

Application of FT-IR Spectroscopy for Investigation of Pink Water Remediation by Pine Bark

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Abstract: This study demonstrates the application of FT-IR spectroscopy for investigating the remediation of pink water with the low cost adsorbent pine bark. The removal of 2,4,6-trinitrotoluene (TNT) from pink water by adsorption to pine bark was accompanied by a reduction in intensities of peaks at 1544 and 1347 cm^{-1} in the spectra of acetonitrile extracts of the pine bark. Hierarchical cluster analysis differentiated samples with high (30-180 mg/L) and low (0-4 mg/L) TNT concentrations, demonstrating the potential of this approach as a quick screening method for the control of the removal of TNT from pink water. The amount of lignin in pine bark was inversely proportional to the size of the pine bark particles, while the concentration of phenolic hydroxyl groups increases with increasing size of pine bark particles. FT-IR spectra showed that as well as TNT, pine bark can also adsorb nitramine explosives such as RDX and HMX.

Keywords: Adsorption, FT-IR spectroscopy, explosives, lignin, low-cost adsorbent, pine bark, pink water, TNT.

INTRODUCTION

Pine bark, a timber industry residue, has been successfully used as a low-cost adsorbent for removing heavy metals from landfill leachates [1], pink water [2] and stormwater [3]. This adsorbent has also been studied for the removal of organic pollutants such as PAHs [4], bisphenol [5], estradiol (E2) [6] and lindane [7] and 2,4,6-trinitrotoluene (TNT) [8] from contaminated water. Nehrenheim *et al.* [2] showed that pine bark can effectively remove TNT from pink water, a wastewater generated during the process of demilitarization of explosives at army ammunition plants.

The most frequently used method for the treatment of pink water in Sweden today is adsorption on activated carbon, which as well as being costly, creates sludge that must be incinerated off-site [2]. Our previous research [8] showed that biotransformation of TNT in the presence of pine bark has potential as a possible substitute for conventional granular activated carbon treatment of pink water. However, additional research is needed to evaluate the economic feasibility of pine bark for the treatment of pink water, its affinity toward compounds other than TNT constituents and the necessity of pine bark pre-treatment to enable higher treatment efficiency and safety. Lignin is anticipated to be the main contributor to adsorption by the pine bark [9]. Acidic leachates of pine bark, caused by desorption of water-soluble phenolic acids, are toxic to some aquatic organisms [10, 11]. Increasing the lignin content and reducing the amount of phenolic acids in pine bark will thus

positively affect both the treatment efficiency and ecotoxicological safety of the adsorbent.

Numerous laboratory and on-site studies and analysis are needed to address the research questions raised by these issues. Therefore there is a need for low cost and environmentally sound analytical tools. Fourier transform infrared (FT-IR) spectroscopy is a time saving method that requires a relatively small amount of sample and no sample pre-treatment, and information about all components is gained simultaneously. The FT-IR spectrum can be used for both qualitative and quantitative analyses of chemical components and is thus highly informative for analysis of multicomponent samples. FT-IR spectroscopy is thus a well-regarded analytical method for evaluation, monitoring and analysis of various biotechnological processes [12-15].

The main objective of this study is to investigate how low-cost FT-IR spectroscopy can partially replace expensive high-performance liquid chromatography (HPLC) in studying pink water remediation by pine bark. The two specific aims are to establish how the amount of lignin and phenol groups in pine bark changes depending on the particle size, and to investigate what explosives other than TNT are removed from pink water by pine bark.

MATERIALS AND METHODS

TNT-contaminated industrial sludge was collected from the treatment plant at the demilitarisation factory Nammo Vingåkersverken AB (Vingåker, Sweden, 59°60'N, 16°56'E) directly before the activated carbon batch adsorption step. The sludge was transported to the laboratory as a 1:5 mix with water for safety reasons. Because of the high solid

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particle content, the solution was decanted and the water phase, subsequently referred to as pink water, was filtered (Vacuum Filtration System, 0.22 μm , VWR, USA) before the batch experiment. Pine bark, provided by Zugol AB (Falun, Sweden), was used as received or sieved to obtain ≤ 1 mm, ≤ 0.045 mm and ≤ 0.025 mm fractions. EPA 8330 Mix A high-performance liquid chromatography (HPLC) standards containing 100 $\mu\text{g}/\text{mL}$ of 2,4,6-trinitrotoluene (TNT), 2-amino-4,6-dinitrotoluene (2-ADNT), 1,3,5-trinitrobenzene, hexogen (RDX), octogen (HMX), 1,3-dinitrobenzene and 2,4-dinitrotoluene in acetonitrile (ACN) were purchased from AccuStandard (New Haven, CT, USA).

