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Pesticide removal in bioaugmented activated sludge using principal component analysis

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Abstract

To meet environmental regulations, it is imperative that industries in Pakistan must use the wastewater treatment system. In this study, the multivariate statistical process control such as Principal Component Analysis (PCA) was used to distinguish the variables that likely control the bioaugmented activated sludge treatment process. During experimental investigation, >88% removal of cypermethrin occurred in short retention time of 48 hours at 30 C temperature and 8 mg/L dissolved oxygen (DO). No significant effect of pH change was noticed and the pH remains between 7.3-8.8. The experimental data when subjected to PCA, indicate that total cypermethrin concentration, organic load as COD and retention time were highly correlated and emerged as variables controlling the first component, whereas pH, DO and temperature governed the second component. The third component repeated the trend exhibited by the first two components. These findings would effectively be applied for the treatment of toxic organics.

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Introduction

In recent years multivariate statistical process control such as principal component analysis (PCA) has been used to monitor the chemical and biological treatment processes (MacGregor and Kourti, 1995). PCA reduces the number of variables in a data set by finding linear combinations of those variables that explain most of the variability and often generates components that have valuable biological meanings (Lukman *et al.*, 1999). Analysis via this technique produces easily interpretable results, and this method has been successfully applied to many industrial treatment processes (MacGregor and Kourti, 1995; Wise and Gallagher, 1996). Moreover, PCA has also been applied to continuous wastewater treatment plants (Rosen and Olsson, 1998; Rosen and Lennox, 2001).

In Pakistan, insecticide, particularly cypermethrin is mainly used to increase cotton crop production. Cypermethrin is classified by WHO as moderately hazardous. It actually acts on the nervous system and is toxic to bees, other beneficial insects, earthworms, fish and shrimps (Stepheson, 1982). Because of its low water solubility, it becomes difficult to remove such compound from the environmental system by conventional means. At present, besides pesticides contamination from agricultural field, the agricultural industries are also contributing relatively high quantities of toxic pesticides into the coastal environment, since most of them have either no treatment facilities or have grossly inadequate arrangement. To meet environmental regulations and to prevent water pollution, environmental friendly method of pesticide waste remediation is therefore needed.

Presently, the bioremediation technology has been found to be a suitable option for the treatment of polluted aquifers containing hazardous waste (Fragoeiro, 2005). It has gained considerable attention as it is ecologically sound, economical when compared with other technologies and has been attempted in many wastewater treatment plants (Grady, 1986; Jilani and Altaf, 2006; Finley *et al.*, 2010). 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-Greeier and Adkins, 1996; Lee *et al.*, 1998; Ramanathan and Lailithakumari, 1999; Karpouzas *et al.,* 2000; Giraud *et al.*, 2001). Generally, the biodegradation of organic pollutant and its rate 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; Hart, 1996; Dua, *et al.*, 2002; Singh and Ward, 2004) and biotic factors such as types of microorganisms and inoculum density (Ramadan *et al.*, 1990).

In this study, the experimental data obtained was subjected to multivariate statistical analysis such as Principal Component Analysis (PCA). The objective was to identify the main parameters that likely control the activated sludge used for the treatment of cypermethrin. The study findings would be valuable to scientists and engineers who are trying to develop methods that can be used for the treatment of toxic organic compounds which are resistant otherwise to conventional treatment.

Materials and methods

Chemicals

The pesticide used in this study belongs to the class pyrethroid and is commercially available as cypermethrin, chemical name (R,S)-α-cyano-3-phenonybenzyl (1RS)-cis, trans-3-(2,2-dichlorovinyl)-2,2- dimethylcyclopropane-carboxylate. Table 1 represents the physical and chemical characteristics of cypermethrin pesticide.

Bacterial Culture

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. The characterization of IES-*Ps*-1 strain was performed by morphological, cultural and biochemical tests using methods described by Colins and Lyne (1985) up to the stage of the genus. Whereas for bacterial growth study, the Miles and Misra technique (1938) was used.

