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An Application of Principal Component Analysis to The Study of Activated Sludge Treatment System Performance Efficiency For the Degradation of Malathion

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Abstract

The biosimulator system (activated sludge system) have proved to be very effective in the treatment of wastewater containing high content of pesticide and Chemical Oxygen Demand (COD) in terms of organic load and the quality of effluent being obtained after treatment was acceptable for landscape irrigation and for growing plants of ornamental value for commercial use. The biosimulator can be operated without being an aesthetic nuisance at a considerably low Dissolved Oxygen (DO). The efficiency of the biosimulator at high organic load (pesticide inobulated) and the inoculation of strain of Pseudomonas capable of degrading malathion, could be used to minimize time for treating wastewater containing high content of hazardous pesticide.The principal component analysis exposed the groups of correlated variables and their importance in the data structure. Mal_{IN} -Mal_{OUT}, TMC, Mal_{OUT}/Mal_{IN} and COD_{OUT}/COD_{IN} were highly correlated with each other and emerged as the variables controlling the first component. DO, pH and retention time governed the second component. The third component of PCA essentially repeated the trend exhibited by the first two components. The presence of pesticides or their metabolites, emphasizes the need for conducting monitoring studies, in order to draw a national picture for overall assessment of the situation.

Introduction

In the recent years there has been increasing concern over the possible transformations of hazardous environmental chemicals such as pesticides. Pesticides are chemicals synthesized in Germany after the Second World War. These are organic substances, which are applied intentionally to terrestrial ecosystems. After accomplishing their intended effects, their residues, which are left in the ecosystem, must be regarded as undesirable environmental chemicals and are known to incur significant economic losses or threaten human life, his health and that of his domesticated animals. The presence of these or their metabolites may scientifically be vouched not only in the environment they are used, but in the entire ecosystem, in the subsoil, in the underwater reservoirs and in the food chain (Boon and Duiniker, 1985; Sheail, 1985). It is estimated that 31 to 48 billion pounds of the organophosphate insecticides are used each year in the United States (Saunders, 1991). Microbial degradation of pesticides is the only way by which the residue level may be brought down. Microorganisms are metabolically active and can degrade a variety of compounds found in the subsurface (Thomas *et al*., 1987). Through species belonging to the genera such as *Achromobacter*, *Alcaligenes*, *Arthrobacter*, *Bordetella*, *Flavobacterium*, *Pseudomonas* and *Xanthobacter* have been isolated from soils and shown to degrade 2,4-dichloroohenoxyacetic acid and MCPS (2-methyl-4-dichlorophenoxy-acetic acid) in liquid nutrient media (Smith and Mortensen, 1991; Greer *et al*., 1992; Ishaq *et al*., 1994). Under aerobic condition alpha, gamma and also more stable beta isomer of Hexachlorocyciohexane is readily metabolized by *Pseudomonas* species isolated from sugar cane rhizosphere (Sahu *et al*., 1990). However, at present the practical application of microorganisms for pesticide degradation is carried out only during waste purification by certain industries (Andreeva, 1985; Liberstein, 1984). Depending on their type and concentration, pesticides can have different effects on the growth of microorganisms. Application of mixed substrates also allows us to understand the mechanisms of microorganisms adaptation to real substrates (Schnoor, 1992; Golovleva and Golovlev, 1980). With increased awareness of toxicological properties, farmers and agricultural workers are now more cautious in the handling and use of various pesticidal chemicals (John, 1992). The World Health Organization (WHO) estimates that 500,000 pesticides poisoning cases occur annually in the World and that 1 percent are fatal (5000 death/year), (McLoughlin and Bellinger, 1993; Rosenstock *et al*., 1991). Organophosphate are toxic to the nervous systems by inhibiting the acetylcholine esterase (AchE) activity. Inhibition of AchE results in accumulation of free acetyl choline in nervous tissues (Michotte *et al*., 1989; Pillans *et al*., 1988; Zwiener and Ginsburg, 1988).

In Pakistan approximately 100 percent of cotton picking in fields is normally done by females. Based on the level of the inhibition of the body enzyme AchE, it was found that 74 percent of the female workers had blood AchE inhibition between 12.5-50 percent and 36 percent had inhibition from 50-87 percent, which is a dangerous level of exposure. Only 1 percent of female workers had satisfactory AchE blood level (SDPI, 1995).

