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TECHNICAL NOTE

A Toxicological Review on Potential Microbial Degradation Intermediates of 2,4,6-Trinitrotoluene, and Its Implications in Bioremediation

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Abstract

In this article, current knowledge on the potential fate, biodegradation, and toxicity of 2,4,6-Trinitrotoluene (TNT) was thoroughly reviewed, focusing on the toxicological evaluation of a variety of potential TNT microbial degradation routes. The present review on microbial degradation pathways and toxicities of biodegradation intermediate products suggests that aerobic TNT degradation pathways may be advantageous from a toxicological perspective, while anaerobic degradation pathways may be preferred over aerobic degradation due to its potential for complete TNT mineralization. Our review on TNT-degrading bacterial and fungal isolates suggests that the ecological understanding of TNT-degrading microbes in subsurface environments must be significantly improved to select an appropriate TNT bioremediation strategy.

Keywords: *bioremediation, biodegradation pathway, intermediate toxicity, TNT*

1. Introduction

Energetic compounds are chemicals that decompose rapidly due to chemical or thermal shock and produce a large amount of heat and gas (Khan *et al*., 2012). The manufacture, detonation and disposal activities of these compounds have severely contaminated vast areas of soil, sediment, and groundwater, especially in the proximity of military installations, which can impact environmental and human health (Juhasz and Naidu, 2007; Muter *et al*., 2012). 2,4,6-trinitrotoluene (TNT) is one of the energetic compounds and is the most widely used explosive for military applications (Ayoub *et al*., 2010). The level of TNT in polluted soil can reach 700,000 mg/kg, and the highest concentrations of TNT have been observed near or on the soil surface (Erkelens *et al*., 2012).

TNT is a potential carcinogen, is toxic to all organisms, binds tightly to soil organic matter, and is recalcitrant to biodegradation (Rylott *et al*., 2010). Therefore, the proper remediation of TNT-polluted environments is crucial for the protection of human health and ecosystems. The conventional methods for removal of TNT, such as thermal oxidation or incineration of soil, are highly expensive and destructive processes, and release a large amount of undesirable greenhouse gases, including CO_2 and NO_x (Snellinx *et al*., 2002). In the recent years, microbial biodegradation of TNT has been considered a cost-effective and eco-friendly approach (Weisse, 2008; Kalderis *et al.,* 2011; Erkelens *et al*., 2012).

Moreover, to select the appropriate bioremediation scheme for a TNT-polluted environment, the biological and physico-chemical properties of a system must be inferred. Firstly, the potential environmental fate of the target contaminant must be taken into consideration. Secondly, obtainment of information on the biodegradability of TNT by natural microorganisms is crucial. Finally, information on the toxicity of metabolites produced during the biodegradation of TNT is important for choosing the appropriate degradation pathway. In risk assessment of TNT bioremediation strategic plan, it is important to notice whether intermediates produced as a result of TNT biodegradation are less toxic than the parent compound. In the past decade, several review articles on the potential fate and biodegradation of TNT in the environment have been published (Juhasz and Naidu, 2007; Stenuit and Agathos, 2010; Kalderis *et al.,* 2011; Singh *et al.*, 2012) however, the toxicities of TNT degradation intermediates have not yet been reviewed. In addition, information related to TNT degradation pathways and the toxicity of intermediates is not available in the literature. In the present review, we focused on microbial degradation and toxicity of TNT and sought to provide information on the linkage between microbial TNT degradation pathways and the toxicity of their

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intermediates.

2. Potential Fate of TNT in the Environment

TNT is solid nitroaromatic compound that is prepared from toluene by nitration. TNT consists of a methyl group and a benzene ring with three nitro groups at the 2, 4, and 6 positions (Fig. S1). The value of the TNT octanolwater partition coefficient (log K_{OW} = 1.6) of TNT (Table S1) indicates that TNT possesses both hydrophobic and hydrophilic properties, which suggests that TNT is not strongly sorbed onto organic particles in soil and sediment. Therefore, in the absence of water-swelling clays, such as montmorillonite, in a subsurface environment, TNT may be relatively mobile via groundwater flow (Ayoub *et al*., 2010). Due to this property of TNT, plants may easily uptake and use dissolved TNT compounds in groundwater, which is an important implication for plant-based remediation strategy (phytoremediation).

TNT has a very low vapor pressure and low Henry's Law constant; thus, TNT and other nitroaromatics associated with munitions have low volatilization rates. Hence, conventional pumping methods using air/gas as a fluid, such as soil vapor extraction and air sparging, are not suitable choices for the remediation of TNT-polluted groundwater.

The fate and toxicity of TNT in the environment are influenced by a number of processes, such as volatilization, dissolution, sorption, bioaccumulation, and abiotic and biotic degradation (Juhasz and Naidu, 2007) (Fig. S2). Due to its low vapor pressure, volatilization is not a significant pathway for most common solid-phase TNTs (Juhasz and Naidu, 2007). Dissolution and solubility in water are the main mechanisms by which solid compounds are diffused in the environment. As presented in Table S1, TNT has low aqueous solubility (130 mg/L) (Kalderis *et al.,* 2011).

3. TNT-transforming Microbes

Several studies on the microbial degradation of TNT have been carried out in the past two decades (Table S2). These studies identified and isolated several enzymes involved in TNT biodegradation and discovered many microbes that can be used to degrade the contaminant (Ayoub *et al*., 2010). Hence, many bacterial and fungal isolates that can metabolize TNT and other explosive compounds have been discovered from polluted environments.

