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Pesticide Contaminated Wastewater using Soil Microorganisms in Bioreactor

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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 needs 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, 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 5 to 50 mg/L gradually decreased the removal efficiency. However, under continuous agitation, complete degradation of cypermethrin (10 mg/L) occurred within a period of 24 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, microbial degradation, environmental conditions, reactor.

INTRODUCTION

Synthetic pyrethroids (SPs) are the chemical analogs of pyrethrin, which are compounds that are present in the flowers of Chrysanthemum cineraria folium. Pyrethrin have been recognized as active insecticide compounds; however, due to their rapid degradation in the environment, they have never been used for plant protection on a large scale in agriculture (Laskowski, 2002; Palmquist et al., 2012). Compared to natural pyrethrin, synthetic pyrethroids

are more stable in direct sunlight and are significantly more effective against a wide range of insects. These properties made them much more suitable for use in agriculture (Laskowski, 2002). Beginning in 2000 when the use of organophosphorus pesticides decreased, the market for pyrethroid pesticides increased significantly. Nowadays, pyrethroids contribute more than 25% of the world's total pesticide market (Laffin et al., 2010; Pérez et al., 2010; Chen et al., 2011a).

Pyrethroids are insecticides that have a high biological activity and are used all over the world to control pest insects in agriculture, public and commercial buildings, animal facilities, greenhouses, and veterinary facilities (Katsuda, 1999). Pyrethroids are also the most common active ingredients in commercially available insect sprays and are the domain pesticide for malaria control. The insecticidal potency of pyrethroids is connected with the induction of a toxic effect in the cells of the nervous system of insects (Burr and Ray, 2004). By permitting a flux of sodium ions, pyrethroids alter the activity of the sodium channels that are responsible for the signal transmissions of nerve impulses. When pyrethroids bind to target channel proteins, they disrupt the proper function of the nervous cells thus leading to paralysis and the eventual death of insects (Burr and Ray, 2004; Davies et al., 2007; Hintzen et al., 2009).pyrethroids are considered to be safer than other insecticides, the common and extensive use of these compounds in a wide variety of fields has resulted in widespread contamination of the environment that is of ecological concern. The results of many studies have revealed that SPs may negatively affect non-target organisms such as fish and aquatic insects (Wendt-Rasch et al., 2003; Antwi and Reddy, 2015), beetles (Desneux et al., 2007), bees (Decourtye et al., 2005), parasitic wasps (Longley and Jepson, 1996) and microorganisms (Widenfalk et al., 2004 and Das et al., 2016). It is thought that some pyrethroids may be responsible for disruptions of the endocrine system, suppression of the immune system, reproductive damage and increased chances of cancer in humans (ATSDR (Agency for Toxic Substances and Disease Registry), 2003; Zhang et al., 2010). According to World Health Organization study (WHO,2004), worldwide three million people suffered from pesticide poisonings with about 250,000 deaths per year. To reduce the environmental and public health risks associated with pyrethroid use, it is necessary to develop rapid and effective methods to remove or minimize the concentrations of insecticides in the environment. Among the variety of methods that are used for the remediation of contaminated environments, the biological approach, which is based on the catabolic activity of pesticide-

degrading bacteria, seems to be the most promising and effective strategy (Chen et al., 2012c; Zhao et al., 2013; Cyco´n et al., 2014; Akbar et al., 2015a).

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 (Zhang, and Bennet,2005). 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 (Chauhan et al.,2008) and biotic factors such as inoculum density (Chowdhury et al., 2008). 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.

EXPERIMENTAL

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^{o}C, 15 psi for 30 minutes) and was used for growth and biodegradation studies.

The bacterial culture *pseudomonas* capable of degrading malathion and cypermethrin were isolated by (Hashmi ,2001, Shanmugananthan and mullai 2011). from agricultural soil using enrichment technique and was used in present study. Cypermethrin degrading culture was obtained through acclimating the *pseudomonas* strain with gradual increased concentration of cypermethrin from 10 to 80 mg/L in nutrient medium. Adapted *pseudomonas* was stored at 4 ℃

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 pseudomonas

Characterizations of *pseudomonas* was performed using morphological, cultural and biochemical tests according to the methods described by (Colins and Lyne,1985) up to the stage of genus. Growth of *pseudomonas* in bioreactor was determined by viable cell enumeration immediately after inoculation and at 24, 48, 72, 96 h later using (Miles and Misra technique, 1938)

Cypermethrin degradation studies using bioreactor

The compact bench scale bioreactor 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 *pseudomonas* for cypermethrin (50 mg/L) degradation was evaluated. Approximately 8.5 litres wastewater sample, inoculated with 350 ml culture and an appropriate quantity of cypermethrin was transferred into the bioreactor. 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.

Analytical procedure

The sample from bioreactor was withdrawn at timed intervals of 8, 24, 32, 48 hours and analysed for pH, temperature, dissolved oxygen and COD as per standard procedure laid down in APHA ,1998

Extraction of Cypermethrin for HPLC analysis

Samples were collected from bioreactor 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.

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.

