ABSTRACT

In this industrialized world, soil contamination is the major concern to environment. Xenobiotic compounds like nitroaromatics, organohposphates are the main source of polluting soil ecosystem as their residues accumulate in soil for prolong period. Therefore, it is necessary to remediate such contaminants from natural environment to restore the ecosystem. Bioremediation is the process to decontaminate and mineralize the contaminants using biological agents like bacteria, fungi or their enzymes to obtain sustainable environment. Present study is an attempt to develop a suitable strategy for bioremediation of soil contaminated by explosives and chemical pesticides.

This study focuses on the use of indigenous bacterial isolates (obtained from explosive contaminated sites) immobilized to develop novel bioformulations, for achieving the remediation goals. Seven bacterial isolates, namely, *Bacillus oceanisediminis, Dydobacter jiangsuensis, Dienococcus misasensis, Arthrobacter subterraneus, Janibacter cremeus, Pseudomonas entomophila* and *Microbacterium esteraromaticum* were kindly provided by Centre for Fire, Explosive and Environment Safety, Defence Research and Development Organisation (CFEES, DRDO) New Delhi (identified by Institute of Microbial Technology, IMTECH, Chandigarh). Initially the interaction study among all bacterial isolates was carried out to check their compatibility among themselves. The results obtained indicated that there was no positive interaction among isolates indicating no compatibility with each other hence all studies were carried out using monoculture system instead of their consortium.

After that these isolates were subjected for examining their bioremedial potential by growing them in presence of Hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) and *Microbacterium esteraromaticum* MTCC 12849, gram-positive, aerobic bacterium was found as an efficient degrader. The strain detoxified 72.6% of 30ppm RDX in minimal nutrient medium in 240 h and in soil, 63.93% of 30 mg RDX/kg of soil degradation was exhibited with viable cell count of $3.2X10^4$ cfu/g within 30 days of incubation.

Subsequently, the different potent strains were formulated in water-dispersible granules (WDG), talcum/charcoal based powder and alginate beads formulations. Developed WDG with the 90% of inert material with the active ingredient in ratio of 1:2, 2% acacia gum as good binder and 8% alginic acid as an efficient dispersing agent gave best results. The tested properties of WDG fulfilled the guidelines of Collaborative International Pesticides Analytical Council (CIPAC). Shelf life of WDG formulation was found to be 120 days when stored at 30^oC, however at 45^oC the bacterial growth was not observed. At low temperature (4^oC) storage condition, the viability of the bacterial isolates in different formulation was found to be quite stable with the loss of only one log unit at the end of 180 days.

Among all developed microbial formulations, WDG showed best degrading efficiency, and formulated *M. esteraromaticum* exhibited highest degradation. However, a mesocosm study of RDX in soil biocolumn reactor indicated 9.88% increment in RDX degradation through developed *M. esteraromaticum* WDG i.e. 73.2%. with the subsequent formation of intermediates N-methyl-N, N′-dinitromethanediamine, and methylenedintramine detected

through LCMS analysis, in soil during the RDX degradation. Interestingly, no significant difference was observed in the rate of RDX degradation due to the formulation process. The first-order kinetics was seen in RDX degradation with a degradation coefficient of 0.04 and 0.0339 day^{-1} by formulated and unformulated strain, respectively. The current investigation implies *M. esteraromaticum* as a potential microbe for RDX degradation and opens up the possibility of exploiting it in its effective WDG form for the remediation of explosive contaminated sites.

Similar studies were conducted for another contaminant i.e. Chlorpyrifos (O, O-diethyl O-3, 5, 6-trichloropyridin-2-yl phosphorothioate), a toxic and chlorinated organic contaminant in soil across the globe. The qualitative testing of all bacterial isolates against CP degradative ability on selective media showed that majorly three bacteria, *D. jiangsuensis, M. esteraromaticum* and *D. misasensis* exhibited change in colour indicating the capability of strains to degrade CP. The effect of chlorpyrifos on bacterial growth was checked and among all three selected strains, the proliferation of *D. jiangsuensis* was highest signifying the ability of this strain to utilize CP as a sole carbon source and also confirmed the utilization of 3,5,6-trichloro-2-pyridinyl (TCP) through silver nitrate assay.

D. jiangsuensis degraded 80.36 and 76.93% chlorpyrifos (CP) in aqueous medium and soil environment, respectively. The trend observed in organophosphorus hydrolase (OPH) activity exposed its localization extracellularly, with highest activity noted in *D. jiangsuensis*, which were in concurrent with the degradation results. The *D. jiangsuensis* WDG achieved 21.13% enhanced CP degradation in soil under microcosm condition as compared to the unformulated one on 15th day of the treatment. The intermediate metabolites namely 3,5,6-trichloro-2-pyridinol (TCP), tetrahydropyridine, thiophosphate and phenol, 1, 3-bis (1,1-dimethylethyl) were detected during the CP degradation.

The current investigation reveals *D. jiangsuensis* as a potential microbe for CP degradation and opens up the possibility of exploiting its formulations to remediate the CP polluted soils. In this study, *D. jiangsuensis* proved as an efficient degrader of chlorpyrifos in soil environment. In *in planta study*, it was notable that WDG of *D. jiangsuensis* was most efficient in enhancing the growth parameters of tomato plants. In pot study, *D. jiangsuensis* WDG showed 25.7% increment in chl a and 59.3 % increase in chl b (total chlorophyll content by 35.84%) in comparison to the control. Also, there was increase of 17.33 and 41.28% in height and fresh weight respectively of tomato plant over control.

The present finding identified a sustainable and environmental friendly technology package for bioremediation of soil contaminated by chemical pesticides and explosives. The use of microbes in formulated form aids in degradation efficacy and enhanced the plant growth.