Oxidation of Gaseous and Volatile Hydrocarbons by Selected Alkene-Utilizing Bacteria

C. G. VAN GINKEL,* H. G. J. WELTEN, AND J. A. M. DE BONT

Department of Microbiology, Agricultural University, Hesselink van Suchtelenweg 4, 6703 CT Wageningen, The Netherlands

Eleven strains of alkene-utilizing bacteria belonging to the genera *Mycobacterium*, *Nocardia*, and *Xanthobacter* were tested for their ability to grow with C_1 to C_6 alkanes, C_2 to C_6 alkenes, alkadienes, and monoterpenes furnished individually as sole sources of carbon and energy in a mineral salts medium. A limited number of alkenes and alkanes supported growth of the bacteria; some bacteria were unable to grow on any of the saturated hydrocarbons tested. Monoterpenes were frequently used as carbon and energy sources by alkene-utilizing bacteria belonging to the genera *Mycobacterium* and *Nocardia*. Washed cell suspensions of alkene-grown bacteria attacked the whole range of alkenes tested, whereas only three strains were able to oxidize alkanes as well. The alkenes tested were oxidized either to water and carbon dioxide or to epoxyalkanes. Few epoxides accumulated in stoichiometric amounts from the corresponding alkenes, because most epoxides formed were further converted to other compounds like alkanediols.

In nature, several gaseous and volatile alkenes are produced. The most predominant of these compounds are the gaseous plant hormone ethene (1, 26), the volatile isoprene from foliage (27), and various monoterpenes that are present in plant oils. Many unsaturated hydrocarbons, especially the lower gaseous alkenes ethene, propene, 1,3-butadiene, and butenes, are produced chemically on a large scale; and inevitably, these compounds are partly released into the environment. It is therefore not surprising that many aerobic microorganisms have been isolated that are able to use these compounds as carbon and energy sources. Isolation substrates included ethene (3, 15); propene (2, 5, 31); 1,3-butadiene (34; C. G. van Ginkel, E. de Jong, J. W. R. Tilanus, and J. A. M. de Bont, FEMS Microbiol. Ecol., in press); 2-butene (33); and monoterpenes, for example, myrcene (24) and α-pinene (20, 36). Other microorganisms, which were isolated by the use of substrates such as alkanes, have also been tested for their ability to grow on alkenes; but growth on unsaturated hydrocarbons was recorded in only a very limited number of instances (9, 10, 22).

Nevertheless, resting cells of alkane-grown bacteria, including methane utilizers, often were able to epoxidate alkenes due to the broad substrate specificity of alkane monooxygenases which are responsible for the initial oxidation of alkanes (16, 17, 19, 25, 28). Alkene utilizers also contain monooxygenases with a broad substrate specificity, but enzymes from these organisms generally do not hydroxylate alkanes (4, 7, 32).

Alkene oxidation by washed cells of either alkane- or alkene-grown cells very often results in the formation and excretion of epoxides. Examples of epoxide-forming alkane utilizers are resting cells of methane- and alkane-grown bacteria that form these compounds from alkenes as a consequence either of the inability of these bacteria to degrade epoxides (18) or of a negligible oxidation rate of epoxyalkanes (25). The excretion of epoxides by alkene-utilizing bacteria is a consequence of a restrictive range of substrates utilized by the epoxyalkane-degrading enzymes (12). Accumulation of epoxides during growth of an organism was shown by Furuhashi et al. (11), who detected 1,2-epoxypropane accumulation during growth of *Nocardia corallina* B-276 on propene.

Epoxyalkane-producing microorganisms have frequently been considered as potential biocatalysts in biotechnological processes for the production of epoxides. Alkene-utilizing bacteria should then be preferred over alkane-utilizing organisms for several reasons. (i) Alkene-utilizing bacteria form epoxides in high enantiomeric excess (13), whereas methane-grown bacteria produce racemic epoxyalkanes (29). (ii) Alkene-grown bacteria only epoxidate and do not hydroxylate alkenes as do some alkane utilizers. (iii) Alkane-grown bacteria are more sensitive to epoxyalkanes than are alkene-utilizing bacteria (14).

