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Degradation of Munition Wastes by *Phanerochaete chrysosporium*

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ABSTRACT

"Pink water" is a waste-water stream generated by munitions LAP (loading, assembly, and packing) operations. The major components of this waste water are trinitrotoluene (TNT) and cyclotrimethylene trinitramine (RDX) at concentrations of 120–175 mg/L and 25 mg/L, respectively. Currently, pink water is treated by activated carbon adsorption. Removal efficiencies of >99.5% have been reported. However, this treatment method suffers a serious limitation in that the carbon cannot be safely regenerated. Loaded carbon is disposed of by incineration after a single use.

We have demonstrated that TNT, RDX, simulated, and actual pink water can be effectively treated by *Phanerochaete chrysosporium* immobilized on the disks of a rotating biological contractor (RBC) in both batch and continuous modes. Greater than 90% removal of TNT from a simulated pink water was observed in a continuous RBC with a residence time of about 24 h. The disk area required was about 10,000 ft^2/gpm (4091 m^2/m^3h) feed. RDX was amenable to treatment, but RDX removal rates were somewhat slower. A full-scale treatment system was designed on the basis of laboratory data, and a cost analysis was performed. This analysis has shown that biotreatment of pink water can be a cost-effective alternative to carbon adsorption.

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Index Entries: Pink water; trinitrotoluene; white-rot fungus; *Phanerobhaete chrysosporium;* munitions wastes.

INTRODUCTION

Pink water is a waste-water stream generated primarily from trinitrotoluene (TNT) loading, assembly, and packing (LAP) operations in munitions plants. The waste stream may also contain other formulation constituents, such as cyclotrimethylene trinitramine (RDX) and cyclotetramethylene tetranitramine (HMX). Pink water derives its name from the pink color that is produced by photochemical reactions of the nitroaromatic compounds present in the water. The concentrations of TNT and RDX vary with the type of operation, but average 120–175 mg/L TNT and 25 mg/L RDX. The current industrial method for treating pink water is adsorption of nitroaromatic compounds on activated carbon. Following particulate removal by multilayer filtration, pink water is passed through two carbon beds in series. Removals of 99.5% for both TNT and RDX have been reported. However, the method suffers from the serious deficiency of the inability to regenerate the carbon. The current practice is to dispose of spent carbon by incineration after a single use (*1*).

Biological treatment of pink water is an attractive alternative to carbon adsorption. However, the nitroaromatic components of pink water are generally recalcitrant to biodegradation, and their toxicity both to microorganisms and to aquatic life in receiving waters makes conventional biological treatment difficult. For example, TNT has been shown to be toxic to fathead minnows and bluegills at concentrations of 2–3 μg/mL (*2*).

This article describes a promising biological treatment of pink water using the white-rot fungus, *Phanerochaete chrysosporium,* immobilized on the disks of a rotating biological contactor (RBC). *P. chrysosporium* belongs to a family of wood-rotting fungi found throughout the Northern Hemisphere. The fungus produces a number of extracellular enzymes that act as peroxidases degrading lignin, which is a complex aromatic polymer that is otherwise very resistant to decay. The complex structure of lignin dictates that these enzymes exhibit low substrate specificity. Not surprisingly, as nonspecific peroxidases or ligninases, these enzymes have found numerous applications in the degradation of compounds that are otherwise considered biologically recalcitrant (*3,4*).

P. chrysosporium is aerobic, and exhibits optimum growth at pH 4.5 and 39–40°C. Ligninases are generally produced under conditions of nitrogen starvation, although carbohydrate, sulfur, and trace element imbalance have also been reported to result in ligninase production (*5*). As noted above, *P. chrysosporium* has been shown to mineralize completely certain recalcitrant compounds, such as chlorohydrocarbons. However, an auxiliary carbon source, such as glucose, is required to sustain viability.

