BIODEGRADATION OF DIFFERENT NITROAROMATIC COMPOUNDS – A REVIEW

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Different nitroaromatic compounds are extensively used in industrial and agricultural activities. Some of them serve as herbicides, others are known as explosives. Mainly, these compounds are composed of recalcitrant molecules which persist in soils and other environments. In this review article the biodegradation of nitroaromatics by mixed microbial populations or by the individual species of bacteria and fungi has been discussed. An emphasis was also paid to the possible degradation of nitroaromatics in chemically contaminated soils. The results of recent studies show that initial steps in the biodegradation of nitroaromatic compounds are accomplished by aerobic and facultatively anaerobic microorganisms, while the complete degradation requires rather anaerobic conditions.

INTRODUCTION

Nitroaromatic compounds are used as raw materials in the manufacture of pesticides, dyes, plastics and pharmaceuticals. Some of them, and especially polynitroaromatics, are known as explosives. Microbial degradation of nitroaromatic compounds is presently studied in many laboratories mainly in view of the negative environmental impacts of these compounds. In the 94th General Meeting of the American Society of Microbiology, and especially in an International Symposium sponsored by the US Air Force Office of Scientific Research, both of which were held in May 1994 in Las Vegas, Nevada (USA), the actual results achieved in this field of environmental microbiology were presented. The aim of this review article is to make some of these results available also to the scientists other than participants, and to all those who are interested in the fate of anthropogenic chemicals in the environment.

DEGRADATION OF NITROAROMATICS BY MIXED MICROBIAL POPULATIONS

The investigations on the biodegradation of different nitroaromatic compounds usually begin by using mixed populations of microorganisms. Cerniglia (USA) who dealt with the mixed populations of soil and intestinal microorganisms has observed, that the reductive capabilities of mixed populations were generally greater than those of pure cultures, for the individual cultures often differ in their ability to produce nitroreductases as a prime enzyme group involved in the degradation process. Averill (USA) pointed out to the role of both denitrifying and nitrifying soil bacteria. Denitrifying bacteria utilize inorganic N-oxides as a terminal oxidant in the absence of dioxygen. The pathway in most organisms proceeds from nitrate to dinitrogen via nitrit, nitric oxide, and nitrous oxide:

$$NO_3^- \rightarrow NO_2^- \rightarrow NO \rightarrow N_2O \rightarrow N_2.$$

Nitrifying bacteria utilize ammonia as a reductant, and convert it to nitrate in only three steps:

$$NH_3 \rightarrow NH_2OH \rightarrow NO_2^- \rightarrow NO_3^-.$$

The enzymes involved in both pathways are metaloenzymes, containing various types of hemes, Mo^+ and Cu^+ in distinct environments. The presence of the metal centers results in the occurrence of certain common types of biochemical transformations, including O-atom transfers and reversible hydration/dehydration reactions, which may be included in the biodegradation of nitroaromatics. According to Preuss and Rieger (Germany), the nitro groups on the aromatic ring are readily susceptible to reductive transformations under aerobic and anaerobic conditions. The rate of nitro reduction depends on the number of nitro groups and their position at the aromatic ring. With respect to the trinitrotoluene (TNT) (I in Tab. I), for example, the reduction of the first two nitro groups can be catalyzed by many aerobic and facultatively anaerobic microorganisms, while the reduction of the third nitro group is strictly confined to anaerobic microorganisms. Therefore complete reduction of TNT to the corresponding triaminotoluene (II) can only be achieved under anaerobic conditions. The initial steps in the anaerobic degradation of TNT, i.e., the reduction of the nitrosubstituents to amino groups occurs preferentially on the para-nitro group; then one of the ortho-nitro groups becomes reduced, thus producing 4-amino-2,6-dinitrotoluene (III), and 2,4-diamino-6-nitrotoluene (IV) in succession (Roberts, USA).

The biodegradability of 2,4-dinitrotoluene (DNT) (V) by denitrifying bacteria was investigated as an alternative process for the treatment of DNT

contaminated wastewaters (Noguera et al., USA). Cultures from a municipal wastewater treatment plant, which was not exposed to DNT and a wastewater treatment plant which continuously received DNT wastes with an average concentration of 8 mg/l were enriched in nitrate (1 000 mg/l as N) as the electron acceptor and in methanol or ethanol (1 500–16 000 mg/l) as the primary source of carbon and energy. About 80% of the initial DNT concentration (30–50 mg/l) was recovered as 2,4-diaminotoluene (DAT) (VI), 2-amino-4-nitrotoluene (VII), and 4-amino-2-nitrotoluene (VIII) transiently accumulated in the culture with the rate of biotransformation being faster for the cultures from the originally DNT contaminated wastewater. The cultures fed with the both intermediate metabolites showed that both of the aminotoluene isomers were biotransformed to diaminotoluene.

