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Alcohol and acid formation during the anaerobic decomposition of propylene glycol under methanogenic conditions

S. Veltman, T. Schoenberg & M.S. Switzenbaum
Department of Civil & Environmental Engineering University of Massachusetts, Amherst, MA, USA

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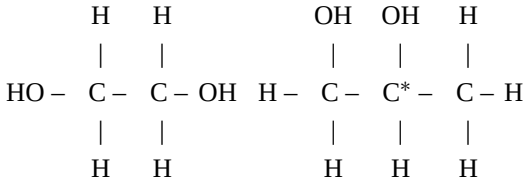
Abstract

Intermediates formed during the anaerobic decomposition of propylene glycol under methanogenic conditions were studied using a serum bottle technique. The pathway is similar to the anaerobic decomposition of ethylene glycol as previously reported. For both compounds, the decomposition is believed to proceed via an initial disproportionation of the glycol to form equal molar amounts of the volatile fatty acid and normal alcohol of the same chain length. In the case of ethylene glycol, disproportionation results in the formation of acetate and ethanol, while disproportionation of propylene glycol produces propionate and n-propanol.

Following disproportionation, the alcohols produced from glycol fermentation are oxidized to their corresponding volatile fatty acid with the reduction of protons to form hydrogen. Ethanol and propionate oxidation to acetate proceeds via a well-established syntrophic pathway that is favorable only under low hydrogen partial pressures. Subsequent degradation of acetate proceeds via acetoclastic methanogenesis with the production of carbon dioxide and methane. Despite the production of hydrogen in the initial steps of glycol degradation, both compounds are completely degradable under the methanogenic conditions tested in this study.

Introduction

Ethylene glycol (1,2-ethanediol) and propylene glycol (1,2-propanediol) are the most widely used aircraft deicing fluids in use today. Until the early 1990's ethylene glycol was the predominant aircraft deicer because of its lower cost. However, in recent years growing concern about the potential toxicity of ethylene glycol led many airports in the United States to switch to propylene glycol-based deicing fluids. Now, propylene glycol is in widespread use in the United States and ethylene glycol is more widely used in Canada.



Ethylene Glycol (1,2-Ethanediol)

Propylene Glycol (1,2-Propanediol)

Both ethylene glycol and propylene glycol are vicinal diols (hydroxyl substituents on adjacent carbon atoms) with a polar structure that makes them completely miscible in water. Propylene glycol has an asymmetric center which indicates that two stereoisomers of propylene glycol are possible, (R)-1,2-propanediol and (S)-1,2-propanediol. In aircraft deicing fluid manufactured for commercial sale, the product is sold as a racemic mixture with the (R) and (S) enantiomers present in equal amounts (S. Harris, ARCO Chemical Company, pers. comm.).

For deicing applications and snow removal, ethylene glycol and propylene glycol are normally applied to aircraft in a 50/50 solution with water and then applied to the aircraft using mechanical sprayers. Anticing, which often follows deicing, involves the use of the same chemicals in concentrated forms with a thickener added to improve retention of the material on the aircraft. The runoff from these operations is now collected at a large number of airports using a variety of collection methods. Depending upon the method of

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Table 1. Relevant reactions in the anaerobic decomposition of ethylene and propylene glycol

