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Biodegradation of Ethylene Glycol by a Salt-Requiring Bacterium[1]

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A gram-negative nonmotile rod which was capable of using *1,2-*^{14}C-ethylene glycol as a sole carbon source for growth was isolated from a brine pond, Great Salt Lake, Utah. The bacterium (ATCC 27042) required at least 0.85% NaCl for growth and, although the chloride ion was replaceable by sulfate ion, the sodium ion was not replaceable by potassium ion. The maximal concentration of salt tolerated for growth was approximately 12%. The bacterium was oxidase-negative when *N,N*-dimethyl-*p*-phenylenediamine was used and weakly positive when *N,N,N',N'*-tetramethyl-*p*-phenylenediamine was used. It grows on many sugars but does not ferment them, it does not have an exogenous vitamin requirement, and it possesses a guanine plus cytosine ratio of 64.3%. Incorporation of ethylene glycol carbon into cell and respired CO_2 was quantitated by use of radioactive ethylene glycol and a force-aerated fermentor. Glucose suppressed ethylene glycol metabolism. Cells grown on ethylene and propylene glycol respired ethylene glycol in a Warburg respirometer more rapidly than cells grown on glucose. Spectrophotometric evidence was obtained for oxidation of glycolate to glyoxylate by a dialyzed cell extract.

Ethylene glycol ($HOCH_2 \cdot CH_2OH$) and sodium chloride are present in certain waste waters resulting from industrial syntheses of polyhydric chemicals. Biodegradative removal of ethylene glycol could be effected provided a bacterium could be obtained which could metabolize ethylene glycol and tolerate high concentrations of sodium chloride. This paper describes a bacterium capable of ethylene glycol metabolism and describes its assimilation and respiration of *1,2-*^{14}C-ethylene glycol.

MATERIALS AND METHODS

Bacterium. Bacterium T-52, deposited with the American Type Culture Collection as ATCC 27042, was isolated in 1969 from water of a solar brine pond, Temple Springs, Great Salt Lake, Utah. The bacterium was maintained on medium S. D. 7 described below.

Culture media. The basal medium S. D. 3.4 consisted of the following constituents, in grams/liter of glass-distilled water: $(NH_4)_2SO_4$, 0.8; K_2HPO_4, 7.5; KH_2PO_4, 1.0; $MgSO_4 \cdot 7H_2O$, 0.1; $FeSO_4 \cdot 7H_2O$, 0.01; $ZnSO_4 \cdot 7H_2O$, 0.0044, $CaCl_2 \cdot 2H_2O$, 0.0060; $CuCl_2 \cdot 2H_2O$, 0.00029; $MnSO_4 \cdot H_2O$, 0.0030; $(NH_4)_6MO_7O_{24} \cdot 4H_2O$, 0.00182; and NaCl, 34. Where indicated, a filter-sterilized solution of vitamins was added to give the following concentrations, in micrograms/liter of medium: biotin, 10; thiamine HCl, 100; pyridoxal, 100; calcium pantothenate, 100; *p*-aminobenzoic acid, 100; nicotinamide, 100; folic acid, 10; riboflavine, 100, and inositol, 1,000. The basal medium was modified where indicated by changing the concentration of NaCl. The number following the letters S. D. refers to percent NaCl (w/v). Medium S. D. 7 contains 70 g of NaCl per liter of medium. Filter-sterilized ethylene glycol was added at a concentration of 10 ml per liter unless indicated otherwise. Sugars and other carbon sources were autoclaved separately or filter-sterilized and added at a rate of 10 g/liter of medium. Cultures were grown either in 33 ml of medium dispensed per 125-ml Erlenmeyer flask or in 250 ml of medium contained in a force-aerated fermentor constructed from a gas washing bottle (14). Air was delivered through a sintered-glass tube at a rate of 0.4 volume of air per volume of medium per minute. Agitation was effected with a magnetic stirring bar by use of a nonheating magnetic stirrer. Respired CO_2 was trapped in 0.25 N NaOH.

Incubation. Cultures grown in 125-ml Erlenmeyer flasks were incubated at 24 C in a PsycroTherm with the platform revolving at 324 rev/min. Aerated fermentors and slant cultures were incubated at room temperature, which ranged from 27 to 29 C.

Growth. Growth was measured by reading optical

[1] This study was part of a thesis submitted by the senior author to the faculty of the graduate college, Texas A & M University, in partial fulfillment of the requirement for the M.S. degree.

density (OD), with a Coleman Junior spectrophotometer, at 640 nm and is expressed as milligrams of dry weight equivalent. A curve relating dry weight of this bacterium to OD was constructed for this purpose. Generation time was calculated graphically from growth curves and from the relationship: $gt = \log_{10} 2(t_1 - t_0)/\log_{10} OD_1 - \log_{10} OD_0$.

