CHAPTER VI

SUMMARY
For years the oceans have been considered as the ultimate sink for all kinds of pollutants. The rationale was that dilution would serve as an effective means to reduce the risks rising from exposure to these chemicals. The spillage of tons of crude oil in the oceans in the last few decades has been of common occurrence and the effects of these spills on marine life and environment cannot be ignored.

Our studies have focussed on the biodegradation of petroleum in the environment with emphasis on essentially the Nitrogen-Heterocyclic Aromatic (NHA) components of oils. Most of the previous studies by numerous workers have mainly concentrated on the aliphatic and aromatic compounds, while very little information is available on the biodegradation of the heterocyclic compounds which are major components of asphaltenes and NSO fractions. While it is correct to emphasize the major components in petroleum it is misleading to limit analysis to them. It has very often been assumed that the nitrogen fraction in oil is unavailable to bacteria. However, many of these polar compounds are more water soluble than their homocyclic analogs and have toxic and carcinogenic properties. They have been shown to be toxic to marine fish and may exert their toxic effects to bacteria as well. This could result in the disruption of the community structure of the microbial population. Thus they may selectively inhibit or kill organisms capable of carrying out ecologically important activities, which may affect the total ecosystem.
Quinoline and its derivatives are the predominant constituents of the neutral and basic fractions of oils. We initiated our studies with the development of quinoline degrading marine bacteria by enrichment culture. Obtaining bacteria selectively enriched on quinoline could result in a population which would attack the N-heterocyclic fraction in crude oil. This is rarely the case with bacteria isolated on whole crude oil because this strategy would serve to select the rapidly growing bacteria attacking the aliphatic fraction and thus the N-heterocyclic fraction would remain unattacked.

Having established a pure culture of a marine bacterium degrading quinoline we found it to have major similarities with genus *Pseudomonas* and designated it as *Pseudomonas* sp. strain GU104. On membrane lipid analysis using computer aided taxonomy (CAT) the strain was shown to be phylogenetically closer to *Ochrobactrum anthropi* (*Achromobacter*) (D. Chandramohan, personal communication). Its growth under different cultural conditions was studied. Strain GU104 was able to utilize many other methyl and hydroxy derivatives of quinoline for growth including some aromatic compounds, crude oil, kerosene, petroleum and diesel. Many quinoline degrading bacteria previously reported are selective with respect to the range of substrates they can utilize for growth. However, the marine strain GU104 was biochemically very versatile. Results generated from different regions of Bay of Bengal showed that quinoline degrading cultures are quite rare in the ocean and the same could be isolated if benzoate is used as substrate for enrichment.
The kinetics of quinoline metabolism was studied by continuous culture in order to determine conditions of maximum rates of quinoline conversion. The $K_i$ value (inhibitory constant for quinoline) and $K_s$ (saturation constant) was also determined by these studies. These studies are especially significant in the case of natural isolates such as marine bacteria that survive in nutrient limiting conditions of the oceans. The immobilization of cells of strain GU104 gave high conversion rates of quinoline and the high concentration used were not inhibitory to the entrapped cells.

It was observed that quinoline was metabolized via 2-hydroxyquinoline. Oxidation of quinoline and its derivatives as also other NHAs and aromatic compounds were studied with cells of strain GU104 grown on quinoline and lactate in ASW medium. Cells grown on quinoline are able to oxidise many monomethyl and hydroxy derivatives of quinoline. However, glucose and lactate grown cells did not show any uptake with quinoline, unless subjected to induction with quinoline as inducer. The inducible nature of quinoline metabolism was also confirmed using chloramphenicol.

The first enzyme quinoline oxidoreductase carrying out the conversion of quinoline to 2-hydroxyquinoline was assayed. The $K_m$ values of the enzyme as well as its pH and temperature optima were investigated. The enzyme was able to give a lowered activity with some methyl- and hydroxy-derivatives of quinoline. Partial purification of the enzyme was attempted and
it was found to be of a high molecular weight as apparent from native polyacrylamide gels.

Strain GU104 was found to contain two high molecular weight plasmids. Curing the larger molecular weight plasmid resulted in the loss of the ability of the strain to utilize quinoline as carbon source. Oxygen uptake and enzyme assay studies with mutants of strain GU104 indicate that the genes for quinoline metabolism are not linked.

Finally the ability of strain GU104 to mineralize various components of crude oil and tarballs was investigated. Growth on crude oil and tarballs was accompanied by a rapid emulsification of the oil and disruption of the tarballs.

Cell free supernatants of strain GU104 grown on crude oil were able to emulsify and solubilize hydrocarbons. All fractions of crude oil were attacked by strain GU104. A prominent feature was the simultaneous depletion of the NPAC fraction of oil which is normally attacked to a very low extent after primary attack on other hydrocarbons and sometimes not at all. Strain GU104 isolated by enrichment culture using quinoline as substrate was able to attack the NPAC fraction as well resulting in a 70% decrease of the same.

Many NSO compounds present in crude oils are known to affect the biodegradation of other aromatic compounds being toxic to many bacteria. Our studies have shown that quinoline affects the biodegradation of
compounds such as xylene, toluene and naphthalene since it prevents the growth of other bacteria degrading these compounds in batch cultures. Presence of strain GU104 lifts these repressive effects by effecting the removal of quinoline from the medium. The above result was demonstrated in binary as well as mixed culture systems.

One of the effects of quinoline was the formation of metabolically blocked mutants of m-toluic acid degradation which resulted in the accumulation of a brown coloured intermediate. Such a phenomenon was also seen when aqueous extracts of tarballs known to contain the remnants of crude oil after weathering and biodegradation were placed in the centre of petriplate plated with the m-toluic acid degrading bacterium. This indicates that such compounds may be present in severely degraded crudes.

The efficacy of a seven membered mixed culture consortium of which strain GU104 was a component was tested in 300l microcosms. The mixed culture worked effectively, all the members maintained stable numbers during the experiment and were able to scavenge and utilize carbon sources derived from tarballs.

In conclusion the biochemical versatility of strain GU104 as well as its ability to degrade the heterocyclic components of crude oil warrants its use in bioremediation of marine environments either singly or as part of a mixed bacterial culture.