ecol. 21: 47–58.

Grbic-Galic D (1990) Anaerobic microbial transformation of nonoxygenated aromatic and alicyclic compounds in soil, subsurface, and freshwater sediments. In: Bollag JM & Stotzky G (Ed) Soil Biogeochemistry (pp 117–189). Marcel Dekker, New York.

Greene AC, Patel BKC & Sheehy A (1997) *Deferribacter thermophilus* gen. nov., sp. nov., a novel thermophilic manganese- and iron-reducing bacterium isolated from a petroleum reservoir. Int. J. Syst. Bacteriol. 47: 505–509.

Harms G, Zengler K, Rabus R, Aeckersberg F, Minz D, Rosello-Mora R & Widdel F (1999) Anaerobic oxidation of *o*-xylene, *m*-xylene, and homologous alkylbenzenes by new types of sulfate-reducing bacteria. Appl. Environ. Microbiol. 65: 999–1004.

Huu NB, Denner EBM, Ha DTC, Wanner G & Stan-Lotter H (1999) *Marinobacter aequaeolei* sp. nov., a halophilic bacterium isolated from a vietnamese oil-producing well. Int. J. Syst. Bacteriol. 49: 367–375.

Ivanov MV, Belyaev SS, Zyakun AM, Bondars V & Laurinivicius K (1983) Microbiological methane formation in oil field development. Geokhimiya 11: 1647–1654.

Jeanthon C, Reysenbach AL, L'Haridon S, Gambacorta A, Pace NR, Glénat P & Prieur D (1995) *Thermotoga subterranea* sp. nov., a new thermophilic bacterium isolated from a continental oil reservoir. Arch. Microbiol. 164: 91–97.

Krekeler D, Sigalevich P, Teske P, Cypionka H & Cohen Y (1997) A sulfate-reducing bacterium from the oxic layer of a microbial mat from Solar Lake (Sinai), *Desulfovibrio oxyclinae* sp. nov. Arch. Microbiol. 167: 369–375.

Krumholz L, Caldwell ME & Suflita JM (1996) Biodegradation of 'BTEX' hydrocarbons under anaerobic conditions. In: Crawford R & Crawford D (Ed) Bioremediation: Principles and Applications (pp 61–99). Cambridge University Press, Cambridge.

L'Haridon S, Reysenbach AL, Glénat P, Prieur D & Jeanthon P (1995) Hot subterranean biosphere in a continental oil reservoir. Nature 377: 223–224.

Lien T & Beeder J (1997) *Desulfobacter vibrioformis* sp. nov., a sulfate-reducer from a water–oil separation system. Int. J. Syst. Bacteriol. 47: 1124–1128.

Lien T, Madsen M, Steen IH & Gjerdevik K (1998a) *Desulfobulbus rhabdoformis* sp. nov., a sulfate-reducer from a water–oil separation system. Int. J. Syst. Bacteriol. 48: 469–474.

Lien T, Madsen M, Rainey FA & Birkeland N-K (1998b) *Petrotoga mobilis* sp. nov., from a North Sea oil-production well. Int. J. Syst. Bacteriol. 48: 469–474.

Leu J-Y, McGovern-Traa, Porter AJR & Hamilton WA (1998) Identification and phylogenetic analysis of thermophilic sulfate-reducing bacteria in oil field samples by 16S rDNA gene cloning and sequencing. Anaerobe 4: 165–174.

Magot M (1996) Similar bacteria in remote oil fields. Nature 379: 681.

Magot M, Caumette P, Desperrier JM, Matheron R, Dauga C, Grimont F & Carreau L (1992) *Desulfovibrio longus* sp. nov., a sulfate-reducing bacterium isolated from oil-producing well. Int. J. Syst. Bacteriol. 42: 398–403.

Magot M, Hurtevent C & Crolet JL (1993) Reservoir souring and well souring. In: Costa JM & Mercer AD (Eds.) Progress in the Understanding and Prevention of Corrosion (pp 573–575). The Institute of Materials, London, UK.

Magot M, Fardeau ML, Arnauld O, Lanau C, Ollivier B, Thomas P & Patel BKC (1997a) *Spirochaeta smaragdinae* sp. nov., a new mesophilic strictly anaerobic spirochete from an oil field. FEMS Microbiol. Lett. 155: 185–191.

Magot M, Ravot G, Campaignolle X, Ollivier B, Patel BKC, Fardeau ML, Thomas P, Crolet JL & Garcia JL (1997b) *Dethiosulfovibrio peptidovorans* gen. nov., sp. nov., a new anaerobic, slightly halophilic, thiosulfate-reducing bacterium from corroding offshore oil wells. Int. J. Syst. Bacteriol. 47: 818–824.

