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The Chemistry and Physicochemical Features of the Microbiological Oxidation of Petroleum Hydrocarbons

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The review examines Soviet and foreign literature on the microbiological synthesis of proteins from petroleum hydrocarbons. The general characteristics of the process and the conditions for its occurrence are described together with the chemical mechanism of the microbiological oxidation of aliphatic, aromatic, and heterocyclic hydrocarbons. Particular attention has been devoted to the physicochemical and topochemical features of the process. It has been shown that the process is similar from the physicochemical standpoint to the oxidation of hydrocarbons in emulsions. The bibliography includes 241 references.

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I. INTRODUCTION

The enzymic synthesis of proteins and vitamins in the yeast cell has been attracting recently an increasing amount of attention from workers in many fields. A new branch of industry, dealing with the microbiological manufacture of amino-acids, vitamins, and other valuable products, has arisen and has been developing vigorously. Certain micro-organisms are known to be capable of utilising pure hydrocarbons[1-5], alcohols, and acids[6-9], and also petroleum and petroleum products[10-16] as sources of carbon and energy. The possibility of the oxidation of petroleum hydrocarbons by micro-organisms was pointed out as early as the 1920's–1930's by the leading Soviet microbiologist Tauson[17]. Certain species of micro-organisms develop very successfully in stagnant waters in petroleum-processing factories[18], in subsurface waters in petroleum deposits, in soils impregnated with petroleum[19], and also under bituminous road surfaces[20,21]. These micro-organisms are mainly aerobic and they metabolise saturated hydrocarbons, mainly with straight-chain structures[22,23]. Since the microbes are living organisms, they have a fairly rich protein store. Thus petroleum can become a source of proteins, which are an essential and the most deficient component in the food of man and animals.

According to McFerson's data[24], to make good this deficit, which is acute as far as the bulk of the world population is concerned, it will be necessary to produce in 1980 an additional amount of about 18 million tons of fats, 9.9 million tons of animal protein, and 11.4 million tons of amino-acids per year. The most convenient and cheapest of the principal raw materials for the synthesis of food products is petroleum and atmospheric nitrogen fixed in the form of ammonia.

The microbiological synthesis of food products is very convenient because of the high rate of multiplication of micro-organisms, their high biochemical activity, the possibility of using relatively cheap starting materials, and also the independence of the manufacture of climatic and weather conditions. Thanks to the progress in the technology of the growing of micro-organisms, favourable conditions have been created for the control of their development under industrial conditions.

At the present time there exists a fairly extensive Soviet and foreign scientific literature on the development of micro-organisms on separate fractions of petroleum and on pure hydrocarbons[25-32]. In particular, one should mention the work of French investigators led by Champagnat[33-37]. By growing micro-organisms (mainly yeasts) on various petroleum fractions, they obtained proteins and valuable water-soluble vitamins. The product was called the protein-vitamin concentrate (PVC). At the same time the removal of paraffins improved the quality of the petroleum, since the solidification temperatures of the fuels and oils obtained from it are thereby reduced[38]. The work of Senez and coworkers[39-42] is of great interest in this respect. Recently extensive studies are being carried out in the USSR on the isolation and selection of micro-organisms assimilating petroleum hydrocarbons, experimental industrial apparatus for the manufacture of PVC has been set up, and tests are being made on the nutritional value and the possible harmful effects of the product[43-54].

The microbiological synthesis of proteins and vitamins from petroleum is based on the enzymic oxidation of hydrocarbons and their derivatives. Little is known at present about the conditions of the occurrence, mechanism, and products of microbiological oxidation, which differs appreciably from the chemical oxidation of these hydrocarbons, and the available information is not systematic and requires revision. Chemists, in close collaboration with microbiologists, biochemists, and workers in other fields, should play the main part in the creation of a theory of the enzymic oxidation of hydrocarbons.

II. GENERAL CHARACTERISTICS OF THE MICROBIOLOGICAL SYNTHESIS OF PROTEINS FROM HYDROCARBONS

The principal raw material for the manufacture of the protein–vitamin concentrate are liquid saturated petroleum hydrocarbons with a straight-chain structure obtained in the process of elimination of paraffins from Diesel fuels with subsequent purification by adsorption. Saturated hydrocarbons obtained in the low-temperature elimination of paraffins and purified with sulphuric acid are also suitable. As a result of such purification, the saturated hydrocarbons contain not more than 0.5% of aromatic

hydrocarbons. Certain species of micro-organisms can be grown on individual fractions of Diesel fuels without preliminary isolation of straight-chain saturated hydrocarbons from the latter. Then the paraffin-free part of the fuel must be separated from the crude protein mass.

In petroleum from different deposits the content of alkanes with different carbon chain lengths varies within wide limits. Different micro-organisms assimulate mainly saturated hydrocarbons with 10–24 (C_{10}–C_{24}) [1-5] and also 1–4 (C_1–C_4) [25-32,49] carbon atoms. Thus in most cases yeasts assimilate hydrocarbons ranging from n-nonane to n-octadecane (C_9–C_{18}) and also unsaturated hydrocarbons with the same carbon chain length[50].

Experiments show that micro-organisms exhibit definite selectivity in their capacity to assimilate a particular substrate[51-65]. Isoalkanes are utilised by micro-organism less satisfactorily than straight-chain alkanes[66]. Aromatic compounds are not very suitable as sources of carbon for micro-organisms of the genus Candida[56,67,68]. An even narrower substrate specificity has also been observed. Micro-organisms can grow on one hydrocarbon but not on another with a similar structure[69].

At the present time search is going on for highly active micro-organism cultures (mainly yeasts) ensuring rapid accumulation of biological material. Most investigators have devoted particular attention to yeasts of the genus Candida, since these are the most active[3,29,70].

Yeasts are rich in B vitamins, the content of which depends on the yeast culture, the nutritional medium, and the method of cultivation (Table 2), [47,48,89,90]. Yeasts grown on petroleum hydrocarbons contain vitamins combined with other growth factors of unknown composition.

Table 2. Content (mg) of water-soluble vitamins and their importance[30].

	Thiamine B_1	Riboflavine B_2	Nicotinic acid PP	Pantothenic acid	Pyridoxine B_6
Daily requirement in man	2	3	15	3	2
Content of vitamins in various products:					
Meat (beef)	1—3	2	40—100	7—21	1—4
Liver	5—10	16	75—275	30—60	5
Milk	0.3—0.7	1—3	1—5	1—4	1—3
Grain	0.5—7.0	1—1.5	10—30	5—20	3—6
Dry yeast	2—20	30—60	200—500	30—200	40—50
Protein-vitamin concentrate	3—16	75	130—200	150—192	23
Functions	carbohydrate metabolism	dehydrogenation, oxidation	carbon transport	acetylation. synthesis of aliphatic acids	transamination
Consequences of vitamin deficiency	nervous disorder, beri-beri	arrest of development	pellagra	arrest of development	dermatitis

Table 1. Content of certain amino-acids in yeast, meat, and wheat grain[30].

Amino-acid	Daily requirement in man, g	Amino-acid content, g/100g		
		in yeast	in animal tissues	in wheat grain
Arginine	3.5	4.3	7.1	3.0
Histidine	2.0	2.8	2.2	1.2
Lysine	5.2	6.4	8.1	2.7
Tyrosine	3.9	4.2	3.1	—
Tryptophan	1.1	1.4	1.2	1.0
Phenylalanine	4.4	4.1	4.5	9.3
Cystine + Methionine	3.8	1.3 traces	1.1 3.3	with tyrosine 4.3
Leucine	9.1	13.2	12.1	13.0
Isoleucine	3.3	3.4	3.4	4.0
Valine	3.8	4.4	3.4	3.5
Threonine	3.5	5.0	5.2	3.3

The nutritional value of yeast was first pointed out by Dellbrook in 1875; this hypothesis was developed in later studies by other workers[71-78]. The nutritional value of protein is determined in the first place by the relative amounts of its constituent amino-acids[79]. The amino-acid composition of yeast protein depends on the yeast species, the nutritional medium, and the method of cultivating the yeast[80,81]. However, different yeast species do not show sharp differences in their amino-acid content[82] and this is the reason for the high biological value of yeast protein[83,84] (Table 1).

Yeast proteins are assimilated by [animal] organisms to the extent of 85–88%; in this respect they occupy an intermediate position between proteins of plant (65–75%) and animal (90–95%) origin[85-87]. Because of their high lysine and valine content, yeasts are a useful additive to cereal proteins and improves their nutritional value[88].

Like any other living organism, yeasts can exist only in the presence of water. Their nutrient must contain carbon, nitrogen, phosphorus, potassium, magnesium, and many micro-elements. Petroleum hydrocarbons are the exclusive source of carbon and energy. Nitrogen, phosphorus, potassium, and magnesium form part of the nutrient medium in the form of a solution of mineral salts in water. Thus microbiological oxidation takes place in an emulsion of the hydrocarbon in the aqueous phase. The elements essential for the growth and development of yeast include in the first place iron, zinc, and manganese[91,92]. Iron enters into the composition of many enzymes and is necessary for the growth of yeasts and the accumulation of biological material[93,94]. Zinc also enters into the composition of carboxyoxidase, cytochrome, cytochrome oxidase, and certain other enzymes[95]. Manganese influences the catalytic activity of many oxidative enzymes. One may assume that other elements, required in smaller doses, such as copper, molybdenum, cobalt, iodine, boron, and others, are present in sufficient amount in tap water and in the mineral salts usually employed in the cultivation of yeasts.

