Biodegradation (2009) 20:551–558
DOI 10.1007/s10532-008-9244-9

ORIGINAL PAPER

Anaerobic ethylene glycol degradation by microorganisms in poplar and willow rhizospheres

D. Carnegie · J. A. Ramsay

Received: 28 July 2008 / Accepted: 23 December 2008 / Published online: 9 January 2009

Abstract Although aerobic degradation of ethylene glycol is well documented, only anaerobic biodegradation via methanogenesis or fermentation has been clearly shown. Enhanced ethylene glycol degradation has been demonstrated by microorganisms in the rhizosphere of shallow-rooted plants such as alfalfa and grasses where conditions may be aerobic, but has not been demonstrated in the deeper rhizosphere of poplar or willow trees where conditions are more likely to be anaerobic. This study evaluated ethylene glycol degradation under nitrate-, and sulphate-reducing conditions by microorganisms from the rhizosphere of poplar and willow trees planted in the path of a groundwater plume containing up to 1.9 mol l^{-1} (120 g l^{-1}) ethylene glycol and, the effect of fertilizer addition when nitrate or sulphate was provided as a terminal electron acceptor (TEA). Microorganisms in these rhizosphere soils degraded ethylene glycol using nitrate or sulphate as TEAs at close to the theoretical stoichiometric amounts required for mineralization. Although the added nitrate or sulphate was primarily used as TEA, TEAs naturally present in the soil or CO_2 produced from ethylene glycol degradation were also used, demonstrating multiple TEA usage. Anaerobic degradation produced acetaldehyde, less acetic acid, and more ethanol than under aerobic conditions. Although aerobic degradation rates were faster, close to 100% disappearance was eventually achieved anaerobically. Degradation rates under nitrate-reducing conditions were enhanced upon fertilizer addition to achieve rates similar to aerobic degradation with up to 19.3 mmol (1.20 g) of ethylene glycol degradation l^{-1} day^{-1} in poplar soils. This is the first study to demonstrate that microorganisms in the rhizosphere of deep rooted trees like willow and poplar can anaerobically degrade ethylene glycol. Since anaerobic biodegradation may significantly contribute to the phytoremediation of ethylene glycol in the deeper subsurface, the need for "pump and treat" or an aerobic treatment would be eliminated, hence reducing the cost of treatment.

Keywords Ethylene glycol · Anaerobic · Bioremediation · Poplar · Willow · Nitrate · Sulphate

D. Carnegie · J. A. Ramsay (✉)
Chemical Engineering, Queen's University, Kingston, ON K7L 3N6, Canada
e-mail: juliana.ramsay@chee.queensu.ca

Introduction

Ethylene glycol is commonly used as a coolant, as a deicing fluid, and to produce plastics such as polyethylene terephthalate. In 2004, its annual worldwide production was estimated to be 18,482 kilotons (Commission of the European Communities 2004). With a log k_{ow} of −1.36, ethylene glycol readily

partitions into the aqueous phase, resulting in high mobility and rapid dispersion through the biosphere. Although the toxicity is low (Staples et al. 2001), and its LD_{100} is 1.4 ml kg^{-1} for humans, acute exposure can result in kidney and brain damage and possibly teratogenesis. Toxic concentrations have been reported in industrial waste streams and airport runoff. Other concerns are the increase in the biological oxygen demand and subsequent disruption of aquatic ecosystems.

Aerobic degradation of ethylene glycol is well documented (Child and Willetts 1978; Gonzalez et al. 1972; Revitt and Worrall 2003; Shieh et al. 1998; Staples et al. 2001). McVicker et al. (1998) suggested that, in soil, anaerobic degradation with acidogenesis plays a more significant role than aerobic degradation. Although anaerobic biodegradation of ethylene glycol has been reported, only methanogenesis (Dwyer and Tiedje 1983; Strab and Schink 1986; McVicker et al. 1998) and fermentation (Schink and Stieb 1983; Strab and Schink 1986) have been clearly demonstrated. Dwyer and Tiedje (1983) have proposed an anaerobic pathway which includes acetaldehyde, ethanol and acetic acid as intermediates for a methanogenic consortium. Despite this evidence of anaerobic ethylene glycol degradation, it remains poorly characterized. Few studies have examined its potential under subsurface conditions and results are conflicting. For example, under simulated subsurface conditions, McGahey and Bouwer (1992) have reported that indigenous soil and water microorganisms degraded ethylene glycol, but concluded that adequate concentrations of oxygen are important and degradation is more likely to occur in upper horizons of the subsurface. However, McVicker et al. (1998) has suggested that anaerobic processes should be more significant under these conditions.