Pesticides can cause cancer, liver disease, hypertension (Sandhu *et al*., 1985; Nriagu and Simmons, 1990) and are neurotoxic (Larsen *et al*., 1986). Barbeau *et al*. (1985) have reported a strong correlation between the incidence of Parkinsons disease and pesticide use in Quebec, Canada. Most poisoning by pesticides occur as a result of misuse or accidental exposure. At home poisoning usually occurs by oral ingestion, occupational users most frequently encounter dermal exposure or inhalation (Gossel and Bricker, 1994). The endocrine disruptions are commonly observed among the effected humans and wild fauna (Hussain, 1998). It is with this aim the present research envisaged to determine (i) the microbial degradation of an organophosphate pesticide (malathion), either from the domestic wastewater or of industrial origin, during activated sludge treatment and to further confirm that innoculation of flocs actively degrading malathion during wastewater further improves the ability of degrading microorganisms to degrade malathion via activated sludge system. (ii) and also to develop a quick, accurate and sensitive HPLC method using U.V visible detector for the analysis of organophosphorous compounds.

Materials and Methods

Sampling Procedure

Site of sample collection: The source for the collection of wastewater samples thoughout the present studies was the Karachi University Campus (KUC).

Isolation, identification and maintenance of bacteria: The bacterial strain capable of degrading malathion was isolated by enrichment technique, using various concentration of malathion. The bacteria isolated showing highest tolerance to was purified and preserved. Gram's staining was performed to identify the bacterial strain. Isolated strains were maintained on nutrient agar (ACUMEDIA) plates containing 1 percent concentration of pesticides. During storage purity of the culture was checked periodically.

Technical Details of Biosimulator: (Design and operation of pilot plant). The technical details and the general layout of the biosimulator are shown in Table 1 and Fig. 1.

Extraction of metabolites for chromatography: Sample (NB) was collected after assuming complete degradation of organophosphate pesticide (malathion) to its metabolites and subjected to n-hexane extraction in a separatory funnel. The hexane layer was separated and evaporated on a water bath maintained at 70°C. The residues was finally dried. After complete drying the residues were recovered using 10 ml of HPLC-grade Sample collection: The samples were collected from the biosimulator after every 2 hours and analyzed. Analysis of sample: The samples for the physical and chemical parameters were analyzed according to the methods described in the Standard Methods for the Examination of Water and Wastewater (APHA, AWWA and WPCF, 1985).

(i) pH (ii) Settleability (iii) Chemical oxygen demand (COD)

Analytical parameters: HPLC and TLC analytical techniques were used, during the activated sludge treatment system, for determining the degradation of the hazardous chemical (malathion).

Table 1: Technical Data Of Biosimulator Used For PCA

1.	Biosimulator	Model ME-114
	(a) DO- Controller	Model DO-81
	(b) pH- Controller	Model PH-22
2.	Vessel capacity	14-Litres
3.	Total Hydraulic load* (RWW (KUC), TMC, Culture inoculum)	8-litres
4.	Retention time	29-hours
5.	Temperature	Room temperature (25-28°C)
6.	Do-operated	4.0 mg/L
7.	Primary treatment (Settleability)	30-minutes
8.	Total malathiion concentration	1010.50 mg/L
9.	Total organic load (In terms of COD)	12.64 gm/L
10.	Sample collection	Every 2-hours
11.	Size of innoculum**	3.0×10^9 bacteria/ml

*RWW(KUC) = Raw wastewater, Karachi University Campus

TMC = Total malathion concentration

**Size of Innoculum = 24-hours old grown culture. (Culture was streaked on nutrient-agar slants and incubated for 24-48-hours. Washings were taken with nutrient broth and matched with McFarland's Index).

Statistical analysis: The data obtained was subjected to principal component analysis (PCA) which has gained much attention than any other ordination technique in the last three decades. PCA is a descriptive tool, which serves the purpose of trend seeking. Feoli (1977) and Nichols (1977) have pointed that PCA can provide unique, objective and parasimonous representations that are predictable and meaningful. The PCA is a variance-oriented technique where the component scores are methanol. The resulting solution was used for TLC and HPLC, directly derived by a linear transformation. The use of PCA permits an objective summarization of the variables 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. Principal Component Analysis: Data Set: For the purpose of present study the biosimulator performance efficiency in relation to inoculation of a strain of *Pseudomonas* with a continued supply of approximately 4.0 mg/L of dissolved oxygen

