

Biodegradation of the Synthetic Pyrethroid, Fenvalerate by *Bacillus Cereus* Mtcc 1305

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Abstract Extensive applications of synthetic pyrethroids not only result in pest resistance to these insecticides, but also may lead to environmental issues and human exposure. The pyrethroid fenvalerate pesticide residues on foods and environmental contamination are a public safety concern. Therefore, the present study investigated the potential of the chosen bacterium, *Bacillus cereus* MTCC 1305 in the degradation of various concentrations of fenvalerate (250, 500, 750 and 1000ppm). The degradation efficiency of the bacterium was tested by the assessment of pH, free carbon dioxide, turbidity and esterase activity. HPLC analysis for 500ppm of fenvalerate degradation by the bacterium at the end of long term and short term treatments showed peaks with different retention times. The degradation was found to be maximum for 500ppm of fenvalerate. These findings suggest that the utilization of fenvalerate by *P. viridiflava* may be a feasible treatment option for the removal of pesticides from soil environment.

Keywords: pesticide, pyrethroid, fenvalerate, bacillus cereus, HPLC

1. Introduction

Over the last 30 years, synthetic pyrethroids (SPs), such as fenpropathrin, cyhalothrin, permethrin, cypermethrin, fenvalerate, cyfluthrin, deltamethrin and bifenthrin, have been widely used as agricultural, home and garden insecticides to replace the more toxic and environmentally persistent organochlorine and organophosphorus pesticides [1]. Synthetic pyrethroids are a group of compounds derived from the natural insecticide pyrethrin, found in the flower heads of certain *Chrysanthemum* species. An annual sale of SPs reaches an estimated 1.3–1.4 billion dollars worldwide and account for 17% of global insecticide sales [2]. Due to their extensive use, SPs residues have been frequently detected in soils, sediments, natural waters and agricultural products (especially in teas) [3,4].

Although SPs generally have lower mammalian toxicity than other classes of insecticides, they affect the central nervous system, cause allergic skin reactions and eye irritation and have high acute toxicity to some non target organisms such as bees, fish and aquatic invertebrates [5]. Many studies have found fenvalerate to be toxic to non-target organisms. It is highly toxic to many fishes [6]. Target insect species are killed at fenvalerate concentrations of 0.015µg/insect, by aerial application (0.11kg/ha), 5.4mg/kg in soil, or 50mg/kg in diet. Fenvalerate is especially toxic to aquatic organisms (e.g., crustaceans died at 0.003-0.03µg/L and fish and amphibians at 0.09-1.1µg/L), and its use in or near aquatic environments should be avoided [7].

In general, SPs are degraded by both abiotic and biotic pathways, including photo-oxidation, chemical oxidation

and biodegradation. Microbial metabolisms play important roles in degrading and detoxifying SPs residues in soils and sediments. The rate of degradation depends on the pyrethroid, soil type, climate, the species of microbes present, and the size of their populations. In soil, degradation of pyrethroids occur via ester cleavage, diphenyl ether cleavage, ring hydroxylation, hydration of the cyano group to amide, and further oxidation of the fragments formed to yield carbon dioxide as a major final product [8]. Many microorganisms are capable of degrading SPs, including *Pseudomonas fluorescens*, *Achromobacter* sp, *Pseudomonas pseudoalcaligenes*, *Trichoderma viride*, *Pseudomonas stutzeri*, and *Micrococcus* sp. [9,10,11].

In the present study, an attempt has been made to determine the efficiency of degradation of fenvalerate with the reference strain, *Bacillus cereus* (No.1305) obtained from MTCC, IMTECH, Chandigarh.

2. Materials and Methods

The reference strain *Bacillus cereus* MTCC 1305 was obtained from the Institute of microbial technology, Chandigarh.

2.1. Degradation Efficiency

The strain was inoculated onto minimal broth containing different concentrations of fenvalerate like 250, 500, 750 and 1000ppm and incubated at room temperature for a period of 16 days (Long term study) and the degradation was confirmed by analyzing various parameters such as pH, biomass, carbon dioxide production, esterase activity and the degradation products. A short term study was done by measuring the above parameters every 24 hours for 4 days (Short term study).

2.2. pH

The pH of the sample was measured on the 4th, 8th, 12th and 16th day of incubation and another set of samples were analyzed on the 1st, 2nd, 3rd and 4th day of the treatment.