Isolation and Culture Conditions of Inocula

A mixed culture was collected from a municipal wastewater treatment plant in mid-Sweden (59°60'N, 16°56'E). Two cultures were enriched for three weeks with glucose (0.2% w/v) in an anaerobic jar on a horizontal shaker at 130 rpm and 28 °C. The first culture, subsequently referred to as In1, co-metabolised TNT (0.4 mM), while the second culture, referred to as In2, co-metabolised the previously described TNT-contaminated industrial sludge mixed with water (1.5 - 2.0% v/v). The cultures were transferred weekly into fresh mineral salt medium M9* [8] containing glucose and TNT in the case of In1 and glucose and sludge particles in the case of In2.

Batch Experiment Setup

100 mL crimp-top serum bottles were filled with 70 mL of pink water and amended with 0.2% w/v glucose solution and 1.75 g pine bark according to the experimental setup shown in Table 1. Anaerobic conditions were initiated by purging the headspace of the bottles with nitrogen. Media (as defined in Table 1) were then inoculated through the septum with liquid cultures (2.0% v/v) of inocula In1 and In2. As In1 and In2 were expected to have quite similar effect in treated batches, In1 was not tested in the presence of pine bark due to experimental space and technical conditions limitations. The bottles were incubated on a horizontal shaker at 130 rpm and 28°C. One of each replicate was taken as a sample on Day 7 and a second was taken on Day 14.

Table 1. Batch experiment treatments. (Abbreviations: PW - pink water; In1, In2 - inocula; Glu - glucose; PB - pine bark).

Batch	Contents
C	PW + PB
D	PW + Glu + PB
E	PW + In2 + PB
F	PW + In1 + Glu + PB
G	PW + In2 + Glu + PB

Pine Bark Affinity Toward Explosives Other Than TNT

At the end of the experiment pine bark from batch C was extracted with acetonitrile (see section Sampling). FT-IR spectra of the ACN extracts of batch C, mixA and TNT

HPLC standard were recorded and the average spectra from two replicates were analyzed.

Comparative Analysis of Different Pine Bark Fractions

0.7 g of each pine bark fraction, i.e. unsieved pine bark, ≤ 1.000 , ≤ 0.045 and ≤ 0.025 mm, was weighed into eppendorf tubes to which 800 μL of ACN was then added. The pine bark constituents were extracted for 4 hours by horizontal shaking at 1000 rpm at room temperature. After centrifugation the supernatants were transferred to new eppendorf tubes and stored at -20 °C until further analysis. Each sample was run in duplicate.

Sampling

Samples for HPLC and Fourier transform infrared (FT-IR) spectroscopy were taken from both liquid and solid phases (pine bark) throughout the experiment. Liquid phase (i.e. supernatant) sampling was performed by piercing the septum with a hollow needle and withdrawing approximately 1 mL of medium after mixing all the components and briefly shaking by hand at the beginning of the experiment (Day 0), and subsequently on Day 1, Day 3, Day 7 and Day 14.

In order to obtain extracts of pine bark (EPB), samples of the solid phase were taken on Day 0, Day 7 and Day 14. For sampling, one of the each triplicate was centrifuged at 5,000 rpm and 4 °C for 10 minutes. The supernatant was removed and 0.5 g (wet weight) of the pine bark from each sample was mixed with 800 μL of ACN. TNT and metabolites were extracted from pine bark by horizontal shaking for 3h at room temperature. All supernatant and EPB samples were stored at -20 °C for further analyses.