Given the widespread use of ethylene glycol and its release to the environment, there is a need for remediation strategies to mitigate its impact. Biological approaches such as bioremediation and phytoremediation are increasingly recognized as being more cost effective than traditional approaches (Erickson 1997). Enhanced degradation of ethylene glycol has been demonstrated by microorganisms in the rhizosphere of shallow-rooted plants such as alfalfa (Rice et al. 1997; Castro et al. 2001), legumes, and grasses (Shupack and Andersen 2000; Marschner et al. 2001). Degradation has been shown to increase after fertilization (Calabrese et al. 1993; Chugunov et al. 2000) and when sufficient water is added to maintain aerobic conditions (Castro et al. 2001). However, phytoremediation using trees with much deeper roots to remediate ethylene glycol at greater depth has not been previously investigated.

The present study focused on microorganisms in rhizosphere soils of poplar and willow trees planted in the path of an ethylene glycol groundwater plume. The water table at the test plot varied with the seasons and ranged from the surface to 1 m below grade. In certain regions, plume concentrations approached 1.9 mol l^{-1} (120 g l^{-1}). Willow trees have been shown to extract water-soluble contaminants, such as ethanol, from an aquifer contaminated with ethanol blended gasoline (Corseuil and Moreno 2001). Poplar trees can evapotranspire dioxane and trichloroethylene (TCE; Shim et al. 2000; Kelley et al. 2001). Information on the ability of poplar and willow rhizosphere microorganisms to degrade anthropogenic compounds is presently limited to TCE, and polyaromatic hydrocarbons. There are no reports on ethylene glycol degradation. One objective of the present study was to evaluate ethylene glycol degradation under nitrate-, and sulphate-reducing conditions by the microorganisms in the rhizosphere of poplar and willow trees from the phytoremediation testplot. Since degradation rates of organic contaminants can be enhanced after fertilization (Calabrese et al. 1993; Chugunov et al. 2000), the effect of fertilizer addition on ethylene glycol degradation was also studied.

Materials and methods

Inoculum source

Poplar (*Populus balsamifera*) and willow (*Salix nigra*) trees were planted in a soil mixture designed to improve exchange with a water table approximately 1 m below grade where ethylene glycol concentrations were up to 1.9 mol l^{-1} (120 g l^{-1}). Rhizosphere soil was augured from beneath the tree crown where the presence of the tree roots was visually confirmed. Soil outside the sphere of influence of the trees (i.e. bulk or non-rhizosphere soil) was collected from the same depths as rhizosphere soils near the phytoremediation site. Soil samples

were collected between the surface and 1 m below grade at 15–20 cm intervals. The auger was cleaned in a solution of soapy water and rinsed with distilled water between sampling intervals. Down to a depth of 1 m, the soil in the phytoremediation plot was relatively homogenous as it had been excavated and mixed before the trees were planted. Unless specified otherwise, the experiments were performed with rhizosphere soil from a depth of 50–70 cm taken in September 2005. Each soil was homogenously mixed before it was first used.

Growth medium

The basal salts medium (BSM; Cote and Gherna 1994) contained per liter: 34.2 g K_2HPO_4, 10 g $NaH_2PO_4 \cdot H_2O$, and 20 g NH_4Cl. The pH of the trace element solution was adjusted to 6.5 and contained per liter 1.5 g of nitrilotriacetic acid, 3.0 g $MgSO_4 \cdot 7H_2O$, 1.0 g NaCl, 0.1 g $FeSO_4 \cdot 7H_2O$, 0.1 g $CoCl_2 \cdot 6H_2O$, 0.132 g $CaCl_2 \cdot 2H_2O$, 0.1 g $ZnSO_4 \cdot 7H_2O$, 8.74 mg $CuSO_4 \cdot 5H_2O$, 10.0 mg $AlK(SO_4)_2 \cdot 12H_2O$, 10.0 mg H_3BO_3, 10 mg $Na_2MoO_4 \cdot 2H_2O$, 27.1 mg $NiCl_2 \cdot 6H_2O$, and 20.0 mg $Na_2WO_4 \cdot 2H_2O$.