2.3. Biomass Estimation

Turbidometric method was followed for estimating the biomass by measuring the turbidity at 600nm.

2.4. Carbon Dioxide Estimation

The release of carbon dioxide during the degradation of various concentrations of fenvalerate was estimated by the method proposed by Eaton *et al.* (1995) [12].

2.5. Esterase Activity

100 μ l of cells or cell extract was incubated with 1.5 ml of 0.42mM 1- naphthyl acetate, 0.5ml Na₂HPO₄ buffer (0.2M, pH 7.0) and 0.4ml glass distilled water at 39 °C for 10 minutes. 500 μ l of 10% lauryl sulfate containing 2.5mg of Fast Garnet GBC was added to the mixture and incubated at room temperature for 15 minutes for color development. Absorbance was measured at 560nm and compared with the absorbance of 1-naphthol curve (linear from 0- 0.08 μ M) [13].

2.6. High Pressure Liquid Chromatography (HPLC)

The samples containing the minimal medium, isolate and 500ppm concentration of fenvalerate taken on the 0th, 4th and 8th day were subjected to HPLC analysis.

2.7. Statistical Analysis

Two way ANOVA was performed for the parameters pH, carbon dioxide released, esterase activity and bio mass using MS Excel for both the isolate and the reference strain. Variability was considered significant only when the statistic value was greater than the tabulated value at P is less than or equal to 0.05.

3. Results and Discussion

The details of the synthetic pyrethroid, fenvalerate is given in the Table 1.

Table 1. Details of Fenvalerate

CAS number	51630-58-1
Chemical formula	C ₂₃ H ₂₂ ClNO ₃
Physical state	viscous liquid
Color	yellow or brown
Odour	mild "chemical" odour
Relative molecular mass	419.9
Boiling point	300 °C at 4.93 kPa (37 mmHg)
Water solubility	2 μ g/Liter
Solubility in organic solvents	Soluble
Relative density (25 °C)	1.175
Vapour pressure (25 °C)	0.037 mPa

Figure 1 exhibits the changes in the pH of the medium during the degradation of fenvalerate by *Bacillus cereus*. The pH decreased during the first 8 days and seemed to be increasing thereafter. Figure 2 illustrates the changes in the pH recorded on the 1st, 2nd, 3rd and 4th days of

treatment with *B.cereus*. In both the cases the pH declined drastically indicating the degradation of fenvalerate.

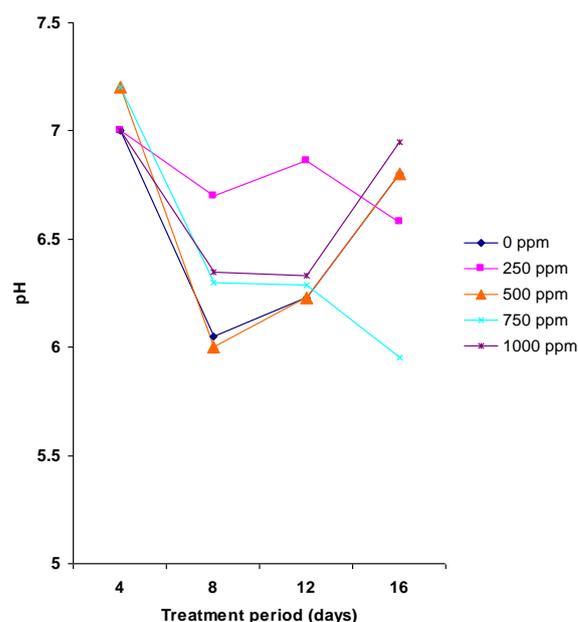


Figure 1. Changes in the pH of the Medium during the Degradation of Fenvalerate by *B. cereus* in the Long Term Treatment

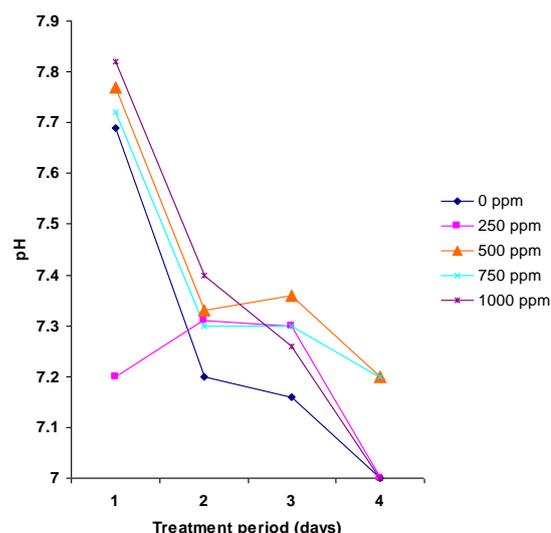


Figure 2. Changes in the pH of the Medium during the Degradation of Fenvalerate by *B. cereus* in the Short Term Treatment

