Bacterial Utilization of Ether Glycols

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ABSTRACT

FINCHER, E. L. (University of Georgia, Athens) AND W. J. PAYNE. Bacterial utilization of ether glycols. Appl. Microbiol. **10**:542–547. 1962.—A soil bacterium capable of using oligo- and polyethylene glycols and ether alcohols as sole sources of carbon for aerobic growth was isolated. The effects of substituent groups added to the ether bonds on the acceptability of the compounds as substrates were studied. Mechanisms for the incorporation of two-carbon compounds were demonstrated by the observation that acetate, glyoxylate, ethylene glycol, and a number of the tricarboxylic acid cycle intermediates served as growth substrates in minimal media. The rate of oxidation of the short-chained ethylene glycols by adapted resting cells varied directly with increasing numbers of two-carbon units in the chains from one to four. The amount of oxygen consumed per carbon atom of oligo- and polyethylene glycols was 100% of theoretical, but only 67% of theoretical for ethylene glycol. Resting cells oxidized oligo- and polyethylene glycols with 2 to 600 two-carbon units in the chains. Longer chained polyethylene glycols (up to 6,000) were oxidized at a very slow rate by these cells. Dehydrogenation of triethylene glycol by adapted cells was observed, coupling the reaction with methylene blue reduction.

In 1959, over 1,300 million pounds of ethylene, diethylene, and triethylene glycols were produced in the USA, in addition to tremendous quantities of polymers of the glycols. These compounds are used in the manufacture of products as diverse as dynamite, shampoos, glue, antifreeze, cosmetics, pharmaceuticals, and synthetic detergents (syndets; Synthetic Organic Chemicals, 1959). Much of this material reaches our sewage disposal systems, and all of it may be expected to return to nature ultimately. The chemical structures of a great number of these compounds make them resistant to oxidative dissimilation by bacteria (Ludzack and Ettinger, 1960) and complicate disposal problems.

Approaching this problem by studying systems simulating biological sewage treatment, Lamb and Jenkins (1952), Mills and Stack (1954), Bogan and Sawyer (1954, 1955), and Sawyer, Bogan, and Simpson (1956) found aerobic dissimilation of a variety of glycols, ether alcohols, and polyethylene glycols to vary greatly in rate and extent. There have been, in addition, frequent reports of physiological and biochemical studies of oxidative catabolism of glycols by pure cultures or cell-free extracts of bacteria (den Dooren de Jong, 1926; Sebek and Randles, 1952; Juni and Heym, 1957; Kersters and De Ley, 1961). However, we found no reports of pure culture study of bacterial utilization of ether alcohol or oligo- or polyethylene glycol compounds as sole carbon sources.

The purpose of this paper is to present results of studies with a bacterium isolated from soil enriched with triethylene glycol. We observed that this isolant can be induced to utilize a number of ether alcohols as sole carbon sources, and that adapted whole cells contained oxidative enzymes for these compounds.

MATERIALS AND METHODS

Bacterium. The isolant used throughout this study was obtained from soil enriched for several days with aqueous solutions of triethylene glycol. The enriched soil was suspended in water and permitted to settle. Afterwards, loops dipped in the supernatant were streaked over minimal salts agar containing 0.25% triethylene glycol. Colonies appearing on plates incubated at 30 C for 5 to 7 days were picked and streaked for pure culture isolation. Detailed descriptions of the aerobic, gram-negative, asporogenous rod selected will be published elsewhere. The bacterium was kept in stock culture on nutrient agar or Trypticase Glucose Extract Agar slants and stored at 4 C. For experimental studies, the organism was cultured on a basal salts medium (pH 7.4) containing K_2HPO_4, 9.28 g; KH_2PO_4, 1,81 g; NH_4Cl, 0.5 g; $(NH_4)_2SO_4$, 0.5 g; Na_2SO_4, 0.5 g; and 0.1 g of $MgSO_4 \cdot 7H_2O$ per liter of distilled water with additions in the form of various glycols and ether alcohols as sole carbon and energy sources. In certain experiments, minimal quantities of yeast extract were added.

