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BIOCHEMISTRY AND MECHANISM OF HYDROCARBON UTILIZATION BY MICROORGANISMS

BY

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Microorganisms are principal agents for recycling of carbon in nature. Over geological times enzyme systems have evolved for the degradation of naturally occuring organic molecules. In fact, metabolic versatality of microbial population has led some microbiologists to believe that for every organic compound there exists somewhere a microorganism that will catalyse its degradation. On this belief works have been undertaken to solve the huge problem of oil crisis by microbial transformation and extraction of oil from possible sources and pollution control through recycling of huge kinds of pollutants through metabolic activities of microorganisms (Cerniglia and Gibson, 1978). It is astounding to think of the vast number of organic compounds that can be formed out of hydrocarbons and the whole organic chemistry, called as chemistry of carbons may better be called as chemistry of hydrocarbons and almost all of them are susceptible to microbial attack (Schlegel, 1976). The way the hydrocarbons are recycled in nature is via initial degradation by microorganisms. There are a very wide range of different bacterial species, filamentous fungi and yeasts responsible for this purpose (Zobell, 1946 ; Fuhs, 1961 ; Klug and Markovetz, 1971).

The present paper discusses recent studies on the microbial metabolism of aliphatic hydrocarbons that have suggested special biochemical traits of such microorganisms and the mechanism involves some unique enzyme systems.

Biochemistry of Hydrocarbon Utilizing Microorganisms : The microorganims which are able to use petroleum hydrocarbons as nutrients are assumed to evolve special biochemical traits to overcome the problems inherent to petroleum hydrocarbons due to their chemical heterogenicity and water insolubility

(Gutnick and Rosenberg, 1977). Three such traits 'are : first, an efficient hydrocarbon uptake system i. e, special receptor site for binding hydrocarbons or production of special chemicals to assist in the emulsification and transport of hydrocarbons into the cells ; second, the organisms must possess group-specific oxygenases to introduce molecular oxygen into the hydrocarbon molecules and with relatively few reactions generate such intermidiates which can enter common energy yielding catabolic pathways ; and third, the organisms must have inducer specificity to induce the first two systems in presence of petroleum hydrocarbons. When oil droplets come in contact with microorganisms, pseudosolubilization of oils in the medium involving uptake of fine droplets take place (Erickson and Nakahara, 1975). Kennedy et al. (1975) demonstrated direct contact of *Acinetobacter* cells to n-alkanes and crude oil (Horowitz et al., 1975) under phase contrast microscopic study. High affinity for the oil droplets of several hydrocarbon degrading yeasts has been shown by various workers (Ludvik et al , 1968 ; Minura et al., 1977) whereas no such affinity has been shown by glucose grown cells of *Escherichia coli*, *Aerobacter aerogenes* and *Bacillus subtilis* (Kennedy et al., 1975). The size of the oil droplets has direct relation to the growth rate of hydrocarbon degrading yeasts. Pesudosolubilization is a function of surface area exposed to water and is expected to increase with the increasing surface area (Gutnick and Rosenberg, 1977).

Extracellular emmulsifying agents of microbial origin have also been reported from hydrocarbon grown cells forming oil-water emulsion. Iguchi et al. (1969) reported an emulsifying agent from *Candida petrophilum* composed of peptides with fatty acid molecules. Suzuki et al. (1969) found a trehalose lipid in the oil phase from culture broth of *Arthrobacter*, *Brevibacterium*, *Corynebacterium* and *Nocardia*. Rhamnolipid as an emulsifier was reported to be produced by different species of *Pseudomonas* (Hisatsuka et al., 1971 ; Itoh and Suzuki, 1972). Recently, Rosenberg et al. (1979a,b) isolated an emulsifying agent of *Arthrobacter* RAGI and reported its specificity for hydrocarbon substrates. Kennedy and Finnerty (1975) and Scott and Finnerty (1976a) showed accumulation of unmodified hydrocarbons in the cells of *Acinetobacter* sp Hexadecane, heptadecane and hexadec-1-ene were found to be localized in specific inclusion bodies within cells growing on these substrates. The inclusion bodies, were found to be in association with the intracytoplasmic membrane systems. They were synthesized specifically during growth on the hydrocarbon (Scott and Finnerty, 1976b). Hydrocarbon oxidation is an inducible system, Thus the ability of a particular substrate to serve as sole source of carbon and energy will be a function of its ability to induce the

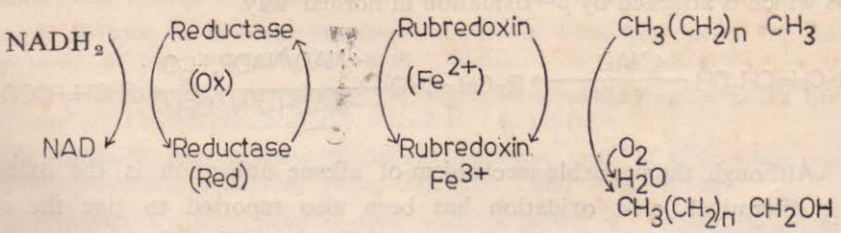
necessary enzymes for its subsequent oxidation. Van der Linden and Huybregste (1967) and Van Eyk and Bartels (1963) demonstrated the inducible alkane oxidising system in a number of *Pseudomonas* sp in which the effective inducers included the alkanes, aliphatic diols. straight chain diethers and dicyclopropyl compounds.

