Genetic manipulations of microorganisms for the degradation of hexachlorocyclohexane

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Abstract

Hexachlorocyclohexane (HCH) is an organochlorine insecticide which has been banned in technologically advanced countries. However, it is still in use in tropical countries for mosquito control and thus new areas continue to be contaminated. Anaerobic degradation of HCH isomers have been well documented but until recently there have been only a few reports on aerobic microbial degradation of HCH isomers. The isolation of these microbes made it possible to design experiments for the cloning of the catabolic genes responsible for degradation. We review the microbial degradation of HCH isomers coupled with the genetic manipulations of the catabolic genes. The first part discusses the persistence of residues in the environment and microbial degradation while the second part gives an account of the genetic manipulations of catabolic genes involved in the degradation.

Keywords: Hexachlorocyclohexane; Catabolic gene; Residue; Persistence; Microorganism; Degradation

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1. Introduction

Pesticides have been extensively used to protect and improve the quality and the quantity of food commodities, building materials, clothing and animal health and to combat certain diseases transmitted by insect vectors to man and animals. However, the indiscriminate use of pesticides has caused serious concern about toxic effects of pesticide residues on non-target organisms. This subject constitutes one of today’s most serious challenges to public health and environmental pollution. Many environmentally important xenobiotics and other chemicals introduced for industrial and agricultural use are halogenated: halogenation is often implicated as the reason for persistence and toxicity of such compounds [1]. These compounds enter the soil, water and foods by several routes e.g., landfill dumping of industrial wastes, by run-off from treated plant surfaces, spillage during application, use of contaminated manure, drift from aerial and ground application, erosion of contaminated soil by wind and water into the aquatic system, accidents in transport of insecticides, etc.

One such halogenated insecticide is hexachlorocyclohexane (HCH), a homocyclic (alicyclic) chlorinated hydrocarbon popularly called benzenehexachloride (BHC). This compound has been used extensively worldwide, before its use was banned. In developing countries such as India, the use of HCH continues due to economic reasons. HCH is mainly used for mosquito control but recently it has been used in different parts of India for anti-flea operations during the so-called outbreak of plague. For instance, in the Beed district of Maharashtra state alone, 30 metric tonnes (MT) of BHC was sprayed (India Today, October 1994).

Theoretically, HCH has eight possible stereoiso-
mers, of which four (alpha, beta, gamma and delta) predominate in the technical product. These isomers differ significantly from each other with respect to their persistence and toxicity to insects, birds, mammals and other non-target organisms. The relative proportions of HCH isomers in an HCH formulation is therefore crucial from a toxicological standpoint. This problem assumes importance in the light of reports that HCH isomers undergo degradation in soil, water, microorganisms, higher organisms, plants and insects, etc. [7]. Chlorinated hydrocarbons have been studied extensively because of the problems associated with DDT, BHC and other pesticides. Hence chlorinated compounds serve as the basis for most of the information available on the biotransformation of synthetic halogenated compounds. Several reviews have been published that deal with various aspects of microbial degradation of xenobiotics [2–15]. It has been shown that microorganisms play an important role in the degradation/detoxification of xenobiotics and the process of microbial degradation has been shown to reduce the toxicity of xenobiotics by 2 to 800 times [16].

Recently the selection of microbes from the natural environment, which can degrade xenobiotics, and genetic manipulations of catabolic genes (present either on plasmids or chromosomally located) responsible for such degradation, has received much attention [17], but less has been published on the genetic manipulations of catabolic genes responsible for the degradation of HCH isomers. The present review deals with the microbial degradation of HCH isomers together with the genetic manipulations of catabolic genes involved in degradation process of HCH. The first part discusses the residue and its
Persistence in the environment. The second part gives an account of genetic manipulations. For an explanation of the abbreviations used, see Table 1.

2. Residue study and persistence of HCH isomers in the environment

The extensive use of HCH, both in agriculture and public health, has resulted in its ubiquitous presence in the environment. The isomers of HCH are generally stable to light, high temperature, hot water, and acidi although they are dechlorinated in the presence of alkali [7]. There is a significant difference in the rate of disappearance of HCH isomers from soil, water and food [18].

