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Treatment of Toxic Organics in Industrial Wastewater using Activated Sludge Process

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ABSTRACT: In Pakistan besides pesticide contamination from agricultural field, the manufacturing industries are also contributing relatively high quantities of toxic pesticides into the environment. These pesticides may be toxic, mutagenic or carginogenic and may be bioaccumulated or biomagnified by the biota. Because of persistent nature of pesticides, its removal from environmental system become difficult. The effective operation of wastewater treatment plant plays an important role in minimizing the release of toxic compounds into the environment. Research on the removal of toxic organics using the biological treatment plants is limited. In this study the bacterial isolate, IES-*PS*-1, was used to assess its potential for Cypermethrin degradation in activated sludge process. The experimental findings indicate that by maintaining the optimum operating conditions in the reactor, the removal efficiency significantly improved and > 88 % degradation observed at 80 mg/L dose. However, the complete removal of Cypermethrin, determined by High Performance Liquid Chromatography (HPLC), occurred at 20mg/L during 48 hour treatment. In addition, a considerable reduction in the sludge volume and chemical oxygen demand (COD) was also observed, producing an acceptable effluent quality with stable residual solids. These findings would be valuable in operating the wastewater treatment system for toxic waste removal.

Key words: Wastewater, Toxic organics, Bacterial isolate, Degradation, Activated sludge

INTRODUCTION

Cypermethrin, a pyrethroid insecticide is mainly used in Pakistan to increase cotton crop production. Being highly insoluble and toxic to aquatic organisms (Stepheson, 1982; Sapiets *et al*., 1984; Kollman & Segawa, 1995), treatment of such compounds generated during manufacturing needs especial attention. Various conventional physical/chemical methods such as volatilization, evaporation., photooxidation, absorption and hydrolysis etc. used for the decontamination of toxic wastes (Park *et al*.,1990). However, the disposal of volatile and other pollutants of significant toxicity are not preferred by these techniques. Moreover, these methods are not efficient and very expensive to employ (Morgan & Watkinson, 1989). Biological means i.e. microbial metabolism provide an excellent alternative to this problem. This is the only means to completely mineralize many toxic compounds (Atlas & Pramer, 1990).

Recently, bioremediation 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 (Young-Gyun *et al*., 2000). Moreover, in most cases, it has been found to be the most cost-effective and environmentally friendly treatment method and attempted in many countries of the world (Frisbie & Nies, 1997; Spain, 1997; Laha & Petrova, 1998). A number of bioremediation field applications have been reported that restores the polluted land, air and water (Grady, 1986; Ritmann, 1988). Research studies have revealed that microbial species isolated from soil, belonging to genus *Pseudomonas, Alcaligenes, Nocardia, Flavobacterium, Arthrobacter, and Corynebacterium* have been shown to degrade organic compounds (Smith & Adkins, 1996; Lee *et al*., 1998; Omar, 1999; Ramanathan & Lalithakumari, 1999; Karpouzas *et al*., 2000b; Martin *et al*., 2000; Giraud, 2001). Bioaugmented activated sludge system was found to be very effective in the treatment of wastewater containing high concentration of toxic organic compounds but the problem which is being faced by the environmental engineers is the difficulty in predicting the performance of such system with respect to the high load of individual organic

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compounds specifically the hazardous compounds. According to the 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 (Comeau *et al*., 1993) and biotic factors such as inoculum density (Ramadan *et al*., 1990). The present study investigated the potential of bacterial isolate for Cypermethrin degradation and provided a reliable prediction of the range of conditions in which pesticide-degrading bacteria may be active. Unfortunately very little information is available concerning the effect of varying influent concentration on the rate of biodegradation when toxic compound like Cypermethrin is present in the treatment system.

MATERIALS & METHODS

The pesticide used in this study belongs to the class pyrethroid and is commercially available as Cypermethrin.The physical and chemical characteristics of Cypermethrin pesticide are listed in Table 1. Due to low water solubility, stock aqueous solution of Cypermethrin (1mg/mL) was prepared in sterile HPLC grade methanol (Merck).

