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Biodegradation of Endosulfan Using Microbial Culture

Priyanka Sharma[1], Santosh K.Sharma[2], Anil Sharma[2], Ashutosh Sharma[2] and Pradeep Paraher[3]

[1]*Ultra International Limited, Ghaziabad*
[2] *University of Rajasthan, Jaipur*
[3] *Govt.P.G College, Kotputli*

***Abstract*- Endosulfan a non systematic insecticide and accaricides with contact and stomach action. It is used in the control of sucking, chewing and boring insects and mites on a very wide range of crops, including fruits. In the present study, the biodegradation of endosulfan has been studied. The bacterial culture isolated from garden soils has been used for degrading the endosulfan. This bacterial culture has degraded up to 70% of alpha and 82% of beta endosulfan with identifying intermediate compound endodiol was identified I the degradation.**

***Keywords* - Endosulfan, Biodegradation, pesticide.**

I. INTRODUCTION

Thousands of manmade chemicals are produced every year by chemical industries, a good number of which are subsequently released into the environment. One of the major problems being faced by the industrialized world today is the contamination with pesticides and their toxic intermediates of the soil, ground water, sediments, surface water and air. Significant environmental contamination has occurred in the past and will probably continue to occur in the future. Regulatory steps have been implemented to reduce or eliminate the production and use of these chemicals [1].

Pests cause a reduction in size, yield, storage and market quality of crops and food, and also serve as vectors in the spread of diseases. To control pests, a series of chemicals are produced called pesticides [2]. According to the Van Nostrand's Scientific Encyclopedia (1958 edition), a pesticide is defined as " any substance or a mixture of substances used in the production, storage or transportation of food which is intended for preventing, developing, destroying, repelling or mitigating any insects, rodents, fungi or weeds and other forms of plant or animal life or viruses.. Pesticides have brought about the green revolution in the world and being widely used to control agriculture pests causing public health problems [3]. Pesticides when applied correctly have enormous benefits in increasing crop yield and improving the quality of agriculture products. Regressively there is a considerable over- use of pesticides which is responsible for environmental contamination [4].

Pesticides are evil of necessity, which at no cost can be excluded from an effective plant protection programme. However, the adverse effect on human health and wild life are not beneficial in the long term. The side effects attributed to the use of these chemicals, are the destruction of natural biotic balance, suppression of bio control agents, insects resurgence, pesticide resistance, aquatic fauna and wild life etc.

The toxicity of pesticides is affecting the whole chain of air water and plants. There is a large body of evidence detailing the side effects of pesticides on biotic component of environment including birds, mammals, fish, reptiles, soil micro-organism and invertebrates. The primary problem arises when organochlorine insecticides transport into the aquatic environment. Most chemicals used are not selective but are generally toxic to many nontarget species including man and other desirable forms of life that co-inhibit the environment. Toxicological monitoring and evaluation of the hazards in the handling and use of pesticides have, for many years, focused primarily on preventing injury to man. The

behaviour and characteristics of pesticide present in the environment are responsible for ecological risk.

A proper solution to pesticide contamination is required. Many physio-chemical treatments like flocculation, photooxidation, reduction etc. are given to damage pestisides but these treatments are not very compatible. In many cases materials are themselves added to the system which damage pesticides. The biotechnological approach i.e. biodegradation, aims at converting organic pollutants into innocuous end products compatible with the ecosystem is currently in practice [5].

Degradation is the reduction in complexity of chemical by physic-chemical and/or biological processes when the degradation is mediated by biological agents, especially microorganisms it is termed as biodegradation. Biodegradation may be considered as the conversion of a complex organic material by biological agencies into one or more simpler substances. Alexander defines biodegradation as "the biologically catalyzed reduction in complexity of chemical". Diverse ranges of pesticides have been degraded using a variety of selectively cultured microorganisms [6].

Endosulfon is synthetic chlorinated cyclodiene that is an environmental endocrine disruptor. It was introduced into the earth's environment in 1956. It possesses a relatively broad spectrum of activity [7]. Technical grade endosulfan is a mixure oof two sterioisomeres Alpha and beta-endosulfan in a ratio of 7:1 [8]. It also acts as a poison to a wide variety of insects and mites ion contact. During the study, the efforts have been made to develop a microbial system for biodegradation of endosulfan and evaluation of genotoxicity potential of endosulfan.

II. MATERIAL AND METHODS

Selection of micro-organism for degradation of endosulfan

About 10 gms of garden soil collected from NEERI, Rose Garden , was added in 100 ml of basal salt medium with 10 ppm endosulfan and kept in orbital shaker for overnight, then centrifuged at 6000g for 10 minutes. The supernatant was collected and was further inoculated in basal salt media with 20mg/l endosulfan. The microorganisms capable of growing in basal salt medium containing endosulfan were further grown in enrichment media containing 25mg/l of endosulfan stock. This was then used for further endosulfan degradation studies.

Culture set up of biodegradation studies

Experiment were performed in flasks containing different concentrations as 50 mg/l and 100 mg/l for endosulfan. These concentrations of endosulfan were inoculated with 0.10 O.D. of mixed culture and incubated at 37^0 C and kept in the shaker at 150 rpm. The cultures were withdrawn at different time intervals and analysed for using UV spectrophotometer at λ max 600 nm. The biodegradation of endosulfan was monitored by GC and GC/MS analysis and the metabolites formed in the process of biodegradation were identified.

