

Utilisation of nitrocompounds

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Abstract: *This paper is a review of methods of biological, chemical and physical utilisation of nitrocompounds, extremely toxic xenobiotics that are abundant in the biosphere and are recalcitrant to biodegradation. Due to their unique physicochemical properties the nature itself has significant problem with complete degradation of nitroxenobiotic substances. There are number of methods developed in order to prevent further contamination of the environment leading to defoliation, inhibition of growth of plants and has adverse health effects on animals. Their abundance, deriving mainly from military industry, poses a serious threat to biosphere and current methods of their utilisation require further optimization.*

Keywords: *nitrocompounds, nitroaromatics, explosives, biodegradation.*

Introduction

Nitrocompounds are a group of the organic substances containing one or more nitro (-NO₂) groups within their aromatic ring or aliphatic chain. They are characterised by, especially these with aromatic character, high toxicity [1], cancerogenicity [2], resistance to degradation and tendency to accumulate in the environment [3-4]. Their abundance in the environment derives from multiple sources, with most important military origin.

Due to the explosive character some compounds, especially 2,4,6-trinitrotoluene (TNT), hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) and octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine (HMX) have been used during wars and maneuvers for several years [5,6,7], as well as in the mining industry [8]. Nitroaromatic compounds are also synthesized as by-products during production of chemicals like the indole-based pharmaceuticals, defoliants (2,4-dinitrophenol – DNP), dyes and during incomplete combustion of polycyclic aromatic hydrocarbons (PAHs).

Some nitrocompounds are also naturally synthesized by plants or other living organisms as pheromones, toxins (nitropropionic acid) or antibiotics (chloramphenicol) [9]. It is their properties what makes them abundant and toxic to biosphere. The development of effective and economical method is therefore a challenge for modern science.

Physicochemical properties of nitrocompounds

The main property making them so recalcitrant to degradation is their low affinity to water. This is what decides about their significantly limited uptake by either bacteria or plants. Usually they are either solid substances or very viscous liquids. The presence of nitro groups attached to the hydrocarbon ring or chain results in their explosophoric properties, which means that their thermal decomposition releases huge amounts of energy. Nitro groups impart also a high electron-withdrawing character of these compounds, what is the cause of their xenobiotic character [10-11]. The representatives of nitrocompounds are shown in Figure 1.

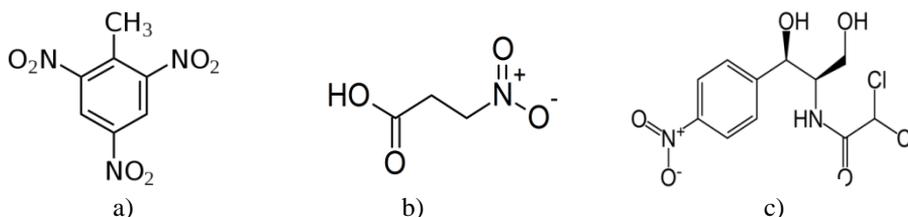


Figure 1. Representatives of nitrocompounds: a) 2,4,6-trinitrotoluene, b) 3-nitropropionic acid, c) chloramphenicol

The mechanism of nitration of aromatic rings is based on electrophilic substitution. Under the effect of nitration mixture (consisting of concentrated nitric and sulfuric acids 3:5 v/v) the created nitronium anion is attacked by aromatic ring, resulting in incorporation of $-\text{NO}_2$ group into the compound [12]. The simplified mechanism is shown in Figure 2.