A nutrient broth 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.

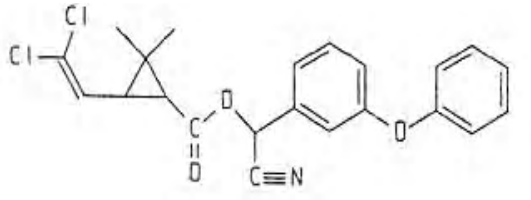
The bacterial culture (IES-*Ps*-1) capable of degrading malathion was isolated by Hashmi (2001) from agricultural soil using enrichment technique and was used in the present study. Cypermethrin degrading culture was obtained by acclimatization of IES-*Ps*-1 strain in a gradually increased concentration of Cypermethrin from 10 to 100 mg/L. Adapted IES-*Ps*-1 was stored at 4°C on nutrient agar slopes containing 0.1 mg/L Cypermethrin and subcultures after every three months.

When a new batch of test was performed with different doses of Cypermethrin, the stock culture was first subculture into 10 ml nutrient broth, aerobically grown and subsequently utilized for characterization, growth and biodegradation studies. Characterization of IES-*Ps*-1 was performed using morphological, cultural and biochemical tests using methods described by Colins & Lyne (1985) up to the stage of the genus. Whereas for bacterial growth study, Miles & Misra technique (1938) was used.

The compact bench scale biosimulator (Model MF-114) as shown in Fig. 1, 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.

Table 1.Physical and chemical characteristics of Cypermethrin

Chemical family Pyrethroid
Chemical name [(R,S)-α-cyano-3-phenonybenzyl (1RS)-cis, trans-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropane-carboxylate]
Chemical formula



Properties	Value
Molecular formula	$C_{22}H_{19}O_3NCl_2$
Molecular Weight	416.3
Appearance	Pure isomers are colorless crystals. Mixed isomers are viscous semisolid or viscous, yellow liquid
Melting point	60-80°C
Water solubility (at 20°C)	0.01 mg/l
Solubility in other solvents	Melthanol, acetone, xylene
Vapor Pressure (at 20°C)	1.3×10^{-9} mm Hg
Partition Coefficient	6.6020
Adsorption Coefficient	100,000
Octanol-water Coefficient	3.98×10^{6}
Hydrolysis half life (at environ expected temperature & pH values)	> 50 days
Field dissipation half life	4-12 days
Aerobic half life	6-20 days
Anaerobic half life	< 14 days

(http://www.cdpr.ca.gov/docs/empm/pubs/atememo/cyperm./pdf and WHO, 1989)

The optimum conditions for biodegradation of Cypermethrin (80 mg/L) by IES-*Ps*-1 strain were 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 (DO) concentration was achieved by diffused aeration using pressure pump and mechanical aeration regulated through continuous agitation of sample. The sample from biosimulator was withdrawn immediately after mixing and at time intervals of 8, 24, 32, 48 hours and analyzed for pH, temperature, dissolved oxygen and COD as per standard procedure laid down in APHA (1998).

