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Temperature and pH Effects on Biodegradation of Hexachlorocyclohexane Isomers in Water and a Soil Slurry

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This study was conducted to monitor the biodegradation of α-, β-, γ-, and δ-hexachlorocyclohexane (HCH) isomers in liquid culture by a *Pandoraea* species and determine the influence of pH and temperature on the biodegradation of α- and γ-HCH in liquid as well as in soil slurry cultures. The *Pandoraea* species degraded 79.4% δ-HCH and 34.3% γ-HCH in liquid culture at 4 weeks of incubation. α- and β-HCH exhibited almost identical rates (41.6 and 42.4%, respectively) of degradation. The highest degradation of α- and γ-HCH (67.1 and 60.2%, respectively) was observed at an initial pH of 8.0 in liquid; 58.4 and 51.7% rates of degradation of α- and γ-HCH, respectively, at an initial pH of 9.0 were found in soil slurry cultures. An incubation temperature of 30 °C was optimum for effective degradation of α- and γ-HCH isomers (62.5 and 57.7%, respectively) in liquid culture, and 54.3 and 51.9% rates of degradation of α- and γ-HCH isomers, respectively, were found in a soil slurry. Increasing the soil/water ratio decreased the extent of degradation of both HCH isomers. Degradation of HCH isomers occurred concomitant with bacterial growth. Byproducts of growth from *Pandoraea* species significantly decreased the pH of the liquid and the soil slurry during the growth on HCH isomers. The results of this study suggest that this bacterial strain may effectively be used for remediating polluted sites and water contaminated with different HCH isomers over a range of environmental conditions.

KEYWORDS: Bioremediation; organochlorine pesticides; hexachlorocyclohexane isomers; pH; temperature; *Pandoraea* sp.

INTRODUCTION

Isomers of 1,2,3,4,5,6-hexachlorocyclohexane (HCH) have been the most extensively used broad-spectrum organochlorine pesticides against a wide range of soil-dwelling and plant-eating (phytophagous) insects (*1*). There are eight isomers of HCH designated β, γ, δ, ϵ, η, θ, and two α-enantiomers (*2*). They differ only in their relative orientation of the chlorine atoms bound to the different carbons (*1*, *3*). The technical product is composed of the following isomers: α-HCH (60−70%), β-HCH (5−12%), γ-HCH (10−15%), δ-HCH (6−10%), and ϵ-(3−4%) (*4*) (**Figure 1**). Although the α-isomer is the major constituent of the technical-grade HCH, the γ-isomer is the most potent insecticidal ingredient. Thus, it is common to refine γ-HCH from the mixture and market it under the name lindane (*5*).

A number of publications depict the health effects of HCH isomers on animals and humans and the occurrence of residues in soil, water, air, plants, plant products, animals, and food commodities (*6*). Although the widespread use of lindane and technical-grade HCH has been discontinued for a long time, the problem of their residues, due to the lengthy persistence of these chemicals in many soils, exists (*7*). Adverse health effects associated with HCH isomers include neurological problems and immunosuppression in humans and liver cancer in rats and mice (*2*, *5*). A study reported by Gerhard et al. (*8*) showed that HCH assimilated through the diet, owing to its carcinogenic and immunotxic properties, played an important role in the

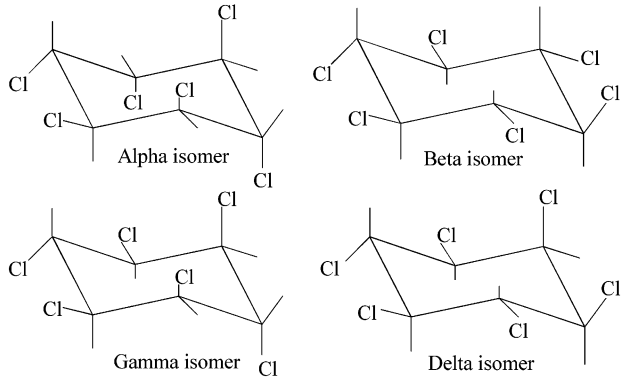

Figure 1. Structures of different HCH isomers studied.

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etiology of human breast cancer. Therefore, there is an urgent need for remedial treatment of sites where HCH isomers were used. Bioremediation offers an attractive cost-effective option to remove HCH isomers.

Environmental parameters, such as pH, temperature, redox potential, moisture, and soil texture and composition, influence biodegradation of chemical pollutants (*9*−*12*). These environmental factors can vary in time and space and affect the proliferation and activity of microorganisms in degrading toxic compounds present in the environment (*13*, *14*).

