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Selective oxidation of gaseous hydrocarbons by bacterial cells

G A Kovalenko

Contents

I.	Introduction	625
II.	Comparative characteristics of the direct selective oxidation reactions of gaseous hydrocarbons	626
III.	Microorganisms as biocatalysts of the direct partial oxidation of gaseous hydrocarbons	626
IV.	Microbiological epoxidation of lower alkenes	632
V.	Microbiological hydroxylation of gaseous alkanes	635
VI.	Conclusion	638

Abstract. Studies on the direct selective oxidation of gaseous C_1-C_4 alkanes and C_2-C_4 alkenes to valuable oxygen-containing products (alcohols, methyl ketones, and epoxides) with the aid of the bacterial cells of gas-assimilating microorganisms are surveyed. The resting bacterial cells of the microorganisms grown on gaseous hydrocarbons possess an enzymic monooxygenase activity: they promote the insertion of an oxygen atom into the hydrocarbon molecule with formation of an oxygen-containing product. This process is effected under very mild conditions and is characterised by high selectivity and stereospecificity. It proceeds without the formation of toxic by-products. Certain problems of the practical implementation of biocatalytic oxidation processes, namely the regeneration of the monooxygenase cofactor, the reduction of the toxic effect of the oxygen-containing product formed, as well as the immobilisation of the cells on solid carriers in order to obtain an active and stable heterogeneous biocatalyst for the direct partial oxidation of hydrocarbons are considered. The bibliography includes 111 references.

I. Introduction

There has been a considerable growth of interest in recent years in the preparation of chemical compounds (the products of large scale organic synthesis) by biotechnological and biocatalytic methods. Environmentally pure processes of this kind, entailing a low energy consumption, constitute a real alternative to chemical synthesis and they are therefore being vigorously investigated and developed in all the industrially developed countries. According to certain predictions,[1] 10%–20% of chemical products will be produced by a biotechnological procedure towards the end of the 1990s. The biochemical process involving the direct selective oxidation of saturated and unsaturated hydrocarbons to valuable oxygen-containing products (chiral epoxides, alcohols, methyl ketones, and dicarboxylic acids) is particularly interesting.

G A Kovalenko Boreskov Institute of Catalysis, Siberian Branch of the Russian Academy of Sciences,
Prosp. akad. Lavrent'eva 5, 630090 Novosibirsk, Russian Federation.
Fax (7-383) 235 57 66. Tel. (7-383) 235 77 89

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The oxidative biotransformation of organic compounds, including hydrocarbon raw materials, occupies one of the leading places among industrial biocatalytic processes. Specific investigations in this field are being undertaken by large chemical companies in Japan (Nippon Mining Co., Nito Chem. Ind.), America (Exxon, Dow Chem., Monsanto, Du Pont), and Western Europe (Hoechst, Bayer, BASF), and also by Western European Universities (Wageningen Agricultural University, the Netherlands). The synthesis of the optical isomers of epoxides derived from unsaturated hydrocarbons (the properties of 31 epoxides have been described), carried out by the Japanese company Nippon Mining Co. on a semi-industrial scale using suspensions of microorganisms of the genus *Nocardia*, is of greatest interest.[2,3] In the microbiological epoxidation of moderately long chains, the yield of the (*R*)-1,2-epoxides is 80% with an optical purity of 80%–90%.[2,3] The optically active epoxides obtained are used in fine organic synthesis for the production of medicinal drugs, biologically active substances, and liquid crystals for electronics. Expert estimates have shown that the biotechnological methods of synthesis of the optical isomers of the epoxides may compete with the chemical methods. For example, the annual profit from the sale of the 'biotechnological' epoxypropane may reach 2–3 billion U.S. dollars.[1]

The present review considers the biocatalytic approaches to the solution of the problems of the direct partial oxidation of gaseous saturated and unsaturated hydrocarbons (methane, ethane, ethene, propane, propene, butane, butene) to valuable oxygen-containing products (epoxides, alcohols, and methyl ketones). Bacterial cells possessing the required enzymic oxygenase activity are used as biocatalysts of these processes in the immobilised state and also in the form of suspensions of resting (not growing) microorganisms. The characteristic features of the microorganisms employing gaseous hydrocarbons for their growth and the intracellular enzyme systems catalysing the oxidative biotransformation reactions of gaseous hydrocarbons will be briefly described in this review. Particular attention will be devoted to heterogeneous biocatalysts based on immobilised bacterial cells for the oxidative biotransformation of propene into 1,2-epoxypropane.

II. Comparative characteristics of the direct selective oxidation reactions of gaseous hydrocarbons

The processes involving the selective oxidation of light gaseous hydrocarbons to valuable oxygen-containing products constitute the key stages in the chemical processing of gas and petroleum. Some of these processes, in particular the direct selective epoxidation of ethene, have been implemented on an industrial scale, whilst others, for example the direct partial oxidation of gaseous alkanes (methane, ethane, and propane) to valuable oxygen-containing products (alcohols and aldehydes) are at the stage of intense laboratory research. Analysis of the literature data on the selective oxidation of C_1-C_4 hydrocarbons has shown that the heterogeneous oxide catalysts widely used in these processes have fairly limited possibilities: the maximum selectivity in the formation of the target product (80%–95%) is attained only for low degrees of conversion of the initial hydrocarbon (not more than 5%).[4–6] In order to attain a selectivity up to 70% for a conversion greater than 5%, either nitrous oxide is employed as the oxidant[5,7] or use is made of catalysts containing precious metals (gold, palladium, and ruthenium).[8] The literature data on the direct selective oxidation of ethane and propane to alcohols, aldehydes, or methyl ketones are very fragmentary and few in number. The main product of the oxidation of ethane is methanol mixed with ethanol,[5] while the oxidation of propane gives rise predominantly to acrylic acid.[9,10]

Biocatalytic processes have attracted the attention of investigators because of their unique features: low energy consumption due to the mild reaction conditions (temperature not exceeding 40 °C), a nearly 100% selectivity regardless of the degree of conversion of the initial substrate, as well as stereospecificity ensuring the formation of chiral compounds. Furthermore, owing to the absence of toxic by-products, biocatalytic processes are environmentally-friendly.

The data presented in Tables 1 and 2 demonstrate the possibilities of biocatalysis in relation to the epoxidation of propene and the hydroxylation of methane by gaseous oxygen as examples. In terms of their principal parameters (activity, yield of products), biocatalysts are at the level of the best heterogeneous chemical catalysts or are superior. The prospects for the oxidative biotransformation of hydrocarbons into valuable oxygen-containing products have been analysed in reviews[19–21] and the possibility of the practical implementation of such a process under chemical industrial conditions has been considered. The only significant disadvantage of biotechnological processes is the low concentration of the final products in the reaction medium (as a rule an aqueous medium). It does not exceed several tens of millimoles per litre, which gives rise to problems in the isolation of the target substances. Similar problems exist also in heterogeneous catalysts for low degrees of conversion of the initial compound. They can be solved, for example, by selecting a suitable reactor design or by employing a two-phase reaction medium for the extraction and concentration of the final oxygen-containing product.

Thus biocatalytic processes exhibit a high selectivity, ensure the possibility of obtaining the stereoisomers of chemical compounds, require a small energy consumption, and are relatively environmentally clean. They are therefore competitive with the chemical processes in basic organic synthesis.

Table 1. Comparative characteristics of the processes involving the direct partial oxidation of propene to 1,2-epoxypropane by molecular oxygen.

Characteristic	Chemical heterogeneous process[11]	Biocatalytic process[12–14]
Temperature / °C	450–550	20–40
Conversion of propene (%)	not above 15	40–80
Selectivity with respect to epoxypropane (%)	25	100
Stereospecificity	Racemic mixture	80%–90% of (*R*)-1,2-epoxypropane
Toxic side products	aldehydes, carbon monoxide, aromatic compounds	absent

Table 2. Comparative characteristics of the processes involving the direct partial oxidation of methane to methanol.

