



# Palladium-Based Catalytic Treatment and a Rhizobacterial-Assisted Detoxification for the Enhanced Removal of Lindane

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## ABSTRACT

This study aimed to assess the efficacy of a bimetallic system consisting of  $Mg^0$ - $Pd^0$  and the bacterium *Acinetobacter* sp. for the complete detoxification of lindane. Our results demonstrate that palladium immobilized on activated charcoal achieved a removal rate of >99% for  $100 \text{ mg.L}^{-1}$  of Lindane within 10 minutes, with the accumulation of trace amounts of intermediates. The reductive transformation of lindane followed 1<sup>st</sup>-order kinetics, with a calculated rate constant ( $k_{\text{obs}}$ ) of  $0.77 \text{ min}^{-1}$ . The bimetallic system resulted in the formation of a non-toxic hydrocarbon as the end-product, indicating complete dehalogenation of lindane. Furthermore, *Acinetobacter* sp. effectively mineralized >98% of  $100 \text{ mg.L}^{-1}$  of Lindane after 26 h of cultivation without any accumulation of toxic metabolite(s) in the reaction medium, demonstrating the efficiency of the biological system. Integrating both chemical and biological systems could provide significant advantages for the treatment of lindane, reducing the treatment time and overall cost. This synergistic approach can significantly enhance the overall removal efficiency of lindane from contaminated soil and water.

## INTRODUCTION

Lindane ( $C_6H_6Cl_6$ ), also known as  $\gamma$ -hexachlorocyclohexane, is a synthetic organochlorine pesticide widely used in agriculture, forestry, and public health sectors for several decades. Due to its poor solubility in water and high lipophilicity, lindane accumulates in the fatty tissues of living organisms, including humans (Alsen et al. 2022). Lindane is a potent neurotoxin and has been found to have harmful effects on human health, even at low levels of exposure. Inhalation of Lindane can cause symptoms such as dizziness, headaches, and confusion, while skin contact with the pesticide can cause irritation, rashes, and seizures (Jayaraj et al. 2016). In addition to its neurological effects, lindane is toxic to the liver and kidneys, potentially leading to long-term health problems. Exposure to lindane over extended periods of time has also been linked to an increased risk of cancer, reproductive and developmental problems, and neurological disorders (Alavanja & Bonner 2012). It was classified as a Group 1 carcinogen by the WHO and IARC, indicating that it was known to cause cancer in humans. This classification was based on extensive research that had been conducted in the past, revealing that exposure to lindane was linked to an increased risk of certain types of cancer, including lymphoma and leukemia (Alsen et al. 2022).

Furthermore, lindane has been shown to have acute toxicity to a variety of animals, including mammals, birds, fish, and invertebrates. Studies have demonstrated that lindane's LD<sub>50</sub> (median lethal dose) varies widely across different animal species. For example, the LD<sub>50</sub> of Lindane for rats is approximately  $88 \text{ mg.kg}^{-1}$ , while for rabbits, it is around  $120 \text{ mg.kg}^{-1}$  (Shakur et al. 2021). The LD<sub>50</sub> for birds is significantly lower, ranging from 12 to  $55 \text{ mg.kg}^{-1}$  (Katagi & Fujisawa 2021). Similarly, the LD<sub>50</sub> for fish varies depending on the species and ranges from 0.26 to  $2.6 \text{ mg.L}^{-1}$  (Tao et al. 2013). The toxic effects of lindane are due to its ability to disrupt the normal functioning of the nervous system by binding to gamma-aminobutyric acid (GABA) receptors, which can lead to seizures, tremors, and convulsions (Islam & Lynch 2012). Numerous studies have highlighted the adverse effects of lindane exposure on aquatic organisms, including genotoxicity and DNA damage in fish (González-Mille et al. 2003). In addition, research has shown that lindane can negatively impact the reproductive success of birds, as evidenced by reduced clutch size, increased nest failure, and decreased egg viability (Etterson & Bennett 2013).

Understanding the fate and transport of lindane in soil, water, and air is crucial for assessing its potential impacts on human health and the environment. The combination

of low solubility and high adsorption to soil particles makes lindane persistent in the environment and difficult to degrade. Research has shown that lindane can persist in soil for many years, with the potential for leaching into groundwater and surface water. Diop et al. (2019) found that lindane residues were detected in soil samples from various agricultural fields in the Niayes zone of Dakar, Senegal, for up to 15 years after application. The study also showed that lindane was detected in groundwater samples, indicating the potential for contamination of drinking water sources. Goufopoulos et al. (2014) measured lindane concentrations in surface water and sediment samples from the rivers of Northern Greece. The study found that lindane concentrations were higher in sediments than in surface water, suggesting that lindane can adsorb onto sediment particles and be transported downstream. In the air, lindane can be transported over long distances through atmospheric transport and deposition. Waite et al. (2005) studied the movement of lindane from the Canadian prairies to the Great Lakes, Arctic, and Rocky Mountains regions. They found that lindane traveled long distances in the atmosphere and was detected in air and precipitation samples from these regions, with peak concentrations in the summer. The study highlights the importance of monitoring and regulating POPs to prevent contaminating remote regions and ecosystems. Lindane is banned or restricted in many countries due to its harmful impact on human health and the environment. Yet, its continued presence poses significant risks, especially in developing countries with poor regulation. Developing effective technologies for removing lindane from contaminated environments is necessary to reduce its risks.