MATERIALS AND METHODS

Organism and Culture

Phanerochaete chrysosporium (ATCC 24725) was obtained from Tom Joyce at North Carolina State University (Rayleigh, NC). The organism was grown supported on the disks of two Plexiglass RBCs. The RBCs consisted of eight 17.8-cm diameter disks mounted on a horizontal shaft and placed coaxially in a horizontal semi-cylindrical tank with a water jacket for temperature control. The RBCs also featured a semi-cylindrical cover to retain moisture and through which pure O_2 was introduced typically at 10–15 mL/min. The disks were rotated at six rpm, while approx 40% of the disk surface was submerged in medium at any given time. Each RBC tank was partitioned into four compartments with each compartment accommodating two disks. The partitions contained 1.2-cm holes, so that in a continuous-flow mode, the medium when introduced in compartment 4 would flow through compartments 3, 2, and 1 in succession and exit the system through an overflow nozzle. The total reactor volume was 8.6 L with a liquid capacity of 2 L. Each disk had an active area of approx 500 cm^2.

Sporulating fungus was grown in 1000-mL Roux bottles on yeast malt agar consisting of 0.1% glucose, 1.0% malt extract, 0.4% peptone, 0.2% yeast extract, 0.01% asparagine, 0.2% KH_2PO_4, 0.1% $MgSO_4 \cdot 7H_2O$, and 1.5% agar at pH 4.5. Suspensions of spores were obtained by washing sporulating fungus with sterile growth medium consisting of (concentrations in m*M*): KH_2PO_4 (14.7), $MgSO_4 \cdot 7H_2O$ (2.0), $CaCl_2$ (0.90), thiamine HCl (0.003), NH_4Cl (2.2), and glucose (55.5) at pH 4.5. Approximately 200 mL of sterile growth medium were used to wash the contents of one Roux bottle. The resulting turbid spore suspension was diluted with 1800 mL of sterile growth medium and dispensed equally into the four compartments of one RBC. After 2 d of growth, the spent growth medium was replaced with fresh medium, and the fungus allowed to grow for two more days. At the end of this time, the disks were covered with the grayish-white mycelial growth of the white-rot fungus about 2–3 mm thick on each side of the support disks.

The two RBCs were operated in parallel initially in a batch mode. In this mode, the four compartments in each RBC were isolated by stoppering the hole in the partitions. After a week of testing in the batch mode, one of the reactors (RBC-1) was converted to a continuous mode, whereas the other reactor continued in a batch mode. After about 11 d of operation, RBC-2 was also converted to a continuous mode and received the effluent of RBC-1 as feed (Fig. 1). As described below, the treatability of a simulated pink water, an actual pink water, and water containing TNT or RDX only was studied.

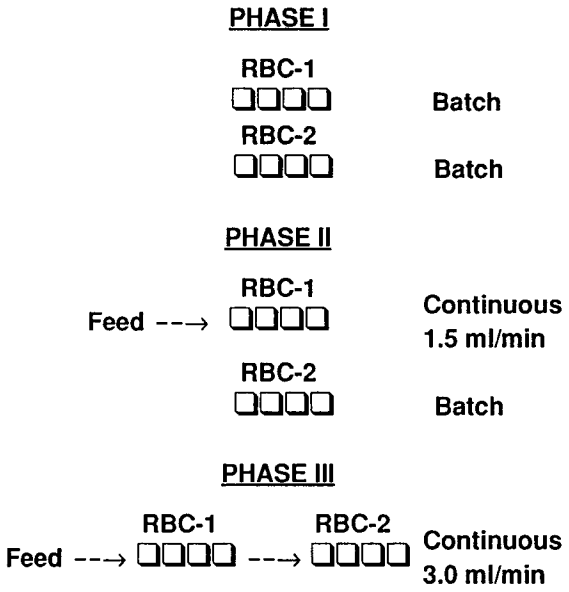

Fig. 1. RBC operating modes.

Chemicals

Practical grade TNT was obtained from Eastman Kodak (Rochester, NY) and recrystallized from CS_2 in our laboratory. The purity of recrystallized TNT (> 99%) was confirmed by a melting point of 80.5°C. RDX was provided through the courtesy of Accurate International (McEwen, TN). Actual pink water was obtained from a US Army munitions plant, and analyzed for TNT and RDX only as described below.

