SUBTASK 1.11 – ANAEROBIC BIOLOGICAL TREATMENT OF PRODUCED WATER

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SUBTASK 1.11 – ANAEROBIC BIOLOGICAL TREATMENT OF PRODUCED WATER

EXECUTIVE SUMMARY

During the production of oil and gas, large amounts of water are brought to the surface and must be disposed of in an environmentally sensitive manner. This is an especially difficult problem in offshore production facilities where space is a major constraint. The chief regulatory criterion for produced water is oil and grease. Most facilities have little trouble meeting this criterion using conventional oil–water separation technologies. However, some operations have significant amounts of naphthenic acids in the water that behave as oil and grease but are not well removed by conventional technologies. Aerobic biological treatment of naphthenic acids in simulated-produced water has been demonstrated by others; however, the system was easily overloaded by the large amounts of low-molecular-weight organic acids often found in produced waters. The objective of this research was to determine the ability of an anaerobic biological system to treat these organic acids in a simulated produced water and to examine the potential for biodegradation of the naphthenic acids in the anaerobic environment.

A small fixed-film anaerobic biological reactor was constructed and adapted to treat a simulated produced water. The bioreactor was tubular, with a low-density porous glass packing material. The inocula to the reactor was sediment from a produced-water holding pond from a municipal anaerobic digester and two salt-loving methanogenic bacteria. During start-up, the feed to the reactor contained glucose as well as typical produced-water components. When glucose was used, rapid gas production was observed. However, when glucose was eliminated and the major organic component was acetate, little gas was generated. Methane production from acetate may have been inhibited by the high salt concentrations, by sulfide, or because of the lack, despite seeding, of microbes capable of converting acetate to methane. Toluene, a minor component of the produced water (0.1 g/L) was removed in the reactor.

Batch tests were conducted to examine naphthenic acid biodegradability under several conditions. The conditions used were seed from the anaerobic reactor, wetland sediments under aerobic and anaerobic conditions, and a sterile control. The naphthenic acid was from a commercial source isolated from Gulf Coast petroleum as was dosed at 2 mg/mL. The incubations were for 30 days at 30°C. The results showed that the naphthenic acids were not biodegraded under anaerobic conditions, but were degraded under aerobic conditions.

Despite poor performance of the anaerobic reactor, it remains likely that anaerobic treatment of acetate, toluene, and, potentially, other produced-water components is feasible.

SUBTASK 1.11 – ANAEROBIC BIOLOGICAL TREATMENT OF PRODUCED WATER

INTRODUCTION

The production of oil and gas typically results in the production of large amounts of water along with the oil and gas. In some situations, the disposal of these produced waters represents a significant environmental challenge. The most difficult environment to handle these produced waters is in the offshore production areas. Disposal of produced water is generally regulated by the oil and grease content. Criteria for oil and grease often cannot be met with oil–water separators, as the components contributing to the oil and grease are soluble, emulsified, or poorly separated by gravity, density, or coalescing methods. These oil and grease components are amenable to aerobic biological treatment; however, produced water, in addition to containing a large amount of salt and oil and grease components, often contains high concentrations of organic acids such as acetate. Although acetate is readily biodegradable, it has a high oxygen demand and is preferentially degraded before other organic compounds. As a result, it is desirable to develop a method to remove acetate and related compounds prior to the aerobic treatment of the oil and grease. Several additional concerns with offshore production are relevant; the high volume of water that must be treated (thousands of barrels per day) and the need to operate in a small footprint. The aerobic biological treatment of the oil and grease components of produced water has been demonstrated (Hickey et al., 1998), but the presence of normally high concentrations of acetate and related acids prevents this process from adequately treating the oil and grease.

Produced water is the largest volume waste stream in oil and gas production operations (Stephenson, 1992). The volume of water produced by a given production facility may vary considerably and has ranged from 500 to 600,000 bbl/day (Jones et al., 2000). Produced water contains a variety of salts of varying types and quantities, soluble hydrocarbons such as benzene, toluene, xylenes, emulsified oils, naphthenic acids and many other compounds (Tibbetts et al., 1992; Shepherd et al., 1992). Toxicity associated with these waters is largely due to the salts, hydrocarbons, and production-associated additives (Sauer et al., 1997; Karman and Reerink, 1998). Despite this, the present regulatory framework for offshore produced waters is based solely on meeting a discharge criteria for oil and grease of 41 mg/L for a daily maximum and 29 mg/L for a monthly average maximum (Jones et al., 2000).

