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Biodegradation Kinetics of Endosulfan by *Fusarium ventricosum* and a *Pandoraea* Species

TARIQ SIDDIQUE,† BENEDICT C. OKEKE,† MUHAMMAD ARSHAD,‡ AND WILLIAM T. FRANKENBERGER, JR.*,†

Department of Environmental Sciences, University of California, Riverside, California 92521, and Institute of Soil and Environmental Sciences, University of Agriculture, Faisalabad, Pakistan

Endosulfan, classified as an organochlorine pesticide, is rated by the U.S. EPA as a Category 1 pesticide with extremely high acute toxicity. This study describes the biodegradation kinetics of endosulfan and the metabolic pathway utilized by *Fusarium ventricosum* and a *Pandoraea* sp. Complete disappearance of both α- and β-endosulfan was observed during 12 days of incubation with *F. ventricosum* in flasks containing 100 mg L^{-1} of endosulfan. The rate constants (*k*) for biodegradation of α- and β-endosulfan by *F. ventricosum* using zero-order kinetics were 14.22 and 6.60 mg L^{-1} day^{-1}, respectively. The *Pandoraea* sp. degraded about 95 and 100% of α- and β-endosulfan, respectively, in 18 days of incubation in flasks spiked with 100 mg L^{-1} of endosulfan. The rate constants (*k*) for biodegradation of α- and β-endosulfan by the *Pandoraea* sp. were 8.19 and 3.78 mg L^{-1} day^{-1}, respectively. Both fungal and bacterial strains formed less toxic endosulfan diol and endosulfan ether as metabolites during metabolism of endosulfan. The results of this study suggest that these novel strains may be used for the bioremediation of endosulfan-contaminated sites.

KEYWORDS: Bioremediation; biodegradation; *Fusarium ventricosum*; kinetics, endosulfan isomers; pesticides; *Pandoraea* sp.

INTRODUCTION

Endosulfan (6,7,8,9,10,10-hexachloro-1,5,5a,6,9,9a-hexahydro-6,9-methano-2,3,4-benzodioxathiepin-3-oxide) is a broad spectrum contact and stomach insecticide and acaricide of the cyclodiene subgroup currently used throughout the world on a wide variety of vegetables, fruits, cereal grains, and cotton as well as shrubs, trees, vines, and ornamentals for use in commercial agricultural regions (*1*, *2*). Endosulfan is currently registered to control insects and mites on 60 U.S. crops. Total average annual use of endosulfan is estimated at ∼1.38 million pounds of active ingredient (*3*).

Endosulfan is a persistent organic pollutant (POP) that enters the air, water, and soil during its use and manufacture. Classified as an organochlorine (the same family as DDT and dieldrin), endosulfan and its breakdown products are persistent in the environment with an estimated half-life of 9 months−6 years (*4*). Endosulfan has been ubiquitously detected in the atmosphere, soils, sediments, surface and ground waters, and foodstuffs (*5*). It is one of the most commonly detected pesticides in U.S. waters (38 states). Endosulfan is a neurotoxin, rated by the U.S. EPA as a Category 1 pesticide with extremely high acute toxicity (*4*). Technical-grade endosulfan comprises two stereoisomers, α- and β-endosulfan, in a ratio of 7:3, and both of the isomers are extremely toxic to fish and aquatic organisms (*6*). Endosulfan affects the central nervous system, kidney, liver, blood chemistry, and parathyroid gland and has reproductive, teratogenic, and mutagenic effects (*7*−*10*).

Detoxification of endosulfan by a bacterial coculture (*11*) and mixed cultures (*12*) has been studied. Certain fungi, *Trichoderma harzianum* (*13*), *Aspergillus niger* (*14*), *Phanerochaete chrysosporium* (*2*), and *Mucor thermohyalospora* (*15*), are also active in the biodegradation of endosulfan. Microbial metabolism of endosulfan often results in the formation of a toxic endosulfan sulfate via oxidation and a less toxic endosulfan diol by hydrolysis (*12*). Endosulfan sulfate, an oxidative metabolite, is shown to be equally as toxic and persistent as endosulfan (*2*). Endosulfan diol can further be transformed by microorganisms to endosulfan ether, endosulfan hydroxyether, endosulfan dialdehyde, and endosulfan lactone (*2*). The problem associated with bioremediation of endosulfan is not only the slow degradation of endosulfan but also the accumulation of endosulfan sulfate. It is, therefore, essential to monitor the formation of metabolites during endosulfan degradation.

