12-31-1991

Biodegradation of TNT (2,4,6-Trinitrotoluene)

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Biodegradation of TNT (2,4,6-Trinitrotoluene)

by

Jin Lou

Thesis submitted to the Faculty of Graduate School of the New Jersey Institute of Technology in partial fulfillment of the requirements for the degree of Master of Science in Civil Engineering - Environmental Option

1991

APPROVAL SHEET

Title of Thesis: Biodegradation of TNT (2,4,6-trinitrotoluene)

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Abstract

This study summaries the results of various methods treating 2,4,6-trinitrotoluene (TNT). Review of available literatures revealed that the most commonly used techniques in full-scale plant for TNT removal are chemical oxidation and carbon adsorption.

From economic point of view, the biological treatment system will be much more cost-effective. Various methods for improving the efficiency of biological system treating TNT wastewater were developed in the laboratory. Current laboratory results show that by isolating appropriate microorganisms under adequate conditions, TNT wastes can be degraded successfully.

At the some time, some pretreatment technologies are recommended in this study for improving the performance of biological system treating TNT.

ACKNOWLEDGMENTS

I wish to thank my advisor, Dr. Yeun C. Wu, for his patience and guidance throughout this study.

I also wish to thank my friends at NJIT for their help during my graduate study. Finally, I wish to thank my parents and wife for their encouragement and support.

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CHAPTER I

Introduction

The compound TNT (2,4,6-Trinitrotoluene) is the predominant conventional explosive used by military force. Unfortunately, past practices for the disposal of TNT-containing wastes generated during the production of TNT and military ordnance which use TNT has led to soil, sediment, and water contamination. The TNT is toxic and results positive Ames Test that is indicative of potential mutagenic-carcinogenic property.

Disposal of waste TNT is a particularly difficult problem. Relatively large volumes of water are required because TNT is only slightly soluble in water (100 ug/ml). TNT is toxic to certain fish at concentrations greater than 2 ug/ml and, in humans, TNT has been shown to cause liver injury and marked changes in the hematopoietic system producing anemia. Accordingly, it is indisputable that treatment of TNT wastes is important if these are to be discharged into receiving water, particularly when recreational or potable water is involved.

TNT can be removed from wastewaters using surfactants, carbon adsorption,incineration, and reverse osmosis. But these processes and disposal of by-product are expensive. As to the efficiency of overall removal of explosives from a multi-component system such as one

containing TNT and other secondary components have not been fully understood. There are some researches using Wet Air Oxidation (WAO) to treat the wastes which contains TNT, but the cost seems be high.

The biological treatment is often seen as an attractive, cost-effective alternative for treatment of hazardous organochemical pollutants. Researchers also found some microorganisms like the white-rot fungi, *Phanerochaete Chrysosporium*, are potentially useful for degrading complex compounds in biological wastewater treatment systems. Because these fungi can degrade a broad spectrum of structurally diverse organic compounds to carbon dioxides. Many compounds degraded by this organism, such as DDT, polychlorinated biphenyls (PCB), benzo[a]pyrene and 2,3,7,8-TCDD, which are considered to be human and environmental health hazards and resistant to biodegradation by most microorganisms. The purposes of this research are to review the various methods treating the TNT wastes and investigate the possibility of biological treatment for TNT wastes.

The overall objectives of this study are as follows:

(1). To review various researches regarding the disposal methods of TNT wastewater.

(2). To identify the biodegradability of TNT.

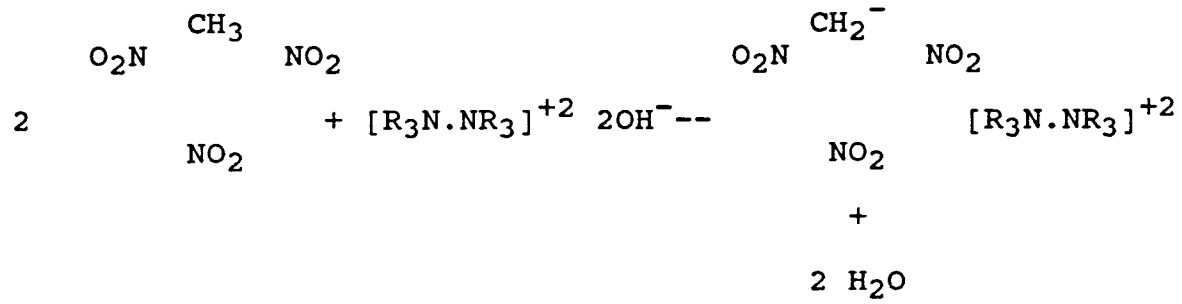
(3). To recommend the best alternatives for treatment of TNT.

