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Treatment of Pesticide Contaminated Wastewater by Soil Microorganisms

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ABSTRACT
Cypermethrin is one of the most widely used pesticides in the country for agriculture crop production. Due to least water solubility and toxicity, its removal need especial attention. Microbial degradation is considered to be an efficient and cost effective method for decontamination of toxic pesticides from the environment. In this study, malathion degrading bacterial isolate, identified as Pseudomonas, was used to assess its biodegradation potential for cypermethrin in aqueous system. The experimental findings indicate that Pseudomonas was able to degrade cypermethrin, if suitable environmental conditions provided in the reactor. Increased concentration from 20 to 80 mg/L gradually decreased the removal efficiency. However, under continuous agitation, complete degradation of cypermethrin (20 mg/L) occurred within a period of 48 hours. These results suggest that the use of potential microorganisms in the treatment system can successfully overcome many of the disadvantages associated with the conventional method used for the degradation of inhibitory compounds.

Key words: Cypermethrin, agriculture, microbialdegradation, environmental conditions, reactor.

1. INTRODUCTION
The intensive nature of modern agricultural practices has led to the development and widespread use of synthetic pesticides in our environment. Pesticides fight against diseases and save crops from pests. However, their indiscriminate use has become a global problem[1]. They have been frequently detected in the water bodies in various regions of the world including Pakistan[2-7] .Presence of these compounds may be toxic, mutagenic or carcinogenic and may be bioaccumulated or biomagnified by the biota[8,9]. The main concern with the use of these compounds is the toxicity threat they pose to public health. It is quite shocking that some pesticides act as endocrine disruptors and many insecticides function by impeding normal nervous system functions[10-12.]According to World Health Organization study[13], worldwide three million people suffered from pesticide poisonings with about 250,000 deaths per year.

In Pakistan, Cypermethrin is mainly used to increase cotton crop production. Cypermethrin actually acts on the nervous system and is toxic to bees, other beneficial insects, earthworms, fish and shrimps[14]. Because of its low water solubility, such compounds are very difficult to be removed from environmental system by conventional means. At present, besides pesticide contamination from agricultural field, the agricultural industries are also contributing relatively high quantities of toxic pesticides into the environment, since most of them have either no treatment facilities or have grossly inadequate arrangement. The Karachi coastal region has become the dumping ground of hazardous waste, receiving huge quantity of untreated domestic, industrial and agricultural wastes. Pesticides waste treatment technologies are therefore to prevent needed to prevent water pollution and to comply with increasing regulatory pressure. A few of the many solutions that have been and are being investigated are containment, incineration, chemical treatment, volatilization, phytoremediation and bioremediation[15].Recently, the bioremediation (biological treatment system) has been proven to be a suitable method for the treatment of polluted aquifers containing hazardous waste that could be implemented either in situ or off-site in specially designed reactors or wastewater treatment plants. Moreover, in most cases, it has been found to be the most cost-effective and environmentally friendly treatment method. According to literature, bioremediation success depends upon the physical and chemical characteristics of the substrate, such as nutrient status and pH, and is influenced by environmental factors such as temperature[16]and biotic factors such as inoculum density[17].The purpose of present study is to assess the microbial potential for cypermethrin degradation in an aquatic environment using biological treatment system. Such studies would be valuable to scientists and engineers who are trying to develop method for the treatment of toxic compounds like cypermethrin which are resistant otherwise to conventional treatment.

2. EXPERIMENTAL

2.1 *Pesticide, medium and culture used*
The pesticide used in this study belongs to the class pyrethroid and is commercially available as cypermethrin. Due to low water solubility, stock aqueous solution of cypermethrin (1mg/ml) was prepared in sterile HPLC grade methanol (Merck).

Nutrient broth and nutrient agar media were prepared according to the manufacturer's instruction (8 gm in 1000 ml purified water, pH 7.2 and autoclaved at 121°C, 15 psi for 30 minutes) and was used for growth and biodegradation studies.

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The bacterial culture (IES-*Ps*-1) capable of degrading malathion was isolated by *Hashmi*[42] from agricultural soil using enrichment technique and was used in present study. Cypermethrin degrading culture was obtained through acclimating the IES-*Ps*-1 strain with gradual increased concentration of cypermethrin from 10 to 80 mg/L in nutrient medium. Adapted IES-*Ps*-1 was stored at 4°C on slopes of nutrient agar containing 0.1 mg/L Cypermethrin and subcultured after every three months.

