

Residual Oil Deposits as a Substrate for Methane Geobioreactors™

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Introduction

Since the discovery of oil in Titusville, Pennsylvania in 1859, the United States has recovered approximately 190 billion barrels of oil from the earth's subsurface (10). This versatile resource has been the primary driver of today's transportation and chemical industry, with more than 90% of the chemicals used today being made from petroleum products (25). Its portability, ease of conversion, flexible chemistries, and overall energy density have made oil a desirable energy source. Unfortunately, only 25-35% of the oil in a typical reservoir can be cost-effectively brought to the surface (7). When enhanced recovery can be employed, total recovery seldom exceeds 50%. While there are also extraordinary exceptions to this norm, like the East Texas Oil Field with an estimated ultimate recovery of 76% (2), routinely more than half of the discovered oil is left in the ground. While technological advances and current high oil prices have allowed oil producers to economically continue to pump oil from many old fields, the above calculations indicate that there are at least 190 billion barrels of uneconomic residual oil remaining in the developed oil fields of the U.S.

Recently, the total Btu value of natural gas produced annually in the U.S. surpassed the Btu value of oil produced annually in the U.S. (8). Natural gas (methane) is one of the most desired and environmentally benign sources of energy in today's world. Its clean-burning properties relative to other fossil fuels have attracted the interests of both consumers and energy producers. Domestic production of natural gas in the United States peaked more than 30 years ago and demand continues to outpace supply. Total natural gas demand in the United States was 22 TCF in 2003 and is expected to grow annually at 3% for the next 20 years (11).

Methane is derived from the decomposition of both terrestrial and marine carbonaceous matter. Much of this methane was commonly believed to have been created from long-buried organic materials subjected to intense heat and pressure, and from microbial processes over millions of years. More recently, scientists have discovered that methane in natural gas reservoirs has been generated in the recent past via the anaerobic microbial breakdown and conversion of complex underground hydrocarbons such as coal, carbonaceous shale and oil to methane (18). Although difficult to quantify, biogenic gases have been credited with contributing at least 20% and perhaps as much as 65% (including hydrates) of the methane known to exist in the earth's subsurface (6, 23). Unconventional gas supplies are projected to make up an increasing proportion of domestic natural gas supplies, with biogenic methane playing an important role. Technological improvements will be essential for expansion of these natural gas supplies and to moderate future natural gas prices (11).

Luca Technologies (Luca) previous research has demonstrated that coal seams in the Powder River Basin (PRB) of Wyoming are methanogenic bioreactors (17). Luca has coined the term “GeobioreactorTM” to describe geologic formations containing hydrogen and carbon that are generating, or have the potential to generate, large amounts of biogenic methane on an ongoing, real-time basis. This methane formation is the result of live, naturally occurring populations of anaerobic microorganisms that exist within these deposits. Field geochemical measurements of carbon isotopes attest to the occurrence of recent methanogenic activity in the coal seams. The native geochemical conditions influence methanogenesis, and, in turn, the distribution of coal bed methane (CBM) in the field. Under the right set of conditions, coal, in conjunction with formation waters removed from these areas, is capable of generating significant amounts of biogenic methane, in the laboratory, in real time. In order for a Geobioreactor to function, the appropriate environmental conditions must be present. For coal, these conditions include abundant substrate, a water saturated environment, a complete absence of oxygen, and the presence of an appropriate consortium of microorganisms.

The biogenesis of methane is a process that concentrates hydrogen. The hydrocarbon substrates partially consumed by this process have varying hydrogen to carbon ratios. Coal and some types of shale kerogen have H:C ratios of less than one whereas oil and other types of shale kerogen have hydrogen to carbon ratios closer to two. Methane has a ratio of 4:1 and clearly represents the most hydrogen-enriched form of a hydrocarbon molecule. Hydrogen availability is the limiting factor in estimating the biogenic resource potential of a particular substrate.

