Available online at www.sciencedirect.com
ScienceDirect
ELSEVIER
Geothermics 36 (2007) 348–361
GEOTHERMICS
www.elsevier.com/locate/geothermics

Biodegradability and groundwater pollutant potential of organic anti-freeze liquids used in borehole heat exchangers

Thimo Klotzbücher, Andreas Kappler *, Kristina L. Straub, Stefan B. Haderlein

Center for Applied Geosciences, Institute for Geosciences, Eberhard-Karls-University Tuebingen, Sigwartstrasse 10, D-72076 Tübingen, Germany

Received 2 October 2006; accepted 22 March 2007
Available online 30 May 2007

Abstract

Ground source heat pump systems are increasingly being used to exploit the energy content of shallow geothermal resources for space heating and cooling. In this study we evaluate the potential for groundwater contamination of the different organic anti-freeze compounds (ethylene glycol, propylene glycol and betaine) used in these pumps, based on a literature review of their biodegradability and the results of our own laboratory experiments on aquifer material.

Ethylene and propylene glycol were found to be readily biodegradable under both oxic and anoxic conditions, without formation of toxic or persistent intermediates. Long-term groundwater contamination by the glycols is therefore not expected. Betaine is also expected to be readily biodegradable in oxic and anoxic groundwater. The potential formation of trimethylamine, an intermediate of anaerobic betaine degradation, is, however, regarded as critical due to its unpleasant odor even at very low concentrations. Additionally, betaine has the potential to complex metal ions and thus may mobilize toxic metals in groundwater. We therefore recommend that betaine not be used in borehole heat exchanger fluids.

In addition to organic anti-freeze compounds such as glycols, borehole heat exchanger fluids also contain additives such as corrosion inhibitors or biocides. We demonstrate that potentially toxic additives in these fluids inhibit biodegradation of the organic anti-freeze compounds. In order to ensure environmental compatibility of borehole heat exchanger fluids, further research should be conducted on the impact of additives on subsurface microbiological activity and on groundwater quality.

Keywords: Geothermal heat pumps; Glycols; Betaine; Additives; Biodegradability; Borehole heat exchangers

* Corresponding author. Tel.: +49 7071 2974992; fax: +49 7071 5059.
E-mail address: andreas.kappler@uni-tuebingen.de (A. Kappler).

0375-6505/$30.00 © 2007 CNR. Published by Elsevier Ltd. All rights reserved.
doi:10.1016/j.geothermics.2007.03.005

1. Introduction

The use of ground source (or geothermal) heat pumps to heat and/or cool buildings has grown significantly during recent years (Curtis et al., 2005; Lund et al., 2005). These heat pumps represent a fast-growing segment of the geothermal market, with >100,000 units already installed in Europe (Sanner et al., 2003). In the widely used system known as a vertical closed loop, the heat exchange fluids are pumped through pipes installed in vertical boreholes that are about 80–120 m deep (Fig. 1). Generally water is used as the heat exchange fluid but, to improve system efficiency and to avoid freezing during winter months, these fluids may also contain anti-freeze compounds (usually approx. 25% by mass). To prevent microbial and fungal growth in the pipes and to improve the long-term stability of these systems, biocides, corrosion inhibitors, etc. may also be added (<5% in total). These anti-freeze compounds and additives represent a potential hazard in terms of groundwater contamination. Ideally, the chemicals used in geothermal heat exchanger loops should be of low toxicity and readily biodegradable under both oxic and anoxic conditions, with no formation of toxic and/or persistent intermediates. The risk of triggering geochemical reactions such as mobilization of toxic metals by complexation or dissolution/precipitation of minerals must also be taken into due consideration.

In this study we have evaluated the potential for long-term groundwater contamination of the different organic anti-freeze compounds commonly used in borehole heat exchanger (BHE) systems: ethylene glycol, propylene glycol and betaine (*N*,*N*,*N*-trimethyl glycine). To this end, we have reviewed the literature available on their biodegradability, focussing on the products and kinetics of anaerobic biodegradation. We have also performed laboratory experiments to identify the products of anaerobic biodegradation of both ethylene and propylene glycol in aquifer materials (i.e. rock samples) collected at a field site during the installation of a vertical BHE system. We also

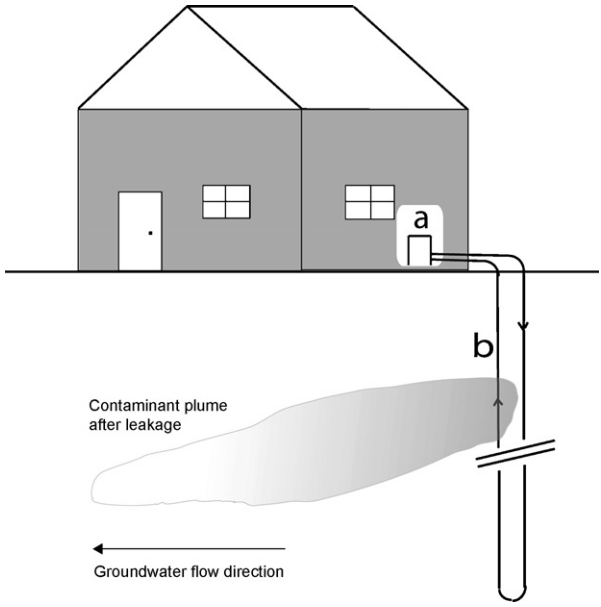

Fig. 1. Sketch of a vertical closed loop heat exchanger system. (a) Heat pump; (b) groundwater contamination after leakage of heat exchanger liquids.

reviewed published studies on the identity and environmental fate of additives such as corrosion inhibitors, biocides, etc., and conducted laboratory experiments to evaluate the effect of these additives on anaerobic glycol degradation. As far as we know, our experiments represent the first published data on anaerobic glycol degradation in aquifer material under fermenting, sulfate-, and iron-reducing conditions.

