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Biodegradation of Aircraft Deicing Fluids in Soil Slurries

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Propylene glycol (PG) is a main component in several commercial formulations of aircraft deicing fluids (ADF). Their use is a source of soil pollution along airport runways. Even though PG is biodegradable by soil bacteria, seasonal overloads can give rise to occasional groundwater contamination. A prevention strategy could be the enhancement of biodegradation rate in the unsaturated zone. The effect of addition of nutrients on the kinetics of PG degradation has been studied in soil slurries. In the absence of added nutrients, the aerobic removal kinetics is zero-order in PG concentration over the range 0.05-1 g/L. There is no biomass growth and PG degradation occurs by maintenance metabolism at constant rate depending on the initial concentration of PG-degraders. In the presence of ammonia as a nitrogen source, biomass exponential growth allows a faster aerobic PG degradation. Biomass growth can be detected by the apparent change in PG removal kinetics.

1. Introduction

Aircraft deicing fluids (ADF) containing propylene glycol (PG) and other additives are used at Northern airport in winter (Switzenbaum et al., 2001). Due to the drips of ADF from the aircrafts during take-off, the ADFs are mixed with snow along the runways during winter. The chemicals infiltrate the soil surface along the runways when the melting of snow begins in spring. It is important to ascertain that these chemicals do not contaminate groundwater and surface waters (Ferguson et al., 2008). The unsaturated zone can act as a natural purification system, because soil bacterial communities easily degrade PG under aerobic conditions (Klečka, 1993; Shupack and Anderson, 2000), whereas anaerobic degradation in groundwater is considerably slower (Wejden and Øvstedal, 2006). ADFs constitute a threat to the groundwater if biodegradation rates are not sufficient compared to infiltration velocities in the unsaturated zone (French and Van der Zee, 2011).
We present experimental data on the biodegradation of PG under aerobic conditions. Soil samples from the Oslo International Airport at Gardermoen have been used as source of degrading microorganisms. Gardermoen Airport is built on Norway's largest rain-fed aquifer and must operate on the condition that the groundwater is not affected (French et al., 2009).
Even though PG is biodegradable by soil bacteria, seasonal overloads can give rise to occasional groundwater contamination. A prevention strategy could be the enhancement of biodegradation rate in the unsaturated zone. The rate and the extent of biodegradation can be severely limited by many factors, such as temperature, biomass concentration, availability of additional nutrients and electron acceptors. Our work has been focused on the effect of addition of nutrients (*biostimulation*) on the kinetics of propylene glycol biodegradation in soil.
All biodegradation tests were carried out in shaken flask reactors containing soil suspensions in PG or commercial ADF solutions. This is an extremely simplified system as compared to what actually occurs at the site, all phenomena related to mass transfer and to soil heterogeneities having been removed.

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Slurry experiments are often used in treatability studies to check if in situ bioremediation will occur (or not). In this study, instead of using slurry tests only for a yes/no answer, further information about the actual remediation process is gathered by its kinetics. In particular, substrate removal kinetics is expected to be markedly different in the presence and in the absence of growth of degrading microorganisms.

2. Materials and methods

2.1 Soil samples

Soil was taken from the site in two different sampling campaigns planned for site characterization. The first one was performed near the West runway, i.e. in a zone affected by deicing fluid pollution in the course of lysimeter excavations performed in October 2008. PG-degrading activity of soil samples L3-1 (first horizon layer from lysimeter 3 excavation at coordinates N60 11.320 E11 04.499) and L7-1 (first horizon layer from lysimeter 7 excavation at coordinates N60 11.330 E11 04.500) from this sampling campaign has been measured in aerobic slurry experiments without addition of nutrients. In a second sampling campaign, a large sample was also taken in an uncontaminated field (Moreppen) nearby the Airport. Fractions of this sample (labeled in the following as “control”) were used in aerobic slurry experiments with and without addition of nutrients. All the soil samples were stored at 4 °C until used.
Soil is acidic loamy sand with low organic matter content and very low enzymatic activities. Soil properties relevant to the discussion are reported in Table 1. PG was not detected in any sample. Thus, the PG spread along airport runways during winter was either degraded or transported to deeper zones until autumn, when sampling has been done.

