

## A COMPARISON OF UINTA BASIN, UTAH CRUDE OIL AND BIODEGRADED PRODUCTS

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### INTRODUCTION

The Green River Formation of Wyoming, Colorado, and Utah contains a significant portion of the world's oil shale (1). In addition, the area contains large deposits of tar sands and bituminous rocks, particularly in the Uinta Basin of Utah. Commercialization of coal, oil and gas deposits are important in the economy of Utah.

The Green River Formation is considered to be rather unique in character because it has been spared extensive disruption from the forces of temperature, pressure, and fracture (2). It has probably been more thoroughly studied by organic geochemists than any other formation in the world. However, the microbiology of the formation and its relationship to hydrocarbon transformations has not been extensively investigated.

The importance of microbial activity to fossil fuel hydrocarbon transformations is widely recognized (3). The metabolic activities of the microflora over a period of time can contribute to changes in pH and redox potential which, in turn, can significantly alter the geological environment (3). These changes need not be directly involved with hydrocarbons. Sulfate-reducing anaerobic bacteria do not metabolize hydrocarbons but can, in the presence of oxygen-consuming aerobic bacteria, actively reduce sulfate to sulfide (4). A case in point is growth of anaerobic *Desulfovibrio* sp. under growth of aerobic *Beggiatoa* sp. Thiobacilli can also contribute significantly to acidification of the environment. As a consequence, sulfur and metal availability can be significantly altered thereby changing the course of subsequent hydrocarbon-generating events.

Most investigators have found that the first hydrocarbons removed from crude oil by bacteria are n-alkanes followed by alicyclics, aromatics, and acyclic isoprenoids (5). Disagreement exist regarding biodegradation of steranes and to a lesser extent hopanes. Nevertheless, it is agreed that biodegradation of a crude oil leads to another oil having a lower API gravity and greater chemical stability (6). Some evidence has been presented that suggests that paraffinic crude oils are precursors of heavy to medium-gravity naphthenic crude oils (7). Observations indicate that primary paraffinic crude oils are expelled from deep source beds. Before, during, or after a generally upward migration, the paraffinic crude is transformed microbiologically into a naphthenic crude oil. In another investigation (8) biodegradation decreased API gravities of a group of common-source crude oils and tar sands 8-fold, increased sulfur 3-fold, and metal content 6-fold. Hydrocarbon content was altered with n-alkanes, isoprenoids light aromatics, and light thiophenes completely removed (8). In

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in severely degraded samples steranes were partially removed while triaromatic steranes and diasteranes were resistant to biodegradation. In a long term study of the effects of a fuel spill on the microbiology of an agricultural soil, biodegradation of the fuel was achieved (9) after three years. Coryneform bacteria and certain fungi were responsible for hydrocarbon degradation. The most active fungi in hydrocarbon degradation were Aspergillus and Penicillium sp.

## EXPERIMENTAL

### Sample Sources and Procedures

Samples of oil shale were collected from Hell's Hole Canyon in Utah, the C-b Tract Mine in Colorado, and the Southman Canyon area in Utah. Tar sands were collected from Asphalt Ridge in Utah. Gilsonite was collected from the Bonanza area of Utah. Petroleum samples were collected from the Red Wash Oil Field and the Altamont-Bluebell Oil Field in Utah. Solid samples were placed in plastic bags and water samples in sterile plastic tubes. The samples were transported to the Idaho National Engineering Laboratory Research Center in Idaho Falls, Idaho for microbiological studies. Samples designated for pyrolysis gas chromatography/mass spectrometry studies were sent to the Center for Micro-Analysis and Reaction Chemistry, University of Utah, Salt Lake City, Utah.

### Microbial Isolation, Identification and Maintenance Procedures

Samples of solid materials were carefully cleaned and the inner surfaces exposed. The inner surfaces were scraped and the scrapings placed on trypticase-soy agar medium in petri dishes. Aqueous samples were streaked on the medium. The medium was incubated aerobically and anaerobically at about 25°C for seven to fourteen days. Colonies were examined for morphology and slides were prepared for microscopic examination. After culture purity was assured, cultures were maintained on trypticase-soy agar.

