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**EFFECTS OF SELECTED THERMOPHILIC MICROORGANISMS ON  
CRUDE OILS AT ELEVATED TEMPERATURES AND PRESSURES**

**1989 Annual Report**

**May 1990**

**Performed Under Contract No. AC02-76CH00016**

**Brookhaven National Laboratory  
Upton, New York**



**National Energy Technology Laboratory  
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**EFFECTS OF SELECTED THERMOPHILIC MICROORGANISMS ON  
CRUDE OILS AT ELEVATED TEMPERATURES AND PRESSURES**

**1989 ANNUAL REPORT**

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**May 1990**

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## Abstract

During the past several years, a considerable amount of work has been carried out showing that microbially enhanced oil recovery (MEOR) is promising and the resulting biotechnology may be deliverable. We are carrying out systematic studies dealing with the effects of thermophilic bacteria on the chemical and physical properties of selected types of crude oils at elevated temperatures and pressures comparable to those of reservoir conditions. Current studies indicate that during the biotreatment several properties of crude oils are affected. The oils are (1) emulsified, (2) acidified, (3) there is a qualitative and quantitative change in light and heavy fractions of the crudes, (4) there are chemical changes in fractions containing sulfur compounds, and (5) the qualitative and quantitative chemical and physical changes appear to be microbial species dependent. In order to analyze for these changes in the crudes, several instruments have been purchased and dedicated to this program. The results generated in the past fiscal year describing (1)-(5) are presented and discussed in this report.

## 1. Objectives

The objective of this program is to determine the chemical and physical effects of thermophilic organisms on crude oils at elevated temperatures and pressures. The information gained from these studies will be used to explore the effects of the same set of thermophilic organisms on samples of oil containing sand cores. Thus experimental conditions will match closely those found in reservoirs, i.e., different temperatures, pressures, salinities and permeabilities. As defined by laboratory studies generated in this program the best MEOR candidates will be used for scale up and technical feasibility studies leading to field applications.

## 2. Background

Over the past several years active groups at universities in Oklahoma, Georgia, Southern California, Texas, National Institute for Petroleum and Energy Research, as well as some workers abroad, e.g., England, Australia, Canada, China, Russia and Romania have laid the groundwork for MEOR (King and Stevens, 1986). The past record indicates that MEOR is a promising technology. However, additional work is needed because most field tests were inconclusive or failures. Further, the chemistry and biochemistry of microorganism-oil interactions is by and large unknown and, most bacteria were not tested in the laboratory under reservoir conditions. Nevertheless, available data allow us to suggest several mechanisms for the action of microorganisms in MEOR biotechnology (Bryant and Douglas, 1987). These include: (1) production of gases ( $\text{CO}_2$ ,  $\text{H}_2$ ,  $\text{N}_2$ ,  $\text{CH}_4$ ) which can increase pressure in the reservoir and reduce oil viscosity; (2) microbial production of low molecular weight acids, which cause rock solubilization; (3) production of biosurfactants which decrease surface and interfacial tensions; (4) microbially mediated changes in wettability; and (5) production of polymers which facilitate mobility. It has been suggested (Gruha, 1986) that some of the desirable properties of bacteria to be used in MEOR should include microorganisms capable of large production of "oil releasing" metabolites (e.g., low molecular weight alcohols, acids, surfactants and gases); they should not require expensive nutrients, they should be anaerobic and/or be capable to grow in the presence of low oxygen concentrations and be able to withstand relatively high pressures and salinities. Also, such microorganisms should be easily grown in facilities above ground, remain viable over extended time and be transportable. Once underground they should continue to be viable

and continue their activity upon re-feeding. At the 1986 First International MEOR Workshop, it was recommended (Donaldson, 1986) that a search be conducted for anaerobic and thermophilic bacteria capable of breaking down heavy oils under reservoir conditions. At the same workshop (1986), it has also been recognized that the use of microorganisms in well bore clean out is also promising, although additional research in the areas dealing with control of sulfate reducing bacteria, corrosion prevention, reduced production of H<sub>2</sub>S and well plugging is still needed.

A number of microorganisms have been found to be present in formation waters and were viable under certain reservoir conditions (Lazar, 1986). The usefulness of these bacteria appears to be limited because, for these organisms to grow, reservoirs have to be within a given depth, salinity, temperature and permeability range and also be resistant to the increases in the concentration of products generated by their action. Thermophilic organisms live under harsh conditions, such as low pH (~1-3) and high temperatures (up to 110°C) and some are known to grow under alkaline conditions (Brock, 1978 and 1985). These organisms can use inorganic and organic energy sources, (e.g., sulfides, elemental sulfur, ferrous ion). Some of these bacteria are also capable of switching from an aerobic to anaerobic metabolism. Further, the natural habitats of these organisms are geothermal brines which means that they are tolerant of high salt concentrations. Thus, the general properties of thermophilic organisms, although not fully explored, already indicate that they possess a number of desirable properties for MEOR as recommended at the 1986 Workshop and further verified in a recent status report (King, 1987).

There are several advantages in using thermophilic chemotrophic organisms:

1. Active at high temperature and pressures
2. Use constituents indigenous to the substrate
3. Withstand pH extremes
4. Tolerate high salt concentrations
5. Biodegrade high molecular weight compounds

In this program, experimental strategies are being developed which allow selected microorganisms to adapt to conditions in which practically the sole source of energy is the indigenous crude oil. We are also conducting a detailed study of the chemistry and biochemistry of interaction between thermophilic, acidophilic, chemotrophic organisms and oils and cores whose chemical and physical properties are known and explore the feasibility of MEOR biotechnology based on thermophilic microorganisms. In this report we shall discuss briefly our earlier findings (Premuzic and Lin, 1989a-1989e) which have indicated that the biotreatment of crude oils at elevated temperatures and pressures causes emulsification, lowering of pH, qualitative and quantitative changes in the composition of low and high molecular fractions of crude oils, as well as in the composition of fractions containing sulfur compounds. Results of recent studies will also be discussed in this report.

### 3. Experimental

Interactions between microorganisms and crude oils are complex and involve multiple processes. In order to understand better the chemistry and biochemistry of these process extensive studies and analyses of several variable parameters are needed. To accomplish some of these requirements we have expanded and/or modified our instrumental capabilities, so that as many variable parameters can be measured at any given time. Expansion and modification of instrumentation capabilities is carried out as necessary. Since such development is an integrated part of the program, the following description of the instrumentation capabilities is warranted.

#### (i) Instrumentation.

A Perkin-Elmer model 8700 gas chromatograph has been purchased and installed as a dedicated instrument for MEOR studies. This instrument is a microprocessor-controlled gas chromatograph with multiramp temperature programming, soft key entry of all temperature and time parameters on color VDU, dual-channel screen graphics, dual-channel integral data handling, and reintegration. Four internal and four external timed events and two RS-232C communications ports are also available. There is an access to ten methods and Automated Bleed Compensation.

Additional features of the gas chromatograph are:

1. Left channel split/splitless capillary injector.
2. Right channel single packed column injector (1/8 in.).
3. Left channel single Flame Ionization Detector. Includes amplifier and combustion gas pneumatics, air, and H<sub>2</sub>.
4. Right channel single Flame Photometric Detector. Includes amplifier and combustion gas pneumatics, air, and H<sub>2</sub>.

5. Left channel pneumatics, capillary pressure control (0-100 psig).
6. Right channel pneumatics, flow controller backed by pressure regulator.
7. The system is also equipped with a Flame Ionization Detector (FID) and a Flame Photometric Detector (FPD).

A Perkin-Elmer Iron Trap Detector (ITD) has also been purchased, installed and interphased with the Perkin-Elmer model 8700 gas chromatograph (GC). The Iron Trap Detector System has a mass range of 20-650 mass units and has the following features:

1. Heated fused silica transfer line
2. Calibrated leak to introduce calibration compounds
3. Air-cooled turbomolecular pump and direct-drive mechanical pump
4. Software package for detector control
5. Data acquisition; display of chromatograms and spectra, quantitation reports and diagnostics
6. Incos library search program and 3000 compound mass spectral library
7. Epson Equity III+ computer system contains: PC/AT class performance with a 80286 CPU; 640 kB RAM; 40 mB Winchester disk drive; 1.2 mB, 5.25 inch floppy disk drive.
8. Color CGA monitor
9. Epson printer/plotter and all required cables and interfaces.
10. The system also encompasses NBS/EPA Mass Spectral Library containing over 42,000 mass spectra.

The interphased system has been installed in a separate room specifically converted for computerized analytical work.

A typical example of a GC/MS analysis of a crude oil is shown in Figure 1 (ITD trace) with selective peak fitting generated by computerized search of the NBS/EPA mass spectral library, shown in Figure 2 for scan number 3702. The GC system is also equipped with a flame ionization detector (FID) and flame photometric detector (FPD). The FID detector is now operative, and a typical trace for the Recluse, WY, untreated oil is given in Figure 3.

(ii) Growth and Adaptation of Microorganisms

Small scale bioreactors have been made out of stainless steel tubes (Premuzic and Lin 1988), can handle a total volume of 20 ml of fluid and can be re-used many times. Typically in each experiment, three bioreactors are used. Thus, one bioreactor is charged with nutrients in water, gases ( $\text{CO}_2$ ,  $\text{N}_2$ ) and the experimental organism only. A second bioreactor is charged with oil, gases ( $\text{CO}_2$ ,  $\text{N}_2$ ), and nutrients in water. A third bioreactor is charged with oil, microorganisms, gases ( $\text{CO}_2$ ,  $\text{N}_2$ ), nutrients and water. All are kept under identical experimental conditions of temperature and pressure.

Culture media consisted of inorganic salts e.g.,  $(\text{NH}_4)_2\text{SO}_4$ ,  $\text{MgSO}_4$ ,  $\text{KH}_2\text{PO}_4$ , and crude oil or yeast extract as a source of carbon. Incubations were carried out at different temperatures (30-80°C) and pressures (200-25000 psi). Through a sequence of different experimental regimes a methodology has been developed (patented) which makes it possible to adapt microorganisms to different pressures and temperatures. Several different species and strains of microorganisms have been used in the work described in this report and are listed with the summary of the appropriate treatment in Table 1.

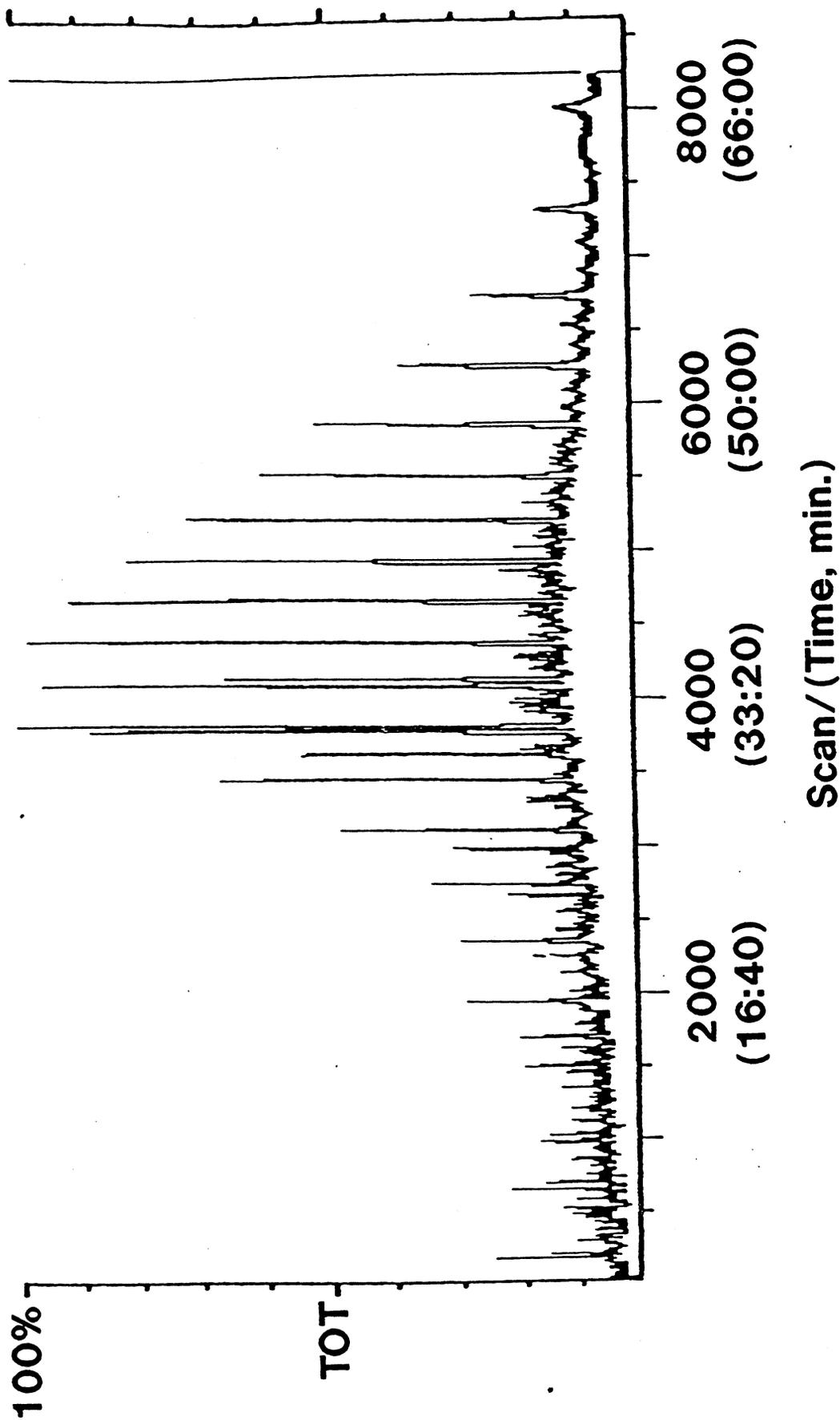


Figure 1. ITD Trace of Recluse WY, untreated crude.

