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Degradation of chlorpyrifos by free cells and calcium-alginate immobilized cells of *Pseudomonas putida*

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ABSTRACT

Pseudomonas putida isolated from an agricultural soil was used for degradation of chlorpyrifos in free and immobilized condition. Free cells of Pseudomonas putida were able to show 76% degradation of pesticide at 2% concentration at pH 7, temperature 35℃, 10 ml of inoculum size, shaking speed of 150 rpm and in presence of 200 mg/l glucose and 300 mg/l yeast extract. With further increase in pesticide concentration efficiency of free cells reduced. Efficiencies of the immobilized cells were better compared to free cells. When Ca-alginate immobilized cells were used for degradation studies, 96% degradation was recorded at 2% pesticide concentration. At 10% concentration of pesticide, free cells could show only 43% degradation, whereas immobilized cells could show 63% degradation of the pesticide.

Key Words: *Pseudomonas putida*., ca-alginate immobilization, chlorpyrifos degradation

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INTRODUCTION

Synthetic organophosphates are widely used to control various pests for agriculture and for public health protection and these account for approximately 38% of total pesticides used globally [1]. Continuous and excessive use of these compounds has led to the contamination of several ecosystems in different parts of the world [2, 3, 4, 5]. Microbial degradation of organophosphate pesticides is of particular interest because of the high mammalian toxicity of such compounds and their widespread and extensive use [6]. Chlorpyrifos [O, O-diethyl *O*-(3, 5, 6-trichloro-2-pyridyl) phosphorothioate] is one of the most commonly and widely used commercial organophosphorous insecticides [7, 8]. It was developed by the U.S. Chemical Company Dow Agro Sciences in 1965, and since then it has been widely used in the cultivation of rice, wheat, sugarcane, corn, cotton, tea, fruit trees, vegetables, flowers, livestock, etc [9]. It is effective in controlling a variety of insects including cutworms, corn rootworms, cockroaches, grubs, flea beetles, flies, termites, fire ants and lice [10]. It is available in emulsifiable concentrate, dust, flowable, pellet, spray, granular and wettable powder formulations [11]. Chlorpyrifos is moderately toxic to humans [12]. Poisoning from chlorpyrifos may affect the central nervous system, the cardiovascular system, and the respiratory system [13]. Recent research indicates that children exposed to chlorpyrifos while in the womb have an increased risk of delays in mental and motor development at age 3 and an increased occurrence of pervasive developmental disorders such as ADHD- attention-deficit hyperactivity disorder [14]. Extensive use of chlorpyrifos contaminates air, ground water, rivers and lakes. The contamination has been found upto about 24 kilometers away from the point of use [15].

Considerable amount of work has been done on chlorpyrifos degradation by bacteria [16, 17, 18, 19, 20, 21] and fungi [22, 23, 24] isolated from agricultural soil and other sources.

There are a few reports on biodegradation of organophosphate pesticides using immobilized cells [25, 26, 27, 28] and immobilized enzymes [29, 30, 31]. Chlorpyrifos degradation has been studied by using an immobilized enzyme from *Fusarium* spp. [32].

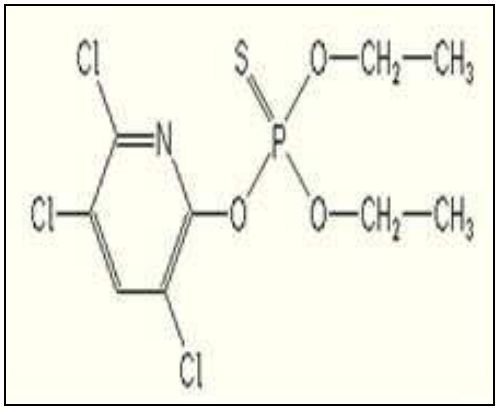
The present study is taken up with the objective of comparing the degradation potential of free and immobilized cells of *Pseudomonas putida*, isolated from a soil amended with the pesticide.

MATERIALS AND METHODS

Pesticide and other chemicals

Commercial-grade insecticide chlorpyrifos (20% EC) was procured from a pesticide selling shop in Bangalore. Other chemicals were procured from Hi-Media Pvt. Ltd. Mumbai.

Chlorpyrifos chemical formula and structure



Growth of the culture

Pseudomonas putida, isolated from an agricultural field with the previous history of pesticide application, identified based on nucleotide sequence and deposited in the gene bank with the accession number JQ701740 was used in the present study. Loopful of the culture was inoculated into 100 ml of mineral salts medium [33] containing 1% chlorpyrifos, 100 mg/lit of glucose and 100 mg/lit of yeast extract with pH 7. Flask was incubated for 18 hours at 37°C in a shaking speed of 150 rpm. After incubation the culture was used as inoculum for degradation studies.

Immobilization of *Pseudomonas putida*

Culture grown in bulk was subjected to centrifugation. 3% wet weight of cell pellet was immobilized using 3% sodium alginate solution. The beads were prepared and stored in 0.2 M $CaCl_2$ solution at 4°C until use [34].