Microcosm setup

Two series of experiments were performed in duplicate. First, ethylene glycol degradation was evaluated under aerobic or anoxic conditions with nitrate or sulphate provided as terminal electron acceptors (TEAs) using poplar or willow rhizosphere soil or non-rhizosphere soil as an inoculum. In the second experiment, the effect of fertilizer addition under nitrate-reducing conditions was determined.

To 15 g soil in 115 ml amber serum bottles, 10% (v/v) BSM, 10% (v/v) trace element solution, and the balance in deionized water were added to a total volume of 100 ml. Concentrated ethylene glycol was added to a final concentration of 96.6 mmol l^{-1} (6 g l^{-1}). In aerobic experiments, the microcosms were covered with a gas permeable foam stopper and placed on a shaker at 170 rpm and 23 ± 1°C. In anaerobic experiments, ten times the stoichiometric quantity of nitrate (sodium nitrate), or sulphate (sodium sulphate) for complete mineralization of ethylene glycol was added from a 1 M standard solution. Theoretical values for TEA usage were determined from stoichiometry as in Cookson (1995). Abiotic controls were identical to the biotic microcosms except they were autoclaved once a day for 3 days.

The headspace of anaerobic microcosms was purged with nitrogen gas (Hungate 1969) and capped with butyl rubber stoppers. The microcosms were kept at 23 ± 1°C in an anaerobic chamber (Nexus One, Vacuum Atmospheres Co., Hawthorn, Calif.) in an oxygen-free nitrogen atmosphere without shaking. Two to seven millilitre samples were taken periodically with a sterile needle and stored at −20°C.

For the fertilization experiments, anaerobic microcosms were prepared as above with 0.5 g of CIL Tree and Hedge Feeder per g of soil and sodium nitrate as the TEA. The fertilizer contained 18% total nitrogen from monoammonium phosphate and urea, 4% phosphoric acid from monoammonium phosphate, and 6% soluble potash from muriate of potash.

Analyses of ethylene glycol and metabolites

Before analysis by gas chromatography, samples were filtered through 0.45 µm nitrocellulose filters (Millipore) and 1-pentanol added as an internal standard, 1 µl was injected onto a 5 m guard column attached to a 30 m × 0.53 mm Restek stabilwax column (Chromatographic Specialties, Brockville, Canada). Quantification was performed with a Varian 3400 and the Star Chromatography software. The flame ionization detector was supplied with a helium flow rate of 4 ml min^{-1}, a hydrogen flow rate of 50 ml min^{-1}, and air at a rate of 300 ml min^{-1}. The oven temperature was held at 55°C for 1 min, followed by an increase of 20°C min^{-1} to 215°C with a 1 min hold. The injector and detector temperatures were constant at 230 and 250°C, respectively. Calibration curves were obtained for ethylene glycol (0.05–6 g l^{-1}), ethanol (0.05–3 g l^{-1}), acetic acid (0.05–3 g l^{-1}) and acetaldehyde (0.05–0.5 g l^{-1}).

Analyses of NO_3^-, SO_4^{2-} and methane

NO_3^-, and SO_4^{2-} were analyzed by ion chromatography using a Dionex DX-300 Gradient Chromatographic System equipped with Ionpac AS4A guard and analytical columns connected to a conductivity detector. The mobile phase was 1.7 mM sodium bicarbonate and 1.8 mM sodium carbonate at a flow rate of 1 ml min^{-1}. Solutions of $NaNO_3$ and

Na_2SO_4 were used as standards for nitrate and sulphate, respectively, with calibration curves in the range of 1–100 mg l^{-1}. Samples were filtered through 0.45 μm polycarbonate filters before injection. Methane headspace analysis of the microcosms was done by injecting 200 μl samples using a gas-tight syringe into a gas chromatograph (HP5890) equipped with a flame ionization detector and a HP-5 column.

Statistical analysis

All data averages were compared by a one-way analysis of variance (ANOVA) at the level of $P < 0.05$ to determine significant differences in ethylene glycol degradation within and between duplicate experiments. The bulk or non-rhizosphere soil microcosms were compared to poplar and willow soil rhizosphere microcosms with oxygen, nitrate or sulphate as TEA. The effect of fertilizer addition when nitrate was provided as a TEA was also evaluated.

