Organochlorines have been used extensively in both agriculture and public health programmes. 1, 1, 1- trichloro- 2,2- bis (*p*-chlorophenyl) ethane (DDT) is one of the most widely used organochlorine pesticide around the globe. It is one of the most toxic and environmentally persistent organic pollutant. It is a highly recalcitrant pesticide with a long residual action. It has broad-spectrum of action and is used extensively in public health programmes (to control insect parasites causing malaria, plague, dengue etc,). It was banned for agricultural applications in 1997 but was reintroduced in countries which have failed to control mosquito related diseases. Tonnes of DDT have already been dumped in the environment and thus it enters human and animal body *via* food chain.

The presence of DDT residues in the environment poses many health problems. Hence, their removal from the environment is of prime importance. Microbial remediation is one method of removing DDT residues from the environment. Microbial remediation is safer and economical. Microbial dehalogenases play an important role in the biodegradation of chlorinated pollutants. Dehalogenases eliminate HCl or Cl^- from their halo-organic substrate, leading to the formation of a double bond. DDT-dehydrochlorinase (EC Number 4.5.1.1) is involved in the catalytic degradation of *p, p'*-DDT by eliminating either HCl or Cl^- to form DDD / DDE. Isolation, purification and characterization of DDT-dehydrohalogenase from a bacterial source was attempted. Ten bacterial cultures belonging to DDT degrading microbial consortium were screened for the DDT- dehydrohalogenase activity. Among these, the cell free extract of *Pseudomonas putida* T_5 showed higher DDT-dehydrohalogenase activity and enzyme was purified to apparent homogeneity with 73% overall recovery. The relative molecular mass of the enzyme estimated by the SDS PAGE method was ~32kDa. Native PAGE revealed the presence of a single band. The purity of the enzyme was confirmed by HPLC and capillary electrophoresis. The enzyme was stable for 4-5 h at pH 7.0 at the temperature optima of 37 °C. The K_m and V_{max}, values for DDT- dehydrohalogenase were 3.7 µM

and 6.8 µM min^{-1}, respectively. The enzyme was a glycoprotein with mannose forming the backbone. AIG- formed the N-terminus chain. Serine and tryptophan appeared to be involved at the active site. The enzyme appeared to be a metalloprotein containing Zn, Mg, and Ca ions. Monovalent and divalent cations (1mM) inhibited the enzyme strongly. The primary sequence of HPLC purified enzyme was deduced by LC-MS-MALDI-ESI.

Urea, sodium dodecyl sulfate (SDS) and guanidine hydrochloride (GdmCl) are often used as excellent denaturing or "unfolding" agents. The effect of these denaturing agents on the structural changes of DDT- enzyme of *Pseudomonas putida* T_5 were studied. There was a progressive loss in catalytic activity of DDT-dehydrochlorinase with increasing concentrations of denaturants, namely urea, SDS and GdmCl. At 10 M urea, 5 % SDS and 1 M GdmCl, the extent of loss in enzyme activity was 98, 78 and 100% respectively. The emission spectrum of urea denatured enzyme did not show shift, but that of SDS and GdmCl treated enzyme showed very marginal shift. The secondary structure analysis of the enzyme by CD spectrum suggested a predominance of β-structure in the untreated enzyme. Urea denatured enzyme revealed complete loss of α-helix and there was substantial reduction in β sheets. SDS treated enzyme showed increase in α-helix, turns and random structure. However, there was a marginal loss in β sheets. GdmCl treated enzyme showed complete absence of α-helix compared to the control enzyme. The β-sheets were also reduced and the number of turns increased. The effects of GdmCl were almost similar to that of urea as observed in CD spectrum. The urea polyacrylamide gel showed only one band whereas SDS polyacrylamide gel showed closely moving two bands.

The effect of few metal ions on DDT-dehydrohalogenase activity was studied and it was observed that the enzyme was inactivated by the addition of metal ions. Monovalent and divalent cations (1mM) inhibited the enzyme strongly. The apparent rate constants of inactivation at different metal concentrations and conformational

changes in the presence of 100 mM metal ions were studied. The conformational changes of DDT-dehydrohalogenase were followed by fluorescence spectra and circular dichoism spectra. There was a progressive loss in catalytic activity of DDT-dehydrohalogenase enzyme with increasing concentrations of metals. The fluorescence spectrum of the enzyme exposed to metal ions showed a shift towards the red region. The CD spectrum of this metal treated enzyme showed significant changes such as reduction in α-helices and β- pleated sheets. Alkali metals, alkaline earth metals, heavy metals and transition metals studied, had almost the same changes in the secondary structure of DDT-dehydrohalogenase.