The metabolic fate of pesticides is dependent on abiotic environmental conditions (pH, temperature, etc.), microbial community and pesticides characteristics. Laskowski (2002) reported that pyrethroids are degraded slowly in acidic and neutral pH, but degradation is more rapid when pH is alkaline [14].

The complete mineralization of fenvalerate results in release of carbon dioxide which is said to be a major decomposition product of fenvalerate degradation [8]. During the degradation of 500ppm of fenvalerate, more amount of carbon dioxide was released and in the case of 250ppm concentration, there was no release of carbon dioxide on the 16th day of treatment (Figure 3). The amount of carbon dioxide released during the first four days of degradation of fenvalerate by *B.cereus* is shown in Figure 4. In both the cases, the amount of carbon dioxide

was the maximum for the 500ppm concentration of fenvalerate.

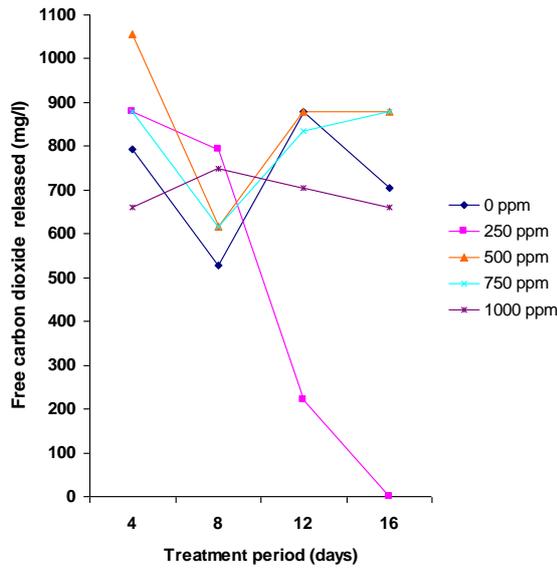


Figure 3. Carbon dioxide released during the Degradation of Fenvalerate by *B. cereus* in the Long Term Treatment

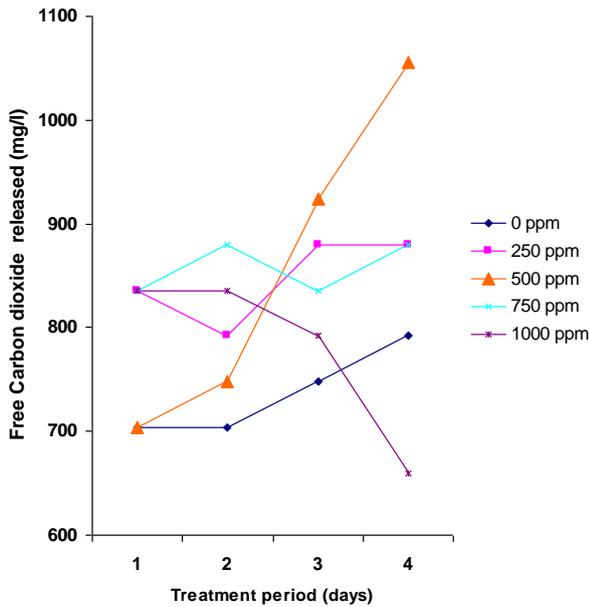


Figure 4. Carbon dioxide released during the Degradation of Fenvalerate by *B. cereus* in the Short Term Treatment

Changes in the turbidity of the medium during the long term treatment of fenvalerate by *B.cereus* are shown in the Figure 5. It seems to be fluctuating but there is a linear increase in the case of 500ppm concentration of fenvalerate indicating an increase in growth. Fluctuations in the absorbance values were observed during the degradation of fenvalerate in the first four days by *B. cereus* (Figure 6).