The principal parameter of process development is temperature. For each micro-organism there exists an optimum temperature at which the time required for cell division is a minimum. All these temperatures lie in the range 25–40°C [42,96,97] and therefore the microbiological oxidation of petroleum hydrocarbons is energetically more favourable than chemical oxidation, which occurs at an adequate rate only at elevated temperatures.

An important factor in the cultivation of micro-organisms on hydrocarbon substrates under aerobic conditions is the supply of oxygen. Oxygen is required not only to synthesise the biological material itself, but also to oxidise the hydrocarbon and to satisfy the energy requirements of the metabolism of yeast cells. The overall

requirement of oxygen in the growing of yeasts on hydrocarbons is higher by a factor of 2–4 than on carbohydrate substrates, since in the latter case oxygen is required only to make good the energy expended on the growth and is not fixed by the cell[30]. The oxygen needed by the micro-organisms must be dissolved in the aqueous medium. The limiting cell growth is determined by the diffusion of atmospheric oxygen into the medium.

At the present time the efforts of scientists and engineers are directed towards the development of a theory and the improvement of apparatus for continuous microbiological oxidation of petroleum hydrocarbons[98-102]. The microbiological oxidation of petroleum hydrocarbons may be carried out in two ways: by growing micro-organisms to be used as such in the form of protein-vitamin concentrates or by extracting from the aqeuous medium the valuable products synthesised by the micro-organisms. The industrial methods for the microbiological synthesis of amino-acids and vitamins used in many countries are based on these properties of micro-organisms[103-110]. The micro-organism species which, after metabolising the carbon-containing substances secrete into the reaction medium predominantly one or other amino-acid, are of particular interest. Glutamic acid and lysine are at present manufactured in this way on an industrial scale from carbohydrates and plant raw material. Scientists in the laboratories of Japan, the USA, the Soviet Union, and other countries are working intensively on the development of methods for the biological synthesis of other amino-acids[111-120].

III. THE CHEMICAL MECHANISM OF THE MICROBIOLOGICAL OXIDATION OF HYDROCARBONS

Numerous experiments have shown that the mechanism of the oxidation of hydrocarbons by micro-organisms differs from that of chemical oxidation. It has been firmly established that in the liquid-phase oxidation of hydrocarbons the primary oxidation product is a hydroperoxide, subsequently responsible for the degenerate branching of the complex chain oxidation process. The main radical propagating the reaction chains is the peroxy-radical ROO·.

The chemical oxidation of hydrocarbons has been investigated in detail by Emanuel' and coworkers[121,122], who elucidated a number of its kinetic features and proposed the following oxidation mechanism:

$$RH + O_2 \rightarrow R\cdot + HO_2$$
$$R\cdot + O_2 \rightarrow RO_2\cdot$$
$$RO_2\cdot + RH \rightarrow ROOH + R\cdot$$
$$ROOH \rightarrow RO\cdot + O\cdot H$$

$$2RO_2\cdot \rightarrow \text{chain termination.}$$
$$R\cdot + RO_2\cdot \rightarrow \text{chain termination.}$$
$$2R\cdot \rightarrow \text{chain termination.}$$

The mechanism of the microbiological oxidation of hydrocarbons differs from that given above and has been much less studied.

The assimilation of hydrocarbons by micro-organisms depends on the following conditions: the penetration of the hydrocarbons into the cell, the capacity of micro-organisms to become adapted to the hydrocarbons, and finally the presence of enzymes necessary for the primary oxidation of the hydrocarbons[123,124]. The penetration of hydrocarbons into the cells of the micro-organisms, which largely determines the topochemistry of the reaction, is to a considerable extent associated with the physicochemical features of the process, which will be examined below.

The adaptation phenomenon, which probably takes place at the level of enzymic systems, is now attracting the attention of many investigators[125-135]. Micro-organisms assimilating hydrocarbons must be adapted to the nutrient medium, which under natural conditions undergoes considerable changes. Some micro-organisms, die, having failed to adapt to the new conditions. Others gradually become adapted, and begin to assimilate the new substrates[136].

The intrinsic oxidation process takes place with the aid of enzymes which lower the activation energy by 16–18 kcal mole^{-1}, while non-biological catalysts result in a lowering of activation energy by only 4–8 kcal mole^{-1}.[137] Both the prosthetic group and certain groups of the protein carrier forming part of the active centre of the enzyme take a direct part in catalysis[138]. It has been shown[139,140] that the configuration of molecules in certain enzymes changes appreciably in the course of interaction with the substrates. Evidently the catalytic groups are then arranged in the order necessary for enzyme action on the substrate[141-143].

In the formation of the enzyme–substrate complex bonds are polarised and this is the cause of the "strained configuration" of the complex. In a complex of this kind the bonds are stretched and weakened[140]. The binding of the substrate by the enzyme induces a change in the conformation of the protein base of the enzyme, and, conversely, under the influence of the enzyme the substrate molecules are distroted and activated. The coordinated action of two of several groups of the active enzyme centre involving the polarisation of definite bonds in the substrate constitutes the basis of the catalytic action of the enzyme[144,145]. The kinetics of enzyme action are described by the Michaelis equation or more complex equations, depending on the number of components in the active centres[146,147].

The data on the role and mechanism of the action of enzymes in the microbiological oxidation of hydrocarbons are inadequate. Only recently interesting studies on the participation of enzymes in these processes have begun to be published[129,132,133,135,148].

The primary step in microbiological oxidation is attack by oxygen on the hydrocarbon molecule. Unfortunately, so far there is no unanimous view in the literature concerning the site of the attack. Many investigators believe that the most likely pathway in the microbiological oxidation of straight-chain paraffins involves the oxidation of one of the terminal methyl groups to a carboxy-group via the intermediate formation of an alcohol and an aldehyde[149-154].

Using the tracer atom method, Stewart and coworkers[155] showed that molecular oxygen is incorporated in the hydrocarbon molecule, the primary oxidation product being a hydroperoxide:

$$R{-}CH_3 \xrightarrow{+{}^{18}O_2} R{-}CH_2{}^{18}O{}^{18}O \rightarrow R{-}CH_2{}^{18}O\,H \rightarrow R{-}C^{18}O\,OH.$$

The presence of a hydroperoxide in the metabolic products is indicated also by other workers[40,156,157]. The possibility of the formation of a hydroperoxide as an oxidation intermediate is confirmed by the fact that synthetic alkyl 1-hydroperoxides are oxidised by micrococci grown on hydrocarbon substrates[136].

The process mechanism described above is not as yet reliably established. Apart from the oxidation of the terminal atom in the straight-chain hydrocarbon, it is believed that the carbon atom in the α-position may be oxidised[158]. Then the formation of aliphatic acids takes place via secondary alcohols and alkyl methyl ketones:

$$R{-}CH_2{-}CH_3 \rightarrow R{-}CHOH{-}CH_3 \rightarrow R{-}CO{-}CH_3.$$

Foster explains the oxidation of the carbon atom in the α-position on the hypothesis that the free radicals within

the chain of the straight-chain hydrocarbon are in equilibrium[165]. Primary activation consists in the formation of a free n-alkyl radical at the terminal carbon atom. However, the hydrogen atom can migrate within the molecule and activate the adjacent carbon atom, i.e. the primary radical isomerises to a radical with the corresponding iso-structure. The initial stable products formed by this mechanism are ketones. These compounds are detected only in certain cultures, grown on saturated hydrocarbons[158-161], but the relative ease with which methyl ketones are assimilated by micro-organisms oxidising hydrocarbons suggests that these substances may behave as intermediates in the oxidation of straight-chain alkanes, particularly those with a short chain[158-162].

In the cultivation of various bacteria (*Pseudomonas aeruginosa*, *Achromobacter* sp., *Nocardia* sp., *Mycobacterium* sp.) on purified hydrocarbons with an even and odd number of carbon atoms (C_6 and C_7), Treccani et al. noted the formation of saturated aliphatic acids with unbranched chains[163-165]. Lower alkanes with an even and odd number of carbon atoms yield various oxidation products[69]. When n-hexane is used as a substrate, caproic, butyric, and acetic acids are formed, while with n-heptane the products are all the [straight-chain] aliphatic acids containing between 1 and 7 carbon atoms in the molecule. This investigation requires additional experimental confirmation. The assimilation of alkanes with an even number of carbon atoms is hindered by the presence in the reaction mass of acids with an odd number of carbon atoms. If, on the other hand, the cells assimilate a hydrocarbon with an odd number of carbon atoms the presence of any aliphatic acids in the reaction medium does not interfere with the reaction[165].

It has been established that *Pseudomonas methanica* converts gaseous hydrocarbons into oxidation products with the same number of carbon atoms as the initial hydrocarbons[166-169]. When ethane, propane, and n-butane were added, the products detected were respectively acetic acid and acetaldehyde, propionic acid and acetone, and butyric acid and 2-butanone. In the microbiological oxidation of methane the extracellular products formed are methanol, formaldehyde, formic acid, carbon dioxide, hydrogen, and polysaccharides[170].