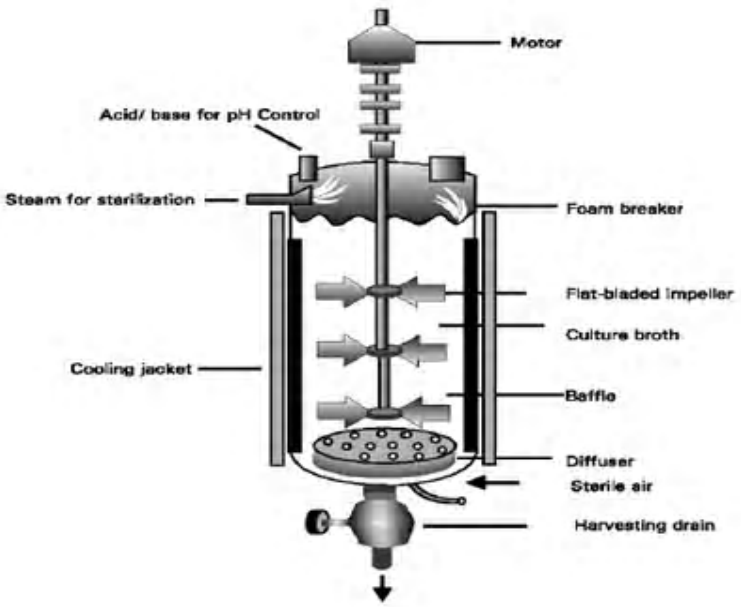


Fig. 1. General layout of a biosimulator (activated sludge treatment system)

For HPLC analysis, samples were collected from biosimulator as per schedule and were extracted two times with n-hexane reagent (75 ml and 50 ml) by vigorous shaking in a separatory funnel for 15-20 minutes. The separated hexane layer was evaporated to dryness at 70 °C using a 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 m filter membrane, an aliquot of 10 µL, was used to determine the concentration of Cypermethrin.

HPLC analysis was performed by isocratic elution with a flow rate of 2.0 ml/min. The mobile phase consisted of methanol (Merck HPLC grade) was filtered through a 0.2 m millipore filter before use and degassed in an ultrasonic bath. 10 µl prepared solutions of samples were injected into the column and quantification was measured at 220 nm. The chromatographic run time was 10 minutes. Each sample was injected 3 times and the mean was calculated.

To characterize the settling properties of solids in reactor, the sludge volume index (SVI) was calculated according to the procedure laid down in the standard method (APHA, 1998) .

RESULTS & DISCUSSION

To determine the performance evaluation of biosimulator for Cypermethrin degradation, COD and HPLC analysis were carried out. The overall data described earlier (Jilani & Khan, 2006, 2008, 2010) and presented in Table 2, explain lower degradation at high Cypermethrin concentration and a good agreement between COD removal and Cypermethrin degradation rates analyzed by HPLC. These results are in accordance with previous findings reported by Berchtold *et al*., (1995), who noticed the same correlation between the COD removal and biodegradation of 2,4-DAT and 2,4 and 2,6 diamino toluene degradation by acclimated bacteria (Pesce & Wunderlin, 1997). Similar correlations were also observed by Ramanathan & Lalithakumari (1999) during biodegradation of hazardous chemicals.

During wastewater treatment, it was observed that even at high organic load (80 mg/L), the biosimulator function satisfactorily when operated under controlled temperature and dissolved oxygen with retention time of 48 hours (Table 3). These findings are in agreement with earlier treatment work conducted by Toprak (1995) who found that the COD removal during treatment mainly depends on temperature and influent COD concentration. Moreover, the study findings were also supported by Schlegel (1969) and Palleroni (1986), who reported the same optimum temperatures (28-30°C) for the growth of *Pseudomonas* in activated sludge process.

From these results it can be concluded that IES-*Ps*-1 can degrade relatively high concentration of toxic organic pollutants, if provided the right environmental conditions in biosimulator. Earlier works on treatment demonstrated the removal of several organic toxicants by activated and trickling filter processes (Hannah *et al*., 1988), but activated sludge process was found to

be quite efficient in decreasing the concentration of many priority pollutants and other xenobiotics to concentration below detection limits (Grady, 1986 & 1990).

In addition to above findings, during the experiment it was observed that water turbidity due to the presence of Cypermethrin at 80mg/L was more than with 40mg/L dose. This may probably decreased the oxygen transfer efficiency of aerators in biosimulator and therefore lower the degradation rate at high concentration. The study assumption was also supported by literature where it is reported that treatment of hydrophobic compounds and other toxic chemicals in aqueous system using specialized microorganism would only be possible if compounds in the reactor are completely mixed by dispersion mechanism (Schonoor, 1992). Therefore the mechanical aeration used during the treatment of Cypermethrin may provided the dispersion of Cypermethrin in wastewater as well as maintained the favorable environmental conditions for biodegradation. Similarly it is reported that several persistent organic toxic pollutants in activated sludge process were biodegraded following the dispersion mechanism (Hannah, 1988).