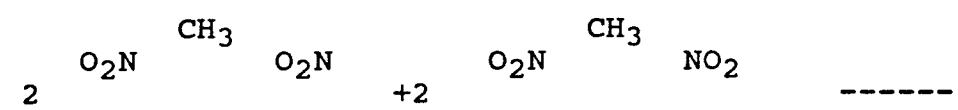
CHAPTER II

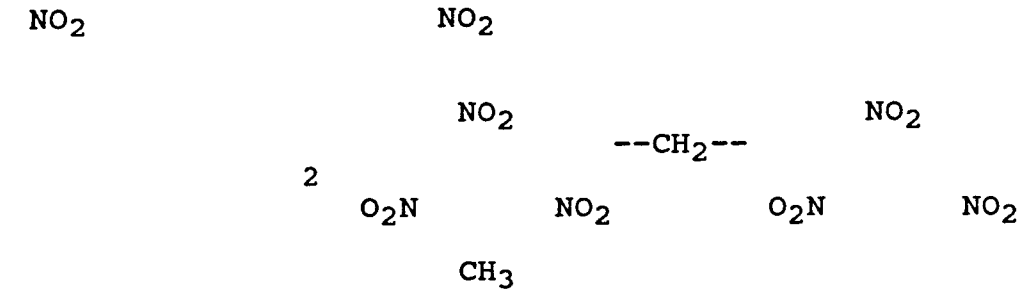
Physico-chemical Methods to treat TNT

A technology for removal of TNT from water with surfactants has been recently developed. Two types of surfactants have been demonstrated to be effective in the precipitation of TNT from solution. One is a surfactant in which the polar group is a primary amine while the other is a quaternary amine. The precipitation of TNT from solution is the result of the reaction between TNT and the surfactant in an alkaline medium to form an intermediate substance identified as the Janocsky Complex that in turn react with the protonated surfactant and precipitates as a salt.

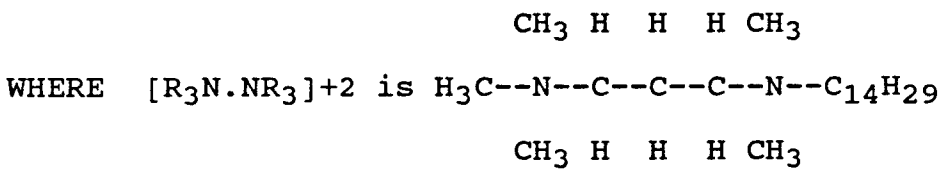
$$[R_3N.NR_3]^{+2}\ 2Cl^- + 2\ NaOH \text{ ----} [R_3N.NR_3]^{+2}\ 2OH^- + 2\ NaCl$$







JANOVSKY COMPLEX



Based on above findings, Freeman (1981) conducted several studies at Hazards Research Corporation, Rockway, New Jersey. Lab results show that the surfactants found most effective were N-tallow 1,3, diaminopropane (Duomeen T) and trimethyl tallow ammonium chloride (Arquad T-50).

In the surfactant treatment experiment, a continuous-flow two-stage reactor surfactant treatment process was used to remove virtually 100 % of TNT from wastewater using the quaternary surfactant Duoquad I-50. In addition, all other identified toxic components, except DNB, were remove by surfactant treatment. Reaction parameters including a PH of 11.5 (with sodium hydroxide solution), reaction temperature of 55 ^{o}C, Surfactant/TNT molar ratio of 0.3 to 0.375, and reactor retention time of one hour per reactor stage.

This process has two by-products: a very small amount of co-precipitated explosive-surfactant complex and excess surfactant, which can be easily incinerated, and a filtrate which, after neutralization with sulfuric acid and filtration, can be discharged to conventional wastewater treatment.

