International Journal of Science and Research (IJSR)
ISSN (Online): 2319-7064
Index Copernicus Value (2013): 6.14 | Impact Factor (2013): 4.438

Optimization Studies on Degradation of Endosulfan by Indigenous Soil Bacterial Isolates

Jayanthi P[1], Prabhu S[2]

[1]Department of Environmental Sciences, Periyar University, Salem – 11, Tamilnadu, India

[2]Department of Microbiology, K S Rangasamy College of Arts and Science, Tiruchengode - 15, Namakkal, Tamilnadu, India

Abstract: ***The study aimed at optimizing suitable conditions which are favorable for effective degradation of the endosulfan residues. The work involved isolation of bacterial species from two different soils (Red and Black soils) which is subjected to repeated applications of endosulfan. The isolates from the soils were differentiated with morphological and biochemical studies and checked for the degradation potential. Four isolates RS1, RS2, BS1 and BS2 were isolated and checked for their degradation potential at different pH (pH 5.5, pH 7.6, pH 8.5), different temperature (Room and Incubation temperature) and different concentrations (80, 100, 120mg/l) respectively. The results showed varying degrees of degradation. The optimal conditions which showed effective degradation with comparison with all the parameters were at pH 7.6 and at incubation temperature over the concentration of 80mg/l. All the four isolates showed the same pattern of degradation and there is no significant variation between them. Thus the outcome of this work may have practical applications in bioremediation of endosulfan contaminated soil.***

Keywords: Endosulfan, Biodegradation, Indigenous Bacteria, Optimization studies.

1. Introduction

Bioremediation has become an important tool to clean up the pesticide contaminated soil and water [1, 2]. In this process microorganisms use xenobiotics (pesticides) as a source of energy and nutrients mediate the transformation of hazardous chemicals to less toxic and more environmentally acceptable compounds. Biological transformation which involve enzymes as catalyst frequently bring about modifications in structure and other toxicological properties of the contaminants and may result in the complete conversion of organic molecules to innocuous inorganic end products.

Though endosulfan is banned for use in India, still its residues pose serious threats to environment. Hence its degradation becomes imperative. Microbial metabolism of endosulfan may lead to form endosulfan sulfate through oxidation or endosulfan diol through hydrolysis [3, 4, and 5]. Endosulfan sulfate is persistent and as toxic as endosulfan [6]. Endosulfan diol is less toxic and may further metabolize to endosulfan ether endosulfan hydroxyether endosulfan dialdehyde and endosulfan lacton [7]. Likewise the persistence and degradation of endosulfon is also affected by the environmental conditions in which it is found. The environmental conditions have profound effects on the resident population of microorganisms, the rate of biochemical transformation and the persistence of the product of biodegradation [8].Environmental parameters like temperature, redox potential, moisture, soil texture and composition have been found to affect the biodegradation of pesticide in soil [9,10]

Thus, Endosulfan is a persistent organic pollutant (POP) that enters the air water and soil during its use and manufacturing. Scientists have been researching ways to eliminate this neurotoxin safely and effectively. Since microbial degradation offers the most attractive and cost effective remediation approach thus this study was undertaken to develop bioremediation strategy to dissipate endosulfan from the contaminated soil and water environments.

2. Materials and Methods

Soil samples (Black and Red soil) were collected randomly from two different sites at Kallakurichi, Tamilnadu, which is subjected to repeated endosulfan applications. These sites are having average temperature range from 25-40ºc. Soil samples were collected from depth of 15cm. The collected samples were dried passed through 5/2mm sieve and subjected to further studies. About 15 gm of the collected soil sample was weighed and then dissolved in 85ml of distilled water from this 0.1ml of the sample was poured in to the nutrient agar plates, spread and incubated at 37ºC for 48 hours. Colony with distinct morphology and repeatability was picked and streaked to get single isolated pure cultures.

The isolated colonies were subjected to Gram staining, biochemical test, and enzymatic reactions to differentiate the organisms with their morphological and biochemical characteristics and were named as RS1, RS2, BS1, BS2, which were maintained in nutrient agar slants.

