APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Apr. 1980, p. 727-733
0099-2240/8004-0727/07$02.000
Vol. 39, No. 4

Microbial Oxidation of Gaseous Hydrocarbons: Production of Methylketones from Corresponding *n*-Alkanes by Methane-Utilizing Bacteria

RAMESH N. PATEL,* C. T. HOU, A. I. LASKIN, A. FELIX, AND P. DERELANKO

Corporate Research Laboratory, Exxon Research & Engineering Co., Linden, New Jersey 07036

Cell suspensions of methane-utilizing bacteria grown on methane oxidized *n*-alkanes (propane, butane, pentane, hexane) to their corresponding methylketones (acetone, 2-butanone, 2-pentanone, 2-hexanone). The product methylketones accumulated extracellularly. The rate of production of methylketones varied with the organism used for oxidation; however, the average rate of acetone, 2-butanone, 2-pentanone, and 2-hexanone production was 1.2, 1.0, 0.15, and 0.025 μmol/h per 5.0 mg of protein in cell suspensions. Primary alcohols and aldehydes were also detected in low amounts as products of *n*-alkane (propane and butane) oxidation, but were rapidly metabolized further by cell suspensions. The optimal conditions for in vivo methylketone formation from *n*-alkanes were compared in *Methylococcus capsulatus* (Texas strain), *Methylosinus* sp. (CRL-15), and *Methylobacterium* sp. (CRL-26). The rate of acetone and 2-butanone production was linear for the first 60 min of incubation and directly increased with cell concentration up to 10 mg of protein per ml for all three cultures tested. The optimal temperatures for the production of acetone and 2-butanone were 35°C for *Methylosinus trichosporium* sp. (CRL-15) and *Methylobacterium* sp. (CRL-26) and 40°C for *Methylococcus capsulatus* (Texas). Metal-chelating agents inhibited the production of methylketones, suggesting the involvement of a metal-containing enzymatic system in the oxidation of *n*-alkanes to the corresponding methylketones. The soluble crude extracts derived from methane-utilizing bacteria contained an oxidized nicotinamide adenine dinucleotide-dependent dehydrogenase which catalyzed the oxidation of secondary alcohols.

In the companion paper (14), we described the production of secondary alcohols from their corresponding *n*-alkanes by cell suspensions of methane-utilizing bacteria. We also demonstrated that particulate fractions derived from methane-utilizing bacteria catalyzed the hydroxylation of *n*-alkanes in the presence of reduced nicotinamide adenine dinucleotide (NADH) as a cofactor. Both primary and secondary alcohols were detected as the products of *n*-alkane oxidation.

In this report, we describe the production of methylketones from their corresponding *n*-alkanes by cell suspensions of various known strains as well as newly discovered strains of the obligate and facultative methane-utilizing bacteria. The effects of various environmental factors on the production of methylketones from *n*-alkanes by cell suspensions of three distinct groups of methane-utilizing bacteria were investigated.

We also demonstrate that soluble crude extracts derived from methane-utilizing bacteria contain an oxidized nicotinamide adenine dinucleotide (NAD^+)-dependent dehydrogenase which catalyzes the oxidation of secondary alcohols.

MATERIALS AND METHODS

Organisms. *Methylosinus trichosporium* (OB3b), *Methylosinus sporium* (5), *Methylocystis parvus* (OBBP), *Methylomonas methanica* (S_1), *Methylomonas albus* (BG8), and *Methylobacter capsulatus* (Y) were kindly provided by R. Whittenbury (School of Biological Sciences, University of Warwick, Coventry, Warwickshire, England). *Methylococcus capsulatus* (Texas) was obtained from A. J. Meyer (University of Miami, School of Medicine, Miami, Fla.). *Methylobacterium organophilum* (ATCC 24886) was obtained from R. S. Hanson (Department of Bacteriology, University of Wisconsin, Madison). Some organisms used in these studies were newly discovered strains of methane-utilizing bacteria isolated in our laboratories from soil and lake water samples by an enrichment culture technique with methane as the sole source of carbon and energy. The methane-utilizing organisms were maintained on mineral salts (3) agar plates in a desiccator under an atmosphere of methane and air (1:1, vol/vol) at 30°C.

Growth of organisms. Small-scale cultures of

methane-utilizing organisms were grown in 300-ml flasks containing 50 ml of mineral salts medium (3) with methane (methane and air, 1:1, vol/vol) as the sole source of carbon and energy.

