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Combined biological–chemical procedure for the mineralization of TNT

Mario Kröger · Gregor Fels

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Abstract Contamination of ground and surface water with 2,4,6-trinitrotoluene (TNT) and its biological and chemical transformation products are a persisting problem at former TNT production sites. We have investigated the photochemical degradation of TNT and its aminodinitro-(ADNT) and diaminonitrotoluene (DANT) metabolites using OH-radical generating systems like Fenton and hydrogen peroxide irradiated with UV, in order to compare the degradation and mineralization rate of ADNT- and DANT-isomers with TNT itself. As a result, we find that the aminoderivatives were mineralized much faster than TNT. Consequently, as ADNTs and DANTs are the known dead-end products of biological TNT degradations, we have combined our photochemical procedure with a preceding biological treatment of TNT by a mixed culture from sludge of a sewage plant. This consecutive degradation procedure, however, shows a reduced mineralization rate of the ADNTa and DANTs in the biologically derived supernatant as compared to the pure substances, suggesting that during the biological TNT treatment by sludge competing substrates are released into the solution, and that a more defined biological procedure would be necessary in order to achieve an effective, ecologically and economically acceptable mineralization of TNT from aqueous systems.

Keywords ADNT · Biological degradation · Chemical degradation · DANT · Radioactivity · TNT

M. Kröger
Freudenberg Forschungsdienste KG, Elastomere
D-69465 Weinheim, Germany

G. Fels (✉)
Faculty of Science, Department of Chemistry,
University of Paderborn, Warburger Str. 100,
Paderborn D-33098, Germany
E-mail: fels@uni-paderborn.de

Introduction

The ultimate clearance of the explosive TNT from former handling and manufacturing sites remains an unsolved problem. Soil and groundwater from these sites still show high and widespread contaminations (Bruns-Nagel et al. 1997; Hampton and Sisk 1997; Heiss and Knackmuss 2002; Lenke et al. 2000). In Germany most contaminations emanate from the vast TNT-production during world war II (Preuss and Eitelberg 1999). TNT as well as its cocontaminants and metabolites, in particular the aminonitrotoluenes, have toxic, mutagenic and carcinogenic properties, and therefore provide a serious environmental hazard (Honeycutt et al. 1996; Lachance et al. 1999; Robidoux et al. 2005). Especially the

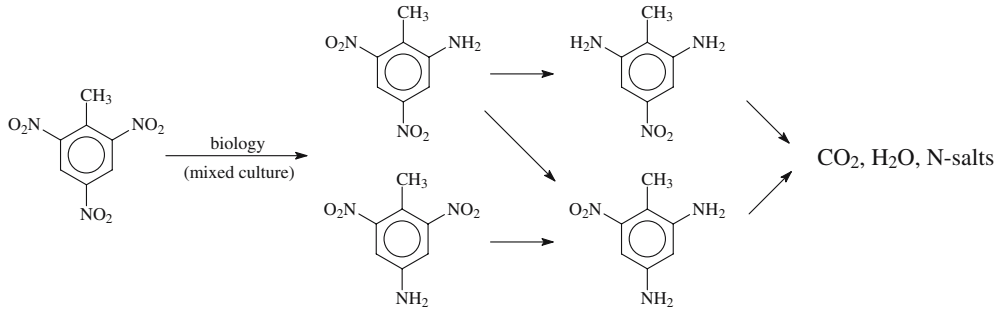
occurance of TNT in wells for public water supply has exposed the significance of the problem (Szöcs 1998). For all of these reasons, the ultimate goal of a remediation should be the complete degradation of TNT into carbon dioxide, water and inorganic nitrogen, i.e. its mineralization.

Although suitable remediation techniques have been investigated since more than two decades, all these methods turned out to have drawbacks that prevent them from being applied on a larger scale. From theses studies we know that bacteria tend to reduce the nitro groups rather than oxidizing the aromatic ring, leading to additional toxic substances (Crawford 1995; Ederer et al. 1997; Esteve-Núñez et al. 2001; Hawari et al. 2000; Lewis et al. 2004; Popesku et al. 2004; Rieger and Knackmuss 1995). Attempts for the bioremediation of TNT by oxidative degradation failed because of the inaccessibility of aromatic compounds to ring opening reactions. The three symmetrically arranged nitro substituents shield the ring and withdraw electrons necessary for an oxidative attack. Only a few examples of organisms capable of TNT mineralization in amounts of more than a few percent have been reported, most of them belong to the white rotting fungi (Hofrichter et al. 1998; Scheibner et al. 1997). However, even in these rare cases, it is assumed, that nitro group reduction and removal preceeds the ultimate mineralization of TNT (Eilers et al. 1999). Therefore, the majority of biological TNT treatments published only reduce TNT to ADNT and DANT metabolites (see scheme 1).