The isolated pure cultures were checked for their biodegradation potential at varying concentration of endosulfan. Based on this the concentration limits for degradation was estimated as 80, 100 and 120mg/l respectively. The optimization studies for these isolates were conducted at different pH (5.5, 7.6, 8.5), different temperature (room temperature and incubation temperature) and at different concentration (80mg/l, 100mg/l, 120mg/l). These studies were carried out by employing Burkes Mineral Medium. The degradation potential has been estimated by the change in absorbance values at 600 nm and expressed as percentage degradation.

3. Results

The biodegradation potential of the four isolates RS 1, RS 2, BS 1 and BS 2 collected from endosulfan soils were studied under different optimizing conditions such as pH, temperature and concentration. The commercial technical grade endosulfan in the name of Endocel was used in the present study. The results showed varying levels of degradation under different optimizing conditions. The results were expressed as percent degradation by observing the difference between the optical density at the initial and final stage of observation. (Fig 1-6).

3.1. Effect of pH

The better degradation of endosulfan by all the isolates were found at the pH 5.5 ranging from 10% to 81% with the lowest at pH 7.6 ranging from 6% to 70% respectively. Among the different isolates RS 1 and RS 2 showed effective degradation when compared to BS 1 and BS2. When the degradation potential of all the isolates were varying considerably, the isolates BS 1 and BS 2 showed the constant degradation between 50% to 90% which is found to be the highest among all. Thus pH is having a varying effect with the different isolates over the degradation of the highly resistant chemical endosulfan.

3.2. Effect of Temperature

Two temperature parameters, Room temperature and Incubation temperature were set to conduct the optimizing studies for endosulfan degradation. Obviously, the incubation temperature showed better degradation than the room temperature ranging from 22% to 90%. Among the isolates BS 2 and RS 2 was found to have potential degradation effect when compared to BS 1 and RS 1. The variation of the degradation percent between the room temperature and incubation temperature is highly significant. Again similar to pH, this isolates RS 1 and RS 2 showed a constant raise in its activity when kept at incubation temperature ranging from 50% to 90%.Thus as a whole incubation temperature was a profounding effect in enhancing the degradation potential by all the cultures.

3.3. Effect of concentration:

Three concentrations such as 80mg/l, 100mg/l and 120mg/l of broth has been prepared to estimate the degradation potential by the selected isolates. These concentrations were selected on the basis of preliminary study conducted to assess the potential of the four isolates. It showed varying degradation pattern at different concentration. The highest degree being 80mg/l which at recorded 90% by the isolate BS 1. Similarly RS1 and RS2 also showed the higher degradation of 80% and 75% respectively at 80mg/l rather than 100mg/l and 120mg/l. Overall the lower concentration range naturally showed better degradation than the higher concentration with respect to all the isolates. But again the pattern is varying greatly within different concentration range. Graphs showing degradation percentages by the isolates