Numerous techniques, comprising physical, chemical, and biological methods, are available for the degradation of lindane in contaminated soil and water. However, the efficacy of these methods may be subject to diverse factors, such as the concentration and spatial distribution of lindane in the soil or water matrix, the physicochemical properties of the soil or water, the prevailing temperature and pH conditions, and the co-existence of other contaminants. Physical remediation, such as soil vapor extraction (SVE), has been explored to remove lindane from contaminated soils with low moisture content. For instance, De Melo Henrique et al. (2022b) successfully removed lindane from contaminated soils using SVE technology, while their subsequent study showed that electrokinetic remediation (EKR) technology was also effective for this purpose (De Melo Henrique et al. 2022a). Also, Fraiese et al. (2020) found that ultrasonic treatment was capable of removing up to 96% of lindane from contaminated water.

In recent years, the field of chemical remediation also made significant advancements, leading to the development

of innovative and effective methods for removing lindane. Begum et al. (2017) reported that Fenton's reagent could effectively degrade lindane from contaminated soil, while Khan et al. (2019) demonstrated the potential of using a combination of nanocrystalline TiO<sub>2</sub> and inorganic oxidants for the photocatalytic degradation of lindane. Metal-based reduction methods have also been studied to detoxify lindane by breaking down the chlorinated bonds and converting them into less harmful products. Zinc, iron, palladium, aluminum, platinum, nickel, and copper had all been investigated for this purpose, either as supported or unsupported catalysts in various reaction systems, such as aqueous, non-aqueous, or solid-state reactions (Adriano et al. 2004, Paknikar et al. 2005, Mertens et al. 2007, Joo & Zhao 2008, Nienow et al. 2008, Singh et al. 2011, Zhao et al. 2016, Jung et al. 2018, Suresh & Thangadurai 2019, Nguyen et al. 2020, Mar-Pineda et al. 2021). Studies have demonstrated the effectiveness of metal-based reduction methods for detoxifying various recalcitrant pesticides with high conversion rates and low toxicity of the reaction products. For example, Shih et al. (2011) studied the effectiveness of Pd/Fe bimetallic nanoparticles in reducing pentachlorophenol (PCP) in water. They found that the nanoparticles were more efficient than individual Pd or Fe nanoparticles. Mohammadi & Sheibani (2019) investigated the effectiveness of a Resin-Au-Pd nanocomposite as a bimetallic photocatalyst for degrading parathion pesticides under visible light. They found that the nanocomposite showed high photocatalytic activity. Ulucan-Altuntas & Debik (2020) compared the effectiveness of Fe/Pd bimetallic nanoparticles and nZVI in degrading DDT. They found that Fe/Pd bimetallic nanoparticles were more effective and proposed a degradation mechanism and pathways. The aforementioned studies underscore the potential of metal-based reduction techniques, specifically bimetallic nanoparticles, as a viable remediation technology for sites contaminated with lindane.

Biological degradation techniques represent a potent and eco-friendly approach to the removal of lindane contamination. This degradation process can be accomplished through multiple metabolic pathways, such as aerobic and anaerobic biodegradation, co-metabolism, and co-oxidation (Singh et al. 1999, Zhang et al. 2020). Numerous bacterial and fungal strains have demonstrated the potential to degrade lindane, such as *Sphingobium indicum* (Zheng et al. 2011), *Pseudomonas putida* (Lal et al. 2008, Jaiswal et al. 2023), *Bacillus subtilis* (Hossain et al. 2018), *Rhodococcus* sp. (Sahoo & Chaudhuri 2022), *Streptomyces* sp. (Benimeli et al. 2006), *Aspergillus niger* (Asemoloye et al. 2017), *Trichoderma viride* (Satish et al. 2017), *Phanerochaete chrysosporium* (Kennedy et al. 1990), and *Pleurotus*

