# Degradation of Endosulfan I and Endosulfan II in the Aquatic Environment: A Proposed Enzymatic Kinetic Model that takes into account Adsorption/Desorption of the Pesticide by Colloidal and/or Sediment Particles

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ABSTRACT

**The rate of degradation of the $\alpha$ and $\beta$ isomers of endosulfan, endosulfan I and endosulfan II in distilled water and river water containing river sediment, were investigated over a period of 90 days. An immediate loss of 18 % endosulfan I and 22 % endosulfan II from the water phase of the river water containing river sediment was observed as a result of adsorption by the sediment. Subsequently biphasic linear rates of degradation were observed for both endosulfan I and II in the water phase, as well as the sediment phase of the experiment. Minimal degradation was observed in the distilled water control. An enzymatic kinetic model is presented showing that the biphasic linear rates are consistent with microbial degradation of free and colloidal particle-adsorbed pesticide in the water phase, and colloidal particle- and sediment particle-adsorbed pesticide in the sediment phase of the experiment. The estimation of the biphasic rates of degradation of the pesticide in the water and sediment phases of the system, and the factors affecting the rates of degradation, are discussed.**

KEY WORDS

**Endosulfan, degradation kinetics, aquatic environment, pesticide, adsorption/desorption kinetics.**

## 1. Introduction

The organochlorine pesticide endosulfan (6,7,8,10,10-hexachloro-1,5,5a,6,9,9a-hexahydro-6,9-methano-2,4,3-benzodioxathiepin3-oxide), CAS Registry No. [115-29-7], is a broad-spectrum non-systematic pesticide of the cyclodiene group. It is used for the protection of several crops, which include cotton, soybean, groundnut, potato and maize.[1–3] It is also used for the control of tsetse fly (*Glossina* spp.) and malaria vectors.[4,5] Although it is relatively non-toxic to mammals ($LD_{50}$ = 18 mg $kg^{-1}$ body mass in rats),[6] it and its metabolite endosulfan sulphate are highly toxic to aquatic organisms, including fish and aquatic vertebrates.[7,8] Ninety-six-hour $LC_{50}$ values of 1.5, 1.4, 1.5 and 1.2 $\mu$g $L^{-1}$ have been reported for rainbow trout, fathead minnow, channel catfish and bluegill sunfish, respectively, while 96-hour $LC_{50}$ values of 5.8 and 3.3 $\mu$g $L^{-1}$ were reported for two aquatic invertebrates, scuds (*G. lacustris*) and stoneflies (*Pteronarcys*), respectively.[7] In humans, endosulfan poisoning is characterized by stimulation of the central nervous system. It has also been shown to have teratogenic, mutagenic and reproductive effects, but no carcinogenic effect has been reported.[9–11]

The persistence of endosulfan in soil and water environments has attracted attention from several research workers. Burns[12] studied the degradation of the pesticide in soils. Kathpal *et al.*[13] and Martens[14] studied the kinetics of its degradation in soils and reported rates of 63 % loss in 2–3 months and 5.4 % in 15 weeks, respectively. Martens also studied the non-microbial degradation of the pesticide in soils under varying conditions of pH, sample wetness and temperature, and found that degradation was faster in wet soil samples with a higher pH. Zaranyika and Mugari[15] studied the persistence in the soil of endosulfan and lindane under subtropical climatic conditions in Zimbabwe and showed that rainfall had a strong influence on the rate of disappearance of the pesticides. Parkpian *et al.*[16] studied the degradation of $\alpha$-endosulfan in the tropical soils of Thailand, and reported that total degradation after 98 days was less than 20 %. Little abiotic degradation was measured in sterile soil samples. Rao and Satyanarayana[17] studied the persistence of the pesticide under natural conditions in India, and found that its residues persisted for about 120 days and 100 days in wet and dry soil, respectively, after application of the same amount of pesticide. El Beit *et al.*[18] studied the degradation of endosulfan in dark laboratory conditions using autoclaved and unautoclaved soil samples and reported the major pathway of degradation to be microbial.