Table 1: Relevant soil properties

	L3-1	L7-1	Control
TOC, g/kg	*nm*	82.4 ± 13.0	1.53 ± 0.30
pH	6.76 ± 0.05	6.60 ± 0.01	5.07 ± 0.15
FDAH, µg fluorescein/(g h)	1.600 ± 0.045	2.500 ± 0.131	*nd*

Means ± standard errors of three replicates; *nd* = not detectable; *nm* = not measured (insufficient sample); TOC = Total Organic Carbon; FDAH = Fluorescein Di-Acetate Hydrolase

2.2 Reagents

Analytical grade propylene glycol (from Sigma-Aldrich, > 99.5 %) has been used for most of biodegradation experiments. The commercial formulation Safewing MP II Flight^{TM} (50 % (w/w) PG, from Clariant, Germany) has been used to test possible inhibitory effects of anti-corrosion additives.

2.3 Analytical methods

Propylene glycol (PG) concentration in water samples has been measured by GC-FID (Shimadzu, model GC-2010) equipped with a capillary column with a PEG stationary phase (BP20, 30 m by 0.32 mm i.d., 0.25 mm film thickness, from SGE). 1 mL samples have been injected with a split-ratio of 1:10. Helium has been feed as carrier gas with a flow rate of 5.9 mL/min. Injector and FID temperatures have been set to 220 °C. Initial column temperature has been set to 40 °C, kept for 1 min, followed by a ramp of 15 °C/min till 145 °C, kept for 1 min. Retention time for PG was about 3.5 min, by comparison with a pure standard.
Soil TOC (total organic carbon) and water-soluble nitrogen were measured by a Shimadzu TOC Analyzer (model TOC-V CSH). The Solid Sample Module SSM-5000A allows direct TOC measures of soil samples. Soluble nitrogen was measured in filtered liquid samples by the same TOC Analyzer with TNM-1 ancillary unit for total nitrogen analysis.
Soil pH was measured in 1: 2.5 soil: water suspensions. The fluorescein diacetate hydrolase (FDAH) activity was assessed as described by Adam and Duncan (2001). FDAH is a generally accepted measure of total catabolic activity of soils. For comparison purpose, the FDAH activity range in an agricultural soil is 40 - 180 µg fluorescein/(g h).

2.4 Aerobic degradation in soil slurries

Aerobic biodegradation in soil slurries was tested in 500 mL shaken flasks filled with 200 mL of 1.0 g/L PG solution in deionized water or mineral media (see Table 2). The flasks and the solutions were sterilized at 121 °C for 15 min before use. After cooling at room temperature, 30 g of soil sample were added under a laminar hood. Flasks were shaken at 140 rpm in a basculating incubator (Minitron, Infors) thermostated at 15 °C. Slurry samples were taken periodically in a sterile way under laminar hood. Prior to analyses, slurry samples were centrifuged at 11,180 g for 15 min at 5 °C and filtered with cellulose acetate membranes with 0.2 µm cut-off (from Sartorius, Goettingen).

Table 2: Mineral media for biostimulation

Label	Medium composition
PG-PBS	PG 1 g/L, Na_2HPO_4 7 g/L, KH_2PO_4 3 g/L, NaCl 0.5 g/L, trace elements
PG-M9	PG 1 g/L, NH_4Cl 1 g/L, Na_2HPO_4 7 g/L, KH_2PO_4 3 g/L, NaCl 0.5 g/L, trace elements
PG-AMM	PG 1 g/L, NH_4Cl 1 g/L
SAFE-M9	Safewing MP II Flight^{IM} 2 g/L, NH_4Cl 1 g/L, Na_2HPO_4 7 g/L, KH_2PO_4 3 g/L, NaCl 0.5 g/L, trace elements.