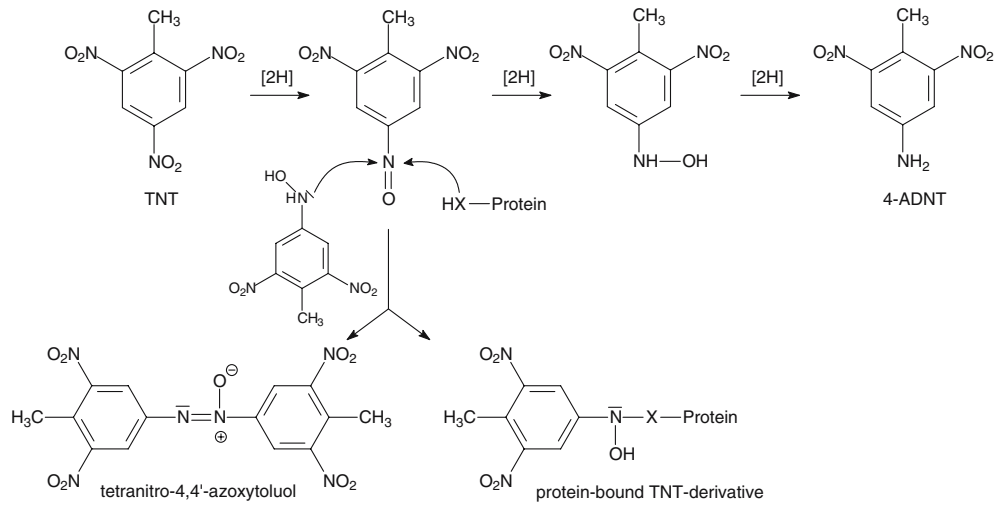
Furthermore, the course of biological degradation procedures is very much depending on the culture condition, i.e. the outcome is depending on whether aerobic or anaerobic conditions are applied. In particular, we could show that for instance using a mixed culture from a sewage plant TNT under anaerobic conditions was converted within 24 h to roughly 40% 4-ADNT, 12% 2-ADNT, 44% 2,4-DANT, and <1% of 2,6-DANT along with a small amount (<1%) of undefined degradation products. In contrast, under aerobic conditions no DANT was formed but rather small amounts of an azooxy-dimer were generated and a substantial amount of TNT metabolites were covalently bound to the cell matrix (presumably protein bound) (Kröger et al. 2004). The latter results can be explained by competing reactions of the intermediates as shown in Scheme 2. Similar results were obtained, when TNT was reduced by the bacteria *Raoultella terrigena* under aerobis conditions (Claus et al. 2006).

In addition to biological procedures, chemical methods have been shown to mineralize TNT, the most important of which, the so called Advanced Oxidation Processes (AOP), use OH-radicals as the oxidizing species (Carey 1992; Rodgers and Bunce 2001). Generation of the radical is achieved by UV-irradiation of hydrogen peroxide (H_2O_2) (Ho 1986; Rodgers and Bunce 2001), ozone (O_3), (Spanggord et al. 2000), or titanium dioxide (TiO_2) (Makarova et al. 2000; Nahen et al. 1997; Schmelling et al. 1997; Son et al. 2004).

In the UV-H_2O_2 system (scheme 3, eq. 1), OH-radicals are generated by homolytic cleavage of H_2O_2 (Legrini et al. 1993), whereas in the Fenton system (scheme 3, eq. 2) they are derived from the reaction of ferrous ion salts with H_2O_2 (Edwards and Curci, 1992; Liou et al. 2003).



Scheme 1: General scheme for the mineralization of TNT



Scheme 2: Covalent binding of TNT metabolites to cell material and formation of dimers from degradation products

$$H_2O_2 \xrightarrow[(254\ nm)]{h\upsilon} 2\ HO\bullet \qquad \text{(eq. 1)}$$

$$Fe^{2+} + H_2O_2 + H^+ \longrightarrow Fe^{3+} + H_2O + HO\bullet \qquad \text{(eq. 2)}$$

$$Fe^{3+} \xrightarrow[(254nm)]{h\upsilon} Fe^{2+} \qquad \text{(eq. 3)}$$

Scheme 3: Mechanism for OH-radical generation on UV-Fenton and UV-H_2O_2 reaction

In the photo-Fenton system these two methods are combined and photons can additionally reduce Fe^{3+} back to Fe^{2+} (Scheme 3, eq. 3). At high concentrations, hydrogen peroxide can compete with organic compounds for hydroxyl radicals, which reduces the effiency of the oxidative degradation of contaminant (Schem 4, eqs 4 and 5).

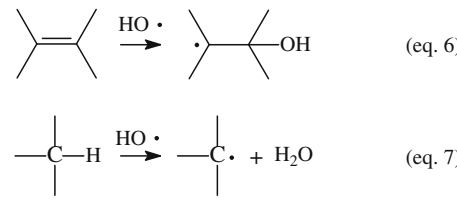
Hydroxyl radicals attack organic material either by addition reaction (Scheme 5, eq 6) or by abstraction of hydrogen (Scheme 5, eq 7).