HCH isomers can be biologically degraded under aerobic and anaerobic conditions (*15*). Various studies reveal that research has mainly focused on the degradation of α- and γ-HCH isomers (*16*−*19*) with little attention on other isomers of HCH (β and δ) present in technical-grade HCH. Among the four isomers, β-HCH has been reported to be comparatively more stable and has a serious problem of persistence and accumulation in the environment (*20*).

In the present study, we report the comparative biodegradation of four HCH isomers (α-, β-, γ-, and δ-HCH) in liquid culture by a *Pandoraea* species isolated in our laboratory from an enrichment culture (*21*). In addition, the influence of pH, temperature, and soil/water ratio on the degradation of α- and γ-HCH in liquid and soil slurry cultures by this *Pandoraea* species is reported.

EXPERIMENTAL PROCEDURES

Reagents and Chemicals. α-Hexachlorocyclohexane (99% pure) and γ-hexachlorocyclohexane (97% pure) were purchased from Aldrich (Milwaukee, WI). β-HCH (98.1% pure) and δ-HCH (98.6% pure) were provided by Reidal-de Haen, Spain. Acetone, hexane (99.9%), ethanol, and a pesticide calibration standard mixture (608 calibration mix) were purchased from VWR Scientific Products (San Diego, CA). Other chemicals were of analytical grade and purchased from commercial sources.

Preparation of Bacterial Inoculum for Biodegradation Studies. The *Pandoraea* species was pregrown in FTW nutrient solution. The medium was sterilized by autoclaving at 121 °C for 20 min and then aseptically spiked with 150 mg L^{-1} of γ-HCH predissolved in acetone/ethanol. The culture was incubated at 30 °C (150 rpm) for 4 days. The FTW medium (*22*) used comprises 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 (*23*). The inoculum was then centrifuged (5000 rpm) for 20 min. To remove residual nutrients and HCH, cells were washed twice by centrifugation (5000 rpm, 20 min) using 40 mL of sterile FTW nutrient solution. Washed cells were resuspended in sterile FTW nutrient solution.

Biodegradation of Hexachlorocychlohexane Isomers in Liquid Culture. Biodegradation studies in liquid culture were performed in 250 mL Erlenmeyer flasks containing 50 mL of FTW nutrient solution, in triplicate. Flasks were autoclaved at 121 °C for 20 min. After cooling to ambient temperature, the flasks were spiked with α-, β-, γ-, and δ-HCH separately, predissolved in acetone/ethanol to give a final concentration of 100 mg L^{-1} of each HCH isomer. Flasks were then inoculated with 500 μL of the inoculum (OD_{600} = 1.19). 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 4 weeks.

Biodegradation of α- and γ-HCH in Liquid and Soil Slurry Cultures at Different pH Values. Biodegradation studies in liquid culture at different pH values were conducted in 50 mL Erlenmeyer flasks, in triplicate. For the liquid culture experiment, 10 mL of FTW nutrient solution was added to each flask. Flasks were autoclaved for 20 min at 121 °C. After cooling to room temperature, the flasks were adjusted to pH values of 4.0, 5.0, 6.0, 7.0, 8.0, and 9.0 by adding predetermined amounts of 0.5 N HCl or 0.5 N NaOH. Sterilized water was used to prepare 0.5 N HCl and 0.5 N NaOH.

For the soil slurry experiment, 10 mL of nutrient solution and 1.5 g of Hanford soil (sandy loam; pH 7.73; 0.07% total N; and 0.52% organic C) collected from the Agricultural Operational Station, University of California (Riverside, CA) were added to 50 mL Erlenmeyer flasks. Flasks were autoclaved at 121 °C for 20 min. The same procedure as for the liquid experiment was adopted to adjust the different pH levels in the soil slurry. No acid or alkali was used to adjust the pH to 7.0 in the soil slurry because autoclaving brought the pH of the soil slurry to near 7.0.

Flasks (liquid and soil slurry cultures) were spiked with α- and γ-HCH (30 μL of acetone/ethanol) at 100 mg L^{-1} of each isomer. Flasks were then inoculated with 500 μL of inoculum (OD_{600} = 1.88). The same volume of nutrient solution was added to the uninoculated controls. The flasks were closed with sterile rubber stoppers, incubated on an orbital incubator (160 rpm) at 30 °C, and aerated weekly in a laminar flow hood.