Matz AA, Borisov AY, Mamedov YG & Ibatulin RR (1992) Commercial (pilot) test of microbial enhanced oil recovery. Proceedings of the 8th SPE/DOE Symposium on Enhanced Oil Recovery. SPE/DOE paper 24208. Society of Petroleum Engineers, Inc., Richardson, TX.

McInnerney MJ & Sublette KL (1997) Petroleum microbiology: biofouling, souring, and improved oil recovery. In: Hurst CJ, Knudsen GR, McInnerney MJ, Stetzenbach LD & Walter MV (Eds.) Manual of Environmental Microbiology (pp 600–607). ASM Press, Washington, DC.

Milekhina EI, Borzenkov IA, Zvyagintseva IS, Kostrikina NA & Belyaev SS (1998) Ecological and physiological characterization of aerobic eubacteria from oil fields of Tatarstan. Microbiology (Engl. Tr.) 67: 170–175.

Moser DP & Nealson KH (1996) Growth of the facultative anaerobe *Shewanella putrefaciens* by elemental sulfur reduction. Appl. Environ. Microbiol. 62: 2100–2105.

Nazina TN & Rozanova EP (1978) Thermophilic sulfate-reducing bacteria from oil strata. Microbiology (Engl. Tr.) 47: 142–148.

Nazina TN, Ivanova AE Kanchaveli LP & Rozanova EP (1988) A new thermophilic methylotrophic sulfate-reducing bacterium, *Desulfotomaculum kuznetsovii* sp. nov. Microbiology (Engl. Tr.) 57: 823–827.

Nazina TN, Ivanova AE, Mityushina LL & Belyaev SS (1993) Thermophilic hydrocarbon-oxidizing bacteria from oil strata. Microbiology (Engl. Tr.) 62: 359–365.

Nazina TN, Ivanova AE, Golubeva OV, Ibatullin RR, Belyaev SS & Ivanov MV (1995) Occurrence of sulfate- and iron-reducing bacteria in stratal waters of the Romashkinskoe oil field. Microbiology (Engl. Tr.) 64: 203–208.

Nealson KH & Saffarini D (1994) Iron and manganese in anaerobic respiration: environmental significance, physiology, and regulation. Annu. Rev. Microbiol. 48: 311–343.

Ng TK, Weimer PJ &Gawel LJ (1989) Possible nonanthropogenic origin of two methanogenic isolates from oil-producing wells in the San Miguelito field, Ventura county, California. Geomicrobiol. J. 7: 185–192.

Nga DP, Cam Ha DT, Hien LT & Stan-Lotter H (1996) *Desulfovibrio vietnamensis* sp. nov., a halophilic sulfate-reducing bacterium from vietnamese oil fields. Anaerobe 2: 385–392.

Ni S & Boone DR (1991) Isolation and characterization of a dimethyl sulfide-degrading methanogen, *Methanolobus siciliae* H1350, from an oil well. Int. J. Syst. Bacteriol. 41: 410–416.

Ni S, Woese CR, Aldrich H.C. & Boone DR (1994) Transfer of *Methanolobus siciliae* to the genus *Methanosarcina* naming it *Methanosarcina siciliae*, and emendation of the genus *Methanosarcina*. Int. J. Syst. Bacteriol. 44: 357–359.

Nilsen RK & Torsvik T (1996) *Methanococcus thermolithotrophicus* isolated from North Sea oil field reservoir water. Appl. Environ. Microbiol. 62: 728–731.

Nilsen RK, Beeder J, Thostenson T & Torsvik T (1996a) Distribution of thermophilic marine sulfate reducers in North Sea oil field waters and oil reservoirs. Appl. Environ. Microbiol. 62: 1793–1798.

Nilsen RK, Torsvik T & Lien T (1996b) *Desulfotomaculum thermocisternum* sp. nov., a sulfate-reducer isolated from a hot North Sea oil reservoir. Int. J. Syst. Bacteriol. 46: 397–402.

Obraztsova AY, Tsyban VE, Laurina Vichus KS, Bezrukova LV & Belyaev SS (1987) Biological properties of *Methanosarcina* not utilizing carbonic acid and hydrogen. Microbiology (Engl. Tr.) 56: 807–812.

Obraztsova AY, Shipin OV, Bezrukova LV & Belyaev SS (1988) Properties of the coccoid methylotrophic methanogen. Microbiology (Engl. Tr.) 56: 523–527.

