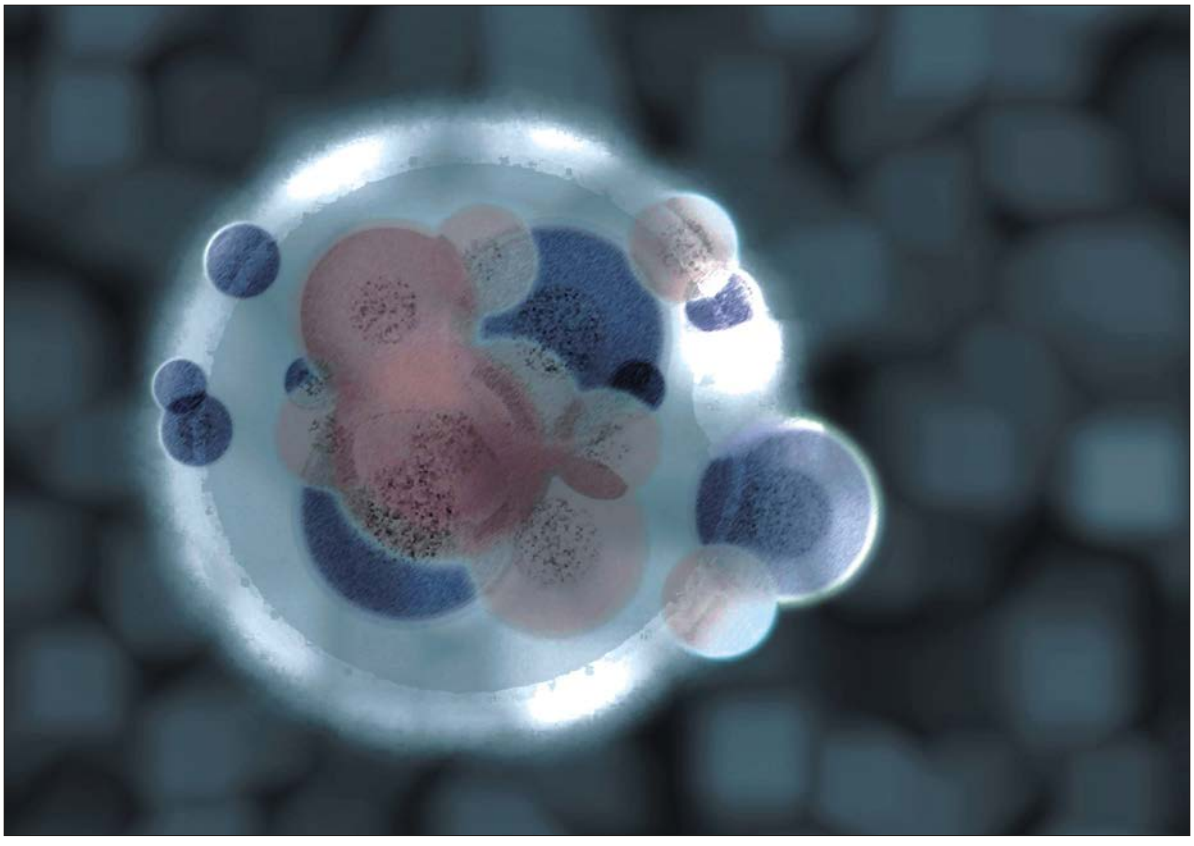

Journal of Biological Sciences

ISSN 1727-3048

science alert

ANSInet
an open access publisher
http://ansinet.com

Journal of Biological Sciences 4 (1): 15-20, 2004
ISSN 1727-3047

Isolation, Characterization and Growth Response of Pesticides Degrading Bacteria

S. Jilani and M.Altaf Khan
[1]Department of Environmental Engineering,
NED University of Engineering and Technology, Karachi, 75270, Pakistan
Dean Faculty of Health Management, Baqai Medical University, Karachi, Pakistan

