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Anaerobic-Aerobic Biodegradation of DDT (Dichlorodiphenyl Trichloroethane) in Soils

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DDT ($C_{14}H_9Cl_5$) is a pesticide that belongs to the group of organochlorines. It has been extensively used since the Second World War all over the world and, even though its use is banned or restricted in many countries because of its deleterious effects, it is still commonly found in the environment, especially in the soil, due to its strong adsorption to solid particles, which results in a great persistency (EPA, 1986). Some investigations have reported that the DDT present in the soil can be degraded in two years, while others have found that the process can take from fifteen to twenty years or more (Alexander, 1994).

The degradation products of DDT are mainly the dechlorination products DDE and DDD. The pathway can be DDT $\rightarrow$ DDE $\rightarrow$ DDD, or from DDT to DDD directly. These routes have not been completely established, even after 40 years of studies (Wayland *et al.*, 1991). In anaerobic marine sediments DDE is readily dechlorinated to DDMU (Quensen *et al.*, 1998).

In less developed countries, the use of DDT still continues in order to control diseases such as malaria and dengue (Wayland *et al.*, 1991; Cebrián, 1998). So far, no chemical compound has been found that brings together DDT's properties and effectiveness. In Mexico, the use of DDT is higher than in any other Latin American country (PAHO, 1994). Mexico used about 1000 tons of DDT in 1992 (Sharpe, 1995). The production of DDT in Mexico is mostly for its own use. Though production has diminished since 1970, it has remained stable since 1985 (López-Carrillo *et al.*, 1996). In a study carried out in the state of Veracruz in Mexico, DDT and DDE were determined in the adipose tissue of people. These compounds were present in all the cases analyzed and the highest levels were found in a suburban zone. Average DDT concentration was 24.82 mg/kg, very high compared to the results in other countries (Waliszewski *et al.*, 1995).

In recent years, there has been renewed research interest in DDT, since it has been associated with several health problems, including the increased risk of breast cancer in women and alterations in reproductive functions in men (Cebrián, 1998).

DDT is a chlorinated organic compound that is highly resistant to degradation by biological, photolithical and chemical means, given that its molecular structure contains chlorinated aliphatic and aromatic structures that impart great chemical stability. Because of this, DDT is a recalcitrant, toxic, persistent pollutant (Kannan *et al.*, 1992). This explains the current interest in developing bioremediation processes for this compound.

Current bioremediation processes typically use aerobic bacteria to degrade petroleum and other chemical wastes by oxidation of the compounds. However, it has been observed that anaerobic systems are often more capable of degrading chlorinated pollutants by reductive dechlorination (Leahy and Brown, 1994). Some compounds, such as polychlorinated biphenyls (PCBs) can be initially dechlorinated and partially degraded by

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anaerobic bacteria and the resulting products can then be more rapidly degraded by aerobic processes. DDT, too, may be amenable to this type of bioremediation approach.

Several bioremediation studies had been carried out in submerged-liquid cultures, using various microorganisms and systems to degrade DDT: *Phanerochaete chrysosporium* (Bumpus and Aust, 1987) and *Alcaligenes eutrophus* (Nadeau *et al.,* 1994) isolated from sewage have been used in aerobic conditions (Pfaender and Alexander, 1972). In the same way, immobilized mixed cultures in anaerobic-aerobic systems have also been employed (Beunink and Rehm, 1988). However, a microorganism that uses the DDT as primary source of carbon and energy for growth has not yet been found.

The objective of this work was to evaluate the degradation of DDT in solid state cultures, by different cultures in anaerobic and/or aerobic systems. The system that yielded the best results was a coupled anaerobic (with a mixed culture)-aerobic (with *P. chrysosporium*) fermentation.

MATERIALS AND METHODS

In order to evaluate the effect of the fermentation system and the use of a pure or mixed culture, a preliminary experiment with four different fermentations was carried out in triplicate, following a 2 X 2 factorial design. The factors were two fermentation systems (anaerobic-aerobic or aerobic) and two biological systems (mixed culture or *P. chrysosporum*). The response variables measured were the concentrations of DDT, DDE and DDD. Four controls were also established, two for each fermentation system. In one no microorganisms were added (only water), and in the other, only nutrients. The fermentations were incubated 28 days in aseptic conditions. Once the fermentation system with the best results of reduction of DDT concentration was determined, the experiment was redone under those conditions but with samples taken by triplicate at different times (0, 6, 10, 14, 16, 20 and 28 days). This was done in order to find out the time in which the process of removal of DDT and its dechlorinated intermediates was more efficient. Data analysis utilized the program *Statistica 6.0* (Statsoft, 1996).

