REMEDIATION OF TNT-CONTAMINATED WATER BY USING INDUSTRIAL LOW-COST RESIDUE PINE BARK

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School of Business, Society and Engineering
REMEDIATION OF TNT-CONTAMINATED WATER BY USING INDUSTRIAL LOW-COST RESIDUE PINE BARK

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Akademisk avhandling

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Abstract

In the process of demilitarization of explosives, army ammunition plants generate a waste stream known as pink water. The principal component of the wastewater is the nitro-aromatic compound 2,4,6-trinitrotoluene (TNT). Although the persistence of TNT when dissolved in surface water is very limited due to its susceptibility to photo- and biotransformation, discharge of pink water to the environment has been prohibited in Sweden, the U.S. and many other countries for decades because of the toxicity of the compound and its metabolites to various ecological receptors.

The most frequently used method for treatment of pink water in Sweden today is adsorption on activated carbon, which as well as being costly, creates a sludge that must be incinerated off site.

In many countries, the timber industry residue pine bark is discarded and has no high value application. The overall aim of this thesis was to investigate the potential of pine bark for the removal of TNT from contaminated water such as pink water. Several batch studies and a column experiment were conducted. Acetonitrile extraction of pine bark and 16S rRNA sequencing for analysis of the indigenous bacterial community of pine bark were used to investigate its performance in the treatment of pink water.

The results show that pine bark has great potential as an adsorbent medium for TNT from contaminated pink water. Simultaneous use of biotransformation and adsorption methods was shown to be an improvement over adsorption alone for the removal of TNT from contaminated water bodies. Pine bark showed higher affinity towards the amino metabolites of TNT than for TNT itself. Molecular analysis of the indigenous microbial community of pine bark and chemical analysis of its acetonitrile extracts provided evidence for its ability to biotransform TNT and its metabolites. The efficiency of the transformation was enhanced by the addition of glucose and/or inoculum.

Overall, this work demonstrates the versatility of this organic industrial residue with respect to pink water treatment. Not only does it have a high affinity towards TNT and its amino metabolites, but its native microbial community even in the absence of external inoculation can also be taken advantage of, opening new possibilities for remediation of pink water.
Summary

In the process of demilitarization of explosives, army ammunition plants generate a waste stream known as pink water. The principal component of the wastewater is the nitro-aromatic compound 2,4,6-trinitrotoluene (TNT). Although the persistence of TNT when dissolved in surface water is very limited due to its susceptibility to photo- and biotransformation, discharge of pink water to the environment has been prohibited in Sweden, the U.S. and many other countries for decades because of the toxicity of the compound and its metabolites to various ecological receptors.

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Keywords: 2,4,6-trinitrotoluene, adsorption, microorganisms, pine bark, pink water
Sammanfattning

I demilitariseringsprocesser, där ammunition oskadliggörs och resursåtervinns, genereras stora mängder överblivna sprängämnen. Processindustrivatten från processerna kallas ”pink water” som till största del innehåller världens vanligaste sprängämne 2,4,6-trinitrotoluene (TNT). Trots att TNT inte är särskilt stabilt i vatten är det väldigt giftigt för de flesta organismer i ekosystemet, därför har man i Sverige, USA och många andra länder förbjudit alla former av TNT-utsläpp till naturen.

Den vanligaste metoden för att behandla ”pink water” i Sverige idag är att använda aktivt kol som adsorbent, vilket dels är kostsamt, dels genereras ett slam som måste köras långt för att destrueras under högtemperaturförbrinnning.

I många länder med skogsindustri bildas restprodukten furubarkflis, däribland i Sverige. Det övergripande syftet med den här avhandlingen har varit att undersöka om det vore möjligt att använda furubarkflis för att avskilja (adsorbera) TNT från förorenade vatten som ”pink water”. Flertalet laboratorieförsök i genomfördes för att reda ut egenskaperna hos furubarkflis och hur väl TNT kan fastna på dess ytor i en filterapplikation. Lakning med acetonitril från furubarkflisen och molekylärmikrobiologiska analyser av mikroberna på flisens ytor användes för att vidare utreda hur starkt TNT binder på ytorna och om TNT kan brytas ner av flisens egen mikrobiologi.

Resultaten visar att furubarkflis har en stor potential som adsorbent för att avskilja TNT från förorenade vatten, t ex ”pink water”. Reningsmöjligheterna med furubarkflis för ”pink water” kan ytterligare förbättras genom att förstärka förutsättningarna för nedbrytning på ytorna och reningseffekten blev bättre än om endast adsorption används som reningsmetod. Furubarkflis visade sig kunna rena TNT-molekylens nedbrytningsprodukter ännu bättre än ursprungsmänet TNT. Molekylärmikrobiologisk analys av furubarkflisens ytor visade tillsammans med lakförsök med acetonitril med mikroberna på furubarkflisens ytor helt kunde bryta ned både TNT och dess metaboliter. Nedbrytningshastigheten på ytorna förbättrades ytterligare av tillsats av glykos med eller utan tillsats av extern ymp.

Den övergripande slutsatsen med avhandlingsarbetet är att furubarkflis visar goda förutsättningar som filtermaterial för behandling av ”pink water”. Försöken visade att furubarkflis har inte bara goda förutsättningar att få TNT och dess nedbrytningsprodukter att fastna på ytorna utan att de mikroorganismer som finns naturligt på furubarkflisens ytor även utan extern ymp kunde bryta ner TNT fullständigt. Detta ger helt nya möjligheter att rena ”pink water”.

Keywords: 2,4,6-trinitrotoluene, adsorption, microorganismer, furubarkflis, pink water
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List of papers


IV Chusova, O., Nehrenheim, E., Odlare, M. Adsorption of trinitrotoluene by pine bark. Manuscript.

Author’s contribution to the appended papers

I – Most of the planning. Co-authors revised the experimental scheme that I sent them and consulted me on how to perform sampling for the molecular analysis. Performed the general experiment. Did the writing except for the molecular analysis related material in Materials and methods and Results and discussion. Co-authors revised the draft manuscript and made suggestions for improvement.

II- Most of the planning. Co-authors revised the experimental scheme that I sent them and consulted me on how to perform sampling for the molecular analysis. Performed the general experiment. The results of molecular analysis were evaluated by the co-authors. Did the writing except for the molecular analysis related material in the Materials and methods and Results and discussion section. Co-authors revised the draft manuscript and made suggestions for improvement.

III – Most of the planning. Co-authors consulted me on how to perform sampling for the FT-IR analysis. Performed the general experiment. Most of the writing; co-authors provided me with
technical text regarding the FT-IR results and the *FT-IR Spectroscopy* part of the *Materials and methods* section.

IV – Most of the planning. Performed the experiments. Did the writing except Abstract, Introduction and Conclusions.

**List of papers not included**


Abbreviations

Buf – buffer
EPB – extracts of pine bark
FT-IR spectroscopy – Fourier Transform Infrared Spectroscopy
HPLC – high-performance liquid chromatography
L/S – liquid/solid ratio
LVI GC-MS – large-volume injection gas chromatography-mass spectrometry
PB – pine bark
PW – pink water
1 Introduction

Due to its low manufacturing costs, safety of handling, low sensitivity to impact and friction, and a fairly high explosive power, 2,4,6-trinitrotoluene (TNT) has been used extensively as an explosive in military and other applications. As a result of problems with environmental contamination at manufacturing sites, from which TNT enters the environment in waste waters and solid wastes, it is no longer produced commercially in North America and has only been produced in limited amounts in Europe for nearly three decades. In Sweden one of the major sources of TNT-containing wastes is the demilitarization industry (such as Nammo Vingåkersverken). Demilitarization processes can result in contamination of surface soils and groundwater by activities such as open burning, open detonation and nondestructive reprocessing of munitions. In addition, these industrial processes result in the generation of large volumes of TNT-saturated waste water, known as pink water. Because of the toxic effect which TNT has been widely reported to have on the human and ecological environment, this waste stream cannot be discharged unless the concentration of TNT and other dissolved explosives in the water is less than 1 mg/L.

The persistence, abundance and resistance to biodegradation of TNT make it one of the most widely studied hazardous organonitro compounds with respect to bioremediation. The biodegradation of TNT is rendered difficult because of the presence of the three nitro groups. However, TNT is not totally refractory to biodegradation, and a number of microorganisms have been isolated that cometabolically biotransform its nitro groups to amino groups. These amino compounds have been demonstrated to be even more toxic than TNT itself and often result in dead-end products.

The use of low-cost adsorbents for water treatment has been recommended for reasons of cost-effectiveness, widespread availability and relative affinity towards metals and organic substances. One example is the forestry by-product pine bark, which has been shown to remove heavy metals and organic substances from contaminated water bodies.