Apart from the OH-radical driven approaches towards TNT degradation, several other techniques have been tested, for example treatment with alkaline solutions (Emmrich 1999; Thorn et al. 2004), or electrochemistry (Rodgers and Bunce 2001). None of these procedures, however, produce an extensive mineralization of TNT with reasonable energetic input.

$$H_2O_2 + HO\bullet \longrightarrow H_2O + H_2O\bullet \qquad \text{(eq. 4)}$$

$$H_2O\bullet + HO\bullet \longrightarrow H_2O + O_2 \qquad \text{(eq. 5)}$$

Scheme 4: Side reaction with OH-radicals in the presence of high H_2O_2 concentrations



Scheme 5: Principle mechanism of hydroxylation reaction of organic compounds

To overcome the drawbacks of biological and photochemical TNT treatment, we here describe a combined biological and chemical treatment that profits from advantages of both methods, while at the same time reduces the disadvantages as for instance low mineralization rates.

Uniformly ring-labelled ^{14}C-starting material, [U-Ring]-^{14}C-ADNTs and [U-Ring]-^{14}C-DANTs (Kröger and Fels 2000, 2002), was employed in order to investigate the mineralization of ADNTs, and DANTs as compared to TNT, utilizing UV-H_2O_2, Fenton and photo-Fenton systems. The optimized photochemical degradation protocol was then applied in a combined biological and chemical process, in which a mixed culture from a sewage plant sludge was used in the biological step as recently described in this journal (Kröger et al. 2004). The literature today only provides a few such combinations of degradation procedures for TNT, and literally all of them had a biological degradation step following a chemical treatment (Hess et al. 1998; Hess and Schrader

2002; Hwang et al. 2000a, b; Kearney et al. 1983; Schrader and Hess 2004). Our complementary approach utilizes a photochemical mineralization after a biological reduction step.

Materials and methods

Chemicals

^{14}C-TNT, TNT, 2-ADNT, 4-ADNT, 2,4-DANT and 2,6-DANT and their ringlabelled analogs [U-Ring]-^{14}C-TNT, [U-Ring]-^{14}C-2-ADNT, [U-Ring]-^{14}C-4-ADNT, [U-Ring]-^{14}C-2,4-DANT, and [U-Ring]-^{14}C-2,6-DANT were synthesized in our laboratory as reported elsewhere (Kröger and Fels 2000, 2002). All ^{14}C labelled compounds had chemical and radiochemical purities >95% as was determined by HPLC. H_2O_2 was obtained as a 30% solution from Fluka (Munich, Germany). For the absorption of ^{14}C-CO_2 we used Carbosorb E (Methoxypropylamin; Packard, Dreieich, Germany).

Biological treatment

Anaerobic and aerobic treatment of ^{14}C-TNT were performed as previously described (Kröger et al. 2004). In short: To a solution of mixed culture (dry mass of 1.88 g/l originating from the sludge of a local sewage plant) in 523 mL of water, 200 mL phosphate buffer (pH 7), 52 mL of basic nutrient and 200 mg sucrose, 225 mL of a TNT stock solution (100.3 mg/L) were added to give a TNT concentration of 22.7 mg/L (0.1 mol/L). Aerobic conditions were adjusted by aerating with 40 l/h of ambient air while for anaerobic conditions 5 l/h of nitrogen were passed through the degradation solution.

Photochemical treatment

Experiments were carried out in a 50 mL quartz glass flask irradiated from outside by a 150 W xenon lamp (XBO, Osram, München, Germany) operated in a lamp case (LAX type, Müller Elektronik-Optik, Essen, Germany) and powered by a special xenon lamp power supply (SVX1450 type, Müller Elektronik-Optik, Essen, Germany). During experiments the quartz flask was aereated with ambient air. Liberated gas was bubbled through two absorber bottles, each filled with 50 mL of Carbosorb E to absorb ^{14}C-CO_2 and it was finally passed over activated carbon to remove potentially remaining volatile organic compounds. For the photochemical experiments aqueous solutions of TNT, ADNT or DANT were prepared from non radioactive and the corresponding ^{14}C-ringlabelled material (50 mL, 0.22 mmol/L), followed by acidification with sulfuric acid (0.5 M) to pH 3. H_2O_2 (1.75 mL) was added immediately after the irradiation was started. The solutions were typically irradiated for 24 h. In experiments under Fenton-conditions, Fe^{2+} (15 mg/L) was added as $FeSO_4 \cdot 7H_2O$. In the chemical degradation step of combined biological/chemical experiments 50 mL of a solution from the biological treatment was employed after acidification to pH 3.

Analytical methods

Quantitative analysis of ^{14}C-labelled compounds and their metabolites was performed by reversed-phase HPLC and by liquid scintillation counting as reported earlier (Kröger et al. 2004). In particular, quantitative analysis of TNT and its metabolites was performed by reversed-phase high-pressure liquid chromatography (HPLC) with a Nucleosil 120–5 C18 column (250 × 4 mm, Macherey-Nagel, Düren, Germany). The injection volume was 20 μl. Nitroaromatics were eluted with a methanol/water gradient from 45/55 (v/v) to 100% methanol at a flow rate of 0.7 mL/min and detected via UV-absorption at 235 nm with a diode array detector. If necessary, samples were concentrated via solid phase extraction chromatography.

