CHAPTER I

GENERAL INTRODUCTION
HISTORICAL HIGHLIGHTS

Hydrocarbons represent compounds with an excellent source of energy. For example, hexane, a straight chain hydrocarbon, yields approximately 1.5 times more calories as compared to that of glucose and aromatic nucleus has a large negative resonance energy. The percentage of carbon in hydrocarbons is about 80-89 indicating that a significant amount of available carbon is combined in them. Moreover, petroleums, petroleum products and synthetic chemicals containing derivatives of aromatic and aliphatic hydrocarbons are added to the environment. Therefore, decomposition of hydrocarbons is essential for maintenance of carbon cycle as their accumulation will lead to serious ecological changes.

However, upto the beginning of the century, hydrocarbons were believed to be stable group of biologically inert compounds highly refractory to biological enzyme attack. Consequently, refined mineral oils, petroleum jelly, vaspar and other hydrocarbon mixtures were commonly used for preserving cultures or for excluding atmospheric oxygen for culturing anaerobes.

The first report on the ability of microorganisms to grow on hydrocarbons appeared in 1895 wherein Miyoshi (1895) described the growth of Botrytis cinerea on paraffin at room temperature and atmospheric pressure. Almost simultaneously in 1906, Kaserer (1906) in Germany and Sohngen (1906a) in the Netherlands reported the isolation of methane oxidizing bacteria from water and soil respectively. Reports on the isolation of bacteria capable of oxidizing toluene, xylol and methane (Stormer, 1908), gasoline, kerosene, paraffin oil and paraffin (Sohngen, 1913a), paraffinic aliphatic compounds (Tausz and Peter, 1919), aromatic compounds
such as naphthalene, toluene, cresol and phenol (Gray and Thornton, 1928), phenanthrene, benzene, xylene and naphthalene (Tausson, 1927, 1928a, 1929) and paraffin wax (Rahn, 1906; Sohngen, 1913a; Gainy, 1917; Buttner, 1926; Haag, 1927; Jensen, 1934) were subsequently appeared.

Further studies on the isolation and characterization of methane oxidizing microorganisms (Munz, 1915, 1920; Aiyer, 1920; Sohngen, 1906b, 1910; Giglioli and Mason, 1914; Tausz and Donath, 1930; Thaysen, 1940), isolation of petroleum oxidizing coccobacillus from petroleum sample collected at a depth of 8700 feet (Lipman and Greenberg, 1932) and microbial utilization of paraffin hydrocarbons (Umbreit, 1939; Sohngen and Pol, 1914; Sohngen, 1910; Jones and Scott, 1939) were reported.

The application of hydrocarbon utilizing abilities of microorganisms in removing aliphatic hydrocarbons and in subsequent purification of cyclic hydrocarbons from a mixture of the two (Tausz and Peter, 1919), quantitative test for aliphatic hydrocarbons in natural oils or artificial mixture (Tausz and Peter, 1919), selective isolation of saprophytic members of Mycobacterium by using mineral salt medium with paraffin wax as sole source of carbon (Buttner, 1926; Haag, 1927; Jensen, 1934), effectiveness of hydrocarbon insecticides (Matthews, 1924), emulsification characteristics of hydrocarbons due to bacterial action (Tausson and Schapiro, 1934), increase in soil fertility over natural gas leaks (Harper, 1939) and microbial methods of petroleum prospecting (Mogilevskii, 1938) were advocated.

These reports clearly demonstrated the hydrocarbon oxidizing abilities of microorganisms. But, despite these investigations,
hydrocarbon utilizing microorganisms were generally regarded as biological curiosities and it was believed that only limited types of microorganisms are able to oxidize hydrocarbons. Profuse microbial growth on hydrocarbons as sole source of carbon, as compared to that on carbohydrates and proteinaceous media, was not considered to be possible.

The review on the role played by microorganisms in formation of oil (Hammer, 1934) focussed the attention of petroleum industry on microbial genesis of oil. The presence of hydrocarbon utilizing bacteria in all marine sediments tested (Zo Bell et al., 1943), investigations on the prevalence of hydrocarbon utilizing microorganisms in soils and their substrate specificity for different hydrocarbons (Stone et al., 1940; Haas et al., 1941; Stravinski and Stone, 1940; Lee and Chandler, 1941) and the respiratory studies on hydrocarbon utilizers (Stone et al., 1942; Johnson et al., 1942) were subsequently reported. Establishment of the fact that bacterial utilization of hydrocarbons is a characteristic common to many types of microorganisms which occurs to a great extent in nature than generally recognised, similarity of hydrocarbon oxidation with oxidation of conventional organic compounds (Bushnell and Haas, 1941) and the abilities of hydrocarbon oxidizing bacteria to use carbohydrates (Johnson and Schwarz, 1944) played significant role in development of the petroleum microbiology in U.S.A. and other countries. The first U.S.A. patent was issued to Blau (1942) for microbial oil prospecting.

By this time, many investigators started studying the microbial mechanism of attack on hydrocarbons and metabolic pathways
(Hayashi and Hashimoto, 1950; Hayashi et al., 1955; Evans et al.,
1951; MacDonald et al., 1954; Stewart et al., 1960; Senez and Azouley,
1961). Since 1955, enumerable reports of microbial attack on hydrocarbons and related subjects appeared in the literature. Microorganisms known to attack hydrocarbons were numbered in thousands (Humphrey, 1967).

The main industrial concern on the microorganisms assimilating hydrocarbons was focussed till 1960 on oil prospecting (Ogilevskii, 1938; Horvitz, 1939; Hassler, 1943), microbial secondary recovery of oils (ZoBell, 1946a, 1947a, b; Updegraff and Allen, 1953), corrosion problems in petroleum industry (Doig and Jachter, 1951; Hadley, 1939; Peat, 1956), microbial growth in aviation turbine fuels (Bakanaukas, 1958; Peat, 1956; Churchill and Leathen, 1961), microbial genesis of oil (ZoBell, 1943, 1958; Davis and Updegraff, 1954), destruction of oil in deposits (Belousov, 1937; Ogilevskii, 1953; Ekhertsov, 1960), plugging of reservoirs (Horvitz, 1943; Fekete, 1959; Allison, 1947; Hart et al., 1960), microbial disposal of petroleum wastes (MacRae et al., 1956; Karelin and Vorobeva, 1957) and microbial increase in soil fertility due to hydrocarbons (Matthews, 1924; Jacobs, 1931; Guseinov, 1958).

Subsequent reports on the possibilities of the microbial dewaxing of crude oils (Raymond and Davis, 1960), production of food and feed grade yeasts from petroleum fractions (Charpagnat, 1963), microbial production of salicylic acid from naphthalene (Hosler, 1963a) and production of large amount of l-glutamic acid from hydrocarbons by microorganisms (Takahashi et al., 1963b) resulted in intensified search for more ways to utilize commercially the capabilities of microorganisms to oxidize hydrocarbons.
**TABLE I.**

**Certain reviews published on hydrocarbon utilizing microorganisms**

<table>
<thead>
<tr>
<th>General aspects of hydrocarbon oxidation.</th>
<th>ZoBell (1946a, 1950); Arnaudi et al. (1955); Shturm (1958); Fuhs (1961); Arnaudi and Treccani (1961); Davis (1956); Foster (1962a); Yamada and Takahashi (1964); Amphlett (1966); Takeda (1966); Mavrina (1966); Durous (1967); Quayle (1967); Nyns (1967); Takahashi (1967); Vamos and Vamos (1967a, b); Nyns and Jiaux (1969).</th>
</tr>
</thead>
<tbody>
<tr>
<td>Application in petroleum industry.</td>
<td>Hammer (1934); Mopilevskii (1940); Davis and Updegraff (1954).</td>
</tr>
<tr>
<td>Microbial utilization of aliphatic hydrocarbons.</td>
<td>Davis (1956); Raymond (1961); Johnson (1964); Ooyama and Foster (1965).</td>
</tr>
<tr>
<td>Microbial utilization of aromatic hydrocarbons.</td>
<td>Evans (1956, 1963); Dagley et al. (1960, 1964); Rogoff (1961); Hayashi (1966); Gibson (1968).</td>
</tr>
<tr>
<td>Metabolism and mechanism of attack.</td>
<td>Quayle (1961); Foster (1962b); Kallio et al. (1963); Zajic (1964); McKenna and Kallio (1965); Treccani (1965); Van der Linden and Thijssse (1965); Flug and Markovetz (1971).</td>
</tr>
<tr>
<td>Bioengineering aspects.</td>
<td>Humphrey (1968a); Coty and Leavitt (1971); Shah et al. (1972b); Sinsele and Fiechter (1972); Prokop and Sobotka (1973).</td>
</tr>
</tbody>
</table>
The importance and the interest developed in petroleum microbiology is evident by large number of reviews presented by many workers (Table I). The publication of books on microbial hydrocarbon utilization and related studies by Davis (1967), Beerstecher (1954) and Sharpley (1961) indicates that petroleum microbiology has come to age. Certain important activities of hydrocarbon utilizing microorganisms are described in the subsequent pages.

**PETROLEUM HYDROCARBON UTILIZING MICROORGANISMS**

The first microbial species reported for growth on paraffin wax as sole source of carbon and energy was *Botrytis cinerea* (Miyoshi, 1895). Other microorganisms were, subsequently, reported for their abilities to utilize hydrocarbons. However, till 1940, it was believed that the abilities to utilize hydrocarbons as source of carbon and energy was restricted to few microbial types only. But, Bushnell and Haas (1941) first reported that the abilities to utilize hydrocarbons is not restricted to few types only but is widespread among microorganisms. They showed that even microbial species from culture collections were able to utilize hydrocarbons. Since then large number of microorganisms were reported for their abilities to utilize various hydrocarbons.