Growth studies. Growth on the various media was determined turbidimetrically with a model-6A Coleman Junior spectrophotometer, at 420 mμ for colorless media and 660 mμ for yellow-tinted media. The bacteria were cultured with continuous shaking at 30 C in 30-ml lots of appropriate media in 125-ml flasks with cuvettes attached for convenience of assay.

Cell-mass production on various media was determined by harvesting cells on tared Millipore filter pads, washing

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with distilled water, drying at 80 C overnight, and weighing.

Manometry. Cells to be assayed for oxidative activity were cultured at 30 C on a shaker for 72 to 96 hr in 0.04 M glycol-basal salts medium. The bacteria were harvested by centrifugation, washed twice in basal salts medium without glycol, and resuspended in the basal medium. The oxygen uptake of the suspensions incubated at 30 C with the various substrates was determined by standard manometric techniques (Umbreit, Burris, and Stauffer, 1957).

Dehydrogenase activity. Dehydrogenase activity of whole-cell suspensions of triethylene glycol-grown bacteria was determined by the Thunberg technique (Umbreit et al., 1957). The pH of the suspending phosphate buffer was varied from 6.4 to 8.0. The substrate was 100 μmoles of triethylene glycol per assay tube.

Results

Growth on glycols and ether alcohols. The results in Fig. 1 indicate that the soil isolant grew on triethylene glycol as a sole carbon source after a prolonged lag. Addition of a minimal quantity of yeast extract resulted in preferential utilization of this complex substrate, followed by a *diauxie* response (Monod, 1942) to the glycol. The bacterium apparently uses natural organic materials in preference to synthetic, but can rapidly adapt to less rich nutritional environments when the natural products are exhausted.

The data in Tables 1, 2, and 3 reveal that the bacterium grew on a number of glycol derivatives. Inspection of these results shows that polyethylene glycols with as many as 400 two-carbon units were acceptable as sole carbon and energy sources. The apparently anomalous growth on polyethylene glycol-1,500 is explained by the fact that it is a blend and contains polyethylene glycol-300. (This information was provided by Union Carbide Chemicals Co., New York, N.Y.)

Addition of various organic groups (other than more glycols or an acetyl group) to the basic ethylene glycol unit provided compounds which did not unequivocably support growth (Tables 2 and 3). Methyl, butyl, and phenyl groups attached to the oxygen of the ether bond impeded growth. However, as an exception, the addition of an ethyl group to the ethylene glycol (to produce ethylene glycol monoethyl ether) did not impede growth. Quite probably, only two of the four carbon atoms of

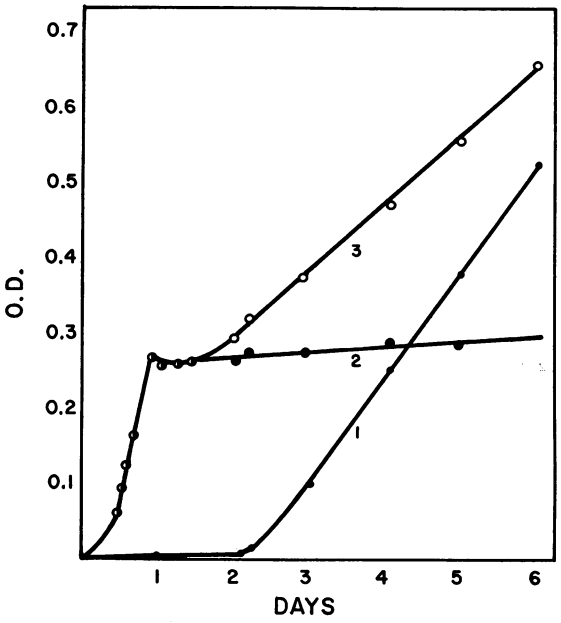

FIG. 1. *Growt h of a soil bacterium on triethylene glycol in minima and supplemented media.* (1) *Basal salts plus 0.25% triethylene glycol*; (2) *basal salts plus 0.1% yeast extract*; (3) *basal salts plus 0.25% triethylene glycol and 0.1% yeast extract.*

