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Biological degradation of explosives and chemical agents

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Abstract

Hazardous energetic organo-nitro compounds are found as contaminants in many environments. A series of nitro aromatics, nitrate esters and nitro amines, all characteristic of this class, has been studied for their susceptibility to biological transformation. Biotransformation pathways for each of these compounds have been identified and are summarized. Implications for these findings in light of current contamination issues is discussed. The detoxification of organophosphate chemical agents focuses on the investigation of organophosphate degrading enzymes from bacteria. Certain of these enzymes, active both in solution and when immobilized onto a solid surface, are very successful in hydrolyzing and detoxifying various organophosphate chemical agents. The relationship of this research to the critical concerns of the agricultural industry regarding detoxification of organophosphorous pesticides is discussed.

Introduction

The scope of environmental contamination from hazardous wastes is overwhelming, with recent estimates for EPA Superfund sites at over 32,000 in the US alone. The cost for remediation of these sites is variably estimated at over a trillion dollars, and even at this cost most sites would not be returned to a 'pristine' condition (Abelson 1992). Most technologies currently considered for remediation are expensive and often do not permanently alleviate the pollution hazard. Bioremediation has recently received renewed consideration as a treatment option due to its potential effectiveness without adding new hazardous burdens to the environment, its comparative low cost, and its adaptability and tailorability to specific treatment scenarios and environments (EPA 1990; Thayer 1991; Fox 1992). Organic energetic compounds are found as contaminants in many environments at sites where these compounds were produced, processed and disposed. Many of these organic energetic compounds are used in the production of explosives, as well as in the synthesis of fungicides, insecticides, herbicides, pharmaceuticals, and dyes. The toxicity and mutagenicity of many of these compounds, along with their relative recalcitrance in the environment lead to concerns regarding environmental fate and impact on human and environmental health. Chemical agents also represent environmental hazards during manufacture, processing, storage, and disposal, particularly with increased emphasis on environmentally-compatible destruction of stockpiles. For both organic energetic compounds and chemical agents, a biodegradation approach may offer a useful option to safe and economic disposal.

Many questions must be answered before a biological approach to the degradation of these compounds can and should be considered. At the basic level, can the target compound(s) be shown to be susceptible to biological degradation such that nontoxic products are produced? Can the organism(s) responsible for the biodegradation, and perhaps the enzymes and genetics involved be identified and characterized? Can genetic enhancement, con-

struction of new genetic pathways, or genetic recruitment be used to improve or tailor the natural process? Can the range and optimum environmental conditions under which the identified organisms or consortia perform be identified? Can laboratory-scale or microcosm studies be conducted to demonstrate the process? Can controlled field studies be successfully run to evaluate performance and to optimize conditions? The intent of this paper is to summarize some of our previous studies on the biodegradation of hazardous organo-nitro explosives/propellants and chemical agents (Kaplan 1991, 1992). These studies only deal with some of the questions raised above, with many questions remaining to be answered before a final assessment of the suitability of biodegradation for a given chemical or set of chemicals at a given site under a given set of conditions can be made.

It will be important to define bioremediation/biodegradation terminology for the purpose of this paper. Biodegradation includes biotransformation and mineralization. Biotransformation indicates some degree of modification of the target chemical, such as a change in functional groups, but not resulting in mineralization. Mineralization is defined as biodegradation of the target chemical where carbon dioxide, methane, water and biomass are the final products. In studies on bioremediation these terms are critical, since two goals for this process are the reduction in hazardous properties of the target compound (biotransformation), and ideally, the complete return of the compounds into the normal geochemical carbon and nitrogen cycles (mineralization).

Explosives and propellants

Organo-nitro explosives and propellants can be divided into at least three categories, (1) nitroaromatics (e.g., 2,4,6-trinitrotoluene, 2,4-dinitrotoluene), (2) nitramines (e.g., nitroguanidine, hexahydro-1,3,5-trinitro-1,3,5-triazine, octahydro-1,3,5,7-trinitro-1,3,5,7-tetrazocine), and (3) nitrate esters (e.g., nitrocellulose, nitroglyerin, ammonium nitrates). General pathways for the biodegradation of explosives and propellants can be summarized as follows (Kaplan 1991):

(1) nitroaromatics:
$\geqslant C\text{-}NO_2 \rightarrow \geqslant C\text{-}NO \rightarrow \geqslant C\text{-}NHOH \rightarrow \geqslant C\text{-}NH_2$
$\geqslant C\text{-}NO_2 \rightarrow \geqslant C\text{-}NH_2 \rightarrow \geqslant C{=}O$
$\rightarrow$ ring cleavage $\rightarrow$ mineralization

(2) nitramines:
$>N\text{-}NO_2 \rightarrow >N\text{-}NO \rightarrow >N\text{-}NHOH \rightarrow >N\text{-}NH_2 \rightarrow$ mineralization

(3) nitrate esters:
$>C\text{-}O\text{-}NO_2 \rightarrow >C\text{-}OH \rightarrow$ mineralization

2,4,6-Trinitrotoluene (TNT)

TNT can be biotransformed, mineralized or conjugated into higher molecular weight complex products. We have previously shown that a reductive pathway exists for the biotransformation of TNT by bacteria (Fig. 1) (McCormick et al. 1976; Carpenter et al. 1978; Kaplan & Kaplan 1982a-e, 1985c; Kaplan et al. 1985; Greene et al. 1985). This pathway has been observed in a number of systems including aqueous, sewage, soil and compost. Nitroaromatic compounds such as TNT are usually considered resistant to oxidation by oxygenase enzymes due to the presence of the electron withdrawing nitro groups on the ring. Recent studies with fungal systems (*Phanerochaete chrysosporium*) have provided the first substantive evidence for mineralization of the aromatic ring of TNT although the details of the mechanism and pathway remain to be elucidated (Fernando & Aust 1990, 1991; Lebron et al. 1992).

2,4-Dinitrotoluene (2,4-DNT)

Until recently, most biodegradation pathways elucidated for 2,4-DNT involved biotransformation but not ring cleavage or substantial mineralization. We have previously elucidated a biotransformation pathway for 2,4-DNT which involves the enzymatic

