Ecotoxicological assessment of anaerobic bioremediation of sludge contaminated by the explosive 2,4,6-trinitrotoluene (TNT)

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BIOREX

Biological Remediation of Explosives

*Project Objectives*
- Develop a method for microbial degradation of explosives
- Remediate sludge and soil contaminated with explosives
- Characterization of the degradation process
Project Partners

- Mälardalen University
- Nammo Demil Division
- FOI (Swedish Defense Research Agency)
- Cesium Innovation Company
- Eurencio Bofors
- Bofors Test Center
- KCEM (Competence Centre for Energetic Material)
PhD Research Project

Aims:
- Chemical characterization of the degradation process
- Ecotoxicological testing during the degradation to identify toxic intermediates in the process
Why microbiological degradation of explosives?

- **Contamination** of soils during the manufacture, employment and disposal of explosives worldwide
- Most explosive substances are *environmentally very stable* compounds
- Many explosives, such as TNT, are very *toxic* to living organisms
- Desirable to develop a *cheap, non-toxic degradation method*
Project Idea

TNT sludge

Bacterial culture Substrate → Bioslurry → Organic material Water CO₂
Current Experimental Set-up

TNT-contaminated sludge mixed with an adapted bacterial culture in a sand slurry

Left picture: Jianjun Hao, UC David Plant Pathology Department, University of California
Current Experimental Set-up

- 16-day degradation of TNT in bioslurry batch experiments (5 & 10% TNT)
- TNT-adapted bacterial culture from a denitrifying activated sludge
- Static, reducing conditions
- Monitoring and adjustment of pH and nutrient levels
Chemical characterisation of the degradation of TNT

- Frequent sampling during the degradation process

- Chemical structural analysis of the extracted samples

- Characterisation of the step-wise degradation of TNT by the bacterial culture
Chemical analysis

Gas chromatography:
- How much of the explosive is degraded?
- What metabolites are formed in the process?
Ecotoxicological testing of metabolites in the degradation process

- Frequent sampling during the degradation process
- Toxicity testing of the samples
- Ecotoxicological characterisation of the degradation process
Ecotoxicity tests

Consumers:
*Heterocypri*s
*incongruens*  
*(Ostracodtoxkit)*

Decomposers:
*Vibrio fischeri*  
*(Microtox)*

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Project Outcomes

- Identifying the metabolites of the degradation process
- Determining the combined toxicity of the metabolites during the degradation
- Optimizing the bacterial degradation of explosives
Results
Chemical characterization

- **Monoaminodinitrotoluene**s (4a-2,6-dnt and 2a-4,6-dnt) and **dinitrotoluenes** (2,6-dnt and 2,4-dnt) found in µg/g levels
- Trace levels of **nitrotoluenes, dinitrobenzenes** and **dinitrotoluenes** (2,5-dnt, 2,3-dnt and 3,4-dnt) were detected

- Concentrations of **trinitrobenzene** could not be quantified
- The presence of **diaminonitrotoluene** (2,6-dia4-nt) could not be verified
- Degradation of **TNT** was low
Chemical characterization

Chromatogram from the analysis of a 5%TNT bioslurry sample taken on day 16 of the degradation
Ecotoxicological characterization

*Heterocypris incongruens Toxicity (Ostracodtoxkit)*

- Mortality of control organisms exceeded the validity limit in all tests

- 100% mortality of all test organisms in slurry samples
Ecotoxicological characterization

Vibrio Fischeri toxicity (Microtox)

Toxicity of slurry leachates to Vibrio Fischeri
Conclusions

- The bacterial culture did not degrade TNT efficiently under the experimental conditions

- The results of the ostracod toxicity test were invalid due to high mortality of the control organisms

- Microtox was used successfully to assess the toxicity of slurry leachates
Future research

- Optimized TNT degradation by environmentally adapted microorganisms in a bioslurry reactor

- Extended ecotoxicity testing using aquatic organisms and enzymatic assays
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