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OPTIMIZATION OF ENDOSULFAN DEGRADATION BY *PSEUDOMONAS AERUGINOSA*

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ABSTRACT

A total of 68 isolates capable of degrading Endosulfan were obtained out of 27 samples collected. Among them 28 isolates showed better efficiencies in degrading Endosulfan as compared to others. Isolate ENS10 which showed 42% degradation of Endosulfan under unoptimized condition was identified as *Pseudomonas aeruginosa.* After optimization process it was able to show 100% degradation of chlorpyrifos at pH 7, temperature 35°C, pesticide concentration of 2%, 10 ml of inoculum size, shaking speed of 150 rpm and in the presence of 200 mg/l glucose and 300 mg/l peptone.

KEYWORDS: Endosulfan degradation, optimization, *Pseudomonas aeruginosa.*

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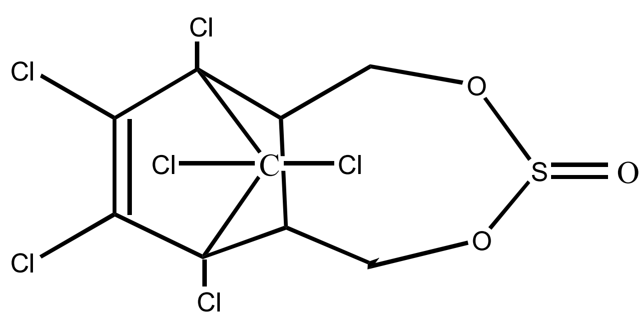
INTRODUCTION

Chlorinated organochlorine pesticides are one of the major groups of chemicals responsible for environmental contamination[1]. These pesticides are chemical pollutants used extensively for agriculture and sanitation purposes in India as these are comparatively cheap and effective[2]. These pesticides are being extensively used in agriculture and disease control purposes for the last more than 50 years across the world and their long persistence in soil has been reported[3]. The most significant properties of the organochlorine insecticides are their extreme lipophilic nature and resistance to biodegradation, which results in their accumulation and concentration in fatty tissues and their extreme persistence in environment[4]. In general, organochlorines cause either CNS depression or stimulation, depending upon the dose[5]. The chief acute toxic action of organochlorine pesticides is on the nervous system, where these compounds induce a hyperexcitable state in the brain[6]. Endosulfan (6,7,8,9,10,10-hexachloro-1,5,5a,6,9,9a-hexahydro-6,9-methano-2,3,4-benzo-dioxathiepin-3-oxide) is a cyclodiene organo-chlorine. It is a mixture of the two isomers, α and β-endosulfan. Both of these isomers are toxic, with the α-isomer being more toxic than β-isomer[7]. It is used extensively throughout the world as a contact and stomach insecticide and as an acaricide on field crops like cotton, paddy, sorghum, oilseeds, coffee, vegetables and fruit crops[8,9]. It is used to control chewing and sucking insects such as Colorado beetle, flea beetle, cabbage worm aphids and leaf hopper[10,11]. It is extremely toxic to fish and aquatic invertebrates[12] and has been implicated in mammalian gonadal toxicity[13], genotoxicity[14] and neurotoxicity[15]. It can provoke chronic symptoms like testicular and prostrate cancer[16], breast cancer and sexual abnormality[17]. There are reports on degradation of endosulfan by bacteria[18,19,20,21,22,23] and fungi[24,25,26,27]. With the aim of isolating efficient strains of bacteria capable of degrading endosulfan and to optimize various parameters for degradation of endosulfan the present investigation has been taken up.

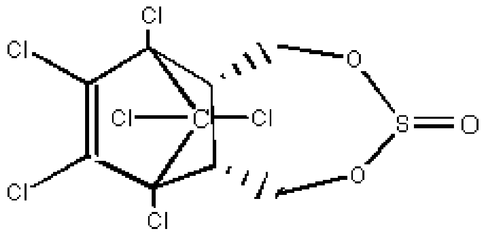
MATERIALS AND METHODS

(i) Pesticide and other chemicals

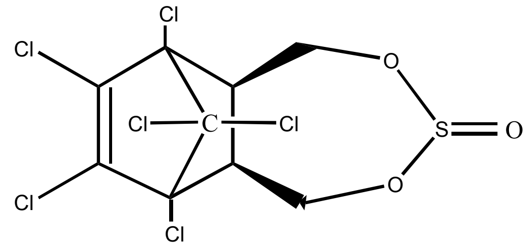
Commercial-grade insecticide Endosulfan was procured from a pesticide selling shop in Bangalore. Other chemicals were procured from Hi-Media Pvt. Ltd., Mumbai.