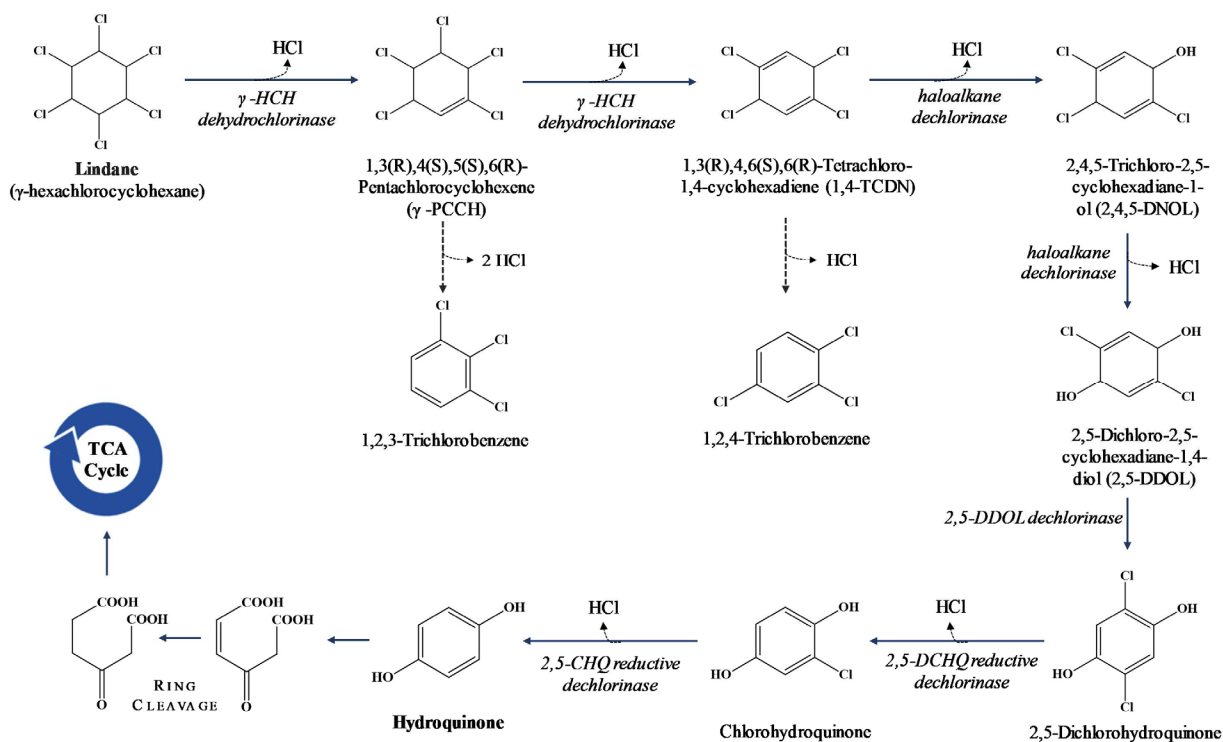


Fig. 1: Pathways involved in the biotransformation of lindane by microorganisms and associated enzymes.

*ostreatus* (Ulčnik et al. 2013). These microorganisms utilize lindane as a carbon source through the degradation pathway of enzymes, such as lindane dehydrochlorinase (Lal et al. 2008, Sowińska et al. 2018) and ligninolytic enzymes like lignin peroxidase manganese peroxidase, and laccase (Singh & Kuhad 2000, Rigas et al. 2005, Kaur et al. 2016, Zang et al. 2020). Microbial degradation of lindane typically proceeds through the formation of less toxic intermediates, namely pentachlorocyclohexene (PCCH), tetrachlorocyclohexene (TCCH), and trichlorobenzene (TCB), before reaching its ultimate degradation products such as  $\text{CO}_2$ . Enzymes crucial in this biodegradation process include dehydrochlorinase,  $\gamma$ -hexachlorocyclohexane dehydrochlorinase, and ligninolytic enzymes. Fig. 1 illustrates the metabolic pathways utilized by microorganisms for the biotransformation of lindane, along with the enzymes involved in the process. Utilizing bacteria and fungi for lindane bioremediation holds immense potential for restoring contaminated sites.

Although biological methods have proven effective in the detoxification of lindane, they are often slow and time-consuming. Conversely, chemical methods offer a more rapid solution but are costly and can lead to secondary pollution issues (Wacławek et al. 2019). In this context, due to their unique catalytic properties, bimetallic systems

have emerged as an effective approach for the reductive transformation of persistent organic pollutants (POPs), such as lindane. In comparison to other chemical methods, bimetallic systems offer several advantages, including higher activity, selectivity, and stability. Bimetallic systems, which consist of two different metals on a single nanoparticle or supported catalyst, have been shown to facilitate electron transfer and enhance the surface area of the catalyst, resulting in faster reaction rates and increased efficiency. Several studies have reported successful reductive transformation of chlorinated pesticides using bimetallic systems. For example, researchers have demonstrated that magnesium-palladium bimetallic nanoparticles effectively degraded endosulfan in an aqueous solution with a high reaction rate and low energy consumption (Thangadurai & Suresh 2013).

Similarly, Nagpal et al. (2010) reported that bimetallic nanoparticles consisting of iron and palladium could degrade lindane more efficiently than individual Fe or Pd nanoparticles. Such findings indicate that bimetallic methods are a promising, cost-effective, and efficient remediation technology for treating contaminated water. Therefore, the present investigation aims to: (a) evaluate the feasibility of bimetallic systems for the reductive transformation of lindane and (b) evaluate the bacterial detoxification of lindane.

## MATERIALS AND METHODS

### Media and Chemicals

Lindane (AS, isomers ( $\alpha:\beta:\gamma:\delta=1:1:1:1$ )) was purchased from Sigma, Missouri, USA. Hexane, cyclohexane, beef extract, peptone, sodium chloride, and acetone were purchased from Merck Ltd. MSM (mineral salts medium) contained  $1.5 \text{ g.L}^{-1} \text{ KH}_2\text{PO}_4$ ,  $3.5 \text{ g.L}^{-1} \text{ K}_2\text{HPO}_4$ ,  $0.03 \text{ g.L}^{-1} \text{ Fe}_2(\text{SO}_4)_3 \cdot 7\text{H}_2\text{O}$ ,  $0.27 \text{ g.L}^{-1} \text{ MgSO}_4$ ,  $1.0 \text{ g.L}^{-1} \text{ NH}_4\text{Cl}$ , and  $0.03 \text{ g.L}^{-1} \text{ CaCl}_2$  and 1 mL of a solution that contained trace elements (TE). The TE stock solution had  $0.136 \text{ mg.L}^{-1} \text{ ZnCl}_2$ ,  $0.198 \text{ mg.L}^{-1} \text{ MnCl}_2 \cdot 4\text{H}_2\text{O}$ ,  $0.18 \text{ mg.L}^{-1} \text{ CuCl}_2 \cdot 2\text{H}_2\text{O}$ ,  $0.025 \text{ mg.L}^{-1} \text{ NiCl}_2 \cdot 6\text{H}_2\text{O}$  and  $0.025 \text{ mg.L}^{-1} \text{ CoCl}_2 \cdot 6\text{H}_2\text{O}$  (Thangadurai & Suresh 2014). To prepare solid plates (media), agar powder was added to the aforementioned MSM at a concentration of 1.5% (w/v).