2. Material and methods

2.1. Anti-freeze compounds

Both ethylene glycol (EG = $HOCH_2CH_2OH$) and propylene glycol (PG = $H_3CCH(OH)CH_2OH$) are high production-volume chemicals used as anti-freeze agents and in the manufacture of polymers, paint, varnish and other products. PG is also utilized in the cosmetics and pharmaceuticals industry (ATSDR, 1997). Both glycols are used to de-ice aircraft and runways. Due to wind drift, large quantities of the glycols enter the surrounding environment, and are regarded as a serious environmental hazard in the vicinity of airports (e.g. Jaesche et al., 2006). Both glycols are completely miscible with water. The octanol–water (K_{ow}) and soil organic carbon–water (K_{oc}) partitioning coefficients of glycols are very low (ATSDR, 1997), with the result that significant bioaccumulation or retardation in groundwater by sorption to organic matter is not expected. The acute toxicity of both EG and PG is very low. LD_{50}-values (i.e. the median lethal dose) for EG vary between 4 and 10 g/kg BW (bodyweight) for rats, while LD_{50}-values for PG are >20 g/kg BW for rats, indicating a higher toxicity of EG compared to PG (OECD, 2001; WHO, 2002).

Betaine (*N*,*N*,*N*-trimethylamine; $CH_3N^+CH_2COO^-$), a methyl-derivative of the amino acid glycine, is also readily soluble in water and occurs naturally in many microorganisms, plants and animals (Stuart, 2004). Experimental studies on interactions of betaine with sediment/soil particles (e.g. K_{oc} value) are not available. From its chemical structure, a certain retardation of betaine in groundwater by sorption processes might be expected.

2.2. Sampling of aquifer material

The aquifer material used in our anaerobic degradation experiments was sampled at the drill site for a vertical borehole heat exchanger system in Wirnsheim (Baden-Württemberg, Germany), from depths of 80 and 100 m. The geologic formation present at these depths is dominated by Triassic carbonates (Lower Muschelkalk). The top of the groundwater table at the drilling site was at about 40 m depth.

Air was used to blow the material from the borehole; at the surface the samples were collected in a sterile plastic vessel as they discharged through a tube inserted at the wellhead. The material was sieved (2 mm) to remove large particles and poured into sterile Müller-Krempel glass bottles. The head space of the bottles was exchanged with N_2/CO_2 (90:10, v/v) and stored at 4 °C until set-up of the experiments. The sedimented, fine-grained material was used directly as bacterial inoculum.

2.3. Medium and growth conditions

An anoxic bicarbonate-buffered mineral medium (Widdel and Bak, 1992) was used for all anaerobic degradation experiments, containing per litre: 0.48 g KH_2PO_4, 0.24 g NH_4Cl, 0.02 g $MgSO_4 \cdot 7H_2O$, 0.32 g $MgCl_2 \cdot 6H_2O$, 0.08 g $CaCl_2 \cdot H_2O$. After autoclaving and cooling under

N_2/CO_2 (90:10, v/v), the bicarbonate buffer (final concentration 30 mM), 1 mL of a vitamin solution (Widdel and Pfennig, 1981), 1 mL of trace element solution, and 1 mL of an Se/Wo-solution (Widdel and Bak, 1992) were added per litre of medium.

2.4. Anaerobic glycol degradation experiments

Experiments (covering a total of 48 different conditions) were set-up anoxically in glass test tubes (head-space exchanged with N_2/CO_2, 90:10, v/v) using the method described by Hungate (1969). EG and PG were each added as sole carbon source (initial concentrations between ~12 and ~31 mM). Twenty-five percent (v/v) of aquifer material from 80 m depth or 10% (v/v) of aquifer material from 100 m depth was added as inoculum. Experiments were set-up without adding an external electron acceptor or with the addition of either poorly crystalline iron(III) hydroxide (ferrihydrite, 10 mM) or sodium sulfate (5 mM). Ferrihydrite was synthesized as described by Straub et al. (2005). The test tubes were incubated either at 12 °C, the presumed aquifer temperature at 80–100 m depth, or at 28 °C. Two parallel tubes were set-up for each combination of conditions and incubated for 8–10 weeks.

2.5. Experimental set-up to test the effect of additives on anaerobic microbial PG degradation

In order to test the effect of the additives in borehole heat exchanger fluids on anaerobic microbial PG degradation in our aquifer samples, we used the commercially available PG-based anti-freeze product Pekasol L (Pro Kühlsole Gmbh, Alsdorf, Germany). This heat exchanger fluid contains ~5–10% additives by volume (information obtained via personal communications with the manufacturer, March 2006).

A bacterial enrichment culture was prepared for inoculation by transferring an aliquot (5%, v/v) of a PG-degrading enrichment culture from the experiments described above (aquifer material from 80 m depth, incubated at 12 °C, no electron acceptor added) to fresh medium containing 20 mM PG. This pre-culture was incubated at 12 °C for 7 days. Experiments with/without additives were set-up anoxically with inoculum (1%) from the pre-culture, with either two different concentrations of pure PG (0.7 and 2.2 mL/L) or with Pekasol L (0.7 and 2.2 mL/L) as substrate. All experiments were run in duplicates for 38 days.

2.6. Analytical methods

Glycols (EG, PG), alcohols (ethanol, propanol), fatty acids (acetate, propionate) and acetaldehyde were analyzed by high pressure liquid chromatography (HPLC) (Shimadzu LC 10A-series, Kyoto, Japan) using an HC-75 Ca^{2+} column (Shimadzu, Kyoto, Japan) in combination with a mobile phase of 20 mM H_2SO_4 or 30 mM H_3PO_4 at a temperature of 80 °C. Sulfide was analyzed spectrophotometrically by the method described by Cline (1969). Iron(II) and total iron content were determined by the ferrozine assay, as described by Stookey (1970).

