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Biodegradation of organochlorine pesticides endosulfan in soil and water environments

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ABSTRACT

Extensive applications of persistent organochlorine pesticides like endosulfan on cotton have led to the contamination of soil and water environments at several sites in India; Microbial degradation offers an effective approach to remove such toxicants from the environment. Several strains of Bacteria and fungi were isolated from endosulfan contaminated soil.

Keywords: - endosulfan in soil, accelerated biodegradation, organochlorine pesticides, aspergillus terricola, soil bacteria, fungal isolates, pseudomonas aeruginosa

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INTRODUCTION

India is actively involved in agriculture practice, planting oil palm, paddy, fruit, vegetables and others for both local consumption and export purposes. In order to achieve the objectives such as to maintain the quantity and quality of agriculture productions, pesticides are used in agriculture sector as a mean of pest control for sustainability of the industry. In India, the annual pesticides sales figure exceeds 300 million. It is estimated that annual crop losses in our country could exceed 30% without pesticides. On the other hand, insects, weeds, fungi, viruses, parasites, bird's and rodents consume or destroy approximately 48% of the world's annual food production.

METERIALS AND METHODS

Enrichment culture technique was used for isolation of microbial strains capable of utilizing endosulfan as a sole source. Microbial inoculate for enrichment studies were prepared by shaking 20 g of soil sample over night in 100 ml nutrient culture medium at 30 °C and 150 rev min^{-1}. The solid particles were allowed to settle for an hour and aliquots of supernatant was separated through paper filtration and used for inoculation of Non Sulfur Medium (NSM)/ endosulfan enrichment medium. The isolated cultures were again inoculated onto sterilized NSM/ endosulfan enrichment medium followed by incubation at 30 ^{0}C temperature and orbital shaking (150 rev min^{-1}) for two weeks. The NSM consisted of (g l^{-1}): K_2HPO_4, 0.225; KH_2PO_4 0.225: NH_4Cl. 0,225: $MgCl_26H_2O$, 0.845:$CaCO_3$, 0.005; $FeGl_2$, $4H_2O$, 0.005; D-glucose. 1.0: and 1 ml of trace element solution per liter. The trace element solution prepared for NSM contained (in mg l^{-1}): $MnCl_24H_2O$, 198; $ZnCl_2$. 136: $CuCl_2$, $2H_2O$, 171; $CoCl_26H_2O$, 24: and $NiCl_2$-$6H_2O$, 24). The pH of NSM was adjusted to 7.0. Nutrient culture media and Erlenmeyer flasks of 50 ml were autoclaved for 20 minutes at 121 ^{o}C temperature separately. To each sterilized flask, endosulfan already dissolved in acetone was added aseptically to attain a final concentration of 100 mg l^{-1} in a laminar flow hood for 10 min to allow acetone to evaporate. Nine milliliters of nutrient culture media and one

milliliter of supernatant solution from source flask were added to spike flasks. These flasks were incubated on orbital shaker (150 rev min^{-1}) at 30 °C for 10 days. There after. 0.1 ml of culture was transferred into 10 ml of fresh sterile NSM enrichment media containing 100 mg 1^{-1} endosulfan and further incubated for 15 days.

The isolates demonstrating prolific growth were investigated for their capability to degrade endosulfan over the time (at 3, 7 and 14 days of incubation). For this purpose, 100 ml Erlenmeyer flasks and medium (NSM) were autoclaved at 121°C for 20 minutes separately. The nutrient medium was adjusted to a pH of 8.2. To each flask, 50 ml of autoclaved media were added. These flasks were spiked with endosulfan to a concentration of 100 mg 1^{-1}. These flasks were inoculated with 800 µl bacterial inocula adjusted to a set optical density of OD595 =0.81. These inoculated flasks (control) were incubated at 30 ^{o}C an orbital shaker at 150 rev min^{-1}. Uninoculaled flasks were also prepared to check the biotic degradation under the same conditions. This procedure was earned out in triplicate and results are means of the three.

Table-1 Biodegradation of spiked α-and β-endosulfan in the broth by various soil bacteria (values are average of 3 repeats: standard error of means).

