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Crude oil degradation by microorganisms isolated from the marine environment[1])

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The utilization of crude oil by microorganisms isolated from marine environments was investigated. Enrichment procedures for isolating crude oil degrading organisms were carried out using samples of mud collected among the estuaries and along the coast of North Carolina. The basal medium was seawater supplemented with nitrogen and phosphate. The fungi isolated under these conditions utilized crude oil more effectively than did the bacteria and several oil utilizing fungi were isolated in pure culture. Among the fungi selected were strains of *Cunninghamella elegans*, *Aspergillus versicolor*, *Cephalosporium acremonium*, and a *Penicillium sp.* These fungi utilized a wide variety of hydrocarbon substrates as a source of carbon and energy. All grew on a mineral salts medium with no requirement for seawater.

The amount of crude oil utilized by growing fungi was determined. The results suggested that of the fungi isolated *C. elegans* and the *Penicillium sp.* were most effective in degrading crude oil. When paraffin base crude oil was added to the seawater basal salts medium (0.2% v/v) and inoculated with *C. elegans* over 90% of the oil was assimilated after 5 days growth. Less than one-half of this amount of asphalt base crude oil was utilized. The optimum temperature for growth of fungi that utilized crude oil differed but all grew at temperatures between 15 and 24 °C. Significant growth of the fungi on crude oil did not occur unless the seawater was supplemented with a source of nitrogen and phosphorus. The results of this study suggested that fungi can effectively assimilate crude oil and that paraffin base crude is more readily degraded than the asphalt base crude oil.

Accidental oil spillage from tankers, off-shore drilling blow-outs, and other mishaps are the cause of a considerable oil pollution problem in marine areas. An estimated one million metric tons of oil is spilled into the waters of the world annually by man (MC CAULL 1969). One of the more publicized recent disasters was the grounding of the Torrey Canyon off the southwest coast of England in 1967 releasing over 110,000 tons of crude oil into the sea. The ultimate cleanup of environments contaminated by such accidents must be accomplished by biodegradation of the pollutant. Although microbes utilizing hydrocarbons are abundant in nature, tar lumps occur widely on the surface of the sea (BACKUS *et al.* 1970, HOULT 1969, MORRIS 1971) and the most readily biodegradable fraction of crude oil, the *n*-alkanes (FREDERICKS 1966), have persisted at the site of an oil spill (BLUMER and SASS 1972) after 2 years. Crude oil is harmful to plant and animal life (ARTHUR and CARTHY 1968, SMITH 1968) and may have unforeseen effects on living systems as hydrocarbons and their oxidative products enter the marine food chains.

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Hydrocarbon utilizing microbes have been studied extensively in recent years (BUSHNELL and HAAS 1941, DAVIS 1967, FOSTER 1962, IIZUKA and KOMAGATA 1964, JOHNSON 1964, PERRY 1968, PERRY and SCHELD 1968, VAN DER LINDEN and THIJSSE 1965). There have been studies on hydrocarbon metabolism in natural systems (HOLCOMB 1969, IIZUKA and KOMAGATA 1964, JONES and EDINGTON 1968, JONES 1969, LADD 1956, NAGUIB and OVERBECK 1970, PILPEL 1968, VOROSHILOVA and DIANOVA 1950) and the presence of hydrocarbon utilizers in soil has been used in oil prospecting (BRISBANE and LADD 1965). ZOBELL and PROKOP (1966) made a study of mineral oil biodegradation in littoral areas and JOBSON *et al.* (1972) investigated the bacterial degradation of crude oil by bacteria. Studies by RAHN in 1906 demonstrated that fungi could be effective utilizers of paraffinic hydrocarbons.

Studies were initiated in this laboratory on the degradation of crude oil constituents by hydrocarbon utilizing stock culture organisms of the genera *Pseudomonas*, *Mycobacterium*, *Corynebacter*, *Streptomyces*, and related forms. Although these organisms grew well on many fractions of crude oil they did not extensively degrade the oil (CERNIGLIA *et al.* 1971). This investigation was undertaken to isolate and study the microorganisms in littoral areas that would effectively degrade the major constituents of crude oil.

Material and methods

Enrichment: Mud and water samples were obtained from littoral areas along the coast of North Carolina. Enrichment culture was carried out by placing 2 cm of mud in a 15 cm crystallization dish covering the mud with seawater and placing 5 ml of selected crude oil on the water surface. The dishes were covered with aluminum foil and seawater was added periodically to prevent drying. The dishes were incubated 3—4 weeks at 25 °C after which samples were removed and streaked on well plates.