Abstract: In Pakistan, insecticides are widely used in agriculture. Despite their biodegradable nature, some are highly toxic and their residues are found in the environment. Moreover, its removal from wastewater generated during manufacture becomes inevitable. Reports on the mineralization of a spectrum of different insecticides by a single potential strain are scarce. In this study, a bacterial strain was isolated from soil using enrichment technique and identified as *Pseudomonas* sp. by microscopic examination and biochemical tests. Growth curve experiments showed that *Pseudomonas* strain was able to grow in nutrient medium containing malathion (35-220 mg L^{-1}), methamidophos (80-320 mg L^{-1}), cartap (60-120 mg L^{-1}) and cypermethrin (40-125 mg L^{-1}) pesticide. However, the optimum concentration which support normal bacterial growth during 24 h was found to be 120 mg L^{-1} malathion, 160 mg L^{-1} methamidophos, 80 mg L^{-1} cartap and 60 mg L^{-1}cypermethrin. When compared with the control test, a significant increased in bacterial population was noted at low concentration of each pesticide, however at high concentration lag phase increased but no zone of inhibition observed. These data indicate that the isolated *Pseudomonas* strain can be used as a microorganism for the bioremediation of pesticide contaminated soil or water.

Key words: Pesticides, agriculture, wastewater, biodegradation, *Pseudomonas* sp.

INTRODUCTION

Pakistan is an agro-based country. Most of its land (82.3%) is meant for cultivation. To meet the need of food requirements for growing population, farmers are using more than 45,000 tones of different types of pesticides. The advantage resulting from pesticides application is generally undisputed, but the residues of the applied pesticides stay in the environment (air, soil, ground and surface water) for variable period of time, which poses serious threats to environment and indeed can lead to acute and chronic effects on human life causing damage to health or even death. According to World Health Organization (WHO) report, two million people suffered from pesticide poisonings with about 40,000 deaths per year. Moreover, the population of the developing countries is known to carry heavy burden of pesticides in their bodies (WHO, 1990).

The agricultural industries in Pakistan are also contributing relatively high quantities of toxic pesticides into the environment, as most of them have either no treatment facilities or have grossly inadequate arrangement. At present the Karachi coastal region has become the dumping ground of hazardous waste, receiving huge quantity of untreated domestic, industrial and agricultural waste. Pesticide waste treatment technologies are needed to prevent water pollution and to comply with increasing regulatory pressures.

Research studies have revealed that microbial degradation process to detoxify pesticides contaminants can be effectively used to overcome the pollution problems (Bhadhade *et al*., 2002; el-Deeb *et al*., 2000; Galli, 1994; and Roy *et al*., 1997). Soil bacteria with the ability to degrade several pesticides have been isolated from soil showing enhanced biodegradation. They include a metamitron – degrading *Rhodococcus* sp. (Parekh *et al*., 1994), a chlorpyrifos – degrading *Flavobacterium* sp. (Mallick *et al*., 1999), atrazine degrading *Pseudomonas* sp. (Ralebits *et al*., 2002) and an iprodione–degrading *Arthrobacter* sp. (Mercadier *et al*., 1996). In further studies with the soil, two *Pseudomonas putida* strains were isolated, which were able to utilize diclofop-methyl as a source of carbon and energy (Karpouzas and Walker, 2000). It has been suggested that cultures of bacteria with the ability to degrade specific compounds can be used for bioremediation of pesticide polluted sites.

Although their have been many reports on pesticide degradation, the ability to degrade different type of insecticide by a single potential strain is scarce. It is because of this reason, the present research study was conducted to isolate and identify a potential microorganism from soil and investigate its biodegradation potential in a medium containing different quantities of phosphatic, carbamate and pyrethroid insecticides.

MATERIALS AND METHODS

Pesticide used: Commercial grade insecticides (malathion, methamidophos, cartap and cypermethrin) were purchased from agricultural chemical dealer and used through out the experimental research studies because it would more closely represent the nature of the active compound that microorganisms are likely to be exposed to in the environment.

Preparation of medium: Nutrient broth and nutrient agar media (Acumedia) was prepared according to the manufacturer's instruction and was used for the isolation, characterization and growth kinetic studies of pesticide degrading bacterial culture. Growth of bacterial isolate was observed in nutrient broth containing different concentration of each pesticide (Malathion, methamidophos, cartap and cypermethrin).