Parameter	Chemical process		Biocatalytic process[17,18]
	homogeneous[a]	heterogeneous[b]	
Temperatures / °C	450–550	250–350	20–40
Pressure / atm	25–65	1	1
Conversion of methane per pass (%)	9–13	4–6	27–61
Selectivity with respect to methanol (%)	38–83	83–96	~ 100
Yield with respect to methanol (%)	2–7	3–6	10–60
Productivity with respect to methanol / mmol g^{-1} h^{-1}		0.001–1.7	1–7

[a] According to data in Refs 5, 6, and 15. [b] According to data in Refs 5, 6, and 16.

III. Microorganisms as biocatalysts of the direct partial oxidation of gaseous hydrocarbons

Evidently the first stage in the development of any biotechnological process is screening for microorganisms possessing the necessary enzymic activity. The partial oxidation of saturated and unsaturated gaseous C_1-C_4 hydrocarbons is effected predominantly by gas-assimilating microorganisms. There are only isolated examples where bacterial cells grown on liquid C_5 and higher hydrocarbons oxidise gaseous substrates. For example, suspensions of the bacterial cells of the microorganism *Nocardia*, grown on isoprene, are capable of effectively oxidising propene and butene to the corresponding epoxides.[22] Other microorganisms employing C_5-C_{18} hydrocarbons for their growth do not oxidise gaseous alkanes and alkenes.

The first gas-assimilating bacterium, using methane as the sole source of carbon for its growth, was isolated in its pure form in 1906 and up to 1954 only five species of such bacteria, called methanotrophs, had been described.[23,24] A profound conviction that microorganisms capable of assimilating chemically inert compounds such as saturated and gaseous hydrocarbons are widely distributed in nature has arisen recently. About 200 strains of methanotrophs have been isolated from their habitats:[25,26] fresh- and salt-water reservoirs, subterranean and waste waters, muds, soils, coal mines, and even from the rumen of ruminants.[23] Soil microorganisms of the genus *Rhodococcus* utilise propane and butane as a source of carbon.[27]

Gas-assimilating microorganisms can be divided into the following groups:

(1) methanotrophs (the principal representatives belonging to the genera *Methylococcus*, *Methylosinus*, and *Methylomonas*) which are highly specialised microorganisms using methane as the sole source of carbon and energy; glucose and other organic compounds inhibit completely their growth;

(2) alkane-assimilating microorganisms (the principal representatives belong to the genera *Rhodococcus*, *Mycobacteria*, *Nocardia*, and *Xantobacter*), which utilise ethane, propane, and

butane are virtually incapable of growing on methane; glucose and certain organic acids support their growth;

(3) alkene-assimilating microorganisms (the principal representatives belong to the genera *Mycobacterium*, *Xantobacter*, and *Nocardia*), which grow on gaseous alkenes (ethene, propene, butene, and butadiene); they are also capable of utilising glucose.

The characteristic differences between the last two groups of microorganisms grown on gaseous hydrocarbons (especially on alkanes) and the same microorganisms grown on glucose or organic acids is the relatively high content of lipids in the cell and the structure of the cell wall, which is a strongly lipophilic formation.[28] The cells of *Mycobacterium vaccae* may serve as a typical example: such cells, grown on ethane (propane or n-butane), contain twice as much lipid as the cells grown on acetate (propionate).[29] These cells have another interesting feature: when acetate is replaced as the growth substrate by propane, the synthesis of lipids precedes the synthesis of nitrogen-containing compounds (proteins) and cell division, which indicates the important role of lipoid materials in the assimilation by the cells of the hydrophobic hydrocarbon substrates necessary for their growth.[29] It has now been demonstrated that passive diffusion of the molecules towards the cell and their sorption on the cell wall take place in the initial stage of the assimilation of the hydrocarbon by the microorganisms.[27,30] A metabolic process involving active (with an expenditure of energy) transport of the hydrocarbon to the cell, where it is oxidised intracellularly on membrane structures (microsomes), takes place in the next stage.[27,28] In *Rhodococcus* sp. grown on propane, an excessive development of the outer membrane of the cell wall is observed and a well developed network of intracellular membrane structures on the periphery of the cells is clearly manifested.[27]

All the processes involved in the oxidative microbiological transformation of hydrocarbons proceed with participation of molecular oxygen and are effected by enzymic oxygenase complexes (monoxygenases), the structure and functional role of which in the cell metabolism have been widely described in the literature.[23,31-33] In the present review, we shall dwell briefly on the structure of the active centre of the key enzyme effecting the primary activation of the gaseous hydrocarbon and the subsequent incorporation into the molecule of one oxygen atom with formation of an alcohol or epoxide. Such enzyme activity of the microorganisms belonging to the second and third groups is induced by the hydrocarbon in the course of cell growth[27,30] (cells then grow relatively slowly and the period for the doubling of the population amounts to tens of hours). The cells grown on glucose or organic acids as a rule do not possess such activity and are incapable of transforming hydrocarbons. The Japanese strain *Nocardia corallina* B-276, the resting cells of which (grown on glucose) are capable of epoxidising alkenes, is an exception.[34,35] However, the yield of the epoxide is then almost twice as small as in a medium with cells grown on propene.[34]

Depending on whether an alkane or an alkene is used as the growth substrate, enzyme complexes having different properties are induced and synthesised in the cell, namely an alkane monoxygenase or an alkene monoxygenase, which are responsible for the primary activation of the gaseous hydrocarbon.[22,36] The alkane monoxygenase has a broader substrate specificity than the alkene monoxygenase: it hydroxylates alkanes and epoxidises alkenes, whereas the alkene monoxygenase is only capable of epoxidising alkenes (but is incapable of hydroxylating alkanes). The microorganisms grown on alkenes are as a rule incapable of growing on alkanes. It is of interest that these two enzyme systems (or the cells of microorganisms with an induced enzyme activity) differ from one another also in their stereospecificity: the methane and alkane monoxygenases as a rule form during the epoxidation of C_3-C_4 alkenes a racemic mixture of optical isomers of the epoxides, whereas in the case where alkene monoxygenases are employed an excess (80%–90%) of the enantiomeric forms is observed. In both cases the configuration of the alkene (butene) is retained (Tables 3–7).[22,41] The Japanese strain *Nocardia corallina* B-276 has exceptional characteristics: regardless of the growth substrate employed (glucose, alkanes, and alkenes), one of the optical isomers of the epoxide is formed in excess within the cells of this microorganism.[2,3]

Two principal types of monoxygenase enzyme systems of microorganisms, capable of assimilating hydrocarbons, including gaseous ones, have now been described and characterised:

(1) monoxygenases containing an oxo- or hydroxo-bridged iron cluster in the active centre are present in the cells of methanotrophs (Fig. 1*a*);[32,33,62]

