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Bioremediation of 2,4,6-Trinitrotoluene (TNT) at munitions sites

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Introduction

TNT is one of the predominant conventional explosives used by military forces (Chaudry, 1994). TNT was first produced on an industrial scale as early as 1891 by Germany, and in 1901 was adopted by all military powers (Kirk and Othmer, 1951). During World War I the production of TNT was limited only by the amount of toluene available as a byproduct of the coke industry (Kirk and Othmer, 1951). After 1940, toluene became readily available as a byproduct of the petroleum industry and during World War II TNT was manufactured extensively (Kirk and Othmer, 1951 and Kirk and Othmer, 1993). This large-scale production of TNT in many World War II armaments factory sites has led to pollution by TNT and its byproducts (Fraunhaufer, 1999). Because of improper disposal of waste and wastewater generated during the production, burning and detonation, and dismantlement of munitions, serious contamination has occurred in many locations worldwide, including the United States and Europe (Chaudry, 1994 and Funk et al., 1996). Contamination often occurred at installations where explosives were produced and handled according to practices considered standard at the time and not known to be inadequate (USAEC, 1999). TNT is well known to have both toxic and mutagenic effects on various organisms, including humans (Chaudry, 1994 and Funk et al., 1996). Exposure to TNT is known to cause rashes, skin hemmorages, and mucus and blood disorders (including pancytopenia, a disorder of blood-forming tissues) (Chaudhry, 1994 and Kirk, 1993). Toxic effects including liver damage (toxic hepetitis) and anemia have been reported by workers engaged in large-scale manufacturing and handling (Chaudhry, 1994 and Kirk, 1993). TNT has been shown to be a potent mutagen as assayed by the Ames test (Won et al., 1976).

A NATO CCMS (Committee on the Challenges to Modern Society) study (1995) has determined there are many countries where remnant explosives, especially TNT, and their degradation products are still present in the soil. Although this contamination is known to be widespread, little information documenting the exact sites of contamination at an international level was found in the literature. Some well-documented sites do exist in Germany, and are currently under investigation (NATO, 1995). In Australia, the Australian Defense Industries (ADI) organization has been given the task of closing down and remediating former munitions factories along the east coast, and has been identifing individual sites (McGee, 1995).

TNT contaminated soils have traditionally been recovered by incineration, but its high cost and the fact that ash from incineration must be treated as hazardous waste has led to a search for other methods (Funk et. al., 1996 and EPA, 1997). The problems with incineration have lead to the growth and further research of bioremediation of TNT (Rittmann, 1994). Bioremediation uses microorganisms and plants to transform these hazardous materials into more benign substances (Rittmann, 1994).

Fundamentals of Bioremediation

Much work has been done to characterize specific bacteria or fungal strains for their effectiveness in biodegrading TNT (Scheibner et al., 1997; Boopathy, 1994; Funk et al., 1996). Strains of microorganisms have been isolated to try to provide the fastest, most complete degradation of TNT (Chaudry, 1994). This effectiveness and completeness of degradation is commonly measured using labeling techniques with carbon-14 to show the actual degradation patterns (Scheibner, 1997 and Chaudry, 1994).

Aerobic degradation pathways consist of microbes in oxygenated conditions breaking down the TNT molecule. In the aerobic degradation of TNT, several species have been promising: *Pseudomonas* and *Phanerchoaete chryosoporium, mycobacterium,* and *Rhodococus erythropolis* (Funk et. al., 1996). Bioremediation of TNT by some aerobic microorganisms is problematic because many common degradation pathways lead to the formation of toxic and mutagenic degradation intermediates (Funk et. al., 1996). TNT, or 2,4,6-trinitrotoluene, consists of a benzene ring, a methyl group, and three nitro groups (see figure 1). When degradation occurs by reducing these nitro groups in a step-wise fashion, leaving the aromatic ring uncleaved. These degradation intermediates, such as Am-DNT, 2,4-DA-6-NT, 2-6-DA-4-NT, 2,4-DNT, 4-A-2-NT, 2-A-4-NT, and 2,4-DAT have been shown to be toxic and/or mutanagenic (Chaudry, 1994; Fraunhofer IGB, 1999; Rittmann, 1994). Usually, aerobic conditions form degradation products of the TNT molecule that are toxic (Davis-Hoover, 1994).