Large-scale cultures of methane-utilizing organisms were grown in 2.8-liter flasks containing 800 ml of mineral salts medium (3) with methane as the sole source of carbon and energy. A 50-ml 24-h culture was used to inoculate large flasks.

Methylketone production. Cells were harvested during exponential growth by centrifugation of 800-ml cultures at 12,000 × *g* for 15 min. Cells were washed twice with 50 mM sodium phosphate buffer, pH 7.0, and the final pellet was suspended in about 12 ml of fresh buffer. A 0.5-ml amount of this washed-cell suspension was placed in 10-ml vials at 4°C which were sealed with a rubber cap. The gaseous phase of the vials was removed by vacuum and then replaced with a gas mixture of the substrate (e.g., propane or butane) and oxygen (1:1, vol/vol). In the case of liquid substrates (*n*-pentane, *n*-hexane), 5 to 10 μl of the substrate was put in the vials. The vials were then incubated at 30°C on a rotary shaker at 200 rpm.

Assay of methylketones. The methylketones (acetone, 2-butanone, 2-pentanone, 2-hexanone) formed by the oxidation of *n*-alkanes by cell suspensions of methylotrophic organisms were assayed by gas chromatography by using a stainless-steel column (20 ft by ⅛ inch [about 61 by 0.3 cm]) packed with 10% Carbowax 20M on 80100 Chromosorb W (The Perkin-Elmer Corp., Norwalk, Conn.). The column temperature was maintained isothermally at 130°C, and the carrier gas flow rate was 35 ml of helium per min. The various methylketone products were identified by retention time comparisons and cochromatography with authentic standards. The amounts of products formed were determined from the peak area, using a standard graph constructed with different concentrations of standard substrate injected in the gas chromatogram. Protein in the cell suspensions was determined by the method of Lowry et al. (7).

Preparation of crude extracts. Methane-grown cells were harvested during exponential growth by centrifugation at 12,000 × *g* for 15 min. The cell pellet was washed twice with 50 mM sodium phosphate buffer, pH 7.0. The final pellet (2 g, wet weight) was suspended in 10 ml of the same buffer. Packed-cell suspensions were disrupted by sonication with a Megason ultrasonic disintegrator at 4°C. The sonicated cell suspensions were centrifuged for 15 min at 30,000 × *g*. The supernatant liquid was termed the crude extract.

Enzyme assay. Oxidation of secondary alcohols by crude extracts was measured spectrophotometrically at 340 nm with NAD^+ as an electron acceptor. The reaction mixture, in a total volume of 3.0 ml, contained 50 mM phosphate buffer (pH 8.0), 5 μmol of NAD^+, crude extract, and substrate. The reactions were started by adding 100 μl of 0.1 M substrate, and the rate of NAD^+ reduction was measured. Protein was determined by the method of Lowry et al. (7).

RESULTS

Production of methylketones by methane-utilizing bacteria. Cell suspensions of various obligate and facultative methane-utilizing bacteria grown on methane catalyzed the oxidation *n*-alkanes to the corresponding methylketones. The product methylketones were estimated by gas chromatography retention time comparison and cochromatography with authentic standards. The rates of conversion of *n*-alkanes (propane and butane) to the corresponding methylketones (acetone and 2-butanone) by cell suspensions of some newly isolated strains of Whittenbury et al. (18) are shown in Table 1. The product methylketones accumulated extracellularly. The rates of production of methylketones varied among the various methane-utilizing bacteria. However, the average rates of acetone, 2-butanone, 2-pentanone, and 2-hexanone production were 1.2, 1.0, 0.15, and 0.025 μmol/h per 5.0 mg of protein in cell suspensions. The oxidation of *n*-alkanes was not catalyzed under anaerobic conditions. Primary alcohols and aldehydes were also detected in low amounts as products of *n*-alkane oxidation (e.g., propane and butane), but were rapdily metabolized further by cell suspensions. Secondary alcohols were detected as intermediates during oxidation of *n*-alkenes to the corresponding methylketones as described in the accompanying paper (14). Cell suspensions of methylotrophic organisms oxidized secondary alcohols to the corresponding methylketones.