FT-IR Spectroscopy

FT-IR absorption spectra were recorded on a VERTEX 70 coupled with the HTS-XT microplate reader extension (Bruker Optik GmbH, Ettlingen, Germany). Samples were dried on a 384 well silicon plate at $T < 50$ °C. Transmission spectra were collected between 4000-600 cm^{-1} and displayed as absorbance spectra. Spectra were acquired at a resolution of 4 cm^{-1} , and 64 spectra were coadded. Each spectrum was baseline corrected by the rubber band method and CO_2 bands were excluded. Only spectra fitting within the absorption limits between 0.25 and 0.80 (where the concentration of a component is proportional to the intensity of the absorption band) were used for data analyses. Measurements for each analytical sample were performed at least in triplicate. Data were processed using OPUS 6.5 software. The second derivative spectra were evaluated and Wards algorithm was used for Hierarchical cluster analysis (HCA).

HPLC Analysis

For detection and confirmation of TNT and its metabolites, HPLC was performed with the Agilent Series 1100 LC/MSD Trap XCT LC-MS system equipped with an in-line degasser, a binary pump, an autosampler and a column thermostat (Agilent Technologies, Santa-Clara, CA, USA). A photodiode array detector (λ 254 nm) and ESI-MS were used for detection. Chemstation (Rev.A.10.02) and LCMSD Trap Control (Version 5.2) software were used for

Table 3. Values of the integrated area of characteristic nitro group absorption bands at 1356-1336 and 1559-1534 cm^{-1} in the second derivative FT-IR spectra and the corresponding sum of the concentrations of total nitroaromatics determined by HPLC.

Batch	Value of integrated area 1356-1336 cm^{-1}	Value of integrated area 1559-1534 cm^{-1}	Total nitroaromatics determined by HPLC, mg/L
C Day0	0.019	0.031	160.00
C Day7	0.007	0.010	49.28
C Day14	0.003	0.005	36.83
D Day0	0.019	0.031	164.15
D Day7	0.009	0.010	69.90
D Day14	0.002	0.002	15.26
E Day0	0.019	0.030	175.28
E Day7	0.002	0.004	16.82
E Day14	0.001	0.002	14.52
F Day0	0.020	0.033	179.30
F Day7	0.004	0.004	16.49
F Day14	0.002	0.002	15.00
G Day0	0.019	0.031	162.00
G Day7	0.001	0.001	13.74
G Day14	0.001	0.001	7.10

Where: C: PW + PB; D: PW + Glu + PB; E: PW + In2 + PB; F: PW + In1 + Glu + PB; G: PW + In2 + Glu + PB

Table 4. Integrated area values of IR absorption bands 1543-1534 and 1556-1544 cm^{-1} of the second derivative spectra and concentrations of ADNTs and DANTs determined by HPLC.

Batch	value of integrated area 1543-1534 cm^{-1}	value of integrated area 1556-1544 cm^{-1}	ADNT, mg/L
E Day7	0.004	0.002	16.82
E Day14	0.002	0.002	11.41
F Day7	0.004	0.002	16.49
F Day14	0.002	0.002	7.80
G Day7	0.001	0.003	3.81
G Day14	0.001	0.002	1.90

Where: E: PW + In2 + PB; F: PW + In1 + Glu + PB; G: PW + In2 + Glu + PB

EPB (Fig. 4). All three spectra, i.e. the spectra of TNT and MixA HPLC standards and the representative spectra of EPB from batch C Day14, showed common absorption bands around 1600, 1545 and 1345 cm^{-1} . The spectra of TNT and MixA were obviously different but showed two strong common absorption bands at 1545 cm^{-1} and 1350 – 1346 cm^{-1} . Thus there was only one common absorption band in the spectra of Mix A and EPB, in the region 1250-1300 cm^{-1} . The absorption maximums were at 1272 cm^{-1} in the EPB spectrum and 1268 cm^{-1} in the Mix A spectrum. The bands in the region 1250-1300 cm^{-1} are assigned to vibrations of N-

NO_2 , NO, C-H, (C-N) of primary aromatic amine and esters of aromatic acids [24-26]. As most common nitramine explosives, such as RDX, HMX and tetryl, are characterised with all these vibrations, the spectra indicate that the pink water contains nitramine explosives, and that these are adsorbed by the pine bark.