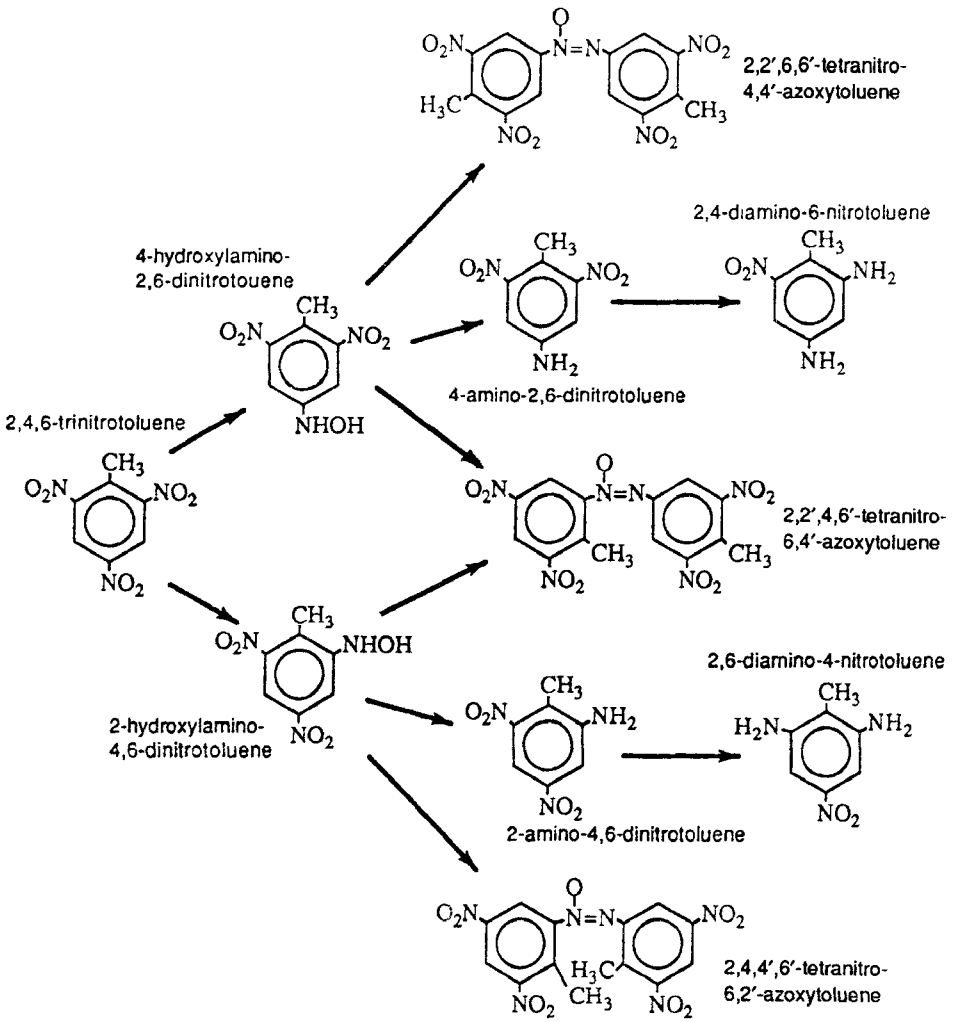

Fig. 1. Reductive biodegradation pathway for TNT.

reduction of both nitro groups to the corresponding amino groups (Fig. 2) (McCormick et al. 1978). This process occurs under aerobic conditions and supplmental carbon is required. The 4-nitro group was preferentially reduced in comparison to the 2-nitro group, similar to the preference noted for TNT. Recently, a reaction mechanism has been elucidated for the mineralization of 2,4-DNT by a reduction-oxidation system involving peroxidase enzymes and the white rot fungus *P. chrysosporium* (Valli et al. 1991).

Nitroglycerin (glycerol trinitrate)

We have shown that glycerol trinitrate is biotransformed through a series of successive denitration steps, including glycerol dinitrate and glycerol mononitrate isomers, with each succesive step slower than the previous step (Fig. 3) (Wendt et al. 1978). Glycerol is mineralized by biological systems. Glycidol and glycidol nitrate, formed by the chemical desensitization of glycerol trinitrate are also mineralized (Kaplan et al. 1982a). The biotransformation pathway of these compounds proceeds from glycidyl nitrate to 1-mononitrate to glycerol, with slower rates at each succeeding step.

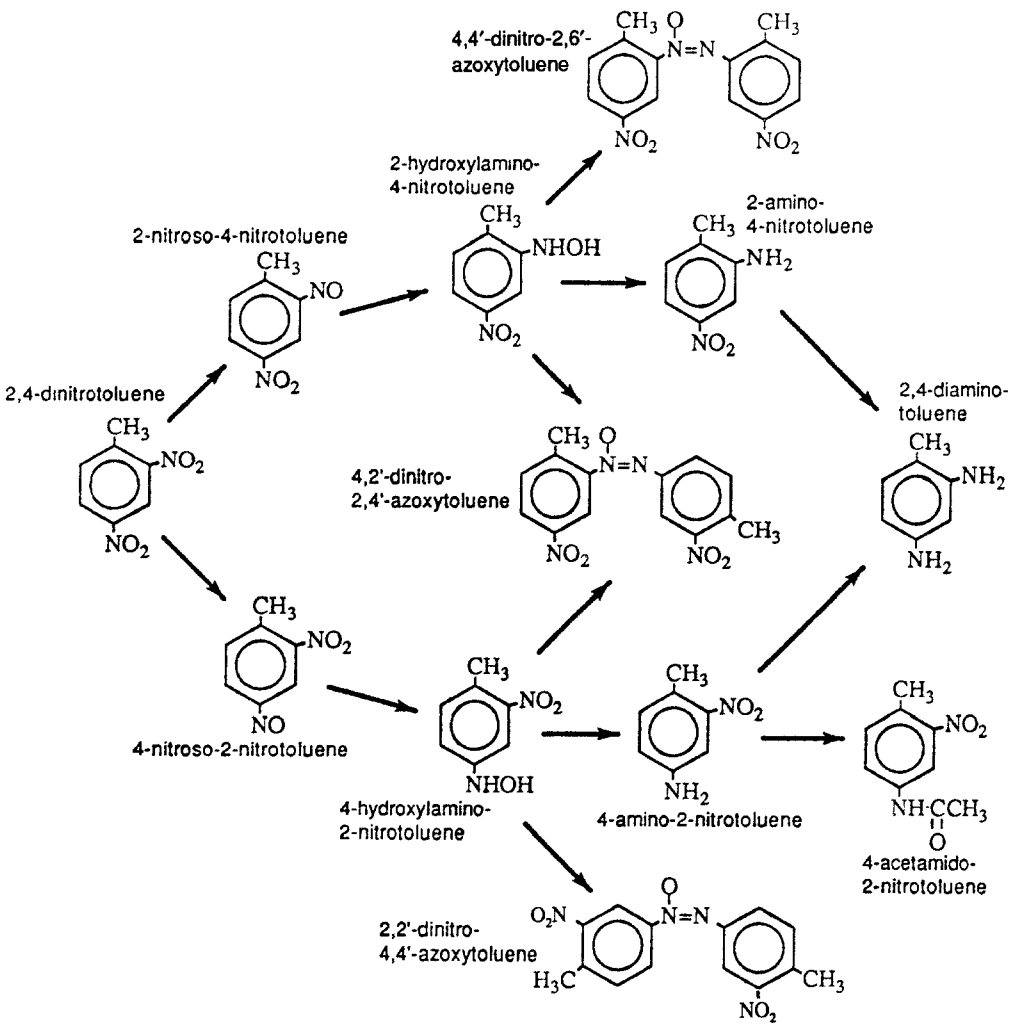

Fig. 2. Reductive biodegradation pathway for 2,4-DNT.

The steps from glycidol to glycerol and glycidyl nitrate to glycerol 1-mononitrate occur spontaneously in aqueous solutions, however it appears that the rates are accelerated by microbial activity, perhaps due to secondary effects.

Nitrocellulose

Nitrocellulose with a high degree of substitution (usually 11.1 to 14.5% nitrogen for cellulose dinitrate to trinitrate) is not subject to direct microbial attack and is generally regarded as persistent in the environment (Riley et al. 1984). In studies where the growth of organisms on nitrocellulose has been observed, growth may be due to contaminants in the preparation, growth on regions of incomplete or low degree of substitution, or possibly due to the effects of secondary metabolites on the chain chemistry (e.g., alkaline hydrolysis of the nitro-groups). We have shown that chemical pretreatment of nitrocellulose by alkaline hydrolysis was necessary to generate a modified denitrated polymer that could be attacked by microorganisms in an anerobic denitrification system with supplemental carbon (Wendt & Kaplan 1976a, 1976b; Kaplan et al. 1987). Importantly, the concentrations of nitrate and nitrite formed in process remained below drinking water limits due to the use of the denitrification process.

