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# Examination of thirteen petroliferous formations for hydrocarbon-utilizing sulfate-reducing microorganisms

A Azadpour[1], LR Brown[2] and AA Vadie[3]

[1]*Dynamac Corporation, Ada, OK;* [2]*Biological Sciences Department;* [3]*Petroleum Engineering Department, Mississippi State University*

**Virgin cores and production fluids were obtained from seven wells, ranging in depth from 805 ft to 14 492 ft, and examined for the presence of sulfate-reducing bacteria (SRB) using Rosenfeld's sulfate-reducing medium modified by using crude oil in place of lactate. Cores from an additional six wells, ranging in depth from 1160 ft to 13 337 ft were tested for SRB using the modified Rosenfeld medium and API-sulfate-reducing medium. Produced waters from five of the six wells were tested also. All of the eleven produced water samples were positive for SRB while $H_2S$ production was not detected from the core samples.**

**Keywords:** core analyses; hydrogen sulfide; petroleum reservoirs; sulfate-reducing microbes

Hydrogen sulfide ($H_2S$) has been a bane of the oil industry for many years [10]. While the problems associated with oil and gas soured by the presence of $H_2S$ are well known [4,11,16,40], the origin of the $H_2S$ is not. For example, $H_2S$ can be generated abiotically [29,35] in a number of ways or it can be produced biologically through the action of sulfate-reducing bacteria (SRB) [12,34,37,45,47]. In fact, the souring of oil [8] from three wells in Indonesia was attributed to *in situ* production of $H_2S$ [42]. SRB are also indigenous members of the microbial community in groundwaters [4,28,34,43], marine environments [24], coastal sediments [2], marine hydrothermal vents associated with volcanic or tectonic activity [35], and hot springs [21,44,45]. It has been reported that the occurrence of SRB in oil-fields is closely associated with the secondary recovery practice of water flooding, where surface water is injected into reservoirs [10,46]. Left open, however, was the question of whether the SRB were indigenous [3,19,25,32] to the oil-bearing formation or whether they were introduced [5,16,38] through exploration activity. Early reports suggest that SRB are indigenous to oil reservoirs [23]. The precautions to prevent contamination of samples by drilling fluids, etc [30] were not clearly delineated, however, and thus it is difficult to assess the validity of these claims. It was generally thought that SRB would not grow on hydrocarbons but recent publications suggest that they can [1,6,15,17,33], increasing the chance that SRB will grow in the petroleum reservoir. Many of the reports of SRB in petroliferous formations are the result of analyses of formation waters [9,19,41,45] rather than of cores from the formation. There is, however, a report by Beck [5] that suggests that SRB are not indigenous to oil reservoirs. He reported that SRB were absent in samples of oil-producing sand obtained as wells were being drilled but were present in over 100 samples of produced water. Regardless of SRB origin in petroleum reservoirs, their deleterious activities, which result in enormous economic loss and health related problems, should be avoided [40]. Obviously, the origins of the SRB are central to the problem. Therefore, the aim of the present work was to examine the distribution of bacteria from fresh cores and production fluids that could produce $H_2S$ in an oil-based environment.

During the course of this work, sections of fresh cores were received as they were pulled from the core barrels, broken into 1-ft sections, wiped with 70% ethanol, and immediately placed in BBL®GasPak®System containers (Cockeysville, MD, USA) under anaerobic conditions. This procedure was completed within minutes, thus the time of exposure to air was minimal and the literature [7] suggests that SRB would survive for a long term if they were exposed to air at 4°C. It should be pointed out also that pressure in the core tends to force fluids and gases outward, further reducing the possibility of exposing the internal section of the core to air and also preventing the entrance of contaminants into the interior of the cores. The containers were placed in ice, transported to the laboratory, stored in a refrigerator at 4°C and analyzed within 48 h.

When the cores were to be analyzed, they were removed from their containers under an atmosphere of nitrogen, wiped again with 70% ethanol, and cut into 4-in sections using a core saw (Raytech Industries, Stanford Spring, CT, USA). To reach the median of the core, 1 inch was cut from all sides of the core using a sterile core saw blade. The median part of the core was then placed in a stainless steel core crusher under nitrogen gas, and subjected to 20 000 psi using a hydraulic press. The crushed core was placed in a bacteriological hood containing a nitrogen atmosphere and assayed for microbial content. No hydrogen sulfide was detected in any of the cores.

