

Comparison of Dinitrotoluene Degradation by A Mixed Culture in Aqueous Batch System

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ABSTRACT

A mixed microbial culture was used to study biodegradation of 2,4-dinitrotoluene (2,4-DNT) and 2,6-dinitrotoluene (2,6-DNT) in aqueous media under aerobic conditions. The study was conducted in shake flasks with mineral salt medium with or without supplementation with either glycerol or succinate as primary carbon and energy source. With glycerol supplementation and 5.2 mg.L⁻¹ initial concentration of DNT, the results showed total disappearance of 2,4-DNT in 6 days and that of 2,6-DNT in 10 days. With succinate as carbon and energy source, total disappearance of 2,4-DNT was observed in 10 days, but the disappearance of 2,6-DNT was poor. One or more metabolites also accumulated in the medium. After six months of a selection pressure by DNTs, the mixed culture was still able to degrade 2,4-DNT almost completely; its ability to degrade 2,6-DNT in basal salt medium also increased. When the dinitrotoluenes were used as the sole source of C-, N- and energy, ~40 % of each DNT was removed within 4 days. However, uptake of 2,4-DNT showed a lag phase and one unidentified intermediate accumulated in the medium. 2,6-DNT uptake did not show any lag-phase and no intermediate accumulated in the medium.

INTRODUCTION

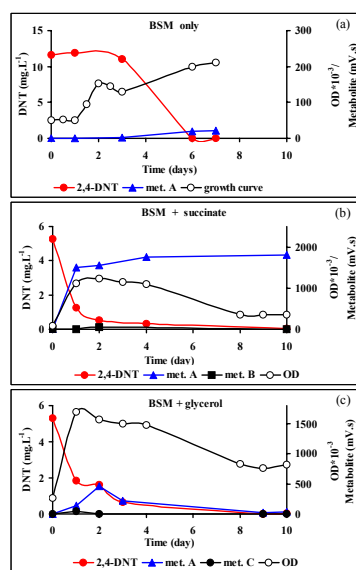
Dinitrotoluenes (DNTs) have been synthesized by successive nitration of toluene for use as explosives (Cooper, 1996) and as raw materials for manufacture of diisocyanate and dyestuffs (Dunlap, 1981). Mainly two isomeric forms of DNT (2,4-DNT and 2,6-DNT) are produced due to the tendency of methyl group to direct substitutions at ortho- and para-positions and of nitro-group to meta-position. The production and usage practices, discharges, and accidental spills have resulted in contamination of large tracks of surface and subsurface soil and groundwater by dinitrotoluenes along with other (mono- and tri) nitrotoluenes (Broder and Westmoreland, 1998). Since nitrotoluenes are categorized as priority pollutants (Kieth and Telliard, 1979) with strict limits on concentrations permitted in soil and water, several researchers have studied clean-up of soil, groundwater, and waste-water contaminated with dinitrotoluenes (Nishino *et al.* 2000; Razo-Flores *et al.* 1999; Davel *et al.* 2000; Mueller *et al.* 1993). Microorganisms with capability to degrade dinitrotoluenes have been isolated from contaminated sites. Spain and coworkers (Nishino *et al.* 2000) isolated pure cultures capable of degrading 2,4- and 2,6-DNT. These cultures were able to metabolize the DNTs as sole carbon, energy, and nitrogen sources. Presence of nitrates in the environment was found to be inhibitory to the cells. 2,6-DNT inhibited 2,4-DNT degraders, and vice-versa. 2,6-DNT was degraded considerably more slowly compared to 2,4-DNT. In a fluidized-bed reactor in which the suspended sand (Smets *et al.* 1999) or granular activated carbon particles (Malcolm Pirnie, 1999) were colonized by the 2,4- and 2,6- degraders, degradation of 2,6-DNT was determined to control the throughput for the reactor for effective clean-up of liquid streams. Given the known general problems of bioaugmentation and the difficulties reported in degradation of nitrotoluenes (Lindemann *et al.* 1998; Fortner *et al.* 2003), it is desirable to characterize the nitrotoluene-degrading microorganisms associated with specific sites of contamination. Hence, this effort involved isolating a mixed culture from an explosives-contaminated site in Czech Republic and investigations of its capability to degrade the different nitrotoluenes. The results dealing with degradations of 2,4-DNT and 2,6-DNT by this mixed culture are presented in this paper.

MATERIALS AND METHODS

Organisms: A mixed culture of nitrotoluene degraders was enriched from soil samples collected from the grounds of Synthesis Company, Pardubice (Czech Republic) in basal salt medium (BSM) and sodium succinate. The mixed culture was subcultured in BSM with a mixture of nitrotoluenes, stored at -80 °C, and propagated on Bacto-agar slants at 4 °C.

Medium: The BSM contained (in g.L⁻¹): KH₂PO₄, 4.30; K₂HPO₄, 3.40; KNO₃, 0.80; MgCl₂·6H₂O, 0.34; and 1 mL.L⁻¹ of trace-element solution. The composition of trace element solution was (g.L⁻¹): FeSO₄·7H₂O, 5.0; ZnSO₄·7H₂O, 5.0; MnSO₄·H₂O, 5.0; CuSO₄·5H₂O, 5.0; CoCl₂·6H₂O, 0.1; Na₂B₄O₇·10H₂O, 0.1; Na₂MoO₄·2H₂O, 0.1. pH of the basal salt medium was 7.2. 1.0 g.L⁻¹ glycerol or sodium succinate was added to the flasks along with the dinitrotoluenes (DNT). Medium containing sodium succinate was supplied with 50 mg.L⁻¹ yeast extract also.

