Effects of Substitutions on the Biodegradation Potential of Benzotriazole Derivatives

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Abstract: Fourteen benzotriazole derivatives were subjected to microcosm tests to study the influence of substitutions on their biodegradation potential. Methylated, nitratated, carboxylated, and propionated benzotriazoles, a heterocyclic triazole, as well as methylated benzimidazoles, were introduced to activated sludge and soil enrichment cultures as the only carbon source. Some of the enrichment cultures were derived from airport soils that had been previously contaminated with aircraft deicing fluids and subsequently enriched with the commercially significant corrosion inhibitor methylbenzotriazole. The 5-methylbenzotriazole and only the carboxylated derivatives were degraded by soil or activated sludge biomass regardless of acclimation conditions. Radiotracer studies of [U-14C] 5-methylbenzotriazole, and [U-14C] 5-carboxybenzotriazole confirmed that relatively high concentrations (25mg L⁻¹) of these derivatives can be completely mineralized in relatively short time frames by microbial consortia regardless of prior exposure. Observations suggested that the growth yield on these compounds is likely low. Biodegradation patterns suggested that carboxylated benzotriazole derivatives are more readily biodegradable than their more popular methylated counterparts.

1. Introduction

Benzotriazole (BT) and its derivatives are produced in large quantities for various well-known purposes such as the production of fuels, detergents and varnish stabilizers [1, 2]. With respect to environmental dissemination routes, methylbenzotriazole (MeBT) has been found as the most commonly occurring triazole-containing pollutant on waterways [3]. This corrosion inhibitor is commonly found in aircraft deicing fluid (ADF) formulations and other freeze point depressing additives. More than 7 billion gallons of stormwater contaminated with deicing chemicals are generated annually in the USA alone [4]. Accordingly, comprehending the mechanisms governing the fate and transport of the deicing components of benzotriaoles is essential for the evaluation of the potential terrestrial environmental impacts [5]. The environmental loadings of benzotriazole derivatives are relatively high and do last for long periods [3, 5, 6]. The storm runoff containing benzotriaoles can impose toxicity to model ecosystem receptors [7, 8]. Surveys in northern Europe [5] and central Europe [9] have already identified the benzotriaole derivatives with significant concentrations in the major drinking water sources.

The commercially significant benzotriazole derivatives are likely will not biodegrade under common water and soil conditions. However, benzotriazole is known to be light sensitive and photodegradable in certain environments [10]. Some direct evidence of biodegradation under specific
environmental conditions is available, but albeit limited. It was claimed that of the two commercially significant methylbenzotriazole isomers, 5-MeBT is actually biodegradable, while 4-MeBT is recalcitrant in oxygen saturated water or soil with active microbial biomass [11, 12]. Benzotriazole (BT), 4-methyl- and 5-methyl-benzotriazole (4- and 5- MeBT) were suggested to not biodegrade under anaerobic conditions in the presence of different electron acceptors. Therefore, presumably, aerobic biodegradation is the major removal mechanism in water systems such as activated sludge systems [13]. 5-MeBT biodegradation was found to be enhanced through acclimation and nutrient supply [14]. Recent study found that the integration of the sunlight-induced processes (with TiO₂) with subsurface-flow treatment wetlands caused 42% further elimination of benzotriazole [15]. Methylbenzotriazole was found to degrade by the lignin peroxidase induced from the fungus Phanerochaete chrysosporium [16]. Therefore, it appears that 4- and 5- MeBT are recalcitrant under conditions void of oxygen and do not serve as substrates for denitrification, sulfate-reduction, or fermentation during conventional (activated sludge) wastewater treatment, or anaerobic digestion [17-19]. Other studies found that BT and 5-MeBT can biodegrade under aerobic and anaerobic conditions and their biodegradation efficiency and biodegradation products were dependent on the predominant terminal electron-accepting condition [20, 21].