In view of the role that alkene-utilizing organisms play in nature, and in view of their potential application in epoxide production, it seems desirable to obtain a more comprehensive knowledge of these bacteria. We therefore compared several available alkene-utilizing bacteria (5, 12, 31, 33; van Ginkel et al., in press) and two new isolates. In this study we particularly dealt with the capacity of these selected organisms to form and excrete epoxyalkanes from alkenes.

MATERIALS AND METHODS

Chemicals. All gaseous alkenes and 1,2-epoxyethane were obtained from Hoek Loos, Amsterdam, The Netherlands. All other chemicals were purchased from Janssen Chimica, Beerse, Belgium.

Microorganisms. The isolation and description of the bacteria used in this study have been reported earlier (5, 12, 31, 33; van Ginkel et al., in press). *Nocardia* sp. strain H8 and *Pseudomonas* sp. strain H1 were isolated by similar methods, except that 1-hexene was used as the sole source of carbon and energy (3).

Cultivation of the microorganism. Organisms were grown at 30°C in 5-dm³ Erlenmeyer flasks containing 500 cm³ of mineral salts medium (35) with the gaseous alkene in air (5%) or 1 cm³ of a volatile alkene as the sole carbon and energy source.

Analyses. Determination of alkenes, 1,2-epoxyalkanes, 1,2-propanediol, and carbon dioxide has been described

---

* Corresponding author.

TABLE 1. Microorganisms isolated from soil samples from 10 different locations on various gaseous and volatile alkenes

Alkene	Isolate genera (no. of strains)
Ethene	*Mycobacterium* (10)
Propene	*Xanthobacter* (9), *Mycobacterium* (1)
1-Butene	*Xanthobacter* (8), *Nocardia* (1), *Mycobacterium* (1)
2-Butene	*Nocardia* (2), *Mycobacterium* (1)
1,3-Butadiene	*Nocardia* (10)
Isoprene	*Nocardia* (10)
1-Hexene	*Pseudomonas* (9), *Nocardia* (1)

previously (4, 7, 35). The protein concentration of washed cell suspensions was determined as described by Habets-Crützen et al. (12). Mycolic acids were determined by thin-layer chromatographic analysis of methanolysates of whole cells, as described by Minnikin et al. (23).

Growth of microorganisms. Microorganisms were grown on slants of mineral salts medium described previously (35). These slants were placed in a dessicator, and the appropriate gas was injected or a volatile alkene in a test tube was placed in the dessicator. After 3 weeks the slopes were examined for growth.

Oxidation of hydrocarbons. Preparation of washed cell suspensions has been described by de Bont et al. (4). The cell suspension (2 cm^3) was placed in 30-cm^3 screw-cap bottles. The appropriate gas (0.5 cm^3) or volatile compound (0.3 × 10^{-3} cm^3) was injected into the screw-cap bottles. The reaction mixture was incubated at 30°C on a water bath rotary shaker at 150 rpm, and alkenes and the products of epoxidation were assayed at regular intervals.

Conversion of 1,2-epoxypropane to 1,2-propanediol. A washed cell suspension of ethene-grown *Mycobacterium* sp. strain E3 (10 cm^3) was incubated with 0.25 mM 1,2-epoxypropane. During 1,2-epoxypropane degradation by washed cell suspensions of ethene-grown *Mycobacterium* sp. strain E3, CO$_2$ formation was measured along with the endogenous CO$_2$ formation rate. At regular intervals the epoxide concentration was determined by analyzing the headspace gas chromatographically. After centrifugation, samples of washed cell suspensions were analyzed for 1,2-propanediol.

RESULTS

Microorganisms growing on gaseous and volatile alkenes. We isolated several alkene-utilizing bacteria by using soil samples from 10 different locations. The enrichment cultures were set up individually with the alkenes used previously (3, 12, 31; van Ginkel et al., in press) or with other alkenes, such as allene, 1-pentene, and 1-hexene. In general, all enrichment cultures showed growth within 1 week, except for incubations with allene, *trans*-2-butene, and 1-pentene. In spite of numerous efforts, a 1-pentene-utilizing bacterium was not isolated; and when allene was used as the carbon and energy source, it was not even possible to obtain a positive enrichment culture. Of the 10 enrichment cultures, only 3 cultures of *trans*-2-butene-utilizing bacteria were isolated. Many *Pseudomonas* spp. and *Nocardia* sp. strain H8 were enriched and subsequently isolated with 1-hexene as the carbon and energy source. All newly isolated bacteria were tentatively classified on the basis of Gram staining, microscopic observation, and mycolic acid analysis. In

Table 1 are summarized the genera to which the new isolates were assigned, and the number of strains isolated is given.