Fuhs (1961) listed the organisms which can grow at the expenses of hydrocarbons. The list comprised over 100 bacteria, yeast, actinomycetes and fungi. It is now known that many species of yeast, bacteria, actinomycetes and fungi are able to utilize various hydrocarbons. Foster (1962a) even suggested the inclusion of hydrocarbons in the list of substrates routinely tested for
identification of microorganisms. Hydrocarbon utilizing abilities of large number of microorganisms were studied in detail (Scheda and Bos, 1966; Markovetz and Kallio, 1964; Komanata et al., 1964; Markovetz et al., 1968) with a view to develop tests for the classification of microorganisms at generic and species levels.

Codification of the numerous microbial species capable of utilizing hydrocarbons is not done probably because of the continuous findings of the new groups of hydrocarbon assimilating microorganisms and meager descriptions of the isolates in many reports. However, such list is highly essential and, therefore, attempts are made to list the known hydrocarbon utilizing microbial species in the subsequent pages. The organisms which were not identified by the workers are not included in the list and only one reference is cited for each species listed. The microorganisms which were classified unto genus level by various workers are listed once only with one reference and the reports by other workers on the same genus are not included.

The list of microorganisms capable of utilizing various hydrocarbons is classified based on the type and nature of the substrates and the microorganisms. The list may not be complete inspite of putting the best efforts to collect all the data. The list is depicted in tables II to XX.
TABLE II.
Bacterial species of genus Bacillus, Methanomonas, Pseudomonas and Methylobacter capable of oxidizing methane and natural gas

Bacillus genus
B. methanica (Sohnren, 1906a), B. hexacarbovorum (Stormer, 1908), B. fluorescens (Aiyer, 1920), B. paraffinicus (Taggart, 1946), Bacillus sp. (Kolnack et al, 1967).

Methanomonas genus

Pseudomonas genus
P. methanica (Dworkin and Foster, 1956), P. methanitrificans (Davis et al, 1964), P. radio bacter (Bogdanova, 1965), Pseudomonas sp. (Vary and Johnson, 1967), P. methanica var. fluva, P. methanica var. fusca and P. methanica var. incolorata (Leadbetter and Foster, 1958).

Methylobacter genus
TABLE III.

Bacteria, actinomycetes and algae capable of oxidizing methane and natural gas

Bacteria


Actinomycetes


Algae and fungi

### Ethane and butane utilizing microorganisms

<table>
<thead>
<tr>
<th>Ethane utilisers</th>
<th>Butane utilisers</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Actinomycetes species</strong></td>
<td><strong>Actinomycetes species</strong></td>
</tr>
<tr>
<td><strong>Bacterial species</strong></td>
<td><strong>Bacterial species</strong></td>
</tr>
<tr>
<td><strong>Fungal species</strong></td>
<td></td>
</tr>
<tr>
<td><em>Fusarium</em> sp., <em>Acremonium</em> sp. (Kester, 1961), <em>Grapmium</em> sp. (Zaici et al., 1969).</td>
<td></td>
</tr>
</tbody>
</table>
TABLE V.

Propane and gaseous hydrocarbons utilizing microorganisms

Propane utilizers

Bacterial species

Pseudomonas propanica (Dostalek, 1954), P. puntotropha, Pseudobacterium subluteum (Tellegina, 1963), Pseudomonas sp. (Kuznetsov and Telegina, 1957), Pseudobacterium sp. (Bogdanova, 1966), Brevibacterium sp. (Vestal and Perry, 1969).

Actinomyces species


Fungal species

Fusarum sp., Acroconium sp. (Kester, 1961).

Gaseous hydrocarbon utilizers

Bacterial species

Brevibacterium ketoglutaricus, Arthobacter roseoparaffinicus, Micrococcus paraffinolyticus (Kyowa Hakko Kogyo Co. Ltd., 1967b).

Yeast species

Candida ripida, C. japonica (Kyowa Hakko Kogyo Co. Ltd., 1968c).

Actinomyces species

Nocardia paraffinica (Kyowa Hakko Kogyo Co. Ltd., 1968d).
TABLE VI.

Solid paraffinic hydrocarbon utilization by members of

Actinomycetes

Actinomyces species

Actinomyces sp. (Buttner, 1926), A. albus (Jensen, 1931), A. violaceus-ruber, A. madurae (Baldacci, 1947).

Proactinomyces species


Mycobacterium species

Mycobacterium album, M. rubrum, M. hyalinum, M. lacticola, M. juteum, M. phlei (Sohnsen, 1913a), Mycobacterium sp. (Buttner, 1926), M. leprae, M. smegmatis (Bushnell and Haas, 1941), M. eos (Buttner, 1926), M. ranae, M. testudinis (Haag, 1926), M. perrugosum var. ethanicum, M. rubrum var. propanicum (Bobova, 1954).

Nocardia species


Streptomyces species

Streptomyces chromogenes (Myamoto, 1952).
TABLE VII.
Fungi and yeast species capable of oxidizing solid paraffinic hydrocarbons

<table>
<thead>
<tr>
<th>Aspergillus species</th>
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</table>

<table>
<thead>
<tr>
<th>Penicillium species</th>
</tr>
</thead>
<tbody>
<tr>
<td>Penicillium camemberti, P. expansum, P. javanicum, P. roqueforti, Penicillium sp. (Miyamoto, 1952), P. notatum (Imai, 1956), P. glaucum (Nalm, 1906).</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Miscellaneous species</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blastomyces cereolytica (Nuttie, 1957), Chaetomium sp. (Pynearson and Peterson, 1965), C. thermophila, Stibella thermophila, Micor puciilllus (Fergus, 1966), Micor sp., Fusarium sp., Cladosporium herbarum (Miyamoto, 1952), Hanascus araneusus, Myrospora sp., Alternaria sp., Curvulatia sp. (Imai, 1956), Petritis cinerea (Miyoshi, 1895), Papulospora sp. (Sohngen, 1913a), Scopulariopsis sp., Citromyces sp. (zikos, 1926), Syncephalastrum sp., Cunninghamella sp. (Pynearson and Peterson, 1965), Verotheca cereophila, Galleria mellonella (Sieber and Metznerkow, 1904).</td>
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<table>
<thead>
<tr>
<th>Candida species</th>
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### TABLE VIII.

Solid paraffinic hydrocarbon utilizing bacterial and yeast species

<table>
<thead>
<tr>
<th>Pseudomonas species</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Pseudomonasfluorescens</em> (Sohngen, 1913a), <em>P. fluorescens denitrificans</em> (Kolesnik and Simonova, 1957), <em>P. pyocyaneus</em>, <em>Pseudomonas</em> sp. (Bushnell and Haas, 1941), <em>P. aeruginosa</em>, <em>P. desmolitica</em> (Yamada and Yogo, 1970).</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Bacterium species</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Bacterium</em> sp. (Buttner, 1926), <em>B. fluorescens liquefaciens</em>, <em>B. pyocyaneum</em>, <em>B. liniolyticum</em>, alpha, gamma, beta and delta, <em>B. stutzeri</em> (Sohngen, 1913a), <em>B. hidium</em> (Gvozdn, 1923), <em>B. aliphaticum</em>, <em>B. aliphaticum liquefaciens</em> (Tausz and Peter, 1919).</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Miscellaneous genera</th>
</tr>
</thead>
</table>

<table>
<thead>
<tr>
<th>Yeasts species</th>
</tr>
</thead>
</table>
TABLE IX.

Liquid n-alkanes and petroleum fractions utilizing yeast species of genus Candida, Pichia and Trichosporon

Candida genus


Pichia genus


Trichosporon genus

Trichosporon sp. (Kvasnikov et al., 1967), T. pullularis (Vadalkar et al., 1969), T. capitatum, T. sericeum (Iizuka et al., 1966).
TABLE X.

Yeast species capable of growth on liquid n-alkanes and petroleum fractions

Debaryomyces genus


Torulopsis genus

Torulopsis sp. (Gatellier and Palliote, 1966), T. apicola (Tulloch and Spencer, 1968), T. magnoliase (Tulloch and Spencer, 1966), T. famata (Kulieve et al., 1967), T. colliculosa (Just et al., 1951), T. dattila (Petit et al., 1970), T. petrophillum (Mizuno et al., 1966), T. haemulonii, T. sake (Scheda and Bos, 1966), T. propengiesseri (Jones and Howe, 1968).