A microbial consortium isolated from sewage from an anaerobic wastewater plant from a soft drink company was employed as the inoculum in the anaerobic fermentation (inoculum, 10% v/v). In the aerobic fermentation, a mixed culture that included five species of *Pseudomonas*, one of *Klebsiella*, four of *Rhodocci* and two strains of fungi was added (inoculum, 5% dry base). This culture has been reported to be capable of degrading aromatic compounds (HAB InterBio LTD., 1990). For the aerobic pure culture system, *Phanerochaete chrysosporium*, strain H-298 (CDBB) from the Collection of Microbial Cultures of CINVESTAV-IPN was employed. The inoculum was a suspension of spores from a fresh culture maintained on potato dextrose agar (PDA) slants, which had been grown for 5 days at 35°C. The spores were collected in 30 ml of Tween solution [0.1 % (v/v)] and were counted by use of a Neubauer chamber at the proper dilution to count from 25 to 50 spores per 0.1 mL (García, 1996). To adapt the mixed culture to DDT, 3 ml of a solution containing DDT (0.5 mg/mL), glucose (1 g/L) and the following minerals (in mg/L): Na_2HPO_4(500), $MgSO_4 7H_2O$ (480), $(NH_4SO_4$(350), KH_2PO_4(300) $Ca(NO)_2$(40), $FeSO_4 7H_2O$ (3), $CoC1_2 6H_2O$ (0.1), $ZnSO_4$(0.07), $NaMoO_4 2H_2O$ (0.03) $MnCl_2 6H_2O$ (0.02), $NiCl_2 6H_2O$ (0.02), H_3BO_4(0.02) and $CuCl_2$(0.01) were added weekly, five times. The adaptation of the anaerobic mixed culture was carried out in as UASB reactor with a capacity of 1.5 litres. For the aerobic mixed culture a glass jar of 2 litres capacity equiped with an air diffusor was used. The incubation temperature in both cases was 35°C.

Ten kg of soil from a plot of land used to produce vegetables in the State of Yucatan in Mexico were collected. The granulometric analysis of the soil reported 40 % silt, 32 % clay and 28 % sand. The organic matter content was 29 %. The DDT and DDE concentrations in the soil prior to treatment are shown in table 1.

Glass tubular reactors (diameter 3.5 cm and length 22 cm) for anaerobic fermentation

were used, and 400 g of soil were introduced in such a way that 75 % of the volumetric capacity of each reactor was occupied. The concentration of nutrients was determined based on the requirements reported by Speece (1996) for anaerobic microorganisms and using Balch medium as a base (Balch *et al*, 1979). There was 50 % humidity and the incubation temperature was 35°C. The production of CH_4 and CO_2 was monitored in a Gow-Mac chromatograph equipped with a thermal conductivity detector and a carbosphere column. The analyses were done at a temperature of 140°C. The fermentations had a duration of 14 days and after this time, the reactors were emptied, and the soil was subject to the aerobic fermentation. These fermentations were carried out in tubular glass fermentators similar to those employed in the anaerobic phase. However, air was supplied to the reactors. It was first humidified by bubbling through water columns placed just below the fermentators. The incubation temperature of 35°C was held using a water bath in which the reactors were submerged. The production of CO_2 during the process of aerobic fermentation was monitored using the Gow-Mac chromatograph.

The substrate for the aerobic reactors was composed of soil, sugar cane bagass (to improve air distribution through the columns), sacarose, urea and potassium phosphate, mixed according to the method of Mendoza (1994) simulating the characteristic composition of compost. A solution of mineral salts (Goodhue 1986) was also added to the formula. The mixture was maintained at 50 % humidity. The fermentation lasted two to four weeks, depending on the system (anaerobic-aerobic or aerobic, respectively).

The quantification of DDT and of its main degradation products (DDE and DDD) were made using gas chromatography according to the technique reported by the United Nations Environmental Program (UNEP, 1992). Two internal standards were added to procedural blanks to assure the quality of the analyses and to calculate the recovery percentage for the samples analyzed. The internal standards employed were PCB 103 and PCB 198.

A Hewlett Packard 5890 Series II gas chromatograph equipped with an electron capture detector (ECD) and a capillary column HP SE-54 (200 mm i.d. x 30 m) was used to identify and quantify the compounds. The peaks of the chromatograms were integrated by means of a HP Chemstation 3365 Series II software. The conditions of the chromatograph were the following: initial temperature of 70°C held for 2 min, and a temperature program with an increment of 3°C/min until 265°C, held for 25 min.

RESULTS AND DISCUSSION

Methane was produced in those systems containing, beside the contaminated soil (S), the inoculum of microorganisms from the sludge (Fig. 1). In the controls containing only water (H_2O) and/or nutrients (N), methane production was practically nil. In the sludge inoculated systems, methane production reached its maximum between days 6 and 8. There was metabolic activity in all the reactors, as shown by anaerobic carbon dioxide production (Fig. 2); however, production was higher in those that were inoculated with sludge.