Degradation of Nitroaromatic Compounds by *P. chrysosporium*

Batch tests were started as follows. Following 4 d of fungal growth, the spent medium was replaced with "working medium" containing various concentrations of TNT and/or RDX in the various compartments of the RBCs. In this manner, a total of 35 batches were tested. The working medium was nitrogen-limiting and was identical to the growth medium, except that the NH_4Cl concentration was only 0.65 m*M*, and the working medium also contained the following trace nutrients and a surfactant (concentrations in μM, except where indicated): nitrilotriacetic acid (712), $MnSO_4$ (298), $FeSO_4$ (59), $ZnSO_4$ (56), $CaSO_4$ (66), $AlK(SO_4)_2$ (3.5), H_3BO_3 (14.6), $NaMoO_4$ (5.0), $CuSO_4$ (5.6), Tween 80 (1.0 g/L), and benzyl alcohol (8000). Benzyl alcohol was used as an enzyme inducer to increase the rate of ligninase production. Tween 80 was used to promote the release of ligninases from the fungus (*6*).

Under continuous-flow conditions, the same working medium (with varying concentrations of TNT and RDX) was used at a flow rate of 1.5 mL/min giving a residence time of approx 24 h in RBC-1. When RBC-1 and RBC-2 were connected in series, the feed rate was increased to 3.0 mL/min. All experiments were conducted at 39–40°C.

Analytical

RBC medium samples were analyzed for TNT and RDX by high-performance liquid chromatography (HPLC) using a Waters HPLC with a Model 440 UV detector and Hewlett Packard automatic peak integrator. UV absorbance at 254 nm was used to detect aromatic and nitro compounds without interference from inorganic salts or other nutrients in the medium. A 60:40 mixture of acetonitrile and water was used as the mobile phase at a flow rate of 3 mL/min on a 3.9 mm × 15 cm Waters (Milford, MA) Bondapak C-18 reverse-phase column. The retention times for TNT and RDX were 4.9 and 3.8 min, respectively.

RESULTS AND DISCUSSION

Out of 35 batch experiments, 24 used only TNT in the feed at initial concentrations of 40–60 mg/L. The balance consisted of three batches with actual pink water, two with RDX only, and seven with simulated pink water containing TNT and RDX. Typical results are shown in Figs. 2–4. In all of these tests, TNT was removed to < 3 mg/L in about 24 h. In experiments that used RDX alone, the RDX was observed to be degraded, although more slowly than TNT. In experiments using actual pink water, however, the RDX concentration appeared to increase with time (while TNT was degraded). This observation has been preliminarily attributed to the production of an intermediate from the degradation of TNT, which had a retention time on HPLC similar to that of RDX. This intermediate appeared only when the initial TNT concentration was high as in the actual pink water. An RDX-like component was also found in batch experiments containing only TNT at concentrations of about 7–10 mg/L (using an RDX standard) after 24 h.

The results of the continuous experiments are summarized in Figs. 5 and 6. The initial feed to RBC-1 contained only TNT in working medium. At a feed rate of 1.5 mL/min, the disk area to flow ratio was approx 10,000 ft^2/gpm (4091 m^2/m^3 h). As seen in Fig. 5, the TNT concentration in RBC-1 was consistently low. After about 78 h, the "RDX-like" component was also evident in the effluent. After 98 h, the TNT feed was replaced with a simulated pink water (TNT and RDX in working medium). Over the next 163 h of operation, TNT removal averaged 91–94%, whereas apparent RDX removal averaged 65–78%. After 261 h of operation, the feed rate

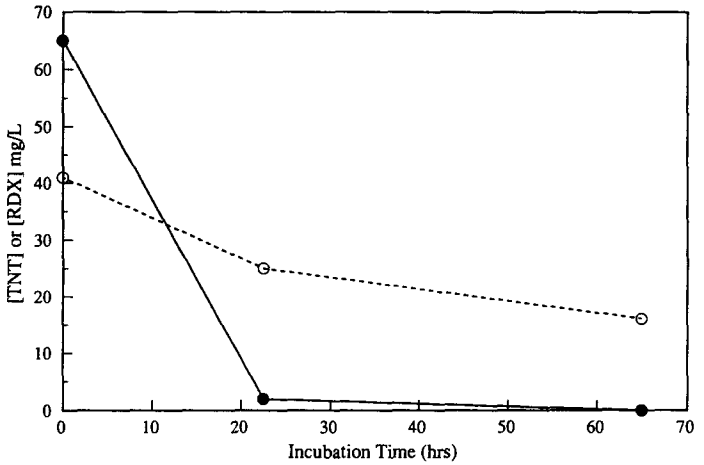

Fig. 2. Results of batch treatment of TNT and RDX alone by *P. chrysosporium* in an RBC. —●— TNT: --○-- RDX.

