Microbial Oxidation of Gaseous Hydrocarbons: Epoxidation of 
C\textsubscript{2} to C\textsubscript{4} \textit{n}-Alkenes by Methylo\-trophic Bacteria

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Over 20 new cultures of methane-utilizing microbes, including obligate (types I and II) and facultative methylo\-trophic bacteria were isolated. In addition to their ability to oxidize methane to methanol, resting cell-suspensions of three distinct types of methane-grown bacteria \textit{(Methylos\textsubscript{5}}inos\textsubscript{5}}us \textit{trichosporium} OB3b [type II, obligate]; \textit{Methylococcus capsulatus} CRL M1 NRRL B-11219 [type I, obligate]; and \textit{Methyl\textsubscript{5}}obacterium \textit{organophilum} CRL-26 NRRL B-11222 [facul\textsubscript{5}}ative]) oxidize C\textsubscript{2} to C\textsubscript{4} \textit{n}-alkenes to their corresponding 1,2-epoxides. The product 1,2-epoxides are not further metabolized and accumulate extracellularly. Methanol-grown cells do not have either the epoxidation or the hydroxylation activities. Among the substrate gaseous alkenes, propylene is oxidized at the highest rate. Methane inhibits the epoxidation of propylene. The stoichiometry of the consumption of propylene and oxygen and the production of propylene oxide is 1:1:1. The optimal conditions for in vivo epoxidation are described. Results from inhibition studies indicate that the same monooxygenase system catalyzes both the hydroxylation and the epoxidation reactions. Both the hydroxylation and epoxidation activities are located in the cell-free particulate fraction precipitated between 10,000 and 40,000 \texttimes g centrifugation.

On the basis of \textsuperscript{18}O incorporation from \textsuperscript{18}O\textsubscript{2} into the cellular constituents of \textit{Pseudomonas methanica}, Leadbetter and Foster (9) suggested that the initial oxidative attack on methane involves an oxygenase. Higgins and Quayle (8) isolated CH\textsubscript{3}\textsuperscript{18}OH as the product of methane oxidation when suspensions of \textit{P. methanica} or \textit{Methanomonas methanooxidans} were allowed to oxidize methane in \textsuperscript{18}O\textsubscript{2}-enriched atmospheres. The subsequent observation of methane-stimulated reduced nicotinamide adenine dinucleotide (NADH) oxidation catalyzed by extracts of \textit{Methylococcus capsulatus} (14, 15) or \textit{Methylomonas capsulatus} (6) suggested that the enzyme responsible for this oxidation is a monooxygenase. These workers relied on indirect enzyme assays, measuring methane-stimulated NADH disappearance spectrophotometrically or methane-stimulated O\textsubscript{2} disappearance polarographically. Recently, methane monooxygenase systems were partially purified from \textit{Methylos\textsubscript{5}}inos\textsubscript{5}}us \textit{trichosporium} OB3b (16, 17) and \textit{Methylococcus capsulatus} (Bath) (3, 4).

The epoxidation of 1-alkenes was first demonstrated by Van der Linden (18), who detected the formation of 1,2-epoxyoctane from 1-octene by heptane-grown resting cells of \textit{P. aeruginosa}. Cardini and Jurshuk (2) found that a cell extract of a \textit{Corynebacterium} sp. oxidized 1-octene to 1,2-epoxyoctane in addition to hydroxylating octane to octanol. Coon and his co-workers (5) isolated an enzyme system from \textit{P. oleovorans} that catalyzed the hydroxylation of alkanes and fatty acids. We have also demonstrated the epoxidation of 1-octene by whole cells and a purified monooxygenase system of \textit{P. oleovorans} grown on \textit{n}-octane (1, 10, 11). The epoxidation system of the octane-grown \textit{P. oleovorans} was found not to be active on gaseous alkenes.

