# Aerobic biodegradation of 2,4- and 2,6-dinitrotoluene in aqueous solution by cultures enriched from aged TNT-contaminated soil


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## ABSTRACT

Mixed cultures enriched from aged trinitrotoluene (TNT) - contaminated soil from Pardubice, Czech Republic and Esterreja, Portugal were used to study 2,4- and 2,6-dinitrotoluene (DNT) biodegradation. Experiments were carried out in shake flasks containing basal salt medium at 26ºC. Degradation was tested with 2,4- and 2,6-DNT as the sole carbon and nitrogen source; in the presence of an additional carbon (glycerol) source only; and in the presence of an additional nitrogen ($(NH_4)_2SO_4$) source only. During experiments optical density, pH of the medium and DNT concentrations were measured. Transformation with the presence of unidentified metabolites was demonstrated at various concentrations of DNT compounds for the *Pardubice* consortium whilst the *Esterreja* consortium showed no significant degradation abilities. Tests to define abiotic disappearance of DNT from media were conducted and showed that this was not of great concern in shake flasks but that some packing materials might have the ability to reduce or adsorb 2,4-DNT and 2-NT. Continuous 2,4-DNT degradation was performed in a packed bed reactor (PBR) with an immobilised catalyst at 30ºC. Even though 2,4-DNT was not completely removed, it was conclusively shown that within an organic loading (OL) range from 22 to 304 mg/L the metabolic elimination capacity (qS) of the cells to biodegrade 2,4-DNT increased with a steady relationship over time. The outcome of this study has been positive for the direct commencement of a bench-scale study of the degradation of 2,4-DNT and 2,6-DNT in the presence of cosubstrates by the *Pardubice* undefined consortium.


## INTRODUCTION

Dinitrotoluenes (Figure 1) are formed from the sequential nitration of toluene and are intermediates in the manufacture of polyurethane, pesticides, rubber chemicals and 2,4,6-trinitrotoluene (Figure 1), once the world's most widely used explosive. Improper handling during the manufacture of these products has resulted in contamination of soils and waters with dinitrotoluenes. These compounds seriously affect living organisms as toxins, mutagens, carcinogens and uncouplers of cellular phosphorylation reactions. The development of bioremediation and risk management strategies for contaminated sites requires an understanding of how the compounds interact with the environment and rehabilitative microorganisms.

In this study the degradation efficiency of pure strains and defined consortia enriched from aged TNT-contaminated soils from Pardubice, Czech Republic and Esterreja, Portugal were examined primarily on 2,4- and 2,6-DNT in shake flasks and a packed bed reactor. The main objective was the evaluation of a bench scale treatment system for the bioremediation of dinitrotoluenes and its potential for scale-up. The adsorption of the compounds to glass and packing materials was also examined.

## METHODS, MEDIA AND CULTURE CONDITIONS

***Batch experiments***

During batch experiments (Figure 2) microorganisms were grown in BSM (initial pH 7.2, $\pm$ 0.2) in 500 ml Erlenmeyer flasks with a working volume of 200 ml on an orbital shaker at 120 rpm at 26°C. Cells were induced on DNT for 48 hours and then washed. These cells were transferred to flasks containing various concentrations of 2,4- or 2,6-DNT as the sole carbon, nitrogen and energy source; with additional carbon only; and with additional nitrogen only.

***Adsorption experiments***

Poraver™, expanded slate and fire clay were added to 100 ml of BSM (Figure 3) in 250 ml Erlenmeyer flasks and autoclaved. Then 20 mg/L of 2,4-DNT or 4-NT was added to each flask from a 100 mg/L stock solution. The flasks were placed on an orbital shaker at 120 rpm at 26°C and compound concentrations were determined periodically by HPLC.

***Packed bed bioreactor experiment***

The continuous biodegradation of nitrotoluene compounds was carried out in a packed bed reactor (Figure 4) at 30°C. Poraver™ packing material covered in a biofilm that had been working for the previous year on the degradation of mononitrotoluene (MNT) was transferred into a new column. This packing material was mixed with fresh packing material at a 1:5 (v/v) ratio. The reactor worked with the *Pardubice* consortium.

***Analytical methods***

The nitrotoluene compounds were determined by reversed phase HPLC with UV detection set at 268 nm and 230 nm.