5. FT-IR Spectral Analysis of the Acetonitrile Extracts of Pine Bark Fractions

FT-IR spectra of ACN extracts of four pine bark fractions with different particle sizes were analyzed to evaluate the

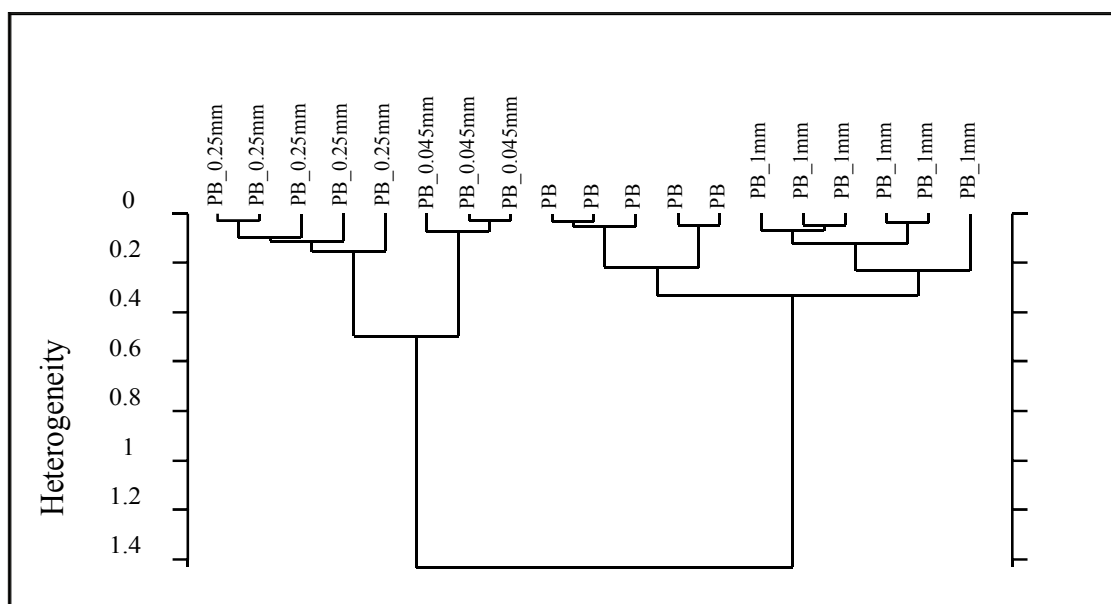


Fig. (6). The dendrogram of HCA of the acetonitrile extracts of four pine bark fractions: unsieved pine bark, ≤ 1.000 , ≤ 0.045 and ≤ 0.025 mm in the absorption region assigned to lignin ($1660\text{-}1405\text{ cm}^{-1}$)

In the second derivative spectra the absorption peak at 1375 cm^{-1} , which is assigned to phenolic hydroxyl groups, was highest in spectra of unsieved pine bark. The intensity of this peak was halved in the spectra of fine-grained pine bark extracts. Thus, the concentration of phenolic hydroxyl groups remaining in the pine bark increases with increasing size of pine bark particles.

CONCLUSION

The study showed that FT-IR spectroscopy applied to the analysis of acetonitrile extracts of pine bark is a useful tool for investigating, optimising and monitoring of pink water remediation by adsorption to pine bark. FT-IR spectra of pine bark extracts showed that as well as TNT, pink water collected at the demilitarisation factory Nammo Vingåkersverken AB also contained one or more nitramine explosives, such as RDX, HMX and tetryl, which were adsorbed by the pine bark. The highest lignin and lowest phenol group content was detected in the pine bark fraction with the smallest particle size of the four tested, which indicates that sieving of pine bark is needed as a pre-treatment method in order to increase the removal efficiency and ecological safety of the adsorbent.

FT-IR spectroscopy could not be used for the analysis of nitroaromatics directly in pink water because the peaks of interest overlap with other components of the multicomponent mixture under study.

Good correlation between HPLC and FT-IR spectroscopy analysis of pine bark acetonitrile extracts show that FT-IR analysis can serve as a quick, green and cheap screening method for the analysis of TNT and nitramine explosives removal from pink water. Overall, FT-IR spectroscopy can serve both to complement and partially substitute HPLC as an analytical tool for studying and monitoring the removal of

nitroaromatics from pink water, thus significantly reducing costs and ensuring environmental soundness of the chemical analysis.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflicts of interest

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