In this thesis a few possible approaches have been studied in which the industrial residue pine bark may be efficient for the removal of TNT from contaminated water bodies. The approaches are based on bioslurry reactor technology, where cometabolic reduction and adsorption of TNT on soil are employed simultaneously; and fluidized bed bioreactor technology, where the particles of an adsorbent provide a surface for biofilm growth.

The current state-of-the-art treatment treats munitions wastewater by adsorption of TNT on granular activated carbon (GAC). This has been a safe and effective method. However, the adsorbent is costly and there are problems associated with the necessity to further treat the spent carbon.

The thesis investigates potentials for pine bark to substitute GAC for the treatment of pink water.
1.1 Aims and objectives

There have been only few studies published where an adsorbent performs several functions simultaneously in the water treatment process. The overall aim of this thesis is therefore to investigate the possible roles of pine bark in the treatment of TNT-contaminated water, particularly for pink water. The objectives of the thesis are as follows:

1. To investigate the influence of temperature, pH, particle size and dose of pine bark on the adsorption-desorption behavior of TNT (Paper IV);

2. To apply Langmuir and Freundlich isotherms to describe the adsorption process (Paper IV);

3. To investigate whether pine bark adsorbs explosives other than TNT (Paper III).

4. To investigate the possibility of biotransformation of TNT on or inside the pine bark particles (Paper II);

5. To study factors that would induce degradation of TNT on the pine bark surface (Paper I and II);
2. Background

2.1 Chemical structure, properties, history and use of 2,4,6-trinitrotoluene

Trinitrotoluene (C₇H₅N₃O₆) can exist as six different isomers. The isomer that is used in the explosives industry is the symmetrical isomer 2,4,6-trinitrotoluene (Figure 1). For convenience, the 2,4,6-isomer is subsequently referred to in this thesis as TNT.

![Figure 1. Structural formula of TNT](image)

The solubility of TNT in water is limited to 81.5-115 mg/L at room temperature (Phelan and Barnett, 2001; Ro et al., 1996). It is sparingly soluble in alcohol and soluble in benzene, toluene and acetone. It darkens in sunlight and is unstable in alkalies and amines (Akhavan, 2004).

The environmental fate of TNT is influenced by biological and abiotic processes, mainly photolysis. The persistence of TNT when dissolved in surface water is very limited; biological degradation by bacterial and fungal species occurs slowly, with slightly higher rates in the presence of other carbon sources. Results of a monitoring study by Talmage et al. (1999) revealed that TNT persists at sites where it was produced or processed. It is present in soil, sediment, surface water and groundwater at military sites.

TNT and its metabolites are toxic to a variety of aquatic organisms (Drzyzga et al., 1995; Liu et al. (vol. I), 1983; Neuwoehner et al., 2007; Nipper et al., 2009; Sims and Steevens, 2008; Smock et al., 1976; U.S. EPA, 1989) and cytotoxic to human cells (Bruns-Nagel, 1999; Cenas et al., 2009; Lachance et al., 1999; Nishino et al., 2000). In trying to assess the environmental hazard of TNT and its reduced metabolites, the mutagenicity of these compounds has been well explored in the literature with varying contradictory results (Einisto, 1991; Jarvis et al., 1998; Inouye et al., 2009; Neuwoehner et al., 2007; Rosser et al., 2001; Whong and Edwards, 1984).

2,4,6-trinitrotoluene was produced extensively both commercially and at government ammunition plants as the standard explosive for all armies in WWI and WWII (Smith, 2007; Steen, 2007; U.S. EPA, 2012). Nowadays, TNT production is limited to military arsenals. It is used in military shells, bombs, and grenades either in pure form or in binary mixtures with other explosives such as RDX and HMX. Besides military use, small amounts of 2,4,6-trinitrotoluene are used for industrial explosive applications, such as deep well and underwater blasting (Akhavan, 2004; ATSDR, 1995).
2.2 Regulations and limits for TNT

Swedish national drinking water regulations provide no limits for TNT (LIVSFS, 2001). Based on available toxicology studies, limits for TNT in drinking water have been set by the Navy Bureau of Medicine (0.05 mg/L) and the U.S. Army (0.03 mg/L) (Committee on Toxicology, 1982). Ryon (1987) developed water quality criteria using the then current guidelines of the U.S. Environmental Protection Agency. He reported 0.56 mg/L as the maximum TNT concentration to protect aquatic life and 0.04 mg/L as the maximum continuous concentration. Hinshaw et al. (1987) reported the following interim limits for munitions compounds for the protection of human health: 0.04 mg/L for TNT, 0.03 mg/L for RDX, 0.03 mg/L for HMX and 0.0007 mg/L for 2,4-DNT. These criteria were developed by the U.S. Army Medical Bioengineering Research and Development Laboratory based on existing methodologies proposed by the U.S. EPA. According to the U.S. Department of Defense (2003), the allowable concentration of nitrobodies in pink water at the pretreatment discharge point is 1 mg/L. “Nitrobodies” is a general term for explosive compounds, which in this case would mean the sum of the concentrations of TNT, RDX, HMX and trinitrobenzene.

The Drinking Water Equivalent Level (DWEIL), a lifetime exposure at which adverse health effects would not be expected to occur, is 0.02 mg/L for 2,4,6-trinitrotoluene (U.S. EPA, 2012). The EPA has assigned 2,4,6-trinitrotoluene a weight-of-evidence carcinogenic classification of C, which indicates that 2,4,6-trinitrotoluene is a possible human carcinogen (U.S. EPA, 1993).

2.3 Demilitarization in Sweden

Demilitarization is the use of various technologies to process munitions so that they are no longer usable for military applications. Methods for demilitarization of munitions can be divided into destructive and nondestructive methods. Destructive methods include incineration, open detonation, and open burning. Nondestructive methods are aimed at recovering various components for reuse or sale (ATSDR, 1995). An example of a demilitarization company that uses both destructive and nondestructive methods is Nammo Vingåkersverken, which is located in mid Sweden. At the factory, mines, abandoned and unexploded explosive ordnance and other explosive remnants of war are deactivated, cleaned, recycled and sold on the civilian market. In the recycling process the ammunition is sunk in hot water (80 °C) in order for the TNT to wash/melt out. The recycling rate at the site is around 90% and the remaining 10% (less than a few kg per day) comprise 1) explosives-contaminated sludge, which is the residual, semi-solid material; and 2) the process water, known as pink water, in which the sludge is suspended. Pink water derived from the sludge is one of the main subjects of research in this thesis. The melted out TNT is sold for civilian purposes, such as the mining industry (Nehrenheim et al., 2010).

2.3.1 Explosives-contaminated sludge

The study by Klee et al. (2004) showed that the main components of sewage sludge samples from a WWTP that received wastewater from explosives manufacturing industries were TNT (≈ 3 % w/w); 2,6-DNT (1 % w/w); TNB (≈ 3 % w/w); toluene (≈ 70 % w/w) and p-cresol (≈ 0.9 % w/w). The reported concentration of TNT in air-dried sludge from Nammo Vingåkersverken was
around 30% (Muter et al., 2009). Aside from TNT, the sludge from the Swedish demilitarization company contained metals, metal ions, dyes, fiberglass, dust, tar and wax.

2.3.2 Explosives-contaminated water (pink water)

Explosives-contaminated process waters are divided into two categories: red water, which comes strictly from the manufacture of TNT, and pink water, which includes any washwater associated with the load, assembly and demilitarization of munitions involving contact with finished TNT. Despite their names, red and pink water cannot be identified by colour (Barth, 1994). Both are clear when they emerge from their respective processes and subsequently turn pink, light red, dark red, or black due to photochemical irradiation of dissolved TNT to form complex dye-like molecules (Yinon, 1990), which are collectively referred to as the TNT colored complex. The rate of pink water formation is especially dependent upon irradiation and pH: alkaline pH enhances the rate and intensity of color development. The chemical composition of pink water varies depending on the process from which it is derived. Red water has a more defined chemical composition. For this reason, it is difficult to simulate either pink or red water in the laboratory. Pink water may also contain varying levels of RDX and HMX, depending on the particular formulation being handled. According to the report by Hinshaw et al. (1987), the nitro-bodies of greatest concern in pink water collected from an Iowa army ammunition plant were TNT (100 mg/L), RDX (30 mg/L), HMX (30 mg/L) and 2,4-DNT (1 mg/L). Barth (1994) reported that, as well as TNT, HMX, RDX and 2,4-DNT, pink water may also contain tetryl, 2,6-DNT, 1,3-DNB, 1,3,5-TNB and nitrobenzene. 2,4-DNT and 2,6-DNT are not strictly explosive munitions compounds, but are by-products in the manufacture of TNT and are normally present at low levels in pink water (Hinshaw et al., 1987).