Structure of Endosulfan



Endosulfan (Alpha Isomer)



Endosulfan (Beta Isomer)

Pseudomonas aeruginosa, with accession number JX204836, isolated and identified from an agricultural soil which showed 42% degradation of endosulfan under unoptimised condition was selected for the present work, the data of which has been presented in our earlier paper[28].

(ii) Optimization of degradation of Endosulfan

Optimization of degradation of endosulfan by *Pseudomonas aeruginosa* with respect to various parameters like pH (between 4 to 10), temperature (30°C to 50°C), inoculum concentration (1 to 25 ml), pesticide concentration (2% to 10%), nutrient sources like carbon (Mannose, Starch, Lactose, Sucrose and Glucose) nitrogen (KNO_3, Peptone, $NaNO_3$, NH_4NO_3 and Yeast extract), aeration and agitation (with different shaking speeds 50 to 250 rpm) was carried out. Degradation was carried out with different concentrations of Glucose (100 mg/lit to 500 mg/lit) and Peptone (100 mg/lit to 500 mg/lit) added to mineral salts medium containing Endosulfan. Degradation efficiency of the culture was obtained by estimating pesticide concentration according to Venugopal and Sumalatha[29]. Degradation of pesticide was carried out under optimized conditions and the result was compared with that under unoptimized conditions.

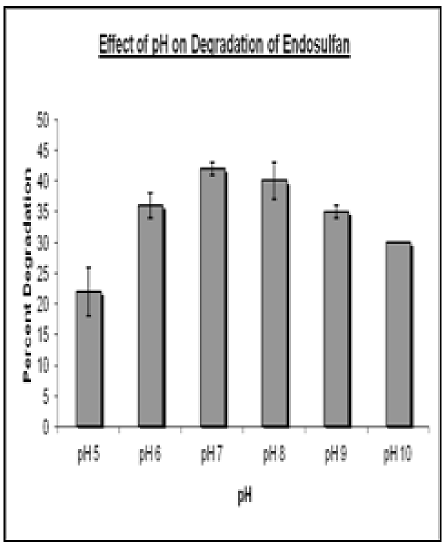
RESULTS

(i) Optimization of degradation of Endosulfan

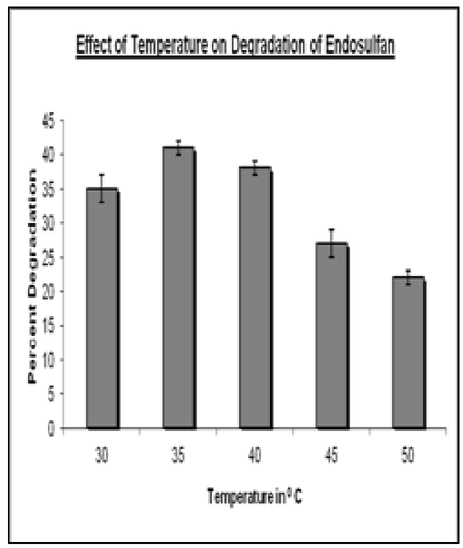
When effect of pH was studied on degradation of Endosulfan by *Pseudomonas aeruginosa* maximum degradation of 42% was seen at pH 7. Least degradation of 22% was seen at pH 5 (Graph 1). *Pseudomonas aeruginosa* showed maximum degradation of 41% at 35^0C. Least degradation of 22% was recorded at 50^0C (Graph 2). Difference in inoculum size had little effect on degradation efficiency of *Pseudomonas aeruginosa*. Maximum degradation of 43% was seen with an inoculum size of 10 ml. A least degradation of 37% was seen with inoculum size of 1 ml (Graph 3). Higher concentration of pesticide resulted in decreased efficiency of *Pseudomonas aeruginosa*. Maximum Degradation of 42% was seen with pesticide concentration of 2%. Least degradation of 15% was seen with pesticide concentration of 10% (Graph 4). With increase in shaking speed percent degradation of chlorpyrifos by *Pseudomonas aeruginosa* also increased upto 150 rpm. Further increase in shaking speed resulted in decreased efficiency of *Pseudomonas aeruginosa*. Maximum degradation of 65% was seen with shaking speed of 150 rpm. Least degradation of 20% was seen with shaking speed of 250 rpm (Graph 5). With the addition of carbon source *Pseudomonas aeruginosa* showed better degradation of endosulfan as compared to that in the absence of any additional carbon source. Maximum degradation of 73% was seen with glucose as the source of carbon. Least degradation of 10% was seen with mannose as the source of carbon (Graph 6). Further when effect of different concentration of glucose was studied, maximum degradation of 97% was seen with 200 mg/l of glucose. With higher a concentration of glucose percent degradation decreased. Least degradation of 38% was seen with 500 mg/l of glucose (Graph 7). Similar results were obtained with the addition of nitrogen source. Maximum Degradation of 78% was seen with peptone as the source of nitrogen. Least degradation of 39% was seen with $NaNO_3$ (Graph 8). When effect of different concentration of peptone was studied, maximum degradation of 100% was seen with 300 mg/l of peptone. Least degradation of 42% was seen with 500 mg/l of peptone (Graph 9).