Aerobic metabolic activity, as shown by carbon dioxide production, was observed in all the systems (Fig. 3). The highest levels were detected in the first day, and the highest values were observed in the fermentations inoculated with the mixed culture. From the second day, CO_2 production remained stable in all systems, and values equal or less to 1 % of CO_2 were registered. In the reactors where no inoculum was added, the production of CO_2 was only detected during the first day. This was also true for the anaerobic fermentation, and for the control to which only a solution of nutrients had been added.

DDT analyses of the samples that underwent fermentation, showed concentrations from 12.88 to 80.69 ng/g (Table 1). Such concentrations are below the concentration found in the DDT-contaminated soil used as substrate in the solid fermentations (82.69 ng/g). The reduction percentages for DDT in all of the treatments are also shown in Table 1; these

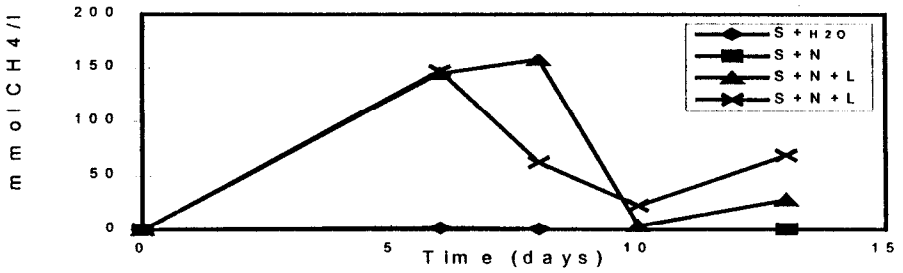

Figure 1. Production of CH_4 under anaerobic conditions. S+H_2O, contaminated soil and water; S+N, contaminated soil and nutrients; S+N+L, contaminated soil, nutrients and sludge.

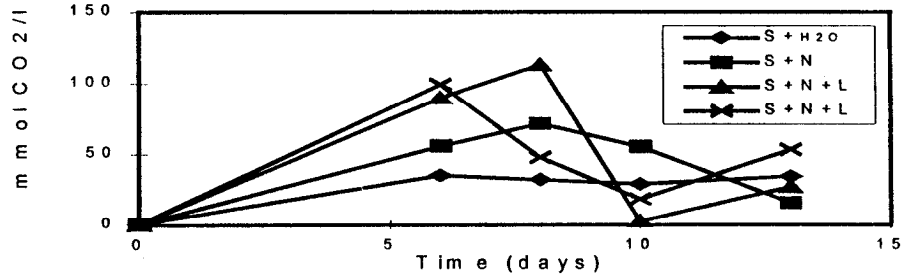

Figure 2. Production of CO_2 under anaerobic conditions. S+H_2O, contaminated soil and water; S+N, contaminated soil and nutrients; S+N+L, contaminated soil, nutrients and sludge.

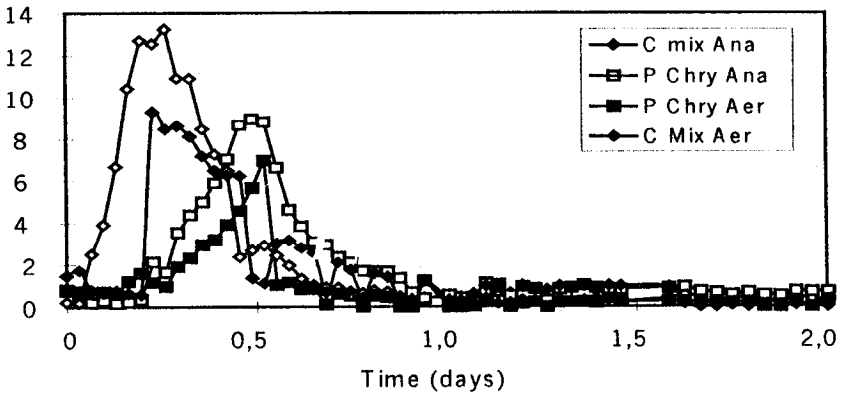

Figure 3. Production of CO_2 under aerobic conditions. C mix Ana, fermentation anaerobic-aerobic with mixed culture; P Chry Ana, fermentation anaerobic-aerobic with *P.chrysosporium*, P Chry Aer, fermentation aerobic with *P.chrysosporium:* C Mix Aer, fermentation aerobic with mixed culture.