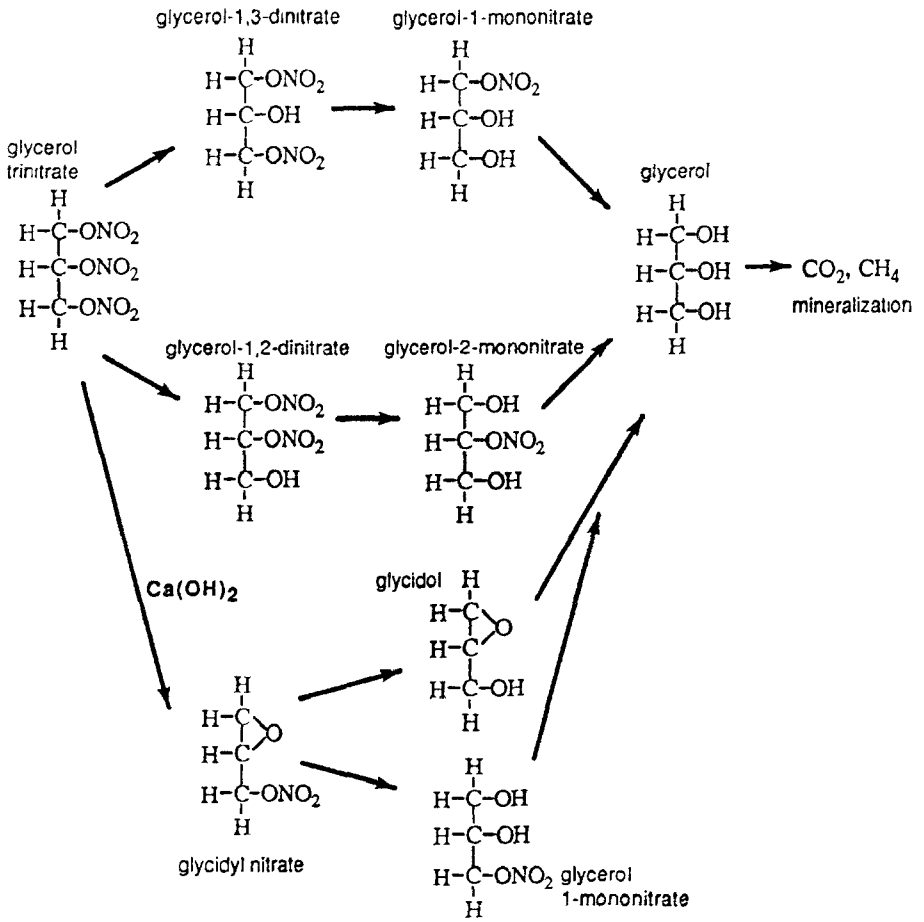

Fig. 3. Biomineralization pathway for glycerol trinitrate.

Propylene-, diethylene- and triethylene- glycol dinitrates and trimethylolethane trinitrate

We have shown that these nitrate esters are microbially transformed in a series of successive denitration steps to the correponding glycols: propylene glycol, diethylene glycol, triethylene glycol, and trimethylolethane glycol (Fig. 4) (Cornell et al. 1981), leading to mineralization apparently through a combination of biological and nonbiological processes. The relative rates of mineralization are propylene glycol > diethylene glycol > triethylene glycol > trimethylolethane glycol (Kaplan et al. 1982c).

Hydroxylammonium-, trimethylammonium-, isopropylammonium-, triethanolammonium-nitrates

We have shown that under aerobic conditions the trimethylammonium-, isopropylammonium-, and the triethanolammonium- nitrates were mineralized (Fig. 5) (Kaplan et al. 1984). Under anaerobic denitrification conditions trimethylammonium nitrate and triethanolammonium nitrate were mineralized and isopropylammonium nitrate was incompletely biodegraded. These studies were conducted with ^{14}C-labeled compounds in aerobic and anaerobic batch studies where the nitrate esters were available individually as the sole source of carbon and nitrogen and in continuous flow denitrification systems. In these studies no accumulation of intermediates was observed. The trimethyl-, isopropyl-

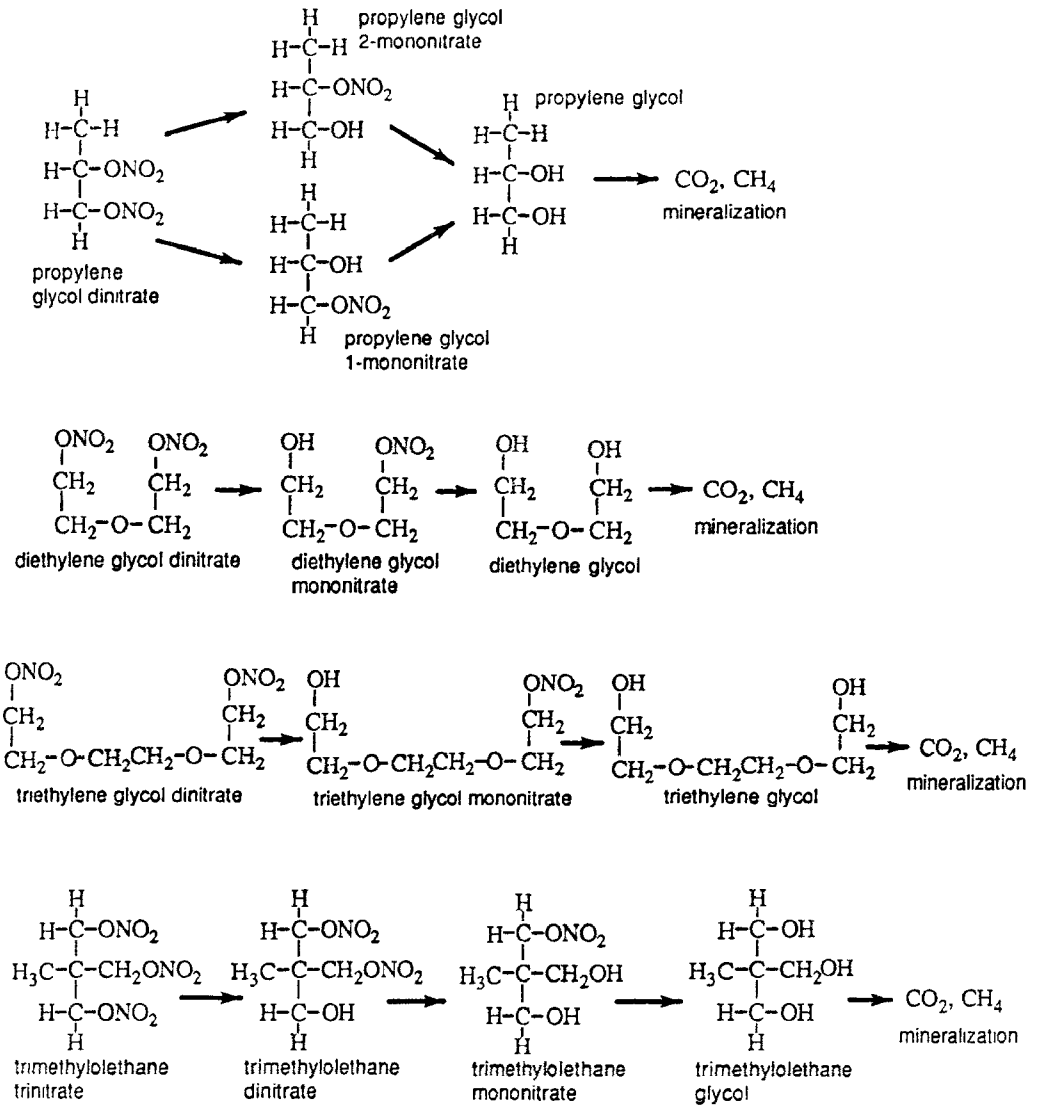

Fig. 4. Biomineralization pathways for a series of glycol nitrate propellants.

and triethanol- ammonium nitrates were mineralized in soil when studied at concentrations from 50 to 5000 mg/l, and hydroxylammonium nitrate was labile under slightly acidic and alkaline conditions. Nitrosamines such as N-nitrosodimethylamine were observed during analysis of culture samples from biodegradation studies and biodegradation studies indicated that this compound is biodegradable and is presumed to be mineralized (Kaplan & Kaplan 1986).

