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# Biological reduction of TNT as part of a combined biological–chemical procedure for mineralization

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*Dedicated to the 60th birthday of my colleague Karsten Krohn*

*Key words:* $^{14}$C-balance, microbial reduction, mineralization, TNT

**Abstract**

The explosive 2,4,6-trinitrotoluene (TNT), one of the most abundant and persistent contaminants at former armament factories and military sites, was cometabolically reduced by sludge (mixed culture) from a sewage plant in order to facilitate mineralization in a subsequent photochemical treatment. Under aerobic conditions, the main reduction products were aminodinitrotoluenes (ADNTs). A greater amount of the nitroaromatics (ca. 30%) was adsorbed by the sludge as was shown by a complete balance of the process using $^{14}$C-TNT. Under anaerobic conditions, TNT was further converted into ADNTs and diaminonitrotoluenes (DANTs) while only negligible adsorption to the sludge occured.

*Abbreviations:* ADNT – aminodinitrotoluene; DANT – diaminonitrotoluene; TNT – 2,4,6-trinitrotoluene

## Introduction

Extensive production of the explosive 2,4,6-trinitrotoluene (TNT) over the last century, particularly during World War II, has caused severe contamination of former TNT-manufacturing and -handling sites (Haas & von Löw 1986; Yinon 1990). The toxic, cancerogenic and mutagenic properties of TNT as well as the chemical persistency have turned it into a major environmental hazard and the effective degradation of TNT is still a challenge for environmental research (Spain & Hughes 2000).

Over the last two decades a large amount of microorganisms have been tested for the ability to metabolize and mineralize TNT. Reviewing this work it turns out that almost all organisms metabolize TNT *via* reductive pathways to amino- or aminonitro-compounds, respectively, rather than processing a complete mineralization (Hawari et al. 2000; Lewis et al. 1997; Spain 1995; Wild et al. 1998). Under aerobic conditions, TNT is reduced to aminodinitrotoluene (ADNT) and diaminonitrotoluene (DANT). Under strictly anaerobic conditions further reduction to triaminotoluene (TAT) is possible. The impossibility of an oxidative attack, which can occur with other nitroaromatics like mono- and dinitrotoluenes, stems from the extremely small electron density on the aromatic ring. The three symmetrically arranged nitro groups of TNT cover the aromatic ring sterically and, more important, reduce the electron density of the ring by resonance. The only one exception reported in the literature are wood-rotting fungi which are able to mineralize TNT (Scheibner et al. 1997). These fungi use extra cellular enzymes, the lignolytic system, to mineralize aromatic rings. However, they need a reduced nitroaromate to start the metabolic pathway. Therefore, even in this case TNT has to be reduced to ADNT before mineralization can proceed (Eilers et al. 1999).

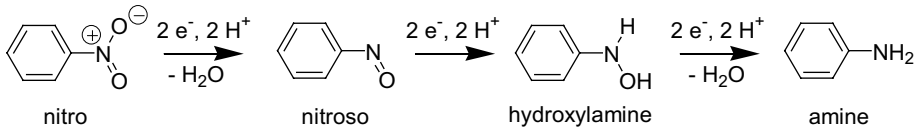


*Figure 1.* Reductive pathway from nitro to amino group.

Reduction of an aromatic nitro group is a six-electron reduction sequence which includes three consecutive two-electron steps *via* a nitroso and a hydroxylamino intermediate. The nitroso group is unstable while the hydroxylamino compound can be isolated, though it is very sensitive towards oxidation and reduction (see Figure 1). Hydroxylamine and nitroso group can react with each other and condense to an azoxy-dimer (Corbett & Corbett 1995).

