February 2010 ISSN : 0974 - 7451 Volume 5 Issue 1

Trade Science Inc.

Environmental Science

An Indian Journal

Current Research Paper

ESAIJ, 5(1), 2010 [38-41]

Assessment and optimization of pesticide degradation by *Pseudomonas putida*

C.Arunachalam*, A.M.Velmurugan

PG and Research Department of Microbiology, Sri Sankara College of Arts and Science, Enathur, Kanchipuram- 631561, (INDIA)

E-mail: agro_arun@rediffmail.com

Received: 26th January, 2010 ; Accepted: 5th February, 2010

ABSTRACT

Number of bacteria species isolated from the highly pesticide pollution soil. Among the several isolate *Pseudomonas putida* grown well on the various pesticide containing medium (Pentachlorophenol and Chlorpyrifos) produced yellow color colonies, pesticide used as sole source of carbon energy and releasing chloride compound. Biodegradation of pesticide were optimized. Maximum degradation was found on the glucose substrate with co-substrate pantothenic acid and pH of 7 was found to be optimum and the *P. putida* degraded 70% and 75%, Pentachlorophenol and Chlorpyrifos respectively at 37ºC±2 for 10 days.

KEYWORDS

Pestide;
Biodegradation;
Pentachlorophenol;
Chlorpyrifos;
Pseudomonas putida.

INTRODUCTION

In an era of modern agriculture where the chemical inputs form the core component of agriculture, the problems arising due to indiscriminate use of chemical ingredients is highly alarming. The modern agriculture has made chemical indispensable. Hence the problems associated with the chemical use are increasing day by day. The various agro-inputs employed include fertilizer, Pesticide including Herbicides, Insecticides and etc, the residual problems and environmental hazards posed by the excess use of these chemical are highly threatening the safety of the ecology. There should be a balance between the use of chemicals and removal of these residues quickly from the environment. Use of chemical need not be banned; but a prudent approach is, to clean the environment from the possible damage caused by these chemicals.

Pesticide when transformed to less compounds after the indented activity, there would not have any problem of residues of the pesticide and the resultant adverse effect on the ecosystem. Two important effect stand out, the food chain effect and biomagnifications. However, the soil quality degradation is of importance. Therefore, the situation warrants detailed investigation on the behavior of pesticides and their residues on plant and soil system from the point of safety to human being as well as protection of environmental degradation due to pesticide pollution. I India, the annual loss caused by pest is estimated to be as much as 50 percent of total crop production including quality deterioration[5]. Several works are available in these lines and this study aims at providing an ecological safe perspective in handling this complicated

issues where in microorganisms are employed to advance and accelerate the detoxification of chemicals and remove the same from the environment within the shortest possible time.

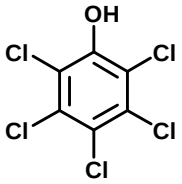
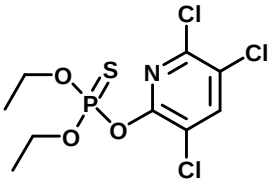
Hence this study is of current interest where in microorganisms are to be employed to degrade the pesticide compounds helping to remove the pesticide residue from the environment. The insecticide chosen for study belonged to various categories of chemical grouping via Pentachlorophenol and Chlorpyrifos.

While there may be 700 or more pesticide compounds that have been synthesized and used over the years, about 50 make up a large percentage of total pesticide usage. However even this number of compounds percents a wide range of physical and chemical properties, of concern to water quality management the pesticide characteristics of most concern from a water & soil quality management standpoint are toxicity to target and other organisms, (since this influence dosage required) solubility in water; persistence (longevity) the environment; and the potential for Bio-magnification. The most trouble some pesticide are those with a high level of toxicity, in environment are not degraded rapidly, and can accumulate in plant and animal tissue, there by allowing bio-magnification of the compound. It is questionable whether pesticide having least over all environment quality hazard.