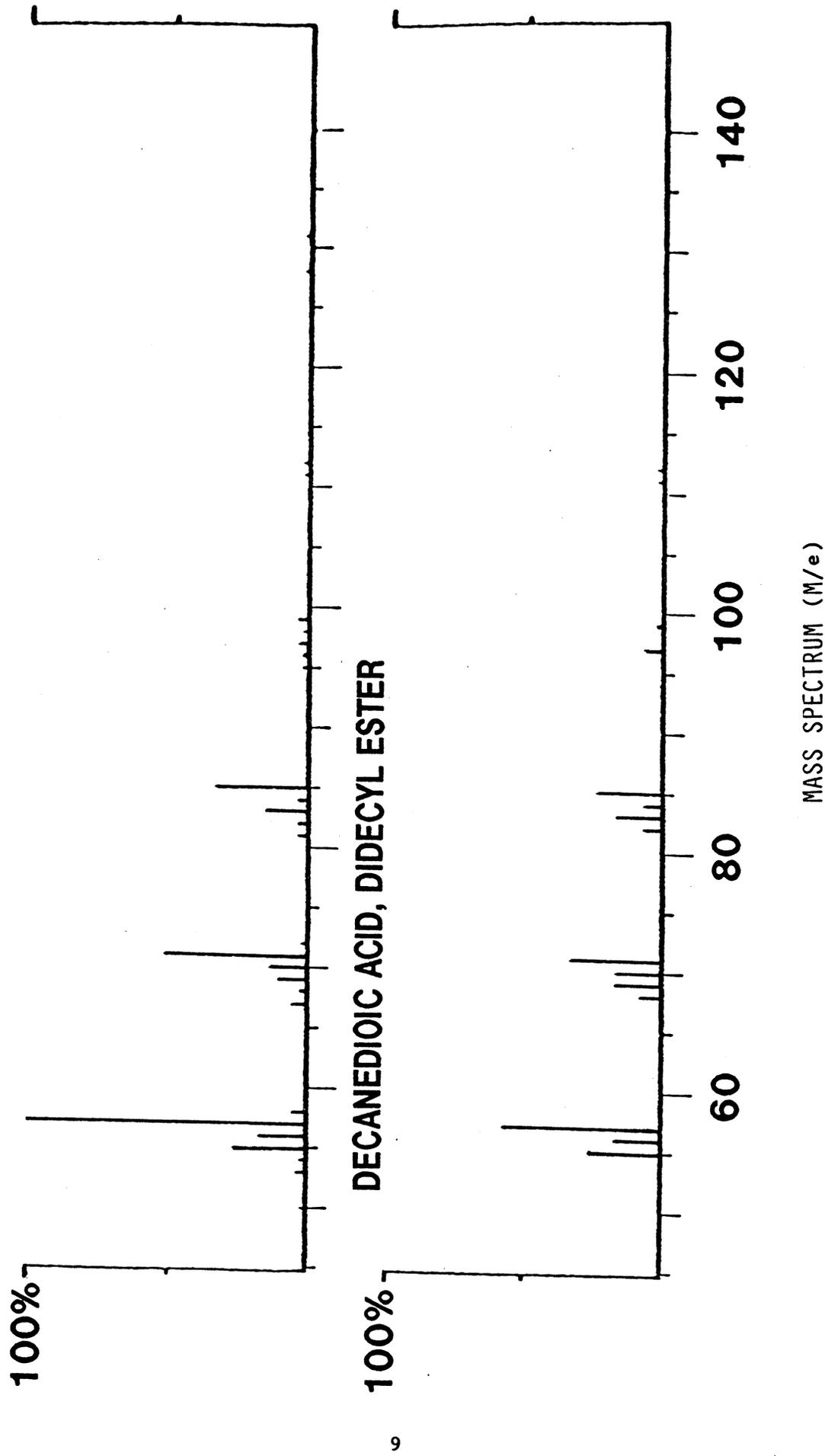


Figure 2. Computerized Library Search of Mass Spectra for Scan 3702.

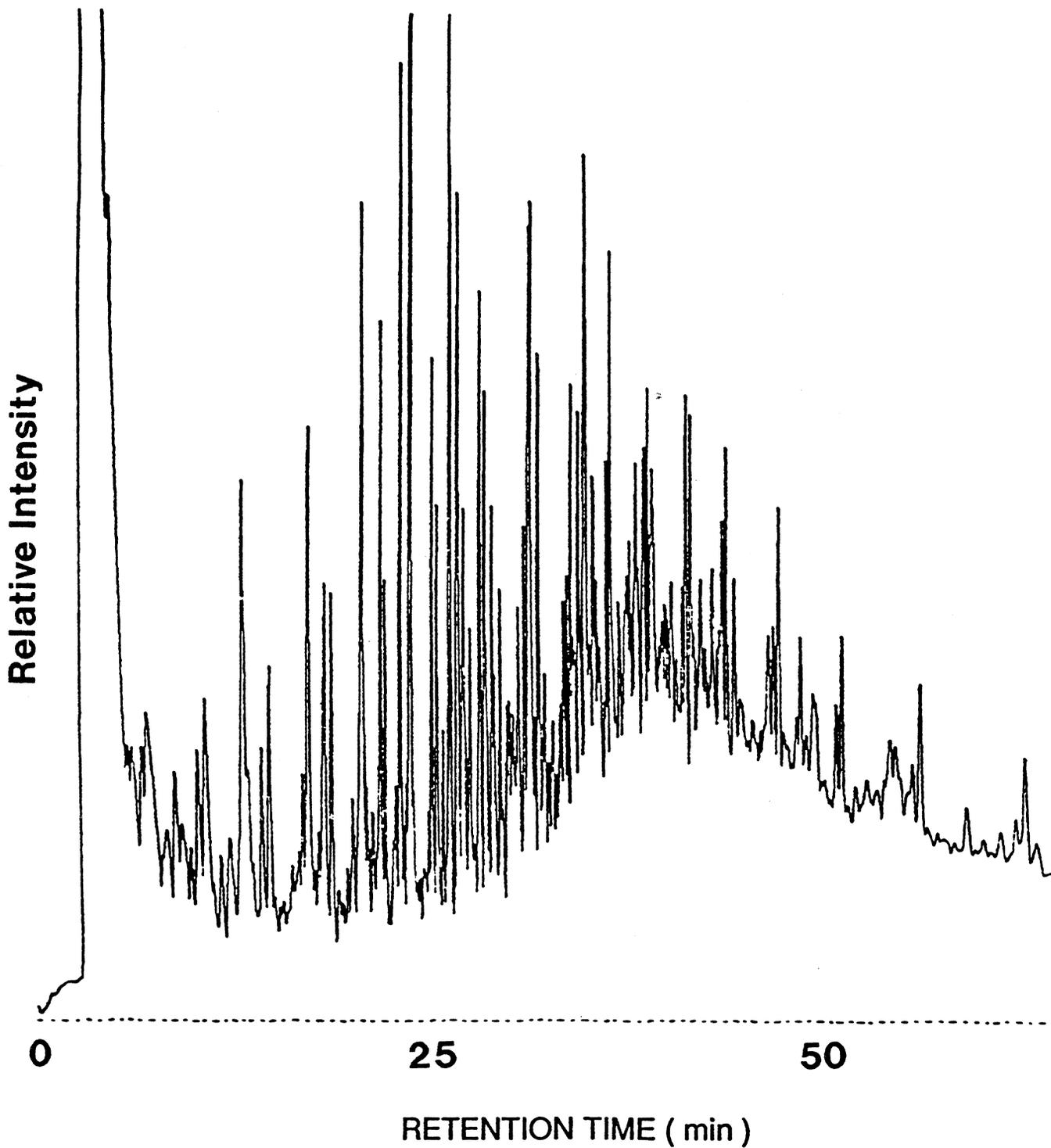


Figure 3. FID Trace of Recluse, WY untreated crude.

Table 1

<u>Microorganism Species</u>	<u>Treatment Conditions</u>			
	<u>Temp. °C</u>	<u>Medium</u>	<u>Pressure psi</u>	<u>pH</u>
BNL-TH-29 <u>Sulfalobus</u>	60-80	A	up to 2000	1.5-4.5
BNL-TH-31 <u>Sulfalobus</u>	60-80	A	up to 2000	1.5-4.5
BNL-4-21 <u>Acinetobacter</u>	25-75	B	Atm.	6-7.5
BNL-4-22 <u>Arthrobacter</u>	25-75	B	Atm.	6-7.5
BNL-4-23 <u>Achromobacter</u>	25-75	B	Atm.	6-7.5
BNL-4-24 <u>Pseudomonas</u>	25-75	B	Atm.	6-7.5
BNL-4-25 <u>Nocardia</u>	30	C	Atm.	7
BNL-5-32 <u>Methanogenium</u>	55-60	D	Atm.	6-7.5
BNL-TH-1 <u>Sulfalobus</u>	60-80	E	up to 2000	1.5-2.5

Medium A is medium 1304, supplemented with a non-peptone modified carbon source, B is a nutrient broth containing beef extract supplemented by a non-peptone carbon source. C medium is a yeast extract medium, D and E media are 1442 and 1256 respectively, also supplemented with non-peptone modified carbon source. In all the cases crude oil becomes the sole carbon source in the final adaptation.

#### 4. Results and Discussion

##### (i) Acidification.

Earlier experimental observations have shown that there is a lowering of pH to 3 (from ~5-7, Premuzic and Lin, 1989c) of the medium during the microbial action on crude oil under our experimental conditions. These results indicated that the aqueous phase should be analyzed for water soluble compounds as possible causes of acidification. An exploratory high pressure liquid chromatographic (HPLC) analysis was carried out with a sample of PR3 (Teapot Naval Petroleum Reserve #3) crude which was treated with BNL-4-22 strain. The result is shown in Figure 4. Preliminary assignments for peaks 8.42, 11.58, 13.84 and 23.87 are lactic, propionic, isobutyric acids and n-butanol, respectively.

Long chain fatty acids may also be produced during the biotreatment of oil. Because such acids are good emulsifying agents, the experiment was repeated and the analyses were directed to the characterization of such acids. This was accomplished by the use of another strain, BNL-TH-1 and PR-3. After the treatment, culture medium was saponified with sodium hydroxide and the free acids methylated with methanol/HCl. The produced methyl esters of fatty acids were extracted and analyzed by GC-MS (See Figure 5). The first three fatty acids identified are heptanoic acid (C-7), decanoic acid (C-10), and octadecanoic acid (C-18).