There were some reports about that TNT was treated by evaporation in rotary kilns, to produce a concentrate of 35 % to 40 % solids. The concentrated liquid is then incinerated to a dry residue. An estimated 0.2 lb of ash is produced per lb of TNT manufactured. The wastewater evaporator condensate represents a disposal problem. The ash may be disposed on land. Due to its high solubility (90 %), significant quantities are washed away by rainfall. To face stringent environmental regulations, another alternative processes should be investigated.

The evaporation/incineration treatment process for TNT suffers from four deficiencies:

(1). The condensate does not comply with accepted standards for nitrobody content.

(2). Energy requirements for concentration and incineration are high.

(3). The ash residue represents a major disposal problem.

(4). There is a potential air pollution problem associated

with the incinerator operation.

The reverse osmosis pilot studies indicated that insufficient TNT concentration could be achieve, and that significant leakage occurred through the membrane.

Activated carbon treatment is effective treatment method. Burrows et.al.(1984) conducted batch carbon adsorption tests under equilibrium conditions using Calgon* F300,-200 mesh. Data were analyzed using the empirical Freundlich relationship

$$Q_e = K * C^{1/n}$$

where Q_e is the amount of organic solute adsorbed per unit weight of carbon (g/g) in equilibrium with concentration C (mg/l) of solute, and K and n are constants. The explosive chemicals present in wastewater, TNT, RDX, HMX, TAX, and SEX, are removed from water by granular activated carbon under batch conditions with fair efficiency when tested individually at concentration levels below 25 mg/l, as shown in Table 1. Tested collectively, removal of each munition chemical is substantially less efficient, since adsorption is competitive; TNT readily desorbs the nitramines and is itself desorbed to a lesser extent.

One of the most serious deficiencies of carbon system is the current lack of capacity to regenerate the exhausted

Table 1. Freundlich constants for carbon adsorption

Chemical	K (L/mg)	n
TNT	0.337	5.429
RDX	0.112	2.938
HMX	0.168	2.169
TAX	0.100	3.498
SEX	0.219	2.853

carbon. Although both chemical and thermal regeneration techniques have been studied, the results are not promising. As a consequence, the carbon is currently disposed by incineration after a single use. This lack of regenerative capacity results in three disadvantage for the carbon system:

(1). The expense of carbon system treating TNT are much higher than those of other industrial wastewater.

(2). There is no potential to recover and reuse the TNT capture by the carbon

(3). Incineration of spent carbon create potential air pollution source.

The ultraviolet (UV) photolysis unit also employed in TNT treatment research by Burrows et al (1984). The results show That TNT is attacked slowly by UV radiation or by ozone alone; in combination, UV radiation and ozone readily attack TNT with complete ring destruction, but with transient appearance of trinitrobenzoic acid. Hydrogen peroxide alone does not attack TNT, nor is the reaction subject to metal ion catalysis or promotion by ultrasound. A maximum rate of disappearance is achieved with UV radiation and 0.01 percent hydrogen peroxide. The combination of UV radiation and ozone can be used to remove TNT from small process stream. It may be economically competitive with activated carbon; for wastewater flows in the large flow rate, power requirements could be prohibitive.

Polymeric resin adsorption has been studied for the TNT wastewater at pilot scale. Polymeric resin, although having a lower adsorption capacity than activated carbon; has the potential advantages of easy chemical regeneration and a long life cycle. One disadvantage is the leakage of color compounds through the polymeric resin column.

Wet air oxidation (WAO) can be used effectively to treat aqueous waste streams which are too dilute to incinerate and yet too toxic to biotreat. The WAO process has been used extensively for the treatment of hazardous

wastes. It refers to the aqueous phase oxidation of organic and inorganic materials at elevated temperature and pressure. Oxidation takes place through a family of related oxidation and hydrolysis reactions at temperatures of 347 to 608 ^{o}F (175 to 320 ^{o}C) and at pressures of 300 to 3000 psig (2169 to 20708 kPa). The enhanced solubility of O_2 in aqueous solution at elevated temperature provides a strong driving force for oxidation. Elevated pressures are required to keep water in the liquid state.