The hydrogen content of oil varies with the constituent molecular composition and gravity. Classic conventional crude oils usually have hydrogen contents exceeding 12% by weight (12). However, heavy oils now being developed in eastern Alberta, Canada, have hydrogen contents of less than 11% (15). Assuming that a typical residual oil in a domestic oil reservoir has a gravity of 35 degrees API and a hydrogen content of 11.5%, then a barrel of residual oil could serve as substrate for the biogenic creation of 3.2 mcf of methane. On this basis, 190 billion barrels of residual oil represent a biogenic “substrate in place” (SIP) for the creation of more than 600 TCF of methane. A conversion factor of only a few percent would yield significant quantities of methane.

Substantial portions of biogenic methane have been found in association with oil deposits (22). While others have reported methanogenic activities associated with oil field samples (27), this report documents the identification of a Geobioreactor for methane creation from residual, and otherwise unrecoverable oil.

Materials and Methods

Sample location and production background. Oil, formation water, and gas samples were obtained from the Monument Butte Field (MB) located in southeastern Duchesne and western Uintah Counties, Utah, approximately 12 miles south of the town of Roosevelt. Geologically, the field is located in the Eocene age, Green River producing trend of the central Uinta basin. The field produces oil and gas from numerous erratic sands deposited in a varying lacustrine and deltaic environment. Oil was sampled from the Nine Mile 4-6 wellhead located in Sec. 6, T9S-

R16E, within the West Point Unit near the northwest corner of the Monument Butte field. The well was drilled in December, 1997, completed in January, 1998, and produces through perforations in the Castle Peak and Green River B and D sands. Through time of sampling in March 2004, this well had produced 8,748 barrels of oil, 23,189 barrels of water and 54,423 mcf of gas.

Field sampling. Oil, gas and water samples were recovered from producing Nine Mile 4-6 wellhead. Wellhead fluids from the Nine Mile 4-6 well were sampled using a short length of rubber hose. One end of the hose was screwed into a bleeder valve on the wellhead. The other end was inserted into a 4-liter glass bottle. Three-phase fluid was collected as semi-solid slugs of oil/water emulsion, some water, and pockets of rapidly-expanding gas. The bottle was filled nearly to the spill point, until remaining bottle capacity was not sufficient to control new fluids. At that point, the bleeder valve was closed, remaining bottle headspace was displaced with argon, and the cap was screwed on tightly.

For a spectrum of samples with minimized field disturbance, other oil and water samples were obtained from Monument Butte injection wells, heater-treaters, storage tanks, or associated piping in nearby wells using various tube sampling devices. While these samples may not be indicative of the downhole ecology or environment, the data and information provided by a group of parallel experiments using these samples proved useful in a variety of comparative and other testing.

Preparation, sampling and analysis of methanogenic activity. The containers of oil and formation water were opened inside an anaerobic glove bag filled with 95% nitrogen and 5% hydrogen under slightly positive pressure with controlled temperature (22°C) and humidity (95%).

Twenty-five ml experimental bottles containing 10 ml of anoxic formation water and 0.1 ml of oil acquired from the same location were prepared inside the anaerobic glove bag. Sodium sulfide (to 0.5 mM) was added to the water to ensure strict anoxic conditions in the oil/water mixtures during long-term incubation. The bottles were sealed with sterile butyl rubber stoppers, removed from the glove bag, vacuumed and purged with nitrogen/carbon dioxide (95/5) for three cycles. A small nitrogen/carbon dioxide overpressure (~ 3 psi) was left in the headspace of the bottles. The experimental bottles were incubated at approximately 10°F lower than the downhole temperature. Sterile controls consisted of samples that were autoclaved (121°C for 20 minutes, three times).

