

## Degradation of Petroleum by an Alga, *Prototheca zopfii*

J. D. WALKER, R. R. COLWELL,\* AND L. PETRAKIS

Department of Microbiology, University of Maryland, College Park, Maryland 20742,\* and Division of Physical Sciences, Gulf Research and Development Company, Pittsburgh, Pennsylvania 15230

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*Prototheca zopfii* is an achlorophyllous alga which degrades oil. It has been found to degrade 10 and 40% of a motor oil and crude oil, respectively, when tested under appropriate conditions. Degradation of the crude oil observed in this study compares well with the amount of degradation accomplished by bacteria. *P. zopfii* was found to degrade a greater percentage of the aromatic hydrocarbons in motor oil than of the saturated hydrocarbons and a greater percentage of saturated hydrocarbons in crude oil than of aromatic hydrocarbons. Resins and asphaltenes were produced during degradation of motor oil, whereas these fractions in crude oil were degraded. *P. zopfii* did not demonstrate preferential utilization of lower homologues of cycloalkanes and aromatics as has been observed with bacteria.

The isolation of a petroleum-degrading alga has been described and the organism has been classified as *Prototheca zopfii* (7). In this study, the degradation of hydrocarbons in petroleum by *P. zopfii*, monitored by mass spectrometry, is described. Compared to reports describing the utilization of pure hydrocarbons by microorganisms, there are relatively few reports describing the utilization of petroleum by pure cultures of bacteria, yeasts, and fungi. This study provides the first attempt to characterize the biodegradation of oil by a pure culture using mass spectrometry and the first investigation of the biodegradation potential of algae.

### MATERIALS AND METHODS

Description of the organism, culture system, and analytical methods have been reported previously (7; J. D. Walker, R. R. Colwell, and L. Petrakis, *Can. J. Microbiol.*, in press). The petroleum-degrading alga *P. zopfii* was isolated from Colgate Creek in Baltimore Harbor of Chesapeake Bay. Two petroleum substrates were selected for study: motor oil (10 to 20 weight, nondetergent), representing a refined product, and a South Louisiana crude oil. After 30 days of growth at 25 C in quiescent culture, uninoculated samples and inoculated cultures overlaid at the time of inoculation with 1% (vol/vol) motor oil or crude oil were extracted with chloroform, as described elsewhere (Walker et al., *Can. J. Microbiol.*, in press). To determine the percentage of oil remaining after degradation, the weight of the total residue, fraction of oil, or hydrocarbon type remaining after degradation was divided by the amount of each component present in the uninoculated sample to correct for weathering.

Results are expressed as percentage and weight of oil remaining to keep the percentage values in proper perspective. The data are means of duplicates.

### RESULTS AND DISCUSSION

The alga was found to utilize 10.7% of the motor oil and 41.4% of the crude oil (Table 1). The motor oil contained a lower percentage of alkanes (9%) than the crude oil (17%) and a higher percentage of aromatics (35%) and cycloalkanes (45%) than the crude oil, which contained 28% aromatics and 39% cycloalkanes. The lower percentage of alkanes, which are most susceptible to microbial degradation, and the higher percentage of cycloalkanes and aromatics, least susceptible to degradation in the motor oil, probably accounts for lower percentage of utilization compared to the crude oil.

Degradation of crude oil by pure cultures of bacteria and fungi has been reported, and the results obtained can be compared with utilization of South Louisiana crude oil by *P. zopfii*. There are, however, differences in the chemical

TABLE 1. Percentage and weight of residue and petroleum components remaining after degradation by *P. zopfii*

Component	Motor oil		Louisiana crude oil	
	%	Weight	%	Weight
Saturates	87.9	402.7	53.1	142.6
Aromatics	82.1	262.1	65.3	80.7
Resins	140.0	78.9	66.0	46.0
Asphaltenes	133.3	0.8	70.0	1.4
Total residue	89.3	744.5	58.6	280.2

composition of crude oils, and this factor should be taken into account. Atlas and Bartha (1) reported 57 and 40% utilization of a Sweden crude oil by a *Flavobacterium* sp. and a *Brevibacterium* sp., respectively, after 12 days of growth in artificial seawater at 28 C. Reisfeld et al. (4) reported 35% utilization of an Iranian crude oil by an *Arthrobacter* sp. after 4 days in a seawater medium supplemented with nitrate and phosphate and incubated at 32 C. Miget et al. (3) reported 35 to 55% utilization of a South Louisiana crude oil by five bacterial isolates after 60 h in seawater supplemented with nitrate and phosphate at 32 C. Cerniglia and Perry (2) reported 85 to 92% utilization of paraffin-base crude oil by the fungi *Cunninghamella elegans* and a *Penicillium* sp. after 10 days in seawater enriched with nitrate and phosphate at 30 C. Despite differences in experimental conditions, *P. zopfii* utilized as much crude oil as the *Brevibacterium* sp., the *Arthrobacter* sp., and three of the five bacterial isolates of Miget et al. (3). However, the fungi isolated by Cerniglia and Perry (2) were more efficient in utilizing crude oil. The capacity for utilizing crude oil demonstrated by *P. zopfii* is highly significant and, therefore, very likely is important in the overall microbial degradation of oil in Baltimore Harbor (7).