In the oxidation of n-hexadecane the final product is cetyl palmitate[165-171]. The cetyl alcohol which esterifies palmitic acid is formed from n-hexadecyl hydroperoxide, which is probably the first intermediate in the oxidation of n-hexadecane. Micro-organisms grown on hexadecane readily oxidised this hydroperoxide. An ester is also formed when micro-organisms are grown on octa- and tetra-decane[172]. The alcoholic component of the ester has the same hydrocarbon skeleton as the alkane in the substrate. On this basis, one may say that the alcoholic component of the ester obtained from the hydrocarbon is formed directly from the alkane by the oxidation of the terminal carbon atom.

On the other hand, according to Treccani[69], the acid component of esters in all cases of bacterial oxidation of alkanes probably contains 16 carbon atoms regardless of the chain length of the straight-chain hydrocarbon used as the substrate.

Gololobov believes[136] that palmitic acid is not always the acid component of the esters. This is also the view of Kallio and Finnerty[173,174], who isolated two esters from the medium in which micrococci were grown with octadecane: octadecyl palmitate $CH_3(CH)_{14}COOCH_2(CH_2)_{16}CH_3$ and octadecyl stearate $CH_3(CH_2)_{16}COOCH_2(CH_2)_{16}CH_3$.

The acid component of octadecyl stearate is formed as a result of the oxidation of the terminal carbon atom in octadecane:

$$CH_3-(CH_2)_{15}-CH_2-CH_3 \rightarrow CH_3-(CH_2)_{15}-CH_2-CH_2OH \rightarrow CH_3-(CH_2)_{15}-CH_2-COOH.$$

In octadecyl palmitate the acid component contains 2 carbon atoms less than the hydrocarbon. The formation of palmitic acid may be explained by assuming the oxidation of the α-carbon atom in octadecane with formation of a secondary alcohol and then an alkyl methyl ketone, the latter being subsequently split by the following mechanism:

$$CH_3-(CH_2)_{15}-\overset{\alpha}{C}H_2-CH_3 \longrightarrow CH_3-(CH_2)_{15}-\underset{OH}{\overset{\alpha}{C}H}-CH_3 \longrightarrow$$

$$\longrightarrow CH_3-(CH_2)_{14}-\overset{H}{\underset{2O}{CH}}-\overset{\|}{\underset{O+O}{C}}-CH_3 \longrightarrow CH_3-(CH_2)_{14}-COOH + CH_3COOH.$$

As stated above, in the microbiological oxidation of hydrocarbons direct binding of oxygen is observed[175,176]. Many investigators suggest that primary enzyme action on the hydrocarbon molecules is possible with direct participation of oxygen. This hypothesis has been proved[169] by the incorporation of oxygen-18 into cetyl palmitate.

The possibility of the microbiological decomposition of hydrocarbons under anaerobic conditions appears to be less likely[177,178], but recent communications describe the development of specific hydrocarbon-oxidising bacteria of the genus *Pseudomonas* on a mineral medium with petroleum under anaerobic conditions[179].

Thus the conversion of aliphatic hydrocarbons consist in the formation during the initial reaction period of saturated aliphatic acids with unbranched chains. Different workers have obtained not entirely consistent results concerning the further breakdown of the aliphatic acids. In the view of the majority[156,159], acids with an even number of carbon atoms break down by a mechanism involving β-oxidation to acetic acid. When the number of carbon atoms is odd, the acids can also undergo α-oxidation and be converted into acids with an even number of carbon atoms, which subsequently undergo only β-oxidation to propionic and acetic acids. Thus, according to Stadman[23], *Methanobacterium suboxydans* converts valeric acid by β-oxidation into propionic and acetic acids, while butyric and caproic acids are converted into acetic acid.

Certain bacterial species are capable of oxidising straight-chain alkane molecules from both ends, forming both mono- and di-carboxylic acids[180]. ω-Oxidation to dicarboxylic acids is evidently a secondary pathway and constitutes the first stages of the subsequent β-oxidation.

The oxidation of metabolic intermediates may be artificially retarded by adding to the reaction medium inhibitors such as sodium azide, sodium cyanide, $\alpha\alpha'$-dipyridyl, heavy metal ions, and others[23,181]. Possibly the use of inhibitors will prove useful in the investigation of the mechanism of the formation and accumulation of valuable products and also for the determination of the role of the enzymes involved in the process.

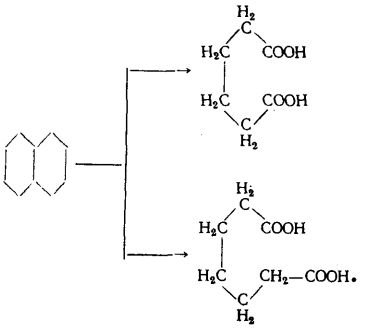
The mechanism of the bacterial oxidation of unsaturated compounds has been little investigated. It is known that micro-organisms are capable of assimilating olefins as sources of carbon and energy. According to Shturm's paper[182], unsaturated hydrocarbons are assimilated by micro-organisms more readily than the corresponding alkanes. Both saturated and double bonds in olefins are attacked. Thus van der Linden and coworkers established that in the decomposition of oct-1-ene by cells of *Pseudomonas aeruginosa* the principal changes take place at the

saturated end of the molecular chain of the olefin, while the double bond is not affected[183].

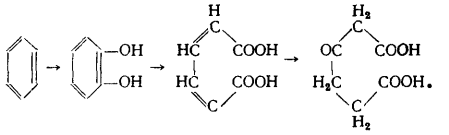
The finding that the oxidation of hexadec-1-ene by the yeast *Candida lipolytica* results in the accumulation of hexadecane-1,2-diol as an intermediate[184] is interesting. This indicates the possibility of the simultaneous oxidation in the olefin at the two carbon atoms linked by a double bond[185]:

$$R{-}CH{=}CH_2 \xrightarrow{O_2} R{-}\underset{OH}{CH}{-}\underset{OH}{CH_2}.$$

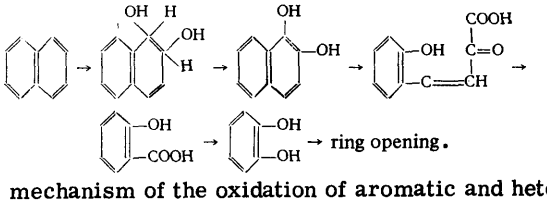
Little is known about the mechanism of the microbiological oxidation of cyclic saturated compounds such as cyclohexane, decalin, perhydrophenanthrene, and perhydroanthracene[186,187]. The formation of a peroxide formaldehyde, and valeric and formic acids when *Pseudomonas fluorescens* is grown on cyclohexane has been demonstrated. When *Flavobacterium* is grown on decalin, adipic acid is an intermediate[189]:



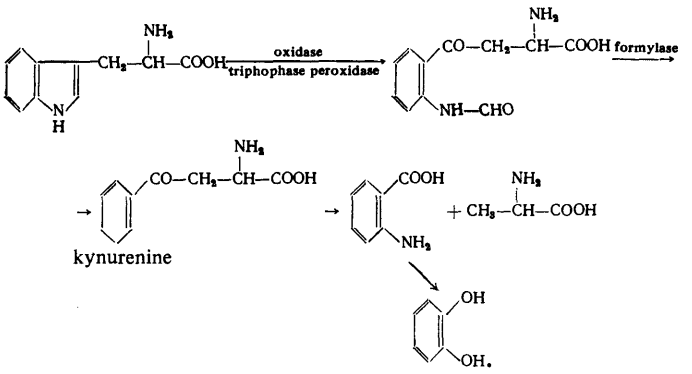
Micro-organisms assimilate aromatic compounds by converting them into *o*- or *p*-dihydroxy phenolic derivatives and then splitting the ring to aliphatic acids[190,191]. In the oxidation of benzene[192,193] catechol is formed as an intermediate; phenol and *cis,cis*-muconic acid also accumulate[194]. Benzene oxidase, which converts benzene into 3-oxoadipic acid, has been isolated from cells grown on benzene[195,196]. The enzymic extract does not contain phenol oxidase and only whole cells grown on phenol (but not adapted to benzene) immediately bind oxygen in the presence of phenol. The oxidation of benzene can be represented by the following mechanism:



Walker and Wiltshire showed in their investigation of the pathways for the decomposition of naphthalene during the growth of five different microorganisms that in all cases the intermediates are naphthalenediol, salicyclic acid, and catechol. The same mechanism has been confirmed by the work of Azoulay and coworkers[198,199]:



The mechanism of the oxidation of aromatic and heterocyclic hydrocarbons has been described in detail in Treccani's review[69]. For example, the decomposition of tryptophan takes place by the following mechanism:



Indole reacts similarly. The microbiological oxidation of indolylacetic and nicotinic acids, nicotine, aromatic nitrocompounds, and compounds of the lignin group have also been studied.

IV. PHYSICOCHEMICAL AND TOPOCHEMICAL FEATURES OF THE PROCESS

Brief examination of the chemistry of the oxidation of petroleum hydrocarbons convincingly demonstrates that only the first steps have been taken in its investigation. The available data are far from adequate.

At present no papers have been published on the physical chemistry of microbiological oxidation and the kinetics of its individual stages. However, in view of the current importance of this process and its application in industry such information is very necessary, since without the knowledge of the physical chemistry and kinetics of the process it is difficult to consider the possibility of its rational control.

