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Bioremediation of endosulfan contaminated soil and water—Optimization of operating conditions in laboratory scale reactors

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Abstract

A mixed bacterial culture consisted of *Staphylococcus sp*., *Bacillus circulans-I* and *-II* has been enriched from contaminated soil collected from the vicinity of an endosulfan processing industry. The degradation of endosulfan by mixed bacterial culture was studied in aerobic and facultative anaerobic conditions via batch experiments with an initial endosulfan concentration of 50 mg/L. After 3 weeks of incubation, mixed bacterial culture was able to degrade 71.58 ± 0.2% and 75.88 ± 0.2% of endosulfan in aerobic and facultative anaerobic conditions, respectively. The addition of external carbon (dextrose) increased the endosulfan degradation in both the conditions. The optimal dextrose concentration and inoculum size was estimated as 1 g/L and 75 mg/L, respectively. The pH of the system has significant effect on endosulfan degradation. The degradation of alpha endosulfan was more compared to beta endosulfan in all the experiments. Endosulfan biodegradation in soil was evaluated by miniature and bench scale soil reactors. The soils used for the biodegradation experiments were identified as clayey soil (CL, lean clay with sand), red soil (GM, silty gravel with sand), sandy soil (SM, silty sand with gravel) and composted soil (PT, peat) as per ASTM (American society for testing and materials) standards. Endosulfan degradation efficiency in miniature soil reactors were in the order of sandy soil followed by red soil, composted soil and clayey soil in both aerobic and anaerobic conditions. In bench scale soil reactors, endosulfan degradation was observed more in the bottom layers. After 4 weeks, maximum endosulfan degradation efficiency of 95.48 ± 0.17% was observed in red soil reactor where as in composted soil-I (moisture 38 ± 1%) and composted soil-II (moisture 45 ± 1%) it was 96.03 ± 0.23% and 94.84 ± 0.19%, respectively. The high moisture content in compost soil reactor-II increased the endosulfan concentration in the leachate. Known intermediate metabolites of endosulfan were absent in all the above degradation studies.

Keywords: Endosulfan; Mixed culture; Biodegradation; S*taphylococcus sp*.; *Bacillus circulans*

1. Introduction

Endosulfan, a sulphurous acid ester of a chlorinated cyclic diol [1] is a mixture of two stereo isomers alpha and beta endosulfan in a ratio of 7:3 (Fig. 1) and registered with several trade marks, Thiodan, Cyclodan, Thimol, Thiofar and Malix. It is extremely toxic to fish and aquatic invertebrates and has been implicated in mammalian toxicity [2], genotoxicity [3], and neurotoxicity [4]. It enters the air, water, and soil environments during its use, and manufacture. Endosulfan is highly insoluble in water. Hence, mostly it will be associated with soil. It is reported that, the half-life of soil bound endosulfan was much higher than that of aqueous forms. However, half-life of endosulfan in sandy loam soil is reported to be between 60 and 800 days and the rate of endosulfan degradation in soil is dependent on soil pH [1], which can be a source of later contamination. These health and environmental concerns have led to an interest in detoxification of endosulfan in the environment.

Bioremediation is employed for the decontamination of many pollutants such as pentachlorophenol [5,6], diesel oil [7], herbicides [8,9], polyaromatic hydrocarbons [10,11]. Endosulfan degradation in aqueous systems by biological methods using many bacterial and fungal cultures was investigated [12–17]. However, the scenario of endosulfan degradation via bacterial

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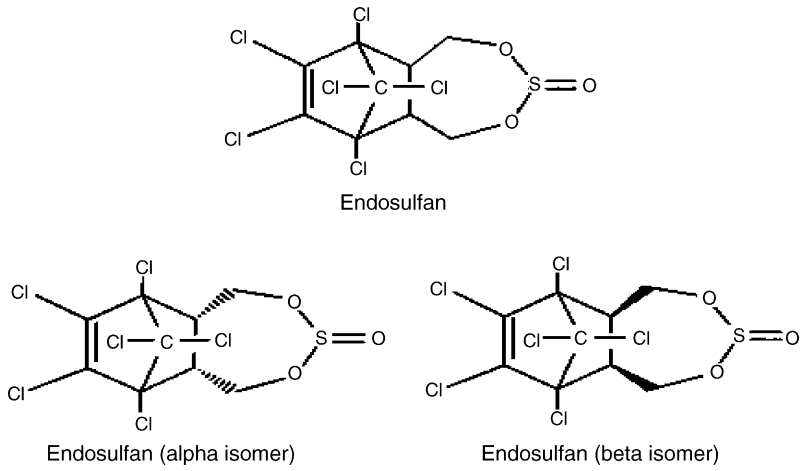

Fig. 1. Molecular structure of endosulfan and its two stereo isomers.

isolates in aqueous and soil environments is different. Properties of pollutants and the characteristics of the soil like clay content, organic matter content and soil texture have influence on the degradation process [18].

In order to develop a suitable field soil bioremediation system, it is essential to have the information regarding the adsorption, desorption characteristics of the concerned pollutant in soil, optimum operating conditions of the bio-system, i.e. pH, effect of co-metabolism, population size of specific microorganism and the performance evaluation through detailed laboratory studies. No detailed study has been conducted to evaluate these parameters for endosulfan degradation using mixed bacterial consortium. Also, the impact of soil properties and the effect of exogenous cells on soil applied endosulfan degradation have not been quantified in detail.

In the present study, endosulfan-degradation potential of a mixed bacterial culture in soil was examined via bench scale soil reactors. The optimization of operating parameters for the degradation of endosulfan by a mixed bacterial consortium was also carried out in laboratory scale reactors.

2. Materials and methods

2.1. Chemicals

High purity (99.4%) endosulfan, endosulfan sulfate, endosulfan ether and endosulfan lactone was purchased from Sigma–Aldrich Ltd., USA, and technical grade endosulfan of 96% purity was purchased from EID Parry India Ltd., Chennai, India. Other chemical reagents and solvents used were of HPLC grade purchased from Ranbaxy Ltd., Chennai, India. The stock endosulfan solution of 1% was prepared in methanol and used for all the experiments. All the glassware used was supplied by Borosil, India, and before every experiment, all glassware were cleaned with distilled water and dried at 110 °C for 5 h.

2.2. Soils for endosulfan degradation studies

Most common Indian soils were selected for the biodegradation experiments and they were classified as clayey soil (CL, lean clay with sand), red soil (GM, silty gravel with sand), composted soil (PT, peat) and sandy soil (SM, silty sand with gravel) [19]. The soils were sieved through IS sieve No. 10 (2 mm aperture as per IS 2720 (Part 4), 1987). The fraction passing through the sieve was collected and preserved in air tight plastic containers for biodegradation studies.