Identification of cultures was based on API Procedures (API Laboratory Products, 8114 Trans Canada Highway, Suite B, St. Laurent, Quebec H4S 1M5).

### Model Compound Utilization Procedures

A basal salts medium was added to screw-cap test tubes and sterilized by autoclaving at 121°C for 20 minutes. The compound to be investigated (Table 2) was added at 0.1% concentration after sterilization. The tubes were inoculated using a pure culture and incubated at room temperature as roller tubes at about 10-20 revolutions per minute. The tubes were examined daily for growth as shown by turbidity in the tubes. Response was graded as 1+ to 4+ (poor to best).

### Petroleum Utilization Studies

These were carried out much as the model compound investigation except that cultures were prepared in Erlenmeyer flasks and petroleum was added at 1% concentration. Cultures were incubated at 30°C on a rotary shaker operating at about 20 revolutions per minute.

### Pyrolysis Gas Chromatography/Mass Spectrometry (Py GC/MS)

Py GC/MS analyses were carried out at the University of Utah Center for Micro-Analysis and Reaction Chemistry, University of Utah, Salt Lake City, Utah.

A basic Curie-point Py GC/MS technique was used with a Curie-point filament temperature of 610°C(10). The gas chromatography was conducted using a Hewlett-Packard 5890 instrument with a 15 meter DB-5 column heated at 40° to 320°C, 10°C/minute. An ion-trap detector was used and a Finnigan Mass Spectrometer.

## RESULTS AND DISCUSSION

### Microbiological Characterization

Genera of bacteria isolated from samples are listed in Table 1. Coryneform bacteria are not listed individually because the members of this group are difficult to classify. At present it is believed that four genera are represented: Arthrobacter, Corynebacterium, Nocardia, and Rhodococcus. However verification is still pending. Only coryneform bacteria could utilize hydrocarbons and heterocyclic compounds provided as carbon sources. All coryneforms were isolated from within bitumen-containing rock and none from water associated with the rock.

Cell counts of coryneforms were at the  $10^4$  to  $10^5$  cfu/gm level. Table 2 shows the performance of the six coryneform isolates which grew best on the model substrates. Not all coryneforms could utilize these materials. In some cases, growth of the isolate was difficult to maintain and some were lost, consequently, some tests could not be completed. It is noteworthy that t-butylcyclohexane was poorly utilized. Apparently, the branched alkyl substituent on the cyclohexane ring inhibited utilization. The result is in accord with experiences with such compounds as branched alkyl benzene sulfonates, notorious in the detergent industry for resistance to biodegradation. The position of the methyl group on the alkyl chain may be significant. Isoprenoid substituents on structures such as steranes are apparently degraded.

During the course of this work, it was observed that certain differences existed between microorganisms and their capability to grow on the model compounds. The wild strains of coryneform bacteria were generally more capable of growing on the unusual substrates provided than laboratory strains obtained from commercial culture collections. The point is significant for those who would study fossil fuel degradation by microorganisms. Figure 1a compares the growth of a laboratory Arthrobacter with oil shale isolate OS-2 on pristane. Figure 1b compares laboratory Nocardia sp. with oil shale-isolate OS-2 and gilsonite-isolate G2 on the heterocyclic compound ethyl nipecotate. Growth was measured by turbidity. In Figure 2a and 2b, a laboratory strain of Arthrobacter is compared with gilsonite-isolate G2 growing on pristane. Growth was measured by oxygen consumption. OS-2, the wild strain, in these and other growth experiments, required several days of adaption before rapid growth occurred. The response suggested that enzyme induction was necessary. In contrast, G-2, also a wild strain, consistently showed immediate growth on substrates provided suggesting constitutive enzyme formation.