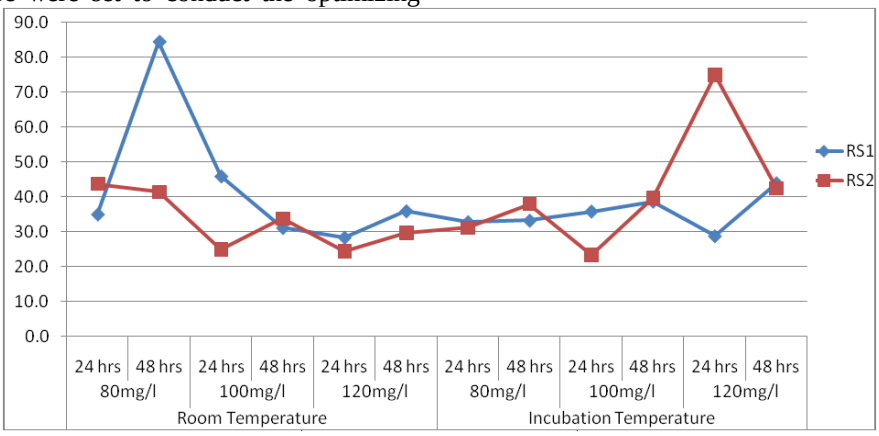

Figure 1: Red soil Isolates at pH 5.5

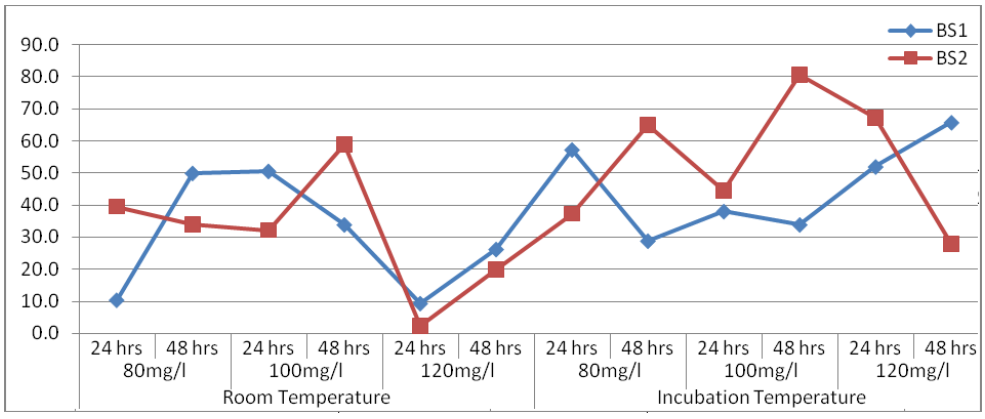

Figure 2: Black Soil Isolates at pH 5.5

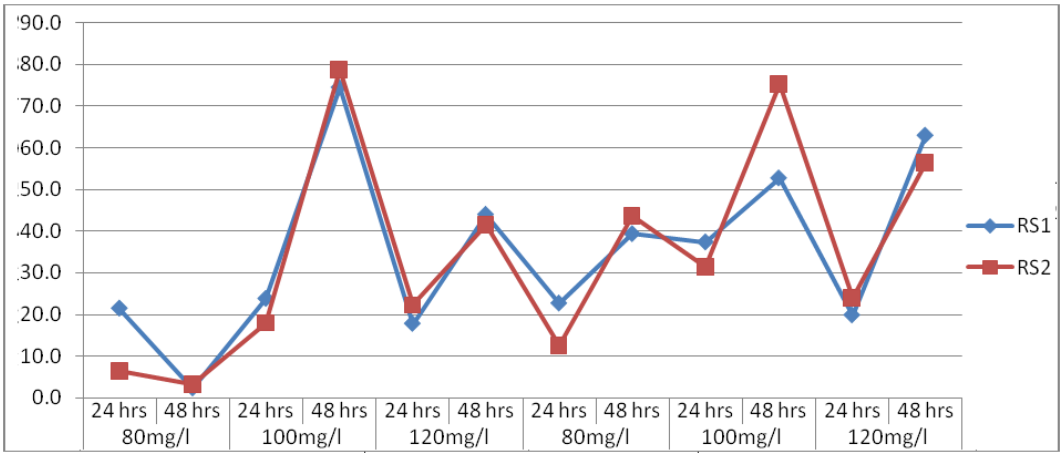

Figure 3: Red Soil Isolates at pH 7.6

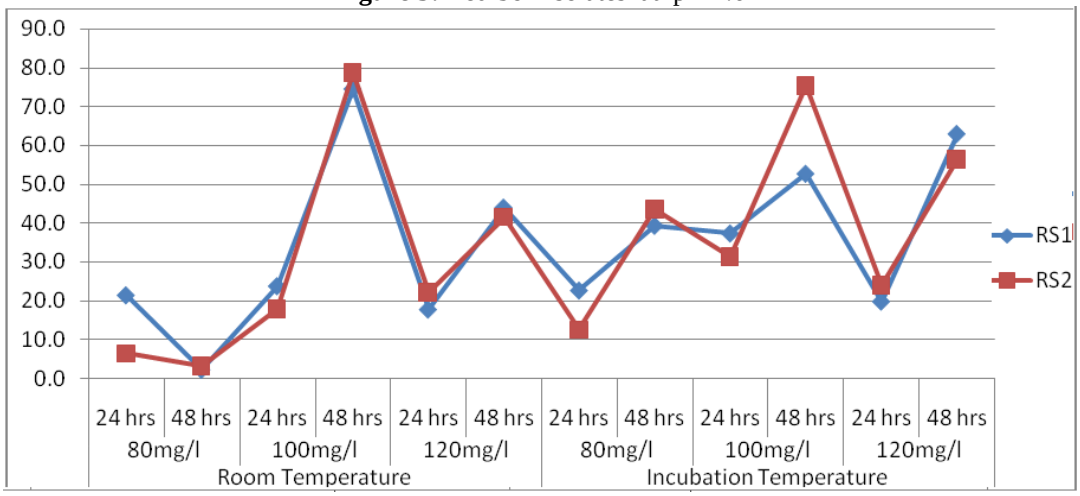

Figure 4: Red Soil Isolates at pH 7.6

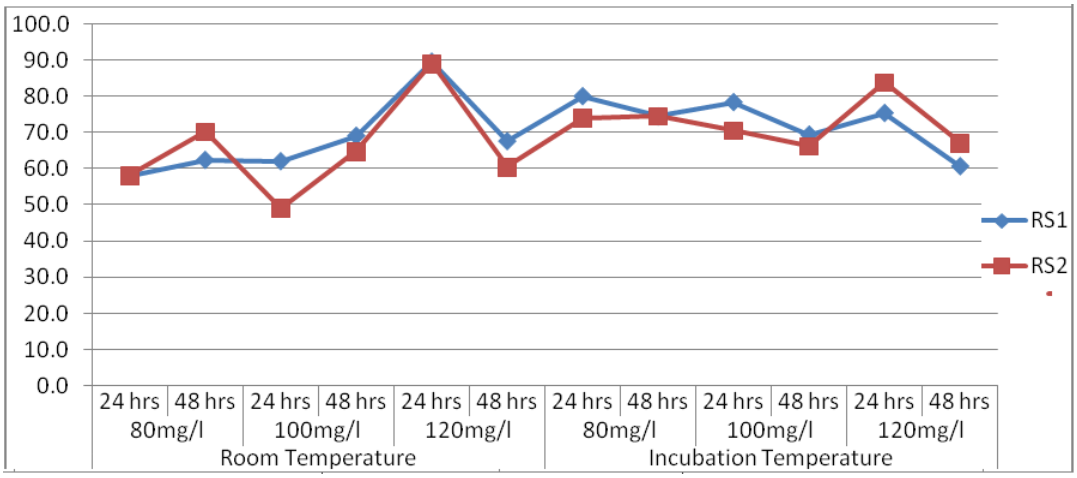

Figure 5: Black Soil Isolates at pH 8.5

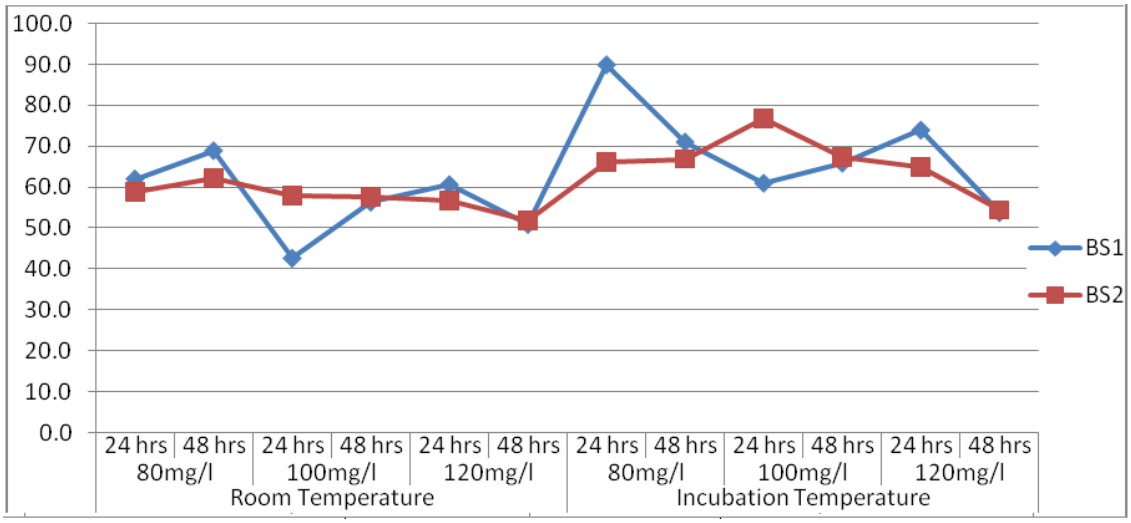

Figure 6: Black Soil Isolates at pH 8.5

Thus all the parameters (pH, temperature and concentration) are having various degrees of biodegradation potential for all the isolates.

4. Discussion

Cleaning of the endosulfan contaminated soil through biological means offers a lost effective and environment friendly strategy. The persistence and degradation of endosulfan is affected by the environmental conditions in which it is found. The environmental conditions have profound effects on the resident populations of microorganisms, the rate of biochemical transformation and the persistence of the product of biodegradation [5]. Environmental parameters like pH, temperature and the concentration of the chemical applied have been found to affect the biodegradation of pesticide in soil. Therefore it is highly desirable to determine optimal environmental parameters for successful bioremediations.

4.1. Effect of pH