Radioactivity in the radiotracer experiments was determined in a Packard TriCarb A4530 liquid scintillation counter. Aqueous samples (0.5 mL) were mixed with Rotiszint eco plus scintillation cocktail (3 mL), while Carbosorb samples (0.5 mL) were taken up in Permafluor E+ scintillation cocktail (3 mL, Packard).

Results

The course of the mineralization was monitored by radioactivity measurements and by HPLC. While HPLC defines the type and amount of remaining starting material in the reaction flask, radioactivity measurements allowed balancing of the material that remained in solution and was trapped as ^{14}C-CO_2 in the absorber bottles, respectively. Loss of radioactivity, mainly due to absorption effects, made up between 5 and 10% of the total activity in all our experiments. The amount of CO_2 absorbed (in the absorber bottles) equates to the amount of complete mineralization.

TNT Mineralization experiments with UV-H_2O_2

In order to optimize the reaction conditions for TNT mineralization, solutions of 50 mg/L [U-ring-^{14}C]-TNT were irradiated at pH 3 for 24 h at various concentrations of H_2O_2. While in the absence of H_2O_2 the TNT concentration decreased to only 24%, addition of peroxide (in concentrations between 0.15 and 2.5%) to the reaction solution resulted in complete decomposition of TNT as monitored by HPLC. At concentrations of 2.5% H_2O_2, for instance, this degradation proceeded within 5 h, while at 0.15% the transformation took 24 h (data not shown).

Photolytic degradation of TNT proceeds via the trinitrobenzyl anion followed by trinitrobenzoic acid as the main product of the photochemical oxidation. These compound can easily decarboxylate to trinitrobenzene (Burlinson et al. 1979; Martinetz and Rippen 1990), an intermediate that is even more difficult to oxidize than TNT. In accordance with this, in the absence of H_2O_2 no mineralization can be detected (data not shown).

In contrast, when H_2O_2 is added to the degradation experiment, a concentration dependend decrease of 30–40% of the radioactivity was observed in the reaction, indicating mineralization of the starting material and elimination of CO_2 from the solution (Fig. 1). At 0.15%–1%H_2O_2 the mineralization rate increased with increasing H_2O_2-concentrations. Further increase from 1% up to 2.5%, however, did not improve the mineralization, which suggests the importance of eqs. 4 and 5 (scheme 4) in the degradation process, as too much H_2O_2 results in competing reactions of the hydroxyl radicals. For the experiment with 1% H_2O_2 Fig. 1 also depicts the amount of radioactivity liberated as ^{14}C-CO_2, shown as a vertical bar after 24 h. The amount of labelled CO_2 turned out to almost quantitatively make up for the loss of radioactivity in the reaction flask during the degradation experiment. For all further experiments, 1% H_2O_2 was chosen as optimal concentration, which is equivalent to a molar

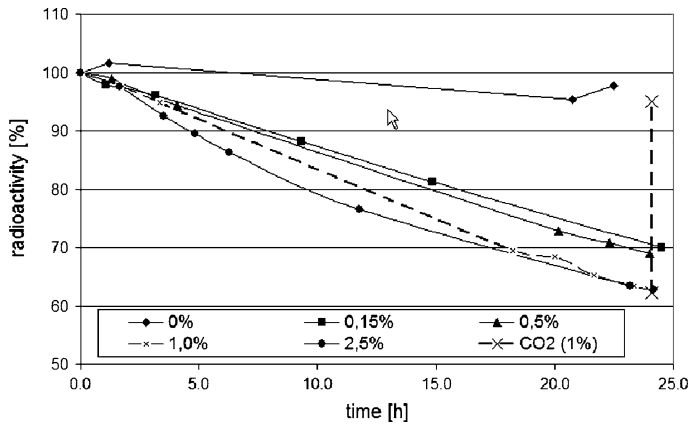


Fig. 1 Mineralization of ^{14}C-TNT as determined by loss of radioactivity from the reaction flask in UV-H_2O_2 experiments at various concentrations of H_2O_2 (50 mL aqueous solution, 50 mg/L [U-ring-^{14}C]-TNT, 1% H_2O_2, pH 3, 150 W Xenon lamp). In addition, for the experiment with 1.0% H_2O_2 the amount of CO_2 liberated during the mineralization process is exemplary shown (vertical bars at 24 h)

H_2O_2/TNT ratio of 1350/1. As shown in Fig. 1 for the mineralization in the presence of 1.0% H_2O_2, mass balances in these experiments always summed up to 90–100% of the initially applied radioactivity.