The substitutions of certain functional groups on compounds containing single or multiple aromatic rings can facilitate the degradation of an otherwise recalcitrant substrate—such substitutions have been observed with other azo-containing compounds [22-24]. An explanation offered for these observations is that some substituted compounds become more accessible to the enzymatic attack of terrestrial microbes or those otherwise enriched in wastewater treatment plants [25]. The contribution of common functional constituents toward higher biodegradation potentials depends on the pathways available through microbial enzyme inventories expressed, as well as local environmental conditions. There have been few reports documenting enhancements to the biodegradation potential of aromatic conjugates with heterocyclic azides, but none have been extended to include those benzotriazoles that are commonly used as industrial corrosion inhibitors. Zimmermann and coworkers showed that some enzyme reaction rates increased with the substitution of electron-withdrawing substituents on phenyl rings; these substitutions, in turn, enhanced the degradation of some common azo dyes [26, 27]. Patil and coworkers [28] observed that the introduction of functional groups of known electro-negative behaviors, particularly electron-withdrawing and lipophilic groups, could enhance the ability of benzyltriazole conjugates to bacterial biodegradation. These observations are in agreement with those reported on the biodegradation of selected azo dyes where electron-donating functional groups, particularly hydroxyl substituents in positions ortho to azo-nitrogen, contributed to increasing biodegradation potential of these compounds [24]. As a gross physical-chemical parameter, water solubility appears to be important to biodegradability, which is clearly impacted by substitution [29, 30]; however, this generality does not appear to apply to heterocyclic azo-containing compounds. In this context, prediction of xenobiotic biodegradation potential, based solely on gross chemical properties, has not been generally successful.

Prevention of environmental hazards and satisfying the impending regulatory pressures entail providing different benzotriazole requiring industries with cost effective and readily biodegradable corrosion inhibitors. In relevant to this, fourteen different benzotriazole derivatives (functional substitutions) were tested for their biodegradation potential using microcosms-containing acclimated and non-acclimated enrichment cultures of soil and activated sludge. In addition, using radio labeled substrates [U-14C], the more readily biodegradable derivatives were examined for their potential to be completely mineralized. Radiography observations were leveraged to estimate effective biomass yields.

2. Material and Methods

2.1. Culture Media

Support media for microcosms slurries consisted of the following (mg per liter of MQ): NH₄SO₄, 600; KH₂PO₄, 440; KH₂PO₄, 340; MgSO₄·7H₂O, 53; CaCl₂·2H₂O, 28; FeCl₃·6H₂O, 8.1; ZnSO₄·7H₂O, 0.68; CuSO₄·5H₂O, 0.17; CoCl₂·6H₂O, 0.17; NaMoO₄·2H₂O, 0.17; MnSO₄·H₂O, 0.15; H₃BO₃, 0.04. The pH of this solution was 7.0. All solutions were sterilized (autoclaved) prior to their use.
2.2. Benzotriazoles Derivatives

[U-^{14}C]-5-Methylbenzotriazole ([U-^{14}C] 5-MeBT) was synthesized from [U-^{14}C]-p-toluidine according to a zinc-catalyzed substitution pathway published in U.S. Patent 4061491. The radiochemical purity of the [U-^{14}C]-5-MeBT using high pressure liquid chromatography (HPLC) was greater than 98%, with a specific activity of c. 500 μCi g⁻¹. [U-^{14}C] 5-Carboxybenzotriazole ([U-^{14}C] 5-CBT) was synthesized from [U-^{14}C] 5-methylbenzotriazole by selective oxidation of the methyl moiety. One-half gram of [U-^{14}C] 5-MeBT was dissolved in 10 ml, 50% NaOH. The mixture was heated to between 50-60 °C, and 1.6 g KMnO₄ was gradually added (stirred) in small portions. The reaction mixture was incubated for 4 hours at 55°C. After this time, the mixture was filtered to remove any reduced manganese, which was present as MnO₂(s). For purification, 0.5 g charcoal was then added to the filtrate and heated between 50-60 °C. The resulting black precipitates were filtered out (Whatman qualitative circles; Fisher Scientific Pittsburgh, PA, USA) and the filtrate discarded. The filter-retained solids were washed in concentrated HCl, and the pH of the wash solution subsequently adjusted to 7. This process yielded [U-^{14}C] 5-CBT with purity determined by HPLC to be > 98 %, and specific activity of c. 300 μCi g⁻¹. The scintillation cocktail used for all radioactivity measurements was Ultima Gold (Packard BioScience Company, Meriden, CT). All other benzotriazole derivatives used in this investigation were synthesized and obtained in cooperation with PMC Specialty Group Inc. (Cincinnati, OH, USA), or Sigma Chemical Company (St. Louis, MO, USA). The benzotriazoles derivatives chemical structures are shown in Figure 1.