Batch degradation with free cells

Batch degradation of pesticide with free cells was carried out using 100 ml of mineral salts medium [33] containing 10 ml of inoculum ($4x10^2$ cfu/ml), 100 mg/lit of glucose and 100 mg/lit of yeast extract with pH 7 at pesticide concentration varying from 2% to 10%. Flasks were incubated for 24 hrs at 150 rpm shaking speed and at 37°C.

Batch degradation with immobilized cells

For batch degradation with immobilized cells 5 g wet weight of beads ($5x10^2$ cfu/bead) were added to 100 ml of minimal mineral salts medium [35] with pH 7 containing different concentrations of chlorpyrifos varying between 2 to 10%. Flasks were incubated for 24 hrs at 150 rpm shaking speed and at 37°C.

Estimation of chlorpyrifos

Amount of chlorpyrifos in media after incubation was estimated by spectrophotometric analysis at 520 nm [36]. Percent degradation was calculated using the given formula.

Percent degradation = $[(C_0-C_t)/C_0]\times100$ where C_0 = initial concentration and C_t = concentration at time 't' i.e. 24 hrs.

RESULTS AND DISCUSSION

In the current study, free cells of *Pseudomonas putida* showed 76% degradation of chlorpyrifos at the end of 24 hrs with 2% pesticide concentration and as the pesticide concentration increased, the degradation decreased and at 10% concentration of pesticide, only 43% degradation of the pesticide was recorded. Ca-alginate immobilized cells of *Pseudomonas putida* were more efficient in chlorpyrifos degradation compared to free cells. When ca-alginate immobilized cells were used for degradation studies, 96% degradation was recorded at 2% pesticide concentration. 85%, 82%, 72% and 63% degradation was recorded at 4%, 6%, 8% and 10% chlorpyrifos concentrations respectively (Fig 1, Plate 1).

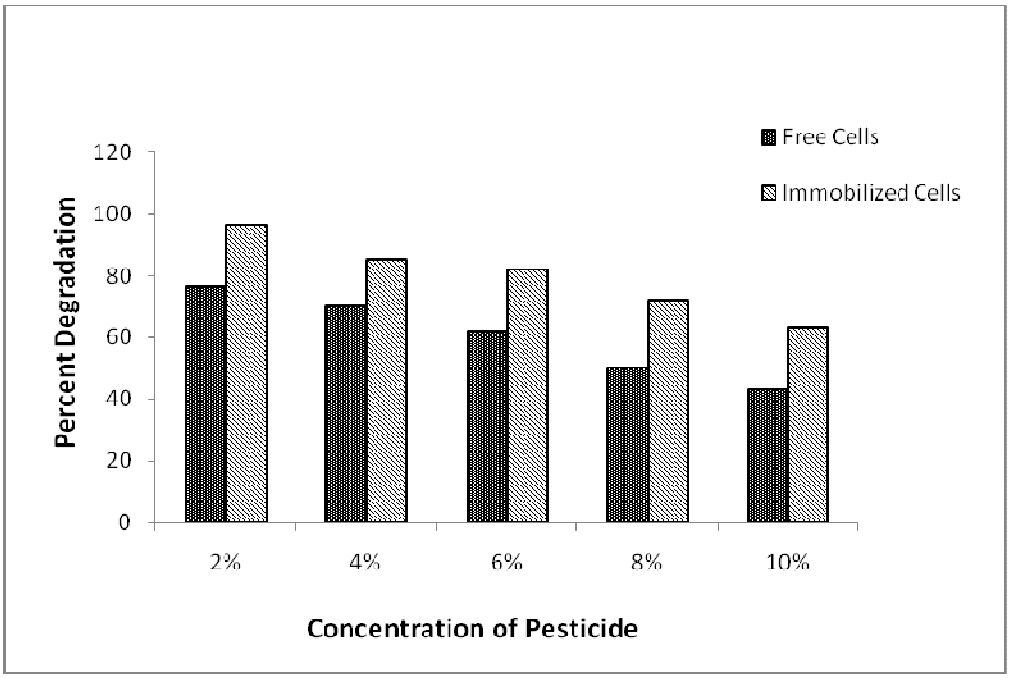


Fig. 1: Batch degradation of chlorpyrifos with free cells and immobilized cells

It was reported by Kim *et al*. [25] that the detoxification rate of organophosphate coumaphos with immobilized cells of recombinant strain of *Escherichia coli* was approximately twice that of freely suspended cells and kinetic studies demonstrated that a higher maximum reaction rate was achieved with the immobilized cell system. The immobilized cells retained their activity over a 4-month period of use and storage, demonstrating both sustained catalytic activity and long-term mechanical stability. Hong *et al.* [27] reported about a genetically engineered strain of *Escherichia coli* that expresses organophosphorus hydrolase (OPH) was immobilized in a polyvinyl alcohol (PVA) cryogel to form a porous biocatalyst that successfully degrades organophosphorous neurotoxins. Immobilization of an enzyme from a fungus *Fusarium* WZ-I for chlorpyrifos degradation was studied by Xie *et al* [24]**.** According to them optimal immobilization of the enzyme was achieved in a solution of 30 g/L sodium alginate at 4ºC for 4-12 hours. The immobilized enzyme showed the maximal activity at pH 8.0, 45ºC.

Plate 1: Ca-alginate beads with immobilized cells of *Pseudomonas putida*

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