Study the Bioremediation of Hazardous Pollutant - Malathion Pesticide in Contaminated soil by Bacterial *pseudomonas sp.*

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Abstract: Pesticides are considered as some of the most serious environmental pollutants, which are being frequently used for the control of agricultural and domestic pests. Though agriculture is vital, pesticides can be harmful to the human health and animals, and have been proved to have a detrimental environmental impact. The widespread use of increasing number of pesticides in agriculture has acquired great importance earlier due to their pest control activities and now due to the deleterious impact of their residues on the human health and environment. Bioremediation is the green route to remove many pollutants from environment. Malathion [S-(1, 2-dicarbethoxyethyl)-O, O-dimethyldithiophosphate], also known as carbophos, maldison and mercaptothion is a no systemic, wide spectrum organ phosphorus pesticide used in public health, residential, and agricultural settings. Malathion is suited for the control of sucking and chewing insects of fruits and vegetables, mosquitoes, flies, household insects, animal parasites. Malathion biodegradation was studied by using bacterial pseudomonas sp. Experiments was carried out at different concentration of malathion from 50 mg/L to 300 mg/L at different temperature and P^H. The maximum degradation was observed at 50 mg/L.

1. INTRODUCTION

Pesticides are considered as some of the most serious environmental pollutants, which are being frequently used for the control of agricultural and domestic pests. Though agriculture is vital, pesticides can be harmful to the human health and animals, and have been proved to have a detrimental environmental impact. The widespread use of increasing number of pesticides in agriculture has acquired great importance earlier due to their pest control activities and now due to the deleterious impact of their residues on the human health and environment. In nature, slow activity of natural micro flora for degradative elimination of these toxic chemicals is proving insufficient to provide needed cure of heavily contaminated pesticide rich environment. Bioremediation is the green route to remove many pollutants from environment. Despite decades of research, it is always a challenge to take lab-scale work into field trials; this study explores the use of molecular tools to understand the catabolic capacity of the target soil and demonstrates the advantage of combining conventional bioremediation techniques with molecular tools to monitor bioremediation. Biodegradation of pesticides is controlled by bioavailability of the pesticide to a pesticide degrading microorganism and its activity. Microbial degradation is an important mechanism controlling the fate of pesticides in soils and is generally considered to be desirable both from an environmental perspective as well as an agricultural point of view. Studies on microbial degradation are useful in the development of strategies for the detoxification of insecticides by selective species of various microorganisms. Malathion [S-(1, 2-dicarbethoxyethyl)-O, O-dimethyldithiophosphate], also known as carbophos, maldison and mercaptothion is a no systemic, wide-spectrum oregano phosphorus pesticide used in public health, residential, and agricultural settings(Xie, S *et.al.*, 2009). Malathion is suited for the control of sucking and chewing insects of fruits and vegetables, mosquitoes, flies, household insects, animal parasites (ectoparasites), and head and body lice. Malathion is used in veterinary medicine (Osweiler G.D.*et al*., 1984) and also as an anti-infective agent (Kamal, Z *et al*., 2008) to control insect vector-borne diseases such as malaria, dengue and yellow fever. Organochlorine pesticides are banned in many countries including India therefore organophosphate pesticides including malathion are largely used for public health and agricultural purposes. Malathion comes in two forms: a pure form of a colourless liquid and a technical-grade solution (brownish-yellow liquid, commonly used in agricultural). Technical grade malathion (may contain up to 11 impurities formed during its production and storage, some of these impurities, such as isomalathion, have been found to be significantly more toxic than malathion (Konrad, J.G *et al*., 1969). Malathion was recognized as the first organ phosphorous insecticide with highly selective (Xie, *et.al.*, 2009; Wester, R.C and Cashman, J.R., 1989; Singh, B. *et al*., 2011). Main target of malathion toxicity in animals is nervous system thus indirectly affecting other organs and their functions. Malathion irreversibly inactivates acetyl cholinesterase enzyme that breaks down acetylcholine, a chemical essential in transmitting nerve impulses across junctions between nerves (Goda, S.K *et al*, 2010).Malathion degrading bacterial isolates have already been reported (Xie,