We selected representatives of three distinct groups of methyltrophic organisms to analyze

TABLE 1. *Oxidation of n-alkanes to methylketones by cell suspensions of methane-utilizing organisms grown on methane*

Microorganism	Conversion rate[a] (μmol/h per 5 mg of protein)	
	n-Propane to acetone	*n*-Butane to 2-butanone
Known strains		
Methylosinus trichosporium (OB3b)	1.5	1.2
Methylosinus sporium (5)	1.1	0.58
Methylocystis parvus (OBBP)	1.8	0.45
Methylomonas methanica (S_1)	1.5	0.30
Methylomonas albus (BG8)	1.7	0.60
Methylobacter capsulatus (Y)	1.6	1.1
Methylococcus capsulatus (Texas)	1.2	0.52
Methylobacterium organophilum (XX)	1.8	1.0
Newly isolated strains		
Methylosinus sp. (CRL-15)	1.5	1.2
Methylomonas methanica (CRL-21)	1.1	1.5
Methylobacterium sp. (CRL-26)	1.4	1.0
Methylobacter sp. (CRL-M6)	1.0	1.0
Methylobacter bovis (CRL-M1Y)	0.5	0.90
Methylococcus capsulatus (CRL-M1)	1.4	2.0

[a] Products were identified by gas chromatography retention time comparisons with authentic standards.

the effect of environmental factors that influence the production of acetone and 2-butanone from oxidation of *n*-propane and *n*-butane, respectively. Washed-cell suspensions of a type I obligate methylotroph, *Methylococcus capsulatus* (Texas strain), a type II methylotroph, *Methylosinus* sp. (CRL-15), and a facultative methylotroph, *Methylobacterium* sp. (CRL-26), were used.

Time course of methylketone production. The oxidation of *n*-propane and *n*-butane to acetone and 2-butanone, respectively, by cell suspensions of the three representative organisms was linear during the first 60 min (Fig. 1 and 2). The rate of production of acetone and 2-butanone decreased upon further incubation. The reactions were carried out in 50 mM phosphate buffer, pH 7.0, at 30°C in a rotary water bath shaker. Heat-killed cell suspensions of each organism did not catalyze the oxidation of *n*-alkanes to methylketones.

Effect of cell concentration on methylketone production. The rate of production of acetone and 2-butanone was linear with cell protein concentration from 1 to 8 mg of cell protein per ml. With increasing cell protein concentrations, the rate of production of acetone and 2-butanone decreased (Fig. 3 and 4). The reactions were carried out in 50 mM phosphate buffer, pH 7.0, at 30°C on a rotary water bath shaker.

Effect of temperature and pH on methylketone production. The optimal temperature for the production of acetone and 2-butanone by cell suspensions of *Methylosinus* sp. (CRL-15)

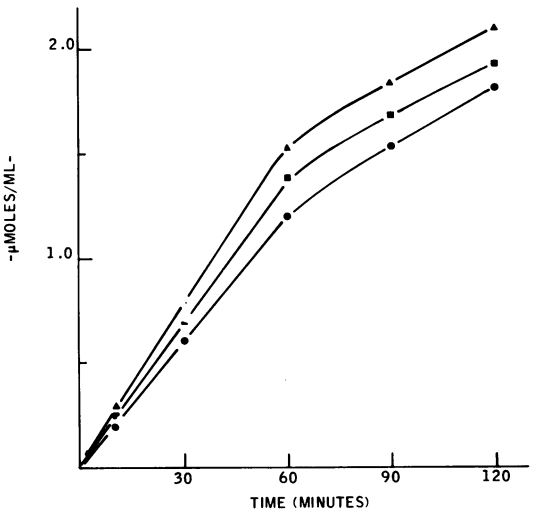

FIG. 1. *Time course of the production of acetone from oxidation of n-propane by cell suspensions of methylotrophs. The products were identified and estimated by gas chromatography. Symbols:* (△) *Methylosinus sp. (CRL-15);* (■) *Methylobacterium sp. (CRL-26);* (○) *Methylococcus capsulatus (Texas). The protein concentrations in cell suspensions of Methylosinus sp. (CRL-15), Methylococcus capsulatus (Texas), and Methylobacterium sp. (CRL-26) were 4.7, 5.0, and 5.1 mg/ml, respectively.*