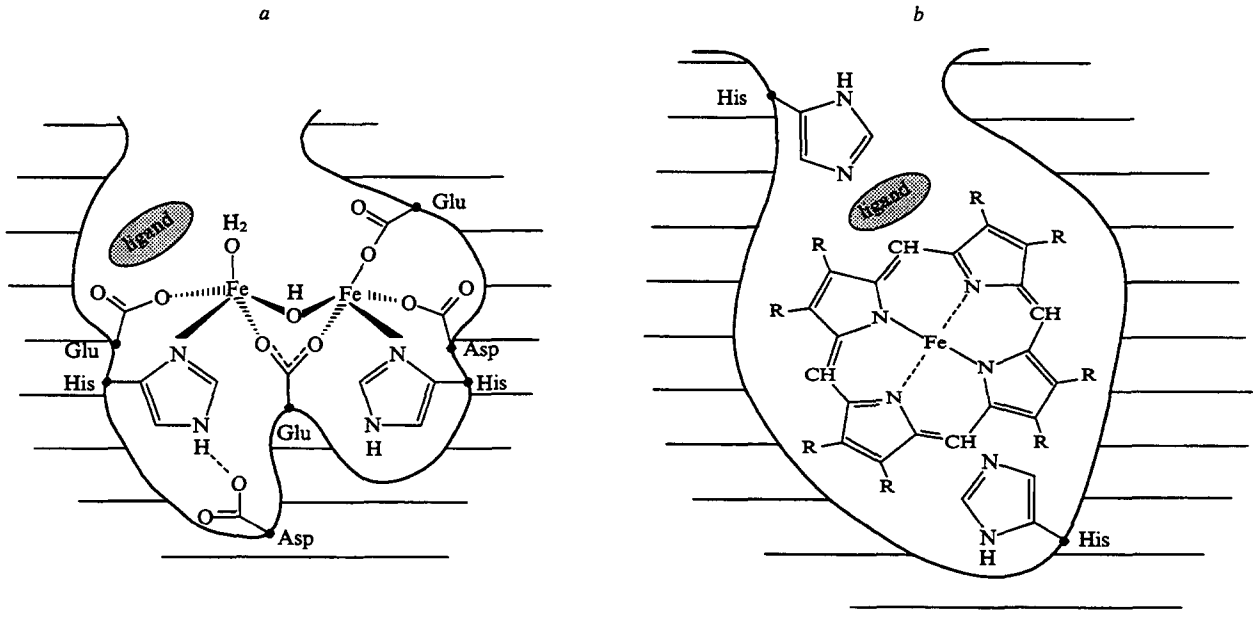


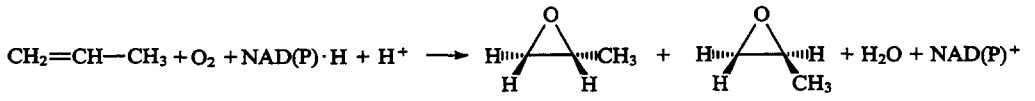
Figure 1. The active centres for the oxidation of hydrocarbon substrates by the monoxygenase of methanotrophs[33] (*a*) and certain other gas-assimilating microorganisms (*b*).
Notation: Asp = aspartic acid residue in the protein component of the monoxygenase; Glu = glutamic acid residue; His = histidine residue; R = hydrocarbon residue in the haem molecule; ligand = low molecular-mass compounds, including substrates (hydrocarbons, oxygen, and inhibitors).

Table 3. Partial oxidation of ethene to epoxyethane by gas-assimilating microorganisms.

Growth substrate	Microorganism (immobilisation method)	a^a	Ref.	Growth substrate	Microorganism (immobilisation method)	a^a	Ref.
Methane	Methanotroph H-2	93	37	Ethene	*Mycobacterium* 2W	4	12
	Methylococcus capsulatus (Bath)	33 [46]	12, 31, 38–40		*Mycobacterium* E44, 12D, Tu1	3	12
					Mycobacterium 32	1	12
	Methylosinus trichosporium OB3b	16 [16]	12, 31, 39–41	Propene	*Mycobacterium* Py1	50	12, 45–47
	Methylobacterium organophilum	[7.5]	31, 40		*Xantobacter* Py2	46	39, 46–48
Ethane	*Brevibacterium fuscum* ATCC 15993	17.5	42		*Mycobacterium* Py8	25	12
	Mycobacterium CRL-69	14	42		*Mycobacterium* Py1 (entrapment in alginate)	6	45
	Arthrobacter CRL-68	8	42		*Mycobacterium* Py1 (adsorption on sand)	5	45
Propane	*Alcaligenes* ATCC 15525	43	43				
	Pseudomonas fluorescens B-1244	28	43				
	Rhodococcus rhodochrous PNKb1	22	44	But-1(2)-ene	*Mycobacterium* By1	19	12
	Brevibacterium CRL-56	16	39, 43		*Xantobacter* By2	11	46
	Rhodococcus erythropolis 3/89	[7]			*Nocardia* By1	9	46
					Nocardia TB1	2	46
n-Butane	*Arthrobacter* CRL-70	38	42				
	Arthrobacter CRL-60	32	42	Buta-1,3-diene	*Nocardia* TB1	17	46
	Pseudomonas CRL-71	20	42		*Mycobacterium* B	11	12
	Mycobacterium E20	15	12				
				CO_2/NH_4	*Nitrosomonas europaea*		39

[a] The activity of the cell suspension expressed in nmol min^{-1} (mg protein)$^{-1}$ [nmol min^{-1} (mg dry cells)$^{-1}$] and determined as the rate of the extracellular accumulation of epoxyethane in the reaction medium. The maximum reaction rates quoted in the literature are presented.

Table 4. Partial oxidation of propene to 1,2-epoxypropane by gas-assimilating microorganisms.



(*R*)-1,2-epoxypropane (*S*)-1,2-epoxypropane

Growth substrate	Microorganisms	a^a	Enantiomeric composition of product (%)		Ref.
			(*R*)-isomer	(*S*)-isomer	
Methane	*Methylomonas* Z 201	[60]			49
	Methylococcus capsulatus	215 [46]	56	44	31, 39, 40, 41, 50–53
	Methylosinus trichosporium OB3b	175 [30]	57	43	22, 31, 39, 40, 41, 52–54
	Methanotroph H-2	100			37
	Methylosinus CRL	37			40, 55
	Methylobacterium organophilum	22 [21]			31, 40
	Methylomonas albus BG8		53	47	22, 40
	Methanomonas GY-J-3	21 [0.1]			52, 56
Ethane	*Mycobacterium* E20		47	53	14
	Arthrobacter CRL-60	33			42
	Mycobacterium rhodochrous	27			42
	Brevibacterium fusscum	20			42
Propane	*Pseudomonas fluorescens* B-1244	102			43
	Nocardia paraffinica	72			43
	Rhodococcus rhodochrous PNKb1	50			44
	Brevibacterium CRL-61	47			43
	Corynebacterium CRL-63	40			43
	Rhodococcus erythrolopis 3/89	[9]			57, 58

Table 4 (continued).

Growth substrate	Microorganisms	a[a]	Enantiomeric composition of product (%)		Ref.
			(*R*)-isomer	(*S*)-isomer	
Propane	*Rhodococcus* 1r	[1]			50
	Scedosporium A-4	[1]			59
	Nocardia carollina B-276	[0.5]	87	13	22, 34, 39
	Mycobacterium P2y		78	22	22
	Pseudomonas P9y		45	50	22, 39
n-Butane	*Arthrobacter* CRL-70	58			42
	Pseudomonas fluorescens B-1244	52			42
	Corynebacterium CRL63	40			42
	Mycobacterium E20	17			12
	Mycobacterium vaccae		56	44	22
	Nocardia TB1		68	32	22, 39
	Nocardia IP1		91	9	22, 39
Ethene	*Mycobacterium* E3	46 [2.5]	93	7	12, 14, 22, 39 46, 50, 60
	Mycobacterium 2W, Eul	11	93	7	12, 14, 22, 46, 60
	Nocardia carollina B-276	[0.5]	86	14	22, 34, 39
	Mycobacterium E20		88	12	14
	Mycobacterium aurum L1		99	1	22, 39
Propene	*Mycobacterium* Py1	9.5[b]			45
	Nocardia corallina B-276	[3.5]			61
	Mycobacterium Py2		97[b]	3[b]	14
trans-But-2-ene	*Mycobacterium vaccae*		61	39	22
	Nocardia TB1	2	60	40	22, 39, 46
Buta-1,3-diene	*Mycobacterium* B	21	93	7	12, 14
	Nocardia TB1	13	93	7	22, 46
	Nocardia IP1		90	10	22, 39
CO_2/NH_4	*Nitrosomonas europeae*	–	32	68	22, 39

[a] The activity of the resting cells expressed in nmol min^{-1} (mg protein)$^{-1}$ [mmol min^{-1} (mg dry cells)$^{-1}$] and determined as the rate of the extracellular accumulation of 1,2-epoxypropane in the reaction medium. The maximum reaction rates quoted in the literature are presented.
[b] In the presence of 10 mM 1,2-epoxybutane as the inhibitor of the further oxidation of the 1,2-epoxypropane formed.