Glucose and ammonia in filtrates. D-Glucose was measured by the Somogyi procedure. Ammonia distilled from filtrates over MgO (4) and trapped in 2% boric acid containing methyl red and bromocresol green (15) was determined by titration.

Respiration. Oxygen consumption and carbon dioxide evolution were measured at 30.6 C by use of a Warburg respirometer and a total liquid volume of 3.2 ml. The gas phase was air. Dissolved CO_2 was driven out of the cell suspension at the end of a measurement with H_2SO_4. Side arms contained 12 μmoles of ethylene glycol, 12 μmoles of propylene glycol, or 6 μmoles of glucose. Cells were washed in 3.4% NaCl solution. Buffer consisted of 0.067 M phosphate, *p*H 6.1, containing 3.4% NaCl.

Radioactivity measurement. Freeze-dried cells or filtrate samples were wet-combusted by use of a modification of the van Slyke reagent (8). The generated CO_2 was trapped in 0.25 N NaOH, converted to $BaCO_3$ with $BaCl_2$ and NH_4Cl, and after washing with acetone and diethyl ether was collected on tared blue ribbon paper no. 589 (Schleicher & Schuell Co.). Radioactivity was measured with a Nuclear-Chicago C-110B counter equipped with a 183B scaler and printing timer (14). Readings were corrected for self-absorption by reference to an experimentally prepared self-absorption curve, background, and daily variation in machine performance. Respired CO_2 was trapped in 0.25 N NaOH and treated as described above.

Chemicals. *1,2-*^{14}*C*-ethylene glycol was purchased from International Chemical Co., Irvine, Calif., and was tested for authenticity by chromatographing on Whatman no. 1 paper sheets with ethyl acetate-acetic acid-water (6:2:2), spraying with alkaline silver nitrate, and comparing spots of authentic ethylene glycol with the radioactivity spot made visible by exposure to X-ray film. The oxidase test was performed with both *N,N*-dimethyl-*p*-phenylenediamine and *N,N,N',N'*-tetramethyl-*p*-phenylenediamine. Chemicals were of the highest grade available.

GC ratio. The guanine plus cytosine (GC) ratio of deoxyribonucleic acid (DNA) extracted from ca. 2 g of cells was measured in collaboration with M. Mandel, M. D. Anderson Hospital and Tumor Institute, Houston, Tex. Reference DNA (density of 1.742 g/cm^3) from bacteriophage 2C (*Bacillus subtilis*, host), the DNA isolation procedure of Mandel (1), the buoyant density procedure of Schildkraut (11), and a Spinco model E analytical centrifuge were used.

Glycolate oxidase assay. Cells grown on ethylene glycol and glycolate were examined for glycolate oxidase (EC 1.1.3.1) by the procedure of Zelitch and Ochoa (17) as modified by Furya and Hayashi (2). Cells grown in 10 liters of S. D. 4 medium containing either ethylene glycol or glycolate were collected with a Sorvall KSB Szent Gyorgyi & Blum continuous-flow system, 20,000 × *g* at 5 C, washed twice with 0.1 M phosphate buffer, *p*H 7.2, suspended in the same buffer, and disrupted with an Artek Ultrasonic Dismembrator with 30 min of treatment. The suspension was centrifuged at 20,000 × *g* for 30 min, and to the supernatant fluid was added solid ammonium sulfate to 30%. The precipitate was resuspended in the same buffer at a concentration of 5 to 10 mg of protein/ml and dialyzed for 6 hr at 4 C against the same buffer. Each cuvette contained 0.2 ml of 0.1 M sodium glycolate, 0.1 ml of 0.1 M KCN in 0.1 M NH_4OH, 0.35 ml of 0.01% 2,6-dichlorophenol-indophenol, 0.1 M phosphate buffer, *p*H 7.2, to a final volume of 2.8 ml, and finally 0.2 ml of enzyme preparation. Absorption was measured at 620 nm by use of a Beckman DU spectrophotometer fitted with a Gilford model 2000 multiple-sample absorbance recorder. One enzyme unit is defined as the amount of enzyme protein which caused a decrease in OD of 0.01 during the first minute.