According to Dietrich et al. (1985) report, bench, pilot, and full-scale performance data have indicated that WAO can be very effective in treating various toxic and hazardous industrial wastewaters. In particular, wastes containing phenols, pesticides, herbicides, and other hazardous organic compounds are amenable to Wet Air Oxidation. Wastewaters with high concentrations of these materials can generally be detoxified to allow subsequent biological treatment, or, in some cases, direct discharge. So, it will be a new alternative to treat the TNT-containing wastes.

CHAPTER III

General conceptions of microbial degradation

Polyaromatic degradation

As we know, disposal of persistent organic pollutants by physico/chemical methods (such as: incineration, carbon system) is very expensive. So, biodegradation is of great interest from the economic point for its potential to cleanup the persistent contaminants.

Fungi is one of the principal degraders of organic compound. Because organic nutrients are often presented to fungi as large, insoluble macromolecular complexes, these complexes must first be degraded to smaller substituents. The white-rot fungus is a potentially useful microorganism in waste treatment system because this fungus is able to degrade a broad spectrum of structurally diverse organic compounds to carbon dioxide.

The white-rot fungi normally grow on decaying wood and forest litter, and appear to be unique among microorganisms in that they can rapidly depolymerize and mineralize lignin. Lignin is a naturally occurring, highly complex, nonrepeating heteropolymer that provides structural support in woody plant.

In order to understand biodegradation of lignin, Kirk et al(1987) have studied the various cultivation conditions affecting the metabolism of lignin by using synthetic ^{14}C-lignins in defined media.

The results are shown as below:

(1). The effect of O_2 concentration in the gas phase above non-agitated cultures indicated essentially complete absence of attack on the lignin polymer at 5 % O_2 in N_2, and a 2- to 3-fold enhancement by 100 % O_2 as compared to air (21 % O_2).

(2). Agitation of the cultures resulting in the formation of mycelial pellets greatly suppressed lignin decomposition.

(3). The optimum culture PH for lignin decomposition was 4 to 4.5 with marked suppression above 5.5 and below 3.5.

(4). The source of nutrient nitrogen had little influence in lignin decomposition, but the concentration of nitrogen was critical; decomposition at 24 mM nitrogen was only 25-30 % of that at 2.4 mM N.

(5). Thiamine was the only vitamin required for growth and lignin decomposition.

To apply this technology in water pollution control, Eaton et al(1982) reported that the ligninolytic fungi can decolorize the first alkali extraction stage (E1) effluent from kraft chlorine bleach plants. Practical aspects of partial decolonization have been studied using *P. chrysosporium*. Biological Oxygen Demand (BOD) and Chemical Oxygen Demand (COD) were reduced by about 40 % during 60 % color reduction.

Color reduction has been scaled up using the rotating biological contactor (RBC). Batch experiments in the RBC with a volume of 2.5 liters of a 35/36 mixture of C1/E1 (3600 color units per liter or 13,590 NCASI units) with glucose as growth substrate has shown that color removal is probably first order. After an initial lag period for growth, color decreased at a rate of approximately 100 color units per hour. Rates of color removal were similar to those achieved on a laboratory flask scale. The fungal mycelia on the discs of one reactor have been used continuously for over 35 days with no apparent loss in decolorizing capacity. The fungus did not slough off the discs; thus, there is no additional disposal burden.

Furthermore, studies showed that numerous chlorinated organics are produced during the bleaching of pulp with chlorine. These compounds are known to be hazards in the environment, and their discharges are regulated by EPA. In

reponse to these potential regulations, the pulp and paper industry has made numerous efforts to reduce chlorinated organic discharges. Huenh et al (1985) used the RBC process to treat this wastewater. E1 effluent is used as the substrate for fungal decolonization. Most of the work has been done with the white-rot fungus, *Phamerochaete chrysosporium*. This fungus is known to have an active enzyme system capable of degrading lignins and modified lignins.

The experimental data (Table 2) shows that most of these chlorinated phenols and other low-molecular-weight components in the effluent are removed by the RBC system.

Studies of the lignin-degrading system of *phamerochaete chrysosporium* have shown that in nitrogen-, carbohydrate, of sulfur-deficient cultures this fungus secrets a unique H_2O_2-dependent extrocellular lignin-degrading enzyme system. Because of its ability to generate carbon-centered free radicals, this enzyme system is able to catalyze numerous nonspecific cleavage reactions on the lignin" lattice". The ability of this fungus to degrade lignin and to metabolize halogenated aromatics suggested to researchers that more recalcitrant organohalides might also be degraded by this organism.

