Research Article Open Acces

Keywords Industrialization; Pesticide; Bioremediation; Pentachlorophenol

Introduction

Industrialization plays a vital role in nation's socio-economic development as well as its political stature. Industries vary according to process technology, sizes, nature of products, characteristics, and complexity of wastes discharged. Ideally citing of industries should strike a balance between socio- economic and environmental considerations. Although industrialization is inevitable, various devastating ecological and human disasters have continuously occurred over the last four decades that made industries responsible for various environmental pollutions. It has been widely reported that industrial e uent has a hazardous e ect on the quality of owing water. Industrial discharge contains toxic and hazardous substances, most of which a ects human health. ese include heavy metals such as lead, cadmium and mercury and toxic organic chemicals such as pesticides, PCBs, dioxins, poly aromatic hydrocarbons (PAHs), petrochemical and phenolic compound [1-5].

Bioremediation is the use of living organisms primarily microorganisms to degrade the environmental contaminants into nontoxic forms. e mechanism of microbial degradation is based on the general principles of physiology and ecology. Biological removal of chemo-pollutants becomes the method of choice since microorganisms can use a variety of xenobiotic compounds including pesticides for their growth, mineralize and detoxify Common soil bacteria and fungi can degrade the majority of the compounds. e most important soil factors that in uence bio-degradation are temperature, moisture, presence or absence of oxygen, organic matter and clay content. Microorganisms possess the capable of degrading a large proportion of chemicals. Consequently many of the man-made pesticides introduced into the environment are microbial degraded, mostly by enzymes evolved in response to the presence of natural substrate [6-8].

In order to enhance the microbial degradation of organic pollutants for remediation of contaminated soils, it is essential to understand the enhancing mechanism, especially the relation between the degradation of chemicals in soil and the behavior of degrading microorganisms.

ere are two types of microbial degradation of pesticides in soil. In the rst, repeated application of a pesticide to soil enhance the degradation by enrichment of the pesticide-degrading microorganisms. e enriched microorganisms o en metabolize the pesticide as carbon and energy source, which is designated as catabolism. In the second type of degradation, the population of degrading microorganisms in soil does not change even when a pesticide is repeatedly applied and no enhancement of degradation occurs. e microorganisms require other carbon sources to degrade the pesticide that are called as incidental metabolism or co-metabolism. Usually, as microorganisms carry out degradation, they obtain carbon and energy required for growth. An increase in the size of the pesticides degrading population leads to the faster rates of degradation. Microbial degradation of pesticides occurs ecologically through various dynamic and complex forces to support aerobic reactions simultaneously leading to more extensive degradation of many complex pesticides. e enhanced degradation occurs due to the repeated application of pesticides. is is favorable for environmental decontamination of toxic residues. e microbial degradation is the best means of detoxi cation of pesticides [9-20].

Pentachlorophenol is a polyhalogenated aromatic hydrocarbon of chlorophenol family. Chlorophenols are phenols carrying one chlorine atom attached to the benzene ring. e chemical formula for PCP is C

have shown that PCP undergoes biodegradation but its biodegradation in the environment is o en slow. is coupled with its extensive use, has led to the contamination of many terrestrial and aquatic ecosystems world-wide.

Material and Methods

All the chemicals used during the course of this investigation were of A.R. grade and were supplied by E. Merck (India), Himedia (India), S.D. Fine chemicals (India), Qualigens (India) or Sigma (U.S.A). All glassware used of corning and borosil made.

Microorganisms

Pseudomonas fluorescence: Pure culture of *P e d m na e cence* was obtained from gene pool (G. B. Pant University of Agriculture and Technology, Department of Microbiology, Pantnagar).

Phanerochete chrysosporium: Pure culture of *Phane che e ch i m* was obtained from Institute of Microbial Technology (CSIR Laboratory), Chandigarh.

Methods

Nutrient agar medium: is medium was used to culture bacterial community of individual strain (Tables 1 and 2).

Bacterial enrichment: Continuous enrichment of bacterial strains was facilitated by minimal salt medium (Table 3).

Fungal enrichment: e fungal community was incubated in Erlenmeyer ask containing a basal minimal medium (Table 4).

microorganisms was analyzed for chloride release and Ring Cleavage at di erent time periods.

Degradation of pentachlorophenol: A er enrichment the bacterial and fungal were inoculated in fresh mineral salt medium with PCP (100 ppm) as a sole carbon source for a few days in a gyratory shaker. e samples were taken out at 4, 8, 16, 32, hours and growth of microbes was measured.

Estimation of chloride ions release (Argentometric method)

24 ml of sample was taken in a beaker and added 5 drops of potassium chromate, an indicator solution. With the help of burette, $AgNO_3$ (0.0141 N) solution was added to the beaker. A red color formed, which disappeared soon. At a point where all the chloride ions were precipitated, a stable red color appeared referring to an end point of the reaction. Calculation of amount of chloride ions in the sample was done.

C_1V_1 (AgNO3)=C_2V_2(Sample)

With the help of this formula C_2concentration was calculated. Amount of chloride ions present in the sample was calculated by multiplying with 35.5 (atomic weight of chloride) with [29-35].