Esterase activity during the long term treatment of fenvalerate by *B.cereus* is shown in Figure 7. The highest activity was observed in 250ppm and 500ppm of fenvalerate and there was a reduction in the esterase activity after the 8th day of treatment. Esterase activity during the first four days of treatment by *B.cereus* increased linearly (Figure 8) and the highest activity was

recorded in the case of 250ppm of fenvalerate. *Pseudomonas fluorescens* and *Achromobacter spp* have been shown to transform fenvalerate employing esterases [15].

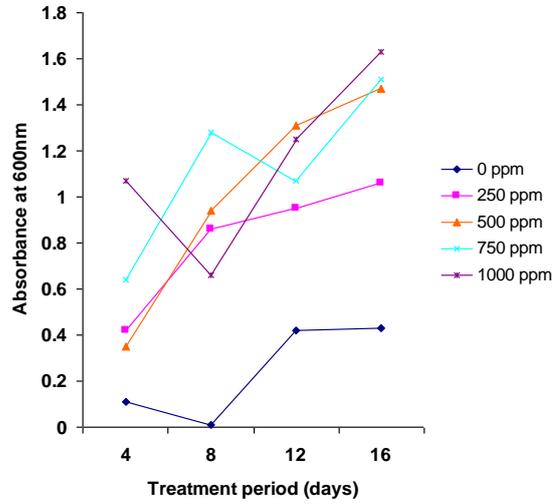


Figure 5. Turbidity during the Degradation of Fenvalerate by *B. cereus* in the Long Term Treatment

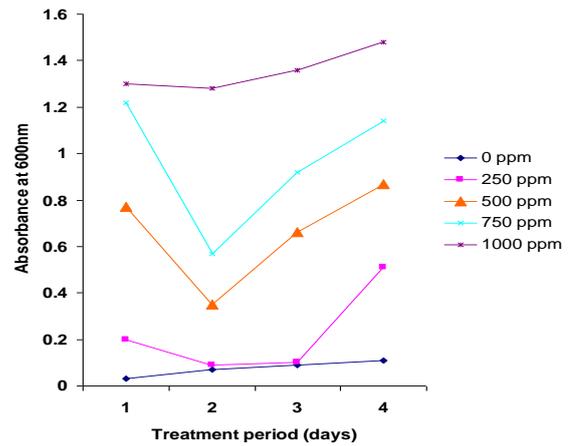


Figure 6. Turbidity during the Degradation of Fenvalerate by *B. cereus* in the Short Term Treatment

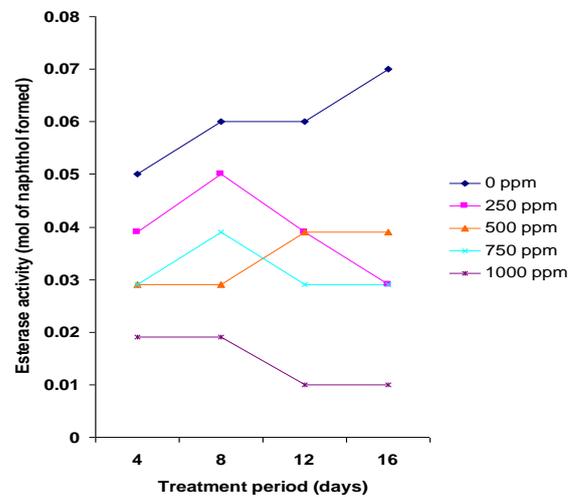


Figure 7. Esterase activity during the Degradation of Fenvalerate by *B. cereus* in the Long Term Treatment

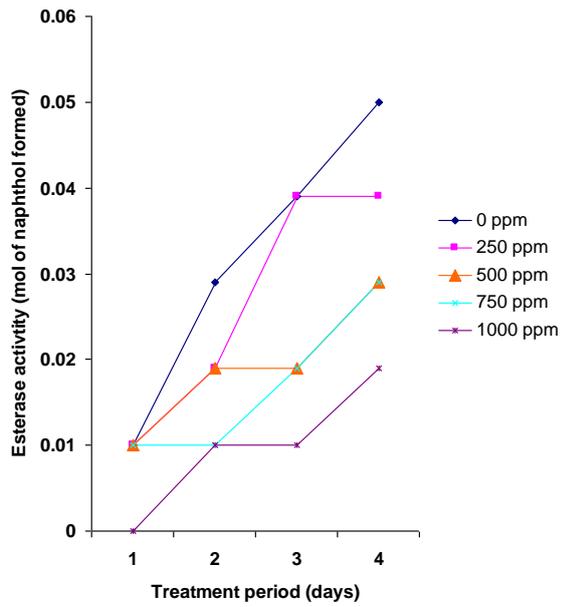


Figure 8. Esterase activity during the degradation of fenvalerate by *B. cereus* in the short term treatment

