**Title and Subtitle**
LABORATORY STUDIES ON BIODEGRADATION OF ORGANICS IN THE SOUTH TANK FARM PLUME (STFP) AQUIFER, SOIL AND MICROBIOLOGICAL ANALYSES OF STFP AQUIFER CORE SAMPLES

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**Performing Organization Name(s) and Address(es)**
SHELL OIL COMPANY
DENVER, CO

**Performing Organization Report Number**
911000R01

**Sponsoring/Monitoring Agency Name(s) and Address(es)**

**Supplementary Notes**
11. SUPPLEMENTARY NOTES

**Distribution/Availability Statement**
APPROVED FOR PUBLIC RELEASE; DISTRIBUTION IS UNLIMITED

**Abstract**
13. ABSTRACT (Maximum 200 words)

THIS REPORT CONTAINS A BASE SET OF DATA ON MICROBIOLOGICAL AND CHEMICAL CHARACTERISTICS OF REPRESENTATIVE SOIL CORES TAKEN FROM THE SOUTH TANK FARM PLUME (STFP). FIVE SOIL CORES WERE ANALYZED FOR VARIOUS BACTERIAL TYPES AND SOIL CHARACTERISTICS (PH, SOIL CLASS, MINERAL NUTRIENTS).

THE BASIC SOIL DATA INDICATE THAT SIGNIFICANT NUMBERS OF BACTERIA AND LEVELS OF NUTRIENTS ARE PRESENT IN THE STFP GROUND WATER TO ENHANCE THE AEROBIC BIODEGRADATION OF ORGANIC COMPOUNDS IN THE AQUIFER.

**Subject Terms**
14. SUBJECT TERMS
GROUNDWATER CONTAMINATION

**Security Classification of Report**
17. SECURITY CLASSIFICATION OF REPORT
UNCLASSIFIED

**Security Classification of This Page**
18. SECURITY CLASSIFICATION OF THIS PAGE
UNCLASSIFIED

**Security Classification of Abstract**
19. SECURITY CLASSIFICATION OF ABSTRACT
UNCLASSIFIED

**Limitation of Abstract**
20. LIMITATION OF ABSTRACT
UNCLASSIFIED

**Number of Pages**
15. NUMBER OF PAGES

**Price Code**
16. PRICE CODE

**Document Number**
NSN 7540-01-280-5500
LABORATORY STUDIES ON BIODEGRADATION OF ORGANICS IN THE SOUTH TANK FARM PLUME (STFP) AQUIFER

Soil and Microbiological Analyses of STFP Aquifer Core Samples
J. P. Salanitro and H. L. Wisniewski

Summary: Soil and microbial analyses were made on samples of aquifer material taken from borings within the organics plume of the South Tank Farm area (STFP). These soils (pH 7.4-8.3) were classified as loams, silty clays or clayey types and contained low levels of organic carbon (<0.1 or 0.2%) and varying concentrations (3-120 ppm) of available nutrients (Fe, NH₄⁺-N, PO₄³⁻-P). Enumeration of culturable microorganisms associated with this aquifer material indicated that aerobic and facultatively anaerobic bacteria were present (10⁵-10⁹/g wet soil) at all depths (19-25 ft) sampled. Anaerobic bacteria (denitrifiers, sulfate-reducers and methanogens) could not be cultured from any sample. These basic soil data indicate that significant numbers of bacteria and levels of nutrients (NH₄⁺, PO₄⁻³) are present in the STFP groundwater to enhance the aerobic biodegradation of organic compounds in the aquifer.

Introduction

It has previously been reported that the predominant mechanism for the natural remediation of aromatic hydrocarbons in an aquifer is the stimulation of aerobic biodegradation by indigenous soil bacteria. Microbial populations present in aquifer material where these transformations have occurred were primarily-aerobic bacteria. It would be important to establish, therefore, the microbial and nutrient status of the RMA - South Tank Farm plume (STFP) aquifer to evaluate the bio-remediation potential of this groundwater. The current report contains a base set of data on microbiological and chemical characteristics of representative soil cores taken from the STFP. Five soil cores were analyzed for various bacterial types and soil characteristics (pH, soil class, mineral nutrients).

Materials and Methods

Field Site Sampling. Subsurface aquifer samples were taken from five borings in the STFP representing areas in which high or low levels of aromatic hydrocarbons were present in the groundwater. Borings were made with a drilling rig and hollow stem auger (4-6 inch diameter). When desired depths were drilled, two inch X five foot cores were taken using a Waterloo saturated sand sampler with a wireline piston core barrel as described previously. The stainless steel core barrel contained a two inch X five foot polybutyrate (Shelby) tube into which the soil was retained. Aquifer cores were retrieved from depths of 1-5 feet below the top of the water table. After cores were obtained they
were cut in half, capped, sealed, and sent on ice within 24-48 hours to Westhollow Research Center for processing. The field sampling was conducted by MK-Environmental Services of Denver, Colorado.

Chemical and Microbiological Soil Analyses of Aquifer Material. Samples from each boring were sent to Soil Analytical Services, Inc. of College Station, Texas for various soil determinations such as pH, CEC (cation exchange capacity), type classification, moisture and mineral nutrients (Fe, NH₄, PO₄³⁻).

Microbial populations in soil were enumerated by tube extinction dilution culture methods. Endpoints of serial 1:10 dilutions of a soil inoculum made into culture media were the highest dilution showing turbid or microscopically discernible cell growth (aerobes, anaerobes), FeS precipitation from H₂S-producing sulfate-reducing bacteria, methane formation in the headspace of culture vials for methanogens or loss of NO₃⁻ for denitrifiers. Denitrifiers, sulfate-reducers and methanogens were estimated by culturing in denitrifying medium (0.8% Difco Nutrient Broth, 0.1% KNO₃, and 0.5% succinate; modified Postgate medium E supplemented with 0.1% sodium acetate and Balch medium I, respectively. Total anaerobes were determined by turbidity and microscopic examination of growth in soil dilutions of modified Postgate E and Balch I media. Soil cultures were incubated at 30-35°C for 14 days.