Over the course of a 10 month incubation period the aqueous phase of the experimental bottles was sampled for organic acids and the headspace sampled for gas composition (methane, hydrogen, and carbon dioxide), using sterile helium purged syringes and needles. Gas concentrations were determined with a Hewlett Packard 5890 gas chromatograph (GC) equipped with a thermal conductivity detector. Organic acids were determined by gas chromatography (Ram Model 3 detector with Alltech 267 column) from the aqueous phase of samples removed from the bottles.

DNA isolation, sequencing and strain identification. DNA was extracted from Monument Butte heater treater samples using state-of-the-art molecular biology techniques, while avoiding contamination by surface microorganisms. Consortia members were identified by sequencing their 16S ribosomal DNA genes using Luca's in-house DNA sequencer (LI-COR model 4300 DNA Analysis System), and comparing these results to a public database containing approximately 125,000 known microorganisms (20).

Results & Discussion

- **Laboratory evidence for recent biogenic methane formation in Monument Butte**

Luca Technologies (Luca) previous research demonstrated that coal seams in the PRB are living Geobioreactors (17). Unamended coal samples in formation water generated new biogenic methane in real-time. Adding certain compounds to either the liquid or the gas headspace either accelerated or inhibited methane generation. In this report we discuss similar results obtained from a different hydrocarbon source, oil, found in the Monument Butte area of the Uinta basin of Utah.

Methane generation in Monument Butte oil/water samples. Direct measurements of methanogenesis in carefully handled and processed production fluids containing oil samples from the Monument Butte Nine Mile 4-6 well indicate that it contains an active consortium of microorganisms capable of converting oil to methane. In Figure 1 on the following page, methane generation in experimental bottles containing just oil and water (unamended) from the Nine Mile 4-6 well rose rapidly during the first 60 days before tapering off to slightly over 10% methane in the headspace as of 297 days. This tapering off trend is commonly found in closed microbial experiments, and may be attributed to nutrient limitation, inhibitor build-up, methane oxidation (16), or a currently unknown phenomenon. As expected, the sterile sample did not generate any methane over the course of the experiment.

The one wellhead sample tested in the Monument Butte field, the Nine Mile 4-6 well, exhibited strong rates of gas generation by Luca's standards. Parallel experiments using samples taken from injection wells, heater-treaters, and storage tanks in other parts of Monument Butte exhibited both stronger and weaker levels of methanogenesis (data not shown).

Oil characterization. Crude oils contain a wide variety of hydrocarbon molecular species. The oil obtained from Monument Butte is waxy, containing a relatively high proportion of straight-chain alkanes, and as a result, the oil has a high pour point temperature (characteristic of waxy crudes) and solidifies at temperatures below 35°C. Alkanes are known to be more susceptible to microbial degradation than the cyclic aliphatic and aromatic molecules typically found in crude oil (1, 14). The waxy composition of Monument Butte oil may have facilitated the robust biogenic response. If so, then areas with large accumulations of waxy oil could prove to be important sites for the bioconversion of residual oil to methane. For example, the Daqing Field in northeast China is thought to contain 25 billion barrels of residual oil with pour points around 27°C (4, 26). This residual oil, left in place after successful waterflood operations, represents a SIP of about 80 TCF. A conversion factor of only a few percent would yield significant

quantities of newly created methane, compared to reported Chinese natural gas reserves of between 46 and 53 TCF (9).