Estimation of ring cleavage

4 ml of cell suspension was dissolved in 0.02 M Tris bu er (0.1) and EDTA (0.1 ml) for lyses of bacterial and fungal cells. e pH was adjusted to 7.8. e mixture was treated with little toluene and 0.1 M catechol (4.0 ml). e development of color was noticed. Yellow color did not appear that suggested absence of meta cleavage. Mixture was shaken for 1 hour at 170 rpm and tested for formation of ß-ketoadipic acid (Roth era Reaction) that indicates the presence of ortho ssion. In this procedure, 10 ml culture uid was acidi ed with 2 ml HCl followed by addition of 1 ml $NaNO_3$(1%). A er 2 minute, concentrated ammonia (15 ml) and 10% ferrous sulfate solution (10%) were added. e development of reddish brown color indicated typical Roth era reaction and the presence of ortho cleavage [29-35].

Soil sample

Soil sample were collected from the College of Basic Science and Humanities ground. Soil was uniformly grinded and screened for any apparent impurities. e collected soil was processed for determining its physico-chemical characteristics such as texture, moisture, organic carbon employing the following methods:

Texture: Particle size analysis of soil used in the experiment was done following the international pipette method using H_2O_2(30%) for the removal of organic matter and sodium hexa meta phosphate as dispersing agent. Soluble salt and calcium carbonate were removed by following Jacksons Method.

Moisture: Moisture content of soil was determined by oven dry and weight loss method.

Potency of Hydrogen (pH): Soil pH was determined in soil extract prepared in a clean 50 ml glass beaker by suspending 20 g soil in 20 ml distilled water and ltered the same through whatman (no.1) lter paper. e ltrate was subjected to measure pH using a pH meter. e standard pH bu er (pH7.0 and pH 4.0) were used to calibrate the pH meter.

Organic carbon: Organic carbon of the soil was determined by wet digestion method of Walkey and Black.

Estimation of organic carbon1.0 g of soil sample was i 10 ml of 1 N $K_2Cr_2O_7$ in a 500 ml conical ask. 20 ml of co H_2SO_4 was gently added to it. e solution was shaken minute or two and allowed to stand for about 30 minutes fo digestion. e content was further diluted by addition of 200 m sodium uoride. ey were then added to the conical ask and against standard ferrous ammonium sulphate solution (0.5 addition of 1 ml Diphenyl amine indicator solution. e end poin detected as the violet color changed to purple and nally to green.

Calculation

% carbon in soil=(x-y) × 0.003/0.76 × w

Where, w=gram weight of soil taken, X=volume in ml of 0.5 N ferrous ammonium sulphate required for reducing 10 ml $K_2Cr_2O_7$ (blank reading), Y=Volume in ml of 0.5 N ferrous ammonium sulphate required for reducing the excess of dichromate (experimental reading) and 0.003=meq weight of carbon [20-35].

Results and Discussion

In the present study an attempt has been made to assess the potentia of these two microorganisms i.e. *Phane chaete im* (fungi) and *P e d m na , e cence* (bacteria) for the degradation of PCP in soil microcosms in order to suggest their degradation e ciency under natural conditions.

Degradation of pentachlorophenol by bacteria and fungi in liquid batch culture

Both the micro-organisms were separately inoculated in fresh minimal salt medium containing 0, 10, 50, 100, 200 and 500 ppm PCP concentrations in separate ask for few hours (4, 8, 16, and 32) and their potentiality to degrade PCP was assessed. e growth of bacteria and fungi and degradation of PCP were measured.

Growth of bacteria

indicates dehalogenation of PCP. With due course of growth, results

for isolation of xenobiotic compounds degrading bacteria and fungi is direct planting of active cultures on mineral salt medium with the toxicant as the only carbon source. e isolation of chlorophenols degrading bacteria has become a problem because of the toxic nature of the compounds and their recalcitrant nature [20-36].

Conclusions

Pentachlorophenol is a wide spectrum biocide with numerous applications in agriculture, industries and public health. It is considered to be an environmental pollutant because of its board toxicity and persistence in soil for long. Biodegradation of PCP is challenging

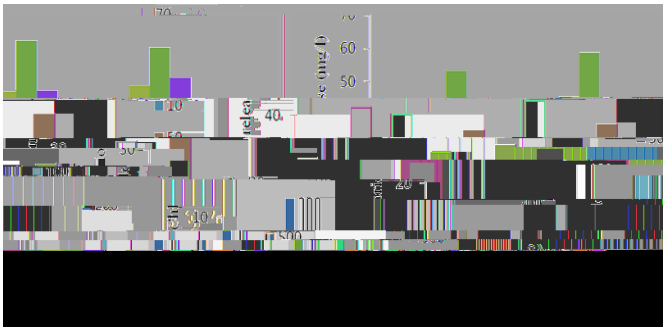

Figure 3: Chloride ion release (mg/l) by 3 F K U \ V R V a t different concentrations of PCP

	4 h	8 h	16 h	32 h
Fungi	-	++	+++	+++
Bacteria	-	-	+	++

(- refers to No cleavage while + indicates Color intensity for ortho-cleavage).

Table 8: 2 U W K R U L Q J F O H D Y D J H E \ E D F W H U L D O D Q G I X Q J D O F X O W X U H V

Time (Hour)	CFU (10^7 cells/g. of soil)					
	0 ppm	10 ppm	50 ppm	100 ppm	200 ppm	500 ppm
4 h	1.45	1.4	1.5	1.63	1.49	1.44
8 h	1.52	1.57	1.42	1.66	1.4	1.4
16 h	1.59	1.52	1.52	1.73	1.3	1.2
32 h	1.1	1.48	1.43	2.2	0.8	0.9

Table 9: Growth pattern of 3 À X R U H V F H Q F H in soil microcosm (30% moisture) at different concentrations of PCP.

Time (Hour)	CFU (10^7					