Oil and grease removal from produced waters is generally accomplished with gravity-based oil–water separators, centrifuges, and hydrocyclones (Bilstad and Espedal, 1996). These technologies are generally effective if the water contains little or no naphthenic acids (Hickey et al., 1998). Naphthenic acids, also known as petroleum acids, are native constituents of petroleum (Davis, 1967). Because of the acid nature of these hydrocarbons, they have significant water solubility, and they will readily form emulsions in water (Seifert and Howells, 1969). The composition of the naphthenic acids is highly variable, but generally will include cyclic and aromatic moieties with aliphatic side chains of varying lengths and containing one or more carboxylate moieties (Seifert and Teeter, 1970; Schmitter et al., 1978; Green et al., 1985; Dzidic et al., 1988; Fan, 1991; Hsu et al., 2000). Naphthenic acids are considered to be biodegradable under aerobic conditions (Herman et al. 1993, 1994; Lai et al., 1996; Hickey et al., 1998).

Anaerobic biological reactors are very simple to operate, can handle a high loading of organic matter, and can be idled for extensive periods with rapid restarts (Schink, 1988; Speece, 1996). Acetate and related low molecular weight organic compounds are readily biodegraded under anaerobic conditions by a variety of microorganisms (Speece, 1996). Anaerobic biodegradation of naphthenic acids has not been reported; however, a number of compounds are structurally similar to some naphthenic acids, including some aliphatics, aromatics, and cyclic compounds, that have been found to be biodegraded (Schink, 1996; Fathepure and Tiedje, 1999). This suggests that there is potential for anaerobic biodegradation of naphthenic acids.

OBJECTIVES

The objectives are to demonstrate the biodegradation of organic acids in produced water under anaerobic conditions in the presence of naphthenic acids and, additionally, to evaluate the biodegradability of naphthenic acids under anaerobic conditions.

MATERIALS AND METHODS

Produced Water

A synthetic or simulated produced water was selected for this project. The simulated produced water allowed ready control of and uniformity of composition and was less expensive to use. The composition of this simulated produced water was of concern. Discussions with representatives from the Gas Research Institute and from published literature (see references cited above, Kharaka et al., 1998) were used to formulate a synthetic produced water. The synthetic produced water consisted of an artificial seawater supplemented with commercially obtained naphthenic acids, toluene, and acetate. Small amounts of phosphate and ammonia are added to provide nutrients for bioactivity. The recipe for this produced water is shown in Table 1. This produced water recipe provides the mixture and concentration of salts that might be expected in a produced water. The artificial seawater recipe, Components 1 through 6 of Table 1, is from Wood's Hole Marine Biological Laboratory. Toluene is added to be representative of condensate hydrocarbons in produced water. The naphthenic acids are a commercial mixture extracted from petroleum that have an advertised acid number of 258 (Merichem Chemicals & Refinery Services, 2701 Warrior Road, Tuscaloosa, AL 35404). Prior to addition of the naphthenic acids to the simulated produced water, they were converted to a stable emulsion by neutralizing the acid groups with sodium hydroxide.

A second media was used as well that was similar to the simulated produced water. This media was used because it was recommended for the growth of salt-tolerant methanogenic bacteria that were seeded into the bioreactor. The second medium, a modified methanogen medium, is based on the medium recommended by the Oregon Collection of Methanogens (OCM, 2000) for MSH medium and is shown in Table 2. The recipe was modified in the following ways: mercaptoethanesulfonate was eliminated; sodium bicarbonate was added rather than saturating a

TABLE 1

Composition of the Synthetic Produced Water	
Component	Amount, g/L
NaCl	24.72
KCl	0.67
CaCl ₂	1.03
MgCl ₂	2.18
MgSO ₄ · 7H ₂ O	6.29
NaHCO ₃	0.18
Na ₂ S · 9H ₂ O	0.75
Na Acetate	0.833
NH ₄ Cl	0.038
K ₂ HPO ₄	0.028
Toluene	0.10
Naphthenic acids	0.05