Nitroreductases from enteric bacteria can transform and reduce TNT to Hydroxylaminodinitrotoluenes (HADNTs), Aminodini-Trotoluenes (ADNTs) and Diaminonitrotoluenes (DANTs) (Rylott *et al*., 2010). Some members of the Old Yellow Enzyme (OYE) family of flavoproteins, such as pentaerythritol tetranitrate reductase (PETNr) from *Enterobacter cloacae* PB2 and xenobiotic reductase B (XenB) from *Pseudomonas fluorescens* I-C, act as nitroreductases and have the ability to transform TNT by adding a hydride to the aromatic ring to form monohydride-Meisenheimer (H^--TNT) or dihydride-Meisenheimer ($2H^-$-TNT) complexes, which results in the release of nitrite (Williams *et al*., 2004; Symons and Bruce, 2006). Fuller *et al*. (2009) showed that xenobiotic reductase A (XenA) in *Pseudomonas putida* II-B and XenB in *P. fluorescens* I-C can transform a wide variety of explosive compounds, especially at low oxygen concentrations. Although transformation occurred when the cells were supplied with both carbon (succinate) and nitrogen (NH_4^+) sources, degradation was not observed when only carbon was supplied (Fuller *et al*., 2009). Cho *et al*. (2009) found that *P. putida* HK-6 was able to use TNT and other nitroaromatic compounds as growth substrates and demonstrated that degradation was enhanced in the presence of supplemental nitrogen sources (urea, NH_4Cl, KNO_3, and $(NH_4)_2SO_4$) and C sources (molasses, glucose, succinate, and citrate).

Van Dillewijn *et al*. (2008b) found that XenB of *P. putida*, PETNr of *Enterobacter cloacae*, and *N*-ethylmaleimide reductase of *Escherichia coli* can reduce TNT. Esteve-Nunez *et al*. (2000) found that *P. putida* JLR11 uses TNT as a N source under anaerobic conditions. Under aerobic and anaerobic conditions, *P. putida* JLR11 reduced TNT to 4-hydroxylamino derivatives in the cytoplasm (Esteve-Nunez and Ramos, 1998). Recently, Claus *et al*., 2007 found that *Raoultella terrigena* removed all of the TNT from the broth solution within only four hrs. In addition, *R. terrigena* was able to degrade considerable amounts of TNT even when small amounts of glucose were supplied to the medium (Claus *et al*., 2007). *P. putida* JLR11 and *R. terrigena* are excellent candidates for the biodegradation of TNT because they are able to metabolize TNT in short periods of time and because relatively low supplies of nutrients are required (Weisse, 2008).

TNT biodegradation have also been found in some yeast and fungi (Zaripov *et al*., 2002; Ziganshin *et al*., 2010) (Table S2). Owing to the ligninolytic systems, basidiodiomycetes, especially white rot fungi, show significant (> 20%) biodegradation of TNT (Nyanhongo *et al*., 2005). The reduction of the nitro moiety, formation of TNT-Meisenheimer complexes, and concomitant release of nitrite have been observed in the white rot fungus *Irpex lacteus* (Kim and Song, 2003) and the yeast *Yarrowia lipolytica* (Ziganshin *et al*., 2007). *Y. lipolytica* NCIM 3589 reduces TNT (particularly in the presence of glucose) to H^--TNT, 2,4-dinitrotoluene (2,4-DNT) (> 0.25 M/M TNT) and ADNTs.

In conclusion, TNT-transforming bacteria are more diverse than TNT-transforming fungi. Therefore, TNT-degrading bacteria may be more available than fungal populations in the field. Among the TNT-degrading bacteria, the predominance of *Pseudomonadaceae* is often observed in TNT-contaminated soils (Stenuit and Agathos, 2010), which suggests that *Pseudomonadaceae* may be of particular interest for TNT bioremediation.

4. Possible Pathways of the Microbial Degradation of TNT

The supply of oxygen is one of the most costly processes in the field. Therefore it is noteworthy that a certain biodegradation

event takes place under aerobic or anaerobic conditions (Khan *et al*., 2012). Also, it would be important for the selection of an optimized respiration conditions for the isolation of TNT-utilizing organisms. From this viewpoint, TNT aerobic and anaerobic biodegradation routes have been reviewed below. In addition, we sought to construct all of the possible pathways of TNT biodegradation.

4.1 Biodegradation Pathways under Aerobic Conditions

The microbial transformation of TNT usually begins with the reduction of one of the nitro groups aerobically (Fig. 1). The enzymes that catalyze these reductions are non-specific NAD(P)H dependent nitroreductases and are largely uncharacterized (McFarlan and Yao, 2011). One exception is a nitrobenzene nitroreductase obtained from *P. pseudoalcaligenes* JS52, which has the ability to transform TNT to mono- and dihydroxylamino intermediates. As its name suggests, this enzyme can reduce nitrobenzene. Aerobic bacteria are able to reduce 2 of the 3 nitro groups of TNT. However, the reduction of the third nitro group requires anaerobic conditions (McFarlan and Yao, 2011).