S.No.	Isolates*	%degradation of α-endosulfan			%degradation of β –endosulfan		
		3d	7d	14d	3d	7d	14d
0	Control	-	9.5±1.5	21.4±2.8	-	7.8± 1.4	19.4±2
1	TJ1.B1	9.3±0.3	58.7 ±4.2	92.9±3.5	9.0±0.5	56.3± 5.4	88.2±4
2	TJ2.B5	6.2±0.5	29.8±3.6	72.6±5.6	5.8±0.3	23.8±2 7	67.8±4
3	TJ2.B6	10.7±0.6	54.7±3.8	93.3±4.7	10.1±0.4	49.4±2.6	87.9±4
4	Sl.l.B	6.3±0.3	22.1±2.8	45.2±3.3	5.9±0.2	20.9±2.1	42.6±3
5	SL1.B1	5.8±0.5	32.2±3.5	52.5±3.4	5.2±0.3	29.4±2.7	49.3±3
6	SL1.B4	4.8±0.2	26.4±3.3	47.3±4.3	4.5±0.3	24.3±2.8	44.9±4
7	SL2.B3	5.6±0.2	21.4±3.7	43.3±4.9	5.4±0.2	19.2±2.6	40.7±3
8	SL3.B1	6.8±0.4	3 1.4±2.9	53.6±4.3	6.3±0.2	31.3±2.5	51.2±4
9	SL3.B3	7.1±0.4	36.2 ±3. 5	83.0±5.2	6.8±0.4	26.5±2.4	82.2±4
10	MN1.B	5.9±0.5	31.9±3.3	50.3±4.5	5.3±0.6	28.2±3.3	48.8±5
11	MN1.B1	5.8±0.2	25.4±3.6	47.3 ±3.3	5.4±0.3	22.7±2.6	46.4±3
12	MN1.B4	6.7±0.3	22.7±2.5	43.7±4.9	6.5±0.2	18.5±2.5	41.6±3
13	MN1.B5	6.8±0.2	33.2±1.7	54.4±3 2	6.4±0.2	29.6±2.9	52.4±2
14	MN2.B3	7.1±0.3	37.7±3.3	56.4±4.4	6.8±0.4	35.3±3.9	51.4±5
15	MN2.B13	7.8±0.4	48.5±3.2	80.7±3.4	7.7±0.3	46.6±3.7	74.2±4
16	MN2.B14	10.7±0.4	60.7±3.2	93.7±3.5	9.4±0.6	57.6±4.4	87.2±3
17	SG1.B2	7.2±0.5	43.9±3.6	77.9±4.4	6.8 ±0.5	40.7±3.6	75.2±4
18	SGI.B6	6.8±0.6	30.6±3. 8	52.3±4.8	6.3±0.7	27.9±2.5	49.7±4
19	SG1.B3	6.2±0.2	21.4±3.4	45.7±4.5	5.9±0.3	21.4±3.3	42.2±3.1
20	SG2.B5	5.8±0.3	33.7±3.5	50.2±3.2	5.7±0.3	31.4±3.7	48.3±4.2
21	SG2.B4	6.5±0.3	28.7±2.7	49.7±5.0	5.7±0.4	26.4±2.7	46.8±4.3
22	SG3.B5	6.3±0.4	34.5±2.8	53.7±3.6	5.8±0.4	3l.7±2.4	51.6±3.2
23	SG3.B7	4.9±0.3	23.8±2.7	44.2±3.4	4.8±0.3	21.2±3.2	40.8±3.5
24	KB2.B1	4.6±0.3	27.5±1.9	47.9±4.4	4.81±0.5	24.6 ±2.4	43.6±4.4
25	KB2.B2	5.8±0.2	32.2±3.5	51.8±4.8	5.7±0.4	29.9 ±2.7	47.9±4.7
26	KB.3.B3	4.6±0.3	27.5±2.1	47.4±3.7	4.8±0.3	25.6±2.8	14.3±3.5
27	KH1.B7	5.9±0.3	43.8 ±3.5	64.8±5.3	4.8±0.3	42.2 ±3.8	61.6±4.7
28	KH2.B5	5.8±0.2	37.6±2.8	56.7±3.8	5.6±0.4	33.6±2.7	53.9±3.8
29	K.H2.B8	4.6±0.3	25.4±2.9	45.5±3.4	4.5±0.4	23.4±2.3	12.5±2.5

RESULTS AND DISCUSSION

The disappearance of endosulfan from the spiked and inoculated broth varied substantially among the total of 29 bacterial and 16 fungal strains were tested for their endosulfan degradation potential. The strains differed substantially in their potential to degrade endosulfan in vitro which ranged from 40 to 93% of the spiked amount (100 mg^{-1}) Biodegradation of endosulfan by these microorganisms also resulted in substantial decrease in pH of the brother from 8.2 to 3.2 within 14 days of incubation. High performance liquid chromatography analyses revealed that endosulfan diol and endosulfan ether were among the products of endosulfan metabolism by these microbial strains while endosulfan sulfates. A persistent and toxic metabolite of endosulfan was not detected in any case. The three strains of each of bacteria (Chaetosartorya stromaloides, Aspergillus terricola and Aspergillus terreus) were the most efficient degraders of both α-and β-endosulfan as they consumed more than 90% of the spiked amount (100mg 1^{-1}) in the broth within 14 days of incubation. The results showed that endosulfan degradation by was most effectively achieved at an initial inoculum size of 600 (OD= 0.86). Incubation temperature of 30 ºC in shaken slurries at pH 8 in loam soil. Biodegradation of endosulfan varied in different textured soils being more rapid in

course textured soils than in fine textured soil. Exogenous application of organic acids (citric acid and acetic acid) and amino acids (L-methionine and L-cystein) had stimulatory and inhibitory effects respectively on biodegradation of endosulfan. The kinetics of endosulfan biodegradation was studied at its different initial concentrations by using different growth and non-growth linked models. It was observed that at initial endosulfan concentrations of 50 to 150 mg $^{-1}$ Mond with growth model was well fitted into the data. The results of these studies may imply that these bacterial/fungal strains could be employed for bioremediation of endosulfan polluted soil and water environments.