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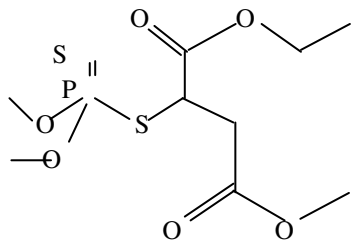
et.al., 2009; Wester, R.C and Cashman, J.R., 1989; Bourquin, A.W. 1977; Durkin, P.R., 2008; Singh, B *et al*.2012)., Fungi, *Penicillium rotatum* and *Aspergillus niger*, metabolized 76% and 59% of the malathion in the medium within 10 days through carboxylesteratic hydrolysis as well as by a demethylation process. Two species of *rhizobium*, *R. leguminnosaru*, *R. trifolii*, were isolated from the Egyptian soil that showed high carboxyesterase activity in the presence of malathion (Howard, P.H. 1991; Uygun, U *et al*., 2007). It was reported that heterogeneous bacterial population (*Flavobacterium men- ingosepticum*, *Xanthomonas* sp, *Comamonas terrigeri*, and *Pseudomonas cepacia)* obtained from river water are capable of degrading malathion (Paris, D.F *et al*., 1975).

Structure of malathion:



Malathion

Slurry bioreactors (SBs) are one of the most important types of ad situ and ex situ technology that can be used for bioremediation of problematic sites such as those characterized by soils with high contents of clay and organic matter, by pollutants that are recalcitrant, toxic, and display hysteretic behavior such as lindane (Robles *et al*., 2006) or when bioremediation should be accomplished in short times under the pressure and monitoring of environmental agencies and regulators (Pandey et al., 2009; Cookson JT, 1995; Collina *et al*.2005). Treatment of soils and sediments in SBs has become one of the best options for bioremediation of soils polluted by recalcitrant pollutants under controlled environmental conditions (Robles *et al*., 2008; Pandey *et al*., 2009). SBs are often applied to determine the feasibility and actual potential of a biological strategy in the final restoration of a contaminated soil or site. In fact, under slurry conditions, the pollutant depletion rates depend mainly on the degradation activity of the microorganisms available in the system, and the results obtained generally reflect the actual biological depuration potential of the soil (Robles *et al.,* 2008; Collina *et al*.2005). Application of aerobic SBs (ASBs) to bioremediation of soils is still predominant. A large number of successful laboratory, pilot and full-scale studies and cases of ASBs for bioremediation of soils polluted with PAHs (Collina *et al*.2005), pesticides (Robles *et al*.2006; Valentin *et al*.2007; Plangklang *et al*.2010), diesel (Manzo *et al.,* 2010), PCBs (Evans *et al*., 1996), and explosives (Daprato *et al*., 2005) have been reported. Studies on anaerobic or anoxic operation of SBs for removal of pollutants are still scarce. Anoxic and anaerobic SBs have been used to remove diesel [13], explosives, pesticides such as 2, 4D (Robles *et al*.2006), chlorpyrifos (Venkata *et al*., 2004), and hexachlorocyclohexane (Bachmann *et al*., 1988; Quintero *et al*.2005). However, little is known regarding the operation of SBs as units with simultaneous electron acceptors (also known as SEASBs), particularly partially aerated methanogenic (PAM) and methanogenicsulfate reducing (MSR) SBs for bioremediation of soils polluted with pesticides such as lindane. Given the success of bioreactors with SEAs in wastewater treatment (Garibay *et al*., 2005), we hypothesized that this approach may also be beneficial to SB operation.

2. MATERIAL AND METHOD

Bacterial species dry culture from MTCC (Microbial Type Community Culture, Chandigarh), soil was collected from farm of Farm Engineering (BHU) Varanasi, malathion from Gyan scientific Laurabir Varanasi.

2.A Soil Characteristics

Soil samples (50 g) were taken in a clean pre weighed 150 mL beaker and kept in an oven at 105°C ± 3°C for 24 h. Difference in the weight of beaker and soil before and after heating was recorded to calculate moisture content. Soil samples (20 g) were taken in a clean dry 150 mL beaker and 50 mL distilled water was added. The contents were thoroughly mixed by vortexing and P^H of the soil suspension was measured with a digital P^H meter.

2.B Calcium Carbonate Determination

Calcium carbonate determination is soil sample was performed using method as follows adding 10 mL of 1 N HCl solution to one gram of air dry soil in 250 mL flask.. The mixture was kept for overnight at 40°C - 50°C. 70 mL of deionised H2O along with 2 - 3 drops of phenol- phthalein indicator (0.5 g phenolphthalein in 100 mL ethanol) was added and titration was done with 1 N NaOH solution until slight pink colour developed.