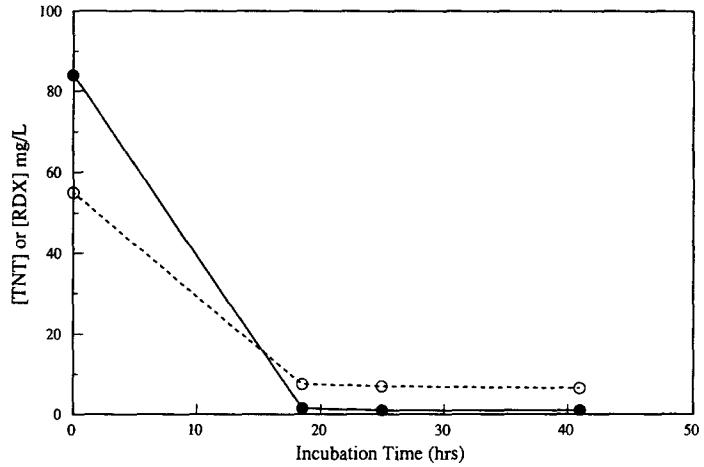

Fig. 3. Results of batch treatment of simulated pink water by *P. chrysosporium* in an RBC. —●— TNT: --○-- RDX.

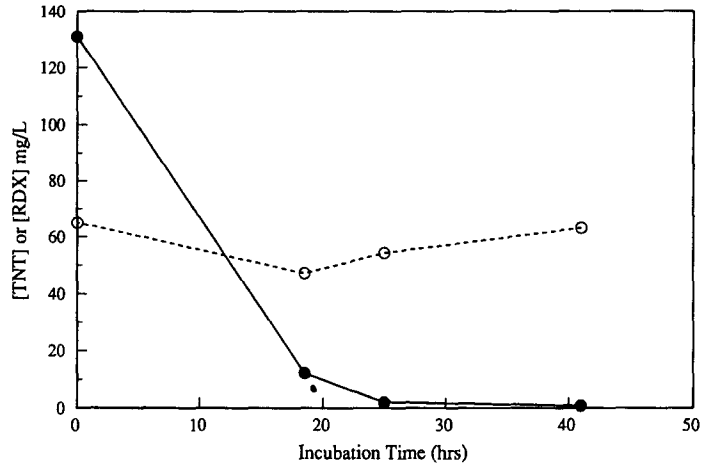

Fig. 4. Results of batch treatment of actual pink water by *P. chrysosporium* in an RBC. —●— TNT: --○-- RDX.

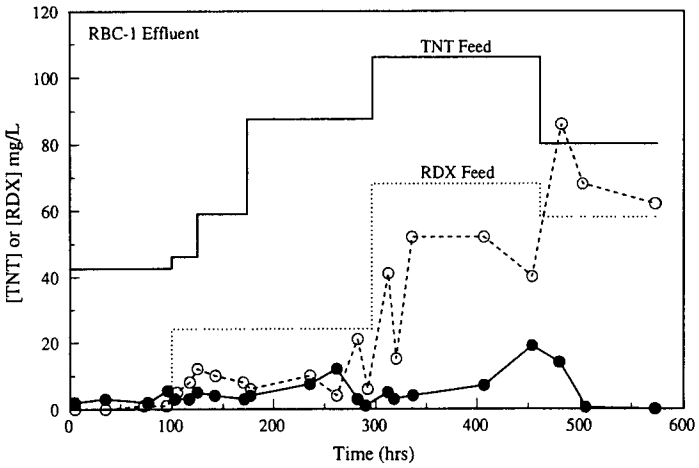

Fig. 5. Results of continuous treatment of TNT and simulated pink water by *P. chrysosporium* in RBC-1. (At $t = 261$ h, the feed rate to RBC-1 was increased from 1.5 mL/min to 3.0 mL/min, and the effluent of RBC-1 fed to RBC-2.) —●— TNT: --○-- RDX.