Reaction	Chemical Equation	$\Delta G°'$, KCal
1) Propylene Glycol → Propionate + n-Propanol	$CH_3CH(OH)CH_2OH \rightarrow 0.5\ CH_3CH_2COO^- + 0.5\ H^+ + 0.5\ CH_3CH_2CH_2OH + 0.5\ H_2O$	−24.4
2) n-Propanol → Propionate	$CH_3CH_2CH_2OH + H_20 \rightarrow CH_3CH_2COO^- + H^+ + 2\ H_2$	+ 2.9
3) Propylene Glycol → Propionate (1+2)	$CH_3CH(OH)CH_2OH \rightarrow CH_3CH_2COO^- + H^+ + H_2$	−22.9
4) Propionate → Acetate	$CH_3CH_2COO^- + 3\ H_2O \rightarrow CH_3COO^- + H^+ + HCO_3{}^- + 3\ H_2$	+ 18.3
5) Ethylene Glycol → Acetate + Ethanol	$HOCH_2CH_2OH \rightarrow 0.5\ CH_3COO^- + 0.5\ H^+ + 0.5\ CH_3CH_2OH + 0.5\ H_2O$	−21.7
6) Ethanol → Acetate	$CH_3CH_2OH + H_2O \rightarrow CH_3COO^- + H^+ + 2\ H_2$	+ 2.3
7) Ethylene Glycol → Acetate (5+6)	$HOCH_2CH_2OH \rightarrow CH_3COO^- + H^+ + H_2$	−20.6
8) Acetate → Methane	$CH_3COO^- + H_2O \rightarrow HCO_3{}^- + CH_4$	− 7.4
9) Hydrogen → Methane	$4\ H_2 + H^+ + HCO_3{}^- \rightarrow CH_4 + 3\ H_2O$	−32.4
10) Ethylene Glycol → Methane (5+6+8+9)	$HOCH_2CH_2OH + 0.25\ H_2O \rightarrow 1.25\ CH_4 + 0.75\ H^+ + 0.75\ HCO_3{}^-$	−36.1
11) Propylene Glycol → Methane (1+2+4+8+9)	$CH_3CH(OH)CH_2OH + H_2O \rightarrow 2\ CH_4 + H^+ + HCO_3{}^-$	−44.5

collection and precipitation at the time of fluid application, glycol concentrations in the collected runoff from deicing operations may range from 2,000 – 300,000 ppm (Veltman et al. 1998). With chemical oxygen demand (COD) equivalents of 1.28 g COD/g and 1.68 g COD/g for ethylene glycol and propylene glycol respectively, deicing wastes have a high oxygen demand. Therefore the collection and treatment of these wastes is necessary to avoid the potential for severe oxygen depletion in waters receiving airport runoff.

The degradation of glycols under anaerobic conditions is believed to involve one or more biological fermentation steps followed by hydrogen oxidation and the cleavage of acetate to form CO_2 and methane (CH_4) in the presence of methanogenic bacteria. The overall reactions would proceed in accordance with the following:

$$HOCH_2CH_2OH + 0.25H_2O \rightarrow 1.25CH_4 + 0.75H^+ + 0.75HCO_3^- \quad (1)$$

$$CH_3CH(OH)CH_2OH + H_2O \rightarrow 2CH_4 + H^+ + HCO_3^- \quad (2)$$

While it is possible to write balanced chemical equations for the complete anaerobic mineralization of ethylene glycol and propylene glycol, and both reactions can be shown to be thermodynamically possible under the conditions present in anaerobic systems, this alone does not prove that the reactions will proceed. In anaerobic environments microorganisms have a high degree of substrate specificity and the complete mineralization of a complex organic compound generally involves a consortium of organisms working in concert with each other (Brock et al. 1995). In these cases, there may be many different steps leading to the formation of end products, intermediate products may be formed, and not all steps may be equivalent energetically (Table 1). Consequently knowledge which helps us to understand how glycols degrade is important to the development and use of anaerobic processes to treat deicing wastes.

Several earlier studies demonstrated that glycols are biodegradable under anaerobic conditions (Cox 1978, Kaplan et al. 1982). In 1983, Dwyer and Tiedje (1983) reported on the anaerobic decomposition of ethylene glycol following a broad investigation into the decomposition of large molecular weight polyethylene glycols. In their work, ethylene glycol was initially degraded to acetate and ethanol via a disproportionation reaction, ethanol was oxidized to acetate, and acetate was subsequently cleaved to form methane under anaerobic methanogenic conditions.