Percent Calcium carbonate was calculated using fol- lowing equation;

$$\% \text{ CaCO3} = [(10 \times \text{NHCl}) - (R \times \text{NNaOH})] \times 100/\text{Wt}$$

where: N HCl = Normality of HCl solution

R = Volume of NaOH solution used (mL)

N NaOH = Normality of NaOH solution

Wt. = weight of air-dry soil (g)

2.1 C Determination of Organic Matter

Organic matter was determined by when 1 g of air dry soil was added 10 mL of 1 N potassium dichromate solution was added followed by addition 20 mL of concentrated sulphuric acid and swirled the beaker to mix the suspension. Solution was left undisturbed stand for 30 minutes and 200 mL

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deionised water and 10 mL concentrated orthophosphoric acid was added. Added 12 drops of diphenylamine indicator (1 g diphenylamine in 100 mL concentrated sulfuric acid) with continuous stirring on magnetic stirrer and finally mixture was titrated with 0.5 M ferrous ammonium sulphate until colour changes from violet-blue to green. Blanks solution was prepared that contained all reagents but no soil. %Organic matter in soil was calculated using following equation:

M = 10/Vblank

% Oxidizable Organic Carbon (w/w)

= [Vblank-Vsample] × 0.3 × M/Wt

% Total Organic Carbon (w/w)

= 1.334% Oxidizable Organic Carbon

% Organic Matter (w/w)

= 1.724 × % Total Organic Carbon

where:

M = Molarity of ferrous ammonium sulfate solution

Vblank = Volume of ferrous ammonium sulfate solution required to titrate the blank (mL)

Vsample = Volume of ferrous ammonium sulfate solution required to titrate the sample (mL)

Wt. = Weight of air-dry soil (g)

0.3 = 3 × 10−3 × 100

where 3 is the equivalent weight of C

2.2. D Soil Pre-Treatment

Soil pretreatment included sieving to select a particle size lower than 0.5 mm and air-drying. The larger soil particles were removed to attain soil homogeneity. The drying was performed to facilitate subsequent grinding and to increase contact between the soil and the organic solvent used for extraction. Drying at elevated temperatures was avoided since this can result in losses of volatile compounds. Prior to its use, soil was autoclaved (121°C for 40 min).

2.3. E Media and Inoculums Preparation

A selective minimal salt medium (M1) was prepared containing 50% malathion as a sole source of carbon in addition to MSM (Mineral Salt Medium) K_2HPO_4, 4.27 g/L; KH_2PO_4, 3.48 g/L; $(NH_4)2SO_4$, 0.34 g/L; $MgSO_4\_7H_2O$, 0.46 g/L; $FeSO_4$, 0.001 g/L; $CaCl_22H_2O$, 0.018 g/L; $CuCl_22H_2O$, 0.01 mg/L;$CoCl_2\_6H_2O$, 0.2 mg/L; $ZnSO_47H_2O$, 0.1 mg/L; $MnCl_2.4H_2O$, 0.03 mg/L; $Na_2MoO_42H_2O$, 0.03 mg/L; and $NiCl_2.6H_2O$, 0.02 mg/L solution was sterilized by added to autoclaved media after cooling bacterial strains *Pseudomonas sp* was added and growth of bacterial species was observed in terms of absorbance by UV spectroscopy at 600 nm, similarly inoculums was inoculated into soil for bioremediation studied.

3. RESULTS AND DISCUSSION

3.1 A Characteristics of Soil

Characteristics of soil used in this study.

Sl. No.	Parameter	Result
1	P^H	6
2	Moisture	32%
3	Calcium carbonate	20%
4	Organic matter	5.5%

3. B Bioremediation of Malathion Contaminated Soil

The following experiment was performing in slurry phase bioreactor at different concentration of malathion at diffe- rent temperature and P^H The degradation was studied and reporting into table the maximum degradation was obser-ved at concentration 50mg/L at 35 ^{0}C temperature and 6 P^H.as shown in table 1.

Sl. No.	Conc Mg/L	Temp ^{0}C	P^H	% Removal of malathion
1	150	30	7	68
2	100	25	5	73
3	200	35	4	64
4	50	25	4	76
5	50	45	8	78
6	250	35	5	59
7	200	40	5	64
8	150	40	4	73
9	250	25	8	63
10	200	45	6	68
11	250	40	6	61
12	50	30	5	82
13	200	25	7	66
14	200	30	8	69
15	50	35	6	84
16	250	30	4	60
17	50	40	7	79
18	100	40	8	74
19	250	45	7	58
20	150	45	5	71
21	100	30	6	77
22	150	25	6	70
23	100	45	4	79
24	100	35	7	75
25	150	35	8	69

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Table 2: Growth of bacteria at different conc