Culture characteristics. Cells were stained with Rhodes (10) flagellar stain, and motility was determined by the hanging-drop technique. Cells negatively stained with phosphotungstate were examined for flagella with a Hitachi HU 11 electron microscope. Oxidase tests were made by applying a 1% aqueous solution of either *N,N*-dimethyl-*p*-phenylenediamine (12) or *N,N,N',N'*-tetramethyl-*p*-phenylene-

TABLE 1. *Utilization of carbon sources for growth*

Carbon source	Growth response[a]	Final *p*H
Acetate (sodium)	+	7.3
Adonitol	−	6.7
L-Arabinose	+	6.6
Citrate (sodium)	+	7.5
D-Glucose	+	6.5
D-Dulcitol	+	6.7
Ethylene glycol	+	6.7
D-Fructose	+	6.5
D-Galactose	−	6.7
Glycerol	+	6.7
Glycolate (sodium)	+	9.0
Glyoxylate (sodium)	+	8.9
m-Inositol	+	6.6
Lactose	+	6.5
Maltose	+	6.5
Malonate (sodium)	−	6.9
D-Mannitol	+	6.6
D-Raffinose	+	6.5
L-Rhamnose	+	6.6
Salicin	−	6.5
D-Sorbitol	+	6.5
Sucrose	+	6.6
Trehalose	+	6.6
D-Xylose	+	6.7

[a] Plus signs indicate turbidity in excess of that present in inoculated media to which no carbon source was added.

diamine (6) to filter paper followed by cells carried with a platinum loop. Biochemical tests were carried out in conventional fashion except that NaCl was added to at least 4% final concentration. Ethylene glycol, sodium glycolate, acetate, glyoxylate, malonate, and disaccharides were sterilized by filtration; all other carbon sources were autoclaved separately and added to media after cooling.

Protein assay. Protein in cell-free preparations was assayed as described by Layne (7).

Paper chromatography. Flash-evaporated samples of culture filtrate were chromatographed on Whatman no. 1 paper with *n*-propanol-water (7:3). Dried papers were sprayed with ninhydrin dissolved in water-saturated *n*-butanol.

TABLE 2. *Diagnostic characteristics of bacterium T-52*

Test or characteristic	Reaction
Casein hydrolysis	Negative
Catalase	Negative
Gelatin hydrolysis	Negative
Hemolysis	Gamma
Indole production	Negative
Litmus milk	Alkaline with slow hydrolysis
Methyl red test	Negative
Nitrate reduction	Positive
Oxidase	
N,*N*-dimethyl-*p*-phenylenediamine	Negative
N,*N*,*N*′,*N*′-tetramethyl-*p*-phenylenediamine	Weakly positive
Oxidative vs. fermentative[a]	Oxidative
Starch hydrolysis	Positive
Temperature allowing growth	
5 C	Positive
25 C	Positive
37 C	Positive
42 C	Positive
52 C	Negative
Triple sugar, iron	Alkaline/alkaline[b]
Urea hydrolysis	Negative

[a] High-Leifson agar. Cultures incubated in open air and in anaerobic jar.

[b] Alkaline reaction on slant and butt.

RESULTS

Characteristics of bacterium T-52. Bacterium T-52 is a gram-negative nonmotile rod, as determined by hanging-drop slide, Rhodes flagellar stain, and transmission electron microscopy. It does not grow anaerobically, does not produce acid or gas by the Durham tube method, and is oxidase-negative when *N*,*N*-dimethyl-*p*-phenylenediamine is used and weakly positive when *N*,*N*,*N*′,*N*′-tetramethyl-*p*-phenylenediamine is used. Several carbon sources are used for growth (Table 1). The results of various diagnostic tests are given in Table 2. The GC ratio is 64.3 moles percent. Approximately 0.85% NaCl was required for growth (Table 3), and growth occurred in the presence of approximately 12% NaCl (Table 4). Sodium ion could not be replaced by potassium ion, although chloride ion was replaced by sulfate ion (Table 5). An exogenous source of vitamins was not required (Table 6). Growth occurred within the *p*H range of approximately 5.2 to 10 (Fig. 1), with most rapid growth occurring close to *p*H 7. Growth occurred within the temperature range of 5 to 42 C (Table 2).

TABLE 4. *Growth in the presence of higher concentrations of sodium chloride*

Time (hr)	NaCl content of the medium						
	0%	3.4%	7.0%	10.0%	12%	15%	18–28%
40	0[a]	1,420	1,270	360	30	0	0
80	0	2,080	1,860	1,670		0	0
96	0	2,250	1,930	1,670	560	0	0
118	0	2,250	1,970	1,760	1,540	30	0
178	0	2,170	2,040	1,820	1,710	60	0
214	0	2,180	2,470	1,870	1,720	220	0
Final *p*H[b]	6.8	6.4	5.6	5.5	5.6	6.8	6.8

[a] Milligrams of dry cells per liter of culture. Average of two flask cultures.

[b] Initial *p*H was 6.8.