It is stated in papers published recently that during the growth of yeast cells free radicals are observed[200,201]. This is confirmed by the presence of ESR signals in the yeast *Saccharomyces cervisiae* and also in the oxidation of NADH by the cells of *Mycobacterium phlei*. In the latter case it is suggested that the ESR signals may be due to radicals similar in structure to semiquinone. The possible participation of free radicals in microbiological oxidation may be established by studying the chemiluminescence arising when free radicals act upon yeast cells[202]. The considerable role of free radicals in enzymic oxidation processes has been confirmed by the work of Emanuel' and coworkers on enzymic oxidation involving succinate dehydrogenase and other enzymes[203-206]. Free radical reactions in enzymic oxidation may be inhibited by inhibitors of chain oxidation processes, such as phenolic compounds and others. All these findings show that one cannot exclude the possibility that individual stages in the microbiological oxidation of petroleum hydrocarbons take place with participation of free radicals.

The microbiological oxidation of petroleum hydrocarbons takes place in a complex system consisting of several phases. The hydrocarbon in a dispersed state and the cells of the micro-organisms are present in the aqueous medium. As a result of the growth of the micro-organisms, surface-active substances responsible for the additional emulsification of the hydrocarbon are produced[36,37]. All this suggests that the system is extremely

complicated. Factors associated with the vital activity of micro-organisms and also with the colloidal-chemical nature of the system are superimposed on the purely chemical processes. From this point of view, it is desirable to examine the mechanism of the penetration of the hydrocarbon into the cells present in the aqueous phases, since the process is impossible without the two component parts of the system coming into contact. There exist various theories explaining the mechanism of the entry of nutrient materials into the cell. Thus Johnson suggests[66] that the penetration of the hydrocarbon into the cell takes place with participation of the lipids in the cell membrane the long alkane chain becoming part of the phospholipid micelle of the cell membrane. This explanation is very general and it leaves many facts obscure. It has been suggested that the initial oxidation of the alkane takes place outside the cell[207]. Then it would be necessary for certain enzymes to be secreted by the cell into the medium[208], but many workers disagree with this postulate[136]. An interesting amount of evidence shows that the enzyme must be incorporated in definite structural formations within the cell in an unchanged form in order to exhibit its activity[209-217].

The intracellular activity of enzymes may be increased by surface-active emulsifying agents, as happens when such agents act upon the yeast *Candida albicans*[218]. In this case the catalase activity of *Candida albicans* increases by virtue of the increase in the permeability of the cell wall under the action of ionic surface-active substances. The interaction of surface-active emulsifying agents with *Saccharomyces ellipsoideus* leads to the formation of complexes with the lipids, lipoproteins, or proteins of the cell surface, whereby metabolic processes are distorted[219].

The complexity of the reaction system suggests that, in order to formulate the general laws governing the process, it is necessary to examine its topochemistry. Since virtually no such investigations of microbiological oxidation have been made, we believe it interesting to consider briefly the oxidation in emulsion of petroleum hydrocarbon, which resembles microbiological oxidation from the physicochemical standpoint. Probably later it will be possible to draw a number of analogies between the two reactions. The kinetics of the oxidation of hydrocarbons in emulsions have been thoroughly studied by Kucner and coworkers, who put forward a hypothesis concerning the topochemistry of the process[220-222].

In the first place they showed that surface-active emulsifying agents constitute an important kinetic factor; they alter the rate of the process (usually increase it) as a result of the stabilisation of the hydrocarbon droplets and also the droplets of the reaction products in the aqueous medium.

When hydrocarbons are oxidised in emulsions, it is quite possible that individual stages of the process take place in the aqueous phase[222]. By postulating the existence of micelles of the emulsifying agents, the nucleus of which consists of the organic part of the molecule and is oleophilic, one may assume that water-insoluble substances will dissolve fairly readily in the nucleus of the micelle. As a result, the loose micellar structure, which is constantly in contact with water, contains a high local concentration of the reactants which are subject to the polarising action of water. Here it is quite reasonable to assume an enhanced reactivity of the solubilised substances.

In this case the hydrocarbon phase is a source of the reactants which undergo solubilisation. Furthermore, the reaction products formed in the aqueous phase diffuse into the hydrocarbon phase. It is quite reasonable that all these processes should be coupled with the diffusion of the reactants from one phase to the other. A somewhat simplified topochemical mechanism of the reaction is presented in Fig. 1.

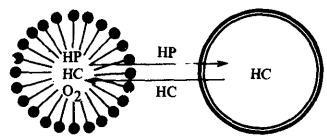


Figure 1. Topochemical mechanism of the oxidation of hydrocarbons in emulsions in the presence of an emulsifying agent. [HP (hydroperoxide) and HC (hydrocarbon) are the tentative interpretation of the Russian abbreviations GP and UVC (Ed. of Translation).]

However, this mechanism is not universal. It postulates the presence of surface-active emulsifying agents in the system and completely disregards the oxidation of hydrocarbons in emulsions not stabilised by such agents.

A number of experiments[223] convincingly demonstrate that the pressure of oxidising oxygen plays a significant role in the oxidation of hydrocarbons in emulsions. Thus in the oxidation of isopropylbenzene in a stainless steel autoclave an increase of pressure up to 10 atm leads to an increase of the reaction rate, which, however, scarcely changes any more when the pressure is raised above 15 atm. All these findings suggest that the oxidation of hydrocarbons in emulsions at atmospheric pressure is diffusion-dependent and is not determined by the kinetics. Then the rate of the process is limited by the solubility of oxygen in the emulsion and particularly in the aqueous phase. It has also been established[224] that the solubility of oxygen in the aqueous phase increases appreciably in the presence of dissolved surface-active emulsifying agents. Fig. 2 shows that the solubility and particularly the rate of dissolution of oxygen are higher in solutions of emulsifying agents than in pure water.

Finally, it has been established by a number of kinetic experiments on the oxidation of hydrocarbons in emulsions that the individual stages of the reaction (in this case it is the initiation reaction, taking place in the decomposition of a hydroperoxide) occur at the interface[225]. In particular, this applies to reactions in which surface-active agents (for example hydroperoxides), which are adsorbed on the surface of hydrocarbon droplets, take part. Here different media act upon different parts of the reactant molecule. The organic (hydrophobic) part of the molecule is drawn into the hydrocarbon medium, while the hydrophilic part is solvated by water molecules.

Thus the difference between the chemical potentials of the substance, different parts of the molecule of which are in different phases, gives rise to an additional polarising effect, which leads to an enhanced reactivity of the reactant. This is particularly evident in the decomposition of the hydroperoxide, the rate of which increases appreciably in the presence of the aqueous phase (Fig. 3).

Turning back now to the consideration of the microbiological oxidation of hydrocarbons, we may note the similarity of a number of its features with the oxidation of hydrocarbons in emulsions. In the first place the individual stages of the reaction take place in different phases or at the interface. The cell membrane consists of diphilic protein molecules, the structure of which resembles the diphilic structure of the molecules of surface-active emulsifying agents[226]. In this case the incorporation of the hydrocarbon between the organic chains of the hydrophobic "tails" of the protein molecules, similar to that occurring in the solubilisation of oleophilic substances in the so called "lamellar" micelles, is quite likely[227].

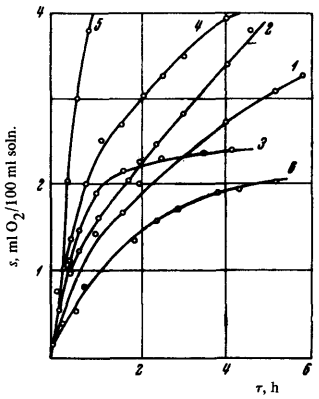


Figure 2. Kinetics of the dissolution of oxygen: 1) in 5% solution of Nekal; 2) in 5% solution of Leukanol; 3) in 0.1 *N* solution of potassium palmitate in 0.1 *N* solution of sodium carbonate; 4) ditto with solubilised isopropylbenzene; 5) in isopropylbenzene at 80°C; 6) in 0.1 *N* solution of sodium carbonate at 25°C.

Probably exceptionally favourable conditions are achieved at the interface, and in the first place on the surface of the cell, for the formation of the enzyme–substrate complex, since in this case a very high local concentration of these species is set up. The rate and kinetics of microbiological oxidation should also be influenced by the degree of dispersion of the reactants. This applies both to the degree of dispersion of the hydrocarbons droplets and the bubbles of the oxidising gas. These questions require systematic investigation. In their solution it is necessary to carry out experiments showing to what extent the rate of the process changes during the mechanical stirring of the reaction mixture. The study of the effect of the mechanical stirring of the oxidising emulsion of the hydrocarbon should make it easier to elucidate the role of diffusion factors in the kinetics of the microbiological oxidation of hydrocarbons, since an increase in the stirring rate produces an increased rate of diffusion of the individual components.

It is quite likely that, when the reaction is carried out at atmospheric pressure, the topochemical mechanism quoted above (Fig. 1) may be used for its explanation to some extent. In this connection it is also interesting to elucidate the effect of the solubilisation of the individual components in the aqueous phase on the process kinetics. We believe that such studies should be performed under conditions of a continually decreasing amount of the hydrocarbon phase until the complete disappearance of the hydrocarbon–aqueous solution interface.

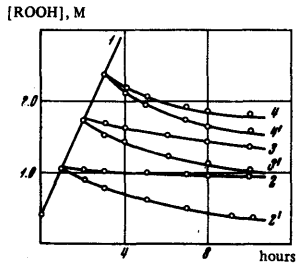


Figure 3. Kinetic curves for the oxidation of isopropylbenzene in emulsion (curve *1*) and for the breakdown of the hydroperoxide in the separated hydrocarbon phase (curves *2*–*4*) and in the emulsion (curves *2′*–*4′*) at 110°C.