Biodegradation of α- and γ-HCH in Liquid and Soil Slurry Cultures at Different Temperatures. Biodegradation studies in liquid and soil slurry cultures at different incubation temperatures were performed in triplicates. For the liquid culture 10 mL of FTW nutrient solution and for the soil slurry experiment 10 mL of FTW and 1.5 g of a sandy loam soil (15% soil slurry) were added to 50 mL Erlenmeyer flasks. Flasks were autoclaved at 121 °C for 20 min. After cooling to ambient temperature, the flasks were spiked with γ- and α-HCH (30 μL of acetone/ethanol) at 100 mg L^{-1} of each isomer. Flasks were then inoculated with 500 μL of the inoculum (OD_{600} = 1.88). The same amount of FTW nutrient solution was added to the uninoculated controls. The flasks were closed with sterile rubber stoppers, incubated on an orbital incubator (160 rpm) at 25, 30, and 35 °C, and aerated weekly in a laminar flow hood.

Biodegradation of α- and γ-HCH at Different Soil/Water Ratios. To study the effect of different soil/water ratios on the degradation of α- and γ-HCH, 1.0, 1.5, or 2.0 g of a sandy loam soil was added separately to 50 mL Erlenmeyer flasks containing 10 mL of FTW nutrient solution. Flasks were autoclaved at 121 °C for 20 min. After cooling to ambient temperature, the flasks were spiked with γ- and α-HCH (30 μL of acetone/ethanol) at 100 mg L^{-1} of each isomer. Flasks were then inoculated with 500 μL of the inoculum (OD_{600} = 1.88). The same amount of FTW nutrient solution was added to the uninoculated controls. The flasks were then closed with sterile rubber stoppers, incubated on an incubator shaker (160 rpm) at 30 °C, and aerated weekly in a laminar flow hood. This study was carried out in triplicates.

Analytical Procedures. Bacterial density in the liquid culture was determined spectrophotometrically by measuring the absorbance at 600 nm. For the soil slurry experiments, the HCH-degrading bacterial biomass was measured by counting the colony forming units (cfu). FTW nutrient solution with washed agar was autoclaved (121 °C, 20 min) and kept warm at 50 °C in a hot water bath. γ-HCH (200 mg L^{-1}) was added to the molten agar and thoroughly mixed before it was transferred to sterile plates. Soil slurry culture samples were serially diluted (10^{-1}−10^{-8}), and 100 μL aliquots were plated on the FTW lindane−agar plates. Plates were incubated at 30 °C, and colony forming units were counted after 7 days.

The HCH isomers in liquid and soil slurry cultures were extracted by the addition of an equal volume of acetone and shaken for 1 h for the liquid culture and for 2 h for the soil slurry using a reciprocating shaker. One milliliter was then transferred to 9 mL of hexane and further shaken for 30 min. The sample was dehydrated by passing it through anhydrous Na_2SO_4 and concentrated using a rotary evaporator. Appropriate dilutions of the sample extract were then analyzed using a Hewlett-Packard gas chromatograph equipped with an electron capture detector (GC-ECD), an autosampler, and a DB-5MS capillary column of 0.25 mm i.d. and 0.25 μm film thickness (J&W Scientific). The oven temperature was programmed at 175 °C for 1 min, followed by a linear increase of 2.43 °C min^{-1} to 260 °C for 5 min. The injector temperature was 250 °C, and the detector temperature was 300 °C. Hydrogen was the carrier gas at a flow rate of 0.8 mL min^{-1}.

Data Analysis. The means and standard deviations (n − 1) of replicates were computed. Biodegradation (percent) was calculated on