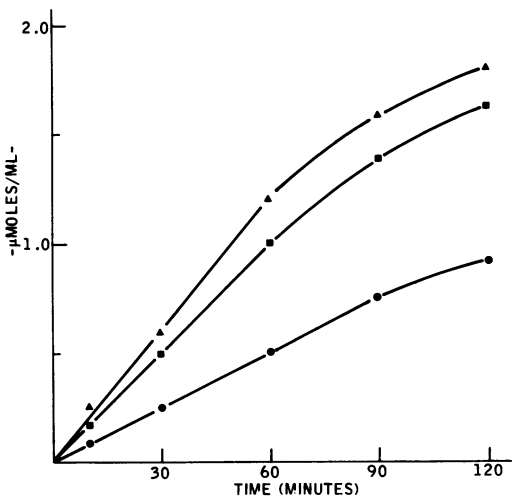

FIG. 2. *Time course of the production of 2-butanone from oxidation of n-butane by cell suspensions of methylotrophs. The products were identified and estimated by gas chromatography as described in Materials and Methods. Symbols are as in Fig. 1. The protein concentrations in cell suspensions of Methylosinus sp. (CRL-15), Methylobacterium sp. (CRL-26), and Methylococcus capsulatus (Texas) were 5.1, 5.0, and 5.2 mg/ml, respectively.*

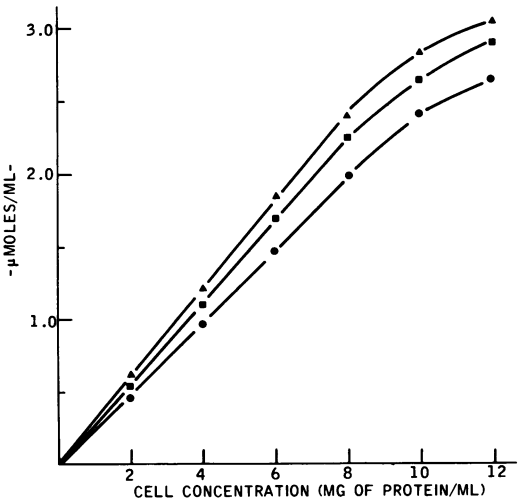

FIG. 3. *Effect of cell mass (protein) concentration on the production of acetone from oxidation of n-propane by cell suspensions of methylotrophs. The products were identified and estimated by gas chromatography as described in Materials and Methods. Symbols are as in Fig. 1.*

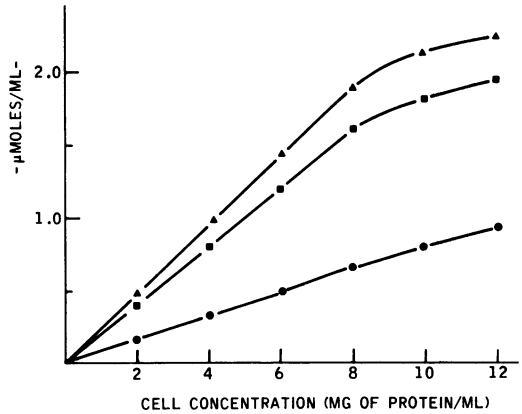

FIG. 4. *Effect of cell mass (protein) concentration on the production of 2-butanone from oxidation of n-butane by cell suspensions of methylotrophs. The products were identified and estimated by gas chromatography as described in Materials and Methods. Symbols are as in Fig. 1.*

and *Methylobacterium* sp. (CRL-26) was found to be around 35°C. Upon increasing the temperature to 40°C, the rate of production of methylketones from oxidation of *n*-alkanes decreased. In the case of *Methylococcus capsulatus* (Texas), the optimal temperature for the production of methylketones was found to be around 40°C (Fig. 5 and 6).

The optimal pH for the production of acetone and 2-butanone from the oxidation of *n*-propane and *n*-butane, respectively, by cell suspensions of *Methylococcus capsulatus* (Texas), *Methylosinus* sp. (CRL-15), and *Methylobacterium* sp. (CRL-26) was found to be around 7.0 (Fig. 7 and 8).

Inhibition studies. It was reported earlier that the oxidation of methane by cell suspensions of methylotrophs was inhibited by various metal-binding agents (11, 15). We examined the effect of metal-binding agents on the oxidation of *n*-propane and *n*-butane by various methylotrophs. Metal-binding agents such as α,α-bipyridyl, 1,10-phenanthroline, thiosemicarbazide, thiourea, and imidazole inhibited the oxidation of *n*-propane and *n*-butane to the corresponding methylketones by cell suspensions of *Methylosinus* sp. (CRL-15) (Table 2). Similar results were obtained with cell suspensions of *Methylococcus capsulatus* (Texas) and *Methylobacterium* sp. (CRL-26). This suggests the involvement of metal ion(s) in the oxidations of *n*-alkanes.