3. Analytical techniques

Endosulfan, its isomers and degradation products, i.e. endosulfan sulfate, endosulfan ether and endosulfan lactone were analyzed by Perkin-Elmer Clarus 500 gas chromatograph with electron capture detection (GC/ECD). Under these conditions the retention time for alpha endosulfan, beta endosulfan, endosulfan sulfate, endosulfan ether and endosulfan lactone was 6.31, 9.36, 11.95, 3.72 and 6.42 min, respectively, and the chromatogram is shown in Fig. 2(a) [19].

Bacterial cells in the systems were measured using a UV digital spectrometer (Techcomp, Hong Kong) in fixed-point measurement at 550 nm [20].

4. Experimental procedure

4.1. Inoculum

A mixed bacterial culture, previously isolated by selective enrichment on endosulfan [20] was used. It consists of three strains, i.e. *Staphylococcus sp*., *Bacillus circulans-I* and *-II* that have been deposited in the Gene Bank (MTCC) at Chandigarh, India as MTCC 6801, MTCC 6802 and MTCC 6803, respectively. The cultures were grown in nutrient broth (NB) [20], centrifuged, washed, suspended in fresh NB, and were used as inoculum for all the biodegradation studies.

4.2. Effect of supplementary carbon source on endosulfan degradation in aqueous system

Technical endosulfan (from 1% stock solution prepared in methanol) was amended in conical flasks containing 200 mL of

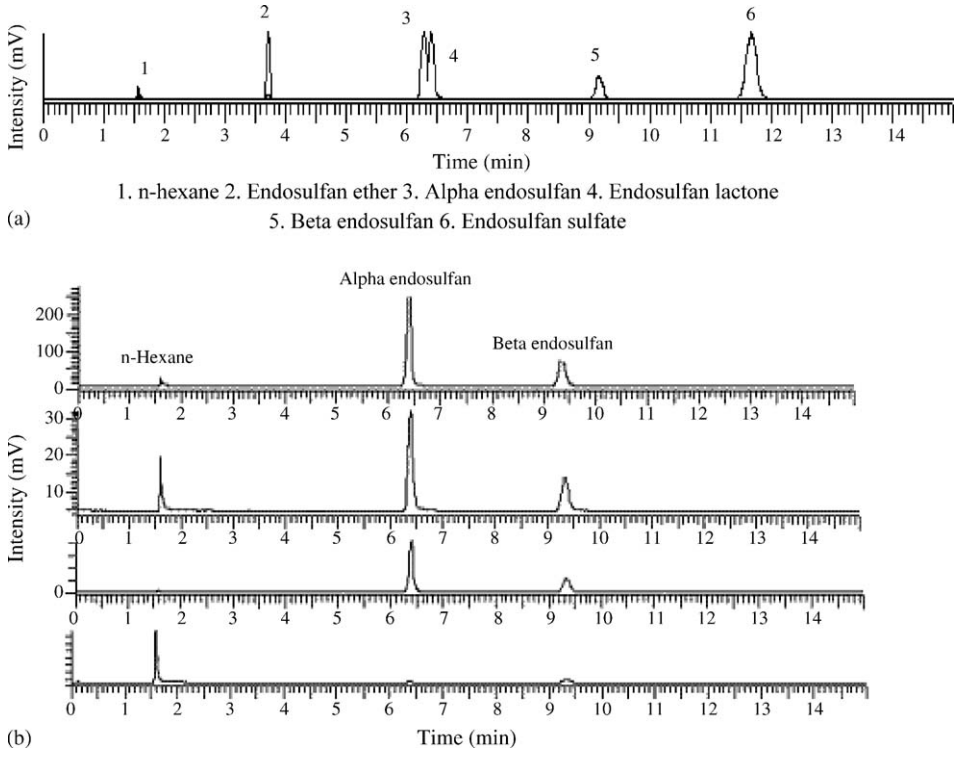

Fig. 2. (a) Chromatogram of endosulfan and its metabolites at standard operating condition. (b) Chromatogram of reactor effluent extracts during biodegradation at 5, 7, 14 and 21 days at standard operating condition.

NB to receive a final endosulfan concentration of 50 ppm. Dextrose was supplied as a supplementary carbon at concentrations of 1, 2, 3 and 4 g/L and the pH of the medium was maintained at 7. Predetermined concentration of bacterial cells corresponds to OD_{550} 0.15 (75 mg/L) were inoculated and the flasks were kept in an orbital shaker at $28 \pm 2\,^{\circ}C$ at 150 rpm. The studies were conducted in both aerobic and facultative anaerobic conditions for 21 days. Nitrogen gas was purged into anaerobic flasks and immediately sealed with air tight septum to maintain the anaerobic condition. At various time intervals (0, 2, 5, 7, 10, 14 and 21 days) samples were withdrawn from the conical flasks and analyzed for residual endosulfan concentration using GC/ECD. After collecting the samples from anaerobic flasks, nitrogen gas was purged again and sealed with air tight septum.

4.3. Effect of inoculum size on endosulfan degradation in aqueous system

Different microbial cell concentrations corresponds to 50, 75, 100, and 150 mg/L were inoculated to eight identical conical flasks containing 100 mL of NB, previously amended with endosulfan concentration of 50 ppm. The pH of the medium was adjusted to 7 and no supplementary carbon was added throughout. The flasks were kept in an orbital shaker at $28 \pm 2\,^{\circ}C$ and 150 rpm. Experiments were conducted in both aerobic and anaerobic conditions. Five millilitres of sample was collected from the flasks at 2, 4, 7, 10, 14 and 21 days and analyzed for endosulfan concentration.

4.4. Effect of pH on endosulfan degradation in aqueous system

NB was prepared as above with an endosulfan concentration of 50 ppm in identical conical flasks. The pH of the medium was adjusted to 4, 6, 7, 8, and 10 with the addition of hydrochloric acid (HCl)/sodium hydroxide (NaOH) solutions. After adjusting the pH, predetermined concentration of bacterial cells (75 mg/L at OD_{550} 0.15) was inoculated. The inoculated flasks were kept in an orbital shaker at $28 \pm 2\,^{\circ}C$ and 150 rpm for 21 days. Blank control reactors were operated simultaneously for all the pH. The samples were collected from the controlled flasks at 0, 2, 5, 7, 10, 14 and 21 days and analyzed for endosulfan concentration using GC/ECD. A blank reactor with distilled water alone was also operated to see the effect of hydrolysis at higher pH (pH 10).