Data from several sources suggest that endosulfan is moderately persistent in the soil environment with a reported average field half-life of 50 days.[19] The two isomers have different degradation rates in soil. The average half-life for the $\alpha$-isomer is 35 days, while it is 150 days for the $\beta$-isomer under neutral conditions. Both isomers persist longer under more acidic conditions. Degradation of endosulfan depends on the nature of the soil, namely its chemical composition, pH, clay content and on the quality and quantity of the biomass (fungi, bacteria).

Knowvenagel and Himmetreich[20] and Miles[21] studied the degradation of the pesticide in river water systems, and found photodecomposition to be a major degradation pathway of the pesticide in water. El Beit[18] studied the rate of leaching of endosulfan in sediments and found that the rate of leaching was slow compared with the rate of degradation by microorganisms. Howard[22] studied the persistence of endosulfan in raw river water exposed to light at room temperature, and reported that both $\alpha$- and $\beta$-endosulfan disappeared in four weeks, and that the breakdown depends on pH. Half-lives of 37.4 and 150.6 days

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were recorded for $\alpha$-endosulfan at pH 7 and 5.5, respectively. Half-lives of 37.5 and 187.3 days were also recorded for $\beta$-endosulfan at pH 7 and 5.5, respectively. Greve *et al.*[23] reported that endosulfan undergoes hydrolysis only under anaerobic conditions. They obtained hydrolysis rates constants of $11.6 \times 10^{-3}$ and $18.5 \times 10^{-3}$ day$^{-1}$ at pH 7, and 0.46 and 0.37 day$^{-1}$ at pH 5.5 for $\alpha$ and $\beta$, respectively. Shivaramaiah *et al.*[3] studied the degradation of endosulfan in river water as a function of pH, and showed that endosulfan is stable to hydrolysis at pH 5, but is subject to hydrolysis at pH 7 and 9. The same authors also showed that endosulfan is subject to conversion to endosulfan sulphate in artificial microcosms containing river water. Pollution of river water and lake sediments by endosulfan has been reported.[8,22,24,25]

Zaranyika and Nyandoro[26] studied the kinetics of the degradation of glyphosate [N-(phosphonomethyl)glycine] in the aquatic environment and observed two linear rates of degradation. The results were explained in terms of a steady state enzymatic kinetic model, which takes into account microbial degradation of both free and colloidal particle-adsorbed glyphosate. According to this model the rate of degradation of glyphosate was given by

$$dP/dt = k_2[G_B] + k_6[GC_B] \quad (1)$$

where G denotes glyphosate, GC denotes glyphosate-colloidal particle complex, the subscript B denotes microbial bound, and $k_6$ and $k_2$ are the rate constants for the degradation of the colloidal particle-adsorbed and unadsorbed glyphosate, respectively, and P denotes products. It was further shown that, provided the concentration of glyphosate in the medium was in excess of the microflora that can bind the pesticide, then a steady state obtains and the rate equation becomes:

$$dP/dt = k_2' + k_6' \quad (2)$$

These experiments were conducted using river water and sediment in order to simulate as closely as possible conditions to be found in the natural aquatic environment.

The aim of the present work was to carry out similar semi-field experiments with endosulfan with a view to elucidating further the kinetics of the degradation of the pesticide in the aquatic environment. The experiment was conducted using river water and sediment contained in plastic drums covered with a clear perforated plastic lid and exposed to sunlight. The rate of degradation of the pesticide was monitored in the water as well as the sediment phase of the experiment.

## 2. Experimental

### 2.1. Equipment

A microprocessor-controlled Varian Model 3300 gas chromatograph equipped with a split/splitless injector and a $^{63}$Ni electron capture detector (ECD) was used in conjunction with a Varian Model 3400 integrator, and a DB 1701 30 m × 0.25 mm refined silica column (J.W. Scientific, Folsom, CA, USA); white plastic tanks, 150 L capacity; 3.7 mL Pyrex glass sample vials with hollow caps and Teflon-lined septa (Supelco SA, Bellefonte, PA, USA); macro Kuderna-Danish (KD) concentrator (Supelco SA); a Kokusan H-103 N Series 5000 rpm centrifuge (Kokusan Corporation, Tokyo, Japan) and a Julabo VSR 8 ultrasonic shaker (Julabo Labortechnik Gmbh, Seelbach, Germany).