### Chemical Detoxification of Lindane Using a Bimetallic System

The experiment involved studying the reaction between Lindane and Pd<sup>0</sup>-Charcoal in a 100 mL reaction mixture, where the concentration of lindane was maintained at  $100 \text{ mg.L}^{-1}$ , and the concentration of Pd<sup>0</sup>-Charcoal was varied. The reactions were carried out using different amounts of palladium, ranging from 1.0 to 3.0 mg.  $100 \text{ mL}^{-1}$  of the reaction mixture. To initiate the reaction,  $5 \text{ mg.mL}^{-1}$  of magnesium was added, and protons were provided by adding 60  $\mu\text{L}$  of acetic acid to enable the corrosion of the base metal. Samples were withdrawn at different time points ranging from 0 to 30 min, and aliquots of 2 mL were taken for analysis. To perform GC-ECD analysis of lindane isomers, we used a  $30 \text{ m} \times 0.25 \text{ mm i.d.}$ ,  $0.25 \mu\text{m}$  film thickness, nonpolar DB-5MS column. The injector temperature was set at  $250^\circ\text{C}$ , and the injection volume was 1  $\mu\text{L}$  in splitless mode. The initial oven temperature was maintained at  $60^\circ\text{C}$  for 2 min, then ramped to  $280^\circ\text{C}$  at a rate of  $20^\circ\text{C}/\text{min}$  and held for 5 min. He (carrier gas) was used at a  $1 \text{ mL.min}^{-1}$  flow rate. The electron capture detector (ECD) was operated at a temperature of  $300^\circ\text{C}$  with a make-up gas flow rate of  $30 \text{ mL.min}^{-1}$  and a voltage of 300 V. Prior to analysis, the samples (2 mL) were extracted using a mixture of cyclohexane and acetone (3:1, v/v) and then cleaned up using solid phase extraction (SPE) with a Florisil cartridge.  $0.2 \mu\text{L}$  of the pooled extracts were injected into GC-ECD to analyze residual lindane and other metabolites released during the reaction.

### Isolation of Lindane Degrading Bacteria

The isolation of bacterial strains from soil that degrade lindane specifically was performed following a rigorous

protocol. Soil samples were collected from Padre village, Kerala, India, an area with a history of pesticide(s) exposure. Sampling was done by removing the top soil layer around the root zone of cashew plants. Multiple samples were combined to form a representative sample, which was transported in sterile plastic bags and stored at  $4^\circ\text{C}$  until processing. An enrichment culture was established using a mineral salt medium with lindane as the only carbon and energy source. Dilution and plating methods were utilized to isolate bacterial strains capable of utilizing lindane as their sole carbon and energy source. The isolated strains were then screened for their ability to degrade lindane by inoculating them into liquid media containing lindane ( $100 \text{ mg.L}^{-1}$ ) as the C source. Samples were taken at regular intervals and analyzed for lindane degradation using GC-ECD. Bacterial strains that showed significant lindane degradation were further characterized using molecular techniques such as 16s rDNA sequencing to identify the strain.

### Identification of Isolated Bacterium Using 16S r-DNA Analysis

Bangalore Genei, India, performed the 16s rDNA analysis of an isolated bacterium. The genomic DNA from the bacterial cells was isolated and purified using commercially available DNA extraction kits. The 16s rDNA gene was amplified using universal primers, and the PCR products were purified and subjected to Sanger sequencing. BLAST and NCBI GenBank were used to analyze the obtained sequences and identify the closest bacterial species. Multiple sequence alignments were performed to compare the obtained sequence with publicly available reference sequences. Phylogenetic analysis was conducted using software (MEGA 3.1) to construct a phylogenetic tree. Finally, the identity of the unknown bacterium was determined based on the results of the 16s rDNA analysis.

### Biological Degradation of Lindane Using a Bacterial Isolate

To investigate the kinetics of *Acinetobacter* sp., flasks containing sterile MSM were spiked with lindane to attain a final concentration of  $100 \text{ mg.L}^{-1}$ . The flasks were inoculated with 2 mL of *Acinetobacter*, which had been grown to an optical density of 0.2 at 600 nm and was subsequently placed in an orbital shaker and incubated at  $30^\circ\text{C}$  while agitated at a speed of 150 rpm. Samples of 2 mL were collected from each flask at different time points between 0-30 h and were analyzed for residual Lindane and bacterial OD. The samples were clarified by centrifugation and then extracted thrice with hexane. Subsequently,  $2 \mu\text{L}$  of the samples were injected into GC-ECD for analysis. The experiment was continued



until complete degradation of lindane was achieved or until a plateau was reached. The progress of the degradation was monitored by comparing the GC-ECD chromatograms of the samples with that of the initial sample.

