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Bioremediation of Chlorpyrifos by *Pseudomonas aeruginosa* using scale up technique

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ABSTRACT
Objective: To assess the bioremediation potential of *Pseudomonas aeruginosa* (NCIM 2074) by improving its adaptability to increasing concentration of chlorpyrifos using scale up process.
Methodology and results: Pseudomonas aeruginosa isolate NCIM 2074 was adapted by subjecting to varying concentrations of chlorpyrifos, i.e. 10, 20, 50, 75 and 100 mg/l in incubator shaker at 37 °C and 150 rpm. An initial 10 mg/l concentration of chlorpyrifos was supplied in minimal salt medium (MSM) under controlled environmental conditions for 14 days. The culture was subsequently scaled up to higher concentrations of chlorpyrifos by transferring one milliliter from the medium with 10mg/L to 25 mg/l of the compound. After every 14 days this process was repeated, each time using medium with higher chlorpyrifos concentration. The entire scale up process continued for a period of 70 days. *Pseudomonas aeruginosa* (NCIM 2074) was adapted to increasing chlorpyrifos upto 50 mg/l, but 75 and 100 mg/l was inhibitory to the organism. The biodegradation of chlorpyrifos, as assessed by GC-MS, showed that chlorpyrifos at 10, 25, 50 mg/l degraded completely over a period of 1, 5 and 7 days, respectively. The intermediate 3, 5, 6 trichloro-2-pyridion, 2, 4-bis (1, 1 dimethyiethyl) phenol and 1, 2 Benzenedicarboxylic acid persised during bioremediation, but in the long run these convert to CO2, biomass and nutrients. *Conclusion and application of findings: Pseudomonas aeruginosa* (NCIM 2074) has been potential use in bioremediation of chlorpyrifos at concentrations upto 50 mg/l, but the organism is inhibited by higher concentrations.

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INTRODUCTION

Chlorpyrifos is a widely used organophosphate insecticide. The manufacture and formulation process of chlorpyrifos generates waste that contains the compound, and this has to be treated by physico-chemical and biological means. Recent advances in bioremediation technology have introduced more effective and efficient methods for handling the pesticide. *Pseudomonas aeruginosa* is the most common Gram negative bacterium found in soil. Isolates of this bacterium have been found to have potential to degrade chlorpyrifos (Fulekar, 2005a, b).

In laboratory experiments, pure culture of *P. aeruginosa* NCIM 2074 grown on minimal medium (FTW medium) was adapted to increasing concentration of chlorpyrifos between 10 and 100 ppm. The adapted *P. aeruginosa* were evaluated for their effectiveness in bioremediation under controlled environmental conditions. Environmental factors such as pH and temperature were monitored and measured to assess their effect on biodegradability of chlorpyrifos by the test microorganism (EPA, 1997).

MATERIALS AND METHODS

Microorganisms: Pure culture of *Pseudomonas aeruginosa* (NCIM 2074), a phenol degrading strain was procured for the bioremediation work from the National Collection of Industrial Microorganisms (NCIM), Pune, India. The culture was maintained on nutrient agar slants. Technical grade Chlorpyrifos was used for the bioremediation study.

Culture medium: The nutrient culture medium was prepared to assess pesticide as a carbon source for microorganism in the enrichment study (Siddique *et al*., 2003). The selected FTW medium (Herman & Frankerberger, 1999) comprised of (in gm/l): 0.255 K_2HPO_4, 0.255 KH_2PO_4, 0.255 $(NH_4)_2SO_4$, 0.05 $MgSO_4.7H_2O$, 0.005 $CaCO_3$ and 0.005 $FeCl_2.4H_2O$ blended with 1 ml of trace elements solution (Focht, 1994). The Focht trace element solution contained (in mg/l): 169 $MgSO_4.H_2O$, 288 $ZnSO_4.7H_2O$, 250 $CuSO_4.5H_2O$, 26 $NiSO_4.6H_2O$, 28$CoSO_4$ and 24 $Na_2.MoO_4.2H_2O$.

Pesticide spiking: Erlenmeyer flasks (250 ml) and nutrient culture media were autoclaved for 20 minutes at 121ºC. Aliquots of 500µl acetone containing the pesticide were aseptically added to the autoclaved and dried Erlenmeyer flasks allowing the acetone to evaporate. After complete evaporation of acetone, 100ml culture media was added aseptically so as to reach the desired pesticide concentration (Brinch, 2002).