Cypermethrin Degradation

The technical details of the compact bench scale biosimulator (NBS; New Brunswick Scientific Company), used for cypermethrin degradation is shown in Table 2. 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 the 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).

HPLC Analysis

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 μm filter membrane, an aliquot of 10 μL, was used for HPLC analysis.

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 μm 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.

Principal Component Analysis

The data obtained from experimental analysis was subjected to principal component analysis (PCA), which is a variance-oriented technique where the component score is directly derived by a linear transformation. The use of PCA permit an objective summarization of the variable in the data matrix by extracting a new set of variables called principal components. Generally the first three components account for a high proportion of total variance in the original data set. The first principal component is the combination of variables that accounts for the largest part of the variance in the sample. It is described as the linear combination, Y_1, of the original variables, of which the total variance is maximized for all vectors $a_{11} \ldots a_{1p}$.

$$Y_1 = a_{11}x_1 + a_{12}x_2 + a_{1p}x_p$$

The second component accounts for the next largest amount of variance and is uncorrelated with the first, etc. To decide how many components are needed to represent data, the percentage of total variance explained by each component is examined (Feoli, 1977; Nichols, 1977). Only components that account for variances greater than the variances of all

variables are included. Hence, the sum of the vectors that represent the variables (Eigenvalue) should be larger than 1.

In the present study, the bench scale bioreactor (activated sludge) performance efficiency in relation to cypermethrin degradation by Pseudomonas (IES-*Ps*-1) strain in the wastewater samples using different concentration of cypermethrin and a retention time of 48 hours was subjected to PCA. Influence of environmental parameters like pH, temperature and dissolved oxygen with respect to pesticide removal were also evaluated. The data set consist of 36 observations and 10 variables related to bioreactor performance (Table 3), including COD_{IN}–COD_{OUT}, COD_{OUT}/COD_{IN}, CYP_{IN}–CYP_{OUT}, CYP_{OUT}/CYP_{IN}, dissolved Oxygen, organic load, total cypermethrin concentration, temperature, pH and retention time. The relationships among and within variables were determined by PCA, using a software package MINTAB 11 for windows. It is important to mention here that many researchers have often been used PCA as a descriptive tool for the purpose of trend-seeking and to provide unique, objective and parsimonious representations that are predictable and meaningful (Victorio *et al.*,1996; Madoni *et al.*,1994).

Results and discussion

Characterization of Bacterial Culture

Bacterial characterization, based on the morphological, cultural and biochemical tests indicate that the IES-*Ps*-1 strain belongs to the genus *Pseudomonas* according to "Bergey's Manual of Systematic Bacteriology" (1994). Further, the experimental results of present study, as well as of other researchers, indicate that bacteria belonging to the genus *Pseudomonas* are gram-negative, rod-shaped, highly oxidative, aerobic and metabolically versatile and have been reported to degrade phenolic compounds (Huges and Cooper, 1996) and other aromatic substances (Christodoulatos *et al.*, 1997; Lee *et al.*, 1998; Ramanathan and Lailithakumari 1999; Martin *et al.*, 2000; Maria *et al.*, 2002).

Table 1. Physical and chemical characteristics of Cypermethrin (WHO,1989).

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

Cypermethrin Degradation

The overall data described elsewhere (Jilani & Altaf, 2006, 2008, 2010) and presented in Table 4, explain the lower degradation (18%) at high cypermethrin concentration and a good agreement between COD removal and cypermethrin degradation rates analyzed by HPLC. These findings are in accordance with previous work reported by Berchtold *et al.* (1995) and Ramanathan and Lalithakumari (1999) 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 and Wunderlin, 1997).

Table 2. Technical data of biosimulator.