Among the organochlorine pesticides, DDT is one of the major cause for food and environmental contamination. To detect this chemical effectively in water samples an immobilized dehydrohalogenase based potentiometric biosensor was developed. The chloride ion released as a result of dehalogenation by immobilized dehydrohalogenase during the degradation of DDT was detected by using an ion selective electrode. The voltage response had a direct linear relationship with the concentration of chloride (Cl^-) released with DDT. The immobilization had an advantage in improving the enzyme property and could be used repeatedly without loosing the activity.

Primers for PCR reaction were designed specifically for DDT-dehydrohalogenase gene. Genomic and plasmid DNA of all the ten individual isolates of the bacterial consortium were screened for the presence of the gene by gradient PCR between 50°C and 70°C and *Pseudomonas putida* T_5 was chosen for further work. Touch-down PCR was standardized by using optimized annealing temperatures. 30 cycles of optimized PCR conditions obtained were: initial denaturation at 94°C for 5 min followed by denaturation at 94°C for 45 sec, annealing at 55°C for 75 sec and extension at 72°C for 45 sec and final extension at 72°C for 10 min. Amplicon size of ~800 bp was obtained when electrophoresis was done on agarose gel (1%). The PCR product of DDT- dehydrohalogenase gene (*dhl* 1) was purified by gel

elution, and ligated to pTZ57R/T vector and cloned in *E. coli* DH5α. The clones were selected on ampicillin Luria agar plates. The insert from cloned plasmid was removed by double digestion with *Eco*RI and *Xho*I. This was further used for gene sequencing analysis. The purified PCR product was ligated to pET 28a vector and cloned in to *E. coli* BL 21 for expression. The expressed protein was purified using His-Tag purification kit. The purified enzyme was analyzed for activity and was confirmed by western blot, and colony PCR. The sequence was translated using Expasy and compared with the published protein sequence of *Anopheles gambiae* and multiple sequence alignment was done using Multalign software.

Bioremediation is an interdisciplinary technology, involving microbiology, engineering, ecology, geology, chemistry, etc. Microbes are the primary stimulants in the bioremediation of contaminated environments. Investigations in to the microbial degradation of DDT are useful in the development of methods for the remediation of the contaminated environments. Laboratory studies have shown that microbes have the machinery to metabolise DDT and other xenobiotics. However, little is known about the conditions, which favour the degradation of DDT and other pollutants. In the computational study, the enzyme sequence deciphored by gene sequence was used for the construction of models employing homology modeling method . Molecular docking of DDT –dehydrohalogenase with DDT substrate was subsequently studied. Docking was also done along with GSH, a co-factor like substrate. These two were found to have different binding pockets regarding the size and the key amino acids involved in binding. Predicted binding modes of these two with DDT – degrading enzyme was compared. The calculated docking interaction energy of DDT showed high affinity, suggesting specificity of the enzyme.

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contaminated environments. Laboratory studies have shown that microbes have the machinery to metabolise DDT and other xenobiotics. However, little is known about the conditions, which favour the degradation of DDT and other pollutants. The microbial communities make remediation a more reliable and safer technology as and when the information hidden within it is deciphered and used in a constructive way.

The pressures of an ever-increasing population and industrial development have led to the addition of an array of man-made chemicals in the environment, leading to tremendous deterioration in environmental quality. Contamination of soil, air, water, and food is one of the major problems facing the industrialized world today. The fate of synthetic chemicals reaching the environment for the most part depends on the microorganisms present in that part of the environment. The capacity of microbes to produce enzymes that recognize xenobiotic compounds and to catalyze reactions that break them decides the extent to which such chemicals can cause damage to the ecosystem. The absence of microorganisms or microbial systems that bring about their degradation will only result in these chemicals being recalcitrant, persistent, and a potent hazard to the ecosystem as a whole. Future research related to biodegradation of chlorinated compounds should focus on both basic and applied aspects of the subject. Since bioremediation is an important tool in detoxifying and eliminating environmental contaminants, a thorough understanding of microbial genetics, biochemistry, and physiology is required. Attempts should be made to bridge the gap between success at laboratory level and success of the same at a field scale.

Further, treatment of hazardous chemicals in the environment also presents the possibility of unknown by-products of biodegradation entering the environment. Consequently, sound knowledge of the degradation products, metabolic pathway, biochemistry, and other details relating to treatability studies should be collected before venturing into a full-scale bioremediation process. Thus a lot has been done and a lot needs to be done in this direction.