TABLE 1. *Utilization of glycols for growth by soil bacterium*

Compound	Structural formula	Growth
Ethylene glycol	$CH_2(OH)—CH_2OH$	++*
Diethylene glycol	$CH_2(OH)CH_2—O—CH_2CH_2OH$	++
Triethylene glycol	$CH_2(OH)CH_2—O—CH_2CH_2—O—CH_2CH_2OH$	++
Tetraethylene glycol	$CH_2(OH)CH_2—O—(CH_2CH_2—O)_2—CH_2CH_2OH$	++
Polyethylene glycol-200	$HO(CH_2CH_2—O)_{200}H$	++
Polyethylene glycol-300	$HO(CH_2CH_2—O)_{300}H$	++
Polyethylene glycol-400	$HO(CH_2CH_2—O)_{400}H$	++
Polyethylene glycol-600	$HO(CH_2CH_2—O)_{600}H$	−
Polyethylene glycol-1,000	$HO(CH_2CH_2—O)_{1,000}H$	−
Polyethylene glycol-1,500	$HO(CH_2CH_2—O)_{1,500}H$	++
Polyethylene glycol-1,540	$HO(CH_2CH_2—O)_{1,540}H$	±
Polyethylene glycol-4,000	$HO(CH_2CH_2—O)_{4,000}H$	−
Polyethylene glycol-6,000	$HO(CH_2CH_2—O)_{6,000}H$	−
Propylene glycol	$CH_3CH(OH)CH_2OH$	+
Dipropylene glycol	$CH_3CH(OH)CH_2—O—CH_2CH(OH)CH_3$	+
Trimethyl glycol	$CH_2(OH)CH_2CH_2OH$	±
2,5-Hexanediol	$CH_3CH(OH)CH_2CH_2CH(OH)CH_3$	±

* Symbols: ++ = rapid, growth observed within 7 days; + = intermediate, growth observed after 7 days but within 30 days; ± = border line, scarcely detectable growth in some experiments, not reproducible; − = negative.

TABLE 2. *Utilization of ether alcohols for growth by soil bacterium*

Compound	Structural formula	Growth
2-Methoxyethanol	$CH_3—O—CH_2CH_2OH$	−*
2-Ethoxyethanol	$CH_3CH_2—O—CH_2CH_2OH$	++
2-Butoxyethanol	$CH_3CH_2CH_2CH_2—O—CH_2CH_2OH$	−
2-(2-Methoxyethoxy)ethanol	$CH_3—O—CH_2CH_2—O—CH_2CH_2OH$	+
2-(2-Ethoxyethoxy)ethanol	$CH_3CH_2—O—CH_2CH_2—O—CH_2CH_2OH$	++
2-(2-Butoxyethoxy)ethanol	$CH_3CH_2CH_2CH_2—O—CH_2CH_2—O—CH_2CH_2OH$	+
2-Phenoxyethanol	$C_6H_5—O—CH_2CH_2OH$	−
3-Methoxy-1-butanol	$CH_3CH(OCH_3)CH_2CH_2OH$	±

* Symbols: ++ = rapid; + = intermediate; ± = borderline; − = negative, as in Table 1.

TABLE 3. *Utilization of ethers for growth by soil bacterium*

Compound	Structural formula	Growth
Ethylene glycol dimethyl ether	$CH_3—O—CH_2CH_2—O—CH_3$	±*
Bis-(2-methoxyethyl)ether	$CH_3(O—CH_2CH_2)_2—O—CH_3$	±
1,2-Bis(2-methoxyethoxy)ethane	$CH_3(O—CH_2CH_2)_3—O—CH_3$	±
Bis-2-(2-methoxyethoxy)ethyl ether	$CH_3(O—CH_2CH_2)_4—O—CH_3$	±
Bis(2-ethoxyethyl)ether	$(CH_3CH_2—O—CH_2CH_2)_2—O$	±
1,1-Dimethoxyethane	$CH_3—CH(O—CH_3)_2$	±
Diethoxymethane	$CH_2(O—C_2H_5)_2$	−
2,2-Diethoxypropane	$CH_3C(O—C_2H_5)_2CH_3$	±
1,4-Dioxane	$O—CH_2CH_2—O—CH_2CH_2$	−
1,2-Diphenoxyethane	$C_6H_5—O—CH_2CH_2—O—C_6H_5$	−
Hydroquinone dimethyl ether	$C_6H_4(OCH_3)_2$	−
2-(Benzyloxy)ethanol	$C_6H_5CH_2—O—CH_2CH_2OH$	−
1,2-Bis(2-chlorethoxy)ethane	$CH_2ClCH_2(O—CH_2CH_2)_2Cl$	−
Bis(2-chloroethyl)ether	$(CH_2ClCH_2)_2—O$	−
N-(2-methoxyethyl)acetamide	$CH_3CONHCH_2CH_2—O—CH_3$	±
Ethoxyacetic acid	$CH_3CH_2—O—CH_2COOH$	++
3-Methoxypropylamine	$CH_3—O—CH_2CH_2CH_2NH_2$	−
2-Methoxyethylamine	$CH_3—O—CH_2CH_2NH_2$	−
2-Ethoxyethyl acetate	$CH_3COOCH_2CH_2—O—CH_2CH_3$	++