### Petroleum Biodegradation

Figure 3a is a GC/MS chromatogram of a waxy crude oil (Bluebell Control), Figure 3b is a chromatogram of the petroleum residue of the oil after degradation by the oil shale-isolate OS-3. The residue primarily consists of isoprenoid components, with almost complete removal of n-alkanes. Figure 3c is a chromatogram of the petroleum residue after degradation by an isolate from oil shale kerogen. The residue consists of isoprenoid and triterpenoid components. Similar results for biodegradation are shown in GC/MS chromatograms for the waxy Red Wash petroleum in Figure 4a. The undegraded petroleum is primarily paraffinic in character. Degradation by the oil shale-isolate OS-3 (Figure 4b) and the gilsonite-isolate G-2 (Figure 4c) gave residue chromatograms showing primarily triterpenoid compounds remaining.

Figure 5a is a GC/MS chromatogram of an asphaltic petroleum. Degradation of the oil by the tar sand-isolate TS-8 (Figure 5b) and the oil shale-isolate OS-2 (Figure 5c) gave residues containing similar components. Pristane and phytane (shown in the 795 and 860 positions) were only partially degraded. The same is true for triterpenoids in the 1400-1600 position. The large peak in the 1760 region corresponds to perhydro- $\beta$ -carotene (2). This substance seems resistant to biodegradation.

### CONCLUSION

Coryneform bacteria can play a significant role in crude oil biodegradation. The capability of these bacteria for hydrocarbon degradation varies between individual bacteria and between the specific forms of hydrocarbon attacked. Tri- and tetraterpenoid compounds are resistant to degradation. Consequently, oil degraded in the laboratory tends to assume the mature character observed under natural conditions.

### ACKNOWLEDGMENT

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TABLE 1. IDENTIFICATION OF BACTERIAL ISOLATES

<u>Source</u>	<u>Group or Genus</u>	<u>Number of Isolates</u>
Oil shale	Coryneform, <u>Aeromonas</u> <u>Flavobacterium</u> <u>Streptomyces</u>	7
Tar sands	Coryneform	6
Gilsonite	Coryneform	3
Oil shale-Mine water	<u>Pseudomonas</u> <u>Aeromonas</u> <u>Flavobacterium</u> <u>Desulfovibrio</u> <u>Beggiatoa</u>	6

TABLE 2. MODEL COMPOUND

Isolated Bacteria ( <u>Coryneform</u> )	<u>Cyclohexanol</u>	<u>Ethyl Nipeotate</u>	<u>Methyl Nipeotate</u>	<u>Ethyl-1 Methylnipeotate</u>	<u>Hexadecane</u>	<u>Pristane</u>	<u>Tert-butyl Cyclohexane</u>
G-2	±	4+		3+	3+	2+	-
OS-2	3+	2+		2+	3+	3+	±
OS-3	3+	ND		ND	ND	3+	ND
TS-6	3+	-		-	1+	-	1+
TS-7	2+	ND		ND	3+	2+	±
TS-8	2+	ND		ND	ND	3+	ND

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ND = Culture not viable for test.