Isolation and maintenance of pesticide degrading bacterial culture: The bacterial culture capable of degrading malathion was isolated from soil using enrichment technique, with varying concentration of malathion in the medium. Wet unsieved soil (2-5 g) from agricultural site was inoculated into 250 ml of wastewater in 500 ml Erlenmeyer flasks containing 100-350 mg L^{-1} malathion. The flasks were incubated on a shaker operating at 240 r.p.m for several days at ambient temperature (25°C). At daily interval one loop full of enrichment culture from the flasks were streaked on to nutrient agar plates supplemented with malathion pesticide and incubated at 35°C for 24 h. Individual colonies were subculture into nutrient agar plates containing malathion until pure cultures were isolated. Bacterial isolates that can handle relatively high concentration of pesticide were subjected to morphological, cultural and biochemical tests. The potential of the isolated strain to utilize other pesticides for their growth was then determined.

The isolated pure bacterial strain was also streaked on nutrient agar slant and slants containing 57 mg malathion pesticide. After incubation at 35°C for 24 h, the culture was maintained at 4°C. The bacterial culture was subculture after every three months. When a new batch of test was performed with different dose and type of pesticide, the stock culture was first subculture to produce active culture and then used for growth kinetic studies.

Enumeration of Viable cell count: The isolated bacterial culture was enumerated with and without adding pesticide. Aliquot (2.5 ml) of 24 h culture grown in nutrient broth was inoculated into 25 ml nutrient broth flask containing different concentration of each pesticide (malathion, methamadiophos, cartap and cypermethrin) and tested their ability to degrade supplemental substrate (the pesticide). Control flasks of equal volume of nutrient broth medium containing culture but no pesticide were run in parallel to confirm that significant die off was not occurring over the period of each test. Three replicate were performed for each dose of pesticide for a total of 42 tests, including the zero dose (control).

Growth of the isolate was determined by viable cell enumeration immediately after inocculation and at 2, 4, 6 and 24 h later. Miles and Misra technique was used for bacterial growth study. Sample of bacterial culture (1 ml) was drawn at regular intervals and serial dilutions (10^{-5} – 10^{-8}) of bacterial culture with and without addition of pesticide (control) was performed using 9 ml sterile saline blank (0.85% NaCl; pH 7). Appropriate dilutions of bacterial samples were plated in triplicate on nutrient agar medium. Each plate divided into four segments and used for several dilutions. Three drops of culture were placed in each section of nutrient agar plate and were allowed to dry followed by incubation at 35°C for 24 h. After incubation viable colonies were counted by the method described by Collins and Lynes (1985) and results were reported accordingly.

RESULTS AND DISCUSSION

Isolation of pesticide degrading bacterial strain: Two different colonies were observed on nutrient agar medium enriched with malathion. One of the largest, most rapidly growing colonies of the bacterial isolate was selected for the present research study. The isolated organism was designated as IES-*Ps*-1 and its ability to mineralize three of the other insecticides (methamidophos, cartap and cypermethrin) were also tested. These insecticides are part of a group of bioresistant compounds, which are not biodegradable by the environment or by conventional treatment in water treatment plants.

Table 1: Characteristics of pesticide degrading strain

Tests	Strain
Shape	Rods
Growth on Nutrient Agar	Round, smooth and convex
Motility	Motile
Gram Stain	gram negative
Gelatin liquification	Positive
Nitrate reduction	Positive
Indole production	Negative
Oxidase and Catalase	Positive
MR and VP reaction	Negative
Citrate utilization	Positive
Acid without gas formation from glucose	Positive
Lactose, mannitol, sucrose	Negative

Table 2: Growth Count of IES-*Ps*-I in nutrient broth and broth containing different concentration of Pesticides

Pesticide concentration (ppm)	Viable Count x 10^7 CFU ml^{-1} at regular Interval				
	0 h	2 h	4 h	6 h	24 h
Control test	11.00	18.16	28.26	93.40	197.0
Malathion					
35	16.7	30.5	41.1	119.00	184.0
50	14.3	35.7	42.8	119.00	124.0
120	12.0	14.3	31.0	50.00	96.0
220	12.0	14.0	6.5	9.60	1.1
Methamidophos					
80	17.0	26.0	36.0	52.00	143.0
160	8.6	17.0	9.5	27.00	263.0
320	16.7	12.0	9.6	14.30	12.0
Cartap					
60	12.0	19.0	24.0	29.00	141.0
80	12.0	17.0	31.0	43.00	190.0
120	3.6	3.6	3.6	3.80	38.0
Cypermethrin					
40	9.5	12.0	8.8	20.00	150.0
60	9.5	14.3	4.8	12.00	170.0
80	11.0	14.0	4.5	7.00	17.0