Mineralization of ADNT and DANT with UV-H_2O_2

Analogous to the TNT-experiment, equimolar amounts of [U-ring-^{14}C]-2-ADNT, [U-ring-^{14}C]-4-ADNT, [U-ring-^{14}C]-2,4-DANT and of [U-ring-^{14}C]-2,6-DANT were mineralized in separate experiments under UV-H_2O_2 conditions with H_2O_2 concentrations of 1%. Starting concentrations of the nitroaromatics were 43.3 mg/L for ADNTs and 36.7 mg/L for DANTs, which corresponds to the same molarity as in the initial TNT solutions. Experiments were performed in duplicates and gave no more than 3% variation of the results. The initial yellow colour of the aminonitrotoluene solutions faded within the first 2 h of the photochemical treatment, indicating the transformation of the starting material. HPLC measurements confirmed this finding, as the concentration of the ADNTs and DANTs decreased to < 10% of the initial value in < 2 h (dashed line in Fig. 2). The experiment was run for 24 h at which time the amount of CO_2 was found to make up 80–90% of the initially applied radioactivity. Fig. 2 shows the time course of the disappearance of radioactivity for the ADNT- and DANT-starting materials along with the amount of radioactivity from CO_2 for the 4-ADNT and 2,4-DANT experiment (vertical bars at 24 h). As can be seen, both compounds show a loss of about 86% of radioactivity, which to the greatest extent can be attributed to CO_2. Roughly 10% more $^{14}CO_2$ is produced in DANT mineralization compared to ADNT mineralization.

As compared to TNT, ADNT degradation proceeded much faster with a conversion of more than 80% rather than 40% after 24 h (see Fig. 2). Degradation of 4-ADNT was slightly faster than 2-ADNT. Interpretation of the results for the DANTs, however, was more difficult, because slightly increased values of radioactivity of up to 10% compared to the initial amount were measured over the first few minutes of the experiment rather than the expected decrease of radioactivity. This finding can possibly be explained by the acidic pK_a of DANTs with a first pK_a-value of 3.4 for 2,6-DANT and of 3.5 for 2,4-DANT, respectively. (Glover et al. 1977). This results in a partial protonation of one aminogroup at the initial reaction condition of pH 3. During a degradation reaction the pH drops slighty to pH-values between 3 and 2. Because the analytical samples were not neutralized with base before HPLC-measurement the protonated compounds will be

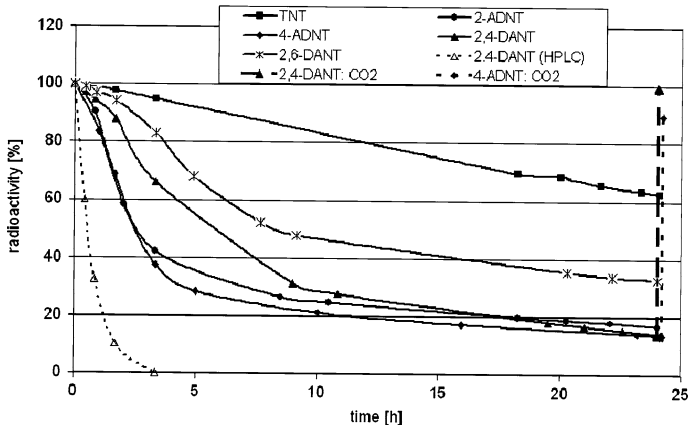


Fig. 2 Decrease of radioactivity in the reaction flask (solid lines) in photodegradation experiments with TNT, ADNT, and DANT (50 mL aqueous solution, 0.22 mmol/L [U-ring-^{14}C]-substrate, 1% H_2O_2, pH 3, 150 W Xenon lamp), and amount of CO_2 liberated, exemplary shown for 4-ADNT and 2,4-DANT, respectively (vertical bars at 24 h). For comparison, the content of 2,4-DANT as measured by HPLC is also shown

underestimated in the analysis. In a control experiment we could show, that at pH 3 only 91% of ^{14}C-2,6-DANT radioactivity could be detected as compared to 100% at pH 7.

Mineralization of ANTs in general turned out to be significantly faster than TNT. Among the ADNTs, the 2,6-DANT was the slowest to mineralize showing only a conversion of 70% after 24 h as compared to more than 85% for 2,4-DANT, and the ADNTs. Compounds with amino groups in *para* position seem to be mineralized faster than their *ortho*-isomers. Attack on the para position is presumably favored because it is less sterically hindered.

TNT Mineralization experiments with UV-Fenton

In order to directly compare the UV-H_2O_2 and the Photo-Fenton degradation, TNT and the ANTs (as described in the following chapter) were treated analogous to the above described experiments with the exemption of employing the Fenton-reagent rather than UV-H_2O_2. The optimal Fe^+ concentration was determined by adding $FeSO_4{\cdot}7H_2O$ in concentrations between 10 and 80 mg Fe/L.

As can be seen from Fig. 3 mineralization of TNT as monitored by the disappearance of radioactivity in the reaction flask was rather independent of Fe-concentrations in the applied range. A mineralization rate of more than 60% was achieved within 24 h with concentrations of 10–40 mg/L Fe^{2+}. At Fe^{2+} concentrations above 20 mg/L the aqueous solutions turned slightly brownish, indicating a complexation of ferrous ions. 15 mg/L Fe^{2+} was, therefore, employed in all further experiments, which corresponds to a molar ratio H_2O_2/Fe^{2+}/TNT of 1350/1.2/1. In similar experiments, Dillert et al. (1997) had used significantly lower concentrations of H_2O_2 and Fe^{2+}, however only degradation rather than mineralization of TNT was determined in those experiments (see Table 1). On the other hand, Li et al. (1998) had come to results comparable to ours, using slightly less H_2O_2 but more iron.