3. Literature review, experimental results and discussion

3.1. Current knowledge on biodegradation of glycols under oxic conditions (literature data)

The capacity to use ethylene glycol (EG) and propylene glycol (PG) as a carbon and energy source is widespread among aerobic microorganisms. Under oxic conditions, several pure cultures

of different bacterial groups proved capable of degrading EG (e.g. Child and Willetts, 1978; Wiegant and de Bont, 1980; Caskey and Tabor, 1981) and PG (e.g. Willetts, 1979; McDonald et al., 1980; Hou et al., 1983). Studies on metabolic pathways showed that degradation of EG in *Flavobacterium* species proceeds via glyoxylate, glycolate and pyruvate (Child and Willetts, 1978), while PG degradation proceeds via lactaldehyde and pyruvate (Willetts, 1979). Pyruvate is further metabolized to acetyl-CoA, which is oxidized to CO_2 by the tricarboxylic acid cycle. These data suggest that microbial degradation of EG and PG under oxic conditions occurs without accumulation of toxic and/or persistent organic intermediates.

Along with the pure cultures of microorganisms, mixed microbial populations were also shown to effectively degrade EG. McGahey and Bouwer (1992) performed ethylene glycol degradation experiments with environmental samples and observed rapid aerobic biodegradation of EG by bacterial communities from wastewater, different soil materials and groundwater. In groundwater, at an initial concentration of 111 ppm, EG was degraded with a half-life time of 0.92 days at 25 °C. A lag period of less than 3 days was observed. The half-life time in soil materials and wastewater was lower, ranging from 0.90 to 0.27 days. The authors also showed that half-life times in soil increased with decreasing temperature and increasing EG concentrations.

As regards PG, there are no data in the literature on the kinetics of aerobic degradation in groundwater. However, rapid aerobic biodegradation was observed in studies with sewage-sludge and soil samples. For example, PG present in soils at concentrations of <6000 ppm was degraded at an average rate of ~2 ppm/day at 2 °C, ~27 ppm/day at 8 °C and ~93 ppm/day at 25 °C (Klecka et al., 1993). Degradation rates in aquifers are lower, probably because of smaller microbial population densities in such oligotrophic systems, but further work is needed to quantify such degradation rates.

3.2. Current knowledge of the biodegradation of glycols under anoxic conditions (literature data)

3.2.1. EG and PG degradation by pure cultures of anaerobic bacteria

Various pure bacterial strains and enrichment cultures with different metabolic capabilities are able to degrade ethylene and propylene glycol anaerobically. These organisms were isolated from very diverse habitats, including nutrient-rich sewage sludge, wastewater (Dwyer and Tiedje, 1983; Obradors et al., 1988), sediments of an oligotrophic lake (Sass et al., 2004) and sandstone from >600 m depth (Sass and Cypionka, 2004). The capacity to biodegrade EG and PG anaerobically is therefore expected to be present in most anoxic environments.

Fermenting bacteria were shown to degrade ethylene and propylene glycol to equal amounts of their corresponding acids (acetate, propionate) and alcohols (ethanol, propanol) (Gaston and Stadtman, 1963; Toraya et al., 1979) (Fig. 2). EG and PG can also be degraded to their corresponding acids (acetate, propionate) as sole organic degradation products (Eichler and Schink, 1985), probably with the concomitant production of methane (CH_4) and/or H_2. Additionally, traces of the corresponding aldehydes (acetaldehyde, propionaldehyde) were found as degradation products (Toraya et al., 1979; Eichler and Schink, 1985). Besides fermenting bacteria, sulfate-reducing bacteria (SRB) were also shown to degrade EG and PG (Quatibi et al., 1991; Ouattara et al., 1992; Sass and Cypionka, 2004; Sass et al., 2004). *Desulfovibrio alcoholovorans* oxidized EG to acetate and PG to propionate and acetate, coupled to reduction of sulfate (Quatibi et al., 1991). Complete oxidation of the glycols to CO_2 by SRB is not reported in the literature. Most of the SRB that were tested for EG and PG degradation were able to use other electron acceptors as well (Sass and Cypionka, 2004; Sass et al., 2004). Therefore, anaerobic

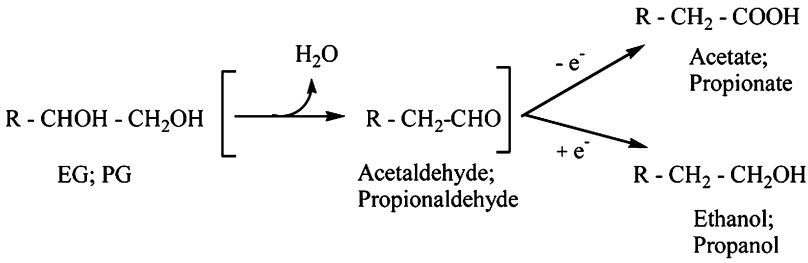

Fig. 2. Scheme of organic products formed from anaerobic degradation of ethylene and propylene (R = H or CH_3).

degradation of the glycols may also be coupled to reduction of nitrate, iron(III), or manganese (IV).

3.2.2. *Anaerobic degradation of EG and PG by mixed microbial populations*

Several published studies have demonstrated that EG and PG are degradable by mixed microbial populations under anoxic conditions in sewage sludge (Kaplan et al., 1982; Dwyer and Tiedje, 1983) and in soil (Sorensen et al., 2000; Jaesche et al., 2006). In this sludge, EG and PG were initially degraded by fermentation to equal amounts of the corresponding acids and alcohols (Dwyer and Tiedje, 1983; Veltman et al., 1998). Further degradation finally yielded methane and CO_2. Jaesche et al. (2006) found that poorly crystalline iron(III) and manganese(IV) oxides were used during microbial degradation of PG in soil columns. The reduction of iron(III) and manganese(IV) may be due either to direct oxidation of PG or to oxidation of the propanol and/or propionate that is produced initially by fermentation of PG (two sequential processes).