percentages ranged from 2.5 to 84.4 %. The latter was obtained in the experiment that consisted of a sequential anaerobic-aerobic fermentation that had the *P. chrysosporium* inoculum. This indicates there was effective DDT reduction (dechlorination) in anaerobic phase by the microorganisms from sewage. This has also been reported by Pfaender and Alexander (1972). Moreover, the use of a fungus in the aerobic second phase of the solid substrate fermentation yielded a higher reduction efficiency (50 % within 30 days of incubation) than that reported by Bumpus and Aust (1987). A two-way analysis of variance of the data obtained from the treatments included in the factorial design showed significant differences between the conditions of fermentation ($F_{1,7}$ = 1215.7; p = 0.00001) and between the inocula ($F_{1,7}$ = 86.016; p = 0.000035). The results clearly established the favorable effect on the degradation process of the sequence of anaerobic-aerobic fermentation and the use of *Phanerochaete chrysosporium* over the aerobic mixed culture.

Table 1. Results of the analysis of concentrations of DDT and DDE in soil subjected to different treatments of solid fermentation.

Treatment	DDT (ng/g) average	DDT Red. (%)	DDE (ng/g) average	DDE Red. (%)
*ana-aer S+H_2O	56.84	31.3	17.78	-
*ana-aer S+N	27.07	57.3	15.0	8.0
ana-aer *P.chry.*	12.88	84.4	5.12	68.6
ana-aer mixed cult.	39.50	52.2	23.70	-
aer *P.chry.*	79.98	3.3	18.73	-
aer mixed cult.	80.69	2.5	18.09	-
*aer S+ H_2O	60.53	26.8	18.35	-
*aer S + N	45.51	45.0	16.18	-
soil without treatment	82.69	0.0	16.30	-

*treatments without the addition of an inocule.
Average recovery 94 %.

In relation to DDE concentrations, these ranged from 5.12 to 23.70 ng/g (Table 1). A decrease in concentration compared to that found in the initial substrate (16.3 ng/g) was observed only in two treatments; however, it is possible to find an accumulation of this compound as a result of the dechlorination of DDT. The analysis of variance considering DDE levels also showed significant differences between the fermentation conditions ($F_{1,7}$ = 8.7469; p = 0.0211) and between the inocula ($F_{1,7}$ = 6.5150; p = 0.0379). The treatment that resulted in a higher reduction of DDE (68.6 %) was the same that showed a higher reduction of DDT.

For DDD, the concentrations found were unacceptably high (50 times over the amount of original DDT concentration). This could be due to a co-elution of this compund with another present in the soil, in such a way that the levels of DDD were overestimated. Therefore, a statistical analysis of these data was not performed.

The eight treatments (including those without an inoculum) were evaluated in order to determine if there were significant differences between the treatments with and without inoculum. A Kruskal-Wallis non-parametric analysis of variance was made. The variables included in the statistical analysis did not fulfill the assumptions of normality and equality of variances required for a parametric analysis. When considering the DDT concentrations, significant differences were found ($H_{7,20}$ = 15.0553; p = 0.0353) between treatments, and the significantly most effective treatment was that with *P. chrysosporium* in an anaerobic-aerobic system.

Table 2 shows the results obtained in the experiment carried out under the optimal conditions obtained in the preliminary experiment, and where samples were taken at different times to analyze the levels of DDT and its dechlorination products. Optimization of the chromatographic procedure resulted in a better quantification of DDT and its metabolites. This experiment was done to determine in which of the two stages

(anaerobic or aerobic) the highest reduction of DDT, DDE and DDD took place.

Table 2. Average values of the concentration of DDT, DDE and DDD at different fermentation times.

FERMENTATION	TIME (day)	DDT (ng/g)	DDE (ng/g)	DDD (ng/g)
	0	15.34	40.54	129.28
anaerobic	6	3.42	35.56	159.09
anaerobic	10	4.10	38.51	177.43
anaerobic	14	1.18	38.16	174.40
aerobic	16	4.81	26.84	108.05
aerobic	20	4.73	29.63	99.40
aerobic	28	4.39	25.86	88.02

Average recovery 80 %.

The statistical analysis of the results obtained for the different fermentation times showed significant differences for DDT concentrations between times 0 and 14 days ($H_{6,21}$ = 13.51527; p = 0.0356). These results prove the efficiency of the anaerobic treatment in the dechlorination of DDT, specially towards the formation of DDD, given that the concentration of this compound increased during this stage of fermentation. In relation to DDE and DDD, no significant differences between times were found. This was already expected, considering that the degradation of DDT involves the formation of DDE and DDD as degradation products. However, there was a trend in reduction of DDE and DDD levels in the following aerobic phase. This supports the efficiency of the coupled anaerobic-aerobic system in the degradation process of DDT.

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