Triaminoguanidine nitrate

We have shown that triaminoguanidine nitrate is biodegraded under aerobic and anaerobic conditions with a requirement for supplemental carbon (Kaplan & Kaplan 1985a). No intermediates were identified during this process. Guanidine, hydrazine, urea, carbohydrazide (1,3-diaminourea), cyanamide, and cyanoguanidine were sought but not detected in culture samples.

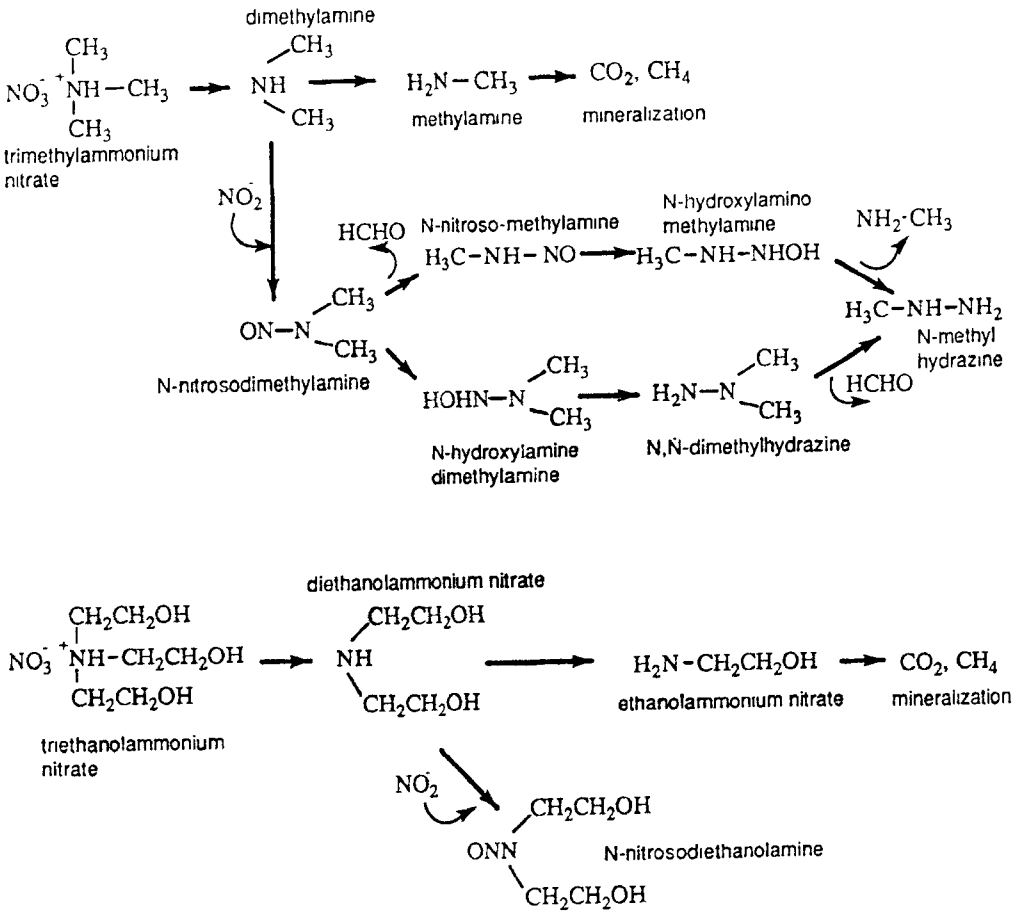

Fig. 5. Biomineralization pathways for ammonium nitrate propellants.

Hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) and hexahydro-1-N-acetyl-3,5-dinitro-1,3,5-triazine (AcRDX)

RDX and the related N-acetylated derivative, AcRDX, were studied in aqueous systems and found to be mineralized by mixed populations of microorganisms under anaerobic conditions when supplemental carbon was provided (Fig. 6) (McCormick et al. 1981, 1985a, 1985b). Intermediates in this pathway included mono-, di-, and trinitroso-compounds formed during the sequential reduction of the nitro groups on the parent compound. Traces of hydrazine, 1,1-dimethylhydrazine, and 1,2-dimethylhydrazine were also detected in the samples after exposure to the mixed microbial populations. No biotransformation of RDX was observed under aerobic conditions. The AcRDX was also biotransformed only under anaerobic conditions with the formation of the analogous series of mono- and di-nitroso compounds and in this case, no hydrazine or dimethylhydrazine.

Octahydro-1-3,5,7-trinitro-1,3,5,7-tetrazocine (HMX) and octahydro-1-N-acetyl-3,5,7-trinitro-1,3,5,7-tetrazocine (AcHMX)

We have reported that HMX and AcHMX were mineralized under anaerobic conditions at 50 mg/l, although rates were slower than for RDX (McCormick et al. 1985a). Biotransformation was incomplete and mono- and di-nitroso intermediates were identified (Fig. 7). In a similar fashion to RDX, supplemental carbon was necessary for the biotransformation process to function. The kinetics of

