Biochemical and Genetic Studies on PAH degradation

Structure of catechol 2,3 dioxygenase
6.0 Summary

- Bacterial consortia capable of degrading crude oil as sole source of carbon were tested for their degradation abilities. Out of the three consortia, Consortium 3 was the most efficient in degrading PAHs.

- A total of 205 bacterial isolates screened for crude oil degradation, of which S19, S30 and P20 were found to be the most efficient crude oil degraders.

- The bacterial isolate P20 was found to be a better PAH degrading bacteria, and could degrade a wide variety of PAHs within 15 days. The bacterial isolates S19 and S30 could degrade only naphthalene, acenaphthene and fluorene.

- S19 and P20 were efficient in degrading alkanes. P20 also showed ability to degrade higher molecular weight alkanes, whereas it was inefficient in degrading lower molecular weight alkanes.

- The bacterial isolate P20 was identified by Institute of Microbial Technology (IMTECH) as *Alcaligenes odorans*, which was found to be resistant to seven different antibiotics.

- *Alcaligenes odorans* had the ability to degrade a variety of PAH compounds, it degraded up to 51% of fluorene, 50.6% of DBT, 37.2% of cyclopentaphenanthrene, 22% of floranthene, 52.8% of pyrene, 81.4% of chrysene and 94.8% of perylene.

- Molecular marker based on PCR-fingerprinting was developed to assess the survivability of *A. odorans*. We standardized the ERIC-PCR conditions for generating species specific DNA fingerprint that could differentiate between various species of *Alcaligenes*. 
Fate of *Alcaligenes odorans* in crude oil contaminated microcosm was monitored using ERIC-PCR in combination with selective plate counting using kanamycin as selectable marker.

Microcosm contaminated with crude oil was augmented with *Alcaligenes odorans*. In our study, we found that the microbial population in the microcosm declined significantly from $10^7$ to $10^3$ over a period of 30 days.

The kanamycin plate counting method resulted in higher rates of survival of *A. odorans* (47.23%) as compared to the survival obtained from PCR fingerprinting (27.22%).

Despite very low survival rates of *A. odorans* it was observed that degradation of PAHs was higher in the treated microcosm (23.49%) as compared to the control microcosm (16.97%).

The quantitative difference in PAH degradation was more striking when the aromatic fractions were analyzed on Gas Chromatograph. Microcosm that was augmented with *A. odorans* degraded naphthalene, pyrene and DBT significantly compared to the control microcosm.

The alkane degradation in the control microcosm was higher (37.73%) as compared to the treated microcosm (36.54%).

*Alcaligenes odorans* when grown in mineral salt medium with pyrene as sole source of carbon and energy, had a doubling time of 8 hours (specific growth rate, $\mu = 0.087 \text{ min}^{-1}$) and reached stationary phase in 72 hours. During this period, approximately 80% of added pyrene disappeared from the medium.

Degradation intermediates of pyrene from *Alcaligenes odorans* were analyzed on Gas Chromatograph coupled with Mass Spectrograph (GC-MS).
Two of the degradation intermediates were identified from the total ion chromatogram peaks at 63.8 and 79.3 minutes.

**Peak I (63.8 min.):** The peak at 63.8 minutes had fragments at 51, 61, 91, 108, 120, 146 and 164 amu. Based on the fragmentation pattern and the abundance of ions, the peak was identified as 1,2-dihydroxy 1,2,3,4-tetrahydro napthalene.

**Peak II (79.3 min.):** The peak at 79.3 minutes had fragments at 53, 59, 91, 129, 141, 183, 199, 212 and 222 amu. Analysis of this fragmentation pattern showed it to be similar to 4-phenanthrene carboxylic acid.

The mass balance of [14C]-pyrene in *Alcaligenes odorans* was following; of the total radiolabelled pyrene added to the whole cell suspension 79% is taken up by the cells in 120 minutes while 21% remains outside the cells. Out of the 79% of pyrene that is taken up by *Alcaligenes odorans* 2.9% is mineralized to 14CO₂ and 75.3% of radiolabelled pyrene remains inside the cells.

Based on the intermediates obtained from *Alcaligenes odorans* grown on pyrene, the pathway for pyrene degradation is proposed in which pyrene is converted to 4-phenanthrene carboxylic acid. Further metabolism of 4-phenanthrene carboxylic acid yield a 1,2,3,4-tetrahydro-1,2-dihydroxy napthalene a two ring containing intermediate which is further degraded to yield 14CO₂.

Inhibitor studies indicate that the uptake of pyrene by *Alcaligenes odorans* is energy dependent contrary to the idea that, aromatic compound enters the cell by passive diffusion.
Alcaligenes odorans had no plasmid, as detected by various techniques for isolation and pulse field gel electrophoresis, therefore it appears that the genes responsible for pyrene degradation in Alcaligenes odorans are present on the chromosomal DNA.

A strategy for subtractive hybridization in prokaryotes has been outlined which involves construction of immobilized cDNA on magnetic bead. The use of E. coli poly(A)polymerase to non specifically polyadenylate the total RNA and construct an immobilized cDNA library has not been reported so far.

Subtractive hybridization, using immobilized cDNA from glucose grown A. odorans and RNA from pyrene grown cells of A. odorans, was performed without much success, suggesting that the genes for pyrene degradation in A. odorans could be expressed constitutively.