Denitration is often a major reaction in the biodegradation of nitro-substituted compounds (Martin *et al*., 1997; Stenuit *et al*., 2009). However, the bacterial denitration of TNT or its reduction products has only been demonstrated in a few cases. For example, as shown in Fig. 2, 2-amino-4-nitrotoluene (2ANT) is produced from 2-amino-4,6-dinitrotoluene (2-ADNT). In addition, Martin *et al*. (1997) showed that *Pseudomonas savastanoi* was unable to mineralize significant quantities of TNT (less than 1%) but was able to generate 2,4-dinitrotoluene and nitrite from TNT. This reaction seems to be enhanced by adding nitrite and removing ammonium from the growth medium. In contrast, the addition of glucose to the growth medium decreased the denitration of TNT and increased the formation of 2-ADNT and 4-amino-2,6-dinitrotoluene (4-ADNT). The formation of Meisenheimer complexes from TNT has been observed in more than three bacterial strains, including *Rhodococcus erythropolis* and *Mycobacterium* sp. (Vorbeck *et al*., 1998). These complexes result from the nucleophilic attack of a hydride ion on the aromatic ring. The formation of hydride complexes could not be identified in recently isolated TNT-enriched strains TNT-8 and TNT-32 or *Pseudomonas* sp. strain A (2NT-). However, these strains are able to reduce the nitro group of TNT. In addition, the slow growth of *Enterobacter cloacae* strains PB2 was observed when TNT was supplied as the sole source of nitrogen.

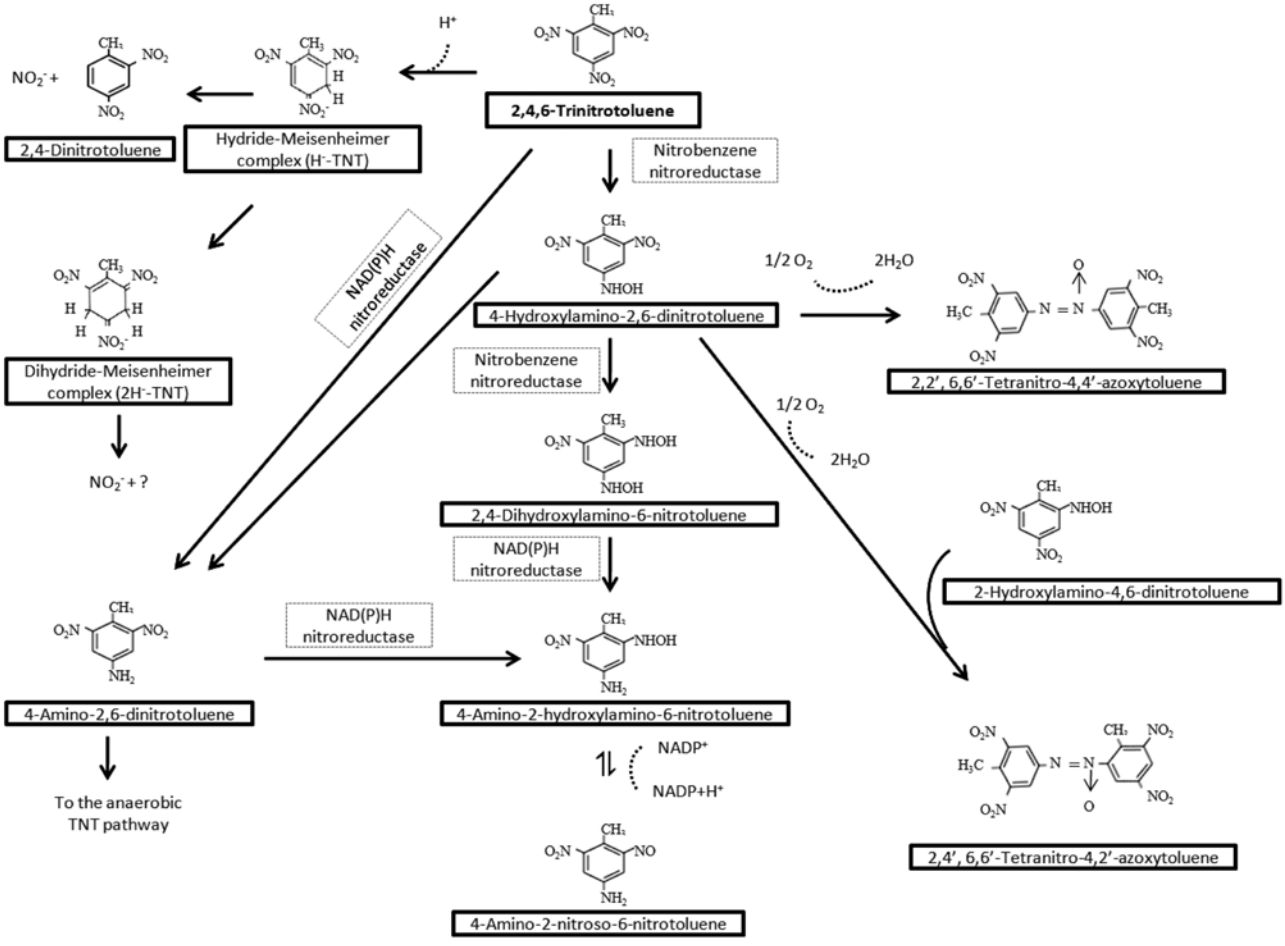

Fig. 1. 2,4,6-Trinitrotoluene Biodegradation Pathway I under Aerobic Conditions (Adapted from McFarlan and Yao, 2011)

