Degradation of lindane from aqueous solutions using iron sulfide nanoparticles stabilized by biopolymers

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Abstract

Lindane (γ-hexachlorocyclohexane) is an organochlorine pesticide and a Persistent Organic Pollutant (POP). Lindane residues have been detected in drinking water sources and development of viable methods for their removal is a global priority. Iron based nanomaterials have been shown to effectively transform chlorinated organic compounds. However, their uses in the treatment of drinking water and beverages have toxicity concerns. In the present study, FeS nanoparticles were synthesized by the wet chemical method and were stabilized using a polymer from the basidiomycetous fungus, Itajahia sp. The stabilized nanoparticles could degrade lindane (5 mg/l) with an efficiency of 94% in 8 h. In a subsequent microbiological treatment, residual lindane, its partially degraded intermediates as well as the stabilizing polymer could be completely degraded in 1 h. The latter process facilitated aggregation of FeS, which could be easily removed by filtration. This novel integrated nano-biotechnological method holds the promise of providing an efficient, safe, and cost-effective solution to the problem of removal of chlorinated pollutants from water sources.

Keywords: Lindane; Nanoparticles; Iron sulfide; Biopolymers

1. Introduction

Lindane (1, 2, 3, 4, 5, 6 - hexachlorocyclohexane, γ-HCH) is a broad-spectrum organochlorine pesticide. It is a Persistent Organic Pollutant (POP), potent carcinogen and teratogen and classified by the World Health Organization (WHO) as ‘moderately hazardous’ (human oral LD50: 840 mg/kg). Residues of lindane have been detected in drinking water sources and beverages as well as in foods [1]. The large-scale uses of lindane coupled with its extreme persistence and slow degradation have led to a global problem [2]. Chemical analysis of branded packaged drinking water (commonly known as bottled water) conducted by the Center for Science and Environment, New Delhi, India, has revealed that bottled water of all the major brands contain high doses of pesticide residues [3]. In order to abide by the stringent regulations, development of viable methods for their removal from water and food sources is a global priority.

Conventionally three methods viz. chemical degradation, physical adsorption, and bioremediation have been reported for the removal of lindane from aqueous solutions. These include use of microwave irradiation [4], degradation with NaOH-modified sepiolite [4] and addition of hydrogen peroxide [5]. Many researchers have demonstrated the use of microbial cultures for bioremediation of lindane [6–10]. These methods could have several operational constraints, especially for the treatment of drinking water. For example, the addition of strong chemicals to drinking water supplies may not be acceptable. Adsorption of lindane on porous inorganic/organic material poses the problem of disposal of the adsorbed lindane. The important considerations in the use of a microbial processes for lindane degradation are, (i) the slow rate of biodegradation requiring long period of time (24–100 h), (ii) possible pathogenic effects of the microorganisms used, and (iii) incomplete degradation of lindane resulting in more harmful degradation products.

A more practical approach that is becoming increasingly popular is the use of zero-valent granular iron or nanoparticles of iron for catalytic reductive dehalogenation
2. Materials and methods

2.1. Stabilizing polymer

A basidiomycetous fungus, *Itajahia* sp. was used as a source of stabilizing polymer (designated as FP1). A total of 5 intact (i.e. non-dehisced) fruiting bodies, each admeasuring 3–6 cm in diameter and weighing 39–50 g were washed carefully to remove attached soil particles. The outer peridium was peeled off using a clean scalpel to expose the inner gelatinous material. The peridium, inner membranous layers and the spore material (gleba) were discarded. The colorless and viscous gelatinous material was mixed with appropriate quantities of water and blended in a homogenizer to get 1 l homogeneous solution. The solution was centrifuged at 5000 rpm for 10 min to remove any particulate matter and the viscous supernatant containing polymer was lyophilized to obtain a dry powder.

2.2. Synthesis of FeS nanoparticles

Fungal polymer FP1 (1 ml containing 0.002 g of dry powder) was added to FeSO₄ solution (0.5 M, 5 ml) and stirred vigorously under nitrogen atmosphere. To this mixture Na₂S (1 M, 5 ml) was added dropwise to get a black colloidal suspension of FeS. Control FeS nanoparticles were also prepared without the addition of FP1.

