Bioremediation of Explosive Contaminants

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Abstract

This paper discusses several methods currently available for the treatment of nitramine-type explosive contaminants in soil environments as well give a brief description of several research projects that were recently conducted in this area. The review begins by first discussing where RDX, HMX, TNT and CL-20 contaminants come from and what avenues through which they can travel to enter the environment surrounding the ammunition production plant. It then goes on to discuss the pathways involved in the treatment of the aforementioned compounds (two electron pathways, denitration, anaerobic and aerobic biodegradation). After providing the reader with this information the paper then discusses an experiment that investigated how to best enhance the aerobic and anaerobic biodegradation of RDX, HMX and TNT. Due to the recent surge in CL-20 production by the military and subsequent increase in CL-20 contamination a synopsis of another experiment is reviewed regarding how to best study the various techniques that are used to remediate this compound.

Keywords: Nitramine, Explosive, Biodegradation, HMX, TNT, RDX, CL-20

Introduction

Near the end of the nineteenth century many compounds were created through the process of nitration. Several of these nitramine compounds were believed to contain practical industrial and military related applications. As the twentieth century progressed through World War I and World War II the synthesis of compounds like 2,4,6-Trinitrotoululene (TNT) and 2,4,6-Trinitrophenol (picric acid) increased exponentially. By the year 1945 it is estimated that a nitramine production line in the United States of America was able to produce approximately 65 tons of TNT every day, leading to approximately 1.2 million tons of soil being contaminated by the waste generated during compound production (Lewes et.al, 2004). Since World War II this high rate of nitroaromatic compound production has continued to the point that the waste generated, which contains traces of the explosive compound produced, is now of major concern to public health. Due to the substantial amount of remediation that must be performed on soil that is contaminated by explosive waste many methods of treatment are being investigated for their respective economical feasibility. Due to the relatively low cost associated with bioremediation, this is the primary technique being analyzed for the remediation of such contaminants. As mentioned before TNT and Picric acid are both explosive compounds commonly found in waste produced by explosive synthesis. Other nitramine compounds include: RDX, HMX, 2, 4, DNT, Tetryl, and CL-20 (Lewes et.al, 2003). This report will summarize several methods used to treat these compounds and describe several recent studies performed in this area. First this paper will discuss how these compounds enter the environment.

How Explosive Compounds Enter the Environment

Many of the compounds listed in the introduction enter the environment suspended in wastewater that leaves the plant after being used to wash the explosive devices. This process, called hydromining, is when hot water is sprayed on the munitions device with the intent of removing the explosive chemicals. Often this “pink water” (named for the color the TNT generates when it is suspended in a fluid) flows to a tank or lagoon, here the TNT is allowed to settle out of the solution. If this process is not allowed to run to completion, or some other problem arises during the explosive chemical removal process then the compound is often allowed to flow to the environment outside the plant where it enters and contaminates the surrounding soil (Lewes et.al, 2004). The next topic in this paper will discuss the primary methods of treatment these compounds undergo.

Treatment of RDX and HMX

Many compounds have been found to reduce RDX and HMX to compounds that are less harmful to the environment. Among these compounds include: aerobic bacteria, anaerobic bacteria and several types of fungi. Due to the readily available sources of RDX and HMX treatment and the fact that these methods can be implemented both in-situ and ex-situ, it is believed that these compounds can be treated in a thorough and economically efficient manner (Crocker et. al, 2006). The biodegradation of RDX compounds will be discussed first.

Biodegradation of RDX

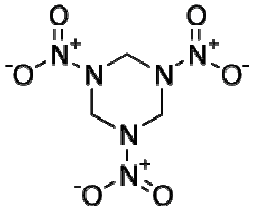


Figure 1 RDX Molecular Structure (Crocker et al., 2006)

Cyclotrimethylenetrinitramine (RDX) is a stable, white crystalline compound commonly used in military explosives. RDX has also been found to be readily degradable, in-situ in a variety of environments including: subsurface soils, aquifers and cold marine sediments. In its degrading process RDX can act as the carbon electron donor in aerobic, anaerobic, nitrate/sulfate/manganese reducing environmental conditions. In this role there are currently three proposed mechanisms for the degradation of this molecule: two-electron reduction, single-electron reduction/denitration (Crocker et al 2006).

Two-Electron Reductive Pathway

In the two-electron reductive pathway RDX is broken down into mono, di and tri-nitroso–RDX as intermediates. These intermediates then breakdown into the following end products of the two-electron reductive pathway: ethanol and formaldehyde. The initiation of this pathway is believed to be caused by *Enterobacteria* using the enzyme: type I nitro-reductase. Recent work by Kitts has also shown that a plasmid in the bacteria *Eschericia Coli* can express the use of this enzyme leading researchers to believe that *E. coli* can be used for the in-situ biodegradation of RDX (Crocker et al 2006).

Another two-electron reductive pathway that has been proposed by researchers is called McCormick's pathway. McCormick's pathway relies on *Clostridium Acetylbutilecum* to transform RDX to MNX, however instead of proceeding in the same fashion as the nitroreductase enzyme, the *C. Acetylbutilecum* transforms MNX into hydroxyl amino and finally 1,3,5-triamino-1,3,5-triazine. This method also differs from the nitroreductase method in that throughout this series of reactions no ring cleavage is believed to occur (Crocker et al 2006).

A third route of the two-electron reductive pathway includes the use of *Aspirgillus Niger*. This bacteria uses nitrate oxireductase to reduce the RDX compound to: ammonium, nitrous oxide and formaldehyde. This method, like the two-electron reductive pathway involving *C. Acetylbutilecum* has MNX intermediate. However it should be noted that in this pathway MNX is not followed by the creation of DNX or TNX (Crocker et al 2006).