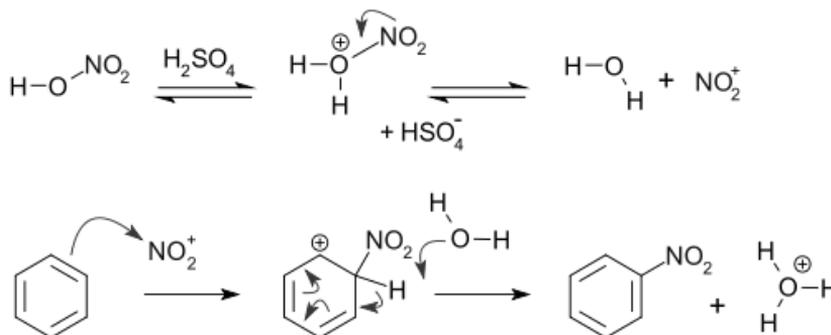


Figure 2. Mechanism of nitration of aromatic ring

The similar system was observed in case of enzymatic nitration of aromatic compounds, which is now thoroughly investigated [13]. It is based on electrophilic substitution catalysed by horseradish peroxidase (HRP), accompanied by NO_2^- and H_2O_2 in the system, providing nitro groups favoured in *ortho*- and *para*- positions (Figure 3).

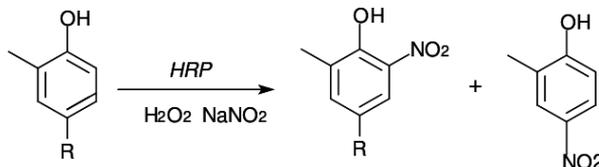


Figure 3. Nitration mechanism of Horseradish Peroxidase. The –R substituent may be either of aliphatic or aromatic character

In case of heterocyclic compounds (like dyes) the synthesis of nitro derivatives is more complicated, nevertheless it also requires utilisation of nitration mixture. In contrary to aromatic compounds, the nitration of aliphatic chains does not run *via* electrophilic substitution – it is based on nucleophilic mechanism. An example of this mechanism is nitration of glycerol with nitration mixture.

Commercial preparations and application

The nitrocompounds are most often used as explosives, due to their physicochemical properties. TNT, RDX, HMX used to be – and still are – widely used demolition charges, either in civil or in military industry. There are several companies specialized in manufacturing of explosive chemicals based on nitroaromatic rings. In the Europe, leading manufacturer of TNT is a NITRO-CHEM company, supplying whole continent with TNT and DNT for military applications and provide range of similar chemicals exclusively to Polish army. [14].

There is a wide range of pharmaceuticals containing one or more nitro groups. An example is mentioned chloramphenicol, an antibiotic synthesized by some *Streptomyces* strains. Usually it is provided as a salve (e.g. Deotrymcyzna® 1%). Another widely used pharmaceutical with nitro groups is well-known nitroglycerin (Nitrocard®, Nitromint®), used as a drug against heart attack or chronic coronary artery disease.

Nitrocompounds were widely used as defoliants, substances causing leaves to fall off. Extensively used during Vietnam War by US forces Agent Orange, Agent Blue or Agent White, not only causing massive deforestation, but also having strong impact on exposed citizens [15-16]. The most toxic preparation was Agent Orange, containing 2,4-dinitrophenol [17].

There is also group of dyes based on nitroaromatic derivatives. These dyes (mainly azo- ones) are for now about 70-80% of all dyes applied in food chemistry. Nevertheless they are also applied in another branches of industry, e.g. in flash photolysis (Disperse Orange 1) or as an recording layer in some DVD/CD disks.

Utilisation – a current problem for environment

A whole range of properties of nitrocompounds was not determined from the beginning. Within the years it was found that not only are they resistant to biological degradation, but also have significant impact on biosphere. Plants exhibit noticeable accumulation of nitro compounds. The presence of TNT and

similar compounds in nature resulted in their accumulation in roots [3]. It varies from a few up to several hundreds of miligrams accumulated per kilogram of a plant investigated, therefore, in some cases, eating crops from contaminated area may lead to severe health problems.