In India, on the country we use cheap (not under patent protection, high-potency (Pentachlorophenol and Chlorpyrifos) insecticide, which are noted for their acute toxicity, non bio-degradability and bio accumulative properties. According to the Ministry of Agriculture, of the 180 registered pesticides in India, 32 (about one sixth of total pesticides used) have been banned in other countries. Pentachlorophenol and Chlorpyrifos a highly toxic insecticides (TABLE 1) whose registration was cancelled in the U.S in 1988 in India's top selling pesticide[1].

The present work pesticide such as Pentachlorophenol and Chlorophyriphos pesticide were purchased at local market Thanjavur. Test organism *Pseudomonas putida* was get from MTCC (Microbial Type Culture Collection) at Chandigarh. The bacterial strain was tested for their stability grown on pentachlorophenol and Chlorophyriphos by growing striking on the medium containing 50ppm each pesticide and the healthy

TABLE 1 : General properties of the selected pesticides

Sr. No.	Particulars	Properties of the selected pesticides	
1	Common name	Pentachlorophenol	Chlorpyrifos
2	Chemical structure		
3	Chemical name (IUPAC)	2,3,4,5,6-Pentachlorophenol	*O,O*-diethyl*O*-3,5,6-trichloro-2-pyridyl phosphorothioate
4	Empherical formula	C_6HCl_5O	$C_9H_{11}Cl_3NO_3PS$
5	Molecular weight	266.34 g mol$^{?1}$	350.59 g/mol
6	Density	1.978 g/cm^3 at 22 °C	1.398 g/cm^3 (43.5 °C)
7	Solubility in water	0.020 g/L at 30 °C	1.398 mg/L
8	Melting point	190-191 °C	42 °C

colony were selected and optimizing the culture parameters for maximum degradation.

MATERIALS AND METHOD

Monitoring growth the analyzing the residual pentachlorophenol and Chlorophyriphos in the medium evaluated the biodegradation potential of the selected strains. Growth was monitored everyday up to 5 days. Analysis of pentachlorophenol and Chlorophyriphos were done following 4-Aminoantipyrine method[9]. Chloride released was estimated by the Argent metric method[2].

Pentachlorophenol and Cholorphyriphos degradation data and maximum chloride released into the broth medium the most efficient isolate was selected for biodegradation ability of *Pseudomonas putida* was optimizing the culture parameters for maximum degradation. Carbon source like glucose, maltose and Manito were tested at 0.5% for their effect on degradation of pentachlorophenol and Chlorophyriphos by *Pseudomonas putida*. All these carbon sources were amended to each mineral salts medium containing pentachlorophenol (50ppm) and Chlorophyriphos and inoculated with *Pseudomonas putida* and incubated for 5 days at 37ºC. To find out the optimum pH range of 5.6 - 7. Effect of vitamins was studied in presence of 10ppm of pantothenic acid.

RESULT AND DISCUSSION

The ability of *P. putida* to depredated the Pentachlorophenol and Chlorpyrifos were assessed in terms of chloride released. *P. putida* depredated both pesticide components at the same time maximum degradation was observed Chlorpyrifos (75%) when compare with Pentachlorophenol (70%). This result were shown (TABLE 2) Chloride release, due to metabolism of pentachlorophenol and Chlorpyrifos have been accepted as an indirect method of measurement of pentachlorophenol and Chlorpyrifos degradation[8,10]

TABLE 2 : Chloride released by the bacterial isolates in mineral salts medium containing pentachlorophenol and chlorpyrifos

S.N	Strain code	Pesticides	Chloride released
1	Pseudomonas putida	Pentachlorophenol	66.71
2		Chlorpyrifos	88.56

The *P. putida* could utilized Pentachlorophenol and Chlorophrephos as a sole source of carbon the growth was very poor, effect of different co-substrates on the growth and pesticide degradation (TABLE 3) were evaluated pantothenic acid best co-substrate. It accelerated the growth of the bacterium (0.56OD) with a concomitant increase in Pentachlorophenol degradation (59.8%) and release[6]. This is in conformity with the findings of other scientists who also found sodium glutamate and pantothenic acid as an additional carbon source to increase the rate of growth *Flavobacterium sp* ATCC 39723 and pentachlorophenol degradation[3]. Flavobacterium capable of pentachlorophenol degradation was found to require sodium glutamate and pantothenic acid as an additional carbon source to decrees the toxicity of Pentacholorphenol[4].