Results

For the anaerobic microcosms, although attempts were made to eliminate oxygen, initial conditions may have been anoxic. The high initial ethylene glycol concentration (6 g l^{-1}) would have resulted in rapid removal of any residual oxygen with subsequent anaerobic conditions being maintained as the microcosms were capped with a butyl rubber stopper. In aerobic microcosms, no attempts were made to remove oxygen and microcosms were covered with a foam stopper which was permeable to air. Ethylene glycol degradation was monitored periodically in all microcosms within an experiment until degradation ceased for the majority of them. There was no degradation in abiotic microcosms (Table 1).

Rhizosphere versus non-rhizosphere soil

Ethylene glycol degradation rates were faster by microorganisms in willow and poplar rhizosphere soils than in bulk (i.e. non-rhizosphere) soil under aerobic conditions (Table 1) with up to 99% ethylene glycol degradation. Significant differences were confirmed by one-way ANOVA at $P < 0.05$ between the bulk and rhizosphere soils (Table 2). On the other hand, when nitrate was provided as the TEA, degradation rates by microorganisms in poplar or willow rhizosphere soil was not significantly different from the bulk soil (Tables 1, 2). There was no significant difference at $P < 0.05$ between tree species under aerobic or nitrate-reducing conditions (Table 2).

Table 1 Ethylene glycol degradation with different TEAs in duplicate microcosms inoculated with rhizosphere soils collected in September 2005 except where indicated

Soil	Degradation rate (mmol l^{-1} day^{-1})		
	Oxygen	Nitrate	Sulphate
Abiotic control	0	0	0
Bulk	8.2 ± 1.1	4.4 ± 1.0	ND
Willow	16.0 ± 4.3	6.5 ± 1.7	0.3 ± 0.8
Willow (2004)	ND	5.4 ± 2.0	1.6*
Willow plus fertilizer	ND	11.4 ± 2.1	ND
Poplar	16.2 ± 3.7	3.8 ± 2.1	1.3 ± 1.2
Poplar (2004)	16.2 ± 0.2	2.2 ± 0.7	2.3 ± 0.6
Poplar plus fertilizer	ND	12.1 ± 6.7	ND

Aerobic and anaerobic microcosms were incubated for 14 and 34 days, respectively

* single microcosm, *ND* not done

Degradation with different electron acceptors

Microcosms were set up with excess stoichiometric quantities of nitrate or sulphate as TEA using poplar or willow rhizosphere soil as inoculum. After 34 days of incubation, ethylene glycol degradation was faster and higher when nitrate was provided as the TEA, with degradation rates of 3.8 ± 2.1 (235 ± 130 mg l^{-1} day^{-1}) and 6.5 ± 1.7 (403 ± 105 mg l^{-1} day^{-1}) mmol l^{-1} day^{-1} for poplar and willow rhizosphere soils, respectively (Table 1). This was confirmed by ANOVA at $P < 0.05$ (Table 2). Although the rate was lower with sulphate, almost complete degradation was achieved with both TEAs at longer incubation times. These experiments were performed with rhizosphere soils taken in September 2005 at a depth of 50–70 cm, similar trends were obtained with rhizosphere soil samples taken in October 2004 and for rhizosphere soil taken at 90 cm.

After only 13 days of incubation with nitrate or sulphate addition, 30 mmol l^{-1} nitrate or 10 mmol l^{-1} sulphate, respectively, was used (Fig. 1). When no TEA was added, a maximum of only 60% of the added

Table 2 Results of ANOVA analysis of ethylene glycol degradation rates by microorganisms in bulk or rhizosphere soils with different TEAs and fertilizer addition using all data from 2004 to 2005 studies

Treatment	Factors	*F* ratio	Fcrit	Probability
Aerobic	Poplar vs. willow	0.0026	4.5	0.96
	Bulk vs. all trees	20.6	5.99	0.00394
Nitrate	Bulk vs. poplar	0.84	5.53	0.43
	Bulk vs. willow	12.9	8.53	0.069
	Poplar vs. willow	4.9	4.5	0.09
	Poplar (no fertilizer vs. fertilizer)	8.4	5.6	0.02
	Willow (no fertilizer vs. fertilizer)	5.97	3.78	0.052
All trees	Aerobic vs. nitrate	39.0	4.96	9.52 E-05
All trees	Aerobic vs. sulphate	102.7	4.74	3.1 E-07
All trees	Sulphate vs. nitrate	20.7	18.5	0.045

ethylene glycol was degraded, most likely using TEAs naturally present in the soil. Although there were initially 0.6 mmol l^{-1} nitrate and 8.67 mmol l^{-1} sulphate without TEA addition, 0.24 mmol l^{-1} nitrate and 2.19 mmol l^{-1} sulphate were utilized after 47 days of incubation (Fig. 1).