Well plates were prepared by adding 0.5 cm of sterile L-salts agar (PERRY 1968) to petri dishes. A sterile cork borer was used to cut a 1.5 cm well in the middle of the plate. About 0.5 ml of crude oil was pipetted into this well. After inoculation the plates were incubated at 25 °C for 10—14 days.

Substrate specifity: 5 ml of the liquid L-salts basal medium was placed in a 150 × 16 mm test tube. Solid and liquid substrates were added at 0.2%. Utilization of gaseous hydrocarbons was determined by replacing 50% of the air in a stoppered flask with the gaseous substrate. Generally 125 ml sidearm flasks containing 30 ml basal medium were employed. Sugars and some other compounds tested were arabinose, chitin, dextrose, fructose, galactose, glucosamine, maltose, mannitol, mannose, sucrose, ribose, starch, sorbose, trehalose dihydrate, turanose, xylose, raffinose, cellobiose, lactose, rhamnose, sorbitol. and melibiose. The intermediates tested were the sodium salts of acetate, lactate, propionate, succinate, butyrate, pyruvate, glucuronate, benzoate, and citrate. Straight chain 1-alkenes were 1-heptene, 1-octene, 1-nonene, 1-undecene, 1-dodecene, 1-tridecene, 1-tetradecene, 1-pentadecene, 1-hexadecene, 1-heptadecene, and 1-octadecene. The alcohols were 1-propanol, t-amyl alcohol, 2-methyl-1-pentanol, 4-methyl-1-pentanol, 1-heptanol, 1-octanol, 1-decanol, 1-undecanol, 1-dodecanol, 1-tetradecanol, 1-hexadecanol, and 1-heptadecanol. The cyclic compounds used were cyclohexane, cycloheptane, cyclooctane, cyclohexene, methylcyclohexane, 1,2-dimethyl cyclopentane, t-1,2-dimethyl cyclopentane, cyclohexanol, cyclopentanone, *o*-xylene, *m*-xylene, and *p*-xylene. Inoculum for the specificity tests were prepared by aseptically homogenizing a 5—10 day old agar plate culture in sterile physiological saline in a blender cup. A drop of the diluted homogenate was added aseptically to the test substrates.

Utilization of crude oil: The amount of crude oil utilized by fungi during growth was determined by adding a weighed sample of crude oil to 500 ml of seawater enriched with 0.25 mg/ml NH_4Cl and 0.03 mg/ml Na_2HPO_4/NaH_2PO_4 (pH 6.8) dispensed in a 2.8 liter FERNBACH flask. The flasks were inoculated with homogenized mycelium and incubated at 30 °C for 10 days in stationary culture. The residual crude oil was extracted from the

medium and mycelia with chloroform. The chloroform was removed by evaporation and the amount of crude oil remaining determined. Control experiments demonstrated that crude oil can be quantitatively recovered in this manner. There was some loss of lighter crudes during evaporation and this volatile material might be utilized by the fungi or would be lost from oil spilled in the environment by evaporation.

A modified SÖHNGEN apparatus (SÖHNGEN 1913) was devised to measure the oxidation rate of the various crude oils. 25 ml of L-salts media were placed in a 4.5×11 cm open mouth bottle and 0.5 ml of crude oil was placed in each bottle. The inoculated bottle was attached by glass tubing to a 50 ml graduated cylinder containing seawater supplemented with nitrogen and phosphate. The inoculum was added as previously described. The organisms were grown in stationary culture at 30 °C. Oxygen uptake was measured in ml at 0, 7, 14 and 21 days. Controls were used in which no inoculum was added, one without added crude oil and a third with n-C_{16} alkane as substrate.

Effect of temperature on growth: The fungi were grown in stationary culture for 10 days in 30 ml of L-salts media placed in a 125 ERLENMEYER flask. Two-tenths percent n-tetradecane was added as substrate. Inoculum was added as previously described and the culture was incubated at prescribed temperatures.

Mycelial mats were harvested by filtration through WHATMAN No. 4 filter paper in a BUCHNER funnel. The mycelial mat was washed 3 times with chloroform and weighed.

Effect of nitrogen on growth: The fungi were grown for 10 days in stationary culture at 30 °C on 80 ml seawater enriched with various concentrations of nitrate or ammonia. Phosphate was kept constant at 0.03 mg/ml Na_2HPO_4/NaH_2PO_4 (pH 6.8). Two-tenths percent n-tetradecane were added as substrate. Them ycelium was harvested after 10 days and the dry weight determined as above.