Researchers agree that there is still much conjecture about the intermediates involved with this pathway and the order of which the reactions occur. Therefore many researchers have recommended the continuing study of the two-electron reductive pathway using in-vivo experiments with whole cells as locations for the enzymatic reactions occur (Crocker et al 2006).

Single-Electron Reductive Pathway/Denitration

The second pathway that RDX can undergo during degradation is the single electron reductive pathway or denitration. Denitration is the formation of a free-radical molecule through the addition of a single electron, which results in the loss of a nitro group from the molecule. According to researchers this is believed to be the primary route of biodegradation undergone by RDX due to the readily created ideal thermodynamic conditions under which the mechanism occurs. Denitration also is the most common degradation route of RDX because it occurs under both aerobic and anaerobic conditions. The first method discussed in this paper will be the anaerobic biodegradation of RDX (Crocker et al 2006).

The anaerobic denitration of RDX is performed by *K. pneumonia* and *C. bifermentans*. Anaerobic denitration occurs when type II nitroreductase adds an electron to an RDX molecule forming RDX^{-}. RDX^{-} then quickly loses a nitro group due to the unstable nature of the molecule.

Once this happens the newly formed ring will destabilize which will ultimately cause the formation of water, nitrous oxide and formaldehyde. Another advantage of using *K. pneumonia* and *C. bifermentans* for this process is that the minor pathway of these organisms is the biodegradation of MNX after it separates itself from the RDX^- allowing for the minimization of environmentally harmful end products (Crocker et al 2006).

The strains of bacteria that use the aerobic mechanism of denitration to biodegrade RDX are: *Rhodococcus rhodochrous, Williamsia and Gordonia*. The primary difference between the mechanism that these bacterium use and the anaerobic bacterium is that under the anaerobic condition, the denitration of RDX occurs by going through a two step process of single electron reduction prior to cleavage of the nitrogen-carbon ring. The final products of this two step process are the creation of: 4-nitro-2, 4-diazabutanal (NBAD), nitrous oxide, ammonium, formaldehyde and carbon dioxide. The NBAD produced by the mechanism can easily be broken down by methylotrophic bacterium and white-rot fungus, for this reason the anaerobic biodegradation of RDX remains a feasible option. (Crocker et al 2006).

The following figure shows an overview of all the possible mechanisms for the biodegradation of RDX that have been discussed in this paper. It should be noted that the mechanisms with dashed lines are hypothetical intermediate processes postulated by researchers in the field.

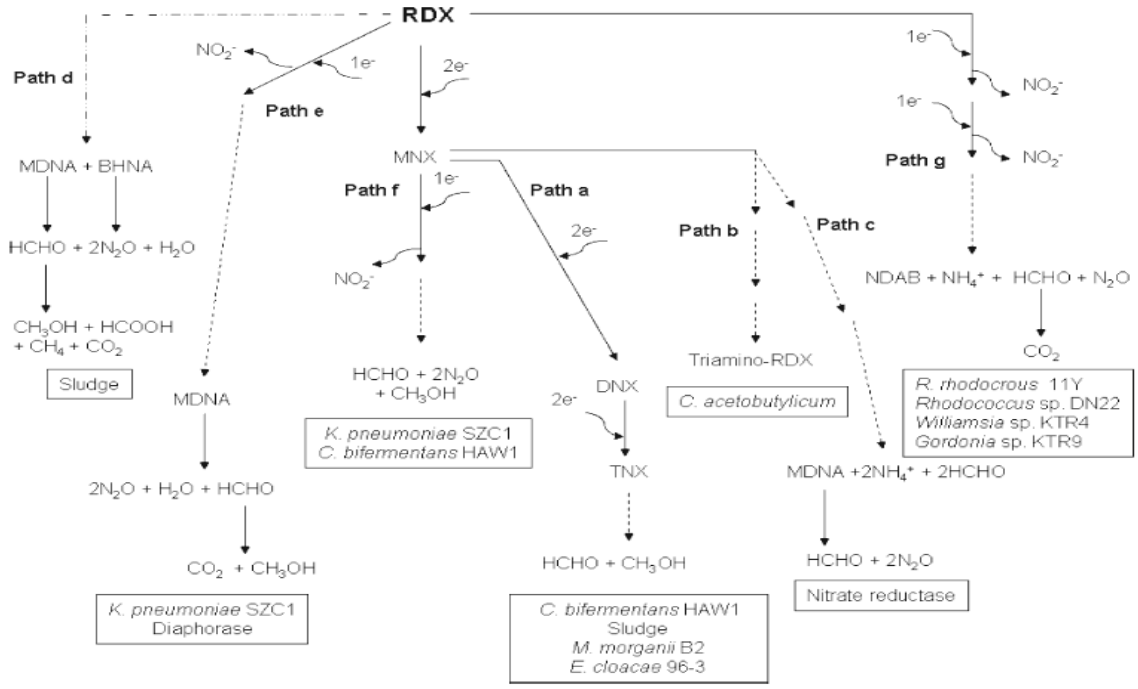


Figure 2 Overview of RDX Biodegradation Pathways (Crocker et al., 2006)

Biodegradation of HMX

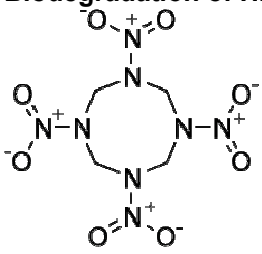


Figure 3 HMX Molecular Structure (Crocker et al., 2006)

Due to their similar molecular structure it is not surprising that HMX and RDX have similar mechanisms of biodegradation. Because of this all mechanisms that biodegrade RDX have also been proven to degrade HMX into simpler molecules. There are however several methods of degradation of HMX that are unique to this molecule. These methods are discussed below (Crocker et al 2006).