Identification and characterization of malathion degrading bacterial isolate: On the basis of morphological, cultural and biochemical characteristics (Table 1), the bacterial isolate was identified as a member of the genus *Pseudomonas* according to, "Bergey's Manual of Systematic Bacteriology" (Palleroni, 1986).

Characterization studies of the isolate from these experiments, 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 (Martin *et al*., 2000; Ramanathan and Lalithakumari, 1999; Lee *et al*., 1998; Ramos *et al*., 1995 and Maloney *et al*., 1988).

Pseudomonads are a vast heterogeneous group of bacteria that occur in substantial numbers in the soil where they are active agents of mineralization of organic matter. Most species can grow well in simple minimal medium with a single organic compounds as carbon and energy source (Palleroni, 1986). Pseudomonads possess a variety of diverse catabolic pathways that enables them to metabolize an equally diverse number of low molecular weight compounds, including chlorinated aliphatic hydrocarbons such as phenoxyalkanoic acid herbicides (Lynch and Hobbie, 1988). It is known for its capacity to degrade phenolic compounds (Hughes and Cooper, 1996) and other aromatic substances and therefore are an ideal choice as the bacteria to be used for degradative biotechnologies. (Karpouz and Walker, 2000; Wenk *et al*., 1994; Zacharias *et al*., 1995; Rani and LalithaKumari, 1994; Singh and Seth, 1989). Because of having an extraordinary range of catabolic pathways; a single species such as *P. cepacia*, utilizes more than 100 different substrates as the only C, N, or S source (Dagley, 1986).

The presence of substantial numbers of nutritionally versatile pseudomonads in soil likely accounts for the reason why these microorganisms are so readily isolated from soil. Furthermore, experimental conditions of standard enrichment culture techniques, as those of this research, are no doubt optimal for the selection of pseudomonads specie. It is possible that under a different set of enrichment procedures, a different group of microorganisms might have been isolated.

Bacterial adaptation: During the process of adaptation, it was observed that in the presence of high concentration of insecticides, the bacteria was greatly stressed and its growth was slowed in consequence. The bacteria changed its normally rod-shaped morphology to that of a coccus when there was an increase in the insecticide concentration. However, this change was temporary, because the cells recovered the original rod form after a few days. Much more difficulty was observed when adapting the bacteria to the nutrient medium with Cypermethrin than with Malathion.

Growth kinetic studies: Depending on their type and concentration, pesticides can have different effects on the growth of microorganisms. A series of growth curve experiments was performed with specific doses of pesticides (Malathion. Methamidophos, Cartap and Cypermethrin) in order to determine the viable count of *Pseudomonas* (IES-*Ps*-1) and to verify whether they could utilize these compounds for their growth. The optimum concentration of each insecticide, which supports bacterial growth, was also evaluated. As growth kinetic studies providing an evidence of mineralization potential of organism, therefore such studies were conducted by several other researchers while performing pesticide degradation using isolated strain of microorganisms (Maria *et al*., 2002; Karpouzas and Walker 2000; Lee *et al*., 1998; Smith and Adkins, 1995 and Haugland *et al*., 1990).

Growth kinetics of *Pseudomonas* in nutrient broth (control): A control test without adding pesticide in nutrient broth was conducted in order to evaluate the mineralization potential of isolated strain when exposed to different concentration and type of pesticides. It is seen from Table 2 and Fig. 1, that the phase of acclimation of *Pseudomonas* (IES-*Ps*-1) continued up to almost 5 h, after the initial inocculation. The count at 0 h was $11x10^7$ CFU ml^{-1} and then started increasing slowly. At 6 h, the total viable count was $93x10^7$ CFU ml^{-1} with generation time of 69 minutes and specific growth rate of 0.014 (generation time and specific growth rate are not shown in Table). At 24 h the total viable counts significantly increased ($191x10^7$ CFU ml^{-1}), indicating that the culture after