* Symbols: ++ = rapid; ± = borderline; − = negative, as in Table 1.

TABLE 4. *Comparative dry cell mass of soil bacterium on triethylene glycol and 2-ethoxyethanol*

Substrate*	Dry cell wt (48 hr)	
	mg/ml	
Triethylene glycol	0.86	0.15†
2-Ethoxyethanol	0.23	0.06†

* At a level of 18.8 mM.
† Expressed as mg/g atom carbon.

the molecule were utilized, since only about one-third of the cell mass per carbon atom was observed with ethylene glycol monoethyl ether as with triethylene glycol (Table 4). If every carbon atom were used, the cell mass should be expected to be two-thirds that with triethylene glycol.

The results of these studies suggest that "two-carbon fragments" enter the metabolism of the bacterium, incorporated perhaps via the tricarboxylic acid cycle. The results in Fig. 2 indicate that acetate was acceptable as sole carbon substrate for the culture of this soil bacterium, and that the organism has mechanisms for incorporating naturally occurring, two-carbon compounds. The data in Table 5 confirmed a functional tricarboxylic

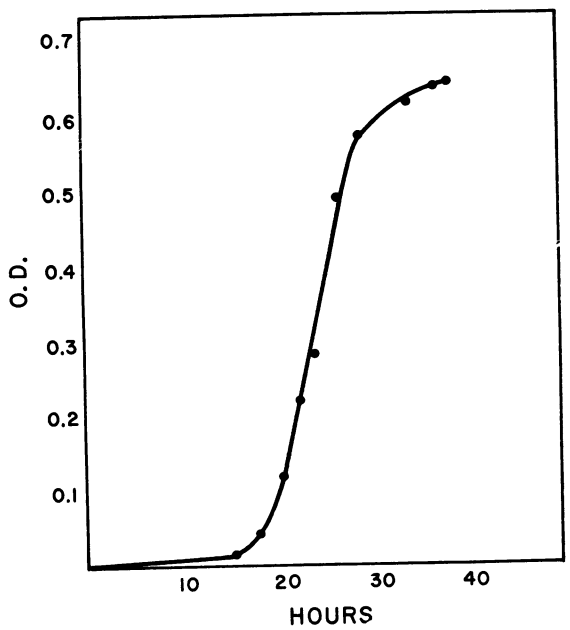

FIG. 2. *Growth of the soil bacterium on minimal salts medium with 0.25% acetate as the sole carbon source.*

acid cycle, since the bacterium grew well on the available intermediates (including glyoxylate), with a notable lag only with citrate.

Differential growth rates and cell mass obtained with glycols. Provision of the series, ethylene, diethylene, triethylene, and tetraethylene glycols, as sole carbon substrates for the growth of the soil isolant permitted measurement of the effects to two parameters simultaneously, i.e., effects of number of ethoxy units per molecule and of inclusion of ether bonds in the molecules. The lag periods and the times required to reach maximal cell concentration for cultures on the ethoxy glycols were shorter than on ethylene glycol (Table 6). However, the mass of cells obtained per gram-atom of carbon diminished as the

TABLE 5. *Utilization of compounds of the tricarboxylic acid cycle by soil bacterium*