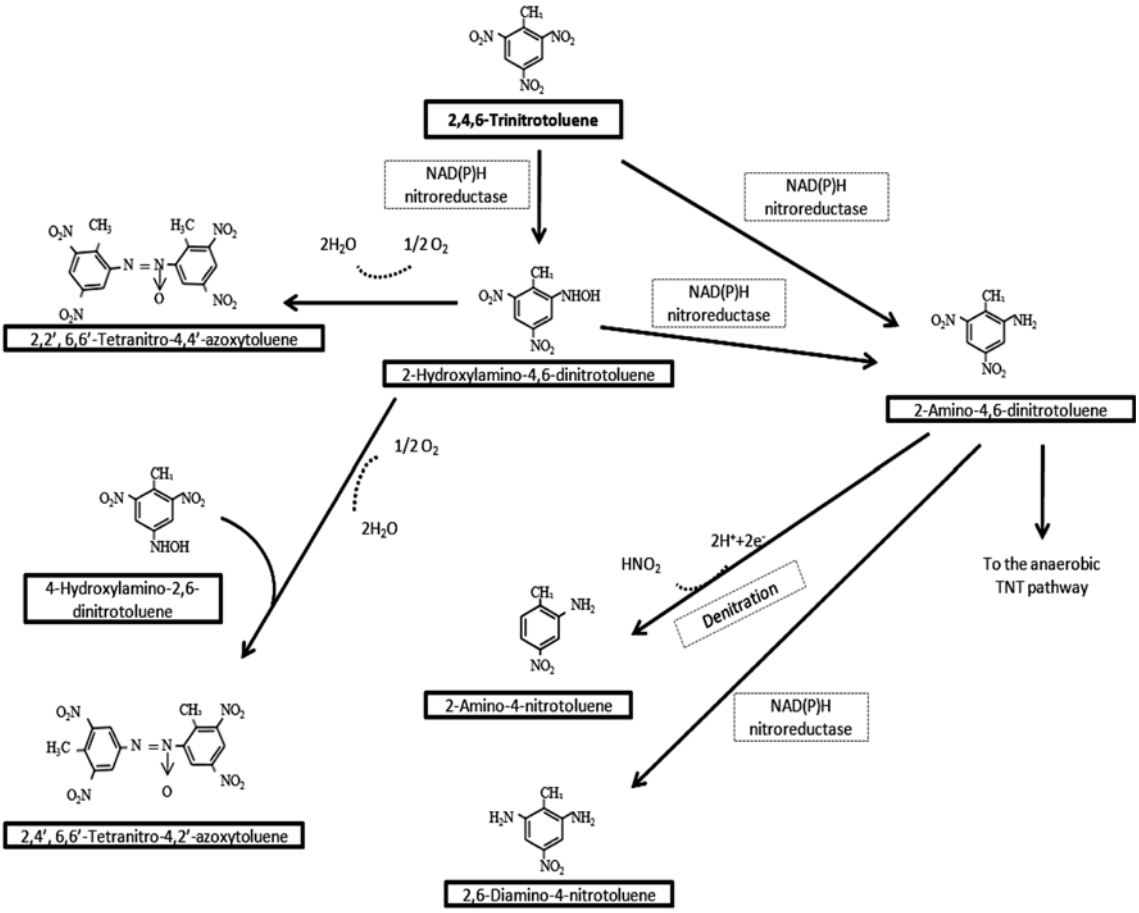

Fig. 2. 2,4,6-Trinitrotoluene Biodegradation Pathway II under Aerobic Conditions (Adapted from McFarlan and Yao, 2011)

Recently, many researchers have obtained in vitro evidence for the reduction of TNT by purified pentaerythritol tetranitrate (PETN) reductase and showed that this enzyme catalyzed the reduction of TNT to hydride- and dihydride-Meisenheimer complexes with concomitant NADPH oxidation and nitrite release (Williams *et al*., 2004; Ramos *et al*., 2005). Won *et al*. (1974) used *Pseudomonos sp.* Y under aerobic conditions in the presence of glucose or yeast extract. This strain was found to transform TNT into 2-ADNT, 4-ADNT, 2,6-dinitro-4-hydroxylaminotoluene (2,6-DHAT), and the corresponding nitrodiaminotoluenes, 2,2',6,6'-tetranitro-4,4'-azoxytoluene and 2,2',4,4'-tetranitro-6,6'-azoxytoluene.

Many studies suggest that partially reduced metabolites (ADNTs, DANTs) and tetranitroazoxytoluenes are the main end products of TNT degradation under aerobic conditions. These compounds present serious obstacles to bioremediation processes due to their recalcitrant nature. Cleavage of the aromatic nucleus of TNT by defined bacteria has not yet been demonstrated or may occur at a very low rate. Promising results on the mineralization of TNT have only been obtained with a few bacterial strains. However, further knowledge of TNT degradation pathways in these organisms is required before they can be used to remediate TNT-polluted soil and water.

4.2 Biodegradation Pathways under Anaerobic Conditions

Anaerobic degradation proceeds by the sequential formation of hydroxylamino intermediates, such as 2-ADNT, 4-ADNT, 2,4-diamino-6-nitrotoluene (2,4-DANT), 2,6-diamino-4-nitrotoluene (2,6-DANT), and triaminotoluene (TAT) as well as other unidentified products (Hawari *et al*., 1998) (Fig. 3). The further anaerobic transformation of TAT to trihydroxytoluene (THT) and toluene remains controversial (Hawari *et al*. 1998).