A greater percentage of aromatics, relative to the saturates, was removed from the motor oil compared with the crude oil (Table 1). Resins and asphaltenes increased during degradation of the motor oil, suggesting these to be products arising from the degradation of motor oil by *P. zopfii*. However, these components decreased in the crude oil during growth of *P. zopfii*, which suggests that they were degraded. An increase in the resins and asphaltenes after degradation has been reported for mixed bacterial cultures on motor oil and South Louisiana crude oil (6; J. D. Walker, R. R. Colwell, and L. Petrakis, *J. Water Pollut. Control Fed.*, in press).

Utilization of specific types of saturated and aromatic hydrocarbons in motor oil and crude oil (Tables 2 and 3) by *P. zopfii* supports the conclusion that greater utilization of the aromatics relative to the saturates was achieved when motor oil was the growth substrate. A significant decrease in the saturate fraction was observed for crude oil. The six-ring cycloalkanes did not appear to be utilized at all. Utilization of cycloalkanes in motor oil and crude oil was found to be less as the number of rings increased from one to four during growth of mixed bacterial cultures (5, 6; Walker et al., *J. Water Pollut. Control Fed.*, in press). However, the cyclo-

TABLE 2. Percentage and weight of saturated hydrocarbons in petroleum remaining after degradation by *P. zopfii*

Hydrocarbon	Motor oil		Louisiana crude oil	
	%	mg	%	mg
Alkanes	77.5	68.5	30.2	23.8
1-Ring cycloalkanes	91.5	93.1	64.4	34.2
2-Ring cycloalkanes	91.0	75.9	64.0	26.3
3-Ring cycloalkanes	89.4	52.1	62.4	17.9
4-Ring cycloalkanes	90.5	54.3	62.2	20.2
5-Ring cycloalkanes	89.3	33.5	46.1	12.3
6-Ring cycloalkanes	89.3	20.8	100.0	7.8

TABLE 3. Percentage and weight of aromatic hydrocarbons remaining after degradation by *P. zopfii*

Hydrocarbon	Motor oil		Louisiana crude oil	
	%	mg	%	mg
Monoaromatics	84.1	108.7	62.4	36.7
Alkylbenzenes	85.5	45.5	59.9	10.9
Naphthene benzenes	84.6	27.5	61.6	10.9
Dinaphthene benzenes	84.0	35.7	65.1	14.9
Diaromatics	80.9	72.2	64.4	28.9
Naphthalenes	86.5	23.1	58.9	9.2
Acenaphthenes-dibenzofurans	79.3	23.8	64.1	9.5
Fluorenes	77.8	25.3	64.4	10.2
Triaromatics	78.6	27.5	69.3	10.6
Phanthrenes	83.1	19.3	67.8	7.8
Naphthene-phenanthrenes	75.9	8.2	73.7	2.8
Tetraaromatics	80.2	13.4	72.6	4.5
Pyrenes	82.9	9.7	72.0	3.1
Chrysenes	74.0	3.7	73.7	1.4
Pentaaromatics	90.9	3.0	78.6	1.1
Perylenes	88.0	2.2	88.8	0.8
Dibenzanthracenes	93.9	0.8	60.0	0.3
Sulfur aromatics	84.8	29.0	68.4	3.9
Benzothiophenes	76.1	8.9	66.6	1.6
Dibenzothiophenes	89.5	17.9	71.4	2.0
Naphthobenzothiophenes	88.0	2.2	60.0	0.3
Unidentified aromatics	76.1	8.9	73.7	4.2

alkanes were degraded to approximately the same extent by *P. zopfii* (Table 2). In general, utilization of aromatics by mixed bacterial cultures decreased as the number of aromatic rings increased from one to five (5, 6; Walker et al., *J. Water Pollut. Control Fed.*, in press). This was observed for *P. zopfii* grown on crude oil, but was not so unequivocal a result when *P. zopfii* was grown on motor oil (Table 3).

In summary, *P. zopfii*, an achlorophyllous alga, possesses the ability to degrade oil. The pattern of oil degradation appears to be different from that of bacteria.

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