TABLE 2

Composition of the Modified Methanogen Medium	
Component	Amount, g/L
NaHCO ₃	8.4
NaS ₂ · 9H ₂ O	0.25
NaCl	43.87
MgCl ₂	5.0
KCl	0.75
NH ₄ Cl	1.0
CaCl ₂ · 2H ₂ O	0.4
K ₂ HPO ₄ · 3H ₂ O	0.4
Yeast Extract	0.2
Sodium Acetate	8.0
Toluene	87 µg

sodium hydroxide solution with carbon dioxide; yeast extract was decreased from 2 to 0.2 g/L; trypticase peptone was eliminated; the indicator resazurin was eliminated; the trace metal solution was eliminated; and toluene was added. The mercaptoethanesulfonate is a vitamin for methanogens and was eliminated as it would not be practical to add it to produced water streams and is probably not necessary when mixed cultures are used. Sodium bicarbonate addition rather than saturating a sodium hydroxide solution with carbon dioxide greatly simplified the medium preparation and still supplied the required bicarbonate ion. Yeast extract is a supplement to provide micronutrients and was not considered practical nor necessary with a mixed culture at the recommended dose. Trypticase peptone is a protein digest that was not considered necessary nor practical. Resazurin is a redox potential indicator that was not considered necessary. The concentration of sodium acetate was decreased to about what might be expected in a produced water. The trace metal solution was eliminated as it would not be practical nor necessary to add these in a commercial operation. Toluene was added to be representative of condensate hydrocarbons that would be expected in produced water. The pH of the modified methanogen medium was ca. 7.

Construction of the Bioreactor

Many types of bioreactor designs and configurations are possible. Two broad types include suspended growth and fixed-film reactors. Fixed-film reactors have the advantage of preventing biomass washout because of insufficient growth, toxicity, or hydraulic overload. The attachment media for a fixed-film reactor could be sand, plastic media (i.e., saddles, berls, etc.), or granular carbon. Although granular carbon is probably the best overall media for these types of applications, it would have confounded the experiment. The naphthenic acids would have adsorbed to the carbon and may have accumulated to toxic levels. After granular carbon was excluded, the choice was reduced to inert materials with a high surface area. A sintered glass bead material was selected. These beads were obtained from Poraver® (Dennert Poraver GmbH, Schlüsselfeld, Germany). The key properties of the beads are shown in Table 3.

The bioreactor was constructed of a polycarbonate plastic cylinder with an internal diameter of 3.8 cm and a height of 52 cm. Other dimensions of the reactor are shown in Table 4. The reactor was arranged vertically, and simulated produced water, or methanogenic media, was pumped into the top of the reactor. The effluent line came out of the bottom of the reactor and looped back up to the top of the reactor before entering an effluent collection vessel. At the top of the effluent line loop,

TABLE 3

Properties of the Support Material	
Property	Value
Grain Size, mm	8 to 16
Bulk Density, kg/m ³	150
Grain Density, kg/m ³	270

TABLE 4

Dimensions of the Bioreactor	
Parameter	Value
Outside Diameter, cm	4.4
Inside Diameter, cm	3.8
Height, cm	52
Volume, L	0.59
Bed Volume, L	0.45
Packed Bed Height, cm	40

a T fitting was inserted as a siphon break. At the top of the reactor, where the feed entered, was an additional T fitting that allowed for pressure relief and for gas produced to be vented. Feed to the reactor was stored in a 4.5-L glass carboy. Feed was pumped into the reactor via a peristaltic pump. About 77% of the reactor internal volume was filled with the bed material. The low density of this media results in it floating in the reactor, resulting in ease of gas movement through the reactor.

Microbial seed for the bioreactors was from the anaerobic digester at the Moorhead, Minnesota, municipal treatment system, from the sludge in a produced water holding pond located near Wamsutter, Wyoming, and included two pure cultures of brine-requiring acetoclastic methanogenic bacteria from OCM. These cultures were *Methanosarcina sicilae* C2J (OCM #653) and *Methanosarcina* WH1 (OCM #750).