Few enzymes that can catalyze the reactions in the aforementioned pathways have been isolated (Fuller *et al*., 2009). Indeed, most of the reactions are catalyzed by non-specific NAD(P)H dependent nitroreductases and are uncharacterized (McFarlan and Yao, 2011). HADNTs are transformed into the corresponding ADNTs and 2,4-dihydroxylamino-6-nitrotoluene (2,4-DHANT). Recently, this metabolite was enzymatically converted to 2-amino-5-hydroxy-4-hydroxylamino-6-nitrotoluene by a *Clostridium sp.* strain (Vorbeck *et al*., 1998). Hence, HADNTs and the unidentified, highly polar metabolites described above may play an important role in the productive breakdown of TNT (Fig. 3). Therefore, the TNT-enriched strain TNT-8 and *Pseudomonas sp.* clone A (2NT2) may utilize TNT as a nitrogen source via the reductive metabolism of the nitro groups of TNT (Vorbeck *et al*., 1998).

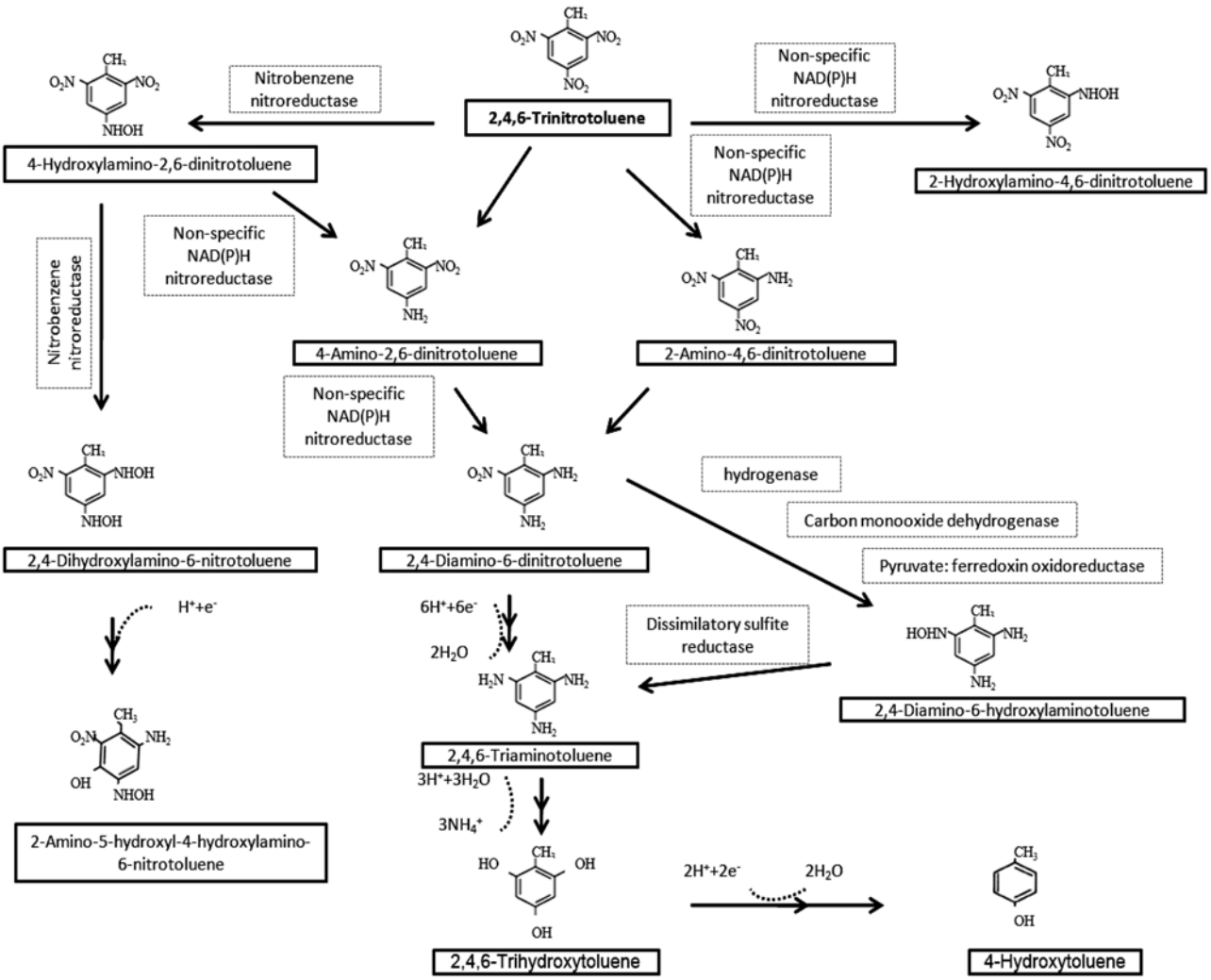

Fig. 3. 2,4,6-Trinitrotoluene Biodegradation Pathway under Anaerobic Conditions (Adapted from McFarlan and Yao, 2011)