## RESULTS AND DISCUSSION

### Synergistic Effect of Bimetallic Systems in Reductive Transformation of Lindane

In Fig. 2(a), the time-dependent profiles illustrate the removal of lindane by a bimetallic system with the Pd-Charcoal (Pd-C) catalyst at different concentrations. It is worth noting that the removal efficiency of lindane increased in proportion to the concentration of the Pd-C catalyst. Specifically, at concentrations of 10 and 20 mg.L<sup>-1</sup> of Pd-C, the removal percentages were approximately 72.5% and 86.6%, respectively. Moreover, using 30 mg.L<sup>-1</sup> of Pd-C, >99% of the 100 mg.L<sup>-1</sup> initial lindane concentration was

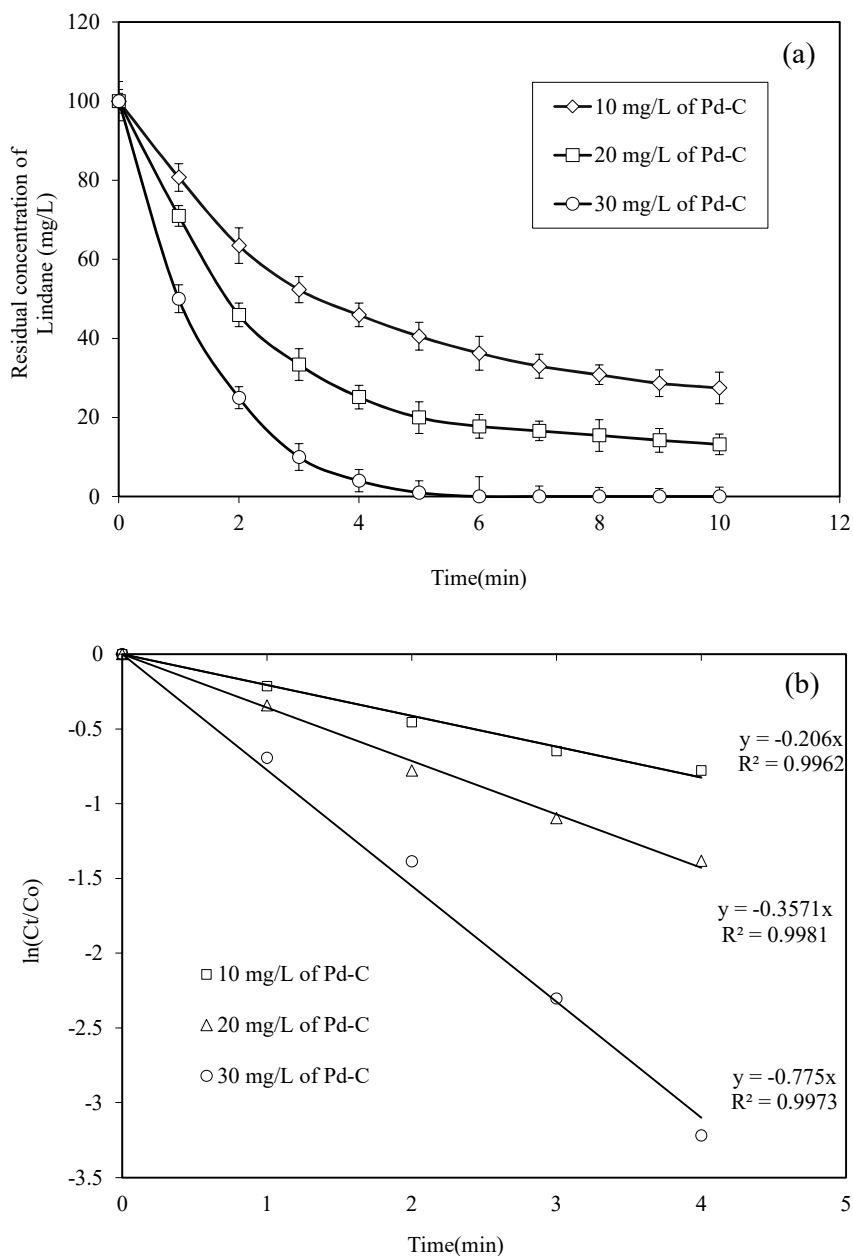


Fig. 2: (a) The time-dependent profiles illustrate the removal of lindane by a bimetallic system, with the Pd-Charcoal catalyst at different concentrations as labeled. (b) presents first-order kinetic plots that showcase the removal of lindane with different concentrations of Pd-Charcoal.