2.1. Residues

There have been reports on the occurrence of HCH residues in soil, water, air, plants, plant products, animals, food commodities, microbial environment and humans. As early as 1974, Melnikov [19] summarized the lindane residue data from soils of USA, England and Japan and found residues to vary from 0.04 to 0.26 mg/kg. Similar reports were then documented from other developed countries, such as the Netherlands [20,21], Sweden [22] and Japan [23]. Residues of HCH were also detected in soils in various parts of India, ranging from 0.125 ppm to 0.5 ppm [24,25]. Residues of HCH have been detected in nearly all water bodies, including both fresh and marine. In the USA, mean levels of HCH in river water ranged from 0.011 to 28 ng/l [26]. Rihan et al. [27] reported maximum values of 0.16 ng/l for lindane from the North Mississippi river. In Japan, lindane residue levels in the Tamagawa river ranged from 5 to 234 ng/l [28]. Recently, residues of HCH were monitored in the Ganga river water in the district of Farrukhabad in Northern India [29]. During 1991–1992 [29], almost all the samples were found to be contaminated with residues of HCH. Alpha-HCH was again the most predominant isomer followed by gamma-HCH in the sample collected. Beta-HCH had the lowest value, indicating accumulation of this isomer in the environment possibly because of its stability and resistance to microbial degradation [29]. In another survey in 1991–1992, Mohapatra et al. [30] found residues of HCH in the ground water near Farrukhabad in the vicinity of the Ganga river in Northern India. Alpha-HCH was the predominant isomer, followed by the gamma and beta isomers [30].

HCH residues have been found in lake waters also [31]. Estuarine waters from Spain showed 210 ppb HCH residues [32]. In 1976, Tanabe and Tatsukawa [33] detected HCH ranging from 4.9 to 22.6 ng/l in surface water samples collected from the Northwest Pacific Ocean. Kumar et al. [34] reported the HCH residues from two lakes in Jaipur, India; HCH residues ranged from 0.08 to 42.8 g/l with the alpha-HCH constituting about 70% of total HCH in rain water collected from Delhi during 1982–1983 [35]. HCH residues ranged from 1.5 to 4.9 $\times$ 10^{-12} g/g have even been found in snow samples near Syowa and Mizuho stations in Antarctica [36].

The occurrence of HCH residues in air from 0.0002 to 0.212 ng/kg has also been reported [37]. Eisenreich et al. [38] found about 2 ng m^{-3} HCH...
residues in air. Tanabe and Tatsukawa [33] measured the levels of HCH in air over the Bay of Bengal; this ranged from 0.14 to 0.95 ng m$^{-3}$, while over the Arabian sea it varied between 0.18 to 3.3 ng m$^{-3}$. Kaushik et al. [39] reported the HCH residues in air from various Delhi sites varied from 0 to 21 797 ng m$^{-3}$.

Due to indiscriminate use of HCH, residues have been found in appreciable amounts in crops and other food materials. The persistence of HCH isomers on two pulse crops, viz. chickpea and pigeonpea [40], revealed that the alpha and gamma isomers dissipated with time, whereas beta isomer persisted and the rate of decay of the delta isomer was also slow [40]. High concentrations of HCH isomers on rice and wheat grains have also been reported from various places in India [41]. Ahuja and Awasthi [42] reported HCH residues in market samples of food grains (rice and wheat) in Bangalore City. All the samples of rice and wheat from different markets were contaminated with HCH residues ranging from 0.0049 to 0.0505 ppm in rice and from 0.0034 to 0.0312 ppm in wheat, the main contaminants being alpha- and beta-isomers of HCH, though gamma-HCH was also recorded from some samples [42]. The presence of alpha- and beta-HCH in most of the samples was attributed to the relative persistence compared to gamma-HCH [42].