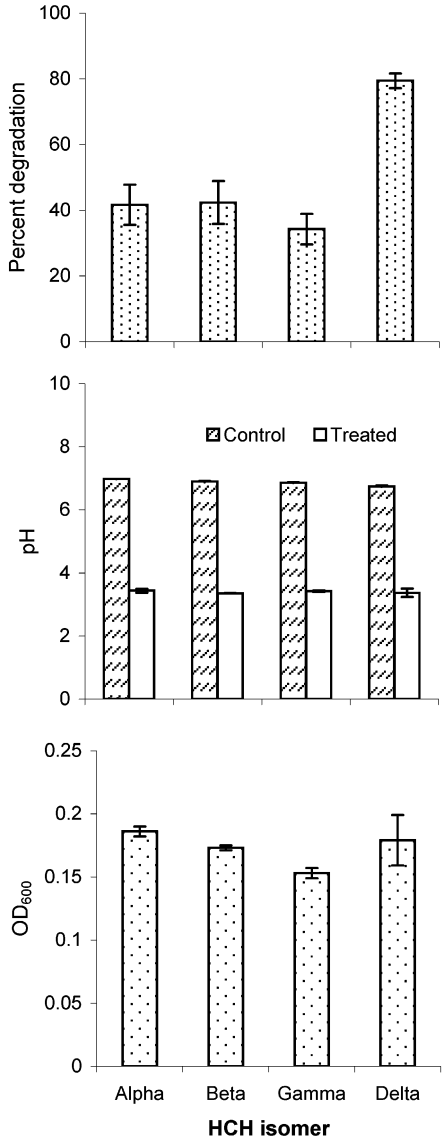

Figure 2. Comparative biodegradation of α-, *β*-, *γ*-, and *δ*-HCH isomers: pH and optical density of liquid culture inoculated with *Pandoraea* sp.

the basis of the difference between remaining HCH in the controls and treated samples.

RESULTS

Comparative Biodegradation of Four Hexachlorocyclohexane Isomers (α, *β*, *γ*, and *δ*) in Liquid Culture. The *Pandoraea* species degraded all four HCH isomers (α, *β*, *γ*, and *δ*) in liquid culture during 2 weeks of incubation (**Figure 2**). The highest degradation (79.4%) was observed in the flasks spiked with *δ*-HCH and the lowest (34.3%) with *γ*-HCH. α- and *β*-HCH isomers were degraded in the liquid culture by as much as 41.6 and 42.4%, respectively, during 2 weeks of incubation by the *Pandoraea* species, indicating almost equal rates of degradation. Thus, the order of degradation of HCH isomers by the *Pandoraea* species was as follows: $\delta > \beta \geq \alpha > \gamma$.

Optical density and pH of the liquid culture were also measured to assess the relationship between growth and metabolic activities of the *Pandoraea* species (**Figure 2**). This bacterial strain grew well on all four HCH isomers (α, *β*, *γ*, and *δ*). The highest OD_{600} was recorded with α-HCH (0.186)

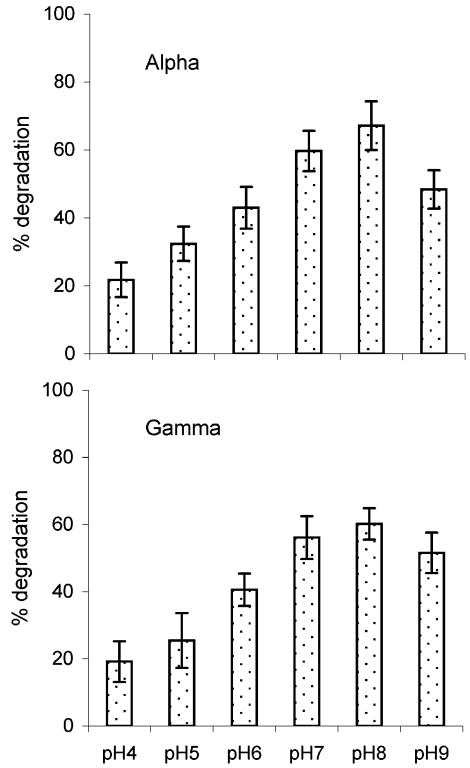

Figure 3. Effect of pH on the biodegradation of α- and *γ*-HCH isomers in liquid culture inoculated with *Pandoraea* sp.

followed by *δ*-HCH (0.179), *β*-HCH (0.173), and *γ*-HCH (0.153) after 2 weeks of incubation.

Changes in pH of the liquid culture after 2 weeks of incubation are also shown in **Figure 2**. Culture pH decreased to an acidic range due to the metabolic activities of the growing bacteria. Degradation of all four HCH isomers drastically decreased the pH of the liquid culture to below 3.50. Inoculated flasks containing α-, *β*-, *γ*-, and *δ*-HCH yielded pH levels of 3.44, 3.35, 3.42, and 3.37, respectively.

Effect of pH on the Biodegradation of Hexachlorocyclohexane Isomers (α- and *γ*-HCH) in Liquid Culture. The *Pandoraea* species was able to grow and degrade α- and *γ*-HCH isomers over a relatively wide range of pH (**Figure 3**). Degradation of α- and *γ*-HCH isomers (21.7 and 19.1%, respectively) was even observed at an initial pH of 4.0 at 4 weeks of incubation. An increase in pH of the liquid culture significantly increased the degradation rate of α- and *γ*-HCH isomers. The highest degradation of α- and *γ*-HCH isomers (67.1 and 60.2%, respectively) was noted at an initial pH of 8.0 at 4 weeks of incubation. Less degradation of α- and *γ*-HCH isomers (48.3 and 51.5%, respectively) was observed at an initial pH of 9.0 as compared to an initial pH of 8.0. Comparatively less recovery of α- and *γ*-HCH isomers from control flasks at an initial pH of 9.0 indicated some chemical hydrolysis of these isomers in the liquid culture.