Results

The well plate procedure was developed as a method for adding hydrophobic substrates to agar plates in a way that growth could readily be observed. Fungal growth predominated on some plates and bacterial growth on others. The superiority of filamentous fungi over bacteria in the ability to utilize substantial quantities of crude oil was illustrated by the profuse growth of the fungi at the circumference of the well and the absence of any residual oil in the bottom of the well. On those plates where bacteria predominated there was no visual evidence of crude oil disappearance. The fungi isolated for further study were strains of *Cunninghamella elegans*, *Aspergillus versicolor*, *Cephalosporium acremonium*, and a *Penicillium sp.*

Table 1

Utilization of various substrates by fungi isolated from the marine environment. Fungi grown in stationary culture for 2 weeks at 30 °C. 5 ml of L-salts to which was added 50/50 (v/v) gaseous substrate, 0.1 ml liquid substrate or 5 mg/ml solid substrate placed in 150×16 mm test tubes. Inoculum for the growth of mycelium was prepared by aseptically homogenizing a 10 day old agar plate culture in 50 ml of sterile saline water using a small Waring blender cup. The homogenate was then added aseptically to the medium

	C. elegans	*Penicillium* sp.	*A. versicolor*	*C. acremonium*
Sugars	+[1])	+	NT	NT
Intermediates	+	+	NT	NT
n-alkanes C_{2-4}	+	+	+	+
C_{5-7}	−	−	−	−
C_8-C_{32}	+	+	+	+
Alkenes C_7-C_9	−	+	+	+
$C_{11}-C_{18}$	+	+	+	+
Alcohols C_3-C_{10}	−	−	−	−
$C_{11}-C_{17}$	+	+	+	+
Cyclics	−	−	−	−

[1]) +: growth, −: no growth, NT: not tested.

Table 2
Utilization of various crude oil samples by fungi isolated from the marine environment. The crude oil samples were tested under the conditions of Table 1. One tenth ml of crude oil added to each tube

Crude Oil Sample	*C. elegans*	*Penicillium* sp.	*A. versicolor*	*C. acremonium*
South Louisiana Crude (T–1)	+[1]	+	+	+
New Mexico Sour Crude (T–2)	+	+	−	+
Mid-Continent Crude Oil (P–1)	+	+	−	+
Bunker 6 (E–1)	−	+	−	−
Brega Crude (E–2)	+	+	−	+
San Andres, West Texas (A–1)	+	+	−	+
Swanson River Field-Alaska (A–4)	+	+	+	+
Off Shore Louisiana (A–6)	−	+	+	+
San Joaquin, Venezuela (A–7)	+	+	+	+
Lagunillas, Venezuela (A–8)	+	+	−	−
West Texas Sour Crude (C–1)	+	+	+	+
No. 6 Fuel Oil (G–1)	−	−	−	−
Mesa Crude (G–3)	+	+	+	+
Nigerian Crude (G–5)	+	+	+	+
Leona Crude (G–6)	−	+	+	+
Pennsylvania Crude (PA–1)	+	+	+	+
Light Off-Shore Crude (S–1)	+	+	+	+

[1]) +: growth, −: no growth.

Table 3
Amount of oil degraded by strains of *Cunninghamella elegans* and a *Penicillium* sp. Fungi grown in stationary culture for 10 days at 30 °C on seawater medium enriched with 0.25 mg/ml NH_4Cl and 0.03 mg/ml Na_2HPO_4/NaH_2PO_4 at pH 6.8 dispensed in a 2.8 l FERNBACH flask. Inoculum was added under the conditions described in Table 1

Crude oil sample	Oil content at beginning (g)[1])	Oil content after 10 days (g)		Amount of oil oxidized by fungi (%)	
		C. elegans	*Penicillium* sp.	*C. elegans*	*Penicillium* sp.
Nigerian Crude (G–5)	0.70	0.21	0.11	70.0	84.3
Pennsylvania Crude (PA–1)	0.70	0.06	0.05	91.5	92.9
Light Off-Shore Crude (S–1)	0.80	0.08	0.11	90.0	86.3
New Mexico Sour Crude (T–2)	0.70	0.40	0.46	42.9	34.3
Mid-Continental Crude (P–1)	0.60	0.10	0.30	83.4	50.0
Brega Crude (E–2)	0.60	0.20	0.26	66.7	56.7
Mesa Crude (G–3)	0.60	0.30	0.21	50.0	65.0
Swanson River Field-Alaska (A–4)	0.70	0.20	0.21	71.5	70.0
West Texas Sour Crude (C–1)	0.60	0.43	0.30	28.4	50.0
San Andres, West Texas (A–1)	0.50	0.21	0.20	58.9	60.0

[1]) Residual crude oil was extracted from mycelial mat and the medium with chloroform. Residual crude oil was determined by dry weight analysis.