However, the aromatic ring can be cleaved without going through some of these dangerous intermediates under anaerobic conditions. Anaerobic degradation used by itself or as part of a two-step aerobic/anaerobic process (aerobic followed by anaerobic) has had a high degree of success (EPA, 1995; Frannhofer IGB 1999; Breitung, 1996). For anaerobic degradation, *Clostridium* spp. were found to degrade TNT-contaminated soils well (Funk et al., 1996). During the anaerobic stage of degradation, a source of sugars, ranging from molasses to sucrose, is often added (Schlyer, 1996 and USAEC, 1999). Anaerobic bacteria with a fermentable food source use this energy to degrade nitrated contaminants (Crawford, 1996). Using an anaerobic consortium of soil microorganisms degrades TNT without forming these toxic products (Davis-Hoover, 1994).

Unlike other microbial degradation systems where degradation occurs by a reduction of the nitrate groups in a step-wise fashion, white rot fungus, or *Pseudomonas fluorescens*, degrades TNT via an oxidation pathway under anaerobic conditions (Gilcrease, 1996 and Fernando et. al., 1990). Using the oxidation pathway eliminates the formation of several of the toxic intermediates of TNT (Fernando et al., 1990).

Using several species of bacteria at once, the ‘consortia’ method, can improve degradation results (Crawford, 1996). *Clostridium* spp. that have been proven to be good choices for TNT degradation are also excellent bases for a consortium (Crawford, 1996). Crawford (1996) proposes that existing ‘natural’ consortia could be stimulated to grow *in-situ* by adding a food

source to a contaminated aquifer. If sufficient numbers of TNT degrading bacteria are not present in the aquifer, they could be added in a foam of spores (Crawford, 1996).

Bioremediation methods can be classified into several strategic categories: composting, bioslurries, and phytoremediaton. An incredible variety of microorganisms and aerobic/anaerobic methods exist for the composting and bioslurry strategies. Also, composting and phytoremediation have distinctions between *in-situ* (on-site treatment) and *ex-situ* (off-site treatment) strategies (during bioslurry the soil is always removed). Currently, *ex-situ* bioremediation is more popular because removal from the site of contaminated soils prevents groundwater contamination (NATO, 1995). Also, *ex-situ* treatment is used to control the process of degradation more closely (Crawford, 1996). However, *in-situ* methods could ultimately yield easier processes and reduce the amount of disturbance of the site.

In the choice between composting, bioslurry, or phytoremediation, the size of TNT contaminant particles is also a consideration. TNT can be present in particles as large as 2.5 cm in soil at some sites (Gilcrease et al., 1996). Larger particles have been slower to degrade in field trials (Gilcrease et al., 1996). Although these larger particles can be crushed or shredded and treated with composting (EPA, 1997), the bioslurry method is often chosen to deal with this complication (Gilcrease et al., 1996). It has been suggested that contaminants need to be in dissolved form to biodegrate, and may be limited by this dissolution rate (Stucki and Alexander, 1987). Gilcrease (1996) suggests that the bioslurry method offers an advantage because dissolution rates can be increased easily with agitation rates. The addition of a neutral solid (Teflon) was studied by Gilcrease (1996) to determine the effect of non-TNT solids on dissolution rates. Teflon promoted TNT particle attrition, creating more surface area for dissolution; although the solid-liquid mass transfer coefficients decreased with higher solid concentrations (Gilcrease, 1996).

Composting

Composting is a centuries-old technology that is being refined to maximize the degradation of hazardous materials such as TNT. During composting, the contaminated soil is mixed with a bulking agent to increase porosity and facilitate air exchange (Funk et al., 1996). The most common type of composting when dealing with TNT-contaminated sites is windrow composting (Funk et al., 1996). During windrow composting, the soil is mixed with compost and spread over a field or other surface in narrow rows called *windrows* (Funk et al., 1996). To facilitate microbial growth, a composting material such as wood chips, straw, alfalfa, manure, or other agricultural products are added (Funk et al., 1996). Windrows are kept aerated with occasional turning, and moisture content, oxygen level and temperature are all carefully monitored (Ritterman, 1996 and Funk, 1996). Windrow composting has been found to have a high degree of treatment success at a low process cost (Funk et al., 1996).