TABLE 3. *Growth in the presence of various concentrations of sodium chloride*

Time (hr)	NaCl content of the medium							
	0–0.5%	0.85%	1.0%	1.5%	1.8%	2.2%	2.5%	3.4%
48	0	36 ± 4[a]	45 ± 0	73 ± 3	73 ± 3	110 ± 0	150 ± 5	158 ± 17
72	0	45 ± 0	140 ± 10	720 ± 20	630 ± 3	580 ± 90	750 ± 78	840 ± 39
96	0	68 ± 35	800 ± 100	2,090 ± 9	2,080 ± 8	1,950 ± 40	2,010 ± 20	1,860 ± 7
104	0	88 ± 24	1,330 ± 41	2,210 ± 8	2,110 ± 20	2,070 ± 0	2,050 ± 20	1,870 ± 0
139	0	270 ± 5	319 ± 0	2,220 ± 0	2,170 ± 8	2,000 ± 46	2,030 ± 18	1,850 ± 7
188	0	1,620 ± 370	2,220 ± 0	—	—	—	2,080 ± 10	1,840 ± 24

[a] Milligrams of dry cells per liter of culture. Arithmetic mean and the standard error of three flask cultures.

TABLE 5. *Efficacy of potassium and sulfate ions in replacing sodium and chloride ions*

Ion content of medium	Cell yield[a]					Na^+ (mM)
	72 hr	96 hr	104 hr	163 hr	212 hr	
NaCl, 0.25%	0	0	0	0	0	42.7
NaCl, 0.85%	0	4 ± 0	4 ± 0	30 ± 1	30 ± 0	101.8
NaCl, 1%	10 ± 0	20 ± 0	10 ± 0	85 ± 39	1,620 ± 155	170
NaCl, 2.5%	235 ± 17	716 ± 53	1,026 ± 298	1,706 ± 6	1,660 ± 0	427
NaCl, 10%	71 ± 7	205 ± 28	280 ± 45	1,532 ± 5	1,610 ± 10	1,079
KCl, 0.25-10%	0	0	0	0	0	—
Na_2SO_4, 0.30%	0	0	0	0	0	42.7
Na_2SO_4, 1.03%	0	0	0	0	0	101
Na_2SO_4, 1.21%	0	10 ± 0	10 ± 0	20 ± 10	10 ± 0	170
Na_2SO_4, 3.0%	20 ± 5	65 ± 10	100 ± 0	1,790 ± 20	1,810 ± 0	427
Na_2SO_4, 12.15%	361 ± 36	1,263 ± 0	1,380 ± 20	1,440 ± 0	1,570 ± 10	1,079

[a] Milligrams of dry cells per liter of culture. Mean and standard error of three replicate cultures.

TABLE 6. *Growth in the presence and absence of added vitamins*[a]

Age (hr)	Cell yield[b]	
	Without vitamins	With vitamins
29	210	260
51	1,050	1,150

[a] See text for list of vitamins.
[b] Milligrams of dry cells per liter of culture.

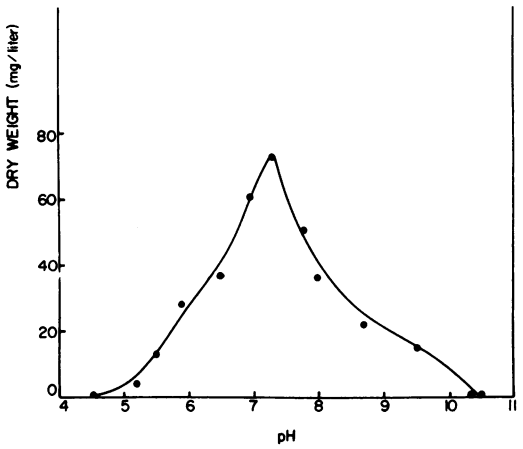
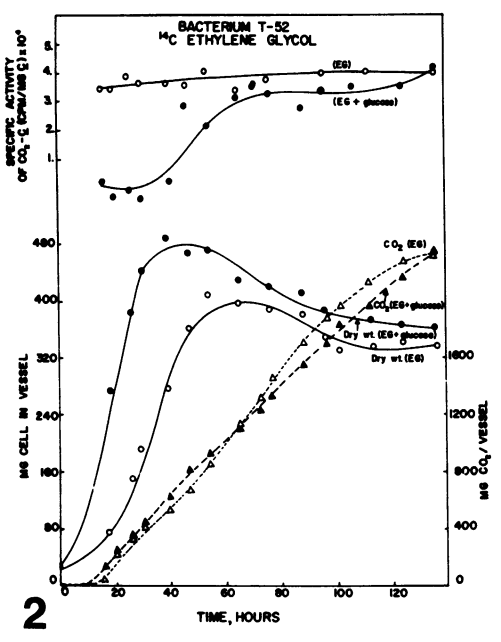


FIG. 1. *Effect of pH on growth at 36 hr. Medium contained ethylene glycol, ammonium sulfate, various proportions of mono-, di-, and tri-basic potassium salts of phosphate, and salts as described in Materials and Methods. Initial pH values were 4.5, 5.2, 5.5, 5.9, 6.5, 6.9, 7.3, 7.8, 8.0, 9.5, 10.3, and 10.5. Final pH values were 4.2, 4.5, 5.0, 5.6, 6.0, 6.5, 6.8, 7.5, 7.6, 9.0, 9.8, and 10.0. Growth was read early to avoid extensive pH changes.*

