

## Biological Degradation of High Concentrations of 2,4- and 2,6-DNT

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**Background/Objectives.** On a former toluene-di-isocyanate production site in Brazil, a variety of contaminants are present, including nitrotoluenes, chlorobenzenes, BTEX and some chlorinated compounds. The main components of concern are 2,4 and 2,6-dinitrotoluene (DNT). Highest maximum concentrations for DNT that were found were 937,174 µg/l 2,4-DNT and 538,508 µg/l 2,6-DNT. Greensoil was given the opportunity to investigate the feasibility of both aerobic and anaerobic biodegradation of the different contaminants with the focus of DNT by means of laboratory degradation tests.

**Approach/Activities.** Based on scientific literature DNT can be biodegraded both aerobically via removal of the nitro groups, leading to the production of nitrite as degradation product and anaerobically, where the nitro groups are being reduced to amino groups. Since diaminotoluene (DAT) is considered more recalcitrant under anaerobic conditions, the anaerobic conditions are typically followed by aerobic conditions, to further biodegrade the DAT. Even though switching from anaerobic to aerobic conditions takes extra effort, costs etc. from a practical point of view, this approach was considered since dichlorobenzene (DCB) was being used as solvent for DNT. In case of aerobic degradation, it is expected that DCB is preferentially degraded over DNT with the potential risk of crystallization of DNT, that has been observed at the site.

**Results/Lessons Learned.** Under aerobic conditions complete degradation of 2,4-DNT (> 99%) was observed in all biological conditions, including the biological control. Degradation rates of 2,4-DNT were however higher at buffered pH and in the presence of additional phosphate. No degradation of 2,6-DNT could be observed under aerobic conditions.

Under anaerobic conditions, both 2,4- and 2,6-DNT were completely reduced (> 99%) within 6 weeks only in the presence of an electron donor. At a natural pH of 6.0, the main degradation products in the presence of an electron donor were the three different aminonitrotoluene (ANT) isomers, while at neutral pH (7.5) they were 2,4-DAT and 2,6-DAT. Only at neutral pH, the degradation products were completely further degraded under subsequent aerobic conditions.

Next generation sequencing showed a strong enrichment in *Dysgonomonas* in both conditions with electron donor and in *Enterococcus* in the presence of an electron donor at neutral pH only, suggesting those genera might be involved in the reduction of DNT and ANT.

Compounds Specific Isotope Analysis showed a clear isotopic shift on the N atom in the anaerobic reactor that was operated.

Based on the laboratory degradation tests, anaerobic degradation of high concentrations of DNT, followed by aerobic degradation of the formed reduced degradation products appears to be a promising in-situ remediation technology. At the end of 2021 a field pilot test will be started where sequential anaerobic reduction and aerobic biological degradation will be applied and the role of the pH will be further investigated.