General detail			
Biosimulator (NBS)		Model MF-114	
(1) DO-controller		Model DO-81 Control through mechanical air pump.	
(2) pH-controller		Model pH-22	
(3) Temperature		Control through thermostat	
Vessel capacity		14 Liters	
Agitation		upto 1000 rpm	
	Performance Evaluation Studies		
Parameters	Effect of Conc.	Effect of Temp.	Effect of DO
i. Total volumetric load (raw wastewater)	8.5 Liter	8.5 Liter	8.5 Liter
ii. Size of inoculum ($1.8x10^9$ bacteria/ml)	350 ml	350 ml	350 ml
iii. Cypermethrin Conc. (mg/L)	40 , 80 , 125 mg/L	80 mg/L	80 mg/L
iv. Working Temperature	Ambient Temp. (18°C - 25°C)	Amb.Temp.(18°-25°C), 30°C & 38°C	30°C
v. Agitation	250 rpm	250 rpm	250 rpm
vi. Dissolved Oxygen	8 mg/L	8 mg/L	6, 8 & 12 mg/L
vii. Sample Collection	Fixed Time intervals (0, 8, 24,32 & 48 h)	Fixed Time intervals (0, 8, 24,32 & 48 h)	FixedTime intervals (0, 8, 24,32 & 48 h)
viii. Total retention time (Hrs)	48	48	48

During wastewater treatment, it was observed that due to low water solubility of cypermethrin, 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 (temperature and dissolved oxygen) as shown in Table 4, the biodegradation efficiency significantly improved and >88 % degradation observed during the retention time of 48 hours. Similar results were also reported by Toprak (1995) who noticed the dependence of COD removal on temperature, influent COD concentration and retention time. Moreover, Schlegel (1969) and Palleroni (1986) recorded the same optimum temperatures (28-30°C) for the growth of *Pseudomonas* in activated sludge process as observed in present study.

Principal component analysis

Figure 2 is based on the principal components I, II and III, which explains 86% of the total variance (Table 5). The highest proportion of the total variance was extracted by first component (PC1), explaining 59.3% of the variance which is primarily a function of COD_{OUT}/COD_{IN}, CYP_{OUT}/CYP_{IN}, $COD_{IN}–COD_{OUT}$, $CYP_{IN}–CYP_{OUT}$, total cypermethrin concentration, organic load and retention time as indicated by Eigen vector coefficient (Table 5). The second PC (PC2) explained 15.4% of the variance and seemed to be governed by pH, dissolved oxygen and temperature (Table 5).

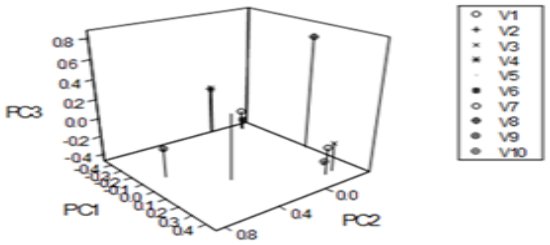

Fig. 1. Three dimensional principle components ordination of the biosimulator using 10 descriptive.
Where: $V1=COD_{IN}-COD_{OUT}$ $V2=COD_{OUT}/COD_{IN}$ $V3=CYP_{IN}-CYP_{OUT}$ $V4=Cyp_{OUT}/Cyp_{IN}$ V5=DO V6=Organic load V7=Total Cyper. Conc. V8 =Temp. V9=pH V10 = Retention time.

Table 3. Basic data of biosimulator used for principal component analyses.