Finally, the last process requiring solution at the present time is the effect of the increasing pressure of the oxidising gas containing oxygen. At an elevated pressure the solubility of oxygen increases. Thus, when the concentration of oxygen in the aqueous phase is raised, the rate of its diffusion into the hydrocarbon droplets or cells saturated by the hydrocarbon also rises. Then the process may be accelerated, as is observed in the oxidation of hydrocarbons in emulsions, but in microbiological oxidation account must be taken of the effect of pressure on the metabolism of the micro-organisms[228]. It is known from the literature that high pressures (up to 200 atm) are destructive to yeast organisms[70]. At a pressure in excess of atmospheric, oxygen may suppress and limit the growth of micro-organisms capable of developing in various nutrient media. Further studies should be directed towards the search for cultures stable under conditions of increased oxygen pressure.

From the kinetic standpoint, microbiological synthesis of proteins (more correctly its individual stages) takes place in an open system, since there is material exchange between the reaction site and the external medium[229]. A change in the amount of substance in an open system results not only from chemical processes but also from material exchange with the surrounding medium, which must be taken into account in the determination of the reaction rate.

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The aim of the present review was to examine the present state of our knowledge of the mechanism of the microbiological oxidation of petroleum hydrocarbons. The importance of this problem is evident. The interest which it has aroused has led to what is actually a new branch of natural science, at the boundary between chemistry and microbiology, which deals with the problems of the preparation of various substances with the aid of micro-organisms. Although microbiological synthesis itself has been known and used industrially for a long time, insufficient

attention has been devoted so far to the processing by this procedure of petroleum hydrocarbons, despite its importance. This applies to the physicochemical features of the process, since its microbiological and biochemical aspects have been vigorously investigated. To avoid overloading the review, many important purely biochemical and microbiological problems, which have been dealt with in other reviews, are omitted.

Much attention was devoted to the biosynthesis of proteins and vitamins at the Eighth and Ninth International Microbiological Congresses held in recent years (Moscow, 1962 and 1966), the Conference on Applied Microbiology, (Stockholm, 1963), the Symposium on Petroleum Microbiology (Czechoslovakia, 1964), and other scientific meetings[230-233]. At the present time most of the work in the field of microbiological synthesis concerns the accumulation of experimental data on the selection of the raw material and the micro-organism cultures, the study and isolation of the oxidation products, the development of methods for the control and investigation of the process, and also the relevant apparatus. Process conditions are selected and the nutritional value of the oxidation product is studied. Some advances have been made in the establishment of the mechanism of the enzymic oxidation of hydrocarbons and the role of trace elements in this process, and the mechanism of the interaction of the living cell with the hydrocarbon is being studied.

However, we believe that the time has come for a more detailed investigation of the chemical (particularly physicochemical) aspect of the microbiological oxidation of hydrocarbons. As in all developing branches of science, there are a number of factors here concerning which there exist only reasonable guesses or the data are contradictory. There has been hardly any work on the physicochemical aspect of the process, although such studies are of primary importance for the improvement of the technology. The chemists studying this process should employ the available literature data on similar processes (liquid-phase oxidation of hydrocarbons, reactions in emulsions, etc.).

The main difficulty in the investigation of the enzymic oxidation of hydrocarbons is in the first place the complexity of the reaction system, which requires the combined efforts of investigators in many fields, including many branches of chemistry. The weak development of our knowledge concerning the purely chemical aspect of enzymic processes, particularly as regards the microbiological oxidation of hydrocarbons, also hinders studies in this field. The development of such investigations promises to create a new technology of the preparation of food products and animal feed and also the synthesis of valuable organic substances (amino-acids, vitamins, etc). There is reason to believe that the development of the theory, the elucidation of the general laws governing the microbiological oxidation of hydrocarbons, and in particular the chemical mechanism of the process, will advance significantly in the next few years. It is quite clear that the extension and deepening of knowledge concerning the mechanism of the enzymic oxidation of petroleum hydrocarbons will lead to a real improvement of the existing technological processes for the synthesis of proteins from petroleum and also the organisation of new processes involving the selective conversion of petroleum hydrocarbons into important products and intermediates in industrial organic synthesis.

During the preparation of this review for the press, new papers on the enzymic oxidation of solid straight chain alkanes by different groups of micro-organisms were published[234,235]. The unsatisfactory growth of certain hydrocarbon-oxidising micro-organisms on solid alkanes can be explained in many cases by the instability of the dispersions of these hydrocarbons. Even in the presence of emulsifying agents, comminuted solid alkanes form large particles in an aqueous medium, which hinders the growth of micro-organisms. Miller and Johnson[234] suggest that solid alkanes be dissolved in liquid hydrocarbons which are not themselves assimilated by a given micro-organisms (or are assimilated to only a slight extent) and can serve as solvents. These workers grew *Candida intermedia* and *Candida lipolytica* on eicosane (C_{20}) and hexadecane (C_{16}) dissolved in pristane (2,6,10,14-tetramethylpentadecane) and noted a high yield of cells. However, the number of alkanes which can be investigated under these conditions is limited by their solubility in pristane.

Recently papers have also been published on the action of surface-active substances on micro-organisms and the study of the relation between the structure and functions of surface-active substances[236,237]. These investigations show that surface-active materials react with the cell wall, altering its permeability and giving rise to a loss of metabolically important components of protoplasm (various amino-acids and nucleic acid derivatives) as a result of the breakdown of osmotic equilibrium. Surface-active substances also influence the localisation of enzymes within the organism and the interaction between the enzymes. Depending on the pH of the medium, there is either an increase in the activity of enzymes in the presence of surface-active materials or their catalytic effect is suppressed.

As before, much attention is devoted to problems of the microbiological conversion of aromatic hydrocarbons[238,239] and also the growth of micro-organisms on gaseous hydrocarbons[240,241].

REFERENCES

1. M. D. Lopatik, Mikrobiologiya, 33, 236 (1964).
2. G. P. Slavnina, Mikrobiologiya, 32, 403 (1963).
3. J. Takahashi and Y. Kawabasta, K. Yamada, Agric. Biol. Chem., 29, 292 (1965).
4. N. D. Ierusalimskii, E. A. Andreeva, S. A. Lirova, and I. T. Ermakova, Prikl. Biokhimiya i Mikrobiol., 1, 601 (1965).
5. K. Kobyashi and K. Yamada, J. Ferment. Assoc. Japan, 21, 407 (1963).
6. V. N. Shapochnikov and I. G. Orlova, Mikrobiologiya, 34, 602 (1965).
7. A. Rieche, G. Hilgetag, A. Martini, M. Thonke, and M. Lorenz, Zb. Bacteriol., Parasitenkunde, Infektionskrankh. und Hyg., 118, 53 (1964).
8. N. B. Gradova and L. K. Kruchinina, Prikl. Biokhimiya i Mikrobiol., 3, 315 (1967).
9. S. A. Lirova, L. M. Glazunova, and N. D. Ierusalimskii, Prikl. Biokhimiya i Mikrobiol., 3, 413 (1967).
10. E. E. Birshtekher, "Neftyanaya Mikrobiologiya" (Petroleum Microbiology), Gostoptekhizdat, Moscow, 1957.
11. V. O. Tauson, "Energetika Zhiznennykh Protsessov" (Energy Relations in Vital Processes), Izd. Akad. Nauk SSSR, Moscow, 1952.
12. V. I. Bilai, N. M. Pidoplichko, V. S. Gutirya, A. S. Bukhalo, G. A. V'yun, P. M. Galich, E. Z. Koval', V. Ya. Masumyan, and O. O. Mil'ko, Mikrobiol. Zhur., 27, 3 (1965).
13. Wang Ta-ch'en and Wang Yu, [Journal name missing (Ed. of Translation)], 4, 532 (1964); Ref. Zhur. Khim., 3F569 (1966).