On account of their high liposolubility, bioaccumulation of HCH residues from aquatic and terrestrial animals has been reported [11,13,43,44]. Man is an omnivorous species with long life expectancy which makes him more vulnerable to frequent exposure to a variety of pesticide residues in more than one way. One important use of HCH is in the treatment of rice crops to control a variety of pests. Amongst the different fractions of rice crop, rice bran contains maximum residues concentrations, followed by straw, husk and rice grains. The use of rice bran and straw from HCH-treated crops as feed for milch cattle results in significant contamination of milk, thereby resulting in substantial human dietary exposure [45]. In Japan, the origin of high levels of beta-HCH in cow milk was traced to straw from HCH-treated rice crops [46–48]. Battu et al. [45] reported residues of HCH in adults and children. The combined theoretical daily intakes of alpha, beta, gamma and delta isomers of HCH for adults came to 3.8, 24.4, 1.4 and 8.8 μg/person/day, while the corresponding values for children were 43, 38.8, 1.2 and 10.2 μg/person/day, respectively [45]. Residues of HCH isomers were also reported from workers at the Indian Agricultural Research Institute (IARI) Pusa, New Delhi, India [49]. Higher amounts of HCH residues were detected in samples of baby milk powder from Gujarat and Bombay than from Rajasthan and New Delhi [50]. Furthermore, apart from its usage on rice crop, technical HCH is also applied to other crops and its intake by human beings can, therefore, take place through other commodities [45]. In addition, most studies related to residue levels have shown the presence of HCH residues in human blood and fat [51–55].

2.2. Persistence

The IUPAC Commission on Pesticide Chemistry has defined persistence as 'the residence time of a chemical species (which could be either the parent compound and/or a metabolite) in a specifically defined compartment of the environment' [56].

Gamma-HCH is known to persist in soil for periods up to 11 years [57]. In a silt loam soil, Lichtenstein and Schulz [58] extracted 10–14% of lindane after 3 years of application. When lindane was rotatilled 4–6 inches deep into sandy loam soils, the residues detected after 4.5 years amounted to 0–18% of the applied amount [59]. The time for 50% loss of lindane in English soil was calculated as 4–6 weeks for surface treatments and 15–20 weeks for incorporation [60]. Edwards [61] estimated the half-life of lindane in soil to be 1.2 years. However, Kearney et al. [62] calculated that 75–100% of lindane disappears from soil in about 3 years. Half-life of BHC was found to be about 2 years after its incorporation deep into the soil [63]. Voerman and Besemer [64] found 3–8% of the applied lindane in soil after 15 years. However, Stewart and Fox [65] could not detect any lindane residues 12 years after surface spray application of Nova Scotian soils in Canada. In tropical countries, there have not been many extensive studies on the persistence of HCH in soil. Agnihotri et al. [66] showed that, in a sub-tropical Indian soil, 98.4% of the applied lindane was lost in 180 days. A few other studies from Indian soils also indicated rapid loss of BHC isomers. The loss of
75–100% BHC was reported in 2–3 months [67]. Kushwaha et al. [68] observed 70–80% loss of BHC in 3 months in a sandy loam soil under the cover of chillies. In another study, 73–90% loss of BHC in 3 months was found in a clay loam soil, covered with maize crop [69]. Virchenko and Bobovnikova [70] reported about 90% decrease in gamma- and alpha-HCH residues within 3 months after surface application to chernozem soil. Lindane undergoes rapid decomposition in submerged soils, especially under tropical conditions [71–73]. Yoshida and Castro [74] reported that in flooded paddy soils in the Philippines, 45–100% of the applied lindane disappeared in 1 month, while in the unflooded soil only 2% was lost during the period. Isotope studies in various Indian rice soils, revealed that both gamma- and beta-HCH disappear rapidly under flooded conditions [75]. Ferreira and Raghu [76] reported the enhanced disappearance of all the isomers of HCH in flooded soil by the application of green manure. Brahmaprakash et al. [77] studied the persistence of HCH isomers in flooded soil, with and without seedlings. The results showed that gamma-HCH decreased to a negligible 2.4% of the initial concentration, after 30 days in both planted and unplanted soils. Benezet and Matsumura [78] isolated a strain, Pseudomonas putida, from the soil, that converted gamma-HCH to alpha-HCH, both in laboratory and aquatic sediments. Thus, they suggested that high levels of alpha-HCH in the environment may be the result of both isomerization reactions mediated by microorganisms and of selective degradation of gamma-HCH, leaving other constituents of technical HCH. Haider [79] studied the degradation of HCH isomers aerobically as well as anaerobically by soil microorganisms. Clostridium spp., Bacillus spp. and representatives of Enterobacteriaceae effectively degrade HCH isomers to nearly chlorine-free, partly volatile metabolites. It was found that gamma-HCH was an easily degraded isomer, while alpha and especially beta and delta were more slowly dechlorinated and these isomers were found as environmental contaminants, partly because of their persistence and partly because of the interconversion of gamma-HCH to alpha-HCH and other isomers during the incubation with microbes [78]. The HCH isomers were considered to be highly persistent in aerobic environments, but they undergo rapid degradation in predominantly anaerobic ecosystem. However, aerobic mineralization of HCH isomers has been recently reported in a soil slurry and by microorganisms isolated from the soil [21,80–82]. Thus, food commodities as well as the environment are contaminated with HCH and its isomers. HCH is a lipophilic compound, and therefore tends to accumulate and concentrate in the body fat of man. Since the toxicity of HCH is well established, it is imperative to develop methods by which HCH and its isomers can be removed from the environment. With the advancement in our knowledge regarding biodegradation of chlorinated hydrocarbons, it may be possible to devise microbes in future which can degrade HCH and its isomers.

