

# *Chapter 7*

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## CONCLUSIONS

The mixed bacterial culture used in the present study was obtained from a soil sample polluted with phenolic compounds and was found to consist of eight different bacterial cultures. The consortium on enrichment and long periods of acclimation was found to degrade all the mononitrophenol isomers (ONP, MNP and PNP) individually as well their mixture, though, to varying degrees after a minimum lag of 6-12h. The rate of MNP degradation being generally the fastest followed by PNP and ONP. Simultaneous degradation also showed rapid disappearance of MNP. Nitrite was released during the degradation of ONP and PNP indicating an oxidative mechanism whereas production of ammonia during MNP breakdown indicated a reductive pathway. This type of activity was observed during degradation of mononitrophenol isomers by the consortium and a single bacterial isolate. Release of nitrite is probably a defense mechanism against the toxicity of the compound. Production of ammonia from nitroaromatic compounds avoids the production of potentially toxic amines in the environment. Toxic concentrations ( $> 0.5\text{mM}$ ) were tolerated by the consortium and successful degradation ranging between  $0.5\text{mM}$ - $5\text{mM}$  were recorded using the consortium as evident by the viability of the cells and the identification of metabolites. The lag period increased with increasing substrate concentration. At very low concentrations, the long acclimation periods may be the result of slow growth of the mineralizing organisms. However at high concentrations, the toxicity of the compound may reduce the number of active organisms and increase the acclimation period and this may also be

accounted for the apparent low growth observed throughout the experimental study. The presence of toxic intermediates, their accumulation or even an inadequate supply of essential nutrients may account for the delay in growth. Induction experiments revealed that pre-exposed cells brought about early substrate disappearance which was a favourable process for rapid biodegradation.

Strong dioxygenase activity was observed in cell extracts of the consortium induced with mononitrophenol isomers. Monooxygenase activity was also observed in partially degraded crude cell extracts of ONP and PNP induced cells. Pronounced catechol 1,2-dioxygenase activity in ONP and PNP induced cell extracts clearly indicated an ortho cleavage of the substrates. In contrast MNP induced cell extracts showed only catechol 2,3-dioxygenase activity which is an enzyme involved in meta cleavage. The ring cleaving enzyme had an absorbance maxima at 375nm consistent with hydroxy muconicsemialdehyde. None of the enzymes involved seemed to require any additional co-enzymes or manganese ions as no such compounds were added during the course of study.

Resolution of the consortium revealed the presence of eight bacterial culture which were characterized and identified as *Bacillus licheniformis* (SNP-1), *Xanthomonas maltophila* (SNP-2), *Serratia liquefaciens* (SNP-3), *Pseudomonas putida* (SNP-4), *Pseudomonas* sp. (SNP-5), *Pseudomonas alcaligenes* (SNP-6), *Pseudomonas* sp. (SNP-7) and *Sarcina maxima* (SNP-8). All the cultures were capable of degrading ONP and PNP and also

brought about simultaneous degradation of a mixture of the mononitrophenol isomers to varying degrees indicating a concerted effort towards breakdown of the substrate. Among them an unreported culture *Sarcina maxima* (SNP-8) was studied in detail for its ability in transforming the substrates. The culture followed both oxidative and reductive mechanisms in degrading the isomers.

Intensive NMR studies revealed the pathways followed by the consortium and the bacterial isolate *Sarcina maxima* based on the metabolites identified. ONP was degraded by the consortium and *Sarcina maxima* with catechol as the initial metabolite asserting the involvement of monooxygenase activity. Muconolactone was a common intermediate though not a dead end metabolite.  $\gamma$ -hydroxy muconicsemialdehyde and  $\beta$ -hydroxy maleylacetate, a new metabolite hitherto unreported was identified in the reaction mixture of the consortium and the individual culture respectively.

A variation between the consortium and *Sarcina maxima* could be observed in the initial catabolism of MNP. The consortium seemed to breakdown MNP to 4-aminocatechol which undergoes enzyme catalyzed removal of nitro group to give  $\beta$ -ketoadipate via 1,2,4-benzenetriol. Alternatively *Sarcina maxima* converted MNP to 2-nitrohydroquinone, not substantially proved in previous reports. The 2-nitrohydroquinone can undergo one-electron reduction to give 2-hydroxyl aminohydroquinone which is further degraded to ammonium and later mineralized by ring cleaving enzymes.

The initial reaction in the degradation of PNP by the consortium was clearly a monooxygenase catalyzed hydroxylation of the ring forming 4-nitrocatechol which undergoes an oxygenase catalyzed removal of the nitro group with the formation of 1,2,4-benzenetriol as evident by the enzyme activity and presence in the reaction mixture. In contrast 4-nitrocatechol was not detected in the reaction mixture of *Sarcina maxima*, instead  $\gamma$ -hydroxy muconicsemialdehyde, maleylacetate and  $\beta$ -keto adipate were observed. Therefore PNP breakdown by this culture was expected to go through the formation of p-hydroquinone.

The investigation aided substantially in understanding the degradative ability and pathway followed by the consortium and the bacterial culture *Sarcina maxima*.

## SCOPE

Because of the environment problems caused by nitroaromatic compounds, a potent microbial consortium or a combination of competent constituent cultures of a consortium could be employed in bioremediation technologies of natural water, soil and in treatment of industrial and sewage waste water.

- ◆ Nitroaromatic degrading microorganisms may also be applied in the biocatalytic production of industrially valuable compounds from relatively cheap substrates which may be difficult to synthesis chemically.
- ◆ Enzymes in the degradative pathway could be employed in degrading related nitroaromatic compounds as some of them possess broad substrate specificity and such reactions may also lead to the formation of selected products
- ◆ Whole cells or cell preparations could be used in the production of biosensors employed in the study of pesticide residue analysis.
- ◆ The substrate range of bacteria may be broadened by genetic manipulation of the degradative pathway in order to treat closely related compounds

- ◆ Adaptation and tolerance to several related compounds could be used as an advantage in the treatment of a particular mixture of pollutants.
- ◆ Cultures able to tolerate varying concentrations and culture conditions could be employed in fermentors with IBT (immobilized bacteria technology) to withstand high chemical loading.
- ◆ Employment of a consortium with two antagonistic activities-reductive and oxidative, is an advantage and can especially be used in treatment of heterogeneous wastes.