Table 5. Partial oxidation of butene to 1,2-epoxybutane by gas-assimilating microorganisms.

$$CH_2{=}CH{-}CH_2{-}CH_3 + O_2 + NAD(P)\cdot H + H^+ \longrightarrow H_2C\overset{O}{\diagup\diagdown}CH{-}CH_2{-}CH_3 + H_2O + NAD(P)^+$$

Growth substrate	Microorganisms	a[a]	Enantiomeric composition of product (%)		Ref.
			(*R*)-isomer	(*S*)-isomer	
Methane	*Methylococcus capsulatus*	[11]			31, 40
	Methylobacterium organophilum	[8]			31, 40
	Methylosinus trichosporium OB3b	[4]	39	64	22, 31, 39, 40, 54
	Methanotroph H-2	64.5			37
Ethane	*Mycobacterium* E20, CRL-69	4	41	59	14, 42
	Brevibacterium fuscum	3			42
	Arthrobacter CRL-68	2			42

Table 5 (continued).

Growth substrate	Microorganisms	a[a]	Enantiomeric composition of product (%)		Ref.
			(*R*)-isomer	(*S*)-isomer	
Propane	*Nocardia paraffinica* NNRRL11326	67			43
	Rhodococcus rhodochrous PNKb1	26			44
	Pseudomonas crucaurae	15			43
	Pseudomonas P9y		45	50	22, 39
	Mycobacterium P2y		77	23	22
	Nocardia corallina B-276		84	16	22, 39
n-Butane	*Pseudomonas* CRL-71	32			42
	Mycobacterium rhodochrous	3			42
	Arthrobacter CRL-60	2			42
	Mycobacterium vaccae		58	42	22
	Nocardia TB1		69	31	22, 39
	Nocardia IP1		87	13	22, 39
Ethene	*Mycobacterium* E3, E4, Eu, 2D	15	84	16	12, 14, 22, 39, 46
	Mycobacterium E44, 2W	11	83	17	12, 14, 22, 46
	Mycobacterium E20		81	19	14
	Mycobacterium aurum L1		90	10	22, 39
	Nocardia corallina B-276		84	16	22, 39
Propene	*Mycobacterium* Py10, Py2		95[b]	5[b]	14
But-2-ene	*Mycobacterium vaccae*		64	36	22
	Nocardia TB1	2	63	37	22, 39, 46
Buta-1,3-diene	*Mycobacterium* B	17	89	11	12, 14
	Nocardia TB1	16	89	11	22, 46
	Nocardia IP1		95	5	22, 39
CO_2/NH_4	*Nitrosomonas europeae*		29	71	22, 39

[a] The activity of the resting cells is expressed in nmol min^{-1} (mg protein)$^{-1}$ [nmol min^{-1} (mg dry cells)$^{-1}$] and determined as the rate of the extracellular accumulation of 1,2-epoxybutane in the reaction medium. The maximum reaction rates quoted in the literature are presented.
[b] In the presence of 10 mM 1,2-epoxypropane as the inhibitor of the further oxidation of the 1,2-epoxybutane formed.

Table 6. Partial oxidation of *trans*-but-2-ene to *trans*-2,3-epoxybutane by gas-assimilating microorganisms.

$$CH_3{-}CH{=}CH{-}CH_3 + O_2 + NAD(P)\cdot H + H^+ \longrightarrow CH_3{-}\underset{\diagdown\,\diagup}{\overset{O}{CH}}{-}CH{-}CH_3 + H_2O + NAD(P)^+$$

Growth substrate	Microorganisms	a[a]	Enantiomeric composition of product (%)		Ref.
			(*R*)-isomer	(*S*)-isomer	
Methane	*Methylococcus capsulatus*		51	49	22, 39
	Methylomonas albus BG8		50	50	22
	Methylosinus trichosporium OB3b		50	50	22, 39
	Methylocystis parvus OBBP		52	48	22
	Methanotroph H-2	17.5			37
Propane	*Nocardia corallina* B-276		73	27	22, 39
n-Butane	*Nocardia* IP1		81	19	22, 39
Ethene	*Mycobacterium* E3	20	85	15	12, 14, 22, 39, 46
	Mycobacterium 2W	13	78	22	12, 14, 22, 46
	Mycobacterium NCIB 11626		79	21	22
	Mycobacterium aurum L1		87	13	22, 39
	Nocardia corallina B-276		80	20	22, 39

Table 6 (continued).

Growth substrate	Microorganisms	a^a	Enantiomeric composition of product (%)		Ref.
			(*R*)-isomer	(*S*)-isomer	
Propene	*Mycobacterium* Py8		93	7	22
	Xantobacter Py2	41	89	11	22, 39, 46
But-1-ene	*Xantobacter* By2	22	82	18	22, 46
	Nocardia By1	11	78	22	22, 39, 46
Buta-1,3-diene	*Nocardia* BT1	19	80	20	22, 46
	Nocardia IP1		81	19	22, 39

[a] The activity of resting cells expressed in nmol min^{-1} (mg protein)$^{-1}$ and determined as the rate of the extracellular accumulation of *trans*-2,3-epoxybutane in the reaction medium. The maximum reaction rates quoted in the literature are presented.

Table 7. Partial oxidation of buta-1,3-diene to 1,2-epoxybutene by gas-assimilating microorganisms.

$$CH_2{=}CH{-}CH{=}CH_2 + O_2 + NAD(P)\cdot H + H^+ \longrightarrow$$

$$\longrightarrow H_2C\!-\!\underset{\diagdown\,\diagup}{O}\!-\!CH{-}CH{=}CH_2 + H_2O + NAD(P)^+$$

Growth substrate	Microorganisms	a^a	Ref.
Methane	*Methylococcus capsulatus*	[37]	31
	Methylobacterium organophilum	[23]	31
	Methylosinus trichosporium OB3b[b]	[21]	31, 40, 54
	Methanotroph H-2	85.5	37
Ethane	*Brevibacterium fuscum* ATCC 159993	1	42
	Arthrobacter CRL-68	1	42
	Mycobacterium CRL-69	1	42
Propane	*Nocardia paraffinica* ATCC 21198	53	43
	Nocardia NRRL 11326	16	43
	Pseudomonas putida ATCC 17453	13	43
	Brevibacterium fuscum ATCC 159993	6	43
n-Butane	*Brevibacterium fuscum* ATCC 159993	3	42
	Mycobacterium rhodochrous	1	42
	Pseudomonas CRL-71	0.5	42
	Arthrobacter CRL-70	0.5	42

[a] The activity of resting cells expressed in nmol min^{-1} (mg protein)$^{-1}$ [nmol min^{-1} (mg dry cells)$^{-1}$] and determined as the rate of the extracellular accumulation of 1,2-epoxybutene in the reaction medium. The maximum reaction rates quoted in the literature are presented.
[b] The ratio (in %) (*R*)-isomer : (*S*)-isomer = 36 : 64.