When stratal materials were examined microscopically, most of the microorganisms in the cores were attached to the stratal material and it was necessary to include some of the suspended stratal matter in every portion of sample employed as inoculum. In order to increase uniformity of inocula, the crushed core material was passed through a

Correspondence: Dr A Azadpour, Dynamac Corporation, 3601 Oakridge Blvd, Ada, OK 74820, USA
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sterile USA Standard Testing Sieve No. 40 (0.4191-mm opening). The medium employed was a modified Rosenfeld sulfate-reducing broth [36] consisting of $FeSO_4 \cdot (NH_4)_2SO_4 6H_2O$, 0.1 g; ascorbic acid, 0.2 g; $K_2HPO_4$, 0.5 g; $(NH_4)_2SO_4$, 1.0 g; crude oil, 10 ml; simulated production water (400%), 25 ml; and distilled water, 965 ml. The simulated production water (400%) was composed of NaCl, 778.0 g; $Na_2SO_4$, 130.0 g; $MgCl_2 \cdot 6H_2O$, 352.0 g; $CaCl_2 \cdot 2H_2O$, 36.0 g; KCl, 11.0 g; $Na_2HCO_3$, 3.2 g; KBr, 1.6 g; $SrCl_2 \cdot 6H_2O$, 0.67 g; $H_3BO_3$, 0.41 g; $Na_2SiO_3 \cdot 9H_2O$, 0.08 g; NaF, 0.05 g; $NH_4NO_3$, 0.03 g; $FePO_4 \cdot 4H_2O$, 0.02 g, and distilled water, 8000 ml. Using the three-tube most-probable-number (MPN) method, three tubes were inoculated with 500 mg, 50 mg, and 5 mg of crushed core samples, respectively. Production fluid samples were prepared by dispensing 0.5, 0.05, or 0.005 ml of reservoir fluid into each of these tubes for the MPN test. Uninoculated tubes and tubes containing an autoclaved production fluid or core sample with a viable SRB culture previously isolated from an oil reservoir brine sample, served as controls. The temperature of incubation was that in the respective reservoirs from which the particular core had been obtained with the exception of core 1 where a temperature of 78°C was employed rather than 115.6°C and core 8 where a temperature of 45°C was employed rather than 87°C. Tubes were observed for a period of 180 days. Sulfide production was shown by the formation of a black iron sulfide precipitate in the broth.

All seven cores had microorganisms capable of growing in Rosenfeld's sulfate-reducing medium containing crude oil (rather than lactate as the carbon source); but none of the core samples produced hydrogen sulfide. Although it is realized that the Rosenfeld medium is not the recommended medium for culturing SRB, samples from other sources (production water, drilling muds, etc) were positive for SRB using this medium. Further, since we were concerned with bacteria that could produce $H_2S$ in an oil-based environment, we used crude oil in Rosenfeld's medium. Also, a recent report indicates that crude oil hydrocarbons were degraded by SRB under anaerobic conditions [41] and SRB can use the byproducts of other organisms that were growing on the crude oil. In previous work in our laboratory, the modified Rosenfeld medium yielded the same results as did API-sulfate-reducing broth [13]. Therefore, it was decided to explore the lack of SRB in cores further. Accordingly, cores from six additional wells that had been collected as described above, were inoculated into API-sulfate-reducing broth prepared in accordance with the formula given in the Difco Manual [13] and into the modified Rosenfeld medium described above. Samples of production water from five of the wells (that came into production shortly after the cores were collected) were used to inoculate tubes of each of these media. Controls were established as described earlier and all tubes were incubated under anaerobic conditions. None of the six core samples was positive for SRB in either the API broth or Rosenfeld's medium while all five samples of production water were positive for SRB in both the API and the Rosenfeld's medium.

While it is virtually impossible to drill a well and obtain cores under completely aseptic conditions, it is possible to drill and to prevent the contamination of the central part of the cores by appropriate methodology [30]. The internal part of a core does not get contaminated if appropriate precautions are employed [48] as described in this paper. Furthermore, only the centermost part of the cores was used for the microbiological examinations. Aside from contamination of cores during drilling activities, three possibilities for the presence of SRB in deep subsurface strata are: (1) introduction during secondary recovery operations [8] since injection water contains viable SRB [18], other microorganisms, and oxygen [16]; (2) introduction by natural fault and oil seep; and (3) survival of the SRB since the formation of the reservoirs. Although studies involving stable isotopes presented evidence on the role of SRB in the precipitation of ancient mineral rocks impregnated with petroleum [14,22,31], survival of ancient SRB in the oil-bearing formations through geologic periods seems unlikely [11]. In regard to the origin of the SRB in the produced waters, it should be pointed out that they are prevalent in most of the drilling muds we have tested and are common inhabitants of many soils.