Substrate specificity. The substrate specificity for the oxidation of various *n*-alkanes was examined in cell suspensions of the three orga-

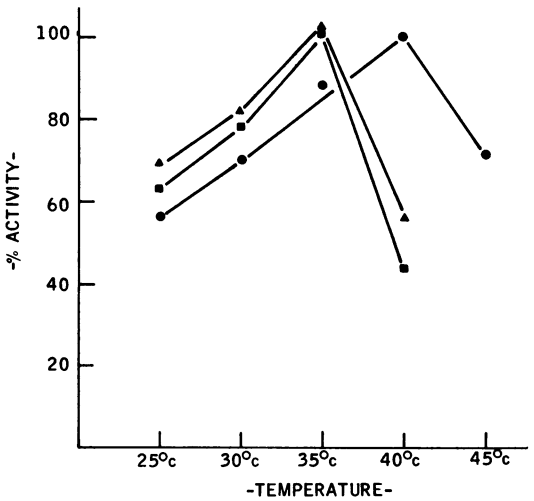

FIG. 5. *Effect of temperature on the production of acetone from the oxidation of n-propane by cell suspensions of methylotrophs. The products were identified and estimated by gas chromatography as described in Materials and Methods. Symbols are as in Fig. 1. 100% Activity = 1.9, 1.7, and 1.8 µmol of acetone produced/h per 5.0 mg of protein concentration in cell suspensions of Methylosinus sp. (CRL-15), Methylobacterium sp. (CRL-26), and Methylococcus capsulatus (Texas), respectively.*

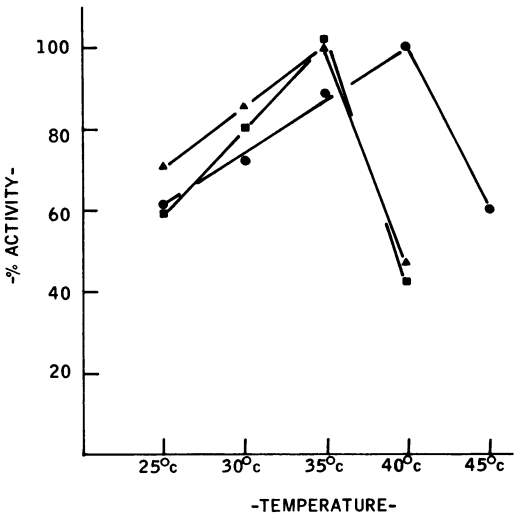

FIG. 6. *Effect of temperature on the production of 2-butanone from the oxidation of n-butane by cell suspensions of methylotrophs. The products were identified and estimated by gas chromatography as described in Materials and Methods. Symbols are as in Fig. 1. 100% activity = 1.5, 1.2, and 0.8 µmol of 2-butanone produced/h per 5.0 mg of protein concentration in cell suspensions of Methylosinus sp. (CRL-15), Methylobacterium sp. (CRL-26), and Methylococcus capsulatus (Texas), respectively.*

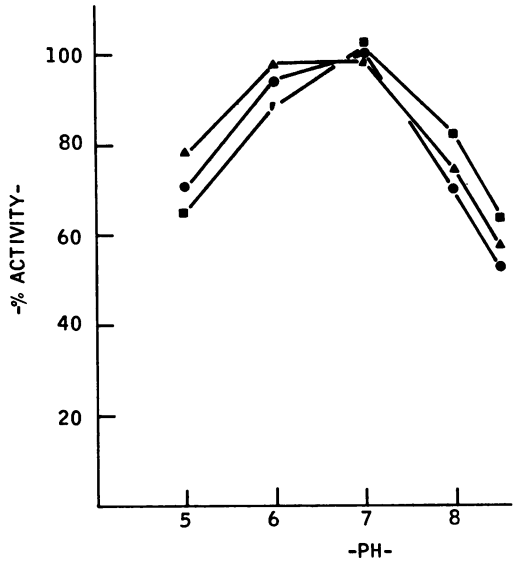

FIG. 7. *Effect of pH on the production of acetone from oxidation of n-propane by cell suspensions of methylotrophs. The products were identified and estimated by gas chromatography. Symbols are as in Fig. 1. 100% activity = 1.5, 1.4, and 1.2 μmol of acetone produced/h per 5.0 mg of protein concentration in cell suspensions of Methylosinus sp. (CRL-15), Methylobacterium sp. (CRL-26), and Methylococcus capsulatus (Texas), respectively.*