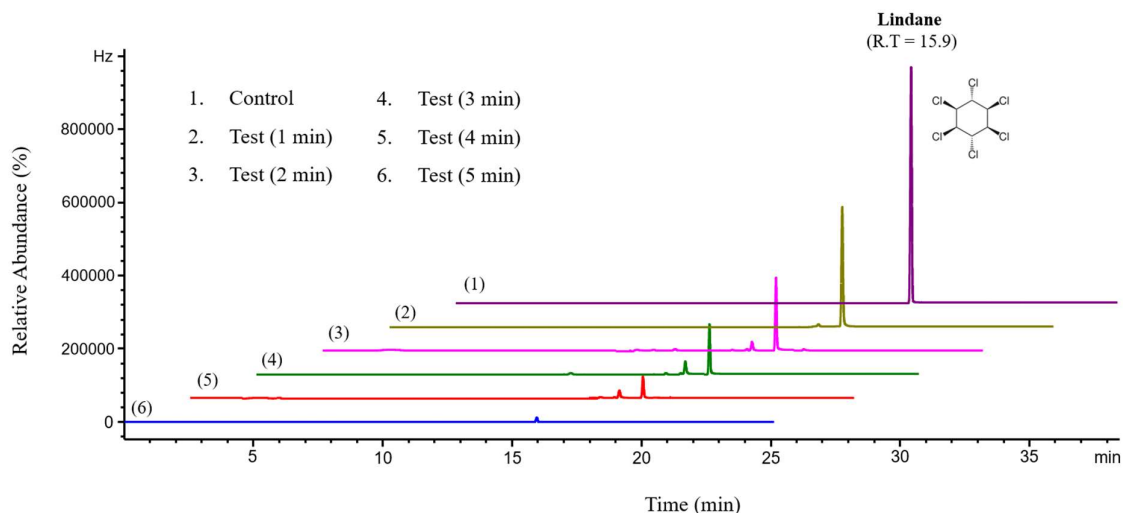


Fig. 3: Comparative analysis of GC-ECD chromatograms obtained at different time intervals for the detoxification of lindane.

removed after 10 minutes of reaction time. The initial phase of the reaction was particularly rapid, with nearly 80% of the lindane removed within 2 min of the reaction. The first-order kinetic plots presented in Fig. 2b demonstrate the removal of lindane using various concentrations of Pd-C, resulting in calculated 1<sup>st</sup>-order rate constants of 0.21 min<sup>-1</sup>, 0.36 min<sup>-1</sup>, and 0.77 min<sup>-1</sup>, corresponding to the 10, 20, and 30 mg.L<sup>-1</sup> Pd-C concentrations, respectively. The estimated 1<sup>st</sup> order rate constant for lindane removal was calculated to be 0.77 min<sup>-1</sup>. The GC-ECD chromatogram depicted in Fig. 3 shows successful baseline resolution of lindane at 15.9 min retention time (RT). The results highlight a clear trend, with the removal of lindane increasing over time, and corresponding partially transformed product(s) accumulating in the reaction medium at lower retention times, with the first eluting at 15.0 min. Within just 5 min of reaction, over 95% of lindane was rapidly removed. In the control experiment (using palladium and acetic acid but no magnesium), there was an insignificant change in the concentration of lindane after 15 minutes of reaction. Additionally, the control sample containing bimetals without acetic acid showed the removal of only 2% of lindane.

Various research studies have investigated the effectiveness of bimetallic systems in reducing chlorinated pesticides and pollutants. Graça et al. (2020) demonstrated that a Pd-Fe bimetallic system effectively reduced chlorpyrifos to less-toxic products, achieving complete removal within 30 min of reaction time. Similarly, Susha et al. (2012) found that a Pd-Ag bimetallic system efficiently reduced atrazine to less toxic products, achieving complete removal in only 2 h of reaction time. Venkateshaiah et al. (2022) showed that bimetallic zero-valent iron nanoparticles (BZNPs) were effective in degrading various chlorinated

organic compounds, with the Fe/Pd system displaying the highest degradation efficiency. Zhao et al. (2021) found that a bimetallic single-atom catalyst (SAC) comprising iron and palladium exhibited improved degradation efficiency for chlorinated pollutants compared to monometallic catalysts. Gunawardana et al. (2019) demonstrated that the addition of nickel coatings on zero-valent iron (ZVI) enhanced the dechlorination efficiency of pentachlorophenol (PCP), while the presence of oxygen hindered PCP dechlorination by ZVI. The various iron oxide phases present on the ZVI surface also played a crucial role in PCP dechlorination. Finally, Lokteva et al. (2023) examined the use of bimetallic Pd/Fe catalysts for the hydro dechlorination of diclofenac. They found that the choice of support material, metal deposition sequence, and reduction conditions significantly influenced the catalytic activity and selectivity of the Pd/Fe catalysts. A comparison of our results with the literature shows that the removal efficiency of lindane increases with increasing catalyst concentration and that the reaction is rapid initially.

Additionally, the estimated first-order rate constant for removing lindane is consistent with the rate constants reported in some of the other articles. The GC-ECD analysis also suggests that the reaction products are partially dechlorinated, a common observation in reducing chlorinated organic pollutants. While the specific catalysts and conditions used in this study differ from those in the other articles, the general trends and observations are consistent with the broader literature on bimetallic system-based reduction.