In this study, we monitored the kinetics of endosulfan degradation by *Fusarium ventricosum* and a *Pandoraea* sp. *F. ventricosum* was isolated through an enrichment technique using endosulfan as a carbon and energy source. This fungal strain

* Corresponding author [telephone (909) 787 3405; fax (909) 787 2954; e-mail william.frankenberger@ucr.edu].
† University of California.
‡ University of Agriculture.

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has shown immense potential for endosulfan degradation during isolation and screening of endosulfan-degrading microorganisms (*16*). The *Pandoraea* sp. isolated (*17*) through enrichment using γ-HCH (lindane) as a carbon and energy source displayed active degradative capabilities toward all four α-, β-, γ-, and δ-HCH isomers (*18*). Endosulfan, like HCH isomers, belongs to the same group of organochlorine pesticides; therefore, the *Pandoraea* sp. was also examined for endosulfan degradation. This bacterial strain produced marvelous results, degrading endosulfan during our screening process (*16*). Because the majority of reported microorganisms produce endosulfan sulfate upon endosulfan degradation (*2*, *12*, *15*), we also determined the formation of metabolites during metabolism of endosulfan.

EXPERIMENTAL PROCEDURES

Reagents and Chemicals. Endosulfan (99.5% pure), endosulfan sulfate, 6,7,8,9,10,10-hexachloro-1,5,5a,6,9,9a-hexahydro-6,9-methano-2,4,3-benzodioxathiepin-3,3-dioxide (98%); endosulfan diol, 1,4,5,6,7,7-hexachloro-2,3-bis(hydroxymethyl)bicyclo[2.2.1]-2-heptene (99%), and endosulfan ether, 1,4,5,6,7,7-hexachlorobicyclo[2.2.1]-5-heptene-2,3-bis(methylene)oxide (99%), were purchased from Chem Services Inc. (West Chester, PA). Acetone, acetonitrile (99.9%), ethanol, and other chemicals (analytical grade) were purchased from VWR Scientific Products (San Diego, CA).

Preparation of Fungal and Bacterial Inocula for Biodegradation Studies. *F. ventricosum* was grown on FTW/endosulfan agar plates. The FTW medium (*19*) used is composed of the following (in g L^{-1}): K_2HPO_4, 0.225; KH_2PO_4, 0.225; $(NH_4)_2SO_4$, 0.225; $MgSO_4 \cdot 7H_2O$, 0.05; $CaCO_3$, 0.005; $FeCl_2 \cdot 4H_2O$, 0.005; and 1 mL of trace elements solution (*20*). FTW/endosulfan agar medium was prepared by adding 2% washed agar to nutrient solution followed by autoclaving (121 °C, 15 min). Thereafter, endosulfan dissolved in 800 μL of acetone/ethanol was aseptically added to 1 L of molten agar kept at 50 °C to give a final concentration of 100 mg L^{-1}.

The *Pandoraea* sp. was pregrown in 50 mL of FTW nutrient solution in 250 mL Erlenmeyer flasks. The medium in each flask was sterilized by autoclaving at 121 °C for 20 min and then aseptically spiked with endosulfan predissolved in 40 μL of acetone/ethanol (1:1) to give a final concentration of 100 mg L^{-1}. The culture was incubated at 30 °C (160 rpm) for 4 days. The culture was then centrifuged (5000 rpm) for 20 min. To remove residual endosulfan, cells were washed twice with 40 mL of sterile FTW nutrient solution and subjected to centrifugation (5000 rpm, 20 min). Washed cells were resuspended in sterile FTW nutrient solution.

Kinetics of Endosulfan Biodegradation. Biodegradation kinetic studies of the liquid cultures were performed in 125 mL Erlenmeyer flasks containing 50 mL of FTW nutrient solution spiked at 100 mg L^{-1} of endosulfan, in triplicate. Flasks were prepared as described in the preceding section. For the inoculation of flasks with *F. ventricosum*, four agar plugs of 0.8 cm diameter bearing uniform growth of the fungal isolates were added to each flask. Flasks used for the bacterial degradation of endosulfan were inoculated with 800 μL of the culture of the *Pandoraea* sp. ($OD_{600} = 3.7$). The same amount of FTW nutrient solution was added to the uninoculated controls. The flasks were closed with sterile rubber stoppers and incubated (30 °C, 160 rpm) for 18 days. Samples of 1.5 mL were taken from each flask daily up to 18 days for the determination of residual endosulfan, bacterial density, and pH.