Growth on 1,2-^{14}C-ethylene glycol. Assimilation and respiration of ethylene glycol carbon was measured by use of the fermentor and medium described in Materials and Methods. One fermentor contained 2 g of ethylene glycol (0.4 μCi/ml); the other contained, in addition to radioactive ethylene glycol, 1 g of unlabeled D-glucose. In both instances, the 250 ml of completed medium was inoculated with the equivalent of 12 mg (dry weight) of cells in 2 ml of saline. Most rapid growth occurred in the fermentor containing glucose (Fig. 2); the minimal generation time was 13 hr. Trace ethylene glycol was metabolized during growth on glucose, as a small amount of radioactivity was present in respired CO_2 (Fig. 2). The specific activity of respired CO_2 increased rapidly after glucose was consumed (Fig. 3), indicating release of either inhibition of metabolism of ethylene glycol or inhibition of uptake of ethylene glycol (Fig. 2). The culture containing ethylene glycol as the sole carbon source grew more slowly, even though both fermentors were inoculated with cells grown on ethylene glycol, and the minimal generation time was approximately 16 hr (Fig. 2). The amount of respired CO_2 from the two cultures was approximately the same (Fig. 2). Both cultures were nitrogen-limited (Fig. 3), and both continued respiring after growth ceased. The cells were 44% C as determined by wet combustion, and they converted most of the carbon supplied by the carbon source to CO_2 (Fig. 2), a desirable feature of a process designed to remove organic matter from water. The distribution of carbon



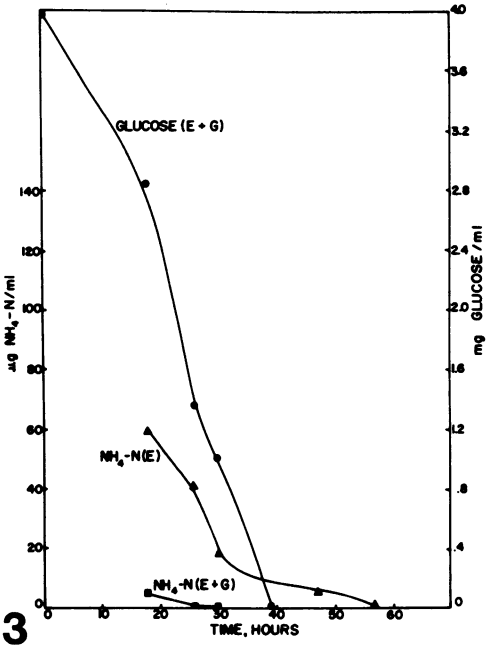


FIG. 2. *Increase in biomass, evolved CO_2, and specific activity of respired CO_2 of culture grown on 1,2-^{14}C-ethylene glycol (EG) and culture grown on radioactive ethylene glycol and unlabeled D-glucose (EG + glucose). Fermentation vessels fashioned from gas washing bottles (14) contained 250 ml of S. D. 7 medium, equivalent to 12 mg (dry weight) of inoculum, and were aerated with compressed air at a rate of 0.4 volume of air per volume of medium per minute. Respired CO_2 was trapped in 0.25 N NaOH. Specific activity of ethylene glycol in carbon in medium at beginning was 5.48 × 10^4 counts per min per mg of C.*

FIG. 3. *Decrease in glucose and ammonia-nitrogen of filtrates from cultures described in Fig. 2. Glucose was measured by the Somogyi method, and ammonia, by titration of ammonia distilled over magnesium oxide into boric acid.*

in cell, respired CO_2, and culture filtrate is given in Table 7. Under these conditions, the culture grown on ethylene glycol removed ethylene glycol at the rate of 7.8 g per liter of liquid per 136 hr (the inoculum consisted of 2 ml of cell suspension [see above]; if the more usual 5 or 10% inoculum concentration had been used, removal would no doubt have been much more rapid). The experiment was terminated on the 136th hr, as respiration by the culture containing ethylene glycol as the sole carbon source was then negligible (Fig. 2). At this time, 7% of the total radioactivity remained in the culture filtrate (Table 7); this radioactivity is assumed to reside in the main in unused ethylene glycol rather than leached out cell constituents, as paper chromatography of 40 ml of filtrate concentrated to 5 ml did not reveal the presence of amino acids. The final culture *p*H was 6.4.

Respiration. Cells grown on glucose respired glucose more readily than ethylene gly-

TABLE 7. *Distribution of ethylene glycol carbon in fermentor containing ethylene glycol as sole carbon source*[a]

Source	Ethylene glycol carbon[b]
Respired CO_2 (accumulative)[c]	82
Cell[d]	20
Remaining in filtrate[e]	7
Accounted for	109

[a] Results shown are for 136 hr. Cell yield was somewhat lower than at 54 hr when cell biomass had peaked (Fig. 2).