Reactor Operation

After the reactor was seeded, it was fed with the simulated produced water that had been supplemented with 1 g/L of glucose at an initial rate of ca. 10% of the reactor volume per day (ca. 60 mL/day). In order to maintain this low flow rate, the pump was connected to a timer and operated at intervals. Over the course of 4 months, the flow rate to the reactor was slowly increased to 500 mL/day, for a hydraulic retention time of ca. 1 day. Fresh media was prepared weekly and stored in sealed glass containers until it was necessary to add to the feed vessel. Samples were taken periodically from the influent container and directly from the effluent line for analysis. Effluent samples were centrifuged at 16,000× G and then frozen at –20°C until analyzed.

Batch Naphthenic Acid Biodegradation Tests

Batch tests were performed to evaluate the potential for biodegradation of naphthenic acids in the reactor. These tests were conducted using borosilicate serum bottles. Fifty-mL bottles were used for anaerobic conditions, and 100-mL bottles were used for aerobic conditions. The medium used for these tests was the modified methanogenic medium. Four conditions were evaluated in

duplicate: sterile controls, biomass covered bed-material from the anaerobic reactor under anaerobic conditions, sediment from a local brackish wetland under anaerobic conditions, and the sediment from the brackish wetland under aerobic conditions. The sterile controls were sterilized by autoclaving the bottles for 15 minutes at 121 °C at 15 psi. The medium to be added to these bottles was filter-sterilized using filters with a pore size of 0.45 µm. Sterile technique was used to prepare these controls. For the anaerobic conditions, 40 mL of medium and the neutralized naphthenic acids were added to the bottles, then the bottles were flushed with ca. 5 headspace volumes of nitrogen. To test the bioreactor bed material, two biomass-covered beads were removed from the reactor and added to the bottles. The wetland sediment was collected in a grab sample from the Kelly's Slough National Wildlife Refuge located northwest of Grand Forks, North Dakota. Approximately 0.5 g of sediment was added to two anaerobic bottles (50 mL, flushed with nitrogen) and to two aerobic bottles (100 mL, flushed with air). The naphthenic acids were added as a concentrated neutralized aqueous emulsion at a dose of 100 mg/bottle. The bottles were all sealed with Teflon-faced butyl-rubber septa and incubated statically at 35 °C for 35 days. After incubation was complete, the bottles were analyzed for naphthenic acids.

Acetate Analysis

Acetic acid was determined in diluted supernatants by high-performance liquid chromatography (HPLC) using an ion exclusion column for organic acid separation and detection by absorption at 210 nm. The organic acid analysis system consisted of a Sierra Separations ion exclusion column (300-mm × 7.8-mm diameter). The eluent was 0.003 N sulfuric acid pumped (ISCO LC-5000 syringe pump) at 0.60 mL/min. The detection was at 210 nm (Kratos model 770R variable ultraviolet detector). Chromatographic data were recorded and integrated with an HP 3390A integrator. An aliquot (0.50 mL) of the supernatant from centrifugation of the sample was diluted with 3.00 mL of water. The diluted sample was loaded from a syringe into a 50-µL injection loop and chromatographed on the described system at ambient temperature. The system was calibrated with fresh standard acetic acid solutions.

Extraction and Analysis of Naphthenic Acid Samples

The aqueous media was poured from the culture bottle into a 250-mL separatory funnel and the bottle rinsed with 10 mL of deionized water, which was added to the separatory funnel. Concentrated HCl (0.5 mL) and the internal standard (0.5 mL of anisole solution in methanol) were added to the separatory funnel. The culture bottle was further rinsed with dichloromethane (35 mL) which was then added to the separatory funnel and shaken with the aqueous media. After allowing the phases to separate, the lower layer consisting of dichloromethane and, frequently, an emulsion were removed. The rinsing and extraction were repeated with two more 35-mL portions of dichloromethane. The combined dichloromethane and emulsion layers were shaken with 25 mL saturated NaCl solution. After settling, the dichloromethane solution was removed and stored over anhydrous Na₂SO₄. This helped remove the remaining emulsion. The dichloromethane was removed in a rotovap at low temperature (45 °C). The residue was redissolved in 5 mL of dichloromethane and analyzed by gas chromatography (GC).