The final reduction steps to produce TAT only take place under anaerobic conditions and enzymes that catalyze these reactions have been identified in *Desulfovibrio* sp., *Clostridium pasteurianum*, and *Moorella thermoacetica*. Many of these reactions (nitro group reductions) are also catalyzed by purified xenobiotic reductase enzyme (Fuller *et al*., 2009). The final reduction to TAT is catalyzed by a disulfite reductase that has only been found in *Desulfovibrio* sp. Moreover, examples of TNT mineralization or transformation to metabolic intermediates by pure bacterial cultures are relatively rare. In general, mineralization is only found with bacterial consortia. Most of TNT biodegradation products are highly reactive and covalently bind to cell organelles and solid supports (such as soil) present in the medium (McFarlan and Yao, 2011). Several studies have shown the formation of unidentified compounds during TNT biodegradation (McFarlan and Yao, 2011). These products have not been shown in either the aerobic or anaerobic pathways.

The bioremediation of TNT using bacteria is advantageous because the bacterial enzymatic reactions are diverse and bacterial enzymes can catalyze a wide array of biochemical reactions (Weisse, 2008; Muter *et al*., 2012). However, in a few cases, significant difficulties were encountered when bacteria were used for TNT degradation. First, bacteria can only utilize TNT present in the immediate area; therefore, once the available TNT is depleted, bacteria are not further involved in the bioremediation process. Second, TNT-degrading bacteria are in competition for nutrients and space with other bacteria that do not metabolize TNT. Bacteria require a steady supply of nutrients and energy for their proper growth and function; TNT-contaminated environments may be unable to provide a steady supply of nutrients (Symons and Bruce, 2006). Weiss *et al*. (2004) demonstrated that the ability of bacteria to access TNT decreases over time. Therefore, future research on the discovery, identification, and isolation of suitable bacterial strains must be conducted to overcome these problems and to enhance bioremediation. However, at present, the major technical obstacle for the discovery of TNT-degrading microbes is the inability to study unculturable terrestrial microbes (Rylott *et al.,* 2010; Khan *et al*., 2012).

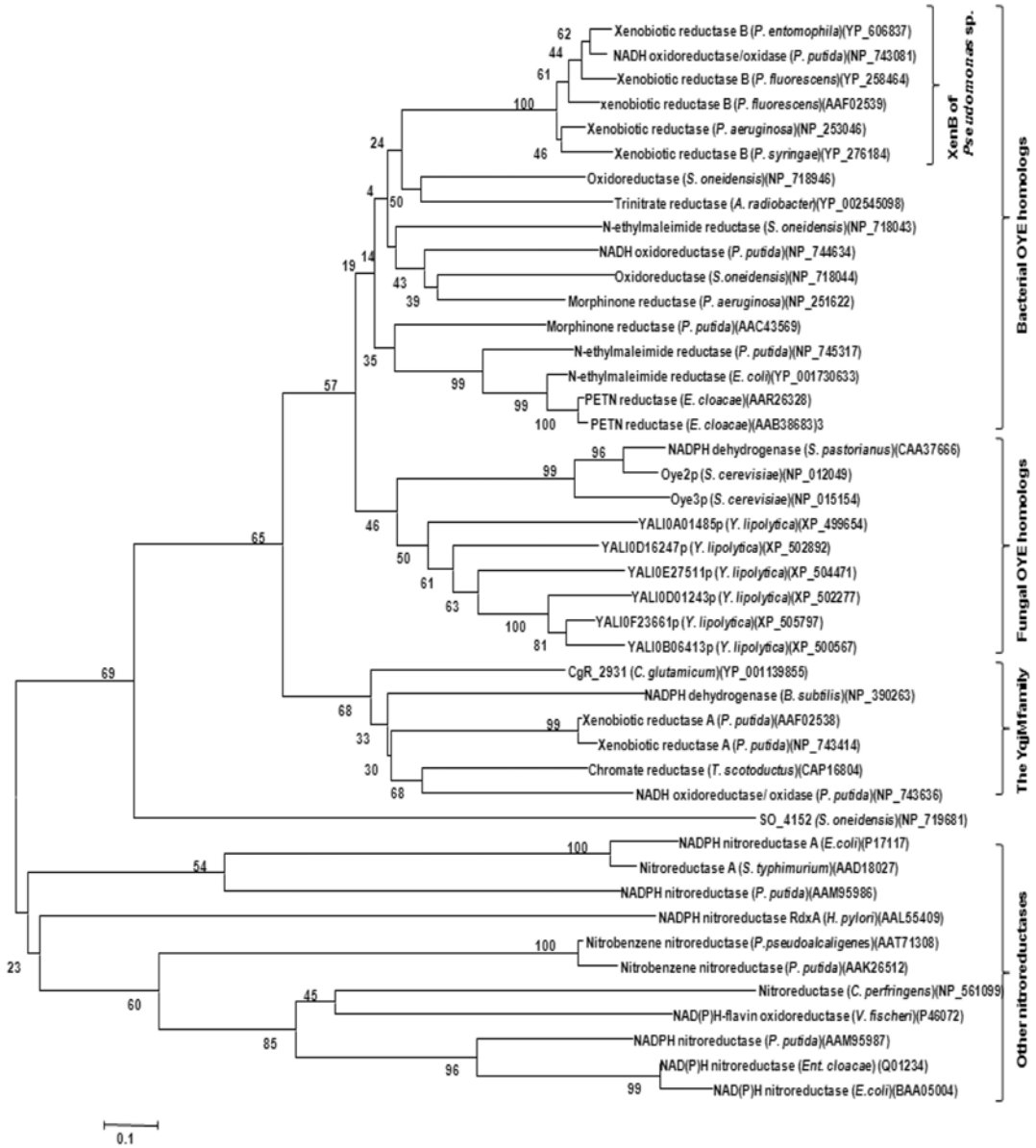

Fig. 4. Phylogenetic Placement of Known TNT-degrading Enzymes and Relatedness among Members of the Old Yellow Enzyme Family (The scale bar represents an amino acid sequence divergence of 10%).