2.3.3 Treatment of wastes

Any wastes generated in the manufacturing and processing of 2,4,6-trinitrotoluene are characterized as hazardous wastes both in Europe (E.C., 2000) and the U.S. (U.S. EPA, 2008). The main technology used to treat munitions wastewater is the adsorption of TNT and other nitro-compounds such as RDX, HMX and 2,4-ADNT (Hinshaw et al., 1987) on granular activated carbon (GAC) (Barth, 1994; Chen et al., 2004; Cervantes, 2009; Jenkins et al., 1986; Maloney et al., 2002; U.S. Department of Defense, 2003). GAC is a non-graphitic processed form of carbon that contains vast internal porosity, offering a surface area of 500-1,500 m²/g (Concurrent Technologies Operation, 1995). At the pink water treatment facility at McAlester Army Ammunition Plant, particle, oil and grease-free pink water passes through one of two GAC adsorbers which operate in parallel. The effluent concentration of the adsorbers is monitored, and when the concentration approaches the breakthrough limit of TNT, the GAC is replaced (U.S. Department of Defense (2003)). The spent GAC is classified as a K045 hazardous waste and must be further treated (U.S. EPA, 2008). Options include incineration, disposal in a secure hazardous landfill, or regeneration by partial oxidation of GAC, during which the contaminants are desorbed and burned. The regenerated GAC is then washed, cooled and mixed with fresh activated carbon and set to adsorb again (Rodgers and Bunce, 2001). The addition of fresh GAC is necessary to maintain the treatment efficiency because the regenerated GAC is known to lose up to 50% of its original adsorption capacity. Alternatively, explosive-laden GAC may be burned as fuel in boilers and cement kilns. Scrubbers must be installed on incineration equipment in order to reduce air
pollution. However, incineration is expensive, permitting can be difficult, and scrubber waste water can be problematic (Concurrent Technologies Operation, 1995). Rodger and Bunce (2001) state that because the bed has a high affinity but finite capacity towards organics, GAC is more suitable as a polishing technique.

At Nammo Vingåkersverken explosives-contaminated sludge is separated from the pink water. This separation is conducted by mechanical settlement in two steps, thereafter shaken for eight hours in GAC, which is then removed by textile filter bags. The dewatered sludge and spent GAC are sent off site for incineration. The treated water is discharged to a recipient lake. As the recipient is a lake that is used as a drinking water source, there is a close to zero tolerance level for any contaminated discharge from the factory (Nehrenheim et al., 2011).

2.4 Methods for the removal of TNT from water

2.4.1 Adsorption

Adsorption is a mass transfer process wherein components of a fluid are deposited on the surface of an adsorbent by physical or chemical forces (Hinshaw et al., 1987).

Static versus dynamic adsorption

Remediation can be performed in static (batch) and dynamic (fixed-bed) systems. Batch systems are flexible to operate, whereas fixed-bed reactors are more convenient to use (Chen et al., 2004). In static equilibrium adsorption, the same solution remains in contact with a given quantity of adsorbent. As the amount of solute adsorbed on an adsorbent increases and the solute concentration in solution decreases, the driving force of adsorption and adsorption capacity also decrease. The adsorption process continues, however, until the equilibrium is reached between the solute concentration in solution and the solute adsorbed per unit weight of adsorbent. This equilibrium is static in character and does not change further in time (Rajagopal and Kapoor, 2001). The advantage of batch experiments is that the performance of different adsorbents in adsorbing a particular compound can be tested relatively quickly in the laboratory and the adsorbent with the best performance is therefore easily identified. Also, the effects of temperature, particle size, and influent solution composition can be readily tested (Hinshaw et al., 1987).

In dynamic column adsorption, solution continuously enters and leaves a column, and so the adsorbent in the column meets fresh solution throughout the process. Depending on the fluid velocity and bed height, the contact time for the adsorption can be varied. However, absolute equilibrium between the solute in solution and the amount adsorbed is never established in dynamic column adsorption (Rajagopal and Kapoor, 2001).

Equilibrium sorption isotherm

The most preliminary information on the performance of any given sorption system comes from equilibrium sorption studies (pH experiment, Paper IV). “Enough time” has to be afforded for contact before sorption equilibrium is reached between the sorbate sequestered on the solid sorbent and the sorbate concentration in the liquid phase (Volesky, 2007). The summary of the sorption system performance is reflected in the sorption
isotherm in which the equilibrium sorbate concentration ($C_e$) is plotted against sorbate uptake by the sorbent solids ($q$).

The calculation of TNT uptake [$\text{mg TNT/g (dry) sorbent}$] is based on the material balance of the sorption system: sorbate which disappears from the solution must be in the solid, i.e.

$$q_e = \frac{V(c_i - c_e)}{m}$$  \hspace{1cm} (1)

where $V$ is the volume of the TNT-bearing solution in contact with the sorbent (L); $C_i$ and $C_e$ are the initial and equilibrium concentrations of TNT in the solution (mg/L) respectively; $m$ is the amount of the added sorbent on dry basis (g). Calculated values of $q_e$ were subsequently used for the quantification of Freundlich and Langmuir isotherm model constants (Paper IV).

The calculation of TNT desorption [$\text{mg TNT/g (dry) sorbent}$] is based on the following equation;

$$q_d = \frac{V(c_i - c_e - c_d)}{m}$$ \hspace{1cm} (2)

Where $q_d$ is the adsorbed TNT amount after desorption (mg/g), $c_i$ is the equilibrium concentration of TNT after desorption (mg/L). Eq. (2) was used to build the TNT desorption isotherms in the pH experiment of Paper IV.

**Adsorption of TNT by different adsorbents**

Although adsorption of TNT on several adsorbents has been reported, the majority of TNT adsorption studies investigate adsorption on GAC (Hinshaw et al., 1987; Lee et al., 2007; Marinovic et al., 2005; Rajagopal and Kapoor, 2001; U.S. Department of Defense, 2003). Current research is focused on developing and testing adsorbents that are more economical and/or efficient compared to GAC, such as pine bark (Nehrenheim and Odlare, 2010; Nehrenheim et al., 2011), activated coke (Zhang et al., 2011), Bamboo charcoal (Fu et al., 2012), metal-impregnated lignite activated carbon (Wei et al., 2011), molecularly-imprinted adsorbent (Meng et al., 2012) and PAM/SIO$_2$ (An et al., 2009), to treat TNT-contaminated water.

**2.4.2 Pine bark**

Pine bark, a timber industry residue, has been used successfully as a low-cost adsorbent for removing heavy metals from landfill leachates (Nehrenheim et al., 2007; Ribé et al., 2012), pink water (Nehrenheim et al., 2011), tannery wastewaters (Alves et al., 1993), stormwater ( Genç-Fuhrman et al., 2007) and water solutions (Blazquez et al., 2011; Goncalve et al., 2012; Martin-Dupont et al., 2002; Mihaiescu et al., 2012; Oh and Tshabalala, 2007). This adsorbent has also been studied for removal of organic pollutants such as hydrocarbons (Haussard et al., 2001) and PAHs (Li et al., 2010), lipids (Haussard et al., 2003), bisphenol (Antunes et al., 2012), endocrine disruptor 17β-estradiol (E2) (Braga et al., 2011), lindane (Ratola et al., 2003; Sousa et al., 2011), pentachlorophenol (Bras et al., 2005), heptachlor (Ratola et al., 2003) and other organochlorine pesticides (Bras et al., 1999). Chemical pretreatment of pine bark with formaldehyde (Antunes et al., 2012), chloric acid (Argun et al., 2005; Li et al., 2010), calcium or sodium salts (Alves et al., 1993), sodium hydroxide, Fenton reactive and graft polymerization (Argun et al., 2009) was shown
to significantly increase the adsorption capacity of the material. The action of these chemical modifiers is aimed at the destruction of cellulosic and hemicellulosic constituents of bark, which suppress the adsorption potential of lignin (Argun et al, 2009; Li et al., 2010), which is thought to be the main adsorption component of the pine bark (Dizhbite et al., 1999).

**Pine bark properties**

As a lignocellulosic material, pine bark is usually characterized in terms of its main constituents cellulose, hemicellulose and lignin (Li et al., 2010). The lignin content of pine bark varies between 29-33 % in different species (Fradinho et al., 2002; Vazquez et al., 1987).

Bark extracts can be roughly divided into lipophilic and hydrophilic constituents (Sjostrom, 2003). The lipophlic fraction, extractable with nonpolar solvents, mainly consists of fats, waxes, terpenoids and higher aliphatic alcohols. The hydrophilic fraction, extractable with water alone or with polar solvents, contains large amounts of phenolic constituents. Many of these phenolic compounds, especially the condensed tannins (often called “phenolic acids”) can be extracted only as salts with dilute solutions of aqueous alkali (Fradinho et al., 2002; Li et al., 2010; Sjostrom, 2003).