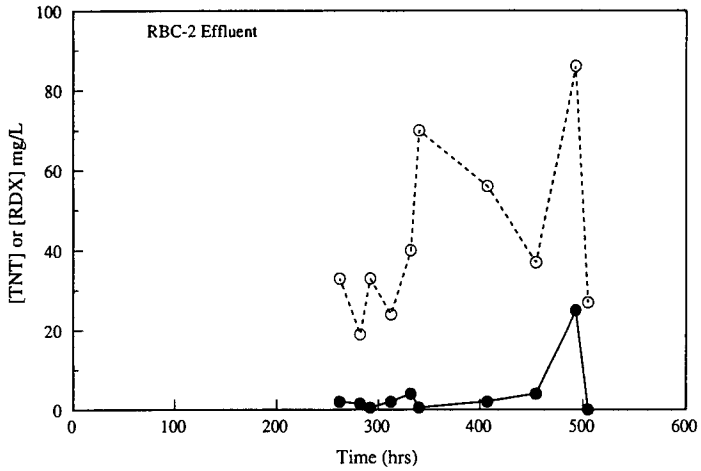

Fig. 6. Results of continuous treatment of TNT and simulated pink water by *P. chrysosporium* in RBC-2. (After $t = 261$, the feed rate to RBC-1 was increased from 1.5 mL/min to 3.0 mL/min, and the effluent of RBC-1 fed to RBC-2.) —●— TNT: --○-- RDX.

(and subsequently the feed concentration) was increased to 3.0 mL/min, and the effluent of RBC-1 used as feed to RBC-2. TNT was consistently removed to very low levels in the final effluent of the treatment system. However, the RDX results, as seen in Fig. 6, are not consistent presumably because of the production of the TNT intermediate, which cochromatographed with RDX in the HPLC. Several attempts to separate RDX and the RDX-like components in these effluents by change of HPLC flow rate, mobile phases, and so forth, failed.

Table 1
Pink Water Composition and Flow Rates
Used as a Design Basis for Bio/Activated Carbon Process

Flow rate, gpd (m^3/d)	Case A—50,000 (189.3)	Case B—170,000 (643.5)
TNT, mg/L	150	
RDX, mg/L	70	
Oil, mg/L	200 max	
COD, mg/L	280	
Temperature	Ambient (approx)	
pH	Neutral (approx)	
On stream factor	8000 h/yr	

Although not shown in Figs. 5 and 6, the different compartments of RBC-1 and RBC-2 were also analyzed periodically for TNT and RDX. In each case, there was no significant difference in the compartment concentrations and the reactor effluent. This indicates that either the flow regime in each reactor was closer to a stirred tank than plug-flow, or in each reactor all degradation occurred in the first compartment.

PRELIMINARY DESIGN AND COST ESTIMATE

Design Basis

The proposed process for treatment of pink water consists of a biotreatment step using *P. chrysosporium* to degrade the bulk of the organic contaminants, followed by an activated carbon polishing step. The active carbon step was designed to produce water quality for either discharge to public waterways or reuse in the plant. The process economics rationale was to reduce the organic load on the carbon-treating step to a level where the biotreatment process became cost-competitive with the existing practice, which is based strictly on carbon adsorption of "pink water" without prior treatment. The design basis for the "pink water" to be treated was derived from a sample received from a US Army munitions plant and is summarized in Table 1. The "pink water" influx rates listed in Table 1 were selected to encompass the minimum and maximum plant capacities likely to be encountered.

Process Description—Biotreatment System

The overall process scheme is shown in Fig. 7. The proposed process consists primarily of a pretreatment system, a bioreactor system, a clarifier, and a sludge dewatering system. As a final polishing step, the effluent water from the bioreactor is treated in an activated carbon adsorption step. This scheme is estimated to be capable of removing TNT to under 1 mg/L and 70% of other organic contaminants (RDX and HMX).