Compound (0.01 M)	Lag	Lag to stationary growth period	Dry cell wt
	hr	*hr*	*mg/ml*
Acetate	13.5	19.5	0.79
Citrate	40.0	25.0	0.87
cis-Aconitate	17.0	37.5	0.42
α-Ketoglutarate	12.0	29.0	0.50
Succinate	17.0	10.5	0.60
Fumarate	10.0	15.0	0.59
l-Malate	18.0	21.0	0.61
Oxaloacetate	12.0	53.0	0.36
Glyoxylate	17.0	18.0	0.19

TABLE 6. *Comparative growth rates and dry cell weights resulting from growth of soil bacterium on several glycols*

Glycol (0.01 M)	Lag	Lag to stationary growth period	Dry cell wt	
	hr	*hr*	*mg/ml*	*mg/C*
Ethylene	32.0	61.5	0.34	0.17
Diethylene	27.5	56.5	0.72	0.18
Triethylene	18.0	46.0	0.86	0.14
Tetraethylene	23.0	46.0	0.84	0.10

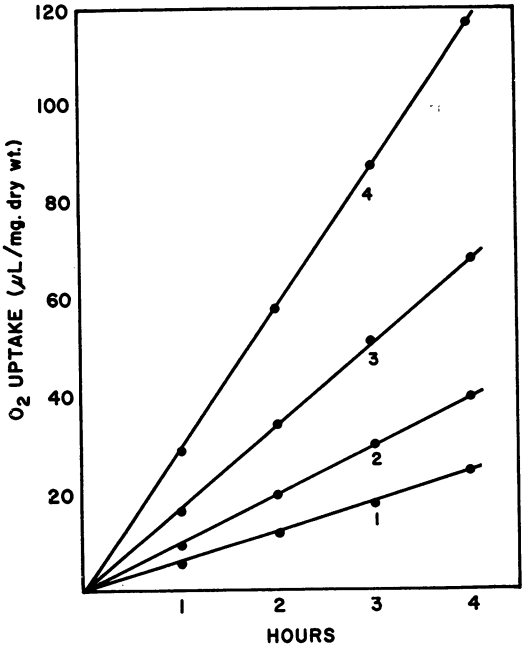

FIG. 3. *Oxidation of ethylene glycols by adjusted resting-cell suspensions of soil bacterium. Cells were cultured on basal salts medium plus the appropriate glycol.* (1) *Ethylene glycol*; (2) *diethylene glycol*; (3) *triethylene glycol*; *and* (4) *tetraethylene glycol. Substrate, 5 μmoles. Endogenous respiration subtracted.*

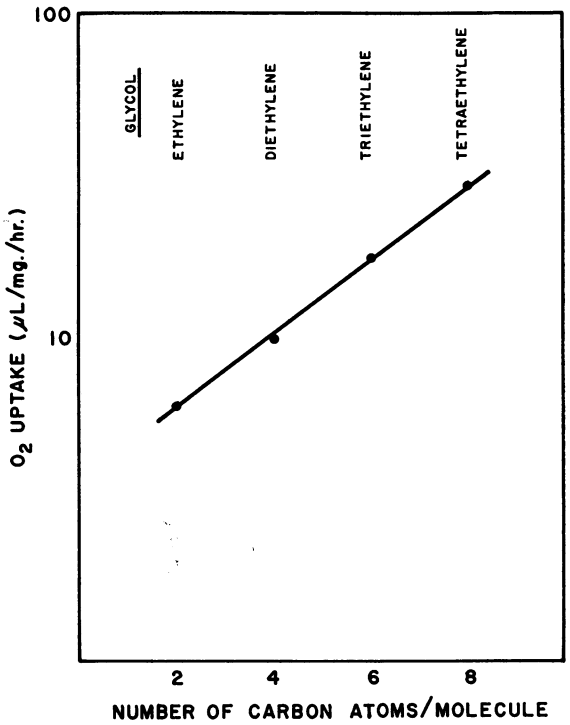

FIG. 4. *Rate of oxidation as effected by increasing chain length of ethylene glycols. Calculated from data in Fig. 3.*

TABLE 7. *Oxygen uptake by adapted cells of soil bacterium on glycol substrates in concentrations equivalent to 1 μmole of carbon*

Glycol	Oxygen uptake*
Ethylene	2.5
Diethylene	3.3
Triethylene	3.5
Tetraethylene	3.6

*Expressed as μliters of O_2 per mg of cells per hr per carbon atom.