Growth on alkenes and alkanes. A representative selection of 11 alkene-utilizing bacteria, mainly strains already described, was made to determine the range of alkenes (ethene, propene, 1-butene, *trans*-2-butene, 1,3-butadiene, 1-pentene, isoprene, 1-hexene), *n*-alkanes (C$_1$ to C$_6$), and monoterpenes used for growth; the substrate specificity of alkene-grown bacteria; and the formation and excretion of epoxides by washed cells.

Eleven strains of alkene-utilizing bacteria investigated grew on only one or two alkenes, except for *Nocardia* sp. strain H8 which utilized all 1-alkenes (C$_2$ to C$_6$). *Mycobacterium* spp. strains E3 and 2W grew only on ethene, whereas *Mycobacterium* sp. strain Py1 utilized propene and 1-butene. *Xanthobacter* spp. strains Py2 and By2 grew on ethene, propene, 1-butene, and 1,3-butadiene. *Nocardia* sp. strain TB1 was only able to utilize 2-butene, whereas *Nocardia* sp. strain By1 used propene and 1-butene. Strains BT1 and IP1 grew on 1,3-butadiene and isoprene. *Pseudomonas* sp. strain H1 grew only on 1-hexene. *Xanthobacter* spp. and *Pseudomonas* sp. strain H1 were not able to grow on any monoterpene tested, but all other alkene-utilizing bacteria were able to grow on several monoterpenes. *Mycobacterium* sp. strain Py1 and *Nocardia* spp. strains TB1 and H8 utilized myrcene, limonene, γ-terpinene, β-pinene, and α-pinene, whereas *Mycobacterium* spp. strains E3 and 2W and *Nocardia* sp. strain By1 grew only on myrcene. *Nocardia* sp. strain BT1 used α-pinene; and *Nocardia* sp. strain IP1 used limonene, γ-pinene, and α-pinene.

Some of the alkene-utilizing bacteria also grew on a limited number of saturated hydrocarbons. *Pseudomonas* sp. strain H1 was able to grow on pentane and hexane, whereas *Nocardia* sp. strain TB1 also grew on these hydrocarbons, as well as on propane and butane. *Mycobacterium* spp. strains 2W and Py1 grew on hexane, while *Nocardia* spp. strains BT1 and IP1 possessed the ability to utilize propane and butane as the sole source of carbon and energy.

Oxidation of alkanes. Resting-cell suspensions of 3 of the 11 strains tested oxidized alkanes when they were grown on alkenes. Isoprene-grown *Nocardia* sp. strain IP1 oxidized butane, pentane, and hexane at rates up to 2 nmol/min per mg of protein. *trans*-2-Butene-grown *Nocardia* sp. strain TB1 oxidized all alkanes tested except for methane, and the oxidation rates were comparable to those of isoprene-grown *Nocardia* sp. strain IP1, except that the rate of oxidation of butane was twice as high. 1-Hexene-grown *Pseudomonas* sp. strain H1 oxidized pentane and hexane at rates of 4 to 6 nmol/min per mg of protein, whereas the gaseous alkanes were only oxidized at negligible rates. The other alkene-grown bacteria tested were not able to oxidize gaseous or volatile alkanes.

Oxidation of alkenes. All alkene-grown bacteria tested oxidized the gaseous and volatile alkenes used. In general, the highest alkene oxidation rates were found with the alkene on which the bacterium was grown (Table 2). 1-Hexene-grown *Nocardia* sp. strain H8 was exceptional, since it oxidized all 1-alkenes at the same rate. Similar oxidation rates were found when the strain was grown on another substrate. Oxidation rates of 1-alkenes by *Nocardia* sp. strain TB1 and *Pseudomonas* sp. strain H1 were low, and after a short period of time the activity of the washed cell suspensions leveled off, whereas alkanes were completely oxidized by *trans*-2-butene-grown *Nocardia* sp. strain TB1 (Fig. 1) and *Pseudomonas* sp. strain H1. All other alkene-grown bacteria oxidized the unsaturated hydrocarbons at

