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Review

Microbial degradation of gamma-hexachlorocyclohexane (lindane)

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Organochloride compounds are known to be highly toxic and persistent, causing serious water and soil pollution. Hexachlorocyclohexane (HCH) is the term which collectively identifies the eight isomers of the hexachlorocyclohexane and they are denoted by the greek letters α, β, γ, δ, etc. Among the eight isomers, gamma-hexachlorocyclohexane (γ-HCH or lindane), is the only one with insecticidal properties. γ-HCH has been mainly used in agriculture and vector control programmes. HCH isomers are recognized for their toxicity, persistence in the environment and potential carcinogenic effects. Lindane is a neurotoxin that interferes with GABA neurotransmittor function. In humans, lindane affects the nervous system, liver and kidneys, and may be a carcinogen. Because of this, lindane, a cheap and effective insecticide, is banned in many other countries, while still being used or have been banned only recently in India. Their extensive use has resulted in a widespread occurrence of residues in the environment and in food products. Residues of γ-HCH have been reported from different soil and water systems in India. Efforts have been made for the remediation of soils and groundwater contaminated with the toxic and persistent HCH isomers through biodegradation processes. Microorganisms capable of degrading HCH isomers have received considerable attention as they provide the possibility to be utilized for *in situ* detoxification. γ-HCH degrading microorganisms, many bacteria and a few fungi, have been isolated and employed for bioremediation of lindane contaminated soil and water systems. The genes and enzymes involved in the γ-HCH degradation pathway have been investigated.

Key words: Biodegradation, gamma-hexachlorocyclohexane (γ-HCH), organochlorine pesticides, toxicity.

INTRODUCTION

Pesticides have benefited modern society by improving the quantity and quality of the world's food production. The use of pesticides to control insects, weeds and pathogens, enables food production to support the world population of over 6 billion people. Due to their relatively low cost, ease of use and effectiveness, they became the primary means of pest control. However, many pesticides are persistent organic pollutants (POPs). POPs are defined as chemical substances that persist in the environment, bioaccumulate through the food web, and pose a risk of causing adverse effects to human health and the environment. Contamination of soil, sediment, ground waters and surface waters by pesticides is nowadays acknowledged world-wide as an important environmental issue (Mertens, 2006).

Lindane is the common name for the gamma isomer of 1,2,3,4,5,6-hexachlorocyclohexane (HCH). Technical HCH is an isomeric mixture that contains mainly five forms differing only by the chlorine atoms orientation (axial or equatorial positions) around the cyclohexane

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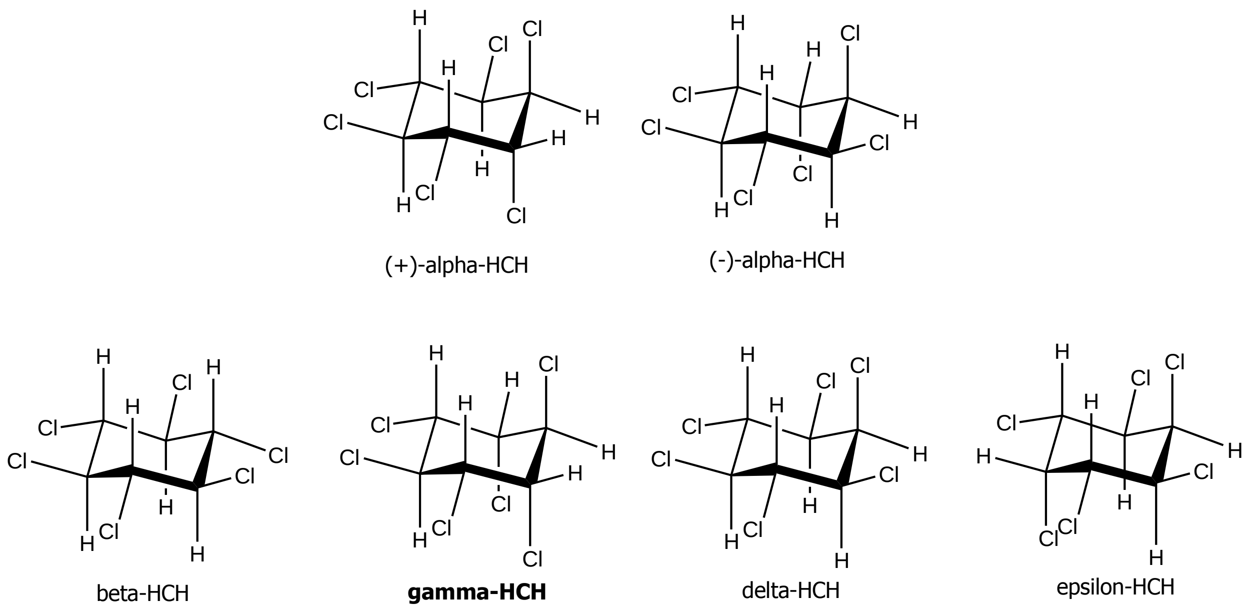