Since the attempts to create a solely microbial degradation procedure to mineralize TNT have failed, some alternatives have been taken into account. One way is to reduce TNT anaerobically to TAT and then bind it irreversibly to the soil by further aerobic treatment (Knackmuss et al. 1998). It is, however, still a matter of discussion, whether binding to the soil really is covalent and irreversible or not. In this respect, various leaching experiments have been performed, and some are still under way collecting data for long term studies (Achtnich et al. 2000; Drzyzga et al. 1999). Other degradation procedures combine microbial and chemical methods to achieve a mineralization (Hess et al. 1998; Hwang et al. 2000).

We have investigated a combined biological and chemical procedure, in which, in a first step, TNT is reduced by a simple and cheap microbial systems, followed by a mineralization of the reduction products in a second step by photochemical techniques with UV-light in the presence of hydrogen peroxide. Here we describe the first part of the procedure, the biological step, that reduces TNT to aminonitrotoluenes, emphasizing the material balance of the entire process by using radiotracer techniques.

## Materials and methods

### *Chemicals*

$^{14}$C-TNT, TNT, 2-ADNT, 4-ADNT, 2,4-DANT and 2,6-DANT were synthesized in our laboratory as reported earlier (Kröger & Fels 2000, 2002). 2,2′,6,6′-Tetranitro-4,4′-azoxytoluene was synthesized according to a procedure of Sitzmann (1974).

Carbosorb E (methoxypropylamin; Packard, Dreieich, Germany) was used for quantitative absorption of $^{14}$C-$CO_2$. For HPLC analysis degassed methanol and water (LiChropur, Merck, Darmstadt, Germany) was employed.

As a basic nutrient solution in aerobic and anaerobic treatments we used a combination of ammonium sulfate (7 mg/l, Fluka, Seelze, Germany), the trace element mixture Fetrilon Combi (2.5 mg/l, BASF, Ludwigshafen, Germany) and Basfoliar 12 + 4 + 6 (0.1 ml/l, BASF). Saccharose from ordinary household sugar was chosen as a cosubstrate.

### *Organisms*

The mixed culture was obtained from the sewage plant in Paderborn-Sande (Germany). The dry mass of the sludge was determined, and before use, the sludge was washed with an isotonic solution of sodium chloride (9 g/l).

### *Aerobic treatment*

All experiments were performed in a 1 l three-neck flask. An appropriate amount of the mixed culture corresponding to 1.88 g/l of dry mass was transferred to the flask into a total volume of 523 ml of distilled water. 200 ml of phosphate buffer (pH 7) and 52 ml of basic nutrient solution were added. Then 200 mg of sugar (saccharose, Südzucker, Mannheim, Germany) was dissolved and in a last step 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). The solution was aerated with 40 l/h of ambient air by a membrane pump (Elite, Germany). In experiments with $^{14}$C the exhaust air was passed through absorption bottles containing 50 ml of Carbosorb E each, to retain $^{14}$C-$CO_2$. The outlet of the second wash bottle was

connected to a tube with activated carbon to potentially adsorb volatile organic compounds (VOCs). 5 ml samples were taken every 4 h from the reaction flask during a 24 h experiment.

*Anaerobic treatment*

The anaerobic treatment was carried out under identical conditions as described above, except for the aeration, which was reduced to 5 l/h of nitrogen or 0.2 l/h of ambient air, respectively.

*Extraction of pellet*

After the aerobic treatment the sludge was given 30 min to sediment. Then 800 ml of the sludge suspension were separated by centrifugation at 4 °C (12,000 rpm, Beckmann, Fullerton, CA, USA).

The resulting pellet was dried overnight at 60 °C, taken up again in 100 ml of distilled water, ultrasonicated for 30 min and then centrifuged and dried again.

Radioactivity (determined by scintillation) and nitroaromatics content (determined by HPLC) of the remaining solution were measured by taking 1 ml samples.

As a second extraction step, the pellet was treated with 100 ml of methanol, sonicated for 30 min and worked up by centrifugation and drying as described before. Finally, 100 ml of dichloromethane was employed in an identical process as a third extraction step. Radioactivity of the resulting final pellet was determined by combustion and measurement of the liberated $^{14}C$-$CO_2$.