3.3. *Current knowledge on EG and PG degradation kinetics (literature data)*

Data on the kinetics of anaerobic glycol degradation in environmental samples (sewage sludge and top soil) were compiled from the literature (Table 1). Glycol degradation occurs within days or

Table 1
Kinetics of ethylene glycol (EG) and propylene glycol (PG) degradation by bacterial communities in environmental samples under anoxic conditions (selected data from the literature)

Inoculum	Experimental conditions	Results	References
EG-degradation			
Sewage sludge	T: 37 °C; C_{ini}[a] = 2.2 g/L	Complete degradation to ethanol and acetate within ~52 days	Dwyer and Tiedje (1983)
Different soils	T: 25 °C; C_{ini} = 1 g/kg soil	26–40% of theoretical CO_2-production after 68 days	Sorensen et al. (2000)
PG-degradation			
Soil (sandy loam)	T: 25 °C; C_{ini} = 1 and 10 g/kg soil	For C_{ini} = 1 g/kg soil: 100% degradation within 14 days; for C_{ini} = 10 g/kg soil: 98% degradation within 105 days	Klier and Goodwin (1997), cited in OECD (2001)
Sewage sludge	T: 37 °C; C_{ini} = 0.1 g/L	Complete removal of PG within 9 days and within 4 days when glucose was added to the medium	Kaplan et al. (1982)

[a] C_{ini}: initial concentration of EG or PG.

up to several months, depending on the initial concentration and/or inoculum used. Note that the data in Table 1 refer to either glycol removal or complete oxidation to CO_2, which probably takes several weeks in all cases. No information was available on the kinetics of glycol degradation in anoxic groundwater. Due to the smaller microbe population densities and lower temperatures, in situ degradation rates in deep aquifers are expected to be much lower and mineralization to CO_2 and H_2O will therefore probably last several months. Field studies will be required in future to predict more precisely the kinetics of microbial glycol removal and/or mineralization in anoxic aquifers.

3.4. Experimental study on biodegradation of glycols in anoxic aquifers

Since no reports on degradation of EG and PG under conditions similar to those present in deep anoxic aquifers were found in the literature, we performed degradation experiments with aquifer samples from a geothermal heat pump site. We incubated aquifer samples with and without the addition of external electron acceptors (ferrihydrite or sulfate) to determine whether the indigenous bacterial communities were able to degrade glycols by fermentation or anaerobic respiration.

Degradation of EG and PG occurred in most experimental set-ups with aquifer material from 80 and 100 m depth, with and without addition of external electron acceptors (iron(III) or sulfate). The results after 8–10 weeks of incubation are shown in Table 2. Degradation of >90% of added EG and PG was achieved in most of the experiments with inoculum from 80 m depth. In aquifer material from 100 m depth, EG was readily degraded whereas in 5 out of 12 experiments with PG, >90% of the added PG still remained after 8–10 weeks. This suggests that: (1) the amount of bacteria capable of metabolizing PG was significantly lower in the aquifer material from 100 m than from 80 m depth, and/or 2) the populations metabolizing EG and PG at 100 m depth are different.

Biodegradation of EG and PG in the aquifer material yielded organic metabolites, as expected from reports in the literature. The corresponding acids (acetate, propionate) predominated and in some experiments were indeed the sole organic products found. The corresponding alcohols (ethanol, propanol) were identified but were usually present in small quantities, with a molar ratio of acids to alcohols of about 8:1. Similar amounts of acids and alcohols were found in a few experiments only. Occasionally aldehydes were also detected (acetaldehyde from EG and propionaldehyde from PG).

In order to distinguish the degradation of EG and PG via fermentation processes from degradation via anaerobic respiration, we also investigated the increase in iron(II) and sulfide in the experiments that were amended with external electron acceptors. We found that in a few assays up to 3 mM of poorly crystalline iron(III) hydroxide was reduced to iron(II), but in most experiments there was a reduction of less than 2 mM of iron(III) (data not shown). From electron balance calculations we expect that oxidation of 1 mM of the glycols to the corresponding acids leads to the formation of 2 mM iron(II). Since we observed in most cases an almost complete degradation of EG and PG and accumulation of only a few mM of iron(II), we concluded that if iron-reducing bacteria were able to use the glycols as substrate, they were responsible for only a small fraction of the overall EG or PG degradation. We hypothesise that EG and PG was degraded via fermentation even in the presence of iron(III), whereas iron(III)-reducing microorganisms utilized the fermentation products of EG and PG, but not EG and PG directly.

Sulfate-reducing activity (revealed by the accumulation of significant concentrations of sulfide in the test tubes) was only observed in experiments with aquifer material from 80 m depth at 12 °C. In these tests, formation of sulfide was coupled with the oxidation of PG to propionate and, possibly, small amounts of acetate. In all other degradation experiments in which sulfate was

Table 2
Results of experiments on anaerobic biodegradation of ethylene glycol (EG) and propylene glycol (PG) in samples of aquifer materials from 80 and 100 m depth after 8–10 weeks of incubation