All of these fungi could utilize a wide variety of hydrocarbon substrates (Table 1). The fungi were particularly effective in utilizing *n*-alkanes, straight chain alkenes, and branched compounds but did not utilize cycloalkanes unless there was a side chain attached to the ring. None of the fungi yielded significant

growth on aromatic substrates. All fungal isolates grew on the mineral salts medium without added seawater.

The fungal isolates were tested for the ability to utilize paraffinic, asphaltic and mixed base crude oil samples as a sole source of carbon (Table 2). Samples of crude were obtained from various sections of the world including South America, Alaska, and domestic crudes from both continental and off shore drilling sites. All of the fungi grew on paraffin and mixed base crude oil. The fungi did not grow as vigorously on the asphalt base crude oils (T-2, E-2, A-7, A-8, C-1). None of the isolates utilized No. 6 Fuel oil (G-1) within 10 days.

The results in Table 3 suggest that strains of *C. elegans* and the *Penicillium sp.* biodegraded 85—92 percent of the paraffin base crude oil (PA-1, S-1). *C. elegans* and the *Penicillium sp.* utilized 28—66 percent of the asphalt base crude oil (T-2, E-2, C-1). Mixed base crudes were utilized at rates between these two extremes.

Since crude oil is a combination of reduced hydrocarbons the amount of oxygen required for the complete utilization of the oil could be compared with the amount of oxygen necessary for the utilization of purified *n*-alkanes. *C. elegans* utilized paraffin base crude oil (PA-1, S-1) more rapidly and degraded

Table 4
Oxygen uptake resulting from crude oil utilization by *C. elegans*. *C. elegans* grown in a closed system (stoppered 150 ml wide mouth bottle) attached by tubing to a 50 ml graduated cylinder filled with L-salts. The bottle contained 10 ml L-salts and 0.5 ml oil. Incubated in stationary culture at 30 °C. Inoculum added as described in Table 1

Crude oil added	Oxygen uptake (ml) after		
	7 days	14 days	21 days
Pennsylvania Crude (PA–1)	10	14	25
Mid-Continent Crude (P–1)	11	14	16
West Texas Crude (A–1)	10.5	15	16.5
No. 6 Fuel Oil (G–1)	0	9	16
Light Off-Shore Crude (S–1)	12	18	31
Lagunillas, Venezuela (A–8)	0	8	10
Heavy Off-Shore Crude (S–2)	10	15	27

Table 5
Dry weight of fungi after growth at various temperatures. Fungi grown in stationary culture for 10 days on 30 ml of L-salts medium +0.2% *n*-tetradecane. Inoculum was added as described in Table 1

Temp. °C	*C. elegans* mg/ml[1])	*Penicillium* sp. mg/ml	*A. versicolor* mg/ml	*C. acremonium* mg/ml
2	0	0	0	0
15	1.00	0.45	0.32	2.25
24	2.85	1.50	0.81	7.45
30	3.21	2.25	0.50	7.52
35	0.23	3.01	0.10	3.25
37	0.15	3.25	0.05	0.25
42	0	0	0	0

[1]) Mycelium was harvested by filtration and extracted with chloroform. Mycelial mass was determined by dry weight.

Table 6
The total growth of *C. elegans* and *Penicillium* sp. at various nitrogen levels
Fungi grown for 10 days at 30 °C on seawater +0.2% *n*-tetradecane. Phosphate concentration constant at 0.03 mg/ml Na_2HPO_4/NaH_2PO_4 at pH 6.8. Inoculum added as described in Table 1