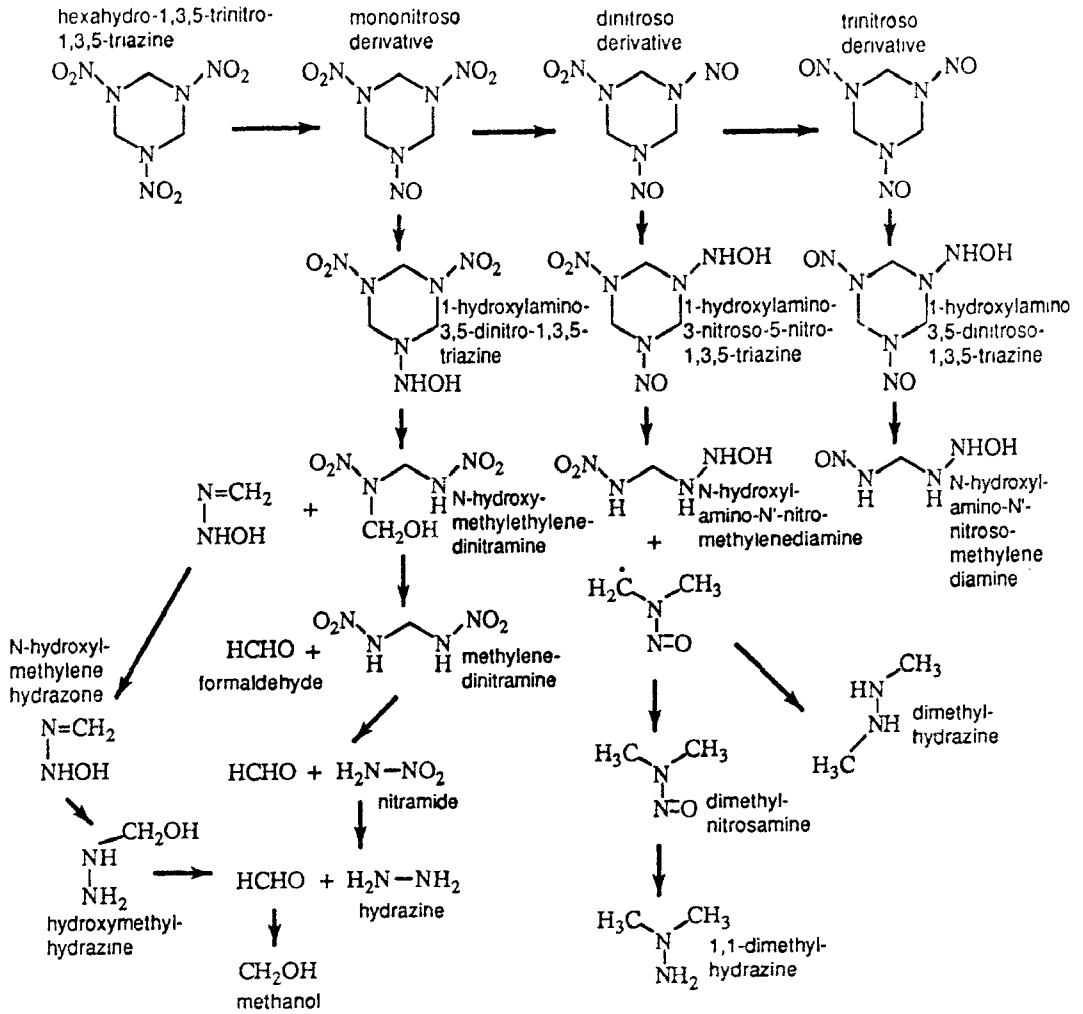

Fig. 6. Anaerobic biomineralization pathway for RDX.

biotransformation of the AcHMX were slower than for HMX, and two isomers of dinitroso-AcHMX were detected. No hydrazine, 1,1-dimethylhydrazine, or 1,2-dimethylhydrazine were detected during the biotransformation of HMX or AcHMX. HMX and AcHMX degraded at a slower rate in continuous culture under anaerobic denitrification conditions than in the batch studies and none of the intermediates were detected. No biotransformation of HMX or AcHMX occured under aerobic conditions.

Nitroguanidine

We have shown that in aqueous systems, nitroguanidine was biotransformed under anaerobic conditions in the presence of cometabolic carbon (Fig. 8) (Kaplan et al. 1982b, 1985b). Biotransformation of nitroguanidine to nitrosoguanidine was observed with subsequent nonbiologically-mediated formation of the intermediates cyanamide, nitrosamide, cyanoguanidine, melamine, and guanidine. Melamine is the trimer-cyanamide product which can also react with ammonia to form guanidine. The nitrosamide is transitory and decomposes to nitrogen gas and water. The cometabolic degradation of nitroguanidine at 150 mg/l in soil was also demon-

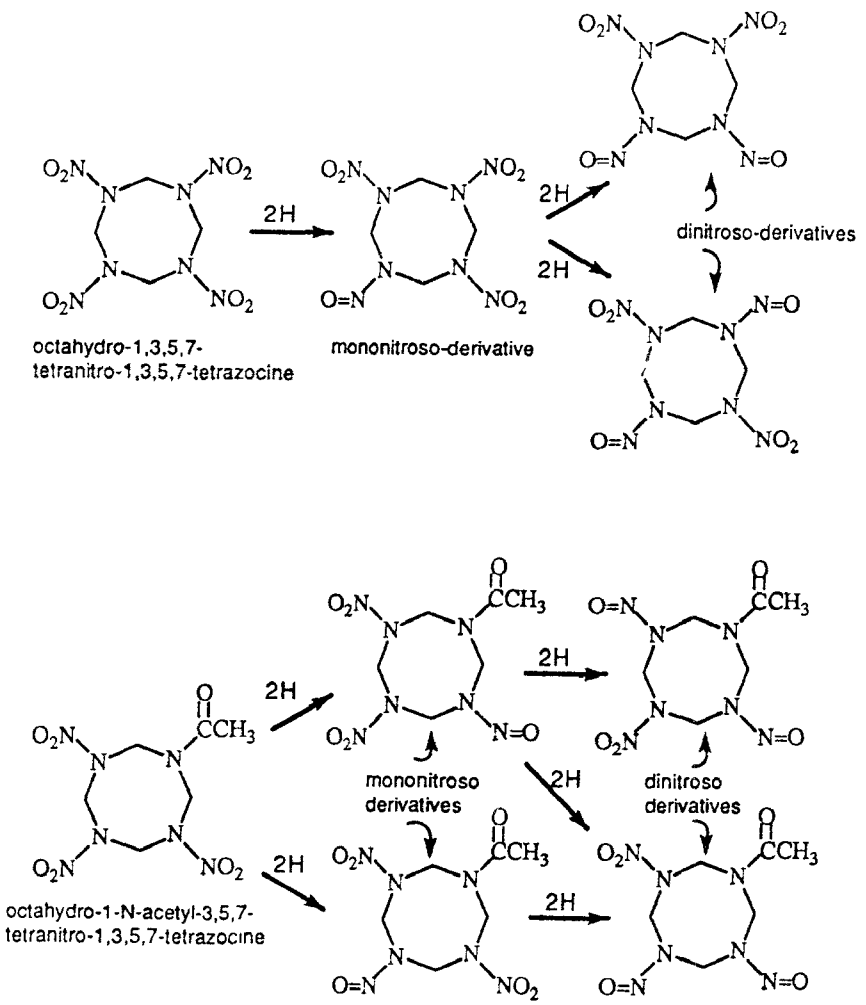

Fig. 7. Anaerobic biotransformation of HMX and acetylated-HMX.

strated (Kaplan and Kaplan 1985). Traces of nitrosoguanidine (< 100 μg/l) were detected in the leachates from the soil, and ammonia was the primary nitrogen species formed. Supplemental carbon, added in the form of glucose, was required for this transformation to occur. The concentration of glucose required for complete mineralization of the nitroguanidine ranged between 1.0 and 0.5%. The ammonia concentration detected in leachates from the soil columns correlated directly with the degradation kinetics of nitroguanidine. Nitrate and nitrite concentrations remained at or below the mg/l range throughout the study. Mass balance studies show an 85% conversion of the nitroguanidine-nitrogen to ammonium-nitrogen in these soil studies.

Chemical agents

Chemical agents are found as enviromental contaminants, particularly in soils, due to past production practices. In addition, international treaties will require the destruction of large quantities of these compounds. Environmentally compatible and cost effective approaches will be required to deal with these problems and a biological approach is one alternative being actively explored. Chemical agents can be divided into the organophosphate

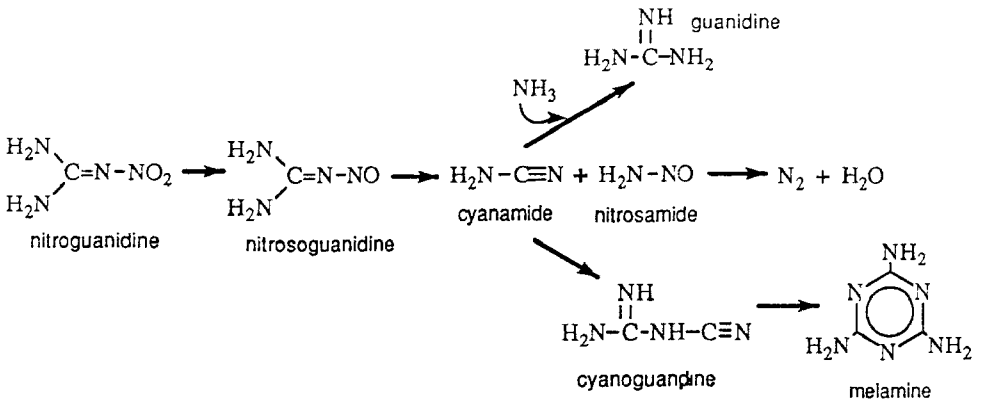

Fig. 8. Biotransformation pathway for nitroguanidine in aqueous systems.

neurotoxins [sarin, VX (malathion-type), paraoxon and parathion-type (P-F) bonds)], and mustards [HT (N-mustard) and HD (S-mustard)]. The mustards can be defined as organic compounds containing at least two chloroethyl groups ($-CH_2CH_2Cl$) linked to either a thioether or amine. The toxicity of mustards is due to alkylating effects on DNA and two chloroethyl groups are required for the toxicity. The toxicity of the organophosphates derives from their interference with neurotransmitter biochemistry such as inhibition of acetylcholinesterase activity.