Scale-up technique: One milliliter of subcultured *Pseudomonas aeruginosa* (in nutrient broth) was inoculated into Erlenmeyer flasks (250ml) containing nutrient culture media with a chlorpyrifos concentration of 10mg/l. The inoculated flasks were incubated on orbital shaker at 160 rpm, 30°C for 14 days. After 14 days, 1ml of the culture media with 10mg/L pesticide was taken and put into culture media with a pesticide concentration of 25mg/l. The flasks were again kept on orbital shaker incubator at 160 rpm, 30°C for 14 days, after which 1 ml was transferred to culture medium with 50mg/l pesticide, and subsequently to 75 and 100mg/l, each stage passing through shaker at 160 rpm, 30°C for 14 days. The entire scale-up period lasted 90 days after which the bacteria were found to be well adapted to chlorpyrifos 25, 50 and 75 ppm, which the bacterium could access and utilize as sole source of carbon. After every 14 days samples were removed and analyzed using GC-MS for biodegradation of the pesticide and their intermediates. Throughout the bioremediation study the microbial growth in flask bioreactors were recorded by measuring absorbance at 550nm.

RESULTS AND DISCUSSION

In the present investigation, bioremediation of the pesticide chlorpyrifos was carried out using pure culture of *Pseudomonas aeruginosa* NCIM 2074 in a scale-up process followed by bioremediation in a shake flask bioreactor under controlled environmental conditions.

The GC/MS data showed that chlorpyrifos was degraded upto 52% in MSM containing 75mg/l chlorpyrifos while in MSM containing100mg/l chlorpyrifos it was degraded upto 25%. In the rest of the concentrations that were evaluated, chlorpyrifos was completely degraded. Thus, the microorganism was found to be well adapted to chlorpyrifos upto a concentration of 50 - 75mg/l (Geetha, 2008).

The adapted *Pseudomonas aeruginosa* was used to study bioremediation of chlorpyrifos in a bioreactor under controlled environmental conditions. The analysis carried out in GC-MS showed that chlorpyrifos was rapidly hydrolyzed to 3, 5, 6 trichloro-2-pyridonol (TCP) in the MSM with chlorpyrifos as the sole carbon and phosphorous source (fig 1). In a previous bioremediation study TCP was detected as the principal metabolite of chlorpyrifos degradation (Baskaran, 2003). The GC-MS data illustrates further that chlorpyrifos was completely degraded within 24 hours when present at 10 mg/l in MSM while at 20 mg/l chlorpyrifos, the pesticide was detected up to the 5th day and for 7 days when applied at 50 mg/l. Transient accumulation of TCP was detected, but during the course of the experiment, its concentration decreased when applied at 10 and 25mg/l chlorpyrifos in growth medium. At 100 mg/l chlorpyrifos, TCP accumulated without further metabolism. The presence of intermediates, e.g. 2, 4-bis (1, 1-dimethyl) phenol and 1, 2 Benzenedicarboxylic acid were also detected at 25 and 50 mg/l chlorpyrifos in MSM.

During the bioremediation study in bioreactor, variation in pH of the inoculated liquid medium was observed (fig 2). In the initial stages of bioremediation, pH decreased until the 5th day and thereafter increased during the later stages of the study. At 10 mg/l chlorpyrifos in MSM, the pH varied from 7 to 6, with a low of 5.75 recorded on the 5th day. At 25 mg/l chlorpyrifos in MSM, the pH decreased from 7 to 5.58 by the last day of the experiment the lowest pH of 5.4 was observed on the 5th day. A decrease in pH was also recorded at 50 mg/l chlorpyrifos from initial 7 to 5.48. In the un-inoculated control flask, the pH remained constant.