4.5. Endosulfan degradation studies in soil

4.5.1. Miniature soil reactor studies

To evaluate the optimum conditions for bioremediation, miniature reactors were employed. Soil employed for all the biotransformation studies were sterilized in a hot air oven at $150\,^{\circ}C$ for 2 h. The miniature soil reactors were of 50 mL capacity conical flasks in which the soil samples, i.e. red soil, sandy soil, clay soil and composted soil of 25 g (oven dried and amended with endosulfan to reach a final endosulfan concentration of 2 mg/g of soil) were placed. Endosulfan amended soil samples were inoculated with 75 mg/g of bacterial cells mixed with NB and added into the reactor. The reactors were operated

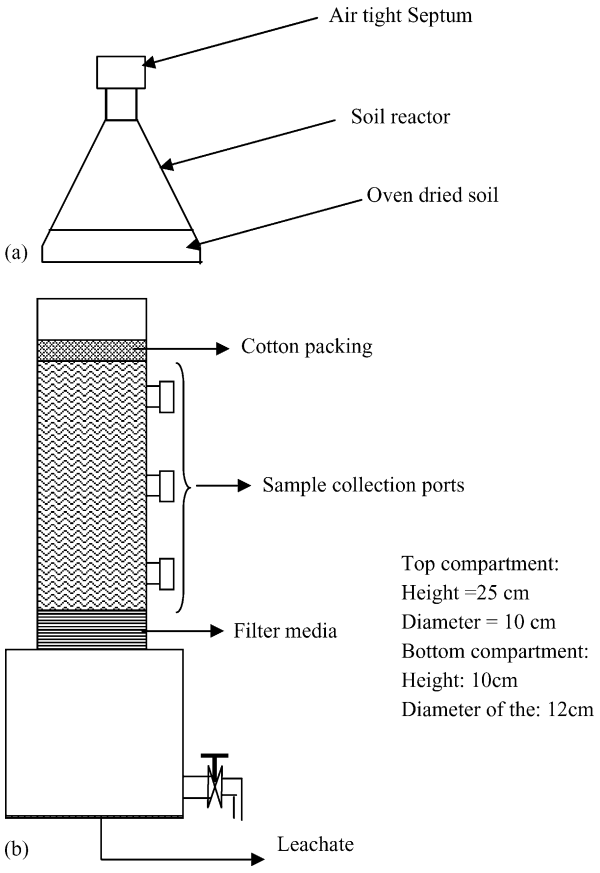

Fig. 3. (a) Schematic diagram of miniature soil reactor; (b) bench scale soil reactor.

in fully saturated condition. Simultaneously, blank rectors (with endosulfan and without bacterial cells) were operated with similar operating parameters in aerobic and anaerobic conditions. The performance of the reactors was monitored regularly under aerobic and anaerobic conditions. The schematic diagram of the miniature reactor is shown in Fig. 3(a). The experiments were conducted in triplicate. At time intervals of 2, 5, 7, 14 and 21 days, the contents of the reactor were mixed well/homogenized in a shaker. From the homogenized contents of the reactors (triplicate), two samples of 0.5 g was collected and analyzed for residual endosulfan concentration. Similar, sampling collection methodology was adopted for leachate collection also. At time intervals of 2, 5, 7, 14 and 21 days, 0.5 mL of the supernatant was also collected from the soil reactor and analyzed for endosulfan concentration. The mean with standard deviation was calculated for each sample (totally six samples at each sampling interval) and was reported in each case.

4.5.2. Bench scale soil reactors for endosulfan degradation

The bench scale reactors were simulated in the laboratory in such a way to represent the contamination of pesticide in an agricultural land. The reactors were made up of 3 mm thick acrylic transparent sheet. The top compartment has 10 cm diameter and 25 cm height with three sample collection ports located at 10, 15, 20 cm from the top. The bottom compartment is 12 cm in diameter and 10 cm in height, which was used as leachate collector. A schematic diagram of the reactor is shown in Fig. 3(b).

Three reactors were started simultaneously, including one red soil reactor and two composted soil reactors. To study the effect of moisture content in bench scale reactors, two composted soil reactors were operated with different moisture contents, one with 38 ± 1% and other with 45 ± 1% named as composted soil reactor-I and -II, respectively. Soils employed for all biotransformation studies were sterilized by keeping it in a hot air oven at 150 °C for 2 h. One hundred millilitres of 1% stock endosulfan solution (in methanol) was mixed with 1000 g of soil. In each soil, final endosulfan concentration was estimated as 0.98 mg/g of soil. Later, microbial cells were added to the soils along with NB and mixed thoroughly. The final microbial concentration was estimated as 75 mg/g of soil (dry wt.) and the final moisture content was adjusted as per the requirements with the use of NB. Glass beads were used as a supporting media, placed in the bottom of the reactor for a depth of 5 mm. Two thousand grams of the above soil ingredients was placed in each reactor and operated with identical conditions. The studies were conducted in duplicate. The performance of the reactor was monitored for 28 days. At the end of 7, 14, 21 and 28 days, two samples of 0.5 g were collected from each port (identified as top, middle and bottom) of the reactors (duplicate reactors). The collected soil samples were mixed well/homogenized in a shaker. The homogenized soil samples were extracted with *n*-hexane and analyzed for endosulfan concentration. Similar methodology was adopted for leachate collection. Exactly, 5 mL of leachate was collected from the bottom chamber, extracted with *n*-hexane and analyzed for endosulfan concentration. The moisture content in the reactor was monitored regularly and the decrease in moisture content due to evaporation/utilization by bacteria was compensated by the supply of NB. The top of the reactor was covered with wet cotton and thereby; the system was maintained with uniform moisture content. The presence of anaerobic condition in the bench scale reactors was checked by the use of 1% resazurin indicator (redox indicator).

5. Results and discussion

5.1. Effect of supplementary carbon source

Batch experiments were conducted at an initial endosulfan concentration of 50 mg/L amended in 200 mL of NB with 75 mg/L (0.15 at OD_{550}) of mixed microbial cells for a period of 21 days. Dextrose was added in the systems at 1, 2, 3 and 4 g/L (supplementary carbon) to check the influence of supplementary carbon. At regular intervals, samples were collected from aerobic and facultative anaerobic systems and analyzed for endosulfan concentration in GC/ECD.

From our earlier studies it was observed that in the absence of dextrose, endosulfan degradation efficiency of the mixed bacterial consortia was 71.58 ± 0.2% and 75.88 ± 0.2% in aerobic and facultative anaerobic conditions, respectively, at the end of 21 days [20]. Addition of 1 g/L of dextrose to aerobic system increased the endosulfan degradation efficiency to 80.62 ± 0.18% (Fig. 4(a)), which corresponds to an increase