Table 2. HPLC Analysis Report for 500ppm Fenvalerate before and after Treatment with *B. cereus*

Sample	Retention Time [min]	Height [mV]	
Before treatment	2.830	12.28	
	3.003	14.10	
	4.560	2.61	
	5.163	9.54	
	5.837	10.06	
	6.540	15.15	
	7.667	4.73	
	8.210	0.94	
Total		69.41	
After 4 days of treatment	2.043	0.98	
	2.827	22.79	
	2.970	25.20	
	4.537	2.03	
	5.150	5.40	
	5.503	10.58	
	7.603	0.64	
	9.727	0.52	
Total		68.14	
After 8 days of treatment	2.110	0.27	
	2.843	26.63	
	3.057	20.92	
	4.480	1.97	
	4.840	1.96	
	5.077	3.84	
	6.377	1.85	
	8.543	0.29	
	Total		57.73

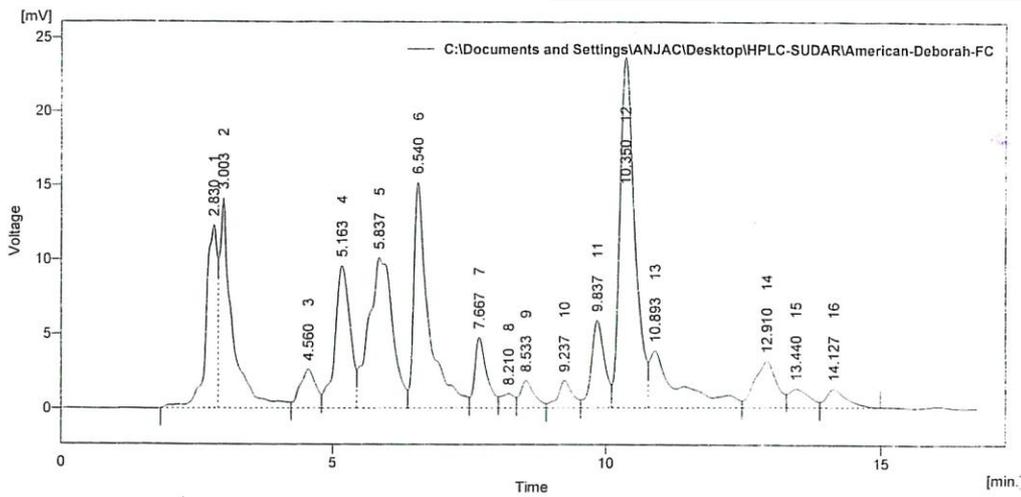


Figure 9. HPLC analysis for 500ppm of fenvalerate before treatment

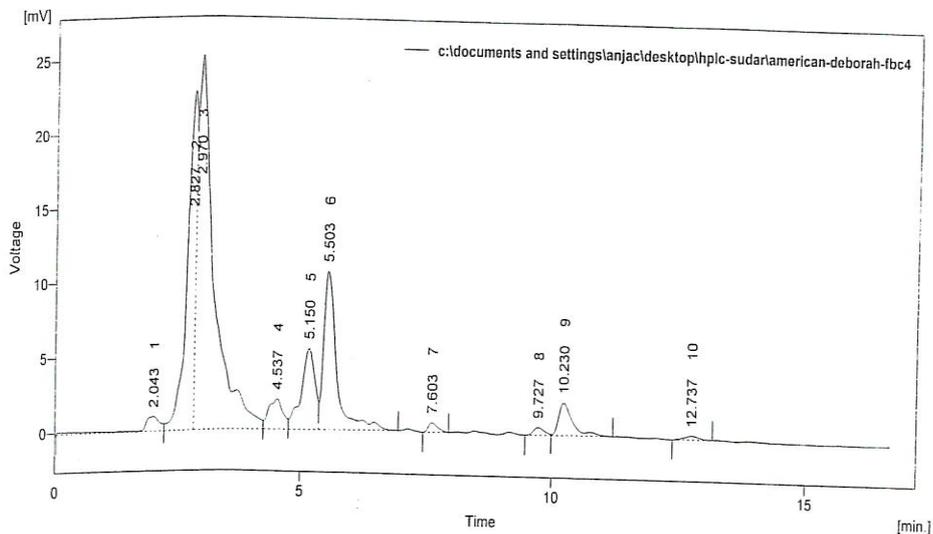


Figure 10. HPLC analysis for 500ppm of fenvalerate treated with *Bacillus cereus* on 4th day