Bumpus(1985) studies with ^{14}C-labeled compounds demonstrated that the tested organpollutants tested [DDT,2,4,5,2′,4′,5′-hexachlors biphenyl](2,4,5,2′,4′,5′-HCB),3,4,3′,4′-tetrachlorobiphenl (3,4,3′,4′-TCB),2,3,7,8-

Table 2. Chloroform extractable aromatics obtained from bleach plant effluent (E1) before and after exposure to the white-rot fungus

Compound	Reference number	RT	Before exposure	After exposure
Benzonic acid	12	5.40	0.12-0.45	none
Dichlorobenzoic acid	13	5.48		none
2,4,6-Tricloro-phenol	1	7.47	0.15-0.33	trace
Vanillin	25	8.05	0.01-0.02	0.05-0.1
4,5 Dichloro-guaiacone	2	9.18	0.08-0.25	trace
Acetoguaiacone	10	9.22	trace	none
6-chlorovanillin	8	10.22	0.05-0.1	trace
Trichloroguaiacol	4	10.67	0.15-0.34	trace
5-Chlorovanillin	7	11.05	trace	none
4,5,6-Trichloro-guaiacol	3	12.74	0.04-0.1	trace
Tetrachloro-guaiacol	5	14.06	0.08-0.15	trace
Vanillyl alcohol	21	8.67	none	0.05-1.2
Veratraldehyde	20	9.11	none	0.08-1.3

- to be continued -

Veratryl alcohol	19	9.37	none	10-20
4,5-Dichloroveratrole	22	9.59	none	0.10-0.2
4,5,6-Trichloro-veratrole	27	11.66	none	trace
3,4-Dimethoxy-acetophenone	28	10.39	none	trace
5-Chloroveratryl alcohol	235	11.84	none	trace
6-Chloroveratryl alcohol	235	12.20	none	0.50-0.8

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tetrachlorodibenzo-p-dioxin (2,3,7,8-TCDD), lindane, and benzol [a]pyrene were oxidized to $^{14}CO_2$ by nitrogen-deficient, ligninolytic cultures of *P. chrysosporium*. Because the radioactive labeling in these compounds was restricted to the ring carbons, researcher is sure that *P. chrysosporium* is able to degrade haloginated aromatic rings. During the 30-day incubation period, $^{14}CO_2$ releasing was first observed between day 3 and day 6, then the production continued at decreasing rates until the end of the incubation.

Bumpus (1987) reported that the white-rot fungus *P. chrysosporium* has the ability to degrade a wide variety of structurally diverse organic compounds, including a number of enviromentally persistant organopollutants. Table 3 lists over fifty compounds that are known to be degraded by *P. chrysosprium*.

Up to now, most biodegradation experiments with this microorganism have focused on its ability to degrade pure compounds. Unfortunately, chemical contamination by mixtures of chemicals is much more common than contamination by a single compound. Thus, in order to apply biological processes in practical use, it must be able to degrade all or most of the individual components of these mixtures.

As we know, PAH_S are widespread environmental pollutants. Bumps (1989) have conducted the experiment to demonstrate the degrading ability of the white rot fungus *P. chrysosporium* toward polycyclic aromatic hydrocarbons (PAH_S) which are present in anthraceme oil (a distillation product obtained from coal tar).

The results showed that at least 22 PAH_S, including all of the most abundant PAH components present in anthracone oil, underwent 70 to 100 % removal during 27 days of incubation with nitrogen-limited nutrient cultures. Because phenanthrene is the most abundant PAH present in anthracene oil, this PAH was selected for further study. During experiments, [^{14}C] phenanthrene was incubated with *P. chrysosporium* culture containing anthracene oil for 27 days. Results shown that 7.7 % of the recovered radiolabeled carbon originally present in [^{14}C] phenanthrene was