2.3. Characterization of FeS nanoparticles

Scanning electron microscopy of the samples was performed using Philips (Netherlands) XL 30 SEM fitted with an Energy Dispersive X-ray Analysis System. The samples were observed at 27,000× magnification with an accelerating voltage of 25 kV. FTIR analysis of the samples was carried out in the mid-IR range using a Perkin Elmer (USA) FT-IR Spectrum 2000 spectrometer.

2.4. Lindane degradation using FeS nanoparticles

Suspensions of FP1-stabilized as well as non-stabilized FeS nanoparticles were used in two separate sets of experiments. The nanoparticles suspensions (1 ml) were added to serum bottles (30 ml capacity) containing 9 ml distilled water supplemented with lindane (5 mg/l). The pH of the reaction mixture was ~7. The bottles were purged with nitrogen and sealed. They were then incubated at room temperature (ca. 30 °C) on a rotatory shaker (150 rpm). Samples (100 µl) were withdrawn from the bottles at an interval of 1 h. Lindane was extracted in HPLC grade hexane (1000 µl) and its concentration was estimated by gas chromatography. Gas Chromatographic system (Perkin Elmer, USA Auto system XL) with ECD detector and capillary column SGE 3780B21 (length, 25 m; type, bonded phase; material, silica; phase, BP5 non polar; film thickness, 0.5µm) was used for lindane analysis. The conditions of analysis were oven temperature, 240 °C; injector temperature, 230 °C and detector temperature, 280 °C. Detection of partially degraded intermediates was also carried out under similar conditions, albeit, at lower oven temperature, viz. 150 °C.

2.5. Biodegradation of residual lindane and other organics

A bacterial culture (designated as Lin1) isolated from a local lindane exposed soil sample was used in the studies. The culture was capable of degrading lindane as well as FP1 biopolymer. Culture suspension (1 ml of 0.1 O.D.₆₀₀) was inoculated in serum bottles (from the experimental sets of lindane degradation using FeS nanoparticles) after 8 h incubation. The bottles were then incubated further and lindane degradation was monitored at an interval of 15 min.
The degradation of other organics, such as FP1 and partially degraded intermediates was monitored in terms of decrease in the Chemical Oxygen Demand (COD). Coalescence and settling of FeS was observed visually and confirmed by separating the particles by filtration through 0.2 μm membrane filter and estimating the iron concentration in the filtrates (using ATI Unicam, UK model 929, atomic absorption spectrophotometer). The presence of any living or non-living bacteria in the filtrate was checked by microscopic examination of the sample and plating the sample (0.1 ml) on Luria agar (casein enzymic hydrolysate, 10 g/l; yeast extract, 5 g/l; agar, 20 g/l; sodium chloride, 10 g/l; pH 7.5 ± 0.2) plates. The plates were incubated at 37 °C overnight and then scored for any visible colonies developing after the incubation.

3. Results and discussion

3.1. Synthesis and characterization of FeS nanoparticles

The yields of FP1-stabilized as well as non-stabilized nanoparticles were identical, i.e. approximately 28 g dry weight per liter of the reaction mixture. This clearly indicated that the quantity of FP1 in the synthesized FeS nanoparticles was negligible, on weight basis. The colloidal suspension was stable at room temperature and could be freeze-dried without altering any physical properties. Scanning Electron Microscopy of the sample showed clusters of rod shaped nanoparticles. In case of FP1-stabilized sample, nanoparticles were clearly seen associated with the biopolymer matrix (Fig. 1). On an average the particles were ~200 nm in width. Thus the synthesized material cannot be considered as nanoparticles, sensu strictu. Nevertheless, our data compares well with most previous studies where iron nanoparticles with diameters of the order of 100–200 nm were used for the degradation of environmental pollutants [11,15]. EDAX analysis of a single nanoparticle from our samples indicated the presence of iron and sulfur as the major constituents (Fig. 2), confirming the presence of iron as FeS. FTIR analyses (Fig. 3) of FP1-stabilized and non-stabilized samples did not show any changes, especially, in the strongly absorbing bands corresponding to C–H bond stretching vibrations (3300–2850 cm\(^{-1}\)), O–H stretching vibrations (3675 cm\(^{-1}\)) and the weaker carbonyl/carboxyl stretching vibrations (1740–1200 cm\(^{-1}\)). These data clearly indicated that there were no chemical interactions between the FeS nanoparticles and FP1. The stabilization of the nanoparticles was achieved probably due to electrostatic interactions between the two constituents, resulting into surface passivation. Such a property of the stabilizing agent, FP1 could be considered highly desirable. The exact chemical composition of FP1 is being determined. However, preliminary studies show that it is a gelatinous material.