### 2.2. Materials

The following were used after redistillation in an all glass system: diethyl ether, hexane, benzene, methanol and nonane. Other materials used include acetic acid, silica gel and Florisil (60–100 mesh, Fluka Chemie AG, Buchs, Switzerland); anhydrous sodium sulphate (99.8 % purity, Acros Organics, Geel, Belgium); doubly-distilled water; river water and sediment collected from Marimba River near the campus of the University of Zimbabwe; high purity nitrogen carrier gas; endosulfan I and II reference standard (99 % pure, U.S. Environmental Protection Agency (EPA) Health Effects Research Laboratory, Environmental Toxicology Division, Research Triangle Park, NC, USA).

### 2.3. Procedures

Volumes of 120 L each of river and distilled water were charged into two separate 150 L tanks and the levels were marked. About 2 kg of sediment was added to the tank containing river water. Equal amounts, 192 $\mu$L of endosulfan solution in hexane, designed to give 320 ng mL$^{-1}$ endosulfan I and 160 ng mL$^{-1}$ endosulfan II, were added to each of the tanks. The contents were thoroughly mixed. Samples were taken at zero time immediately after the system had settled. The tanks were covered with transparent perforated polyethylene and left exposed to sunlight on the roof of the Department of Chemistry building. Thereafter samples of water and sediment were collected periodically for a period of 90 days, each time compensating for evaporation 24 hours prior to sampling. Water and sediment samples were taken without disturbing the system. The new level was marked after each sampling, then the system was stirred and left to settle.

Once collected all samples were stored in a freezer in plastic bottles with screw caps until required for analysis. All the samples were thawed and mixed thoroughly prior to analysis.

### 2.4. Extraction, Clean-up and Concentration

Water samples were analyzed in duplicate. In order to avoid any loss through adsorption, for the water samples filtration was done by centrifugation. The supernatant was slowly decanted and collected. A volume of 100 mL of it was extracted with 4 × 25 mL hexane using a separating funnel. The hexane fraction was concentrated to 1 mL using the KD apparatus maintained at 75 °C in a water bath.

For clean-up the concentrate was transferred to a chromatographic column plugged with glass wool and containing 5 g of Florisil and 5 g of anhydrous $Na_2SO_4$ pre-cleaned by eluting with hexane and 5 % diethyl ether in hexane successively. The column was then eluted twice using 20 mL of hexane and 70 mL 5 % diethyl ether in hexane. The two fractions were collected and concentrated to 1 mL using the KD apparatus and injected into the GC-ECD separately.

Sediment samples were extracted after the excess water in the sample had been removed by centrifugation. The moisture content of the centrifuged sediment samples was determined after thoroughly mixing the sample. About 1 g equivalent of dry mass was weighed and placed in a 50 mL beaker and 1 mL of acetic acid was added. The mixture was stirred with a glass rod, then 1 mL of nonane was added to the slurry and stirred. The slurry was subjected to ultrasonic shaking for 30 min, then 5 g of silica gel (60–100 mesh) was added and the mixture was stirred. The sample was then quantitatively transferred to a cellulose thimble containing 3 g of silica gel. The thimble was placed in a Soxhlet extraction apparatus and extracted with 200 mL of a 2:1 (v/v) hexane-benzene mixture. After extraction, the crude extract was concentrated to 1 mL in the KD apparatus. The concentrate was cleaned using the Florisil column, eluting twice with 20 mL of hexane and 70 mL 5 % diethyl ether in hexane. The two fractions were collected and concentrated to 1 mL using the KD apparatus and injected into the GC-ECD separately.

**Table 1** Gas chromatographic conditions employed.

| | |
|---|---|
| Initial column temperature | 150 °C |
| Initial hold time | 5 min |
| Final column temperature | 230 °C |
| Temperature program rate | 4 °C $min^{-1}$ |
| Final column hold time | 25 min |
| Detector temperature | 300 °C |
| Injector temperature | 200 °C |
| ECD attenuation | 32 on auto zero |
| Relays | –1 to 1 in 0.5 min |
| Detector range | 1 |
| Flow rate: carrier gas | 5 mL $min^{-1}$ |
| Flow rate: makeup gas | 25 mL $min^{-1}$ |

### 2.5. Gas Chromatography

Gas chromatographic conditions employed are given in Table 1. A volume of 1 $\mu$L of the concentrated extract was injected each time. The $\alpha$- and $\beta$-endosulfan isomers were well resolved, see Fig. 1.