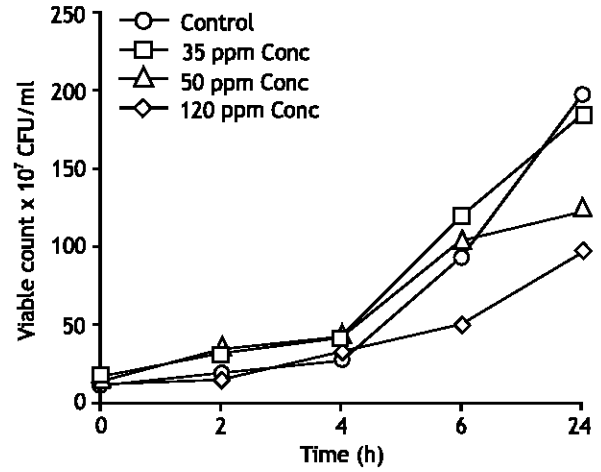

Fig. 1: Growth of *Pseudomonas* in the presence of malathion

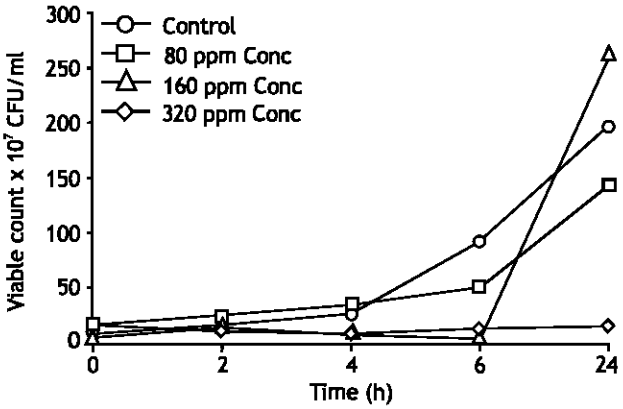

Fig. 2: Growth of *Pseudomonas* in the presence of methamidophos

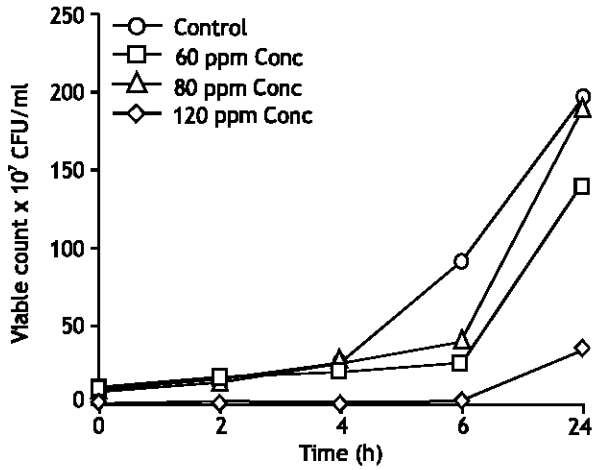

Fig. 3: Growth of *Pseudomonas* in the presence of cartap

remaining in lag phase (phase of adjustment) for 5 h entered into the phase of positive acceleration. The total viable count from $28x10^7$ CFU ml^{-1} at 4 h significantly increased to $93x10^7$ CFU ml^{-1} at 6 h and $197x10^7$ CFU ml^{-1} at 24 h, respectively showing that the culture entered the lag phase after 5 h and remain in that phase during 24 h of incubation.

Growth kinetics of *Pseudomonas* in nutrient broth supplemented with Malathion: Commercial grade malathion in the range (35 mg L^{-1} to 300 mg L^{-1}) was used to determine the growth response of bacterial isolate. The stimulatory and inhibitory responses of *Pseudomonas* were observed when exposed to varying concentration of malathion. The growth pattern are shown in Fig. 1 and recorded in Table 2. A significant reduction in the viable cell number was observed after 6 h of incubation at 300mg L^{-1} malathion dose and 450 mg L^{-1} malathion dose proved to be inhibitory for bacterial isolate (data not shown).