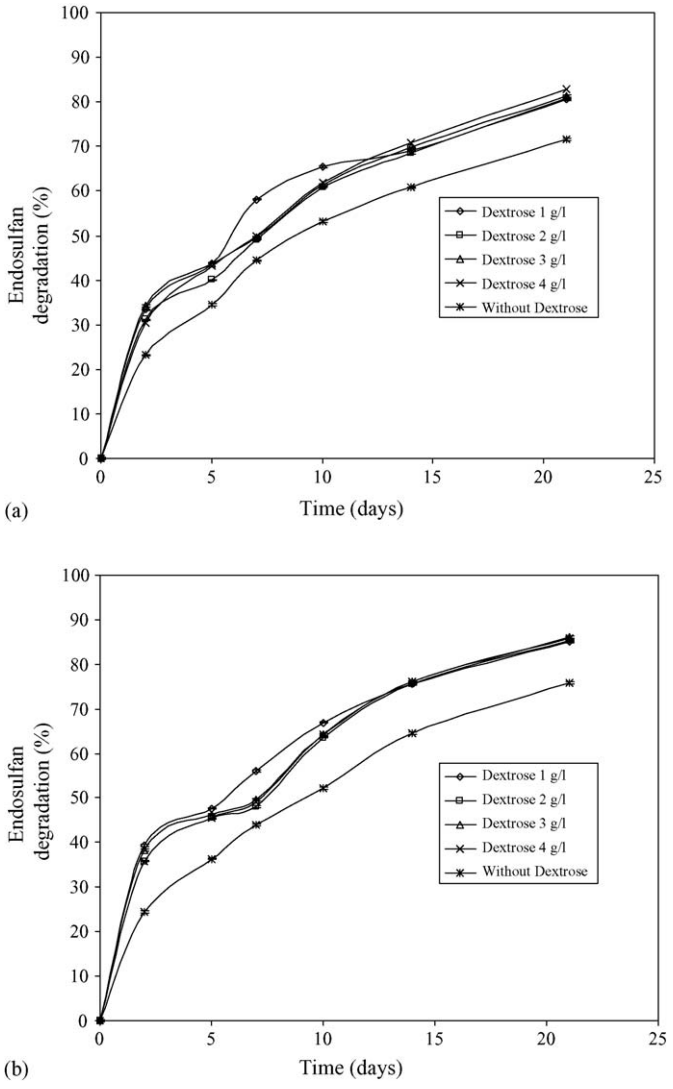

Fig. 4. (a) Degradation of endosulfan at various supplementary carbon concentrations in aerobic system; (b) in facultative anaerobic system.

Table 1
Degradation of alpha and beta endosulfan in the presence and absence of dextrose at the end of 21 days

Dextrose addition (g/L)	Degradation at the end of 21 days (%)			
	Aerobic system		Anaerobic system	
	Alpha endosulfan	Beta endosulfan	Alpha endosulfan	Beta endosulfan
0	71.29 ± 0.09	72.27 ± 0.02	76.40 ± 0.13	74.67 ± 0.03
1	81.66 ± 0.15	78.20 ± 0.03	85.17 ± 0.13	85.00 ± 0.05
2	81.71 ± 0.08	78.40 ± 0.03	85.43 ± 0.11	85.13 ± 0.03
3	82.94 ± 0.11	77.80 ± 0.02	85.97 ± 0.15	86.27 ± 0.05
4	84.91 ± 0.11	78.33 ± 0.05	85.77 ± 0.13	86.46 ± 0.04

in endosulfan degradation efficiency of 12.63 ± 0.80%. Many researchers observed that the addition of auxiliary carbon to the system having xenobiotic compounds increased the biodegradation potential of bacterial and fungal cultures. These findings were in well agreement with the present study results. Thereafter, no significant increase in endosulfan degradation efficiency was observed due to the addition of dextrose, i.e. 2, 3 and 4 g/L. Degradation efficiency of beta endosulfan (72.27 ± 0.02%) was slightly more compared to alpha endosulfan (71.29 ± 0.09%) in the absence of dextrose in aerobic system whereas the addition of dextrose increased the degradation efficiency of alpha endosulfan more compared to beta endosulfan (Table 1).

In anaerobic condition, addition of 1 g/L of dextrose increased the endosulfan degradation efficiency by 12.20 ± 1% (75.88 ± 0.2% to 85.14 ± 0.18%). At concentrations of 2 and 3 g/L the increase in endosulfan degradation was 12.47 ± 0.85% and 13.42 ± 0.70%, respectively (Fig. 4(b)). Interestingly, in anaerobic system, addition of dextrose increased the degradation of beta endosulfan more compared to alpha endosulfan (Table 1). From these results it can be concluded that co-metabolic process increased the endosulfan degradation and 1 g/L of dextrose can be used as an optimum supplementary carbon dose.

Throughout the present study, the intermediate metabolites (endosulfan sulfate, endosulfan lactone, endosulfan diol, endosulfan ether, endosulfan hydroxy ether and endosulfan monoaldehyde) reported by previous researchers were not

Table 2
Growth of mixed and pure bacterial cultures during degradation of endosulfan in the presence and absence of dextrose

Time (days)	Growth of mixed culture without dextrose (mg/L)[a]		Growth of mixed culture with dextrose (mg/L)[a]		Growth of pure culture in aerobic condition with dextrose (mg/L)[b]		
	Aerobic	Anaerobic	Aerobic	Anaerobic	Aerobic	Anaerobic	Aerobic
0	75 ± 4	75 ± 3	75 ± 4	75 ± 6	75 ± 4	75 ± 3	75 ± 5
2	120 ± 4	90 ± 5	140 ± 8	110 ± 5	100 ± 5	120 ± 7	115 ± 8
5	210 ± 3	155 ± 7	245 ± 5	180 ± 6	175 ± 5	195 ± 4	190 ± 8
7	255 ± 6	210 ± 7	280 ± 5	220 ± 8	210 ± 4	240 ± 9	240 ± 11
10	295 ± 10	245 ± 7	315 ± 6	260 ± 8	245 ± 6	265 ± 7	270 ± 8
14	320 ± 8	275 ± 10	335 ± 9	295 ± 10	270 ± 6	275 ± 7	280 ± 9
18	345 ± 8	300 ± 5	360 ± 9	320 ± 7	–	–	–
21	355 ± 7	320 ± 7	375 ± 6	340 ± 8	–	–	–
28	350 ± 6	325 ± 6	365 ± 6	340 ± 5	–	–	–

1 g/L of dextrose was added to all systems and measured at OD_{550}.
[a] Degradation studies conducted at an endosulfan concentration of 50 mg/L.
[b] Degradation studies conducted at an endosulfan concentration of 5 mg/L.

observed. Also, the loss due to dissipation was checked with the help of control reactors (without bacterial culture and with endosulfan). It was observed that abiotic loss in the system was negligible. The chromatogram of reactor effluent extracts at 5, 7, 14 and 21 days during degradation is shown in Fig. 2(b).

The growth of mixed and pure bacterial cultures during degradation of endosulfan with the presence and absence of endosulfan was shown in Table 2. The maximum specific growth rate of the mixed culture was $0.0395 \pm 0.002\ h^{-1}$ while using dextrose as carbon source, where as the specific growth rate was only $0.0137 \pm 0.001\ h^{-1}$ when methanol was used as the auxiliary carbon source. This observation shows that, methanol and dextrose can be used by the microorganisms for their cell growth. When both were available, dextrose was preferred over methanol [20].