TABLE 2. Oxidation of ethene, propene, 1-butene, 1,3-butadiene, cis-2-butene, trans-2-butene, 1-pentene, and 1-hexene by washed cell suspensions of alkene-grown bacteria

Strain	Growth substrate	Substrate oxidation rate (nmol/min per mg of protein) on the following substrate:							
		C_2H_4	C_3H_6	C_4H_8	C_4H_6	cis-C_4H_8	trans-C_4H_8	C_5H_{10}	C_6H_{12}
Mycobacterium sp. strain E3	Ethene	50	17	12	19	20	20	14	13
Mycobacterium sp. strain 2W	Ethene	23	6	6	11	12	13	6	7
Mycobacterium sp. strain Py1	Propene	15	20	17	ND[a]	ND	ND	8	5
Xanthobacter sp. strain Py2	Propene	50	81	62	17	70	60	20	14
Nocardia sp. strain By1	1-Butene	19	23	26	9	21	17	12	11
Xanthobacter sp. strain By2	1-Butene	45	70	61	24	67	29	24	16
Nocardia sp. strain TB1	2-Butene	2	2	3	3	6	5	1	1
Nocardia sp. strain BT1	Butadiene	18	16	17	57	19	19	ND	ND
Nocardia sp. strain IP1	Isoprene	11	14	12	13	ND	ND	ND	ND
Pseudomonas sp. strain H1	1-Hexene	0	0	1	1	1	1	3	5
Nocardia sp. strain H8	1-Hexene	16	19	19	ND	ND	ND	16	16
Nocardia sp. strain H8	Propene	17	18	16	ND	ND	ND	17	16

[a] ND, Not determined.

rates of 10 to 80 nmol/min per mg of protein (Table 2), and no decrease in activity was detected during the time course of the experiment.

Formation of epoxyalkanes. Washed cell suspensions of the 11 strains of alkene-utilizing bacteria were able to accumulate epoxyalkanes from one or more alkenes. However, no significant excretion of epoxyalkanes was detected from alkenes on which the bacteria were grown (Table 3). Epoxyalkanes also did not accumulate from alkenes, which are potential growth substrates of the bacteria. For instance, Nocardia sp. strain H8, which was able to grow on all tested 1-alkenes, did not form 1,2-epoxyalkane. 1,2-Epoxyethane formation by alkene-grown Xanthobacter spp. was an exception, since these bacteria are able to grow on ethene at very slow rates (31). No formation of epoxides was observed from 1,3-butadiene by alkene-grown bacteria. The formation of 1,2-epoxypentane and 1,2-epoxyhexane was demonstrated with Mycobacterium spp. strains E3 and 2W. The epoxyalkane formation rates varied from 1 to 50 nmol/min per mg of protein, and the highest rates were found with ethene- and propene-grown bacteria (Table 3). When alkene oxidation rates from Table 2 and epoxyalkane formation rates from Table 3 are compared, it can be seen that epoxides are formed stoichiometrically in only a few cases. 2,3-Epoxybutanes were not degraded by ethene-grown Mycobacterium spp. and 1,3-butadiene-grown Nocardia sp.

strain BT1, and consequently, they accumulated stoichiometrically in the supernatant. Nocardia sp. strain TB1 formed 1,2-epoxyalkanes only stoichiometrically from ethene and propene. Finally, Mycobacterium sp. strain Py1 did not degrade 1,2-epoxyethane. From most nongrowth alkenes, however, only a portion of the alkene oxidized by the alkene-grown bacteria was recovered as epoxyalkane during the time course of the experiment.

Utilization of epoxyalkanes. During the degradation of 1,2-epoxypropane by ethene-grown Mycobacterium sp. strain E3, no additional CO_2 was formed over the CO_2 that was formed endogenously. Subsequently, it was shown that 1,2-epoxypropane was hydrolyzed to 1,2-propanediol by washed cell suspensions of ethene-grown Mycobacterium sp. strain E3 (Fig. 2).

DISCUSSION

Gaseous and volatile alkenes can serve as sole carbon and energy sources for microbial growth. In particular, taxonomically related gram-positive bacteria were isolated when these compounds were used as the growth substrate (Table 1). Gram-negative Pseudomonas spp. were isolated with enrichment cultures with 1-hexene as the sole carbon and energy source. This alkene is probably a borderline growth substrate between lower gaseous and higher liquid alkenes. Pseudomonas spp. have been described as liquid 1-alkene utilizers (8, 21).