Picric acid (2,4,6-trinitrophenol) (IX) is formed as a toxic and explosive byproduct during manufacture of nitroaromatics. Presence of electron-withdrawing nitro groups in its structure has made this compound difficult to biodegrade. Rajan et al. (USA) reported the isolation of a microbial consortium, capable of degrading 1 000 ppm of picric acid in 48–60 hours. The authors were also successful in isolating some unidentified single cultures from this consortium that completely mineralize picric acid. The appearance and disappearance of 2,4-dinitrophenol (X) and release of $^{14}CO_2$ were observed. Since the studies with single cultures of different microorganisms usually allow a better insight in the mechanism of the biodegradation processes and also may indicate the use of microorganisms as a detoxifying agents, they usually attract a high attention by the researchers as shown below.

DEGRADATION OF NITROAROMATICS BY SINGLE BACTERIA

As repeatedly indicated, anaerobic conditions are required to complete the biodegradation of different nitroaromatic compounds. The ecological observations suggest sulfate reducing and methanogenic bacteria might metabolize these compounds under anaerobiosis if appropriate electron donors and electron acceptors are present in the environment, but there has been no demonstration of this ability until recently. Kulpa and Booparthy (USA) examined the ability of these bacteria to metabolize TNT and various other compounds. *Desulvovibrio* sp., e.g., metabolized TNT, 2,4-dinitrotoluene, 2,6-dinitrotoluene (XI), nitrophenol (XII), and aniline (XIII). All the nitroaromatics tested served as the sole source of nitrogen, however, the *Desulvovibrio* sp. did not use the compounds as the sole source of carbon. Thus, the transformation of nitroaromatics was accomplished by a co-metabolic process. The *Desulvovibrio* sp. was able to use a variety of carbon sources

including pyruvate, ethanol, formate, lactate and $H_2^+CO_2$. In addition to serving as nitrogen sources, the nitroaromatics also served as electron acceptors in the absence of sulfate. The major intermediates produced from TNT were 4-amino-2,6-dinitrotoluene, 2-amino-4,6-dinitrotoluene (XIV), 2,4-diamino--6-nitrotoluene, and toluene (XV).

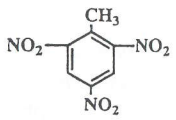
Numerous authors performed their studies on the biodegradation of nitroaromatic compounds using different strains of *Pseudomonas* spp. which are capable of growing and metabolizing organic substrates under either aerobic and/or anaerobic conditions. Haidour and Ramos (USA) isolated *Pseudomonas* sp. from soil and demonstrated its capability in utilizing TNT as the sole N-source under aerobic conditions. Accumulation of nitrite and dinitrotoluenes, mononitrotoluenes, and toluene in culture supernatants suggested the progressive elimination of nitro groups from the aromatic ring. An oxidative attack with subsequent release of the nitro groups as nitrite represents the predominant mechanism initiating mineralization of nitroaromatic compounds according to Spain (USA). Two mechanisms have been demonstrated for the oxidative removal of the nitro group. Monooxygenase enzymes catalyze the insertion of one atom of oxygen with elimination of nitrite from a variety of nitrophenols. Recent evidence strongly indicates that one mole of NADH is required for the reaction and that the product of the reaction is a benzoquinone (XVI) which is subsequently reduced to form a diphenol (XVII). The second mechanism involves the dioxygenase catalyzed insertion of two atoms of molecular oxygen on the ring of less polar compounds such as 2-nitrotoluene (XVIII), 1,3-dinitrobenzene (XIX), and 2,4-dinitrotoluene. The proposed product of the reaction is a dihydroxy nitrocyclohexadiene (XX) which is thought to spontaneously rearomatize with elimination of nitrite. Recent evidence indicates that the dioxygenase involved in the removal of the nitro group may be closely related to naphthalene dioxygenase. The diphenols resulting from either monooxygenase or dioxygenase catalyzed reactions are subsequently degraded by well established pathways of aromatic metabolism. Spain (USA) also found that a strain of *Pseudomonas pseudoalcaligenes* can degrade nitrobenzene (XXI) by reducing the nitro group to form hydroxylaminobenzene (XXII), which is converted to 2-aminophenol (XXIII) by an unusual Bamberger type of internal rearrangement. The ring of 2-aminophenol is opened by a dioxygenase mechanism analogous to that catalyzed by catechol-2,3-dioxygenase. Subsequent reactions release ammonia and allow the ring fission product to serve as a source of carbon. DNT, a byproduct of the manufacture of TNT, and toluene diisocyanate (XXIV), are US EPA priority pollutants. Reardon and Whitty (USA) demonstrated an oxidative degradation pathway of DNT which was utilized as a sole source of carbon and energy by *Pseudomonas* sp. strain PR7. When immobilized on diatomaceous earth pellets in a packed-bed bioreactor, the bacterium removed DNT at rates as high as 470 µmol/l/h.