When compared with the control test (growth without pesticide), the growth pattern of *Pseudomonas* in the medium containing 35 mg L^{-1} to 50 mg L^{-1} malathion is very much similar during 24 h of incubation, the viable count remains same at 6 h (109 CFU ml^{-1}) where as at 24 h the viable count, $184x10^7$ CFU ml^{-1} and $104x10^7$ CFU ml^{-1} was observed. The generation time was calculated to be 75 min. with 35 mg L^{-1} malathion dose and 80 min. with 50 mg L^{-1} malathion dose after 6 h of inocculation. The results of the analysis indicate that malathion concentration in the range of 35 mg L^{-1} to 50 mg L^{-1} stimulated the growth of isolates, however a marked reduction in bacterial count at 24 h was noted when 220 mg L^{-1} malathion dose was used with generation time of 212 min., this indicates that bacterial enzymes at high concentration suppressed and the growth rate thus decreased. Similar results were reported by Singh and Seth (1989), who found a significant decreased in the rate of degradation and growth of microorganisms at 200 ppm malathion concentration.

Growth kinetics of *Pseudomonas* in nutrient broth supplemented with methamidophos: Growth response of bacterial isolate in the presence of methamidophos was found to be stimulatory in the concentration range of 80 mg L^{-1} to 200 mg L^{-1} during 24 h of incubation. Results are shown in Table 2 and Fig. 2. At 800 mg L^{-1} concentration, the growth showed resistance and at 1200 mg L^{-1}, the growth of orgamisms inhibited and significant die off was observed (data not shown). These results

indicate that methamidophos concentration at 200 mg L^{-1} did not affect the growth of microorganisms when compared with the control experiments (growth without pesticide). The isolates can also survive at 320 mg L^{-1} to 400 mg L^{-1} concentration but the viable count significantly decreased at 24 h.

Growth kinetics of *Pseudomonas* in nutrient broth supplemented with cartap: Growth curve experiment with different concentration of cartap pesticide indicates that cartap in concentration of 60 mg L^{-1} to 80 mg L^{-1} provoked an increase in bacterial growth. However, a limited growth was observed at 24 h with culture containing 160 mg L^{-1} cartap pesticide and a marked reduction in viable cell count at 400 mg L^{-1} was noted during 6 h of incubation and no growth seen at 24 h.

These results indicate that cartap at high concentration become toxic to microorganisms and therefore bacterial isolate was unable to utilize cartap pesticide at high concentration to support their growth

Growth kinetics of *Pseudomonas* in nutrient broth supplemented with cypermethrin: A bacterial strain was also tested to determine its potential for cypermethrin degradation, the insecticide which is being widely used by the farmers for agricultural production. Nutrient broth containing varying concentration of cypermethrin was inoculated with bacterial strain. The extent of growth during 24 h of incubation was recorded in Table 2 and shown in Fig. 4.

When comparing the growth of IES-*Ps*-1 in the presence of cypermethrin pesticide with that of malathion, it is clear from Table 2 and Fig. 1 that the bacteria grows faster and to a higher number of cells, when malathion was used. These results are an indication of the difficulty that the microorganism has to adapt to this type of pesticide. The growth of organism in a medium modified with 40 and 60 ppm cypermethrin pesticides markedly increase at 24 h. However at high concentration of cypermethrin (80 to 125 ppm) the growth count significantly decreased and the lag phase increased, but no zone of inhibition was observed. At 6 h the viable count was noted to be $20x10^7$ CFU ml^{-1} when 40 mg L^{-1} cypermethrin dose was used and $10x10^7$ CFU ml^{-1} at 60 mg L^{-1} cypermethrin concentration. At 24 h the total viable count at 40 and 60 mg L^{-1} became $150x10^7$ and $170x10^7$ CFU ml^{-1}, respectively. The generation time with 40 and 60 mg L^{-1} cypermethrin dose was 90 min. with specific growth rate of 0.011 at 6 h. At 80 mg L^{-1} cypermethrin concentration, the generation time was