Only a few bacteria that have been isolated on gaseous alkenes can also grow on saturated gaseous hydrocarbons, namely, Nocardia corallina B276 (11), Mycobacterium sp. strain E20 (5), and other Mycobacterium spp. (10). Furthermore, only a very limited number of gaseous alkane-utilizing bacteria can grow on gaseous alkenes (9, 22). In general, these observations have now been confirmed by the results obtained with the alkene-utilizing bacteria that were selected because only three strains were capable of growth on some gaseous alkanes. In particular, 1-hexene-utilizing Pseudomonas sp. strain H1 and trans-2-butene-utilizing Nocardia sp. strain TB1 grew more abundantly on some alkanes than on alkenes, and results of additional experiments showed that both bacteria resembled alkane-utilizing bacteria more so than did their alkene-utilizing counterparts.

In view of the low alkene concentrations found in nature, it is likely that alkene-utilizing bacteria also utilize other naturally occurring carbon and energy sources to sustain life. The ability to utilize gaseous alkenes might have
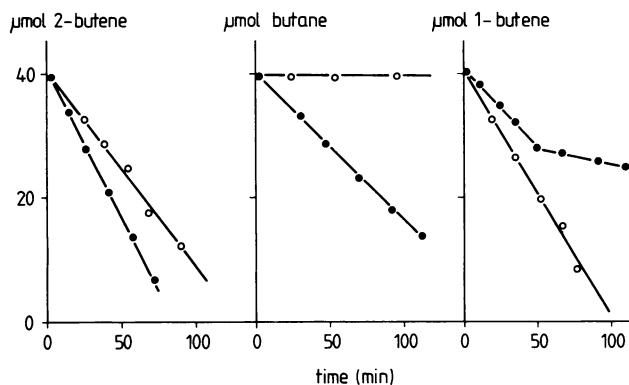

FIG. 1. Oxidation of C_4 hydrocarbons by washed cell suspensions of Nocardia sp. strain By1 (18 mg of protein) grown on 1-butene (○) and Nocardia sp. strain TB1 (95 mg of protein) grown on 2-butene (●). The culture volume was 10 dm^3.

μmol 2-butene   μmol butane   μmol 1-butene

time (min)

TABLE 3. Formation of epoxyalkanes from ethene, propene, 1-butene, cis-2-butene, and trans-2-butene
by washed cell suspensions of alkene-grown bacteria

Strain	Growth substrate	Product formation rate (nmol/min per mg of protein) for the following product[a]:				
		C_2H_4O	C_3H_6O	C_4H_8O	cis-C_4H_8O	trans-C_4H_8O
Mycobacterium sp. strain E3	Ethene	0	15	11	20	20
Mycobacterium sp. strain 2W	Ethene	0	5	4	12	13
Mycobacterium sp. strain Py1	Propene	15	0	0	ND[b]	ND
Xanthobacter sp. strain Py2	Propene	46	0	0	17	41
Nocardia sp. strain By1	1-Butene	9	0	0	7	11
Xanthobacter sp. strain By2	1-Butene	11	0	0	27	22
Nocardia sp. strain TB1	2-Butene	2	2	2	0	0
Nocardia sp. strain BT1	Butadiene	17	13	16	19	19
Nocardia sp. strain IP1	Isoprene	10	13	11	ND	ND
Pseudomonas sp. strain H1	1-Hexene	0	0	1	1	1
Nocardia sp. strain H8	1-Hexene	0	0	0	ND	ND
Nocardia sp. strain H8	Propene	0	0	0	ND	ND

[a] None of the strains tested excreted 1,2-epoxy-3-butene from 1,3-butadiene. Both ethene-grown mycobacteria excreted 1,2-epoxypentane and 1,2-epoxyhexane from the respective 1-alkenes at rates of up to 2 nmol/min per mg of protein.

[b] ND, Not determined.

evolved from the potential to degrade the saturated hydrocarbons that are more abundantly present in nature. As stated above, Nocardia sp. strain TB1 and Pseudomonas sp. strain H1 are better described as alkane utilizers, and for these organisms saturated hydrocarbons may be more important substrates in nature. The high incidence of monoterpene utilization by Mycobacterium and Nocardia strains suggests that these bacteria may use these naturally occurring compounds as carbon and energy sources in soil ecosystems.