The biodegradation of nitrobenzene by *P. pseudoalcaligenes* strain JS45 proceeded by the reduction of nitrobenzene through nitrosobenzene (XXV) and hydroxylaminobenzene, followed by rearrangement to 2-aminophenol, which then underwent meta-ring cleavage (Nishino and Spain, USA). The authors also isolated a gram positive bacterium that uses an oxidative pathway for the complete mineralization of nitrobenzene by using the compound as a sole carbon, nitrogen and energy source. Extracts of nitrobenzene-grown cells showed high catechol-2,3-dioxygenase activity that was not abolished by heating the extracts to 60 °C for 10 min. From *P. pseudoalcaligenes* JS45, Somerville et al. (USA) separated and purified a nitrobenzene nitroreductase which specifically catalyzed the production of hydroxylaminobenzene from nitrobenzene under exclusion of aniline as an intermediate product.

Pseudomonas sp. strain JS51 was found to grow on m-nitrobenzoic acid (m-NBA) (XXVI). This compound is a component of a metal strippers and is resistant to biodegradation in waste treatment facilities. Nadeau and Spain (USA) determined the degradation pathway of m-NBA by using respirometry, HPLC and UV/VIS spectrophotometry. The oxidation of m-NBA to protocatechuate required 1 mol O_2 per mol of substrate which indicates the reaction is catalyzed by a dioxygenase. A stoichiometric amount of nitrite was released from the degraded m-NBA. *Pseudomonas fluorescens* a frequent inhabitant of the plant rhizosphere was isolated from soil containing TNT by Murphy and Gilcrease (USA). In the anaerobic shake flask cultures with a minimal salt medium containing 1 g/l KNO_3 as the electron acceptor and 2 g/l ethanol or acetate as the carbon source, TNT was quantitatively reduced to diaminonitrotoluenes (80 % 2,4-, and 20 % 2,6-diaminonitrotoluene – XXVII) in 200 hours. *P. fluorescens* was also shown to degrade p-nitrophenol with the concomitant accumulation of nitrite in the medium, indicating an oxidative route for degradation. *Pseudomonas pickettii* degraded both p-aminobenzoate (XXVIII) and p-nitrobenzoate (XXIX) with the simultaneous accumulation of ammonia in the culture medium, indicating a reductive way for degradation (Hrywna et al., USA). Kalafut et al. (USA/Russia) reported the isolation of *Pseudomonas* sp., *Bacillus* sp. and *Staphylococcus* sp. that were effective as biodegradation agents for solubilized TNT under aerobic conditions. Alvarez et al. (USA) performed the isolation of microorganisms on a monoaminoaromatic (anthranilate) in an attempt to find organisms with the ability to deaminate aminonitrotoluenes, and therefore allow for the complete mineralization of TNT. The isolation yielded a *Pseudomonas aeruginosa* strain MAo1, able to degrade

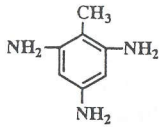
I. Structure of chemical compounds quoted in this review