Health effects, in humans, include symptoms of acute poisoning like irritation, dizziness, nausea and headaches, especially when inhaled. Long-term exposure may bring about the failure of several organs, including liver and pancreas [18] also cancer and decrease sperm quality [19]. They may also provoke allergies and have genotoxic features [20, 21]. The effect of aliphatic nitro compounds is not completely known yet, nevertheless their hazard is mostly associated with explosive capabilities and toxic gases released upon combustion (mainly NO_x). Therefore the investigation on degradation methods of nitrocompounds, with current tendencies indicating increase the pollution of the environment, is required in order to preserve the biosphere from being further damaged. Current methods of degradation are described below.

Physicochemical degradation of nitrocompounds

Physicochemical degradation of nitrocompounds used to be main way of their elimination from the environment. Usually pure physical and chemical methods are not applied, as they were found exclusively inefficient.

The explosives, coming from shells or another misfires are transported to military areas and just blown up, unless spread within certain area. Otherwise they need to be treated in completely other way. Along with another type of compounds (e.g. dyes) the situation is more complicated. Apart from most expensive methods like incineration, or landfilling, costing up to 800\$/m³ of soil [40]. The physicochemical alternatives include UV radiation leading to photocatalytic process, accompanied by vast number of chalcogenides (various oxides and sulfides), or oxidizing agents like H₂O₂ or O₃. Also Fenton's reagent can be clearly applied in a photodegradation process [22, 23], as well as sonolysis, based on sound waves providing high temperature and pressure for a short time. Nevertheless, this technique used exclusively is inefficient, therefore is usually utilised with another, more advanced techniques [24]. Similarly, one of pure physical methods is based on thermal degradation. Such method can be applied directly, by flushing the soil with hot gas or another carrier, or indirectly, by contacting the debris coming from soil with for example hot surface. However, despite decent efficiency the costs of such process are very high, like the costs of all listed methods. It is also essential to include proper pretreatment of soil before remediation processes.

More complicated physicochemical processes include coagulation (for poor water-soluble substrates, at least if used solely), combined with ozonation when it comes to more recalcitrant substances, adsorption on carbon-based supports and membrane processes. While the latter is based on separation of dyes from water on film surfaces, the other provides highly efficient, relatively low-cost

adsorption on special materials, including clay, coal or oxides. Worth mentioning is that both adsorption and membrane filtering processes can be significantly enhanced by specified before ozonation or UV-H₂O₂ oxidation [25, 26, 27].

The most significant problems concerning utilisation of nitrocompounds using physicochemical methods are the cost and sometimes limited efficiency. Therefore there are methods developed based on cooperation of physicochemical and biological processes. While physicochemical processes *via* e.g. oxidation or photocatalysis reduce toxicity or recalcitrance of the substrates, the biological treatment decomposes remaining substances. An example of such method is activated sludge treatment in wastewater plants. Nevertheless, finding an optimal solution is dependent on several variables, like costs, efficiency, toxicity of compounds, their potential concentration etc. The biological treatment of nitrocompounds is described in a following section.

Biological decomposition of nitrocompounds

The microbial and plant degradation of nitrocompounds is strongly dependant on the structure and a number of nitro groups contained within the aliphatic chain or aromatic ring. Nitroaromatics with one nitro group are much less resistant to biodegradation than more nitrated derivatives, therefore are more likely to be degraded by bacteria or plants [28, 29]. Biological degradation of nitrocompounds is the most carefully investigated branch of bioremediation of such pollutants because not only are they cheap and easy to be applied in comparison to physicochemical methods, but also they are able to degrade wide range of compounds, usually without producing additional pollutants.

Biological denitrification process is a key to biodegradation. It is based on synergetic work of several enzymes belonging usually to oxidoreductases group. The most important ones are: nitroreductases, peroxidases, laccases and azoreductases [30].

Bacterial degradation

In case of bacteria, the complex nitroaromatics are degraded in oxidative or reductive pathway. The main problem with them is once the nitro groups are arrange symmetrically, high-electron deficiency practically exclude any oxidation processes. Therefore, reduction pathway is necessary, what is presented in Figure 4 [31].