*Analytical methods*

Redox potential of the culture were measured with a Pt 5900A platinum redox electrode (Schott, Mainz, Germany).

All samples (5 ml) from the reaction flask were drawn through a syringe filter (0.45 $\mu$m, Roth, Karlsruhe, Germany). Before HPLC-analysis, each sample was concentrated *via* solid phase extraction chromatography. For this procedure the column (Bakerbond spe, J.T. Baker, Phillipsburg, NJ, USA) was conditioned with water, and the aqueous sample (4 ml) was applied followed by elution with methanol (1 ml).

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 $\mu$l. 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. The azoxytoluenes were eluted isocratically with methanol/water 80/20 (v/v).

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 and discussion

*Optimization of biological treatment*

The ultimate aim of our biological TNT-waste water treatment was to achieve fast and complete reduction of the TNT nitro groups. The resulting reaction mixture should then be subjected to further degradation by photochemical procedures in a separate process. In order to establish standard conditions for our sewage sludge based experiments, we first optimized the concentrations of sludge and the cosubstrate saccharose. The results (see Table 1) indicated that nitro group conversion increases with higher amounts of sludge, while a higher concentration of TNT slows the process down. From these experiments a sludge/TNT ratio of 100/1 seems to be ideal for our procedure and was maintained throughout our further experiments.

Saccharose was chosen as a cosubstrate and carbon source, because it is readily available, chemically pure and cheap.The optimum amount of cosubstrate was found to be a tenfold excess over TNT concentration (see Table 2). These conditions were used in the subsequent investigation of aerobic and anaerobic conditions for TNT transformation. For the supply of nitrogen, phosphorus and other trace elements, we used a

*Table 1.* Biological conversion of TNT with varying concentrations of sludge

| Experiment | S1 | S2 | S3 | S4 | S5 | S6 | S7 |
|---|---|---|---|---|---|---|---|
| $c(TNT)_0$ [mg/l] | 2.0 | 4.0 | 3.9 | 4.2 | 3.4 | 42.4 | 20.0 |
| c(sludge) [mg/l] | 0 | 79 | 158 | 315 | 394 | 1880 | 1880 |
| Ratio sludge:TNT | 0 | 20 | 40 | 80 | 100 | 45 | 94 |
| c(TNT) after 24 h [% of $c_0$] | 81.6 | 16.4 | 1.8 | 0.2 | 0 | 47.7 | 10.6 |

*Table 2.* Biological conversion of TNT with varying concentrations of saccharose

| Experiment | Z1 | Z2 | Z3 | Z4 | Z5 | Z6 | Z7 |
|---|---|---|---|---|---|---|---|
| $c(TNT)_0$ [mg/l] | 21.0 | 20.0 | 21.1 | 20.1 | 4.1 | 3.4 | 4.4 |
| c(Saccharose) [mg/l] | 0 | 26.0 | 130.0 | 200.0 | 0 | 26.0 | 100.0 |
| Ratio sugar:TNT | 0 | 1.3 | 6.2 | 10.0 | 0 | 7.6 | 22.7 |
| c(TNT) after 24 h [% of $c_0$] | 44.2 | 10.6 | 5.0 | 1.5 | 1.2 | 0 | 0.5 |

basic nutrient solution which has been optimized in earlier experiments (Schlesselmann 1996).

*Aerobic treatment*

Aerobic conditions were established by bubbling air through the solution at a rate of 50 l/h which resulted in redox potentials above 250 mV. Under these conditions, reduction of TNT was completed within 24 h. Figure 2 shows the TNT and the metabolite concentrations during a typical aerobic experiment. More than 95% of inital TNT concentration were reduced after 16 h. The main reduction product was 4-ADNT, while 2-ADNT only occurred in much smaller amounts. No DANT-isomers were detected.