Analytical Procedures. Reversed-phase liquid chromatography (LC) was used to analyze the residual concentrations of endosulfan and its major metabolites in liquid culture (*21*). Briefly, acetonitrile was used to extract the residues of endosulfan and its metabolites from the liquid culture. LC was performed with a Waters (Milford, MA) liquid chromatograph consisting of binary HPLC pumps (Waters 1525) with a pressure of 6000 psi and a flow capacity of 10 mL min^{-1}. Compounds were monitored using a UV−vis dual absorbance detector (Waters 2487) and separated on a μBondapak C_{18} (30 cm × 3.9 mm i.d.) column. The mobile phase was acetonitrile/water (70:30 v/v) at a flow rate of 1 mL min^{-1}, and solutes were detected at 214 nm.

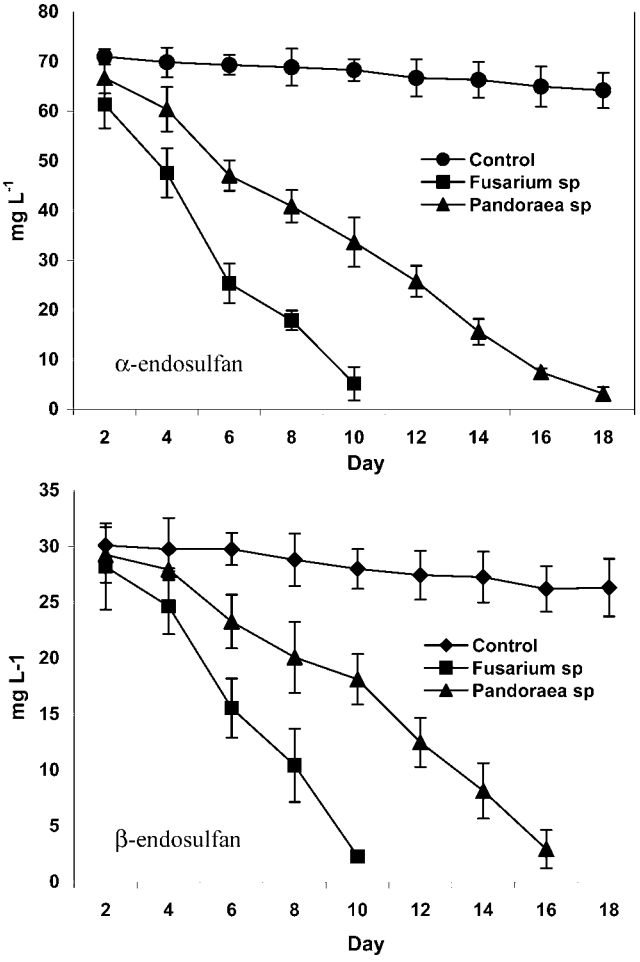

Figure 1. Time course biodegradation of α- and β-endosulfan in liquid culture initially spiked at 100 mg L^{-1} of endosulfan by *F. ventricosum* and the *Pandoraea* sp.

Bacterial densities in liquid cultures were determined spectrophotometrically by measuring the absorbance at 600 nm.

RESULTS

Kinetics of α- and β-Endosulfan Degradation by *F. ventricosum*. The degradation of α- and β-endosulfan was determined by monitoring their disappearance and the appearance of metabolites. Rapid degradation of endosulfan isomers by *F. ventricosum* was observed in liquid culture initially spiked at 100 mg L^{-1} of endosulfan (**Figure 1**). *F. ventricosum* degraded 100% of α- and β-endosulfan isomers in 12 days of incubation. The concentration of α-endosulfan in the liquid culture decreased to 5.2 mg L^{-1} in 10 days (**Figure 1**), and no residue of this isomer could be detected at day 12 upon analysis. Similar results were observed with β-endosulfan degradation by *F. ventricosum*. HPLC analysis showed a concentration of 2.3 mg L^{-1} of β-endosulfan in the liquid culture at day 10. Beyond this sampling interval, no residue of β-endosulfan was found in liquid culture. No significant degradation of α- and β-endosulfan occurred in the control flasks (**Figure 1**). A zero-order kinetics model was used to determine the rate constants (k) for α- and β-endosulfan degradation at 100 mg L^{-1} of endosulfan. The rate constants (k) determined for α- and β-endosulfan by *F. ventricosum* were 14.22 and 6.60 mg L^{-1} day^{-1}, respectively (**Figure 2**).

