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

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Project Partners

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

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PhD Research Project

Aims:

- Chemical characterization of the degradation process
- Ecotoxicological testing during the degradation to identify toxic intermediates in the process

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Why microbiological degradation of explosives?

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

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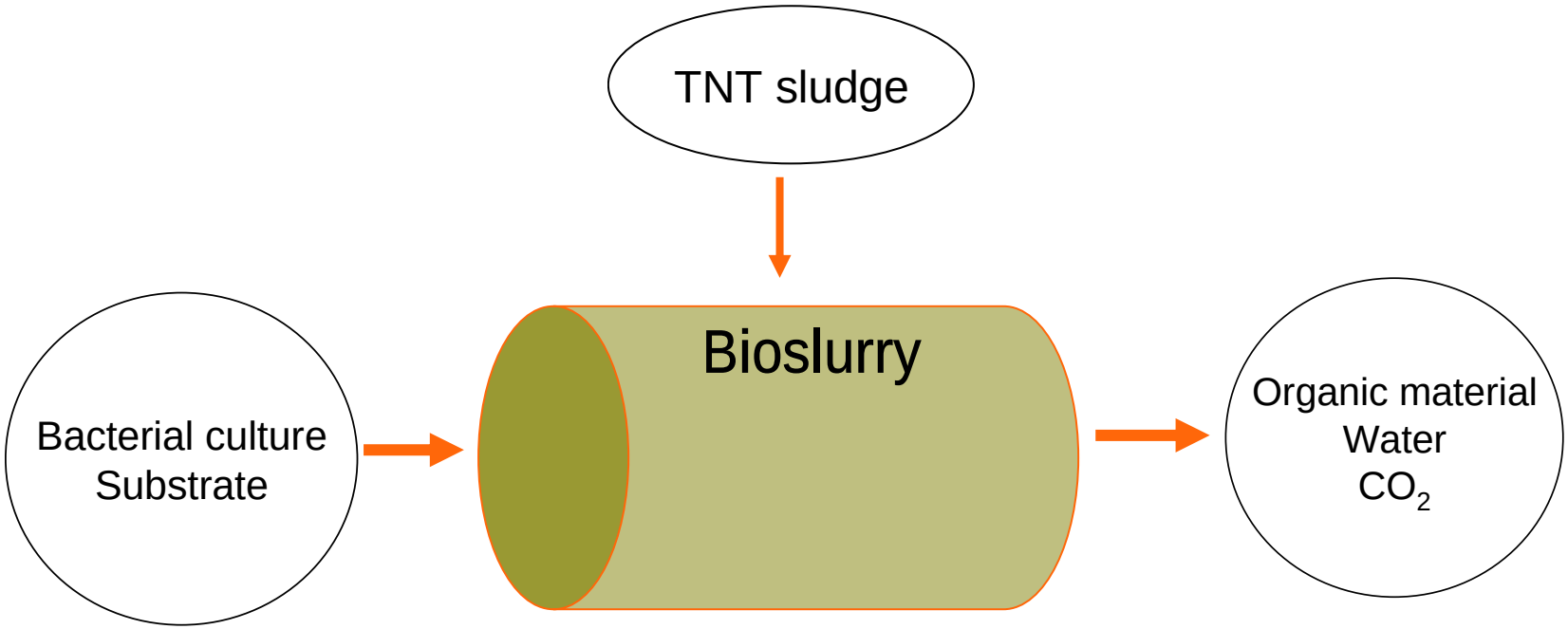
Project Idea



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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

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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

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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

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Chemical analysis

Gas chromatography:

- How much of the explosive is degraded?
- What metabolites are formed in the process?