## RESULTS

- The Pardubice undefined consortium demonstrated the best transformation of 2,4-DNT when $(NH_4)_2SO_4$ was present in the medium (Figure 2).
- Tests to define abiotic disappearance of DNT from media showed that 2,4-DNT losses by adsorption to glassware and Poraver™ packing material were insignificant creating more certainty about the role of the consortium in the degradation of 2,4-DNT from the packed bed reactor. Poraver™ was the least complex packing material compared to expanded slate and fired clay (Figure 3).
- The bioreactor study (Figure 4) showed correlation between organic load (OL) and elimination capacity (qS) to be extremely significant ($P > 0.01$). The metabolic ability of the cells to biodegrade the 2,4-DNT load increased with a steady relationship over time (Figure 4c).

## CONCLUSION

The correlation between organic load and the degradation efficiency (Figure 4c) demonstrated the selective enrichment of organisms with high affinity substrate uptake systems for 2,4-DNT although more work needs to be done to establish this and the same potential for the degradation of 2,6-DNT. The progression of the bench-scale packed bed bioreactor to the next level of research for the degradation of 2,4-DNT as the sole source of carbon and nitrogen will not be immediate. The outcome of this project has been positive for the direct commencement of a bench-scale study into the degradation of 2,4-DNT and 2,6-DNT in the presence of additional nitrogen and cosubstrates by the *Pardubice* undefined consortium.

## BIBLIOGRAPHY


Crawford, R.L., 1995. The microbiology and treatment of nitroaromatic compounds, *Current Opinion in Biotechnology*, 6, 329-336

Nishino, S. F., J. C. Spain, and Z. He. 2000. Strategies for aerobic degradation of nitroaromatic compounds by bacteria: process discovery to field application, p. 7-61. In J. C. Spain, J. B. Hughes, and H.-J. Knackmuss (ed.), Biodegradation of nitroaromatic compounds and explosives. Lewis Publishers, Boca Raton.

Sturman, P.J., Stewart, P. S., Cunningham, A.B., Bouwer, E. J. and Wolfram, J. H., 1995. Engineering scale-up of in situ bioremediation processes: a review, *Journal of Contaminant Hydrology*, 19, 171-203


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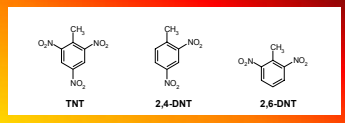
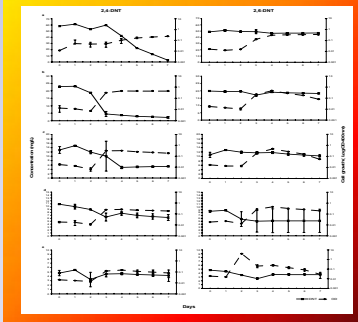
### Figure 1



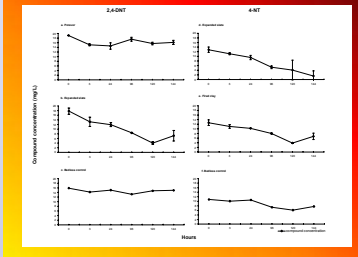

Chemical structures of 2,4,6-trinitrotoluene (TNT), 2,4-dinitrotoluene (2,4-DNT) and 2,6-dinitrotoluene (2,6-DNT).

### Figure 2



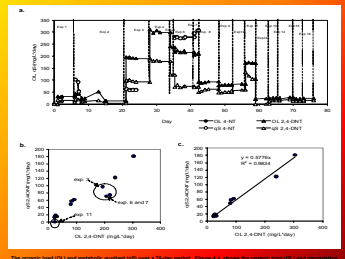

The degradation of varying concentrations of 2,4- and 2,6-DNT as the sole carbon and nitrogen source by the *Pardubice* undefined consortium.

### Figure 3




Adsorption of 2,4-DNT and 4-NT to packing material.

### Figure 4




The organic load (OL) and metabolic quotient (qS) over a 76-day period. Figure 4 a. shows the organic load (OL) and degradation velocity (qS) over a period of 76 days. Figure 4 b. shows a scatter plot of the corresponding average results for OL plotted against qS. Figure 4 c. shows the same scatter plot after the data points for experiments 3, 4, 7 (4-NT was present) and 11 (highest concentration of 2,4-DNT present) are removed.