3. Microbial degradation of HCH isomers

In recent years, extensive literature on the microbial degradation of HCH isomers has been published and general schemes indicating mechanisms of degradation of HCH isomers have been proposed [7,11,13,81,83].

3.1. Major degradation reactions of HCH isomers

Microorganisms can degrade HCH isomers by reductive dechlorination, dehydrochlorination, oxidation and isomerization of the parent molecule, these various mechanisms for HCH degradation are described below.

3.1.1. Reductive dechlorination

This reaction proceeds by replacing a chlorine atom, on a nonaromatic carbon, with a hydrogen atom. Degradation of insecticide lindane to gamma-3,4,5,6-tetrachlorocyclohexane (gamma-TeCCH) by Clostridium rectum and P. putida exemplifies the process of reductive dechlorination. Bacteria that are able to degrade lindane anaerobically produce gamma-TeCCH [84–87]. Clostridium and several other representatives of Bacillaceae and Enterobacteriaceae actively degraded lindane under anaerobic conditions [85]. A complete dechlorination of 14C-labelled lindane was shown to occur in 4–6 days by Clostridium butyricum, C. pasteurianum and Citrobacter freundii, while other facultative anaerobic
species were less active [85]. Lindane degradation is associated with the membrane fraction in C. sphenoïdes and this activity was shown to require reduced glutathione [84]. Aerobically grown facultative anaerobes also dechlorinated lindane during subsequent anaerobic incubation with glucose, pyruvate or formate as substrates. The alpha, beta and delta isomers were also dechlorinated but at slower rates (gamma > alpha > beta ≥ delta-HCH). All species which were active in the anaerobic degradation of lindane formed gamma-TeCCH [85]. Small amounts of tri- and tetrachlorinated benzenes have been also found [85]. Some other metabolites like chlorinated benzenes or phenols have also been observed during the degradation of lindane by fungi and aerobic microbes [88–90].