Ollivier B, Caumette P, Garcia JL & Mah RA (1994) Anaerobic bacteria from hypersaline environments. Microbiol. Rev. 58: 27–38.

Ollivier B, Cayol JL, Patel BKC, Magot M, Fardeau ML & Garcia JL (1997) *Methanoplanus petrolearius* sp. nov., a novel methanogenic bacterium from an oil producing well. FEMS Microbiol. Lett. 147: 51–56.

Ollivier B, Fardeau ML, Cayol JL, Magot M, Patel BKC, Prensier G & Garcia JL (1998) Characterization of *Methanocalculus halotolerans* gen. nov., sp. nov., isolated from an oil-producing well. Int. J. Syst. Bacteriol. 48: 821–828.

Oremland & King (1989) Methanogenesis in hypersaline environments. In: Cohen Y & Rosenberg (Ed) Microbial Mats: Physiological Ecology of Benthic Microbial Communities (pp 180–190). American Society for Microbiology, Washington, DC.

Patel BKC, Andrews KT, Ollivier B, Mah RA & Garcia JL (1995) Reevaluating the classification of *Halobacteroides* and *Haloanaerobacter* species based on sequence comparisons of the 16S ribosomal RNA genes. FEMS Microbiol. Lett 134: 115–119.

Philippi GT (1977) On the depth, time, and mechanism of origin of the heavy to medium gravity naphtenic crude oil. Geochim. Cosmochim. Acta 41: 33–52.

Premuzic E & Woodhead A (1993) Microbial enhancement of oil recovery-Recent advances. Proceedings of the 1992 Conference on Microbial Enhanced Oil Recovery. Elsevier, Amsterdam.

Rainey FA, Zhilina TN, Boulygina ES, Stackebrandt E, Tourova TP & Zavarzin GA (1995) The taxonomic status of the fermentative halophilic anaerobic bacteria: description of *Haloanaerobiales* ord. nov., Halobacteroidaceae fam. nov., *Orenia* gen. nov., and further taxonomic rearrangements at the genus and species level. Anaerobe 1: 185–199.

Ravot G, Magot M, Fardeau ML, Patel BKC, Prensier G, Egan A, Garcia JL & Ollivier B (1995a) *Thermotoga elfii* sp. nov., a novel thermophilic bacterium from an African oil-producing well. Int. J. Syst. Bacteriol. 45: 308–314.

Ravot G, Ollivier B, Magot M, Patel BKC, Crolet JL, Fardeau ML & Garcia JL (1995b) Thiosulfate reduction, an important physiological feature shared by members of the order *Thermotogales*. Appl. Environ. Microbiol. 61: 2053–2055.

Ravot G, Ollivier B, Fardeau ML, Patel BKC, Andrews KT, Magot M & Garcia JL (1996) L-alanine production from glucose fermentation by hyperthermophilic members of the domains *Bacteria* and *Archaea*: a remnant of an ancestral metabolism? Appl. Environ. Microbiol. 62: 2657–2659.

Ravot G, Magot M, Ollivier B, Patel BKC, Ageron E, Grimont PAD, Thomas P & Garcia JL (1997) *Haloanaerobium congolense* sp. nov., an anaerobic, moderately halophilic, thiosulfate- and sulfur-reducing bacterium from an african oil field. FEMS Microbiol. Lett. 147: 81–88.

Redburn AC & Patel BKC (1994) *Desulfovibrio longreachii* sp. nov., a sulfate-reducing bacterium isolated from the Great Artesian Basin of Australia. FEMS Microbiol. Lett. 115: 33–38.

Rees GN, Grassia GS, Sheehy AJ, Dwivedi PP & Patel BKC (1995) *Desulfacinum infernum* gen. nov., sp. nov., a thermophilic sulfate-reducing bacterium from a petroleum reservoir. Int. J. Syst. Bacteriol. 45: 85–89.

Rees GN, Patel BKC, Grassia GS & Sheehy AJ (1997) *Anaerobaculum thermoterrenum* gen. nov., sp. nov., a novel, thermophilic bacterium which ferments citrate. Int. J. Syst. Bacteriol. 47: 150–154.

Rengpipat S, Langworthy TA & Zeikus JG (1988) *Halobacteroides acetoethylicus* sp. nov., a new obligately anaerobic halophile isolated from deep surface hypersaline environment. Syst. Appl. Microbiol. 11: 28–35.