In a dark Fenton experiment, using 15 mg/L Fe^{2+} and H_2O_2 without UV-irradiation, no mineralization occured (see Fig. 3). This can probably be attributed to the fact that under these conditions oxalic acid as a degradation product after ring opening can not be further oxidized to carbon dioxide as the last step in the mineralization chain (Li et al. 1997a). Also Dillert did not find degradation of TNT at all under these conditions (Dillert et al. 1997). We have, therefore, not followed a dark Fenton protocol any further in our investigations.

Mineralization of ADNT and DANT with UV-Fenton

Conditions were maintained as in the mineralization experiments with UV-H_2O_2 with the

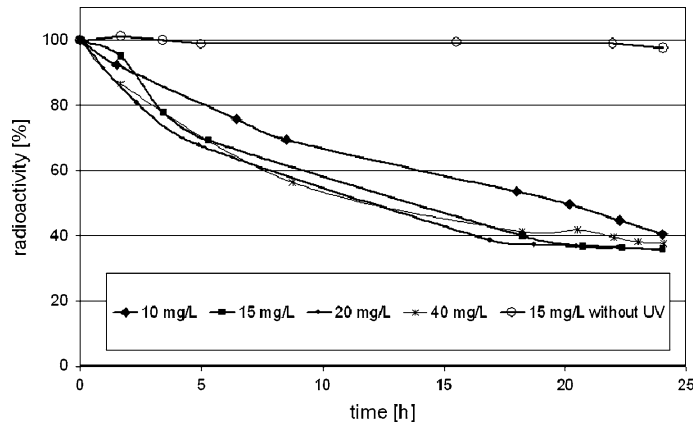


Fig. 3 Decrease of TNT-radioactivity in the reaction flask in a Photo-Fenton reaction at various concentrations of Fe^{2+} (50 mL aqueous solution, 50 mg/L [U-ring-^{14}C]-TNT, 1% H_2O_2, pH 3, 150 W Xenon lamp)

Table 1 Comparison of conditions for optimized UV-Fenton degradation

	TNT (mg/L)	H_2O_2 (%)	Fe^{2+} (mg/L)	molar ratio TNT : H_2O_2 : Fe^{2+}	mineralization (%)
this work	50	1	15	1 : 1350 : 1.2	64
Lie[1]	70	1	80	1 : 970 : 4.6	70
Dillert[2]	22.7	0.07	5.5	1 : 200 : 1	n.d.

[1] Li et al. 1998

[2] Dillert et al. 1997

exception of 15 mg/L iron (II) which were additionally applied. Loss of radioactivity from the reaction solution was attributed to mineralization of the starting ANT-derivative to CO_2.

When UV-Fenton conditions were applied to the ADNTs and DANTs, the results was comparable to the UV-H_2O_2 experiment, except that the degradation proceeded faster, with all ANTs showing more than 60% mineralization to CO_2 after only two hours. HPLC measurements verified that no ANT-starting material was left after 15 min (dashed line in Fig. 4 shows 2,4-DANT as a representative example). As in the UV-H_2O_2 experiment, 2,6-DANT is degrading slower than the 2,4-isomer, presumably due to its partial protonation. Mineralization of 4-ADNT is again superior to 2-ADNT, which reacts with similar reaction rates as 2,4-DANT.

Combined biological and chemical procedure

In a recent publication we have described a simple biological system for the complete reduction of TNT to ANTs within 24 h or less (Kröger et al. 2004) using a mixed culture from a sludge of an ordinary sewage plant. We showed that an aenaerobic treatment proved to be faster and occurred with no absorption to the sludge, while under aerobic condition degradation proceeds considerably slower and is accompanied by massive absorption of organic material to the organic matrix, part of which turned out to be covalently bound. Together with the results from photochemical degradation of ADNTs and DANTs described in this paper, we now propose a combined biological and chemical procedure for the degradation of TNT and its metabolites. This should first employ a biological treatment by sludge to generate ANTs from TNT, followed by a photochemical degradation of the reduction products with an advanced oxidation procedure, as for instance with an UV-H_2O_2 system.

Combined biological and photochemical degradation procedure for TNT

Aqueous ^{14}C-TNT solutions were aerobically and anaerobically metabolized by a mixed culture from sludge, followed by UV-H_2O_2 treatment of the biological supernatants after 24 h incubation.