3.1.2. Dehydrochlorination

This involves the simultaneous removal of hydrogen and chlorine from organochlorine insecticides. Typically, the reaction takes place between the saturated chlorinated carbon and the adjacent hydrogen on the neighbouring carbon. The formation of gamma-PCCH from lindane is the most familiar example of this reaction and has been reported in bacterial cultures [78,82,90–93] (Fig. 1). In P. paucimobilis UT26, gamma-HCH was converted by two steps of dehydrochlorination to the chemically unstable intermediate 1,3,4,6-tetrachloro-1,4-cyclohexadiene (1,4-TCDN) which in turn is converted to 2,5-dichloro-2,5-cyclohexadiene-1,4-diol (2,5-DDOL) by two steps of hydrolytic dehalogenation via the chemically unstable intermediate 2,4,5-trichloro-2,5-cyclohexadiene-1-ol (2,4,5-DNOL) [83]. Studies were conducted on the biodegradation of alpha-HCH and beta-HCH in a soil slurry under different redox conditions: aerobic, methogenic, denitrifying and sulfate-reducing and it was observed that aerobic conditions proved to be the best for the microbial conversion of alpha-HCH [21]. This alpha-HCH was mineralized within about 18 days, at an initial rate of 23 mg/kg of soil per day, by the mixed microbial populations in the soil. The dry soil initially contained 400 mg of alpha-HCH/kg. The only intermediate that was detected during the breakdown was PCCH. Under methogenic conditions, alpha-HCH was degraded at a rate of 13 mg/kg of soil per day. Mass balance calculations showed that about 85% of the initial alpha-HCH was converted to monochlorobenzene (MCB), dichlorophenol (3,5-DCP), and a trichlorophenol (TCP) isomer, possibly 2,4,5-TCP. No significant degradation was found under denitrifying and sulfate-reducing conditions in the case of beta-HCH; however, it was found that it is recalcitrant under four redox conditions [21]. Apart from microorganisms, temperature, auxiliary carbon source, substrate concentration, etc., were identified as important factors for the aerobic degradation of alpha-HCH in a soil slurry. Temperature in the range of 20–30°C, was determined to be the most favourable for degradation of alpha-HCH. No alpha-HCH degradation occurred at 4°C or above 40°C. The addition of auxiliary organic carbon compounds showed repressive effects on alpha-HCH biomineralization. High concentrations of dichlorobenzene (1,4-DCB), 1,2-DCB, trichlorobenzene (1,2,4-TCB), and tetrachlorobenzene (TeCB) were found. On the basis of these results and previous work [21], a hypothetical pathway of alpha-HCH degradation in a soil slurry was proposed. First, alpha-HCH is converted to PCCH, subsequently PCCH is converted to TeCB and TCB, which in turn are further dechlorinated to DCB [80]. The aerobic degradation of DCB could then continue via dichlorocatechol and dichloromuconic acid to CO₂ and H₂O as proposed by Schraa et al. [94]. However, these data do not predict whether all PCCH was converted to TCB via TeCB or by direct dehydrochlorination or whether TeCB was converted to DCB via TCB or by direct reductive dechlorination.

3.1.3. Oxidation

The oxidation reaction is an important reaction of organochlorine degradation in higher organisms but is less common during microbial degradation. Probably this is because of lack of the mixed-function oxidase system in microorganisms [95]. The degrada-
tion of gamma-HCH in *Pseudomonas putida* through the process of oxidation was reported by Benezet and Matsumura [78]. However, these studies were not subsequently confirmed.

### 3.1.4. Isomerization

HCH is prepared by chlorination of benzene in the presence of UV light [96]. The crude extract, thus obtained is a mixture of stereoisomers. Of the 16 theoretically possible stereoisomers of HCH, chain interconversions reduce the number to only eight [97]. Out of them, four isomers viz. alpha, beta, gamma and delta, are predominant in the technical product, probably because they have relatively strainless bonds. Among these four isomers gamma-HCH is highly insecticidal. The technical product contains about 2% gamma, 16% alpha, 12% beta and 7% delta isomers [98]. There are several reports of interconversion of HCH isomers by heating, change in temperature and by the action of UV light, etc. [99–102]. However, biological isomerization appears to be a rare event. Although reports have appeared on biological isomerization but they have not been confirmed subsequently. For instance, biological interconversion of lindane to other isomers of HCH and formation of other metabolites was reported by many workers in plants, animals, and in microbes [7,78,103,104]. Srimathi et al. [103] also found that when alpha-HCH was incubated with a crude enzyme abstract of 10-day-old tomato seedlings, it results in the formation of beta- and gamma-HCH and PCCH. Benezet and Matsumura [78] isolated a strain *P. putida* from soil capable of producing alpha-HCH from gamma-HCH in laboratory condi-