However, besides the expected ADNT-products an azoxy-dimer (TN-4,4′-AzT = tetranitro-4,4′-azoxytoluene) also appeared in small quantities. This dimeric nitro compound results from condensation of the intermediates of the reduction process outlined in Scheme 1, i.e. from a dimerization of nitroso- and hydroxylamino-dinitrotoluene.

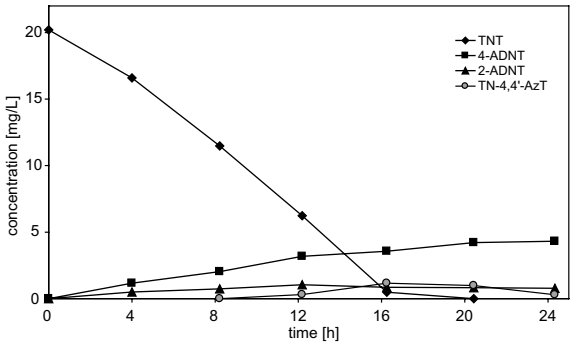


*Figure 2.* Aerobic reduction of TNT.

Although the reduction of TNT seemed to be complete after the reaction period of 24 h (see Figure 2), it was never possible to obtain a quantitatively adequate amount of reduction products with respect to the originally applied TNT concentration. Only about 30% of initial TNT could be detected in form of reduced ANTs. In order to elucidate the discrepancy, we employed $^{14}$C-ring-labelled TNT to detect the missing quantities and to determine the balance of the entire aerobic treatment.

*$^{14}$C-balance*

Two similar aerobic transformation experiments (A1 and A2, see Tables 3 and 4) were carried out using $^{14}$C-TNT as the substrate, in experiments, otherwise identical to the metabolisation of non radioactive TNT described above. As in the unlabelled experiments, the dominating transformation product was 4-ADNT (see Table 3), accompanied by small amounts of 2,4-DANT. Extraction of the pellet liberated some further amounts of ADNT- and DANT-compounds, which (as in the unlabelled experiments) could, however, only account for transformation of about half of the initially

*Table 3*. Quantities of metabolization products resulting form experiment A1 (in% of initial TNT concentration, as detected by HPLC)

| | 4-ADNT | 2-ADNT | 2,4-DANT | Sum |
|---|---|---|---|---|
| Aerobic treatment solution [%] | 33.1 | 5.8 | 2.7 | 41.6 |
| Water-extract [%] | 1.6 | 0.2 | 1.1 | 2.9 |
| Methanol-extract [%] | 0.5 | – | – | 0.5 |
| $CH_2Cl_2$-extract [%] | – | – | – | – |
| Sum [%] | 35.2 | 6.0 | 3.8 | 46.0 |

*Table 4*. Overall $^{14}C$ balance in aerobic treatments for experiments A1 and A2 (as detected by liquid scintillation counting)

| Radioactivity [% of initial] | A1 | A2 |
|---|---|---|
| After aerobic treatment [%] | 62.6 | 60.5 |
| Separated by centrifugation [%] | 13.5 | 12.7 |
| Water-extract [%] | 4.9 | 2.8 |
| Methanol-extract [%] | 1.9 | 3.7 |
| $CH_2Cl_2$-extract [%] | 1.5 | 0.5 |
| Combustion [%] | 15.7 | 16.3 |
| $CO_2$ [%] | 0.7 | 0.1 |
| Sum | 100.8 | 96.6 |

employed TNT. Together with the finding that no significant mineralization had occurred, as $^{14}CO_2$ formation was determined to be less than 1% (see Table 4), the results suggest the existence of additional metabolites that cannot be detected under the applied HPLC-conditions, and which therefore presumably have a more polar character than the amino-nitro-aromatics.

Because of the negligible amounts of $^{14}CO_2$ liberated in these experiments, one should expect the vast amount of radioactivity to be still contained in the reaction vessel. However, in contrary, only slightly more than 60% of radioactivity were still detectable in solution after 24 h of aerobic treatment. We therefore investigated the solid biological material in the reaction vessel in more detail to look for absorbed radioactivity. The resulting data is outlined in Table 4.