Nitrogen Concentration (mg/ml N)	$NaNO_3$		NH_4Cl		NH_4PO_4		NH_4NO_3	
	C. elegans	*Penicillium* sp.	*C. elegans*	*Penicillium* sp.	*C. elegans*	*Penicillium* sp.	*C. elegans*	*Penicillium* sp.
	mg/ml[1])		mg/ml		mg/ml		mg/ml	
0	0.23	0.41	0.20	0.36	0.23	0.31	0.22	0.34
0.08	0.30	0.54	1.40	2.72	1.00	4.83	1.00	3.36
0.16	0.50	1.52	1.61	4.00	2.40	5.15	1.20	3.82
0.25	1.30	2.20	2.10	6.21	1.50	5.91	2.30	3.92
0.32	1.32	2.32	2.00	7.67	0.80	4.19	1.40	4.63
0.50	0.50	2.30	1.40	6.83	0.23	2.27	1.40	2.01

[1]) Mycelium harvested and extracted as described in Table 5.

this crude more vigorously than the asphalt base crude oil (A-8) (Table 4) (note that G-1 is oxidized extensively after the prolonged lag).

The optimal temperature varied with the organism but all grew at temperatures between 15 and 24 °C. The rate of growth of the fungi is affected more than total growth at lower temperatures. The optimal temperature for *C. elegans* was 30 °C, 37 °C for the *Penicillium sp.*, 24 °C for *A. versicolor* and 30 °C for *C. acremonium* (Table 5).

All of the fungi grew well in a mineral salts medium without increased salinity. The optimum pH (6—8) for growth would render them capable of growth in most marine habitats.

The degradation of any increased quantity of carbonaceous material, such as hydrocarbons, fuel oil, or crude oil, in marine environments would depend on corresponding increased amount of microbial growth. Since nitrogen is a limiting factor in productivity in marine habitats (GUNKEL 1967), the effect of the limited nitrogen levels in seawater on the total growth of the fungi was tested (Table 6). The results suggested that 0.25—0.32 mg N per ml of liquid culture was sufficient for maximal growth of both *C. elegans* and the *Penicillium sp.*

Discussion

The way that oil pollutants are recycled in nature is via initial degradation by microorganisms. The marine environment contains a very wide range of different bacterial species, fungi and yeasts that degrade hydrocarbons. The experiments described in this report demonstrated that filamentous fungi degraded crude oil much more vigorously than did the bacteria. The elimination of crude oil polluting the marine environment might occur if organisms that degrade hydrocarbons were grown in bulk and added at the site of the oil spill. Substrate specificity results suggest that no single organism can attack all the component parts of crude oil and for the degradation of hydrocarbon pollutants several different kinds of microorganisms would be necessary. The fungi isolated during these studies were most effective against paraffin base crude oil. It is essential that organisms should be isolated that can degrade the more recalcitrant parts of asphalt base crude oil.

The rate of degradation of crude oil is markedly influenced by a range of environmental conditions. The hydrocarbon degrading filamentous fungi grew well in a mineral salts medium without added seawater. The salinity varies within very narrow limits in the open ocean but is seasonally quite variable in the estuarine waters where most oil spill accidents occur. These fungi would grow at any salinity from that of fresh water to that of the oceans. The fungi grew in a wide pH range (4—9) which would render them capable of growing in most marine habitats. The optimal temperature varied with the organism but all of the fungal isolates grew abundantly between 15 and 37 °C, therefore, the rate of oxidation of crude oil would proceed rapidly in warmer regions. Below 15 °C microbial degradation of crude oil would preceed slowly and the rate of oxidation of crude oil would not be great in latitudes beyond 75° N or S.

The levels of minerals, particularly nitrogen (GUNKEL 1967) in marine environments is insufficient to support the amount of microbial growth that would be necessary to eliminate any large oil spill. It would be necessary, therefore, to add some nitrogen and phosphate source to the waters in harbors and estuaries to accelerate the biodegradation of crude oil.

To rid the biosphere of oil pollutants by microbial degradation we might (1) augment the growth of indigenous hydrocarbon degrading microorganisms in the affected environment and/or (2) add significant numbers of organisms that utilize hydrocarbons (as well as adding N and P) at the site of the oil spill.

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References

ARTHUR, D. R. and CARTHY, J. D. 1968. The biological effects of oil pollution on littoral communities. London Field Studies Council London.

BACKUS, R. H., HORN, M. H. and TEAL, J. M., 1970. Petroleum lumps on the surface of the sea. Science (New York), **168**, 245—246.

BLUMER, M. and SASS, J., 1972. Oil pollution: persistence and degradation of spilled fuel oil. Science (New York), **176**, 1120—1122.

BRISBANE, P. G. and LADD, J. N., 1965. The role of microorganisms in petroleum exploration. In: Annual Rev. Microbiol., Vol. 19 (edited by C. E. CLIFTON). Annual Reviews Inc. Palo Alto, California.