Quantification was performed by the external standard technique. Preliminary studies with the spiked sediment and river water samples showed the average extraction efficiency to be 92 ± 2 % and 97 ± 2 %, respectively, when samples containing 10 ng $g^{-1}$ of each were extracted and determined as described above. Endosulfan was not detected when blank determinations were done on the sediment and river water samples. The results obtained are shown in Table 2. The loss in endosulfan I and II after a given time period t in days was calculated and plotted as a function of t in Fig. 2. The slopes of the linear portions of the curves in Fig. 2 were obtained using regression analysis.

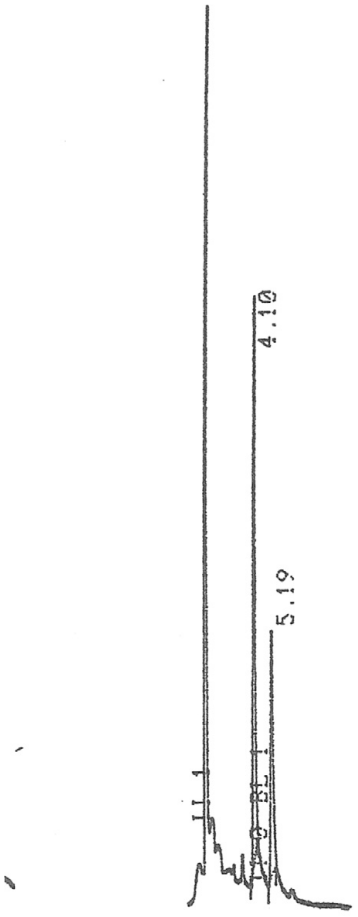


**Figure 1** Typical chromatograms for the sediment sample collected on day 34 showing the retention times for endosulfan I and endosulfan II at 4.10 and 5.19 min, respectively.

**Table 2** Degradation of endosulfan I and II in distilled water, river water and sediment phases of the experiment.

| Day of sampling | Concentration/ng $g^{-1}$ [a] | | | | | |
|---|---|---|---|---|---|---|
| | Distilled water | | River water | | Sediment | |
| | E I | E II | E I | E II | E I | E II |
| 0 | 305.0 | | 255.5 | 142.2 | 57.65 | 36.10 |
| 3 | 287.2 | | 241.7 | 135.7 | 70.65 | 28.20 |
| 7 | 282.7 | | 193.1 | 99.4 | 37.60 | 20.00 |
| 14 | 271.1 | | 121.6 | 90.1 | 25.51 | 16.16 |
| 21 | 252.1 | | 141.7 | 67.1 | 23.83 | 17.63 |
| 34 | 250.0 | | 117.5 | 62.0 | 21.64 | 14.68 |
| 48 | 238.2 | | 101.2 | 50.0 | 14.74 | 15.20 |
| 62 | 228.2 | | 88.9 | – | 18.74 | 11.63 |
| 76 | | | 93.0 | 13.2 | 11.27 | 13.18 |
| 90 | | | 81.3 | – | 8.17 | 8.40 |

[a] Concentrations before charging were all zero.

## 3. Results and Discussion

Figures 2A to 2D show that the rates of decrease of both isomers of endosulfan, both in the water and sediment phases of the experiment, are similar, i.e. an initially fast rate of disappearance, followed by a slower rate. The rapid disappearance in the water phase lasted for about 25 days for endosulfan I, and about 10 days for endosulfan II. In the sediment phase, the rapid disappearance lasted for about 10 days for both isomers. The slower rate of disappearance lasted up to the end of the experiment period for all cases for both isomers.

Figure 2 shows that both the fast and slow rates of degradation of endosulfan in the water phase, as well as the sediment phase of the experiment, are linear, pointing to steady state kinetics. These degradation trends are similar to those obtained by Zaranyika and Nyandoro[26] for glyphosate as explained above. Degradation of endosulfan in the sediment phase also shows two linear rates.