2.3. Microbial Enrichments

Enrichments consisted of both non-acclimated and acclimated consortia. The acclimated cultures for these experiments were enriched from shallow subsurface soils within two meters of the central deicing pad at Denver International Airport, Colorado, USA. Soils removed from this area are geotechnical grade fills, and have been comprehensively analyzed using accepted geotechnical methods [31]; soil fill characteristics, sampling, and microbial enrichment have been previously described [11]. This culture has been maintained...
in batch reactors and exposed to 4-and 5-MeBT for extended periods (months at room temperature under aerobic conditions).

Non-acclimated cultures were obtained from a bench-scale sequencing batch activated sludge reactor. This system was initially inoculated from a local municipal wastewater treatment plant (Boulder, CO). The reactor was operating under aerobic conditions, at a residence time of 5 days with a glucose substrate, when biomass samples were removed for these experiments.

2.4. Biodegradation Potential Assays
Inocula for these experiments consisted of either acclimated or non-acclimated biomass, which was normalized by widely accepted gravimetric analysis [32] immediately prior to metabolic and radiotracer assays. Cells were harvested by centrifugation at 3500 rpm for 30 min, and washed three times in sterile 0.85% NaCl, before final suspension in culture media. All microcosm assays were initiated with a biomass concentration of 500 mg L⁻¹ total suspended solids (TSS) including 25 mg L⁻¹ of each benzotriazole derivative in sterile one-liter Erlenmeyer flasks. All flasks were covered with sterile gauze and incubated on a rotary shaker table at 180 rpm at 25 °C ± 2°C. Control flasks consisted of autoclaved inocula to quantify any potential abiotic transformations, or sorptive losses, but were maintained under otherwise identical conditions.

Samples for benzotriazole derivative analysis were periodically withdrawn with a sterile polypropylene pipette and centrifuged at 10,000 x g in polypropylene micro centrifuge tubes for 5 minutes to separate biomass. After centrifugation, the supernatant was transferred to 2.0 ml amber crimp top high pressure liquid chromatography (HPLC) vials (Agilent Technologies, # 5181-3376), stored at 4°C prior to analysis, and analyzed by HPLC.

2.5. Chemical and Physical Parameter Analysis
Benzotriazole Derivative Assay. All benzotriazole derivatives were measured using a HPLC fitted with an UV detector (λ = 254 nm) except for H₄TT (λ = 220 nm). Separation of the isomers was performed isocratically using two Zorbax Rx-C8 4.6 x 250 mm columns in series (MacMod Analytical, Inc., Chadds Ford, PA, USA). The eluent for this method consisted of a phosphate buffer mixed in a 70:30 ratio with HPLC grade acetylnitrile at a flow rate of 1.5 ml minute⁻¹ with a sample injection volume of 200 μL. All samples were centrifuged at 10,000 x g for 5 minutes prior to injection. The method detection limit was 0.2 mg L⁻¹, using accepted USEPA QA/QC methods [32].

2.6. Gravimetric Biomass Measurements
Total suspended solids (TSS) were measured according to standard method for the analysis of water and wastewater 2540D [32], with the exception that a 45-μm Durapore filter (Fisher Scientific, Pittsburgh, PA) was used instead of glass fiber filters.