Some of the selected strains grown on alkenes were able to oxidize both saturated and unsaturated hydrocarbons, and therefore, it can be assumed that an alkane-type monooxygenase is present in these cells. Indeed, Pseudomonas sp. strain H1 is a bacterium which resembles a Pseudomonas strain used by Thijsse and van der Linden (30), and it was shown by these investigators that the initial attack on 1-hexene is preponderantly via the methyl group. Nocardia sp. strain TB1 grows more abundantly on saturated than on unsaturated gaseous hydrocarbons, and evidence is presented elsewhere (33) that Nocardia sp. strain TB1 metabolizes trans-2-butene via crotonic alcohol.

Resting-cell suspensions of bacteria grown on alkenes readily oxidized all alkenes and alkadienes tested (Table 2). This suggests that the monooxygenases involved do not have a high degree of specificity toward alkenes. Such a broad substrate specificity toward hydrocarbons is not unique because alkane-grown bacteria act in the same way (16, 17, 19, 25, 28). 1-Hexene-grown Pseudomonas sp. strain H1 is an exception, however, because this bacterium did not oxidize the gaseous alkenes to any significant extent.

Although Pseudomonas sp. strain H1 and Nocardia sp. strain TB1 oxidized 1-alkenes, the oxidizing activity leveled off after a short period of time. This decreasing activity was probably due to the toxic effect of the epoxyalkanes that were formed, and such susceptibility toward epoxides is found in alkane-utilizing bacteria but not in alkene-utilizing bacteria (14). In this respect, Nocardia sp. strain TB1 and Pseudomonas sp. strain H1 also resemble the alkane utilizers. The general differences observed between alkane and alkene utilizers, with respect to both substrate specificity and epoxide toxicity, are illustrated in Fig. 1. Activities toward C_4 hydrocarbons of washed cells of 1-alkene-grown Nocardia cells were compared with these activities of 2-butene-grown Nocardia cells (Fig. 1).

No epoxides accumulated from alkenes on which the bacterium was grown. However, alkene-grown bacteria, in general, accumulate epoxyalkanes from nongrowth alkenes. This epoxide formation by alkene-utilizing bacteria is a consequence of the restrictive range of epoxides converted by 1,2-epoxyalkane-degrading enzymes (12). Stoichiometric formation of epoxyalkane from 2-butene was found with ethene-grown Mycobacterium spp. and with 1,3-butadiene-grown Nocardia sp. strain BT1, whereas propene-grown Mycobacterium sp. strain Py1 cells accumulated stoichiometric amounts of 1,2-epoxyethane from ethene (7). Neither Nocardia sp. strain TB1 nor Pseudomonas sp. strain H1 metabolized 1,2-epoxyalkanes or oxidize epoxyalkanes at negligible rates, and in this respect, these bacteria again acted in the same way as alkane-utilizing bacteria (25).

Nevertheless, most epoxyalkanes derived from nongrowth alkenes are degraded further by alkene-utilizing bacteria. Ethene-grown Mycobacterium sp. strain E3 catalyzed the hydrolysis of 1,2-epoxypropane to 1,2-propanediol, but 1,2-propanediol formation by Mycobacterium sp. strain E3 is probably not mediated by an enzyme of the degradative pathway of ethene (35). Such diol formation from epoxyalkanes is not catalyzed by methane-grown bac-
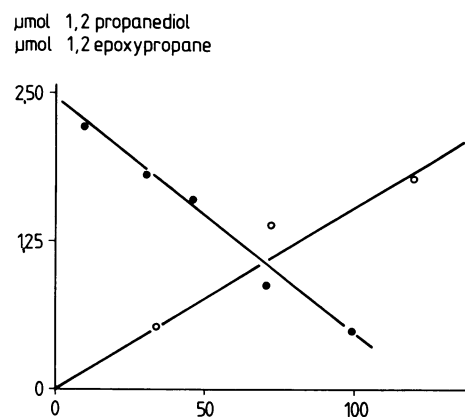
μmol 1,2 propanediol
μmol 1,2 epoxypropane

FIG. 2. Formation of 1,2-propanediol (O) from 1,2-epoxypropane (●) by washed cell suspensions of ethene-grown Mycobacterium sp. strain E3 (9.5 mg of protein). The culture volume was 10 dm³. The abscissa is time, in minutes.

teria either (18), and information about this reaction in bacteria is scarce (6).