Examination of the data for the fast and slow rates of degradation in Table 3 suggests that the biphasic rates of degradation of endosulfan I and endosulfan II correspond to microbial degradation of free and colloidal particle-adsorbed pesticide in the water phase of the experiment. In the sediment phase, the rates are much slower, and correspond to degradation of free and sediment particle-adsorbed pesticide in the pore water of the sediment phase. The difference in the rates of the fast degradation of the pesticides in the water and sediment phases may be explained by the reduced mobility of the free pesticide, the reduced mobility of microbial organisms, or the reduced density of microbial organisms in the pore water of the sediment phase. Accordingly, the model shown in Table 4 is proposed for the

**Table 3** Rates of degradation (ng $g^{-1}$ $day^{-1}$) of endosulfan I and endosulfan II in the sediment and water phases of the river water and sediment experiment.

| Phase | Fast/slow | Endosulfan I/ng $g^{-1}$ $day^{-1}$ | Endosulfan II/ng $g^{-1}$ $day^{-1}$ | Designation |
|---|---|---|---|---|
| Water | Fast | 9.93 | 6.27 | $k_{E(WP)}'$ |
| | Slow | 0.68 | 1.18 | $k_C'$ |
| Sediment | Fast | 3.16 | 2.29 | $k_{E(SP)}'$ |
| | Slow | 0.22 | 0.11 | $k_{S1}'$ |