S.No.	COD_{IN} - COD_{OUT} mg/L	COD_{OUT} / COD_{IN} mg/L	Cyp_{IN} - Cyp_{OUT} mg/L	Cyp_{OUT} / Cyp_{IN} mg/L	Dissolved Oxygen mg/L	Organic load gm/L	Cyper Conc. mg	Temp. °C	pH	Retention time (Hours)
				Data at different cypermethrin concentration						
1	1000	0.83	6	0.86	7.5	44	306	21.2	8.20	8
2	2734	0.56	20	0.52	7.8	29	187	22.4	8.50	24
3	3884	0.38	25	0.4	8.2	20	145	22.6	8.47	32
4	5087	0.18	34	0.2	8.5	9	68	22.8	8.30	48
5	600	0.94	6	0.93	7.0	78	680	22.4	7.53	8
6	1934	0.80	18	0.79	8.0	67	578	23.1	8.30	24
7	3100	0.68	28	0.67	8.0	57	493	23.0	8.37	32
8	5267	0.46	44	0.49	8.0	38	357	23.2	7.81	48
9	150	0.99	4	0.97	6.2	53	1190	23.0	7.60	8
10	1700	0.91	8	0.94	6.6	140	1156	23.3	7.80	24
11	2050	0.89	14	0.90	7.0	137	1105	23.2	7.77	32
12	800	0.74	32	0.78	6.8	117	952	23.1	7.83	48
				Data at different temperature						
1	600	0.94	6	0.93	7.0	78	680	23.0	7.53	8
2	1934	0.80	18	0.79	8.0	67	578	23.2	8.30	24
3	3100	0.68	28	0.67	8.0	57	493	23.1	8.37	32
4	5267	0.46	44	0.49	8.0	38	357	23.3	7.81	48
5	900	0.89	10	0.87	7.6	62	578	28.2	7.9	8
6	3334	0.59	38	0.51	7.8	41	340	28.4	8.0	24
7	4500	0.45	48	0.38	7.5	31	255	29.6	7.53	32
8	7234	0.11	69	0.12	7.6	7.9	77	30.2	7.33	48
9	1166	0.86	9	0.9	7.5	61	612	38.1	7.57	8
10	2166	0.74	21	0.74	7.8	52	510	38.4	7.67	24
11	3000	0.64	25	0.7	7.8	45	476	38.3	7.57	32
12	4333	0.48	39	0.52	7.8	34	357	38.2	7.5	48
				Data at different dissolved oxygen						
1	500	0.96	4	0.95	5.0	61	612	28.1	8.30	8
2	1167	0.85	8	0.89	5.5	55	561	28	8.40	24
3	1867	0.76	14	0.81	5.5	49	510	28.2	7.83	32
4	2500	0.67	24	0.68	5.5	44	434	28.3	7.87	48
5	900	0.89	10	0.87	7.6	62	578	28.6	7.9	8
6	3334	0.59	38	0.51	7.8	41	340	29.2	8.0	24
7	4500	0.45	48	0.38	7.5	31	255	29.4	7.53	32
8	7234	0.11	69	0.12	7.6	7.9	77	29.6	7.33	48
9	700	0.91	6	0.92	10.5	58	612	30.2	8.4	8
10	2200	0.71	24	0.69	10.8	45	459	29.5	8.4	24
11	4300	0.43	36	0.54	10.8	27	357	29.8	8.3	32
12	6200	0.17	61	0.22	10.8	11	145	29.4	8.2	48

Table 4. Comparative performance evaluation of IES-Ps-1 for Cypermethrin degradation after 48 hrs.

Parameters	pH	COD removal		Cypermethrin degradation	
		Conc.(mg/l)	% removal	Conc.(mg/l)	% degradation
Cyper. Conc. (mg/L)		Effect of Cypermethrin Concentration (mg/l)			
20	8.60	80	97	-	No Peak
40	8.30	1080	82	8.2	81
80	7.81	4500	54		
125	7.83	13767	24	42	51
				118	18
Temperature °C		Effect of Temperature (°C) at 80 mg/L dose			
Ambient Temp.(18-25)	7.80	4500	54	42	51
28-30	7.33	867	89	9.0	88
38-40	7.50	4000	52	42	48
Dissolve Oxygen (mg/L)		Effect of Dissolved Oxygen (mg/L) at 80 mg/L dose			
8-9 mg/L	7.81	4500	54	42	51
8-9mg/L(30°C)	7.33	867	89	9.0	88
11-12 mg/L	8.20	1300	83	17	78

*Data indicate average values of three experiments.

Table 5. Results of principal component analysis.