3. Substrate uptake models

PG removal kinetics is expected to be markedly different for: i) maintenance metabolism only, ii) substrate uptake with biomass growth.

3.1 Maintenance metabolism only

This is the case when primary substrate (carbon/energy source) concentration is very low or when a secondary substrate (N or P source) is limiting. Biomass cannot be synthesized due to energy or material limitation.

If m is the specific rate of substrate uptake for maintenance (g substrate per g biomass by dry weight and per h) and x_0 the (constant) biomass concentration (g/L by dry weight), the time course of residual PG concentration is given by

$$[\mathrm{PG}] = [\mathrm{PG}]_0 - mx_0t = [\mathrm{PG}]_0 - kt \qquad (1)$$

(cfr. Model I in Simkins and Alexander, 1984).

3.2 Substrate uptake with biomass growth

In the case of substrate removal with biomass growth, if the specific growth rate μ (h^{-1}) and the biomass yield on substrate Y (g biomass by dry weight produced per g of substrate used) can be considered constant, the time course of residual PG concentration is given by

$$[\mathrm{PG}] = [\mathrm{PG}]_0 - \frac{x_0}{Y}\left(e^{\mu t} - 1\right) \qquad (2)$$

(cfr. Model VI in Simkins and Alexander, 1984).

In the derivation of Equation 2, it has been supposed that μ is constant throughout the course of batch growth. This is in seeming contrast with the standard Monod model for growth kinetics in the presence of one limiting substrate. The Monod law gives the best fit for chemostat fermentation data. However, since the Monod constant is generally in the ppm range, it is almost impossible to obtain an estimate from batch fermentation data alone (see discussion in Nielsen et al., 2003, §9.1.1). Therefore, we have chosen to approximate the Monod law with a zero-order kinetics in substrate concentration with μ = constant if [PG] > 0, μ = 0 if [PG] > 0.

4. Results and discussion

4.1 Aerobic slurries without nutrient addition

Typical results of aerobic degradation tests carried out with different soil samples at 15 °C are shown in Figure 1a-b.

By inspection of Figure 1a, it appears that the rate of PG degradation does not depend on substrate concentration, even though the latter might vary from 1 g/L to almost nil. This result can be explained if the Monod constant is lower than the detectable concentration limit for PG (about 0.05 g/L for the adopted analytical conditions). On the other hand, constant degradation rate can only be explained if no cell growth takes place: PG-degraders concentration x_0 is constant, as well, since the rate of degradation is first-order in x_0. Therefore, the degradation of PG is only due to maintenance metabolism of the original population, further biomass growth being limited by the lack of some other key substrate.

Zero-order kinetics constants k were calculated by linear regression on concentration vs. time data (Equation 1). Degradation rate is almost the same in slurries with soil samples from L3 - 1 and L7 - 1 (Figure 1a) with average $k = 8.9 \cdot 10^{-3}$ g/(L h), whereas it is substantially lower in slurries with control soil samples (Figure 1b) with average $k = 4.2 \cdot 10^{-5}$ g/(L h). Such difference can be explained by markedly different original concentrations of PG-degrading microorganisms (x_0 in Equation 1) in soil samples from a previously contaminated zone (L3 - 1, L7 - 1) and from uncontaminated zone ("control"). By using FDAH activity as an indicator of overall active biomass concentration in soil, such explanation is consistent with the findings of comparable FDAH values for L3 - 1 and L7 - 1 samples and of activity below assay detection limits for control sample (see Table 1).