Composting was used in the cleanup of a Superfund site at Umatilla Army Depot, Oregon. (USAEC, 1999 and EPA, 1997). Umatilla was contaminated over a 15-year period when workers used water and steam to clean TNT and other explosives out of decommissioned bombs This

'cleaning' sent more than 80 million gallons of pink water into two 10,000 sq. ft. lagoons (Weston, 1993). These evaporative ponds created a giant soil contamination site, with TNT levels upwards of 4800 ppm (Weston, 1993 and EPA, 1997). Potato waste and manure was mixed with the contaminated soil using windrow composting (Weston, 1993). To insure optimum composting conditions, large temporary mobile buildings were placed over the piles (EPA, 1997). After 40 days of treatment, TNT reductions were as high as 99.7%. Composting reduced initial average contaminant concentrations from 1574 ppm TNT to 4 ppm (Craig, 1998 and EPA, 1997). The cost savings over traditional incineration methods was estimated at $2.6 million for the Umatilla site (EPA, 1993 and USAEC,1999).

Another site at the U.S. Naval Submarine Base in Bangor, Washington was contaminated by the open burn and detonation of munitions over a 19-year period. Composting methods to the Umatilla site were used; TNT levels were reduced 96.7-99.43% after 30 days. The intermediates, 2,4-DNT and 2,6-DNT and 1,3,5-TNB, were all degraded at very fast rates (NFESC, 1999). A feasibility study estimated treatment cost of $206 to $766 per ton, which is 40-50% less than on-site incineration.

The effectiveness of anaerobic/aerobic two-phase composting was studied by Breitung et al. (1996). Continually aerated compost was compared to compost aerated after an anaerobic 'pre-phase' of 65 days. The continuous aeration system rapidly degraded 92% of the TNT and contaminating byproducts. Although slower, the two-phase system was able to achieve an almost 100% degradation of TNT and contaminating byproducts (levels were undetectable). The two-phase system works by reducing TNT almost entirely to Am-DNT during the anaerobic 'pre-phase', then using the aerobic phase to degrade the Am-DNT (Breitung et al., 1996).

Composting can be problematic because the exact fate of explosives can not be determined easily. If TNT or its degradation intermediates are bound to the compost mix these could leach back into the soil. Also, because of the bulking agents used, the soil returns at 5 to 30 times its original volume.

Bioslurry

Bioslurries use a large containment tank or field to process a slurry of soil, microbes, filler, and nutrients to degradade TNT. Slurry reactors have been used to treat municipal sewage for years, and the technique of using bioslurries is readily applied and modified for TNT degradation. Bioslurry reactors are preferred if high levels of agitation, aeration, or soil pretreatment are necessary to reach a certain degradation rate (Crawford, 1996). They also provide a controlled environment for optimal degradation levels of TNT, and prevent further leeching into the surrounding soils (Crawford, 1996). Limiting factors, such as pH, temperature, and nutrient availability that can prevent successful degradation are easier to monitor in a controlled environment (Funk et al., 1996 and EPA, 1995).

Optimum conditions for TNT removal from a soil-slurry using one bacterial consortium at pH of 6 and at 20 to 22 degrees Celsius (Boopathy et al., 1997). This consortium could not use TNT as a nitrogen source so optimal growth occurred after adding ammonium chloride (0.25 g/L).

The bioslurry method was tested at the Joliet Army Ammunition Plant, USA (Illinois) for the remediation of an explosives contaminated area. The bioslurry used an alternating cycle between aerobic and anaerobic conditions and achieved removal of more than 99% TNT and related contaminants. Again, the two-phase aerobic/anaerobic treatment showed virtually complete breakdown of TNT into CO2, simple organic acids, and carbon fragments (USAEC, 1999).

A bioslurry method has also been tested and marketed under the Fraunhaufer IGB Company (1999). They also incorporate a two stage anaerobic/aerobic treatment process, and have done large-scale testing at Hessisch Lichtenau-Hirschhagen, a former TNT production site in Germany. Using a TERRANOX (registered trademark) system anaerobic sludge reactor, they were able to reduce contaminants by more than 99% (Fraunhaufer IGB, 1999). Fraunhaufer (1999) ammended the bioslurry with a sugar (sucrose) supplement.

The J.R. Simplot Company developed and tested an effective bioslurry process called J.R. Simplot Anaerobic Bioremediation (SABRE) (EPA, 1995). The contaminated soil is excavated, screened, mixed with a carbon source (potato starch), buffered with phosphate, and formed into a slurry (EPA, 1995 and Davis-Hoover, 1984). In the SABRE process, selected TNT-degrading microorganisms are added to the slurry after the mixture becomes anaerobic if they are not indigenous to the soil (EPA, 1995). TNT toxic intermediate compounds are evolved during the anaerobic phase, but are eventually destroyed by the process (EPA, 1995 and Davis-Hoover, 1994). Using the SABRE process, the reduction efficiency of TNT was found to be 99.4% at the test site at Weldon Spring Ordnance Works (EPA, 1995).