Rhodotorula genus

Rhodotorula sp. (Gatellier and Palliote, 1966), R. glutinis, R. mucilaginosa (Vasnikov et al., 1967), R. rubra (Komagata et al., 1964), R. gracilis (Markovetz and Kallio, 1964), R. aurantiaca, R. glutinis var. rubescens (Hsiu-yuan and Schwartz, 1961)

Miscellaneous genera

Mycotorula japonica (Mimura et al., 1971), Torula sp. (Gatellier and Palliote, 1966), T. utilis (Gatellier, 1963), Lipomyces sp., Endomyces sp. (Gatellier and Palliote, 1966), E. magnusii (Iizuka et al., 1966), Sporoholomyces sp., Cryptococcus sp. (Vasnikov et al., 1967), Zygoschizia sp., Endomycopsis sp. (Ierusalimsy and Skryabin, 1966), Saccharomyces sp., Schizosaccharomyces sp., Trettanomyces sp. (Nyns et al., 1968), Saccharomyces cerevisiae, S. acidifaciens, Trigonopsis paraffinica (ESSO Res. Eng. Co., 1968), Saccharomyces elangospora (Scheda and Bos, 1966), Trettanomyces lambicus (Takahashi et al., 1963a), Hansenula anamola, H. saturnus (Markovetz and Kallio, 1964), Endomycopsis javanensis (Ionsane et al., 1971a), E. fibuliger (Ionsane et al., 1971b), Saccharomyces fructuvm (Ionsane et al., 1972), S. chevalieri, S. uvarum, S. maxima (Ionsane et al., 1971b), Schwanniomyces occidentalis (Ionsane et al., 1973a), Saccharomyces lipolytica (Ionsane et al., 1973b).
TABLE XI.

Liquid n-alkanes and petroleum fractions utilizing bacterial species of *Bacterium*, *Bacillus*, *Corynebacterium* *Pseudomonas* genus

<table>
<thead>
<tr>
<th><strong>Bacterium genus</strong></th>
</tr>
</thead>
</table>
| *B. berzoli* (Jann, 1914), *B. fluorescens* *liquefaciens*, *B. fluorescens* non-*liquefaciens*, *B. pyocyaneum*, *B. punctatum*, *B. lipolyticum* (Sohngen, 1913a), *B. aliphaticum*, *B. aliphaticum* *liquefaciens* (Tausz and Peter, 1919).

<table>
<thead>
<tr>
<th><strong>Bacillus genus</strong></th>
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<table>
<thead>
<tr>
<th><strong>Corynebacterium genus</strong></th>
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<thead>
<tr>
<th><strong>Pseudomonas genus</strong></th>
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</table>
TABLE XII.

Micrococcus, Arthrobacter, Brevibacterium and other bacterial species utilizing n-alkanes and petroleum fractions

<table>
<thead>
<tr>
<th>Micrococcus genus</th>
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<table>
<thead>
<tr>
<th>Arthrobacter genus</th>
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</thead>
</table>

<table>
<thead>
<tr>
<th>Brevibacterium genus</th>
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</thead>
</table>

<table>
<thead>
<tr>
<th>Miscellaneous genera</th>
</tr>
</thead>
</table>
TABLE XIII.

Fungal species of *Fusarium* and *Aspergillus* genera and actinomycetes utilizing liquid n-alkanes and petroleum fractions

<table>
<thead>
<tr>
<th>Fungal species</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Fusarium genus</strong></td>
</tr>
<tr>
<td><strong>Aspergillus genus</strong></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Actinomycetes species</th>
<th>Streptomyces genera</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Actinomyces</em> sp. (Imamura, 1948), <em>A. oligocarbohylus</em> (Lantzsch, 1922), <em>A. chromogenes</em> (Haag, 1927), <em>Streptomyces</em> sp. (Zaïc, 1964)</td>
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<table>
<thead>
<tr>
<th>Mycobacterium genus</th>
<th></th>
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</thead>
</table>

<table>
<thead>
<tr>
<th>Nocardia genus</th>
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</thead>
</table>
TABLE XIV.

Petroleum fractions and liquid n-alkanes utilizing fungal species other than Aspergillus and Fusarium genera

Penicillium genus


Cephalosporium genus

\( \text{Cephalosporium sp. (Markovetz et al, 1968), C. roseum (Foster, 1962a), C. acremonium (Perry and Carniglia, 1973).} \)

Cunninghamella genus

\( \text{Cunninghamella sp., C. blackesleeanus (+), C. blackesleeanus (-) (Markovetz et al, 1968), C. elegans (Carniglia and Perry, 1973).} \)

Miscellaneous genera

Yeast species

Candida genus


Miscellaneous genera

Cryptococcus laurentii, Debaryomyces kleckeri, D. membreaefaciens, Hansenula anamola, H. satunus, Rhodotorula glutinis, R. gracilis, R. mucilaginosa, Schizobasostporitum starkeys-

Bacterial species

Miscellaneous genera

Pseudomonas sp. (Birkinshaw et al., 1945), P. oleovorans (Peterson et al., 1966), P. aeruginosa (Markovetz et al., 1967), Micrococcus cerificans (Stewart et al., 1960), Desulfiovibrio sp. (Zobell, 1949).

Actinomycetes species

Mycobacterium sp. (Haag, 1927).
### TABLE XVI

**Fungal species utilizing olefins and microorganisms utilizing alkynes**

<table>
<thead>
<tr>
<th>Olefin utilizing fungal species</th>
</tr>
</thead>
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<table>
<thead>
<tr>
<th>Alkynes utilizing microorganisms</th>
</tr>
</thead>
</table>
### TABLE XVII.

**Cycloparaffin, phenylalkanes, cycloalkylalkanes and isoparaffin utilizing microorganisms**

<table>
<thead>
<tr>
<th>Group</th>
<th>Bacterial species</th>
<th>Actinomycetes species</th>
<th>Yeasts species</th>
</tr>
</thead>
</table>
### TABLE XVIII.

#### Benzene, catechol, benzoate and phenols utilizing microorganisms

<table>
<thead>
<tr>
<th>Bacterial species</th>
<th>Yeast, fungal and actinomycetes species</th>
</tr>
</thead>
<tbody>
<tr>
<td>Micromococcus sphaeroides</td>
<td>Rhodotorula glutinis (Martins and Drews, 1966), Trichosporon sp. (Itoh and Doi, 1969), Torula sp., Penicillium sp., Micor sp. (Perrier, 1913), Proactinomycetes sp. (Moore, 1949)</td>
</tr>
<tr>
<td><em>M. urea</em> (Takayama et al, 1955)</td>
<td></td>
</tr>
<tr>
<td>Rhodospirillum sp., Rhodopseudomonas sp. (Proctor and Scher, 1960)</td>
<td></td>
</tr>
<tr>
<td>Actinomycetes, yeast and fungal species</td>
<td></td>
</tr>
<tr>
<td>Nocardia sp. (Veel and et al, 1952), Mycobacterium rhodoehrosee (Marr and Stone, 1961), Torula sp., Micor sp., Penicillium sp. (Perrier, 1913)</td>
<td></td>
</tr>
</tbody>
</table>

#### Phenols

<table>
<thead>
<tr>
<th>Bacterial species</th>
<th>Yeast, fungal and actinomycetes species</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Bacillus benzoicus</em> (Perrier, 1913), Flavobacterium helvorum (Fowler et al, 1911), <em>Micromonaspora</em> sp. (Erikson, 1949), Achromobacter sp. (Czekalowski and Skarzynski, 1948), <em>Vibrio</em> sp. (Evans, 1947), Chromobacter sp. (Puttilina, 1959), <em>Pseudomonas fluorescens</em> (Sleeper and Stanier, 1950)</td>
<td></td>
</tr>
</tbody>
</table>
TABLE XIX.

Toluene, xylene and other aromatic hydrocarbons utilizing microorganisms

Toluene

Bacterial species

- Micromonospora sp. (Erikson, 1941), Micrococcus sp., Spirillum sp., Bacillus sp., Bacterium sp. (Gray and Thornton, 1928), B. toluicum, B. benzoli (Tausson, 1929), Pseudomonas putida (Gibson et al., 1967), P. mildenbergti (Nozaka and Kusunose, 1969), P. aeruginosa (Kitagawa, 1956), P. desmolvtica (Yamada et al., 1965), P. aeruginosa var. achenobic (Hori et al., 1967), Achromobacter sp. (Claus and Jalker, 1964), Azotobacter aromaticum (Coty, 1967), Bacillus hexacarbovorum (Stormer, 1908)

Actinomycetes species

- Nocardi a corallina (Forro, 1966), Mycobacterium sp., K. agraste (Gray and Thornton, 1928)

Xylene utilizing bacterial species

- Bacillus hexacarbovorum (Stormer, 1908), Bacterium benzol (Tausson, 1929), Pseudomonas aeruginosa (Otori and Yamada, 1969), P. mildenbergti (Nozaka and Kusunose, 1969), P. desmolvtica (Yamada et al., 1965).

Terpenes, dibenzothiophene, benzopyrene, cinnamic acid and other aromatic hydrocarbons utilizing microorganisms

- Azotobacter chroococcum (Coty, 1967), Bacillus megaterium (Poglazova et al., 1966), Actinobacter sp., Pseudomonas sp. (Knecht, 1961), P. pseudomallei (Hayashi et al., 1969a), P. iianni, P. ahikonensis (Kodama et al., 1970), Rhodotorula sp. (Uchiyama et al., 1969), Trichosporon sp., Streptomyces sp. (Research Association of Petroleum Fermentation, 1970)
TABLE XX.

Microorganisms capable of utilizing aromatic polycyclic hydrocarbons

Bacterial species

*Micrococcus* sp., *Bacillus* sp., *Spirillum* sp., *Bacterium* sp.


Actinomycetes and yeast species

METABOLISM OF PETROLEUM HYDROCARBONS

Through the works of various investigators, certain patterns of metabolism in microorganisms have been suggested for various hydrocarbon compounds. The metabolic pathways of hydrocarbons were investigated by applying the principle of sequential induction for elucidation of adaptive metabolic patterns, by identification and isolation of intermediate metabolites, by using isotopic tracer technique and by identification and isolation of enzymes catalysing the reactions.