(ii) Degradation under optimized condition

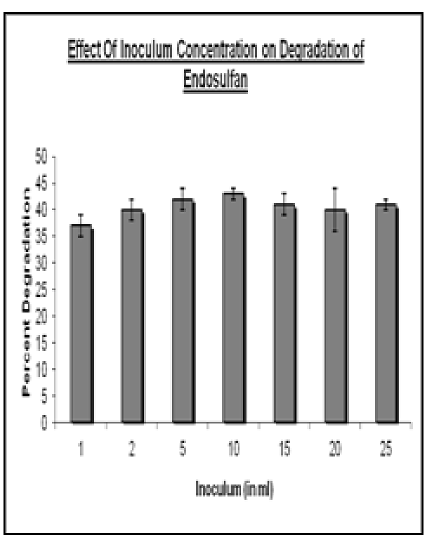
Degradation of 100% was recorded at the end of 24 hrs with 2% pesticide concentration, 10 ml inoculum size, 200 mg/l of glucose and 300 mg/l of peptone, at 150 rpm shaking speed, at 35^0C and at pH 7 as compared to 42% degradation recorded at the end of 120 hrs under unoptimized condition of pH 7, temperature 35°C, 2% pesticide concentration, 5 ml of inoculum size, under static condition and in absence of additional carbon and nitrogen source (Graph 10 and 11).



