ABSTRACT

Rapid industrialization and urbanization have led to the indiscriminate use of xenobiotics across the world. Xenobiotic compounds like nitramines, and carbamates, due to their high recalcitrancy, reside in the environment for longer durations and thus contribute to the pollution of soil and water bodies. Thus, there is an imperative demand for feasible approaches for the removal of these xenobiotics from soil and water for sustainable rejuvenation of the surrounding ecosystem. Bioremediation is the most efficient approach for the clean-up of environmental bodies contaminated with hazardous xenobiotics. Several bacteria, fungi, and their respective metabolic enzymes are prime agents used in the bioremediation process.

Considering all these facts, the present work entitled "Bioremediation of xenobiotics (RDX and Carbofuran) polluted soils" focuses on the rejuvenation of explosives and pesticides contaminated soils using integrated bioaugmentation and biostimulation approach.

The study started with screening native bacterial strains isolated from explosives-contaminated sites for RDX degrading potential in nitrogen-limited minimal salt media. Four different bacterial isolates viz., *Bacillus oceanisediminis, Pelomonas aquatica*, *Kinneretia asachharophila* and *Arthrobacter subterraneous* were initially isolated and identified by CSIR-Institute of Microbial Technology, IMTECH, Chandigarh, and kindly provided by Centre for Fire, Explosive and Environment Safety, Defence Research and Development Organisation (CFEES, DRDO) New Delhi. Of the four microbial isolates, *P. aquatica* and *K. asachharophila* were the most efficient RDX degraders with 82 and 74 % RDX (10 mg l^{-1}) degradation within 240 h of incubation with the highest microbial growth $8.7 \pm 0.52 \times 10^8$ and $3.1 \pm 0.07 \times 10^7$ CFU ml^{-1} respectively. Both *B. oceanisediminis* and *A. subterraneous* were poor RDX degraders with only 32 and 29 % RDX degradation after 240 h, thus left out for further steps. Alongside, six different carbofuran degrading bacterial strains were isolated from contaminated soils and screened for carbofuran degrading potential in minimal salt media.

Bacterial isolates, *O. intermedium*, and *B. albus* were found to be the most effective carbofuran degraders, with 75.8 and 58.4 % carbofuran (300 mg l–1) degradation within 120 h of incubation and thus selected for further steps.

Next, the interaction study was conducted between potent RDX (*P. aquatica* and *K. asachharophila*) and Carbofuran (*O. intermedium* and *B. albus*) degrading isolates to check compatibility for consortia development. However, no positive interaction was observed between RDX degraders and carbofuran degraders. Hence, no microbial consortium was developed, and monocultures of the potential RDX and carbofuran degrading strains were used in further studies.

After that, aqueous phase degradation studies were conducted to determine the RDX and carbofuran degradation kinetics and intermediate metabolites. *P. aquatica* and *K. asachharohila* isolated from explosives contaminated sites removed 80 and 75% RDX (30 mg l^{-1}) from MSM I within 240 h. The degradation of RDX was rapid and highly effective with RDX half-lives of 4.17 and 4.89 days with *P. aquatica* and *K. asachharophila* resp. The LCMS analysis of the aqueous extracts confiemed the presence of MNX, MEDINA, NDAB in case of *P. aquatica*, while only MEDINA and NDAB were detected as intermediates with *K. asachharophila*. In aqueous phase degradation of carbofuran, *O. intermedium* and *B. albus* degraded 92 and 68% carbofuran (100 mg l^{-1}) from aqueous media resp. *O. intermedium* showed rapid carbofuran removal with degradation rate constant 0.69 day^{-1} and half-life of 1 day while a half-life of 29 days was observed in control treatments. GCMS analyses of the aqueous extract confirmed the formation of carbofuran-7-phenol (M.W. 164 Da) as major intermediate metabolite detected at RT of 11.4 min. Other intermediate 1,2-benzenecarboxylic acid, monoethyl ester (194 Da) was detected at RT of 15.2 min.

Followed by this, different potent bacterial strains were formulated into water-dispersible granules (WDG), talcum and charcoal-based dust and encapsulated beads formulation. The developed formulations were further checked for shelf life and contaminant (RDX and carbofuran) degrading potential over a storage period of six months at three different temperatures (4, 30 and 40 ˚C). The WDGs retained >80 % of initial viability, while a loss of >50 % in initial viable cell counts was recorded in talcum, charcoal and beads formulation after 6 months. However, at 4 ˚C all the developed formulations remained viably stable till 6 months of storage. The bacterial counts were greatly reduced within 2 months at 40 ˚C storage temperature.

WDGs of *P. aquatica* and *K. asachharophila* retained 95 and 96 % of the initial RDX degrading efficiency after storage period. While, WDGs of *O. intermedium* retained ~ 94 % carbofuran removing potential at the end of 6 months of storage at 30 ˚C. The remediating potential of powder and beads formulation was significantly decreased by >15 % within 3 months which further reduced to >50 % at the end of 6 months. Thus, the WDGs were finally selected for soil microcosm studies to study the impact of bioaugmentation and biostimualtion on RDX and carbofuran degradation.

P. aquatica WDGs in sucrose amended microcosms showed a 77.57 % RDX removal while a 66.49 % RDX degradation was recorded in microcosms stimulated with wheat straw after one month of storage. The biostimulated microcosms recorded reduced RDX soil half-lives of 13.9 and 19.04 days resp. over control treatment with RDX half-life of 185.5 days. In case of carbofuran degradation in soil microcosms, the *O. intermedium* cells in WDGs stimulated by biogas slurry (BGS) recorded a 95 % carbofuran degradation within 30 days of treatment. The carbofuran half-life in control set was 78.3 days which was critically reduced to 8.04 days in microcosms treated with integrated bioaugmentation and biostimulation approach.