Phytoremeditaion

Phytoremediation, or bioremediation using higher plant species, is just starting to be researched for degradation of TNT (NATO, 1994). During phytoremediation, plants either degrade TNT or immobilize it through incorporation into vacuoles and cell walls (Mueller et. al., 1995 and Rittmann, et al., 1994). Also, plant root structures foster rhizobial and mycorrhizal communities that can detoxify and mineralize TNT (Mueller et al., 1995). Immobilization of TNT can stop the downward leaching of TNT contaminants into the water table (Mueller et al., 1995). This immobilization is used as an intermediate phase to allow the mycorrhizal communities time to break down all of the contaminants. There were no cases found of the plant material being harvested to remove immobilized TNT contaminants. Phytoremediation is seen as a good cleanup method for low to intermediate levels of contamination or to further reduce/remove contamination after composting highly-contaminated soils (Mueller et. al., 1995).

Mueller et al. (1995) studied *Datura innoxia* (Jimson weed) and *Lycopersicon peruvianum* (wild tomato species) for their ability to uptake and biotransform TNT into non-toxic degradation products. Both plant species were found to be suited for the removal of low levels of TNT (less than 1000 ppm). Also, the degradation of TNT did not necessarily stop at the Am-DNT level, but would go on to produce a variety of non-toxic degradation products. TNT levels in experimental soils in greenhouse conditions were reduced to less than 10% TNT in two weeks.

Some research has been accessing toxicity thresholds of TNT for other plant species (Gong, et al., 1999). Findings suggest that low levels of TNT, 5-25 mg/kg for *Lepidium sativum* (cress) and

Brassica rapa (turnip) and 25-50 mg/kg for *Acena sativa* (oat) and *Triticum aestivum* (wheat) actually stimulated seedling growth (Gong, et. al., 1999). Also, *Acena sativa* was capable of tolerating as much as 1600 mg TNT/kg and demonstrated a potential ability to detoxify soil. With these qualities, *Acena sativa* looks to be a promising bioremediation tool in low-level TNT contaminated soils (Gong, et al., 1999).

Phytoremediation of TNT-contaminated water was explored by Best et al. (1996), who compared the ability of aquatic plant species to remove TNT and RDX contaminated water. In tests at Department of Defense munitions plants in Milan, Italy and Iowa, USA, all plant species significantly increased the amount and rate of TNT removal (Best et al., 1996). Some of the most effective species were *Elodea, Potamageton pectiratic* (sago pondweed), *Myriophyllum spicatum* (Eurasion watermilfoil), *Potamageton crisym* (curlyleaf pondweed), *Heteranthera dubia* (waterstargrass), and *Phalaris arundinacea* (reed canary grass) (Best et al., 1996).

Conclusion

Bioremediation is a valuable and viable method of removal of TNT and related degradation products. When compared to incineration, the previously most common method of biodegradation, bioremediation is both less expensive and less environmentally hazardous (NATO, 1994). Incineration can result in ash that must be handled and disposed of as hazardous residue, but composting produces a nutrient-rich product comparable to an enriched top soil used for landscaping and agricultural applications (EPA, 1997).

The different strategies of bioremediation of TNT are definitely specific to the site. Composting lends itself to low levels of TNT and is suitable where it is not necessary to completely eradicate all of the degradation products. Bioslurry has the advantage of being rapid, minimizing the likelihood contaminants will leech into the groundwater. Phytoremediation, although not widely used now, has potential to become a major strategy. It is best suited where contaminant levels are very low (where toxicity to plants is not an issue) and as a cleanup method after other remediation techniques such as composting or bioslurry have already taken place.

NATO (1994) analyzed several ‘representative sample’ scenarios during a conference on the destruction of chemical weapons. Their first-choice technology to clean up a TNT-contaminated munitions site consisted of removing the soil to a site were it cannot reach the groundwater, then treatment with composting or other bioremediation. The second choice was a bioslurry reactor, while the last choice was recommended as incineration. One of the reasons given by NATO (1994) was "Bioremediation of the soil will produce a material that can be returned to the original site", something that in itself is worth the effort.

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