Chapter VI:

Conclusions and future directions

6.1. Conclusions:

1. As many as 72 bacterial strains were isolated from enrichment culture of crude oil contaminated soils of Borholla Oil Field, ONGCL using n-hexadecane as sole carbon source in mineral salt media. The 35 of them were screened based on cytochrome P450 induction study. Only four of them Viz. TM14001, TM14007, TM14023, TM14030 having CYP 450 content of 4.21,2.6, 3.1 and 2.5 µmol were further screened for growth optimisation and cytochrome P450 induction study.
2. The two bacterial strains namely TM14001 and TM14023 were subjected to 16S rRNA sequencing and phylogenetic analyses which confirmed their identities to be *Bacillus pumilus and Bacillus stratosphericus* having 99% and 98.75% similarities respectively.
3. The specific content of the CYP was found to be 4 to be 1.3789E-08, 3.0527E-06 and 8.0113E-06 µM for spheroplast, AEC and GFC respectively.
4. SDS-PAGE analysis of the purified protein sample showed the presence of two bands, one between 68 kDa and 53 kDa, and the other between 10 kDa and 25 kDa, suggesting that the purified protein belongs to the class II monooxygenase system.
5. The detection of iron in the purified component in Atomic Absorption Spectroscopy (AAS) analysis indicated its metalloprotein nature.

6. The SDSPAGE analysis of the purified protein indicated molecular masses of 13.681 kDa and 68.183 kDa for band 1 and band 2 respectively.
7. Both the purified components are further subjected to de novo sequencing using MALDI MS. For the 68.1 kDa band, the predicted sequence showed a top score of 80, hitting a cytochrome P450 of *Bacillus megaterium*. Whereas the smaller band showed similarities with a transcriptional regulatory protein with a lesser significance.
8. The limited experimental analyses of the purified components done by using SDS-PAGE and Mass Spectrospy indicated its proximity to the class II monooxygenase system.
9. When used as biological recognition element, the smaller band (band1) didn't show any significant machine output. The larger band (band 2) showed some activity, however the sensitivity was maximum for the crude.
10. The average sensitivity of the ENFET device was calculated to be 54.34 mV/molar.
11. The device is stable for 48 hours with 99% accuracy in reproducibility.
12. The average error in sensitivity due to hysteresis is calculated to be ±0.015%.
13. The detection limit of the sensor is found to be .01 molar.

14. From the transfer and output characteristics, the threshold voltage for ENFET is found to be 2.08 and 2.095 V respectively for substrate concentration of 0.4 and 0.5 M.
15. For a current 0.5μA, the standard curve for determining unknown substrate concentration is y = 0.0653x + 2.6553 with correlation coefficient of 0.9848 (X-values assays the concentration of n-hexadecane depending on the Y-values)
16. Use of polyaphrons as support matrix for enzyme immobilization had stabilising effect for their storage in room temperature and pressure for more than two months with a spreading coefficient of 0 and PVR value of 20.
17. The average sensitivity of the ENFET device was calculated to be 67 mV/molar, with 35% increase in sensitivity.
18. The stability of the device has been increased by 250%.
19. The device reported here possess a high reproducibility of 99% accuracy till seven days.
20. The average error in sensitivity due to hysteresis is calculated to be ±0.01%.
21. With the increase in temperature there was a decrease in output VGS.
22. With the increase in pH the there is an increase in V_{GS} up to pH 8.2. However, At pH 9.2 there was not any significant change in V_{GS} for change in concentrations.

23. The response time was found to be 180-200 seconds.
24. Without NADPH there is no reaction, and hence there is no change in output V_{GS}.
25. The variation of output with respect to different concentrations of the NADPH shows that the concentration of 1 μm/ml is optimum for sensitivity.

6.2. Future directions

1. An enzyme engineering approach may be required for better stabilisation of the enzyme leading to more device stability.

2. The prototype can further be refined for industrial use in a cost effective manner.

3. An array of such enzymes can further be used for development of a multi-substrate hydrocarbon sensor for more pragmatic uses.