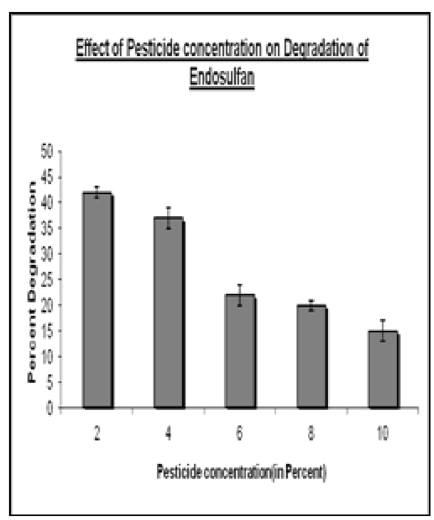
Graph 1: Effect of pH



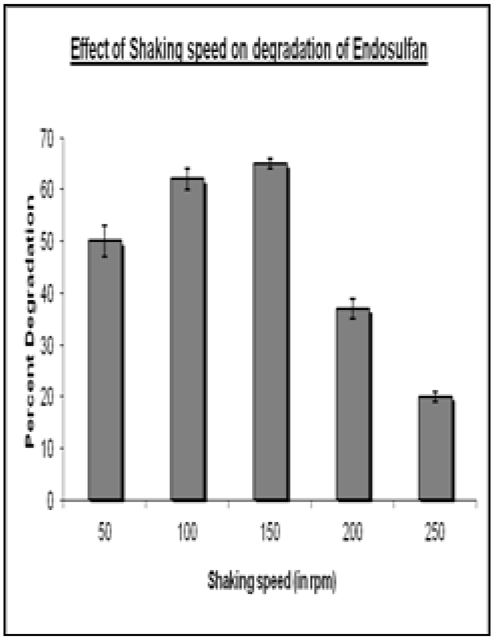
Graph 2: Effect of Temperature



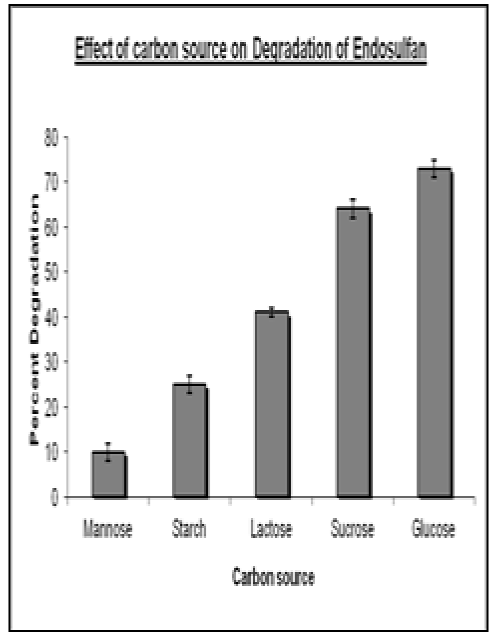
Graph 3: Effect of Inoculum Concentration



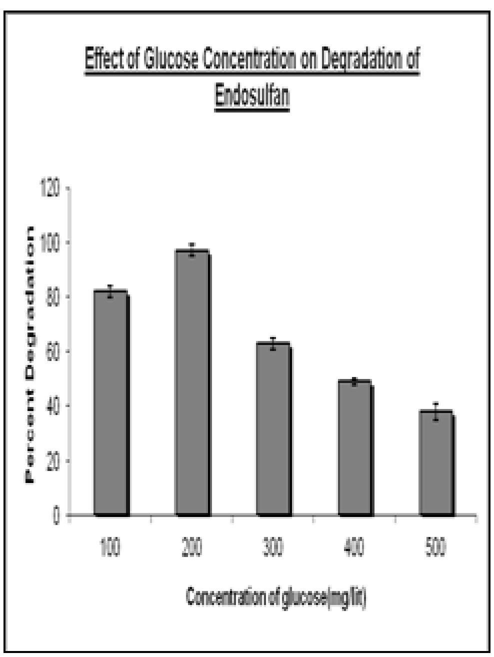
Graph 4: Effect of Pesticide Concentration