Experiment	Temperature											
	12 °C						28 °C					
	–[a]		Ferrihydrite (e-acceptor)		Sulfate (e-acceptor)		–[a]		Ferrihydrite (e-acceptor)		Sulfate (e-acceptor)	
	1	2	1	2	1	2	1	2	1	2	1	2
EG degradation in material from 80 m depth												
C_{ini}[b] (mM)	20	24	22	24	nd	nd	22	25	20	20	nd	nd
Degradation[c]	+++	+++	+++	+++	+++	+++	++	++	+++	+++	+++	+++
Product formation[d]	Acet Prop[e]	Acet Prop[e]	Acet Prop[e]	Acet	Acet Etol	Acet	Acet Etol Acal	Acet Etol Acal	Acet Prop[e]	Acet Prop[e]	Acet Etol	Acet Etol
EG degradation in material from 100 m depth												
C_{ini}[b] (mM)	nd	18	18	nd	nd	nd	17	18	17	17	nd	nd
Degradation[c]	++	++	++	+++	+++	+++	+++	+++	+++	+++	+++	+++
Product formation[d]	Acet	Acet	Acet	Acet	Acet Etol Acal	Acet Etol	Acet Etol[e]	Acet	Acet Etol[e]	Acet	Acet Etol Acal	Acet Etol
PG degradation in material from 80 m depth												
C_{ini}[b] (mM)	13	13	13	13	27	29	13	15	13	13	28	28
Degradation[c]	+++	+++	+++	+++	++	++	+++	+++	+++	+++	+++	+++
Product formation[d]	Prop Acet[e]	Prop Acet[e]	Prop Acet[e]	Prop Acet[e]	Prop Acet[e,h]	Prop Acet[e,h]	Prop Acet[e] Prol[e]	Prop Acet[e]	Prop Acet[e] Prol[e]	Prop Acet[e] Prol[e]	Prop Prol[g]	Prop Prol[g]
PG degradation in material from 100 m depth												
C_{ini}[b] (mM)	13	13	13	13	29	30	13	13	12	12	30	32
Degradation[c]	+	+	++	++	+	+	+++	+	+	+	+++	+++
Product formation[d]	–[f]	–[f]	Prop	Prop Prol[e]	Prop[e]	Prop[e] Acet[e]	Prop Prol[e]	–[f]	–[f]	Prop	Prop Prol[l]	Prop Prol

[a] No electron acceptor added.
[b] Initial concentration of EG/PG.
[c] Degree of degradation: (+) >80% of added EG/PG recovered; (++) 20–80% of added EG/PG degraded. (+++) >80% of added EG/PG degraded.
[d] Organic products formed: Acet: acetate; Prop: propionate; Etol: ethanol; Prol: propanol; Acal: acetaldehyde.
[e] Concentration of ≤3 mM.
[f] No organic degradation products detected.
[g] Propionate, propanol and, probably, propionaldehyde found.
[h] Production of sulfide. n.d.: not determinable because of analytical problems in the presence of sulfate.

added, SRB were obviously out-competed by glycol-fermenting bacteria. From these results we conclude that fermentation was the dominant process responsible for glycol degradation in our aquifer samples from 80 and 100 m depth. As acids (acetate, propionate) were found to be the main degradation products formed, the bacterial community in our samples was probably dominated by homo-acidogenic, glycol-degrading bacteria that are, as regards their glycol metabolism, similar to bacterial strains isolated by Eichler and Schink (1985) from various freshwater sources.

As a summary of the literature review and our experimental study, the following conclusions can be drawn:

(1) glycols are expected to be biodegradable in most anoxic environments, including anoxic groundwater systems;
(2) fermentation appears to be the main process for glycol degradation under anoxic environmental conditions, while anaerobic respiration processes (reduction of iron(III) and sulfate) are of minor significance;
(3) anaerobic glycol degradation will most probably yield CO_2 and small organic compounds such as aldehydes (acetaldehyde, propionaldehyde), acids (acetate, propionate), and alcohols (ethanol, propanol) as the main degradation products (shown schematically in Fig. 2);
(4) none of the organic degradation products that are generated by anaerobic EG or PG degradation (see Fig. 2) is expected to accumulate in groundwater. They are common intermediates produced during mineralization of organic compounds and have a very low toxicity. Many studies have shown that such compounds are rapidly degraded under both oxic and anoxic conditions. Therefore, mineralization to CO_2 and H_2O is expected ultimately for both EG and PG without accumulation of harmful intermediates.

3.5. Experimental study on the effect of additives on the biodegradability of glycols and evaluation of the identity and environmental fate of additives

Commercially available anti-freeze compounds used in borehole heat exchangers usually contain additives (approx. 5% of the added volume of organic compounds) such as corrosion inhibitors, biocides, wetting agents, odorants, and colorants. Information on the chemical identity of individual components is very limited, since manufacturers regard the composition of the additive mixtures as proprietary data. However, there are reports in the literature on the additives present in glycol-based aircraft de-icing fluids (ADFs) (e.g. Kent et al., 1999; Cancilla et al., 1997; Castro et al., 2004), which presumably contain components similar to those used in anti-freeze mixtures in geothermal loop systems.

Kent et al. (1999) reviewed the literature on the ecotoxicology of ADF mixtures. Their compilation clearly shows that EG- and PG-containing ADFs are more toxic to the aquatic biota than pure EG or PG. Cancilla et al. (1997) identified tolyltriazole additives as the most toxic fraction in ADF mixtures. ADFs contain benzotriazole and/or tolyltriazole compounds as corrosion inhibitors in concentrations of 0.5–2% by volume (Castro et al., 2004). Furthermore, benzotriazole is probably highly persistent in soil and groundwater due to its low biodegradability (Breedveld et al., 2003). Other corrosion inhibitors that may be used include phosphates, nitrites, nitrates and borax (Kent et al., 1999). Johnson et al. (2001) identified ethylene oxide, acetaldehyde, dioxane, high molecular weight polymers and polyamines as additives in ADFs.

The presence of additives inhibited PG degradation by anaerobic bacteria in sewage sludge (Johnson et al., 2001) and soil bacteria under oxic conditions (Cornell et al., 2000) when higher initial concentrations were used.

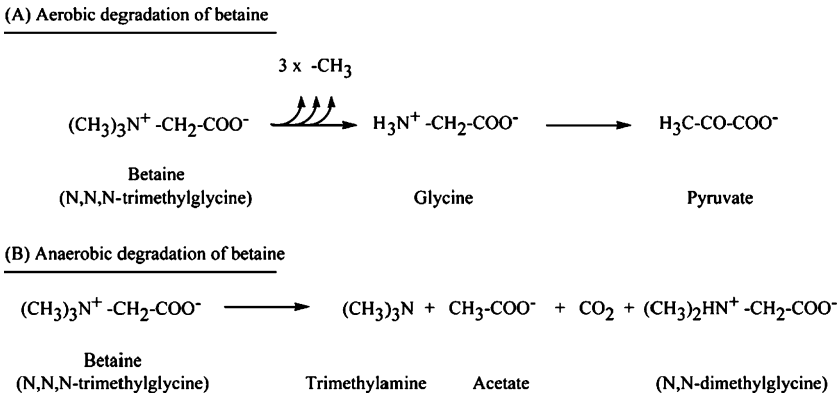

Fig. 3. Scheme of organic products formed from: (A) aerobic and (B) anaerobic degradation of betaine (*N*,*N*,*N*-trimethyl glycine).