Rosnes JT, Torsvik T & Lien T (1991) Spore-forming thermophilic sulfate-reducing bacteria isolated from North Sea oil field waters. Appl. Environ. Microbiol. 57: 2302–2307.

Rozanova EP & Khudyakova AI (1974) A new nonspore-forming thermophilic sulfate-reducing organism, *Desulfovibrio thermophilus* nov. sp. Microbiology (Engl. Tr.) 43: 1069–1075.

Rozanova EP & Nazina TN (1979). Occurrence of thermophilic sulfate-reducing bacteria in oil-bearing strata. Microbiology (Engl. Tr.) 48: 907–911.

Rozanova EP & Pivovarova TA. (1988) Reclassification of *Desulfovibrio thermophilus* (Rozanova, Khudyakova, 1974). Microbiology (Engl. Tr.) 57: 102–106.

Rozanova EP, Nazina TN & Galushko AS (1988). Isolation of a new genus of sulfate-reducing bacteria and description of a new species of this genus, *Desulfomicrobium aspheronum* gen. nov. sp. nov.. Microbiology (Engl. Tr.) 57: 634–641.

Rueter P, Rabus R, Wilkes H, Aeckersberg F, Rainey FA, Jannasch HW & Widdel F (1994) Anaerobic oxidation of hydrocarbons in crude oil by new types of sulphate-reducing bacteria. Nature 372: 455–458.

Semple KM & Westlake DWS (1987) Characterization of iron-reducing *Alteromonas putrefaciens* strains from oil field fluids. Can. J. Micobiol. 33: 366–371.

Stetter KO, Hoffmann A & Huber R (1993a) Microorganisms adapted to high temperature environments. In: Guerrero R & Pedros-Alio C (Ed) Trends in Microbial Ecology (pp 25–28). Spanish Society for Microbiology.

Stetter KO, Huber R, Blöchl E, Kurr M, Eden RD, Fielder M, Cash H & Vance I (1993b) Hyperthermophilic archaea are thriving in deep North Sea and Alaskan oil reservoirs. Nature 365: 743–745.

Tardy-Jacquenod C, Caumette P, Matheron R, Lanau C, Arnauld O & Magot M (1996a) Characterization of sulfate-reducing bacteria isolated from oil-field waters. Can. J. Microbiol. 42: 259–266.

Tardy-Jacquenod C, Magot M, Laigret F, Kaghad M, Patel BKC, Guezennec J, Matheron R & Caumette P (1996b) *Desulfovibrio gabonensis* sp. nov., a new moderately halophilic sulfate-reducing bacterium isolated from an oil pipeline. Int. J. Syst. Bacteriol. 46: 710–715.

Tardy-Jacquenod C, Magot M, Patel BKC, Matheron R & Caumette P (1998) *Desulfotomaculum halophilum* sp. nov., a new halophilic, spore-forming, sulfate-reducing bacterium isolated from oil production facilities. Int. J. Syst. Bacteriol. 48: 333–338.

Telang AJ, Ebert S Foght JM, Westlake DWS, Jenneman GE, Gevertz D & Voordouw G (1997) Effect of nitrate injection on the microbial community in an oil field as monitored by reverse sample genome probing. Appl. Environ. Microbiol. 63: 1785–1793.

Voordouw G, Voordouw JK, Jack JK, Foght J, Fedorak PM & Westlake DW (1992) Identification of distinct communities of sulfate-reducing bacteria in oil fields by reverse sample genome probing. Appl. Environ. Microbiol. 58: 3542–3552.

Voordouw G, Armstrong SM, Reimer MF, Fouts B, Telang AJ, Shen Y & Gevertz D (1996) Characterization of 16S rRNA genes from oil field microbial communities indicates the presence of a variety of sulfate-reducing, fermentative, and sulfide-oxidizing bacteria. Appl. Environ. Microbiol. 62: 1623–1629.

Zeikus JG, Dawson MA, Thompson TE, Ingvorsen K & Hatchikian EC (1983) Microbial ecology of volcanic sulphidogenesis: isolation and characterization of *Thermodesulfobacterium commune* gen. nov. and sp. nov. J. Gen. Microbiol. 129: 1159–1169.

Zvyagintseva IS, Belyaev SS, Borzenkov IA, Kostrikina NA, Milekhina EI & Ivanov MV (1995) Halophilic archaebacteria from the Kalamkass oil field. Microbiology (Engl. Tr.) 64: 83–87.

Zvyagintseva IS, Kostrikina NA & Belyaev SS (1998) Detection of halophilic in a upper devonian oil field in Tatarstan. Microbiology (Engl. Tr.) 67: 688–691.