After the transformation experiment the biological material of the mixed culture was separated from the aqueous phase by centrifugation. This physical procedure already liberated more than 10% of labelled material from the sludge, which, together with the radioactivity originally detected in the solution, sums up to about 73%. Therefore, a quarter of the initially applied $^{14}C$-TNT radioactivity must still be bound to the sludge pellet after this procedure.

The sludge was then subjected to three successive extraction steps, using water, methanol and finally dichloromethane as solvents. The extraction procedures were assisted by ultrasonication. By this process 8.3% and 7%, respectively, of overall radioactivity was removed from the sludge, which accounts for 35% of the total radiolabel in the pellet. The effect of the different solvents in the extractions varied somewhat, though the last step, using dichloromethane, turned out to be the least effective. It should be noted that we purposely extracted the pellet under neutral conditions, as we were aiming at a distribution of the unaltered transformation products rather than reacting covalently bound metabolites on the pellet for instance with alkaline solution in order to maximize the liberation of radioactivity from the pellet.

As can be seen from Table 3, the overall amounts of ANTs from sludge extraction were very small with 4-ADNT again being the prevailing component. In the water extract, however, the concentration of 2,4-DANT was comparatively high, which indicates that the more polar DANT is more prone to adsorption than the less polar ADNTs. The total amount of radioactive material that could be extracted from the sludge by the three step extraction procedure (8.3% and 7.0%, respectively, see Table 4) was twice as high as determined by HPLC-analysis (3.4%, see Table 3). This again is an indication for the accumulation of other metabolites than ADNTs and DANTs also adhering to the biological matrix.

The radioactive balance was completed by analyzing the remaining pellet after extraction. For both of the separate transformation runs (A1

and A2), the pellet contained about 16% of the overall radioactivity as determined by combustion. Since this fraction of $^{14}$C-labelled material had not been removed from the sludge by the various extraction procedures, we must conclude an irreversible adsorption of the labeled material to the biological matrix by chemical bonding rather than by physical association. Similar findings were also reported by Carpenter et al. (1978) who found that the radioactivity of metabolized $^{14}$C-TNT was irreversibly bound particularly to the lipid and protein fractions of the sludge.

In summary, aerobic treatment of TNT is characterized by a considerable binding of metabolites, particularly ADNTs, to the biological matrix which therefore is not accessible for further transformation. These results raise the question of how to achieve an appreciable decontamination of TNT polluted waste water in a sewage plant. TNT metabolites will probably massively adsorb to the biological system and thereby presumably reduce the metabolization capability of the sludge to a large extend. In order to answer such questions, future transformation experiments have to be investigated in continuous flow reactors.

*Anaerobic treatment*

Since the aerobic conditions resulted in a substantial reversible and irreversible binding of TNT metabolites to the sludge, we were interested to see, if a similar behavior is also found under anaerobic conditions. Nitrogen rather than air was bubbled through the solution which resulted in a redox potential of −50 mV as compared to more than +250 mV in aerated solutions. All other conditions remained unaltered. The change in redox conditions, however, turned out to have a drastic effect on the reduction of TNT.

Figure 3 shows the result of a representative anaerobic treatment of TNT solution with sludge over 24 h. Transformation of TNT is faster than under aerobic conditions, yielding over 95% turnover of TNT within only 8 h. After 12 h no more TNT could be detected. As expected, ADNTs are the first metabolites to arise. The *para*-reduction product, 4-ADNT (40%), is once again the dominant metabolite with a threefold excess over the *ortho*-product 2-ADNT (12%). However, in contrast to the aerobic transformation, both ADNTs are reduced further to 2,4-DANT, which was the