Aerobic and Anaerobic Biodegradation of HMX

Researchers have shown that several species of *Methylobacterium* are able to aerobically cometabolize HMX with

carbon dioxide at a ratio of 100:61, despite the fact up until the publishing of these findings researchers were reluctant to subscribe to the idea that HMX could be aerobically biodegraded (Van Aken et al., 2004). As mentioned above the anaerobic pathway for the biodegradation of HMX is also similar to that of RDX. It involves the reduction of two nitro groups from the carbon-nitrogen ring. This destabilizes the HMX molecule causing cleavage and breakdown of the compound. It should be noted however that the anaerobic McCormick pathway that functions as a method of anaerobic biodegradation of RDX has not been found to apply to the HMX molecule. The pathways discussed in this section of the paper for the degradation of the HMX molecule are summarized in the figure below (Crocker et al 2006).

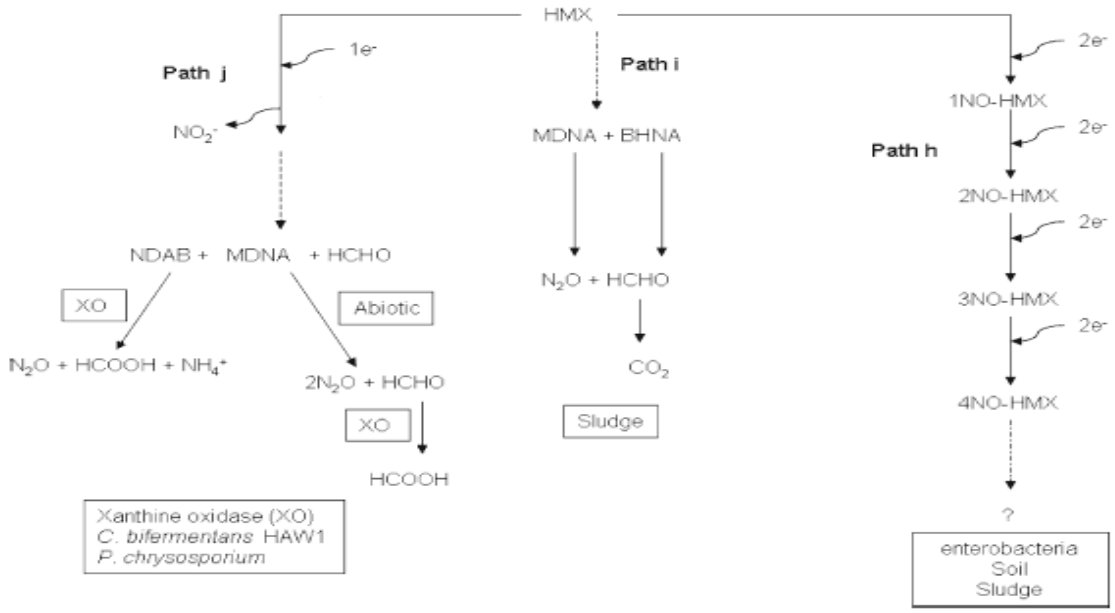


Figure 4 Overview of HMX Biodegradation Pathways (Crocker Et Al 2006)

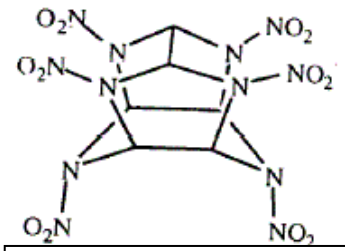


Figure 5 CL-20 Molecular Structure (Crocker et al., 2006)

Biodegradation of CL-20

Hexanitrohexaazaisowurtzitane (CL-20) is a recently developed nitramine explosive that is believed to be approximately 20% more powerful an explosive than HMX. The methods of biodegradation are discussed below.

Aerobic/Anaerobic Biodegradation of CL-20

CL-20 has been found to be degradable in both surface and subsurface soil stratum. *Clostridium* is responsible for the anaerobic utilization CL-20 (as well as RDX and HMX) as a nitrogen source for cell growth. While it is possible to biodegrade CL-20, at low concentrations (essentially unsaturated condition) bacterium who metabolize CL-20 see little to no aerobic degradation. At sufficient concentrations however it has been found that white rot fungus appears to be responsible for the mineralization of CL-20. The fact that fungi are responsible for the breakdown of CL-20 also pays heed to the observation made by researchers that the rate of biodegradation increased with the addition of starches and carbohydrates to the soil where degradation is taking place. There have been several degradation mechanisms proposed by researchers on this topic. The first pathway is similar to the ones discussed in the RDX and HMX sections of this paper in that it involves the denitration of the molecule in question. Cleavage of the C-N bonds of the CL-20 molecule occurs after two successive reductions of the molecule causing two successive removals of nitro groups. As discussed before, this removal of the nitro groups causes the destabilization of the C-N bonds causing the breakdown of the CL-20 molecule to two intermediates and ultimately acetic acid, glyoxal and nitrous oxide. Two other proposed mechanisms occur when CL-20 is in the presence of *Clostridium* and are both catalyzed by the dehydrogenase enzyme. This mechanism results in

the production of acetic acid, glyoxal, nitrous oxide and nitrogen dioxide. The final method of CL-20 biodegradation occurs when CL-20 is located in an environment where white rot fungus can flourish where the said fungus mineralizes the compound and thus removing the threatening contaminant from the environment. The first three mechanisms described above are summarized in the figure located below (Crocker et al 2006).