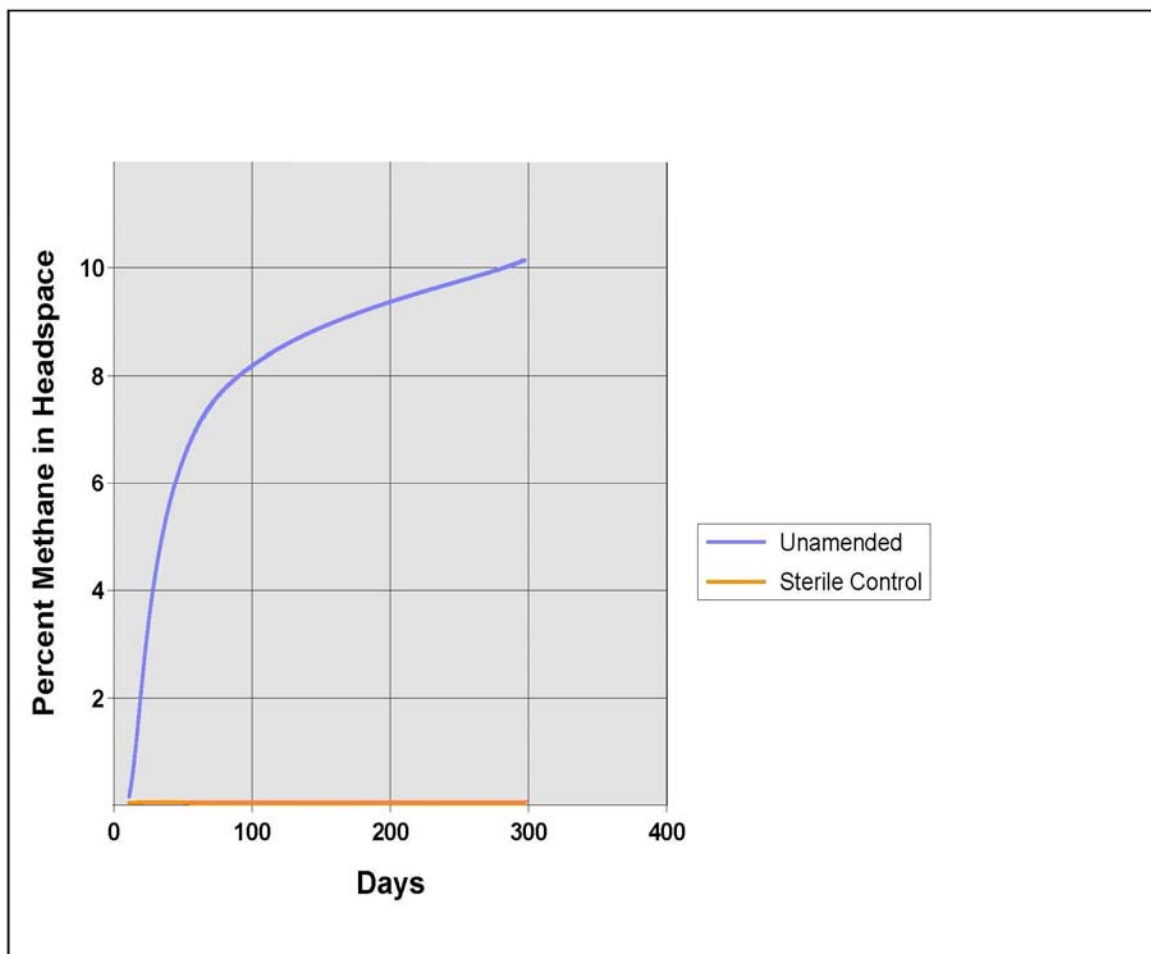


Figure 1. Monument Butte Nine Mile 4-6 Average Methane in Oil/Water Bottles

Water chemistry analysis. Sodium, chloride, calcium, nitrogen, phosphate, magnesium, and bicarbonate levels were determined for all the samples. A standard suite of organic acids was also measured. In general, the measured organic acid concentrations were unremarkable and unchanging, with the exception of acetic acid concentrations which decreased by at least a factor of two in most Monument Butte samples over the first two months of the experiment. Acetic acid is one of the products of hydrocarbon biodegradation and its simple molecular structure allows it to be readily converted to methane by a variety of methanogens (24). The brief rise in acetic acid concentration followed by a steady decline is suggestive of this organic acid being used as a carbon and hydrogen backbone for methane formation. Mass balance calculations indicate that the acetic acid alone could not account for the quantity of methane produced in the experiments. This indicates that active biodegradation of more complex substrates occurred with acetate as an important intermediate.