**Ecotoxicological safety**

Although the water treatment efficiency of pine bark is high, one of its reported disadvantages for water treatment is potential leaching of organic compounds, e.g. water-soluble phenols (Ali, 2010; Bailey et al., 1999). The safety of unused and untreated pine bark as alternative to GAC was investigated in batch leaching experiments with deionized water (Ribé et al., 2009) which demonstrated desorption of metals and DOC from the pine bark. Phenols represented 7 % of the DOC. During the leaching the pH of the leachate decreased from neutral to 4.6. This is in line with Bras et al. (2005), who found that the pine bark surface behaved like an acid and acted as a buffer, resisting change in pH from pH 4 to 10. An acute toxicity test of the leachate showed that it was toxic to Daphnia magna (Ribé et al., 2009). However, since the pH-adjusted leachate samples showed no toxicity to the tested organism and the phenol concentrations after the pH adjustment remained the same, it was concluded that the decreased pH caused by desorption of organic acids was the primary cause of toxicity. The suggestions provided by Ribé et al. (2009) for avoiding the release of DOC from the adsorbent were either to pre-treat it chemically prior to use or to ensure buffering of the first effluent. Chemical stabilization of pine bark has been described by several researchers (see reviews by Ali, 2010 and Bailey et al., 1999), and can be accomplished by the same modifiers that are used to enhance its adsorption capacity (Argun et al., 2009).

**2.4.3 Biotransformation of TNT**

Despite considerable effort, microorganisms that can utilize TNT as a sole source of carbon for growth have remained elusive (Lewis et al., 1997; Rylott et al., 2011). The reason for the lack of bacteria that can grow on TNT are that TNT is xenobiotic, i.e. it does not exist in nature and has a unique electronic structure. The inclusion of substituents (xenophores) such as the nitro group that are rare or even absent among natural compounds renders a nitroaromatic molecule less susceptible to oxidative processes, which is a major mode of microbial catalysis of aromatic hydrocarbons (Gibson, 1976). Therefore, the most common reaction of the nitro group of TNT in biological systems is reduction. Reduction of aryl nitro groups (R-NO₂) to corresponding amines
(R-NH₂) through nitroso (R-NO) and then hydroxylamino (R-NHOH) (McCormick et al., 1976) intermediates is often referred to as the TNT transformation pathway in many systems (Boopathy and Kulpa, 1992; Ederer et al., 1997; Preuss et al., 1993; Spain, 1995). The rate of reduction of each successive nitro group decreases dramatically because amino groups deactivate the molecule for further reduction (Spain, 1995). Therefore, the reduction of one nitro group of TNT is very rapid under a variety of conditions including those prevalent in growing cultures of aerobic bacteria. Contrastingly, reduction of 2-amino-4,6-dinitrotoluene (2-ADNT) and 4-amino-2,6-dinitrotoluene (4-ADNT) to form 2,4-diamino-6-nitrotoluene (2,4-DANT) and 2,6-diamino-4-nitrotoluene (2,6-DANT) requires a lower redox potential. Finally, a reduction 2,4-diamino-6-nitrotoluene (DANT) requires a redox potential below -200 mV (Funk et al., 1993) and a reductant such as glucose as an auxiliary substrate, because the electron-donating properties of the amino group lower the electron deficiency of the molecule (Spain, 1995). While the mineralization of TNT has not yet been demonstrated unambiguously (Ramos et al., 2005; Rylott et al., 2011), biotransformations of TNT have been studied in many organisms (Faull et al., 2004; Schoenmuth and Pestemer, 2004), and especially in bacteria (Esteve-Nunez et al., 2001; Kulkarni and Chaudhari, 2007; Lewis et al., 1997; Rosser et al., 2001; Smets et al., 2007; Stenuit and Agathos, 2010; Symons and Bruce, 2006).

Cometabolic reduction of TNT in the presence of glucose

In anaerobic systems all three nitro groups may be reduced to produce triaminotoluene (Funk et al., 1993; Lenke et al., 2000), whereas in aerobic systems, partially reduced products accumulate (Johnson et al., 2001; Lewis et al., 1997). The predominant products under aerobic conditions are the ADNTs, and to a lesser extent DANTs and azoxytetranitrotoluenes (Gilcrease et al., 1995; Johnson et al., 2001).

Preuss et al. (1993) showed that sulfate reducing bacteria were able to completely reduce dissolved TNT to TAT when fed with pyruvate as an easily degradable carbon source under anaerobic conditions. This metabolic pathway was later confirmed and widely used by a group of German scientists (Achtthin et al., 1999a; Achtthin et al., 1999b; Daun et al., 1995; Daun et al., 1998; Daun et al., 1999; Lenke et al., 1998): an anaerobic consortium from a local sewage treatment plant, grown on glucose, formed TAT by reducing all the nitro groups on TNT. Figure 2-a shows schematically how some of the reduction equivalents [H] generated during the fermentation of glucose are transferred to an aromatic nitro group, which is thereby reduced in three steps via nitroso and hydroxylamino intermediates to an amino group. Figure 2-b shows the metabolites of the complete anaerobic reduction of TNT which accumulate to detectable amounts: hydroxylaminodinitrotoluenes (HADNTs) and aminodinitrotoluenes (ADNTs) as isomeric mixtures, 2,4-diaminonitrotoluene (DANT) and the final reduction product triaminotoluene (Daun et al., 1999).
2.4.4 Combination of biotransformation and adsorption

Adsorption to clay minerals and soil organic matter

Binding of reduction products of TNT to the mineral (Daun et al., 1998; Pennington and Patrick, 1990) and organic (Achtnich et al., 1999b; Li et al., Drzyzga et al., 1998; Drzyzga et al., 1999; Knicker et al., 1999; Thorn and Kennedy, 2002; Thorn et al., 2002) fractions of soil form the basis of the strategy for bioelimination of TNT from contaminated soils by using composting or bioslurry technology. Daun et al. (1998) concluded that after soil treatment under anaerobic conditions, neither TAT nor HADNTs could be desorbed from soil by methanolic extraction or by alkaline or acidic hydrolysis. These products are therefore considered as the key metabolites for this cost-effective remediation solution, where an irreversible chemical reaction of fixation to an inert matrix leads to detoxification (Daun et al., 1998; Lenke et al., 2000).

GAC-FBB

A fixed-film fluidized bed bioreactor (GAC-FBB) consists of immobilized microbes on hydraulically fluidized media particles. The particles provide a large surface for biofilm growth. In the FBB pilot studies by Maloney et al. (2002) and the U.S. Department of Defense (2003), a microbial biofilm on GAC created an anaerobic environment for cometabolic reduction of TNT to amines in the presence of ethanol. Although there was little data on the transformation products detected in the column effluent, the authors claimed that the products were either completely mineralized under anaerobic conditions or with subsequent aerobic treatment (U.S.
Department of Defense (2003), or degraded to undetectable non-toxic end products (Maloney et al., 2002).
3 Materials

3.1 TNT-contaminated sludge from Nammo Vingåkersverken

Sludge was sampled at the factory prior to the active carbon batch filtration step and transported to the lab. The tank was stored in a dark room at 4 °C for two weeks in order to allow it to saturate the water with TNT. The water phase, henceforth referred to as pink water, was then separated from the sludge for use in the experiment. The pink water sample was analysed by TerrAttesT® which includes qualitative and quantitative measurement by LVI GC-MS of more than 200 compounds, including seven groups of chemical compounds: metals, aromatic compounds (mono aromatic hydrocarbons, phenols, PAHs), halogenated hydrocarbons (volatile halogenated HCs, chlorinated benzenes, chlorinated phenols, PCB, chloronitrobenzenes, miscellaneous chlorinated HCs), pesticides (chlorine pesticides, phosphor pesticides, nitrogen pesticides, miscellaneous pesticides), miscellaneous HCs, phthalates, and total petroleum hydrocarbons. The results of this test showed that the C12-C16 fraction of total petroleum hydrocarbons (TPH) was the only group of substances of possible concern (Table 1). TPH is a term used to describe a large family of several hundred chemical compounds that originate from crude oil. The test also showed that aside from TPH and metals (Table 2), the pink water contained trace levels of toluene and pentachlorophenol. In earlier studies, pink water derived from sludge sampled at Nammo Vingåkersverken was reported to contain metals (Nehrenheim et al., 2001; Table 2) and trace concentrations of other explosives, namely HMX and RDX (Nehrenheim and Odlare, 2010).