Figure 1. Structure of alpha, beta, gamma, delta and epsilon HCH isomers (Source: Buser and Muller, 1995).

ring (Figure 1). The five principal isomers are present in the mixture in the following proportions: alpha-hexachlorocyclohexane (53 to 70%) in two enantiomeric forms [(+) alpha-HCH and (-) alpha-HCH], beta-hexachlorocyclohexane (3 to 14%), gamma-hexachlorocyclohexane (11 to 18%), delta-hexachlorocyclohexane (6 to 10%) and epsilon-hexachlorocyclohexane (3 to 5%). The gamma isomer is the only isomer showing strong insecticidal properties (Kutz et al., 1991).

Lindane is a halogenated organic insecticide largely used as an insecticide for the control of agricultural pests over the past five decades (Prakash et al., 2004). Dutch scientist Dr. Teunis van der Linden discovered its insecticidal properties (Hardie, 1964). Commercial production of lindane started in 1945. The insecticide is synthesized by chlorination of benzene in the presence of ultraviolet light (IARC, 1973), which yields a mixture of five main isomers. This mixture of isomers is subject to fractional crystallization and concentration to produce 99% pure lindane, with only a 10 to 15% yield. The production of lindane is therefore inefficient as for each ton of lindane (gamma isomer) obtained, approximately 6 to 10 tons of other isomers are also obtained (Vijgen, 2006).

Lindane is a colorless crystal compound having a molecular weight of 290.85 and melting point of approximately $113^{o}C$. Because of its broad range of action, lindane has known numerous applications. The use of lindane as an insecticide began in the 1940s. The insecticide has been used on crops and as a public health measure to control insect-borne diseases. Lindane has been used for seed and soil treatment, foliar applications, tree and wood treatment and against ectoparasites in both veterinary and human applications (Humphreys et al., 2008). The forestry industry also used lindane to control pests on cut logs (Donald et al., 1997). It is estimated that between 1950 and 2000, about 6,00,000 tonnes of lindane were produced globally, and the vast majority of which was used in agriculture (Vijgen, 2006).

LINDANE IN THE ENVIRONMENT

The production and agricultural use of lindane are the primary causes of environmental contamination. When lindane is used in agriculture, an estimated 12 to 30% of it volatilizes into the atmosphere (Shen et al., 2004), where it is subjected to long-range transport and can be deposited by rainfall (Donald et al., 1997). A mean global concentration of 580 pg γ-HCH $(m^3)^{-1}$ in air has been reported (Walker et al., 1999). A half-life for lindane in air of 2.3 days was estimated, based on the rate constant for the vapor-phase reaction with hydroxyl radicals in air (Mackay et al., 1997). Brubaker and Hites (1998) estimated a lifetime in air of 96 days for lindane.

Once in the soil, lindane adsorbs strongly to organic matter and is therefore relatively immobile in the soil. The plants growing in such contaminated soils accumulate HCH in their tissues. The most likely mechanisms of HCH accumulation in plants were sorption of soil HCH on roots and sorption of volatilized HCH on aerial plant tissues (Abhilash et al., 2008; Pereira et al., 2008). Lindane in soil with especially low organic matter content or subject to high rainfall can leach to surface and even ground water (Wauchope et al., 1992). Soil microflora and aquatic microflora are adversely affected by γ-HCH (Ray, 1983; Babu et al., 2001). Lindane significantly reduced population of nitrifying bacteria (Martinez-Toledo et al., 1993). The growth and activity of denitrifying bacteria was adversely affected by lindane (Sáez et al., 2006). A reduction of 50% in bacterial cell concentration was observed in lindane-amended soil microcosms (Rodríguez and Toranzos, 2003). HCH also reduces plant seed germination and seedling viguor (Bidlan et al., 2004). Lindane is highly to very highly toxic to fish and aquatic invertebrate species (Johnson and Finley, 1980). It accumulates in the food chain (Deo et al., 1994) on account of its lipophilic properties, which leads to toxicity. Lindane has half lives of 3 to 30 days in rivers and 30 to 300 days in lakes. Other studies report calculated experimental hydrolysis half lives ranging from 92 to 3090 h; a persistence of about 2 to 3 years in soil is also reported (Mackay et al., 1997).