We investigated the effect of the additives present in a glycol-based commercial heat exchanger fluid (Pekasol®) on the anaerobic microbial degradation of PG in samples of aquifer materials collected from a borehole at a geothermal drilling site. When either 0.7 mL/L of pure PG (approx. 10 mM) or 0.7 mL/L PG-containing Pekasol® (containing additives as well as the PG) were used as organic substrate, the PG was degraded within 38 days to concentrations below detection limit. However, when PG and Pekasol® were added in higher concentrations (2.2 mL/L PG or 2.2 mL/L Pekasol®, corresponding to approx. 30 mM PG), significant concentrations of PG still remained in the Pekasol® experiments after 38 days, indicating inhibition of PG-degrading bacteria by the additives in Pekasol®. Further research is, however, needed to investigate in detail the potential impact of additives in anti-freeze compounds on groundwater quality.

3.6. Current knowledge on biodegradation of betaine under oxic conditions (literature data)

Literature data indicate that betaine undergoes rapid biomineralization to CO_2 and H_2O in oxic environments: various microorganisms were shown to be able to use betaine as sole carbon and nitrogen sources under oxic conditions (see Müller et al., 1981; Oren, 1990). The pathway of betaine degradation was studied in the halophilic bacterium *Rhizobium melioti* (Smith et al., 1988). Betaine was demethylated stepwise to glycine ($H_3N^+CH_2COO^-$), which was further metabolised to pyruvate (Fig. 3) (e.g. via hydroxymethyltransferase into serine and to pyruvate through serine dehydratase). Pyruvate is a common intermediate in metabolic pathways. It is further metabolized to acetyl-CoA, which enters the tricarboxylic acid cycle and is degraded to carbon dioxide.

No information is available in the literature on the kinetics of aerobic degradation of betaine in soils or groundwater. However, according to the OECD protocol (OECD, 1993), betaine was shown to be easily biodegradable in biodegradability tests, with >90% of the theoretical CO_2 production achieved within 28 days (BfB Oil Research SA, 2003). In these tests sewage sludge was used as source of bacteria.

3.7. Current knowledge on biodegradation of betaine under anoxic conditions (literature data)

Anaerobic degradation of betaine has been studied extensively with pure cultures, and several degradation pathways are described in the literature: anaerobic betaine degradation yields

trimethylamine as one of its end products (Fig. 3). Trimethylamine is regarded as a critical component because of possible adverse effects on groundwater quality caused by its unpleasant rotten fish smell at very low concentrations (∼1 ppb in water). *Sporomusa* strains fermented betaine to trimethylamine, acetate, CO_2 and *N*,*N*-dimethylglycine (Möller et al., 1984) (Fig. 3). Strains of *Eubacterium acidaminophilum* cleaved betaine in a Stickland-type reaction to trimethylamine and acetate with other amino acids and/or hydrogen serving as electron donors (Hormann and Andreesen, 1989).

The significance of trimethylamine formation from betaine degradation in anoxic aquifers has not been studied. King (1984) investigated anaerobic degradation of betaine in marine sediments and suggested that most of this compound was degraded in a first step to trimethylamine and acetate in a 1:1 stoichiometry. However, it is also known that other anaerobic bacteria do not produce trimethylamine from betaine. For example, sulfate-reducing strains of *Desulfobacterium autotrophicum* produce *N*,*N*-dimethylglycine, CO_2 and sulfide from betaine-degradation coupled to sulfate reduction (Heijthuijsen and Hansen, 1989). Further research will be required to quantify: (1) to what extent trimethylamine is formed by betaine-degrading bacteria in anoxic groundwater systems, and (2) how fast trimethylamine can be further degraded.

3.8. Interactions between betaine and metals

Interactions between betaine and metal ions may occur in subsurface systems and wells, resulting in mobilization of toxic organo-metal compounds. Craig and Rapsomanikis (1985) observed that Pb(II) and Sn(II) were converted to toxic methyllead and methyltin derivatives in presence of betaine in aqueous media. Moreover, betaine may act as a complexing agent and increase the mobility of toxic metals in groundwater. To our knowledge, this process has not yet been studied. Further research could provide a better understanding of the fate and environmental behaviour of betaine in the subsurface. We therefore recommend that betaine not be used in heat exchanger fluids until a more comprehensive data set is available on its evolution and effects in groundwaters.

4. Conclusions and outlook for the future

In this study we evaluated the potential for long-term groundwater contamination of the antifreeze compounds (EG, PG and betaine) and additives used in ground source heat pump systems. With the increase in the number of heat pump units being installed, the risk of heat exchanger liquids leaking into groundwater aquifers might also be expected to increase in the future. Our focus was on microbial EG and PG degradation and product formation under anoxic conditions, which are most relevant in deeper aquifers.

Literature studies and laboratory experiments using aquifer material showed that both ethylene and propylene glycol are readily biodegradable in many oxic and anoxic environments, including soil and aquifer samples, without the occurrence of toxic or persistent organic intermediates. Although no field data on the kinetics of anaerobic degradation in groundwater are available, long-term contamination is not expected.

Considering that only small quantities of the glycols are used in borehole heat exchanger systems, the risks to groundwater quality are very low. In the case of betaine, the potential adverse impact on groundwater quality is higher. Trimethylamin, a potential degradation product formed by anaerobic betaine-degradation, is a critical component because of its unpleasant odor in very low concentrations in water. Furthermore, betaine has the potential to interact with metals, result-

ing in mobilization of toxic metal species. Since these processes are poorly studied, further studies are recommended to better understand the evolution of betaine in subsurface environments.