Degradation rates with both rhizosphere soils were significantly much faster under aerobic conditions (Tables 1, 2) producing mostly acetic acid, little ethanol and no acetaldehyde as intermediates (Fig. 2a). Regardless of whether nitrate or sulphate was added as a TEA, the relative trends for substrate consumption and metabolite production were similar (Fig. 2b) except that under sulphate reducing conditions twice as much acetaldehyde was detected. Production of acetaldehyde, ethanol and acetic acid corresponded with the disappearance of ethylene glycol, and the subsequent consumption of the metabolites paralleled each other, albeit at different levels. Although the final pH after aerobic degradation was always acidic, the pH increased from 6.8 to 8 in some anaerobic microcosms. This increase did not appear to inhibit degradation.

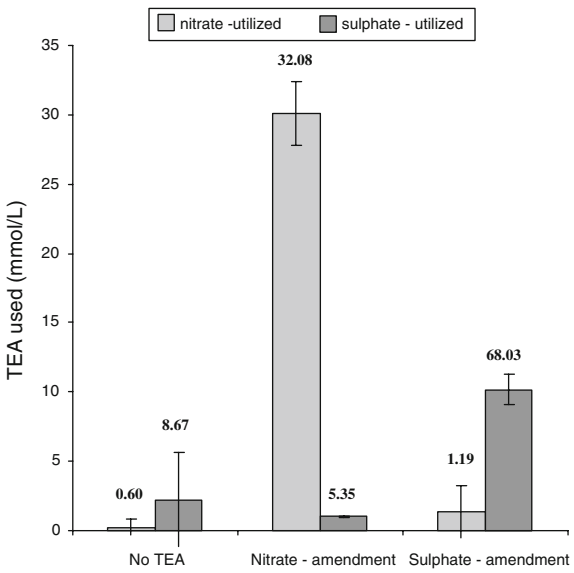

Fig. 1 TEA consumed after 13 days of incubation when nitrate or sulphate were added as a TEA with willow rhizosphere soil as inoculum. Values above *each bar* are the initial nitrate or sulphate concentration in mmol l^{-1}. *Error bars* represent the range of values in duplicate microcosms

Fertilizer addition

ANOVA analysis showed that fertilization significantly ($P < 0.05$) enhanced ethylene glycol degradation in poplar rhizosphere soils but was not significant for willow rhizosphere soil ($P < 0.05$) where $P = 0.052$. Poplar-soil microcosms amended with nitrate as TEA and 0.5 g of fertilizer per gram of soil achieved higher degradation rates of 12.1 ± 6.7 mmol l^{-1} day^{-1} (750 ± 415 mg l^{-1} $day^{-}1$; Table 1) compared to 3.8 ± 2.1 mmol l^{-1} day^{-1} (235 ± 130 mg l^{-1} day^{-1}) in unfertilized microcosms. A maximum of 19.3 mmol (1.20 g) of ethylene glycol degradation l^{-1} day^{-1} in fertilized poplar soils was obtained.

Discussion

Although reasonable care was taken to have reproducible test conditions, the single largest factor which

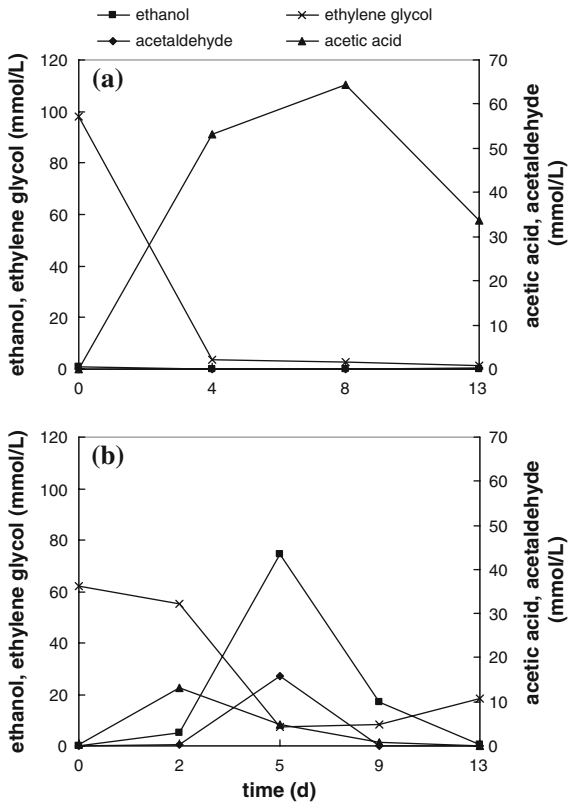

Fig. 2 Typical degradation profiles of ethylene glycol under (**a**) aerobic and (**b**) nitrate-reducing conditions