When a new batch of test was performed at different environmental conditions using varying dose of cypermethrin, the stock culture was first subcultured into 10 ml nutrient broth, aerobically grown and subsequently utilized for characterization, growth and biodegradation studies.

Characterization and growth of IES-Ps-1

Characterizations of IES-*Ps*-1 was performed using morphological, cultural and biochemical tests according to the methods described by *Colins & Lyne*[43] up to the stage of genus. Growth of IES-*Ps*-1 in biosimulator was determined by viable cell enumeration immediately after inocculation and at 24, 48, 72, 96 h later using *Miles and Misra technique*[44].

2.2 *Cypermethrin degradation studies using biosimulator (activated sludge)*

The compact bench scale biosimulator (Model MF-114) consists of a stainless steel reactor with a heavy wall glass jar of borosilicate glass equipped for monitoring and controlling rate of agitation and aeration was used.

The effect of cypermethrin concentration and environmental conditions (pH, temperature, and dissolved oxygen) on the performance of IES-*Ps*-1 for cypermethrin (80 mg/L) degradation was evaluated. Approximately 8.5 liters wastewater sample, inoculated with 350 ml culture and an appropriate quantity of cypermethrin was transferred into the biosimulator. The sample was strongly agitated by impeller with flat stirring paddles and by four vertical baffles. The required temperature was maintained by the built in thermostat and the dissolved oxygen concentration of 8-9 mg/L was achieved by mechanical aeration regulated through continuous agitation.

2.3 *Analytical procedure*

The sample from biosimulator was withdrawn at timed intervals of 8, 24, 32, 48 hours and analyzed for pH, temperature, dissolved oxygen and COD as per standard procedure laid down in *APHA*[45].

2.4 *Extraction of Cypermethrin for HPLC analysis*

Samples were collected from biosimulator as per schedule and were extracted two times with n-hexane (75 ml and 50 ml) by vigorous shaking for 15-20 minutes in a separatory funnel. The hexane layer was separated and evaporated to dryness at 70 °C using vacuum rotary evaporator (BUCHI Rotavapor R- 200/205). The dried residue was then dissolved in 10 ml HPLC grade methanol. After gently vortexing and filtering through a 0.2 μm membrane filter, an aliquot of 20 μL, was used for HPLC analysis. Each sample was injected 3 times and the mean was calculated.

2.5 *High Pressure Liquid Chromatography (HPLC)*

HPLC (Shimadzu, Japan) chromatographic system consisted of a solvent delivery pump LC-10 AS, connected with an autoinjector model SIL-6A and a rheodyne injection valve fitted with a sample loop (20 μl). The chromatographic separation was achieved on a reverse phase C_{18} column with a guard column and monitored by UV-detector (visible spectrophotometer detector SPD-10A) set at 220 nm. The output of the detector was connected to a chromatopack (CR6A). The mobile phase consisted of methanol (Merck HPLC grade) since cypermethrin is miscible in alcohol. The filtered methanol was degassed prior to use by sonication. The flow rate was adjusted at 2 ml/minute with total elution time of 10 minutes for each run. The column was flushed with deionized distilled water and methanol whenever required for removing impurities and was allowed to equilibrate between runs.

3. RESULTS AND DISCUSSION

3.1 *Characterization and adaptation of bacterial isolate*

On the basis of morphological, cultural and biochemical characteristics, the bacterial isolate was identified as a member of the genus *Pseudomonas* according to "*Bergey's Manual of Systematic Bacteriology*" [18]. Characterization studies of the isolate from experimental results, as well as of those by other researchers, indicate that bacteria belonging to the genus *Pseudomonas* are gram-negative, rod-shaped, highly oxidative and metabolically versatile, able to degrade aromatic hydrocarbons, oil, petroleum products and pesticides[19-27].

3.2 *Bacterial growth in biosimulator*

The results as shown in Figure 1, clearly indicate that cypermethrin had pronounced effect in promoting better growth of IES-*Ps*-1. As in the presence of cypermethrin, the bacteria grow fast and a higher number of cells were observed when compared with the control (without cypermethrin). The maximum count at 24 hours with 40mg/L cypermethrin

was 13±1.73x10^7 CFU/ml and with 80mg/L, it was 19±2.65x10^7 CFU/ml respectively. However, the generation time at these concentrations (40 and 80mg/L) were noted to be 57 and 53 minutes. On the other hand in the control experiments, the cell count at 24 hours was relatively low (7±1.73 x10^7 CFU/ml) with marked increase in generation time (98 minutes).