14. T. L. Miller and M. J. Johnson, Biotechnol. Bioengng., 8, 567 (1966).
15. M. Alexander and B. Lustigman, J. agric. and food chem., 14, 410 (1966).
16. T. Aida and K. Yamaguti, J. agic. chem. Soc. Japan, 40, 119 (1966).
17. T. A. Tauson, Mikrobiologiya, 8, 530 (1939).
18. M. S. Matveev, Khim. i Tekhnol. Topliv i Masel, 5, 34 (1966).
19. I. G. Nette, N. N. Grechushkina, and I. L. Rabotnova, Prikl. Biokhimiya i Mikrobiol., 1, 167 (1965).
20. R. W. Traxler, "Bituminous Materials", Interscience, New York–London–Sydney, 1964, Vol. 1, p. 323.
21. R. W. Traxler, J. Robinson, and D. Wetmore, J. appl. chem., 16, 266 (1966).
22. D. K. Kersten, Mikrobiologiya, 33, 31 (1964).
23. T. C. Stadman and U. A. Barker, J. Bacteriol., 6167 (1951).
24. A. McFerson, Chem. Engng. Progr., 61, 101 (1965).
25. J. W. Foster, J. Microbiol. and Serol., 28, 3 (1962).
26. W. J. Evans, J. Gen. Microb., 32, 2 (1963).
27. F. Just and W. Schabel, Die Brauerei, wiss. Beillage, 4, 95 (1961).
28. N. Fritsche, "V Mezhdunarodnyi Biokhimicheskii Kongress, 2 Sektsiya, Sbornik Referatov" (Fifth International Biochemical Congress, Section 2, Abstracts), Izd. Akad. Nauk SSSR, Moscow, 1961, p. 76.
29. L. D. Shturm and E. P. Rozanova, Mikrobiologiya, 32, 1103 (1963).
30. A. Champagnat, C. Vernet, B. Laine, and J. A. Filosa, Neftekhimiya, 3, 5 (1963).
31. A. Champagnat, Industrie, 17, 242 (1963).
32. L. A. Gorskaya and R. A. Rogacheva, Symposium, "Mikrobiologicheskii Sintez" (Microbiological Synthesis), 1967, No. 3, p. 6.
33. A. Champagnat, C. Vernet, B. Laine, and J. A. Filosa, "Microbiological Elimination of Paraffins for the Preparation of the Protein-Vitamin Concentrate" (Translated into Russian), Sixth International Congress on Petroleum, Frankfurt, 1963.
34. A. Champagnat, Sci. Americain, 213, 13 (1965).
35. L. Lefrancois, Inds. agric. et aliment., 6 (1964).
36. A. Champagnat and B. Laine, Bull. Assoc. franc., 163 (1964).
37. A. Champagnat, Rev. Inst. franc. petrole, 1372 (1962).
38. M. Grimberg, Seifen-Ole-Fette-Wachse, 92, 109 (1966).
39. J. Senez and E. Azoulay, Ann. Inst. Pasteur, 38, 868 (1960).
40. J. Senez and E. Azoulay, "Symposium on Marine Microbiology (Edited by C. C. Thomas), Springfield, 1963, p. 464.
41. E. Azoulay, P. Couchoud-Beaumont, and J. Senez, Ann. Inst. Pasteur, 107, 520 (1964).
42. E. Azoulay, Bull. soc. franc. physiol. véget., 2, 299 (1965).
43. A. P. Kryuchkova and R. N. Bravicheva, Symposium, "Mikrobiologicheskii Sintez" (Microbiological Synthesis), 1965, No. 3, p. 1.
44. M. G. Kolomiitseva, A. S. Skoropostizhnaya, G. K. Osipov, A. K. Zaporozhskaya, and A. K. Rudenko, "Mikrobiologicheskii Sintez" (Microbiological Synthesis), 1966, No. 13, p. 6.
45. I. N. Pozmogova and N. S. Kuryatov, Prikl. Biokhimiya i Mikrobiol., 2, 640 (1966).
46. A. N. Grigorian, N. D. Gulyaeva, and A. P. Kovalev, Symposium, "Mikrobiologicheskii Sintez" (Microbiological Synthesis), 1967, No. 5, p. 6.
47. E. I. Kvasnikov, D. M. Isakova, and V. T. Vaskivnyuk, Mikrobiologiya, 36, 932 (1967).
48. T. V. Finogenova, A. B. Lozinov, V. M. Belikov, I. T. Ermakova, L. N. Muntyan, and E. N. Safonova, Mikrobiologiya, 37, 38 (1968).
49. Z. P. Telegina, Mikrobiologiya, 36, 67 (1967).
50. R. Scheda, Branntweinwirtschaft, 106, 373 (1966).
51. E. W. Hansen and R. E. Kallio, Science, 125, 1198 (1957).
52. B. Magasalik, in "Regulyatsiya Kletochnogo Obmena" (Regulation of Cell Metabolism), Izd. Akad. Nauk SSSR, Moscow, 1962, p. 117.
53. V. N. Gershanovich, Vestnik Akad. Med. Nauk SSSR, 12, 74 (1963).
54. I. S. Popov, Trudy V Leningradskoi Mikologicheskoi Konferentsii" (Proceedings of the Fifth Leningrad Mycological Conference), Leningrad, 1960, p. 35.
55. A. S. Spirin, Vestnik Akad. Nauk SSSR, 4, 41 (1965).
56. E. M. Gubarev, "Osnovnye Protsessy Obmena Veshchestv u Mikrobov" (Fundamental Metabolic Processes in Microbes), Izd. Akad. Nauk SSSR, Moscow, 1961, p. 11.
57. N. D. Ierusalimskii and G. K. Skryabin, Izv. Akad. Nauk SSSR, Ser. Biol., 125 (1965).
58. R. E. Kallio and W. R. Finnerty, see Ref. 40, p. 769.
59. A. P. Kryuchkova and G. I. Vorob'eva, Mikrobiologiya, 32, 856 (1963).
60. N. T. Semushkina and N. I. Monakhova, Gidroliznaya i Lesokhim. Prom., 35 (1962).
61. G. P. Slavnina, Mikrobiologiya, 33, 851 (1964).
62. L. D. Shturm and E. P. Rozanova, Mikrobiologiya, 35, 138 (1966).
63. T. L. Miller and M. J. Johnson, Biotechnol. and Bioengng., 6, 299 (1964).
64. G. W. Fuhs, Arch. Microbiol., 39, 374 (1961).
65. J. W. Foster, "Doklad na Zasedanii Moskovskogo Otdeleniya Vsesoyuznogo Mikrobiologicheskogo Obshchestva pri Akademii Nauk SSSR, 1964" (Reports at the 1964 Session of the Moscow Division of the All-Union Microbiological Society at the USSR Academy of Sciences), Izd. Vses. Mikrobiol. Obshchestva, 1964, p. 41.
66. M. J. Johnson, Chem. Ind., 36, 272 (1964).
67. N. P. Elinov, Doctoral Thesis, 1963, Kirov State Institute for the Advanced Training of Physicians, Leningrad.
68. N. P. Elinov, "Trudy V Leningradskoi Mikologicheskoi Konferentsii" (Proceedings of the Fifth Leningrad Mycological Conference), Leningrad, 1960, p. 45.
69. V. Treccani, Progr. ind. microbiol., 4, 2 (1964).
70. N. P. Elinov, "Patogennye Drozhzhepodobyne Organizmy" (Pathogenic Yeast-like Organisms), Izd. Meditsina, Moscow, 1964, p. 59.
71. E. Schmidt, Unasylva, 7, 152 (1953).
72. R. Kautzmann and M. Lindenmann, "Die Hefen", Nurnberg, 1962, Vol. 2, Herausg. Dr. Ferdinand.
73. A. F. Wiley, F. M. Holderby, and K. W. Fries, "Food and Feed Yeast in the USA", 1943.
74. R. V. Givartovskii, "Pishchevye Drozhzhi i Ikh Primenenie" (Food Yeast and Its Applications), Pishchepromizdat, Moscow–Leningrad, 1960, p. 38.
75. A. M. Lazarev, E. A. Plevako, and P. N. Fisher, "Belkovye Pishchevye Drozhzhi" (High-Protein Food Yeast), Pishchepromizdat, Moscow, 1942, p. 27.
76. V. I. Sharkov and I. A. Belyavskii, "Proizvodstvo Pishchevykh Drozhzhei iz Drevesiny" (Manufacture of Feed Yeast from Wood), Leningrad, 1943, p. 76.