Organophosphorous hydrolases (OPH)

Organophosphorous hydrolases (OPH) [EC 3.1.8.1] previously termed organophosphorous acid anhydrases or OPA anhydrases, DFPases, somanases, sarinases, paroxonases, parathion hydrolase,and phosphotriesterase have been identified in bacteria (mesophilic, thermophilic and halophilic), protozoa, invertebrates and mammals. Mazur (1946) first described a P-F splitting enzyme that he termed DFPase. Over the following decade a number of papers appeared on the general topic of the DFPases and the developments of this period have been summarized by Mounter (1963). In 1965, as an unexpected result of bathing squid giant axons in diisopropylfluorophosphate (DFP) solutions, squid nerve and indeed cephalopod nerve in general was found to have a high level of DFPase by Hoskin (1966) and coworkers. The term 'squid-type DFPase' was introduced by Garden et al. (1975) to denote that the Hoskin enzyme hydrolyzed DFP more rapidly than it hydrolyzed tabun (ethyl N,N-dimethyl-phosphoramidocyanidate) or sarin (isopropylmethylphosphonofluoridate). The squid enzyme was shown to have a molecular weight close to 26 kDa. Hoskin (1982) was able to demonstrate that the squid type DFPase has a degree of stereospecificity with respect to the phosphorous chiral center of Soman (1,2,2-trimethylpropyl methylphosphonofluoridate), that is, the enzyme preferentially hydrolyzed the toxic enantiometers of soman.

OPHs are especially plentiful in mammalian kidney and liver, and are capable of hydrolyzing DFP, tabun and sarin. Hoskin (1984) stated that there appears to be two distinctly different classes of OPH. The first class is of the 'Mazur' type OPH. They hydrolyze Soman 10 to 20 times faster than DFP, are stimulated three to five fold by manganous ion , are inhibited by Mipafox (N,N-diisopropylphosphodiamidofluoridate), and are inactivated by ammonium sulfate. Mazur type OPH have molecular weights in the range of 50 to 100 kda. This class includes OPH from mammalian tissues as well as from bacteria. The second class is called 'Squid' type OPH. It is a much narrower class than the Mazur type and includes not only squid but cephalopods in general. Unlike the Mazur type, they hydrolyze DFP five times faster than soman,

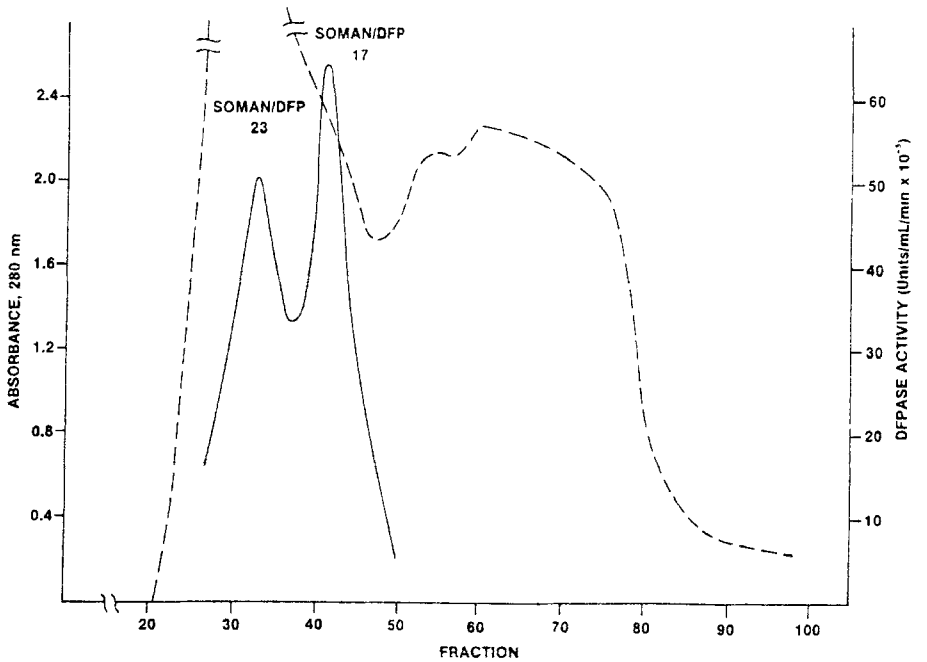

Fig. 9. Chromatogram of *E. coli* extract on g-150 column. (---) protein; (——) activity.

are indifferent to Mipafox, and tolerate ammonium sulfate precipitation as a first purification step.

Escherichia coli *OPH*

Zech & Wigand (1975) were the first investigators to report that a cell extract of *E. coli* exhibited two chromatographically separable forms that could hydrolyze DFP. Walker et al. (1986) showed that these fractions had a DFP/Soman activity ratio of 23 and 17, respectively. Using gel filtration (Fig. 9) and ion exhange chromatography on DEAE

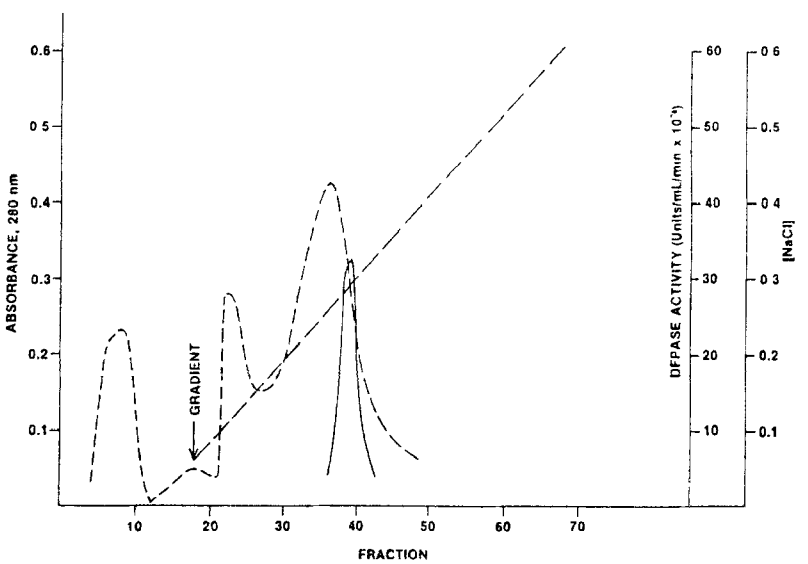

Fig. 10. A-50 chromatogram of *E. coli* fraction-1. (---) protein; (——) activity.

