

MICROBIOLOGICAL OXIDATION OF A COAL TAR FRACTION*

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A process for the production of edible protein by the microbiological oxidation of petroleum hydrocarbons has recently been developed to pilot-plant scale. By fermentation with yeasts, n-alkanes are selectively oxidized in the presence of other types of saturated hydrocarbons in lubricating-oil fractions, and are converted almost quantitatively to high-protein cellular material.¹

The use of other forms of fossil organic matter as sources of carbon for the production of protein, amino acids, and other supplementary edible materials is of interest, and it has been found in this laboratory that certain types of coal derivatives and humic materials will support the growth of microorganisms. The microbiological treatment of some by-products of coal processing has lately been investigated² and it has been shown that C₁₁–C₁₅ paraffins and olefins present in low-temperature coal tar fractions will support the growth of the yeast *Candida*, giving cell yields similar to those obtained from petroleum fractions boiling in the same range. Also, the biochemical degradation of hydrocarbons and other compounds present in oils, tars, effluents, and industrial wastes is a research topic of growing importance because of the increasing pollution of rivers, lakes, and beaches and the vital necessity, in many industrial areas, of more efficient conservation and purification of water supplies.

In a preliminary investigation of the microbiological oxidation of materials of this type, a continuous-vertical-retort coal tar was extracted with aqueous acid and alkali to remove bases and phenols. A fraction of the neutral tar (20.2 g, b.p. 220–250°/20 mm) was stirred in a nutrient salt medium (1500 ml) containing ammonium sulphate (0.1%), magnesium sulphate (0.02%), dipotassium phosphate (0.2%), sodium dihydrogen phosphate (0.3%), sodium carbonate (0.01%), calcium chloride (0.001%), and ammonium nitrate (0.1%) at 25–35°. The mixture was inoculated with enriched cultures of paraffin-consuming and 2-methylnaphthalene-consuming soil microorganisms, consisting mainly of rod-shaped bacteria.

Aliquot samples were removed at intervals during a total incubation period of 40 days. Unconverted neutral material was removed from each fraction by extracting with ether at pH 8.0 and the cell material was collected by centrifuging and washed with ether. Acidic and phenolic oxidation products were recovered by adjusting the pH to 3.0 and re-extracting with ether. The aqueous liquors were evaporated to dryness in a rotary evaporator under vacuum at 100°. The solid residue was extracted with acetone to recover other water-soluble products.

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¹ Champagnat, A., Vernet, C., Lainé, B., and Filosa, J., *Nature*, 1963, **197**, 13.

² Silverman, M. P., Novak, J. M., and Wender, I., 150th National Meeting, Am. chem. Soc., Division of Fuel Chem., Vol. 9, No. 4, p. 55.

The neutral material from each aliquot was fractionated by chromatography on Fluorisil. The non-fluorescent first fraction eluted by light petroleum was analysed by gas chromatography on 2 m by 2 mm columns having 5% of polyphenyl ether dispersed on Chromosorb G, maintained at 210°, and 5% silicone SE 30 on Chromosorb G, at 225°.

For the original tar fraction the chromatogram showed a regular series of peaks superimposed on shallow unresolved background. The main peaks were shown to be

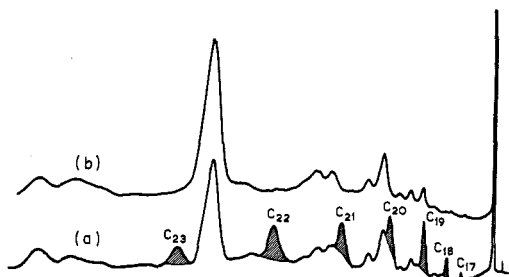
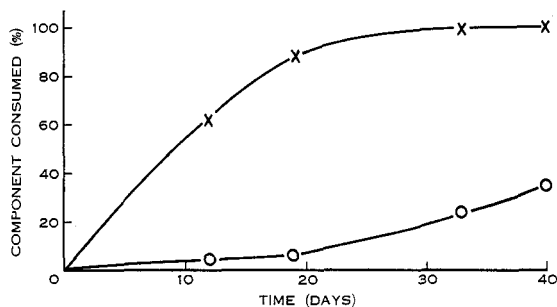