Gas Chromatography

The GC of naphthenic acids was performed with a J&W 60-m $\times$ 0.25-mm DB1701 (1- μ m phase thickness) column, with a split injection ratio of 20:1, and the following oven temperature heating profile: initial 80°C for 2 min; then a rate of 2°/min to 250°C at a column flow of 3 mL/min at 80°C with flame ionization detection. The GC was calibrated with anisole as the internal standard. The area ratio for the calibration was obtained by dividing the total of the peak areas of the carboxylic acids by the peak area of the anisole. This gave a linear calibration curve when the naphthenic acid concentration was kept in the range 15–25 mg/mL. When the naphthenic acid concentration of the sample is in this range, the relative standard deviation is about 10%. The relatively narrow usable concentration range for the samples result from the incomplete resolution of the many acid components. The anisole elutes earlier than the acid components. Thus there is always some concentration-dependent baseline slope that limits the precision of the calibration.

RESULTS AND DISCUSSION

Bioreactor Operation

The adaptation phase of bioreactor operation was initiated in June of 2000. In September 2000, the feed to the reactor was switched from daily batch to continuous at 430 mL/day (HRT ca. 1.4 days). During this phase, good quantities of gas were evolved from throughout the reactor bed. During this adaptation time, the feed to the reactor contained about 1 g/L glucose aid in stimulating microbial growth. At that time, the feed was gradually switched from 100% simulated produced water to 50% simulated produced water and 50% modified methanogenic media (Table 2). The addition of the modified methanogenic media was to allow for better growth of the salt-requiring methanogens that had been seeded into the reactor.

In November of 2000, solubilized naphthenic acids were added to the feed. These naphthenic acids were solubilized by neutralizing the acidity (reported as 258) with potassium hydroxide and vigorously mixing them with water. Near the end of this adaptation phase, glucose was eliminated from the feed, resulting in a loss of visible gas evolution. This was not of concern at the time, as the amount of methane expected from acetate alone is much less than that for glucose plus the acetate (Equations 1 and 2 show the methanogenic stoichiometry for glucose and acetate, respectively). After a few months of operation without any sign of gas production, the feed was changed from 50% simulated produced water and 50% modified methanogenic media to 100% methanogenic media. This was done to see if the level or type of salts in the simulated produced water, or the naphthenic acids, might be inhibiting methanogenesis. In addition, the reactor was reinoculated with the two salt-requiring methanogenic strains (see methods above). Despite these changes, only very small quantities of gas were noted.

Periodically, from January to May of 2001, the influent and effluent of the bioreactor were analyzed for acetate. These data are shown in Figure 1. Until about the middle of February (Day 260), the acetate concentration of the modified methanogenic media was 8.2 g/L (as the sodium salt). Because this concentration was much higher than is expected in produced water, the concentration was decreased to 1 g/L. Some increases in acetate might be expected early in the analyses, because of fermentation of the glucose. However, real losses of acetate did not appear to occur. Even after a short period of adaptation from when the acetate concentration was decreased, the losses of acetate are small. These small losses or increases in acetate are apparent in Figure 2, which shows the difference between influent and effluent acetate concentrations. Although toluene was not analyzed for in this project, the influent had about 87 $\mu\text{g/L}$ and smelled strongly of toluene. Effluent samples had the same sulfide odor as the feed, but had no discernible odor of toluene.

The results have shown that gas production in simulated produced water can occur when glucose is present, but did not occur when it was removed. This suggests that, under the test conditions, acetate-utilizing methanogens were absent or inhibited, while those that can use glucose were not.

Biodegradation of Naphthenic Acids

The results of the batch experiments for naphthenic acid biodegradation are shown in Table 5. The data show that no loss of naphthenic acids occurred either in the sterile control or in the anaerobic treatments. The aerobic treatment did show a loss in total naphthenic acids by GC analysis

Figure 1. Influent and effluent acetate concentrations in the anaerobic bioreactor.