In the course of our studies of gaseous hydrocarbon-utilizing microbes, we discovered that resting cell suspensions of methylo\-trophic bacteria oxidize gaseous 1-alkenes to their corresponding 1,2-epoxyalkanes. Independently from our studies, Colby et al. reported the ability of a nonspecific, soluble methane monooxygenase from \textit{Methylococcus capsulatus} (Bath) to oxygenate \textit{n}-alkanes, \textit{n}-alkenes, ethers, and alicyclic, aromatic, and heterocyclic compounds (4). The present paper deals with the epoxidation of gaseous alkenes by resting cell suspensions of representative groups of methylo\-trophic bacteria. These are: \textit{M. trichosporium} OB3b (an obligate methylo\-troph with type II membrane structure); \textit{Methylococcus capsulatus} CRL M1 (an obligate methylo\-troph with type I membrane structure), and \textit{Methyl\textsubscript{5}}obacterium \textit{organophilum} CRL 26 (a facultative
methylotroph). Because of the instability of the monooxygenase system, the optimal conditions for the epoxidation were studied in cell suspensions of methylotrophs with particular reference to the epoxidation of propylene oxide. Subsequent studies revealed that the activity for both the epoxidation of propylene and the hydroxylat-

MATERIALS AND METHODS

Bacterial strains. Methane-utilizing cultures were isolated from lake water of Warinanco Park, Linden, N.J., and lake and soil samples of Bayway Refinery, Linden, N.J. (Table 1). These methylotrophs were identified by the classification given by Whittenbury et al. (19) and Patt et al. (13). M. trichosporium OB3b, Methyloinosinus sporum 5, Methylocystis parvus OB2P, Methylococcus methanica S, Methyloconas albus BG 8, and Methylobacter capsulatus Y were kindly provided by R. Wittenbury (School of Biological Sciences, University of Warwick, Coventry, the United Kingdom). M. organophilum XX ATCC 24886 was kindly provided by R. S. Hanson (University of Wisconsin, Madison). The organisms were maintained on mineral salt plates in a desiccator jar under an atmosphere of methane and air (1:1, vol/vol) at 30°C. Organisms were grown at 30°C in 300-ml flasks containing 50 ml of mineral salt medium (7) with methane and air (1:1, vol/vol) as the sole carbon and energy source. When methanol (at 0.3%) was used, the gaseous phase in the flask was air.

Chemicals. Gaseous hydrocarbons (methane, ethylene, propylene, 1-butene, and butadiene) and ethyl-

tene oxide were obtained from Matheson Gas Products (East Rutherford, N.J.). 1,2-Epoxypropane, 1,2-epoxy-

Propylene 1,2-epoxybutane, and 1-pentene were pur-

Preparation of cell-free particulate fraction. Organisms were harvested by centrifugation (5,000 ×

g, 30 min, 4°C) and washed twice with 0.05 M sodium phosphate buffer (pH 7.0) containing 5 mM MgCl2.

cells were suspended in the same buffer and disrupted by passing twice through a French pressure cell

Activity assay. When whole cells were used, the harvested cells from shake-flask cultures were washed twice with 0.05 M phosphate buffer (pH 7.0). The final pellet was resuspended in fresh buffer to obtain an optical density at 660 nm of 0.5. A 0.5-ml amount of this washed cell suspension (containing a given amount of dry cell mass) was placed in a 10-ml vial.

The vial was sealed with a rubber cap to minimize evaporation. The gaseous phase of the vial was re-

placed with a gas mixture containing 50% gaseous alkene and 50% pure oxygen. The reaction mixture was incubated at 30°C on a water bath rotary shaker (New Brunswick Scientific Co., Edison, N.J.) at 300 rpm. A 3-μl sample was removed with a syringe and was assayed by using a stainless-steel column (20 ft by

Retention time (msec)

retaining power of the column was maintained at 100°C, and the carrier gas flow was 35 ml of helium per min. The various epoxy products were identified by retention time comparisons and cochromatography with authentic standards. This identification was supplemented by observing the presence and absence of product peaks before and after bromination and acid hydrolysis. The amount of epoxy was determined from the peak area by using a standard curve which had been constructed with authentic epoxides. Duplicate measurements were performed for each assay. A typical gas chromatogram with authentic C3-hydrocarbons is shown in Fig. 1A.

The conditions used in Fig. 1A could not separate propylene oxide from propanol. To rule out the possibility that propanol was an oxidation product of propylene, the reaction mixture was assayed with a glass column (6 feet long [ca. 183 cm], 2 mm ID) 60/90 Tenax G.C. (Supelco, Inc., Bellefonte, Pa.). The column temperature was maintained isothermally at 180°C; carrier gas flow was 35 ml of helium per min. The retention times for propylene oxide and propanol were 13.5 and 15.7 min, respectively. Propylene glycol was assayed with this same Tenax G.C. glass column with column temperature maintained at 200°C.