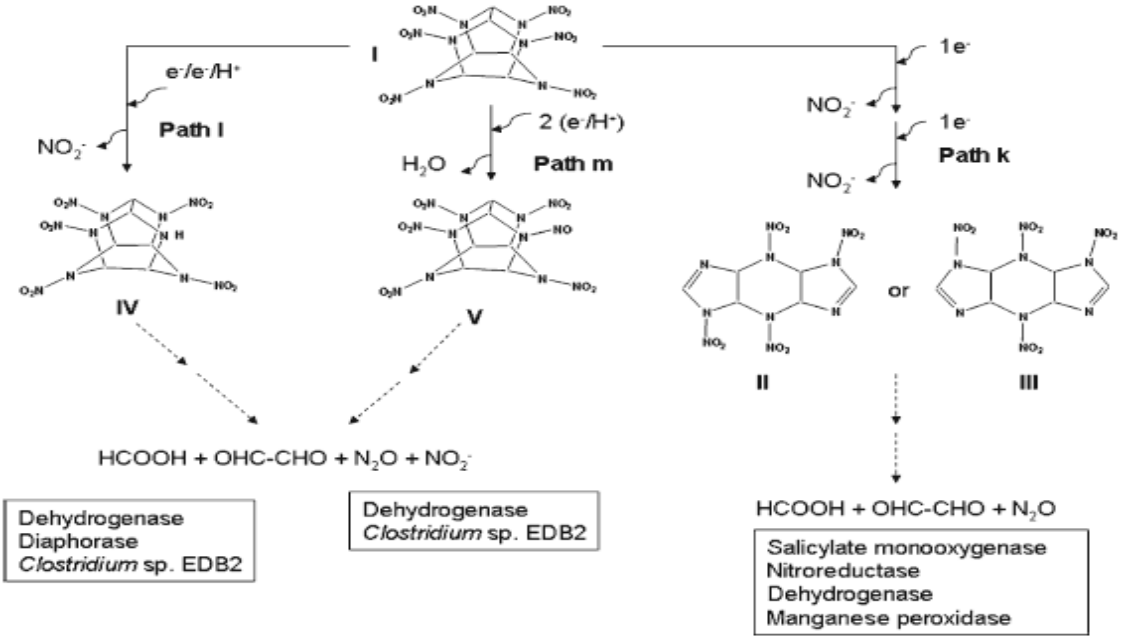


Figure 6: Overview of CL-20 Biodegradation Pathways (Crocker et al, 2006)

Recent Developments in the Field of Nitramine Biodegradation

Now that the primary degradation methods of the four common nitramines have been discussed, recent developments made by researchers in how the treatment methods of RDX, HMX, TNT and CL-20 can be enhanced for more the efficient breakdown of the molecules will be analyzed.

Enhancing the Anaerobic Biodegradation of RDX, HMX and TNT

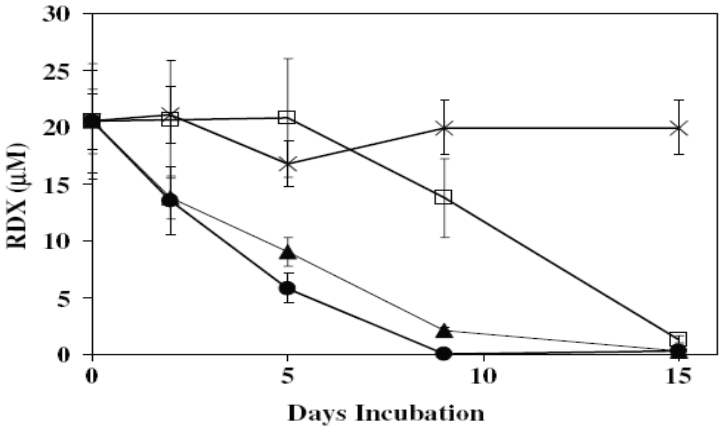
As mentioned before the anaerobic breakdown of nitramines is the most common method of nitramine degradation. This process is initiated when a single electron is donated to the nitramine causing a destabilization of the C-N ring ultimately resulting in the cleavage of the ring and simplification of the nitramine in question (Crocker et al., 2006). Currently the limiting factor in the anaerobic biodegradation process is the availability of a substrate for the microbes to attach and grow on as the explosive molecule itself is currently unable to fulfill this responsibility. Because of this a nutrient must be added to the soil. Historically a starch has been supplied to the contaminated environments to successfully facilitate the transformation of TNT, HMX and RDX. The problem that has been encountered when introducing this starch into the environment is that there are many other heterotrophic microorganisms that also utilize starches for cellular growth. This means that additional substrate will need to be added to the injection point of the contaminants to ensure that the bacteria that promote nitramine transformation can obtain sufficient resources to initiate the process. Another problem caused by flooding substrate into the ecosystem is that block forming of the heterotrophic biomass in the area can occur due to the sudden availability of resources. This increase in concentration of compounds will eventually increase the volume of substrate that must be added at the point of injection, resulting in a higher cost for the nitramine transformation process. Because of this much research is being done to find an alternate electron donor that can be introduced to the environment without the negative consequences described above to develop (Adrian et al., 2006).

Recent research has shown that the addition of reduced electron donors along with a methanogenic bacterium can result in increased biodegradation of nitramine compounds (Adrian et al., 2003). This research was performed by introducing 1, 2-propanediol (propylene glycol) or ethanol with the bacterium *Acetobacterium malicum* to a nitramine culture. The theory behind this research was that the addition of propylene glycol to a culture of bacterium would initialize the metabolism of this compound. Once metabolized the bacterium releases hydrogen gas into the surrounding environment which can then be readily used by explosive degrading organisms as the electron donor in the anaerobic transformation of nitramines (Adrian et al., 2006).