FIGURE 1a Growth Curve for Arthrobacter #15590 and OS2 + Pristane

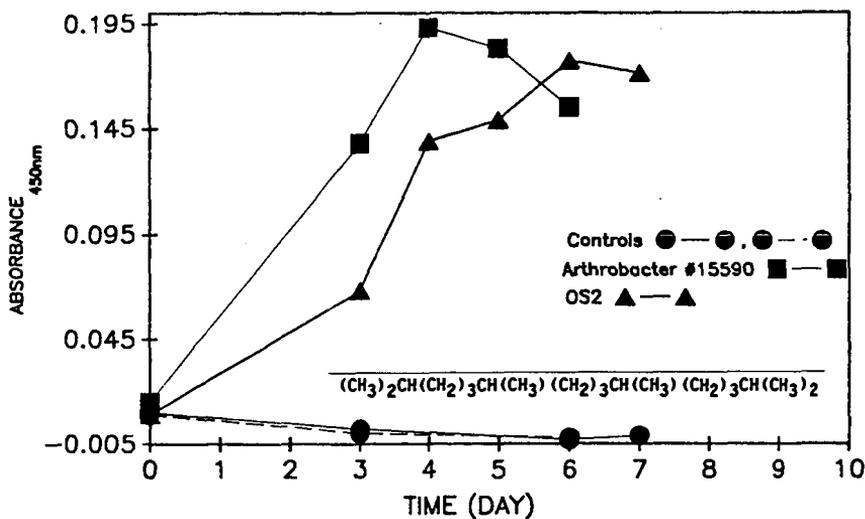
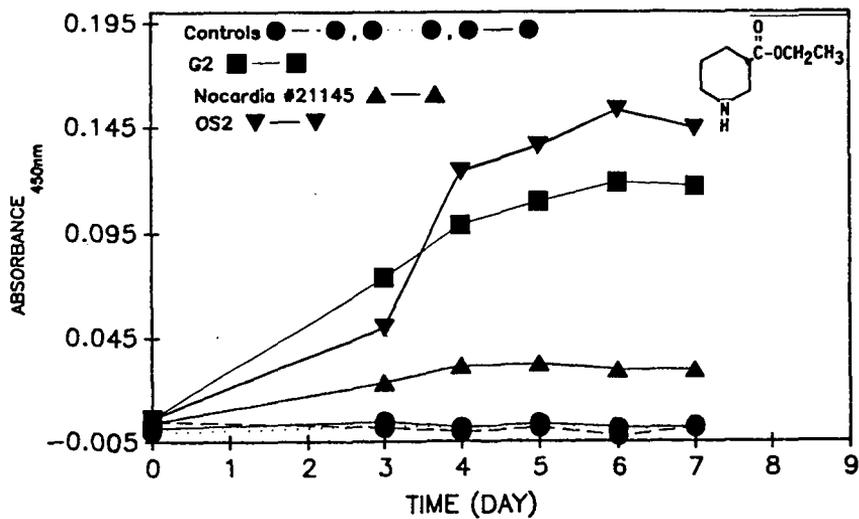