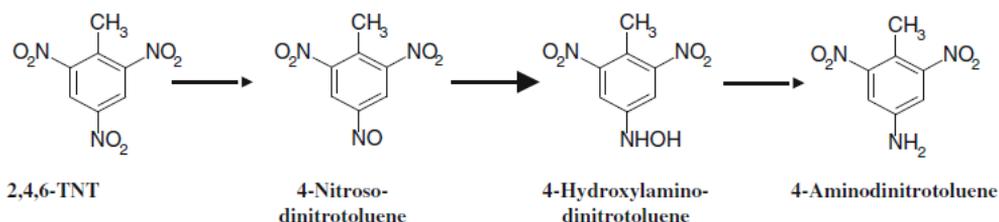


Figure 4. Reduction of nitro groups by aerobic bacteria

There is wide range of bacteria capable of decomposition of nitrocompounds. From the aerobic group one may list the representatives from *Pseudomonas*, *Rhodococcus* or *Clostridium* genera, like *Pseudomonas aeruginosa*, *Rhodococcus erythropolis* or *Clostridium acetobutlicum* [32, 33, 34].

In case of explosive compounds like TNT and its similar products the nitro groups are reduced through nitroso- and hydroxylamino- derivatives, presented, for DNP in Figure 5. However, during such reactions some by-products may be even more toxic than the initial substrate – for example 2,4-dinitrotoluene (DNT)[35]. Another problem is a decent efficiency of the process, but it may be significantly enhanced by immobilizing the bacteria. For example, *Bacillus mycoides*, suspended freely in media shown weaker biodegradation capabilities in comparison to immobilized culture [36].

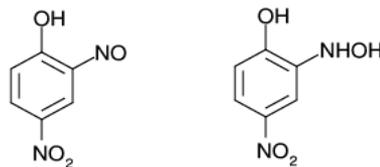


Figure 5. Hydroxyamino- and nitroso-derivatives of DNP

Fungal decomposition of nitrocompounds

It is essential also to mention about range of fungi that are similar in their efficiency in biodegradation of nitrocompounds. Special attention is nowadays given to fungi secreting enzymes of low specificity and high potential to degrade a wide range of substrates. These microorganisms belong to white-rot fungi and are primary microbes decomposing extremely recalcitrant lignin [37, 38, 39]. Their effectiveness in its degradation is based of cooperation of three enzymes: Manganese peroxidase (MnP; E.C. 1.11.1.13), lignin peroxidase (LiP; E.C. 1.11.1.14) and laccase (E.C. 1.10.3.2). They are secreted by white rot fungi when the environment is short in nutrients because decomposition of lignin enables an access to polysaccharides that are the sources of glucose and other simple sugars [29]. The most intensively studied representatives of these microorganisms are basidiomycetes *Phanerochaete chrysosporium*, *Pleurotus ostreatus* and *Trametes versicolor*. The fungi are capable of complete degradation of nitrocompounds, primarily due to reduction of nitrogroups and further decomposition via mentioned oxidoreductases. A fragment of such pathway is presented in Figure 6.