In 2005, the production and agricultural use of lindane was banned under the Stockholm Convention on Persistent Organic Pollutants (Hanson, 2005). The use of lindane had been banned in more than 50 countries and restricted in 33 countries (Humphreys et al., 2008). However, restricted use of lindane still continues in India due to low cost and popularity of these formulations among farmers (Murthy and Manonmani, 2007). In India, the residues of HCH have been detected in surface and subsurface soils (Agnihotri et al., 1996; Titus et al., 2001; Nawab et al., 2003), in food products (Kannan et al., 1992), and dairy milk (John et al., 2001), having concentrations several folds higher than permissible limits. Ground-water, drinking water (Mukherjee and Gopal, 2002; Fatoki and Awofolu, 2004) and commercial brands of drinking water were found to contain residues of HCH isomers (Prakash et al., 2004). Even soft drinks were found to contain very high levels of HCH residues (Narain, 2003). Although the use of γ-HCH for control of agricultural pests has been discontinued, run-offs from the already contaminated agricultural soils or from the dumping sites of adjoining regions can result in high levels of contamination (Prakash et al., 2004).

Effects on humans

Gamma-HCH is generally considered to be the most acutely toxic of the isomers following single administration (Smith, 1991). The primary routes of potential human exposure to lindane and other hexachlorocyclohexane isomers are ingestion, inhalation and dermal contact. Major potential dietary sources of lindane include milk, eggs, dairy products, and to a lesser extent, seafood (ROC, 2004). Absorbed by the respiratory, digestive or cutaneous pathways, it accumulates in tissues in the following order: fat > brain > kidney > muscle > lung > heart > spleen > liver > blood (Srinivasan and Radhakrishnamurty, 1983). Exposure to large amounts of lindane can harm the nervous system producing a range of symptoms from headache, dizziness, seizures, diarrhoea, sickness and irritation of skin, nose, throat and lungs (Smith, 1991). Lindane is a neurotoxin that interferes with GABA neurotransmitter function by interacting with the $GABA_A$ receptor-chloride channel complex at the picrotoxin binding site (Pomes et al., 1994; Narahashi, 1996). In humans, lindane affects the nervous system, liver and kidneys, and is carcinogenic (Smith, 1991; Sauviat and Pages, 2002). Marked deleterious effects of lindane on liver of treated rats were evident by increased activities of enzymes of liver function tests such as aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP) and lactate dehydrogenase (Prasad and Soni, 2005). Lindane produces histological alterations of cardiac tissues and a cardio-vascular dystrophy (contracture, degenerescence and necrosis) mainly in the left ventricular wall and a hypertrophy of the left ventricle (Sauviat and Pages, 2002). DNA binding and mutagenicity of lindane and its metabolites was reported by Gopalaswamy and Nair (1992). Lindane was considered as possible human carcinogens (IARC, 1973; USEPA, 1999). Lindane induces oxidative stress; it modifies the activity of the scavenger enzymes (Sauviat and Pages, 2002). Reproductive effects of lindane have been recorded in laboratory animals. In rats, doses of 10 mg/kg/day for 138 days resulted in marked reductions in fecundity and litter size. Doses as low as 0.5 mg/kg/day over 4 months caused observable disturbances in the rat estrus cycle, lengthened gestation time, decreased fecundity, and increased fetal mortality. Lindane was found to be slightly estrogenic to female rats and mice, and also caused the testes of male rats to become atrophied. Semeniferous tubules and Leydig cells (important for production of sperm) were completely degenerated at doses of 8 mg/kg/day over a 10-day period. Reversible decreases in sperm cell production were noted in male mice fed approximately 60 mg/kg/day for 8 months (Smith, 1991). An important toxicity aspect of HCH is the bioconcentration or biomagnification to high levels following uptake. Following exposure to 5 mg kg^{-1} of the compound for up to 8 weeks, the earthworms bioconcentrated γ-HCH by a factor of 2.5 (Viswanathan et al., 1988).