As there are several nitramines to be transformed through an anaerobic process the change in concentration of RDX, HMX and TNT were all measured. The transformation effectiveness for all three molecules were analyzed under four different conditions:

- Addition of Propylene Glycol
- Addition of Ethanol
- No Electron Donor Added (Control
- Sterile Control

Though the addition of propylene glycol and ethanol did increase the rate of RDX transformation, by the end of the incubation period (15 days) the concentration of RDX in the propylene glycol and ethanol specimens was the same as that in the condition that did not experience any addition of electron donating molecules. This finding lead researchers to the conclusion that there are currently enough electron donors available in the environment to allow RDX reducing microbes to transform all RDX contaminants to a less threatening state without any addition of reaction facilitating molecules necessary. The results of this experiment are summarized in the figure below (Adrian et al., 2006).

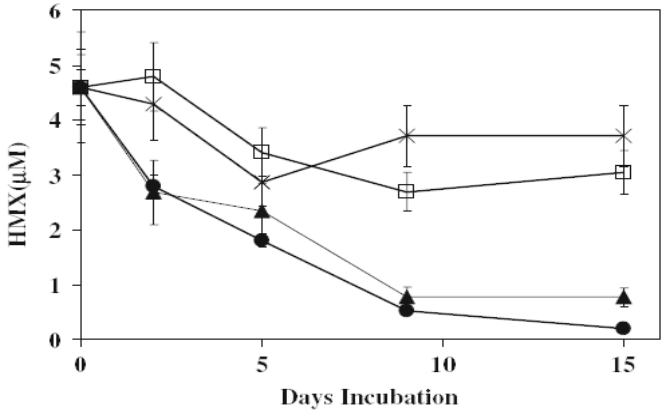


. Biodegradation of hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) in anoxic serum bottles containing 50 g explosive-contaminated soil and 75 ml of groundwater. The serum bottles were amended with ethanol or propylene glycol on day zero to a final concentration of ~6 mM. Values are the means of three replicates ±standard deviations. Error bars are not shown for standard deviations that are less than 10% of the value of the point. Symbols: ethanol amended, ●; propylene glycol amended, ▲; electron donor unamended control, □; sterile control, ∗.

Figure 7: Biodegradation of RDX Using Propylene Glycol, Ethanol (Adrian et al., 2006)

Due to the increased stability of the HMX molecule, it was determined that there did not exist enough electron donating strength in the contaminated environment surrounding molecules to initiate its transformation. Because of this it was concluded that some breakdown amending molecule must be added in situ to the surrounding environment for the reduction of the HMX molecule to occur. While it was concluded that some amending substrate must be added to the solution, the concentrations of HMX at the end of the incubation period were very similar under both the propylene glycol and ethanol, leading researchers to conclude that one is not more

effective than the other. The results obtained from the transformation of HMX using ethanol or propylene glycol experiments are summarized in the figure at the top of the following page (Adrian et al., 2006).

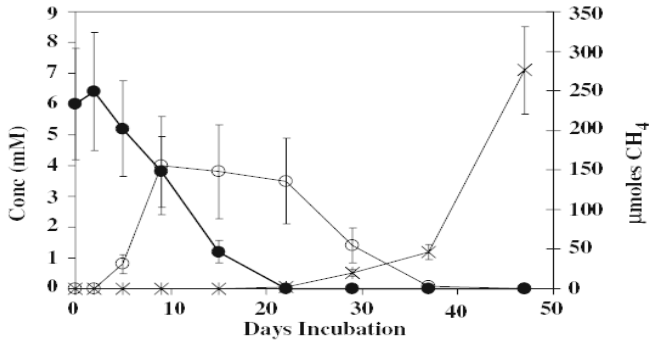


2. Biodegradation of octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine (HMX) in anoxic serum bottles containing 50 g explosive-contaminated soil and 75 ml of groundwater. The serum bottles were amended with ethanol or propylene glycol to a final concentration of ~6 mM. Values are the means of three replicates ±standard deviations. Error bars are not shown for standard deviations that are less than 10% of the value of the point. Symbols: ethanol amended, ●; propylene glycol amended, ▲; electron donor unamended control, □; sterile control, ∗.

Figure 8: Biodegradation of HMX Using Propylene Glycol and Ethanol (Adrian et al., 2006)

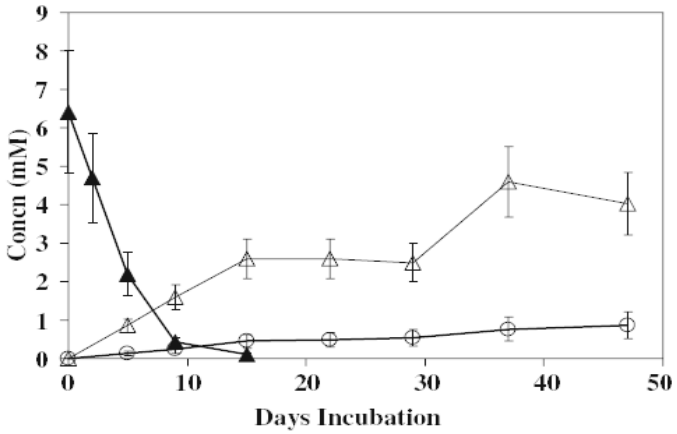
In the section of the experiment investigating the transformation of TNT it was concluded that in all three conditions being investigated (no addition of electron reducing compound, addition of ethanol and addition of propylene glycol) the concentrations of TNT was rapidly removed within 24 hours of experiment initiation. Because of these results it was concluded that TNT can be readily reduced without the addition of any reduction assisting compounds (Adrian et al., 2006).

The following figures display the concentration changes of primary additive (ethanol and propylene glycol respectively), process intermediate (acetate and propionate, respectively) and each process' mineralized product (methane and acetate) with the progression of time in the experiment (Adrian et al., 2006).



Ethanol, acetate and propionate concentrations in anoxic serum bottles containing 50 g explosive-contaminated soil and 75 ml of groundwater. The microcosms were amended with ethanol on day zero to a final concentration of ~6 mM. Values are the means of three replicates ±standard deviations. Error bars are not shown for standard deviations that are less than 10% of the value of the point. Symbols: ethanol, ●; acetate, ○; methane, ∗.