Figure 1: Biodegradation of 2,4-DNT



Biodegradation experiments: All the experiments were conducted in 500 mL shake flasks (100 mL working volume) incubated at 26 °C on an orbital

DISCUSSION

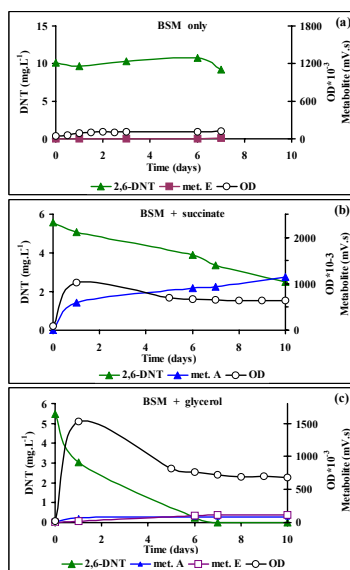
Cell Growth: In basal salt medium, cells grew well on 2,4-DNT but not on 2,6-DNT. As a result, degradation of 2,4-DNT was observed in BSM, but not of 2,6-DNT. Cell grew equally well in BSM supplemented with succinate or glycerol in presence of 2,4-DNT and 2,6-DNT. In a mixture of several nitrotoluenes, cell growth in succinate supplemented medium was less than in glycerol supplemented medium.

Biodegradation of 2,4-DNT: 2,4-DNT disappeared rapidly in all the three media (BSM only, BSM + succinate, BSM + glycerol). In BSM only, 2,4-DNT degradation had a long lag. In each case, biodegradation of 2,4-DNT appeared to be associated with cell growth. Only one major metabolite (A) accumulated in the broth, although two other metabolites (B and C) were also observed in trace quantities. Accumulation of Metabolite A was most significant in succinate-supplemented BSM.

Biodegradation of 2,6-DNT: 2,6-DNT disappearance in BSM-only medium was insignificant. Even in supplemented media, rate of disappearance of 2,6-DNT was considerably slower than that of 2,4-DNT. In succinate-supplemented medium, a significant amount of 2,6-DNT remained in the broth even after 10 days of incubation. In glycerol-supplemented medium, however, 2,6-DNT disappeared completely by the sixth day.

RESULTS

Figure 2: Biodegradation of 2,6-DNT



shaker at 120 rpm. Samples were collected either once or twice a day, and were analyzed for cell density, DNTs, and intermediate metabolites.

Biodegradation in a mixture of nitrotoluenes:

The rates of disappearance of both, 2,4- and 2,6-DNT, were slower in a mixture of mono- and dinitrotoluenes than when the DNTs were present alone. 2,4-DNT was still completely degraded by the sixth day in succinate- as well as in glycerol-supplemented BSM. In both the media, however, degradation of 2,6-DNT was incomplete even after 10 days.

Effect of storage on biodegradation capabilities of the mixed culture: The mixed culture was stored in presence of a mixture of nitrotoluenes in refrigerator for six months. After this period of storage, the cells' ability to degrade 2,4-DNT in un-supplemented BSM remained unaffected. The stored cells showed an ability to degrade 2,6-DNT in BSM-only medium. This acquired ability to degrade 2,6-DNT has been reported also by Nishino *et al.* (2000).

Figure 3: Biodegradation in a mixture of nitrotoluenes

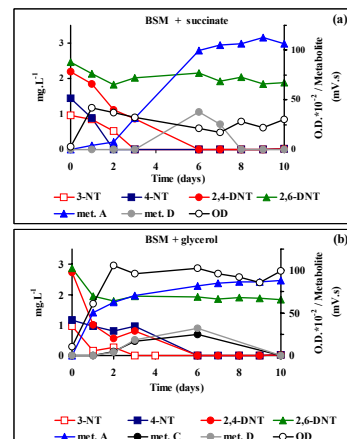
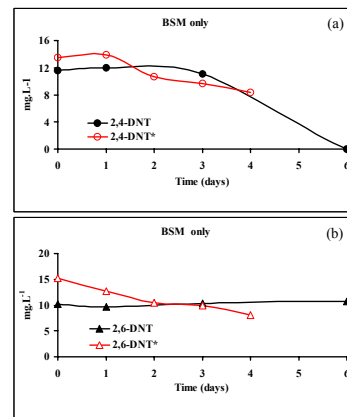


Figure 4: Effect of storing cells in a deep freezer on their ability to biodegrade nitrotoluenes



CONCLUSIONS

The mixed culture isolated from the Pardubice site was capable of biodegrading both the nitrotoluenes when properly supplemented with additional carbon source. It was able to utilize 2,4-DNT as a sole source of carbon and energy all the time, starting from the beginning. However, it acquired an ability to use 2,6-DNT as sole carbon and energy source only after a considerable selection pressure. Storage of cells in a mixture of nitrotoluenes was, thus, helpful.

ACKNOWLEDGMENT

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