##### (ii) Emulsification.

In addition to acidification, emulsification of the reaction mixture was also observed. An example of a spectrophotometric analysis of BNL-TH-29 treated PR3 crude oil is shown in Figure 6. This experiment has shown that

INTENSITY (arb. units )

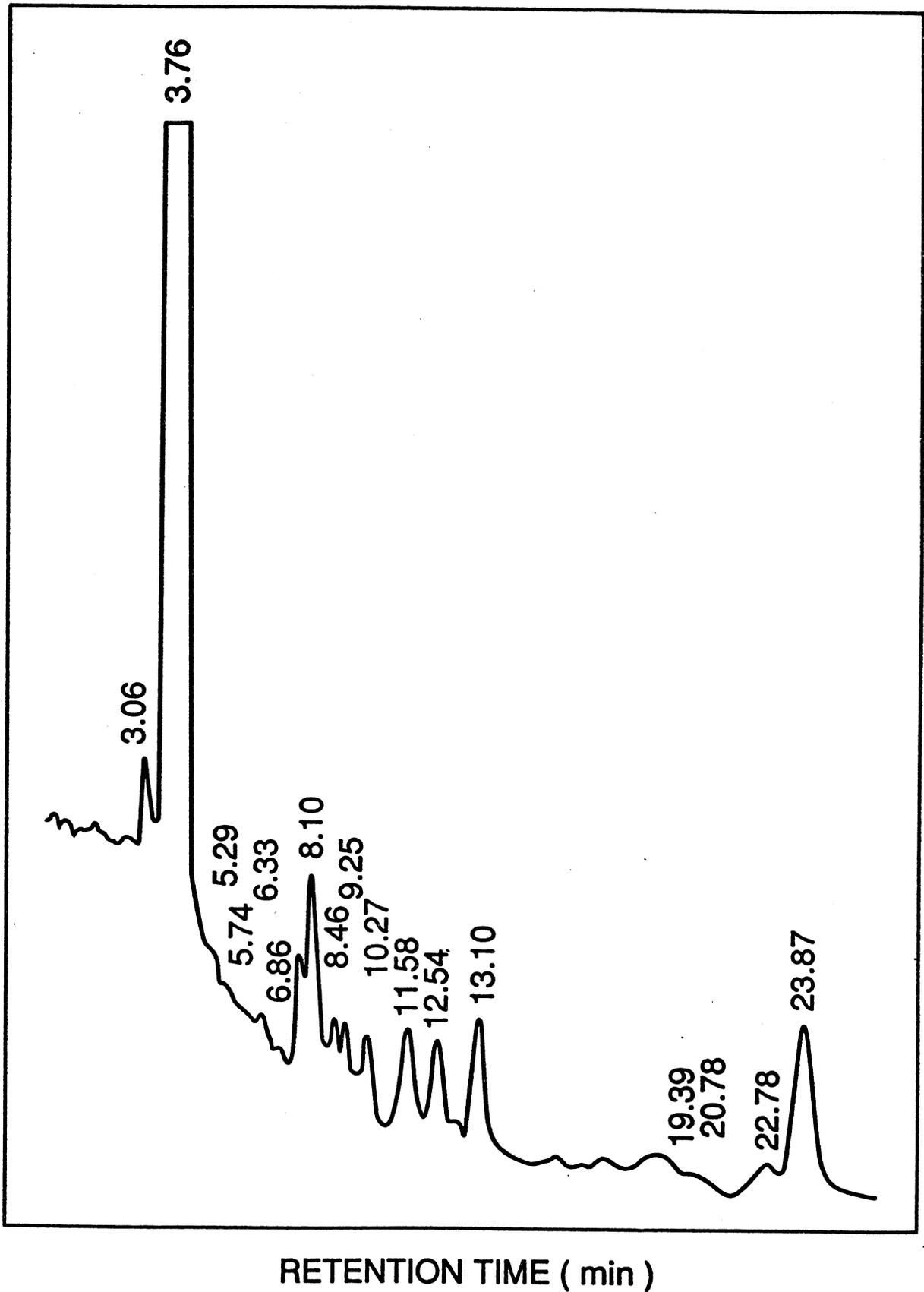


Figure 4. HPLC trace of PR3 + BNL-4-22 + medium.

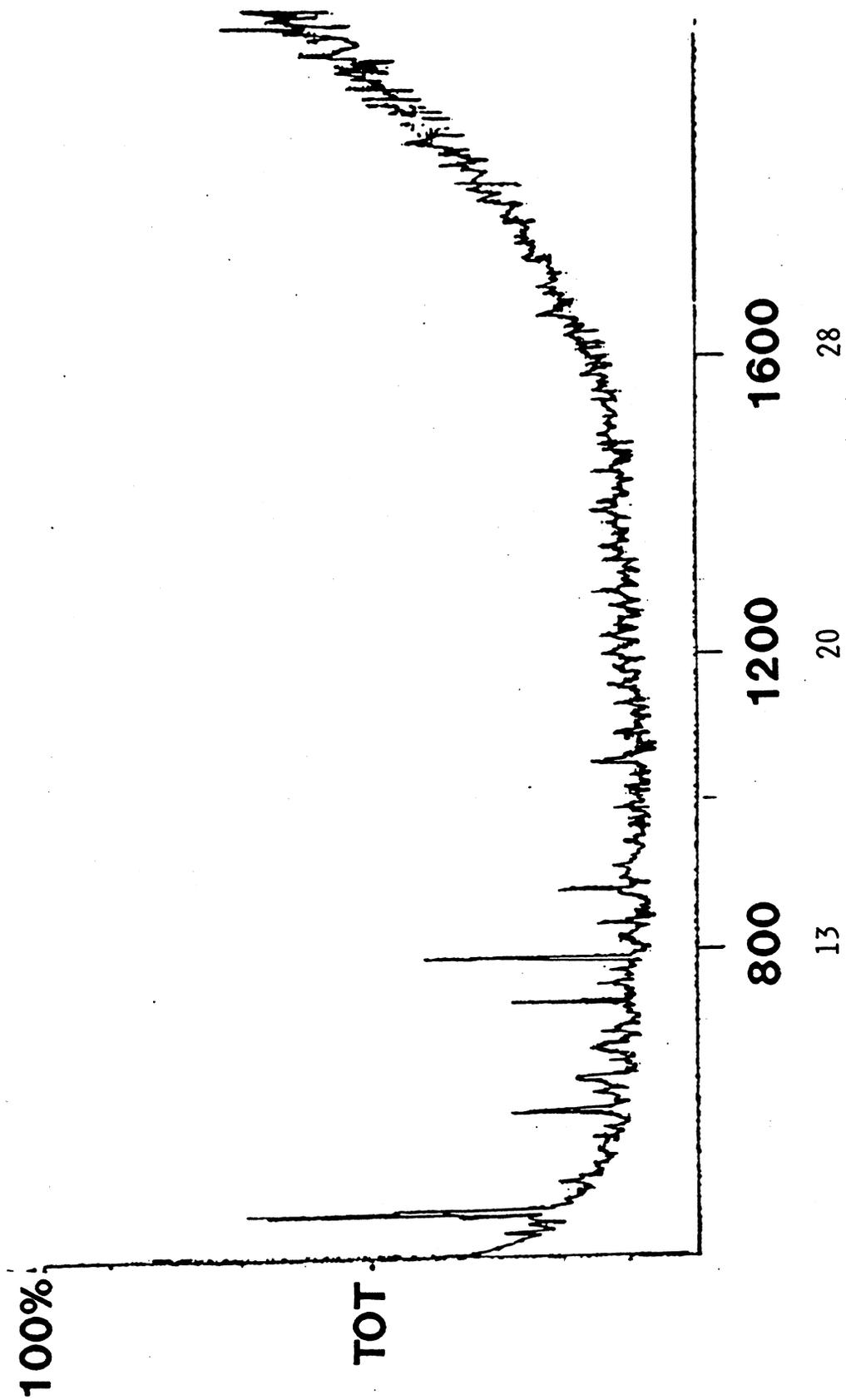


Figure 5. GC-trace of fatty acid methyl esters isolated from the PR3/BNL-TH-1 culture. Scan number (upper), retention time in mins. (lower).

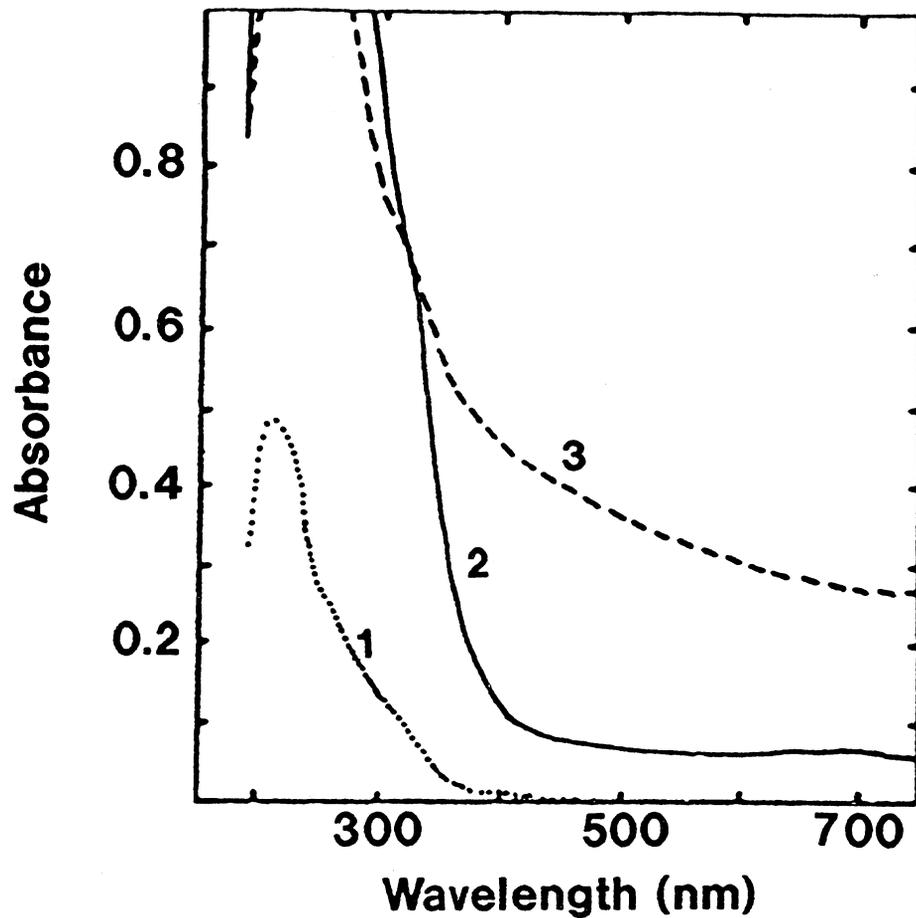


Figure 6. Extent of emulsification due to action of BNL-TH-29 on PR3 at 70°C and 200 psi.

1. PR3 oil + medium (inorganic salts + yeast extract)
2. PR3 oil + medium (inorganic salts only) + bacteria
3. PR3 oil + medium (inorganic salts + yeast extract) + bacteria

considerable biochemical reactions occur when crude oil is the only carbon source in the medium (Spectrum #2).

In order to determine the effects of different microorganisms on PR3 and other crudes under our experimental conditions, we have initiated a series of experiments in which different types and/or strains of microorganisms are allowed to act upon the same oil. These studies will be further expanded to other oils as well. The object is to develop a data base for efficient "emulsifiers" and "acidifiers" and relate this to experimental conditions and chemical changes. Figure 7 shows spectra of control (PR3 + culture medium) and the results of treatment with two additional and different strains of microorganisms. A comparison of data presented indicates significant differences in the extent of emulsification which may occur due to the action of different microorganisms. In very small samples of oil, emulsification is expressed in Klett units (Rosenberg et al., 1979) given by:

$$\frac{1000 \times D}{2} = R \text{ Klett units,}$$

where D is the absorbance determined at 545 nm. The difference between the three samples shown in Figure 7 are given in Table 2.