Fig. 1. A typical gas chromatogram for authentic C3 hydrocarbons. The conditions for gas chromatog-

raphy were as described in the text. (A) 10% Carboxaw 20 M on 80/100 Chromosorb W column. (B) 60/80 Tenax GC glass column. Numerals: (1) n-propane; (2) propylene; (3) propylene oxide and propanol; (4) acetone; (5) isopropanol; (6) 1-propanol; (7) isobutanol; (8) allyl alcohol; (9) propylene oxide; (10) propanal.
For the cell-free system, the methane hydroxylation and propylene epoxidation activities were measured by determining methanol and propylene oxide formation by gas-liquid chromatography (GLC). A 0.2-ml portion of the particulate fraction (1 mg of protein) was placed in a 5-ml vial. The vial was sealed with a rubber cap to minimize evaporation. The gaseous phase of the vial was replaced with a gas mixture containing 50% methane or propylene and 50% pure oxygen. In the case of methane hydroxylation, the buffer concentration of the reaction mixture was raised to 0.15 M sodium phosphate to inhibit further oxidation of methanol (17). The reaction mixture was incubated at 30°C on a water bath rotary shaker as described above. A 3-μl sample was removed with a syringe and was assayed by GLC.

RESULTS

Resting cell suspensions of methane-grown cells of M. trichosporium OB3b, Methylococcus capsulatus CRL M1, and M. organophilum CRL 26 oxidized propylene to 1,2-epoxypropane, which accumulated. Methanol-grown cells showed no activity for either epoxidation of propylene or hydroxylation of methane. After the reaction, the cell suspensions were centrifuged to remove the cells. The product propylene oxide was found totally in the supernatant fraction in all the three strains tested, i.e., the product epoxide accumulated extracellularly. No product peak other than propylene oxide from propylene was detected. The possibility of propanal as an oxidation product of propylene was ruled out on the basis of GLC analysis (Fig. 1B). A comparison of the activity for epoxidation of propylene by methane-grown microbes is listed in Table 1. Epoxidation activity was found in all of the strains tested.

The optimal conditions for the production of propylene oxide were compared among three distinct types of methylotrophic bacteria.

Time course of epoxide formation. Control experiments with heat-killed cells indicated that the epoxide was produced enzymatically. With the standard assay system, the propylene oxide concentration reached a maximum after 3 h of incubation (Fig. 2). The rate of propylene oxide production was linear for the first 120 min for strains CRL M1 and OB3b and for the first 60 min for strain CRL 26. Therefore, epoxide production was measured within 1 h whenever the effect of a variable was tested.

The possibilities that the slower reaction rate after 1 to 2 h of incubation was due to enzymic or nonenzymic degradation of propylene oxide or due to product inhibition was examined. A 4.8-μmol amount of propylene oxide was added to viable or heated-killed cell suspensions in the presence or absence of substrate (propylene) and incubated under standard assay conditions. No disappearance of propylene oxide or formation of propylene glycol was detected during 6 h of incubation in either viable or heat-killed cell suspensions. However, after a prolonged incubation (24 h), a slight disappearance of propylene oxide and the formation of propylene glycol was observed. The amount of the further oxidation of propylene oxide was identical in both viable and heat-killed cell suspensions. Data shown in Fig. 3 indicate that there was no enzymic degradation of propylene oxide, and the nonenzymic degradation of propylene oxide was negligible under our assay conditions. The viable cells incubated with both propylene and propylene oxide showed further production of propyl-

| Table 1. Epoxidation of propylene to propylene oxide by methane-grown microbes* |
|-----------------|-----------------|-----------------|
| Methylophroths  | Epoxidation      | Epoxidation     |
|                 | rate (μmol/h)    | per mg of protein |
| Obligate, type II | CRL 15          | 2.2             |
| Methylosinus sp. | OB3b            | 1.8             |
| Methylosinus trichosporium | CRL 16 | 1.6             |
| Methylosinus sp. | 5               | 1.0             |
| Methylocystis sp. | CRL 18         | 0.7             |
| Methylocystis parvus | OBBP         | 0.8             |
| Obligate, type I  |                 |                 |
| Methylophroths  |                 |                 |
| Methylophroths  |                 |                 |
| Methylophroths  |                 |                 |
| **The reactions were conducted as described in the text. Strains with CRL numbers are newly isolated cultures in our lab.**
Propylene oxide was estimated by gas chromatography. Three milligrams of cells (dry weight) was used: Methylosinus trichosporium OB3b (●); Methylococcus capsulatus CRL M1 (■); Methylbacterium organophilum CRL-26 (▲).