Table 1. Total petroleum hydrocarbons in pink water

<table>
<thead>
<tr>
<th>Compound</th>
<th>Concentration (µg L⁻¹)</th>
<th>Reporting limit (µg L⁻¹)</th>
<th>Concentrations of concern for health in drinking water (mg/L) ²</th>
</tr>
</thead>
<tbody>
<tr>
<td>TPH C10-C12</td>
<td>&lt;10</td>
<td>15</td>
<td>~ 0.1</td>
</tr>
<tr>
<td>TPH C12-C16</td>
<td>160</td>
<td>15</td>
<td>~ 0.1</td>
</tr>
<tr>
<td>TPH C16-C21</td>
<td>&lt;15</td>
<td>15</td>
<td>~ 0.09</td>
</tr>
<tr>
<td>TPH C21-C30</td>
<td>25</td>
<td>15</td>
<td>~ 0.09</td>
</tr>
<tr>
<td>TPH C30-C35</td>
<td>&lt;20</td>
<td>15</td>
<td>~ 0.09</td>
</tr>
<tr>
<td>TPH C35-C40</td>
<td>&lt;20</td>
<td>15</td>
<td>—</td>
</tr>
<tr>
<td>TPH C10-C40</td>
<td>210</td>
<td>100</td>
<td>—</td>
</tr>
</tbody>
</table>

²WHO, 2005.
Table 2. Metal and TNT concentrations in pink water and U.S. EPA MCL guideline values

<table>
<thead>
<tr>
<th>Compound</th>
<th>Concentration (µg L⁻¹)</th>
<th>U.S. EPA MCLc (µg L⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Al</td>
<td>290a/—b</td>
<td>50-200</td>
</tr>
<tr>
<td>As</td>
<td>3.2a/＜3b</td>
<td>10</td>
</tr>
<tr>
<td>Ba</td>
<td>9.2a/13b</td>
<td>2,000</td>
</tr>
<tr>
<td>Be</td>
<td>－/＜1b</td>
<td>4</td>
</tr>
<tr>
<td>Cd</td>
<td>2.0a/＜0.4b</td>
<td>5</td>
</tr>
<tr>
<td>Co</td>
<td>2.5a/＜1b</td>
<td>－</td>
</tr>
<tr>
<td>Cr</td>
<td>3.7a/＜2b</td>
<td>100</td>
</tr>
<tr>
<td>Cu</td>
<td>22.3a/＜5.5b</td>
<td>1,300</td>
</tr>
<tr>
<td>Hg</td>
<td>0.0a/＜0.04b</td>
<td>2</td>
</tr>
<tr>
<td>Ni</td>
<td>1.7a/＜2b</td>
<td>－</td>
</tr>
<tr>
<td>Se</td>
<td>－/＜5b</td>
<td>0.05</td>
</tr>
<tr>
<td>Pb</td>
<td>7.1a/＜3b</td>
<td>15</td>
</tr>
<tr>
<td>Zn</td>
<td>3,970a/160b</td>
<td>5,000</td>
</tr>
<tr>
<td>TNT</td>
<td>29,500a/—b</td>
<td>－</td>
</tr>
</tbody>
</table>

aData taken from Nehrenheim et al., 2011
bTerrAttesT®
cMaximum contaminant level (MCL): The highest level of a contaminant allowed in drinking water

3.2 Pine bark

The material (Figure 3) is a commercial product named Zugol™, which is supplied by Zugol AB in Falun, Sweden. According to the suppliers, it consists of approximately 85-90% dried and granulated pine bark and 10-15% wood fibers. It has a particle diameter range of ＜0.25 mm (7.5%), 0.25-5.0 mm (76.2%) and ＞5 mm (16.3%). Pine bark is commonly used as an adsorbent for liquid spills such as petrol and oil (http://www.zugol.se/produkter.html).

Figure 3. Pine bark
4 Experimental design

Laboratory experiments were conducted with either artificial TNT solutions or pink water. The majority of the experiments were performed in batch systems, primarily because it is the cheapest and most practical way to test various adsorption affecting parameters, such as temperature and particle size. Secondly, batch adsorption for the treatment of pink water by GAC has been used by Nammo Vingåkersverken for many years. The method proved to be easy to perform and the disadvantages are mainly associated with the high cost of GAC and the difficulties faced in utilizing the spent adsorbent. Thus, the main challenge for the company has been to find an adequate substitute for GAC. Finally, the results of laboratory batch experiments can be upscaled relatively easily.

Laboratory column tests, which are performed in continuous/dynamic mode, are potentially far more challenging than batch experiments in terms of cost and laboriousness as there are various parameters which directly affect the adsorption process, such as breakthrough, hydraulic loading and pressure drop, in addition to parameters which are frequently tested for the optimization of adsorption, such as pH and the adsorbent dose. In order to estimate the effect of a parameter on the adsorption capacity, a breakthrough curve should be obtained for each value of the tested parameter. Plotting such a curve necessitates the analysis of relatively many samples, which increases the cost of the experiment. On the other hand, laboratory column experiments are valuable as they simulate on-site filter systems, where contaminated water is passed through packed filter material. The main use of the column experiment in the present thesis was to compare the adsorption capacities of pine bark and GAC, evaluate the dynamic desorption of organic acids from pine bark, and for ecotoxicological screening of the effluent.

4.1 Batch experiments

4.1.1 Biotransformation of TNT in the presence of pine bark

Batches were divided into two groups according to the respective TNT removal method: 1) adsorption on pine bark (subsequently referred to as Group I), 2) biotransformation of TNT in the presence of pine bark (subsequently referred to as Group II) (Paper I and II). Group II batches were either spiked with glucose solution or TNT-degrading inoculum or with both inoculum and glucose. One of the aims of adding inoculum to Group II batches was to investigate how TNT-derived amines interact with pine bark. The reduction of TNT to amines could also be achieved by chemical agents, such as nanoscale zero-valent iron (Zhang et al., 2009) and sulfide (Qiao et al., 2010). However, since pine bark is an adsorbent with its own microbial community (Davis et al., 1992), it was particularly interesting to investigate how its native bacterial community was affected by the addition of TNT-degrading inoculum. The aim of incubating the inoculum and pine bark without glucose was to evaluate the effect of external inoculation on the adsorption of TNT. Incubations with glucose and pine bark but no inoculum were aimed at investigating whether the
native microbial community of pine bark was activated by addition of glucose. The details of the experiments are summarized in Table 3.

Table 3. Experimental design for Paper I and II

<table>
<thead>
<tr>
<th>Experimental parameters</th>
<th>Paper I</th>
<th>Paper II</th>
</tr>
</thead>
<tbody>
<tr>
<td>TNT-contaminated media</td>
<td>Water spiked with TNT</td>
<td>Pink water</td>
</tr>
<tr>
<td>Concentration (TNT)</td>
<td>90 ± 4 mg/L</td>
<td>31 ± 2 mg/L</td>
</tr>
<tr>
<td>Processes examined</td>
<td>1. Adsorption</td>
<td>1. Adsorption</td>
</tr>
<tr>
<td></td>
<td>2. Biotransformation</td>
<td>2. Biotransformation</td>
</tr>
<tr>
<td>Type of biotransformation</td>
<td>Cometabolic reduction</td>
<td>Cometabolic reduction</td>
</tr>
<tr>
<td>Substrate</td>
<td>Glucose</td>
<td>Glucose</td>
</tr>
<tr>
<td>Concentration (glucose)</td>
<td>0.2 % w/v</td>
<td>0.2 % w/v</td>
</tr>
<tr>
<td>Additional nutrients</td>
<td>M9* mineral medium, M8* mineral medium</td>
<td>None</td>
</tr>
<tr>
<td>Inoculum (source)</td>
<td>Mixed culture from WTT</td>
<td>Mixed culture from WTT</td>
</tr>
<tr>
<td>Concentration (inoculum)</td>
<td>1.5 % v/v</td>
<td>1.5 % v/v</td>
</tr>
<tr>
<td>Culture conditions</td>
<td>1. Glucose, TNT, M9* medium, micro-oxic</td>
<td>1. Enrichment with Glucose and TNT;</td>
</tr>
<tr>
<td></td>
<td>conditions;</td>
<td>2. Enrichment with Glucose and TNT-</td>
</tr>
<tr>
<td></td>
<td>2. Glucose, TNT, M9* medium, anaerobic</td>
<td>contaminated sludge</td>
</tr>
<tr>
<td></td>
<td>conditions;</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3. Glucose, TNT, M8* medium, anaerobic</td>
<td></td>
</tr>
<tr>
<td></td>
<td>conditions.</td>
<td></td>
</tr>
<tr>
<td>Environment</td>
<td>Anaerobic</td>
<td>Anaerobic</td>
</tr>
<tr>
<td>Incubation conditions</td>
<td>28°C, 130 rpm</td>
<td>28°C, 130 rpm</td>
</tr>
<tr>
<td>Volume (solution)</td>
<td>70 mL</td>
<td>70 mL</td>
</tr>
<tr>
<td>Dose (PB)</td>
<td>1.75 g</td>
<td>1.75 g</td>
</tr>
<tr>
<td>Fraction (PB)</td>
<td>≤ 0.125 mm</td>
<td>≤ 0.125 mm</td>
</tr>
<tr>
<td>Duration</td>
<td>14 days</td>
<td>14 days</td>
</tr>
</tbody>
</table>

In previous research on the adsorption of TNT by pine bark, pine bark (3 g) demonstrated affinity towards TNT in moderately contaminated pink water (200 mL, 22-30 mg/L) in a 24 h incubation (Nehrenheim et al., 2011). However, complete removal was not achieved. Thus, one of the aims of the experiment described in Paper I was to evaluate the extent of removal of TNT from a saturated solution using a finer fraction of pine bark.