---

Table 2

Extent of emulsification of Teapot Naval Reserve, PR3 Crude Oil

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Sample	Klett Units
PR3 + culture medium	10
PR3 + BNL-4-21	50
PR3 + BNL-4-22	200

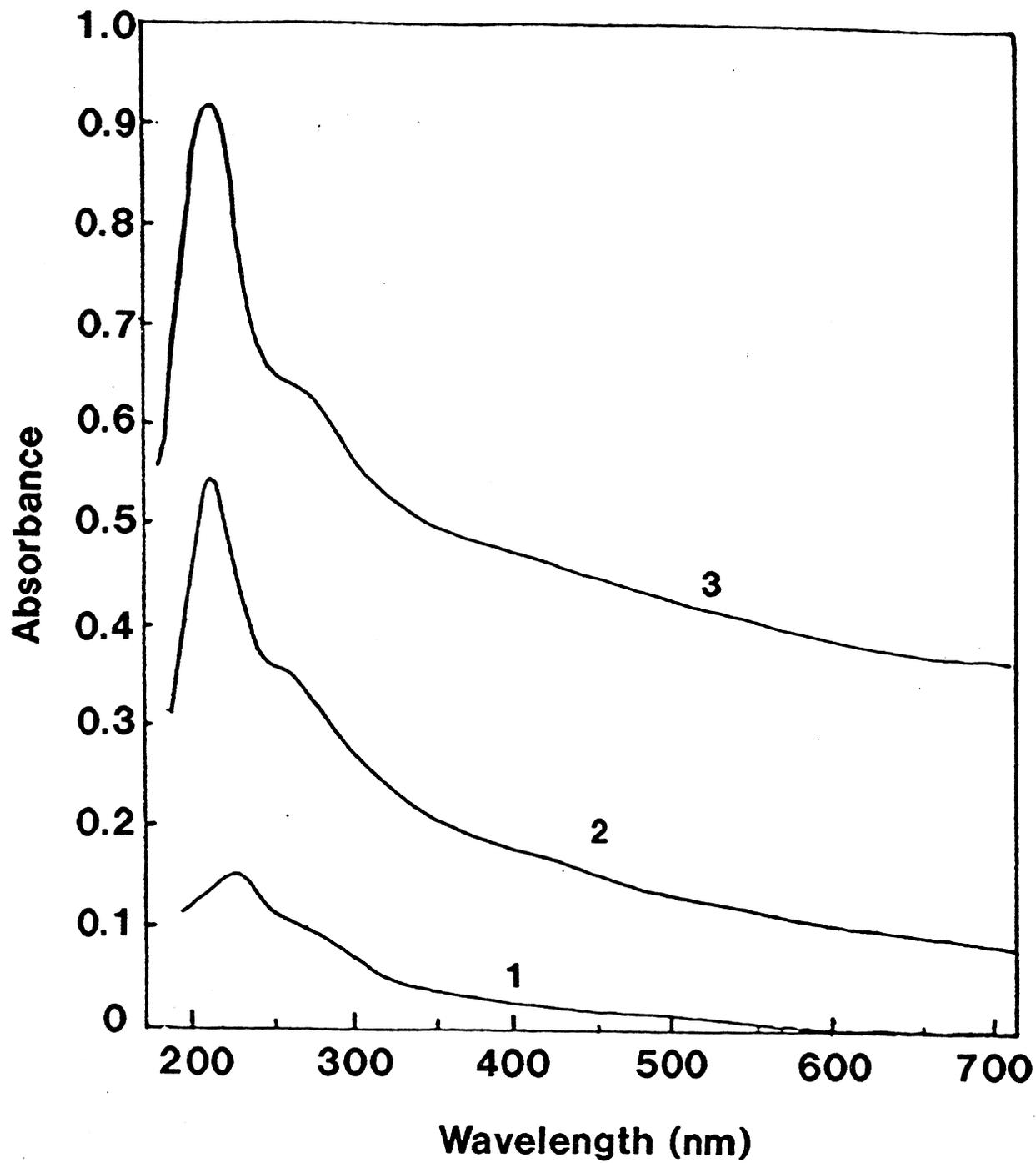


Figure 7. Extent of emulsification due to action of BNL-4-21 and BNL-4-22  
1. PR3 oil + medium; 2. PR3 + BNL-4-21 and 3. PR3 + BNL-4-22.

It has been reported (Premuzic and Lin, 1989c, d) that the extent of emulsification during the biotreatment varies and, simultaneously, there is a loss of lower molecular weight components with some qualitative changes in the higher molecular weight fraction of the crude (PR3). In order to further investigate these experimental observations, several heavy fractions of a suite of crude oils have been treated by different microorganisms under identical experimental conditions. The results are shown in Table 3 and Figure 8.

Table 3. Extent of emulsification due to the action of various microorganisms on heavy fractions of crude oils. The results are expressed in Klett units

Microorganisms	Heavy Oil Fraction				
	Asphalt <sup>1</sup>	Wilmington <sup>2</sup> (Calif)	Goch Saran <sup>2</sup> (Iran)	Recluse <sup>2</sup> (Wyo)	Prudhoe <sup>2</sup> (Alaska)
BNL-4-24	35	115	168	250	215
BNL-4-23	250	290	238	225	195
BNL-4-22	275	252	320	175	285
BNL-4-21	475	515	142	600	615

<sup>1</sup>Commercially available asphalt

<sup>2</sup>Heavy fractions (>200°C) of crude distillate

### (iii) Changes in the Chemical Composition of Crudes

#### A. High and Low Molecular Weight Components

In a typical experiment the organisms are cultured in the presence of 10- 18% by volume of crude oil with no other carbon source in the media. Analyses of bacterial growth are done by examination and measurement of turbidity at 600 and 660 nm (Beckman grating spectrometer or spectronic 20). These analyses are carried out before and after incubation on samples taken from bioreactors. The bioreactors have been described in earlier reports (e.g., BNL reports 42048, October 1988). Changes in the chemical composition

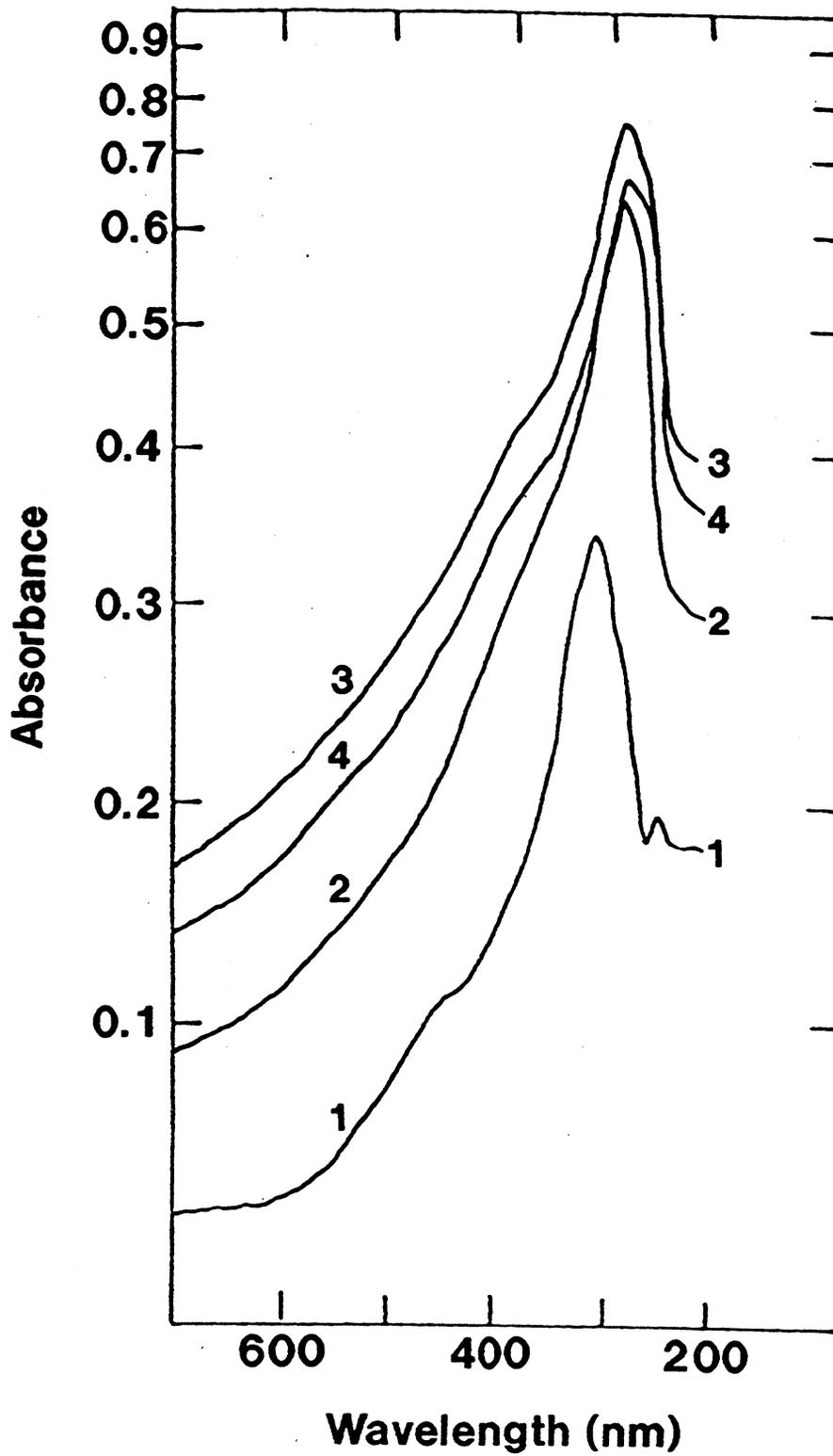


Figure 8. Extent of emulsification (700-200 nm spectral region) of PR3 by BNL-4-24 (1); BNL-4-23 (2); BNL-4-22 (3); BNL-4-21 (4).

of oils are monitored by GC/MS analyses. It is to be noted that establishment of viable cultures and optimum conditions such as the temperature range, pH, biomass to substrate ratio (i.e., oil), is an essential, however, time consuming task.

Currently, a two to four week treatment appears to be an average time required for evidence of biochemical activity. For example, treatment of PR3 crude with BNL-4-21 and BNL-4-22 resulted in emulsified products measuring 50 and 200 Klett units (KU) respectively. The GC traces (flame ionization detector) of untreated and treated PR3 are shown in Figs. 9 and 10.

Clearly in the 800-2400 (C6-C13) scan region there is a major change in PR3 composition after treatment with either of the microorganisms used. In the 2400-4200 (C13-C28) scan region, i.e., in the area of higher molecular weight components of the crude, there are significant qualitative changes. In both cases the duration of the biotreatment was three weeks. These are preliminary findings and do not represent optimum conditions. Suffice it to say that these highly promising results already indicate a difference in the effect of two different microbial strains on the same oil, particularly in the extent of alteration of the higher molecular weight components of the crude. Systematic qualitative and quantitative studies are in progress. A peak-by peak comparison of relative intensities in the 2400-4200 scan region (Figs. 9 and 10) suggest that BNL-4-22 may have caused a larger alteration in the heavy end of the crudes, a result consistent with the earlier observation expressed in Klett units (Table 2). This result is also consistent with our overall concept based on the chemical and physical complexity of crude oils and reservoirs. In fact it is feasible that no single microbial and/or biochemical system may satisfy all the requirements of an efficient process.