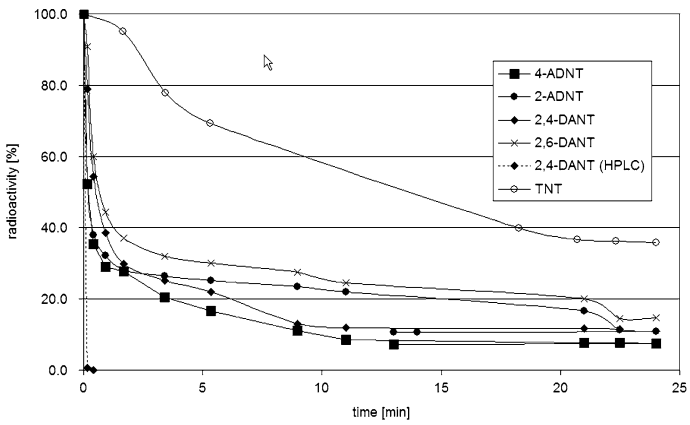


Fig. 4 Decrease of radioactivity in the reaction flask in UV-Fenton experiments with TNT, ADNT and DANT (50 mL aqueous solution, 0.22 mmol/L [U-ring-^{14}C]-substrate, 1% H_2O_2, 15 mg/L Fe^{2+}, pH 3, 150 W Xenon lamp. For comparison, TNT data from Fig. 3 and the content of 2,4-DANT as measured by HPLC is also shown

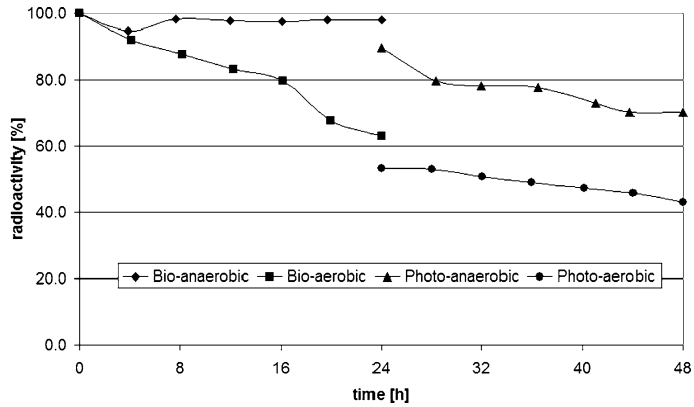


Fig. 5 Degradation of ^{14}C-labelled TNT in a combined biological (Bio) and photochemical (Photo) experiment employing aerobic and anaerobic conditions, respectively Loss of radioactivity is attributed to mineralization of starting material to CO_2. The reaction progress is monitored as % of initial radioactivity (Bio: 20 mg/L [U-ring-^{14}C]-TNT, 1,88 g/l sludge, 200 mg sucrose, nutrients, pH 7; K1: 5 L air/h, K2: 5 L N_2/h; Photo: 1% H_2O_2, pH 3, 150 W Xenon lamp)

During anaerobic treatment more than 95% of the radioactivity remained in solution (Fig. 5 Bio-anaerobic). An aliquot of the supernatant was transferred from the biological to the photochemical setup and treated as described for the UV-H_2O_2 degradation starting initially at pH 3. As can be seen from Fig. 5, radioactive material is only mineralized to a degree of 22% (Fig. 5 Photo-anaerobic).

In contrast, if aerobic conditions were employed in the biological step, more than 30% of the initial radioactivity was adsorbed to the sludge (Fig. 5 Bio-aerobic). Photochemical degradation of the resulting solution only gave 10% of mineralization (Fig. 5 Photo-aerobic). In terms of mineralization the anaerobic procedure, therefore, is more effective, as it shows twice as much decrease of radioactivity in the photochemical step than the aerobic procedure.

HPLC monitoring of the biological process (data not shown) confirmed the published results of aerobic and anaerobic sludge TNT treatment (Kröger et al. 2004), i.e. degradation under anaerobic conditions yields both ADNTs along with a major content of 2,4-DANT which all together make up for almost 100% of the originally applied TNT quantities. Aerobic conditions however give only minor amounts of the same three metabolites, which at least partially can be attributed to adsorption of material to the sludge. Investigation of the photochemical process revealed that 2- and 4-ADNT are degraded at comparable rates while 2,4-DANT is slightly faster degraded (data not shown).

Variations of the combined degradation procedure, using anaerobic conditions were investigated with the aim of increasing the over all mineralization. In particular

- in order to reduce the matrix effects of the medium the phosphate buffer was omitted in the biological step. The buffering capacity of the biological matrix turned out to be enough to keep the pH in a neutral range.
- the pH of the biological supernatant was not acidified to pH 3 prior to the photochemical degradation but rather left at the original neutral pH. During the UV-H_2O_2 treatment of this supernatant the solution acidifies to pH 3 and 2, which resembles the pH-value usually seen in - the photochemical treatment when starting at an initial pH of 3.
- the efficiency of H_2O_2 was improved by adding the reagent continuously in amounts of 0.2 mL/h rather than all at once at the start of the experiment, which avoids the competition reaction.