**Methane.** Microbial oxidation of methane by microorganisms with autotrophic growth (Johnson and Temple, 1962; Brown, 1958) and with heterotrophic growth (Johnson and Paiye, 1965; Brown and Strawinski, 1958) was reported. Methane is oxidized to methanol incorporating molecular oxygen. Methanol is further oxidized to formaldehyde and then to formate as shown in Fig.1. Alcohol peroxidase which requires hydrogen peroxide for catalytic formation of formaldehyde from methanol and NAD-linked alcohol, aldehyde and formic dehydrogenases have been demonstrated in cell free preparations of methanol grown bacteria (Coty, 1969).

**Gases alkanes other than methane** (C₂ - C₄). Application of simultaneous adaptation techniques gave indications that ethylene, methanol, acetaldehyde and acetic acid might be intermediates in the oxidation of ethane by bacteria (Davis et al., 1956). Leadbetter and Foster (1960) used the obligate methane oxidizer *Pseudomonas methanica* to co-oxidize ethane, propane and butane while growing on methane and obtained various products reflecting a primary attack on the terminal as well as on the neighbouring carbon atoms. Lukins (1962), using mycobacteria growing at the expense of
**Methane**

\[ CH_4 + O_2 \rightarrow CH_2OH \]

Methane \[ \rightarrow \] Methanol \[ \rightarrow \] Methanol

\[ H_2O_2 \rightarrow CH_2O \]

Formate \[ \rightarrow \] Methanol

\[ CH_2OH \]

**Propene**

\[ \text{Propene} \rightarrow \text{Propanol-2} \rightarrow \text{Propanol-1} \]

\[ \text{O}_2 \rightarrow \text{Acetone} \rightarrow \text{Acetate} \rightarrow \text{Propionic acid} \]

**Liquid n-alkanes**

\[ \text{R} \rightarrow \text{CH}_2 \rightarrow \text{CH}_2 \rightarrow \text{COOH} \]

\[ \text{CH}_3 \text{(CH}_2)_n \text{CH} \rightarrow \text{COOH} \]

**Pathways of Microbial Degradation of Gaseous and Liquid n-Alkanes**

**FIG. I**
n-propane, n-butane, n-pentane or n-hexane, obtained corresponding methyl ketones as oxidation products suggesting a methyl ketone pathway in the oxidation of short-chain alkanes. Fig.1 shows the possible metabolic pathway of propane oxidation which may be applicable to ethane and butane also.

Liquid n-alkanes. From the works of various investigators using bacteria, yeasts and filamentous fungi, it has been established that the terminal methyl group of n-alkane is first oxidized with the incorporation of molecular oxygen into corresponding primary alcohol which is subsequently converted into corresponding fatty acid through an aldehyde intermediate (Treccani et al., 1955; Heringa et al., 1961; Baptist et al., 1963; Davis, 1964, Singh et al., 1972).

It has been shown that (Baptist et al., 1963; Peterson et al., 1966) initial attack on the alkane molecule consists of a hydroxylation reaction with the participation of molecular oxygen catalysed by the enzyme alkane dehydroxylase in which rubredoxin or cytochrome P-450 (Cardini and Jurtshunk, 1968), NADH, NADH reductase, flavin adenine dinucleotide are involved as electron carriers.

There are, however, considerable experimental evidences for variation of the above general metabolic pathway in a number of microorganisms. Initial formation of alpha-olefin in the oxidation of n-alkane under the action of NAD dependent dehydrogenases (Azoulay et al, 1963; Chouteau et al, 1962, Iizuka et al, 1968, 1969a), sub-terminal oxidation of n-alkanes leading to the formation of methyl ketones and secondary alcohols (Fredricks, 1967; Allen and Markovetz, 1970), terminal oxidation of fatty acids with the formation of omega-hydroxy fatty acids and diol acids (Kester and Foster, 1963; Alikhan et al, 1963, 1964) and alpha-oxidation accompanied by one
carbon cleavage from fatty acids (Socler, 1962; Vano et al., 1971) have been reported.

Fig. I shows the pathways of \( \eta \)-alkane oxidation in microorganisms.

**Olefins.** Oxidative attack on \( \alpha \)-olefins seems to occur at several positions as shown by oxidative products obtained in various microorganisms. However, main degradation pathways seem to be methyl group oxidation leading to the formation of omega unsaturated fatty acid (Thijsse and Van der Linden, 1963; Klug and Markovetz, 1968) and double bond oxidation leading to formation of epoxide, diol, 2-hydroxy acid followed by decarboxylation with the formation of saturated fatty acid with one carbon less than the substrate (Ishikura and Foster, 1961; Klug and Markovetz, 1968, 1969). Fig. II shows the metabolic pathways of \( \alpha \)-olefin oxidation in microorganisms.

**Phenylalkanes and cycloalkylalkanes.** Metabolic studies (Webley et al., 1956; Davis and Raymond, 1961) showed preferential oxidative attack on the alkyl chain of the phenylalkanes and cycloalkylalkanes in most of the microorganisms. The alkyl chain seems to be oxidized in a similar fashion as in the case of \( \eta \)-alkanes. Fig. II shows the metabolism of phenylalkanes and cycloalkylalkanes.

**Isoparaffins.** It has been reported that microorganisms attack isoparaffins containing sufficiently long unbranched chain and only one methyl side chain but not alkanes with a branched alkyl group longer than methyl or multiple methyl groups (Finney et al., 1962b; Thijsse and Zwilling de Vries, 1959). Studies by Thijsse and Van der Linden (1961, 1963) on the oxidation of 2-methyl hexane by Pseudomonas aeruginosa showed the degradation of the isooalkane through
**Olefins**

\[
H_3C-(CH_2)_n-CH=CH_2 \rightarrow H_3C-(CH_2)_n-CH_2-CH_2OH
\]

\[
\text{chain elongation and desaturation}
\]

\[
\text{13-oxidation}
\]

**Cycloalkanes**

- Cyclohexane
  - Cyclohexanol
  - Methylcyclohexane
  - 4-Methylcyclohexanone

**Pathways of Olefins and Cycloalkanes Degradation by Microorganisms**

**Fig. II**
ISOPARAFFINS

2-Methylhexane

\[ \text{C}_1 \text{ scheme} \]

2-methyl hexanoic acid

\( \beta \) oxidation

Propionic + Butyric acid

\[ \text{C}_6 \text{ scheme} \]

5-methyl hexanoic acid

\( \beta \) oxidation

Isovaleric + Acetic acid

\( \text{CO}_2 \) fixation

3-oxobutyric + Acetic acid

Phenylalkanes and cycloalkylalkanes

3-Phenyllicosane

\[ \text{CH}_3\text{CH}(\text{CH}_2)_{16}\text{CH}_3 \]

Phenylethylacetic acid

\[ \text{CH}_3\text{CH}_2\text{CHCOOH} \]

1-(\( \alpha \)-naphthyl)-hendecane

\[ \text{CH}_2(\text{CH}_2)_{9}\text{CH}_3 \]

3-(\( \alpha \)-naphthyl)-propionic acid

\[ \text{CH}_2\text{CH}_2\text{COOH} \]

3-(\( \alpha \)-naphthyl)-acrylic acid

\[ \text{CH}_2\text{CH}_2\text{COOH} \]

\[ \text{CH}_2\text{COOH} \]

n-Butyl cyclohexane

\[ \text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_3 \]

cyclohexane acetic acid

\[ \text{CH}_2\text{COOH} \]

PATHWAYS OF MICROBIAL DEGRADATION OF ISOPARAFFINS, PHENYLALKANES AND CYCLOALKYLALKANES

FIG. III
Mode of ring splitting

Further oxidation

Phenanthrene
Mandelate
Naphthalene
O-cresol
Benzate
Phenol
Salicylate
p-cresol
p-hydroxy benzate
Catechol
α-keto acids
β-keto adipate
Protocatechuate
δ-keto acids
Phthalate
m-hydroxy benzoate
Gentisate
fumaryl pyruvate

General mode of ring splitting and metabolic routes of aromatic hydrocarbons

Fig. IV
C_1 and C_6 carbon atoms leading to conclusion that 2-methyl hexane was preferentially oxidized via the long-chain end of the molecule. Fig. III shows the pathway.

**Cycloparaffins.** In general, these are poorly utilized by microorganisms. Cycloparaffins seem to be oxidized via alcohol (Imelik, 1948a; Elliott et al., 1957) and ketone derivatives (Ooyama and Foster, 1965). Colla and Treccani (1960), however, found that oxidation of Cis-decalin by Flavobacterium gave rise to adipic acid and pimelic acid (Fig. III).

**Aromatic hydrocarbons.** The oxidation of aromatic hydrocarbons in microorganisms through a diol intermediate followed by ring splitting has been generally accepted (Marr and Stone, 1961; Hayashi and Hashimoto, 1950; Walker and Hiltshire, 1953; Treccani et al., 1954). Davies and Evans, 1964). Mode of ring splitting and general metabolic route of aromatic hydrocarbon oxidation are shown in Fig. IV.

**BIOENGINEERING ASPECTS OF MICROBIAL CULTIVATION ON HYDROCARBONS**

The chemical and physical nature of hydrocarbons poses many problems in their effective utilization by microorganisms and it is necessary to overcome them for obtaining optimum growth.