Kinetics of α- and β-Endosulfan Degradation by a *Pandoraea* Species. A significant decrease in the concentrations of

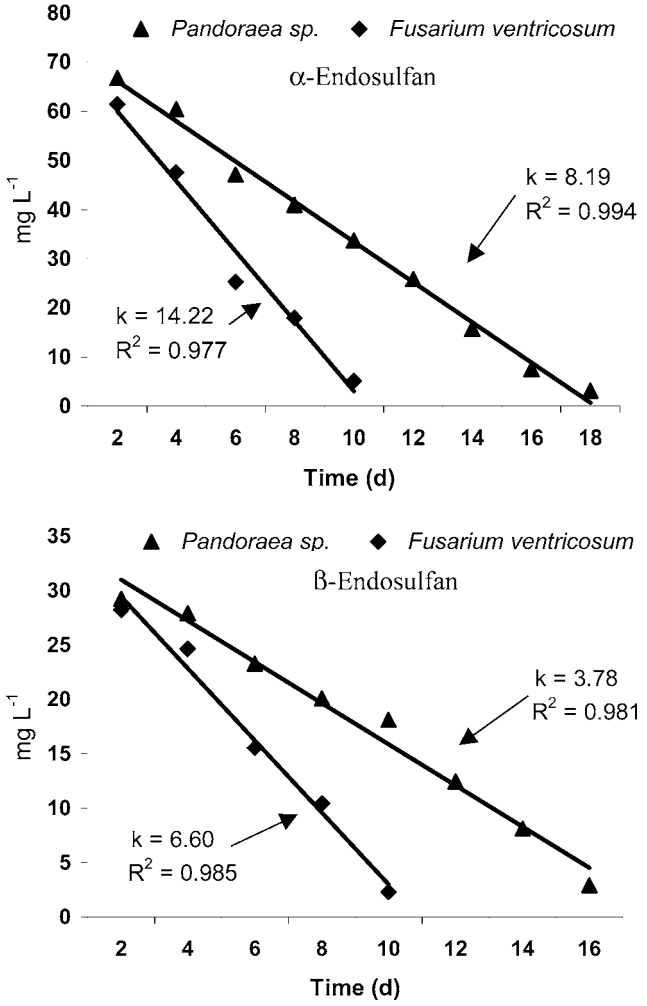

Figure 2. α- and *β*-Endosulfan biodegradation kinetics by *F. ventricosum* and the *Pandoraea* sp. Unit of rate constant (*k*) is mg L^{-1} day^{-1}.

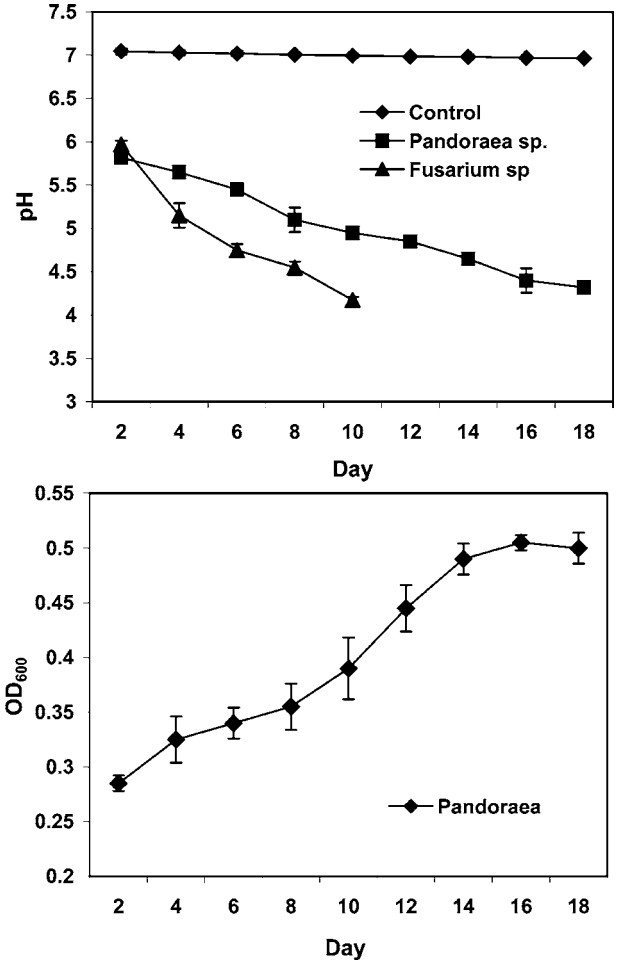

Figure 3. Change in pH by *F. ventricosum* and the *Pandoraea* sp. and bacterial density of the *Pandoraea* sp. during metabolism of endosulfan at 100 mg L^{-1} over a period of 18 days.