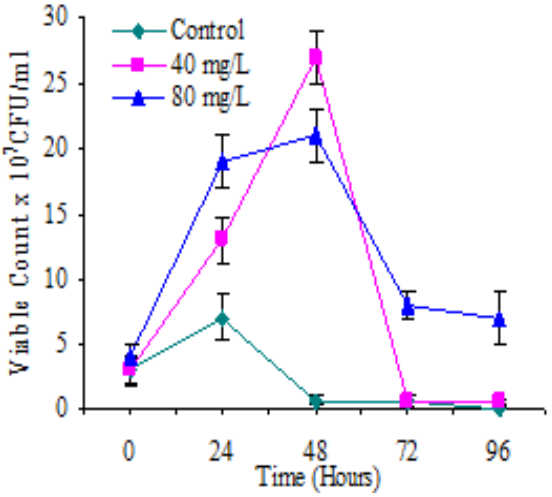

Fig-1: Growth of bacteria in biosimulator containing Cypermethrim.

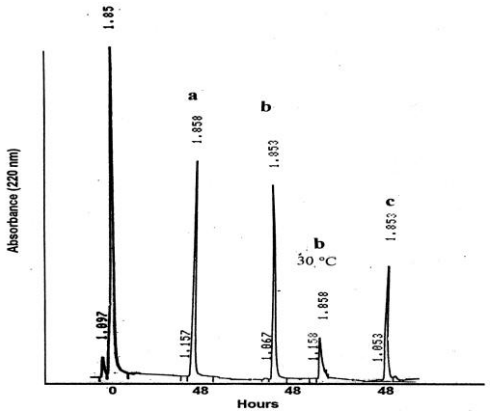

Fig-2: HPLC chromatograms showing comparative effect of Cypermethrin (80mg/L) degradation. a: 6 mg/L DO; b: 8 mg/L DO& 8 mg/L DO at 30 °C; c: 10 mg/L DO.

It was further noted that the growth at 40mg/L cypermethrin dose significantly increased after 48 hours incubation. But the growth at 80mg/L dose was slightly less but continued to grow till 96 hours incubation and a count of 7x10^7 CFU/ml was observed. This may be due to availability of nutrients and favorable environmental conditions in biosimulator which allow the cells to survive till 96 hours. In contrast, the population density in control experiment (no pesticide) was comparatively less (0.1x10^7 CFU/ml). This may be because of the presence of limited concentration of nutrient in wastewater sample (no cypermethrin), which does not allow the cells to grow to higher numbers.

Since 78-88% degradation of cypermethrin observed after 48 hours of aerobic treatment in biosimulator, these result suggests that IES-*Ps*-1 has the potential to degrade cypermethrin in wastewater samples. The bacterial cells in log phase during the period of biodegradation clearly indicate that the substrate conversion rate would be at its maximum as also described by *Gray*[28,29].

3.3 *Cypermethrin degradation in biosimulator*

Cypermethrin degradation was evaluated by conducting experiments at different temperature, dissolved oxygen and using different concentration of cypermethrin. Results as shown in Figure 3, clearly indicate that due to low water solubility of cypermethrin[30], at ambient temperature (18-25°C) and 38 $\pm$ 1°C using mechanical aeration (8-9mg/L dissolved oxygen), the degradation ability of IES-*Ps*-1 significantly decreased with increased concentration (80 mg/L) and the removal rate was only 48% - 51%. But under ambient temperature (18-25°C) using 20 mg/l concentration, a complete degradation of cypermethrin occurred after 48 hours of aerobic treatment. However, at other dosages (40, 80 and 125 mg/L), it was 82%, 50% and 17% respectively (data not shown). In contrast, at optimum temperature (28-30°C) using 80 mg/L cypermethrin concentration, biodegradation efficiency significantly improved and >88%degradation was observed (Figure 3).These findings were supported by Schlegel (1969) and Palleroni (1986), who reported the same optimum temperatures (28-30°C) for the growth of *Pseudomonas*. During treatment, it was also noticed that higher dissolved oxygen concentration (10 mg/L) had no more pronounced effect on cypermethrin