**Substrate transport.** Solubilities of \( n \)-alkanes in water are very low and are determined up to \( n \)-octane by McAuliffe (1963) who found exponential decrease in solubilities with increasing number of carbon atoms in \( n \)-alkanes. Baker (1956) noted that solubilities of \( n \)-alkanes from \( n \)-octane onwards did not follow the extrapolated solubility curves made by Johnson (1964) from McAuliffe's data.
Fairly small contribution of growth on dissolved substrate (Shah et al., 1972a; Erickson et al., 1970; Prokop et al., 1971) and merely 0.5 per cent of total growth on dissolved n-alkanes (Alba et al., 1969) revealed that the uptake of dissolved substrate cannot fully account for microbial growth on hydrocarbon.

The improvement in biomass yields by increasing the interface area of the substrate particles was noted by many workers. The increase in the interface area of the substrate was affected by increased mechanical agitation (Humphrey, 1968a) and by using surface active agents (Alba et al., 1969; Whiteworth et al., 1973; Tanaka and Fukui, 1971). The abilities of certain hydrocarbon utilizing microorganisms to produce hydrocarbon emulsifying factors (Mimura et al., 1971a, b; Iwachi et al., 1969) further indicated the importance of the emulsification of hydrocarbons for microbial attack.

Finely divided submicron particles of hydrocarbons as a primary source of substrate (Srdtsieck and Rietama, 1969; Johnson, 1964) and subsequent transport due to physical adsorption on cell surface (Ludvik et al., 1968, 1966), liquid hydrocarbon uptake via gas phase (Yoshida and Yamane, 1971), uptake of solid hydrocarbons in the form of solute in water (Chakravarty et al., 1972; Jodzinski and Bertolini, 1972), were suggested as means of substrate uptake by microbial cells.

Hydrocarbon penetration into cells by passive diffusion across lipid layers (Kotyk and Janacek, 1970), by enzyme transport systems (Mitchell, 1957) and through direct incorporation by forming a part of the phospholipid micelle of the cell membrane (Johnson, 1964) are suggested. Direct penetration of hydrocarbon molecules in cell wall and their accumulation on the cytoplasmic membrane was proved.
by employing tritium as well as $^{14}$C labelled hydrocarbons (Ludvik et al. 1968, 1966). This was also observed by using methods such as electron microscopy (Meisel et al. 1968), column chromatography (Rachinskii et al. 1971), paramagnetic probes (Bininkov et al. 1972) and fluorescence microscopy as well as spectrometry (Meisel et al. 1971). It was reported that the hydrocarbon penetrated into the cell very rapidly saturating the cell within 40-60 seconds. The substrate was found deposited in the outermost internal structures of the cells or in the cytoplasmic membrane (Ludvik et al. 1966, 1968).

After diffusion of hydrocarbon through cell wall, the substrate is oxidized to fatty acids by enzymes located at the outermost side of cytoplasmic membrane (Liu and Johnson, 1971) and the fatty acids then pass through cytoplasmic membrane due to active transport (Einsele and Fiechter, 1972). Further oxidation of the substrate takes place in mitochondria, localized in the cytoplasm (Lebeault et al. 1970).

Oxygen transport. Hydrocarbon utilizing microorganisms require three times greater oxygen for oxidation as compared to carbohydrate utilizers (Guenther, 1965; Darlington, 1964) due to chemical nature of the substrate. The maximal specific oxygen uptake rate in the range 9.6-14 m mol/g/h was reported for hydrocarbon utilizing microorganisms (Mimura et al. 1971a; Non-Young et al. 1971). On this basis, Mimura et al. (1971a) calculated that as much as 150 to 200 m mol/1-hr molecular oxygen must be supplied to a fermentor operating with cell concentration of 15 to 20 g/l. The dissolved oxygen level beyond which no further increase in specific oxygen uptake rate could be obtained was termed "critical" level. This was
found to be in the range of 0.5 - 1.2 ppm (Mimura and Takeda, 1972; Moo-Young et al, 1971). The oxygen demand i.e., the amount of oxygen required to produce unit biomass was reported to be in the range of 1.95 - 2.24 g O$_2$/g biomass for hydrocarbon utilizing yeast (Mimura et al, 1971a; Katinger, 1972; Einsele et al, 1972; Lenz and Katinger, 1971). It was also reported that the oxygen demand is dependent on the biomass yield and molecular weight of the substrate (Maitles, 1971; Darlington, 1964).

Mimura et al (1972) found that the volumetric oxygen transfer coefficient rates decreased continuously with the progress of fermentation and the values calculated for the dissolved oxygen consumption rate were found to be markedly lower than actual values. Therefore, they concluded that most of the oxygen is taken up by the cells directly from the gas phase. Prokop and Sobotka (1973) reported the oxygen transfer from air to water in the initial stages of the fermentation. The oil droplets at the air-water interface partially slow down the diffusion of oxygen due to resistance by an oil phase. But during the course of fermentation, the oxygen transfer takes place from air to oil when the interfacial tension of oil/water in the presence of cells is approaching zero (Prokop and Erickson, 1972). It was further stated that the utilization of oxygen by cells in hydrocarbon fermentation may be through water, oil and gas phases functioning simultaneously (Prokop and Sobotka, 1973).

Oxygen transfer was found to increase continuously with increase in hydrocarbon volume fractions (Mimura et al, 1969b). Coty et al (1971) reported four times higher oxygen transfer rates with water in oil emulsions containing high oil fractions. Higher oxygen transfer with high hydrocarbon volume fractions was ascribed to the
higher solubility of oxygen in hydrocarbons as compared to that in water (Prokop et al., 1971; Matsumura et al., 1972).

**Heat evolution.** In the thermodynamic evaluation of the aerobic fermentation of hydrocarbons, Guenther (1965) showed that the net energies gained in production of 100 g. yeast from hydrocarbons and carbohydrates were 750 and 383 Kg. cal respectively. Further it was calculated that the heat evolution, with 150 per cent biomass yields from hydrocarbons, was equivalent to that evolved on carbohydrates. However, the biomass yields, generally obtained with petroleum hydrocarbon substrates in fermentors, were about 95 per cent for yeasts and bacteria (Miller and Johnson, 1967a; Raymond and Davis, 1960). Hence, the production of biomass by aerobic hydrocarbon fermentations needs adequate cooling arrangement in order to maintain the optimum temperature in fermentors. It has been stated that the costs involved in refrigeration of the fermentors can reach the point where it would probably be uneconomical (Guenther, 1965).

Attempts were made by many workers to use thermophilic bacteria (Mikhlin and Trofieeva, 1966; Baruah et al., 1967; Mateles et al., 1967; Klug and Markovetz, 1967a) and yeasts (Pozmogova et al., 1967; Andrusenko and Lakhonina, 1969) which could make circulating waters at ambient temperatures to be effective in fermentor cooling.

**Growth kinetics.** Various attempts have been made to derive kinetic models for growth of microorganisms on hydrocarbons in order to obtain better understanding of the system and to workout optimal process design for this kind of fermentation. But because of the nature of the substrate, the fermentation results in the formation
of a four phase system consisting of air, oil, cell and water with consequent difficulty in arriving to a satisfactory kinetic model.

In batch cultures, two growth regions have been observed i.e. initial exponential phase followed by a linear phase of generally longer duration (Erickson et al., 1970; Katinger and Meyrath, 1969; Prokop et al., 1971; Binsele et al., 1972; Goma et al., 1971). It has been reported that the specific growth rate in the exponential region of the batch growth is a function of the impeller speed which has been shown to control the surface area of the oil droplets (Moo-Young and Shimizu, 1971; Moo-Young et al., 1971; Jang and Ochao, 1972; Aiba et al., 1971; Whitworth, 1971). On the other hand, other group of workers (Katinger and Meyrath, 1969; Binsele et al., 1972; Blanch and Binsele, 1973) have reported that batch growth proceeds the exponential phase at maximum specific growth rate, only the duration of the exponential phase being affected by the impeller speed.

Several models have been proposed, to explain the observed growth kinetics on pure alkanes as the dispersed phase (Dunn, 1968; Aiba et al., 1969; Erickson and Humphrey, 1969a; Moo-Young and Shimizu, 1971; Porzani et al., 1971; Blanch and Binsele, 1973), on alkanes dissolved in a dispersed phase (Erickson et al., 1969) and on solid alkanes (Chakravarty et al., 1972).

In the case of growth on pure alkanes as the dispersed phase, Erickson and Humphrey (1969a) considered the hydrocarbon uptake through direct contact as well as from the aqueous phase. They concluded that in batch cultures at low cell concentration or in the earlier phase of growth, the uptake of hydrocarbon through the
aqueous phase was important. With the increase in cell concentra-
tion, however, direct contact between the cells and hydrocarbon
droplets was the predominating factor and the extent of the linear
growth was dependant upon the amount of drop surface area available.
They showed good agreement between the model and the experimental
data available in the literature for batch growth as well as for con-
tinuous culture (Erickson and Humphrey, 1969b).

Alba et al (1969) considered growth as occurring through fine
droplets of oil which were "accommodated" and ranging in size between
dissolved hydrocarbon and microscopically visible droplets. They
concluded that hydrocarbon taken up from aqueous solution constituted
but an insignificant fraction of the total uptake. The model pro-
posed by them was consistent with their observations on the exponential
growth phase but the linear growth phase was not dealt with.

Moo-Young and co-workers (1971), considered cell growth occu-
rning by direct attachment and proposed that the potential for the
accommodation of cells on oil drop surfaces limited the growth via
a Monod type of relationship. They felt that very fine oil droplets
smaller in size than the yeast cells might play a predominant role.
Their proposed model was consistent with the results obtained by
them for both batch and continuous cultures but the model was ade-
quate only for explaining the exponential growth phase.