Finally, from the results presented in this report, almost every epoxyalkane can be formed by alkene-grown bacteria when an appropriate combination of bacterium and alkene is used.

LITERATURE CITED

1. Abeles, F. B. 1973. Ethylene in plant biology. Academic Press, Inc., New York.
2. Cerniglia, C. E., W. E. Blevins, and J. J. Perry. 1976. Microbial oxidation and assimilation of propylene. Appl. Environ. Microbiol. 6:764–768.
3. de Bont, J. A. M. 1976. Oxidation of ethylene by soil bacteria. Antonie van Leeuwenhoek J. Microbiol. Serol. 42:59–71.
4. de Bont, J. A. M., M. M. Attwood, S. B. Primrose, and W. Harder. 1979. Epoxidation of short-chain alkenes in Mycobacterium E20; the involvement of a specific mono-oxygenase. FEMS Microbiol. Lett. 3:89–93.
5. de Bont, J. A. M., S. B. Primrose, M. D. Collins, and D. Jones. 1980. Chemical studies on some bacteria which utilize gaseous unsaturated hydrocarbons. J. Gen. Microbiol. 117:97–102.
6. de Bont, J. A. M., J. P. van Dijken, and C. G. van Ginkel. 1982. The metabolism of 1,2-propanediol by a propyleneoxide-utilizing bacterium Nocardia A60. Biochim. Biophys. Acta 714: 465–470.
7. de Bont, J. A. M., C. G. van Ginkel, J. Tramper, and K. C. A. M. Luyben. 1983. Ethylene oxide production by immobilized Mycobacterium Py1 in a gas/solid bioreactor. Enzyme Microbiol. Technol. 5:55–60.
8. de Smet, M. J., H. Wijnberg, and B. Witholt. 1982. Synthesis of 1,2-epoxyoctane by Pseudomonas oleovorans during growth in a two-phase system containing high concentrations of 1-octene. Appl. Environ. Microbiol. 42:811–816.
9. Fuhs, G. W. 1961. Der mikrobielle Abbau von Kohlenwasserstoffen. Arch. Microbiol. 39:379–422.
10. Fujii, T., T. Ogawa, and H. Fukuda. 1985. A screening system for microbes which produce olefin hydrocarbons. Agric. Biol. Chem. 49:651–657.
11. Furuhashi, K., A. Taoka, I. Karube, and S. Suzuki. 1981. Production of 1,2-epoxyalkanes from 1-alkenes by Nocardia corallina B276. Eur. J. Appl. Microbiol. Biotechnol. 20:39–45.
12. Habets-Crützen, A. Q. H., L. E. S. Brink, C. G. van Ginkel, J. A. M. de Bont, and J. Tramper. 1984. Production of epoxides from gaseous alkenes by resting-cell suspensions and immobilized cells of alkene-utilizing bacteria. J. Appl. Microbiol. Biotechnol. 20:245–251.
13. Habets-Crützen, A. Q. H., S. J. N. Carlier, J. A. M. de Bont, D. Wistuba, V. Schurig, S. Hartmans, and J. Tramper. 1985. Stereospecific formation of 1,2-epoxypropane, 1,2-epoxybutane and 1-chloro-epoxypropane by alkene-utilizing bacteria. Enzyme Microb. Technol. 7:17–21.
14. Habets-Crützen, A. Q. H., and J. A. M. de Bont. 1985. Inactivation of alkene oxidation by epoxides in alkene- and alkane-grown bacteria. Appl. Microbiol. Biotechnol. 22:428–433.
15. Heyer, J. 1976. Mikrobielle verwertung von Äthylene. Z. Allg. Mikrobiol. 16:633–637.
16. Higgins, I. J., R. C. Hammons, F. S. Saraislani, D. J. Best, M. M. Davies, S. E. Tryhorn, and F. Taylor. 1979. Biotransfor-
mations of hydrocarbons and related compounds by whole organisms of Methylosinus trichosporium OB3b. Biochem. Biophys. Res. Commun. 89:671–677.
17. Hou, C. T., R. N. Patel, and A. I. Laskin. 1979. Microbial oxidation of gaseous hydrocarbons by methylotrophic bacteria. Appl. Environ. Microbiol. 38:127–134.