TABLE 3. Organic compounds degraded by *P. Chrysosprium*

Aromatic compounds	Polycyclic aromatic compounds
Vanillic acid	Benzo[a]pyrene
Ferulic acid	
2,4-Dihydroxybenzoic acid	
4-Hydroxy-3-methoxybenzaidehyde	
Isovanillic acid	Biopolymers
Syringic acid	
Curcumin	Lignin
4-Hydroxy-3-methoxyphenylacetic acid	Cellulose
7-hydroxy-4-methylcoumarin	Kraft lignin
2,6-Dihydroxybenzoic acid	3-Chloroaniline-
2′-Hydroxy-3′-methoxyacetophenone	lignin conjugate
4′-Hydroxy-3′-methoxyacetophenone	3,4-Dichloroani-
6.7-Dimethoxycoumarin	line-lignin conjugate
7-Hydroxycoumarin	
Gentisic acid	
Guaiacol	
4-Hydroxy-3-methoxymandelic acid	
Protocatechuic acid	
3′,4′-Dihydroxyacetophenone	
Gallic acid	
2′,3′-Dihydroxy-4′-methoxyacetophenone	
6.7-Dihydroxy-4-methycoumarin	

(- to be continue -)

3.5-Domethylcatechol

2′,3′,4′-Trigydroxyacetophenone

Pyrogallol

Catechol

3-Methylcatechol

3,4-Dimethylcatechol

4-Methylocatechol

Benzoic acid

Acetoguaiacone

Vanillin

Veratryl alcohol

Veratraldehyde

Vanillyl alcohol

Lignin model compounds

Veratrylglycerpl- -(O-methoxyphenyl) ether

Guaiacylglycerol- - coniferyl alcohol ether

Dehydrodiconiferyl alcohol

Dehydrodivanillin

Chlorinated armomatic compounds

4-Chlorobenzoic acid

Dichlorobenzoic acid (isomer unknown)

2,4,6-Trichlorophenol

(- to be continue-)

4,5-Dichloroguaiacol

6-Chlorovanillin

Trichloroguaiacol(isomer unknown)

5-Chlorovanillin

4,5,6-Trichloroguaiacol

Tetrachloroguaiacol

3-Chloroaniline

3,4-Dichloroaniline

Polycyclic chlorinated aromatic compounds

DDT (1,1-bis(4-chlorophenyl)-2,2,2-trichloroenthane)

2,3,7,8-Tetrachlorodibenzo-p-dioxin

3,4,3′,4′-Tetrachlorobiphenyl

2,4,5,2′,4′,5′-Hexachlorobiphenyl

Arochlor 1254

Non-aromatic chlorinated compounds

1,2,3,4,5,6-Hexachlorocyclohexane (Lindane)

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metabolized to $^{14}CO_2$ and 25.2 % was recovered from the aqueous fraction, while 56.1 and 11.0 % were recovered from methylene chloride and particulate fraction, respectively. These results suggest that this microorganism may be useful for decontamination of sites contaminated with PAHs.

Photodegradation by sunlight or ultraviolet light (UV) has also been considered as a potential technology to detoxify the contaminated site. Katayama and Matsumura (1991) conducted the experiment with the idea of combining photochemical and microbial technologies. The initial study was using [u-^{14}C] TCDD established conclusively that simultaneous application of UV irradiation (at 300 nm, 2 hr/day) and *P. chrysosporium* in a nitrogen-deficient medium caused a much more accelerated rate of mineralization of this compound than those achieved by either photochemical action alone or microbial action. These results clearly showed that UV irradiation could act synergistically with the microbial degradation activities.

The rates of removal of DDT, dieldrin, heptachlor, 3,4,3′,4′-tetrachlorobiphenyl, toxaphenl, and TCDD were studied in their series experiment. The removal of the original compounds were monitor by gas chromatography.

CHAPTER IV

Biological Methods to treat TNT

To evaluate the feasibility of using biological processes for the treatment of TNT wastewater, Marshall and Clifford (1974) had conducted Biochemical Oxygen Demand(BOD) testing procedures to investigate the degree of biodegradability and microbial toxicity of the waste. Experimental results are listed in Table 4.