Principal Components	Eigenvalue %	Cumulative Variance	Ranked Eigenvector	Associated variables
I	59.34	59.34	-0.403	COD_{OUT}/COD_{IN}
			-0.401	Cyp_{OUT}/Cyp_{IN}
			0.395	COD_{IN}-COD_{OUT}
			0.383	CYP_{IN}-CYP_{OUT}
			-0.351	Total Cyp. Conc.
			-0.338	Organic load
			0.314	Retention time
			0.169	Dissolved Oxygen
			0.092	Temperature
			-0.043	pH
II	15.42	74.76	0.740	pH
			0.436	DO
			-0.318	Temperature
			-0.220	Total Cyp. Conc.
			-0.212	Organic load
			-0.183	Retention time
			-0.163	CYP_{IN}-CYP_{OUT}
			-0.086	COD_{IN}-COD_{OUT}
			0.015	COD_{OUT}/COD_{IN}
			0.006	CYP_{OUT}/CYP_{IN}
III	11.56	86.32	0.774	Temperature
			-0.373	Retention time
			-0.304	Organic load
			0.259	DO
			-0.204	Total Cyp. Conc.
			-0.124	COD_{IN}-COD_{OUT}
			-0.115	CYP_{IN}-CYP_{OUT}
			0.106	COD_{OUT}/COD_{IN}
			0.103	CYP_{OUT}/CYP_{IN}
			-0.103	pH

Note: Eigen values and eigen vector elements together with associated variables for the first three principal component.

The third PC (PC3) accounts for 11.6% of the variance and is mainly based on temperature and retention time (Table 5). The percentage of total variance explained by principal component of each of the 10 variables are shown in Figure 3. The PCA results indicate that the main factors governing the biosimulator performance are cypermethrin concentration, organic load (COD) and retention time. Other factors are operational parameters such as pH, temperature and DO. The statistical analysis confirmed that COD_{OUT}/COD_{IN}, CYP_{OUT}/CYP_{IN}, COD_{IN}–COD_{OUT}, CYP_{IN}–CYP_{OUT}, total cypermethrin concentration, organic load and retention time were highly correlated with each other and emerged as important variables controlling first component. pH, DO and temperature governed the second component where as the third component represents the temperature and retention time.

These findings explain that the high values of effluent COD are directly related to cypermethrin concentration. It is likely that complete removal of cypermethrin as high organic load in short retention time would only be possible, if the system operates at optimum pH, temperature and with extended aeration. In this study, the presence of high cypermethrin concentration in the reactor even after treatment was actually associated with reduce metabolic activity of microorganism (IES-*Ps*-1) at high organic load. However, at optimum temperature and dissolved oxygen, the biodegradation rates were greatly enhanced and >88% removal of cypermethrin was observed. Similarly, many researchers also reported the significant degradation of Naphthenic Acids at optimized environmental conditions (Mandelstam *et al.* 1968; Tanapat 2001; Paslawski 2008). In fact, it has been noted that the changes in these variables were found to be highly correlated with quantitative and qualitative changes in the plant operation. Quail *et al.* (1991) demonstrated that biodegradation can be greatly improved if treating the pollutants at optimum environmental conditions and a better designed and controlled bioreactor.

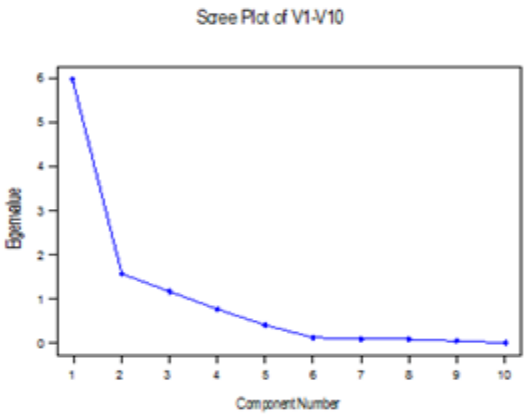

Fig. 2. Percentage of total variance explained by principal component of each of the 10 variable ordination.

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