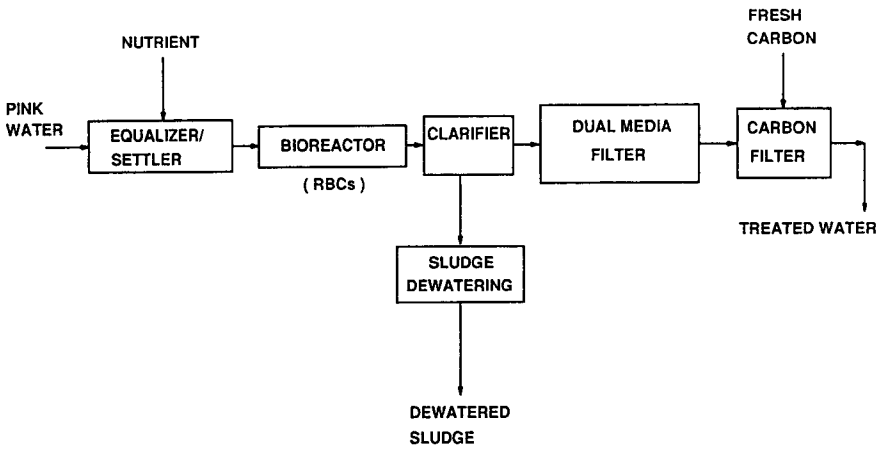

Fig. 7. Block flow diagram of proposed bio/activated carbon process for treatment of pink water.

Based on discussions with the US military, the production and quality of pink water vary considerably within the same operating plant. When this water is produced in weekly washdown of equipment and the plant in general, the water could contain gritty suspended solids and oil. The pretreatment step, therefore, is essentially a holding tank of 24-h capacity with provisions for screening out gritty suspended matter. This tank also serves as a mixing and conditioning tank to add the necessary nutrients for fungal growth (such as glucose, reduced nitrogen, trace minerals) and to adjust the pH to 6. (Oxidation of glucose in the bioreactor results in further decreases in pH.) The pretreatment system will permit equalization of flow and contaminant variations, and result in more stable operation of the biotreater. After conditioning in the pretreatment system, pink water is fed to a continuous RBC system using *P. chrysosporium* for biodegradation of the TNT and RDX.

The RBC system consists of standard units in a series/parallel configuration as required to meet the capacity. The RBC tank has a retention time of 24 h. The system is sized for 85% COD reduction, exclusive of nutrients added. A summary of the biotreater operating conditions is given in Table 2.

The next step of the process is the separation of biosludges from the treated water. Thus, the biotreater effluent is fed to the clarifier. The clarifier bottoms (sludge) are pumped to the filter press for dewatering to 20% solids content. The daily wet cake production is 200 kg (0.20 m^3)) for Case A and 682 kg (0.68 m^3) for Case B. The sludge is water-washed in the filter prior to discharge to remove traces of TNT and RDX. The dewatered sludge is disposed of by truck to a nonhazardous landfill.

The clarifier effluent is pumped through a standard sand/coal dual media filter for suspended solids removal. The filtered water is passed through two carbon beds in series. The carbon adsorption system is sized

Table 2
Bioreactor Operating Conditions for Bio/Activated Carbon Process

	Inlet mg/L	Effluent mg/L
TNT	120	<2
RDX and other organics (exclusive of nutrients)	56	25
COD (calculated)	660	30
BOD	550[1]	25[2]
pH	6.0	4.8
Biodisk area		10,000 ft^2/gpm (4091 m^2/m^3 h)

[1]Estimated based on added nutrients.
[2]Residual nutrients.

for removal of the residual organics (including metabolic waste products of *P. chrysosporium*) to 1 mg/L level. The spent carbon is discharged periodically and trucked to an approved solid-waste incineration service. In modifying a currently operating "pink water" treatment plant, it is possible to use the existing dual media (or equivalent) filters and the carbon beds. This option is included in cost estimates.