5.2. Effect of inoculum size

The addition of 50 mg/L of bacterial cells to the aerobic system reduced the alpha and beta endosulfan concentration from 35 to 9.38 ± 0.001 ppm and 15 to 5.48 ± 0.003 ppm, respectively, at the end of 21 days. The increase in bacterial cell concentration to 75 and 100 mg/L increased the endosulfan degradation efficiency of the system from $70.28 \pm 0.18\%$ to $71.58 \pm 0.13\%$ and $72.38 \pm 0.30\%$, respectively (Fig. 5). However, maximum endosulfan degradation efficiency of 77.38% was observed at an inoculum size of 150 mg/L after 21 days of incubation.

In anaerobic condition, the initial alpha and beta endosulfan concentrations were reduced to 9.3 ± 0.002 and 4.1 ± 0.002 ppm, respectively, with an inoculum size of 50 mg/L within 21 days. The increase of bacterial cells to 75 and 100 mg/L increased the endosulfan degradation efficiency marginally (Fig. 6). However, the maximum endosulfan degradation efficiency of $77.1 \pm 0.28\%$ was achieved at an inoculum size of 150 mg/L. Though it is reported that the increase in inoculum size had no significant effect on the degradation of soil applied endosulfan [21], in the present study, it was observed that, the inoculum size is a rate limiting factor in the degradation of endosulfan in aerobic process compared to anaerobic process. When the bacterial concentration in the system was 75 mg/L, better performance of the system was observed in both aerobic and facultative anaerobic conditions. Hence, it can be concluded that an inoculum size of 75 mg/L can be used as an optimum dose for endosulfan degradation experiments.

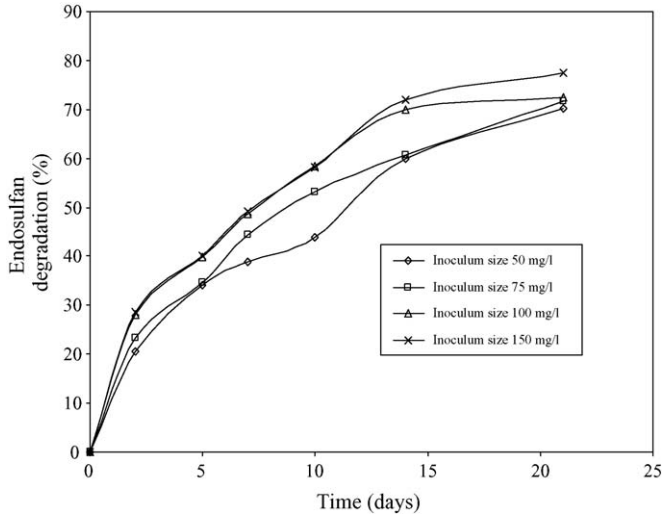

Fig. 5. Effect of inoculum size on endosulfan degradation in aerobic system.

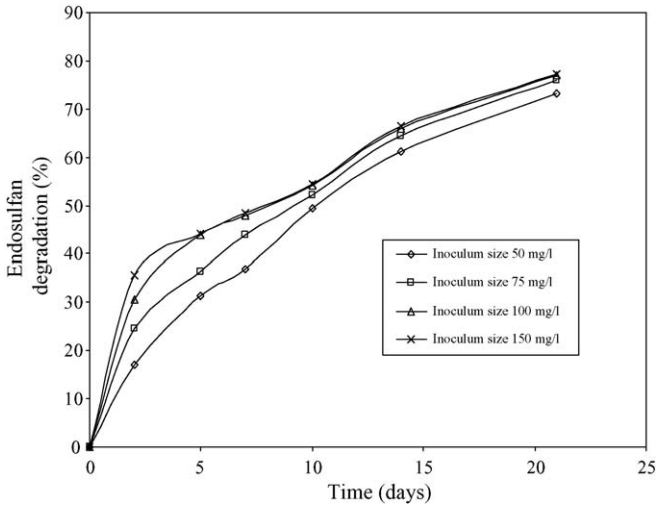

Fig. 6. Effect of inoculum size on endosulfan degradation in facultative anaerobic condition.

5.3. Effect of pH

Batch experiments were conducted in aerobic and facultative anaerobic conditions to study the influence of pH on endosulfan degradation by the microbial consortium. In the present study, endosulfan was amended directly into nutrient broth (NB) and endosulfan degradation experiments were conducted at various pH, i.e. 4, 6, 7, 8 and 10. These pH values are corresponds to the initial pH of the NB. Later, the pH of the system was decreased. At the end of the study, pH of the systems was 4.4, 6.2, 7.1, 7.9 and 8.3, respectively. In aerobic condition, at the end of 21 days, endosulfan degradation efficiency (at pH 4) was $55.7 \pm 0.22\%$. The increase in pH value to 6 and 7 increased the degradation efficiency to $71.3 \pm 0.19\%$ and $71.58 \pm 0.27\%$, respectively. Further increase in pH value to 8 reduced the efficiency marginally ($71.28 \pm 0.2\%$) and at pH 10 it reduced drastically ($37.56 \pm 0.3\%$). Decrease in pH to 4 and increase in pH to 10 reduced the endosulfan degradation efficiency by $22.18 \pm 1.4\%$ and $47.53 \pm 1.45\%$ (compared to neutral pH) at the end of 21 days (Fig. 7(a)). Similar trends were observed by Siddique et al. [17] while studying the endosulfan degradation by enriched fungal and bacterial strains. This may be due to the decreased growth of microbes at extreme pH. It is reported that at high pH, hydrolysis of endosulfan was faster [22,23]. In the present system, nutrient medium was present along with endosulfan in control reactors. Though the initial pH was increased to 10, the final pH reduced to 8.3 at the end of 21 days, whereas, no significant change in the pH value (pH 9.9 ± 0.1) was observed in the blank reactor (operated at pH 10) at the end of 21 days.