2.7. Mineralization of 5-MeBT and 5-CBT
During radiotracer biotransformation assays, an overall mass balance of the sole organic carbon source supplied (as MeBT or CBT) was compiled by measuring the gaseous, soluble, and biomass fractions in a time series according to widely-accepted methods [33]. As a function of time, the degraded carbon source [U⁻¹⁴C] 5-Methylbenzotriazole or [U⁻¹⁴C] 5-Carboxybenzotriazole, Cₜ:

\[ Cₜ = Cₚ + C_{CO₂} + Cₛ \]

Where,

- \( Cₜ \) = total amount of \( ^{14} \)carbon added to the culture solution
- \( Cₚ \) = biomass \( ^{14} \)carbon
- \( C_{CO₂} \) = carbon recovered as \( ^{14} \)CO₂
- \( Cₛ \) = soluble \( ^{14} \)carbon (soluble biomass + HCO₃⁻ in solution + other soluble products)
The initial benzotriazole mass was normalized by its β-particle emissions, which were traced through metabolism (if it occurred), sorptive processes, gas phase partitioning, and biomass incorporation. In 250 mL borosilicate biometer flasks (Bellco Glass, Inc, Vineland, NJ), both 14C labeled 5-MeBT and 14C labeled 5-CBT (25 mg L⁻¹) were incubated under sustained aerobic conditions. Each microcosm was prepared in triplicate using acclimated and non-acclimated biomass as previously described. The reaction was held on a shaker table at room temperature 25°C ± 2°C. The potential for non-biological CO₂ evolution was evaluated by aerating a sterile control, which consisted of biomass inactivated with 0.2% sodium azide. Side arms of 14CO₂ traps contained 5 ml of 1M NaOH. At each sampling interval, all the NaOH solution was removed from the trap and replaced with fresh NaOH. The radioactivity retained by the NaOH in the trap was determined by diluting 1 ml aliquot in 15 mL of scintillation cocktail. The quantity of 14CO₂ evolved and subsequently trapped was measured by accepted liquid scintillation counting methods in accordance with the protocol outlined [34] with a Packard Instrument Model Tri-Carb 2300 TR Liquid scintillation counter (LSC) (Meriden, CT). Counts per minute (cpm) were converted to disintegrations per minute (dpm) by using an external standard (t-SIE) quench curve available from the scintillation manufacturer. When the rates of radiolabeled carbon dioxide evolution indicated that mineralization process reached a plateau, 14C, which was respectively incorporated into biomass and present in solution, was determined by the following procedure: a 1 mL sample of suspended biomass was obtained from each biometer and centrifuged for 15 minutes at 12,000 x g. After centrifugation, the supernatant was carefully separated from the biomass pellet. The activity of the supernatant (C₅) was measured by LSC as described above. To the biomass pellet, 250 μL of a proprietary alkaline-surfactant tissue solubilizer (ScintiGest, Fisher Scientific, Pittsburgh, PA) was added to aid in sample digestion and solubilization of biomass components. The resulting mixture was then sonicated on ice for 2 hours, after which the β-emissions of the pellet (C₆) were also determined by LSC as described above. β-emissions from all experimental dilutions were measured within an hour of preparation.

3. Results and Discussion

3.1. Biotransformation of Benzotriazole Derivatives

Among the benzotriazoles tested, only those derivatives conjugated to a methyl group in the 5-position, or a carboxyl group in either the 4- or 5- positions were labile for the biodegradation under the conditions described. All three of these isomers were susceptible to biodegradation by either acclimated or non-acclimated microbial biomass, although a slower degradation rate (lag phase) was observed for the degradation of 4-CBT (Figure 2 and Figure 3). Replacement of a 5-methyl group by a carboxylic acid moiety increased biodegradation potential (rate and onset) of respective CBT and MeBT disappearance under identical conditions.

The substituted groups of nitro on benzene ring, the propionic acid on the triazole ring, and replacement of benzene ring with non-aromatic ring (cyclohexane) has been found to be inhibitory to biodegradation. The biodegradation patterns observed here suggest that the biodegradation potential depends mainly on the biodegradation pathways [24]. More derivatives should be tested in the future to explore the quantitative relationships between biodegradability of benzotriazole and their chemical structures.
Figure 2. Concentrations of commercially significant and noble benzotriazole isomers in soil enrichment cultures acclimated to aircraft deicing fluids. CBT = carboxybenzotriazole, MeBT = methylbenzotriazole, H₄TT = tetrahydro tolyltriazole, TT-AA = 1-propionyl-5-methylbenzotriazole, NO₂TT=methyl-6(7)-nitrobenzotriazole, BT = benzotriazole, TI = methylbenzimidazole. Slight increases in concentration are due to evaporative losses.