Microbial degradation of lindane

Bioremediation, the removal of environmental pollutants by living organisms, has become a viable and promising means of restoring contaminated sites. Therefore, bacteria capable of degrading HCH isomers have received considerable attention as they provide the possibility to be utilized for *in situ* detoxification. *In situ* bioremediation by bioaugmentation method requires large amounts of inoculum that can be obtained by growing the microorganisms in cheap carbon sources as co-substates along with a known concentration of HCH (Afsar et al., 2005). Lindane biodegradation has been observed in both anaerobic and aerobic ecosystems. The fate of HCH in the environment and the efficiency of its microbial degradation, depend on certain biotic and abiotic factors like availability of HCH degrading microbes, temperature, pH, moisture, texture and organic content of soil, etc. When HCH is adsorbed onto the soil, the degradation rate is much slower due to mass transfer limitations (Rijnaarts et al., 1990). The ability of microbes to degrade HCH can be optimized by the process of induction and acclimatization of these microbes, when the enzyme systems of the biodegradation pathway(s) get induced, facilitating effective removal of the pollutant (Girish et al., 2000; Elcey and Kunhi, 2010).

Clostridium sphenoides, Clostridium rectum and several other representatives of *Bacillaceae* and *Enterobacteriaceae* actively degraded γ-HCH under anaerobic conditions (Heritage and Mac Rae, 1977; Haider, 1979; Ohisa et al., 1980). Francis et al. (1975) and Matsumura et al. (1976) first reported aerobic degradation of HCH by *Escherichia coli* and *Pseudomonas* strain, respectively. Degradation of HCH by a *Pseudomonas paucimobilis* strain (later reclassified as *Sphingomonas paucimobilis* strain SS86) was reported in upland experimental fields in Japan where γ-HCH had been applied once a year for 12 years (Wada et al., 1989). Another strain of *P. paucimobilis* SS86, which is now *Sphingomonas paucimobilis* UT26, that was able to use γ-HCH as the sole source of carbon and energy was isolated from an experimental field to which γ-HCH had been applied (Senoo and Wada, 1989). *Flavobacterium* spp., *Pseudomonas* spp. and *Acromobacter* spp. isolated from the gut of earthworms treated with hexachlorocyclohexane were capable of degrading α, β and γ isomers of HCH (Ramteke and Hans, 1992). γ-HCH degrading *S. paucimobilis* was also isolated from French soils (Thomas et al., 1996). A strain of *P. paucimobilis* isolated from paddy field rhizosphere soil was demonstrated to degrade γ-HCH (Sahu et al., 1990). About 98% of γ-hexachlorocyclohexane was aerobically degraded by *S. paucimobilis* after 12 days of incubation (Johri et al., 1998). *Rhodanobacter lindaniclasticus,* a bacterium from the Rhine River in France had capability to degrade γ-HCH (Nalin et al., 1999). Four sulfate-reducing bacteria (SRB) were reported for their transformation potential of γ-HCH from anaerobic marine sediments (Boyle et al., 1999).