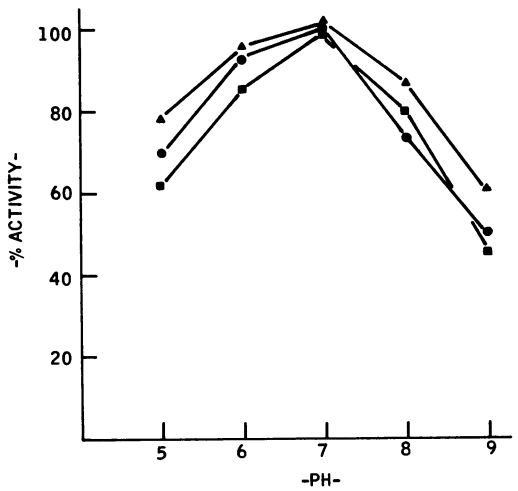

FIG. 8. *Effect of pH on the production of 2-butanone from oxidation of n-butane by cell suspensions of methylotrophs. The products were identified and estimated by gas chromatography. Symbols are as in Fig. 1. 100% activity = 1.2, 1.1, and 0.55 μmol of 2-butanone produced/h per 5.0 mg of protein in cell suspensions of Methylosinus sp. (CRL-15), Methylobacterium sp. (CRL-26), and Methylococcus capsulatus (Texas), respectively.*

nisms. Among *n*-alkanes, propane and butane were oxidized at higher rates than pentane and hexane. The products of oxidation of *n*-alkanes were identified as the corresponding methylketones by gas chromatography retention time comparison and cochromatography with authentic standards (Table 3).

Substrate competition experiments. The question of whether a methane monooxygenase that catalyzes the oxidation of methane in methylotrophs is involved in the oxidation of *n*-propane or *n*-butane was examined further by substrate competition experiments. The experiment consisted of determining the effect of methane on the production of acetone and 2-butanone from *n*-propane and *n*-butane, respectively. There was a twofold reduction in the amount of acetone and 2-butanone produced by cell suspensions of *Methylosinus* sp. (CRL-15) in the presence of methane (Table 4). Similar results

TABLE 2. *Inhibition of oxidation of n-alkanes to corresponding methylketones in cell suspensions of Methylosinus sp. (CRL-15)*

Inhibitor	Concn (M)	Inhibition (%)[a]	
		n-Propane to acetone	*n*-Butane to 2-butanone
α,α-Bipyridyl	10^{-3}	85	87
1,10-Phenanthroline	10^{-3}	84	89
Thiosemicarbazide	10^{-3}	66	69
Thiourea	10^{-3}	87	90
Imidazole	10^{-3}	92	90
Potassium cyanide	10^{-3}	95	98
Control	0	0	0

[a] Uninhibited rates of conversion of *n*-propane and *n*-butane to corresponding methylketones (acetone and 2-butanone) were 1.6 and 1.2 μmol/h per 5 mg of protein, respectively.

TABLE 3. *Substrate specificity for n-alkane oxidation by methane-utilizing organisms*

Microorganism	Conversion rate[a] (μmol/h per 5 mg of protein)			
	n-Propane to acetone	*n*-Butane 2-butanone	*n*-Pentane to 2-pentanone	*n*-Hexane to 2-hexanone
Methylosinus sp. (CRL-15)	1.5	1.2	0.2	0.03
Methylococcus capsulatus (Texas)	1.2	0.52	0.11	0.02
Methylobacterium sp. (CRL-26)	1.4	1.0	0.15	0.024

[a] Products were identified by gas chromatography retention time comparisons with authentic standards.

were obtained with cell suspensions of *Methylococcus capsulatus* (Texas) and *Methylobacterium* sp. (CRL-26). This suggests that the oxidation of methane and of the other *n*-alkanes is catalyzed by the same or a similar enzyme system.