TABLE 4. Summary of Results of BOD Tests on TNT Wastes

Dilution of TNT Waste (%)	5-Day BOD (mg/l)	K1 (1/day)	Ultimate BOD (mg/l)	TNT-IN (mg/l)	TNT-OUT (mg/l)	TNT-Removal (mg/l)
2.5	450	0.277	610	1.0	0.1	0.9
5	220	0.377	249	2.0	1.4	0.6
7.5	190	0.269	230	3.0	2.2	0.8
10	180	0.261	217	4.0	3.3	0.7
10	79	0.399	82	4.0	3.6	0.4
20	48	0.340	56	8.0	7.3	0.6
30	40	0.343	41	12.0	11.5	0.5
40	36	0.321	38	16.0	15.0	1.0

The results show that the TNT waste does exert a BOD, but it is extremely concentration dependent. Because the ultimate,

first-stage carbonaceous BOD decreased as the dilution factor or food to microorganism (F/M) ratio increased in magnitude, it seems that TNT does exhibit a toxic or inhibitive effect on the biomass. The low TNT removal percentage achieved indicate that the oxidation of TNT requires a long period of time.

After the BOD testing has been completed, treatability study of the TNT combination waste were also conducteded by using acclimated activated sludge in continuous-flow reactor. Treatability experiments with the TNT wastewater were accomplished by mixing the waste with the plant's domestic waste. From series experiments, an important phenomenon was noticed that the TNT concentration in the mixed liquid suspended solids (MLSS) increased during continuous operation, demonstrating that adsorption rate is greater than the oxidation rate. Experiments also shown that in a contiouous-flow biological treatment system, as the TNT wastewater is neutralized, the color increase and the wastewater become more resistant to biological degradation. Consequently, for successful biological treatment of TNT wastes, it would be necessary to modify the conventional actvated sludge system (may use extended-aeration activated sludge system) or find another effective alternatives.

Won et al.(1974) conducted the experiment regarding metabolic disposition of 2,4,6-trinitrotoluene (TNT). The

results of studies indicate that biological treatment can be an effective and efficient method for disposing of TNT wastewater before it is discharged from the processing plant to receiving water. Accordingly, three pseudomonas-like organisms have been shown to metabolically oxidize 2,4,6-trinitrotoluene. Results of respirometrioc studies indicate that TNT is a biologically oxidizable substrate. Furthermore, those microorganisms grown without a lag period.

TNT degradation at PH 6.5 to 7.2 appeared to be more efficient than those of lower or higher PH values. To accelerate TNT degradation, addition of glucose or a nitrogenous substance was essential. It has been shown that TNT disappeared most rapidly in cultures supplemented with yeast extract. Within 24 hours, the TNT concentration was reduced from 100 ug/ml to less than 1 ug/ml, whereas a substantial amount of TNT persisted up to 96 hours in the corresponding control and glucose-supplemented cultures. It was also mentioned that TNT was metabolized to yield (in descending order) 2,2′,6,6′-tetranitro-4-azoxy toluene, TNT's isomer 2,2′,4,4′-tetranitro-6-azoxytoluene, 4,6-dinitro-2-aminotoluene, 2,6-dinitro-4-hydroxyl-aminotoluene, and nitrodiaminotoluene. After depletion of TNT (24 hours) the azoxy compounds are shown to degrade gradually, approaching complete disappearance at 96 hours.

Information from above research have far-reaching possibilities in the solution of TNT waste disposal problem. Further studies on the metabolism of these and other isolate organisms, including artificially induced mutants, may lead to a more rapid and complete TNT degradation process.

Kearney et al (1983) first treated [^{14}C]-TNT by ultraviolet (UV) ozonation and then subjected the products to microbial degradation by *Pseudomonas putida*. They found that the former treatment helped the metabolic degradation of TNT. The effect of concentration on TNT degradation after 10 minutes irradiation using the 450 W lamp in the presence of O_3 is reported in this paper. Experiments were conducted at various [^{14}C]-TNT concentration and loss of [^{14}C]-TNT due to degradation was measured before and after the 10 minutes irradiation. It is clear that the removal of TNT during UV-O_3 is decreased as the concentration increases. However, there has been no successful demonstration of simultaneous application of these two technologies (i.e., use of isolated microbial and ultraviolet treatments) for the degradation of TNT.

Kanekar and Godbole (1984) isolated some microorganisms, *Pseudomonas trinitrotoluenophila*, *Citrobacter freundii* and *Bacillus polymyxa*, from soil exposed to explosive waste for over 50 years were found to be effectively degrading TNT in synthetic medium. The

reduction in COD of the explosive waste brought about by these organisms ranged from 20 to 50 %. It was further seen that the degradation of TNT was optimum at PH 5, incubation temperature of 28 ^{o}C under stationary culture condition.