I



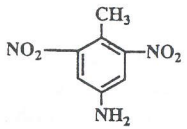
Trinitrotoluene

II



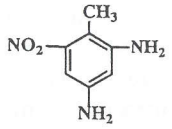
Triaminotoluene

III



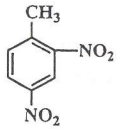
4-Amino-2,6-dinitrotoluene

IV



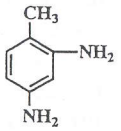
2,4-Diamino-6-nitrotoluene

V



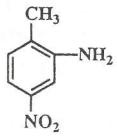
2,4-Dinitrotoluene

VI



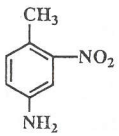
2,4-Diaminotoluene

VII



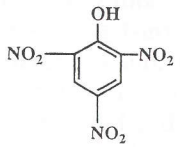
2-Amino-4-nitrotoluene

VIII



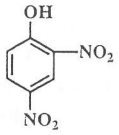
4-Amino-2-nitrotoluene

IX



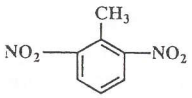
2,4,6-Trinitrophenol

X



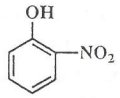
2,4-Dinitrophenol

XI



2,6-Dinitrotoluene

XII

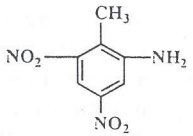


2-Nitrophenol

XIII

Aniline

XIV



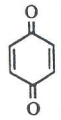
2-Amino-4,6-dinitrotoluene

XV

Toluene

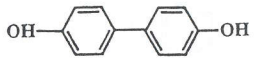
Continuation of Tab. I

XVI



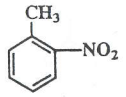
Benzoquinone

XVII



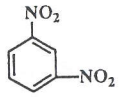
Diphenol

XVIII



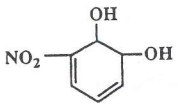
2-Nitrotoluene

XIX



1,3-Dinitrobenzene

XX



Dihydroxy-nitro-cyclohexadiene

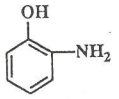
XXI

Nitrobenzene

XXII

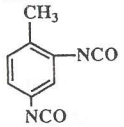
Hydroxyl-aminobenzene

XXIII



2-Aminophenol

XXIV

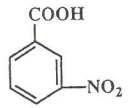


Toluene-diisocyanate

XXV

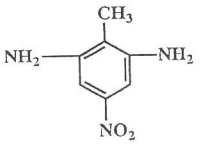
Nitrosobenzene

XXVI



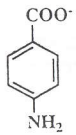
3-Nitrobenzoic acid

XXVII



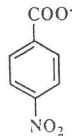
2,6-Diamino-4-nitrotoluene

XXVIII



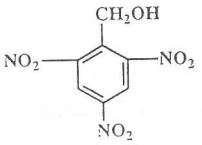
p-Aminobenzoate

XXIX



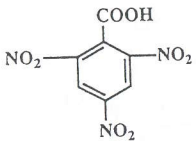
p-Nitrobenzoate

XXX



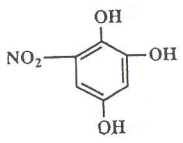
Trinitrobenzylalcohol

XXXI



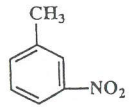
Trinitrobenzoic acid

XXXII



Hydroxy-nitrocatechol

XXXIII



3-Nitrotoluene

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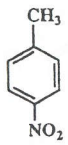
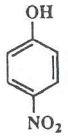
XXXIV



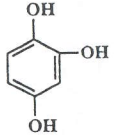
4-Nitrotoluene

XXXV



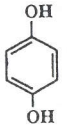
p-Nitrophenol

XXXVI



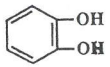
1,2,4-Benzenetriol

XXXVII



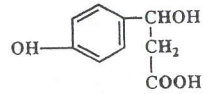
Hydroquinone

XXXVIII



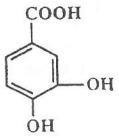
Catechol

XXXIX



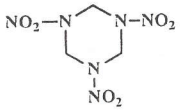
3-Hydroxy-3-(p-hydroxy-phenyl)-propionic acid

XL



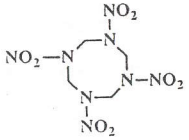
Protocatechuic acid

XLI



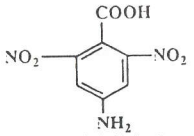
1,3,5-Trinitro-1,3,5,-hexahydrotriazine (RNX)

XLII



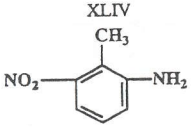
1,3,5,7-Tetranitro-1,3,5,7 tetrazocine (HMX)