Fig. 2. Time course of propylene oxide production by resting cell suspensions of methylotrophic bacteria. The reactions were conducted as described in the text. Propylene oxide was estimated by gas chromatography. Three milligrams of cells (dry weight) was used: Methylosinus trichosporium OB3b (●); Methylococcus capsulatus CRL M1 (■); Methylbacterium organophilum CRL-26 (▲).

Fig. 3. Degradation of propylene oxide by resting cell suspensions of Methylosinus capsulatus CRL M1. A 4.8-μmol amount of propylene oxide was added to each reaction mixture before incubation. Viable cell suspension plus propylene oxide (○); heat-killed cell suspension plus propylene oxide (▲); viable cell suspension plus propylene oxide and propylene (●). Four milligrams of cells was used in each experiment.

The initial oxygen partial pressure in the gaseous phase was kept constant (50%, vol/vol). Helium gas was used to balance the rest of the gaseous phase. The amount of propylene oxide produced was assayed after 30 min of incubation. A propylene concentration, in the gaseous phase, of ca. 15% (66 μmol) supported maximum propylene oxide production (Fig. 6). Higher propylene concentration did not stimulate nor inhibit the production of propylene oxide. The reaction rate appears to be dependent upon the solubility of both substrates, propylene and oxygen.

Optimum conditions for the production of propylene oxide. (i) pH. The effect of pH on propylene oxide production by resting cells of the three strains of methylotrophs was examined. Sodium phosphate buffer (0.05 M) was used for pH values from 5.5 to 8.0, and tris(hydroxymethyl)aminomethane buffer (0.05 M) was used for values from 8.0 to 10.0. A pH between 6.0 and 7.0 appeared to be optimum for epoxide production for all three of the methylotrophs (Fig. 4). The initial and final pH readings in these experiments differed by less than 0.5 pH unit, and similar amounts of epoxide were produced in either buffer at pH 8.0. Authentic samples of propylene oxide (final concentration, 4 μmol/ml) were added to heat-killed cell suspensions of Methylococcus capsulatus CRL M1 at pH 5.5, 7.0, and 10.0 to test for nonenzymatic degradation of propylene oxide at these pH values. The propylene oxide concentration in these suspensions did not decrease during 3 h of incubation, indicating that nonenzymatic oxidation or hydrolysis of propylene oxide was negligible under these assay conditions.

(ii) Temperature. The temperature optimum for the epoxidation of propylene by resting cell-suspensions of the three strains was about 35°C (Fig. 5). Strain OB3b showed a broader temperature optimum. At 40°C, there was an apparent decrease in the amount of epoxide accumulated. This is possibly due to both the instability of the monooxygenase system at higher temperature and the volatility of the product propylene oxide (bp 35°C).

(iii) Propylene concentration. Various concentrations of propylene were used to examine the production of propylene oxide by resting cell suspensions of Methylococcus capsulatus CRL M1. The initial oxygen partial pressure in the gaseous phase was kept constant (50%, vol/vol). Helium gas was used to balance the rest of the gaseous phase. The amount of propylene oxide produced was assayed after 30 min of incubation. A propylene concentration, in the gaseous phase, of ca. 15% (66 μmol) supported maximum propylene oxide production (Fig. 6). Higher propylene concentration did not stimulate nor inhibit the production of propylene oxide. The reaction rate appears to be dependent upon the solubility of both substrates, propylene and oxygen.
Stoichiometry of propylene oxidation. The stoichiometry of epoxidation of propylene by a cell suspension of *M. trichosporium* OB3b was examined. The amount of oxygen consumed during the reaction was determined polarographically with a Clark oxygen electrode. The propylene consumed and the propylene oxide formed were estimated by gas chromatography. The stoichiometry of consumption of propylene and oxygen and the production of propylene oxide was found to be approximately 1:1:1.