Bacillus circulans and *Bacillus brevis*, isolated from soil contaminated with HCH and acclimatized to different concentrations of HCH for more than 2 years, degraded hexacholorocyclohexane isomers including γ-HCH at a significantly high rate (Gupta et al., 2000). *Pandoraea* sp. substantially degraded γ- HCH at concentrations of 10 to 200 mg l^{-1} in liquid cultures. After 8 weeks of incubation in liquid culture, 89.9 of the γ- HCH isomer declined at an initial concentration of 150 mg l^{-1} (Okeke et al., 2002). Four different bacteria, identified as *Sphingobacterium spiritivorum*, *Ochrobactrum anthropi*, *Bosea thiooxidans* and *S. paucimobilis*, were isolated. Pure strains of *B. thiooxidans* and *S. paucimobilis* degraded lindane after 3 days of aerobic incubation (Pesce and Wunderlin, 2004). A bacterial consortium of 10 bacterial species containing 7 *Pseudomonas* spp. and one each of *Flavobacterium, Vibrio and Burkholderia* was developed. This consortium grown on wheat bran hydrolysate and 25 ppm HCH showed best ability degrading nearly 90% of γ-HCH within 72 h of incubation (Afsar et al., 2005). A gram-positive *Microbacterium* sp. strain, ITRC1, that is capable of degrading all four major isomers of HCH was isolated and characterized (Manickam et al., 2006). *Pseudomonas aeruginosa* ITRC-5 degraded >98% *γ*-HCH after 15 days of incubation under 15% water content, pH 8.0, temperature 28°C and inoculum density 10^6 colony forming unit g^{-1} soil. Addition of *P. aeruginosa* ITRC-5 enhanced the degradation of soil-applied HCH-isomers in ‘open field’ conditions as well, and 94% of *γ*-HCH was degraded after 12 weeks of incubation (Manish et al., 2006). Chaudhary et al. (2006) reported >80% degradation of *γ*-HCH *by P. aeruginosa* ITRC-5 after 24 days of incubation.

Three lindane degrading bacterial cultures *viz., Pseudoarthrobacter* sp., *Pseudomonas* sp. and *Klebsiella* sp. were isolated from lindane-exposed soils by enrichment culture technique which exhibited a maximum lindane degradation efficiency of ~50% (Nagpal and Paknikar, 2006). Benimeli et al. (2008) reported bioremediation of lindane-contaminated soil by *Streptomyces* sp. M7. *Streptomyces* sp. M7 was grown in sterile soil with different initial pesticide concentrations (100, 150, 200 and 300 μg kg^{-1}), when a decrease of the residual lindane concentration was detected in soils samples (29.1, 78.03, 38.81 and 14.42%, respectively). A hexachlorocyclohexane (HCH) degrading bacterial strain *Sphingobium ummariense* sp. nov. was isolated from an HCH dump site located in the northern part of India (Singh and Lal, 2009). *Azotobacter chroococcum* JL 102 was screened for lindane degradation by a chloride estimation method. Maximum degradation of lindane was recorded at 10 ppm concentration in Jensen's medium. A pot culture experiment conducted to study *in situ* degradation potential of this strain for a period of 8 weeks showed increased degradation over the days with maximum degradation observed on the 8th week of incubation (Anupama and Paul, 2010).

The cyanobacteria *Anabeana* sp. PCC7120 and *Nostoc ellipsosporum* metabolized lindane producing a mixture of 1,2,4- and 1,2,3-trichlorobenzenes (Kuritz and Wolk, 1995). Degradation of lindane by cyanobacterial species isolated from the Egyptian Lakes Qaroun (*Qaroun* spp.- *Oscillatoria* sp. 12, *Oscillatoria* sp. 13, *Synechococcus* sp., *Nodularia* sp., *Nostoc* sp., *Cyanothece* sp. and

Synechococcus sp.) and Mariut (*Mariut* spp.- *Microcystis aeruginosa* MA1, *Anabaena cylindrica*, *Microcystis aeruginosa* MA15, *Anabaena spiroides* and *Aphanizomenon flosaquae)* was studied and percentage of lindane removal efficiency (RE), was calculated. Lindane was removed by all the species, either as individuals or mixtures. The lindane RE percentage of Qaroun species ranged between 71.6 and 99.6% with a maximum of 98.0 to 99.6% at 5 ppm, 83.9 and 99.7% at 10 ppm. *Mariut* species showed an RE percentage of 45.23 to 100.0% with maximum between 99.23 and 100.0% at 5 ppm and 43.15 and 100.0% at 10 ppm (El-Bestawy et al., 2007).

The bracket-like polypore fungus, *Ganoderma australe*, was selected for its potential to degrade lindane in liquid agitated sterile cultures. The maximum lindane biodegradation (3.11 mg g^{-1} biomass) was obtained with nitrogen content of 1.28 g l^{-1}, lindane concentration of 7.0 ppm, temperature of 18°C, and 5 days of cultivation time (Dritsa et al., 2009). *Conidiobolus* 03-1-56, a phycomyceteous fungus isolated from litter, completely degraded lindane on the 5th day of incubation in the culture medium (Nagpal et al., 2008). The degradation of the insecticide lindane by two white-rot fungi, *Cyathus bulleri* and *Phanerochaete sordida*, was studied. *C. bulleri* degraded lindane more efficiently than *P. sordida* (Singh and Kuhad, 2000). Maximal degradations of 94.5% was attained after 30 days for *γ*-HCH isomer by the white-rot fungus *Bjerkandera adusta* in a slurry batch bioreactor (Quintero et al., 2007). Biodegradation of lindane up to 85 to 95% by white rot fungus, *Pleurotus ostreatus, Plerotus sajorcaju* and *Trametus hirsutus,* were reported (Arisoy and Kolankaya, 1997; Singh and Kuhad, 1999; Papadopoulou et al., 2006).