[b] Percentage of ethylene glycol carbon (774 mg [2 g × 38.7%]).

[c] Calculated from weight of $BaCO_3$.

[d] Calculated from cell dry weight and measured carbon content of 44%.

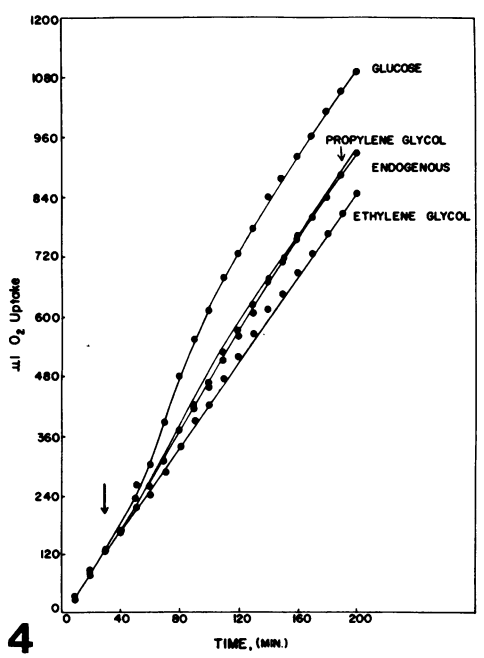
[e] Calculated from radioactivity remaining in culture filtrate (2.8680 × 10^6 counts per min divided by 4.2496 × 10^7).

col or propylene glycol (Fig. 4), and cells grown on either ethylene glycol or propylene glycol respired ethylene glycol more readily than glucose (Fig. 5 and 6). Q_{O_2} values calculated from these data are shown in Table 8. Clearly, rapid oxidative removal of ethylene glycol from waters would be facilitated by prior growth of the cells on ethylene glycol. Cells grown on propylene glycol oxidized ethylene glycol more rapidly than propylene glycol. Repetitions of this experiment confirmed the preferential oxidation of ethylene glycol over propylene glycol. The respiratory quotient (RQ) associated with oxidation of ethylene glycol was approximately 1 when the calculation was based on 3 hr of respiration and the CO_2 that was released after addition of acid at the end of 3 hr was included; the RQ was 0.77 when calculation was based on the first hour of respiration after tipping in ethylene glycol (Table 9).

Glycolate oxidase. Stouthamer et al. (13) studied ethylene glycol metabolism of *Acetobacter aceti* and speculated that it would be oxidized to glycolate. Bacterium T-52 can grow on glycolate and glyoxylate (Table 1), and it would seem reasonable to suspect that metabolism might involve oxidation of ethylene glycol to glycolate, oxidation of glycolate to glyoxylate, and entrance of the latter into the glyoxylate bypass or into glycerophosphate via tartronic semialdehyde (9). The enzyme glycolate oxidase was sought, as the presence of it in the cell would possibly afford a clue to ethylene glycol metabolism. Cells grown on ethylene glycol and on glycolate oxidized glycolate (Fig. 7).

DISCUSSION

Bacterium T-52. Bacterium T-52 was first thought to be a species of *Acinetobacter* because it is nonmotile and oxidase negative (16). However, the GC ratio (64.3%) is unlike that of *Acinetobacter* but is similar to that of *Pseudomonas* (16). Further, this bacterium does not produce acid oxidatively (16). Bacterium T-52 may be a previously undescribed bacterium, and hence it may not be possible to assign it to a known genus. Possibly this bacterium may be a nonmotile variant of a *Pseudomonas* species. Perhaps the brine environment



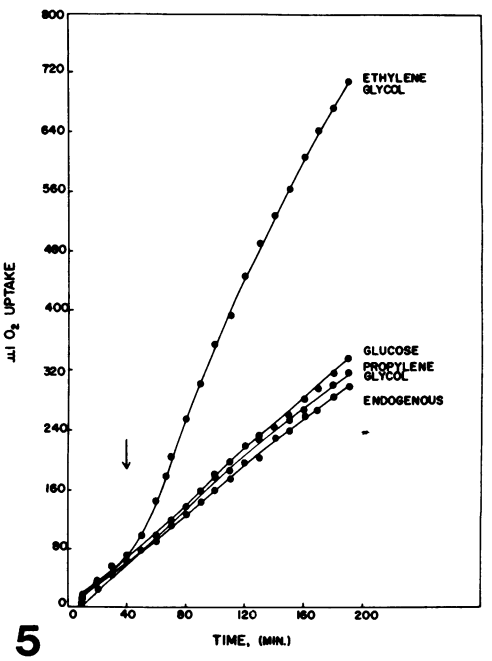


FIG. 4. *Oxygen uptake by glucose-grown cells in the presence of ethylene glycol, glucose, and propylene glycol. Endogenous uptake is uptake in absence of added substrate. Each flask received the equivalent of 12 mg (dry weight) of cells. Buffer contained 3.4% NaCl.*