77. O. P. Molchanova, Symposium, "Materialy Konferentsii po Lechebnomu Pitaniyu" (Proceedings of the Conference on Therapeutic Diets), Izd. Meditsina, Moscow,, 1942, p. 41.
78. A. Zalit, Molochnaya Prom., 56 (1959).
79. Yu. I. Kremer and A. A. Shmidt, Voprosy Pitaniya, 32,(1961).
80. A. A. Stafeichuk, "Trudy Dnepropetrovskogo Instituta po Gidrolizu Rastitel'nykh Otkhodov Sel'skogo Khozyaistva" (Transactions of the Dnepropetrovsk Institute for the Hydrolysis of Agricultural Plant Waste), Sel'khozgiz, 1940, p. 287.
81. E. I. Medvedeva, R. B. Zaidenberg, S. Z. Khait, and Z. V. Levina, Prikl. Biokhimiya i Mikrobiol., 2, 372 (1966).
82. A. P. Kryuchkova and P. N. Fisher, Khim. Nauka i Prom., 2, 86 (1957).
83. R. J. Block and D. Bolling, "Amino-acid Composition of Proteins and Foods" (Translated into Russian), Inostr. Lit., Moscow, 1949, p. 216.
84. J. White, "Yeast Technology" (Translated into Russian), Pishchepromizdat, Moscow, 1957, p. 112.
85. C. Vernet, Ind. chim. belge, 30, 213 (1965).
86. O. P. Molchanova, "Osnovy Ratsional'nogo Pitaniya" (Fundamentals of Rational Nutrition), Medgiz, Moscow, 1958, p. 18.
87. I. Leopold, Ceskosl. biol., 3, 235 (1945).
88. H. J. Burker, in "Global Impacts of Applied Microbiology", New York–London–Sydney, 1964, p. 234.
89. E. A. Plevako and R. V. Givartovskii, "Tekhnologiya Drozhzhevogo Proizvodstva, (Technology of Yeast Production), Pishchepromizdat, Moscow, 1949, p. 56.
90. M. N. Meisel', Uspekhi Biol. Khim., 1, 390 (1950).
91. G. P. Slavnina, Trudy Vses. NII Geolograzved. Neft. Inst., 41, 308 (1964).
92. G. I. Vorob'eva, L. P. Samoilova, L. M. Chaplina, and M. L. Berkovich, Symposium, "Mikrobiologicheskii Sintez" (Microbiological Synthesis), 1965, No. 9, p. 1.
93. A. M. Malkov, "Tekhnologiya Khlebopekarnykh i Drozhzei " (Technology of Baker's Yeast and Feed Yeast), Pishchepromizdat, Moscow, 1962, p. 134.
94. M. Dixon and E. C. Webb, "Enzymes" (Translated into Russian), Izd. Mir., Moscow, 1966.
95. B. H. Olson and M. J. Johnson, J. Bacteriol., 57, 235 (1949).
96. I. N. Pozmogova, Prikl. Biokhimiya i Mikrobiol., 2, 276 (1966).
97. I. N. Pozmogova, Prikl. Biokhimiya i Mikrobiol., 1, 606 (1965).
98. N. V. Stepanova, Yu. M. Romanovskii and N. D. Ierusalimskii, Dokl. Akad. Nauk SSSR, 163, 1266 (1965).
99. N. D. Ierusalimskii, Mikrobiologiya, 30, 818 (1961).
100. I. N. Pozmogova, I. E. Lomova, and M. I. Lakoza, Prikl. Biokhimiya i Mikrobiol., 2, 505 (1966).
101. J. Malek and J. Ričica, Folia microbiologica, 10, 301 (1965).
102. L. Lefrancois, Inds. agric. et aliment, 61 (1964).
103. A. Toshinobu, "Recent Progress in Microbiology", Toronto University Press, 1963, p. 325.
104. E. L. Dulaney, "Recent Progress in Microbiology", Toronto University Press, 1963, p. 317.
105. Z. G. Rozumovskaya, Mikrobiologiya, 31, 172 (1962).
106. L. Nachev and R. Gesheva, Mikrobiologiya, 3, 739 (1964).
107. I. T. Ermakova, Symposium, "Mikrobiologicheskii Sintez" (Microbiological Synthesis), 1967, No. 3, p. 11.
108. S. F. Dronov, L. M. Lupova, and V. V. Belova, "Mikrobiologicheskii Sintez" (Microbiological Synthesis), 1967, No. 3, 4.
109. M. I. Verkhovtseva, E. L. Ruban, and N. N. Suvorov, Izv. Akad. Nauk SSSR, Ser. Biol., No. 3, 374 (1967).
110. Y. Imada, J. Takanashi, and K. Yamada, Biotechnol. and Bioengng., 9, 45 (1967).
111. A. M. Bezborodov, S. S. Urusova, D. N. Chermenskii, and L. M. Shul'ts, Mikrobiologiya, 32, 385 (1963).
112. J. Senez, see Ref. 88, p. 363.
113. K. Yamada, J. Takahashi, and K. Kobyashi, Agric. and Biol. Chem., 27, 773 (1963).
114. A. P. Sims and B. F. Folkes, Proc. Poy. Soc., B159, 479 (1964).
115. A. P. Kryuchkova, G. I. Vorob'eva, and L. M. Bobyr', Prikl. Biokhimiya i Mikrobiol., 1, 78 (1965).
116. Y. Imada, O. Takahashi, K. Yamada, K. Uchida, and K. Aida, Agric. and Biol. Chem., 30, 487 (1966).
117. N. Brot and H. Weissbach, J. Biol. Chem., 240, 3064 (1965).
118. H. Huang, Progress in industr. microbiol., 5, 56 (1966).
119. K. Arima, S. Ogino, K. Yano, and D. Tamura, Agric. and Biol. Chem., 29, 1004 (1965).
120. V. M. Belikov, S. V. Gordienko, and E. N. Safonova, Symposium, "Mikrobiologicheskii Sintez" (Microbiological Synthesis), 1965, No. 10, 2.
121. N. M. Emanuel', E. T. Denisov, and Z. K. Maizus, "Tsepnye Reaktsii Okisleniya Uglevodorodov v Zhidkoi Faze" (Liquid-Phase Chain Oxidation Reactions of Hydrocarbons), Izd. Akad. Nauk SSSR, Moscow, 1965.
122. I. V. Berezin, E. T. Denisov, and N. M. Emanuel', "Okislenie Tsiklogeksana" (Oxidation of Cyclohexane), Izd. Moskov. Gos. Univ., Moscow, 1962.
123. C. de Duve, "Structural Components of the Cell" (Translated into Russian), Inostr. Lit., Moscow, 1962, p. 65.
124. C. de Duve, "The Structure and Function of the Cell" (Translated into Russian), Inostr. Lit., Moscow, 1964, p. 18.
125. A. G. Sereda, Candidate's Thesis, Institute of Biochemistry of the Academy of Sciences of the Ukrainian SSR, Kiev, 1964.
126. Yu. N. Karasevich, Mikrobiologiya, 31, 2 (1962).
127. K. V. Kosikov, Dokl. Akad. Nauk SSSR, 80, 105 (1951).
128. A. I. Oparin, N. S. Gel'man, and I. G. Zhukova, Dokl. Akad. Nauk SSSR, 99, 593 (1954).
129. J. C. Senez, see Ref. 65, p. 67.
130. I. O. Harris, Arch. Biochem. Biophys., 457, 70 (1957).
131. T. A. Pederson, Physiologia plantarum, 16, 151 (1963).
132. E. Azoulay and J. C. Senez, Ann. Inst. Pasteur, 107, 520 (1964).
133. E. Azoulay, J. Chentean, and J. Davidovics, Biochim. Biophys. acta, 77, 554 (1963).
134. R. L. Schuman, M. A. Farrell, and R. W. Stone, J. Bacteriol., 45 (1943).
135. J. Senez and E. Azoulay, Biochim. biophys. acta, 47, 2 (1961).
136. A. D. Gololobov and N. K. Gracheva, Symposium, "Mikrobiologicheskii Sintez", (Microbiological Synthesis), 1966, No. 1, 5.
137. V. A. Yakovlev, "Kinetika Fermentativnogo Kataliza" (Kinetics of Enzymic Catalysis), Izd. Nauka, Moscow, 1965, p. 27.