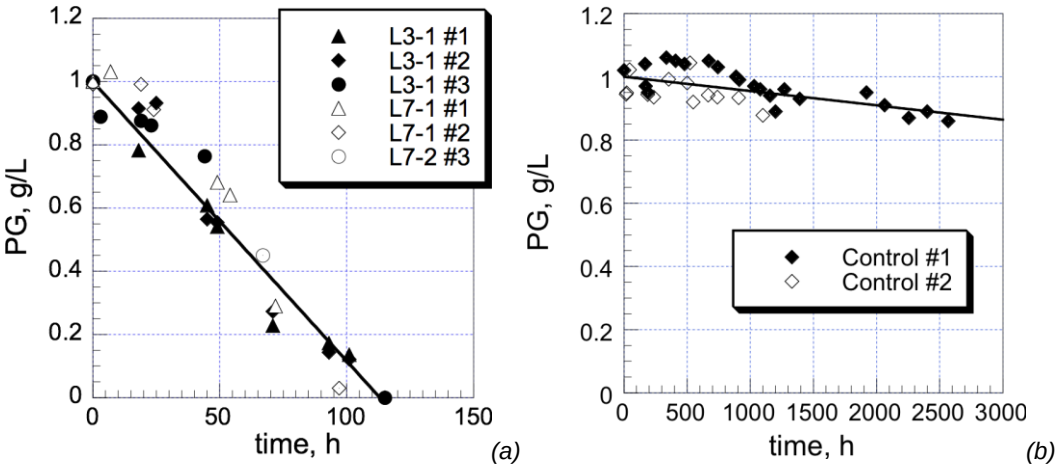

Figure 1: Aerobic PG degradation in: (a) L3 - 1 and L7 - 1 soil slurries (three replicas), (b) control soil slurries (two replicas)

4.2 Aerobic slurries with nutrient addition

As stated above, apparent zero-order kinetics followed throughout all experimental runs suggests that no biomass growth takes place, PG consumption being only due to maintenance energy requirements of the existing population of PG-degraders. This can be attributed to the lack of a key substrate, such as nitrogen source. Lack of nitrogen source is here a reasonable possibility since all Gardemoen soils are rather poor in organic matter content.

Figure 2a depicts PG aerobic degradation in control soil slurries, in the presence of different nutrient mixtures. Almost no PG removal is obtained in the time span of the experiments, if only phosphate and trace elements are added (PG-PBS medium). The data compare with those of Figure 1b, obtained without addition of nutrients. A dramatic, exponential decrease in PG concentration is apparent whenever the nutrient mixture contains 1 g/L of ammonium chloride. The removal courses are the

same for the complete synthetic medium (PG-M9 medium) and for the minimal one with PG and ammonia only (PG-AMM medium). Incidentally, the use of a commercial deicing fluid preparation as a source of PG (SAFE-M9 medium) does not change the rate of degradation, indicating no inhibition by anti-corrosion additives.

The time course of PG removal in the presence of a nitrogen source suggests that first-order kinetics in biomass concentration holds (see derivation of Equation 2). By performing a non-linear regression on data with Equation 2, the estimated parameter values (± standard error) are μ = 0.052 (± 0.013) h^{-1} and x_0/Y = $1.8 \cdot 10^{-6}$ (± $5.9 \cdot 10^{-6}$) g/L (large estimation errors are common in pre-exponential factors).

The biomass yield Y can be estimated by measurement of residual soluble nitrogen. It has been found that about 75.6 mg N/L have been assimilated into the biomass upon removal of 0.97 g/L of PG. By assuming an average biomass raw formula $C_5H_7O_2N$ with 10 % ash content by weight, the production of about 0.68 g/L of dry weight biomass is computed from the nitrogen assimilation. The biomass yield is then about 0.70 g of biomass dry weight per g of PG. From the estimated value of x_0/Y, x_0 = $1.26 \cdot 10^{-6}$ g/L of dry weight biomass, much less than the final amount even with a large estimation error.

In order to check qualitatively the consistency of the model, a further test has been performed by recycling the soil after full removal of PG from solution. The soil has been separated by centrifugation at 1,780 g for 5 min and then re-suspended in a fresh PG-M9 medium. As it can be seen from Figure 2b, the lag phase is seemingly absent due to the higher initial biomass concentration (the test is not fully quantitative due to the incomplete recovery of planktonic biomass by centrifugation).