OD_{600} measurements after 4 weeks of incubation (**Table 1**) revealed the growth rate of the *Pandoraea* species in the liquid culture at different pH levels. The slightly alkaline pH range favored the growth of this bacterial strain, and the highest OD_{600} (0.775) was observed in the liquid culture at an initial pH of 9.0, whereas the lowest (0.194) was noted at an initial pH of 4.0, after 4 weeks of incubation. An increase in pH, from acidic to alkaline, substantially increased the bacterial growth in the liquid culture.

Table 1. Viable Bacterial Count in Soil Slurry and Optical Density of Liquid Culture at Various pH Values after 4 Weeks of Incubation

pH	bacterial count (cfu mL^{-1})	OD λ_{600}
4	9.15×10^6 ($\pm 0.78 \times 10^6$)[a]	0.194 (±0.003)
5	1.30×10^7 ($\pm 0.28 \times 10^7$)	0.196 (±0.006)
6	3.40×10^7 ($\pm 0.43 \times 10^7$)	0.262 (±0.020)
7	4.65×10^7 ($\pm 0.35 \times 10^7$)	0.296 (±0.011)
8	6.45×10^7 ($\pm 0.50 \times 10^7$)	0.556 (±0.124)
9	9.80×10^7 ($\pm 0.14 \times 10^7$)	0.775 (±0.064)

[a] Values in parentheses show standard deviation ($n - 1$).

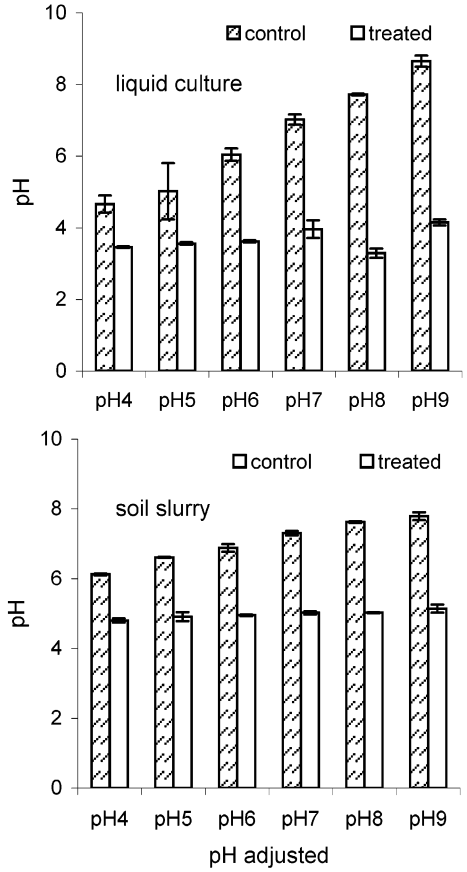

Figure 4. Changes in pH of liquid culture and soil slurry after 4 weeks of incubation.

Bacterial growth in the liquid culture drastically changed the pH of the culture during 4 weeks of incubation (**Figure 4**). The lowest pH (3.29) was noted in the inoculated flasks initially adjusted to pH 8.0. A decrease in pH was observed in all of the inoculated flasks initially adjusted to a pH range of 4.0−9.0.

Effect of pH on the Biodegradation of Hexachlorocyclohexane Isomers (α- and γ-HCH) in the Soil Slurry. In the soil slurry inoculated with the *Pandoraea* species, substantial degradation of α- and γ-HCH isomers (37.2 and 27.1%, respectively) was observed at an initial pH of 4.0 at 4 weeks of incubation (**Figure 5**). An increase in the initial pH significantly increased the degradation of α- and γ-HCH isomers in the soil slurry, and the highest degradation (58.4% α-HCH and 51.7% γ-HCH) was noted at an initial pH of 9.0 at 4 weeks of incubation. The α- and γ-HCH isomers followed a similar pattern of degradation in relation to pH levels.