FIG. 5. *Oxygen uptake by ethylene glycol-grown cells in the presence of ethylene glycol, glucose, and propylene glycol. Endogenous uptake is uptake in absence of added substrate. Each flask received equivalent of 6 mg (dry weight) of cells. Buffer contained 3.4% NaCl.*

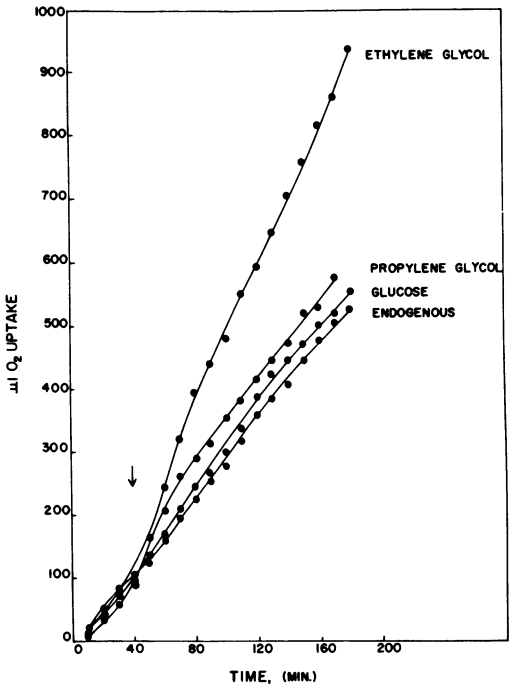


FIG. 6. *Oxygen uptake by propylene glycol-grown cells in the presence of ethylene glycol, glucose, and propylene glycol. Endogenous uptake is uptake in absence of added substrate. Each flask received the equivalent of 7 mg (dry weight) of cells. Buffer contained 3.4% NaCl.*

TABLE 8. *Oxidation of* D*-glucose, ethylene glycol, and propylene glycol*

Cells previously grown on	Carbon source oxidized	Q_{O_2}[a]
D-Glucose	Glucose	12.3
	Ethylene glycol	3.8
	Propylene glycol	6.2
Ethylene glycol	Glucose	3.6
	Ethylene glycol	21.5
	Propylene glycol	2.9
Propylene glycol	Glucose	6.9
	Ethylene glycol	28.7
	Propylene glycol	6.4

[a] Above endogenous.

from which it was isolated did not select for preservation of flagellar synthesis. Bacterium T-52 has been deposited with the American Type Culture Collection as ATCC 27042.

Growth on 1,2-^{14}C-ethylene glycol. Bacterium T-52 utilized ethylene glycol, although the rather long generation time (i.e., ca. 16 hr) when growing on synthetic medium indicates that it was not a preferred carbon source. Under conditions of nitrogen limitation, most of the ethylene glycol carbon appeared in respired CO_2 (Fig. 2). Use of nitrogen-limited waters in

TABLE 9. *Gas exchange and respiratory quotient associated with oxidation of ethylene glycol*

Parameter	Exogenous (endogenous subtracted)	Endogenous
O_2, μliters	266.9	112.8
CO_2, μliters	273.1	125.9
RQ[a]	1.02 (0.8)[b]	

[a] RQ is ratio of microliters of CO_2 to microliters of O_2.

[b] Calculated from first-hour gas exchange. Does not include CO_2 driven out with acid after 3 hr of incubation.

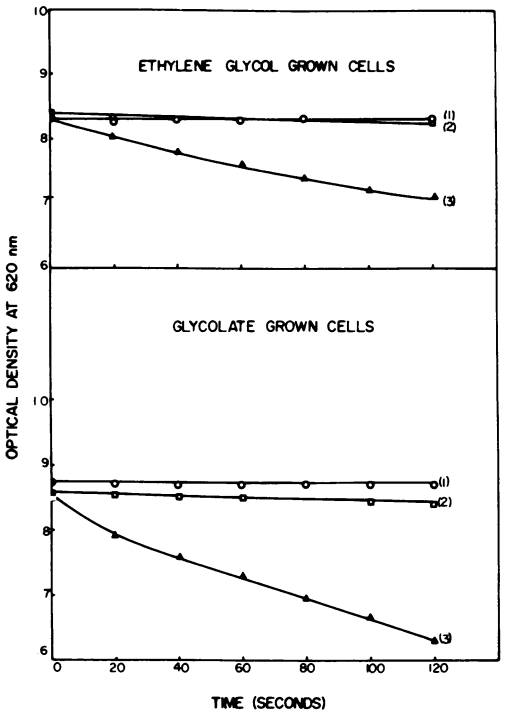


FIG. 7. *Spectrophotometric assay for the presence of glycolate oxidase in cells grown on ethylene glycol and on glycolate. The reaction cuvette contained reagents in the amounts listed in Materials and Methods. The dialyzed extract from cells grown on ethylene glycol contained 0.905 mg of protein in 0.2 ml, and that from glycolate-grown cells contained 0.805 mg of protein in 0.2 ml. Symbols:* ○, *enzyme preparation heated for 30 min at 60 C;* □, *cuvette with no glycolate added; and* △, *complete system.*