Figure 3. Concentration (mg/L) of methylbenzotriazole and carboxylated benzotriazole isomers introduced to activated sludge biomass (non-acclimated). CBT=carboxybenzotriazole, MeBT=methylbenzotriazole. Slight increases in concentration are due to microcosm evaporative losses.

3.2. Mineralization Potential of Methylbenzotriazole

Soil enrichment cultures, previously exposed to aircraft deicing components, including MeBT, demonstrated a significant aerobic mineralization response by degrading up to 25 mg L⁻¹ [U-¹⁴C]-5-MeBT within 5 days, most of which was recovered as ¹⁴C CO₂. On average, final recoveries of 65% as ¹⁴CO₂ and 18% in biomass were observed for soil enrichment cultures (Table 1). Degradation of [U-¹⁴C]-5-MeBT to ¹⁴CO₂ was entirely attributable to biological activity because no mineralization was observed in otherwise identical systems that had been inactivated with sodium azide. When using [U-¹⁴C]-5-MeBT, the ¹⁴C radioactivity left in solution accounted for 11% of the total ¹⁴C beta emission originally added to the biometers (Table 1). As judged by HPLC, this ¹⁴C could not be associated with any measurable amounts of 5-MeBT (< 0.2 mg L⁻¹), and is likely some other unknown byproduct of bacterial metabolism and/or colloidal, unfilterable biomass fragments and/or ¹⁴CO₂ (as bicarbonate alkalinity) in solution.
3.3. Mineralization of Carboxybenzotriazole

Replacement of a methyl group with a carboxylic acid group in either the 4- or 5- positions resulted in the enhancement of biodegradation potential with respect to the methylated derivatives due to the present carboxyl group (COOH) which enhances the primary degradation of aromatic rings under aerobic conditions. Regardless of acclimation condition or consortia of soil or sewage origin, significant aerobic biodegradation of carboxybenzotriazole was observed. Fifty-eight and fifty-seven percent of the radiolabeled carbon added as [U-14C] 5-CBT were recovered as 14CO2, whereas 15% and 14% were recovered in biomass. No mineralization was observed for otherwise identical systems containing sodium azide confirming biological activity. The amount of 14C left in solution was 17% and 16% of the total carbon introduced to acclimated and non-acclimated biomass, respectively (Table 1).

The differences in biodegradation responses and the intermolecular architecture of 5- and 4- substituted benzotriazoles carry some resemblance to biodegradation and substitution patterns reported before when investigating a class of poorly degradable sulfonated azo-dyes [35]. Among the sulfonated azo dyes, the hydroxyl group in para position relative to the azo linkage were the most susceptible to degradation, such that there was no steric hindrance or potential for intramolecular azo(N)—hydrogen bonding. As with some azo dyes, differences in biodegradation potential of 4 and 5 substituted benzotriazoles are due to the steric hindrance of methyl and carboxy group, 4-MeBT was not biodegradable and 4 CBT was less susceptible to degradation than 5-CBT and lag times for degradation of 4 CBT was longer than 5 CBT. The group substituted closer to azo bond tends to hinder the steric hindrance that could prevent or slow the interactions between microbial enzymes and the methyl or carboxyl group in the substrate.

The results agree with Kulla and coworkers [36] who reported analogous patterns for Orange I and Orange II sulfonated and carboxylated azo dyes. Also, Pasti-Grigsby et al. [37] reported an enhancement of overall degradability by replacing selected moieties with a carboxyl group. In general oxygen—containing molecular fragments (such as OH, carboxy, and carbonyl groups) have been previously reported to enhance the biodegradability regardless of the molecular skeleton [38]. The biodegradation patterns observed here suggest that at least one enzyme system associated with the biodegradation of 4-carboxybenzotriazole is inducible. There is no direct evidence here, or in the literature, documenting the biodegradation potential of 4-methyl substituted benzotriazole [13].