Since ethylene and propylene glycols are widely used for various purposes, the metabolism of both is well understood (Pasternak 1993). It is known that propylene glycol is metabolized in the liver by alcohol dehydrogenase to lactic acid, and then to pyruvic acid. Both of these metabolites are normal consitituents of the citric acid cycle and are further metabolized to carbon dioxide and water. Ethylene

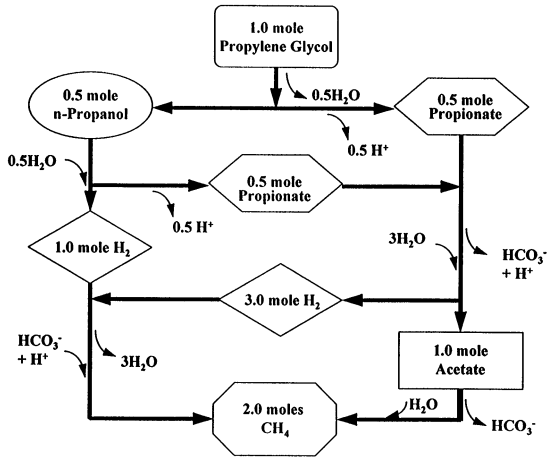

Figure 1. Proposed pathway for the anaerobic decomposition of propylene glycol.

and propylene glycol are also known to be degradable under aerobic conditions (Verschueren 1996). However the anaerobic biodegradation of propylene glycol has not been widely studied. In this paper we propose that propylene glycol degrades, under anaerobic methanogenic conditions, by means of a similar disproportionation reaction to that found by Dwyer and Tiedje (1983) as shown in Figure 1. In addition, we demonstrate propylene glycol may be completely degraded to form methane and carbon dioxide in an anaerobic methanogenic environment.

Materials and methods

Source of inoculum

The inoculum used in the serum bottles tests described in this paper were taken from a separate suspended growth, semi-continuous flow, fill and draw reactor. The reactor contained a mixed methanogenic culture that had been acclimated to the degradation of propylene glycol. Inoculum was drawn prior to feeding to minimize the amount of degradable COD introduced to the serum bottles with the inoculum. A 20% by volume inoculum was used in the serum bottles resulting in the addition of approximately 16 mg TSS to each serum bottle. The inoculum for the reactor was originally derived from several municipal wastewater digesters and the organisms were slowly transitioned from sucrose to propylene glycol. At the time of the study the reactor was operating under steady state conditions at a SRT of 30 days. The inoculum reactor was kept in the dark at a constant temperature of 35 °C and had been in operation for over 6 months at the start of this study.

Serum bottle test procedure

The serum bottle test procedure used in this work was an adaptation of a method that was originally developed by Hungate (1969), and later modified by Wolin and Miller (1974) and Owen et al. (1979). Serum bottles (160 ml) were filled with a mixture of seed, mineral salts, bicarbonate buffer, and sample to a volume of 100 ml, leaving a headspace of 60 ml. Resazurin was added to the bottles at a concentration of 1 mg/l to detect possible oxygen contamination, and $Na_2S \cdot 9H_2O$ was added to maintain a reducing environment. The transfer of all media was done in a manner to minimize oxygen contamination and the sample bottle headspace was gassed out using a mixture of 70% Nitrogen/30% CO_2 prior to sealing. Seals consisted of a butyl rubber septum (Wheaton 224100-193) with an aluminum crimp cap (Wheaton 224182-01).

The concentrations of glycol used in the serum bottle tests were carefully selected to avoid the accumulation of inhibitory levels of hydrogen or the volatile fatty acids formed during glycol degradation. To accomplish this, a total COD of 2000 mg/l was used in the tests involving propylene glycol (1190 mg/l of Fischer USP/FCC grade (+/-) -1,2-propanediol).

All serum bottle incubations were carried out in the dark at a temperature of 35 °C. During each test a sample blank was run to correct for the degradable material introduced with the seed. In all instances the COD introduced with the seed was less than 1% of the total sample COD introduced to the serum bottle at the start of each test. Samples and sample blanks were run in triplicate.

Liquid sample preparation and analysis

Serum bottles were sampled using a 1-cc disposable tuberculin syringe (Becton-Dickinson Model 5602) fitted with a 19 mm, 23 gauge needle (Becton-Dickinson Precision Glide^{TM}, Model 5156) and a 0.45 μm pore size disposable Corning 13 mm polypropylene syringe filter with cellulose acetate membrane. After shaking the serum bottle, 490μL of filtered effluent was withdrawn and transferred into Target Micro-Sert^{TM} vial inserts placed in Target DP^{TM} 12x32 mm vials (National Scientific Company). Samples were refrigerated at 1.8 °C until analy-

sis. After analyzing for alcohol and glycol, 10ml of 10N sulfuric acid was added to drop the pH below 2.