Time (day)	Absorbance (50mg/L)	Absorbance (100mg/L)	Absorbance (150mg/L)
0	0.127	0.068	0.168
1	0.121	0.064	0.188
2	0.179	0.068	0.258
3	0.144	0.089	0.296
4	0.098	0.066	0.242
5	0.086	0.054	0.162

Time (day)	Absorbance (200mg/L)	Absorbance (250mg/L)	Absorbance (300mg/L)
0	0.278	0.294	0.418
1	0.262	0.274	0.418
2	0.395	0.362	0.815
3	0.362	0.432	1.043
4	0.306	0.382	0.865
5	0.231	0.302	0.605

3.C Growth of bacteria

Growth of bacterial was observed in terms of absorbance Vs time

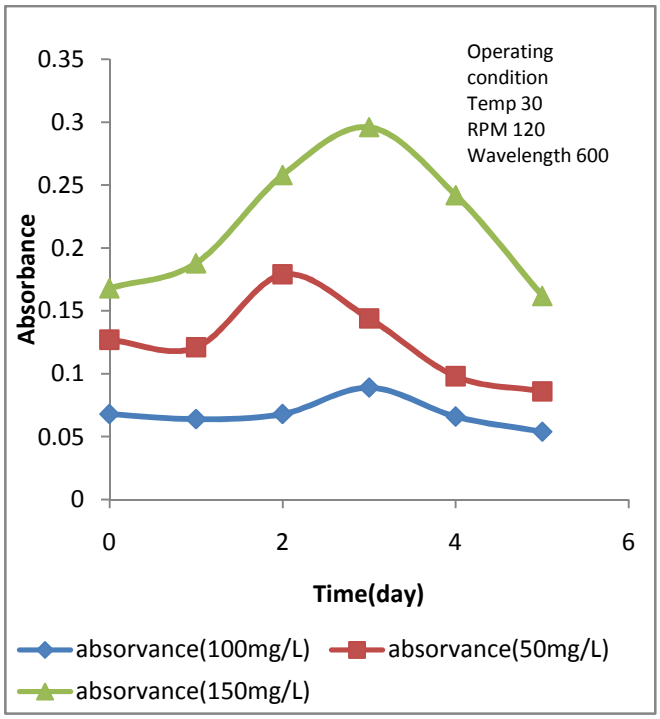

Fig. 1. Growth of bacteria vs time(Hr)

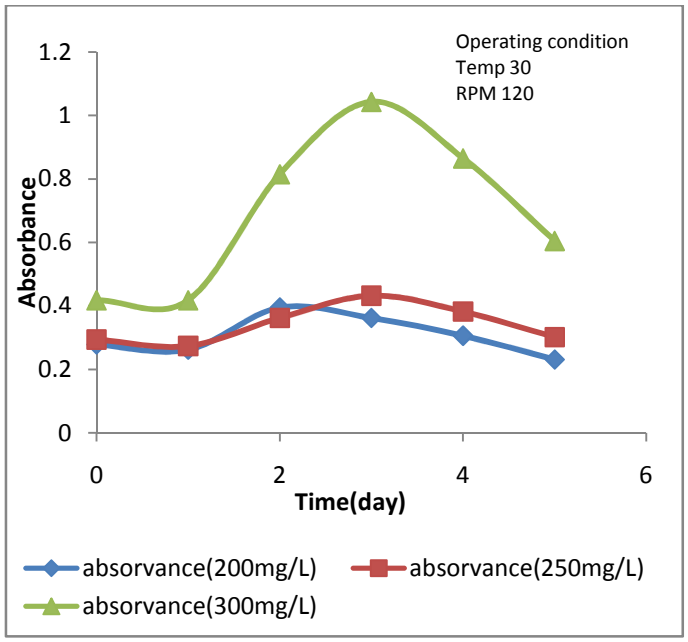

Fig. 2. growth of bacteria vs time (Hr)

4. CONCLUSION

Pesticides are considered as some of the most serious environmental pollutants, which are being frequently used for the control of agricultural and domestic pests. Though agriculture is vital, pesticides can be harmful to the human health and animals, and have been proved to have a detrimental environmental impact. Malathion is a no systemic, wide-spectrum organ phosphorus pesticide used in public health, residential, and agricultural settings. Malathion is suited for the control of sucking and chewing insects of fruits and vegetables, mosquitoes, flies, household insects, animal parasites. Malathion biodegradation was studied by using bacterial *pseudomonas sp.* Experiments was carried out at different concentration of malathion from 50 mg/L to 300 mg/L at different temperature and P^H. The maximum degradation was observed of concentration 50 mg/L at temperature 35 ^{0}C and P^H 6.

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