XLIII



4-Amino-2,6-dinitrobenzoic acid

XLIV

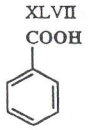


2-Amino-6-nitrotoluene

XLV

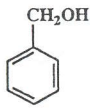
Propane

XLVII



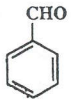
Benzoic acid

XLVII



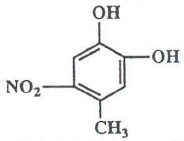
Benzylalcohol

XLVIII



Benzaldehyde

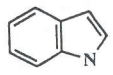
XLIX



4-Methyl-5-nitrocatechol

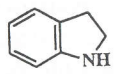
Continuation of Tab. I

L



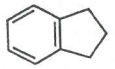
Indole

LI



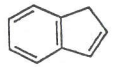
Indoline

LII



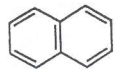
Indane

LIII



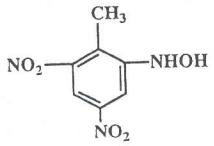
Indene

LIV



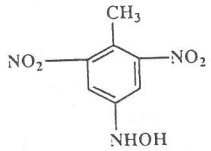
Naphthalene

LV



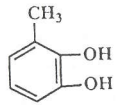
2-Hydroxylamino-4,6, dinitrotoluene

LVI



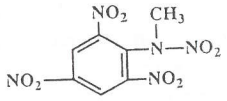
4-Hydroxylamino-2.6-dinitrotoluene

LVII



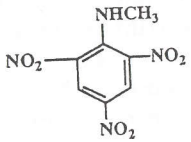
3-Methylcatechol

LVIII



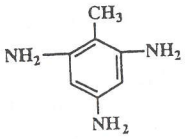
2,4,6-Trinitrophenylmethyl-nitramin (Tetryl)

LIX



N-methylpicramid (N-Methyl-2,4,6-Trinitroaniline)

LX



2,4,6-Triaminotoluene

LXI

Acetone

TNT while producing only low concentration of aminodinitrotoluenes and azoxydimers. The same strain was also capable of utilizing some aminotoluenes as a source of nitrogen. However, nitrogen limitation experiments indicated adding exogenous nitrogen yields a more complete degradation of TNT and its intermediates. Uniformly ring labeled (^{14}C) TNT studies showed the majority of TNT was degraded to polar intermediates, which appear to be ring breakage products. Ali-Sadat et al. (USA) described a new pathway for the biotransformation of TNT in *Pseudomonas putida*. This includes

the following sequence of steps: TNT → trinitrobenzylalcohol (XXX) → trinitrobenzoic acid (XXXI) → trinitrophenol → hydroxynitrocatechol (XXXII) → ring cleavage. The oxidation of 2- and 4-nitrotoluene occurred by the direct insertion of an oxygen atom in the benzene ring, and reduction of the nitro group of 2-, 3-, and 4-nitrotoluene (XXXIII and XXXIV) led to the formation of their respective aminotoluenes.

Dreisbach et al. (USA) showed an other typical soil bacterium *Arthrobacter* sp., strain JS443, to degrade p-nitrophenol (PNP) (XXXV) with stoichiometric release of nitrite. A mutant which was obtained by treatment of JS443 with ethyl methane sulfonate, accumulated 1,2,4-benzenetriol (XXXVI) in stochiometric amounts. JS443 converted 1,2,4-benzenetriol to maleyacetate which was metabolized through β-ketoadipate pathway. The authors also reported on *Moraxella* sp. and *Pseudomonas* sp. to degrade p-nitrophenol with release of nitrite and formation of hydroquinone (XXXVII). Meulenberg et al. (The Netherlands) have detected a novel degradative pathway for nitroaromatics in *Comamonas acidovorans* leading to a catechol (XXXVIII).

The importance of anaerobiosis for the microbial degradation of nitroaromatic compounds inspired several scientists to turn their attention also to the enteric bacteria. Haidour and Ramos (USA) isolated an enteric bacterium JLR11 that used TNT under anaerobic conditions. As final products of TNT metabolism were isolated 3-hydroxy-3(p-hydroxyphenyl)-propionic acid (XXXIX) and protocatechuic acid (XL). *Morganella morganii*, an enteric bacterium, cometabolized the nitramine explosives 1,3,5-trinitro-1,3,5-triazine (RDX) (XLI) and 1,3,5,7-tetranitro-1,3,5,7-tetrazocine (HMX) (XLII) in experiments carried out by Kitts et al. (USA). The metabolism of both explosives required a microaerobic environment and proceeded through reduction of the nitramine moities. *M. morganii* also required either yeast extract or beef extract for growth and nitramine explosive degradation was optimal in a medium containing 0.8% yeast extract. Addition of TNT to a culture metabolizing RDX resulted in a five fold increase in the initial rate of nitramine reduction by *M. morganii*.