Reports on the degradation of γ-HCH by *Phanerochaete chrysosporium* showed percent decompositions between 10.6 (Arisoy, 1998) and 90% (Singh and Kuhad, 1999); and mineralization values between 3.9% (Mougin et al., 1996, 1997) and more than 90% (Bumpus et al., 1985). White rot fungi species, *Bjerkandera adusta, Irpex lacteus, Lentinus tigrinus, Phanerochaete chrysosporium, Phanerochaete sordida, Phlebia radiata, Pleurotus eryngii, Poliporus cialatus,* and *Stereum hirsutum*, were studied for their ability to degrade lindane. The γ-HCH isomer was degraded between 15.1 and 70.8% by six of the nine fungal species, *B. adusta, P. ciliatus, L. tigrinus, S. hirsutum, P. eryngii*, and *I. lacteus* (Quintero et al., 2008).

GENES AND ENZYMES

Genes for γ-HCH degradation are highly conserved in diverse genera of bacteria, including the Gram-positive groups, occurring in various environmental conditions. The genes and enzymes which trigger the degradation of γ-HCH have been characterized mostly from *S. paucimobilis* UT26 which converts γ-HCH to β-ketoadipate through the action of six enzymes: LinA (dehydrochlorinase), LinB (halidohydrolase), LinC (dehydrogenase), LinD (reductive dechlorinase), LinE (ring cleavage dioxygenase) and LinF (reductase) (Nagata et al., 1999; Endo et al., 2005). The genes were either partially (Thomas et al., 1996; Kumari et al., 2002) or completely (Nagata et al., 1999) cloned or sequenced.

The *linA*-to-*linF* genes in UT26 are dispersed on the three large circular replicons: the *linA*, *linB*, and *linC* genes on the 3.6-Mb chromosome I; the *linF* gene on the 670-kb chromosome II; and the *linDE* operon with its regulatory gene (*linR*) on a 185-kb plasmid, pCHQ1 (Nagata et al., 2006). Nearly identical *lin* genes have also been identified in other HCH-degrading bacterial strains, such as *Sphingobium indicum* B90 (Kumari et al., 2002) and B90A (Dogra et al., 2004) from India and *Sphingobium francense* Sp+ from France (Ceremonie et al., 2006). In addition to these catalytic enzymes, a putative ABC-type transporter system encoded by linKLMN is also essential for the γ-HCH utilization in UT26. It has been suggested that the distribution of lin genes is mainly mediated by insertion sequence IS6100 and plasmids (Ceremonie et al., 2006; Lal et al., 2006). Two dehalogenases, LinA and LinB, have variants with small number of amino acid differences, and they showed dramatic functional differences for the degradation of HCH isomers, indicating these enzymes are still evolving at high speed (Nagata et al., 2007). Two nonidentical copies of the *linA* gene encoding HCH dehydrochlorinase, which were designated *linA1* and *linA2*, were found in *S. paucimobilis* B90*.* The *linA1* and *linA2* genes could be expressed in *Escherichia coli*, leading to dehydrochlorination of γ-HCH (Kumari et al., 2002).

PATHWAYS OF LINDANE DEGRADATION

The aerobic degradation pathway of γ-HCH was extensively revealed in bacterial strain *S. japonicum* (formerly *S. paucimobilis*) UT26. γ-HCH is transformed to 2,5-dichlorohydroquinone through sequential reactions catalyzed by LinA, LinB and LinC, and then 2,5-dichlorohydroquinone is further metabolized by LinD, LinE, LinF, LinGH and LinJ to succinyl-CoA and acetyl-CoA, which are metabolized in the citrate/tricarboxylic acid cycle (Nagata et al., 2007). The degradation patways of γ-HCH in *S. japonicum* proposed by Endo et al. (2006) is presented in Figure 2.