FIGURE 1b Growth curve for Nocardia #21145, G2, and OS2 + Ethyl Nipacotole



RESPIROMETER STUDIES

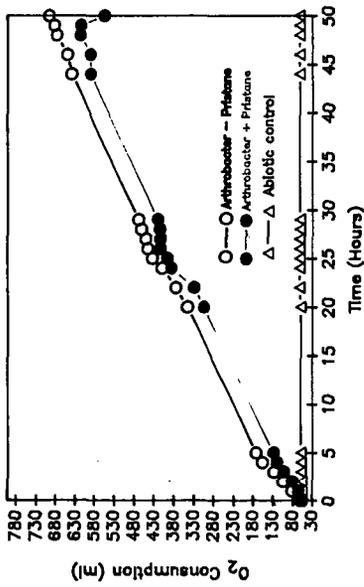


Figure 2a

RESPIROMETER STUDIES

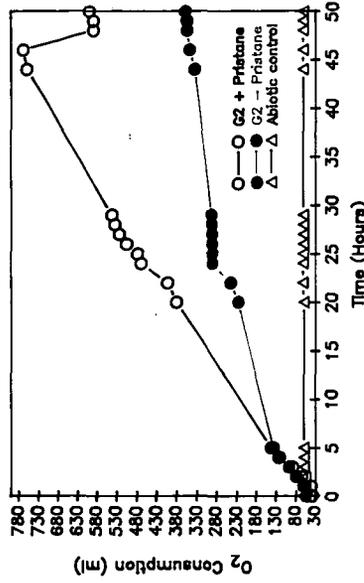


Figure 2b

FIGURE 3

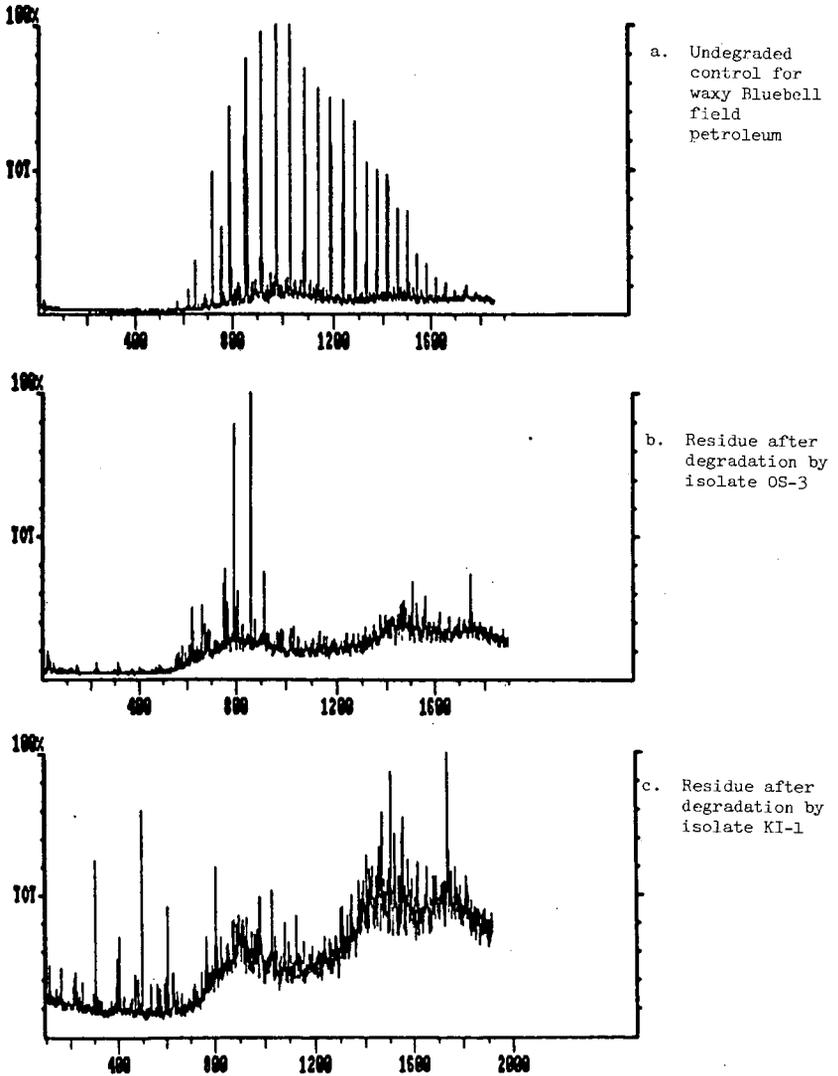


FIGURE 4