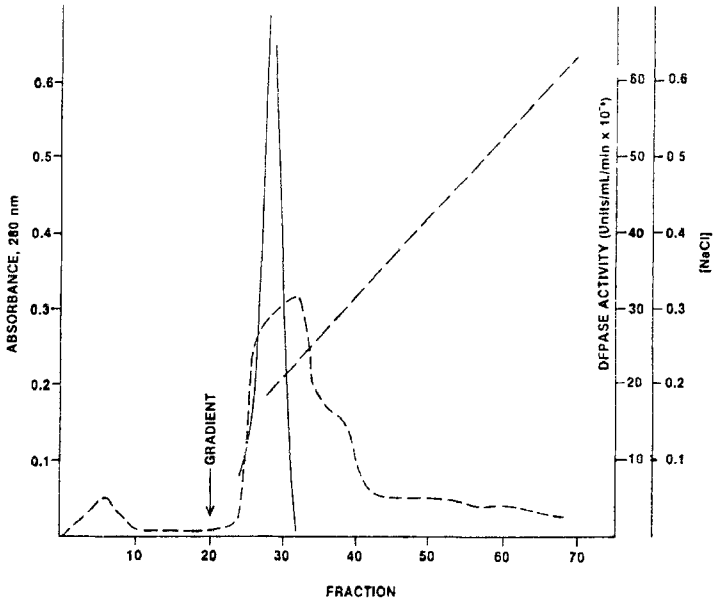

Fig. 11. A-50 chromatogram of *E. coli* fraction-2. (---) protein; (——) activity.

A-50 (Pharmacia) (Figs. 10 and 11), two active fractions were isolated.

SDS gel electrophoresis of the DEAE separated OPHs gave monomer molecular weights of 50 and 40 kDa respectively (Fig. 12). Recently Hoskin et al. (1993) reported that *E. coli* OPHs react differentially in their ability to detoxify the toxic and nontoxic pairs of soman isomers. One form hydrolyzes and detoxifies all the isomers equally, while the other preferentially hydrolyzes the nontoxic pair initially (Table 1). Akkara & Kaplan (1991) have immobilized the *E. coli* enzyme to cotton fabric and showed that it is reactive against DFP in solution (Fig. 13). These authors have shown that the immobilized *E. coli* enzyme can react against soman in the vapor state (Fig. 14).

Table 1. Enzymatic degradation of soman by *E. coli* fractions determined by two methods.

Fraction	Hydrolysis by F-electrode (%)	Detoxication by AChE inhibition (%)
G-150-I	50	48
G-150-II	50	42
A-50-(G-150-I)	50	49
A-50-(G-150-II)	50	18

Bacillus stearothermophilus *OPH*

Walker et al. (1988) isolated an enzyme from a strain of *B. stearothermophilus* (JD-100) that possesses no hydrolyzing activity against DFP but hydrolyzes and detoxifies both the toxic and non toxic isomers of soman at the same rate (Table 2). The enzyme has a molecular weight close to 80 kDa as determined by HPLC on a calibrated TSK-400 (Bio-Rad) column and by SDS gel electrophoresis, and has monomer molecular weights of 52, 40, and 36 kDa (Fig. 15). The characteristics of the enzyme are given in Table 3. The enzyme has been immobilized by covalent attachment onto cotton and hydrolyzes soman in solution (Fig. 16). On-going work is proceeding to test the immobilized thermophilic enzyme against soman vapor. The enzyme appears to retain its activity in short term heat stability tests (Table 4).

Tetrahymena thermophilia *OPH*

Landis et al. (1987) reported that *T. thermophilia* synthesizes an intracellular OPH capable of degrading DFP. Gallo et al. (1991) isolated two enzymes from *T. thermophilia* bryant which has high

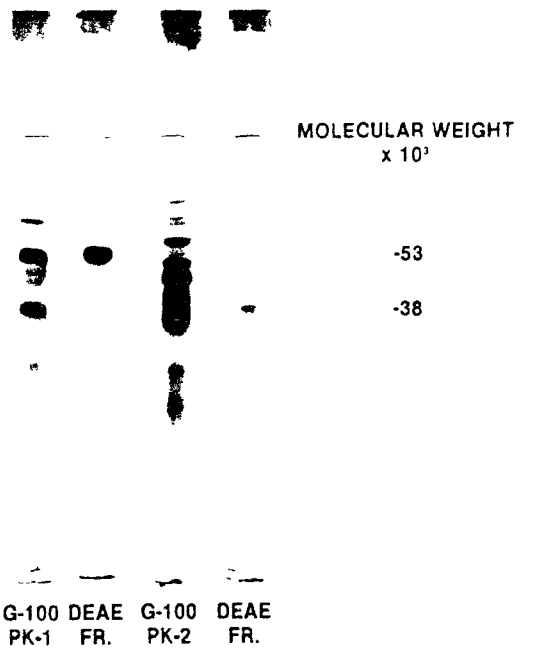

Fig. 12. SDS gel electrophoresis of A-50 fractions of *E. coli*.

OPH activity when compared to both the OPH from *B. stearothermophilus* and *E. coli* (Table 5). The enzyme could be separated into two forms by hydrophobic chromatography on phenyl sepharose (Pharmacia). Both chromatographically separated forms hydrolyzed and detoxified all isomers of soman (Table 6). SDS gel electrophoresis of the enzyme gives a molecular weight of 45 kDa.

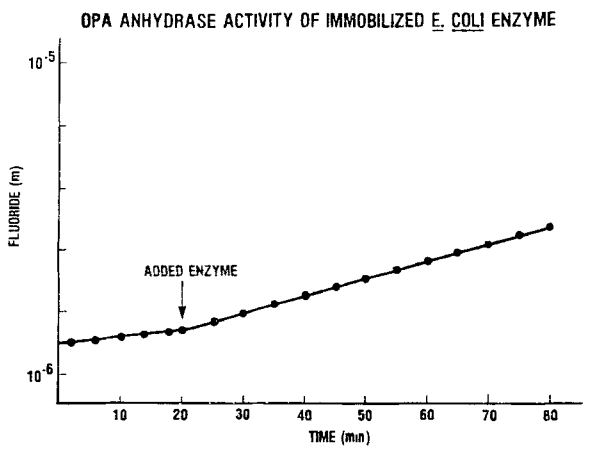

Fig. 13. Activity of immobilized *E. coli* OPH against soman in solution.

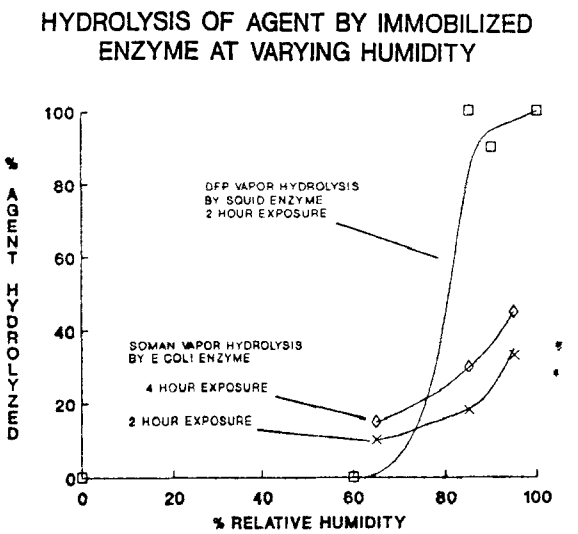

Fig. 14. Activity of immobilized *E. coli* OPH against soman vapor.