Substrate specificity. The substrate specificity for the epoxidation of 1-alkenes by the three strains of methylotrophs was determined. Epoxides were produced from gaseous alkenes only (Table 2). The highest production of epoxide was for propylene in all three strains.

Inhibition studies. The epoxidation of propylene to propylene oxide and the hydroxylation of methane to methanol by the three types of methane-utilizing bacteria were inhibited by metal-binding and metal-chelating agents such as potassium cyanide, 1,10-phenanthroline, a,a'-bipyridyl, thiourea, and imidazol (Table 3). This suggests the involvement of metal ion(s) in both the epoxidation of propylene and the hydroxylation of methane.

The effect of methane on the oxidation of propylene to propylene oxide was also studied. The production of propylene oxide from propylene by a cell suspension of *M. trichosporium* OB3b was assayed in the presence of a given amount of methane. The initial partial pressure of propylene in the gaseous phase was kept constant in all of the experiments. Methane inhibits the propylene oxidation reaction by the whole cell system of strain OB3b (Table 4).

(iv) Cell concentrations. The cell concentration also influences the rate of propylene oxide production. The amount of propylene oxide accumulated after 1 h of incubation increased as the cell concentration was increased up to about 9 mg/ml (Fig. 7). No further increase in propylene oxide production was observed at higher cell concentrations. This indicated that at cell concentrations higher than 9 mg/ml, the solubility of the gaseous substrates becomes the rate-limiting factor in the production of propylene oxide.
strated by will be pressed). (30%)

40,000 x both capsulatus epoxidation further centrifugation was found. were collected.

All of the activity in the crude extract was collected as a particulate fraction by further centrifugation of the crude extract at 40,000 x g for 90 min at 4°C. NADH stimulated both the epoxidation (20%) and the hydroxylation (30%) reactions. Further information on epoxidation of propylene by cell-free systems will be published (Patel et al., J. Bacteriol., in press).

**DISCUSSION**

Both the system of *P. aeruginosa* demonstrated by Van der Linden (18) and the system of *P. oleovorans* demonstrated in our laboratory (1, 10, 11) epoxidized liquid 1-alkenes from C6 to C12, but not gaseous alkenes.

In this report, we describe the epoxidation of ethylene, propylene, 1-butene, and butadiene by cell suspensions of all three distinct groups of methane-utilizing bacteria. The epoxidation of alkenes and the hydroxylation of methane were

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**TABLE 2. Oxidation of gaseous alkenes and methane by resting cell suspensions of methane-grown bacteria**

<table>
<thead>
<tr>
<th>Strains</th>
<th>Rate of oxidation (µmol/h per assay)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Methane to methanol</td>
</tr>
<tr>
<td>Obligate, type II membrane structure</td>
<td></td>
</tr>
<tr>
<td><em>Methylosinus trichosporium</em> OB3b</td>
<td>1.6</td>
</tr>
<tr>
<td>Obligate, type I membrane structure</td>
<td>2.5</td>
</tr>
<tr>
<td><em>Methylococcus capsulatus</em> CRL M1 NRRL B11219</td>
<td></td>
</tr>
<tr>
<td>Facultative</td>
<td></td>
</tr>
<tr>
<td><em>Methylobacterium organophilum</em> CRL 26 NRRL B-11222</td>
<td>0.7</td>
</tr>
</tbody>
</table>

* Two milligrams of cell (dry weight) per assay was used.

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**TABLE 3. Effect of inhibitors on the epoxidation of propylene and the hydroxylation of methane**

<table>
<thead>
<tr>
<th>Inhibitor</th>
<th>% Inhibition</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Epoxidation</td>
</tr>
<tr>
<td></td>
<td>Methylosinus trichosporium OB3b</td>
</tr>
<tr>
<td>Thiourea</td>
<td>100</td>
</tr>
<tr>
<td>1,10-Phenantrone</td>
<td>90</td>
</tr>
<tr>
<td>p,p'-Bipyridyl</td>
<td>100</td>
</tr>
<tr>
<td>Imidazole</td>
<td>95</td>
</tr>
<tr>
<td>Potassium cyanide</td>
<td>100</td>
</tr>
</tbody>
</table>

* The reactions were conducted as described in the text. The products were estimated by gas chromatography after 1 h of incubation at 30°C. Each inhibitor was added at a final concentration of 1 mM.