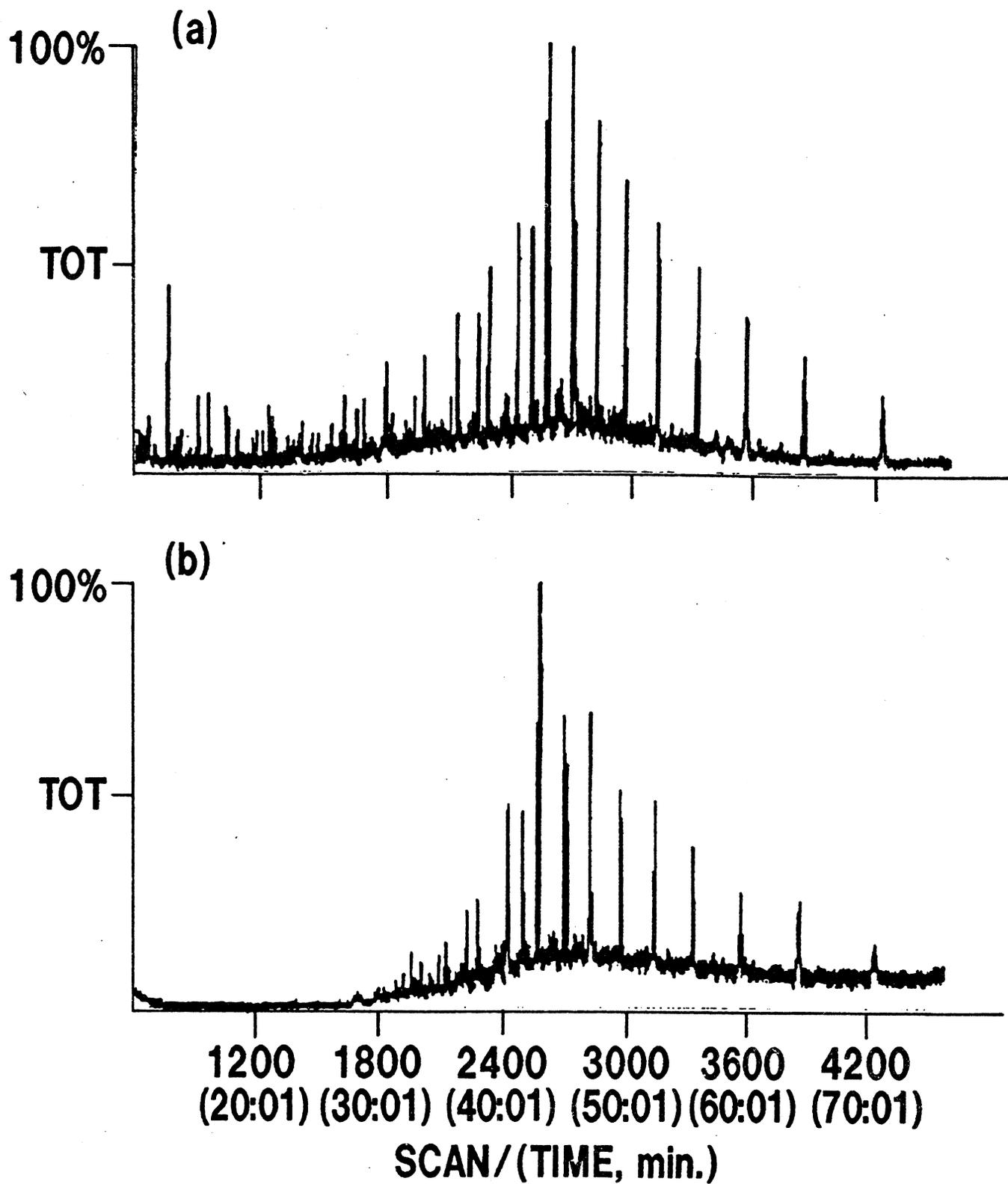


Figure 9. (a) PR3 untreated; (b) PR3 treated with BNL-4-21.

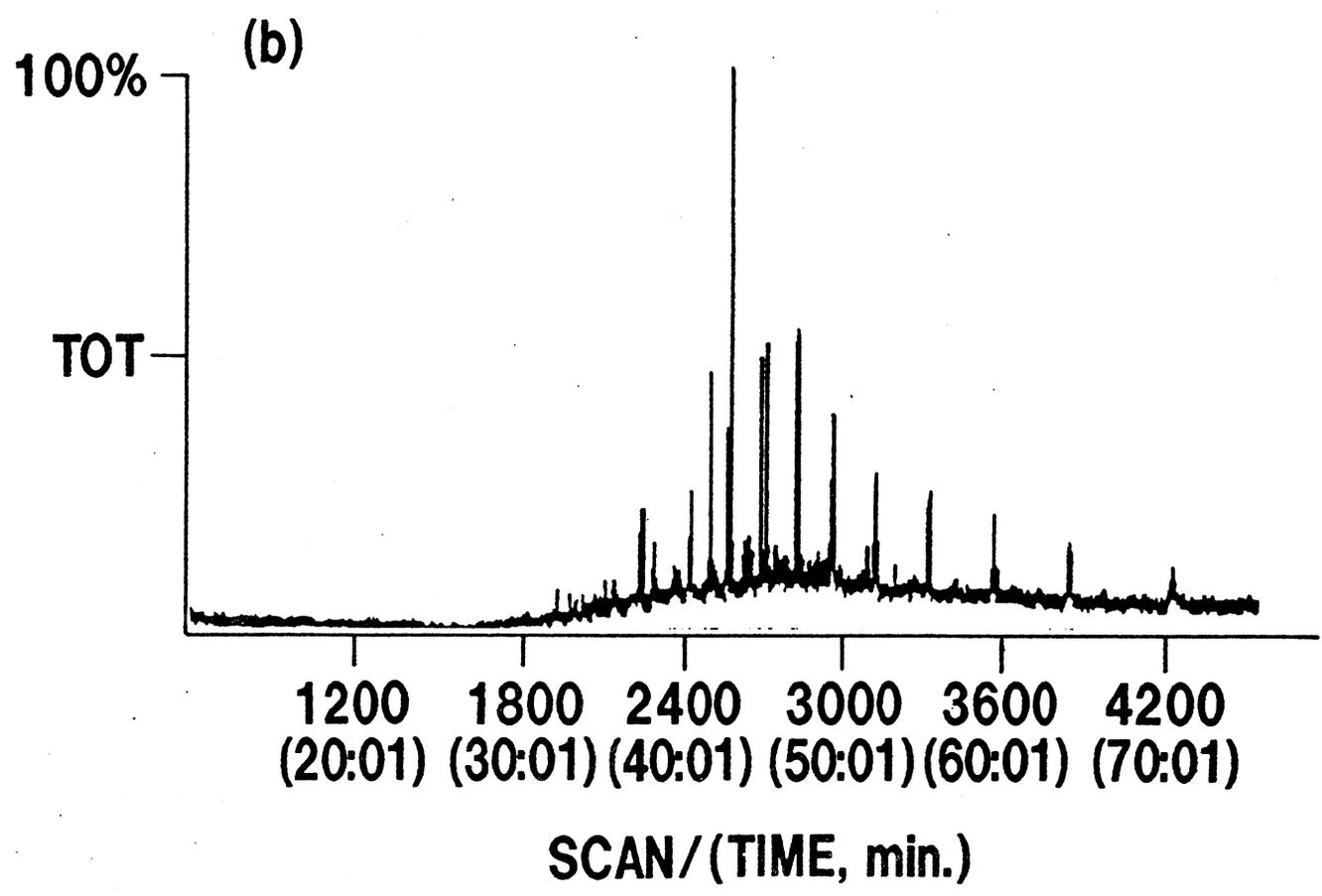
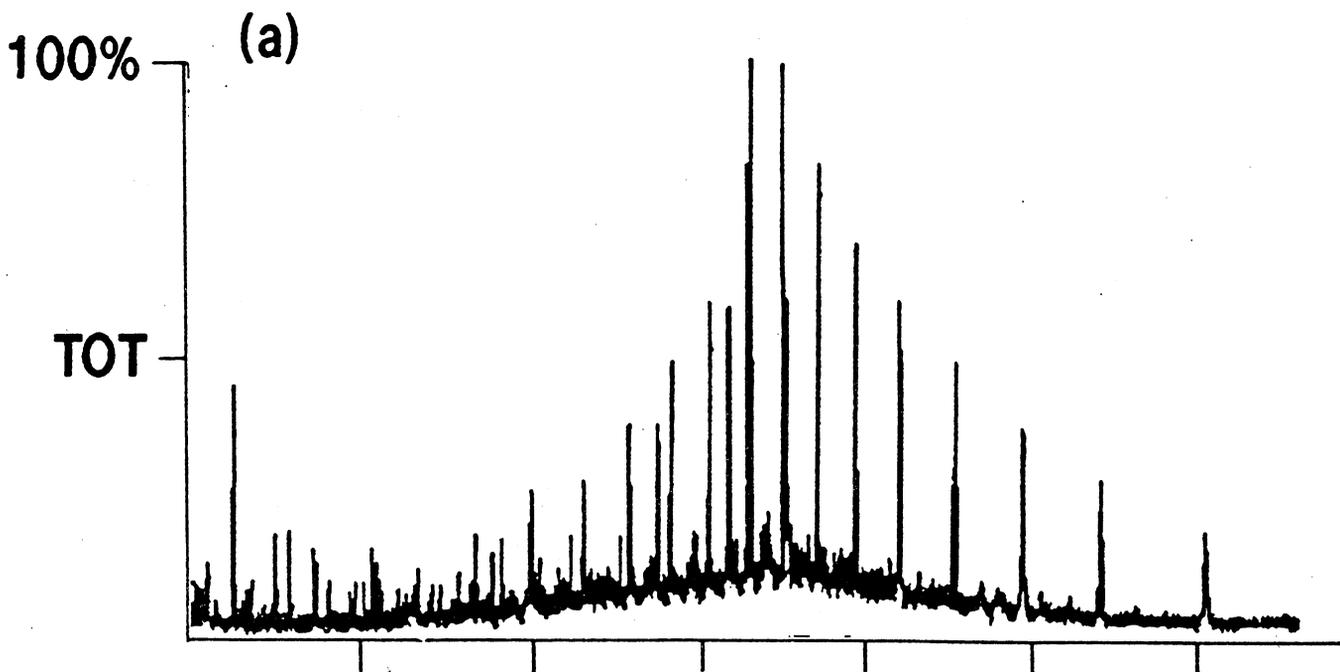


Figure 10. (a) PR3 untreated; (b) PR3 treated with BNL-4-22.

It is quite possible that microbial cultures, combined with other recovery technologies may ultimately prove to be the most efficient process strategies.

#### B. Sulfur Containing Components of Crudes

We have reported (Annual Report 1988, BNL 42048) that when Recluse, WY, crude is treated with BNL-TH-1 thermophilic microorganisms at 70°C and 2000 psi, the GC-MS scan for mass 32 signals is significantly different in the treated oil when compared to that of the untreated oil, indicating that there is a change in the nature and/or composition of sulfur compounds present. In order to study this observation further we have installed and calibrated a flame photometric detector (FPD). FPD works as a flame spectrophotometer and for certain elements, e.g., sulfur, phosphorous and chlorine can be used as a specific, highly sensitive detector. In this program, the FPD detector is used for the detection of sulfur compounds, including those which may be trapped in the gas phase present in our bioreactors. An understanding of the changes brought about by biochemical action on the sulfur compounds will enable us to determine whether indeed sulfur compounds are modified or degraded biochemically in a manner which does not produce volatile products such as H<sub>2</sub>S gas.

Typical sulfur scans of crude oils are shown in Figs. 11 and 12. It is anticipated that a combination of GC equipped with an FPD and mass-spectrometry will enable us to understand the chemical-structural changes which may occur in organic sulfur constituents of crude oils under our experimental conditions. Application of the FPD detector for the identification of small molecular weight volatile sulfur containing compounds (including H<sub>2</sub>S) is now being explored. Further, the Perkin-Elmer 8700/ITD