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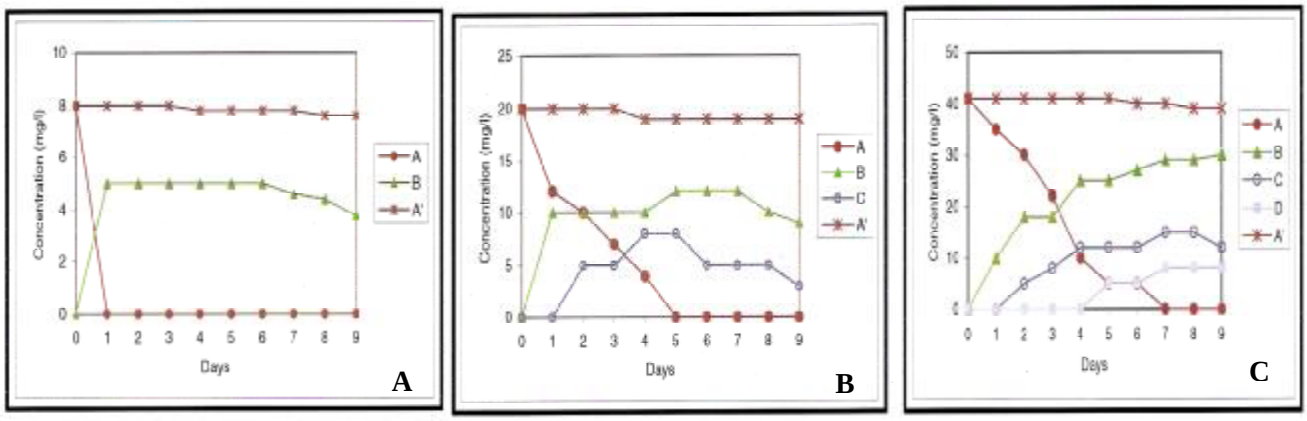
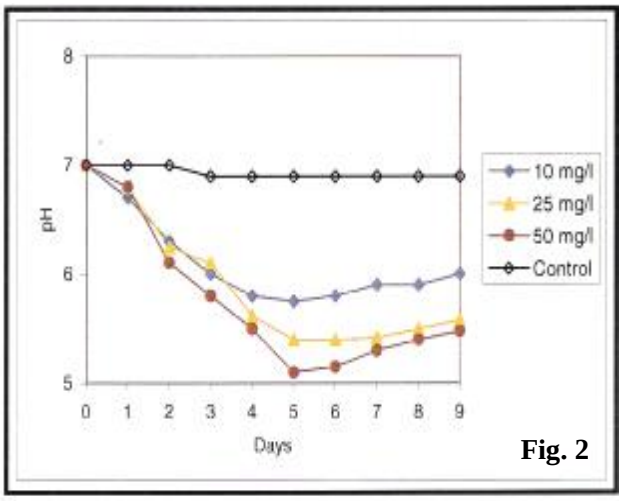


Figure 1: Concentration of intermediates found during the bioremediation of Chlorpyrifos containing MSM (a) 10mg/L; (b) 25mg/L; (c) 50 mg/L chlorpyrifos where A = chlorpyrifos; B=TCP; C=2,4 – bis (1,1 dimethylethyl) phenol; D = 1,2 benzenedicarboxylic acid and A' = Chlorpyrifos control. Values are expressed as mean ± SD of experiments in triplicate.



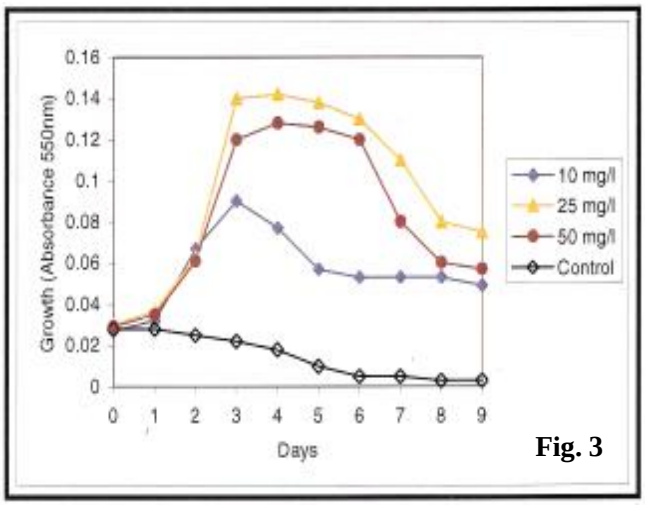


Figure 2: Variation in pH during bioremediation of Chlorpyrifos in MSM. Values are expressed as mean ±SD of experiments in triplicate.

Figure 3: Variation in bacterial growth during bioremediation of chlorpyrifos in MSM. Values are expressed as mean ± SD of experiments in triplicate.

During the bioremediation study, growth of *Ps. aeruginosa* reached exponential phase on the 1st day regardless of the chlorpyrifos concentration in growth medium. At 10 mg/l chlorpyrifos, maximum growth was reached on the 3rd day and the stationary phase on the 6th day. At 25 and 50 gm/l chlorpyrifos, maximum microbial growth was attained on the 5th day and thereafter growth declined (Huang, 2000).

Although *Ps. aeruginosa* was well adapted to chlorpyrifos upto a concentration of 75 mg/l in MSM, higher concentrations were detrimental to the growth and survival of the microorganism. Intermediates such as 2,4-bis(1,1-dimethylethyl) phenol and 1,2 benzenedicarboxylic acid persisted for longer duration at higher concentration of Chlorpyrifos in MSM. The bioremediation of pesticides by scale up technique using specific microorganism could be effectively used for remediation of pesticide contaminated soil and water.

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