Table 2: Basic data of biodimulator used for principal component Analysis

S.No.	COD_{IN}-COD_{OUT} mg/L	COD_{IN}/COD_{OUT} mg/L	MAL_{in}-MAL_{out} mg/L	MAL_{out}-MAL_{in}- mg/L	Dissolved oxygin mg/L	Organic load mg/L	Total malathion concentration mg/L	pH	Retention time hours
1.	160	0.898	2.603	0.979	4.12	11.36	989.977	7.42	2
2.	280	0.822	6.988	0.944	3.95	10.4	954.902	7.46	4
3.	400	0.746	8.175	0.935	3.98	9.44	945.401	7.53	6
4.	520	0.670	30.792	0.756	3.86	8.48	764.46	8.02	8
5.	640	0.594	71.894	0.430	3.75	7.52	435.654	8.16	10
6.	960	0.392	112.604	0.108	4.11	4.96	109.975	8.40	24
7.	980	0.379	112.628	0.108	4.21	4.8	109.772	8.53	25
8.	1100	0.303	112.73	0.107	4.10	3.84	108.964	8.51	27
9.	1240	0.215	113.981	0.097	3.96	2.72	98.957	8.54	29

Table 3: List of variables used for PCA

S.No.	Variables	Symbols	Units
1.	Efficiency as COD_{IN}-COD_{OUT}	-	mg/L
2	Efficiency as COD_{OUT}/COD_{IN}	-	mg/L
3	Efficiency as MAL_{IN}-MAL_{OUT}	-	mg/L
4.	Efficiency as MAL_{OUT}/MAL_{IN}	-	mg/L
	Independent Variables		
5.	Dissolved Oxygen	DO	mg/L
6.	Organic load	O	mg/L
7.	Total malathion concentration	x	mg/L
8.	pH	pH	-
9.	Retention time	Rt	hours

and a retention time of 29-hours was subjected to PCA. The data set consists of 9-observations and 9-variables related to biosimulator performance, including retention time, pH, dissolved oxygen (DO), organic load in terms of COD and total malathion concentration calculated in terms of per centage degradation of organophosphate (Table 2).

List of variables used for the PCA analysis are presented in Table 3.

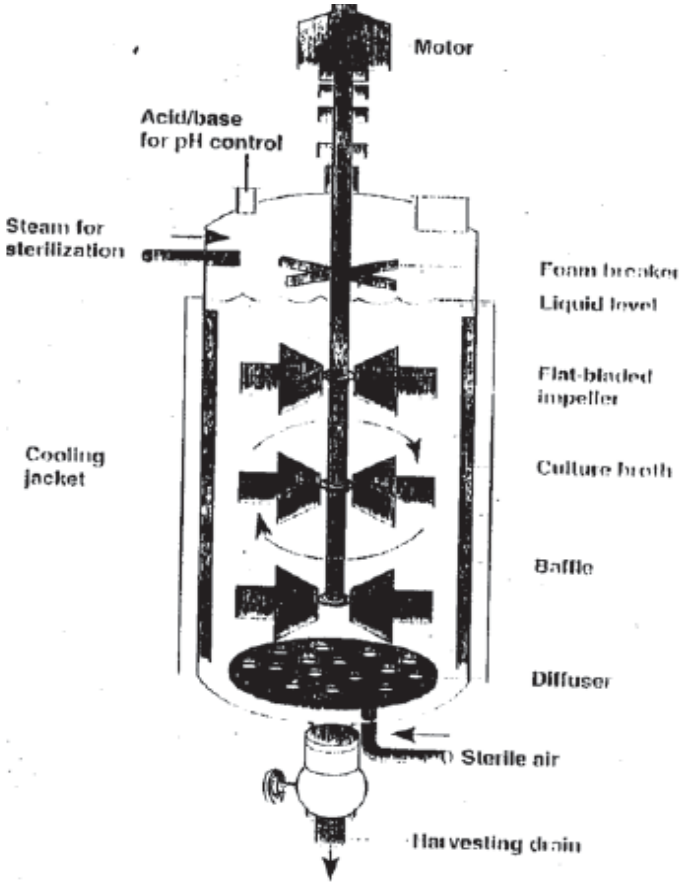

Fig. 1: Layout of a biosimulator used for principal components analysis

Table 4: Results of PCA. Eigenvalues and eigenvector elements together with associated variables for the first three principal components