The metabolic ability to utilize hydrocarbons is controlled by transmissible plasmids as demonstrated by Wheelis (1975). Introduction of plasmids and plasmid fusion by UV irradiation resulted in an increased ability of a *Pseudomonas* strain to grow on crude oil (Chakravorty, 1972 : Chakravorty et al. 1973) and the specificity for hydrocarbon degradation may be regulated by transmissible plasmid DNA segments (Dunn and Gunsalus, 1973 ; Rheinwald et al. 1973 ; Chakravorty et al., 1978).

Mechanism Of Hydrocarbon Utilization : The mode of attack on n-alkanes has been adequately reviewed (van der Linden and Thijsse, 1965 , Mckenna and Kallio, 1965 ; Klug and Markovetz, 1971). There are differences among various microorganisms, but the initial attact on the hydrocarbon is usually a hydroxylation reaction generally at c_1 and catalysed by a mixed function oxidase (monooxygenase).

$$R\text{-}CH_2\text{-}CH_3 \xrightarrow[-H_2O]{+NADH_2 \; + \; O_2} R\text{-}CH_2\text{-}CH_2OH \quad \text{(Foster)}$$

The enzyme system has been studied in cell-free extracts and probably involves a series of electron carriers ; in *Pseudomonas oleovorus* the following system occurs (Peterson et al., 1967).

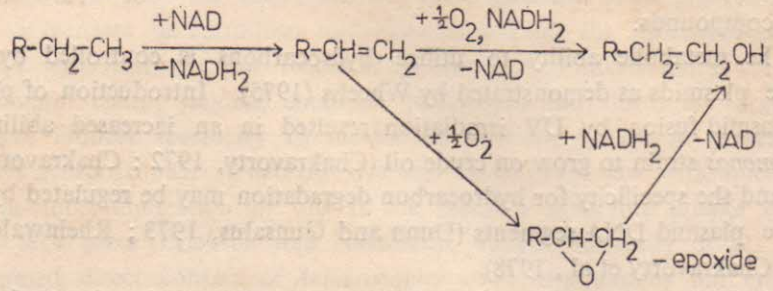


In other organisms a cytochrome P-450 functions as an intermediate electron carrier (Cardini and Jurtshuk, 1968).

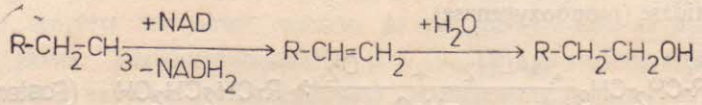
The initial oxygenases reaction may involve a hydroxy peroxidation stage (Stewart and Kallio, 1959).

$$R\text{-}CH_2\text{-}CH_3 \xrightarrow{+O_2} R\text{-}CH_2\text{-}\underset{\underset{O\text{-}OH}{|}}{CH_2} \xrightarrow[+H_2O]{+H_2} R\text{-}CH_2\text{-}CH_2OH$$

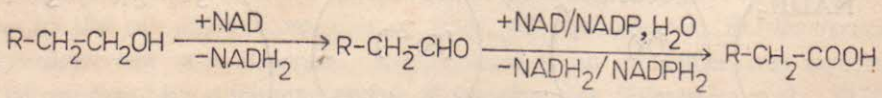
Another possibility which has great attractions for an economic industrial process of alkane utilization is dehydrogenation to the correspoding alk-1-ene (Senez and Azoulay, 1961).



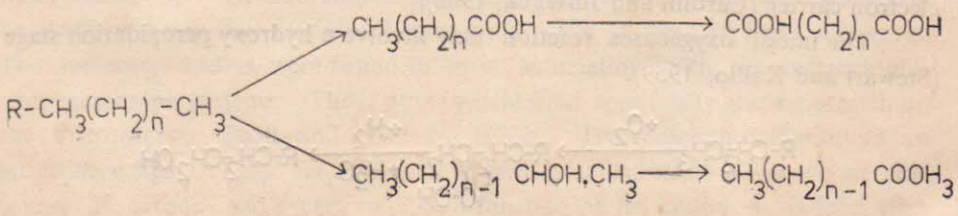
This reaction as shown above either followed by the addition of water to the double bond or by oxygenation to give a 1, 2, epixide which could be reduced to primary alcohol. The anaerobic fermentation of alkanes has also been proposed by Iizuka (1968) and confirmed by Traxler and Bernard (1969).



Anyway the initial oxidation is usually accompanied by incorporation of molecular oxyzen as has been shown by Leadbetter and Foster (1959) by $^{18}O_2$ experiments. The initial oxidation to corresponding alcohol is normally followed by two NAD linked dehydrogenation steps to the corresponding acids which is attacked by β—oxidation in normal way.



Although the probable mechanism of alkane utilization is the oxidation of c_1, diterminal ω, w oxidation has been also reported to give the corres ponding dicarboxylic acid. Another possibility is to give rise to corresponding secondary alcohols and ketones.



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