As a final evidence for growth, soil-free mixed cultures of PG degraders have been obtained by a sequential enrichment procedure in PG-M9 medium. Estimated values of μ and Y for soil slurries match those of direct measures in soil-free cultures of PG-degraders selected from the site (Toscano et al., 2012).

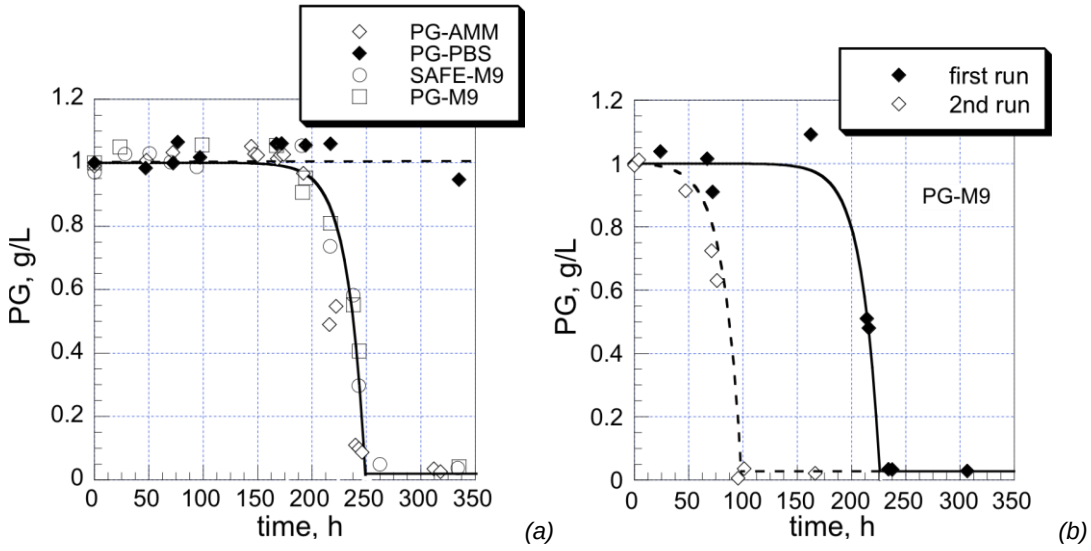

Figure 2: Aerobic PG degradation in control soil slurries: a) effect of different nutrient additions; b) second run performed by recycling the soil from the first run.

5. Conclusions

Slurry reactors are frequently used in the assessment of feasibility of biodegradation in natural soil systems. The rate of contaminant removal is usually quantified by zero- or first-order kinetics decay constants. The significance of such constants for the evaluation of removal rate in the field could be questioned, because the slurry reactor is a water-saturated, well-stirred system without resemblance with an unsaturated fixed bed of soil. Nevertheless, a kinetic study with soil slurry reactors can still be

useful by means of only slightly more sophisticated kinetic models than zero-/first-order decay. The use of kinetic models taking into account the role of degrading biomass, even in the absence of reliable experimental methods for its quantification, provides further insight into the effect of nutrient additions. A real acceleration of biodegradation processes is obtained only when the degrading biomass is in the growth condition. The apparent change in contaminant removal kinetics, as shown in our work, can be useful to diagnose biomass growth without direct biomass amount measurement.
In the case of PG degradation, the zero-order kinetics with respect to PG concentration was found to hold, at least to the extent that can be evaluated in the current experimental setup. The addition of a nitrogen source changes the time-course profile of PG concentration from linear (Equation 1) to inverted exponential (Equation 2), signaling the switch from maintenance metabolism to unconstrained growth.
Slurry experiments suggest that a suitable *in situ* enhancement technique could be the soil fertilization with ammonium salts. The availability of ammonium would accelerate PG removal by promoting the growth of autoctonous, aerobic PG-degrading biomass in the unsaturated zone.

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