TABLE 3 : Effect of different co-subject rates penta and chlorophyraphos by *Pseudomonas putida*

Sr. No.	Co-substrate	%pesticide degradation		Chloride released in broth (ug/l)
		A	B	
1	Glucose	60.2	64.7	80.99
2	Mannitol	48.3	49.9	49.9
3	Maltose	51.7	54.3	48.00
4	Fructose	50.00	49.00	45.00

Assessment of vitamins enhanced degradation and dechlorination of pentachlorophenol and Chlorpyrifos (TABLE 4). Similarly Kafkewitz and Armenate observed increased growth of *Pseudomonas picketti* leading to higher bioconversion of 4chlorobiphenyl to 4chlorobenzeoic acid and also the degradation of the latter to chloride ion[7].

TABLE 4 : Effect of vitamins on degradation of pentachlorophenol and chlorpyrifos by *Pesudomonas putida*

Sr. No.	Vitamins	% Pesticide degradation		Chloride released in to broth (ug/l)
		A	B	
1	Without vitamins	44.98	45.77	87.70
2	Ascorbic acid	59.4	59.44	109.22
3	Pantothenic acid	58.55	61.77	137.66

At pH 7.0 significantly higher growth of *Pseudomonas putida* was produced resulting in enbhanced Penetachlorophenol and Chlorpyrifos degradation and chloride releases into medium (TABLE 5). When pH was set to above 7.5 there was a decrees in growth as well as pentachlorophenol and Chlorpyrifos degradation.

TABLE 5 : Effect of different pH of medium on degradation of pentachlorophenol and chlorpyrifos by *Pesudomonas putida*

Sr. No.	pH of the Medium	% Pesticide degradation		Chloride released in pH adjusted
		A	B	
1	pH adjusted to 6.0	5.3	6.1	39.7
2	pH adjusted to 7.0	54.1	56.2	152.3
3	pH adjusted to 8.0	49.2	51.2	111.2
4	pH adjusted to 9.0	2.7	3.1	60.2

The tolerance of *P. putida* to diffenternt pentachlorophenol and Chlorpyrifos concentration was tested. Above 50ppm of Pentachloropheno and Chlorpyrifos led to slight degrease growth of *P. putida* . Pentachlorophenol and Chlorpyrifos at 75ppm in the soil have been reported to be inhibitory to bacterial consortium degradation.

ACKNOWLEDGEMENTS

I wish to sincerely record my deepest gratitude to Dr. K.R. Venkatesan, M.A., M.Phil., Ph.D., Principal, Sri Sankara Arts and Science College, Enathur,

Kanchipuram, for his valuable suggestions and insightful advices my scientific endeavors.

REFERENCES

[1] W.B.Bollen; A.Rev.Microbilogy, **15**, 69-92 **(1961)**.
[2] Calif-Scientists; Journal of Environmental Quality (JEQ), 37-40 **(2003)**.
[3] S.K.Gautam, R.Sharma, A.H.Ahmad, I.S.Thskut; World J.Microbiol Biotechnol., **19**, 73-423 **(2003)**.
[4] Y.Gu, R.A.Korus; Appl.Microbiol.Biotechnol., **43**, 274-378 **(1995)**.
[5] H.R.Kureger; 'Pesticide Residues in the Environment in India'. Eds: C.A.Edwards, G.K.Veeresh, Krueger Bangalore, India, 7-16 **(1978)**.
[6] J.Y.Lee, L.Xun; J.Bacteriol., **179**, 1521-1524 **(1997)**.
[7] M.S.Nandish, K.S.Jagadeesh; Indian Journal of Microbiology, **46**, 25-29 **(2006)**.
[8] G.K.Narde, A.Kapley, H.J.Purohit; Curr.Microbiol., **48**, 419-423 **(2004)**.
[9] N.Prasanna, N.Saravanan, P.Geetha, M.Shanmugaprakash, P.Rajasekaran; Advanced Biotech., 20-24 **(2008)**.
[10] D.L.Saber, R.I.Crowford; Appl.Environ.Micobiol., **50**, 1512-1518 **(1985)**.