3.2. Lindane degradation using FeS nanoparticles

Gas Chromatographic analysis of the hexane extracted samples showed reduction in the peak area for lindane.
(chromatograms not shown) corresponding to 94% degradation of lindane in 8 h by FP1-stabilized FeS nanoparticles. The non-stabilized FeS could degrade merely 25% lindane in the same period. Appearance of a new peak (corresponding to 1,2,4-trichlorobenzene or TCB, a key intermediate of lindane degradation pathway) in the chromatograms obtained with FP1-stabilized FeS nanoparticles clearly indicated that these nanoparticles catalyzed a rapid reductive dehalogenation reaction. The stochiometry of this reaction, however, needs to be established. Nevertheless the degradation efficiency of FP1-stabilized nanoparticles was quite high and comparable to that obtained by Assaf-Anid and Kun-Yu [17] for carbon tetrachloride. However, it must be emphasized that in contradistinction to the present study, Assaf-Anid and Kun-Yu used higher concentrations (70–500 mM) of FeS, alkaline pH and an organic amendment to get increased rates of degradation. Even though our results were highly promising, the degradation efficiency obtained was not adequate to meet the acceptable standards of lindane residues in aqueous streams. Moreover, the degradation was not complete as partially degraded intermediates such as trichlorobenzene were detected in the gas chromatographic analysis of the samples. Therefore, an additional treatment was found to be necessary.

3.3. Biodegradation of lindane and other organics

In the present studies, it was observed that residual lindane as well as its partially degraded intermediate viz. TCB were completely removed in 60 min after bacterial culture Lin1 was inoculated. This was evidenced from the loss of the peaks corresponding to residual lindane as well as TCB. COD levels of <50 mg/l in the samples indicated that all the organics including FP1 were completely mineralized by the culture. Since FP1 was closely associated with FeS nanoparticles, upon its degradation, the nanoparticles aggregated and settled down (Fig. 4). Settled FeS as well as bacterial cells could be easily separated by filtration through 0.2 μm membrane filters. The filtrates were found to be completely free of iron as seen by atomic absorption spectrophotometric analysis of the supernatant that showed absence of iron. Microscopic examination of the supernatant sample as well as plating of the sample on Luria agar plates showed complete absence of dead as well as live bacteria.

Methods employing purely biological systems for difficult-to-degrade compounds such as chlorinated pesticides are often not attractive on account of slow rates of the reaction and requirement of large quantities of biomass. In the present studies, with FP1 as a nutrient, low levels of residual lindane (as a result of FeS nanoparticles-catalyzed degradation) were rapidly co-metabolized by a small population of bacteria. The biodegradation of FP1 also led to coalescence of FeS nanoparticles, thus ensuring their easy and fast removal. Thus, in order to develop a viable technology for lindane removal, an integrated approach, viz. combination of FeS nanoparticles-catalyzed degradation followed by biodegradation appears to be highly promising. It is also acknowledged that there is a further scope for improving the overall efficiency of the system by using smaller sized nanoparticles. Moreover, there is a need to scale-up the process so that a realistic cost analysis could be done. Efforts in this direction are presently underway.

4. Conclusions

Using a novel, integrated nano-biotechnological approach comprising catalytic dechlorination using FeS nanoparticles followed by microbial degradation, it was possible to achieve complete removal of lindane (5 mg/l) from aqueous solutions in about 9 h. The process also ensured complete removal of FeS nanoparticles. The new method could be an efficient, highly safe, and cost-effective way for the removal of various chlorinated pesticides from aqueous streams.

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Fig. 4. Biodegradation of FP1 associated FeS nanoparticles leads to aggregation and settling of FeS. (A) Control showing FeS nanoparticles in suspension, (B) Growth of Lin1 culture and settled FeS.
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