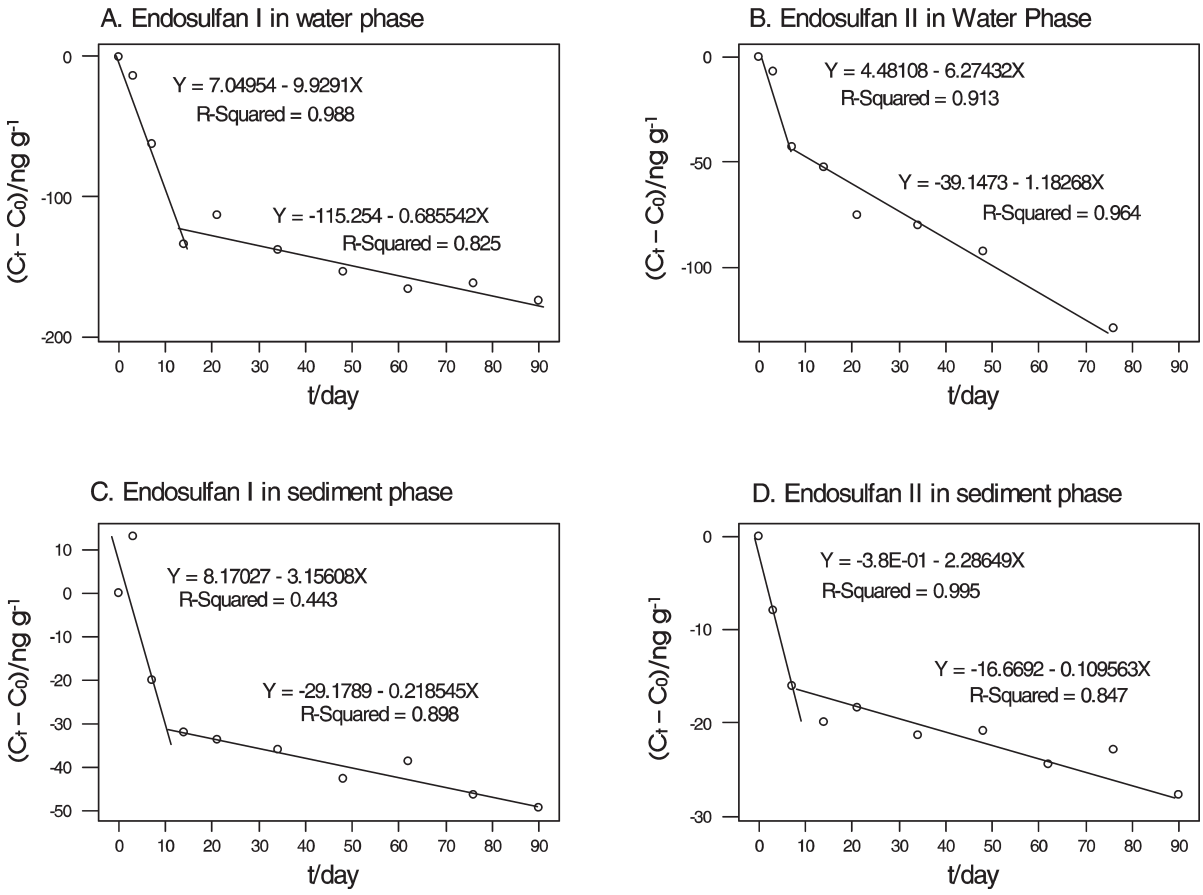


**Figure 2** Rates of degradation of endosulfan I and II in the water and sediment phases of the river water and sediment experiment: $C_o$ = concentration at t = 0 and $C_t$ = concentration at day t.

degradation of endosulfan in the aquatic environment.

From step 2, it can be shown that

$$\frac{dP}{dt} = \frac{k_2 k_3 [I_B][E]}{k_{-2} + k_3} \tag{3}$$

where

$$k_{E(WP)} = \frac{k_3 k_2 [E]}{k_{-2} + k_3} \tag{4}$$

and is the apparent rate constant for the enzymatic degradation of microbial bound insecticide in the water phase of the experiment.

**Table 4** Steps in the microbial degradation of free insecticide (I) and adsorption site–insecticide (IS) complex.

| Step | Water phase | | Sediment phase | |
|---|---|---|---|---|
| | Process [a] | Rate constant | Process [a] | Rate constant |
| 1(a) | $I + M \rightarrow I_B$ | $k_1$ | $I + M \rightarrow I_B$ | $k_1$ |
| | $I_B \rightarrow I + M$ | $k_{-1}$ | $I_B \rightarrow I + M$ | $k_{-1}$ |
| 2 | $I_B + E \rightarrow IE$ | $k_2$ | $I_B + E \rightarrow IE$ | $k_2$ |
| | $IE \rightarrow I_B + E$ | $k_{-2}$ | $IE \rightarrow I_B + E$ | $k_{-2}$ |
| | $IE \rightarrow P + E$ | $k_3$ | $IE \rightarrow P + E$ | $k_3$ |
| 1(b) | $I + C \rightarrow IC$ | $k_4$ | | |
| | $IC \rightarrow I + C$ | $k_{-4}$ | | |
| 1(c) | | | $I + S_1 \rightarrow IS_1$ | $k_5$ |
| | | | $IS_1 \rightarrow I + S_1$ | $k_{-5}$ |

[a] M = microorganism; IE = insecticide–enzyme complex; E = enzyme; P = products; subscript B = 'microbial-bound'; C = colloidal particle; S = adsorption site.

When [I] is in large excess of [M], the concentration of bound insecticide at any instant, $[I_B]$, is constant, hence Eq. 3 reduces to

$$\frac{dP}{dt} = k'_{E(WP)} \tag{5}$$

Since the $k_3$ step is fast, [IE] = 0. Thus from step 1a and step 2, assuming a steady state with respect to $I_B$, it can be shown that

$$[I_B] = \frac{k_1 [I][M]}{k_{-1} + k_2 [E]} \tag{6}$$

In the organism [E] is excess of microbial bound insecticide, hence [E] = 1, and Eq. 3 becomes

$$\frac{dP}{dt} = \frac{k_3 k_2 k_1 [I][M]}{k_{-2}(k_{-1} + k_2)} \tag{7}$$

From step 1(b), and assuming a steady state with respect to IC, it can be shown that

$$[I] = \frac{k_{-4}[IC]}{k_4 [C]} \tag{8}$$

Hence Eq. 7 becomes

$$\frac{dP}{dt} = \frac{k_3 k_2 k_1 k_{-4} [IC][M]}{k_{-2} k_4 [C](k_1 + k_2)} \tag{9}$$

[M] and [C] are constant for a given system, hence Eq. 9 becomes

$$\frac{dP}{dt} = k_C [IC] \tag{10}$$

where

$$k_C = \frac{k_3 k_2 k_1 k_{-4} [M]}{k_{-2} k_4 (k_1 + k_2)[C]} \tag{11}$$

and is the apparent rate constant for the microbial degradation of colloidal particle-adsorbed insecticide in the water phase of the experiment.