Anion concentrations exhibited considerable variability with bromide, bicarbonate, and sulfur registering at moderate levels (1-10 g/l). The sodium concentrations in the formation water were also moderate at 2-9 g/l. Samples from the Nine Mile 4-6 well contained intermediate levels of sodium and formed intermediate levels of methane in our experiments. Interestingly, when the sodium content of Nine Mile 4-6 formation water was compared with water samples obtained from injection wells, heater treaters and tanks from 10 other areas in Monument Butte, an inverse correlation was noted between methane generation and sodium concentrations. As depicted in Figure 2, of the eleven areas sampled, the three areas from the Monument Butte field whose samples produced the most methane in Luca's experiments had three of the lowest sodium concentrations.

Microbial characterization. The microorganisms involved in the degradation of oil and its subsequent bioconversion to methane are part of a complex microbial community known as a consortium. Through partial DNA sequence analysis of oil and water samples, we have identified *Clostridia* and *Thermotoga* as two of the key members of this consortium at Monument Butte. *Clostridia* form a broad genus known for its diverse metabolic pathways. *Clostridia* frequently thrive in anaerobic environments and many species are known for their thermotolerance (3). *Thermotoga* microorganisms are known to play a role in the anaerobic oxidation of hydrocarbons to alcohols, organic acids (e.g., acetic acid), and carbon dioxide (21). In addition, *Thermotoga* also thrive in high temperature environments, such as those found in sub-surface oil wells.

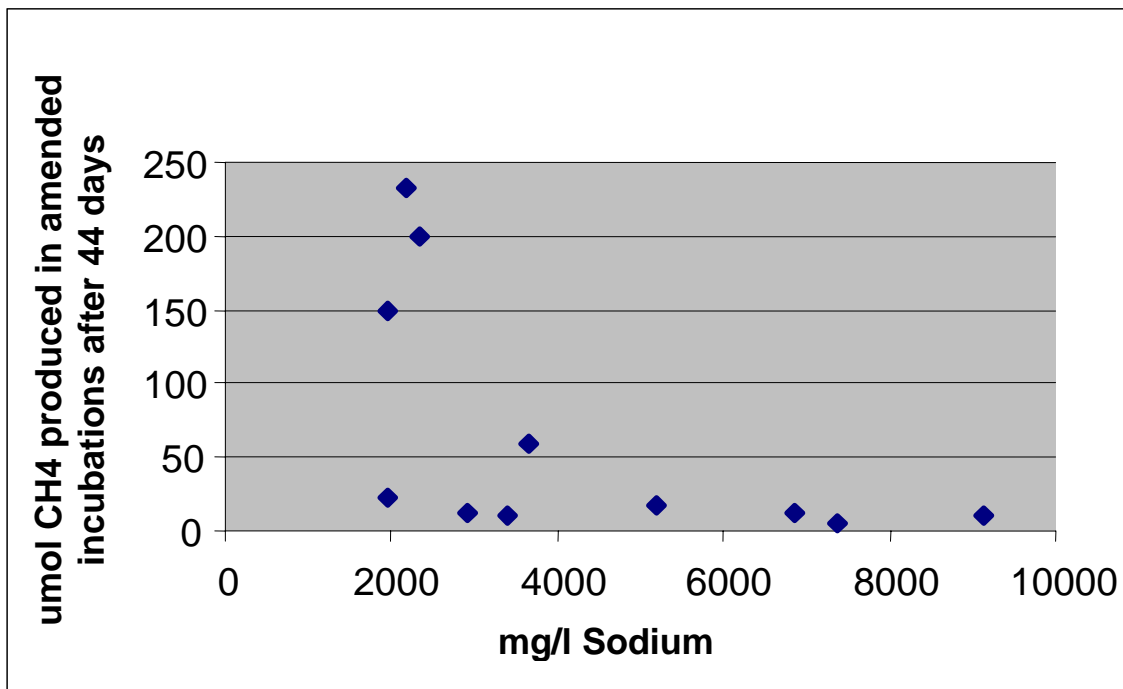


Figure 2. Relationship between sodium concentrations in formation waters and methane productivity in the Monument Butte unit