Figure 9: Change in Concentration of Additive, Intermediate and Product of Ethanol Degradation (Adrian et al., 2006)



. Propylene glycol, propionate, and acetate concentrations in serum bottles containing 50 g explosive-contaminated soil and 75 ml of groundwater. The microcosms were amended with propylene glycol on day zero to a final concentration of ~6 mM. Values are the means of three replicates ±standard deviations. Error bars are not shown for standard deviations that are less than 10% of the value of the point. Symbols: propylene glycol, ▲; propionate, △; acetate, ○.

Figure 10: Change in Concentration of Additive, Intermediate and Product of Propylene Glycol Degradation (Adrian et al., 2006)

Ultimately the conclusion reached by researchers conducting this experiment supported their hypothesis. The addition of a stimulant that increases the concentration of hydrogen gas develops an increase in the rate of anaerobic degradation of soil containing RDX, HMX and TNT. The addition of these compounds however is not necessary for the reduction of RDX and TNT as these compounds can be degraded by resources already available in the environment. Having said this the addition of either ethanol or propylene glycol are practical methods of going about enhancing the degradation of these three compounds (Adrian et al., 2006).

Analysis of the Biodegradation of CL-20

There is a lot of research currently being conducted on CL-20 due its recent development as a powerful explosive compound that will soon replace RDX, HMX and TNT as the primary explosive used by military's around the world. Though CL-20 has been found to have a low rate of diffusion through soil and is immediately degraded in environments that have a high pH, CL-20 must still be removed in acidic environments by some process that is often dependent on microbial activity. Recent research has been conducted on the ability of various growth substrate additions to enhance the degradation CL-20, over the course of this experiment researchers also analyzed how the decomposition of CL-20 related to the growth of the microbial cell. This was done to determine if the degradation of CL-20 will provide any benefit (promotion of cell growth, etc.) to the microbial cell (Panikov et al., 2006).

Effect of Substrate Addition on the Degradation of CL-20

The researchers determined that the addition of a carbon compound as a growth substrate did increase the degradation rate. The substrates tested were: sucrose, pyruvate, yeast, acetate, glucose and starch the results of the experiment showed that the effect a carbon compound addition had on the rate of CL-20 degradation decreased in that order, respectively. See Figure 8 for a graphical representation of these results. It should be noted however, that the addition of these various substrates had little to no effect on the growth of the bacterial cell (Panikov et al., 2006).

g. 2 Effect of auxiliary substrates on the rate of CL-20 degradation in the soil. Dynamics of residual CL-20 concentration was followed in the soil samples amended with mineral nitrogen and various organic substrates (see legend). Control stands for autoclaved soil without co-substrates. The vertical bar here and below shows sample SD

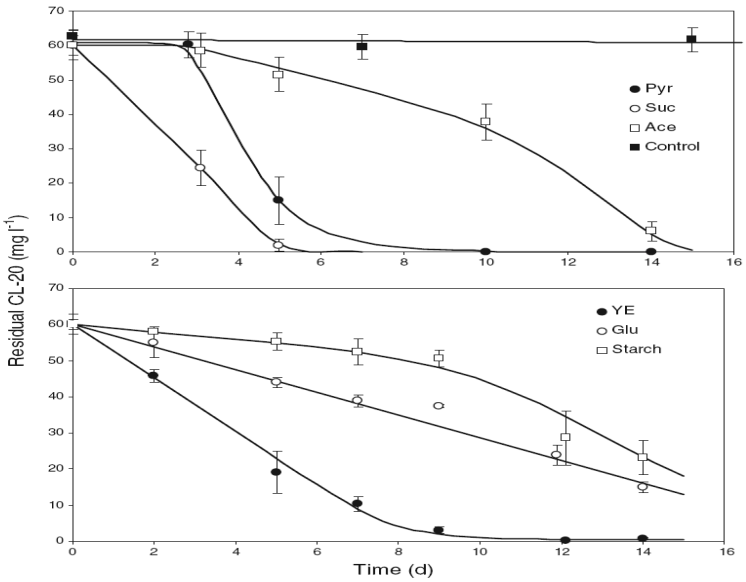


Figure 11: Effect of Substrate on the Degradation of CL-20 (Panikov et al., 2006)

The experiment also concluded that CL-20 could be degraded by microbial cells under anoxic conditions. This result was reached by analyzing the concentration of sucrose in aerobic and anoxic conditions. It should be noted however that the highest rate of CL-20 was achieved through the addition of sucrose under the oxic condition. Figure 9 displays a summary of these results (Panikov et al., 2006).

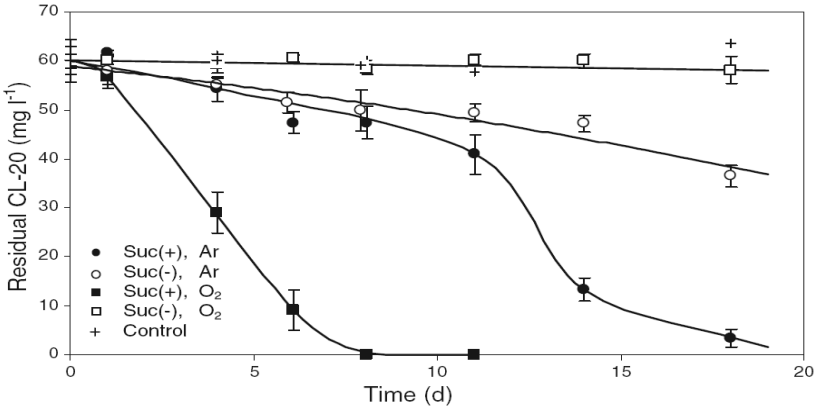


Fig. 3 CL-20 decomposition dynamics in soil under oxic and anoxic conditions. CL-20 was added to soil in combination with succinate (Suc(+), filled circles and squares) or without auxiliary substrate (Suc(–), open circles and squares) and incubated under air (O_2, squares) or argon (Ar, circles) in headspace. No mineral nitrogen was added. Control vials (+), contained CL-20 in sterile mineral solution instead of soil