degradation instead 8 mg/L DO using mechanical aeration at the temperature range of 28-30°C proved to be stimulatory and sufficient for effective biodegradation (Figure 2). *Zacharias et al.*,[31], also observed that higher oxygen supply in the treatment system had no pronounced effect on biodegradation rates of chlorinated aromatic hydrocarbons. This would mean that if optimum operating conditions (28-30 ºC temperature, 8-9 mg/L DO, mechanical aeration) not maintained in biosimulator, cypermethrin degradation still continue but at a reduced rate.
The study findings were supported by previous work on biodegradation of recalcitrant compounds[32], where the biodegradation rates were significantly reduced due to low aqueous solubility of chemical compounds and the presence of an inappropriate environmental conditions. It is also reported that in spite of their high resistant nature, pentachlorobiphenyls (PCB_S) and pentachlorophenols (PCP), were biodegraded when the right microorganisms and environmental conditions were present in the system[33-35]. Thus, comprehensive knowledge of the range of contaminants present, their fate mechanisms and environmental conditions under which treatment proceed being considered essential for effective biodegradation.

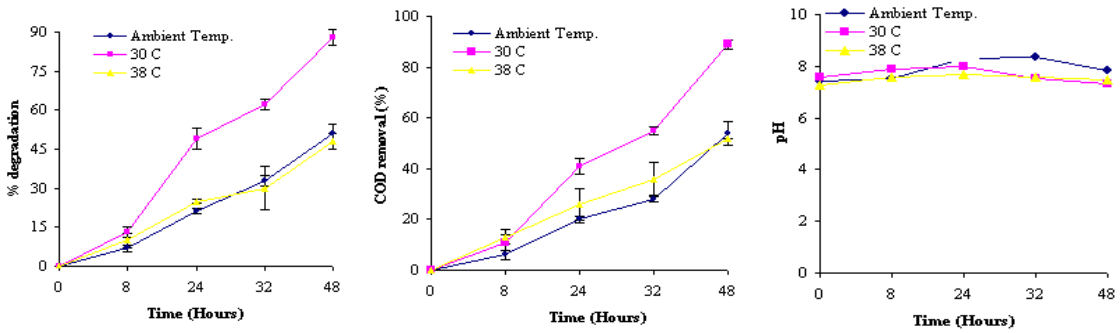

Fig-3: Effect of temperature on Cypermethrin (80 mg/L) degradation at 8 mg/L dissolved oxygen

It is interesting to note that during biodegradation, the COD removal was found to be proportional to the disappearance of cypermethrin. The corresponding decreased in COD values further provided an evidence of cypermethrin removal from the system. These results are in accordance to the previous findings reported by *Berchtold et al.*[36], who noticed the same correlation between COD removal and biodegradation of 2,4-DAT and 2,4 and 2,6 diamino toluene degradation by acclimated bacteria[37].

During the experiment, it was observed that the IES-*Ps*-1 retained their biodegradation capability at a wide range of pH (pH 7.3 – pH 8.8), therefore the alkaline pH which was achieved during treatment need no further adjustment. Several research studies reported similar results of pH variation without affecting the growth and biodegradation performance in the reactor[38-39]. Moreover, according to literature the tolerable limits for pH in the activated sludge aeration tank ranged between pH 6.0 to 9.0 and even the influent pH values outside this range are of little or no practical significance[40].

The present research findings described that this may be the first instance in which high concentration of cypermethrin degradation was achieved in short retention time of 48 hours. Earlier, *Maloney et al.*,[19], reported the transformation of permethrin (50mg/L) by pure culture of *Pseudomonas fluorescence* in the presence of tween 80 under aerobic conditions with a half-life of less than 5 days. *Grant et al.*,[41], reported that technical grade cypermethrin can be reduced from 60mg/L to 6mg/L by *Pseudomonas* sp. in 20 days.

From the research study, it can be concluded that biodegradation performance are highly dependent on cypermethrin concentration. However, optimizing treatment conditions in activated sludge process can effectively reduce inhibition at higher concentration. Moreover, during treatment optimal residence time need to be assessed while taking into account the cypermethrin concentration but it appeared that 2 days would be a convenient time to reach satisfactory biodegradation at low concentration of cypermethrin (< 20 mg/L) in the presence of acclimated IES-*Ps*-1 culture. These findings suggest that activated sludge process using IES-*Ps*-1 culture would be a feasible option for the treatment of pesticide wastes.

4. ACKNOWLEDGMENTS

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