Extrapolation of experimental results. The volumes of methane created during Luca's experiments can be extrapolated to scaled-up field dimensions using gas percentage composition measurements and the Ideal Gas Law. For example, with appropriate cautions, assume Monument Butte oil wells might be drilled on 40 acre spacing to produce from a 20-foot thick sand with 12% porosity and 40% residual oil saturation after waterflood. The predicted methane extrapolations are shown in Table 1 for Nine Mile 4-6.

Table 1. Extrapolated Peak Rates of Methane Production

Nine Mile 4-6 Wellhead Unamended Samples		
	<u>Laboratory Bottle Result</u>	<u>Scaled-up Field Rate</u>
87 days	8.4 % Methane	132 MCF/Day/Well (avg)
297 days	10.1% Methane	50 MCF/Day/Well (avg)

The experiments discussed in this report consist of samples containing just oil and water (unamended). Previous work by Luca has demonstrated that methane creation in coal and water slurries could be enhanced by the addition of various nutritional amendments (17). Preliminary work suggests that a similar level of methane enhancement can be obtained when specific amendments are added to Monument Butte experimental bottles (data not shown).

The above extrapolation rates of laboratory analysis yield very impressive rates of methane creation. Luca believes that under optimized conditions the rates of methanogenesis can be extended considerably beyond their current values.

Other Oilfields. In addition to the above described Monument Butte field, Luca has investigated oil reservoirs in four other states. The Geobioreactor classification for these other areas range from strong to weak to inactive. Interestingly, in at least one instance, a light, non-paraffinic crude oil as opposed to a waxy crude, was able to support substantial methanogenesis.

Conclusions

Because their environment is hostile to familiar forms of surface life, oil reservoirs were originally thought to be devoid of life. However, more recent research has revealed that many oil reserves contain a variety of active and diverse microorganisms (19). In general, these microorganisms have been studied in the context of fouling, souring, and degradation of oil

reservoirs. Various gases are frequently associated with oil wells, and Luca's data indicate that methanogenesis, the creation of methane, is another biological process occurring in some oil reservoirs. In addition to identifying these active systems (Geobioreactors), it will be important to understand the variables that control this overall methanogenic process.

Because oil is a liquid, it is likely to be an easier substrate for the microbial consortia to contact, biodegrade, and convert to methane compared to solid-phase substrates such as coal and the kerogen in shale. Biodegradation is carried out by the consortia, and it has been shown that a mixed group of microorganisms is more effective at biodegrading organic compounds than any of the component strains acting alone (5). These microorganisms utilize the hydrocarbons as both a carbon and energy source, and the process most likely takes place at the oil/water interface (13). The enzymatic diversity within these microorganisms required to carry out the myriad of metabolic steps involved in methanogenesis is extensive. The ability to influence and control these microbial reactions *in situ* has major economic implications.