Using a 7000 Series Modified Microliter^{TM} syringe (Hamilton Company) fitted with a Chaney adapter, 2 μL was withdrawn from sample vials and injected into a Varian 3300 gas chromatograph through a split/splitless injector (in splitless mode) fitted with a Varian unpacked split glass inlet sleeve. The injector temperature was 220 °C. Zero-grade nitrogen (Merriam Graves Corp., West Springfield, MA) flowing at around 18 mL/min carried the sample through a 15 m Nukol^{TM} capillary column (Supelco, Inc.) with 0.53 mm inside diameter and 0.50 μm film thickness. Compound elution was detected with a flame ionization detector (FID) set at 250 °C. Data were plotted on a Spectra-Physics SP4270 integrator.

Samples were analyzed for alcohol and glycol before acidification out of concern that strong acid would chemically transform the hydroxyl-bearing compounds. The column temperature was held at 40 °C for one minute, then increased at 15 °C/min to 145 °C, at which point the analysis was complete. Samples were then acidified to ensure that volatile fatty acids were predominantly in their protonated form. To analyze for VFAs (specifically, acetate, propionate, and butyrate), an initial column temperature of 90 °C was increased immediately at 7.5 °C/min to 105 °C, held for one minute, then increased at 10 °C until all compounds eluted, at which point the run was manually concluded.

Standards were run each time that samples were analyzed. Three sets of standards were used: one for alcohols, one for VFAs, and one for glycols. Standards were run in duplicate and spanned the range of concentrations present in the samples. The concentration in a standard was plotted versus the average area of replicate runs for that concentration. Standard curves for each compound analyzed were produced. Curves were forced through the origin, which still allowed for good regression fits.

Gas analysis

Gas production in the serum bottles was measured periodically, usually every two to three days. Excess gas was extracted from the serum bottles and wasted using a Perfectumþ fitted glass hypodermic syringe with a 19 mm, 23 gauge needle (Becton-Dickinson Precision Glide^{TM}, Model 5156). The syringe was lubricated with distilled water prior to analysis to provide a gas tight seal and permit free movement of the syringe plunger. Syringes were held horizontally and allowed to equilibrate with atmospheric pressure to determine gas volume. The total volume of methane produced by each serum bottle was computed from measurements of total gas volume and gas composition, and corrected for seed contribution.

Gas analysis was completed immediately following gas volume determination to insure that the headspace pressure in the serum bottles was at atmospheric pressure. One milliliter of headspace gas was withdrawn using a 1-cc gastight syringe (Hamilton #1001) equipped with a 25.4 mm, 20 gauge needle and immediately injected into a GOW Mac Series 550 Gas Chromatograph for analysis. Separation and detection of the gas components was accomplished by using a 1.83 m $\times$ 6.35 mm Poropak Q column (Supelco, Inc.) and a thermal conductivity detector. Ultra-pure helium (Merriam Graves Corp.) was used as a carrier gas at a flowrate of 30 ml/min with an injector port temperature of 110 °C, a column temperature of 80 °C, and a detector temperature of 70 °C. Methane content and CO_2 content for the samples were determined by comparing the observed peak height response of the sample to calibration curves prepared during each run using methane and CO_2 calibration gases of known purity (Merriam Graves Corp.).

Results and discussion

The results of intermediate monitoring and methane measurements for the propylene glycol serum bottle tests as a function of time are summarized in Figure 2. The data represent the mean values of three replicates tested.

Analysis of the data in Figure 2 confirms the postulated disproportionation of propylene glycol to form n-propanol and propionate. This initial step occurred rapidly with all of the glycol disappearing from the serum bottles within 48 hours, at which time n-propanol concentrations reached their peak value. During this first 48 hours propionate and n-propanol were produced in nearly equal molar amounts.