Cell-free system. In the companion paper (14), we demonstrated that the particulate fractions derived from various methane-grown bacteria catalyzed an NADH-dependent hydroxylation of propane and butane. Both primary and secondary alcohols were detected as the products of *n*-alkane oxidation.

The soluble crude extracts derived from various methane-utilizing bacteria grown on methane catalyzed an NAD^+-dependent dehydrogenation of secondary alcohols (C_3 to C_6) to the corresponding methylketones. Primary alcohols were not oxidized by the NAD^+-linked secondary alcohol dehydrogenase. The rates of oxidation of 2-butanol with crude extracts of various organisms are shown in the Table 5.

DISCUSSION

By using a cooxidation technique, Leadbetter and Foster (5, 6) first reported the production of methylketones from *n*-alkanes during growth of *Pseudomonas methanica* on methane.

In this report we demonstrate the oxidation of *n*-alkanes to the corresponding methylketones directly by using washed-cell suspensions of various methylotrophic bacteria. The production of methylketones was observed only with cells that had been grown on methane. Methanol-grown cells did not oxidize *n*-alkanes.

Primary alcohols and aldehydes are oxidized rapidly by cell suspensions of methane-utilizing bacteria (8, 9, 11) and hence are not accumulated in high levels. Methane-utilizing organisms generally contain a methanol dehydrogenase (8, 9) which catalyzes the oxidation of primary alcohols. Secondary alcohols are not oxidized by methanol dehydrogenase. Oxidation of aldehydes is catalyzed by an aldehyde dehydrogenase (10, 11). Secondary alcohols have been detected as intermediates during *n*-alkane oxidation (14). We have also demonstrated that cell suspensions of methylotrophic organisms grown on methane or methanol catalyze the oxidation of secondary alcohols to the corresponding methylketones (4, 12). The soluble crude extracts derived from various methane-utilizing bacteria contain an NAD^+-linked secondary alcohol dehydrogenase as described previously (4, 12) and in this report. Primary alcohols are not oxidized by secondary alcohol dehydrogenase.

The soluble methane monooxygenase (1) for *Methylococcus capsulatus* (Bath) catalyzes the NADH- and oxygen-dependent oxidation of various *n*-alkanes (C_2 to C_8). Both primary and secondary alcohols are produced from *n*-alkanes. Unlike *Methylococcus capsulatus* (Bath), it has been reported that *Methylosinus trichosporium* (OB3b) (17), *Methylococcus capsulatus* (Texas) (15), and *Methylomonas methanica* (2) contain particulate methane monooxygenase activity which catalyzes the oxidation of *n*-alkanes. We have demonstrated the hydroxylation of *n*-alkanes to the corresponding alcohols (primary and secondary) in a cell-free system derived from methane-utilizing organisms (13).

The oxidation of *n*-alkanes to the corresponding methylketones was inhibited by metal-binding agents. These results suggest that the oxidation of methane and of other *n*-alkanes may be catalyzed by a metal-containing methane monooxygenase. The inhibition of oxidation of *n*-alkanes to the corresponding methylketones in the presence of methane suggests this possibility.

Recently, Thompson et al. (16) reported the production of acetone during the oxidation of ethane by cell suspensions of the methane-utilizing bacterium *Methylosinus trichosporium* (OB3b). The acetone production occurred by a

TABLE 4. *Effect of methane on the oxidation of n-alkanes to the corresponding methylketones by cell suspensions of Methylosinus sp. (CRL-15)*

Substrate	Product formed[a]	Concn (μmol/h per mg of protein)
n-Propane-helium-O_2 (25:25:50)	Acetone	1.6
n-Propane-methane-O_2 (25:25:50)	Acetone	0.85
n-Butane-helium-O_2 (25:25:50)	2-Butanone	1.2
n-Butane-methane-O_2 (25:25:50)	2-Butanone	0.72

[a] Products were identified and estimated by gas chromatography retention time comparison with authentic standards.

TABLE 5. *Oxidation of 2-butanol by crude extracts of organisms*

Organism	Sp act (nmol of NAD^+ reduced/min per mg of protein)
Methylosinus sp. (CRL-15)	4.0
Methylococcus sp. (CRL-24)	3.0
Methylobacterium sp. (CRL-26)	2.1
Methylosinus trichosporium (OB3b)	4.2

different mechanism (via 3-hydroxybutyrate and acetoacetate) from that proposed here.

ACKNOWLEDGMENT

We are grateful to Nancy Barnabe for her skillful technical assistance.