Further research should also be conducted on the identity, toxicity and environmental impact of the additives in anti-freeze mixtures. Certain components used as biocides or corrosion inhibitors in additives may have adverse effects on subsurface microbiology and inhibit the degradation of the anti-freeze agents.

Acknowledgments

The authors would like to thank Katja Amstätter for her assistance with the HPLC analysis. Comments by Prof. Peter Grathwohl and Dr. Philipp Blum helped to improve the quality of the manuscript. This work was supported by the State Ministry of Environment Baden-Wuerttemberg and by an Emmy Noether fellowship from the German Research Foundation (DFG) to AK.

References

ATSDR, 1997. Toxicological profile for ethylene glycol and propylene glycol. US Department of Health and Human Services, Agency for Toxic Substances and Disease Registry, Atlanta, GA, USA, 287 pp., published online as pdf.

BfB Oil Research SA, 2003. Biodegradability test report according to OECD 301B. Test report FO39962.01.01, Belgium.

Breedveld, G.D., Roseth, R., Sparrevik, M., Hartnik, T., Hem, L.J., 2003. Persistence of the deicing additive benzotriazole at an abandoned airport. Water, Air Soil Pollution, Focus 3, 91–101.

Cancilla, D.A., Holtkamp, A., Mattassa, L., Fang, X., 1997. Isolation and characterization of microtoxTM-active components from aircraft de-icing/anti-icing solutions. Environ. Toxicol. Chem. 16, 430–434.

Caskey, W., Tabor, W.A., 1981. Oxidation of ethylene glycol by a salt-requiring bacterium. Appl. Environ. Microbiol. 42, 180–183.

Castro, S., Davis, D.C., Erickson, L.E., 2004. Natural, cost-effective, and sustainable alternatives for treatment of aircraft deicing fluid waste. Environ. Progr. 24, 26–33.

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

Cline, J.D., 1969. Spectrophotometric determination of hydrogen sulfide in natural waters. Limnol. Oceanogr. 14, 454–458.

Cornell, J.S., Pillard, D.A., Hernandez, M.T., 2000. Comparative measures of the toxicity of component chemicals in aircraft deicing fluids. Environ. Toxicol. Chem. 19, 1465–1472.

Craig, P.J., Rapsomanikis, S., 1985. Methylation of tin and lead in the environment: oxidative methylation as a model for environmental reactions. Environ. Sci. Technol. 19, 728–730.

Curtis, R., Lund, J., Sanner, B., Rybach, L., Hellström, G., 2005. Ground source heat pumps—geothermal energy for anyone, anywhere: current worldwide activity. In: Proceedings of the World Geothermal Congress, Paper No. 1437, Antalya, Turkey, April 24–29, 9 p.

Dwyer, D.F., Tiedje, J.M., 1983. Degradation of ethylene glycol and polyethylene glycols by methanogenic consortia. Appl. Environ. Microbiol. 46, 185–190.

Eichler, B., Schink, B., 1985. Fermentation of primary alcohols and diols and pure culture of syntrophically alcohol-oxidizing anaerobes. Arch. Microbiol. 143, 60–66.

Gaston, L.W., Stadtman, E.R., 1963. Fermentation of ethylene glycol by *Clostridium glycolicum*, sp. nov. J. Bacteriol. 85, 356–362.

Heijthuijsen, J.H.F.G., Hansen, T.A., 1989. Anaerobic degradation of betaine by marine *Desulfobacterium* strains. Arch. Microbiol. 152, 393–396.

Hormann, K., Andreesen, J.R., 1989. Reductive cleavage of sarcosine and betaine by *Eubacterium acidaminophilum* via enzyme systems different from glycine reductase. Arch. Microbiol. 153, 50–59.

Hou, C.T., Patel, R.N., Lasking, A.I., Barnabe, N., Barist, I., 1983. Purification and properties of a NAD-linked 1,2-propanediol dehydrogenase from propane-grown *Pseudomonas fluorescens* NRRL B-1244. Arch. Biochem. Biophys. 223, 297–308.

Hungate, R.E., 1969. A roll tube method for cultivation of strict anaerobes. In: Norris, J.R., Ribbons, D.W. (Eds.), Methods in Microbiology. Academic Press, Inc., New York, NY, USA, pp. 117–132.

Jaesche, P., Totsche, K.U., Kögel-Knabner, I., 2006. Transport and anaerobic biodegradation of propylene glycol in gravel-rich soil materials. J. Contam. Hydrol. 85, 271–286.

Johnson, J.J., Varney, N., Switzenbaum, M.S., 2001. Comparative toxicity of formulated glycol deicers and pure ethylene glycol and propylene glycol. Report submitted to the University of Massachusetts, Amherst, MA, USA, Water Resources Research Center, August 2001, 60 pp.

Kaplan, D.L., Walsh, J.T., Kaplan, A.M., 1982. Gas chromatographic analysis of glycols to determine biodegradability. Environ. Sci. Technol. 16, 723–725.

Kent, R.A., Andersen, D., Caux, P.Y., Teed, S., 1999. Canadian water quality guidelines for glycols—an ecotoxicological review of glycols and associated aircraft anti-icing and deicing fluids. Environ. Toxicol. 14, 481–522.

King, G.M., 1984. Metabolism of trimethylamine, choline, and glycine betaine by sulfate-reducing and methanogenic bacteria in marine sediments. Appl. Environ. Microbiol. 48, 719–725.

Klecka, G.M., Carpenter, C.L., Landenberger, B.D., 1993. Biodegradation of aircraft deicing fluids in soil at low temperatures. Ecotoxicol. Environ. Safety 25 (3), 280–295.

Klier, N.J., Goodwin, P.A., 1997. Anaerobic biodegradation of propylene glycol in soil. Unpublished report, The Dow Chemical Company, Midland, MI, USA (cited in OECD, 2001).