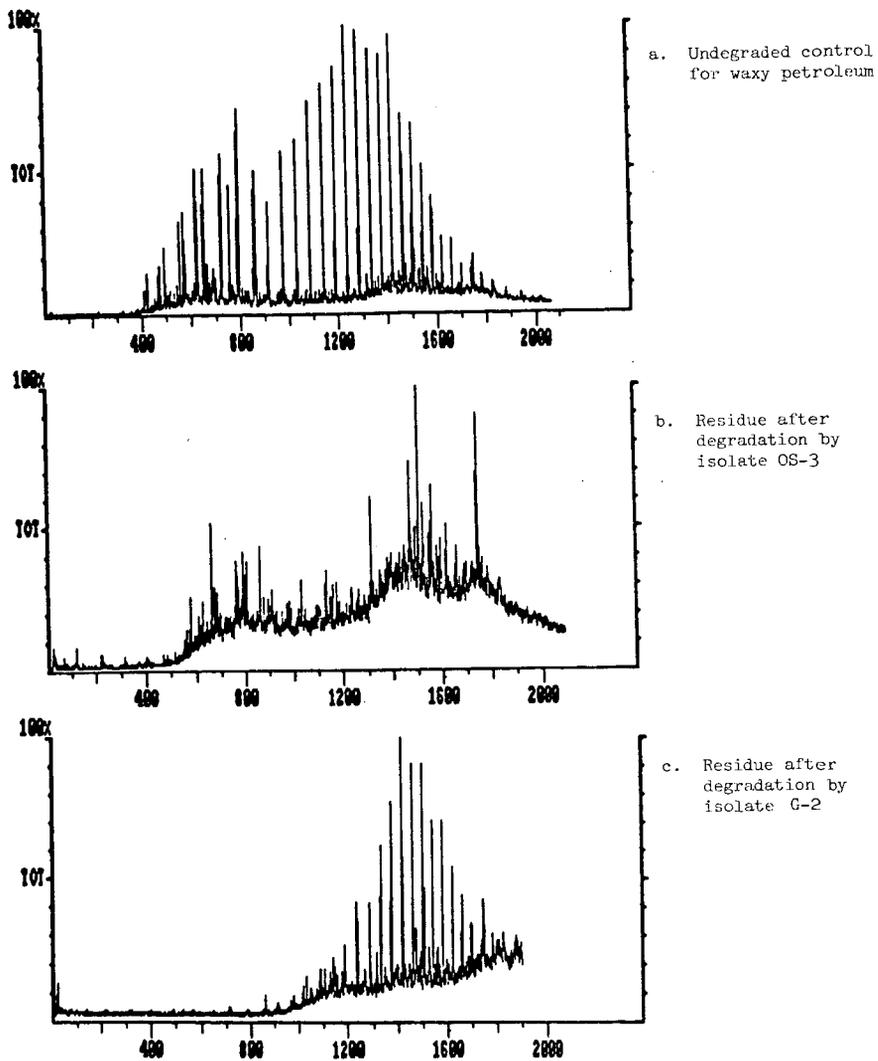
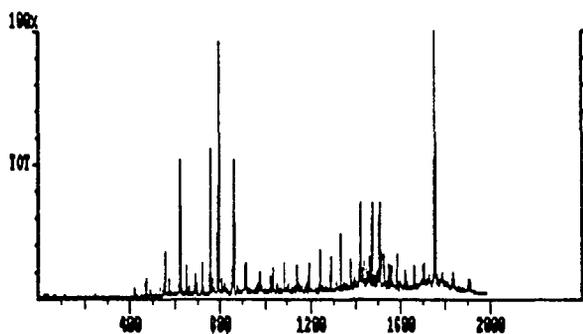
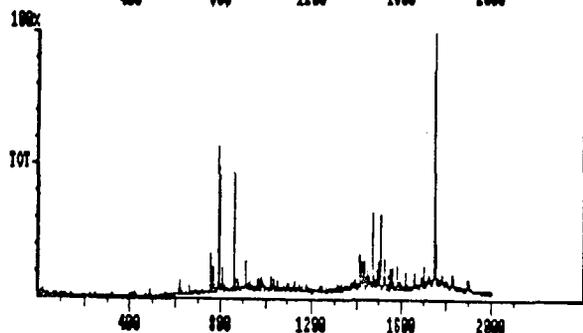


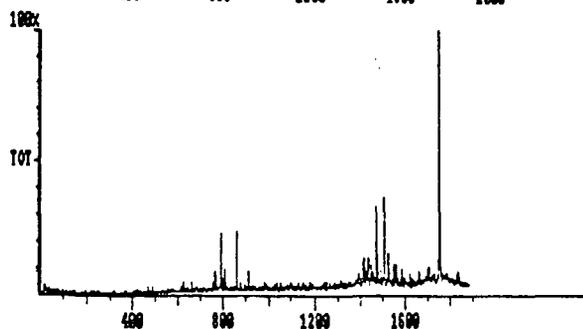
FIGURE 5



a. Undegraded control for asphaltic petroleum



b. Residue after degradation by isolate TS-8



c. Residue after degradation by isolate OS-2