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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

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Ecotoxicity tests

Consumers:

Heterocypris 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

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Results

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Chemical characterization

- Monoaminodinitrotoluenes (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

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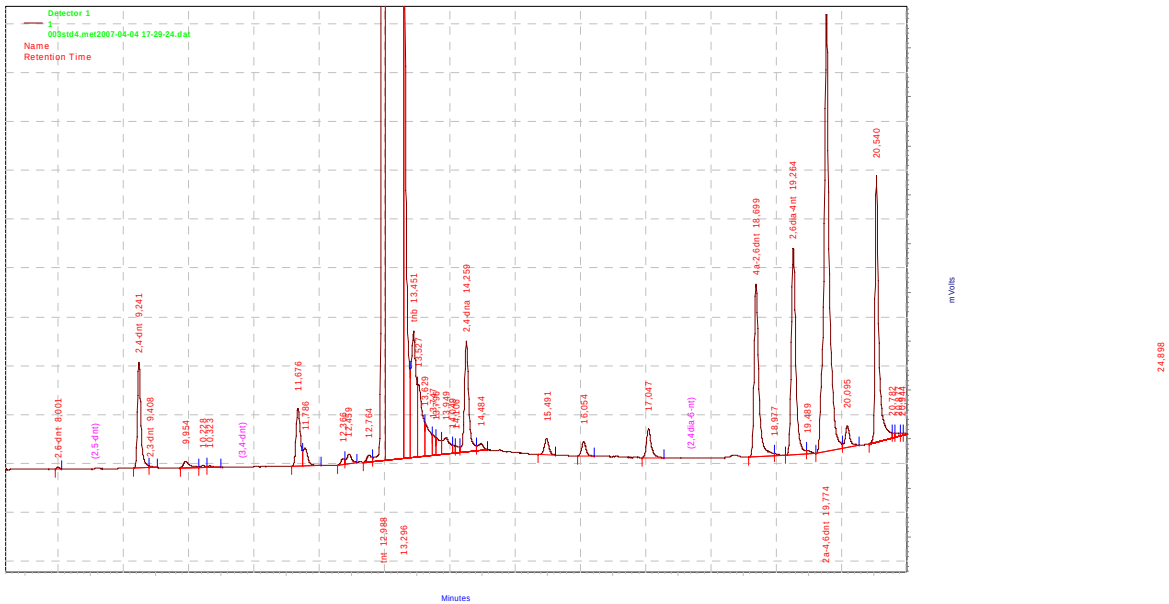
Chemical characterization

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



Ecotoxicological characterization

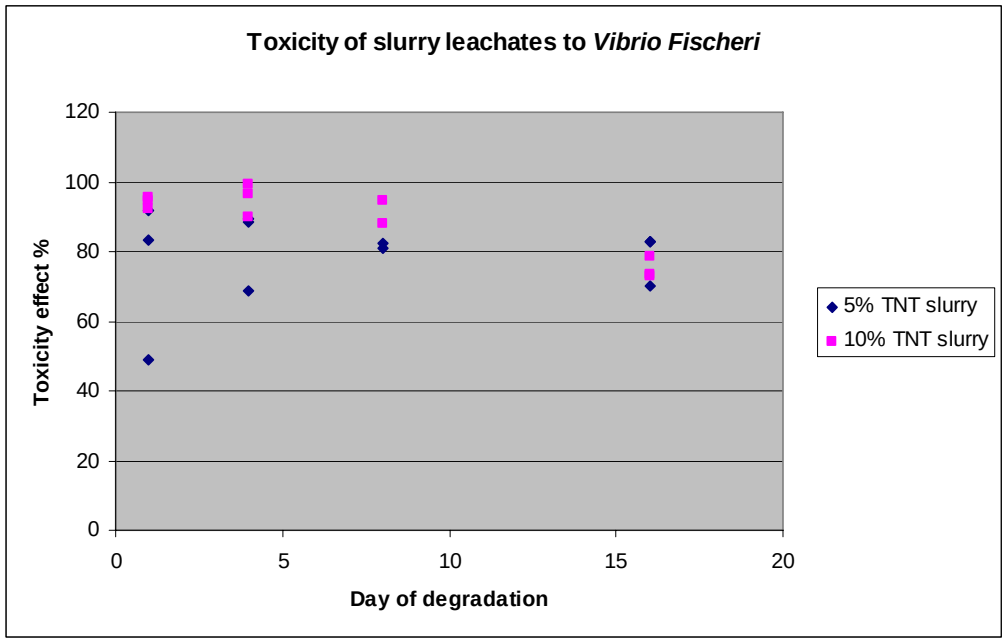
Heterocypris incongruens Toxicity (Ostracodtoxkit)

- 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)



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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

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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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Nammo Demil Division

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