References

1. Altken, C.M., Jones, D.M., and S.R. Larter (2004). "Anaerobic hydrocarbon biodegradation in deep subsurface oil reservoirs." *Nature* 431:291-294.
2. Bureau of Economic Geology. <http://www.beg.utexas.edu>
3. Burrows, W. (1968). *Textbook of Microbiology*, 19th Edition. W.B. Saunders Company, Philadelphia.
4. Central News Agency (2004). "Daqing oilfield cuts oil production by seven percent." March 29, 2004.
5. Clarke, P.H. (1984). "The Evolution of Degradative Pathways, in *Microbial Degradation of Organic Compounds*" Vol. 13, Marcel Dekker, Inc., New York, pp.435-444.
6. Claypool, G. (2004). Personal Communications.
7. Dake, L.P. (1978). "Fundamentals of Reservoir Engineering." Elsevier Scientific Publishing Company, Amsterdam. pp 86-94.
8. Energy Information Agency (2001). "Annual Energy Review" <http://tonto.eia.doe.gov/FTPROOT/multifuel/038401.pdf>
9. Energy Information Agency (2002). "Annual Energy Review" <http://tonto.eia.doe.gov/FTPROOT/multifuel/038402.pdf>
10. Energy Information Agency (2003). "Annual Energy Review" <http://www.eia.doe.gov/aer/contents.html>
11. Energy Information Agency (2005). "Annual Energy Review" <http://tonto.eia.doe.gov/FTPROOT/multifuel/mer/00350502.pdf>
12. Frick, T.C. (1962). *Petroleum Production Handbook*. Vol II Reservoir Engineering. Society of Petroleum Engineers of AIME. Dallas, Texas.
13. Head, I.M., Jones, D.M., and S.R. Larter (2003). "Biological activity in the deep subsurface and the origin of heavy oil." *Nature* 426:344-352.
14. Hou, B.K., Ellis, L.B.M., and L.P. Wackett (2004). "Encoding microbial metabolic logic: predicting biodegradation." *Journal of Industrial Microbiology and Biotechnology*, 31:261-272.
15. Hoy, G.J. (2005). Personal communications.
16. Lieberman, R.L. and A.C. Rosenzweig (2005). "Crystal structure of a membrane-bound metalloenzyme that catalyses the biological oxidation of methane." *Nature* 434:177-182.

17. Luca Technologies, LLC (2004). "Active Biogenesis of Methane in Wyoming's Powder River Basin"
http://www.lucatechnologies.com/resources/files/Web_Files/PRB%20Report%20112004%20version.pdf
18. Martini, A.M., Budal, J.M., Walter, L.M., and M. Schoell (1996). "Microbial generation of economic accumulations of methane within a shallow organic-rich shale." *Nature* 383:155-158.
19. McInerney, M.J. and K.L. Sublette (1997). "Petroleum Microbiology: Biofouling, Souring, and Improved Oil Recovery", in *Manual of Environmental Microbiology*, C.J. Hursted, ed., ASM Press, Washington, D.C. pp. 600-607.
20. Michigan State University. <http://rdp.cme.msu.edu/index.jsp>
21. Orphan, V.J., Taylor, L.T., Hafenbradl, D., and E.F. Delong (2000). "Culture-dependent and culture-independent characterization of microbial assemblages associated with high-temperature petroleum reserves." *Applied and Environmental Microbiology*, 66:700-711.
22. Pallasser, R.J. (2000). "Recognizing biodegradation in gas/oil accumulations through the delta-¹³C composition of gas components." *Organic Geochemistry*, 31:1363-1373.
23. Rice, D.D. and Claypool, G.E., 1981, Generation, accumulation, and resource potential of biogenic gas. *AAPG Bulletin*, 65: 5-25.
24. Valentine, D.L. (2001). "Thermodynamic ecology of hydrogen-based syntrophy. In: *Symbiosis: Mechanisms and Model Systems.*" J. Seekbach, ed., Kluwer Academic Publishers. Dordrecht, Netherlands.
25. Wilson, Elizabeth K. (2005). "Engineering cell-based factories." *Chemical & Engineering News*, 83:41-44.
26. Yang, W. (1985). "Daqing oil field People's Republic of China: A giant field with oil of non-marine origin." *American Association of Petroleum Geologists Bulletin*, 69:110-111.
27. Zengler, K., Richnow, H.H., Rossello-Mora, R., Michaelis, W. and F. Widdel (1999). "Methane formation from long-chain alkanes by anaerobic microorganisms." *Nature* 401:266-269.