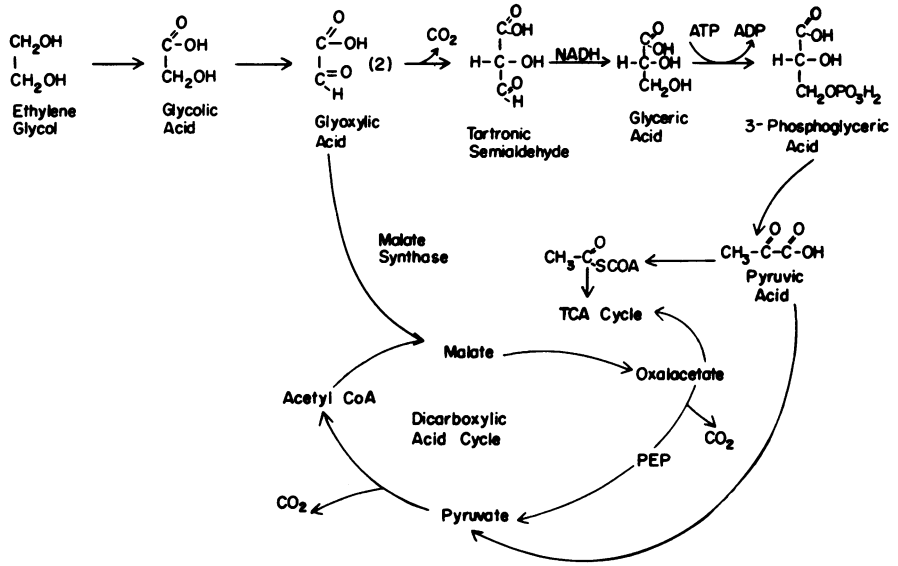


FIG. 8. *Possible pathway of ethylene glycol metabolism (adapted in the main from Ornston and Ornston [9]).*

the biodegradative removal of ethylene glycol could result in conversion of most of the carbon to carbon dioxide, which could be vented to the atmosphere. The presence of glucose, and perhaps of other readily utilized carbon sources, would in effect prevent biodegradation of ethylene glycol, as radioactivity in respired CO_2 was almost nil until glucose had been consumed (Fig. 2).

Respiration by washed cells. Cells grown on glucose, and then washed, oxidized ethylene glycol slowly (Fig. 4 and Table 8). Cells grown on either ethylene glycol or propylene glycol oxidized ethylene glycol more rapidly than glucose (Fig. 5 and 6). If ethylene glycol were to be scavenged from unsupplemented waters by preformed cells, they should be grown on one of these glycols. Oxidation of 1 mole of ethylene glycol could be expected to require 2.5 moles of O_2: $C_2H_6O_2 + 2.5O_2 \rightarrow 2CO_2 + 3H_2O$. The expected RQ value would be 0.8. The calculated value based on 3 hr of respiration and including CO_2 driven out of buffer with acid was 1.02. The value based on the first hour of respiration after tipping was 0.8. These data support the conclusion that ethylene glycol metabolism is oxidative and that ethylene glycol was no doubt converted to a conventional metabolite and oxidized via conventional electron transport.

Possible initial metabolism of ethylene glycol. Ornston and Ornston (9) and Kornberg and Sadler (5) have proposed pathways for metabolism of two-carbon molecules that could be available for ethylene glycol metabolism, provided that ethylene glycol could be oxidized to glycolate (Fig. 8). Stouthamer et al. (13) have proposed that *Acetobacter aceti* metabolizes ethylene glycol via glycolate. Bacterium T-52 can utilize both glycolate and glyoxylate as sole carbon sources for growth (Table 1). Although oxidation of ethylene glycol to glycolate was not examined during this investigation, spectrophotometric evidence was obtained for oxidation of glycolate to glyoxylate (Fig. 7). As currently visualized by Hansen and Hayashi (3) and Ornston and Ornston (9), two-carbon compounds would be converted to malate and glycerate, and the dicarboxylic acid cycle (5) would not be required. Further studies are required to elucidate the pathway of ethylene glycol utilization by bacterium T-52, and in particular to determine whether ethylene glycol is oxidized to glycolate.

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