Bumps (1987) studies has shown that the wood-rotting fungus, *Phanerochaete chrysosporium*, is able to degrade a wide variety of environmentally persistent pollutants to carbon dioxide. The refractory pollutants include a number of chlorinated hydrocarbons such as DDT [1,1,1,-trchlori-2,2-bis(4-chlorophenyl) ethane], lindane (1,2,3,4,5,6-hexachlorocy-clohexane), chloroanilices, and polychlorinated biphenyls (PCB). Recent evidence shows that the white-rot fungus has the ability to degrade a diverse group of compounds is dependent on the nonspecific and nonstereoselective lignin-degrading system which is expressed by this organism under nutrient (nitrogen, carbon, or sulfur)-limiting conditions.

It becomes a great concern whether *P. chrysosporium* could grow in the presence of a highly toxic contaminant like TNT at concentrations that occur in the environment and whether it would degrade the TNT. Because *P. chrysosporium* has the ability to degrade a wide variety of environmental persistent organopollutants to carbon dioxide. We have assumed that this organism may be useful in certain hazardous waste treatment systems.

Fernando et al(1990) conducted this experiment to determine whether *P. chrysosporium* could degrade the TNT waste. In this study, the TNT concentration was adjusted equivalent to contamination levels found in the environment and was tested for its ability to mineralize [^{14}C] TNT in liquid at level that may be encounted in the environment (TNT=100 mg/l). Rate of mineralization were obtained, and mass balance analyses were performed after 30, 60, and 90 days for the liquid culture. As a mass balance analysis was performed, the data showed 35.4 % of the total radioactivity was evolved as ^{14}-CO_2, 25.1 % was found in the methylene chloride fraction, and 17.3 % was associated with the mycelial fraction. A total mass recovery of 93.5 % was achieved.

This study shows that the wood-rotting (white rot) fungus *P. chrysosporium* is able to cause extensive degradation of [^{14}C]TNT in a reasonably short period of time. Degradation was demonstrated by mineralization of [^{14}C]TNT, metabolite formation, and mass balance analyses. The results of this study indicate that biological treatment systems which utilize *P. chrysosporium* could be used as an effective method for remediation of TNT-contaminated water.

CHAPTER V

Results and Discussion

All researches have shown that TNT is quite resistant during biodegradation. It is because the aromatic ring is not very easy break. But this is not to say that TNT is totally refractory to biodegradation. Because the specific growth rate (u) of those TNT degrading microorganisms is very small, which means that long retention time is required for those microorganisms to degrade the TNT. A number of microorganisms have already been isolated that mediate substantial biodegradation of this compound. For example, Won et al.(1974) have reported that pseudomonas-like bacteria could cause extensive degradation of TNT to several intermediates and even totally degraded.

Similarly, a number of fungi have been shown to be able to degrade TNT as assayed by TNT disappearance from culture. It shall be note that the fungus *Rhizopus stolonifer* was able to mediate near complete disappearance of TNT from cultures containing 100 mg of TNT per liter.

A major objective of this research is to identify the biodegradability of TNT and which can recommend to treat water, soils, sediments, and other materials that are contaminated with TNT. In this study, we have also found that *P. chrysosporium* is able to extensively degrade TNT.

CHAPTER VI

Conclusions and Recommendation

The results of these studies indicate that TNT wastes can be degraded by appropriate microorganisms under adequate conditions.

Numerous strategies have been used in the aerobic treatment of TNT contaminated waste. Among these are activated sludge, aerated lagoons, aerobic digestion, trickling filters, rotary biological contactors (RBC), and aerobic composts. The effectiveness of these systems is ultimately dependent upon the microorganisms present in the system. Thus it is critical that the most appropriate organisms be selected. It has been shown that the following organisms can effectively degrading TNT:

(1). *Pseudomonas putida*.

(2). *Rhizopus stolonifer*.

(3). *Phanerochaete chrysosporium*

Recommendation for biological treatment TNT wastes including the following:

(1). apply photooxidation prior to biological system.

(2). apply wet air oxidation (WAO) prior to biological treatment.

(3). apply extended aeration system.

CHAPTER VII

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