Following the disproportionation step, n-propanol began to disappear with a corresponding increase in the amount of propionate as predicted by Reaction 2 in Table 1. At 375 hours all of the n-propanol was gone and propionate reached its peak value, after which it slowly began to disappear from the serum bottle with a corresponding increase in methane production. Small

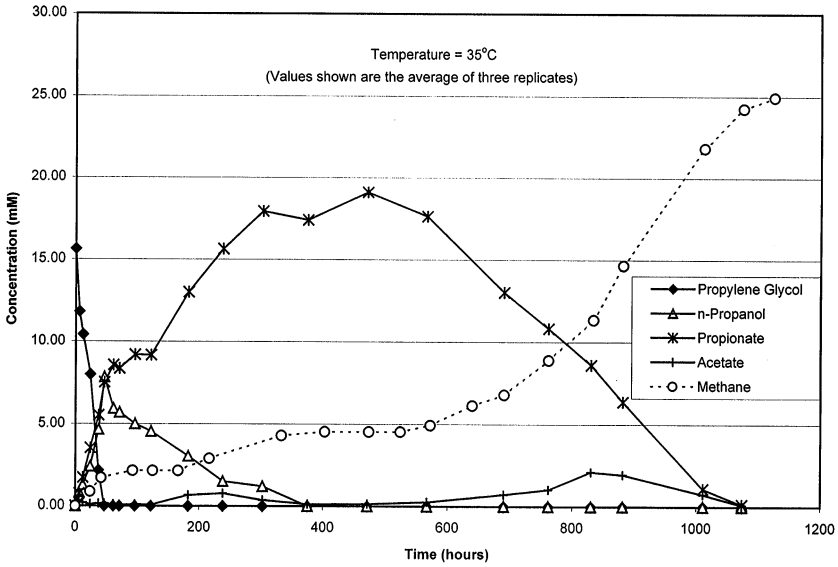

Figure 2. Propylene glycol decomposition vs. time.

amounts of acetate were observed. By 1100 hours all of the COD initially placed into the serum bottle was completely converted and the total methane production reached a final value of 24.9 mm, or 1.6 moles of CH_4 per mole of propylene glycol added at the start of the test. This value very nearly approaches the theoretical yield of 2 moles CH_4 per mole of propylene glycol oxidized (Figure 1) when cell yield and the COD removed from the serum bottles during sampling are accounted for.

Single enantiomer tests

The test data reported in Figure 2 are for a racemic mixture of (+/-) 1,2-propandiol. Results of our tests on the pure enantiomers, (R) – (−) – 1,2-propoandiol and (S) – (+) – 1,2-propandiol (Lancaster synthesis) reveal no difference in the degradation of the two enantiomers.

Other diols

The results of the testing conducted on propylene glycol and the previous study on ethylene glycol might lead one to hypothesize that higher molecular weight diols (such as 1, 2-butanediol) degrade via an initial disproportionation reaction in a manner analogous to ethylene glycol and propylene glycol. To test this theory we conducted serum bottle tests using a mixed seed with 1,2-butanediol, 1,3-butanediol and 1,4-butanediol. The butanediols tested (Lancaster Synthesis) were added to the serum bottles at a concentration sufficient to provide a total degradable COD of 500 mg/l. In our analysis of intermediates formed during these tests, butyrate and acetate appeared as intermediates in the bottles containing 1,2-butanediol and 1,3-butanediol, but n-butanol was not observed in any of the bottles. Consequently we have no evidence to suggest that higher molecular weight diols degrade via an initial disproportionation reaction.

Conclusions

Dwyer and Tiedje showed that the anaerobic decomposition of ethylene glycol under methanogenic conditions proceeds via an initial disproportionation of the glycol to ethanol and acetate in a microbially mediated fermentation process that is highly exergonic. The degradation of propylene glycol under methanogenic conditions also appears to begin with a highly exergonic disproportionation reaction. In this first step the glycol is transformed into equal molar amounts of n-propanol and propionate. Propionate is the oxidized species resulting from this fermentation and n-propanol is the reduced product. The n-propanol thus formed may, in turn, be oxidized to propionate with the simultaneous reduction of protons to form hydrogen.

The oxidation of this propionate requires a syntrophic association between a propionate oxidizer and a hydrogen oxidizing methanogen and hydrogen and

acetate are produced as a result of the reaction. Under favorable anaerobic methanogenic conditions the acetate formed may be cleaved to form methane and carbon dioxide, hydrogen is oxidized to methane, and propylene glycol may be completely biodegraded.

Acknowledgments

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