LITERATURE CITED

1. **Colby, J., D. I. Stirling, and H. Dalton.** 1977. The soluble methane monooxygenase of *Methylococcus capsulatus* (Bath). Its ability to oxygenate n-alkanes, n-alkenes, ethers, and alicyclic, aromatic and heterocyclic compounds. Biochem. J. **165**:395–402.
2. **Ferenci, T., T. Strom, and J. R. Quayle.** 1975. Oxidation of carbon monoxide and methane by *Pseudomonas methanica*. J. Gen. Microbiol. **91**:79–91.
3. **Foster, J. W., and R. H. Davis.** 1966. A methane-dependent coccus, with notes on classification and nomenclature of obligate methane-utilizing bacteria. J. Bacteriol **91**:1924–1931.
4. **Hou, C. T., R. N. Patel, A. I. Laskin, N. Barnabe, and I. Marczak.** 1979. Microbial oxidation of gaseous hydrocarbons: production of methylketones from their corresponding secondary alcohols by methane and methanol-grown microbes. Appl. Environ. Microbiol. **38**:135–142.
5. **Leadbetter, E. R., and J. W. Foster.** 1958. Studies on some methane-utilizing bacteria. Arch. Microbiol. **30**: 91–118.
6. **Leadbetter, E. R., and J. W. Foster.** 1960. Bacterial oxidation of gaseous alkanes. Arch. Mikrobiol. **35**:92–104.
7. **Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall.** 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. **193**:265–275.
8. **Patel, R. N., and A. Felix.** 1976. Microbial oxidation of methane and methanol: crystallization and properties of methanol dehydrogenase from *Methylosinus sporium*. J. Bacteriol. **128**:413–424.
9. **Patel, R. N., C. T. Hou, and A. Felix.** 1978. Microbial oxidation of methane and methanol: crystallization of methanol dehydrogenase and properties of holo- and apo-methanol dehydrogenase from *Methylomonas methanica*. J. Bacteriol. **133**:641–649.
10. **Patel, R. N., C. T. Hou, and A. Felix.** 1978. Microbial oxidation of methane and methanol: isolation of methane-utilizing bacteria and characterization of a facultative methane-utilizing isolate. J. Bacteriol. **136**:352–358.
11. **Patel, R. N., C. T. Hou, and A. Felix.** 1979. Microbial oxidation of methane and methanol: purification and properties of a heme containing aldehyde dehydrogenase from *Methylomonas methylovora*. Arch. Microbiol. **122**:241–247.
12. **Patel, R. N., C. T. Hou, A. I. Laskin, P. Derelanko, and A. Felix.** 1979. Microbial oxidation of methanol: oxidation of secondary alcohols to methylketones by yeasts. Appl. Environ. Microbiol. **38**:209–223.
13. **Patel, R. N., C. T. Hou, A. I. Laskin, A. Felix, and P. Derelanko.** 1979. Microbial oxidation of gaseous hydrocarbons: hydroxylation of *n*-alkanes and epoxidation of *n*-alkenes by cell-free particulate fractions. J. Bacteriol. **139**:675–679.
14. **Patel, R. N., C. T. Hou, A. I. Laskin, A. Felix, and P. Derelanko.** 1980. Microbial oxidation of gaseous hydrocarbons: production of secondary alcohols from corresponding *n*-alkanes by methane-utilizing bacteria. Appl. Environ. Microbiol. **39**:720–726.
15. **Ribbons, D. W.** 1975. Oxidation of C_1 compounds by particulate fractions from *Methylococcus capsulatus*: distribution and properties of methane-dependent reduced nicotinamide adenine dinucleotide oxidase (methane hydroxylase). J. Bacteriol. **122**:1351–1363.
16. **Thompson, A. W., J. G. O'Neill, and J. F. Wilkinson.** 1976. Acetone production by methylobacteria. Arch. Microbiol. **109**:243–246.
17. **Tonge, G. M., D. E. F. Harrison, and I. J. Higgins.** 1977. Purification and properties of the methane monooxygenase enzyme system from *Methylosinus trichosporium* OB3b. Biochem. J. **161**:333–344.
18. **Whittenbury, R., K. C. Phillips, and J. F. Wilkinson.** 1980. Enrichment, isolation, and some properties of methane-utilizing bacteria. J. Gen. Microbiol. **61**:205–218.