3.4. Observed Yields

When challenged with [U-14C]-5-MeBT, the amount of 14C recovered from acclimated biomass was 18% of the total 14C added (Table 1). This agrees with yield observations estimated using standard suspended solids measurements and biomass phospholipid increases (effective yield c. 0.20). When challenged with [U-14C]-5-CBT, 15% and 14% percentages (Table 1) were recovered from the acclimated and the non-acclimated enrichment cultures respectively. This agrees with the yield observations estimated using the standard suspended solids measurements and the biomass phospholipid increases (effective yield c. 0.1). These observed yields are markedly lower than those commonly observed for the aerobic biodegradation of phenyl compounds and/or conjugates containing methyl or carboxyl moieties [39]. This may be due to the great deal of energy needed to break the azo ring before the substrate can be metabolized to produce energy.

Table 1. Triplicate means of the recovery of 14C as percentage of total carbon added as either 14C-MeBT or 14C-CBT to soil and activated sludge enrichment cultures.

|                        | Acclimated to aircraft deicing fluids (Soil enrichment cultures) | Non-acclimated to aircraft deicing fluids (Activated sludge cultures) |
|------------------------|-------------------------------------------------------------------|-----------------------------------------------------------------------|
| 14C-MeBT               | 18%                                                               | 15%                                                                   |
| 14C-CBT                | 17%                                                               | 14%                                                                   |

7
4. Conclusions
Using acclimated soil enrichment cultures and non-acclimated activated sludge, only 5-MeBT, 4-CBT and 5-CBT appear labile to biodegradation at different degradation rates, and under aerobic conditions. Replacement of a methyl group with a carboxylic acid group in either the 4- or 5- positions resulted in the enhancement of biodegradation potential with respect to the methylated derivatives. The carbon in these benzotriazole derivatives was found to be mineralized to nontoxic products (CO₂) in both acclimated and non-acclimated cultures; Therefore, carboxylate derivatives may be a good industrial alternative to methylated counterparts and can lower environmental exposures and ecosystem risks. No clear correlation was found between aerobic biodegradation of benzotriazoles and their chemical structure.

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References
[1] Huntscha S, Hofstetter T B, Schymanski E L, Spahr S and Hollender J 2014 Environ. Sci. Technol. 48 4435-4443.
[2] Dummer N M 2014 Rev. Environ. Sci. Biotechnol. 13 53-61.
[3] Kolpin D W, Furlong E T, Meyer M T, Thurman E M, Zaugg S D, Barber L B and Buxton H T 2002 Environ. Sci. Technol. 36 1202-1211.
[4] USEPA 2000 Preliminary Data Summary - Airport Deicing Operations, US Environmental Protection Agency, Washington DC, p. 448.
[5] Weiss S, Jakobs J and Reemtsma T 2006 Environ. Sci. Technol. 40 7193-7199.
[6] Kiss A and Fries E 2009 Environ. Sci. Pollut. Res. 16 702-710.
[7] Cancilla D A, Holtkamp A, Matassa L and Fang X 1997 Environ. Toxicol. Chem. 16 430-434.
[8] Cornell J S, Pillard D A and Hernandez M T 2000 Environ. Toxicol. Chem. 19 1465-1472.
[9] Voutsa D, Hartmann P, Schaffner C and Giger W 2006 Environ. Sci. Pollut. Res. 13 333-341.
[10] Hem L J, Weideborg M and Schram E 2000 Degradation and Toxicity of Additives to Aircraft De-Icing Fluids; The Effect of Discharge of Such Fluids to Municipal Wastewater Treatment Plants., WEF and Purdue University Industrial Wastes Technical Conference., Oslo, Noreay.
[11] Cornell J S 2002 The Environmental Chemistry of Aircraft Deicing Fluid (ADF) Component Chemicals, Civil, Environmental and Architectural Engineering, University of Colorado, Boulder.
[12] Rao N, M., Lu F F and Nhgiem N P 1996 Method of Preventing Yellow Metal Corrosion in Aqueous Systems with Superior Corrosion Performance in Reduced Environmental Impact, Nalco Chemical Company, USA.
[13] Herzog B, Lemmer H, Huber B, Horn H and Müller E 2014 Environ. Sci. Pollut. Res. 21 2795-2804.
[14] Herzog B, Yuan H, Lemmer H, Horn H and Müller E 2014 Bioresour. Technol. 163 381-385.
[15] Felis E, Sochacki A and Magiera S 2016 Water Res. 104 441-448.
[16] Wu X, Chou N, Lupher D and Davis D 1998 Benzotriazoles: Toxicity and Degradation, in: Erikson L E, Rankin M M (Eds.), 13th Annual Conference on Hazardous Waste Research, Kansas State University, Manhattan, KS.