Figure 2. Plot of the difference between influent and effluent acetate concentrations in the anaerobic bioreactor.

TABLE 5

Results of the Batch Naphthenic Acid Biodegradation Tests	
Condition	Naphthenic Acid, mg
Sterile Control	104.5 ± 10.6
Anaerobic Bioreactor	112.5 ± 16.3
Anaerobic Sediment	112.0 ± 4.2
Aerobic Sediment	92.0 ± 9.9

of about 12.5 mg. Figure 3 shows a GC chromatogram of the sterile control. Compare this with the chromatogram for the aerobic treatment, shown in Figure 4. These data show the loss of a series of large peaks from Figures 3 to 4 and the appearance of a few early eluting peaks. To investigate this, a quantity of naphthenic acid extract from the sterile control was methylated with diazomethane and analyzed by GC with mass spectrometry detection (Finnigan ion trap mass spectrometer). This showed that the large peaks that disappeared with aerobic treatment consisted of a set of C9 to C14 fatty acids. Considering that the bulk of the naphthenic acids are said to be composed of cyclic and substituted aromatic carboxylic acids (see references above), the biodegradation observed is probably

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not significant. However, the results of this experiment showed that the naphthenic acids are probably stable under anaerobic conditions.

CONCLUSIONS

The results of these experiments have shown that an anaerobic bioreactor can be bioactive. The reactor showed bioactivity in terms of growth of biomass that was greater at the influent end of the reactor, in terms of loss of toluene in the reactor, and in that when glucose was used, rapid gas production was noted. Apparently, however, acetate methanogenesis was either inhibited under these conditions or appropriate microorganisms were not present, despite seeding.

The experiments on biodegradation of the naphthenic acids showed that the naphthenic acids were not reduced in the sterile controls or in both anaerobic conditions. Loss of naphthenic acids was noted in the aerobic experiment, although it appeared to be loss of straight-chain fatty acids, rather than cyclics or aromatics.

Despite poor performance, it remains likely that anaerobic treatment of acetate, toluene and, potentially, other produced water pollutants is technically feasible. This possibility is based on the literature of methanogenesis in many other saline environments. Also, gas production did occur when glucose was present.

REFERENCES

- Bilstad, T. and E. Espedal. 1996. Membrane Separation of Produced Water. *Wat. Sci. Technol.* 34:239–246.
- Davis, J.B. 1967. *Petroleum Microbiology*, Elsevier Publishing, NY.
- Dzidic, I., A.C. Somerville, J.C. Raia, and H.V. Hart. 1988. Determination of Naphthenic Acids in California Crudes and Refinery Wastewaters by Fluoride Ion Chemical Ionization Mass Spectrometry. *Anal. Chem.* 60:1318–1323.
- Fan, T. 1991. Characterization of Naphthenic Acids in Petroleum by Fast Atom Bombardment Mass Spectrometry. *Energy Fuels* 5:371–375.
- Fathepure, B.Z. and J.M. Tiedje. 1999. Anaerobic Bioremediation: Microbiology, Principles and Applications, p. 339–396, In D.C. Adriano, J.-M. Bollag, W.T. Frankenburger, Jr., and R.C. Sims (eds.), *Bioremediation of Contaminated Soils*, American Society of Agronomy, Madison, WI.
- Green, J.B., B.K. Stierwalt, J.S. Thomson, and C.A. Treese. 1985. Rapid Isolation of Carboxylic Acids from Petroleum Using High-Performance Liquid Chromatography. *Anal. Chem.* 57:2207–2211.