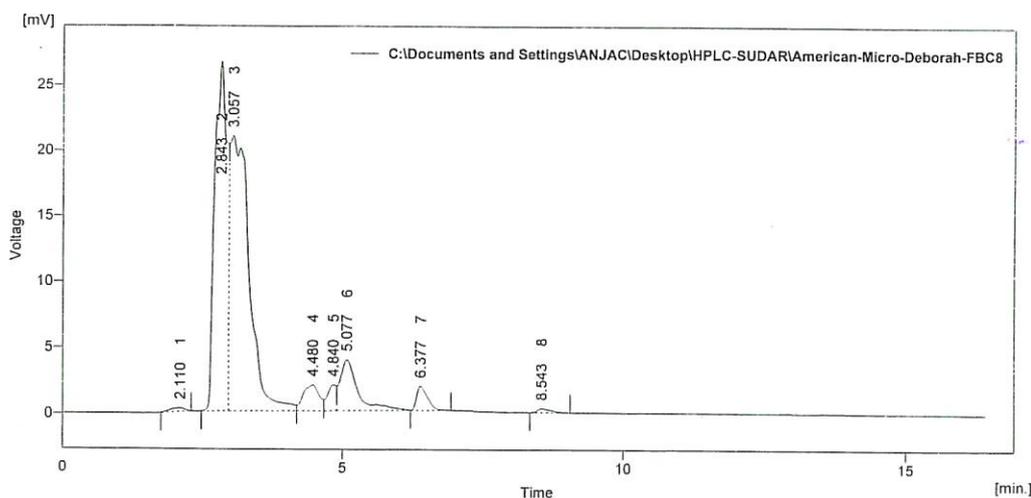


Figure 11. HPLC analysis for 500ppm of fenvalerate treated with *Bacillus cereus* on 8th day

Table 2 shows the HPLC analysis report for 500ppm fenvalerate which explores the retention time and heights of peak obtained during the treatment with *B. cereus*. Figure 9 shows the HPLC analysis for 500 ppm of fenvalerate on the 0th day of treatment. The peaks observed here were missing in the HPLC analysis of the same concentration of fenvalerate on the 4th day of degradation by *B.cereus* and there were few new peaks with different retention times indicating the formation of intermediates (Figure 100). Figure 111 exhibits the

degradation of 500ppm of fenvalerate on the 8th day of treatment by *B. cereus*. There was formation of several intermediates as well as disappearance of compounds.

Table 3 and Table 4 exhibits the two way analysis of variance for the factors such as pH, carbon dioxide, turbidity and esterase activity with the variables, fenvalerate concentration and treatment period for *B.cereus* in the long term and short term treatment respectively. The variations due to the concentration and treatment period were statistically significant at 5% level.

Table 3. Two Way Analysis of Variance (ANOVA) for the Factors with the Variables, Treatment Period and Fenvalerate Concentration for *B. cereus* during Long Term Treatment

Factor	Source of variation	df	MS	F value	Table value at 5% level	Level of Significance
pH	Concentration	4	0.072558	0.887943	0.500279	Significant
	Treatment period	3	0.62946	7.703193	0.003929	Significant
Carbon dioxide	Concentration	4	87265.2	1.68138	0.218515	Significant
	Treatment period	3	50690.9	0.97668	0.43595	Significant
Turbidity	Concentration	4	0.56258	10.79877	0.000601	Significant
	Treatment period	3	0.46281	8.883742	0.002249	Significant
Esterase activity	Concentration	4	0.001073	22.32986	1.74	Significant
	Treatment period	3	3.34E-05	0.69476	0.57	Significant

Table 4. Two Way Analysis of Variance (ANOVA) for the Factors with the Variables, Treatment Period and Fenvalerate Concentration for *B. cereus* during Short Term Treatment