18. Hou, C. T., R. N. Patel, and A. I. Laskin. 1980. Epoxidation and ketone formation by C_1-utilizing microbes. Adv. Appl. Microbiol. 26:41–69.
19. Hou, C. T., P. Patel, A. I. Laskin, N. Barnabe, and I. Barist. 1983. Epoxidation of short-chain alkenes by resting-cell suspensions of propane-grown bacteria. Appl. Environ. Microbiol. 46: 171–177.
20. Hungund, B. L., P. K. Bhattachaharyya, and P. N. Rangachari. 1970. Terminal oxidation pattern of a soil pseudomonad (PL-strain) Arch. Microbiol. 71:58–70.
21. Huybregtse, R., and A. C. van der Linden. 1964. The oxidation of α-olefins by a Pseudomonas reactions involving the double bond. Antonie van Leeuwenhoek J. Microbiol. Serol. 30:185–196.
22. Lukins, H. B., and J. W. Foster. 1963. Utilization of hydrocarbons by mycobacteria. Z. Allg. Microbiol. 3:251–264.
23. Minnikin, D. E., L. Alshamaohy, and G. Goodfellow. 1975. Differentiation of Mycobacterium, Nocardia and related taxa by thin layer chromatographic analysis of whole organisms methanolysates. J. Gen. Microbiol. 88:200–204.
24. Narushima, H., T. Omori, and Y. Minoda. 1980. Microbial oxidation of β-myrcene. Adv. Biotechnol. 3:525–531.
25. Patel, R. N., C. T. Hou, A. I. Laskin, A. Felix, and P. Derelanko. 1983. Epoxidation of n-alkenes by organisms grown on gaseous alkanes. J. Appl. Biochem. 5:121–131.
26. Primrose, S. B. 1979. Ethylene in agriculture: the role of the microbe. J. Appl. Bacteriol. 46:1–25.
27. Rasmussen, R. A. 1970. Isoprene: identified as a forest-type emission to the atmosphere. Environ. Sci. Technol. 4:667–671.
28. Stirling, D. I., and H. Dalton. 1979. The fortuitous oxidation and co-metabolism of various carbon compounds by whole cell suspensions of Methylococcus capsulatus (BATH). FEMS Microbiol. Lett. 5:315–318.
29. Subramanian, V. 1986. Oxidation of propene and 1-butene by Methylococcus capsulatus and Methylosinus trichosporium. J. Ind. Microbiol. 1:119–127.
30. Thijsse, G. J. E., and A. C. van der Linden. 1963. Pathways of hydrocarbon assimilation by a Pseudomonas as revealed by chloramphenicol. Antonie van Leeuwenhoek J. Microbiol. Serol. 29:89–100.
31. van Ginkel, C. G., and J. A. M. de Bont. 1986. Isolation and characterization of alkene-utilizing Xanthobacter spp. Arch. Microbiol. 145:403–406.
32. van Ginkel, C. G., H. G. J. Welten, and J. A. M. de Bont. 1986. Epoxidation of alkenes by alkene-grown Xanthobacter spp. Appl. Microbiol. Biotechnol. 24:334–337.
33. van Ginkel, C. G., H. G. J. Welten, S. Hartmans, and J. A. M. de Bont. 1987. Metabolism of butane and trans-2-butene by Nocardia TB1. J. Gen. Microbiol. 133:1713–1720.
34. Watkinson, R. J., and H. J. Sommerville. 1976. The microbial utilization of butadiene, p. 35–42. In J. M. Sharply and J. A. M. Kaplan (ed.), Proceedings of the 3rd International Biodegradation Symposium. Applied Science, Essex, England.
35. Wiegant, W. M., and J. A. M. de Bont. 1980. A new route for ethylene glycol metabolism in Mycobacterium E44. J. Gen. Microbiol. 120:325–331.
36. Wright, S. J., P. Caunt, D. Carter, and P. B. Baker. 1986. Microbial oxidation of alpha-pinene by Serratia marcescens. Appl. Microbiol. Biotechnol. 23:224–227.