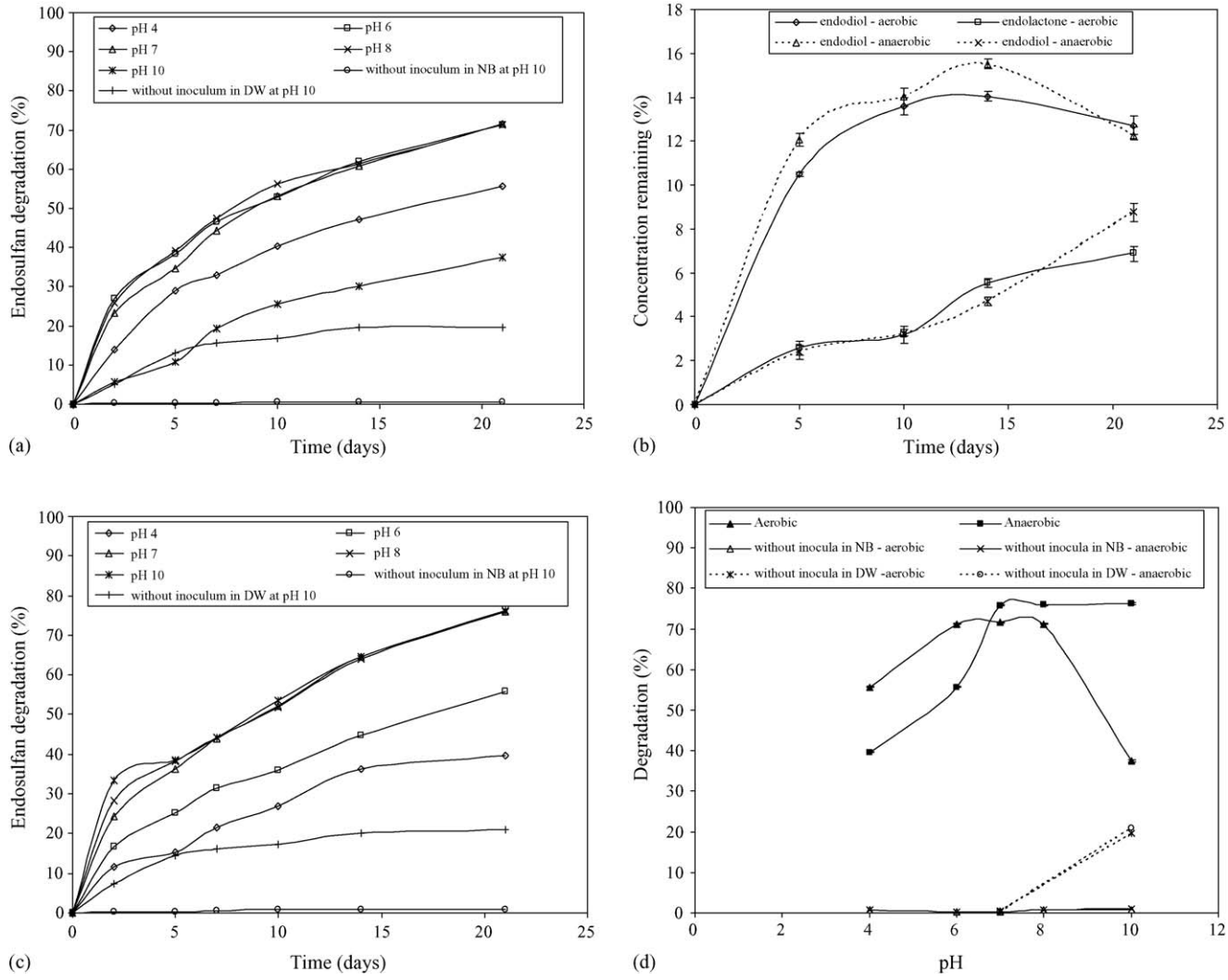

Fig. 7. (a) Degradation of endosulfan at various pH in aerobic system. (b) Variation of endosulfan metabolites in blank reactor (distilled water) at pH 10. (c) Degradation of endosulfan at various pH in facultative anaerobic system; (d) in aerobic and facultative anaerobic system at the end of 21 days.

The decrease in pH might have retarded the alkaline hydrolysis. From the control studies (endosulfan amended directly in NB), it was observed that no intermediate metabolites of endosulfan were detected in any of the system and the abiological loss of endosulfan in the controlled flasks was negligible. Later, the control studies were repeated in distilled water (endosulfan amended in distilled water without NB) where endosulfan diol and endosulfan ether was observed due to alkaline hydrolysis in both aerobic and facultative anaerobic conditions. Around 20% of endosulfan was hydrolyzed to endosulfan diol and endosulfan ether. This value was converted to parent compound equivalents and it was around 99%. This emphasize the fact that endosulafan is not getting removed from the system by volatilization. The accumulation of endosulfan diol and endosulfan ether, in blank reactors, operated in aerobic and facultative anaerobic systems is shown in Fig. 7(b).

In facultative anaerobic process (at pH 4) endosulfan degradation efficiency was $38.64 \pm 0.28\%$ and it was increased to $55.72 \pm 0.21\%$ at pH 6. The reduction in efficiency of the system due to decrease in pH to 4 and 6 was $47.76 \pm 1.3\%$ and $26.57 \pm 1.1\%$ (compared to the neutral pH), respectively. But, increase in pH to alkaline side, i.e. pH 8 and 10 enhanced the endosulfan degradation efficiency to $75.92 \pm 0.18\%$ and $76.24 \pm 0.2\%$, respectively (Fig. 7(c)). Endosulfan degradation efficiency of mixed culture at the end of 21 days at various pH was shown in Fig. 7(d). Conversely, the supplementary carbon source increased the endosulfan degradation efficiency from $71.58 \pm 0.2\%$ to $80.62 \pm 0.18\%$ in aerobic condition and from $75.88 \pm 0.2\%$ to $85.14 \pm 0.18\%$ in anaerobic condition at pH 7. For all the pH ranges studied, percentage degradation of alpha endosulfan was less compared to beta endosulfan in aerobic condition and vice-versa in anaerobic condition. The growth of *Staphylococcus sp*. is more favorable in facultative anaerobic condition compared to other *Bacillus* cultures. In facultative anaerobic system endosulfan might be utilized mainly by *Staphylococcus sp*. where as in aerobic system majority of endosulfan was utilized by *Bacillus circulans-I* and *-II*. From our earlier study, it was observed that *Staphylococcus sp.* utilized more beta endosulfan and other *Bacillus* strains utilized more of alpha endosulfan [20].

These observations were in good agreement with the present study.

5.4. Soil applied endosulfan degradation studies

Endosulfan degradation in soil is partially different from degradation in water. Soil adsorption, presence of organic matter, clay content, silt content and many other factors, which directly influence the degradation of soil applied endosulfan. Though, the operating parameters of biodegradation experiments were optimized in aqueous systems it may not be same for the soil system. The miniature soil reactors were employed to check the degradation of endosulfan in different soils with the optimized operating parameters obtained from the liquid phase studies.