Using a similar approach, Borzani et al (1971) developed a
model which allowed the simulation of an exponential growth phase
followed by a phase of declining growth. They assumed that the
growth rates of cells not attached to oil droplets were zero and
that such cells were present only when all the drop surfaces were
fully covered.
The mechanism common to all the growth models described above is the quantitification of the effect of interfacial area on the growth of the organism. Blanch and Einsle (1973) proposed an entirely new mechanism of growth. By using two different types of fermentors, they showed two distinct phases of growth, i.e. the initial exponential phase which was independent of stirrer speed and the latter linear phase, the onset of which was determined by stirrer speed. They further showed that the drop size of the dispersed phase was not primarily responsible for the observed kinetics. They proposed that the formation of biological flocs consisting of cells, hydrocarbon droplets and air bubbles determined the observed growth kinetics. This was substantiated by the results of their experiments on continuous cultures.

In the case of growth on substrate dissolved in the dispersed phase, such as growth on gas oil, it may be expected that the substrate concentration in the dispersed phase will change with time but the surface area of the dispersed phase may not. Growth kinetics in this case may be expected to be different from that on entirely consumable dispersed phase such as pure alkanes. Erickson et al (1969) constructed a growth model on the assumption that all growth occurred at the surface of the dispersed phase which showed fair agreement between the model and relatively long linear phase of growth observed by others. Erickson and co-workers (Erickson et al, 1970; Prokop et al, 1971; Shah et al, 1972a; Prokop and Erickson, 1972) improved the model by taking into account the drop size distribution, the rate of adsorption of cells on the drop surface, the rate of desorption of cells from the drop surface, substrate transport between phases, phase equilibrium, coalescence and dispersion of oil drops in the system.
Because of different physical nature, growth kinetics on solid hydrocarbons may be expected to be different from that on liquid hydrocarbons. Chakravarty et al (1972) observed that in the growth of a *Pseudomonas* species on emulsified solid paraffins, the cells did not attach to the paraffin particles. They constructed a growth model on the assumption that the cells utilized only the soluble hydrocarbon substrates for growth and that the growing cells produced a metabolite which helped in the solubilization of the solid substrates in the aqueous medium. They showed that the model could satisfactorily explain the experimentally observed linear growth on solid paraffins.

**Fermentor design.** It is economically essential that the productivity of the fermentor should be high which in turn is the function of the fermentor design. Ideal fermentor design should ensure high oxygen transfer, effective heat removal and greater uptake of the substrate in addition to the maintenance of aseptic conditions.

Fully baffled fermentor equipped with open blade turbine mixer is not generally suitable for hydrocarbon fermentation because of the homogeneity problems. Modification of such fermentor by vortexing was recommended by Blakebrough *et al* (1967) for use in hydrocarbon fermentations. Jaldhöf fermentor consisting of an open turbine with a draught tube was used for hydrocarbon fermentation (Prokop and Erickson, 1972; Einsele and Frichter, 1969; Kattinger, 1972). Non-mechanically agitated air-lift fermentors, which are generally preferred, were used by many workers (Iyengar and Baruah, 1968; Jang *et al.*, 1971; Coty *et al.*, 1971). A modified air-lift fermentor with mechanical mixing device is reported to be used by British Petroleum.
Co. Ltd. (Prokop and Sobotka, 1973). Certain modifications such as a venturi contractor for air and oil introduction (Blakebrough et al., 1967), excentrically aerated slant bottom vessel (Liu, 1972) were also reported.

A local turbulence is reduced in favour of circulation in all these fermentor designs to obtain intensive mixing to generate sufficient oxygen transfer and interfacial area between oil and oxygen phase. However, in such fermentors, possibilities of reduced concentrations of individual particles of the substrate could occur due to enhanced coalescence. It is well known that the type of hydrocarbon substrate determines the design of the fermentor (Kvasnicka, 1972a,b). Gas oil needs a higher axial/radial ratio of the flow of mixer than \( n \)-alkanes which can be achieved by combination of axial and radial mixers on a single shaft. Similarly, a cyclone column fermentor was used by Mueller (1969) with natural gas.

**SINGLE-CELL PROTEIN FROM HYDROCARBONS**

The world-wide shortage of food and feed stuffs, especially deficiency of protein, is well recognized (Sukhatme, 1961). Efforts for augmentation of traditional protein sources have received much attention for solving the deficiencies of food and proteins. However, these sources are limited and their effective utilization is based on the vagaries of the nature. Hence, considerable attention has been given in recent times to unconventional protein sources such as yeast, bacteria and algae grown on carbohydrates and hydrocarbon with a view to meet the projected shortage of foods and feeds in the near and distant future.

Though the potentialities of the use of hydrocarbons for the production of single-cell protein (SCP) was realised in 1948
(Just and Schnabel, 1948), petroleum hydrocarbons received a worldwide attention after the first proposal by Champagnat et al. (1963a) on the use of microbial hydrocarbon fermentation for dewaxing of the waxy crude oil coupled with the production of protein-vitamin concentrates for use as animal feed supplement. The experimental work was started by British Petroleum Co. Ltd. in 1960 (Champagnat et al., 1963b) for commercial production of SCP on pilot plant scale at the laboratories set up at Lavera (France) and Grangemouth (Scotland).

Subsequently, attempts have been made by the workers in several laboratories and oil companies to grow yeasts and bacteria on hydrocarbons for the production of single-cell protein (Klass et al., 1967; Evans, 1968; Johnson, 1967; Mateses et al., 1967; ESSO Engineering Co., 1967b; McNab and Rey, 1966). Semicommercial-stage plant for production of SCP from hydrocarbons are in existence in several countries (McNab and Rey, 1966; Anon, 1967; Champagnat et al., 1963b; Evans, 1968; Iyengar and Baruah, 1968; Ko et al., 1967). Soviet Union has at least three plants with fermentors varying in size from 300–850 cubic meters (Chepigo et al., 1967a) and produced 10,000 tons of SCP in 1967 (Humphrey, 1969). The pilot plant that will eventually be expanded to full-scale plant with 100,000 tons/year capacity in Czechoslovakia (Dostalek et al., 1968b), pilot plant with a capacity to produce 700 tons/year SCP in Taiwan (Ko et al., 1967) and a large pilot plant operating on Mongolian crude, located near Shanghai in Red China (Humphrey, 1968b) have been reported. British Petroleum Co. Ltd. has been operating demonstration plant at Grangemouth, Scotland, with 4,000 ton/year capacity and 16,000 tons/year plant at Lavera, France by using n-alkane feed stock and heavy gas oil (Bennet et al., 1969; Evans, 1968; Llewellyn, 1967).
Esso in collaboration with Nestle are operating large pilot plant at the Nestle Nestreco plant (Esso Research Engineering Co, 1967a; McNab and Rey, 1966). A number of companies in Japan has plan for commercial production in the scale of 60,000-1,00,000 tons/year (Hoshal, 1972). Documentation of large number of publications and patents on the subject (Bhuyan, 1971; Iyengar, 1971) gives the idea of the efforts made for the production of SCP from hydrocarbons.

Most of the work carried out on the production of SCP from petroleum hydrocarbons is by batch fermentations but recently continuous fermentation has been given prominence. It was stated that with continuous culture at optimal conditions, 5-10 fold increase in productivity could be obtained (Humphrey, 1968c). Continuous cultivation of yeasts on n-alkanes (Rodinova et al, 1969; Glikman, 1969; Alekseyeva et al, 1969) and gas oil (Dostalek et al, 1968a; Munk et al, 1966) for the production of SCP and for other related studies (Golobov et al, 1969; Gradova et al, 1969) were reported. The continuous runs were carried out from one stage continuous run (Dostalek et al, 1968a) to 700 hours (Llevelyn, 1967).

The chemical composition of SCP, produced from hydrocarbons by using bacteria and yeasts, is more or less similar or somewhat better to that produced on conventional carbohydrates (Pillai et al, 1972a). Extensive feeding trials of SCP grown on hydrocarbons in pigs, chicks, laying birds, rats and other poultry animals were carried out by many workers (Shacklady, 1970, 1969, 1967; Shacklady et al, 1969; van Weerden et al, 1969; van der Dall et al, 1969; de Groot et al, 1970; Narayanaswamy et al, 1971; Pillai et al, 1972b; Hoshal, 1972). It was reported that
hydrocarbon grown yeasts can substitute fish meal or other poultry feeds provided adjustment is made to the methionine content of the ration (Shacklady, 1969; Narayanaswamy et al., 1971; Pillai et al., 1972b). Hydrocarbon grown yeast has not shown any appreciable difference in performance during reproduction, suckling and growing periods as compared to conventional high protein sources (van der Wal et al., 1969). Studies on chronic toxicity, digestibility, protein efficiency ratio and absence of carcinogenic factors in hydrocarbon grown yeasts gave encouraging evidence of the safety and utility of the product (van der Wal et al., 1969; Pillai et al., 1972b).