Table-2 Change in pH and optical density of broth as a result of endosulfan degradation by soil bacteria (values are average of 3 repeats : standard error of means).

Isolates	pH			Optical densities		
	3d	7d	14d	3d	7d	14d
Control	8.11±0.08	7.98±0.13	7.77±0.12	-	-	-
TJ1 B1	7.83±0.17	6.73±0.11	3.66±0.21	0.121±0.011	0.378±0.034	0.875±0.0
TJ2.B5	7.96±0.21	7.13±0.14	4.80±0.19	0.097± 0.009	0.302 ±0.038	0.698±0.0
TJ2.B6	7.79±0.23	6.96± 0.1 6	3.63±0.21	0.126±0.014	0.361±0.019	0.841±0.0
SL1 B	8.03 ±0.17	7.21±0.11	4.21 ±0.16	1.105±0.011	0.2 84 ±0.035	0.607±0.0
SL1 B1	7.98 ±0.20	7.26 ±0.19	4.56±0.25	0.0790±0.10	0.268 ±0.041	0.563 ±0.0
SL1 B4	8.01 ±0.15	7.33±0.23	4.76±0.24	0.089± 0.009	0.262±0.03 1	0.543±0.0
SL2 B3	7.96±0.20	7.22±0.15	4.13±0.23	0.094± 0.007	0.284±0.017	0.591 ±0.0
SL3 B1	7.98 ±0.20	7.18±0.20	4.33±0.19	0. 091±0.008	0.278±0.029	0.561 ±0.0
SL3 B3	7.91 ±0.19	6.88±0.19	5.00±0.18	0.111±0.010	0.32 1± 0.031	0.693±0.0
MNI B	7.99±0.16	7.13±0.14	4.43±0.19	0.091±0.009	0.298±0.023	0.562±0.0
MNI B1	8.08±0.11	7.22±0.21	4.10±0.23	0.098±0.007	0.263±0.034	0.532 ±0.0
MN1 B4	8.10±0. 14	7. 10 ±0.17	4.51±0.16	0.0971±0.010	0.278±0.029	0.543±0.0
MN1 B5	8.06±0.17	7. 19±0.1 9	4.39±0.21	0.084± 0.011	0.273±0.021	0.54±0.0
MN2 B3	8.01 ±0.18	7.16±0.23	4.52±0.24	0.098± 0.009	0.315±0.022	0.567±0.0
MN2 B13	7.98 ±0.12	6.76±0.18	4.40±0.19	0.095±0.015	0.318± 0.013	0.710±0.0
MN2 B14	7.81±0.12	6.63±0.21	3.76±0.18	0.127±0.011	0.4 15± 0.027	0.890±0.0
SG1 B2	7.94±0.17	6.92±0.15	4.62±0.16	0.096±0.010	0.265±0.027	0.532±0.0
SGI B6	7.97 ±0.14	7.03±0.25	4.63±0.18	0.078±0.008	0.3121±0.018	0.671±0.0
SG1 B3	8.08 ±0.12	7.28±0.2.3	4.45±0.21	0.0 79± 0.010	0.285±0.03 1	0.583±0.0
SG2 B5	7. 94±0.1 4	6.98±0.17	4 .37± 0.22	0.09±0.008	0.269±0.018	0552±0.01
SG2 B4	7.99 ±0.11	6.85±0.12	4.53±0.19	0.083±0.009	0.267±0.021	0.543±0.02
SG3 B5	7.92±0.13	7.07±0.21	4.44±0.16	0.082±0.010	0.279±0.024	0.565±0.03
SG3 B7	8.04±0.18	7.41±0.18	4.12±0.19	0.089±0.10	0.287±0.023	0.563±0.02
KB2 BI	7.93 ±0.12	7.22±0.24	4.37±0.21	0.085± 0.009	0.261 ±0.022	0.561±0.03
KB2 B2	7.97 ±0.1 5	7.11±0.21	4.47±0.23	0.086±0.010	0.26l±0.019	0.584±0.02
KB3 B3	7.94 ±0.12	6.96±0.19	4.39±0.18	0.091±0.011	0.283±0.024	0.545±0.02
KHI B7	7.88±0.19	6.82±0.16	4.35±0.21	0.087± 0.009	0.290 ± 0.031	0.547±0.02
KH2 B5	7.9 1±0.21	7.06±0.23	4.52±0.19	0.083±0.009	0.284±0.021	0.563± 0.02
KH2 B8	8.04 ±0.12	7.13±0.21	5.16±0.18	0.079± 0.010	0.287±0.018	0.678±0.02

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