Figure 12: Decomposition of CL-20 Under both Oxic and Anoxic Conditions (Panikov et al., 2006)

The results of this experiment conclude that CL-20 biodegradation can be accelerated under anoxic conditions by adding various amounts of substrate. The study also concluded that the aerobic process of CL-20 degradation of a co-metabolism type reaction is independent of the growth of the degradation microorganism. This fact has been overlooked by many researchers in past experiments involving CL-20. The final major conclusion reached by researchers conducting this experiment is that nearly half of the microorganisms “randomly selected” for use in this experiment were able to degrade CL-20 when grown on succinate. This leads researchers to believe that this metabolic skill is very common in many naturally occurring microorganisms.

The researchers involved in this experiment believe that if this avenue of research is investigated more closely a substantially amount of money could be saved due to the fact that no importing of microorganisms or removal of polluted material would need to be performed, as the researchers believe that all CL-20 related remediation work could be performed through in-situ techniques (Panikov et al., 2006).

Table 1: Microbial Degraders of CL-20 (Panikov et al., 2006)

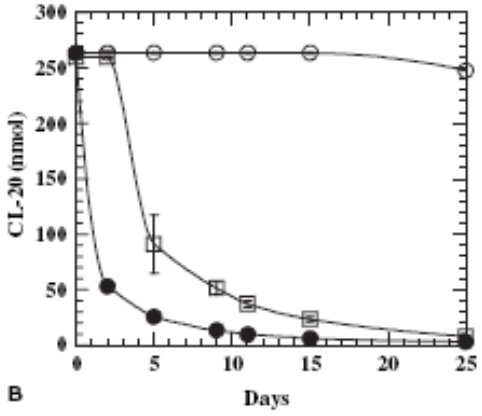
Organism, strain	Cell morphology	CL-20 degradation rate, day^{-1}[a]	BLAST search for related phylotypes (similarity)	The confidence threshold, % (Cole et al. 2003)
Pseudomonas sp. MS-P	Rod-shaped gram-negative motile asporogenous bacteria, 0.8–2.0 × 0.3–0.7 μm	0.06–0.11	*Pseudomonas putida* (99%)	*Pseudomonas*, 100
Rhodococcus sp. MS-1	Pleomorphic (irregular rods-to-coccoid) non-motile and asporogenous gram-positive aerobic bacteria. Produces abundant extracellular slime	0.01–0.09	*Rhodococcus opacus* (97%) *R. equi* (97%) *R. erythropolis* (97%)	*Rhodococcus*, 100
Rhodococcus sp. MS-6	The same but no slime production	0.05–0.07	*R. erythropolis* (99%)	*Rhodococcus*, 100
Ochrobactrum sp. MS-8	Motile, clock-wise rotating rods with rounded ends, 0.8–1.8 × 0.5–1.0 μm; aerobic asporogenous gram-negative or gram-variable (in old culture)	0.07–0.09	*Ochrobacrum* sp. AY576683 (99%) *Ochrobacrtum* sp. AF229879 (99%)	*Brucellaceae*, 100 *Ochrobactrum*, 74
Mycobacterium sp. MS-11	Pleomorphic (Y- and V-shaped, rods and ovals) non-motile and asporogenous cells 0.7–2.2 × 0.3–0.9 μm; gram-positive aerobic; form aggregates and biofilms in shake flasks	0.02–0.04	*Mycobacterium fortuitum* (99%) *M. porcinum* (99%)	*Mycobacterium*, 100
Ralstonia sp. MS-10	Motile and asporogenous rod-shaped cells 0.6–1.4 × 0.3–0.6 μm; gram-negative aerobic	0.07–0.10	*Ralstonia pickettii* (99%)	*Ralstonia*, 100

[a] The initial CL-20 and Na-succinate concentration were respectively 10 mg l^{-1} and 2 g l^{-1}, analysis of residual CL-20 was made on days 7 and 14, other conditions see in Materials and methods. The specific degradation rate of CL-20 was calculated from eq. 1 for two replicate vials

The Use of White-Rot Fungi for the Degradation of CL-20

A recent study was performed where CL-20 was added to a culture containing spores of *P. Chrysosporium or white-rot fungi***.** These cultures were monitored for their ability to degrade the CL-20 molecule over the course of eight days under the aerobic degradation mechanism of CL-20. The results obtained from this experiment show that in the observed cultures containing the spores of white rot fungus, all traces of CL-20 molecules remaining were essentially non-existant. Over the course the experiment the concentrations of nitrous oxide were also monitored. It was concluded that the concentration of nitrous oxide increased to about 45% the initial concentration of CL-20. In the experiment $NaNO_2$ was used as the primary nitrogen source. Using these conditions fungal growth of white-rot fungi was observed to have occurred. Over the course of the experiment no intermediate molecules of the CL-20 breakdown process were observed to develop in the culture. This is believed to be due to the existing condition's supernatant ability to break these molecules down as soon as they had formed. These results show that the use of white-rot fungi for the biodegradation of the CL-20 nitramine molecule is an extremely economical solution when the contaminants occur in an environment that supports the growth of lignolytic fungi similar to the white-rot fungi used in this experiment (Fourneir et al., 2006).

A graph of the researcher's results is displayed at the top of the next page.