Fig. 1.—Gas chromatograms of neutral tar fraction
(a) before
(b) after incubation for 19 days.

the n-paraffins C₁₇–C₂₃ by comparison with standard mixtures run before and after the sample; the peaks gave a perfectly linear graph of log(retention time) against carbon number. Quantitative estimations of the n-alkanes were made by measuring the areas under the corresponding peaks.

In the light petroleum eluate from the material remaining after 19 days' incubation the amount of n-paraffins was small; the gas chromatogram showed that peaks which were almost certainly due to iso-paraffins were much more prominent

Fig. 2.—Microbiological oxidation of paraffins and non-paraffins in a neutral coal tar fraction.
× n-Paraffins.
○ Non-paraffins.



than in the original tar fraction. It is well known that these hydrocarbons are much more resistant to microbiological oxidation than the n-paraffins.

During the experiment the rate of consumption of the tar fraction was approximately linear. The changes that had occurred in its composition after 19 days are indicated in Figure 1. Initially, bacterial growth on the aliphatic hydrocarbons was preferential. After 19 days 88% of the original n-alkanes had been converted to cell material and other products, but only 5% of the non-paraffinic compounds had been attacked. In this tar fraction the aliphatic hydrocarbons (21.8%) consisted almost

entirely of the n-alkanes, with only small amounts of olefins and branched-chain paraffins. In the non-paraffinic part of the tar fraction the main constituent was phenanthrene as shown by gas chromatography, with and without added standard compound. Extraction of the tar fraction with saturated methanolic potassium hydroxide gave 12% extract consisting of hindered phenols which had not been removed by the aqueous alkali extraction of the original tar. In the later stages, the rate of conversion of the non-paraffinic constituents rose steeply and had not begun to diminish at the end of the 40 days' incubation (Fig. 2).

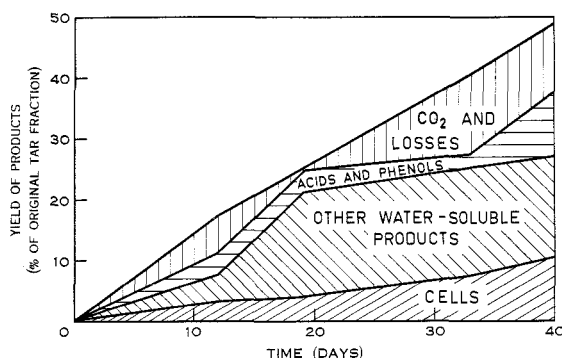


Fig. 3.—Products of the microbiological oxidation of a neutral coal tar fraction.

Figure 3 shows the yields of the products of the microbiological degradation. At the end of the experiment the dry cell yield was 25% of the tar fraction consumed. The yield of water-soluble products was 33%, and of ether-soluble acids and phenols 22%. The remainder of the tar fraction was presumably oxidized to carbon dioxide; evolution of gas was noticeable towards the end of the experiment.

It was reported by Traxler³ that a paraffin-consuming *Pseudomonas* micro-organism was unable to utilize the n-alkanes known to be present in petroleum bitumens if the aromatic hydrocarbon content of the bitumen was high. In the investigation reported here, however, the oxidation of the n-alkanes proceeded freely in the presence not only of polynuclear aromatic hydrocarbons but also of phenolic compounds. The n-alkanes and the aromatic compounds were oxidized successively.

More detailed investigations of the microbiological treatment of coal by-products are in progress.

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³ Traxler, R. W., 150th National Meeting, Am. chem. Soc., Division of Fuel Chem., Vol. 9, No. 4, p. 268.