- Herman, D.C., P.M. Fedorak, and J.W. Costerton. 1993. Biodegradation of Cycloalkane Carboxylic Acids in Oil Sands Tailings. *Can. J. Microbiol.* 39:576–580.
- Herman, D.C., P.M. Fedorak, M.D. MacKinnon, and J.W. Costerton. 1994. Biodegradation of Naphthenic Acids by Microbial Populations Indigenous to Oil Sands Tailings. *Can. J. Microbiol.* 40:467–477.
- Hickey, R.F., R.V. Rajan, J. Shi, and J. Cook. 1998. Treatment of Off-Shore Produced Water Using Biological and Coupled Bio-Ozone Processing, p. 1195–1208, *In Proceedings of the 5th International Petroleum Environmental Conference*, Oct. 20–23, 1998, Albuquerque, NM.
- Hsu, C.S., G.J. Dechert, W.K. Robbins, and E.K. Fukuda. 2000. Naphthenic Acids in Crude Oil Characterized by Mass Spectrometry. *Energy Fuels* 14:217–223.
- Jones, F.V., A.J.J. Leuterman, and I. Still. 2000. Discharge Practices and Standards for Offshore Operations Around the World, p. 903–938, *In Proceedings of the 7th International Petroleum Environmental Conference*, Albuquerque, NM, November 7–10, 2000.
- Karman, C.C. and H.G. Reerink. 1998. Dynamic Assessment of the Ecological Risk of the Discharge of Produced Water from Oil and Gas Producing Platforms. *J. Haz. Mat.* 61:43–51.
- Kharaka, Y.K., L.Y.C. Leong, G. Doran, and G.N. Breit. 1998. Can Produced Water be Reclaimed? Experience with Placerita Oil Field, California, p. 1145–1169, *In Proceedings of the 5th International Petroleum Environmental Conference*, Oct. 20–23, 1998, Albuquerque, NM.
- Lai, J.W.S., L.J. Pinto, E. Kiehlmann, L.I. Bendell-Young, and M.M. Moore. 1996. Factors That Affect the Degradation of Naphthenic Acids in Oil Sands Wastewater by Indigenous Microbial Communities. *Environ. Toxicol. Chem.* 15:1482–1491.
- Oregon Collection of Methanogens. <http://caddis.esr.pdx.edu/OCM/media.html>. Accessed on 8/4/00.
- Sauer, T.C., H.J. Costa, J.S. Brown, and T.J. Ward. 1997. Toxicity Identification Evaluations of Produced-Water Effluents. *Environ. Toxicol. Chem.* 16:2020–2028.
- Schink, B. 1988. Principles and Limits of Anaerobic Degradation: Environmental and Technological Aspects, p. 771–846, *In J.B. Zehnder (ed.), Biology of Anaerobic Microorganisms*, John Wiley and Sons, NY.
- Schmitter, J.M., P. Arpino, and G. Guiochon. 1978. Investigation of High-Molecular Weight Carboxylic Acids in Petroleum by Different Combinations of Chromatography (gas and liquid) and Mass Spectrometry (electron impact and chemical ionization). *J. Chromatog.* 167:149–158.
- Seifert, W.K. and W.G. Howells. 1969. Interfacially Active Acids in a California Crude Oil. *Anal. Chem.* 41:554–562.

- Seifert, W.K. and R.M. Teeter. 1970. Identification of Polycyclic Aromatic and Heterocyclic Crude Oil Carboxylic Acids. *Anal. Chem.* 42:750–758.
- Shepherd, M.C., F.L. Shore, S.K. Mertens, and J.S. Gibson. 1992. Characterization of Produced Waters from Natural Gas Production and Storage Operations: Regulatory Analysis of a Complex Matrix, p. 163–173, *In* J.P. Ray and F.R. Engelhart (eds.), *Produced Water: Technological/Environmental Issues and Solutions*, Plenum Press, NY.
- Speece, R.E. 1996. *Anaerobic Biotechnology*, Vanderbilt University, Nashville, TN.
- Stephenson, M.T. 1992. A Survey of Produced Water Studies, p. 1–11, *In* J.P. Ray and F.R. Engelhart (eds.), *Produced Water: Technological/Environmental Issues and Solutions*, Plenum Press, NY.
- Tibbetts, P.J.C., I.T. Buchanan, L.J. Gawel, and R. Large. 1992. A Comprehensive Determination of Produced Water Composition, p. 97–112, *In* J.P. Ray and F.R. Engelhart (eds.), *Produced Water: Technological/Environmental Issues and Solutions*, Plenum Press, NY.
- Wood's Hole Marine Biological Laboratory. <http://www.mbl.edu/html/BB/COMPENDIUM/Comp-Appl.html>. Accessed on 5/4/00.