Due to the high microbial diversity of terrestrial environments, full sequencing of the soil/sediment metagenome would not be appropriate for TNT biodegradation investigations. The Stable Isotope Probing (SIP), as an alternative, could be employed to isolate a metagenome of TNT-metabolizing populations (Gallagher *et al*., 2010). In addition, gene-targeted metagenomic techniques combined with next generation sequencing could be used to detect specific biodegradative genes in the environmental samples (Iwai *et al*., 2010; Lee *et al*., 2011; Khan *et al*., 2012). The combined-use of TNT-SIP and gene-targeted metagenomics is a promising approach for the selection of an appropriate bioremediation method. The present impuissance and main hindrance in gene-targeted metagenomics is that a universal functional degradative-gene for discovering TNT-degrading bacteria in the environment has not yet been identified. To overcome this weakness, we suggest that the OYE family of flavoproteins could be used as biomarkers for monitoring TNT-degrading microbes *in situ*. Uncommonly, OYE family proteins are distributed in diverse organisms, including bacteria, fungi, and plants, while *Pseudomonadaceae* populations that are predominant in TNT-polluted soils contain the highly conserved type II OYE homologs (Fig. 4).

5. TNT and Its Intermediates Toxicities

The amount, potential exposure and toxicity of a contaminant are the important factors for the risk assessment of a compound (Khan *et al*., 2012). Extensive research studies have been conducted

Table 1. Toxicity of TNT Degradation Intermediates

Metabolites	Toxicity	Exposure time	Organism tested	Relative toxicity	Sources
4-Hydroxylamino-2,6-dinitrotoluene (4-HADNT)	Mutation	24 h	Cricetulus griseus	TNT	Honeycutt *et al.,* 1996
2-Hydroxylamino-4,6-dinitrotoluene (2-HADNT)	Growth	12-36 h	*P. aeruginosa* strain MX	< TNT	Oh *et al.,* 2003
2-Amino-4,6-dinitrotoluene (2-ADNT)	Mutation	24 h	Cricetulus griseus	< TNT	Honeycutt *et al.,* 1996
	Mortality	14 d	Eisenia andrei	TNT	Lachance *et al.,* 2004
	Survival	96 h	Hyalella azteca	<TNT	Sims and Steevens, 2008
	Reproduction	30 d	Acheta domesticus	> TNT	Karnjanapiboonwong *et al.,* 2009
4-Amino-2,6-dinitrotoluene (4-ADNT)	Mutation	24 h	Cricetulus griseus	- TNT	Honeycutt *et al.,* 1996
	Mortality	14 d	Eisenia andrei	TNT	Lachance *et al.,* 2004
	Survival	96 h	Hyalella azteca	<TNT	Sims and Steevens, 2008
	Reproduction	30 d	Acheta domesticus	> TNT	Karnjanapiboonwong *et al.,* 2009
2,4-Dinitrotoluene (2,4-DNT)	Cell viability	12-36 h	*P. aeruginosa* strain MX	> TNT	Oh *et al.,* 2003
	Reproduction	30 d	Acheta domesticus	> TNT	Karnjanapiboonwong *et al*., 2009
2,6-Diamino-4-nitrotoluene (2,6-DANT)	Survival, Mutation	5 h	Cricetulus griseus	< TNT	Kennel *et al*., 2000
2,4-Diamino-6-nitrotoluene (2,4-DANT)	Mutation	24 h	Cricetulus griseus	TNT	Honeycutt *et al.,* 1996
	Survival, Mutation	5 h	Cricetulus griseus	< TNT	Kennel *et al.,* 2000
	Survival	96 h	Hyalella azteca	>TNT	Sims and Steevens, 2008
2,4,6-Triaminotoluene (TAT)	Survival, Mutation	5 h	Cricetulus griseus	< TNT	Kennel *et al.,* 2000
2,2', 6,6'-Tetranitro-4,4'-azoxytoluene	Mutation	24 h	Cricetulus griseus	TNT	Honeycutt *et al.,* 1996
	Survival, Mutation	5 h	Cricetulus griseus	< TNT	Kennel *et al.,* 2000
	Cell viability	12-36 h	*P. aeruginosa* strain MX	< TNT	Oh *et al.,* 2003
2,4', 6,6'-Tetranitro-4,2'-azoxytoluene	Survival, Mutation	5 h	Cricetulus griseus	< TNT	Kennel *et al.,* 2000

on the toxicities of TNT and its intermediate degradation products employing a variety of reporter organisms, including vertebrates, invertebrates, plants, algae, and microorganisms (Table S3 and Table 1). The different biological assays yielded diverse toxicity values that indicate the diverseness of the sensitivities of different bioindicator organisms. Toxic dosages can also be different depending on time of exposure and conditions; however, these data can provide a reference dose for human exposure (Khan *et al*., 2012). The US Environmental Protection Agency has recommended 1.0 μg of TNT per liter of drinking water for human health (Martel *et al*., 2009).

