CHAPTER - VI

Summary and Conclusions

Biodegradation studies were carried out for anthracene, pyrene and chrysene in bioslurry reactors. As PAH compounds are highly insoluble in water and they adsorb tightly to the sediment particles, slurry phase remediation can be considered as an effective way of treating them. Bioslurry phase systems (liquid slurry treatment) utilize naturally occurring bacteria (native microflora) or inoculated strains having specific metabolic capabilities to convert hazardous organic compounds present in solid, liquid or sorbed forms to carbon dioxide and water. Bioslurry processes foster a heterogenous, auto-catalytic reaction, in which the microorganisms act as catalysts in metabolizing the substrate. Bioslurry systems can be maintained under aerobic or anaerobic conditions and an inoculum can be added if the soil being treated has low populations of organisms capable of degrading the chemicals of interest. Further, bioslurry reactors are easy to handle and are more efficient than conventional reactors. Therefore, in the present investigations, in depth studies were carried out for the degradation of three PAH compounds viz., anthracene, pyrene and chrysene using bioslurry reactors.

The selected soil was spiked by anthracene, pyrene and chrysene by dissolving them in dichloromethane. Five different slurry phase reactors were operated with different soil loading rates. Reactor A was operated as killed control under sterile conditions. It had sterile soil (autoclaved twice at 121^0C at 15 lbs pressure) and sterile water. Negligible degradation was observed in this reactor for all the three PAH compounds studied. It was operated to observe any abitotic losses taking place in the reactor. No variation was observed in the biochemical parameters viz., pH, ORP, DO and OCR. As there was no microbial growth CFU variation was also negligible.

Reactor B was the non- augmented reactor which had only native microflora of soil along with the spiked soil. Degradation to a small extent was observed in this reactor which can be attributed to the fact that native microorganisms include millions of microorganisms that are present in the soil which to some extent can degrade the contaminant. Reactors C, D and E are the augmented reactors. They had spiked soil and augmented domestic sewage. The effect of augmentation on different substrate concentrations was evaluated in these reactors. Reactor C was operated with a concentration of 30 g/Kg, reactor D at 60 g/ Kg and reactor E at 90 g/Kg. Bioaugmentation is a way to enhance the biodegradative capacities of contaminated sites by inoculation of bacteria with the desired catalytic capabilities. It is a method to improve degradation and enhance the transformation rate of xenobiotics by the injection of specific microbes, which are able to degrade the xenbiotics of interest. The degradation was found to be rapid at a concentration of 30 g/Kg followed by 60 g/Kg and 90 g/Kg. Initial slow degradation observed in the case of reactors (D and E) operated at higher substrate loading rate compared to reactor C might be due to adaptation requirement prior to acclimatization of the microflora to the higher substrate concentrations. It can be concluded that augmented reactors showed better and higher degradation efficiency compared to non- augmented reactors. When the consortium of soil and sewage microflora acted together as in case of reactors C, D and E the degradation was rapid and higher. It can be suggested that the microflora of soil and sewage were compatible with each other and thus effective bioaugmentation was observed.

Degradation of the PAH compounds at ambient soil temperatures in soil slurry systems for 30 g/Kg, 60 g/Kg and 90 g/Kg of substrate concentrations was studied. The biochemical parameters viz; pH, ORP, DO and OCR were comparatively consistent with the respective degradation profiles and the CFU values for all of the reactors increased by several orders of magnitude from time zero, indicating successful development of the microbial biomass in the soil slurry except in the case of 90 g/Kg of substrate concentration. The analysis showed that degradation must have taken place in the soil phase suggesting the fact that PAHs are highly hydrophobic compounds.

The degradation was found to be highest for pyrene and anthracene followed by chrysene. As the molecular weight of the compound increases, the compound tends to become recalcitrant [Harvey, 1997; Zander, 1983]. Maximum degradation for anthracene was found to be 89.6% in 144 h, 90% for pyrene in 120 h and 61% of degradation for chrysene in 168 h. Eventhough anthracene is a three ring compound, pyrene was degraded rapidly which might be due to high hydrophobicity of anthracene (0.065 mg/l for anthracene and 0.135 mg/l for pyrene).

Experiments were carried out taking augmented inocula alone with pyrene. The reactor performed well than the non- augmented reactor but the degradation was much less than the bioaugmented reactors. Thus, it can be said that bioaugmentation enhanced metabolic function of the microorganisms. This strategy also helped to improve the process performance due to the transfer of the requisite property to native bio- film through horizontal plasmid transfer between the microorganisms.

Microbial diversity offers environment- friendly options for mineralization of contaminants or their transformation into less harmful non- hazardous compounds. Efforts were made to characterize bacterial communities in the bioslurry reactor for the respective PAH compounds. PCR- DGGE technique provides a "snapshot" of community structure, an approximation of the number of populations and their proportional representation within the total community. Strength of the PCR- DGGE relies on its use in determining the effect of different soil treatments on community structure. It was observed that treatments that selectively enrich specific populations resulted in community structure differences, which could be seen in the PCR- DGGE profiles.

Genomic DNA from the reactor was extracted and then the purity of the DNA was checked on agarose gel. The DNA bands from the agarose gel were eluted and the DNA was amplified using PCR. The amplified DNA was checked for accuracy on agarose gel. DGGE was performed for the obtained bands on the agarose gel. Running the DGGE for collected band and the original sample side by side in a new DGGE gel checked the

accuracy of the process. For sequencing analysis, PCR products were sent to MWG Biotech Ltd., Bangalore.

The sequences obtained were aligned using CLUSTAL software. Phylogenetic trees were constructed for the PAH compounds.

The following conclusions were drawn from the present study

- Efficient degradation of three PAH compounds viz., anthracene, chrysene and pyrene was achieved using bioslurry reactors by optimization of various experimental parameters.
- Bioaugmentation enhanced the degradation of PAH compounds under study as the augmented reactors performed well than the augmented reactors.
- The consortium of native microflora and augmented inoculum worked efficiently towards degradation of PAH compounds compared to the treatments done by either native microflora or augmented inoculum alone.
- As the substrate concentration was increased, the degradation was found to be decreasing indicating toxicity of either the substrate or the metabolites to the microflora.
- Among the PAH compounds, the degradation of anthracene and pyrene was higher compared to chrysene suggesting the fact that as the molecular weight of the compound increased, recalcitrant nature increased, thereby decreasing the degradation ability.
- *Pseudomonas saccharophila* and *Pelomonas puraquae* were found to be common in all the reactor samples suggesting their ability to degrade all the three PAHs [pyrene, chrysene and anthracene].
- As PCR- DGGE involves genomic DNA extraction, many OTUs were identified for the PAH compounds under investigation, many of which were found to be suitable for the degradation of pyrene but for chrysene and anthracene it was not yet reported.