α- and *β*-endosulfan in the liquid culture by *Pandoraea* sp. was observed during 18 days of incubation (**Figure 1**). The concentration of α-endosulfan declined from 66.75 mg L^{-1} at day 2 to 3.14 mg L^{-1} at day 18, with an overall biodegradation of 95% in 18 days. Biodegradation of *β*-endosulfan in the liquid culture revealed 100% removal in 18 days as no residue of *β*-endosulfan was found at day 18 (**Figure 1**). The rate constants (*k*) determined for α- and *β*-endosulfan degradation were 8.19 and 3.78 mg L^{-1} day^{-1} by the *Pandoraea* sp., respectively (**Figure 2**).

Bacterial Density and Culture pH. Optical density (λ_{600}) and culture pH of the liquid culture were also determined to assess the relationship between growth and metabolic activities of the microorganisms (**Figure 3**). OD_{600} of the liquid culture inoculated with the *Pandoraea* sp. increased in the first 14 days followed by a stationary phase from 14 to 18 days of incubation. Flasks assigned as a positive control (inoculated with the *Pandoraea* sp. and spiked with the same amount of acetone/ethanol without endosulfan) did not show any increase in bacterial population with time (data not shown). Variation in pH of the liquid culture with the metabolic activities of the growing microorganisms (*F. ventricosum* and *Pandoraea* sp.) is presented in **Figure 3**. Biodegradation of α- and *β*-endosulfan drastically decreased the pH of the liquid culture to an acidic range. *F. ventricosum* reduced the culture pH from neutral to 4.17 in 10 days of incubation while growing on the isomers of endosulfan. A decrease in pH was also noted in flasks inoculated with the *Pandoraea* sp. Culture pH was lowered to a value of 4.32 in 18 days of incubation with bacterial growth metabolizing both the α- and *β*-isomers of endosulfan.

Formation of Endosulfan Metabolites by the Cultures. HPLC analysis indicated the disappearance of both isomers of endosulfan with the formation of major metabolites of endosulfan. **Table 1** reveals that the oxidation product, endosulfan sulfate, was not formed during the metabolism of both α- and *β*-endosulfan by these two fungal and bacterial strains. Two metabolites detected were endosulfan diol and endosulfan ether with authentic standards and matching retention times by HPLC. No residue of endosulfan sulfate was found in the cultures of both fungal and bacterial strains. In the control flasks, concentrations of endosulfan diol and endosulfan ether increased very slightly with time upon insignificant chemical degradation of endosulfan. The nonaccumulation of these two metabolites in liquid cultures inoculated with the fungal and bacterial strains throughout the incubation period indicated that both metabolites were transient intermediates and precursors to subsequent metabolic products. Almost the same quantities of both the metabolites were observed in the cultures of *F. ventricosum* and the *Pandoraea* sp. The formation and decay of these metabolites led us to propose a pathway of endosulfan metabolism by the microbial strains (**Figure 4**). An initial hydrolysis of endosulfan results in the formation of an intermediate metabolite, endosulfan diol, which is further metabolized to endosulfan ether.

Table 1. Concentration of Endosulfan Metabolites (Milligrams per Liter) in Control Flasks as well as Flasks Inoculated with *F. ventricosum* and the *Pandoraea* Species[a]

	control			*F. ventricosum*			*Pandoraea* sp.		
day	ES	ED	EE	ES	ED	EE	ES	ED	EE
2	ND	2.69 (±0.014)	0.125 (±0.007)	ND	2.80 (±0.057)	0.165 (±0.035)	ND	2.54 (±0.170)	0.155 (±0.064)
4	ND	2.93 (±0.071)	0.125 (±0.007)	ND	2.83 (±0.014)	0.135 (±0.007)	ND	2.45 (±0.014)	0.130 (±0.014)
6	ND	3.09 (±0.042)	0.145 (±0.007)	ND	2.81 (±0.071)	0.160 (±0.028)	ND	2.46 (±0.085)	0.185 (±0.078)
8	ND	3.20 (±0.078)	0.155 (±0.007)	ND	2.92 (±0.113)	0.185 (±0.007)	ND	2.49 (±0.099)	0.140 (±0.028)
10	ND	3.56 (±0.057)	0.165 (±0.007)	ND	0.79 (±0.467)	0.115 (±0.007)	ND	2.61 (±0.156)	0.155 (±0.021)
12	ND	3.64 (±0.028)	0.165 (±0.007)				ND	2.53 (±0.042)	0.115 (±0.007)
14	ND	3.77 (±0.021)	0.175 (±0.007)				ND	2.56 (±0.007)	0.125 (±0.007)
16	ND	3.90 (±0.141)	0.175 (±0.007)				ND	1.81 (±0.177)	0.094 (±0.023)
18	ND	4.15 (±0.042)	0.175 (±0.007)				ND	1.05 (±0.071)	0.053 (±0.011)

[a] Initial concentration of endosulfan used was 100 mg L^{-1}. ES, endosulfan sulfate; ED, endosulfan diol; EE, endosulfan ether; ND, not detected. Values in parentheses show standard deviation.