It is well known that the detoxification mechanism for hazardous organic compounds vary widely depending upon the compound, the microbial species involved, and the environmental conditions present in engineering systems. Therefore, in this study, the optimum conditions for Cypermethrin degradation in biosimulator was evaluated from the results of the experiments conducted at different temperature, dissolved oxygen and using different concentrations of Cypermethrin. These results have been explained earlier, however the optimum conditions for Cypermethrin degradation by IES-*Ps*-1 in activated sludge are reported in Table 4.

During experimental study, it was observed that due to low water solubility of Cypermethrin compound, at ambient temperature (18-25°C) and 38°C using mechanical aeration, the degradation by IES-*Ps*-1 at 80 mg/L dose was markedly lower. However, by maintaining the optimum operating conditions (pH, temperature and dissolved oxygen) as shown in Table 5, the biodegradation efficiency significantly improved and >88 % degradation observed at 80 mg/L dose.After evaluating the optimum conditions for Cypermethrin degradation, it was felt appropriate to determine that concentration of Cypermethrin which can be removed completely from wastewater. Aim of this treatment was to produce Cypermethrin free effluent because the presence of even low concentration of Cypermethrin is extremely toxic to aquatic organisms (WHO, 1989).

During earlier experiments of this study, it was observed that increased concentration of Cypermethrin from 40mg/L to 125mg/L had a significant adverse effect on the rate of degradation (Jilani & Khan, 2006). Even after 72 hours of aerobic treatment, its complete removal from wastewater was not achieved (data not shown). Therefore to obtain complete elimination of Cypermethrin from wastewater, 20mg/L concentration was used. Results are reported in Table 4. It was noted during the experiment that after 48 hours of aerobic treatment, the isolated strain (IES-*Ps*-1) was capable to degrade Cypermethrin completely and therefore no peak was detected after 48 hours of aerobic treatment. In contrast, over this time period, the cell could degrade only 81% of Cypermethrin when the initial added concentration was 40mg/L. These degradation rates were confirmed by HPLC and COD analysis. Since COD is a parameter of organic load assessment, its corresponding decreased also indicate the removal of Cypermethrin from wastewater.

It is interesting to note that during biodegradation of Cypermethrin none of the metabolites and intermediate of Cypermethrin were detected by HPLC analysis in the samples drawn from biosimulator. These results described that the concentration of metabolites formed during the degradation may be very low and which may be beyond the detection limits of HPLC analysis calibrated for Cypermethrin detection. Although a direct proof of Cypermethrin metabolites and intermediate was not obtained, but the absence of metabolites together with COD removal suggest that treatment is at least 97% effective in removing Cypermethrin from wastewater. These findings were also supported by literature where it is reported that under aerobic conditions, Cypermethrin metabolites may undergo further breakdown to CO_2 (Kaufman *et al*., 1981; Bacci *et al*., 1987). Moreover, the persistence of the metabolites is reported unknown [36] (Walker & Keith, 1992).

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. Although Maloney *et al*., (1988), 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*., (2003), reported that technical grade Cypermethrin can be reduced from 60mg/L to 6mg/L by *Pseudomonas* sp. in 20 days.