Current Practiced Activated Carbon Process

Current practice is to adsorb the TNT, RDX, and other organics contained in "pink water" on activated carbon. The spent carbon is disposed of by incineration. The "pink water" is first passed through a dual-media filter containing sand and coal to remove suspended solids, which would otherwise reduce the life of the activated carbon. The filtered water then flows through two carbon adsorbers in series. When the carbon in the first adsorber is spent, it is taken out of service, and the carbon is replaced. During this period, the second adsorber will function as the primary unit.

Equipment List and Plant Cost

Process equipment and sizings for the bio/activated carbon process are given in Tables 3 and 4. Equipment sizing was based on a residence time of 24 h and the bio-disk area of 10,000 ft^2/gpm (4091 m^2/m^3 h). Atomic absorption analysis of the "pink water" received from military sources indicated that many of the metallic elements added as nutrients in lab experiments are already present in the waste water. Accordingly, in determining the operating costs, only the necessary nutrients have been considered. It is further assumed that all of the nutrient additives are completely consumed, so that the final polishing step with activated carbon

Table 3
Equipment List for Bio/Activated Carbon Process
Treating Pink Water at 50,000 gpd (189.3 m^3/d)—Case A

Item	Quantity	Specs
Settler/equalizer	1	15 ft dia×40 ft TT‡—CS† (4.6 m×12.2 m)
Biotreater	1	347,000 ft^2 (32,236 m^2) biodisks in four stages
Clarifier	1	12 ft×14 ft TT‡, CS† (3.7 m×4.3 m)
Filter press	1	41 kg/d dry solids, 20% wet cake solids content, automatic, CS†
Auxiliary equipment	-	Pumps, chemicals, and nutrients feed systems
Dual media filter	2	Sand/coal, lined CS† pressure vessel, automatic, CS† 3 ft dia×6 ft (0.9 m×1.8 m)
Carbon column system	2	Existing columns in series* (alternate: 2-5 ft dia×10 ft (1.5 m×3.1 m) columns in series; 1364 kg carbon bed in each)

*Assumes carbon columns already existing on installation site.
†CS = carbon steel.
‡TT = tangent to tangent.

can be designed for removal of trace quantitites of TNT and RDX. Based on literature data, the activated carbon requirements were estimated to be approx 8 kg/kg of TNT removed. The carbon requirements for RDX removal were assumed to be the same (*1*).

Equipment sizing for a corresponding carbon treatment process as currently practiced is given in Table 5. Estimated costs of the conventional activated carbon process (without prior biotreatment) are also presented here for comparison with the biotreatment process. This activated carbon process was also designed for a requirement of 8 kg of carbon/kg of TNT and RDX removed. The carbon beds were sized for 45 d of operation.

In-house equipment cost data (previously obtained from vendors) and standard multipliers were used to estimate the total installed cost (TIC). The capital costs are summarized in Table 6. Operating costs consist of variable cost items: chemicals, nutrients, inocula, activated carbon, utilities, sludge disposal and spent carbon disposal; and fixed cost items: labor, maintenance (labor and materials), and overhead. Cost of supervision, analytical and office services are included in the overhead. The operating costs are summarized in Table 7.

Table 4
Equipment Lists for Bio/Activated Carbon Process
Treating Pink Water at 170,000 gpd (643.5 m^3/d)—Case B

Item	Quantity	Specs
Settler/equalizer	1	30 ft dia×35 ft—CS† (9.1 m×10.7 m)
Biotreater	1	1,200,000 ft^2 (111,480 m^2) biodisks in four stages (16 units in series/parallel arrangement)
Clarifier	1	21 ft dia×14 ft TT‡, CS† (6.4 m×4.3 m)
Filter press	1	136.4 kg/d dry solids, 20% wet cake solids contents, automatic, CS†
Auxiliary equipment	–	Pumps, chemicals, and nutrients feed systems
Dual media filter	2	Sand/coal, lined CS† pressure vessel, automatic, 6 ft dia×10 ft (1.8 m×3.1 m)
Carbon column system	2	Existing columns in series* (alternate: 2-7 ft dia×13 ft (2.1 m×4.0 m) columns in series; 4545 kg carbon bed in each)

*Assumes carbon columns already existing on installation site.
†CS = carbon steel.
‡TT = tangent to tangent.