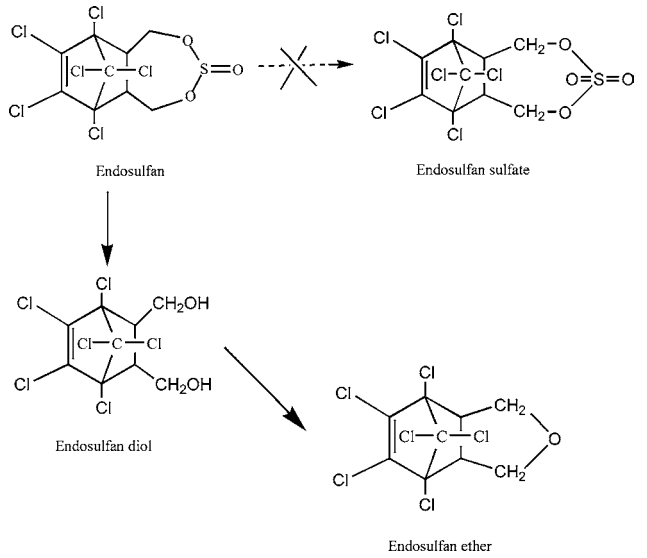

Figure 4. Proposed pathway for metabolism of endosulfan by *F. ventricosum* and the *Pandoraea* sp.

DISCUSSION

Previous studies have reported the biodegradation of endosulfan by a bacterial coculture and mixed cultures or with fungi that are known to degrade other xenobiotics in the environment (*2*, *11*−*15*). Some of these studies revealed that endosulfan could be used only as a sulfur source and considered endosulfan as a poor biological energy source because it contains only six potential reducing electrons. Others described the production of a toxic metabolite, endosulfan sulfate, upon degradation. We have isolated pure cultures of microorganisms (*16*) through an enrichment technique using endosulfan as a carbon and energy source. *F. ventricosum* and the *Pandoraea* sp. are very active in degrading endosulfan. This study describes the biodegradation kinetics of endosulfan and metabolic pathway utilized by *F. ventricosum* and the *Pandoraea* sp.

F. ventricosum was highly active in degrading endosulfan with 100% degradation of α- and β-endosulfan in flasks initially spiked at 100 mg L^{-1} of endosulfan in 12 days. The degradation rate shown by *F. ventricosum* is higher than rates reported in the earlier scientific literature. Shetty et al. (*15*) studied the ability of a fungus, *Mucor thermo-hyalospora* (MTCC 1384), to degrade endosulfan in a culture medium. Their results indicated that ∼78% of the endosulfan isomers were degraded in flasks spiked at 5 mg L^{-1} after 20 days of incubation. Mukherjee and Gopal (*14*) reported that β-endosulfan dissipated in the presence of *Aspergillus niger* to 98.6% by day 15, compared to 78.4% in the control medium without *A. niger*. Kim et al. (*22*) studied the biodegradation kinetics of endosulfan by *Phanerochaete chrysosporium*. They reported a degradation rate of 0.23 mg L^{-1} day^{-1} in flasks spiked at 2.5 mg L^{-1} of endosulfan. The findings of this study support the role of the *Fusarium* sp. in bioremediation of xenobiotics. Mitra et al. (*23*), Jirku et al. (*24*), and Yagafarova et al. (*25*) revealed that other *Fusarium* spp. (*F. solani* and *F. proliferatum*) are active in the bioremediation of DDT and hydrocarbons in oil-contaminated soils as well as other xenobiotics in water.

The *Pandoraea* sp. degraded 95 and 100% of α- and β-endosulfan, respectively, in flasks containing 100 mg L^{-1} of endosulfan during 18 days of incubation. Awasti et al. (*11*) studied the degradation of endosulfan by a bacterial coculture and observed that nearly 50% degradation of both isomers of endosulfan occurred in flasks spiked at 50 mg L^{-1} in 7 days. Zero-order kinetics was also used to determine the rate constants (k) for α- and β-endosulfan degradation by the *Pandoraea* sp. Both *F. ventricosum* and the *Pandoraea* sp. performed well in degrading endosulfan, with *F. ventricosum* yielding higher degradation rate constants than the *Pandoraea* sp.