The mass of pine bark, the flask volume and TNT concentration were selected based on the previous research (Nehrenheim et al., 2011), which itself applied the pink water/GAC ratio which is used at the Nammo Vingåkersverken treatment plant. In the same paper by Nehrenheim et al. (2011), it was established that equilibrium was reached within 8 hours of incubation with shaking (120 rpm). Hence, the experiments described in Paper I and II were run for an additional two
weeks with the aim of investigating whether the pine bark would keep adsorbing TNT after this equilibrium was established.

4.1.2 Extracts of pine bark

Extracts of the pine bark (subsequently referred to as EPB) were obtained by acetonitrile extraction (Paper II and III) and used to address the following questions: 1) What happens to the TNT adsorbed on pine bark during the experiment, is it extractable and how does the extractability change during the course of the experiment? 2) Does pine bark participate in biotransformation of TNT on/inside its particles? (Paper II); 3) Can the lignin and phenolic acids content in pine bark be increased/decreased by sieving? (Paper III) 4) Can explosives other than TNT and its metabolites be adsorbed on pine bark? (Paper III).

In order to answer Question 2, the analysis of EPB samples in Paper II also included detection of the reduction products of TNT, such as ADNTs and DANTs. In order to answer Question 3, EPB of four different pine bark fractions, i.e. unsieved pine bark, ≤ 1.000, ≤ 0.045 and ≤ 0.025 mm, obtained by sieving were recorded and analyzed. Since the reduction of particle size of adsorbent is known to have a positive effect on the adsorption capacity of both metals (Al-Ashen and Duvnjak, 1997; Chong et al., 2013) and organic pollutants (Bras et al., 2005), it was interesting to investigate if it also has effect on the content of lignin and phenolic acids. Question 4 was addressed by recording and analyzing FT-IR spectra from three different samples: EPB from a batch containing only pink water and pine bark, the HPLC standard for TNT, and mix A, i.e. the EPA 8330 HPLC standard containing a set of the most common military explosives (Paper III).

4.1.3 The influence of pH, temperature, glucose, particle size and dose of pine bark on adsorption/desorption of TNT

Adsorption isotherms were studied using spiked TNT solutions and pink water in three different pH environments: pH 4, pH 7 and in an unbuffered system. After 2 days of shaking, tubes containing pine bark were filled with sterile water and returned to the shaker. The TNT from the pine bark was left to desorb for 6 days at room temperature. The details of the pH experiment are listed in Table 4.

The temperature experiment consisted of two stages. In the first stage (referred to as “I stage” in Table 4) the effects of temperature, pine bark dose (0.23g and 0.45 g) and particle size (≤ 1 mm and ≤ 0.125 mm) on the adsorption capacity were studied using pink water at 6, 22 and 37°C with contact times of 8 hours and 5 days on a table shaker. Desorption of TNT was studied for pine bark which had been subjected to adsorption for 5 days. The details of the temperature experiment are summarized in Table 4.

The second stage (referred to as “II stage” in Table 4) of the temperature experiment is a small extension of the first stage, which was based on its results. In the second stage of the study, the dose of the adsorbent was increased 5-fold, thus investigated L/S was reduced 5-fold. Apart from the different L/S applied, this test also examined the impact of glucose addition and pine bark particle size on the TNT removal efficiency.
Table 4. Experimental design for Paper IV

<table>
<thead>
<tr>
<th>Experimental parameters</th>
<th>Experiments</th>
<th>Temperature</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>1) Water spiked with TNT; 2) Pink water</td>
<td>Pink water</td>
</tr>
<tr>
<td>Concentration (TNT), mg/L</td>
<td>1) 40 ± 2; 25 ± 1; 8 ± 1; 2) 33 ± 2; 16 ± 2; 8 ± 1</td>
<td>I stage. 78 ± 4; II stage. 69 ± 3</td>
</tr>
<tr>
<td>Processes examined</td>
<td>Adsorption/desorption</td>
<td>I stage. Adsorption/desorption II stage. Adsorption</td>
</tr>
<tr>
<td>L/S</td>
<td>67</td>
<td>I stage. 66; 130 II stage. 13</td>
</tr>
<tr>
<td>Adsorption pH</td>
<td>pH 4; pH 7; no buffer system</td>
<td>Both stages: No buffer system</td>
</tr>
<tr>
<td>Adsorption temperature, °C</td>
<td>22</td>
<td>I stage. 6; 22; 37 II stage. 22</td>
</tr>
<tr>
<td>Shaking speed, rpm</td>
<td>150</td>
<td>Both stages: 150</td>
</tr>
<tr>
<td>Dose (PB), g</td>
<td>0.45</td>
<td>I stage. 0.23; 0.45 II stage: 2.25</td>
</tr>
<tr>
<td>Pretreatment (PB)</td>
<td>Sieving</td>
<td>Both stages: Washing, drying, sieving</td>
</tr>
<tr>
<td>Fraction (PB), mm</td>
<td>≤ 1 mm</td>
<td>Both stages: ≤ 1 mm; ≤ 0.125 mm</td>
</tr>
<tr>
<td>Duration</td>
<td>Adsorption: 2 days; Desorption: 6 days</td>
<td>I stage. Adsorption: 8 hours; 5 days Desorption: 6 days II stage. Adsorption: 3 days</td>
</tr>
</tbody>
</table>

4.2 Comparative column study

The detailed column setup is described and presented in Paper IV. Briefly, a volumetric pump was used to pump pink water from a buffer tank upwards through a plastic column filled with 1) 10 g of pine bark; 2) 10 g of GAC; and 3) 100 g of pine bark (Figure 4). The column comprised three
layers; the middle layer was the adsorbent bed, while the top and bottom layers were comprised of sand, i.e. an inert material. The function of the top and bottom beds was to prevent escape of the adsorbent particles through the inlet and outlet of the column. Fractions of the treated water, in which TNT was subsequently measured by HPLC, were collected at the column exit. The specific aim of the study was the comparison of the adsorption capacities of pine bark and GAC during continuous column operation.

Figure 4. Experimental column and volumetric pump
5 Results and discussion

5.1 Solubility of TNT in pink water

Although the extraction of pink water from the demilitarization sludge followed the same procedure throughout the different experiments, the concentration of dissolved TNT in the pink water varied between 28 mg/L (Paper II-III) and 70 mg/L (Paper IV). The concentration of TNT in pink water is obviously limited by its solubility and is dependent on the sludge matrix. Literature values of TNT solubility at room temperature (20-23 °C) vary widely in the range 81.5-115 mg/L (Phelan and Barnett, 2001; Ro et al., 1996). The most likely reason for the range of TNT concentrations is the composition of the sludge from which the pink water was derived. Firstly, if several compounds with limited solubility are simultaneously present in solution, none of them can dissolve to the full extent of their reported aqueous solubility. According to the TerrAttesT® results (chapter 3, section 3.1) pink water contains trace levels of many organic substances as well as HMX and RDX (Nehrenheim and Odlare, 2010). Secondly, the concentration of explosives in water depends on the size and surface area of individual explosive particles (Speitel et al., 2002).

The particles of the sludge from which the pink water with higher dissolved TNT concentrations were extracted were visually finer than those from which the pink water with lower dissolved TNT concentrations was extracted. It is therefore likely that the extraction process from the finer explosive particles was more efficient and resulted in higher TNT concentration in the pink water.

The study by Ro et al. (1996) concluded that TNT solubility was pH and temperature dependent, i.e. it increased with increasing temperature. pH dependency was reported as less predictable, but solubility generally increased with pH within the room temperature range 21-25 °C. Nevertheless, pH and temperature are unlikely to explain the relatively large variations in the TNT concentrations in the pink water derived from different sludge samples since both samples were handled at room temperature, stored at 4 °C and had similar pH values, i.e. 7.5 and 7.2 respectively.