(2) haem-containing monoxygenases of the type of cytochrome P-450 are present in the cells of *Nocardia*,[63] *Rhodococcus*,[63] *Mycobacterium*,[63] *Xantobacter*,[64] and *Corynebacterium*[65] (Fig. 1*b*).

It is noteworthy that the term 'monoxygenase' is applied not to an individual enzyme but to the whole enzyme system, which consists of at least two to three components performing different functions (the electron transport, regulation, and oxidative functions).[32] Fig. 1 illustrates the structure of the active centres of the enzyme performing the oxidative function, which consists in the incorporation of an oxygen atom into the hydrocarbon molecule with formation of an alcohol or an epoxide, while the other atom enters into a water molecule (Fig. 2).

The mechanism of the microbiological oxidation of hydrocarbons to alcohols, in particular of methane to methanol, has been thoroughly investigated. There are data showing that the rate-limiting stage of this process is the dissociation of a C–H bond.[25,32,33] The methane monoxygenase in different strains of methanotrophs has a broad substrate specificity: it hydroxylates C_1-C_8 alkanes with formation of primary and secondary alcohols,[32,66] epoxidises C_2-C_4 alkenes,[32,54] and oxidises a series or aromatic (benzene, toluene, and styrene) and alicyclic (cyclohexane) hydrocarbons.[32,67] The ability of methanotrophs to oxidise chloroalkenes (dichloroethene, trichloroethene, and vinyl chloride) with the aid of methane monoxygenase gives rise to prospects for their use in the purification of waste waters: in the first stage, methanotrophs oxidise the chlorinated alkene to the epoxide, which subsequently undergoes degradation with participation of the population of microorganisms, giving rise to nontoxic products — carbon dioxide and chloride ions.[68-70]

The so called cofactor (or coenzyme), performing the function of an electron donor, is consumed in the course of the bioxidation of hydrocarbons by a monoxygenase (Fig. 2). The natural cofactors for the monoxygenase are the reduced forms of high molecular-mass derivatives of nicotinamide [nicotinamide-adenine dinucleotides NADH or NAD(P)H].

When whole viable cells (and not cell-free enzyme extracts) are used for the oxidative biotransformations, the natural cofactors may be regenerated intracellularly as a result of the occurrence of consecutive enzyme reactions with participation of low molecular-mass substrates. For example, for the methane monoxygenase the effective electron donors are the intermediates products of methane metabolism, namely methanol,[71] formaldehyde,[41,55] and formate,[41,55] which are oxidised by intracellular enzymes with formation of the reduced form NAD(P)H. The latter is in its turn consumed by the methane monoxygenase on the oxidation of the hydrocarbon molecule (Fig. 2).[25,33]

It has been observed that in certain cases the addition of ethanol and ethyl acetate to the reaction medium prolongs the bioepoxidation of propene by mycobacteria. In addition, the rate of formation of epoxypropane increases in their presence.[60] Apparently these compounds participate in the regeneration of the natural cofactor of the monoxygenase in the above bacteria.

For *Rhodococcus* sp. it has not so far been possible to detect a low molecular-mass electron donor.[50] Glucose, added to a cell suspension of the Japanese strain *Nocardia corallina* B-276, promotes the regeneration of the monoxygenase cofactor.[35,72,73] Evidently the solution of the problem of the replacement of the expensive and virtually inaccessible natural cofactors by low molecular-mass substrates may render the oxidative biocatalysis based on resting cells promising from the standpoint of its practical application.

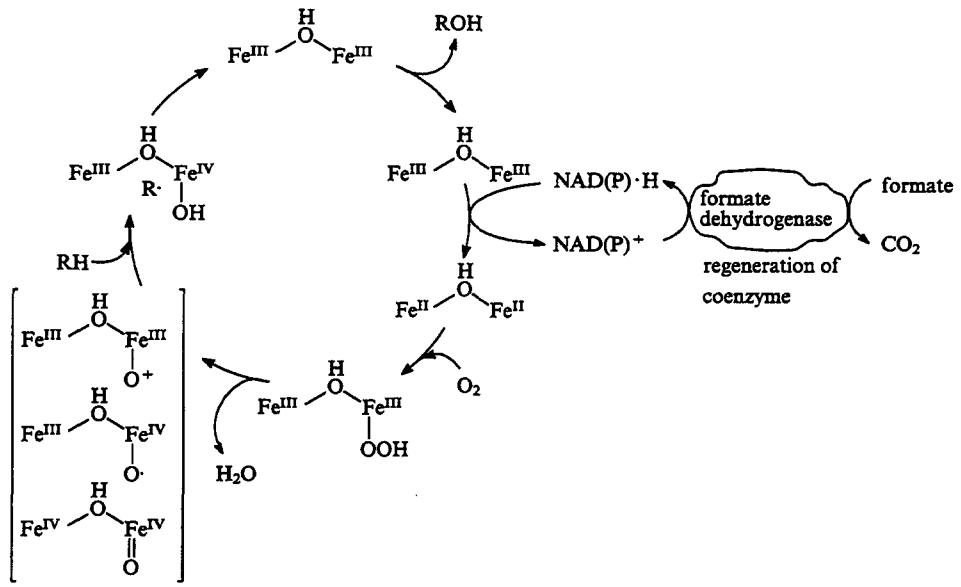


Figure 2. The mechanism of the hydroxylation of the alkanes RH by the monoxygenase of methanotrophs [33] and a possible pathway leading to the regeneration of the coenzyme NAD(P)H as a result of the consecutive enzymic reaction.

Thus the direct selective oxidation of gaseous hydrocarbons can be achieved by biocatalytic methods involving gas-assimilating microorganisms possessing a monoxygenase activity, the process being characterised by a high selectivity and stereospecificity and occurring under mild conditions without the formation of toxic side products (the only possible product of this kind is carbon dioxide).

IV. Microbiological epoxidation of lower alkenes

The microbiological epoxidation of lower alkenes was first achieved in 1963 when it was possible to obtain 1 mg of 1,2-epoxyoctane from oct-1-ene with the aid of *Pseudomonas aeruginosa* cells grown on heptane.[74] During the last decade, the studies in this field have been carried out by scientific teams from many countries (Table 8). As already noted, certain 'biotechnological' methods of synthesis of epoxides from alkenes have been implemented on a semi-industrial scale.[2,3] The epoxidation of gaseous alkenes is effected by microorganisms grown both on gaseous alkanes (methane, ethane, and propane) and on gaseous alkenes (ethene, propene, and butene) (Tables 3–7), the monooxygenase of the alkane-assimilating microorganisms having the ability not only to epoxidise alkenes but also to hydroxylate the terminal CH_3 group with formation of unsaturated alcohols.[78]

It is of interest to note that, in the reactions involving the oxidative biotransformation of alkenes effected by *Pseudomonas oleovorans*,[78] there is no relation whatever between the chemical reactivity of the bonds being oxidised and the oxidative activity of the microorganisms. For example, oct-2-ene is not epoxidised, whereas oct-1-ene is oxidised with formation of 1,2-epoxyoctane; propene is also not epoxidised, but is transformed into allyl alcohol.

The epoxides formed during oxidative biotransformation accumulate extracellularly in the reaction medium, provided that they are not intermediates in the metabolism of the main growth substrate but are formed by virtue of the relatively broad substrate specificity of the monoxygenase. For example, ethene-assimilating mycobacteria, which effectively oxidise propene and butene, effect the extracellular accumulation of their epoxides (Tables 4–6), whereas epoxyethane undergoes further metabolic transformations, which are necessary for cell growth and biosynthesis. In order to prevent the metabolic transformations of the epoxide formed on oxidation of the growth substrate, one may add an inhibitor to the medium. For example, the inhibitor of the further oxidation of 1,2-epoxypropane, formed by propene-assimilating cells, is the related 1,2-epoxybutane, which undergoes degradation, enabling the epoxypropane to accumulate in the medium.[45,79]

The epoxides accumulated in the medium have a distinctive toxic activity, since they are capable of alkylating protein molecules and of decomposing membranes (this permits their use as sterilising agents in medical practice, epoxyethane being the most toxic). The inhibition by epoxides is irreversible and specific in relation to monoxygenases, while other vitally important enzymes are only slightly inactivated.[79] Various microorganisms are unequally susceptible to the action of epoxides: the most stable are the alkene-utilising microorganisms of the genus *Mycobacterium* (they withstand a concentration up to 100 mM), while the

Table 8. Microbiological epoxidation of alkenes.