The relative toxicity of TNT degradation intermediates compared to the parental compound is dependent on the individual intermediate (Table 1). Before 2009, TNT was believed to be more toxic than its metabolites (2-ADNT, 4-ADNT, 2,6-DANT, 2,4-DANT, 2,2', 6,6'-tetranitro-4,4'-azoxytoluene). This conclusion was based upon mutagenesis assays using CHO (Chinese Hamster Ovary) and macroalgae (*Ulva fasciata*) systems, and survival assays using aquatic invertebrate *Hyalella azteca* (Kennel *et al*., 2000; Carr and Nipper, 2003; Sims and Steevens, 2008). However, in 1999, Dodard *et al*. (1999) raised doubts over the hypothesis that the reductive metabolism of nitroaromatics is associated with detoxification and demonstrated that the partially reduced product 2,4-DNT was more toxic than the parent compound in Microtox tests. Later, Lachance *et al*. (2004) proved that TNT metabolites (4-amino-2,6-dinitrotoluene, 2-amino-4,6-dinitrotoluene) are as toxic as TNT itself based upon their investigation on the toxicity and bioaccumulation of TNT metabolites in *Eisenia andrei* exposed to amended forest soil. Recently, Karnjanapiboonwong *et al*. (2009) showed that the relative toxicity of TNT and its metabolites in soil display the following trend: TNT < 2A-DNT < 4A-DNT < 2,4-DNT. This observation was based upon the reproductive toxicity of TNT and its metabolites to crickets (*Acheta domesticus*). In addition, 2,4-DANT and 2,4-DNT were also found to be more toxic than TNT to *H. azteca* and *Rana catesbeiana,* respectively (Sims and Steevens, 2008; Paden *et al*., 2011). According to these studies, the degradation of toxic TNT metabolites is as important as the degradation of the parent compound (TNT). Hence, complete mineralization/degradation or transformation of TNT to non-toxic final products must be the goal of any bioremediation process.

Based upon their reproductive toxicities, TNT is relatively less toxic than its intermediates (Karnjanapiboonwong *et al*. 2009). However, the mutation-inducing toxicities of TNT and its intermediates are comparable (Kennel *et al*., 2000; Carr and Nipper, 2003). The toxicities of 2-hydroxylamino-4,6-dinitrotoluene and 4-hydroxylamino-2,6-dinitrotoluene are similar to that of TNT (Table 1). 2,6-diamino-4-nitrotoluene, 2,4-diamino-6-nitrotoluene, 2,4,6-triaminotoluene, and 2,2', 6,6'-tetranitro-4,4'-azoxytoluene are less toxic than TNT because they lead to less reproductive and mutation toxicities and have higher chemical stabilities. Most of the other intermediates produced

during TNT degradation (Figs. 1-3) can be classified as uncharacterized because their relative toxicities are not well reported in the literature.

In the present study, the toxicity-based classification of TNT intermediate products was applied to examine the risk of potential TNT biodegradation pathways (Figs. 1-3). In aerobic TNT degradation pathways (Figs. 1-2), sub-pathways to tetranitro-azoxytoluenes via hydroxylamine-dintrotoluene exhibit less toxicity than those to dinitrotoluene or amine-dinitrotoluene. The sub-pathways to tetranitro-azoxytoluenes dead-end products require the presence of oxygen. The relative toxicities of the products of other possible sub-pathways in aerobic pathways currently remain uncharacterized. The complete mineralization of TNT can be achieved in the anaerobic TNT degradation pathway (Fig. 3). From a toxicological perspective, all of the intermediate products formed are mono-aromatics, which are generally more toxic than the condensed di-aromatic dead-end products produced under aerobic conditions. The sub-pathways to amino-dinitrotoluenes and diamino-dinitrotoluene by non-specific NADH nitroreductases may provide compounds with greater toxicities than that of the other anaerobic sub-pathways. However, this sub-pathway eventually results in complete TNT mineralization via the anaerobic degradation of 4-hydroxyltoluene. The sub-pathway to 2,4-dihydroxylamino-6-nitrotoluene via 4-hydroxylamino-2,6-dinitrotoluene by nitrobenzene nitroreductases may be toxicologically preferred due to the relatively low toxicity and stability of these reaction products.

6. Conclusions

In the present study, we mainly focused on the potential fate, biodegradation, and toxicity of TNT and attempted to provide information on linkage between TNT biodegradation pathways and its intermediate toxicities. The present review suggests that TNT is moderately mobile through groundwater in most soil/sediment environments, especially in the absence of water-swelling clay particles. Furthermore, due to the relatively high hydrophilicity, TNT may be available for plant uptake, affirming the practicability of plant-based remediation option. The results of this review on TNT biodegradation pathways and their potential intermediate products suggests that aerobically stimulated microbial degradation of TNT may be advantageous in a toxicological perspective, while anaerobic stimulation of TNT microbial degradation may promote complete mineralization of the pollutant. Hence, microbes that can expeditiously mineralize toxic intermediates of TNT must be identified. Functional metagenomics combined with next generation sequencing is a powerful probe for this emerging field of biodegradation studies.

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