Mycobacteria are generally known as cellulose degraders. Vorbeck et al. (USA/Germany) reported the bioconversion of TNT by a *Mycobacterium* sp., strain HL4NT-1, which was isolated for its ability to grow on 4-nitrotoluene. During conversion of TNT a dark red intermediate accumulated transiently. A brown pigment was transiently produced during catabolism of TNT by *Mycobacterium vaccae* in experiments carried out by Vandenberg et al. (USA). The metabolite was identified by NMR-spectroscopy as 4-amino-2,6-dinitrobenzoic acid (XLIII). Cometabolism of TNT by *M. vaccae* with toluene as carbon and energy source resulted in an accumulation of 2-amino-6-nitrotoluene (XLIV). When incubated with ^{14}C TNT and propane (XLV), 50% of the labeled carbon was incorporated into the cellular lipid fraction of *M. vaccae*. The ring cleavage also occurred during cometabolism of TNT.

The genetic manipulation on bacteria seems to enhance the effectiveness of the nitroaromatic degradation. The *P. putida* TOL plasmid, e.g., is a self-transmissible plasmid that replicates in other *Pseudomonas* sp. and in some *Enterobacteriaceae* belonging to rRNA I group. This plasmid encodes a catabolic pathway for metabolism of toluene to benzoic acid (XLVI) via benzylalcohol (XLVII) and benzaldehyde (XLVIII). Transfer of the TOL plasmid PWWO-Km to *Pseudomonas* sp. clone A, an isolate that used TNT as a N-source via its conversion into toluene, led to transconjugants able to use TNT as C- and N-source (Ramos et al., USA). The hybrid and parental strains also reduced TNT to several monohydroxylaminodinitrotoluenes, monoaminodinitrotoluenes and diaminomononitrotoluenes, which were identified in culture supernatants. Furthermore four azoxydimers resulting from condensation of reduced TNT form were found by Haidour and Ramos (USA). To elucidate the mechanism of nitrogroup removal from 4-methyl-5-nitrocatechol (MNC) (XLIX), Suen et al. (USA) have cloned and localized the gene (dntA) encoding DNT dioxygenase within a 6.8 Kb NsiI-NsiI DNA fragment. Sequence analysis revealed four open reading frames with extensive homology (59 to 78% identity) to the genes encoding naphthalene dioxygenase (NDO) from three *Pseudomonas* sp. strains. DNT dioxygenase has a broad substrate specifity similar to that of the NDO enzyme system and can attack such substrates as indole (L), indoline (LI), indan (LII), and indene (LIII). An *Escherichia coli* clone that expressed DNT dioxygenase converted DNT to MNC and also converted naphthalene (LIV) to the corresponding cis-dihydrodiol.

DEGRADATION OF NITROAROMATIC COMPOUNDS BY FUNGI

Extensive biodegradation of TNT by the white rot fungus *Phanerochaete chrysosporium* has been observed (Stahl and Aust, USA). Significant mineralization of TNT occurs but only under lignolytic conditions. The first step of TNT metabolism like by other organisms appears to be reduction of TNT to aminodinitrotoluenes. Reduced metabolites accumulated under non-lignolytic conditions but they were quickly metabolized when manganese peroxidases were present and mineralization of TNT only occurred after ligninases were detected. The rate of mineralization was reduced 50% when the fungus was grown in the absence of manganese. Michels and Gottschalk (Germany) also detected 2-hydroxylamino-4,6-dinitrotoluene (LV) and its isomer 4-hydroxylamino-2,6-dinitrotoluene (LVI) in cultures of

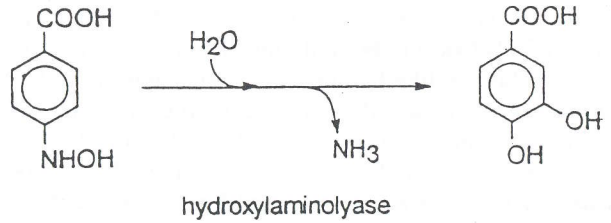
P. chrysosporium. Both compounds transiently accumulated and were responsible for the inhibitory effect of high concentrations (20 mg/l) of TNT to the fungus.

ENZYMATIC STUDIES ON THE DEGRADATION OF NITROAROMATICS

Somerville et al. (USA) purified nitrobenzene nitroreductase to homogeneity from the crude extract of *P. pseudoalcaligenes* in order to elucidate the mechanism of nitrobenzene reduction. The crude extract was precipitated by ammonium sulfate followed by Q-Sepharose^R anion exchange and Sephacryl S-200^R gel filtration chromatography. A single 33 kDa polypeptide was detected by denaturing gel electrophorese. Hydroxylaminobenzene was the only detectable product after incubation of the purified enzyme with nitrobenzene and NADPH.