Together, these changes increased the total mineralization to amounts of 30–35%, which, however, represents a much lower mineralization

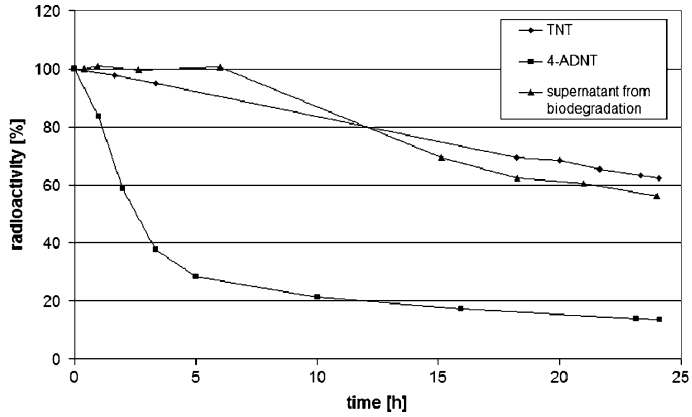


Fig. 6 Comparison of UV-H_2O_2 mineralization of TNT, 4-ADNT and an ANT mixture from biological pretreatment)

rate than could be expected from the experiments in which the pure ADNTs and DANTs were employed in photochemical degradation. In addition, use of Photo-Fenton instead of the UV-H_2O_2 conditions turned out to be disadvantageous, as UV-H_2O_2 under all conditions gave the better results. Fig. 6 compares the mineralization rate of TNT, 4-ADNT and the supernatant from an optimized anaerobic biological degradation step as determined by loss of radioactivity from the reaction flask. It is obvious that within a reaction time of 24 h a pure ADNT-solution is mineralized twice as much as an ANT-mixture from a biological pre-treatment. The latter is rather comparable to the photochemical degradation of TNT itself, perhaps because of a lag phase that can be seen for at least the first seven hours of the experiment.

Discussion

We have investigated the degradation of TNT and its major metabolites of the ADNT- and DANT-type by photochemical procedures employing UV-H_2O_2 conditions and the Photo-Fenton reagent, respectively, with the aim of designing a remediation process that allows complete elimination of hazardous material from aqueous systems contaminated with TNT and its metabolites. Employing solutions of purified ADNTs and/or DANTs, our experiments prove that these TNT metabolites are degraded much faster than TNT itself. This indicates that the attack of a hydroxyl radical on the aromatic ring is the rate determining step in the entire mineralization process. An electrophilic substitution at the aromatic ring is facilitated when the ring has a higher electron density as in the ADNT and DANT derivatives and in particular when the amino group is *para* to the methyl group. As a consequence, all ANTs were mineralized much better than TNT. Similar results for the degradation of ANTs with UV-Fenton systems have been described earlier (Schmidt and Butte 1999).

Various biological processes are known to convert TNT to the isomeric 2- and 4-ADNT as well as further on to the 2,4- and 2,6-DANT (Esteve-Núñez et al. 2001; Lewis et al. 1998; Yin et al. 2005; Zaripov et al. 2004), which now suggest a combined biological/chemical procedure as an ecologically and economically favorable remediation technique for TNT contaminated ground and surface water. In a recent publication we have described such a suitable biological system for TNT reduction using a mixed culture sludge from an ordinary sewage plant which showed complete conversion of TNT within 4 h or less (Kröger et al. 2004). However, when applying this procedure as the biological step in a combined biological and (photo) chemical degradation procedure, mineralization turned out to be slower than the high mineralization found with pure ADNT- and DANT-solution. In addition, as can be deduced from Fig. 6, a two-phase course of the mineralization process of biologically derived ANTs seems to occur. At the start of the photochemical procedure there is a lag phase of a few

hours, in which the TNT metabolites at least are not predominantly mineralized. This is probably due to the presence of additional organic material liberated form the sludge during the biological treatment, the so called dissolved organic matter, like humic acids and other constituents (Baker and Spencer 2004; Simjouw et al. 2005). Humic acid, for instance, is known to inhibit reactions of aromatic compounds by radicals (Lindsey and Tarr 2000), while on the other hand; it may also be substrate for mineralization (Li et al. 1997b). This can explain the delayed release of radioactive CO_2 in the first few hours (see Fig. 6). Another hindering effect of the dissolved organic matter could be binding of TNT and its metabolites to humic acid (Fukushima et al. 2001).

Combined degradation procedures have already been described in the literature (Hess et al. 1998; Hess and Schrader 2002; Hwang et al. 2000a, b; Kearney et al. 1983; Schrader and Hess 2004), which, however, in contrast to our procedure all start with a photochemical treatment followed by a microbiological transformation. From a comparison of those results with the data presented here, the concept of a biological reduction of TNT prior to an oxidative photochemical treatment by advanced oxidation procedures (AOP) seems to be the most appropriate approach towards mineralization of TNT. However a more effective biological procedure would be needed to make such a procedure economically and ecologically advantageous over the presently used adsorption of the contaminants by charcoal. In this respect our recently described TNT degradation by the bacteria strain *R. terrigena* seems to be a good alternative (Claus et al. 2006). This bacterium was shown to scavenge TNT from aqueous media and to maintain most of the material inside the cell in form of azooxy-dimers the formation of which is explained in Scheme 5. In these experiments only 10–20% of degraded material was excreted to the media, mostly as ADNTs and DANTs. Therefore, experiments using this biological step as part of a combined biological/chemical procedure for cleaning of TNT contaminated water are presently under investigation in our laboratories, employing photocatalytic procedures in chemical treatment of the biologically derived material.

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