**Table 1** Viable counts in cores and presence of SRB in production fluids

| Core no. | Reservoir temp (°C) | Viable count in cores (MPN per gram) | Sulfate reducers in production fluids (+,ND)[a] |
|---|---|---|---|
| 1 | 116 | 240 | ND |
| 2 | 32 | 48 | + |
| 3 | 40 | 32 | + |
| 4 | 39 | 1 | + |
| 5 | 32 | >220 | + |
| 6 | 38 | 1 | + |
| 7 | 52 | 92 | + |
| 8 | 92 | <100 | + |
| 9 | 31 | 24 | + |
| 10 | 38 | 15 | + |
| 11 | 57 | <100 | + |
| 12 | 34 | 32 | ND |
| 13 | 34 | 93 | + |

[a]'+' indicates presence of SRB; ND, not determined.

The limited number of tests reported herein are not sufficient to conclude that SRB are absent in most petroleum reservoirs and, indeed, it was not the objective of the project to explore this possibility. The lack of detection of SRB does not mean that they do not occur in some oil reservoirs. For example, there could have been SRB that did not grow on lactate [20] as in the API medium or on oil as in Rosenfeld's medium. Some SRB require acetate, branched fatty acids, or elaborate organic compounds for growth [19]. However, similar numbers of SRB from formation waters of an oil field in the Apsheron were reported on media containing different energy sources including lactate, acetate, propionate, or sodium butyrate [26]. It also is possible that a larger inoculum might have generated different results [27]. Also, the SRB might have been in the ultramicrobacterial form as postulated by some investigators [8,10]; therefore, use of an enrichment culture procedure using sub-normal concentrations of nutrients may have resulted in growth. The presence of viable microorganisms in the samples was not a question since it was observed that each

**Table 2** Characteristics of core samples

| Core no. | Location of well | Type of formation | Cored pay depth (feet) | Porosity (%) | Permeability (md)[a] |
|---|---|---|---|---|---|
| 1 | Monroe Co[b], AL | Dolomite/limestone | 14 492 | 8.43 | <1 |
| 2 | Kern Co, CA | Clay/sandstone | 805 | 32 | 1000–1500 |
| 3 | Andrews Co, TX | Dolomite | 4725 | 8–10 | 0.1–10 |
| 4 | Ector Co, TX | Sandy/dolomite | 4050 | 10–17 | 5–11 |
| 5 | Lea Co, NM | Dolomite | 4300 | 8–10 | 3 |
| 6 | Crane Co, TX | Dolomite | 2705 | 8–10 | 3 |
| 7 | Johnson Co, WY | Sandstone | 6568 | 13.3 | 3.3 |
| 8 | Conecuh Co, AL | Smakover | 13 337 | 1–5 | 5–11 |
| 9 | Kern Co, CA | Dolomite | 1160 | 55 | 1000–2000 |
| 10 | Mitchell Co, TX | Dolomite | 3022 | 5–10.9 | 0.8–9.2 |
| 11 | LA Co, CA | Sandstone/clay sale | 2964 | 25–30 | 1200 |
| 12 | Lamar Co, AL | Sandstone | 2250 | 5–19 | 10–110 |
| 13 | Lamar Co, AL | Limy sand | 2500 | 12 | 2–22 |

All of the cores were taken from wells drilled into known oil fields but none of the fields had been exposed to EOR procedures.
Porosity and permeability values resulted from three replications.
[a]Millidarcy.
[b]County.

core had viable microorganisms (Table 1) recoverable using normal microbiological procedures. Essentially most (92%) of the isolates from the cores could use crude oil as a sole carbon source and produced one or more of the following by-products: gases, acids, fatty acids, and surfactants. Some of the isolates could use *n*-hexadecane, acetate, or molasses as a carbon source and ammonium ion, nitrate ion, or urea as a sole nitrogen source (Azadpour *et al*, in preparation). It is also interesting that the cores examined in this investigation were obtained from a wide variety of geographical locations, depths (Table 2), and types of formations, but none contained hydrogen sulfide.

Before now, SRB have been considered to be normal inhabitants of oil reservoirs and as a consequence their deleterious activities are simply an expected part of the oil and gas business. Preventing their activities or repairing damage they have caused is a major expense to the industry. However, the data presented in this paper coupled with that of Beck [5] and Nazina [39], suggest that the absence of SRB in petroliferous formations may be commonplace. If true, the SRB causing the problems are introduced into the reservoir through drilling activities, maintenance operations, or waterflooding. Thus by developing strategies to prevent the introduction of the SRB into the formations, the expensive problems they cause can be obviated.

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