Alkenes epoxidised	Microorganisms	Research groups
C_2-C_4	Methanotrophs: *Methylococcus capsulatus*, *Methylosinus trichosporium*	C T Hou (EXXON Res. & Eng. Co., USA); H Dalton, J C Murrell (University of Warwick, UK)
C_2-C_4	*Mycobacterium* sp.	J A M de Bont (Agriculture University of Wageningen, The Netherlands)
C_2-C_{18}	*Nocardia corallina B-276*	K Furuhashi (Nippon Mining Co. Ltd, Japan)
C_2-C_3	*Caldariomyces fumago*, *Flavobacterium Cetus* sp.	S L Neidleman (CETUS Corp., USA)[75–77]
C_6-C_{12}	*Pseudomonas oleovorans*	M J de Smet (University of Groningen, The Netherlands)

most sensitive are the methanotrophs (the epoxide exerts a distinct toxic effect on the latter at a concentration less than 10 mM).[79]

The following approaches have been proposed for the practical solution of the problem of reducing the toxic effect of the epoxides and of the concentration and isolation of the valuable oxygen-containing product from the reaction medium: the immobilisation of the cells on/in solid carriers, as a result of which the resistance of the microorganisms to the action of the toxic reagents as a rule increases; the removal of the epoxide formed from the reaction zone, which is achieved either by its extraction into the solvent phase of a two-phase water–immiscible organic solvent system or by passing a gas stream with subsequent isolation of the target product by absorption in a suitable organic phase.

These approaches may be clearly illustrated in relation to the epoxidation of propene (Table 9). The gas-utilising microorganisms are immobilised mainly via their entrapment into

Table 9. Epoxidation of propene by the immobilised cells of gas-assimilating organisms.

Microorganism	Immobilisation method	Process characteristics		Bioreactor[b]	Ref.
		a[a]	other parameters		
Methanomonas GYJ-3	adsorption on sand, zeolite, aluminium oxide, polypropylene paraffin	0.25 (sand) 0.19 (polypropylene)	adsorption: 1.5 (sand), 3.4 mg g^{-1} (polypropylene)	G–S, flow type with stationary bed	56
	incorporation in alginate gel	1.2		L–S, periodic with stirring	80
Methylosinus CRL 31, *Methylococcus* CRLM1, *Methylosinus trichosporium* OB3b	adhesion in the form of a biofilm on porous glass	8–15	stability: 18 h of continuous operation (regeneration by methanol vapour after each 10 h); conversion of propene: 2.7%	G–S, flow type with stationary bed	31, 55, 71
Methylosinus trichosporium OB3b *Methylococcus capsulatus* M1	adhesion on glass beads, activated charcoal, silica gel		yield of product: 250–300 nmol min^{-1}; stability: 7 h of continuous operation	G–S	81
Rhodococcus Mycobacterium E3, *Methylosinus trichosporium*	adsorption on silica gel, carbon, or carbonsaturated aluminium oxide; double immobilisation in the pores of carbonsaturated aluminosilicate	10–20	adsorption, mg g^{-1}: 40 (rhodococci), 3 (methanotrophs) stability: after 14 h of continuous operation, 46% of initial activity is retained	L–S, flow type with stationary bed	50
Mycobacterium E3	lyotropic surfactant mesophase in the water–hexane twophase system	26.3			82
	incorporation in alginate gel	0.07	stability: 7 days; productivity: 0.03 g of (*R*)-epoxypropane per 1 g of cells after 2 days	L–S, flow type with recirculation and stationary bed in a two-phase system water–organic solvent	83, 85, 86, 89
	incorporation in alginate gel	2.3		G–L–S, flow type with fluidised bed	86
Mycobacterium E3, E4, 2W	incorporation in alginate and carrageenan	2.5	stability: 3 h of continuous operation	L–S, periodic with stirring	12, 87, 88
Mycobacterium Py1	on sand		productivity: 0.25 mmol after 4 days	G–S	89
Mycobacterium Eu1	adhesion on lava	2.6[c]	stability: 20 days of operation (16 cycles 15 h each); conversion of propene: 90%	G–S	60

Table 9 (continued).

Microorganism	Immobilisation method	Process characteristics		Bioreactor [b]	Ref.
		a [a]	other parameters		
Nocardia corallina B-276	emulsion in liquid paraffin	7.1	stability: 9 days of continuous operation, $t_{1/2}$ = 200 h; conversion of propene: 95%–100%; productivity: 2.3 g of epoxypropane per 1 g of cells	L–L, continuous bubbling of the gas stream	72
Nocardia corallina B-276	adsorption on polypropylene and polyethylene	5.0–6.8	adsorption: 1.0–2.8 mg g^{-1}; stability: 2–3 days of operation, $t_{1/2}$ = 36 h	G–S, flow type with stationary bed	13
	incorporation in alginate, carrageenan, and urethane matrix	0.9	stability: $t_{1/2}$ = 78 h	G–S, flow type with stationary bed	13
	incorporation in polyacrylamide gel	1.3–3.7	stability: 7 days without loss of activity	L–S, flow type	90

[a] Activity expressed in nmol min^{-1} (mg dry cells)$^{-1}$ and determined as the rate of the extracellular accumulation of epoxypropane in the reaction medium.
[b] Notation: G = gas phase; L = liquid phase; S = solid phase.
[c] The activity expressed in nmol min^{-1}(mg protein)$^{-1}$.

natural organic gels (alginate, carrageenan), in the matrix of which the cells remain virtually intact (integrity, metabolic activity) and retain a fairly high transforming activity and stability.[12, 13, 60, 80, 83–88, 90] The principal deficiency of organic gels is their swellability, inducing a high hydrodynamic resistance to the flow, and also their decomposition during use by chemical and biological factors.

Another method for the immobilisation of bacterial cells is adsorption (adhesion) on the surfaces of inorganic carriers (silicates, oxides, and carbon materials).[31, 50, 55, 56, 60, 71, 81, 89] Under these conditions, biofilms are formed as a rule. Covalent immobilisation is used rarely, since the cross-linking agents used for the formation of covalent bonds (dialdehydes and carbodiimides) are usually toxic to cells.

The immobilisation of microorganisms in the mesophases of nonionic surfactants (of the type of polyoxyethylene n-dodecyl ethers with different molecular masses), formed in a water–organic solvent (hexadecane) two-phase system, leads to interesting prospects.[82] The *Mycobacterium* E3 cells immobilised in this way exhibit the maximum epoxidising activity (Table 9).[82]

Another nontrivial approach to the immobilisation of enzymes and bacterial cells involves the sealing of the biologically active material with the aid of a gel-forming system within the pores of an inorganic carrier.[91] In essence, this is a double immobilisation method, which combines the advantages of the incorporation of the cells into gels (high biocatalytic activity and stability) and of the use of inorganic carriers (high mechanical strength and resistance to degradation). The heterogeneous biocatalyst obtained in this way exhibits a relatively high enzyme activity and is stable both in storage and under operating conditions (Table 9).[50]

One of the main problems in the immobilisation of enzymes and cells is that of the selection of the optimum carrier and immobilisation procedure. Nowadays the immobilisation of enzymes and cells is approached purely empirically, predominantly on the basis of the tradition which has arisen in a particular research group. However, analysis of a large volume of literature data and our own results enables us to formulate certain general principles governing the immobilisation and stabilisation of enzymes and microbial cells on/in solid matrices.