Squid OPH

The squid OPH has received the most attention as a potential enzymatic decon material against G-type agents. Extensive stability studies by the US Navy under contract with IIT research Institute (Rajan et al. 1989) have shown that the enzyme retains its activity as a lyophilate after two years storage at 4 °C. A phosphate buffered solution of the enzyme (100 mg/ml) can decontaminate 200–250 mg of soman/square centimeter of material surface. The enzyme retains >96% of its initial activity after each decontamination experiment. Walker et al. (1990) have studied the secondary and tertiary structure of the enzyme (Table 7). The OPH from squid has a calcium ion requirement for expression of activity. Circular di-

Table 2. Relative rates of hydrolysis of soman by immobilized DFPases from various sources.

Enzyme source	Racemic	Rp (AChE inhib)	Sp
Hog Kidney	100	8.6	91.4
E. coli	100	0.0	100.0
Squid	100	44.1	55.9
JD-100	100	38.4	61.1

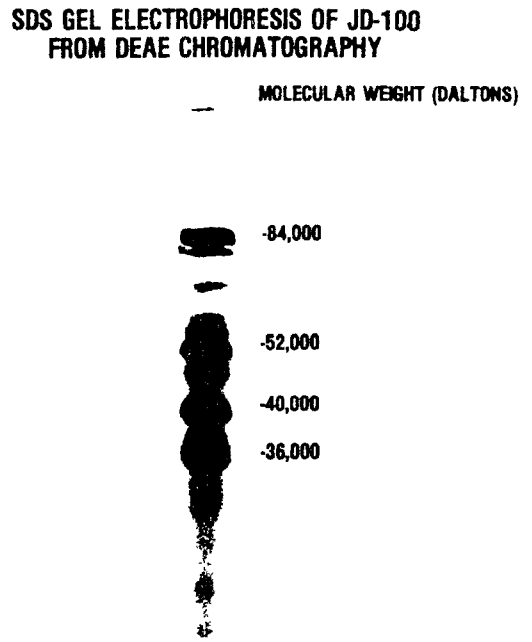

Fig. 15. SDS gel electrophoresis of *B. stearothermophilia* OPH.

chroism spectra as well as fluorescence life time studies of tryptophan residues, indicate that the enzyme undergoes a structural change in the presence of calcium. On-going studies are focusing on the sequence of amino acids in the active site region and the binding sites for calcium.

Biodegradation of mustard

A number of possible routes for the biodegradation of mustard have been considered, including dehalogenation, reduction and oxidation. Since mustard (dichlorodiethyl sulfide) is a strong DNA alkylating agent, it was expected to be lethal to bacteria and therefore isolation of microorganisms capable of degrading mustard has been unsuccessful. Chemostats were recently used to select for mustard degraders in a mineral salts medium with thiodiglycol as a carbon source (Harvey et al. 1991). Post selection, the thiodiglycol could be used as a sole carbon source at 10 mM. *Pseudomonas picketii* and *Alcaligenes xylosoxidans* sp. have been putatively identified as the responsible organisms. The organisms can grow on thiodiglycol, thiodiglycol sulfoxide and thioether compounds, producing carbon dioxide and biomass. The mustard is in equilibrium with the thiodiglycol and sulfonium ion intermediate in the mineral salts medium.

Table 3. Characterization of JD-100.

Molecular weight:	80,000 Daltons
Oligomeric enzyme:	Subunits; 52,000 40,000 and 36,000 Daltons
Isoelectric point:	4.0
Detoxifies all isomers of Soman	
Not inhibited by Mipafox	
Manganous ion requirement	

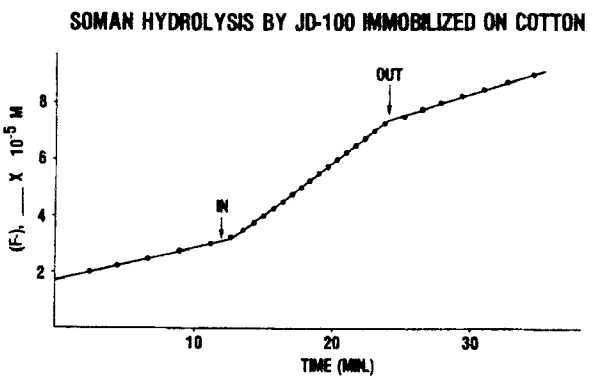

Fig. 16. Activity of immobilized *B. stearothermophilia* OPH against soman in solution.

Discussion

In general, many of the explosives/propellants and

Table 4. Stability of JD-100.

	Activity (Units/mL)	% Loss of activity
Initial		
Solution	0.60	–
Freeze dried	0.60	–
23 °C for 2 weeks		
Solution	0.18	60%
Freeze dried	0.54	10%
55 °C for 2 weeks		
Solution	0.45	25%
Freeze dried	0.66	0.0%
4 °C for 2 weeks		
Solution	0.51	15%
Freeze dried	0.60	0.0%

Table 5. Specific activity of partially purified OPA anhydrase from 3 diverse microbial strains.[a]

Microbe type	Species	Strain	Specific activity[b]
Protozoan	*Tetrahymena thermophilia*	Brynat	0.73
Bacterium	*Baccilus stearothermophilus*	I5D	0.13
Bacterium	*Escherichia coli*	ATCC 25922	0.06

[a] Partially purified OPA anhydrase resulting from Sephadex G150 and then Phenyl-Sepharose CL-4B columns chromatography.
[b] uM PNMPP hydrolyzed/min*mg^{-1} soluble protein.

chemical agents are amenable to biodegradation. The activity of biological systems on these compounds can lead to products that represent reduced toxicity hazards when compared with the parent compound, and in many cases can lead to mineralization to return the carbon, hydrogen and oxygen to natural geochemical cycles. This can be the scenario in many instances provided the appropriate conditions can be maintained. In many cases, the reaction kinetics and the products formed in these reactions depend on the conditions under which the target compound is studied. Pathways outlined in this chapter for explosives/propellants and chemical agents form only a starting point, to indicate that a potential exists to treat the compound in a biological system.

It would be easy to generalize the pathways and reactions described. However caution must be used in the careful assessement of each compound and the conditions under which it is to be studied. In most cases, biological approaches to the degradation or biotransformation of these types of compounds can offer significant benefits such as the low process costs and the formation of less toxic compounds. Often, chemical pretreatments in combination with the biological system, as in the case of the alkaline pretreatment of nitrocellulose or glycerol trinitrate and the hydrolysis of mustard are useful and perhaps in some cases essential steps, to accelerate the follow-on biological process.

Table 6. Enzymatic hydrolysis and detoxification of soman by two Phenyl-Sepharose CL-4B OPA anhydrase fractions from *Tetrahymena thermophilia.*

Activity peak	% Soman hydrolysis[a]	% Soman detoxification[b]
Fraction F-1	50	39
Fraction F-2	50	59

[a] Soman hydrolysis was determined by measuring the release of fluoride ions by a fluoride specific electrode.
[b] Soman detoxification was determined by measuring the degree of inhibition of acetylcholinesterase by soman (Conducted at IIT).

Table 7. Physico-chemical properties of squid OPA anhydrase.

Narrow distribution; Squid nerve, saliva, hepatopancreas
Molecular weight; 26,000 Daltons
Isoelectric point; 5.5
Soman/DFP = 0.25
Km = 2.5×10^{-3} M
Hydrolyses all isomers of soman
Ca^{+2} requiring, not Ca^{+2} stimulated. Ca^{+2} may be involved in intramolecular bonding
Mipafox indifferent
Em 280 = 38,500
Disulfide bridges not essential for enzyme activity
2 of the 6 Trp residues are exposed to the microenvironment

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