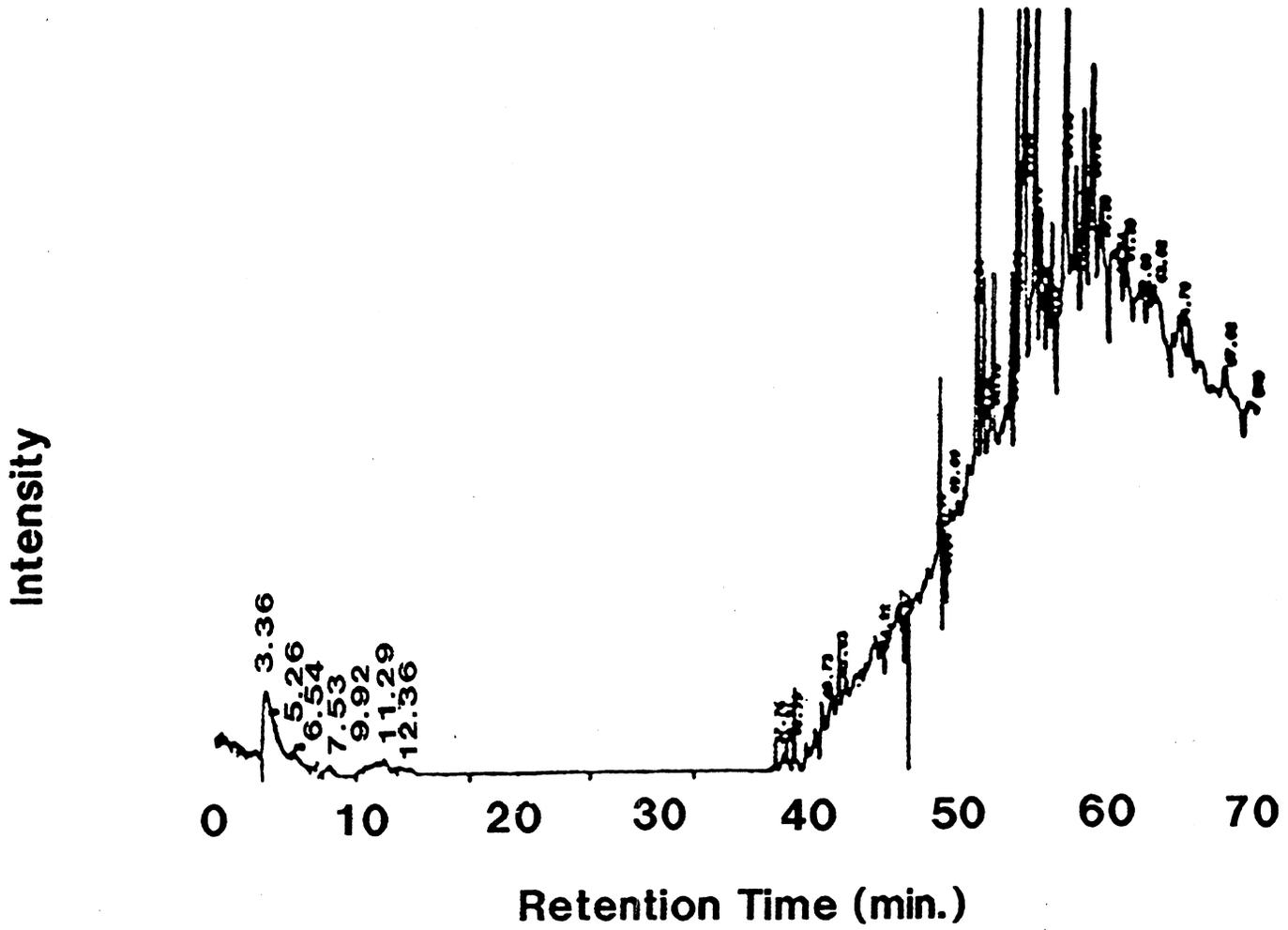


Figure 11. AGC scan of a sample of PR3 (Teapot Naval Petroleum Reserve #3) crude with a flame photometric detector installed.

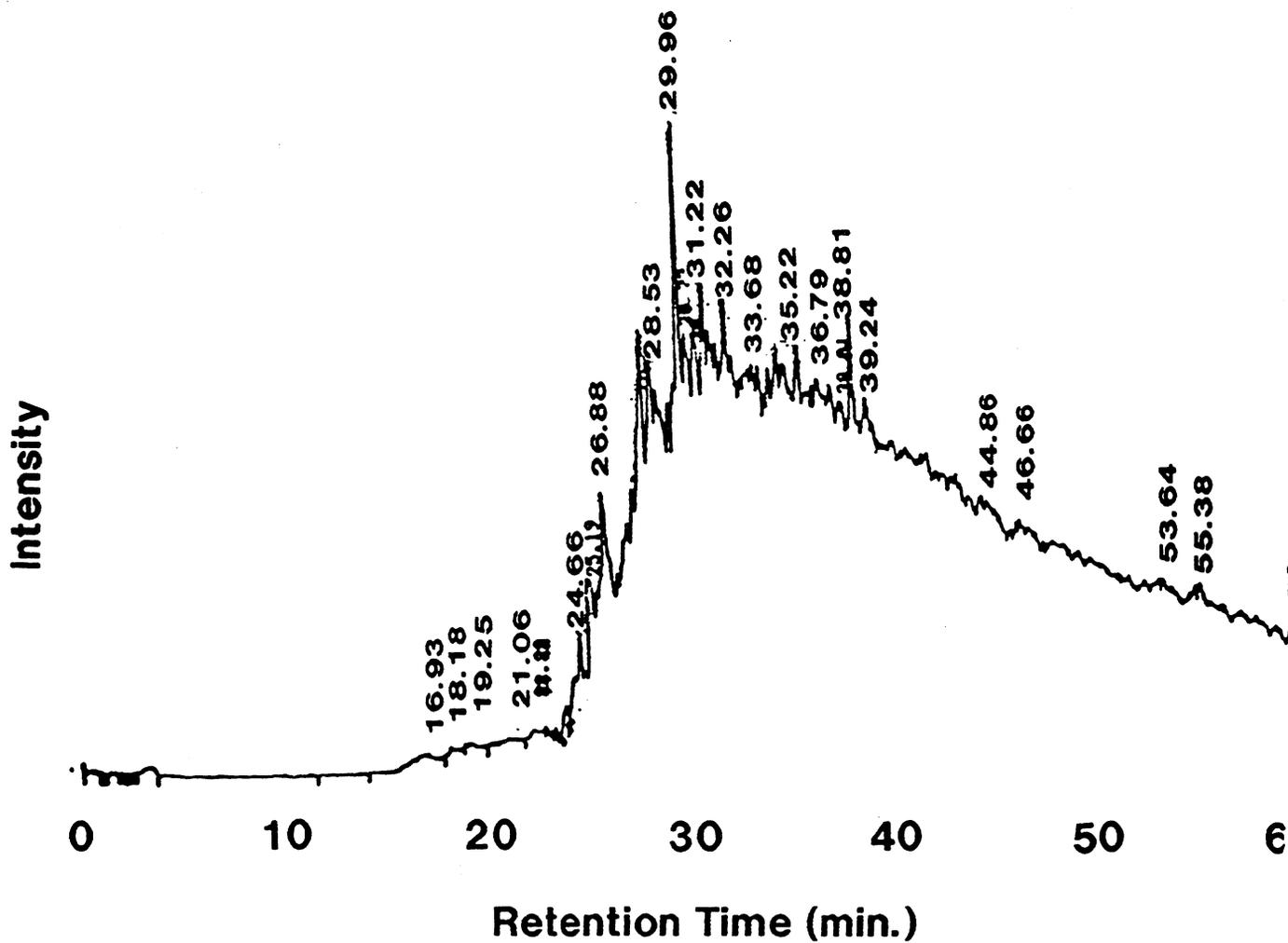


Figure 12. GC scan of a sample of Wilmington crude with flame photometric detector installed.

system has been equipped with a splitter, which allows a portion of the sample to be analyzed by GC and the flame photometric detector (FPD), specifically for sulfur. Simultaneously a portion of the same sample is analyzed by mass spectrometry. This capability makes it possible to follow changes in the chemical composition of sulfur containing compounds caused by microbial treatment. This will be of particular interest in the study of oils with a high sulfur content. For this purpose we have obtained through the courtesy of W.D. Peters and P.W. Woodward of NIPER, two samples of high sulfur content Venezuelan oils (Boscom and Cerro Negro). These oils will be subjected to the biotreatment and particular attention will be paid to the changes in sulfur components.

Analyses of the effects caused by different microorganisms on heavy crudes are continuing. The first experiments, in which the above described system was used have been completed. For example, treatment of Teapot Naval Petroleum Reserve #3 crude (PR3) with a temperature and pressure adapted organism, BNL-4-24 at 65-70°C and 1400 psi, resulted in considerable changes in the composition of sulfur compounds. A typical FPD sulfur trace is shown in Figure 13A before treatment and in Figure 13B after treatment; considerable qualitative and quantitative changes are evident. The corresponding mass spectra are shown in Figure 14. In Figure 14C the GC/MS trace corresponding to Figure 13A is shown. Figure 14D shows the trace for the control sample and Figure 14E is the GC/MS trace chloroform extract of the treated sample. At the present time, high instrument gain has to be used because the splitter is such that only small (2% to MS, 98% to FPD) samples are injected. Efforts are now under way to modify this experimental condition. It is to be noted that when detectors other than FPD are used, the crude oils are injected as carbon

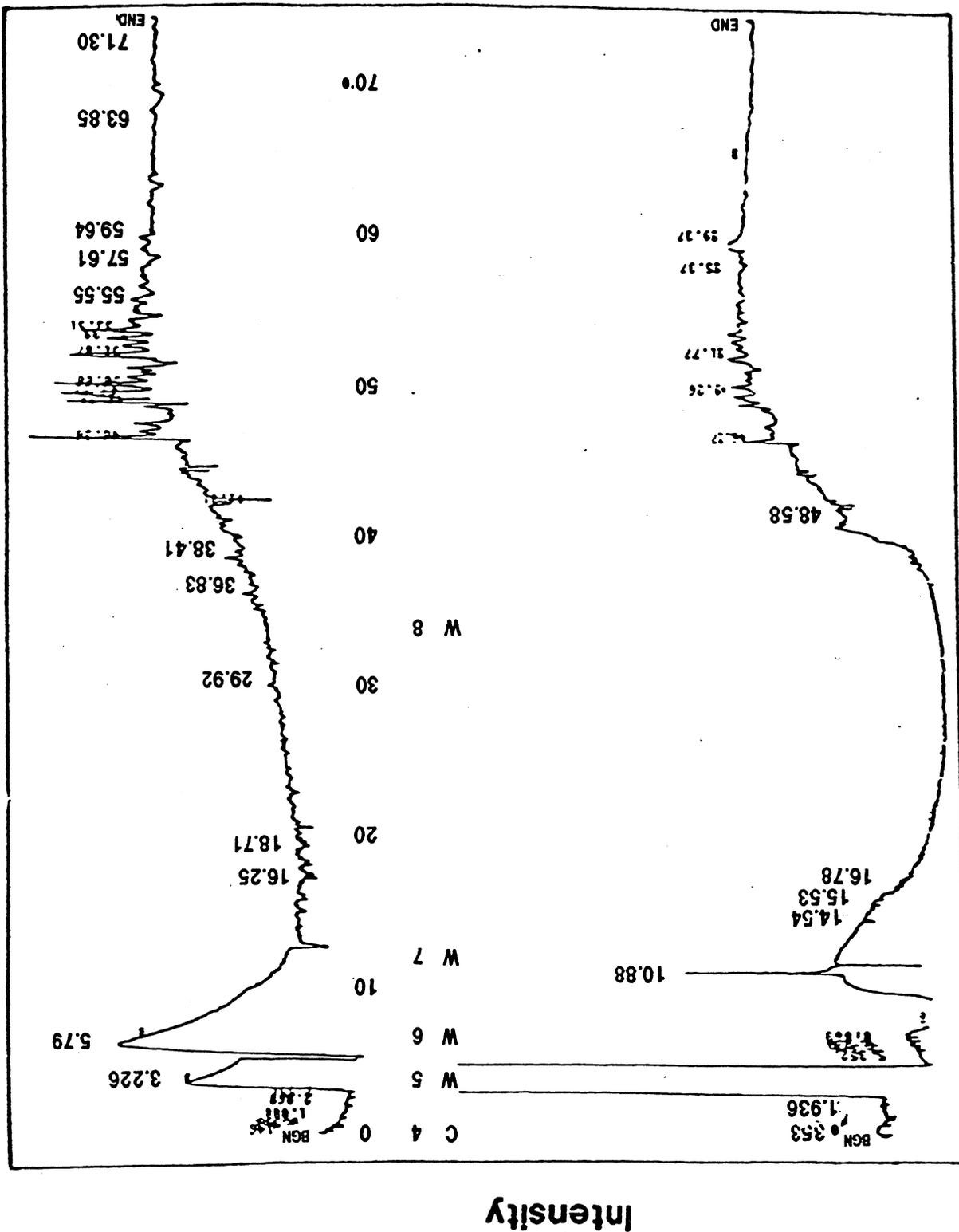


Figure 13. GC/FPD sulfur trace of BNL-4-24 treated PR3. 13A before treatment and 13B after treatment.