### Phylogenetic Identification of Bacterial Isolate Through 16S rDNA Analysis

Fig. 4 depicts a phylogenetic tree illustrating the evolutionary

relationship of lindane mineralizing bacterial isolate with other members of the *Acinetobacter* genus. This informative tree highlights the taxonomic position of the isolate and its genetic relatedness to other *Acinetobacter* species based on molecular markers such as 16S rRNA gene sequences. The analysis provides insights into the evolutionary history of the isolate and its potential ecological niche. It could contribute to our understanding of the diversity and biogeography of lindane-degrading bacteria in the environment. *Acinetobacter* is a genus of ubiquitous bacteria commonly found in various environments, including soil. Research has demonstrated that *Acinetobacter* species present in soil have numerous beneficial properties, such as plant growth promotion, bioremediation of pollutants, and biodegradation of organic compounds. For instance, Cai et al. (2021) found that *Acinetobacter* isolates from contaminated oil sludge efficiently bioremediate petroleum hydrocarbons. Furthermore, Kang (2009) demonstrated that *Acinetobacter* can enhance plant growth and development. *Acinetobacter* has also been shown to be effective in the biodegradation of pesticides and other organic pollutants, as demonstrated by Silambarasan and Vangnai (2016) and Chaudhry and Chapalamadugu (1991). Additionally, *Acinetobacter* species produce various enzymes, such as chitinase, cellulase, and protease, which have potential applications in bioremediation and other biotechnological processes (Poomai et al. 2014, Ranganath et al. 2016, Muhammed et al. 2021, Akram et al. 2022). Collectively, these studies highlight the promising potential of *Acinetobacter* in various environmental and biotechnological applications.

#### Biodegradation of Lindane Using *Acinetobacter*

The bacterial culture was cultivated in a mineral salt medium (MSM) supplemented with 100 mg.L<sup>-1</sup> of Lindane, and the biodegradation profile was then closely monitored.

Fig. 5 depicts the growth profile of *Acinetobacter* sp., which showed a consistent rise in cell density over time. The rate of lindane degradation was initially slow until 6 h after inoculation, following which it increased rapidly. This observation is consistent with the process of bacterial acclimation to the presence of a high concentration of lindane, which is an essential prerequisite for initiating the process of biodegradation. After 30 h of bacterial growth, the concentration of lindane decreased from 100 mg.L<sup>-1</sup> to >1 mg.L<sup>-1</sup>, resulting in an almost 99% reduction. The kinetics of lindane biodegradation were evaluated, and the results showed that the process followed 1<sup>st</sup> order kinetics, with a calculated rate constant of 0.12 h<sup>-1</sup>. This observation implies that the rate of lindane degradation is proportional to the concentration of lindane in the medium and decreases exponentially over time. The GC-ECD analysis confirmed the complete degradation of Lindane by *Acinetobacter* sp. without the presence of any toxic intermediates or metabolites. This observation strongly suggests that the biodegradation pathway involves the complete mineralization of lindane into simpler compounds that can be efficiently utilized as a source of energy and carbon by the bacterial species. The absence of toxic intermediates or metabolites further supports the potential application of *Acinetobacter* sp. as a promising bioremediation agent. It is also important to note that the toxicity of lindane to root-associated bacteria, which perform vital ecosystem functions like nutrient cycling, nitrogen fixation, and plant growth promotion, is a significant concern. Thus, the ability of *Acinetobacter* sp., a rhizosphere resident, to degrade lindane is of great ecological significance. This claim is supported by the empirical data in Fig. 5, which provides compelling evidence of the bacterium's ability to degrade lindane. Consequently, *Acinetobacter* sp. has immense potential as a bio-augmenting agent for remediating sites contaminated with lindane,

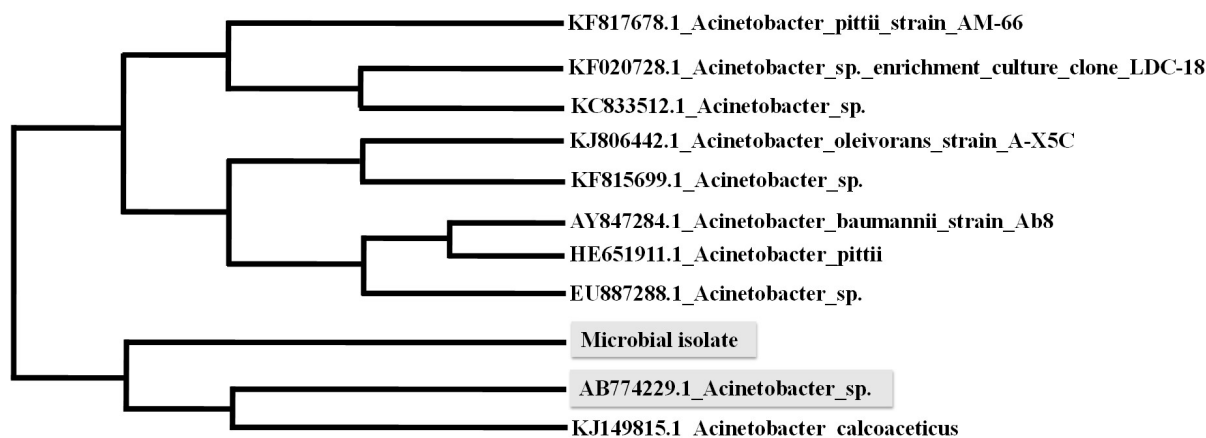


Fig. 4: Phylogenetic tree showing the relationship of lindane mineralizing bacterial isolate to other *Acinetobacter* species.