The pH factor plays a critical role in determining the degradation pattern. Manonmani [11] has investigated the effect of wide range of pH on biodegradation of HCH which is similar to endosulfan by the microorganisms. They reported that slightly acidic to neutral pH (6.0 to 8.0) favoured the growth and biodegradation. The same factor was obvious in the present study. Awasthi *et al.*, [10] also reported the biodegradation of endosulfan is better pronouncing at neutral pH 7.0 and alkaline pH 8.5. The same condition was observed for the isolates RS1, BS1 in the present study. However RS2 and BS2 also recorded higher degradation potential at the pH of 7.6 and 8.5 rather than at the pH 6.5. Thus, neutral pH and increase towards alkaline pH favours the bacterial degradation of endosulfan which is evident from the present study.

4.2. Effect of temperature

Similar to pH, temperature also has its own significant over the degradation of endosulfan. In the present study, room temperature and incubation temperature were set for degradation. Okeke *et al.*, [12] reported that the temperature factor is the main cause for the redox reaction which is resulting in structural change and there by resulting in degradation or conversion of its metabolites. He also reported that about 64% of degradation was possible at 25^0c whereas it can be increased at incubation temperature. Guerine *et al* [13] has explained the effectiveness of microbial metabolism at incubation temperature to degrade any persistent chemical. In line with that the present study also indicates the maximum degradation has effected at incubation temperature for all the isolates.

4.3. Effect of Concentration

The persistence of endosulfan in the soil has a high correlation with the concentration applied to the soil. As reported earlier 99.9% of the applied chemical finds its way to the soil and results in contamination [14, 15]. Aswathi *et al.*, [10] has reported that the rate of biodegradation progressed with the increase in endosulfan concentration up to $5mg/g^{-1}$ of soil. But the present study involves 0.018 to $0.12mg/g^{-1}$ soil concentration of the pesticide. It is also reported that the inoculums size is also a determining factor in this process. Kumar and Philip [5] has reported that the bacterial consortium is able to degrade 71.5% of endosulfan with a concentration of 50mg/l. In comparison, the present study showed higher degradation present at 80mg/l followed by 100 and 120mg/l to all the isolates. Thus higher the concentration the lesser the degradation potential was observed within the present study. Thus the environmental factors such as pH, temperature and concentration can plays a vital role for the effective bacterial degradation the more persistent endosulfan.