TABLE 8. *Theoretical and actual oxygen uptake by adapted cells of soil bacterium on various substrates*

Substrate	Oxygen uptake (liters)		
	Theoretical	Actual	Per cent theoretical*
Ethylene glycol	56	38	67
Diethylene glycol	112	128	114
Triethylene glycol	168	155	92
Tetraethylene glycol	224	222	99

*Based on theoretical conversion of substrate to CO_2 and water.

number of two-carbon units in the molecules increased after the first addition.

Comparative rates of oxidation of glycols by resting cells. Culture of the soil isolant on ethylene, diethylene, triethylene, and tetraethylene glycols induced enzymes for the rapid oxidation of these substrates, whereas cells from broth culture were unable to dissimilate them (Fig. 3). The rate of oxidation varied directly and logarithmically with the length of the substrate chains (Fig. 4). However, when the oxygen consumption of the cells was calculated for each single carbon atom, it can be seen that the gas consumption with the three oligoethylene glycols was uniform and greater than that with ethylene glycol (Table 7). This interpretation is further substantiated by the data in Table 8. Whereas the actual uptake of oxygen in the oxidation of the ethoxy glycols was very nearly that theoretically required for complete oxidation of the substrates to CO_2 and water, only 67% of the theoretical was consumed with ethylene glycol.

Additional results, consistent with the assumption that there are differences in the catabolism of these oligoethylene glycols and ethylene glycol, are presented in Fig. 5. It is apparent that resting cells, adapted to tetraethylene glycol in culture, oxidized oligo- and polyethylene glycols ranging in chain length from 2 to 600 units, whereas approximately 10 μliters of oxygen were consumed in 4 hr with ethylene glycol. Furthermore, it can be seen that polyethylene glycols with chain lengths up to 6,000 were very slowly oxidized by these cells. With only slight variations, curve 3 (Fig. 5) could represent the very slow oxidation by the cells of other poor or unacceptable growth substrates such as dipropylene glycol, trimethyl glycol, 2,5-hexanediol, and 2-methoxyethanol.

Ethoxyethanol derivatives served as oxidizable substrates for the soil isolant. The results in Fig. 6 show that resting cells taken from cultures on the homologous substrates oxidized both 2-ethoxyethanol and 2-(2-butoxyethoxy)ethanol rapidly and extensively; the latter was oxidized despite its being a poor substrate for growth.

Dehydrogenase for triethylene glycol. A preliminary study with adapted cells revealed that the dehydrogenation of triethylene glycol can be coupled to methylene blue reduction. The results in Fig. 7 indicate that the reactions occurred more rapidly in alkaline medium than in acid medium. We were unsuccessful in attempts to determine pyridine nucleotide-linked dehydrogenation in experiments with cell-free extracts of these bacteria.

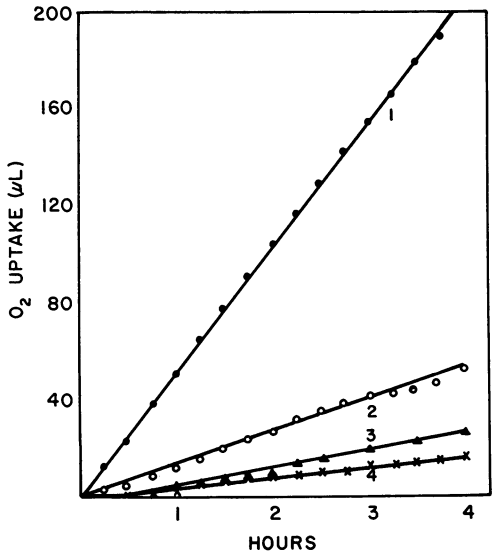

FIG. 5. *Oxidation of a variety of ethylene glycol derivatives by resting cells of the soil isolant grown on tetraethylene glycol.* (1) *Diethylene glycol*; (2) *polyethylene glycol-600*; (3) *po yethylene glycol-1,000, 4,000, or 6,000*; (4) *ethylene glycol. Substrate, 5 μmoles. Endogenous respiration subtracted.*

