

Summary

SUMMARY

Nitroaromatic compounds (NAC) are pollutants resulting from numerous industrial and agricultural activities. Some compounds are produced by incomplete combustion of fossil fuels, others are used as synthetic intermediates in the manufacture of dyes, plasticizers, pesticides, explosives and solvents. As a consequence, nitroaromatic compounds have become pollutants in rivers, wastewaters, groundwaters, soils and the urban atmosphere.

The presence of the nitro group causes such compounds to be more resistant to biodegradation than the unsubstituted analogs and they tend to accumulate in the environment and cause deleterious effects to the biological systems due to their toxicity. Fresh and marine waters, sewage and soils possess highly diverse microbial communities that exhibit degradative capacities and species within these communities transform many organic compounds aerobically or anaerobically. A mixed bacterial system isolated from a contaminated soil sample was employed during the present study on individual and simultaneous degradation of mononitrophenol isomers ONP, MNP and PNP. The study is recorded in the following seven chapters as follows-

Chapter 1 consists of a brief introduction to nitroaromatic compounds and detail review of literature regarding the biodegradation of these compounds both aerobically and anaerobically by various microorganisms and specially

bacteria. Emphasis is also laid on the different mechanisms involved in degradation. Factors that affect biodegradation such as carbon and nitrogen sources, inorganic nutrients, acclimation period, inoculum size, substrate concentration, adaptation, varying pH and temperature conditions have been dealt with. It includes a brief introduction the characteristics of the mononitrophenol isomers and the objective of studying their degradation.

Chapter 2 encompasses all the materials and methods employed during the degradative studies of mononitrophenol isomers. Analytical procedures like calibrations and estimation of growth of cultures, estimation of phenol, cresols, nitrophenol isomers, ammonia and nitrite have been dealt with. A separate section, Bacteriology, deals with the enrichment, isolation of mixed bacterial system and its constituent cultures, their characterization and identification.

Chapter 3 deals mainly with degradation using a consortium. The mixed bacterial culture successfully degraded individual isomers of mononitrophenol and their mixture. The consortium degraded subtoxic concentrations ($< 0.5\text{mM}$) of ONP, MNP and PNP by 54h, 12h and 36h on short periods of induction. Nitrite was released during ONP and PNP degradation indicating an oxidative mechanism whereas production of ammonia during MNP breakdown suggested a reductive mechanism. Simultaneous degradation of a mixture of the isomers occurred by 18h during which more than 90% substrate disappearance was observed. Differential rates of degradation of the three isomers from a mixture

indicated that MNP was catabolized faster than PNP and ONP as evident by HPLC. Only nitrite release could be recorded during simultaneous degradation of subtoxic concentrations probably due to the presence of two sources of nitrite production in the form of ONP and PNP in the mixture. Pre-growth of the consortium on related aromatic compounds such as isomers of cresol and phenol drastically affected the rate of degradation of all the mononitrophenol isomers. Pre-growth on non-aromatic compound such as sodium acetate resulted in retardation of degradation of ONP, MNP and PNP. Pre-exposed cells of the consortium degraded ONP, MNP and PNP by 24h whereas non-exposed cells could catabolize only 50% in the same period. Presence of an additional nitrogen source (NH_4NO_3) did not affect the degradation rates. The consortium could tolerate toxic concentrations (0.5mM-3mM) and bring about complete degradation of 1.5mM concentrations.

Chapter 4 highlights the degradative abilities of the individual bacterial cultures of the consortium. Eight bacterial cultures were isolated from the consortium and they were identified as *Bacillus licheniformis* (SNP-1), *Xanthomonas maltophila* (SNP-2), *Serratia liquefaciens* (SNP-3), *Pseudomonas putida* (SNP-4), *Pseudomonas* sp. (SNP-5), *Psuedomonas alcaligenes* (SNP-6), *Psuedomonas* sp. (SNP-7) and *Sarcina maxima* (SNP-8) of which *Sarcina maxima*, an unreported culture was employed for degradation studies.

All the cultures except SNP-4 and SNP-7 showed around 50% reduction in substrate concentration by 96h. Cultures SNP-2, SNP-5 and SNP-6 brought around 90% reduction in substrate concentration within 96h followed by SNP-3, SNP-8 and SNP-1 which degraded 50% of the mixture. SNP-4 and SNP-7 could degrade only about 20% of the isomers. Well induced cells of *Sarcina maxima* exhibited both oxidative and reductive mechanisms in degrading mononitrophenol isomers as evident by the release of nitrite and ammonia respectively. Interestingly cells induced for a long periods could degrade around 50% of 0.5mM of a mixture of the isomers while cells induced for comparatively short periods showed only 34% reduction in substrate concentration.

Chapter 5 includes the assays adopted to study the enzymatic activities of cell extracts of the mixed bacterial culture. Dioxygenase activities were observed in cell extracts of the consortium induced with ONP, MNP and PNP separately. Pronounced catechol 1,2-dioxygenase activity was observed in ONP and PNP induced cell extracts indicating an ortho cleavage pathway in their degradation during which catechol was oxidized to cis, cis-muconic acid. Catechol 2,3-dioxygenase activity could be recorded only in MNP induced cell extracts and an absorbance maxima at 375nm consistent with the formation of 3-hydroxy muconicsemialdehyde was observed

Chapter 6 is an extensive study on the biochemistry of the pathways employed by a mixed bacterial culture and one of its constituent culture *Sarcina maxima*, hitherto unreported, using NMR spectroscopy. Intensive ¹HNMR and 2D HMQCT studies revealed the pathways followed by the

presence of metabolites such as catechol, cis, cis-muconic acid, γ -hydroxy muconicsemialdehyde, maleylacetate and β -keto adipate. The spectra of ONP reaction mixture degraded by *Sarcina maxima* showed that formation of maleylacetate from γ -hydroxy muconicsemialdehyde should go through a new metabolite β -Hydroxy maleylacetate, hitherto unreported. A deviation in MNP degradation of the consortium and the single culture was observed. The consortium seemed to breakdown MNP to 4-aminocatechol indicating it came from 3-hydroxyl aminophenol, 1,2,4-benzenetriol and β -keto adipate were the other metabolites. *Sarcina maxima* seemed to convert to 2-nitrohydroquinone as indicated by its presence along with γ -hydroxy muconicsemialdehyde, muconolactone and maleyl acetate. The pathway followed by the consortium during PNP degradation was by the formation of 4-nitrocatechol, maleylacetate and β -keto adipate which was confirmed by the ^1H NMR spectra. Both ^1H NMR and Carbon-13 signals from 2D HMQCT confirmed the presence of maleylacetate, γ -hydroxy muconicsemialdehyde and β -keto adipate in the PNP reaction mixture of *Sarcina maxima*. The pathway is expected to go through the formation of p-hydroquinone as the initial metabolite as no 4-nitrocatechol was detected.

Chapter 7 draws conclusions from the degradation studies of mononitrophenol isomers carried out by the mixed bacterial culture and the individual culture *Sarcina maxima* (SNP-8) and lists the scope for future research in the field of biodegradation studies.