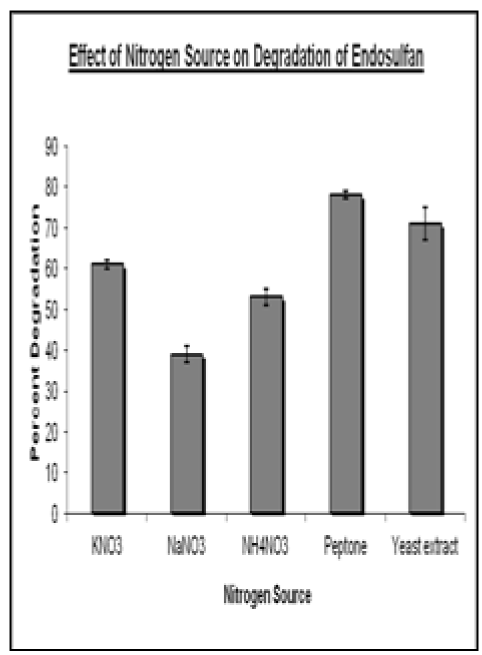
Graph 5: Effect of Shaking speed



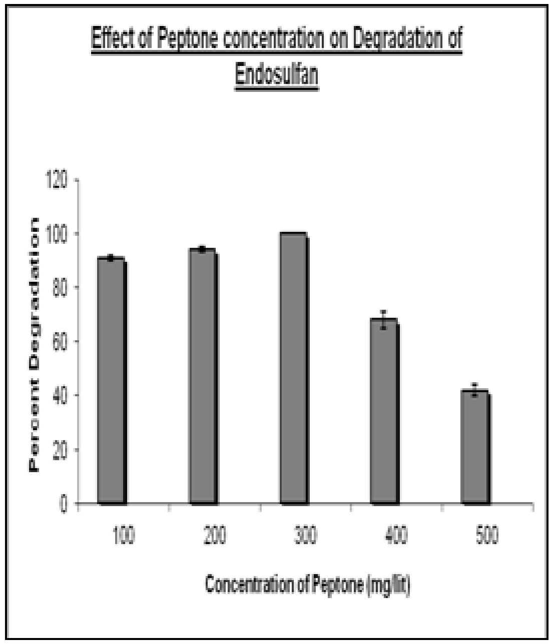
Graph 6: Effect of Carbon source



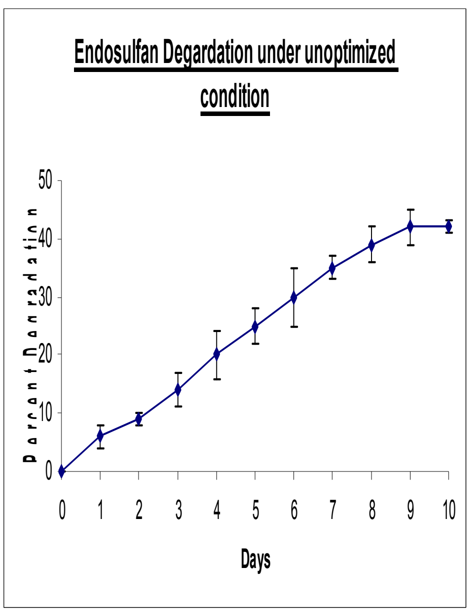
Graph 7: Effect of Glucose Concentration



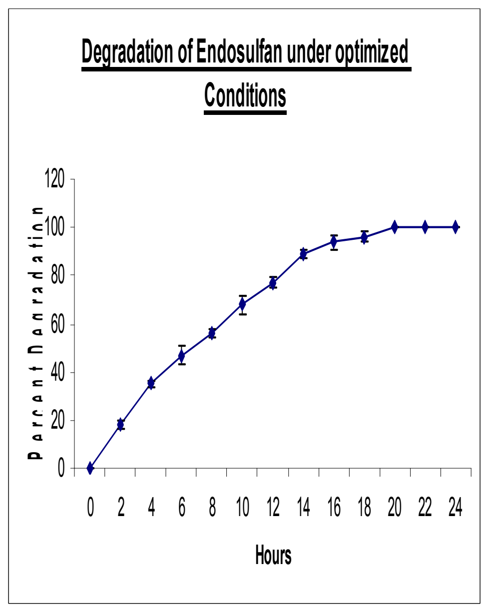
Graph 8: Effect of Nitrogen Source



Graph 9: Effect of Peptone concentration



Graph 10: Degradation under unoptimized condition



Graph11: Degradation under optimized conditions

DISCUSSION

Several species of *Pseudomonas* have been studied for degradation of endosulfan[30]. Efficient degradation of Endosulfan using *Pseudomonas aeruginosa* has been carried out[23,30, 31,32]. *Pseudomonas aeruginosa* grows best at neutral pH and at mesophilic range of temperature. Optimum degradation of endosulfan by *Pseudomonas aeruginosa* was observed at pH 7, temperature 35 °C, pesticide concentration of 2%, 10 ml of inoculum and shaking speed of 150 rpm. Degradation was enhanced in the presence of 200 mg/l of glucose and 300 mg/l of yeast extract. Arshad *et al*[23], carried out Optimization of environmental parameters for biodegradation of alpha and beta endosulfan in soil slurry by *Pseudomonas aeruginosa*. Parameters that were investigated included soil texture, soil slurry: water ratios, initial inoculum size, pH, incubation temperature, aeration, and the use of exogenous sources of organic and amino acids. The results showed that endosulfan degradation was most effectively achieved at an initial inoculum size of 600 µl (OD = 0.86), incubation temperature of 30^0C, in aerated slurries at pH 8, in loam soil. Under these conditions, the bacterium removed more than 85% of spiked α and β endosulfan (100 mg/1) after 16 days. Decrease in degradation efficiency of *Pseudomonas aeruginosa* was observed at higher speed of shaking condition. This could be due to less contact between the pesticide and the culture. Similar results in higher concentration of glucose and yeast extract could be due to preferential utilization of the same as compared to that of the pesticide. Under unoptimized condition *Pseudomonas aeruginosa* showed highest degradation in 10 days whereas under optimized condition culture was able to show better efficiency within 24 hrs.

CONCLUSION

Pseudomonas spps. are efficient organisms for degradation of xenobiotics. There are a few reports to say that *Pseudomonas* spps. can degrade organochlorine pesticides including endosulfan. In the current investigation *Pseudomonas aeruginosa* is an efficient degrader of endosulfan and can be used for bioremediation of pesticide contaminated soils.

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