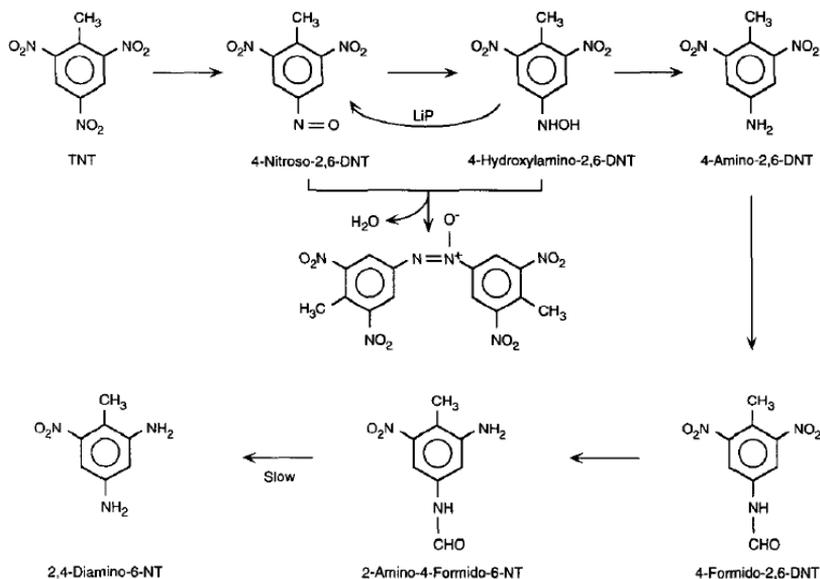


Figure 6. Metabolic reduction of 2,4,6-trinitrotoluene by *Phanerochaete chrysosporium* [40]

Phytoremediation

In case of plants, the biodegradation of nitrocompounds is based on phytoremediation processes. As these substances (explosives, dyes) clearly bioaccumulate in plants, this method seems to be suitable in most cases, although sometimes very time-consuming. It was found that some substances (TNT, RDX) not only were accumulated, but also transformed and mineralized [41, 42]. Such pathway – where 2,4,6-trinitrotoluene is transported, modified accumulated is shown in Figure 7 [43].

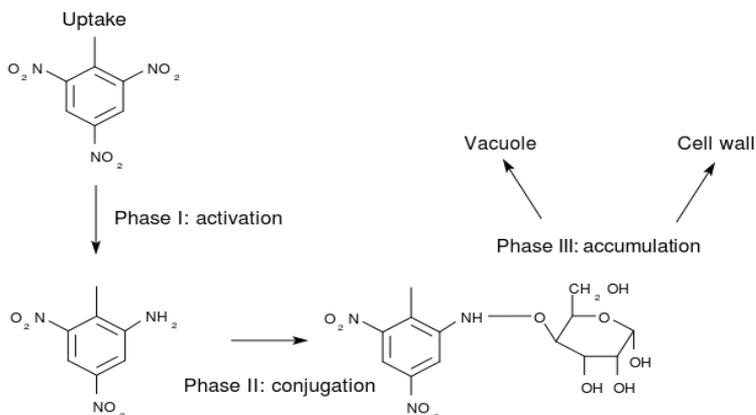


Figure 7. Uptake and transformation of TNT inside the plant cell

The aquatic plants, like *Typha latifolia*, *Juncus glaucus*, *Scirpus cyperinus* or *Myriophyllum spicatum* showed noticeable accumulation within a few days of incubation [33, 44, 45]. Nevertheless, ground plants also exhibit both accumulation and transformation, but the process is significantly slower, unless the plants applied are not transgenic [33].

When certain bacterial enzymes are introduced into genotype of some species, the efficiency of the process significantly increases. For example, comparing wild and transgenic tobacco in their phytoremediation process, it was found that the plant with introduced nitroreductase gene was capable of total accumulation and transformation of TNT from soil, what was impossible for the wild type [46]. Similar, although not identical, results were found for RDX [47]. It is essential to mention a fact, that not only does introduction of bacterial genes increase efficiency of phytoremediation, but also enhances resistance of plants to the toxicity of contaminating substances [39, 48].

Summary

The contamination of the biosphere with nitrocompounds and their growing abundance pose a serious threat to both human and environment. Accumulation in living organisms, toxicity and recalcitrance to biodegradation are reasons for intensive and accurate investigation. There is a number of methods that may be applied for degradation of the nitro-based organic substances, starting with pure physical methods (sonification, thermal decomposition), chemical technologies (advanced oxidation processes, Fenton's reaction), biological, combined physicochemical (coagulation with ozonation) or biological/physicochemical hybrids (oxidation prior to activated sludge treatment). Each has its pros and cons and cannot be applied in every situation. However, these methods need an optimization and enhancement to meet the requirements of efficient utilisation of nitrocompounds.

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