Meulenberg et al. (The Netherlands) described the conversion of 4--hydroxylaminobenzoate to 3,4-dihydroxylbenzoate and ammonium as catalyzed by a hydroxylaminolyase from *Pseudomonas* sp. (Fig. 1). Similar activity was found by five strains of other bacteria tested. With all bacteria, the production rate of ammonium and catechol from 4-hydroxylaminobenzoate was the highest. An et al. (USA) isolated a new multicomponent enzyme system from *Pseudomonas* sp., which was designated 2-nitrotoluenedioxygenase. An extract prepared from cells grown with tryptic soy broth yielded three protein fractions which were all required for the oxidation of 2-nitrotoluene to 3-methylcatechol (LVII) and nitrite.

Van Beelen and Burris (The Netherlands/USA) were able to find TNT reducing enzymes in different fresh water or salt water sediments. The amount of protein isolated varied from 2 to 31 mg/kg sediment dry weight (dw) and the activity varied from > 1 to 53 nmol TNT/min/kg sediment (dw). Shah and Spain (USA) found that the reduction of the explosive 2,4,6-



1. Conversion of 4-hydroxylaminobenzoate to 3,4-dihydroxybenzoate and ammonium, catalysed by a hydroxylaminolyase (after Meulenberg et al., 1994)

-trinitrophenylmethylnitramin (Tetryl) (LVIII) by ferredoxin NADP oxidoreductase from spinach (EC 1.18.1.2) and NADP led to the elimination of NO_2^- and the formation of N-methyl picramide (LIX in Tab. I) as the major products. For every mole of tetryl reduced, about one mole of N-methyl picramide and nitrite were produced. The rate of release of nitrite from tetryl was inhibited by about 75–80% under aerobic conditions.

DEGRADATION OF NITROAROMATICS IN SOIL

Lenke et al. (Germany) pointed out on the extensive contamination of soil in their country as a result of the large scale manufacturing and handling of polynitroaromatic compounds as explosives, and especially during World War II. The authors recommended a sequential anaerobic/aerobic treatment for TNT contaminated soil in a bioreactor. Under anaerobic conditions TNT is subject to complete reduction of all nitro groups leading to 2,4,6-triaminotoluene (LX). This metabolite appears to be rather reactive and thus disappears readily in a subsequent aerobic treatment, especially in soil. It can also become strongly bond to the soil particles. According to Rieger and Knackmuss (Germany), the oxygenolytic mechanisms as initial nitrite liberating reactions are well known for mono- and dinitroaromatics, while reductive initial reactions as part of a productive catabolic pathway have been described only recently. Hydrogenations of the aromatic nucleus, e.g., were identified as key reactions for complete mineralization of picric acid and 2,4-dinitrophenol. In principle, such a catabolic route appears to be applicable also to TNT. However, a major part of TNT is misrouted yielding toluenes with some of the nitro groups being reduced. These are dead end products under aerobic conditions. Under sulfidogenic or methanogenic conditions, TNT and its partially reduced derivatives are completely converted into triaminotoluene. This compound can be further degraded under anaerobic conditions, or if soil and air are present, it can be irreversibly bound by humic substances.

Roberts (USA) reported that levels of up to 1% (w/v) TNT contaminated soil (12 000 mg/kg) could be remediated using an anaerobic procedure developed for the removal of the herbicide Dinoseb from soil. The TNT removal rate decreased drastically when levels of the metabolite 4-amino-2,6--dinitrotoluene were above 40 mg/l. Based on their laboratory studies, Stahl and Aust (USA) tested the ability of the white rot fungus *P. chrysosporium* to remediate environmentally contaminated soil containing 200, 2 000 and 10 000 mg/kg of TNT. The fungus was able to degrade TNT from 200 and 2 000 mg/kg to less than 10 mg/kg within 21 and 100 days, respectively. The TNT concentration was reduced from 10 000 to 3 500 mg/kg in

100 days. The principle metabolites were aminodinitrotoluenes. Their concentrations decreased to less than 10 mg/kg by day 28 in the 200 mg/kg TNT soil microcosm and to 80 and 1 000 mg/kg by day 100 in the 2 000 and 10 000 mg/kg TNT microcosm. The effectiveness of mixing the soil microcosm, addition of fungal inoculum, or addition of inoculated substrate to the 2 000 and 10 000 mg/kg microcosm when TNT degradation ceased was also evaluated. Addition of inoculum or inoculated substrate were effective in the former microcosm but in the latter only the addition of inoculated substrate proved effective. Microscopic examination of the microcosm demonstrated that filamentous fungal mycelia were only present when TNT was degraded.