Some of the advantages offered by petroleum hydrocarbons over conventional carbohydrate substrates are the world-wide distribution system, comparatively lower price, nearly double biomass yield as compared to carbohydrates and possibilities of the use of the substrate to maximum level due to insolubilities in water. But, petroleum has also serious disadvantages such as its non-returnable nature, control of its production by oil producing countries and unstable price observed in recent times. It was estimated that about 50 million tons of protein could be produced by diverting ten per cent of the present world production of the crude (Iyengar et al., 1969) and 5 million tons of the protein with a potential figure of 20 million tons in 2000 AD by diverting one third of the wax (Charpagnat, 1965). In fact, Humphrey (1969) is of the opinion that the world protein deficiency between now and the year 2000 AD could be overcome with the utilization of high waxy crudes in microbial fermentations.
A variety of hydrocarbons are used by many workers for the production of SCP. The selection of the substrate is important as it offers advantages as well as disadvantages over other substrates and the nature of process technology is influenced to a greater extent by the type of the substrate.

Waxy crude oil. Dewaxing of the waxy crude oil is coupled with the production of SCP in its use as a substrate. However, it is difficult to separate cells from the unused crude and the dispersion of the nearly semi solid crude in the nutrient medium poses difficulties. Yeast such as Candida ruillier mondii (Deley et al., 1970), C. intermedia (Chepigo et al., 1967b), C. lipolytica (Takeda et al., 1965) and C. roposa (Deley et al., 1970) were generally used though Pseudomonas, Micrococcus and Actinomyces were also suggested (Societe Francaise des Petroles BP, 1965a). Microbial processes for the production of SCP from crude oil are developed and recommended by many workers (Champagnat et al., 1963a; Gatellier, 1966; Molinski, 1964).

Gas oil. It is obtained as byproduct stream from a cracking process and is the petroleum fraction which distills between diesel oil and lubricating oils. It contains 15-30 per cent n-alkanes in addition to aromatic and naphthenic hydrocarbons. Gas oil (bp 150-400°C) is available in abundance and its use couples SCP production with a modified raw material of improved quality (deparaaffinised gas oil) suitable for certain uses. Advantages of the use of gas oil over pure n-alkanes are lower viscosity leading to better dispersion of hydrocarbon phase in aqueous medium, better
solubility of oxygen in large hydrocarbon phase, presence of growth promoting factors and trace elements in gas oil, comparatively cheaper cost and possibilities of recycling unused gas oil left over after fermentation with addition of waxes. However, it requires careful controls and efficient solvent treatment units for removal of the traces of unused oil adhering to the cell surfaces.

Yeast and bacteria such as *Micrococcus* (Ertola and Mazza, 1969), *Pseudomonas* (Ko and Yu, 1968), *Nocardia opaca* (British Petroleum Co. Ltd., 1965a), *Candida lipolytica* (Munk et al., 1969), *Candida tropicalis* (Goux and Laine, 1968) and *Trichosporon pullulans* (Vadakkar et al., 1969) were used with gas oil. The process technology of SCP production based on the use of gas oil is developed by many workers and organisations (Chepigo et al., 1968; British Petroleum Co. Ltd., 1969; Iyengar et al., 1967).

**Diesel oil.** The advantages and disadvantages offered by diesel oil are similar to those by gas oil. Yeasts such as *Candida guilliermondii* (Zhdannikova et al., 1967), *Candida* sp. (Chepigo et al., 1967a), *C. tropicalis* (Filosa, 1966), and *Torulopsis famata* (Kulieve et al., 1969) are used in diesel oil processes.

**Kerosene.** The microorganisms studied for the production of biomass from kerosene include *Candida* sp. (Societe Francaise des Petroles BP, 1965b), *Achromobacter delmaeata* (British Petroleum Co. Ltd., 1967), *Mycohacterium japonica* (Yamaguchi, 1963), *Bacillus megaterium*, *B. subtilis* and *Pseudomonas aeruginosa* (Societe Francaise des Petroles BP, 1965b). The advantages and disadvantages of the use of kerosene are similar to those of gas oil. The presence of low boiling aromatic fractions in kerosene demands the
use of strains with great tolerance for them. The process technology of SCP production from kerosene was studied and developed by various workers (British Petroleum Co. Ltd., 1965b, 1967; Societe Francaise des Petroles BP, 1965b; Chinese Petroleum Corp., 1970).

n-Paraffins. The cost of n-paraffins is much higher than other petroleum fractions due to the extra step required for their separation from the fractions by molecular sieve or urea adduct methods. However, 100 per cent utilization is possible with n-paraffins which results in simplified separation of the cells from spent medium without subsequent solvent treatment. Bacteria and yeasts employed in the processes based on the use of n-paraffins include Candida lipolytica (British Petroleum Co. Ltd., 1965c), C. zeyploides (Kyowa Hakko Kogyo Co. Ltd., 1965), Candida sp. (British Petroleum Co. Ltd., 1963), Arthrobacter paraffinicus (Kyowa Hakko Kogyo Co. Ltd., 1965e), species of Alcaligenes, Brevibacterium, Cellulomonas, Corynebacterium and Pseudomonas (Esso Research and Engineering Co. Ltd., 1966), Micrococcus cerificans (Dourous et al., 1970), Micrococcus lactis (Poznrovova and Lognova, 1970), Pseudomonas (British Petroleum Co. Ltd., 1965d) and P. lipaestri, P. pseudomalley as well as P. orvillia (Esso Research and Engineering Co. Ltd., 1966).

Methane and natural gas. These are regarded as ultimate hydrocarbon substrates for the production of SCP by many workers because of their abundant availability, low cost, relative purity and ease of removal of unused substrate from the fermentation broth. However, high partial pressures of oxygen necessary for good growth on methane present hazardous possibilities of explosion. The
growth rates of the microorganisms on these substrates are also generally low. Moreover, a built up static charges or even overheated bearing of the fermentation systems may be of ignition danger due to inflammabilities of the mixture of methane and oxygen.

The studies on the fermentation of methane for the production of cell biomass have been carried out by many workers using *Methanomonas* sp. (Hammer et al., 1967), *Pseudomonas* (Chinese Petroleum Corp., 1970), unidentified gram-negative bacilli (Jinak et al., 1967) and mixed bacterial culture (Vary and Johnson, 1967). The processes based on the use of methane and natural gases for the production of SCP are patented and described by various workers (Belot and Barat, 1969; Chinese Petroleum Corp., 1970; Fukucka, 1972).

Gaseous hydrocarbons other than methane and natural gas. The use of propane for biomass production was studied with *Mycobacterium* (Smirnova and Golubeva, 1968), *Proactinomyces* (Smirnova and Golubeva, 1969), *Nocardia* (Jegner, 1970) and *Brevibacterium* as well as *Candida japonica* (Tanaka et al., 1970). The microorganisms employed for the production of SCP from butane include *Nocardia paraffinicum* (Kyowa Fermentation Industry Co. Ltd., 1970), *C. salmonicolor* (Mobile Oil Corp., 1968) and *Candida rigidida* (Kyowa Fermentation Industry Co. Ltd., 1969a).

Solid paraffins. These substrates pose problems in proper dispersion in the medium and, hence, are studied to a limited extent only. Processes for the production of microbial cells with solid hydrocarbons dissolved in solvents were developed (Jegner, 1967; British Petroleum Co. Ltd., 1962). Solid paraffins were subjected to preliminary degradation, with the help of high temperature and pressure, before incorporation into the media in the process developed by Katrush et al. (1968). Laboratory investigations on the biomass production from solid alkanes dissolved in solvent using Candida intermedia and C. lipolytica (Miller and Johnson, 1966a) and from emulsified paraffin wax with Candida tropicalis and Corynebacterium hydrocarboclastus (Yamada and Yogo, 1970) were reported.

Slack wax. It is a mixture of paraffin wax and lubricating oil with melting point in the range 45-65°C and is obtained as by-product during the manufacture of lubricating oil. Though it is available in large quantities and many microbial species are known to utilize solid hydrocarbons, it is not been used for the production of SCP probably due to solid nature of the substrate. However, emulsification of the slack wax for proper dispersion in the medium, may prove its use in production of SCP.

MICROBIAL PRODUCTS FROM HYDROCARBONS

During the course of studies on the utilization of hydrocarbons by microorganisms, a variety of products were encountered by various investigators. Abundant availability of hydrocarbons at comparatively cheaper cost was, therefore, resulted in concentrated studies leading to the formulation of many processes based on the
use of hydrocarbons.

**Enzymes and coenzymes.** Formation of considerable quantities of coenzyme Q (Shimizu et al., 1969), coenzyme A (Nakao et al., 1968), lipase (Takahashi et al., 1963a), cytochrome C (Tanaka et al., 1967a) and protease (Morihara et al., 1965) have been reported during growth of microorganisms on hydrocarbons and some of these processes are patented (Takahashi et al., 1965a; Morihara, 1966; Nakao et al., 1969).

**Organic acids.** A wide variety of organic and keto acids (Finogenova et al., 1968), alpha-keto glutaric acid (Tanaka et al., 1969a), fumaric acid (Yamada et al., 1969) and citric acid (Tanaka et al., 1969d) are formed by yeast and bacteria during assimilation of hydrocarbons. Consequently, processes for microbial conversion of petroleum hydrocarbons to organic acids were patented by many workers (Tanaka and Kimura, 1970; Arima and Okino, 1970; Tanaka et al., 1968b, 1969b).