5.2 Adsorption of TNT on pine bark

5.2.1 Batch experiments

TNT from the spiked water and pink water were adsorbed on pine bark for two weeks (Paper I and II). Despite the differences in the initial concentration of TNT (90 mg TNT/L in the spiked water and 31 mg TNT/L in the pink water) and the environmental matrix, the patterns of the adsorption process demonstrated similar behavior (Figure 5). The spontaneous uptake of TNT by the pine bark was 61 % from the spiked water and 53 % from the pink water. TNT uptake
continued in the subsequent week, to 78 %, 80%, 84 % on Day 1, Day 3 and Day 7 from the spiked water and 75 %, 88 %, 87.5 % on Day 1, Day 3 and Day 7 from the pink water. The uptake value on Day 14 was only measured for the pink water and was 98 %. The continued uptake after equilibrium had been reached provides indirect evidence for the ability of pine bark to biotransform TNT. This evidence was subsequently supported by chemical analysis of the pine bark extracts (section 5.5) and molecular analysis. The 16S rDNA amplicon sequencing of the pine bark bacterial community revealed that the pine bark contained bacteria capable of growing in the presence of TNT that could enhance TNT biotransformation (Paper I).

Figure 5. Adsorption on pine bark of TNT from the pink water and spiked water. (The lines between the dots are added for visualization of the trend, and do not represent a curve fit)

As mentioned in the previous paragraph, 75% of TNT was adsorbed on pine bark after 24 h of shaking (Paper II). In a similar batch experiment performed by Nehrenheim et al. (2011), the uptake of TNT from pink water with the same initial TNT concentration was only 50% after the same incubation time. Several factors may have contributed to the increased removal of TNT achieved in Paper II: 1) the application of the ground pine bark (Nehrenheim et al. (2011) used untreated pine bark); 2) the L/S ratio of 40, compared to the L/S ratio of 67 used by Nehrenheim et al. (2011); and 3) an incubation temperature of 28 °C, compared to 22 °C used by Nehrenheim et al. (2011). The later batch experiment provided answers regarding which of the three parameters had the most profound effect on the removal of TNT from pink water (Paper IV). According to these findings, a smaller L/S ratio (i.e. a larger dose of pine bark) and smaller pine bark particle size had positive effects on TNT removal efficiency. Of the two parameters, the L/S ratio had a more significant impact than particle size. The temperature did not seem to play a significant role on the uptake of TNT by pine bark (Paper IV).

The results show that in order to maintain a high TNT adsorption efficiency, the pH in the batch should be maintained around neutral. A good fit was obtained between the Langmuir and
Freundlich models and the data (Table 5). However, the Freundlich model provided a better fit with the pink water data.

Table 5. Langmuir and Freundlich isotherms constants and Pearson correlation

<table>
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<td>$b$</td>
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<td>$k$</td>
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5.2.2. Column experiment

The color of the pink water at the inlet of the column was distinctively red-yellow, and after passing through the adsorbent bed it changed from colorless (first fraction), to pale yellow (second to fourth fraction), yellow, and back to red-yellow again (Figure 6).

Figure 6. The collected fractions of pink water after passing through the pine bark bed (10 g). From left to right: untreated pink water (0) followed by treated fractions (1 to 10, 15)

Breakthrough curves were obtained for both pine bark and GAC (Figure 7). The experiment was stopped after 40*25 mL fractions had been collected at the column exit, i.e. at 67% and 23% breakthrough for the pine bark and GAC bed respectively. In order to compare adsorption capacities of GAC and pine bark the area above the curve was calculated and two bed service times were selected as breakthrough times: 10% and 23% (Paper IV). The results show that at 10% breakthrough, the TNT adsorption capacity of GAC is less than twice that of pine bark. At 23% breakthrough the TNT adsorption capacity of GAC is five times that of pine bark.
Figure 7. The concentration of TNT in the pink water at the outlet of the column after passing the pine bark bed and GAC bed.

In the third experiment the mass of the pine bark bed was increased ten fold. The experiment was stopped after 9*25 mL fractions of the treated pink water had passed through the column twice. Unlike the experiment with lower adsorbent mass, where only the first fraction was colorless, in this experiment all nine collected fractions were colorless (Figure 8). The TNT concentration in the treated pink water did not exceed 0.7 mg/L after all 9*25 mL had passed through the 100g PB bed.

Figure 8. Collected fractions of pink water after passing through the pine bark bed (100 g). Untreated pink water (0), and fractions 1,2,3,8 and 9.

5.3 Desorption of the adsorbed TNT

Desorption increased with increasing amounts of TNT adsorbed on the pine bark. TNT that was adsorbed on pine bark at 37 °C exhibited less than half the desorption of TNT that was adsorbed at 6 °C or 22 °C, which most likely indicates that stronger interactions were established between TNT and pine bark after 5 days of incubation at 37 °C (Paper IV). Desorption of TNT from pink water was generally higher than that of TNT from TNT solution. The most desorption was
observed from the pine bark that had adsorbed TNT in unbuffered media, i.e. where pH was determined by the elution of organic acids from the pine bark. No TNT desorbed from the pine bark that had adsorbed TNT at neutral pH.

5.4 Biotransformation of TNT in the presence of pine bark

Group II batches showed more efficient removal of TNT from the contaminated media compared to the “adsorption” batches. All TNT was removed from solution by Day 1 (Paper I and II).

TNT removal was the same for batches containing inoculum with and without glucose after the first 24 h of treatment (Figure 9). Thus, since the removal of TNT from the spiked water from the batches with and without inoculum took place within the first day of the treatment, no conclusions could be drawn on the effect of the added glucose in Paper I. The appearance of some batches from this study is shown in Figure 10.

Figure 9. The absence of the effect of glucose on the removal of TNT from the artificial solution [TNT = water spiked with TNT; Glu = glucose; In = inoculum and PB = pine bark]

Figure 10. Batches on the 3rd day of the biotransformation experiment. Left to right: the control batch containing TNT in M9* mineral medium; the batch with glucose and inoculum grown in
nitrogen-limiting M8* mineral medium; the batch with glucose and inoculum grown in M9* mineral medium; and the batch with glucose, inoculum and pine bark

The effect of glucose was investigated further in Paper II. Figure 11 compares removal of TNT from the pink water with and without glucose. On Day 1 the extent of TNT removal in both batches was the same. However, by Day 3 there was no TNT remaining in the batch containing glucose, while the batch without glucose still contained traces of TNT on Day 14 of the experiment. Thus, it is likely that after an adaptation period of up to 3 days, indigenous microorganisms on the pine bark started to co-metabolize TNT with glucose, enhancing its removal from the water.

Figure 11. The effect of glucose on the removal of TNT from pink water [PW = pink water; Glu = glucose, and PB = pine bark] (The lines between the dots are added for visualization of the trend, and do not represent a curve fit)

The results of a second batch adsorption experiment showed that on Day 3 the uptake of TNT from the batches without glucose was no different from those with glucose (Paper IV), contradicting the earlier results (Paper II). Thus, it can be concluded that the addition of glucose in the various experiments had different effects on TNT adsorption. The reasons for these differences could be: 1) the L/S ratio (L/S = 40 in Paper II; L/S = 13.3 in Paper IV); 2) the concentration of TNT in the pink water in Paper IV was double that in Paper II, and 3) the study in Paper II was conducted at 28 °C, while the study in Paper IV was conducted at 22 °C. The lower temperature may have extended the adaptation period of the microbes inhabiting the pine bark, i.e. while 3 days were sufficient for the microbes to start cometabolizing TNT and glucose at 28 °C, it was not long enough at 22 °C.

5.5 Pine bark extracts

The progressive decrease in TNT concentration in EPB of all batches along with the appearance of metabolites of TNT during the second half of the experiment suggests that TNT is biotransformed on the surface of the pine bark as well as in the liquid phase. The discrepancy between the input (i.e. the TNT concentration detected in the acetonitrile extracts of EPBs on Day
0) and summarized output molar concentrations of TNT and its metabolites (ADNTs and DANTs) increased between Day 0 and Day 7, and in most cases up to Day 14. The output molar concentrations of TNT and its metabolites were on average 80% lower than the input concentration, indicating contributions by processes other than biotransformation. A proportion of TNT and its metabolites was apparently bound sufficiently tightly to the adsorbent surface through chemisorption so that it could no longer be extracted with acetonitrile. Binding increased as biotransformation progressed. TNT was extracted more readily than ADNTs and ADNTs were generally extracted in a larger proportion than DANTs, indicating that ADNTs were bound more tightly than TNT, and DANTs were bound more tightly than ADNTs (Paper II).