5.4.1. Miniature soil reactor studies

The optimum condition for bioremediation of endosulfan-contaminated soils was evaluated through miniature soil reactors. Initially, endosulfan concentration in all soils was maintained uniformly as 2 mg/g of soil and operated in saturated condition with a bacterial concentration of 75 mg/g of soil. The reactors were operated in aerobic and facultative anaerobic conditions and the performance of the reactors was monitored for 28 days. Anaerobic soil reactors gave better results compared to aerobic soil reactors. Among the soil reactors studied, maximum endosulfan degradation was observed in sandy soil reactors in both aerobic and facultative anaerobic conditions. The endosulfan degradation efficiency of the sandy soil reactor in aerobic condition was $31.5 \pm 0.23\%$, whereas, in anaerobic condition it was $32.57 \pm 0.18\%$. Similar trends in the degradation pattern were observed in all other reactors. Endosulfan degradation efficiency in the soil reactors were in the following order. Sandy soil followed by red soil ($24.75 \pm 0.18\%$ and $29.12 \pm 0.22\%$), composted soil ($22.81 \pm 0.18\%$ and $27.19 \pm 0.15\%$) and clay soil ($19.17 \pm 0.32\%$ and $19.63 \pm 0.24\%$) in aerobic and facultative anaerobic conditions, respectively (Fig. 8(a) and (b)). The efficiency of endosulfan degradation was less in clay soil ($19.17 \pm 0.32\%$ and $19.63 \pm 0.24\%$ in aerobic and anaerobic conditions, respectively). This may be due to the presence of more clay and silt content in the soil. The miniature soil reactors

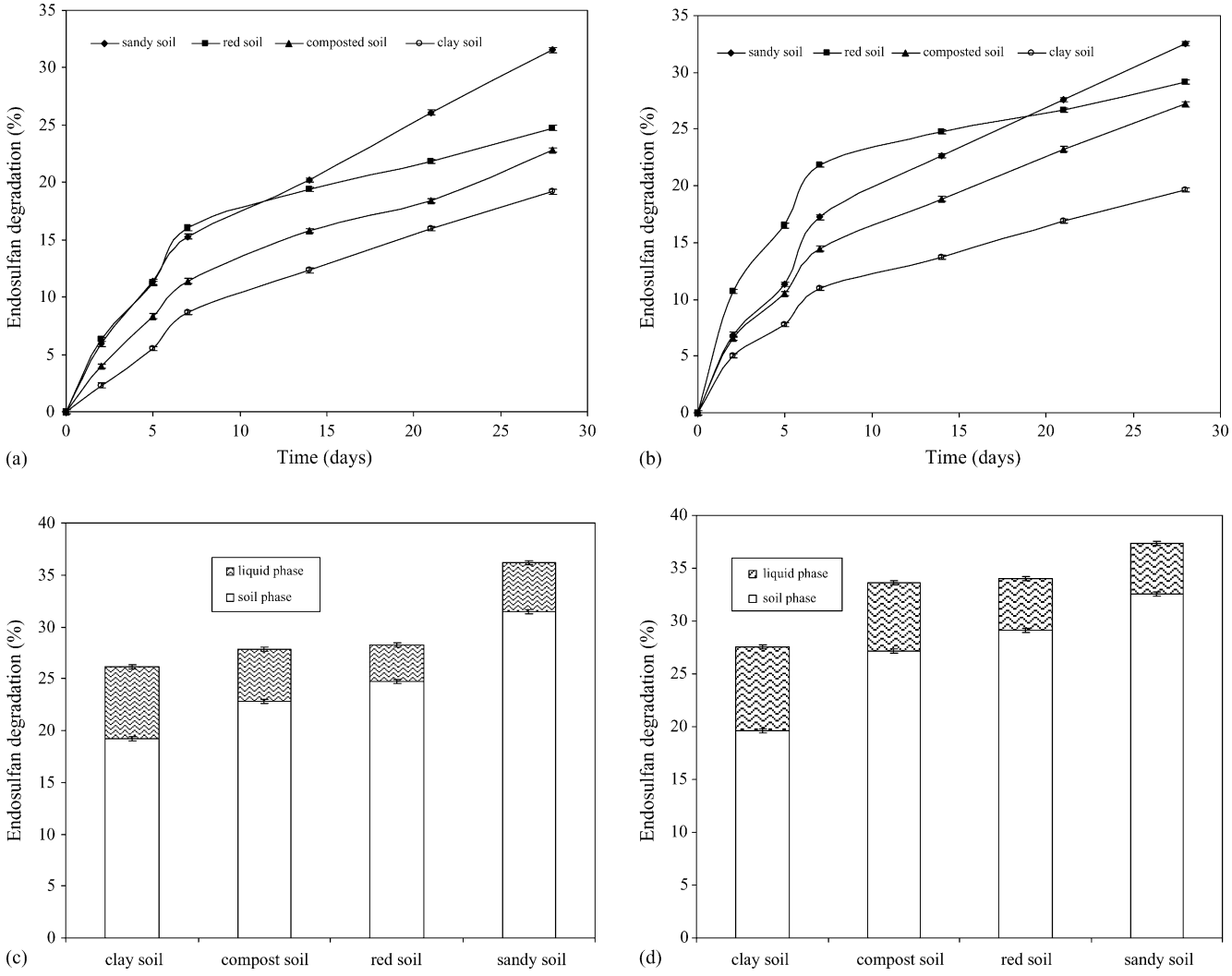

Fig. 8. (a) Kinetics of endosulfan degradation in miniature soil reactors in aerobic condition; (b) in facultative anaerobic condition. (c) Endosulfan degradation in miniature soil reactors at the end of 28 days in aerobic condition; (d) in facultative anaerobic condition.

Table 3
Endosulfan degradation in bench scale soil reactors for different soils

Type of soil	Moisture content (%)	Endosulfan degradation after 28 days in different ports of the reactors (%)			Endosulfan remaining in the leachate (mg/L) (%)	Endosulfan remaining in the blank reactor after 28 days (%)			
		Top	Middle	Bottom		Top	Middle	Bottom	Leachate (mg/L) (%)
Red soil	38 ± 1	92.13 ± 0.16	94.80 ± 0.22	95.48 ± 0.17	3.7 ± 0.027 (0.185)	81 ± 0.13	103 ± 0.26	110 ± 0.30	4.2 ± 0.025 (0.21)
CS-I[a]	38 ± 1	89.46 ± 0.21	84.61 ± 0.17	96.03 ± 0.23	1.9 ± 0.012 (0.095)	89 ± 0.21	99 ± 0.24	105 ± 0.17	3.41 ± 0.018 (0.17)
CS-II[a]	45 ± 1	91.41 ± 0.24	94.83 ± 0.20	94.84 ± 0.19	2.67 ± 0.022 (0.133)	84 ± 0.24	96 ± 0.22	113 ± 0.25	4.68 ± 0.021 (0.23)

[a] Composted soil.

were operated in saturated condition; hence the concentration of endosulfan in the liquid phase was also analyzed to observe the endosulfan degradation in the liquid by the microbial consortium. Initially, liquid phase endosulfan concentration (leachate) was observed more in sandy soil followed by red soil, compost soil and clay soil reactors whereas during operation the endosulfan degradation in the liquid phase was observed in the reverse order. Endosulfan degradation (soil phase and liquid phase) in the miniature soil reactors at the end of 28 days in aerobic and anaerobic conditions are given in Fig. 8(c) and (d), respectively.

It was observed from our earlier studies that the attraction/influence of endosulfan molecules towards the clay particles was high which might have reduced the availability of endosulfan for the microorganisms [19]. This may be the reason for the decrease in endosulfan degradation efficiency in clayey soil reactors.