138. O. R. Polyanovskii, in "Fermenty" (Enzymes), Izd. Akad. Nauk SSSR, Moscow, 1964, p. 56.
139. D. Koshland, in "Horizons in Biochemistry" (Translated into Russian), Izd. Mir., Moscow, 1964, p. 83.
140. A. E. Braunshtein, M. Ya. Karpeiskii, and R. M. Khomutov, see Ref. 138, p. 142.
141. A. A. Balandin, Biokhimiya, 23, 655 (1958).
142. A. A. Balandin, Uspekhi Khim., 31, 1265 (1962) [Russ. Chem. Rev., No. 11 (1962)].
143. V. L. Kretovich, Izv. Akad. Nauk SSSR, Ser. Biol., 525 (1961).
144. V. L. Kretovich, Biokhimiya, 23, 335 (1958).
145. A. A. Balandin, Biokhimiya, 23, 475 (1958).
146. K. Laidler, "The Chemical Kinetics of Enzyme Action", Oxford, 1958.
147. K. Laidler, Trans. Faraday Soc., 51, 550 (1955).
148. M. J. Johnson, Chem. Ind., 36 (1964).
149. A. P. Kryuchkova and G. I. Vorob'eva, Gidroliznaya i Lesokhim. Prom., 86 (1964).
150. R. W. Traxler, Yale Scient. Mag., 38, 18 (1938).
151. M. V. Pomortseva and K. A. Solov'eva, Mikrobiologiya, 34, 598 (1965).
152. T. V. Finogenova, V. M. Belikov, I. T. Ermakova, and L. M. Muntyan, Prikl. Biokhimiya i Mikrobiol., 2, 156 (1966).
153. N. V. Pomortseva and K. A. Solov'eva, Mikrobiologiya, 34, 598 (1965).
154. R. J. Ertola and M. D. Lilly, Biotechnol. and Bioengng., 7, 309 (1965).
155. J. E. Stewart, W. R. Finnerty, and R. E. Kallio, Science, 132, 3475 (1960).
156. L. Canonica, Riwista Italiana delle sostanze grasse, 41, 505 (1964).
157. J. E. Stewart and R. E. Kallio, J. Bacteriol., 78, 441 (1959).
158. J. W. Foster, Arch. Microbiol., 92, 35 (1960).
159. V. M. Solonin, Mikrobiol. Zhur., 28, 90 (1966).
160. A. C. van der Linden and G. J. E. Thijsse, "Advances in Enzymology and Related Subjects", Vol. 27, "Biochemistry", Interscience, New York–London–Sydney, 1965, p. 469.
161. R. Shaw, Nature, 209, 1369 (1966).
162. E. R. Leadbetter and J. W. Foster, Arch. Mickrobiol., 35, 92 (1960).
163. V. Treccani and L. Canonica, Ann. Mircobiol., 5, 162 (1953).
164. V. Treccani and L. Canonica, Atti Congr. int. Mircobiol., 1, 274 (1953).
165. V. Treccani, L. Canonica, and M. Girolamo, Ann. Mircobiol., 6, 183 (1956).
166. E. R. Leadbetter and J. W. Foster, Bact. Proc., 118 (1958).
167. E. R. Leadbetter and J. W. Foster, Arch. Microbiol., 30, 91 (1958).
168. E. R. Leadbetter and J. W. Foster, Arch. biochem. biophys., 82, 491 (1959).
169. E. R. Leadbetter and J. W. Foster, Arch. Microbiol., 35, 92 (1960).
170. E. Zajic, Developments in industrial microbiology, 6, 16 (1965).
171. D. Schissler, J. Bact., 78, 441 (1959).
172. J. E. Stewart and R. E. Kallio, Bact., Proc., 118 (1959).
173. R. E. Kallio and W. R. Finnerty, see Ref. 40, p. 149.
174. W. R. Finnerty and R. W. Kallio, J. Bacteriol., 87, 1261 (1964).
175. R. W. Hansen and R. E. Kallio, Science, 125, 1198 (1957).
176. E. R. Leadbetter and J. W. Foster, Nature, 184, 1498 (1959).
177. F. M. Muller, A. van Leenwenhock J. Microbiol. and Serol., 23, 369 (1957).
178. D. M. Updegraff and G. B. Wren, Appl. Microbiol., 2, 309 (1954).
179. V. A. Kuznetsova and V. M. Gorlenko, Prikl. Biokhimiya i Mikrobiol., 1, 623 (1965).
180. A. S. Kester and J. W. Foster, J. Bacteriol., 85, 4 (1963).
181. G. J. E. Thijsse, Biochim. biophys. acta, 84, 2 (1964).
182. L. D. Shturm, Mikrobiologiya, 27, 783 (1958).
183. R. Huybregste and A. C. van der Linden, A. van Leenwenhock J. Microbiol. and Serol., 30, 185 (1964).
184. J. Bruyn, Meded. vlaamsche Acad., C57, 41 (1954).
185. J. W. Foster, A. van Leenwenhock J. Microbiol. and Serol., 28, 242 (1962).
186. R. Wieland, G. Griss, and B. Haccins, Arch. Microbiol., 28, 383 (1958).
187. D. Ooyama and J. W. Foster, A. van Leenwenhock J. Microbiol. and Serol., 31, 45 (1965).
188. B. Imelik, Compt. rend., 226, 2082 (1948).
189. C. Colla and V. Treccani, Ann. Microbiol., 10, 77 (1960).
190. H. H. Tabak, C. W. Chambers, and P. W. Kabler, J. Bacteriol., 87, 910 (1964).
191. K. Yamada and D. Takahashi, Agric. and biolog. chem., 28, 943 (1965).
192. C. Arnaudi, L. Canonica, and V. Treccani, Ricerca scient., 25, 3244 (1955).
193. S. Dagley, P. J. Chapman, D. T. Gibson, and J. M. Wood, Nature, 202, 7775 (1964).
194. R. Wieland, G. Griss, and B. Haccins, Arch. Microbiol., 28, 383 (1958).
195. E. K. Marr and R. W. Stone, Bact. Proc., 123 (1958).
196. V. Treccani and B. Bianchi, Atti Congr. Naz. Microbiol. IX, Biochem. J., 41, 373 (1959).
197. N. Walker and G. H. Wiltshire, J. Gen. Microbiol., 8, 273 (1953).
198. M. Malesset-Bras and E. Azoulay, Ann. Inst. Pasteru, 109, 894 (1965).
199. M. Malesset-Bras and E. Azoulay, Ann. Inst. Pasteru, 109, 106 (1965).
200. R. M. Nalbandyan, A. F. Vanin, and L. A. Blyumenfel'd, "Trudy Moskov. Obshch. Ispyt. Prirody, Otd. Biol., 16, 187 (1966).
201. T. C. Hollocher and M. M. Weber, J. Biol. Chem., 240, 1783 (1965).
202. Yu. M. Petrusevich and A. G. Konoplyannikov, "Trudy Moskov. Obshch. Ispyt. Prirody, Otd. Biol., 16, 59 (1966).
203. M. M. Emanuel', "Trudy VIII Mezhdunarodnogo Protivorakovogo Kongressa" (Proceedings of the Eighth International Anti-Cancer Congress), Medgiz, Moscow, 1963, Vol. II, p. 117.
204. N. M. Emanuel' and L. P. Lipchina, Dokl. Akad. Nauk SSSR, 121, 141 (1958).
205. N. M. Emanuel' and L. P. Lipchina, Dokl. Akad. Nauk SSSR, 124, 1157 (1959).
206. N. M. Emanuel' and L. P. Lipchina, Dokl. Akad. Nauk SSSR, 130, 221 (1960).
207. V. O. Tauson, "Osnovnye Polozheniya Rastitel'noi Bioenergetiki" (Fundamental Postulates of Plant Bioenergetics), Izd. Akad. Nauk SSSR, Moscow, 1950, p. 443.
208. D. Koshland, Adv. Enzymol., 22, 45 (1960).
209. N. S. Gel'man, Doctoral Thesis, Bakh Institute of Biochemistry, USSR Academy of Sciences, Moscow, 1963.

210. M. N. Meisel', Trudy Inst. Mikrobiol. Akad. Nauk SSSR, 6, 185 (1959).
211. A. Rotstein, "Trudy V Mezhdunarodogo Biologicheskogo Kongressa, Symposium II" (Proceedings of the Fifth International Biological Congress, Symposium II), Izd. Akad. Nauk SSSR, Moscow, 1961, p. 243.
212. L. E. Ruban, Izv. Akad. Nauk SSSR, Ser. Biol., 461 (1962).
213. F. Sikevich, "O Znachenii Vnutrikletochnoi Strukturi dlya Regulyatsii Obmena" (The Importance of the Intracellular Structure for the Regulation of Metabolism), Izd. Akad. Nauk SSSR, Moscow, 1962.
214. M. V. Fedorov, Izv. Akad. Nauk SSSR, Ser. Biol., 112 (1957).
215. D. R. Biggs and A. W. Limane, Biochim. biophys. acta, 78, 785 (1963).
216. A. W. Limane and J. L. Still, Biochim. biophys. acta, 16, 305 (1955).
217. P. Mitchell, Nature, 180, 134 (1957).
218. S. S. Stroev, Trudy Leningrad. Khim.-Farm. Inst., 18, 109 (1965).
219. M. Okuda and H. Takada, J. Biol. Osaka City Univ., 14, 141 (1963).
220. R. V. Kucher, A. I. Yurzhenko, and M. A. Kovbuz, Dokl. Akad. Nauk SSSR, 117, 638 (1957).
221. R. V. Kucher, A. I. Yurzhenko, and M. A. Kovbuz, Symposium, "Okislenie Uglevodorodov v Zhidkoi Faze" (Liquid-Phase Oxidation of Hydrocarbons), Izd. Akad. Nauk SSSR, Moscow, 1959, p. 212.
222. R. V. Kucher, S. D. Kaz'min, and A. I. Yurzhenko, Sbornik Rabot Inst. Fiziko-organicheskoi Khimii Akad. Nauk Beloruss. SSR, 8, 132 (1960).
223. R. V. Kucher and M. A. Kovbuz, Zhur. Fiz. Khim., 33, 429 (1959) [Russ. J. Phys. Chem., see Russ. J.
224. Phys. Chem., Abs., Vol. 33, Nos. 1–6 (1959)].
R. V. Kucher, M. A. Kovboz, E. M. Bugrova, and I. M. Vasil'kevich, Zhur. Prikl. Khim., 35, 170 (1962).
225. R. V. Kucher and S. D. Kaz'min, Neftekhimiya, 3, 371 (1963).
226. A. V. Pasynskii, "Biofizicheskaya Khimiya" (Biophysical Chemistry), Moscow, 1963.
227. J. W. McBain, "Solubilisation in Detergent Action", 1942.
228. J. P. Hennesy, J. Inst. Brew., 70, 337 (1964).
229. A. G. Pasynskii, Uspekhi Sovrem. Biol., 43, 1263 (1957).
230. "O Pervom Mezhdunarodnom Simpoziume v Italii, 1960" (The First International Symposium on Microbiology in Italy, 1960; Mikrobiologiya, 30, 371 (1961).
231. "Konferentsiya po Prikladnoi Mikrobiologii, Stokgol'm, 1963" (Conference on Applied Microbiology, Stockholm, 1963), Mikrobiologiya, 32, 1105 (1963).
232. "IX Mezhdunarodnyi Kongress po Mikrobiologii, Moskva, 1966, Tezisy Dokladov", (Ninth International Congress on Microbiology, Moscow, 1966. Abstracts of Reports), Izd. Meditsina, Moscow, 1966.
233. M. V. Ivanov, G. A. Mogilevskii, and G. P. Slavnina, Izv. Akad. Nauk SSR, Ser. Geol., 5, 799 (1965).
234. T. Miller and M. Johnson, Biotechnol. and bioengng., 8, 549 (1966).
235. E. Z. Koval', V. Ya. Masumyan, and A. A. Grushevs'ka Mikrobiol. Zhur., 30, 309 (1968).
236. A. Obajasi, J. agric. chem. Soc. Japan, 40, 53 (1966).
237. S. S. Stroev and N. P. Elinov, Mikrobiologiya, 36, 451 (1967).
238. E. M. Yurovskaya, L. E. Botvinova, and L. F. Erusalimskaya, Mikrobiologiya, 37, 655 (1968).
239. D. Gibson, J. Wood, P. Chapman, and S. Dagley, Biotechnol. and bioengng., 9, 33 (1967).
240. P. S. Vary and M. J. Johnson, Appl. microbiol., 15, 1473 (1967).
241. E. I. Kvasnikov, Yu. R. Malashenko, V. A. Romanovskaya, and E. F. Zapotyl'ko, Mikrobiologiya, 37, 662 (1968).

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