Lund, J.W., Freeston, D.H., Boyd, T.L., 2005. Direct application of geothermal energy. Geothermics 34, 691–727.

McDonald, H.C., Takeguchi, M.M., Detar, C.C., Simon, P.A., Livsey, K.A., Odstrchel, G., Kaplan, N.O., Weetall, H.H., 1980. The isolation and characterization of 1,2-propanediol oxidoreductase from *Neisseria gonorrheae*. J. Gen. Microbiol. 119, 451–458.

McGahey, C., Bouwer, E.J., 1992. Biodegradation of ethylene glycol in simulated subsurface environments. Water Sci. Technol. 26, 41–49.

Möller, B., Ossmer, R., Howard, B.H., Gottschalk, G., Hippe, H., 1984. *Sporomusa*, a new genus of gram-negative anaerobic bacteria including *Sporomusa sphaeroides* spec. nov. and *Sporomusa ovata* spec. nov. Arch. Microbiol. 139, 388–396.

Müller, E., Fahlbusch, K., Walther, R., Gottschalk, G., 1981. Formation of *N*,*N*-dimethylglycine, acetic acid, and butyric acid from betaine by *Eubacterium limosum*. Appl. Environ. Microbiol. 42, 439–445.

Obradors, N., Badia, J., Baldoma, L., Aguilar, J., 1988. Anaerobic metabolism of the L-rhamnose fermentation product 1,2-propanediol in *Salmonella typhimurium*. J. Bacteriol. 170, 2159–2162.

OECD, 1993. OECD guidelines for testing of chemicals. OECD 301 B-CO^2 evolution test. Organization for Economic Cooperation and Development, Paris, France.

OECD, 2001. 1,2-dihydroxypropane. Organisation for Economic Cooperation and Development-SIDS Initial assessment report for 11th SIAM. Organization for Economic Cooperation and Development, Washington, DC, USA.

Oren, A., 1990. Formation and breakdown of glycine betaine and trimethylamine in hypersaline environments. Antonie van Leeuwenhoek 58, 291–298.

Ouattara, A.S., Cuzin, N., Traore, A.S., Garcia, J.S., 1992. Anerobic degradation of 1,2-propanediol by a new *Desulfovibrio* strain and *D. alcoholovorans*. Arch. Microbiol. 158, 218–225.

Quatibi, A.I., Niviere, V., Garcia, J.L., 1991. *Desulfovibrio alcoholovorans* sp. nov., a sulfate-reducing bacteria able to grow on glycerol, 1,2- and 1,3-propandiol. Arch. Microbiol. 155, 143–148.

Sanner, B., Karytsas, C., Mendrinos, D., Rybach, L., 2003. Current status of ground source heat pumps and underground thermal energy storage in Europe. Geothermics 32, 589–602.

Sass, H., Cypionka, H., 2004. Isolation of sulfate-reducing bacteria from the terrestrial deep subsurface and description of *Desulfovibrio cavernae sp. nov*. Systemat. Appl. Microbiol. 27, 541–548.

Sass, H., Overmann, J., Rütters, H., Babenzien, H.-D., Cypionka, H., 2004. Desulfosporomusa polytropa gen. nov., sp. nov., a novel sulfate-reducing bacterium from sediments of an oligotrophic lake. Arch. Microbiol. 182, 204–211.

Smith, L.T., Pocard, J.A., Bernard, T., Le Rudulier, D., 1988. Osmotic control of glycine betaine biosynthesis and degradation in *Rhizobium meliloti*. J. Bacteriol. 17, 3142–3149.

Sorensen, J.A., Gallagher, J.R., Hawthorne, S.B., Aulich, T.R. 2000. Final report (2000-EERC-10-04) for the Gas Industry Groundwater Research Program. Energy & Environment Research Center, University of North Dakota, Grand Forks, ND, USA, 80 pp.

Stookey, L.L., 1970. Ferrozine—a new spectrophotometric reagent for iron. Anal. Chem. 42, 781–799.

Straub, K.L., Kappler, A., Schink, B., 2005. Enrichment and isolation of iron(III) and humic acid reducing bacteria. Meth. Enzymol. 397, 58–77.

Stuart, A.S.C., 2004. Betaine in human nutrition. Am. J. Clin. Nutr. 80, 539–549.

Toraya, T., Honda, S., Fukui, S., 1979. Fermentation of 1, 2-propandiol and 1, 2-ethanediol by some genera of *Enterobacteriaceae*, involving coenzyme B12-dependent diol dehydratase. J. Bacteriol. 139, 39–47.

Veltman, S., Schoenberg, T., Switzenbaum, M.S., 1998. Alcohol and acid formation during the anaerobic decomposition of propylene glycol under methanogenic conditions. Biodegradation 9, 113–118.

WHO, 2002. Ethylene glycol—human health aspects. World Health Organization—*Concise International Chemical Assessment Document* 45, Genf, Switzerland, 44 pp.

Widdel, F., Bak, F., 1992. Gram-negative mesophilic sulfate-reducing bacteria. In: Balows, A., Trüper, H.G., Dworkin, M., Harder, W., Schleifer, K.-H. (Eds.), The Prokaryotes, 2nd ed. Springer, New York, NY, USA, pp. 3352–3378.

Widdel, F., Pfennig, N., 1981. Studies on dissimilatory sulfate-reducing bacteria that decompose fatty acids. I. Isolation of a new sulfate-reducer enriched with acetate from saline environments. Description of *Desulfobacter postgatei* gen. nov. sp. nov. Arch. Microbiol. 12, 395–400.

Wiegant, W.M., de Bont, J.A.M., 1980. A new route for ethylene glycol metabolism in *Mycobacterium* E44. J. Gen. Microbiol. 120, 325–331.

Willetts, A., 1979. Bacterial metabolism of propane-1,2-diol. Biochim. Biophys. Acta 588, 302–309.