The viable bacterial counts of the *Pandoraea* species as a function of pH of the soil slurry after 4 weeks of incubation are presented in **Table 1**. Increasing the initial pH levels favored

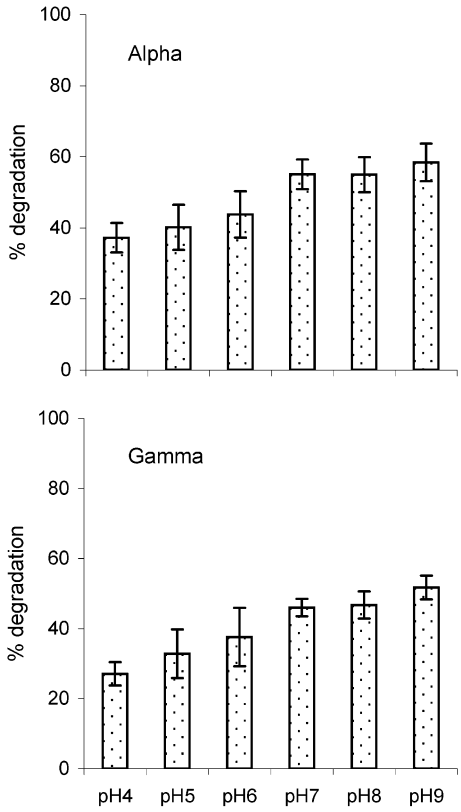

Figure 5. Effect of pH on the biodegradation of α- and γ-HCH isomers in soil slurry inoculated with *Pandoraea* sp.

the bacterial growth in the soil slurry. The results showed an increase in the viable bacterial cells from 9.15×10^6 cfu mL^{-1} at the initial pH of 4.0 to 9.80×10^7 cfu mL^{-1} at an initial pH of 9.0.

The growth of the *Pandoraea* species at the expense of α- and γ-HCH isomers in the soil slurry significantly decreased the pH of the soil slurry culture to an acidic range (**Figure 4**). Slurry pH reduction was noted in all of the inoculated flasks initially adjusted to a pH range of 4.0−9.0.

Effect of Temperature on the Biodegradation of Hexachlorocyclohexane Isomers (α- and γ-HCH). The *Pandoraea* species degraded appreciable amounts of α- and γ-HCH isomers in liquid as well as soil slurry cultures at all incubation temperatures (25, 30, and 35 °C) at 4 weeks of incubation (**Figure 6**) with the optimal at temperature 30 °C. About 49.4 and 47.4% rates of degradation of α- and γ-HCH isomers, respectively, in the liquid culture were observed in the inoculated flasks incubated at 25 °C at 4 weeks of incubation. Increasing the incubation temperature to 30 °C significantly increased the degradation of α- and γ-HCH isomers (62.5 and 57.7%, respectively) in the liquid culture at 4 weeks of incubation. A further increase in incubation temperature to 35 °C decreased the degradation of both isomers in liquid culture.

The influence of temperature on the degradation of α- and γ-HCH isomers in the soil slurry and liquid cultures (**Figure 6**) by the *Pandoraea* species followed a similar pattern. The highest degradation of α- and γ-HCH isomers (54.3 and 51.9%, respectively) was observed in the inoculated flasks incubated at 30 °C at 4 weeks of incubation.

The highest OD_{600} of 0.252, after 4 weeks of incubation, was noted in the liquid culture at 30 °C (**Table 2**) followed by 0.190 and 0.174 at incubation temperatures of 25 and 35 °C, respectively. The viable counts of *Pandoraea* species at different incubation temperatures (**Table 2**) indicated that 30 °C was the

Table 2. Viable Bacterial Count in Soil Slurry and Optical Density in Liquid Culture at Various Temperatures after 4 Weeks of Incubation

bacterial count (cfu mL^{-1})			optical density (λ_{600})		
25 °C	30 °C	35 °C	25 °C	30 °C	35 °C
3.15×10^7 ($\pm 0.49 \times 10^7$)[a]	8.75×10^7 ($\pm 1.06 \times 10^7$)	0.98×10^7 ($\pm 0.25 \times 10^7$)	0.190 (±0.014)	0.252 (±0.012)	0.174 (±0.008)

[a] Values in parentheses show standard deviation ($n - 1$).