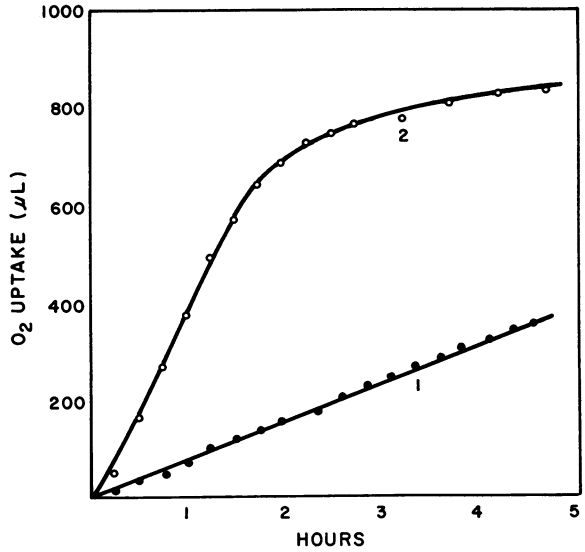

FIG. 6. *Oxidation of ethoxyethanols by adapted resting cells of the soil bacterium.* (1) *2-Ethoxyethanol;* (2) *2-(2-butoxyethoxy)ethanol. Substrate, 5 μmoles. Endogenous respiration subtracted.*

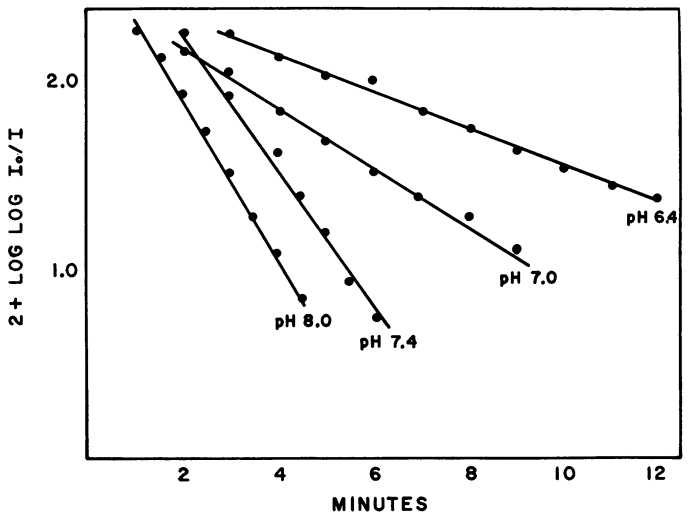

FIG. 7. *Effect of pH on dehydrogenation of triethylene glycol by adapted resting cells of the soil bacterium as indicated by methylene blue reduction.*

Discussion

An important biochemical problem was generated by this study: the mechanism by which bacteria break ether bonds in metabolizable compounds. This investigation was hampered by the lack of sensitive tests for the bond which could be used to determine cleavage.

From a practical viewpoint, it is perhaps most important to note that the bacterium isolated for this study was induced to grow on triethylene glycol only after available natural organic material was exhausted. Therefore, if a bacterium of this type should be put into a disposal system to oxidize synthetic organic compounds, on the basis of these findings it would be necessary to extend the usual period of digestion after the bulk of the natural organic products had been dissimilated.

It seems additionally significant that observations be made (as in this study) of the limitations to the versatility of bacteria which may be selected to accomplish the disposal of biologically persistent compounds. Clearly, any single isolant will be limited to oxidative dissimilation of those compounds having only certain chemical groups and bonds: those for which the organism has genetic information which will support formation of the appropriate induced enzyme. Our experiments indicate that the number of repeating units of a given, metabolizable group becomes an additional factor determining rate of degradation of polymers. Although a highly polymerized substrate may not serve as a growth substrate, it may yield to a slow oxidation by bacteria adapted to utilization of its subunits. Experimental determinations of the extent to which each of these factors operates might prove to be valuable criteria for collecting bacteria as inocula for disposal systems with specific, difficult degradation requirements.

However, the most significant contribution of this study is the affirmation that bacteria capable of oxidizing synthetic organic molecules can be isolated by the enrichment technique with the representative substrate as a sole source of carbon. The isolation of aerobic bacteria seems most appropriate in this instance, since the ultimate usefulness of the organisms is diminution of the biological oxidation demand in the disposal of wastes as rapidly as possible.

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