The stabilisation of immobilised enzymes and cells is ensured by the mutual correspondence of the characteristics of the biologically active material and the carrier. In the first place, the biomolecule (cell) must correspond exactly to the size of the support pores, as a result of which a multipoint interaction is achieved. For the stabilisation of bacterial cells and the maintenance of their viability in the immobilised state, it is essential that the morphology of the porous space of the carrier should definitely correspond to the biologically active material: the ratio of the pore size to the size (length) of the microorganism should range from 1:5 and the pores of the given size should occupy ~ 70% of the porous space.[92] In other words, 1–10 μm macropores should predominate in the support texture.

Another principle is the mutual chemical correspondence of the biomolecule (cell) and the carrier (gel) surface, particularly from the standpoint of their hydrophilic–hydrophobic characteristics.[93] For example, microorganisms with a hydrophobic cell wall are sorbed most strongly on a hydrophobic carrier surface (or in a hydrophobic mesophase) and exhibit a relatively high biocatalytic activity and stability.[82] The practical implementation of the mutual correspondence principles, particularly in the case of the immobilisation of individual enzymes, as a rule promotes the formation of active heterogeneous biocatalysts, which are more stable by a factor of tens and thousands than their homogeneous analogues.

Unfortunately, on immobilisation of whole bacterial cells the stabilisation of the enzyme activity by virtue of the interaction of the microorganisms with the surface of optimum carriers (selected taking into account the mutual correspondence) is not quite so marked and striking as in the case of enzymes. The enzyme activity of whole bacterial cells apparently depends to a large extent on the intracellular microenvironment of the enzymes, which is determined only indirectly by the state of the immobilised bacterial cells.

The mechanisms of the initiation and stabilisation of enzyme activity in immobilised microorganisms may be different in each specific case. For example, an increase in activity may be

promoted by the improvement in gas exchange and in the distribution of nutrient substances,[94,95] by the structural rearrangement of the membranes leading to an increase in permeability and the acceleration of transport of substrates into the cell,[96,97] by the change in the physiology of the cells under the influence of the stress accompanying the immobilisation procedure,[98] etc. Evidently the main advantage of the immobilisation of microbial cells consists in the possibility of obtaining biocatalysts in a heterogeneous form, which makes it possible to carry out biocatalytic processes in a continuous regime in bioreactors of different design and with repeated employment of the biologically active material. The matrix stabilises fairly frequently the enzyme activity of the immobilised bacterial cells and also increases the resistance of the microorganisms immobilised on the carrier surface to the effect of toxic compounds.

Another approach to the solution of the problem of overcoming the inhibiting effect of epoxides on the cells of gas-utilising microorganisms consists in the removal of the epoxide from the reaction zone. Two-phase water–organic solvent (as a rule, n-hexadecane) systems with extraction of the epoxide into the organic phase[61,83,84,86] and/or the removal of the epoxide, for example epoxypropane, from the gas stream (where its fraction at 35 °C is ~ 3% of all the molecules[86]) by absorption in di-n-octyl phthalate or by acid hydrolysis in sulfuric acid solution (pH 1, 30 °C) are used for this purpose.[84] The immobilisation of microorganisms promotes an increase in the resistance of bacterial cells to the action of the organic solvent apparently as a result of the presence of an aqueous microenvironment in the vicinity of the solid carrier surface.

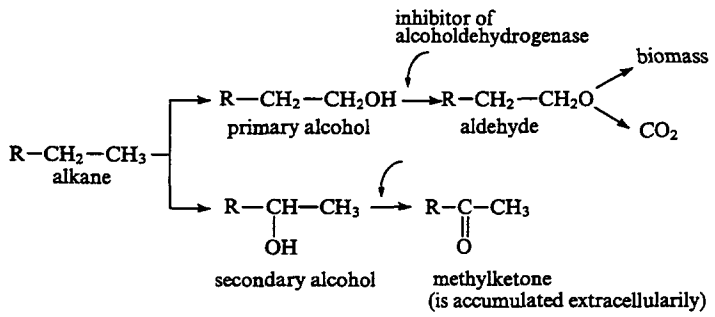
In conclusion, it must be emphasised yet again that certain gas-assimilating microorganisms of the genera *Mycobacteria* and *Nocardia* are capable of epoxidising unsaturated hydrocarbons stereospecifically, so that only one of the optical isomers is formed in excess, accumulating extracellularly in the reaction medium (Tables 4–7). Another procedure for the preparation of optical isomers of epoxides is based on the fact that certain microorganisms of the genera *Xantobacter*[99] and *Nocardia* H8[100] are capable of the degradation (utilisation) of only one of the stereoisomers of the racemic mixture, whereas the other isomer of the epoxide accumulates in the medium.

Thus the epoxidation of light alkenes (ethene, propene, and butene) can be achieved by means of the enzymic monoxygenase complexes of alkane- and alkene-utilising microorganisms in free or immobilised forms (Tables 4–7 and 9). One of the main ways of increasing the effectiveness of the alkene bioepoxidation processes is the reduction of the inhibiting effect of the epoxides, which accumulate in the reaction medium and exhibit a very pronounced toxicity. For this purpose, the processes are optimised by employing, for example, immobilised microorganisms in water–organic solvent two-phase systems.

V. Microbiological hydroxylation of gaseous alkanes

As already mentioned, the direct selective oxidation of saturated hydrocarbons to valuable oxygen-containing products (alcohols, aldehydes, ketones, and acids) is the main problem in the chemical processing of natural gas by homogeneous and heterogeneous catalytic methods. The partial oxidation of alkanes is traditionally carried out on oxide catalysts (for example, catalysts based on V_2O_5 or MoO_3), the key parameter of the processes being selectivity. The biotechnological approach to the direct single-

Table 10. Partial single-stage oxidation of gaseous hydrocarbons to alcohols and methyl ketones by atmospheric oxygen with participation of gas-assimilating microorganisms.



Initial hydrocarbon	Product	Microorganisms (growth substrate)	a^a	Inhibitor (concentration)	Ref.
Methane	methanol	*Methylococcus capsulatus* (methane)	42	none	41
	"	*Methylosinus trichosporium* (methane)[b]	[149]	phosphate ions (80–100 mM)	18
	"	*Methylococcus capsulatus* (methane)	[131]	EDTA (10 mM)	52
	"	*Methylosinus trichosporium* (methane)	[79]	phosphate ions (80–100 mM)	18
		"	[50]	EDTA (10 mM)	52
	"	*Methylomonas* GY-J-3 (methane)	[31]	"	52
	"	*Methylosinus trichosporium* OB3b (methane)	25 [24]	treatment of cells with cyclopropane; cyclopropanol (0.3 mM)	41, 101, 102
	"	"	[8]	NaCl (0.2 M), EDTA (2 mM), H_2 (10%)	17

Table 10 (continued).