**Amino acids.** Extracellular production of amino acids by microorganisms growing on hydrocarbons was observed by many workers (Yamada et al., 1963b; Shah et al., 1967b). Production of l-glutamic acid (Imada and Yamada, 1969), l-ornithine ("chit et al., 1967), dl-alanine (Imada and Yamada, 1969), o-acetyl-l-homoserine, a precursor of methionine (Nakayama et al., 1969) and l-phenylalanine (Takoro et al., 1970) in hydrocarbon fermentations were studied in detail and the processes were patented (Shiio et al., 1970; Kinoshita et al., 1966; Nakayama and Hagiwara, 1970; Tanaka et al., 1968d; Abe and Takayama, 1968; Yamada, 1969).
Vitamins and carotenoids. Microbial production of vitamin B\(_12\) (Morikawa and Kamikudo, 1969), vitamin B\(_6\) (Tanaka et al., 1967c), biotin vitamer (Tsuboi et al., 1966), riboflavin (Sato et al., 1966), carotenoids (Tanaka et al., 1968c) and B group vitamers (Popova, 1968) were reported during growth of microorganisms on hydrocarbons.

Sugars and polysaccharides. Accumulation of glucose and trehalose in culture broth of \(n\)-alkane utilizing bacteria was reported by Suzuki et al. (1969a) and subsequently a patent was filed (Tanaka and Suzuki, 1970). Production of saccharides (Suzuki et al., 1968), acid heteropolysaccharide (Kanamura and Yamatodani, 1969) and polysaccharides (Raymond and Davis, 1960; Dworkin and Foster, 1956) were also reported.

Nucleic acids and nucleotides. The production of inosine (Iguchi and Tanaka, 1966), orotic acid and orotidine (Kawamoto et al., 1970) by hydrocarbon assimilating bacteria was reported. Commercially feasible processes for the production of 5-inosinic acid, 5-xanthyllic acid, 5-guanylvic acid (Osawa et al., 1967), 5-adenyllic acid and inosine (Takeda et al., 1970) by fermentation of hydrocarbons were also developed.

Fats and fatty acids. Hydrocarbon utilizing microorganisms were reported for their abilities to produce trehalose lipids (Suzuki et al., 1969b), phospholipids (Kikuchi et al., 1969), fatty acids (Mizuno et al., 1966; Davis, 1964) and poly-\(\beta\) -hydroxybutyrate (Davis et al., 1964). Considerable work was carried out on the production of microbial fat (Iwamoto et al., 1960; Ratledge, 1970) and fatty acids (Romero and Brenner, 1966; Ratledge, 1970) and the processes were considered as of economic value.
Sterols and esters. Extracellular production of large amount of cetyl palmitate (Stewart et al., 1959), production of ergosterol (Diknaskaya et al., 1966) and ester (Hayashi et al., 1969a) by microorganisms growing on hydrocarbons were reported. Oxidation of sterols in hydrocarbon fermentation (Iizuka et al., 1969c, b) revealed the possibilities of the microbial transformation of sterols by hydrocarbon utilizing microorganisms.

Miscellaneous products. Various products such as benzoylphenyl glycerine (Takeda, 1970), porphyrins (Tanaka et al., 1969a), pigments (Mikitina, 1966), 1-phenazine carboxylic acid (Higashihara and Sato, 1970) and emulsifying factors (Kobayashi et al., 1967) were formed during microbial fermentation of hydrocarbons.

Products from aromatic hydrocarbons. Microbial production of salicylic acid from naphthalene (Hosler, 1963a, b), coumarin and catechol from naphthalene, anthracene, phenanthrene and benzene (Fernley and Evans, 1958), cis-cis muconic acid and 3-keto adipic acid from catechol (Evans, 1963) were reported. Synthesis of trans-4-ethylcyclohexanol from ethylcyclohexane (Arai and Yamada, 1969), trans-cinnamic acid from alkylbenzene (Dourous and Frankenfeld, 1968a) benzalalcohol, benzaldehyde and benzoic acid from toluene (Kitagawa, 1956) and m- as well as p-cresol from toluene (Raymond, 1961) were reported in microbial assimilation of aromatic hydrocarbons.

Omori et al. (1967) and Omori and Yamada (1969) reported the formation of p-toluic acid from p-xylene, m-toluic acid and 3-methyl salicylic acid from m-xylene, 3-4 dimethyl benzoic acid and 3-4 dimethyl phenol from pseudocumene by Pseudomonas aeruginosa. Similarly, production of cuminic acid from p-cymene (Yamada et al., 1965) was
also observed during microbial oxidation.

**BENEFICIAL MICROBIAL ACTIVITY TO PETROLEUM INDUSTRY**

Microbial petroleum prospecting. Studies on the soil bacterial flora for detecting and contouring petroleum gas emanating areas was first introduced by Mogilevskii (1938) which was further worked out and patented by many workers (Harvitz, 1939; Hassler, 1943; Updegraff and Chase, 1958). It was pointed out the application of microbial petroleum prospecting methods along with geological and geophysical methods will at least help in minimizing the drilling of dry holes (Davis and Updegraff, 1954).

Microbial secondary recovery of petroleum. The use of bacteria for increasing or aiding secondary oil recovery from reservoir was advocated and worked out by many investigators (LoBell, 1946a, 1947a,b; Updegraff and Bren, 1953). Field tests were carried out in many countries (Dostalek et al, 1957; Kuznetsov et al, 1963; Jeranyi et al, 1963) and were indicative of appreciable oil release from reservoir rocks.

Microbial disposal of petroleum waste. Microbiological methods for the treatment of water soluble petroleum wastes and petroleum oil wastes are used in most of the countries (Karelin and Vorobeva, 1957; McRae et al, 1956; Sontheimer, 1963; Samsel and Hawkins, 1960). The methods used include activated sludge (Sontheimer, 1963), oxidation ponds (McKinney, 1963) and trickling filters (Nau, 1954).

Microbial genesis of oil. The chemical and physical investigations on ancient and recent sediments (Trask et al, 1932) indicated microbial genesis of oil in sediments. Hypothesis of the biogenic
Origin of oil was elaborated (LoBell, 1943; Davis and Updegraft, 1954) and the data supporting the hypothesis was reported by various workers (LoBell, 1958; Ernery and Rittenberg, 1952; Oakwood, 1946).

**Microbial increase in soil fertility.** Increased soil fertility and marked increase in bacterial population of soil to which hydrocarbons were added was noted by various workers (Matthews, 1924; Jacobs, 1931; Harper, 1939). The increase in nitrogen contents of the soil exposed to gas leakage, naphthenic acid and other hydrocarbons was attributed to the activities of hydrocarbon utilizing microorganisms capable of fixing atmospheric nitrogen (Coty, 1967; Davis et al, 1964; Guseinov, 1958).

**Miscellaneous activities.** The hydrocarbon utilizing abilities of microorganisms led Yurovskii et al (1939) in suggesting the application of methane oxidizing bacteria to the walls and roofs of coal mines for effective removal of methane. This also led Davis and Yarbrough (1962) and Solari et al (1962) in constructing ethane fuel cell and in developing method to reveal the presence of *Pseudomonas aeruginosa* in clinical samples respectively. The ability of bacteria capable of using paraffinic compounds but inert toward cyclic hydrocarbons was utilized by Tausz and Peter (1919) in removing the former and purifying the latter compounds in a mixture of the two.

**DETRIMENTAL MICROBIAL ACTIVITY TO PETROLEUM INDUSTRY**

**Destruction of oil in deposits.** Experimental results for the microbial destruction of petroleum oil in deposits, resulting in loss of oil, were reported by Belousov (1937), Mogilevskii (1953), Ashirov and Maksimov (1958) and Ezkertsev (1960).
Corrosion of metals. Serious consequences and economic losses to the petroleum and aviation industries due to bacterial corrosion of oil-well casing (Doig and Iachter, 1951), oil-pipeline (Hadley, 1939), air-craft fuel tanks (Iard, 1963) and air-craft fuel system (Peat, 1956) are well known.

Plugging of petroleum reservoirs. It was attributed to the growth of microorganisms in flood waters and on reservoir rocks (Merkt, 1943; Hart et al., 1960; Feket, 1959) and also to microbial products such as polysaccharides (Allison, 1947) and hydrogen sulphide (Myers and Slabyj, 1962).

Degradation of drilling mud additives. These additives commonly used for increasing viscosity during drilling are decomposed by microorganisms resulting in further demand of these compounds for proper drilling, loss of time, poor drilling and serious contamination of borehole resulting in bacterial corrosion of completed well.

Deterioration of petroleum products. A great deal of attention has been shown to the wide spread occurrence of microorganisms in aviation turbine fuel systems (Bakanaukas, 1958; Churchill and Leathen, 1961; Peat, 1956). These microbial activities were responsible for the formation of microbial sludge and highly corrosive organic sulphides resulting in fuel-system filter plugging, fuel-gauge malfunction and fuel tank corrosion. Microbial deterioration of gasoline resulting in the formation of peroxides and the precipitation of tetra-ethyl lead was noted by Allen (1945). Microbial attack on polymerised inert organic lining of metallic storage tanks meant for storing petroleum products (Allen and Fore, 1953) and the microbial spoilage of cutting oil emulsions (Bennett, 1957) are other examples of the
Degradation of asphalts. Microbial degradation of asphalts, a main building material, was reported by Phillips and Traxler (1963) and Harris et al (1956). Degradation of asphalts by microorganisms is of economic significance to petroleum industry in connection with coating of pipeline with asphalt and subsequent corrosion of metal.

The aim of the present studies has, therefore, been to obtain petroleum hydrocarbon utilizing microorganisms from natural habitat and to study the various aspects of cellular morphology, growth and product formation so as to obtain basic knowledge about these microorganisms and also to find industrial use for them.