When [IC] is in large excess of [M], the concentration of colloidal particles adsorbed insecticide, [IC], is virtually constant, hence Eq. 10 reduces to

$$\frac{dP}{dt} = k'_C \tag{12}$$

The overall rate of degradation in the water phase of the experiment is given by

$$\frac{dP}{dt} = k_{E(WP)}[I_B] + k_C[IC] \tag{13}$$

and

$$\frac{dP}{dt} = k'_{E(WP)} + k'_C \tag{14}$$

in agreement with Figs. 2A and 2B. The values of $k'_{E(WP)}$ and $k'_C$ are given by the slopes of the plots of – [ΔI] *versus* t, see Figs. 2A and 2B. The values obtained are shown in Table 3.

Similarly for the adsorption sites, $S_1$, in the sediment, i.e., step 1(c), it can be shown that

$$\frac{dP}{dt} = k_{S1}[IS_1] \tag{15}$$

and

$$\frac{dP}{dt} = k'_{S1} \tag{16}$$

where

$$k_{S1} = \frac{k_3 k_2 k_1 k_{-5}[M]}{k_{-2} k_5 (k_1 + k_2)[S_1]} \tag{17}$$

and is the apparent rate constant for the microbial degradation of sediment particle-adsorbed insecticide in the sediment phase of the experiment.

Hence the overall rate of degradation in the sediment phase of the experiment is given by

$$\frac{dP}{dt} = k_{E(SP)}[I_B] + k_{S1}[IS_1] \tag{18}$$

and

$$\frac{dP}{dt} = k'_{E(SP)} + k'_{S1} \tag{19}$$

in agreement with Figs. 2C and 2D. The values of $k'_{E(SP)}$ and $k'_{S1}$ are given by the slopes of the plots of – [ΔI] *versus* t, see Figs. 2C and 2D. The values obtained are shown in Table 3. In Eq. 19 $k'_{E(SP)}$ is the apparent rate constant for the enzymatic degradation of microbial-bound insecticide in the sediment phase of the experiment.

Equations 7, 9 and 17 define the factors on which the overall rate of degradation of endosulfan I and II depend in the aquatic environment. In the water phase as well as the sediment phase, the rate of degradation is directly proportional to the concentration of free insecticide in solution and the density of microflora in the phase, and is inversely proportional to the density of colloidal particles or adsorption sites in the medium. Of particular note is the fact that the rate of degradation is directly proportional to the desorption rate constants, $k_{-4}$ and $k_{-5}$, and inversely proportional to the adsorption rate constants, $k_4$ and $k_5$, suggesting that the stronger the adsorption bond, the slower will be the degradation of the pesticide.

The actual rates of degradation observed in the environment will depend on the density of the microbial population and microbial types present in the specific environment. The actual rates of degradation observed will also depend on the composition of the water and sediment, temperature and pH, inasmuch as these will affect the populations of the different microorganisms in the study medium.

Persistence data on endosulfan from the literature were reviewed in the introduction section of this paper. It is apparent that these data, expressed in terms of half-lives, are variable, in contradiction to the prediction of true first order kinetics. Variable rates of degradation are consistent with the steady state kinetic model proposed in this paper as discussed above, suggesting that the model we have proposed is phenomenologically closer to what actually happens in the environment rather than the traditional pseudo-first order approach.

## 4. Conclusions

From the foregoing discussion, it can be concluded that the degradation of endosulfan in the aquatic environment is biphasic, and appears to be primarily due to microbial decomposition. In the water phase, the biphasic rates of degradation can be explained in terms of an enzymatic kinetic model, which takes into account microbial degradation of both free and colloidal particle-adsorbed endosulfan. In the sediment phase, the biphasic rates of degradation can be explained in terms of an enzymatic kinetic model, which takes into account microbial degradation of both free and sediment particle-adsorbed endosulfan. Provided the concentration of endosulfan in the medium is in excess of the microflora present that can bind the pesticide, the endosulfan will be lost at a constant rate which depends on the microflora count of the specific medium, the colloidal particle content (in the case of the degradation of endosulfan in the water phase), and the adsorption/desorption kinetics of the pesticide on colloidal or sediment particles.

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