The degradation of α- and γ-HCH is initiated with a dechlorination to form pentachlorocyclohexane (PCCH), from which the 1,2- di- chlorobenzene (DCB) and 1,3- di-chlorobenzene (DCB) isomers and finally mono-chlorobenzene (CB) are formed (Figure 3) (Quintero et al., 2005). In the degradation of lindane, intermediate metabolites such as tetrachlorocyclohexene (TCCH) and

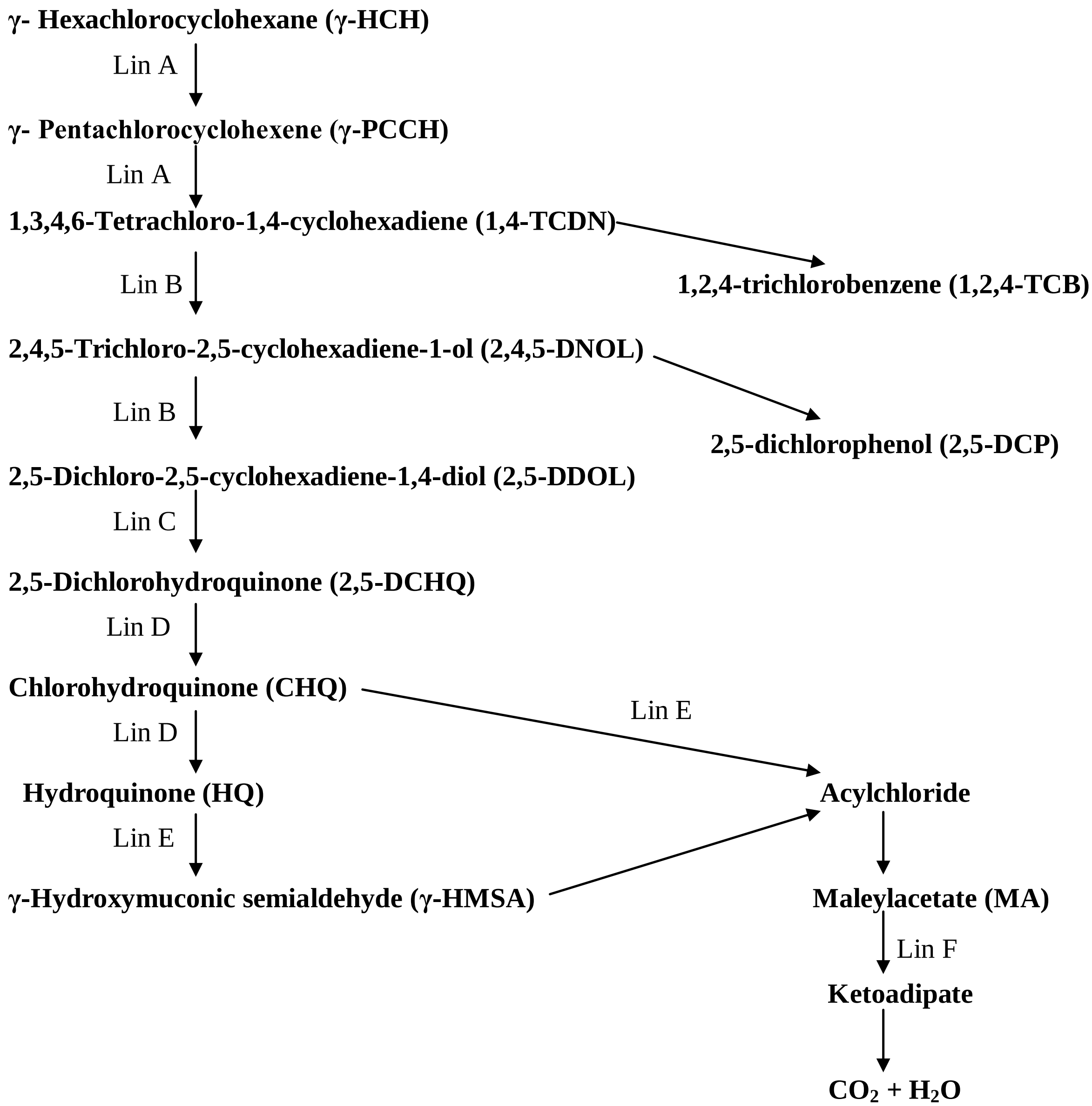

Figure 2. Degradation Pathways of γ-HCH in *S. japonicum* UT26 (Source: Endo et al., 2006).