introduced significant variability between experiments and within the same experiment was the rhizosphere soil inoculum. All soils were uniformly mixed but the number and diversity of microorganisms introduced into each microcosm were not identical due to the heterogeneous nature of soil. Since the sampling intervals were widely spaced, the degradation rates may have been underestimated. Therefore, measured values were not absolute and consequently, only major trends were noted in the interpretation of the results.

Ethylene glycol degradation was clearly enhanced in poplar and willow rhizosphere soils when compared to the non-rhizosphere soil. Although anaerobic degradation of ethylene glycol has already been shown to be coupled to methanogenesis (Dwyer and Tiedje 1983; Strab and Schink 1986) and anaerobic fermentation (Schink and Stieb 1983; Strab and Schink 1986), its link to nitrate, or sulphate as a TEA has not been previously reported. The present study did clearly show ethylene glycol degradation with these TEAs. Methanogenesis, sulphate reduction and fermentation yield less energy for microbial growth than anaerobic respiration coupled to nitrate as electron acceptor (Cookson 1995). Consequently, the former processes result in slower bioremediation rates.

Anaerobic metabolism was different (Fig. 2) from aerobic degradation which was characterized by greater acetic acid production and no acetaldehyde formation. Anaerobic substrate consumption and metabolite production were similar under nitrate, and sulphate reducing conditions. Significantly higher ethanol production than what was obtained under aerobic conditions, the formation of acetaldehyde, and final alkaline conditions were distinct differences of anaerobic degradation. The formation of high concentrations of acetaldehyde suggest that the predominant microorganisms in the rhizosphere were not fermenting organisms like *Pelobacter venetianus*, which produce nearly equal amounts of ethanol and acetate, and trace amounts of acetaldehyde from ethylene glycol (Strab and Schink 1986). The metabolites of the rhizosphere consortia in this study were more similar to those of an enriched methanogenic consortium (Dwyer and Tiedje 1983; 1986) which had produced acetaldehyde, ethanol, acetic acid and methane.

The rhizosphere consortia utilized nitrate or sulphate in quantities comparable to the theoretical amounts required for their use as TEAs in ethylene glycol mineralization (Table 3). In some microcosms, more than the theoretical stoichiometric amount of ethylene glycol was degraded than the molar amount of nitrate or sulphate consumed, indicating that other TEAs may be involved. A resazurin dye indicated that oxygen was not present. Methanogenesis was not expected since CO_2 gas was not added to the headspace or bicarbonate added to the basal medium. However, CO_2 arising from ethylene glycol degradation coupled to another TEA could result in methanogenesis to yield methane. Although CO_2 consumed or methane produced was not quantified, methane was detected in the headspace of some microcosms suggesting that CO_2 was also used as a TEA. This suggests multiple species using different TEAs as pH and redox conditions changed with the initial degradation linked to nitrate or sulphate reducing microorganisms with subsequent proliferation of methanogens

Table 3 Comparison of the theoretical stoichiometric ratio of TEA required for ethylene glycol mineralization to CO_2, H_2O and growth ($C_5H_6O_2N$ represents biomass) to the experimentally obtained ratio

TEA	Theoretical		Experimental
	Stoichiometric reaction for ethylene glycol mineralization	Molar ratio of TEA to ethylene glycol	Molar ratio of TEA consumed to ethylene glycol degraded
Nitrate	$C_2H_6O_2 + 0.25\ NH_3 + 0.83\ NO_3^- \rightarrow 0.25\ C_5H_6O_2N + 0.42\ N_2 + 2.5\ H_2O + 0.75\ CO_2$		
Microcosm 1		0.83	0.73
Microcosm 2			0.65
Sulphate	$C_2H_6O_2 + 0.25\ NH_3 + 0.625\ SO_4^{-2} \rightarrow 0.25\ C_5H_6O_2N + 0.63\ S^{-2} + 2.5\ H_2O + 0.75\ CO_2$		
Microcosm 1		0.625	0.68
Microcosm 2			0.21

Equations developed according to Cookson (1995)

as CO_2 concentrations increased. Conditions became more alkaline (pH increased from 6.8 to 8.0) as H^+ ions were consumed and degradation rates indicated that acidogenesis might not be as important in anaerobic ethylene glycol degradation, as proposed by McVicker et al. (1998).