III. RESULTS & DISCUSSION

The bacterial densities in the liquid medium were monitored spectrophotometrically at every 24 hrs. There was considerable increase in OD at 600 nm upto 7^{th} day suggesting the growth of microorganisms due to glucose as carbon source. However, from 8^{th} day onwards, there was a decrease in ODs as only those microorganism will remain alive which are capable to of utilize in endosulfan as the sole source of carbon. Optical densities of culture measured at λ max 600 nm ranged between 0.030 to 0.0856 for 50 ppm and 0.032 to 1.821 for 100 ppm which are shown in Table-I.

As endosulfan and its chlorine containing metabolites are strongly electronegative. Therefore GC with electron-capture detector was used for monitoring the degradation. On analyzing the samples on GC initially on the day two peaks were seen. The larger peak was seen at the retention time of 1.58 minute with the smaller peak at 2.08 minutes. The peak at 1.58 minute was found to be that alpha-endosulphan and the smaller peak at 2.08 minute that of beta-endosulfan on comparing with respective standards. On injecting the 7^{th} day samples, there was a drastic reduction in the peak heights of both the peaks as shown in Fig. 1.

Whether reduction was because of degradation or adsorption was further studied as it has been reported that endosulfan is strongly adsorbed by microorganisms, with the majority of the insecticide being associated with the cell membrane [9].

Awasthi *et.al.* [10] studied the biodegradation of endosulfan by an isolated bacterial co-culture. Substantial i.e. about 50% degradation of both the isomers was seen in 7 days and almost all the pesticide was degraded by 15^{th} day. Degradation of beta-endosulfan appears to be slower than alpha-endosulfan. Increase in chloride ions was also observed. 50% degradation of alfa-endoslfan, beta-endosulfan and endodiaol individually was observed in 7 days. The degradation of soil bound endosulfan was slower by nearly fourfold than in culture medium. Endosulfan degradation was observed upto 3 weeks. Awasthi et al. also did further studies on biodegradation of the soil applied endosulfan in the presence of biosurfactant [11].

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Table I

Growth of micro-organisms at OD 600 nm

Time (Days)	50 mg/l	100 mg/l
0	0.030	0.032
1	0.100	0.129
2	0.521	0.690
3	0.742	1.271
4	0.986	1.441
5	1.120	1.702
6	1.171	2.100
7	1.180	2.201
8	0.986	2.111
9	0.671	1.700
10	0.527	1.444
11	0.621	1.698
12	0.670	1.818
13	0.802	1.993
14	0.881	2.099
15	0.856	1.821

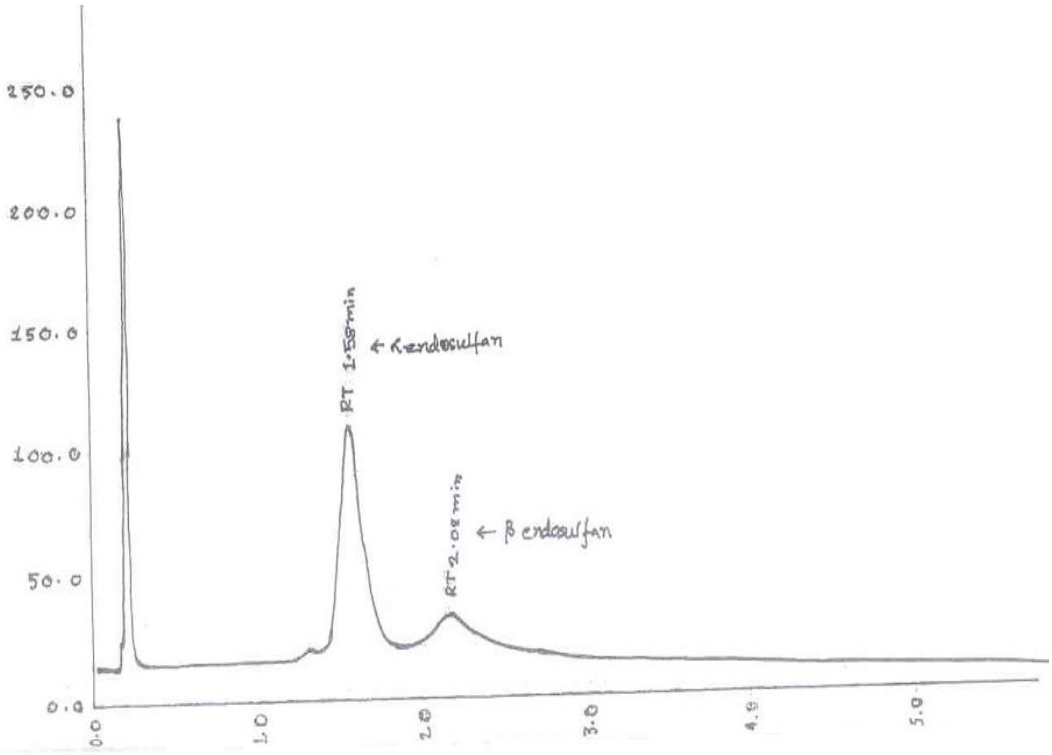

Fig.1 : 7 days samples reduced peak of α-endosulfan and β- endosulfan