Factor	Source of variation	df	MS	F value	Table value at 5% level	Level of Significance
pH	Concentration	4	0.032058	1.79619	0.194462	Significant
	Treatment period	3	0.269893	15.12219	0.000223	Significant
Carbon dioxide	Concentration	4	11954.8	1.50304	0.262545	Significant
	Treatment period	3	5775.7	0.72616	0.555702	Significant
Turbidity	Concentration	4	1.105901	58.62477	8.93	Significant
	Treatment period	3	0.107693	5.70888	0.01152	Significant
Esterase activity	Concentration	4	0.0003	9.66433	0.00098	Significant
	Treatment period	3	0.00058	18.72776	8.03	Significant

4. Conclusion

Due to only 10% of applied pesticides reach to the target organism, a high percentage is deposited on non target areas (soil, water, sediments) leading to pollution, besides affecting public health. For this reason, it is necessary to generate strategies for waste treatment and/or for the bioremediation of polluted sites. Therefore, the

findings from the present study suggest that the utilization of fenvalerate by *Bacillus cereus* would be an effective treatment technology for the removal of pesticide from the soil.

Acknowledgement

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References

- [1] Katsuda, Y., "Development of and future prospects for pyrethroid chemistry", *Pesticide Science*, 55. 775-782. 1999.
- [2] Khambay, B. and Jewess, P., "Pyrethroids", *Molecular insect science*, Elsevier publication, Oxford. pp 1-29. 2005.
- [3] Weston, D.P., You, J. and Lydy, M.J., "Distribution and toxicity of sediment-associated pesticides in agriculture-dominated water bodies of California's central valley", *Environ Sci Technol*, 38.2752-2759. 2004.
- [4] Gu, X.Z., Zhang, G.Y., Chen, L., Dai, R.L. and Yu, Y.C., "Persistence and dissipation of synthetic pyrethroid pesticides in red soils from the Yangze river delta area", *Environ Geochem Hlth*, 30. 67-77. 2008.
- [5] Kumar, A., Shama, B. and Pandey, R.S., "Cypermethrin and Icyhalothrin induced alterations in nucleic acids and protein contents in a freshwater fish, *Channa punctatus*", *Fish Physiol Biochem*, 34. 331-338. 2008.
- [6] Tilak, K.S., Satyavardhan, K. and Thathaji, P.B., "Biochemical changes induced by fenvalerate in the freshwater fish *Channa punctata*", *J. Ecotoxicol. Environ. Monit*, 13(4). 261-270. 2003.
- [7] Eisler, R., Fenvalerate hazards to fish, wildlife and invertebrates: A synoptic review contaminant hazard reviews. Report. 24. 1992.
- [8] Kelley, K. "Environmental fate of esfenvalerate. Environmental monitoring branch, Department of pesticide regulation, California environmental protection agency. 2000.
- [9] Halden, R.U., Peters, E.G., Halden, B.G. and Dwyer, D.F., "Transformation of mono- and dichlorinated phenoxybenzoates by phenoxybenzoate-dioxygenase in pseudomonas pseudoalcaligenes POB310 and a modified diarylether-metabolizing bacterium", *Biotechnol Bioeng*, 69.107-112. 2000.
- [10] Saikia, N., Das, S.K., Patel, B.K.C., Niwas, R., Singh, A. and Gopal, M., "Biodegradation of beta-cyfluthrin by Pseudomonas stutzeri strain S1", *Biodegradation*, 16. 581-589. 2005.
- [11] Tallur, P.N., Megadi, V.B. and Ninnekar, H.Z., "Biodegradation of cypermethrin by Micrococcus sp. strain CPN 1", *Biodegradation*, 19. 77-82. 2008.
- [12] Eaton, A.D., Clesceri, L.S. and Greenberg, A.L., *Standard Examination of Water and Waste water*; 19th Edition, p 4.17. 1995.
- [13] Lanz, W.W. and Williams, P.P., "Characterization of Esterases Produced by a Ruminant Bacterium Identified as *Butyrivibrio fibrisolvens*", *Journal of Bacteriology*, 113. 1170-1176. 1972.
- [14] Laskowski, D.A., "Physical and chemical properties of pyrethroids", *Rev. Environ. Contam. Toxicol*, 174. 49-170. 2002.
- [15] Maloney, S.E., Maule, A., Smith, A.R.W., "Microbial transformation of the pyrethroid insecticides: Permethrin, Deltamethrin, Fastac, Fenvalerate and Fluvalinate", *Applied and Environment Microbiology*, 2874-2876. 1988.