CHAPTER - V
5.0. Summary and Conclusions

The microbial consortia degrading chlorophenols were recovered from chlorophenol contaminated soil under anaerobic conditions. The individual members of the consortium were isolated and identified. One of the isolate was characterized to be *Rhodopseudomonas palustris* on the basis of its biochemical, morphological characteristics and also by FAME-GC analysis. The other members of the consortium were identified as *Bacillus subtilis* and *Providencia sp.CJ-3* on the basis of their biochemical, morphological and on the basis of rDNA gene analysis.

The characteristic feature of the photobacterium is presence of bacteriochlorophylls and carotenoids. The production of photopigments bacteriochlorophyll-α and carotenoids were determined by measuring the *In vivo* light absorption by *Rhodopseudomonas palustris* culture. The absorption spectra of cell suspensions exhibited characteristic maxima at 380, 590, 805, and 865 nm, which indicated the presence of bacteriochlorophyll α. Absorption maxima at 465, 490, and 525 nm indicated the presence of carotenoids.

The identified photobacterial member of the consortium, *Rhodopseudomonas palustris* degraded 2-CP. *R. palustris* metabolized 2-CP as sole source of carbon and energy through a pathway that involved a reductive dechlorination. This conclusion was supported by the intermediate metabolite analysis by TLC as well as by GC-MS. The TLC analysis showed that one of the metabolite produced during degradation was phenol with an *Rf* value 0.72. This was further confirmed as phenol by GC-MS study. Based on the above observations a tentative biodegradation pathway for 2-CP in *R. palustris* is proposed (Fig. 3.22).
Fig. 3.22. A tentative pathway for the biodegradation of 2-CP by R. palustris

The biodegradation of 4-CP by the same R. palustris however followed through utilization of a hydrolytic dechlorination mechanism. This conclusion was due to the identification of metabolite hydroquinone in TLC with an R_f value of 0.78 matched with the authentic sample. This was again confirmed by the GC-MS analysis, which showed the hydroquinone as well as the cleavage product 4-hydroxy muconic semialdehyde as intermediary products. This indicates the different modes of halophenol metabolism in R. palustris.
Summary and Conclusions

The cell free extract of the *R. palustris* degrading 2-CP showed CD with a specific activity of 0.114 µmoles min\(^{-1}\) mg\(^{-1}\) protein as well as CNOR with a specific activity of 0.26 µmoles min\(^{-1}\) mg\(^{-1}\).

The cell free extract of the *R. palustris* degrading 4-CP showed a specific activity 1.996 µmoles min\(^{-1}\) mg\(^{-1}\) protein for CD and 0.51 µmoles min\(^{-1}\) mg\(^{-1}\) for CNOR. Since the SDS-PAGE protein profile exhibited induction of several proteins these could be the products of the genes coding for those enzymes involved in 4-CP degradation. A tentative pathway for the biodegradation of 4-CP in *Rhodopseudomonas palustris* is proposed (Fig.4.13).

![Pathway Diagram]

**Fig.4.13. A tentative pathway for the biodegradation of 4-CP in *R. palustris***

A plasmid of same size was noticed in both 2-CP and 4-CP grown cells as well as in LB grown cells indicating that plasmid may not be involved in the degradation. This however hints on the constitutive nature of the chlorophenol metabolism in *R. palustris*.
Fig. 3.21. Comparison of the degradation of 2-chlorophenol by *R. palustris* entrapped in alginate and agar

Fig. 4.12. Comparison of degradation of 4-chlorophenol by *R. palustris* entrapped in alginate and agar
The bacterium *R. palustris* was immobilized in alginate and agar matrices. Further the immobilized *R. palustris* cells demonstrated the degradation of chlorophenols. The rate of chlorophenol metabolism by immobilized cell system was enhanced by 3 to 50 folds. The degradation kinetic analysis with the immobilized *R. palustris* cells demonstrated that alginate is a good matrix for immobilization of bacterial cells in comparison to agar (Fig.3.21 & 4.12).

This study has thus explored and contributed the potential of *R. palustris* for the degradation of toxic environmental pollutants. This work provides evidence that *R. palustris*, is a good bioremediation agent for chlorophenols. Therefore this ability of bacteria would allow complete detoxification of the wastewater and soil contaminated with chlorophenols. The evidence of organisms that is capable of removing chlorophenols and mineralizes the resulting aromatics under the same operational conditions is scanty. The strain *R. palustris*, was used in the study has proved to be the most efficient degrader of chlorophenols; 2-CP and 4-CP. Biodegradation of these chlorophenols by *R. palustris* is thus an attractive alternative due to its flexibility in adapting to the environment, lower cost ecofriendly technology and the capability of degradation and mineralization of the chlorophenols under phototrophic conditions, slow sludge generation (low growth rates) with no disposal problem. Since bacterial biomass is readily available and an inexpensive bio-resource with potential for chlorophenol remediation, it has potential application in degrading chlorophenols. Their capabilities can thus be harnessed for developing biotechnologies for decontamination of industrial waste water bodies. The immobilized *R. palustris* cells adapted to higher chlorophenol concentrations could also become important components for engineering bioreactors that could be useful for the removal of chlorophenols from contaminated effluents.