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Oxidation of Hydrocarbons by Marine Bacteria.*

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Using oxygen consumption as a criterion it has been demonstrated that various kinds of pure and mixed hydrocarbons are oxidized by bacteria found in sea water and marine mud. The bacteria were grown in sea water enriched with hydrocarbons as the only source of energy. In the initial experiments the manometric technic was used for following oxygen consumption but later it was found that more accurate results were obtainable by determining the dissolved oxygen content of water.

Sea water inoculated with enrichment cultures was thoroughly shaken to insure uniformity in its composition and its saturation with oxygen, after which it was siphoned into 150 ml glass-stoppered bottles. While holding the bottles at an angle to provide for trapping the hydrocarbon in the shoulder of the bottle, 0.1 ml of hydrocarbon was introduced with a pipette. The bottle was then stoppered, care being taken not to entrap any air bubbles. Duplicate bottles of water were analyzed at once for oxygen content using the Winkler technic, and other bottles were analyzed after different periods of incubation in a water bath at 22°C.

The protocol of a representative experiment in which raw sea water was used is summarized in Table I. Similar results were obtained with aged sea water which was virtually organic matter-free as indicated by the fact that its B.O.D. was less than 1.0 mg/l.

It is especially noteworthy that toluene which is sometimes used as a preservative of biological materials was attacked by certain marine bacteria. Soil bacteria which oxidize toluene have been described by Gray and Thornton[1] and by Tauson.[2] Incidentally in these studies we found marine microörganisms which utilize phenol and cresol. Some of them tolerate a saturated aqueous solution of tricresol and as much as 1.0% phenol.

Besides the hydrocarbons listed in Table I, we have noted the bacterial oxidation of petroleum ether, paraffin wax, paraffin oil, vaseline, benzene, xylene, n-hexane, pyridine and naphthalene as indicated by oxygen consumption and by bacterial multiplication. Enough bacteria develop in aged sea water enriched with certain hydrocarbons to render it turbid. Pronounced differences have been noted in the rate of utilization of different kinds of hydrocarbons but due to differences in solubilities and other technical difficulties, many factors which influence utilizability of the various classes of hydrocarbons are still obscure. In general, it appears that aliphatic hydrocarbons are attacked more readily than cyclic or aromatic compounds, and long chains are more susceptible to bacterial oxidation than hydrocarbons of small molecular weight.

All 60 ml samples of sea water and all 0.1 g samples of mud examined show the presence of bacteria which are able to utilize hydrocarbons. From enrichment cultures several species of *Proactinomyces, Pseudomonas* and *Mycobacterium* have been isolated. Bacteria which oxidize various kinds of petroleum hydrocarbons have been isolated from soil by Tauson,[2] Haas *et al.*,[3] Stone *et al.*[4] and others. The marine hydrocarbon-oxidizing bacteria differ greatly in their ability to attack different hydrocarbons.

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1 Gray, P. H. H., and Thornton, H. G., *Centralbl. f. Bakt.*, Abt. II, 1928, **73**, 74.

2 Tauson, V. O., *Planta*, 1929, **7**, 735.

3 Haas, H. F., Yantzi, M. F., and Bushnell, L. D., *Trans. Kansas Acad. Sci.*, 1941, **44**, 39.

4 Stone, R. W., Fenske, M. R., and White, A. G. C., *J. Bact.*, 1942, **44**, 169.

TABLE I.
Oxygen Consumed by Bacteria in Raw Sea Water Treated with Hydrocarbons.

Hydrocarbon	Initial dissolved O_2 content, mg/l	Dissolved O_2 after 14 days, mg/l	O_2 consumed 14 days, mg/l	O_2 consumed oxidizing hydrocarbon, mg/l
None (Control)	8.46	5.99	2.47	—
Crude oil	8.50	0.00	8.50	6.03
Petroleum ether	8.57	0.00	8.57	6.10
Kerosene	8.60	1.72	6.88	4.41
Gasoline	8.60	0.00	8.60	6.13
Toluene	8.64	0.00	8.64	6.17

They do not require hydrocarbons for their growth.

Marine hydrocarbon-oxidizing bacteria may influence the formation, accumulation and storage of petroleum or its products. Hydrocarbons which may be produced by the reduction of sapropel in marine sediments may be oxidized by such bacteria *in situ* as rapidly as they are formed, thereby preventing their accumulation in detectable quantities. As a matter of fact, traces of complex compounds such as polyene pigments and their allies are about the only hydrocarbons found in recent sediments with the exception of methane. The abundance of hydrocarbon-oxidizing bacteria in storage tank water suggests that they may play a role in the decomposition of petroleum products. Marine bacteria which oxidize natural and synthetic rubber, $(C_5H_8)_x$, have been described elsewhere by the authors.[5]

It is still indeterminate whether hydrocarbons are utilized in an anærobic environment although there is some evidence that certain substances such as nitrate and possibly sulfate may serve as hydrogen-acceptors in the absence of free oxygen. Low concentrations of hydrocarbons appear to be oxidized quantitatively to carbon dioxide and water, but with larger concentrations, methane and organic acids have been detected as end-products. Adsorbing the hydrocarbons on sand has proved to be a satisfactory method of providing for the dispersion of the hydrocarbons which are virtually insoluble in water thereby rendering them more susceptible to bacterial attack.

[5] ZoBell, C. E., and Grant, C. W., *Science*, 1942.

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Amino Acid Fermentations by Anaerobic Bacteria.

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Although many anærobic bacteria decompose proteins and the products of protein hydrolysis, only a few species are known to be able to satisfy their energy requirements by the fermentation of single amino acids or other nitrogenous compounds. These species are *Clostridium tetanomorphum* and *Cl. cochlearium*[1,2] which ferment glutamic acid and *Cl. acidi-urici* and *Cl. cylindrosporum*[3,4] which ferment uric acid and some other purines. Possibly *Cl. botulinum*,[5] *Cl. sporogenes*,[6] and *Cl. tetani*[7] should also be in-

[1] Barker, H. A., *Enzymologia*, 1937, **2**, 175.

[2] Barker, H. A., *Arch. f. Mikrobiol.*, 1939, **10**, 376.

[3] Barker, H. A., and Beck, J. V., *J. Bact.*, 1942, **43**, 291.

[4] Barker, H. A., and Beck, J. V., *J. Biol. Chem.*, 1941, **141**, 3.

[5] Clifton, C. E., *J. Bact.*, 1940, **39**, 485.

[6] Hoogerheide, J. C., and Kocholaty, W., *Biochem. J.*, 1938, **32**, 949.

[7] Clifton, C. E., *J. Bact.*, 1942, **44**, 179.