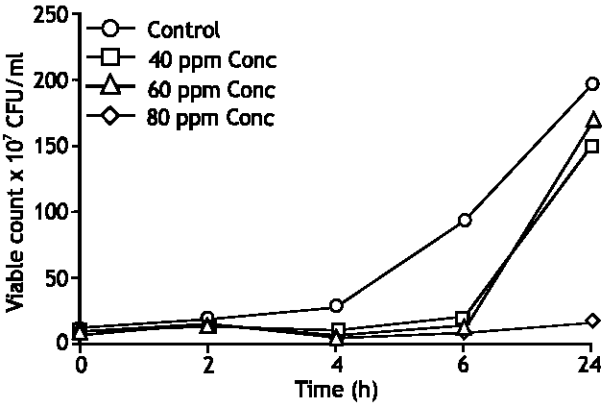

Fig. 4: Growth of *Pseudomonas* in the presence of cypermethrin

calculated to be 180 min. at 6 h after inoculation and rate of substrate utilization was found to be 0.0055. These observations indicate that the bacterial growth resulted because of the utilization of cypermethrin and proved that cypermethrin even at high concentration is not toxic to microorganisms. However the rate of substrate utilization decreased which prolonged the lag phase.

The growth responses revealed the degree of bacterial sensitivity or resistance and the amount of growth stimulation when exposed to different type and concentration of insecticides. Taken together our studies suggested that bacterial strain, which was selected as a malathion degrading microorganism is able to utilize wide range of pesticides. It was further noted that *Pseudomonas* organism showed a higher number of counts at low concentration of each pesticide. However at higher concentration, the number of organisms significantly decreased or very slightly increased during 24 h of incubation, when compared with the control tests (no pesticide). This implies that at high concentration the appropriate catabolic enzymes may be repress. Another plausible explanation is that microorganism may need an acclimation period to induce the necessary degradative enzymes and may be because of this reason the prolonged lag phase observed at high concentration of each pesticides. From these findings it can be concluded that the isolated bacterial strain could be useful for the treatment of pesticides contaminants in industrial effluent and can detoxify agricultural waste.

ACKNOWLEDGEMENT

This research work was partially supported by United Nation Development Project (UNDP) funds. The authors wish to express their gratitude for this support.

REFERENCES

Bhadhade, B.J., S.S. Sarnaik and P.P. Kanekar, 2002. Bioremediation of an industrial effluent containing monocrotophos. Curr Microbiol. 45(5):346-9.

Collins, C.H. and P.M. Lyne, 1985. Microbiological Methods. 5th Edition. Butterworth and Co (Publishers) Ltd.

Dagley, S., 1986. Biochemistry of aromatic hydrocarbons degradation in Pseudomonads. In the bacteria. Vol. 10. Edited by J.R. Sokatch. Academic Press. New York, pp: 527.

el-Deeb, B.A., S.M. Soltan, A.M. Ali and K.A. Ali, 2000. Detoxication of herbicide diuron by *Pseudomonas* sp. Folia Microbiol (Praha), 45: 211-6.

Galli, E., 1994. The role of microorganism in the environment decontamination. In: Contaminants in the Environment – A multidisciplinary assessment of risks to man and other organisms, Renzoni A., Mattei N., Lari L. and Fossi M.C., Ed. CRC Press Boca Raton, U.S., pp: 235-296.

Haugland, R.A., D.J. Schlemm, R.P. Lyons, P.R. Sferra and A.M. Chakrabarty, 1990. Degradation of chlorinated phenoxy acetate herbicides 2,4-dichlorophenoxy acetic acid and 2,4,5-tricholorophenoxy acetic acid by pure and mixed culture. Appl. Environ Microbiol., 50: 1357-62.

Hughes, S.M. and D.G. Cooper, 1996. Biodegradation of phenol using the self-cycling fermentation (SCF) technique. Biotechnol. Bioengg., 51: 112-119.

Karpouzas, D.G., J.A. Morgan and A.Walker, 2000. Isolation and characterization of 23 carbofuran-degrading bactertia from soils from distant geographical areas. Lett Appl Microbiol., 31: 353-8.

Lee, S.G., B.D. Yoon, Y.H. Park and H.M. Oh, 1998. Isolation of a novel pentacholorophenol-degrading bacterium, Pseudomonas sp. Bu 34. J. Applied Microbiol., 85: 1-8.