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**TABLE 4. Effect of methane on the epoxidation of propylene**

<table>
<thead>
<tr>
<th>Composition of gaseous phase</th>
<th>Propylene oxide formed (µmol)</th>
<th>% Inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Propylene + helium + O₂ (25:25:50, vol/vol)</td>
<td>1.6</td>
<td>0</td>
</tr>
<tr>
<td>Propylene + methane + O₂ (25:25:50, vol/vol)</td>
<td>0.8</td>
<td>50</td>
</tr>
</tbody>
</table>

* Reactions were conducted as described in the text except that various gaseous compositions were used to maintain a constant propylene partial pressure. Cell suspensions of methane-grown *M. trichosporium* OB3b (3.6 mg) were used. Propylene oxide was estimated by gas chromatography after 15 min of the incubation.
not found under anaerobic condition or in methanol-grown cells, suggesting that the enzyme system is inducible. The product 1,2-epoxides accumulated extracellularly. The nonenzymic degradation of propylene oxide in our standard assay system was not significant even after a prolonged incubation time (Fig. 3). Van der Linden (18) demonstrated the production of 1,2-epoxyoctane from 1-occtene by heptane-grown cells of Pseudomonas sp. and also stated that the epoxide was not further oxidized enzymatically. However, May and Abbott (10, 11) reported that when 1-occtene was supplied as a substrate to the $\omega$-hydroxylation enzyme system of P. oleovorans, both 8-hydroxy-1-occtene and 1,2-epoxyoctane were formed. In addition, Abbott and Hou (1) found that the methyl group of the latter compound was also susceptible to hydroxylation. The present results obtained from the studies of viable cell suspensions of the methane-utilizing bacteria, however, indicated that propylene oxide was not further metabolized enzymatically (Fig. 3).

Epoxide accumulation from 1-occtene by P. aeruginosa was accompanied by the metabolism of a large quantity of 1-occtene via methyl group oxidation (18). In the epoxidation of propylene by cell suspensions of methane-utilizing bacteria, however, no formation of 3-hydroxy propene-1 was detected.

Both the epoxidation of 1-alkene and the hydroxylation of methane were inhibited by various metal-binding and metal-chelating agents, indicating the involvement of metal(s)-containing enzyme system(s). The epoxidation of propylene to propylene oxide by a cell suspension of methane-grown strain CRL M1 was inhibited (50%) in the presence of the hydroxylation substrate methane (Table 4).

The optimum conditions for the in vivo epoxidation of propylene by the three distinct groups of methane-utilizing bacteria are quite similar. The pH optima were around 6 ~ 7, and the temperature optimum was around 35°C. The apparent decrease in epoxidation above 40°C may be due to both the instability of the mono-oxygenase system and the volatility of the product propylene oxide (bp, 35°C).

Both the hydroxylation and epoxidation activities are located in the cell-free particulate fraction precipitated between 10,000 and 40,000 $\times$ g centrifugation. Tonge et al. (16, 17) have reported the purification of a membrane-bound methane mono-oxygenase from the particulate fraction (precipitated between 10,000 and 150,000 $\times$ g centrifugation) of M. trichosporium OB3b. Recently, Colby et al. (4) demonstrated a unique soluble methane mono-oxygenase from Methyllococcus capsulatus (Bath strain) which catalyzes the oxidation of $n$-alkanes, $n$-alkenes, ethers, and alicyclic, aromatic, and heterocyclic compounds. The strains from the three distinct groups of methane-utilizing bacteria that we have examined all catalyze the epoxidation of gaseous alkenes (C2 to C6) and the hydroxylation of gaseous alkanes (C1 to C4). In contrast to Methyllococcus capsulatus (Bath), these methylo-trophs did not oxidize liquid alkanes and alkenes.

ACKNOWLEDGMENTS

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LITERATURE CITED

14. Ribbons, D. W. 1975. Oxidation of C, compounds by...