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![Diagram](image_url)

**Fig. 2.** Proposed aerobic degradation of HCH isomers by microorganisms.
tions. This reaction was catalyzed by nicotinamide adenine dinucleotide (NAD) and to a lesser extent by flavin adenine dinucleotide (FAD). The high levels of alpha-HCH and beta-HCH in the environment may possibly be due to the isomerization of gamma-HCH. A general scheme for the degradation of HCH isomers by microorganisms is proposed (Fig. 2).

4. Genetic manipulations of catabolic genes

The development of recombinant DNA techniques has brought about a revolution in the field of biological sciences. Although the application of r-DNA techniques to identify and characterize catabolic genes in microbes started as early as in 1975, the expression of major catabolic genes for the degradation and detoxification of xenobiotics have been partially completed for benzene toluene xylene (BTX) [105-129], naphthalene, anthracene and phenanthrene [130-132], chlorobenzoates [133-155], chlorophenoxyacetates [156-166], polychlorinatedbiphenyls [167-175] and parathion [176-178]. However, not much has been done on genetic manipulations of catabolic genes responsible for HCH isomers degradation, although the genetic manipulations of microorganisms for the degradation of gamma-HCH has been recently reported for P. paucimobilis by Imai et al. [81] and Nagata et al. [83,179]. No such information is available for other isomers of HCH.

4.1. Hexachlorocyclohexane (HCH)

As mentioned earlier, HCH and its isomers are still in use in tropical countries. Anaerobic degradation of HCH isomers has been extensively studied. However, only a few reports of aerobic degradation of HCH isomers are available [21,81-83,90]. The isolation of microbes for the aerobic degradation of gamma-HCH made it possible to clone catabolic genes responsible for the degradation of this compound. Imai et al. [81] initially characterized the degradative pathway of lindane in Pseudomonas UT26. A genomic library of P. paucimobilis UT26 was constructed in P. putida by using the broad-host-range cosmid vector, pKS13. One of the clones was further characterized for subcloning the lindane degradative gene. This clone was shown to contain a recombinant plasmid (pKSR1) which was actually pKS13 with a 25-kb insert. From pKSR1, a 5-kb HindIII fragment was subcloned into pUC118 (pMAI). E. coli cells containing pMAI retained the ability to convert lindane to 1,2,4-TCB. This result showed that 5-kb HindIII fragment was responsible for the transformation of lindane to 1,2,4-TCB. A series of deletions were introduced in the 5-kb insert of pMAI by nuclease digestions, and these showed that a 500-kb fragment contained the region for the activity that converted lindane to 1,2,4-TCB. The nucleotide sequence of this region and its flanking regions was determined. Only one open reading frame

\[ \text{Fig. 3. Proposed metabolic pathway of aerobic degradation of gamma-HCH in Pseudomonas paucimobilis UT26 (from Nagata et al. [179]). Reproduced with permission from J. Bacteriol. 176, 3117, copyright the American Society for Microbiology.} \]
of 465 bp was found within the 500-kb region that encodes for gamma-HCH dehydrochlorinase, which confers the dehydrochlorination yielding 1,2,4-TCB from lindane [81].