RESULTS AND DISCUSSION

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,1986. 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. (Palleroni, and Genus,1917; Maloney et al.,1988; Ramos et al.,1995; Zhang et al.,2005; Chauhan et al.,2008)

Bacterial growth in the bioreactor

The results as shown in Figure 1, clearly indicate that cypermethrin had pronounced effect in promoting better growth of *Pseudomonas*. 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 20 mg/L cypermethrin was $12\pm1.73x10^7$ CFU/ml and with 50 mg/L, it was $17\pm2.65x10^7$ CFU/ml respectively. However, the

generation time at these concentrations (20 and 50mg/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). It was further noted that the growth at 30mg/L cypermethrin dose significantly increased after 48 hours' incubation. But the growth at 50mg/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 favourable environmental conditions in bioreactor which allow the cells to survive till 96 hours in contrast, the population density in control experiment (no

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

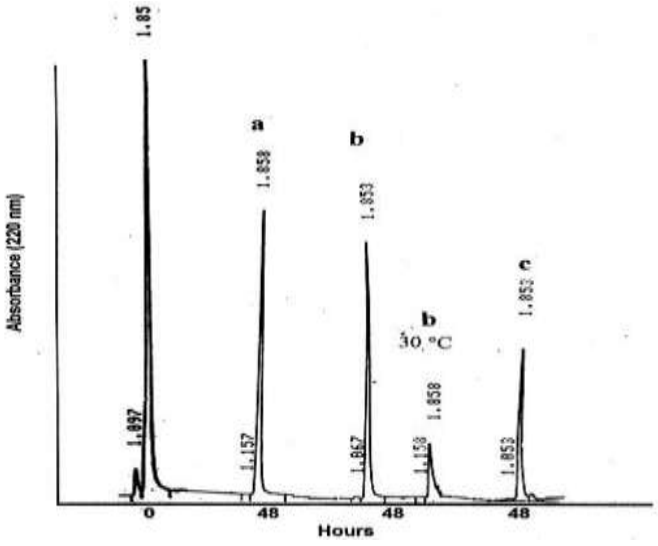

Fig-2: HPLC chromatograms showing comparative effect of Cypermethrin (50mg/L) degradation. a: 4 mg/L DO; b: 6 mg/L DO and c: 8mg/L DO at 30 C;

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 bioreactor, these results suggest that *Pseudomonas* 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 Chowdhury etal.,2008; Gray 1989.

Cypermethrin degradation in bioreactor

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 Gray (1989), at ambient temperature (18-25^{o}C) and 38 + 1C using mechanical aeration (6-8 mg/L dissolved oxygen), the degradation ability of *Pseudomonas* significantly decreased with increased concentration (50 mg/L) and the removal rate was only 48% - 51%. But under ambient temperature (18-25^{o}C) using 5 mg/l concentration, a complete degradation of cypermethrin occurred after 48 hours of aerobic treatment. However, at other dosages (20, 50 and 80 mg/L), it was 82%, 50% and 17% respectively (data not shown). In contrast, at optimum temperature (28-30^{o}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^{o}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 6 mg/L DO use mechanical aeration at the temperature range of 28-30^{o}C proved to be stimulatory and sufficient for effective biodegradation (Figure 2). Zacharias et al.,1995, 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 (28-30ºC temperature, 8-9 mg/L DO, mechanical aeration) not maintained in bioreactor, 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 (PCBS) and pentachlorophenol's (PCP), were biodegraded when the right microorganisms and environmental conditions were present in the system Strands et al (1998); Vogel et al (1987); Boyle et al (1992). 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. 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.(1995) who noticed the same correlation between COD removal and biodegradation of 2,4-DAT and 2,4 and 2,6 diaminotoluene degradation by acclimated bacteria.

During the experiment, it was observed that the *Pseudomonas* 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 Pesce, and Wunderlin, (1997); Rael,and Frankenberger(1996); Mayo,and Noike,(1996). 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 Hanel (1988).

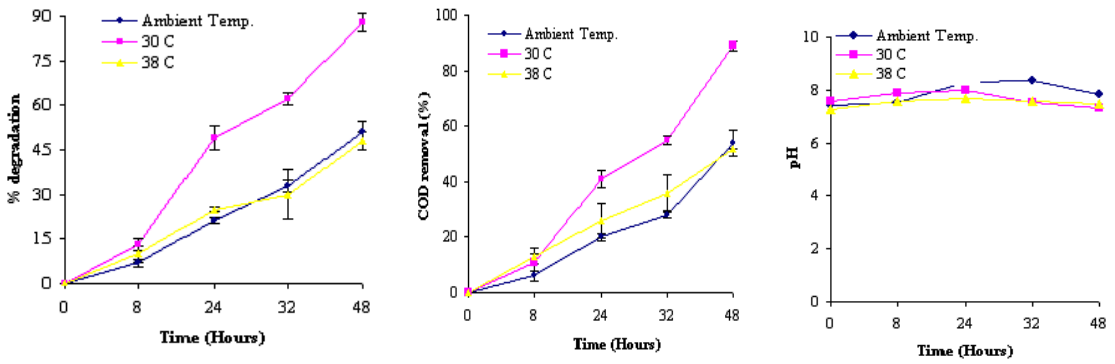

Fig-3:

Effect of temperature, PH, COD removal on Cypermethrin (50 mg/L) degradation at 8 mg/L dissolved oxygen

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. (1988), reported the transformation of permethrin (50 mg/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. (2003), 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 is 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 *Pseudomonas* culture. These findings suggest that activated sludge process using *Pseudomonas* culture would be a feasible option for the treatment of pesticide wastes.

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