Table 5
Equipment List for Conventional Activated Carbon System
for Treatment of Pink Water

Item	Quantity	Specs
Dual media filter	2	Sand/coal, lined CS pressure vessel, automatic, CS CaseA—3 ft dia×6 ft (0.9 m×1.8 m) Case B—6 ft dia×10 ft (1.8 m×3.1 m)
Carbon column	2	Case A—12 ft dia×14 ft (3.7 m×4.3 m) CS columns with 15,000 kg activated carbon bed Case B—4 parallel 12 ft dia×12 ft CS columns with 12,730 kg carbon bed in each
Auxiliary equipment	–	Pumps, feed systems

Table 6
Capital Cost Summaries

	Direct cost*	TIC†
	$1000	$1000
Case A—50,000 gpd (189.3 m^3/d) pink water facility		
Biotreatment process with existing carbon treatment	465	1000
Biotreatment process with new carbon treatment	537	1178
Carbon treatment (base case) only	248	410
Case B—170,000 gpd (643.5 m^3/d) pink water facility		
Biotreatment process with existing carbon treatment	1320	2910
Biotreatment process with new carbon treatment	1460	3210
Carbon treatment (base case) only	870	1440

*Direct cost includes packaged equipment and labor for setting the equipment.
†Total installed cost (TIC) includes, in addition to direct cost, material, and labor costs for activated carbon inventory, structures, civil, all nonpackaged piping, instrument, electrical, and other engineering costs, but excludes costs of land, access roads, and so on.

Table 7
Operating Costs Summaries in Dollars/1000 Gal (3.8 m^3) of Pink Water Treated†

	Bio/activated carbon process	Activated carbon process base case
Variable costs		
Inoculum, nutrients and chemicals	1.84	-
Activated carbon, @ $1.98/kg (delivered)	1.21	13.24
Sludge disposal, @ $22/mton (inc. frt.)	0.09	-
Spent carbon incineration, @ $1200/$m^3$ (excl. frt.)	1.90	20.78
Spent carbon transportation, 1600 km round trip @ $37.90/$m^3$	0.06	0.66
Utilities, @ $.05/kWh and $4.00/MM BTU ($3.79/10^6 kJ)	0.25	neglig.
Subtotal variable costs	5.35	34.68
Fixed costs*		
Operating labor		
Maintenance, L&M		
Overhead (incl. supervision and lab)		
Subtotal fixed costs	2.67	1.03
Total operating costs	8.02	35.71

*Fixed operating costs are considered as constant in this study.
†A more detailed study should recognize the variation in the fixed costs with plant capacity (i.e., effect of decreasing costs with increasing capacity).

CONCLUSIONS

Both TNT and RDX were observed to be degraded by *Phanerochaete chrysosporium* immobilized on the disks of an RBC in both batch and continuous modes. Removal efficiency of TNT was constantly high. Similar results have been reported by Fernando et al. (*7*) in batch reactors and Joyce (*8*) in an RBC. At high TNT concentration (and/or short residence times), an apparent intermediate of TNT degradation accumulated in the reactor. These results are encouraging, and suggest that this organism can be useful in the treatment of munitions wastes and other wastes containing nitrobodies. However, in pink water applications this intermediate must be identified and its eventual degradation assured either in the *P. chrysosporium* reactor or by postfungal treatment. In future work, we will study the chemistry of TNT degradation and investigate alternative reactor designs.

Preliminary design and cost estimates for a bio/activated carbon process for pink water treatment at plant capacities of 50,000 gpd (189.3 m^3/d) and 170,000 gpd (643.5 m^3/d) have also been prepared. The cost data show that the total installed cost of the plant, including a new activated carbon polishing step, is 3–4 times the cost of the currently practiced activated carbon adsorption process. However, the operating costs for the bio/activated carbon plant are only 20–25% of the activated carbon process. The resulting payback for the biotreatment process, within the range of capacities studied, over the carbon process is about 12–18 mo.

ACKNOWLEDGMENT

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