[17] Gruden C L 2000 Fate and Toxicity of aircraft deicing fluid additives through anaerobic digester, Department of civil, environmental, and architectural engineering, University of Colorado, Boulder.

[18] Gruden C L, Dow S M and Hernandez M T 2001 *Water Environ. Res.* **73** 72-79.

[19] O’Brien I 2002 The Environmental Chemistry of Aircraft Deicing Fluid (ADF) Component Chemicals, Civil, Environmental and Architectural Engineering, University of Colorado, Boulder.

[20] Liu Y-S, Ying G-G, Shareef A and Kookana R S 2013 *J. Contam. Hydrol.* **151** 131-139.

[21] Liu Y-S, Ying G-G, Shareef A and Kookana R S 2011 *Water Res.* **45** 5005-5014.

[22] Pasti-Grigsby M B, Burke N S, Goszczyński S and Crawford D L 1996 *Appl. Environ. Microbiol.* **62** 1814-1817.

[23] Pasti-Grigsby M B, Paszczynski A, Goszczyński S, Crawford D L and Crawford R L 1992 *Appl. Environ. Microbiol.* **58** 3605-3613.

[24] Suzuki T, Timofei S, Kurunczi L, Dietze U and Schuurmann G 2001 *Chemosphere* **45** 1-9.

[25] Paszczynski A, Pasti M B, Goszczyński S, Crawford D L and Crawford R L 1991 *Enzyme microb. technol.* **13** 378-383.

[26] Zimmermann T, Kulla H G and Leisinger T 1982 *Eur. J. of Biochem.* **129** 197-203.

[27] Zimmermann T, Gasser F, Kulla H G and Leisinger T 1984 *Arch. Microbiol.* **138** 37-43.

[28] Patil S G, Nicholls P H and al e 1998 *Pestic. Sci.* **22** 333-342.

[29] Howard P H, Saxena J and Sikka H 1973 *Environ. Sci. Technol.* **12** 398-407.

[30] Alexander M 1973 *Biotechnol. Bioeng.* **15** 611-647.

[31] AASHTO 2003 Handbook Standard Recommended Practice for Conducting Geotechnical Subsurface Investigations, Thomson, Ann Arbor.

[32] APHA 1995 Standard Methods for the Examination of Water and Wastewater, American Public Health Association, Washington, DC.

[33] Raghavan D, Wagner G C and Wool R P 1993 *J. Environ. Polym. Degrad.* **1** 203-211.

[34] Schmidt S K and Scow K M 1997 Use of soil bioreactors and microcosms in bioremediation research, in: Hurst C J (Ed.) Manual of Environmental Microbiology, ASM Press, Washington, DC, pp. pp. 822-829.

[35] Pasti-Grigsby M B, Lewis T A, Crawford D L and Crawford R L 1996 *Appl. Environ. Microbiol.* **62** 1120-1123.

[36] Kulla H G, Klausener F, Meyer U, Lüdeke B and Leisinger T 1983 *Arch. Microbiol.* **135** 1-7.

[37] Pasti-Grigsby M B, Burke N S, Goszczyński S and Crawford D L 1996 *Appl. Environ. Microbiol.* **62** 1814-1817.

[38] Alexander M 1999 Biodegradation and bioremediation, Gulf Professional Publishing.

[39] Grady L C P, Daigger G L and Lim H C 1999 Biological Wastewater Treatment, Marcel Dekker, New York.