Initial hydrocarbon	Product	Microorganisms (growth substrate)	a [a]	Inhibitor (concentration)	Ref.
Methane	methanol	*Methylobacterium organophilum* (methane)	7	none	41
	"	*Methanonas methanooxidans* (methane)	–	iodoacetate (0.003 M)	103
Ethane	ethanol	*Methylosinus trichosporium* OB3b (methane)	22 [12]	EDTA (2 mM), H_2 (10%)	17, 41
	"	*Methylococcus capsulatus* (methane)	33	none	41
	"	*Methylobacterium organophilus* (methane)	7	"	41
	"	*Scedosporium* A-4 (propane)	[6]	pyrazole (10 mM)	59
Propane	propan-1-ol	*Scedosporium* A-4 (propane)	[5]	"	59
	"	*Rhodococcus rhodochrous* (propane)	–	none	104
	propan-2-ol	Methanotroph H-2 (methane)	25	"	37
	"	*Methylosinus trichosporium* CRL15 (methane)	18	"	105
	"	*Methylococcus capsulatus* (methane)	18	"	105, 106
	"	*Methylobacterium organophilum* (methane)	12.5	"	41, 105–107
	"	*Methylosinus trichosporium* OB3b (methane)	8 [3]	EDTA (2 mM)	17, 105, 106
	"	*Scedosporium* A-4 (propane)	[2]	pyrazole (10 mM)	59
	"	*Rhodococcus rhodochrous* (propane)	–	–	104
Propane	acetone	Methanotroph H-2 (methane)	50	none	37
	"	*Methylococcus capsulatus* CRL M1 (methane)	42	"	41, 105, 108
	"	*Nocardia paraffinica* (propane)	33	"	43
	"	*Methylobacterium organophilum* (methane)	30	"	41, 105, 108
	"	*Methylosinus trichosporium* OB3b (methane)	25 [3]	"	17, 41, 105, 108
	"	*Methylobacter* CRL M6 (methane)	17	"	105, 108
	"	*Pseudomonas fluorescens* (propane)	17	"	43
	"	*Methylocystis parvus* (methane)	15	"	105, 108
	"	*Scedosporium* A-4 (propane)	[0.6]	"	109
	"	*Mycobacterium smegmatis* (propane)	–	–	110
n-Butane	butan-1-ol	*Scedosporium* A-4 (propane)	[8]	pyrazole (10 mM)	59
	"	*Methylosinus trichosporium* OB3b (methane)	5	none	41
	"	*Methylococcus capsulatus* CRL M1 (methane)	2	"	41
	butan-2-ol	Methanotroph H-2 (methane)	33	"	37
	"	*Methylococcus capsulatus* CRL M1 (methane)	20	"	41, 106
	"				
	"	*Methylosinus trichosporium* OB3b (methane)	10	"	41, 105, 106
	"	*Methylomonas* CRL (methane)	10	"	105–107
	"	*Methylobacterium organophilum* (methane)	3	"	41
	"	*Scedosporium* A-4 (propane)	[2]	pyrazole (10 mM)	59

Table 10 (continued).

Initial hydrocarbon	Product	Microorganisms (growth substrate)	a [a]	Inhibitor (concentration)	Ref.
n-Butane	butan-2-one	*Nocardia paraffinica* (propane)	73	none	43
	"	*Brevibacterium* CRL 56 (propane)	43	"	43
	"	*Methylococcus capsulatus* CRL M1 (methane)	33	"	41, 105, 108
	"	*Methylobacterium organophilum* (methane)	25	"	41, 105, 108
	"	*Pseudomonas fluorescens* (propane)	20	"	41, 105, 108
	"	*Methylosinus trichosporium* OB3b (methane)	20	"	41, 105, 108
	"	*Methylomonas* CRL 8 (methane)	12	"	105
	"	*Scedosporium* A-4 (n-butane)	[0.3]	"	109
	butan-2-one	*Mycobacterium smegmatis* (propane)	–	–	110
Isobutane	2-methyl-propan-1-ol	Methanotroph H-2 (methane)	89	none	37
	2-methyl-propan-2-ol	Methanotroph H-2 (methane)	89	"	37
Cyclopropane	cyclo-propanol	*Methylosinus trichosporium* OB3b (methane)	–	"	102
Propene	allyl alcohol	*Pseudomonas oleovorans* (n-octane)	–	"	78
	acrylic acid	*Mycobacterium convolutum* (propane)	–	"	111
But-1-ene	but-3-en-1-ol	*Pseudomonas oleovorans* (n-octane)	–	"	78

[a] The activity is expressed in nmol min^{-1} (mg protein)$^{-1}$ [mol min^{-1} (mg dry cells)$^{-1}$] and is determined as the rate of the extracellular accumulation of the oxidation product in the reaction medium. The maximum reaction rates quoted in the literature are presented.
[b] Immobilised by covalent binding to DEAE cellulose.

stage oxidation of alkanes by atmospheric oxygen to alcohols and methyl ketones with the aid of the cells of gas-assimilating microorganisms is demonstrated in Table 10.

In the microbiological hydroxylation of alkanes, certain additional problems, which do not exist in the epoxidation of alkenes, arise. One of them involves the search for an effective inhibitor of the further bioconversion of the alcohol formed in the reaction (see the Scheme in Table 10). The point is that, during the growth of microorganisms on saturated hydrocarbons, the primary activation of a C–H bond of the hydrocarbon by the bacterial monoxygenase results in the formation of molecules of a primary or secondary alcohol (Fig. 2), which are converted by alcohol dehydrogenase into an aldehyde or methyl ketone respectively.[23,25,27] The aldehyde molecules then undergo further metabolic transformations, leading to the incorporation of carbon into the biomass, whereas the methyl ketones accumulate in the reaction medium. The problem of the search for an effective universal inhibitor of alcohol dehydrogenase, which would interrupt the cell metabolism at the stage involving the formation of a primary or secondary alcohol, has still not been solved and it apparently should be solved individually in each specific case. Cyclopropanol, pyrazole, and the sodium salt of ethylenediaminetetraacetic acid (EDTA) are the most effective of the inhibitors of bacterial alcohol dehydrogenases investigated (Table 10).

As in the microbiological epoxidation of alkenes, the concentration of the final oxygen-containing product (an alcohol or a methyl ketone) in the reaction mixture does not exceed several tens of millimoles per litre. The hydroxylation process is retarded as a function of time. The causes of this retardation may be as follows: an increase in the rate of bioconversion of the alcohol formed; the consumption of the cofactor (electron donor) required for the oxidative biotransformation of the hydrocarbons; the toxic effect of the alcohol or methyl ketone, accumulated in the reaction medium, on the microbial cell.

Thus the problems of reducing the toxic effect of the oxygen-containing product formed, of the regeneration of the monoxygenase cofactor with the aid of available low molecular-mass substrates of the coupled enzymic reactions, and the isolation of the final oxygen-containing product from dilute buffer solutions arise when the hydroxylation process is carried out. In addition, it is necessary to solve also the problem of inhibiting the further bioconversion of the alcohol formed. The procedures for overcoming some of these difficulties were described above.

There is so far no need for the practical implementation of the biocatalytic alkane hydroxylation processes, since in the basic industrial organic synthesis traditional chemical procedures for the synthesis of alcohols (of methanol from the synthesis gas and of ethanol by the hydration of ethene) are widely employed. In terms of their economic parameters, in particular in terms of the cost of the final oxygen-containing product, they cannot at the moment be competed with.

VI. Conclusion

The alternative biotechnological procedures for the direct selective oxidation of gaseous C_1-C_4 alkanes and C_2-C_4 alkenes make it possible to obtain valuable oxygen-containing products — alcohols and epoxides. The single-stage oxidative transformations of hydrocarbons can occur with participation of the bacterial cells of gas-assimilating microorganisms with the necessary monoxygenase enzyme activity. The biocatalytic processes are distinguished by a low energy consumption, because they are carried out under mild conditions and at low temperatures (below 40 °C), by a high selectivity (close to 100%) independent of the degree of conversion of the initial substrate, and, what is most important, by stereospecificity. One optically active stereoisomer may predominate in the oxygen-containing product, which is impossible when the analogous chemical processes are performed. It is at present still too early to consider the replacement of the large scale organic synthesis in industry by oxidative biocatalytic processes, but the scientific investigations performed in this field, which are at the boundary between microbiology and organic chemistry, are quite timely and promising.

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