In *P. paucimobilis* UT26, lindane is converted by two steps of dehydrochlorination to a chemically unstable intermediate, 1,4-TCDN, which is then metabolized to 2,5-DDOL, by two hydrolytic dehalogenation steps via the chemically unstable intermediate 2,4,5-DNOL. To clone a gene encoding the enzyme responsible for the conversion of 1,4-TCDN and 2,4,5-DNOL, a genomic library of *P. paucimobilis* UT26 was constructed in *P. putida* PpY101 LA, into which *lin a* gene had been introduced by Tn5. An 8-kb BglII fragment from one of the cosmid clones, which could convert lindane to 2,5-DDOL, was subcloned [83]. Subsequent deletion analysis revealed that a 1.1-kb region was responsible for the activity. Nucleotide sequence analysis revealed an open reading frame (designated the *lin b* gene) of 885 bp within the region. The deduced amino acid sequence of Lin B showed significant similarity to hydrolytic dehalogenase, DhlA [180]. The protein product of the *lin b* gene is a 32 kDa protein, as detected by SDS-PAGE. Not only 1-chlorobutane, but also 1-chlorodecane (C10) and 2-chlorobutane, which are poor substrates for other dehalogenases, were good substrates for LIN B, suggesting that LIN B may be a member of haloalkane dehalogenases with broad-range specificity for substrates [83]. Further, Nagata et al. [179] cloned *lin c* gene encoding 2,5-DDOL dehydrogenase, which converts 2,5-DDOL to 2,5-DCHQ in *P. paucimobilis*. Pathway of gamma-HCH degradation was reconstructed as shown in Fig. 3.

### 5. Conclusions

Environmental contamination may be viewed as an ecosystem malaise, while bioremediation can be regarded as a kind of environmental medicine, which can be used to treat the contaminated sites. Investigation of a variety of microorganisms, which are involved in the degradation of xenobiotics have demonstrated that these microorganisms can be used to clean-up the contaminated sites. More recently, considerable attention has been directed towards exploitation of diverse microbial species for enzyme production as well as for the synthesis and degradation of xenobiotics. Our knowledge of the enzymatic degradation of HCH isomers is still incomplete and contains many inconsistencies. In future work greater emphasis should be paid on the isolation and synthesis of enzymes involved in the degradation of HCH isomers. However, the applications of microorganisms and their enzymes has been limited due to several problems, including low product yield, poor growth or pathogenicity. A major advantage of the recombinant DNA technology is that it provides the way to circumvent these barriers and has greatly extended the potential for using the existing microbial diversity.

### 6. Future prospects, survival and effectiveness of genetically engineered microorganisms (GEM’s)

A major reason for the construction of genetically engineered microorganisms capable of detoxifying toxic and persistent xenobiotics, is to examine their effectiveness for degrading xenobiotics in contaminated sites. Although to date no engineered microbes have been released for the detoxification of xenobiotics. Various arguments have been put forward as to why genetically engineered microorganisms may not produce desired effects in terms of removing the recalcitrant pollutants such as 2,4,5-T from contaminated sites [181]. The major argument states that: (1) most genetically engineered microorganisms would be unable to compete with indigenous microorganisms and would not therefore survive for too long to utilize the toxic chemicals; and (2) the genetically engineered microorganisms would have no incentive to attack the toxic chemicals because of the presence of large amounts of other easily available carbon sources. It was demonstrated that *P. cepacia* a laboratory developed strain was capable of utilizing 98% of 2,4,5-T (100 ppm) in a week [182]. The same strain took 6 weeks in removing the 2,4,5-T (10000 ppm 20 000 ppm) from the contaminated sites [183]. Kilbane et al. [183] observed that AC100 could not compete with indigenous microflora in the absence of 2,4,5-T and declined rapidly, becoming undetectable in about six weeks. It was concluded that there was little possibility of any ecological disaster
due to the use of AC100, since the strain could not survive in the absence of 2,4,5-T. The major outcome was the fact that only the target chemical was completely removed.

Until now only a few reports have been published regarding the cloning of gamma-HCH degradative gene. Our work has led to the identification of a gene for the degradation of gamma-HCH in the Sphingomonas paucimobilis. This strain can now be further studied to characterize the genes responsible for the degradation of HCH and to reconstruct the actual pathway of degradation of HCH and its isomers [184].

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