CHAPTER 7

Conclusion
Biodegradation of petroleum crude oil depends on the composition of the oil, the prevalent environmental factors and the microbial population. Both the crude oils, BHCO and SGCO used in this study had relatively high saturate content and low amounts of the aromatic, resin and asphaltene fractions which made them highly susceptible to biodegradation. Of the various bacterial isolates obtained from hydrocarbon contaminated soil/water samples, *Acinetobacter* sp. A3 proved to possess the best crude oil degradative potential as assessed by its growth on crude oil and rate of crude oil oxidation. Moreover, it could tolerate and grow at BHCO concentration of 20%, thus making it potential candidate for degradation of high crude oil concentrations. A test for growth and degradation of individual hydrocarbons showed that *Acinetobacter* sp. A3 could utilize alkanes with greater than ten carbon atoms (C₁₀) but not cycloalkanes (cyclohexane), monocyclic (benzene, xylene, toluene) or polycyclic (naphthalene, anthracene, phenanthrene) aromatic hydrocarbons.

Analysis of the residual crude oil after biodegradation by *Acinetobacter* sp. A3 revealed that while the saturate fraction was most rapidly and extensively degraded, degradation of the resin fraction occurred more slowly. The aromatic and asphaltene fractions on the other hand remained undegraded. Thus, total biodegradation of crude oil by *Acinetobacter* sp. A3 was limited by the inability of this culture to utilize aromatics and asphaltenes.

The growth of *Acinetobacter* sp. A3 during BHCO degradation was not accompanied by the production of any biosurfactants/bioemulsifiers, but the cells showed a highly hydrophobic cell surface with the induction of at least two outer membrane proteins (OMPs) of molecular weights of 26.5 and 56 kD. Moreover, *Acinetobacter* sp. A3 grown with BHCO were much smaller in size and spherical in shape compared to nutrient broth grown cells; an adaptation to increase the cell surface area so as to allow greater cell-oil interaction. Thus, in the absence of production of any surface active agents to promote crude oil emulsification/dispersion, the hydrophobic cell surface, the induced OMPs and the cell morphology played an important role in cell-oil interaction for facilitating crude oil uptake and degradation.

With respect to the optimum conditions for crude oil degradation by *Acinetobacter* sp. A3, it was observed that medium pH of 7-8 with incubation temperatures of 30-40 °C and high aeration rates favoured maximum biodegradation. In addition, N and P were essential to the biodegradation process and C:N and C:P ratios of 40 and 200 respectively supported best degradation rates. Similarly, while the presence of iron enhanced degradation, NaCl concentration of 3% (average salinity of oceans) inhibited degradation. Moreover, no enhancement of biodegradation was observed on
supplementation with chemical or bio-surfactants to the medium. Thus, these studies indicates that Acinetobacter sp. A3 was a potential crude oil degrader under optimum condition of $O_2$, pH temperature and nutrients, but its inability to tolerate high salt concentrations limits its use to non-marine environments.

Acinetobacter sp. A3 not only effectively degraded crude oil in shake flask experiments, but also in unsterile soil, which was representative of the actual oil contamination scenario. Bioaugmentation with Acinetobacter sp. A3 to BHCO contaminated soil resulted in its proliferation accompanied by enhanced oil degradation. Enhanced crude oil degradation in the treated soil reduced the phytotoxic effect exerted by the crude oil, thereby allowing almost normal rate of germination and growth of Mung (Phaseolus aureus) plants. These experiments thus conclusively proved the ability of this culture to not only remain viable, but also multiply utilizing oil from the contaminated soil, thereby making it a potential agent for bioremediation purposes.

To overcome the shortcomings of water soluble nutrients (fertilizers) of being leached away or diluted when applied for bioremediation, paraffinized fertilizers were prepared which supported crude oil biodegradation by slow nutrients release as degradation progressed. Also, formulations of freeze dried Acinetobacter sp. A3 cells and paraffinized fertilizers were equally effective in supporting biodegradation. Such formulations consisting of microbial cells and nutrients would make them more effective for bioremediation since the added microorganisms would enhance biodegradation rates while the fertilizers would remain associated with oil and release nutrients as degradation progressed.

With the exception of microbial consortium, OSL, all the other microbial consortia isolated from hydrocarbon contaminated samples were found to be better crude oil degraders than consortium FCON which was prepared by mixing cultures with known hydrocarbon degradative abilities. This emphasizes the importance of interaction between the microorganisms constituting a consortium for effecting maximum degradation. Such microbial interactions coupled with the ability of the individual microorganisms to degrade various types of hydrocarbons makes the use of microbial consortium much more effective when used for bioremediation.

This study also revealed that there was no involvement of plasmids and that the genes responsible for alkane degradation in Acinetobacter sp. A3 were chromosomal. Moreover, the initial steps of alkane degradation proceeded via the generally observed route of the step wise conversion of the alkane to the corresponding alcohol, aldehyde and carboxylic acid. As expected, transformation of Acinetobacter sp. A3 was not
successful, but fusion of protoplasts of *Acinetobacter* sp. A3 and *P. putida* DP99 resulted in hybrids capable of degrading alkanes and naphthalene and with enhanced BHCO degradation capability compared to either of the parent strains. Also, the fusants were morphologically similar to *Acinetobacter* sp. A3 but preferentially utilized naphthalene over tetradecane. The events at the genetic level responsible for imparting such unique characteristics to the fusant need to be worked out. Microorganisms with such multiple degradative abilities are better suited for bioremediation, since a microbial consortium might prove to be difficult to maintain and monitor in the environment.

Thus, during the course of this study, a potential crude oil degrading bacterium, *Acinetobacter* sp. A3 was isolated and various conditions for it to degrade crude oil were optimized. Also its ability to degrade crude oil under natural conditions were assessed and its degradative capabilities enhanced by protoplast fusion technique. In addition, formulations suitable for environmental application (bioremediation) were prepared and tested.