5.4.2. Bench scale soil reactors for the performance evaluation of endosulfan biodegradation

The purpose of bench scale reactor study was to represent the contamination of pesticide in an agricultural land. Though, endosulfan degradation was promising in the miniature soil reactors, the actual field condition would be different from the laboratory conditions. In miniature soil reactor studies, the reactors were operated separately in aerobic and anaerobic condition, which gave an idea about the efficiency of endosulfan degradation in the above conditions. But in real life situations, prevailing environmental condition may be a combination of both. This often leads to the failure of a laboratory-designed system. Hence, it is always better to simulate a reactor, which can represent actual field condition. To investigate this aspect, bench scale reactors were designed and the degradation studies were carried out.

It was observed from the aqueous batch experiments that the supply of external carbon source increased the endosulfan degradation efficiency and the pH of the system has a role on endosulfan degradation. Though, dextrose increased the degradation efficiency of the system, in bench scale reactor no dextrose was added. Endosulfan was amended to soils directly from stock solution, which is prepared in methanol. Methanol also acted as a carbon source for the microbes, though it is a less preferred substrate for the microbial consortia compared to dextrose [20]. The selected soils showed a very narrow pH variation (7.2–8.48). Hence, no attempt was made to change the soil pH during the bench scale soil reactor studies.

During the operation, the simulated bioreactor has shown two distinct zones, i.e. top of the reactor was in aerobic condition, whereas, bottom layers were in anaerobic condition. The entry of air to the bottom layers may be restricted due to the compaction of soil and no attempts were made to supply the air in the bottom zone of the reactors. The loss in moisture in the reactor was

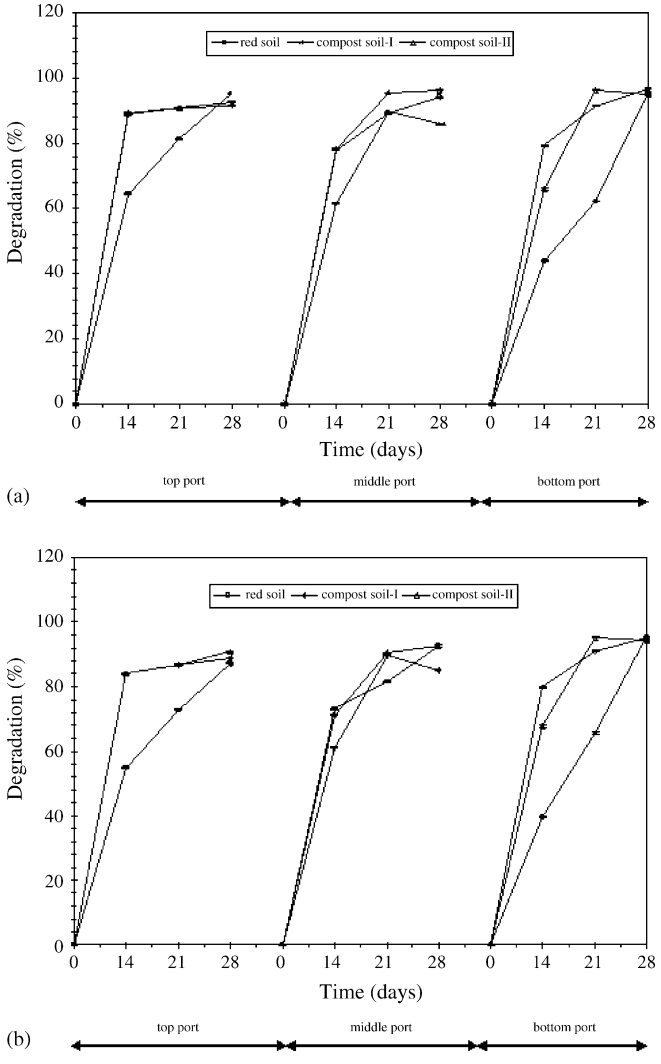

Fig. 9. (a) Alpha endosulfan degradation in bench scale reactors. (b) Beta endosulfan degradation in bench scale reactors.

compensated by the supply of nutrients from the top layer of the reactor. Due to this, an increase in endosulfan concentration was observed in the middle and bottom layers of the reactors in the fourth week. Red soil reactor exhibited an endosulfan degradation efficiency of around $95.48 \pm 0.17\%$ and $92.13 \pm 0.16\%$ in the bottom and top layers after 28 days of operation. On the other hand, 3.7 ± 0.027 ppm (0.185%) of endosulfan was found in the leachate collection compartment. The percentage of endosulfan degradation in each port of the soil and blank reactors was shown in Table 3. From the control reactors it was observed that no endosulfan was disappeared from the system at the end of 28 days. This reflects that the half-life of endosulfan in composted soil and red soil was high. Degradation of endosulfan in the bottom layers was more compared to top layers, which proved that the workability of the culture was high in facultative anaerobic condition.

Presence of anaerobic condition in the reactor was confirmed by the use of 1% resazurin indicator (redox indicator). The indicator solution was boiled and 16 M hydrochloric acid (HCl) was added into it. The contents were well mixed (solution becomes colour-less) and applied immediately in the bottom layers of the reactor through a surgical syringe. Due to the prevailing anaerobic condition the colour-less solution was not turned into pink, which confirms the absence of oxygen in the bottom layers. These findings were in good agreement with the aqueous endosulfan degradation experiments. The degradation of alpha and beta endosulfan at different ports of the reactors is given in Fig. 9(a) and (b). After 28 days, maximum endosulfan degradation efficiency of $96.03 \pm 0.23\%$ was observed in composted soil reactor-I whereas in composted soil reactor-II it was $94.84 \pm 0.19\%$. More endosulfan concentration was observed in the leachate chamber of the composted soil reactor-II (0.133%) than composted soil reactor-I (0.095%). This may be due to the high moisture content ($45 \pm 1\%$) in reactor-II compared to reactor-I. However, high moisture content did not affect any significant increase in degradation of endosulfan. From the results it may be concluded that, bioremediation of endosulfan in the field can effectively be carried out with moisture content of around 35–40%.

6. Conclusion

The mixed bacterial consortium was able to mineralize endosulfan in both aerobic and facultative anaerobic conditions. Addition of external carbon source increased the degradation efficiency. The pH of the system has a significant effect on endosulfan degradation. The performance of anaerobic soil reactors was better compared to aerobic soil reactors irrespective of the soil types. Maximum endosulfan degradation efficiency was observed in sandy soil. In bench scale soil reactors, endosulfan degradation efficiency was more in bottom layers due to the prevailing anaerobic condition. Throughout the endosulfan degradation study, no intermediate metabolites were accumulated in the system. The optimum moisture content for the system was 35–40%. The results showed that, the enriched mixed bacterial consortium used in the study can be effectively used for the treatment of endosulfan contaminated water and soil.

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