Overall findings, indicate that biodegradation efficiency are highly dependent on Cypermethrin concentration but by optimizing the treatment conditions in activated sludge, IES-*Ps*-1 can effectively

Table 2. Comparative performance evaluation of Cypermethrin degradation at 48 hours using biosimulator (activated sludge system)

Cypermethrin		COD Values		HPLC Data	
Conc.	pH	Conc.(mg/l)	% removal	Conc.(mg/l)	% degradation
40	8.30	5087	82	34	81
80	7.81	5267	54	44	51
125	7.83	4433	24	26	18

Table 3. Comparative performance evaluation of Cypermethrin (80mg/L) degradation at different temperature and dissolved oxygen after 48 hours using biosimulator

Dissolved Oxygen (mg/L) & Temperature (^{o}C)		COD Values		HPLC Data	
	pH	Conc. (mg/L)	% removal	Conc. (mg/L) %	Degradation
-6 mg/L DO at ambient temp. (18-25 ^{o}C)	7.87	5000	31	50	32
8-9 mg/L DO at ambient temp. (18-25 ^{o}C)	7.81	5267	54	44	51
8-9 mg/L at 30 °C temp89	7.33	850	89	9	88
8-9 mg/L DO at ambient temp. (38-40 ^{o}C)	7.50	4333	52	39	48
11-12 mg/L at ambient temp. (18-25 ^{o}C)	8.20	1300	83	17	78

*Results based on mean of three replicates

Table 4. Optimum conditions evaluated for Cypermethrin degradation in biosimulator

Essential factors	Optimum conditions
Bacteria	Cypermethrin adapted soil isolate (IES-*Ps*-1)
Aeration mechanism	Mechanical aeration (250 rpm)
pH	Ranged from 7.33 to 8.2
Temperature	28-30°C
Oxygen content	8-9mg/L
Cypermethrin Conc.	20 mg/L (allowable limits) 80mg/L (maximum allowable limits)
Retention time	48 hours
Agitation rate	Continuous at 240 rpm
Degradation	Complete degradation at 20mg/L > 85% degradation at 80mg/L

Table 5. Performance evaluation of IES-*Ps*-1 for Cypermethrin (20mg/L) degradation in biosimulator

Time(Hours)		COD Values		HPLC data	
0	pH	Conc.(mg/l)	% removal	Conc.(mg/l)	% degradation
24	7.9	2300	-	23	-
48	8.6	440	81	4.77	79
	8.6	80	97	No peak detected	

*Data indicate average values of three experiments

Table 6. Sludge volume index of wastewater samples containing Cypermethrin after 48 hours treatment

		Wastewater with IES-*Ps*-1 and Cypermethrin		
Experiment Number	Wastewater with IES-*Ps*-1 culture	40 mg/L (28-30^{o}C)	80 mg/L (28-30^{o}C)	80 mg/L (Amb. Temp)
1	94	50	36	12
2	98	63	38	14
3	102	74	46	16
Mean	98	62	40	14

reduce the higher concentration of Cypermethrin. These findings are also supported by Strands (1998), who found the lower degradation due to less aqueous solubility of chemical compounds and the presence of inappropriate environmental conditions in the reactor.It is further reported that in spite of high resistant nature of pentachlorobiphenyls ($PCB_{S)}$ and pentachlorophenols (PCP), these were biodegraded when the right microorganisms and environmental conditions were present in the system (Vogel *et al*., 1987; Boyle *et al*., 1992; Mc Allister *et al*., 1996). 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.

The activated sludge process, comprising a biological reactor and a secondary settler, is widely used as a secondary treatment for both municipal and industrial waste water treatment (Liu, 2003). For efficient treatment work both the aeration basin and secondary clarifier must function satisfactorily. Very few research studies looked at the interaction between these two units (Dupont & Henze, 1992; Cote *et al*., 1995). In the present study both biological oxidation and solid separation were determined to evaluate the overall performance efficiency of biosimulator used as an activated sludge process. The results are shown in Table 6.

Generally, SVI for a conventional activated sludge process is reported between 40<SVI<150 (Cakici & Bayramoglu, 1995). A high SVI (>150 ml/g) indicates bulking conditions, whereas an SVI below 70 ml/g indicates the predominance of pin (small) flocs (U.S.EPA, 1987). In these flocs, the filamentous bacteria are absent or occur in low numbers. This results in small flocs that do not settle well and the secondary effluent remains turbid despite the low SVI (Bitton, 1994).