An increase in optical density of the liquid culture with disappearance of endosulfan demonstrates that the *Pandoraea* sp. consumes endosulfan as a carbon and energy source. Awasti et al. (*11*) and Sutherland et al. (*12*) also observed a substantial disappearance of endosulfan with a simultaneous increase in bacterial mass. *F. ventricosum* and the *Pandoraea* sp. significantly decreased the pH of the liquid culture. pH reduction in the culture medium may be due to either the dehalogenation of endosulfan, resulting in the formation of HCl, or organic acids produced by microorganisms during their metabolic activities. Sutherland et al. (*12*) reported that the positive-ion chemical ionization (PCI) mass spectrum of a metabolite resulting from endosulfan degradation displayed fragment ions indicating consecutive losses of two molecules of HCl from the molecular parent ions $[M + H]^+$.

Microbial metabolism of endosulfan was monitored by substrate disappearance and product formation. The results suggest that both *F. ventricosum* and the *Pandoraea* sp. metabolized endosulfan to endosulfan diol. In comparison to previous reports on the microbial metabolism of endosulfan, our results indicate that *F. ventricosum* and the *Pandoraea* sp. are unique in their capabilities to hydrolyze the parent form of this pesticide. Several soil fungi have been shown to metabolize endosulfan and produce endosulfan sulfate as the major metabolic product. Kullman and Matsumura (*2*) studied the metabolic pathway of *P. chrysosporium* for the degradation of endosulfan. They revealed that this organism utilized both oxidative and hydrolytic pathways for the degradation of endosulfan. GC-ECD and GC-MS identified endosulfan sulfate,

endosulfan diol, and endosulfan hydroxyether in the culture. Sutherland et al. (*12*) enriched an endosulfan-degrading mixed bacterial culture capable of degrading endosulfan as a sulfur source. They reported that endosulfan was both oxidized and hydrolyzed by the mixed bacterial culture. Martens (*26*) reported that soil bacteria were capable of metabolizing endosulfan and produced predominantly endosulfan diol, favoring hydrolysis. The amount of endosulfan ether determined in the liquid culture of *F. ventricosum* and *Pandoraea* sp. was less than that of endosulfan diol, suggesting further metabolism of endosulfan diol to endosulfan ether. Miles and Moy (27) proposed a succession of oxidation reactions converting endosulfan diol to endosulfan ether, endosulfan hydroxyether, and endosulfan lactone.

In summary, *F. ventricosum* and the *Pandoraea* sp. degraded endosulfan at a rapid rate. Both strains metabolized endosulfan, forming nontoxic metabolites, which were subject to further degradation. The results of this study have valuable applications for endosulfan bioremediation in polluted sites.

LITERATURE CITED

(1) Lee, N.; Skerritt, J. H.; McAdam, D. P. Hapten synthesis and development of ELISAs for the detection of endosulfan in water and soil. *J. Agric. Food Chem*. **1995**, *43*, 1730−1739.

(2) Kullman, S. W.; Matsumura, F. Metabolic pathways utilized by *Phanerochaete chrysosporium* for degradation of the cyclodiene pesticide endosulfan. *Appl. Environ. Microbiol*. **1996**, *62*, 593−600.

(3) EPA's National Service Center for Environmental Publications. *Endosulfan RED Facts*; Cincinnati, OH, 2002; http://www.epa.gov/pesticides/reregistration/endosulfan/.

(4) PAN-UK News Release. Endosulfan concerns are global. *PAN's Pesticide Database* **2001** (http://www.pesticideinfo.org).

(5) Turner, K. O.; Syvanen, M.; Meizel, S. The human acrosome reaction is highly sensitive to inhibition by cyclodiene insecticides. *J. Androl*. **1997**, *18*, 571−575.

(6) Sunderam, R. I. M.; Cheng, D. M. H.; Thompson, G. B. Toxicity of endosulfan to native and introduced fish in Australia. *Environ. Toxicol. Chem*. **1992**, *11*, 1469−1476.

(7) *Farm Chemicals Handbook*; Meister Publishing: Willoughby, OH, 1992.

(8) Paul, V.; Balasubramaniam, E. Effect of single and repeated administration of endosulfan on behaviour and its interaction with centrally acting drugs in experimental animals: a mini review. *Environ. Toxicol. Pharmacol*. **1997**, *3*, 151−157.

(9) U.S. Department of Health and Human Services. *Toxicological Profile for Endosulfan (Update)*; Agency for Toxic Substances and Disease Registry (ATSDR): Atlanta, GA, 2000.