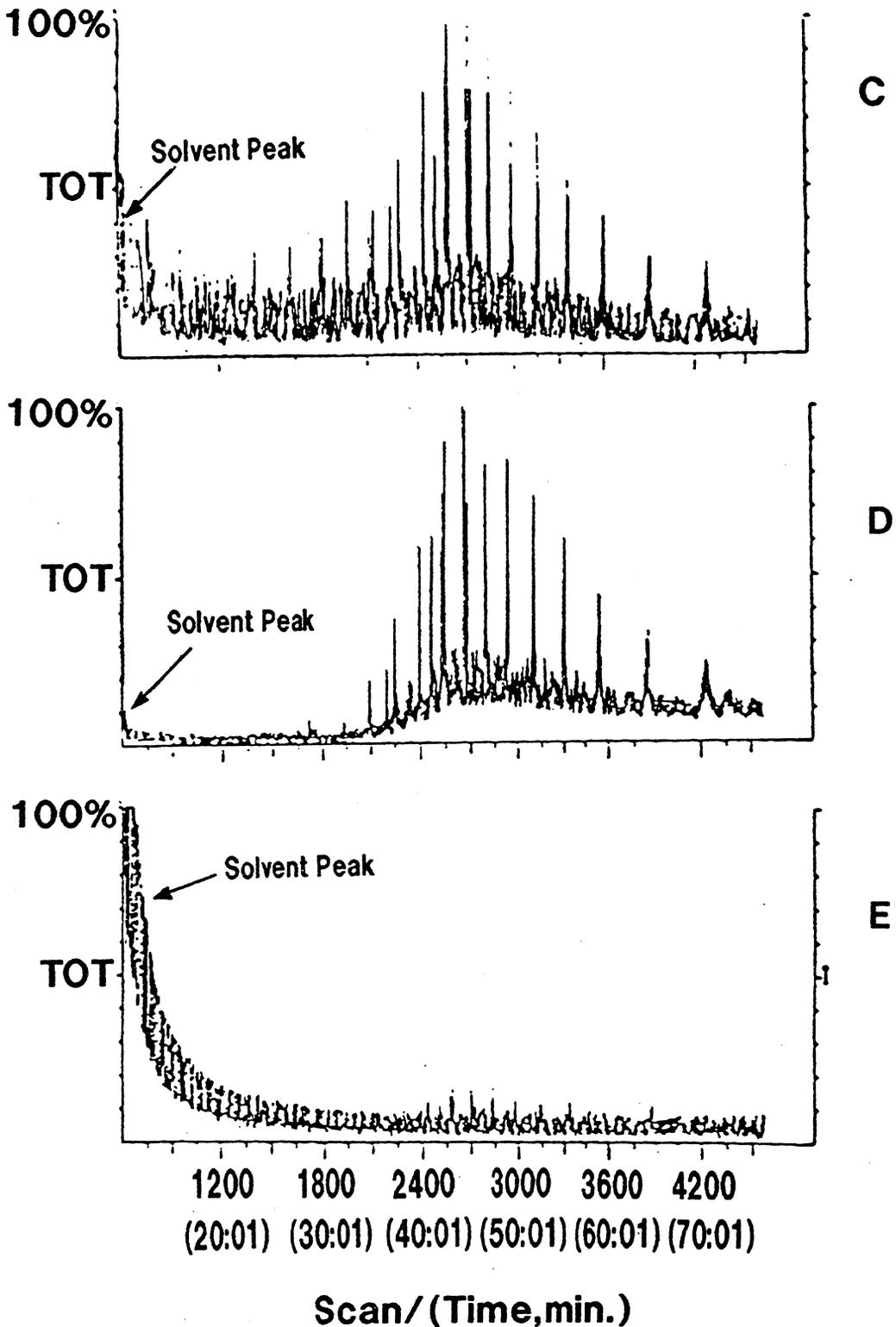


Figure 14. GC/MS traces of the BNL-4-24 treated PR3 corresponding to the sulfur compounds before treatment, control (14C), PR3 treated in the medium and under identical conditions, but without inoculation with bacteria (14D), and PR3 after treatment with microorganisms (14E).

disulfide solutions. This solvent is not suitable for FPD, therefore, chloroform extracts of oils are used routinely in these experiments.

### C. Preliminary Studies of an Emulsified Phase

It has been mentioned earlier in this report that the extent of emulsification during the biotreatment varies and differs for different microorganisms. We have initiated the GC/MS analyses of the emulsified heavy fractions of the crude oils listed in Table 3. Thus, Prudhoe Bay heavy fraction of the crude treated with BNL-4-24 yielded an aqueous emulsion measuring 215 units on the Klett scale. The emulsion was extracted with chloroform and analyzed by GC/MS. The result is shown in Figure 15. Preliminary analysis indicates that hydrocarbons in the range of C13 to C26 are present in the emulsion. Concurrently, analyses of methylated fatty acids present in the aqueous phase after treatment are being continued.

#### (iv) Expansion of Microbial Reference Library

On the assumption that the chemical composition of a crude oil makes it a complex matrix, unlikely to be degraded and altered by a single type of microorganism, we are exploring under our experimental conditions the effects on crude oils of both aerobic and anaerobic organisms. Work with strictly anaerobic microorganisms requires adaptation of culturing techniques for anaerobic thermophiles to our particular needs, such as pressure and the presence of oils as a sole carbon source. Currently, several anaerobic cultures are growing with 10-18% total volume of crude oil present in each culture. A full time microbiologist is responsible for maintenance of stock cultures as well as adaptation and optimization of conditions which favor emulsification, acidification and biochemical degradation of oils.

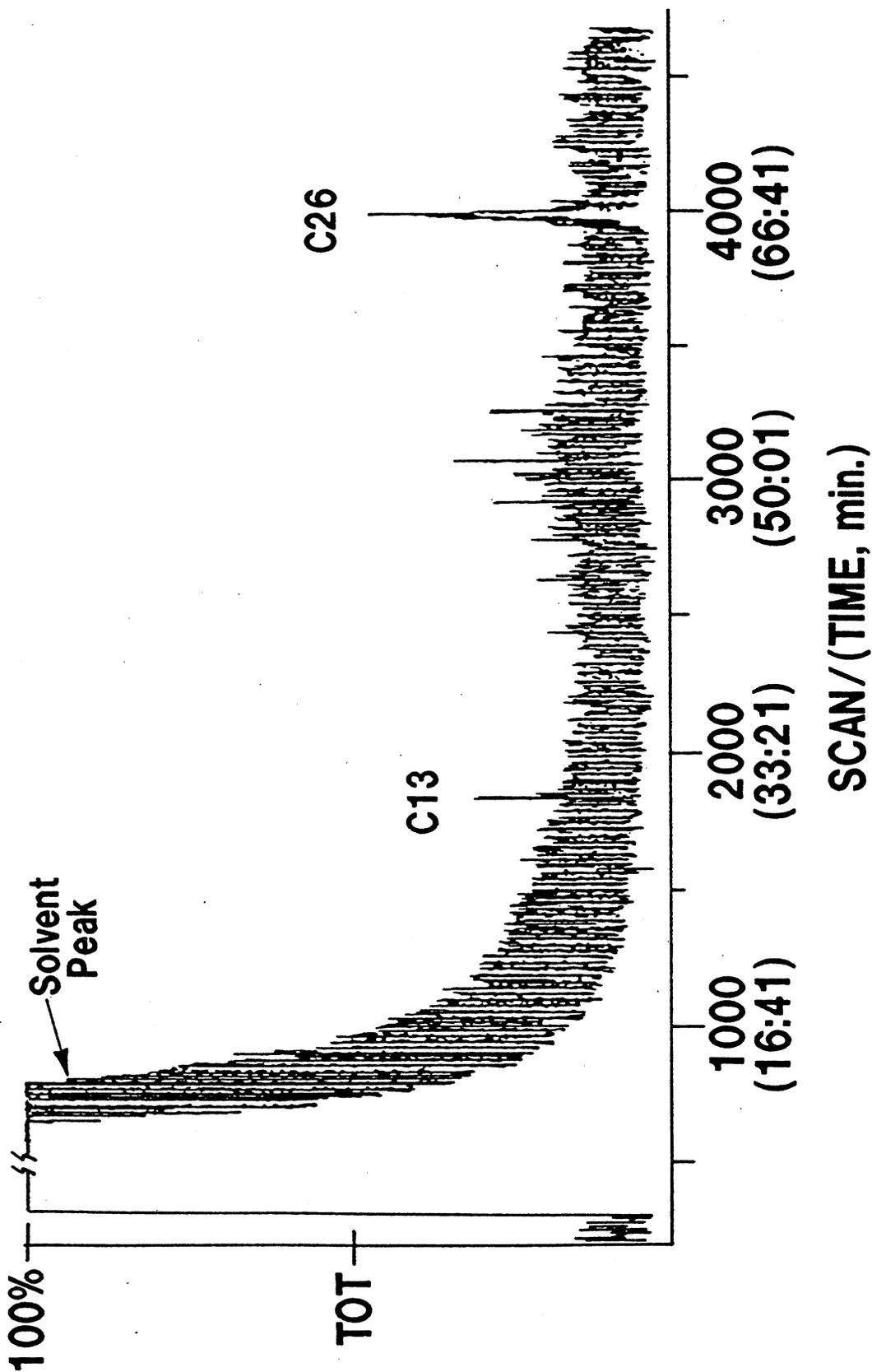


Figure 15. Heavy fraction for Prudhoe Bay crude treated with BNL-4-24. GC/MS trace of the chloroform extracted emulsion (high instrument gains because of small sample).

Forty-one different microbial cultures from the Brookhaven National Laboratory (BNL) collection (Premuzic and Lin, 1989) are now actively growing. These include aerobic and anaerobic microorganisms which can grow and/or adapt to elevated temperatures and pressures. The selected microorganisms are also able to grow in the presence of crude oils, salt brines and at pH extremes.

The reference library of aerobic and anaerobic microorganisms has been further expanded. Exploratory temperature-pressure adaptation studies have been initiated. Particular attention is being given to microorganisms which are not initially strictly thermophilic and barophilic. If the adaptation experiments are successful, then the consortium of microorganisms available for MEOR will become considerably larger. It is anticipated that, in some cases, under field conditions combined MEOR and water flooding at elevated temperatures may have distinct advantages. In such circumstances the aerobic/anaerobic conditions become critical. In Figures 16, 17, and 18 an untreated PR3 and PR3 treated with an aerobic microorganism, BNL-4-25, and a methanogenic thermophilic microorganism, BNL-5-32, are compared. As expected, there are significant changes in the 1200 to 2500 scan range as indicated by the lack of lower molecular weight species. In the >2500 scan region differences in the peak intensities and lack of some high molecular weight peaks are also evident. It is premature to conduct systematic kinetic studies, because neither optimum conditions nor most promising microbial types have as yet been determined. However, the available data indicate that BNL-4-25 may be a faster acting microorganism than BNL-5-32.

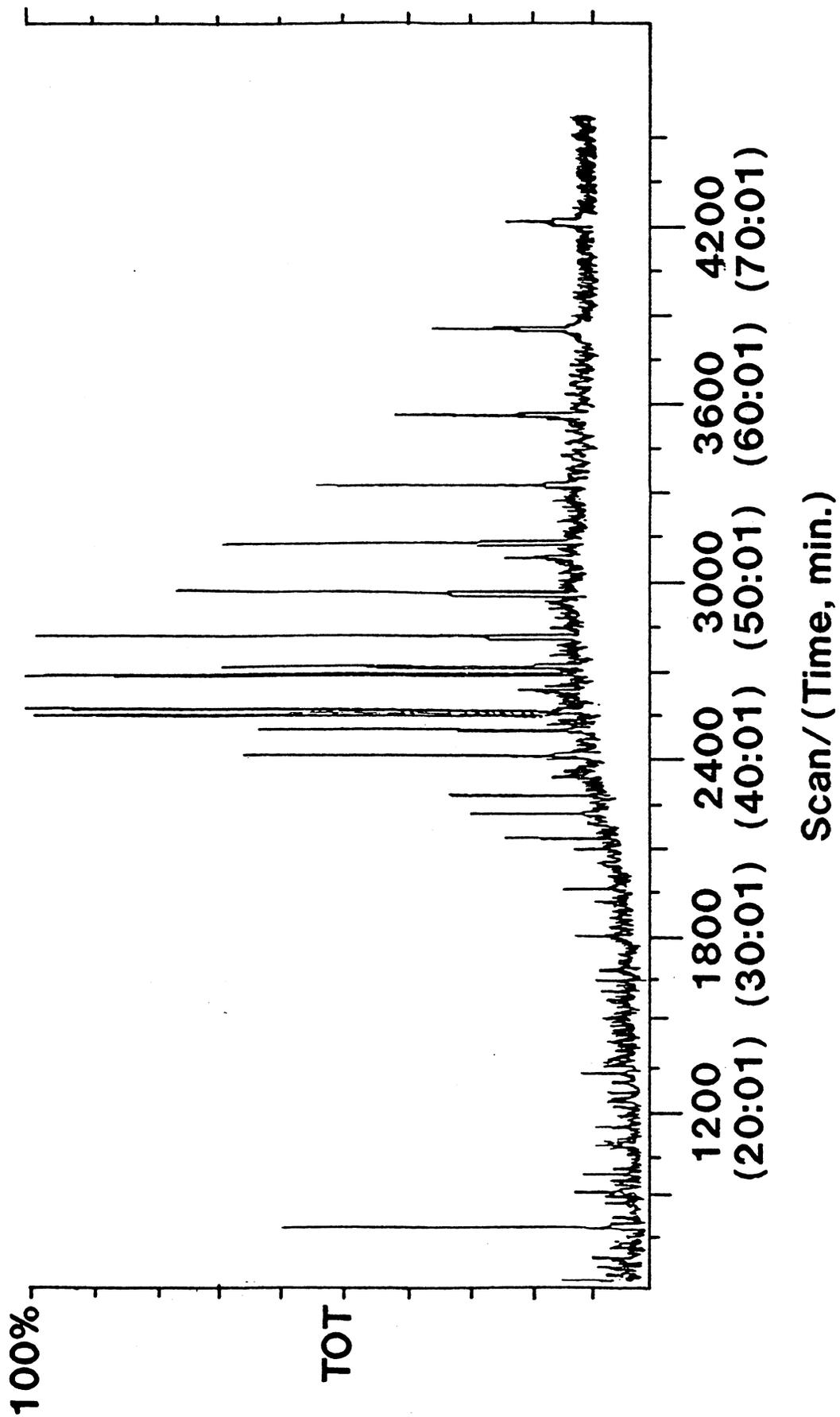


Figure 16. Untreated PR3 control.