Principal Components	Eigen value %	Cumulative Variance	Ranked eigenvector element	Associated variables
I	84.15527	84.15527	0.969497	MAL_{in}-MAL_{out}
			-0.969497	Total mal. Conc.
			-0.969464	MAL_{in}/MAL_{out}
			-0.968054	COD_{out}/COD_{in}
			0.968032	COD_{in}/COD_{out}
			-0.968032	Organic load
			0.967612	Retention time
			0.953727	pH
			0.311605	Dissolved Oxygen
II	10.49351	94.64878	0.949159	Dissolved Oxygen
			-0.128240	pH
			0.095074	Retention time
			0.076088	COD_{out}/COD_{in}
			0.075920	Organic load
			-0.075920	COD_{in}/COD_{out}
			0.015561	MAL_{out}/MAL_{in}
			-0.015329	MAL_{in}/MAL_{out}
			0.015327	Total mal. Conc.
III	1.84795	96.49672	0.150298	MAL_{out}/MAL_{in}
			0.150182	MAL_{in}-MAL_{out}
			-0.150182	Total mal. Conc.
			0.148160	Organic load
			-0.148060	COD_{in}/COD_{out}
			0.147718	COD_{out}/COD_{in}
			-0.131466	Retention time
			0.125161	pH
			0.001866	Dissolved Oxygen

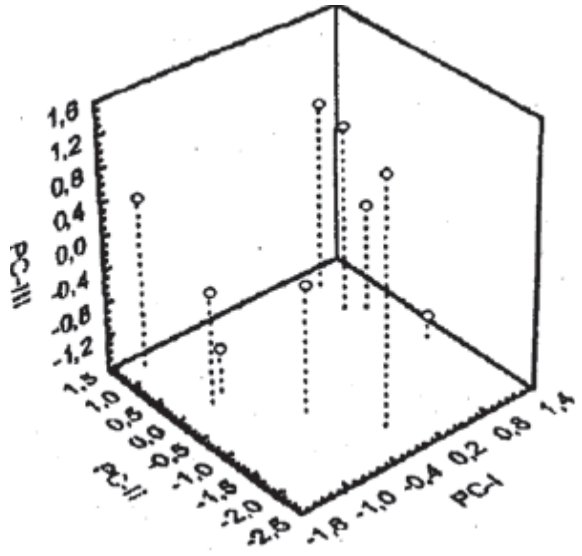

Fig. 2: Three dimentional principal comonents ordination o the biosimulator using 9 descriptors (see text for exapanation)

Table 5: Correlation coefficients between principal component and the variables

Variables	Components		
	PC-I	PC-II	PC-III
COD_{in}-COD_{out}	0.988814	-0.07581	-0.12817
COD_{out}/COD_{in}	-0.98884	0.075976	0.127874
MAL_{in}-MAL_{out}	0.990311	-0.01531	0.130007
MAL_{out}-MAL_{in}	-0.99028	0.015538	-0.13011
DO	0.318295	0.947761	0.001615
Or/L	-0.98881	0.075809	0.12817T
MC	-0.99031	0.015304	-0.13001
pH	0.974220	-0.12805	0.108346
Rt	0.988385	0.094934	-0.1138

DO = Dissolved oxygon
Or/L = Organic load
TMC = Total malathion concentration
Rt = Retention time

Results and Discussion

The microorganism isolated showed mucoid surface appearance with convex elevation and entire margin. The

colonies were regular and pinpoint. They were Gram's negative, thin-rods, shorts and scattered and showed motility. In order to confirm the ability, whether the isolated microorganism could utilize or degrade the pesticide to its metabolites that were formed as a result of degradation HPLC was performed. The results confirmed the degradation of the pesticide to its metabolites or the pesticide was completely degraded.

As soil is rich with microorganisms and microbial activities occur in many niches, they represent the pare force of environmental alteration of pesticidal residues because of their ability to adapt and proliferate the total biomass and surface area. Furthermore it was also contributed due to the heavy tolerance of microorganism for pesticide degradation and is also evident by the heavy growth of microorganism in the sample which represent the stability and efficacy of the isolated microorganisms to degrade pesticide with respect to the control.

Statistical analysis: Fig. 2 is based on the principal components I, II and III which explains 96.49 percent of the total variability (Table 4). The first component I explaining 84.15527 percent is primarily a function of MAL_{IN}-MAL_{OUT}, TMC, MAL_{OUT}/MAL_{IN} and COD_{OUT}/COD_{IN} as indicated by eigenvector coefficient (Table 4). The principal component II explains 10.49351 percent variability which is mainly governed by DO, pH, Rt and COD_{OUT}/COD_{IN} (Table 4). The principal component III accounts for 1.84795 percent variability of the total variance and it is also mainly governed by Mal_{IN}-Mal_{OUT} TMC and organic load (Table 4). Table 5 provides the correlation coefficients between the 9-variables and the first three principal components.

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