Zappi et al. (USA) favorized a bioslurry treatment as an innovative approach to the application of biotreatment technology toward contaminated soils. Soil samples from a former military installation and containing about 18 000 mg/kg TNT were placed in five liter bioslurry reactors. The co-metabolite selection experiments indicated that simple aromatic compounds such as toluene seemed to promote the microbial activity toward TNT in terms of disappearance. Some more simple organic compounds such as acetate and succinate also promoted microbial activity but yielded lesser degradational kinetics. The evaluation of various microbial consortia indicated that the microbial populations native to the site had as good or better activity than any other consortia evaluated. Radiolabeled TNT was used to determine that carbon dioxyde evolution as high as 20% was observed indicating appreciable degrees of TNT mineralization. The surfactant and solvent evaluations determined that the addition of small quantities of acetone (LXI) (3% w/v) did not significantly enhance TNT desorption. However, the addition of several commercial surfactants at same levels (3% w/v) enhanced TNT desorption by over 400% over water extraction. TNT removal in excess of 99% was observed in the surfactant (Tween 80) and acetate amended bioreactors within nine weeks of incubation. In this period of time an increase then a subsequent decrease in aminodinitrotoluenes was also observed. The poisoned controls (using mercuric chloride) delivered several usually biotically based intermediates indicating also some abiotic degradation of TNT in the aerobic soil slurry to occur.

Acknowledgement

Tab. I was kindly compiled by Miss S. Urmann a PhD student in the author's laboratory.

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Received for publication June 22, 1994

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Biodegradace nitroaromatických látek - přehledná studie.

Scientia Agric. Bohem., *26*, 1995 (2): 145–161.

Nitroaromatické látky zahrnují rozsáhlou skupinu sloučenin známých jako výchozí suroviny, meziprodukty a finální produkty různých chemických výrobních procesů. Řada z nich nachází použití i v zemědělství (jako herbicidy), ve výrobě barviv a ve farmaceutickém průmyslu. Mnohé nitroaromatické látky jsou známy jako výbušniny. Velmi často se jedná o sloučeniny s vlastnostmi toxickými nebo kancerogenními, které jsou rovněž odolné vůči přirozenému biologickému a chemickému rozkladu. Zejména tyto vlastnosti jsou příčinou negativních účinků nitroaromatických látek na prostředí, zvláště na půdu a kvalitu podzemních vod.

Problematika mikrobiálního rozkladu nitroaromatických látek se proto stala velmi aktuální z hlediska často nezbytné asanace půdy a vodních zdrojů. Ve Spojených státech amerických a mnoha dalších zemích světa existuje velký počet stanovišť vojenského charakteru a průmyslově využívaných ploch (některé ještě z doby druhé světové války), které vykazují vysokou koncentraci nitroaromatických látek. Mnohé intenzívně chemicky ošetřované polní a lesní plochy netvoří v tomto ohledu výjimku. Mikrobiální aktivity by mohly napomoci odstranit nebo zmírnit rizika plynoucí z přítomnosti těchto škodlivin.

V práci je podán přehled o aktuálních výsledcích výzkumu, jak byly představeny na monotematickém sympoziu o biodegradaci nitroaromatických látek, pořádaném v květnu 1994 v Las Vegas (Nevada, USA) Úřadem pro vědecký výzkum amerických vzdušných sil.

V jednotlivých příspěvcích bylo poukázáno na význam komplexních mikrobních populací (zejména půdních mikroorganismů) pro rozklad např. trinitrotoluenu, dinitrotoluenu, kyseliny pikrové a jiných sloučenin. Mechanismy rozkladu jsou však často lépe osvětlovány za využití jednotlivých druhů bakterií nebo mikroskopických hub. Zejména bakterie rodů *Pseudomonas, Arthrobacter, Mycobacterium* a *Desulfovibrio* vykazují v tomto ohledu významnou aktivitu. Lignolytická houba *Phanerochaete chrysospoprium* je rovněž středem pozornosti. Kromě mikrobních kultur je z hlediska rozkladu nitroaromatických látek intenzívně studován i účinek mikrobiálních enzymů.

Dosavadní výsledky nasvědčují tomu, že při biotechnologické optimalizaci rozkladného procesu je možné dosáhnout výrazného snížení koncentrace nitroaromatických látek v půdě. Jejich biodegradace je zpravidla započata účinky aerobních a fakultativně anaerobních mikroorganismů; efektivní rozklad však v závěru vyžaduje navedení podmínek anaerobních. Nitroaromatické sloučeniny, o nichž se v práci pojednává, jsou pro názornost uvedeny strukturními vzorci v tab. I.

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