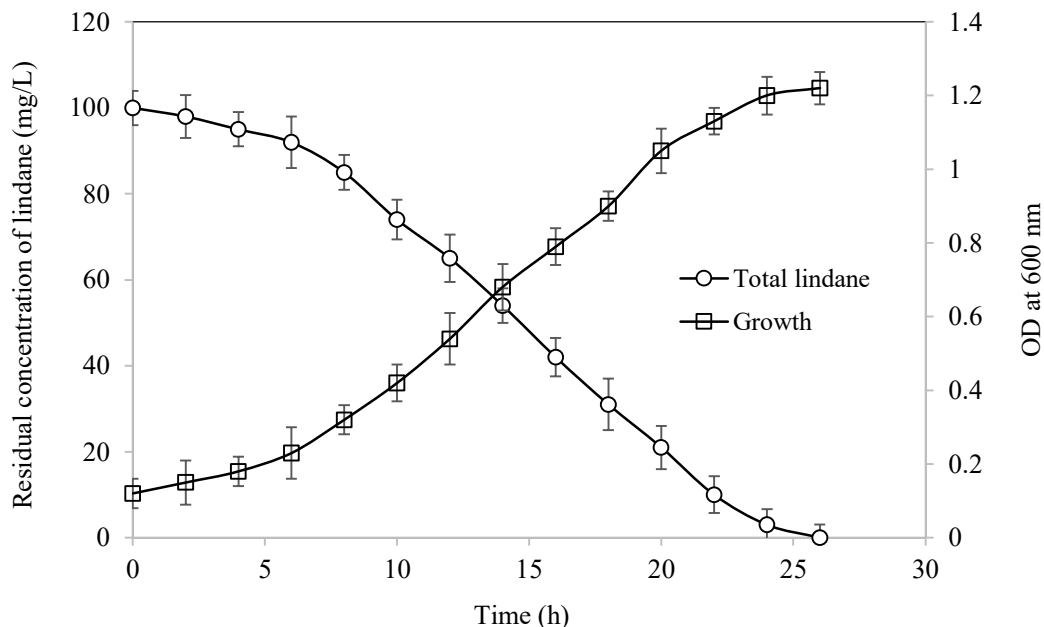


Fig. 5: Growth profile of *Acinetobacter sp.* in MSM containing 100 mg L<sup>-1</sup> of Lindane and corresponding biodegradation profile.

making it a valuable tool for environmental restoration efforts.

The degradation of organochlorine pesticides by microorganisms is a burgeoning research area, and one group of microorganisms that shows great promise in this regard is *Acinetobacter*, which has a well-established ability to degrade diverse organic compounds. Numerous studies have explored the potential of *Acinetobacter* in biodegrading pesticides and other organic pollutants. For example, Ozdal and Algur (2022) isolated *Acinetobacter schindleri* B7 from a grasshopper species and found that it was able to degrade  $\alpha$ -endosulfan and  $\alpha$ -cypermethrin pesticides. Kumar et al. (2021) investigated the degradation of profenofos by *Acinetobacter sp.*33F and developed mathematical models to predict the degradation kinetics. Zhan et al. (2018) investigated the degradation of permethrin by *Acinetobacter baumannii* zh-14 and identified a novel degradation pathway. Jiang et al. (2018) studied the co-biodegradation of pyrene and other polycyclic aromatic hydrocarbons (PAHs) by *Acinetobacter johnsonii*, while Chen et al. (2018) examined the biodegradation and metabolic mechanism of cyprodinil by *Acinetobacter sp.* isolated from contaminated agricultural soil. Finally, Wu et al. (2022) isolated and characterized *Acinetobacter sp.* ZX01, which was able to efficiently degrade cyromazine. Recently, Li et al. (2022) investigated the biodegradation of dibutyl phthalate (DBP) by a newly isolated *Acinetobacter baumannii* DP-2 and found that it could degrade DBP and

identify its metabolites. *Acinetobacter* strains have been found to effectively degrade various POPs, including HCH, dioxins, PCBs, PCP, chlorpyrifos, PAHs, and benzo[a]pyrene, by producing specific enzymes. These findings suggest that *Acinetobacter* is potentially a valuable tool for bioremediation in contaminated environments. Nonetheless, further research is needed to optimize *Acinetobacter* growth conditions and activity across different contaminated environments to guarantee the successful application of this bacterium in bioremediation.

## CONCLUSIONS

Overall, this research highlights the effectiveness of bimetallic systems in reducing lindane contamination. Despite the cost implications of using palladium, the reuse of palladium immobilized on carbon offers potential economic benefits. In addition to their higher efficiency, bimetallic systems produce fewer harmful by-products than traditional treatment methods. The flexibility of the bimetallic systems to optimize various parameters also increases their potential for maximum degradation efficiency. However, further research is necessary to optimize the bimetallic system-based approach for lindane removal, particularly under varying environmental conditions. The biodegradation studies conducted in this research demonstrate that *Acinetobacter sp.* can be a highly effective biocatalyst for lindane remediation, with a removal rate of >99%. Further research is needed to optimize conditions for its growth and evaluate its efficacy



in large-scale bioremediation applications. Overall, this study provides valuable insights into the potential application of bimetallic systems and biocatalysts for the remediation of environmental lindane contamination.

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