Results and Discussion

Soil Analyses. Table 1 is a summary of physical and chemical characteristics of the soil cores taken from the STFP aquifer. These saturated soils are slightly alkaline (pH 7.4-8.0), contain low levels of organic carbon (<0.1 or 0.2%) and are classified as a loam, silty clay loam or clayey types. The CEC for the samples varied from 14-53 meq cations/100g and was similar to those reported for loams, silty clays and clayey soils. 7) The soluble Fe and Mn content varied among the five soil cores from 10-86 ppm. NH₄⁺-N was 2.5 - 9.6 ppm and available PO₄³⁻-P concentrations were 2.5 - 117 ppm. The total phosphorus (P) content was variable (155 - 1274 ppm) between samples. The NO₃⁻-N levels were less than the detectable level of 1 ppm and the organic-nitrogen (TKN) content was also low (0.07-0.2%).

Soil Microbial Populations. Soils cultured on various media for viable aerobic and anaerobic bacteria showed (Table 2) that aerobes and facultative anaerobes (microbes capable of growth under high or low dissolved oxygen conditions) were present at levels 10³-10⁴/g wet soil. Similar levels of these microbes were determined from aquifer material taken where the groundwater containing high (≥ 40 ppm) or low (ppb) concentrations of aromatic hydrocarbons. These results indicate that high levels of hydrocarbon in the groundwater have not adversely affected the viability of microbes in the saturated zone. It should be noted that very similar levels of aerobic bacteria have been cultured from sandy aquifer material from Michigan and Florida. 3, 8) Significant numbers of
other anaerobic and obligately anaerobic bacteria such as denitrifiers, sulfate-reducers and methane-formers could not be cultured from any soil core. These findings also confirm that the STFP is predominately an oxygenated aquifer containing primarily aerobic microorganisms.

References

1) Salanitro, J. P. and H. L. Wisniewski. Laboratory Studies on Biodegradation of Organics in South Tank Farm Plume Aquifer Samples. RMA Project Status Report 6-90.


Table 1
Physical and Chemical Soil Analyses on Aquifer Core Material from the STFP

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>7.8</td>
<td>7.9</td>
<td>7.7</td>
<td>7.4</td>
<td>8.3</td>
</tr>
<tr>
<td>% Moisture</td>
<td>18</td>
<td>28</td>
<td>29</td>
<td>29</td>
<td>23</td>
</tr>
<tr>
<td>CEC, meq/100g</td>
<td>24</td>
<td>53</td>
<td>52</td>
<td>48</td>
<td>14</td>
</tr>
<tr>
<td>Texture %</td>
<td>13/53/33</td>
<td>42/30/27</td>
<td>44/30/26</td>
<td>43/33/23</td>
<td>39/18/43</td>
</tr>
<tr>
<td>Sand/Silt/Clay</td>
<td>silty clay</td>
<td>loam</td>
<td>loam</td>
<td>loam</td>
<td>clay</td>
</tr>
<tr>
<td>Organic Carbon, %</td>
<td>&lt;0.1</td>
<td>&lt;0.1</td>
<td>&lt;0.1</td>
<td>&lt;0.1</td>
<td>&lt;0.2</td>
</tr>
<tr>
<td>Minerals, ppm</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fe</td>
<td>19</td>
<td>62</td>
<td>86</td>
<td>50</td>
<td>10</td>
</tr>
<tr>
<td>Mn</td>
<td>29</td>
<td>53</td>
<td>31</td>
<td>61</td>
<td>22</td>
</tr>
<tr>
<td>NH₄⁺-N</td>
<td>9.5</td>
<td>6.5</td>
<td>8.3</td>
<td>2.5</td>
<td>5.3</td>
</tr>
<tr>
<td>PO₄³⁻-P</td>
<td>2.4</td>
<td>79</td>
<td>117</td>
<td>62</td>
<td>42</td>
</tr>
<tr>
<td>Total P</td>
<td>177</td>
<td>155</td>
<td>1274</td>
<td>1146</td>
<td>371</td>
</tr>
</tbody>
</table>

* In all soil samples NO₃-N was <1 ppm and the TKN varied 0.07-0.2%.
Table 2

Enumeration of Microbial Populations in Aquifer Core Material from the STFP

<table>
<thead>
<tr>
<th>Core Sample (ft) a)</th>
<th>No. of Bacteria/g Wet Soil</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Aerobes</td>
</tr>
<tr>
<td>1) 01552-B (19-21)</td>
<td>$10^4$</td>
</tr>
<tr>
<td>2) 01588-B (19-21)</td>
<td>$10^3$</td>
</tr>
<tr>
<td>3) 01588-B (21-23)</td>
<td>$10^4$</td>
</tr>
<tr>
<td>4) 02506-B (23-25)</td>
<td>$10^7$-$10^8$</td>
</tr>
<tr>
<td>5) 02579-B (17-19)</td>
<td>$10^4$</td>
</tr>
</tbody>
</table>

a) Depth below ground level. Cores were taken 1-5 ft below the top of the water table.

b) Other anaerobic bacteria (denitrifiers, sulfate-reducers and methane-forming organisms) could not be cultured from any sample (less than the detection level of $10^4$/g).

c) Total aromatic hydrocarbons in ground water samples were $\geq$ 40 ppm (high) or ppb levels (low).