FT-IR analysis of EPB of different pine bark fractions established that the lignin content was inversely proportional to the size of the pine bark particles, while the concentration of phenolic hydroxyl groups increased with increasing size of pine bark particles. FT-IR spectra showed that as well as TNT, pine bark also adsorbed nitramine explosives such as RDX and HMX (Paper III).

### 5.6 Desorption of organic acids from the pine bark

Since toxic effects of the pine bark leachates to the ecological receptors, such as luminescent bacteria *V. fisheri* and green algae *P. subcapitata* (Ribé (2012), Ribé et al. (2012) and Ribé et al. (2009)) were attributed to low pH, the pattern of desorption of organic acids was analyzed in greater detail. Figure 12 shows the dynamics of organic acids elution from the pine bark in batches consisting of spiked water and pine bark (Paper I) and pink water and pine bark (Paper II). Two things are worth stressing: there is 1) a sequential decrease in pH throughout the experiments; and 2) a relatively high pine bark pH at the end of the experiments compared to that observed in the pH experiment in Paper IV and studies performed by other researchers. The pH of the solutions after addition of pine bark fell to 6.9 (Figure 13). The sequential decrease in pH through the course of experiment can be explained by a progressive increase in desorption of organic acids from the pine bark. Nevertheless, the pH did not go below 6.8 and 6.7 by the end of the spiked water and pink water studies respectively. This is generally higher than the pH values observed in the pH experiment in Paper IV, where the pH of the batch containing pink water mixed with water (80/20 v/v) fell to 4.9 after two days of incubation. Other researchers have also reported pH of pine bark leachates below 5. For instance, in a study by Ribé et al. (2009) the pH value of leachate at the end of a 24 hour long leaching test was 4.6. In another study the pH of pine bark watersoluble extract, which was prepared by 15 hours of shaking in water, was 3.8 (Spencer and Benson, 1982). Notably, all of these experiments were conducted at room temperature and for a shorter period than the present studies. The differences in pH of the leachates could be due to: 1. Higher buffering capacity of the pink water compared to water; 2. Particle size of the pine bark: the content of phenolic hydroxyl groups decreases with decreasing size of pine bark particles (Paper III). The particle size of the adsorbent was ≤ 0.125 mm in Paper I-II and ≤ 1 mm in Paper IV (the pH experiment). The organic acids content in the leachate of the pine bark with particle size ≤ 1 mm was thus higher, and its pH was lower; or 3. The source of the pine bark. The latter hypothesis was also discussed in the studies by Spencer and Benson (1982), who accounted for the differences in pH with differences in pine species, age of tree, height of the tree where the bark was collected, or duration of aging time after collection. In addition, Ribé et al. (2011) speculated that leaching properties depend on the texture of the bark, which in turn, varies seasonally. For
instance, bark from trees cut in the late summer or early autumn is firmer. This type of bark is likely to be less prone to desorption of organic material.

Figure 12. Desorption of organic acids from the pine bark in the batch studies with the spiked water (Paper I) and pink water (Paper II)

Figure 13 shows desorption of organic acids from the pine bark bed during the dynamic adsorption of pink water TNT. It is noteworthy that pH of the early fractions of the treated water was sharply lower at 4.1, compared to the pH of the untreated pink water, which was 7.3. In a column study by Nehrenheim et al. (2008) and a batch study by Ribé et al. (2012) where pine bark was examined for the removal of heavy metals from different landfill leachates, pH of the treated leachates was not lower than that of the untreated leachates. Conductivity, and therefore ionic strength of the leachates was high. Ionic strength and buffering capacity are closely linked: increasing ionic strength increases buffering capacity (Reijenga et al., 1996). Thus, Ribé et al. (2012) accounted for the relatively constant pH values of the leachates before and after the treatment with high buffering capacity of the leachates against reducing pH. Indeed, in another column study by Ribé (2012), the pine bark bed reduced the pH of storm water below 5 even after 10 bed volumes had been pumped through the bed. One possible explanation for this is that the buffering capacity of the storm water, whose electrical conductivity was 10-30 times less than that of the landfill leachates, was not sufficient to withstand pH reduction. Thus, aside from the source of the pine bark used in the column experiment, low buffering capacity of pink water may be the cause of the significant reduction of pH in the early fractions of the treated pink water.
Figure 13. Desorption of organic acids from the pine bark bed in the column study

The graph also shows that the pH of the treated water began to increase after 125 mL of pink water had been pumped through the column. This means that desorption of organic acids from pine bark is finite. This is in contrast to the studies by Ribé et al. (2011) and Ribé (2012), which showed that serial leaching of pine bark did not cause the pH reducing effects of the bark on the leachate to decrease.
This thesis has investigated the multiple functions of the industrial residue pine bark when applied to the removal of TNT from solutions and from pink water.

- The first objective of the thesis (chapter 1.1) was to investigate the influence of temperature, pH, particle size and dose of pine bark on the adsorption-desorption behavior of TNT: use of smaller L/S ratio and smaller pine bark particle size had a positive effect on TNT removal efficiency. Of these two parameters, the L/S ratio had a greater influence on TNT removal efficiency than particle size. Temperature did not have a significant influence on the uptake of TNT by pine bark. However, increasing temperature above 22 °C seemed to strengthen interactions between TNT and pine bark.

- The adsorption of TNT on pine bark showed a good fit with both Freundlich and Langmuir models. However, the Freundlich model provided a slightly better fit for adsorption of TNT from pink water. This conclusion relates to the second objective of the thesis.

- Aside from TNT, pine bark adsorbs nitramine explosives such as RDX, HMX and tetryl from the pink water. This conclusion relates to the third objective of the thesis.

- The fourth objective was to investigate the possibility of biotransformation of TNT on or inside the pine bark particles. The ability of pine bark to biotransform TNT on its surface was supported by three findings:
  - Slow adsorption of TNT from solution continued after the equilibrium time between the adsorbent and solute had elapsed;
  - 16S rDNA amplicon sequencing of the pine bark bacterial community revealed that pine bark harbors bacteria capable of growth in the presence of TNT;
  - The progressive decrease in TNT concentration in acetonitrile extracts of pine bark together with the appearance of its metabolites throughout the experiment suggest that TNT is biotransformed on the surface of the pine bark as well as in the liquid phase.

- Both separate and joint addition of glucose and TNT-degrading inoculum enhanced the rate and extent of biotransformation of TNT on the pine bark surface. A small increase in the rate was achieved by separate addition of glucose. There was a greater increase when TNT degrading inoculum was added. However, the largest increase in the rate of biotransformation of TNT on the pine bark surface was observed in when both glucose and inoculum were added. This conclusion relates to the fifth objective of the thesis “To study factors that would induce degradation of TNT on the pine bark surface”.

6 Conclusions
The ability of the pine bark to biotransform TNT on its surface has a potential to be used on-site. Two things, aside from pine bark, are needed: glucose or another carbon source, and pH control. The results showed that the addition of glucose enhanced the removal of TNT by pine bark since bacteria present in pine bark would cometabolize TNT by consuming glucose. However, an adaptation period of about 3 days and an elevated temperature (28 °C) were needed for indigenous microorganisms of the pine bark to begin co-metabolizing TNT with glucose. Noteworthy, TNT will get biotransformed by pine bark without an external carbon source as well, but the rate of the process will be slower. pH maintenance around neutral, however, would perform three things: 1. provide vital conditions for bacteria; 2. increase the adsorption efficiency and also appear to facilitate stronger adsorption between TNT and pine bark; 3. reduce/eliminate toxicity of pine bark extractives, which also can be decreased by using finer fractions of the adsorbent.
7 Suggestions

7.1 Nammo Vingåkersverken pink water
As described above, pine bark can be used as a basic adsorption media before the activated carbon polishing step. The TNT removal efficiency and ecological safety of pine bark can be enhanced by maintaining a neutral pH and using a finer fraction of the adsorbent.

If optimal conditions were established for this method, a two-step treatment process could be created. Rapid adsorption would occur in the first step, where the molecules are attracted (adsorbed) to the particle surfaces in a shaking batch mode for eight hours. In the second step, anaerobic digestion of the pine bark slurry could be used to degrade the adsorbed components. There are two options for sustainable disposal of the TNT-laden pine bark:

- It may be a viable local source of energy through methane production if co-digested with other more easily degradable substrates. This methane could be used in a gas engine, and in the long run, the electricity produced could significantly reduce electricity costs at the treatment site;
- It can be composted and used as a growing medium for plants.

7.2 Application of pine bark for water treatment
Since the results presented in the thesis indicate that pine bark has stronger affinity towards aromatic amino groups than towards nitro groups, pine bark may also have potential applications in adsorbing amines and their derivatives such as ammonia or carbamate insecticides from contaminated water.


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