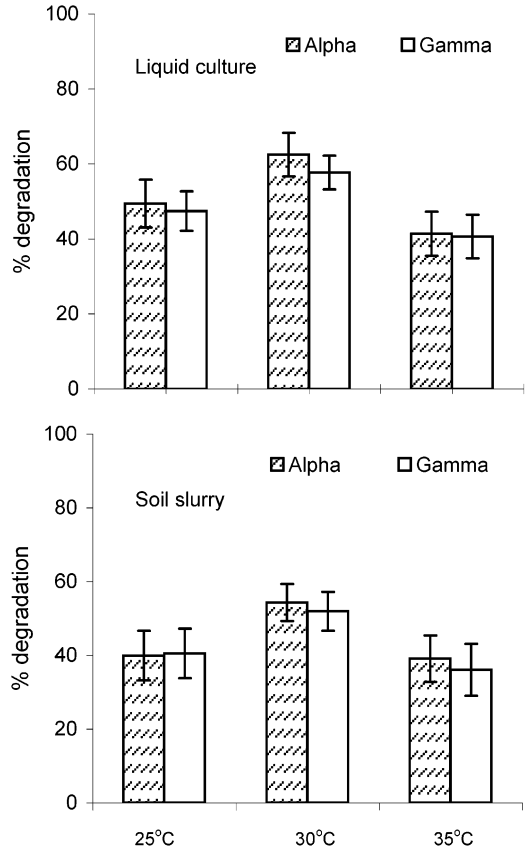

Figure 6. Effect of temperature on the biodegradation of α- and γ-HCH isomers in liquid culture and soil slurry inoculated with *Pandoraea* sp.

Table 3. Residual α- and γ-HCH Isomers in a Soil Slurry at Different Soil/Water Ratios after 4 Weeks of Incubation with an Initial Concentration of 100 mg L^{-1} of Each Isomer

		soil slurry		
HCH isomer	treatment	10%	15%	20%
α	control	86.2 (±2.6)[a]	79.6 (±6.1)	73.1 (±2.0)
	treated	31.0 (±1.3)	35.1 (±4.8)	38.1 (±2.9)
γ	control	83.8 (±1.8)	76.5 (±3.5)	73.3 (±4.6)
	treated	36.6 (±5.2)	36.7 (±2.9)	40.7 (±5.2)

[a] Values in parentheses show standard deviation ($n - 1$).

most favorable temperature for bacterial growth. The highest bacterial count (8.75×10^7 cfu mL^{-1}) was observed in the soil slurry culture incubated at 30 °C after 4 weeks of incubation. The lowest count (0.98×10^7 cfu mL^{-1}) was observed at 35 °C.

Effect of Soil/Water Ratio on the Biodegradation of Hexachlorocyclohexane Isomers (α- and γ-HCH). The soil/water ratio significantly decreased the rate of degradation of α- and γ-HCH isomers (**Table 3**). The highest degradation of α- and γ-HCH (62.9 and 56.4%, respectively) occurred in a 10% soil slurry, whereas the lowest (47.9 and 44.4%, respectively) was noted in a 20% soil slurry at 4 weeks of incubation. About 56% of α-HCH and 52.0% of γ-HCH were degraded in a 15% soil slurry at 4 weeks of incubation.

DISCUSSION

α-, β-, γ-, and δ-HCH isomers constitute >97% of technical-grade HCH, and their persistence in the environment poses a serious environmental threat. In this study, we examined the comparative degradation of these four HCH isomers in liquid culture by a *Pandoraea* species. Although this bacterial strain was isolated through enrichment using γ-HCH as a sole carbon source, it performed equally well in degrading other HCH isomers. γ-HCH used in the enrichment study was the least degraded isomer in this work. The *Pandoraea* species degraded the highest amount of δ-HCH in liquid culture at 4 weeks of incubation. The rate of degradation of HCH isomers by the *Pandoraea* species was in the order $\delta > \beta \geq \alpha > \gamma$. These results differ from the work of Johri et al. (*20*), who reported the degradation of these four HCH isomers in liquid culture by *Sphingomonas paucimobilis*. They observed complete degradation of α-HCH after 3 days and 98% degradation of β- and γ-HCH after 12 days of incubation in flasks spiked with HCH isomers at 5 mg L^{-1}. They reported the rate of degradation of HCH isomers in the order $\alpha > \beta \geq \gamma > \delta$. This is possibly due to differences in the bacterial strains. Gupta et al. (*24*) also reported the degradation of HCH isomers by *Bacillus circulans* and *Bacillus brevis* and found β-HCH as the most recalcitrant to degradation.