5. Conclusion

The residues of endosulfan are major environmental contaminants in several countries of the world. They are potentially hazardous to living systems because of their inclination to bioaccumulation and resistance to biodegradation. It is hence imperative develop biodegradation method to cleanup the contaminated soil. The optimal conditions which showed effective degradation of

endosulfan by the indigenous bacterial isolates, when compared with all the parameters were at pH 7.6 and at incubation temperature over the concentration of 80 mg/l. All the four isolates showed the same pattern of degradation and there is no significant variation between them. Thus the outcome of this work may have practical applications in bioremediation of endosulfan contaminated soil.

References

[1] Karpouzas.D.G., J.A.Morgan and A.Walker. 2000. Isolation and characterization of 23 carbofuran-degrading bacteria from soils from distant geographical areas. Lett. Appl. 31: 353-8.
[2] Jilani.S. and M.A. Khan.2004. Isolation, Characterisation and growthresponse of pesticides degrading bacteria. J.Biol Sci. 4(!):15-20
[3] Sutherland .T.D.,I.Horne .M.J.Laccy .R.L.Harcourt .R.L.Harcourt, R.J. Russell and J.G.Oakeshott, 2000. Environment of endosulfan – degrading mixed bacterial culture, Appl. Environ. Microbial, 66: 2822-2828.
[4] Kaur.I., R.P. Mathur . S.N.Pandon.P.Dureja.1998. persistence of endosulfan (technical) in water and soil. Environ.Technol.19:115-119.
[5] Kumar.M and L.Philip .2006. Bioremediation of endosulfan contaminated soil and water – optimization of *operating* condition in laboratory scale reactors.J. Hazard. Material.136: 354-64.
[6] Sutherland .T.D.,I.Horne, K.M.Weir, R.J. Russell and J.G.Oakeshott, 2004. Toxicity and residues of endosulfan isomers. Rev. Environ. Contam. Toxicol. 183: 99-113
[7] Katagi.T.2006. Behavior of pesticides in water-sediment systems. Rev.Environ.contam.Toxicol.87:133-251.
[8] Kumar.M. and L.Philip. 2006a. Adsorption and desorption characteristic of hydrophobic pesticides endosulfan in four Indian soils. Chemosphere.62: 1064-1077
[9] Wang, T.Y., Y.L. Lu, R. W. Dawson, Y.j. Shi, H. Zhng, Y, Xing, 2006b. Effects of environmental factors on organochlorine pesticide residues in soils of the Guanting reservoir area. China. J. Environ. Sci. Health. Part B: Pestic . Food Contam. Agric. Wastes. 41: 309-321
[10] Aswathi. N., A. K. Singh, R. K. Jain. B. S. Khangarot and A. Kumar. 2003. Degradation and detoxification of endosulfan isomers by a defined co-culture of two Bacillus strain. Appl. Microbiol. Biotchnol. 62: 279-283.
[11] Manonmani K. 2011. Bioremediation of Hexachlorocyclohexane Contaminated Soil: Field Trials *In:* Pesticides in the Modern World - Pesticides Use and Management Edited by Dr. Margarita Stoytcheva; Intech publishers:475-504.
[12] Okeke IN, Lakinmanra A and Edelman R. 2001 Dissemination of antibiotic-resistant bacteria across geographic borders. Clinical Infectious Diseases. ;33:364–369
[13] Guerin.T.P and I.R. Kennedy.1992.Distribution and dissipation of endosulfan and related cyclodienes in sterilr aqueous systems: implications for studies on biodegradation. J.Agric.Food Chem.40: 2315-2323.
[14] Wilkerson. R., L.D. Northington and W. Fisher, 2005. Ingestion of toxic substances by infants and children what we don't know can hurt. Crit. Care Nurse. 25(4):35-44.
[15] Lee.S.E., J.S.Kim, I.R.Kennedy. J.W. Park. G.S. Kwon, S.C.Koh and J.F.Kim. 2003. Biotransformation of an organochlorine insecticide endosulfan.by *Anabena* sp. J.Agric. Food Chem.51: 1336-1340.