. (B) Degradation of CL-20 (260 nmol) by 7 days-old *P. chrysosporium* (●), *T. lacteus* (□), or noninoculated cultures (○).

Figure 13: Degradation of CL-20 by Various Fungi

Explosive Remediation Case Study: Louisiana Army Ammunition Plant

In Minden, Louisiana there is an army ammunition production plant that currently disposes of its waste through dumping or waste incineration. As the initial and maintenance costs of these waste disposal methods is typically fairly substantial the plant has been investigating alternative methods. The two most common alternative methods implemented are soil slurry or land farming. It should be noted that both these methods have their own advantages and disadvantages. The soil slurry degradation technique is typically much faster than land farming, but also has a more substantial maintenance cost associated with it. This being said researchers have recently performed a case study to analyze the effectiveness of these two biodegradation methods on soil surround the ammunition pant in Louisiana that has been contaminated by TNT waste (Clark et al., 2007).

Soil Slurry Reactor

For the soil slurry culture portion of the experiment the researchers used molasses as the provided growth substrate for the bacteria. The researchers also developed a control condition where no molasses was added to the culture. The researchers found that after 182 days of incubation 7,000mg-TNT/kg soil the cultures that received molasses showed 99% removal efficiency where the control cultures showed little to no TNT degradation. This report led researchers to the finding that while a soil slurry is a viable option for the degradation of the contaminated soil, a carbon source would need to be added if TNT degradation were to be accomplished. The researchers also found that the soil slurry cultures led to the removal of other explosive contaminants like RDX and HMX, but at a slower removal rate. For the reason researchers believe that if the culture were to be allowed to incubate for a longer period of time these contaminants could also be removed. The decrease in concentration of TNT with time is shown on the graph at the top of the next page (Clark et al., 2007).

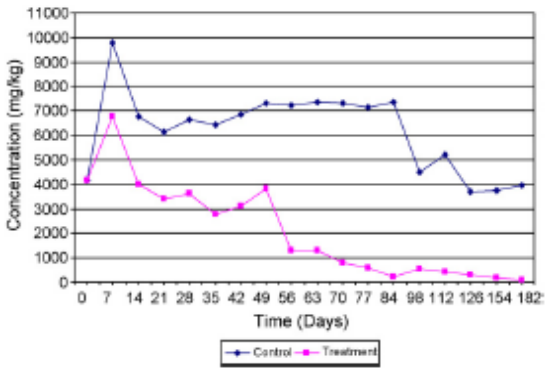


Figure 14: Change in Concentration of TNT with Time in Soil Slurry Reactor (Clark et al., 2007)

Land Farming

In the land farming section of the experiment researchers repeated many of the conditions that were exhibited in the soil slurry portion of the experiment, ie: soil samples were taken from the same area, molasses was also used as the carbon source for the bacteria. With these conditions the researchers found that after 182 days of incubation, the cultures that received 7,000mg-TNT/kg soil showed approximately 82% removal efficiency. The results also showed that this technique produced little to no removal of HMX or RDX. A graph of TNT concentration vs. time obtained from this experiment is displayed below (Clark et al., 2007).

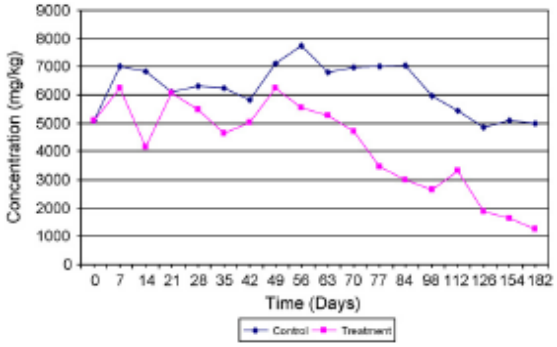


Figure 15: Change in Concentration of TNT with Time in Land Farming Pan (Clark et al., 2007)

Experiment Conclusion

With these results researchers feel that for the ammunition plant in Louisiana, a soil slurry TNT biodegradation technique is the most advantageous. The researchers felt that the typically high cost of this method can be kept to a minimum if researchers use molasses as the carbon source and simply cycle an oxygen supply through the cultures. If this is done correctly it would be possible for the plant to take advantage of the soil slurry's much faster rates of nitramine degradation (Clark et al., 2007).

Conclusion

The fact that explosive compound production is only likely to increase in the future means that the hazards these compounds produce in the environment to which they are released will only become more severe with time if no remedial action is taken soon. For this reason there are many studies currently being conducted in this area of environmental engineering. Particularly concerning the breakdown and degradation of the nitramine compounds discussed in this paper. Due to the general cost effectiveness of in-situ bioremediation it comes as no surprise that this is the primary method of treatment currently being investigated to accomplish this goal. The purpose of this paper was to provide its reader with a review of the possible avenues that can be traveled that would ultimately lead to the breakdown of HMX, RDX, CL-20 and TNT and provide the reader with samples of research that are currently being conducted to determine the optimum method of degradation of these various compounds. For convenience the following table is a summary of the different degradation pathways for the respective molecules.

Table 2 Summary of Biological Tretment Methods of Explosive Compounds

Molecule	Name of Degradation Method	Bacteria Involved
RDX	Two-Electron Reductive Pathway	*Enterobacteira, E. Coli, Clostridium Acetylbutilecum, Aspirgillus Niger*
RDX	Anaerobic Denitration	*K. pneumonia, C. bifermentans*
RDX	Aerobic Denitration	*Rhodococcus rhodochrous, Williamsia and Gordonia*
HMX	Aerobic Degradation	*Methylobacterium*
HMX	Anaerobic Denitration	*K. pneumonia, C. bifermentans*
CL-20	Aerobic Degradation	*P. Chrysosporium*
CL-20	Anaerobic Degradation	*Clostridium*

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