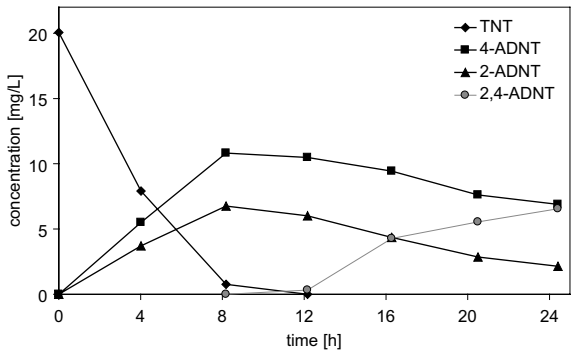


*Figure 3.* Anaerobic reduction of TNT.

main detectable product of the reduction process after 24 h, and which made up for 44% of the initially employed TNT. On the other hand, no 2,6-DANT could be detected. Unlike the TNT transformation under aerobic conditions, more than 95% of the initial TNT concentration was converted to reduced products and was available in solution. Thus almost no adsorption of metabolites to the sludge occurred under theses anaerobic conditions and therefore no extractions were necessary to complete the overall transformation balance.

## Conclusions

The anaerobic transformation process results in a faster reduction of TNT than treatment under comparable aerobic conditions. Complete reduction was achieved within the first 12 h, while 20 h were necessary under aerobic conditions. This is a consequence of the redox potential since the reducing power of a solution is, by definition, proportional to its redox potential. Although under strictly anaerobic conditions a redox level of lower than −200 mV would be achievable, we have chosen a medium that resulted in a redox potential of −50 mV, because it provided sufficiently good reducing power while at the same time being a comparatively cheap medium. Under these conditions reduction was faster and more complete than at an aerobic potential of 200 mV. This result also explains, why only under anaerobic conditions ADNTs is further reduced to 2,4-DANT. The reduction step from ADNT to DANT is more time and energy consuming than the reduction of TNT to ADNT. This can be deduced from the one-electron redox potentials, because the initial transfer of one electron to give a radical is the rate

limiting step of these reductions. The potential is higher for TNT (120 mV) than for 2-ADNT (30 mV) and 4-ADNT (−10 mV); 2,4-DANT has the lowest potential (−95 mV, Riefler & Smets 2000). Hence the reduction of 2,4-DANT was not observed under the aerobic conditions employed and is only possible under anaerobic conditions on larger time scales.

A second finding that makes the anaerobic treatment more suitable for our intended combined biological-chemical treatment of TNT, is the lack of adsorption to the biological matrix of the reaction system. In contrast, in aerobic experiments almost 40% of material was initially bound to the sludge. Although a part of this material could be liberated by physical separation and chemical extractions, about 16% stuck irreversibly to the sludge. A covalent binding of TNT metabolites to living cultures is supposed to be indicated by the appearance of the intermediate transformation product OH-ADNT. Under aerobic conditions it can be reoxidized to the very reactive nitroso species. This highly electrophilic group can then attack nucleophilic sites, for example cystein groups of proteins. By the same mechanism it can also attack the nucleophilic site of a hydroxylamino group and thereby condense to an aromatic azoxy dimmer (e.g. TN-4,4′AzT). Since we detected this azoxy dimer only under aerobic conditions, this compound can serve as an indicator for aerobic metabolization conditions that cause covalent binding of nitroaromatics to the sludge.

In summary, the anaerobic treatment does not show adsorption effects of the metabolization material to the biological matrix and, in addition, it yields almost complete transformation of TNT to the desired ADNTs and DANTs. We therefore propose this procedure as the method of choice for a combined biological and chemical treatment of aqueous TNT contaminations, in order to ultimately achieve complete mineralization of TNT to non hazardous compounds like carbon dioxide, water and inorganic nitrogen salts. Studies along this line are presently under investigation in our laboratory.