The addition of a chemical fertilizer did not increase nitrate or sulphate concentrations or appear to cause a shift in the microbial population to organisms which utilized nitrate or sulphate preferentially. Enhanced degradation rates were most likely due to growth arising from the increase in nitrogen and phosphorous nutrients with the increase in fertilizer concentration. The use of nitrate as a TEA with fertilization can be recommended as it achieved degradation rates comparable to aerobic degradation. However, the appropriate fertilizer concentration should be determined to avoid any inhibition. Organic fertilizers such as compost may also enhance degradation rates. While compost would provide nutrients to the trees and rhizosphere microorganisms, it should be avoided with hydrophobic contaminants which could sorb to its organic component or covalently link to humic materials thus reducing bioavailability (Alexander 1994).

The substantial rates and extent of ethylene glycol degradation under anaerobic conditions with different TEAs, by microorganisms in both poplar and willow rhizospheres, demonstrated the potentially significant contribution of anaerobic microbial degradation in phytoremediation. Such conditions may occur in rhizospheres where contaminant concentration is high (i.e. high biological oxygen demand) and/or where O_2 transfer conditions are poor such as in a saturated zone with low permeability. This is important considering that ethylene glycol uptake by poplar or willow trees is probably negligible. In unpublished studies, negligible ethylene glycol was taken up by willow trees (Jacob Sun personal communication) while Shupack and Andersen (2000) have shown that grasses and legumes did not uptake radiolabelled propylene glycol. Although ethylene glycol degradation is enhanced in the rhizosphere of shallow-rooted plants like alfalfa (Rice et al. 1997; Castro et al. 2001), legumes and grasses (Shupack and Andersen 2000; Marschner et al. 2001) where the soil horizon is potentially aerobic, this is the first study which demonstrates that rhizosphere microorganisms of deeper rooted trees like willow and poplar could degrade ethylene glycol using nitrate, sulphate and potentially CO_2 as TEAs. The potential application of these trees in phytoremediation to treat contaminated groundwater is advantageous as it avoids the complexities of a pump and treat system or the need for an in situ aerobic treatment requiring oxygen amendment. This is especially relevant to sites where treatment time is not crucial and the economies of a lower maintenance in situ approach may make remediation viable where it would otherwise not be given serious consideration.

Conclusions

Anaerobic degradation of ethylene glycol was demonstrated by microorganisms in willow and poplar rhizosphere soils using nitrate or sulphate as a terminal electron acceptor. The bacterial consortia used multiple TEAs resulting in complete (up to 99%) ethylene glycol degradation. Use of nitrate as TEA and fertilization improved rates of anaerobic degradation comparable to rates of aerobic degradation.

Acknowledgments The authors acknowledge the financial support of Malroz Engineering, Kingston, ON, and Ontario Centers of Excellence, Centre of Excellence for Earth and Environmental Technologies and Queen's University for financial support to D. Carnegie.

References

Alexander M (1994) Biodegradation and bioremediation. Academic Press, New York

Calabrese VGM, Elsavage RE, Bissonnette GK, Sexstone AJ (1993) Mobility and enhanced biodegradation of a dilute waste oil emulsion during land treatment. J Ind Microbiol 12:13–20

Castro S, Davies LC, Erickson LE (2001) Plant-enhanced remediation of glycol-based aircraft deicing fluids. Pract Period Hazard Toxic Radioact Waste Manag 5:141–152. doi:10.1061/(ASCE)1090-025X(2001)5:3(141)

Child J, Willetts A (1978) Microbial metabolism of aliphatic glycols bacterial metabolism of ethylene glycol. Biochim Biophys Acta 538:316–327

Chugunov VA, Ermolenko ZM, Zhigletsova SK, Martovetskaya II, Mironova RI, Zhirkova NA, Kholodenko VP, Urakov NN (2000) Development and application of a liquid preparation with oil-oxidizing bacteria. Appl Biochem Microbiol 36:577–581. doi:10.1023/A:1026696506947