BUSHNELL, L. D. and HAAS, H. F., 1941. The utilization of certain hydrocarbons by microorganisms. J. Bacteriol., **41**, 653—673.

CERNIGLIA, C. E., HUGHES, T. J. and PERRY, J. J., 1971. Microbial degradation of hydrocarbons in marine environments. Bacteriol. Proc., 56.

DAVIES, J. B., 1967. Petroleum Microbiology. Elsevier Publ. Co. New York.

FOSTER, J. W., 1962. Hydrocarbons as substrates for microorganisms. Antonie van Leeuwenhoek, **28**, 241—274.

FREDERICKS, K. M., 1966. Adaptation of bacteria from one type of hydrocarbon to another. Nature (London), **209**, 1047—1048.

GUNKEL, W., 1967. Experimentell-ökologische Untersuchungen über die limitierenden Faktoren des mikrobiellen Ölabbaues im marinen Milieu. Helgoländer wiss. Meeresuntersuchungen, **10**, 246—256.

HOLCOMB, R. W., 1969. Oil in the ecosystem. Science (New York), **166**, 204—206.

HOULT, D. P., 1969. Oil on the Sea. Plenum Press London.

IIZUKA, H. and KOMAGATA, K., 1964. Microbiological studies on petroleum and natural gas. I. Determination of hydrocarbon utilizing bacteria. J. gen. appl. Microbiol., **10**, 207—221.

JOBSON, A., COOK, F. D. and WESTLAKE, D. W. S., 1972. Microbial utilization of crude oil. Appl. Microbiol., **23**, 1082—1089.

JOHNSON, M. J., 1964. Utilization of hydrocarbons by microorganisms. Chem. and Ind. (London), **36**, 1532–1537.

JONES, J. G. and EDINGTON, M. A., 1968. An ecological survey of hydrocarbon oxidizing microorganisms. J. gen. Microbiol., **52**, 381–390.

JONES, J. G., 1969. The determination of microbial hydrocarbon metabolism in natural environments. Arch. Mikrobiol., **67**, 397–407.

LADD, J. N., 1956. The oxidation of hydrocarbons by soil bacteria. I. Morphological and biochemical properties of a soil diptheroid utilizing hydrocarbons. J. Austral. biol. Sci., **9**, 92–104.

MCCAULL, J., 1969. The black tide. Environment, **11**, 2–16.

MORRIS, B. F., 1971. Petroleum: tar quantities floating in the Northwestern Atlantic taken with a new quantitative neuston net. Science (New York), **173**, 430–432.

NAGUIB, M. and OVERBECK, J., 1970. On methane oxidizing bacteria in fresh waters. I. Introduction to the problem and investigations on the presence of obligate methane oxidizers. Z. Allg. Mikrobiol., **10**, 17–36.

PERRY, J. J., 1968. Substrate specificity in hydrocarbon utilizing microorganisms. Antonie van Leeuwenhoek, **34**, 27–36.

PERRY, J. J. and SCHELD, H. W., 1968. Oxidation of hydrocarbons by microorganisms isolated from soil. Canad. J. Microbiol., **14**, 403–407.

PILPEL, N., 1968. The natural fate of oil on the sea. Endeavour, **27**, 11–13.

RAHN, O., 1906. Ein Parrafin-zersetzender Schimmelpilz. Zbl. Bakteriol., II. Abt., **16**, 382–384.

SÖHNGEN, N. L., 1913. Benzin, Petroleum, Paraffinöl und Paraffin als Kohlenstoff- und Energiequelle für Mikroben. Zbl. Bakteriol., II. Abt., **37**, 595–609.

SMITH, J. E., 1968. 'Torrey Canyon' Pollution and Marine Life. Cambridge University Press London.

VAN DER LINDEN, A. C. and THIJSSE, G.J.E., 1965. The mechanisms of microbial oxidation of petroleum hydrocarbons. In: Advances in Enzymology and Related Subjects of Biochemistry, Vol. 27, pp. 469–546 (edited by F. F. NORD). Interscience Publishers New York.

VOROSHILOVA, A. A. and DIANOVA, E. V., 1950. Bacterial oxidation of oil and its migration in natural waters. Mikrobiologia, **19**, 203–210.

ZOBELL, C. E. and PROKOP, J. F., 1966. Microbial oxidation of mineral oil in Barataria Bay bottom deposits. Z. Allg. Mikrobiol., **6**, 143–162.

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