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## References

Achtnich C, Lenke H, Klaus U, Spiteller M & Knackmuss H-J (2000) Stability of immobilized TNT derivatives in soil as a function of nitro group reduction. Environ. Sci. Technol. 34: 3698–3704

Carpenter DF, McCormick NG, Cornell JH & Kaplan AM (1978) Microbial transformation of $^{14}$C-labelled TNT in an activated-sludge system. Appl. Environ. Microbiol. 35: 949–954

Corbett MD & Corbett BR (1995) Bioorganic chemistry of the arylhydroxylamine and nitrosoarene functional groups. In: Spain JC (Ed) Biodegradation of Nitroaromatic Compounds. Plenum Press, New York

Drzyzga O, Bruns-Nagel D, Gorontzy T, Blotevogel K & von Löw E (1999) Anaerobic incorporation of the radiolabelled explosive TNT and metabolites into the organic soil matrix of contaminated soil after different treatment procedures. Chemosphere 38: 2081–2095

Eilers A, Rüngeling E, Stündl UM & Gottschalk G (1999) Metabolism of TNT by the white-rot fungus *bjerkanda dausta* dsm 3375 depends on cytochrome P-450. Appl. Microbiol. Biotechnol. 53: 75–80

Haas R & von Löw E (1986) Grundwasserbelastung durch eine Altlast. Die folgen einer ehemaligen Sprengstoffproduktion für die heutige Trinkwassergewinnung. Forum Städte-Hygiene 37: 33–43

Hawari J, Beaudet S, Halasz A, Thiboutot S & Ampleman G (2000) Microbial degradation of explosives: biotransformation versus mineralization. Appl. Microbiol. Biotechnol. 54: 605–618

Hess TF, Lewis TA, Crawford RL, Katamneni S, Wells JH & Watts RJ (1998) Combined photocatalytic and fungal treatment for the destruction of 2,4,6-trinitrotoluene. Water Res. 32: 1481–1491

Hwang H-M, Slaughter LF, Cook SM & Cui H (2000) Degradation of TNT in a freshwater environment. Bull. Environ. Contam. Toxicol. 65: 228–235

Knackmuss H-J, Daun G, Lenke H & Reuss M (1998) Biological treatment of TNT-contaminated soil 1. Anaerobic cometabolic reduction and interaction of TNT and metabolites with soil components. Environ. Sci. Technol. 32: 1956–1963

Kröger M & Fels G (2000) $^{14}$C-TNT synthesis revisited. J. Labelled Cpd. Radiopharm. 43: 217–227

Kröger M & Fels G (2002) Microbiotic synthesis of $^{14}$C-ringlabelled aminodinitrotoluenes (ADNT) and diaminonitrotoluenes (DANT). J. Labelled Cpd. Radiopharm. 45: 249–255

Lewis TA, Ederer MM, Crawford RL & Crawford DL (1997) Microbial transformation of 2,4,6-trinitrotoluene. J. Industr. Microbiol. Biotechnol. 18: 89–96

Riefler RG & Smets BF (2000) Enzymatic reduction of TNT and related nitroarenes: kinetics linked to one-electron redox potentials. Environ. Sci. Technol. 34: 3900–3906

Scheibner K, Hofrichter M, Herre A & Michels J (1997) Screening for fungi intensively mineralizing TNT. Appl. Microbiol. Biotechnol. 47: 452–457

Schlesselmann E (1996) Diploma Thesis, University of Braunschweig, Braunschweig

Sitzmann M (1974) Chemical reduction of TNT – initial products. J. Chem. Eng. Data 19: 179–181

Spain JC (1995) Biodegradation of nitroaromatic compounds. Annu. Rev. Microbiol. 49: 523–555

Spain JC & Hughes J (2000) Biodegradation of Nitroaromatic Compounds and Explosives. CRC Press, Boca Raton

Wild JR, Kalafut T, Wales ME, Rastogi VK, Naumova RP & Zaripova SK (1998) Biotransformation patterns of 2,4,6-TNT by aerobic bacteria. Curr. Microbiol. 36: 45–54

Yinon J (1990) Toxicity and Metabolism of Explosives. CRC Press, Boca Raton