Commission of the European Communities (2004) Case No 3467—DOW Chemicals. Office for Official Publications of the European Communities

Cookson JT (1995) Bioremediation engineering design and application. McGraw-Hill, New York, pp 474–475

Corseuil HX, Moreno FN (2001) Phytoremediation potential of willow trees for aquifers contaminated with ethanol-blended gasoline. Water Res 35:3013–3017. doi:10.1016/S0043-1354(00)00588-1

Cote R, Gherna R (1994) Nutrition and media. In: Gerhardt P, Murray RGE, Wood WA, Krieg NR (eds) Methods for general and molecular bacteriology. American Society for Microbiology, Washington, DC, pp 155–178

Dwyer DF, Tiedje JM (1983) Degradation of ethylene glycol and polyethylene glycols by methanogenic consortia. Appl Environ Microbiol 46:185–190

Dwyer DF, Tiedje JM (1986) Metabolism of polyethylene glycol by two anaerobic bacteria, *Desulfovibrio desulfuricans* and a *Bacteriodes* sp. Appl Environ Microbiol 52:852–856

Erickson LE (1997) An overview of research on the beneficial effects of vegetation in contaminated soil. Ann N Y Acad Sci 829:30–35. doi:10.1111/j.1749-6632.1997.tb48563.x

Gonzalez CF, Taber WA, Zeitoun MA (1972) Biodegradation of ethylene glycol by a salt- requiring bacterium. Appl Microbiol 24:911–919

Hungate RE (1969) A roll-tube method for cultivation of strict anaerobes. Methods Microbiol 3B:117–132. doi:10.1016/S0580-9517(08)70503-8

Kelley SL, Aitchison EW, Deshpande M, Schnoor JL, Alvarez PJJ (2001) Biodegradation of 1,4-dioxane in planted and unplanted soil: effect of bioaugmentation with *Amycolata* sp. cb1190. Water Res 35:3791–3800. doi:10.1016/S0043-1354(01)00129-4

Marschner P, Yang CH, Lieberei R, Crowley DE (2001) Soil and plant specific effects on bacterial community composition in the rhizosphere. Soil Biol Biochem 33:1437–1445. doi:10.1016/S0038-0717(01)00052-9

McGahey C, Bouwer EJ (1992) Biodegradation of ethylene glycol in simulated sub-surface environments. Water Sci Technol 26:41–49

McVicker L, Duffy D, Stout V (1998) Microbial growth in a steady-state model of ethylene glycol contaminated soil. Curr Microbiol 36:136–147. doi:10.1007/PL00006757

Revitt DM, Worrall P (2003) Low temperature biodegradation of airport de-icing fluids. Water Sci Technol 48:103–111

Rice P, Anderson T, Coats J (1997) Evaluation of the use of vegetation for reducing the environmental impact of deicing agents. In: Kruger F, Anderson T, Coats J (eds) Phytoremediation of soil and water contaminants. ACS Symposium Series No. 664, American Chemical Society, Washington, DC, pp 162–176

Schink B, Stieb M (1983) Fermentative degradation of polyethylene glycol by a strictly anaerobic, Gram-negative, nonsporeforming bacterium, *Pelobacter venetianus* sp. nov. Appl Environ Microbiol 45:1905–1913

Shieh WK, Lepore JA, Zandi I (1998) Biological fluidized bed treatment of ethylene and propylene glycols. Water Sci Technol 38:145–153. doi:10.1016/S0273-1223(98)00523-X

Shim H, Chauhan S, Ryoo D, Bowers K, Thomas SM, Canada KA, Burken JG, Wood TK (2000) Rhizosphere competitiveness of trichloroethylene-degrading, poplar-colonizing recombinant bacteria. Appl Environ Microbiol 66:4673–4678. doi:10.1128/AEM.66.11.4673-4678.2000

Shupack DP, Andersen TA (2000) Mineralization of propylene glycol in root zone soil. Water Air Soil Pollut 118:53–64. doi:10.1023/A:1005178219430

Staples CA, Williams JB, Craig GR, Roberts KM (2001) Fate, effects and potential environmental risks of ethylene glycol: a review. Chemosphere 43:377–383. doi:10.1016/S0045-6535(00)00148-X

Strab A, Schink B (1986) Fermentation of polyethylene glycol via acetaldehyde in *Pelobacter venetianus*. Appl Microbiol Biotechnol 25:37–42