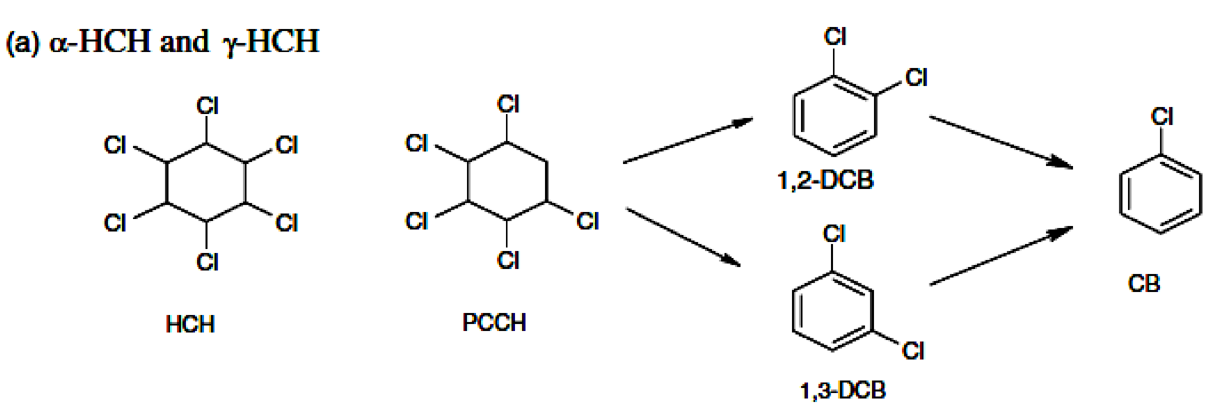

Figure 3. Proposed degradation routes for γ- HCH isomers under anaerobic conditions (Source: Quintero et al., 2005).

tetrachlorocyclohexenol (TCCOL) have been detected (Mougin et al., 1996; Singh and Kuhad, 2000). During the degradation of γ-HCH by *Xanthomonas* sp. ICH12, formation of two intermediates, γ-2,3,4,5,6-pentachlorocyclohexene (γ-PCCH) and 2,5-dichlorobenzoquinone (2,5-DCBQ), were identified by gas chromatography-mass spectrometric (GC-MS) analysis. While γ-PCCH was reported previously, 2,5-dichlorohydroquinone was a novel metabolite from HCH degradation (Manickam et al., 2007).

Organochloride compounds such as ethanone 1-(3-chloro-4-methoxyphenyl)- and 1-benzenecarbonyl chloride, 2,4-dichloro-3- methoxy were detected due to fungal biosynthetic capacity by Quintero et al. (2008). A modified γ-HCH degradation pathway by *Sphingobium indicum* B90A in which γ-PCCH is converted to 2,5-cyclohexadiene-1,4-diol via 3,4,5,6-tetrachloro-2-cyclohexene-l-ol and 2,5,6-trichloro-2-cyclohexene-1,4-diol was reported by Raina et al. (2008).

CONCLUSION

Several soil microorganisms capable of degrading, and utilizing HCH as a carbon source, have been reported. In selected bacterial strains, the genes encoding the enzymes involved in the initial degradation of lindane have been cloned, sequenced, expressed and the gene products characterized. Studies on microbial biodegradation of lindane in liquid cultures and soil have been done. The comparison between soil and liquid assays showed that in soil medium, the degradation rates were slower than those found in liquid media due to the mass transfer limitations associated with the soil. The slurry system with anaerobic sludge appears as an effective alternative in the detoxification of polluted soils with HCH. However, it should be noted that the compounds which were biodegraded in the laboratory were present in relatively high concentrations *in situ*. Further, the soil characteristics such as temperature, pH, moisture content, soil texture and organic matter appear to influence degradation. Thus, the degradation potential observed under laboratory conditions should be studied further under *in situ* conditions to assess the success of a bioremediation. There is a need of large scale, more in-depth, evaluation of bioremediation protocols using soil with high HCH concentrations.

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