As can be seen from Table 2, the SVI values at low concentration of Cypermethrin (40 mg/L) are found within an acceptable range ensuring reliable stability and good plant operation. In contrast, at 30°C using 80mg/L Cypermethrin, the SVI mean values were found to be low (40 ml/g). This may be due to the presence of residual amounts of Cypermethrin in the reactor that may cause a low sludge settleability. It is worth mentioning here that although the SVI values were found to be low at this high concentration but the degradation efficiency were satisfactory. However, in the absence of Cypermethrin (control experiments), a better oxygen transfer efficiency of aerators in the reactor favors floc formation and good sludge settleability. In contrast, the experiments with 80 mg/L Cypermethrin dose at ambient temperature, the SVI values significantly decreased to 14 ml/g, which was markedly low, compared to SVI values obtained at optimum operating conditions (40 ml/g). This shows that the residual Cypermethrin concentration and bacterial biomass in the biosimulator after treatment may have a significant adverse effect on sludge settleability. These findings are also supported by literature where it is reported that a high F/M ratio is conducive to poor sludge settleability. Moreover, the presence in high number as dispersed growth is associated with the failure of flocforming bacteria. This phenomenon occurs as a result of high BOD loading and oxygen limitation. Toxicity may also cause a dispersed growth of activated sludge bacteria. As it is reported that sudden changes in physical parameters like temperature, pH, absence of nutrient and presence of toxicants can also cause a partial deflocculation of activated sludge (Chudoba, 1989). In the present study as the experiments were performed under controlled conditions, the only reasons for low sludge settleability may be the presence of residual Cypermethrin concentration in wastewater. These results were further supported by Nowak *et al*., (1986), who reported that higher organic loading in activated sludge leads to the loss of their selective advantages over floc forming bacteria and thus causing poor sludge settling.

During the experimental study it was noted that due to low water solubility of Cypermethrin (0.01 mg/L), at high concentration (80mg/L), the wastewater turn turbid (milky white) and this results in poor sludge settling in the reactor. However, even at this high concentration the settling of solids reasonably improved when maintaining the optimum operating conditions in the reactor. In contrast, at 40 mg/L Cypermethrin concentration, the SVI value and settling was comparatively better. The overall findings as shown in Table 2, suggesting that it would be possible to enhance the overall performance efficiency of an activated sludge by further treatment of effluent using the physicochemical methods, including the action of flocculating agents (e.g. polyelectrolytes, iron and alum salts) or activated carbon treatment (Bitton, 1994).

CONCLUSION

The results finding indicates that the use of potential microorganism in the treatment system can successfully overcome many of the disadvantages associated with the conventional batch culture bioreactor used for the degradation of inhibitory compounds. Such study would be a valuable addition in the improvement in design and operation of a biomechanical treatment system used for degradation of hydrophobic compounds like Cypermethrin which

are resistant otherwise to biological degradation in conventional activated sludge system.

Following conclusions can be drawn from the results of present study:

- Malathion degrading bacterial isolate, IES-*Ps*-1, can be used for the degradation of pesticide wastes, as IES-*Ps*-1 showed potential to grow in the presence of Cypermethrin.
- Removal of organic load in terms of COD was found to be proportional to the disappearance of Cypermethrin analyzed by HPLC. Further confirming the degradation of Cyperrnethrin by IES-*Ps*-1.
- Because of the low aqueous solubility of Cypermethrin, mechanical aeration in biosimulator proved to be very effective in reducing the concentration of Cypermethrin. As it was observed that mechanical aeration in the reactor not only provided the maximum dispersion of Cypermethrin in wastewater but also maintained the sufficient dissolved oxygen required for the growth of IES-*Ps*-1.
- The overall performance of biosimulator in terms of settling of solids and the complete removal of Cypermethrin (20 mg/L) would only be possible if an appropriate organism (IES-*Ps*-1) and optimum conditions like pH, temperature, dissolved oxygen, mechanical aeration be maintained in biosimulator.

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