Lynch, J.M. and J.E. Hobbie, 1988. Microorganisms in action: concepts and applications in microbial ecology. Blackwell Scientific Publication, Oxford, pp: 322-347.

Mallick, K., K. Bharati, A. Banerji, N.A. Shaki and N. Sethunathan, 1999. Bacterial degradation of chlorpyrifos in pure cultures and in soil. Bulletin of Enviroumental contamination and Toxicol., 62: 48-54.

Maloney, S.E., A. Maule and A.R.W. Smith, 1988. Microbial transformation of the pyrethroid insecticides. Permethrin, Deltamethrin, Fastac, Fenvalerate and Fluvalinate. Applied and Enviroumental Microbiol., 54: 2874-2876.

Maria, K., C. Graciela and F. Zauscher, 2002. Biodegradation of two commercial herbicides (Gramoxone and Matancha) by the bacteria Pseudomonas putida. EJB J. Env. Biotech., 5: 0717-3458.

Martin, M., G. Mengs, E. Plaza, C. Garbi, M. Sanchez, A. Gibello, F. Gutierrez and E. Ferrer, 2000. Propachlor removal by Pseudomonas strain GCH1 in an immobilized cell system. Appl Environ Microbiol., 66: 1190-1194.

Mercadier, C., D. Garcia, D. Vega, J. Bastide and C. Coste, 1996. Metabolism of iprodione in adapted and nonadapted soils; effect of soil inoculation with iprodione-degrading Arthrobacter strain. Soil Biol. and Biochem., 28: 1791-1796.

Palleroni, N.J., 1986. Family I Pseudomonaceae Winslow, Broadhurst, Buchanan, Krum wiede, Rogers and Smith 1917, 555^{AL}. In Bergey's Manual of systematic Bacteriology, Vol.1 Ed. Sneath, P.H.A. Baltimore: Williams and Wilkins, pp: 140-199.

Parekh, N.R., A. Walker, S.J. Roberts and S.J. Welch, 1994. Rapid degradation of triazinone herbicide metamitron by a *Rhodococcus* sp. Isolated from treated soil. Applied Bacteriol., 77: 467-475.

Ralebits, T.K., E. Senior and H.W. Van Versevell, 2002. Microbial aspects of atrazine degradation in natural environments. Biodegradation, 13: 11-19.

Ramanathan, M.P. and D. LailithaKumari, 1999. Complete mineralization of methyl parathion by *Pseudomonas* sp. A_3 Appl. Biochem. Biotechnol., 80: 1-12.

Ramos, T.L., E. Duque, M.J. Huertas and A. Haidour, 1995. Isolation and expansion of the catabolic potential of a Pseudomonas putida straion able to grow in the presence of high concentration of aromatic hydrocarbons. Bacteriol., 177: 3911-916.

Rani, N.L. and D. LalithaKumari, 1994. Degradation of methyl parathion by *Pseudomonas* putida. Can J. Microbiol., Dec., 40: 1000-6.

Roy, D., H. Monstafa and K. Maillacheruvu, 1997. Analine degradation in a soil slurry bioreactor. J. Environ. Sci. Health, 32: 2364-2377.

Singh, A.K. and P.K. Seth, 1989. Degradation of Malathion by microorganisms isolated from industrial effluents. Bull Environ Conatam. Toxicol., 43: 28-35.

Smith-Greeier, L.L. and A. Adkins, 1996. Isolation and Characterization of soil microorganisms capable of utilizing the herbicide dichloro-p-methyl as a sole source of carbon and energy. Can J. Microbiol., 42: 221-6.

World Health Organization, 1990. Public Health Impact of Pesticides in Agriculture. Geneva; WHO.

Wenk, M., T. Baumgartner, T. Doborsek Fuchs, J. Kuesera, J. Zopfi and G. Stuchi, 1998. Rapid atrazine degrading *Pseudomonas* sp. Strain. Appl. Microbiol. Biotechnol., 49: 624-30.

Zacharias, B., E. Lang and H.H. Hanert, 1995. Biodegradation of chlorinated aromatic hydro-carbons in slowsand filters simulating conditions in contaminated soil-Pilot study for in situ cleaning of an industrial site. Water Res., 29: 1663-1671.