The *Pandoraea* species grew well, yielding a high OD_{600} at 2 weeks of incubation on all four HCH isomers (α, β, γ, and δ) studied in the liquid culture. Johri et al. (*20*) observed that *S. paucimobilis* grew well on α- and δ-HCH and showed moderate growth on β- and γ-HCH isomers. Although δ-HCH was degraded at a higher rate compared to the other isomers, the OD_{600} did not reflect this trend. There is a possibility that while degrading δ-HCH at a relatively fast rate, the *Pandoraea* species completed all of the phases of its growth cycle and started declining with decreasing concentration of δ-HCH. At a lower substrate concentration, the strain most likely derived energy for respiration to maintain its viability rather than for biosynthetic purposes. The growth of the *Pandoraea* species on the HCH isomers in the liquid culture drastically decreased the culture pH to an acidic range. This change in pH might be due to the production of acidic metabolites during the metabolism of HCH isomers in liquid culture.

The fate of organic pollutants in the environment is influenced by environmental factors, such as pH and temperature, affecting the activity of microorganisms. The *Pandoraea* species was able to degrade α- and γ-HCH isomers over a fairly wide range of pH in liquid and soil slurry cultures. This strain performed well in degrading α- and γ-HCH isomers in both liquid and soil slurry cultures in slightly alkaline conditions. The degradation of α- and γ-HCH isomers was optimal at an initial pH of 8.0 in the liquid culture and at an initial pH of 9.0 in the soil slurry. Manonmani et al. (*19*) examined the influence of pH on the degradation of the α-HCH isomer in a basal mineral medium by an acclimated consortium of microorganisms. They found

that a pH range of 6.0−8.0 was most favorable for growth and effective degradation of α-HCH. Lower recovery of α- and γ-HCH isomers from the control flasks at an initial pH of 9.0 indicated that some chemical hydrolysis of these isomers occurred at this pH. Manonmani et al. (*19*) also observed chemical hydrolysis of the α-HCH isomer in liquid medium in control flasks at pH 9.0.

Manonmani et al. (*19*) reported a relationship between bacterial growth and degradation of the α-HCH isomer in liquid culture over a pH range of 3.0−11.0. They observed a decline in bacterial population beyond pH 8.0. However, in our study OD_{600} measured after 4 weeks of incubation in the liquid culture over a pH range of 4.0−9.0 showed that an increasing pH from acidic to an alkaline range rapidly increased the bacterial population in the liquid culture, reaching the highest OD_{600} at an initial pH of 9.0.

The *Pandoraea* species degraded appreciable amounts of α- and γ-HCH isomers in the liquid culture as well as in the soil slurry at all incubation temperatures (25, 30, and 35 °C) at 4 weeks of incubation and displayed optimal degradation at 30 °C. These results are in accordance with the work of Bachmann et al. (*25*), who reported that temperatures in the range of 20−30 °C were most favorable for the degradation of the α-HCH isomer in a soil slurry by a natural microbial population under aerobic conditions. Bhuyan et al. (*16*) reported enhanced degradation of the γ-HCH isomer in acclimated soil at a temperature range of 20−35 °C. Manonmani et al. (*19*) also observed the degradation of the α-HCH isomer under a wide range of temperatures (4−40 °C) in the liquid culture medium and found 30 °C as the most favorable for α-HCH degradation.

The OD_{600} of the liquid culture and viable bacterial counts of the soil slurry at different incubation temperatures after 4 weeks of incubation showed an increase in bacterial growth with an increase in incubation temperature up to 30 °C. A decline in bacterial population was found in the cultures (liquid and soil slurry) incubated at 35 °C. These results agree with the findings of Manonmani et al. (*19*), who reported a sharp decline in bacterial population in liquid culture when the incubation temperature was raised beyond 35 °C.

Degradation of α- and γ-HCH isomers in the soil slurry was significantly influenced by the soil/water ratio. Increasing the soil/water ratio decreased the degradation of the α- and γ-HCH isomers in the soil slurry at 4 weeks of incubation. This might be due to the adsorption of α- and γ-HCH isomers to the soil particles, decreasing their bioavailability to the microorganisms. Wu et al. (*26*) studied the sorption of HCH in a sediment/water system and calculated the adsorption coefficient (K_d) using the Freundlich equation. The adsorption coefficient ($K_d = 15.13$) showed that HCH was strongly adsorbed to the sediment.

In summary, the *Pandoraea* species isolated through enrichment is an active HCH degrader. This bacterial strain performed well in degrading all HCH isomers in liquid culture. With its high degrading ability over a fairly wide range of pH and temperature, the *Pandoraea* species is ideally suited for bioremediation of HCH-contaminated soils, waste dump sites, water bodies, and industrial effluents.

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