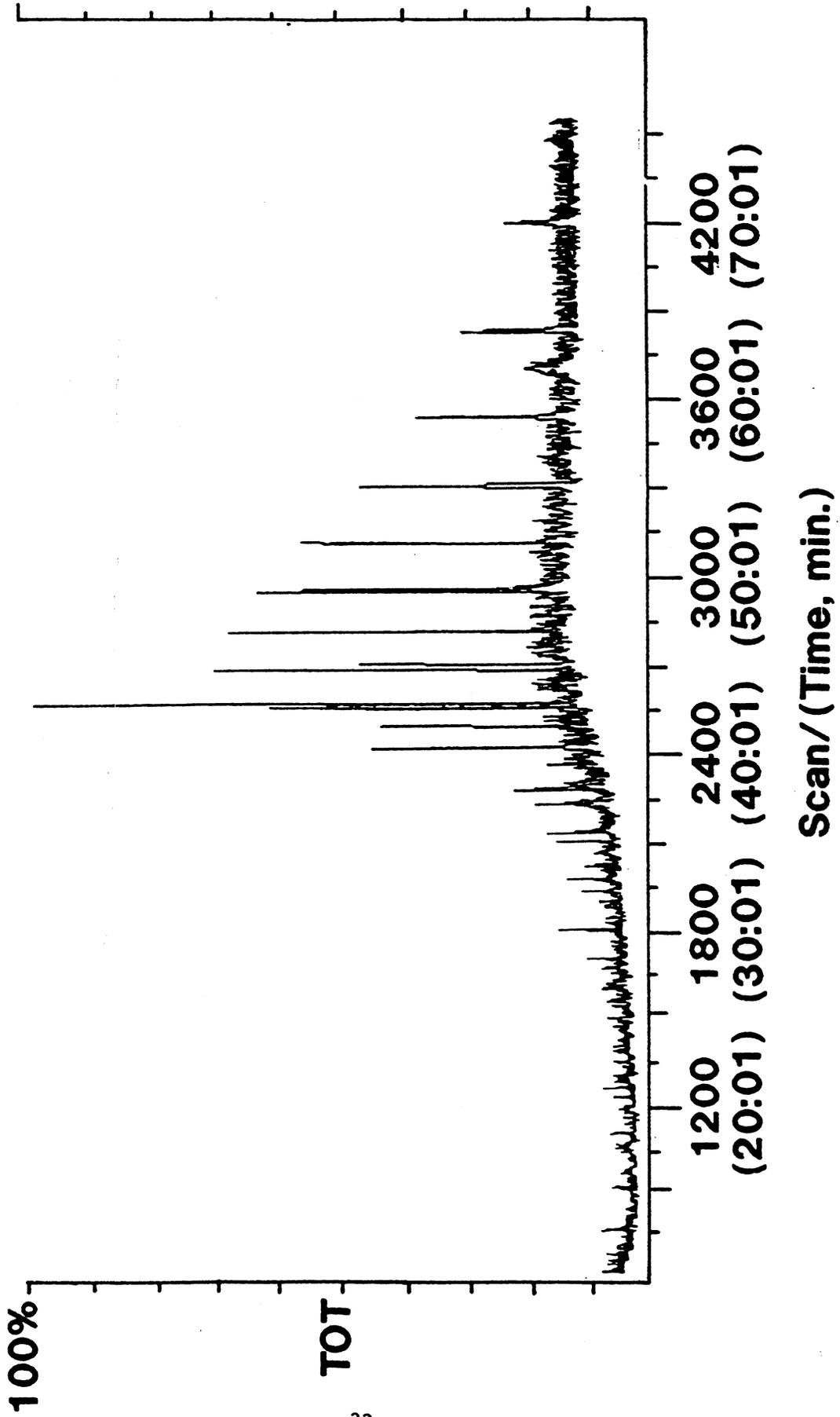


Figure 17. PR3 treated with aerobic BNL-4-25 strain.

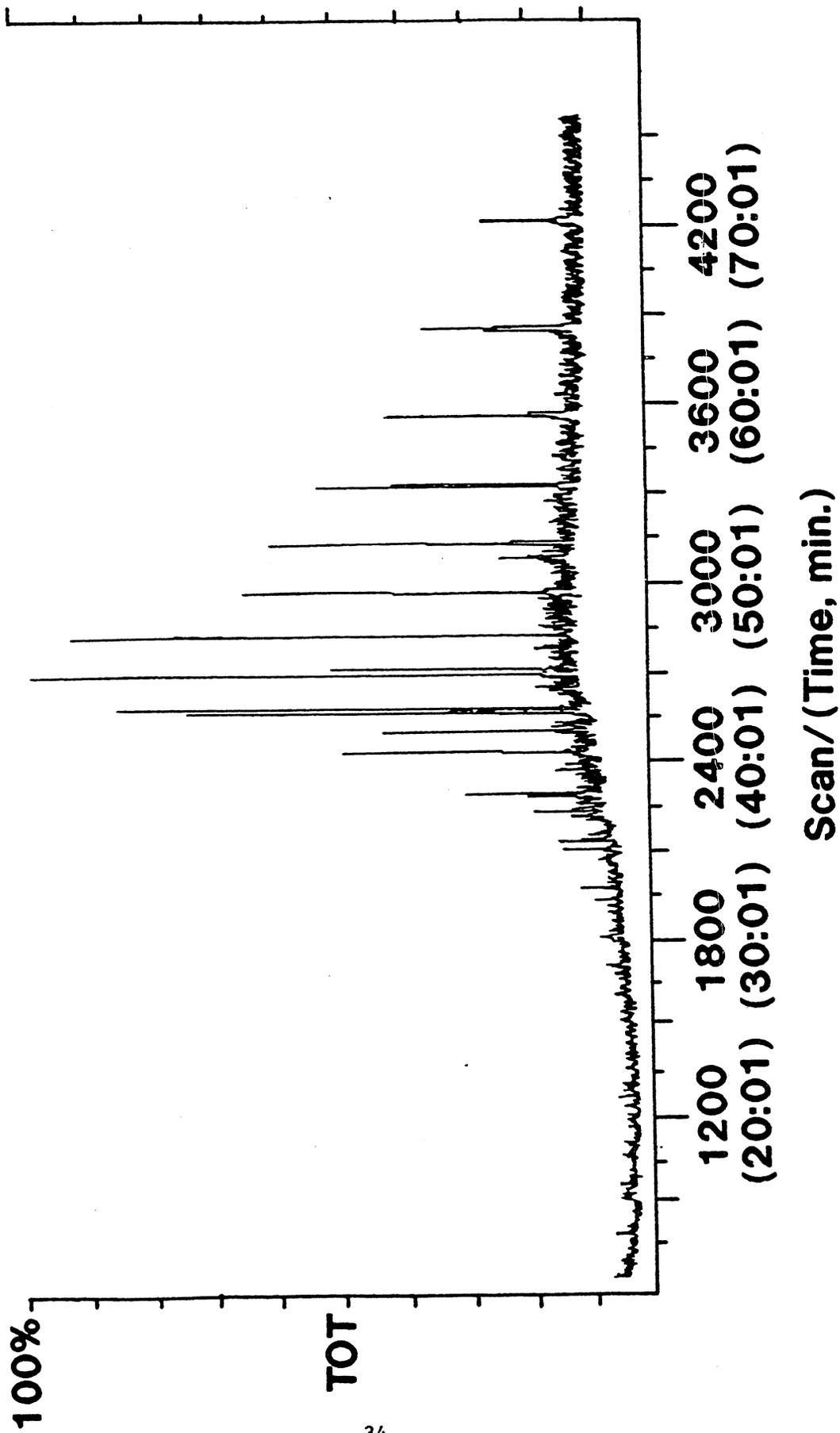


Figure 18. PR3 treated with an anaerobe, methanogenic thermophilic BNL-5-32 strain.

## 5. Presentations

1. Premuzic, E.T. and M.S. Lin. Interaction between thermophilic microorganisms and crude oils: Recent Developments. Bioprocessing of Fossil Fuels Workshop, Vienna, Virginia, August 8-10, 1989.
2. Premuzic, E.T. Biochemical mechanisms of microbial transformation of energy related materials. Symposium Progress 89, Biotechnology on Long Island, New York, October 18-20, 1989.

## 6. Publications

1. Premuzic, E.T. and M.S. Lin. Interaction between thermophilic microorganisms and crude oils: Recent Developments. Bioprocessing of Fossil Fuels Workshop, Proceedings, in press, 1989.

## 7. Reports

1. Premuzic, E.T. and M.S. Lin. Microbial Enhanced Recovery of Oil at Elevated Temperatures and Pressures. Patent pending, 1989.
2. Premuzic, E.T. and M.S. Lin. Effects of Selected Thermophilic Microorganisms on Crude Oils at Elevated Temperatures and Pressures, Enhanced Oil Recovery, 55, 79, DOE/BC-88/3 (DE89000730), 1989.
3. Premuzic, E.T. and M.S. Lin. Effects of Selected Thermophilic Microorganisms on Crude Oils at Elevated Temperatures and Pressures, Enhanced Oil Recovery, 56, 106-107, DOE/BC-88/4 (DE89000742), 1989.
4. Premuzic, E.T. and M.S. Lin. Effects of Selected Thermophilic Microorganisms on Crude Oils at Elevated Temperatures and Pressures, Enhanced Oil Recovery, 57, 71, DOE/BC-89/1 (DE89000755), 1989.

5. Premuzic, E.T. and M.S. Lin. Effects of Selected Thermophilic Microorganisms on Crude Oils at Elevated Temperatures and Pressures. Informal Report BNL-43185, 1989.

6. Premuzic, E.T. and M.S. Lin. Effects of Selected Microorganisms on Crude Oil at Elevated Temperatures and Pressures. Annual Report 1988, BNL 42048.

## 8. Highlights

Results presented in this report have indicated that:

1\*. Compared to controls there are overall changes in hydrocarbon content of heavy crudes. Preliminary, qualitative analyses indicate that biodegradation of crudes occurs in heavy and lighter fractions of the oils.

2. There are changes in the composition of the organic sulfur components of the crudes, with a net overall effect being a decrease in sulfur compounds.

3. During the biotreatment a number of organic acids are produced ranging from small molecular weight acids such as lactic acid to larger molecular weight acids, e.g., oetadecanoic acid (C-18).

4. Studies of an emulsified phase have shown that hydrocarbons in the range of C13 to C26 are dispersed during the biotreatment.

5. Based on the experimental data thus far available, the physico-chemical changes brought about by biotreatment are organism specific and vary with different types of microorganisms.

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\*Record of Invention on file with BNL Patent Office.

## 9. Recommendations

Based on the current experience, continuing effort in this program should aim for:

1. Study effects of other thermophiles from the BNL collection under aerobic and anaerobic conditions.
2. Continue temperature and pressure adaptative studies.
3. Expand further the data base in terms of different types of organisms and their effects on crudes under our experimental conditions and explore the diagnostic utility of the chemical data base.
4. Explore further the chemical changes in organic sulfur compounds brought about by biotreatment.
5. Consider more versatile bioreactors

## 10. References

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