(10) Lu, Y.; Morimoto, K.; Takeshita, T.; Takeuchi, T.; Saito, T. Genotoxic effects of α-endosulfan and β-endosulfan on human HepG2 cells. *Environ. Health Perspect*. **2000**, *108*, 559−561.

(11) Awasthi, N.; Manickam, N.; Kumar, A. Biodegradation of endosulfan by a bacterial coculture. *Bull. Environ. Contam. Toxicol*. **1997**, *59*, 928−934.

(12) Sutherland, T. D.; Horne, I.; Lacey, M. J.; Harcourt, R. L.; Russel, R. J.; Oakeshott, J. G. Enrichment of an endosulfan-degrading mixed bacterial culture. *Appl. Environ. Microbiol*. **2000**, *66*, 2822−2828.

(13) Katayama, A.; Matsumura, F. Degradation of organochlorine pesticides, particularly endosulfan by *Trichoderma harzianum*. *Environ. Toxicol. Chem*. **1993**, *12*, 1059−1065.

(14) Mukherjee, I.; Gopal, M. Degradation of β-endosulfan by *Aspergillus niger*. *Toxicol. Environ. Chem*. **1994**, *46*, 217−221.

(15) Shetty, P. K.; Mitra, J.; Murthy, N. B. K.; Namitha, K. K.; Savitha, K. N.; Raghu, K. Biodegradation of cyclodiene insecticide endosulfan by *Mucor thermo-hyalospora* MTCC 1384. *Curr. Sci*. **2000**, *79*, 1381−1383.

(16) Siddique, T.; Okeke, B. C.; Arshad, M.; Frankengerger, W. T., Jr. Enrichment and isolation of endosulfan-degrading microorganisms. *J. Environ. Qual*. **2003**, *32*, 47−54.

(17) Okeke, B. C.; Siddique, T.; Arbestain, M. C.; Frankenberger, W. T., Jr. Biodegradation of γ-hexachlorocyclohexane (lindane) and α-hexachlorocyclohexane in water and a soil slurry by a *Pandoraea* sp. *J. Agric. Food Chem*. **2002**, *50*, 2548−2555.

(18) Siddique, T.; Okeke, B. C.; Arshad, M.; Frankenberger, W. T., Jr. Temperature and pH effects on biodegradation of hexachlorocyclohexane isomers in water and a soil slurry. *J. Agric. Food Chem*. **2002**, *50*, 5070−5076.

(19) Herman, D. C.; Frankenberger, W. T., Jr. Bacterial reduction of perchlorate and nitrate in water. *J. Environ. Qual*. **1999**, *28*, 1018−1024.

(20) Focht, D. D. Microbiological procedures for biodegradation research. In *Methods of Soil Analysis, Part 2—Microbiological and Biochemical Properties*; Weaver, R. L., Angle, J. S., Bottomley, P. S., Eds.; Soil Science Society of America Book Series 5; SSSA: Madison, WI, 1994; pp 407−426.

(21) Siddique, T.; Zahir, Z. A.; Frankenberger, W. T., Jr. Reversed-phase liquid chromatographic method for analysis of endosulfan and its major metabolites. *J. Liq. Chromatogr. Relat. Technol*. **2003**, *26*, 1069−1082.

(22) Kim, Y.-K.; Kim, S.-H.; Choi, S.-C. Kinetics of endosulfan degradation by *Phanerochaete chrysosporium*. *Biotechnol. Lett*. **2001**, *23*, 163−166.

(23) Mitra, J.; Mukherjee, P. K.; Kale, S. P.; Murthy, N. B. K. Bioremediation of DDT in soil by genetically improved strains of soil fungus *Fusarium solani*. *Biodegradation* **2001**, *12*, 235−245.

(24) Jirku, V.; Masak, J.; Cejkova, A. Significance of physical attachment of fungi for bio-treatment of water. *Microbiol. Res*. **2001**, *156*, 383−386.

(25) Yagafarova, G. G.; Gataullina, E. M.; Barakhnina, V. B.; Yagafarov, I. R.; Safarov, A. Kh. A new oil-oxidizing micromycete *Fusarium sp. Prikladnaya Biokhim. Mikrobiol.* **2001**, *37*, 77−79.

(26) Martens, R. Degradation of [8,9-^{14}C]endosulfan by soil microorganisms. *Appl. Environ. Microbiol*. **1976**, *31*, 853−858.

(27) Miles, J. R. W.; Moy, P. Degradation of endosulfan and its metabolites by a mixed culture of soil microorganisms. *Bull. Environ. Contam. Toxicol*. **1979**, *23*, 13−19.

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