Chemicals of manmade origin, used as pesticides, herbicides, solvents, textile dyes refrigerants, etc have also caused considerable environmental pollution and human health problems. Of particular mention are, chlorinated pesticides such as γ-hexachlorocyclohexane (gammexane, γ-HCH, lindane), endosulfan and 1,1,1-trichloro-2,2-bis(4-chlorophenyl) ethane (DDT) that have been extensively used for crop protection and prevention of vector borne diseases for several decades. Lindane is the common name of the γ-HCH, one of eight stereoisomers of 1,2,3,4,5,6-hexachlorocyclohexane (C$_6$H$_6$Cl$_6$) with a molecular weight of 290.83 grams. It is a white crystalline solid which is stable in light, heat, air, carbon dioxide, and strong acids. HCH isomers are manufactured by chlorinating benzene and allowing the photochemical reaction which results in the formation of technical HCH (CAS No. 608-73-1). This technical mixture comprises of five different stereoisomers of HCH isomers; α-HCH (53-70%), β-HCH (3-14%), γ-HCH (11-18%), δ-HCH (6-10%), and ε-HCH (3-5%) (Howard 1989). Gamma-HCH was first used in the 1940’s as an efficient and effective insecticide and thereon it was immensely popular as it was cheap with broad spectrum efficacy against various insecticides. These isomers are hydrophobic, persistent, and ubiquitously distributed in the environment and due to their lipophilic properties they accumulate in the food chain and impart toxicity. During their presence in the environment they also volatilize, get transported to settle in remote and geographically distant colder regions, Thus, HCH isomers are one among the most persistent and frequently encountered pollutant in nature and its residues are being detected in various environments from most part of the world. In India, the residues of HCH were detected in surface and subsurface soils, in food products, dairy milk and recently in packaged drinking water having concentrations many fold than the permissible limits. Current practices to detoxify organochlorine pesticides rely on chemical treatment, incineration, and landfills. Such commonly practiced methods of disposal including incineration met with serious public opposition because of potential toxic emissions besides being economically restrictive.
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. Although, HCH isomers are known for their persistence in aerobic environments, they undergo substantial degradation in anaerobic ecosystems such as flooded soils and lake sediments. Several bacteria with the ability to biodegrade HCH-isomers have been isolated and characterized. From anaerobic environment many pure bacteria have been identified with ability to convert γ-HCH to less chlorinated metabolites via TeCCH. Aerobic microorganisms, adapted to biodegrade HCH were also isolated. In this case, the biodegradation involved dehydrochlorination pathway to produce γ-pentachlorocyclohexene (γ-PCCH) which is further metabolized and mineralized.

Traditionally, the method to isolate a xenobiotic-degrading bacterial population from a contaminated environment depends on “selective enrichment” based on culture techniques. These methods usually take inordinate time and often only the fast growing bacterial species were selected and the best degraders even with higher capacities are lost due to its slow growing property. Therefore, various alternate methods are being explored to isolate the bacteria for the degradation of particular pollutants. Usually the isolated pure cultures have been characterized for their identification, degradation kinetics, and metabolites formation, genes and enzymes responsible for the pathway and their utilization in natural contaminated environments.

**Objectives of the Present Study**

Enrichment methods so far resulted in isolation of diverse microorganisms capable of biodegrading varieties of persistent chemicals. The application of enzyme based assays and other molecular-biology-based techniques in biodegradation are being increasingly used. Such methods have provided faster screening, information for improving of bioremediation strategies and assessing the impact of bioremediation treatments on ecosystems. These techniques also provide rapid, sensitive, and accurate methods of analyzing bacteria and their catabolic genes in the environment for the degradation of various xenobiotics. Enumeration and monitoring of xenobiotic-degrading and other microbial communities are important for bioremediation processes.
Phylogenetic information is also needed together with estimates of metabolic potential in order to link specific members of the community to xenobiotic biodegradation and for the biogeochemical processes. However, the phylogenetic information present in HCH contaminated soils are very little or missing. Phylogenetic analysis results may also indicate the type of microbial populations present despite some extreme contaminant levels in a mixed-waste contaminated site.

It is widely accepted that the bioavailability of pollutants in the subsurface is limited to that portion of the compound dissolved in the aqueous phase. Numerous studies have investigated the enhanced solubilization of hydrophobic organic compounds (HOCs) in the presence of surfactants at concentrations above their critical micelle concentration (CMC). Organochlorine pesticides such as HCH have low aqueous solubility, high hydrophobicity and tendency to stay sorbed in soil. Surfactants, at concentrations above their CMC, can enhance solubilization of hydrophobic compounds in soil and have been successfully used in soil washing or soil flushing for remediation of contaminated sites. Although there are many reports about the application of surfactants for the enhanced degradation of polyaromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs) role of surfactants on HCH biodegradation has not been defined. The purpose of this research is also to assess the effect of biosurfactants on the degradation of HCH isomers by a Sphingomonas sp strain NM05.

Therefore the objectives of the study were to:

1) Isolate and characterize hexachlorocyclohexane (HCH) biodegradation bacteria by employing direct functional methods and characterization of isolated bacterium.

2) Characterize the metabolites formed during the biodegradation of HCH.

3) Elucidate the genes and enzymes involved in biodegradation of γ-HCH.

4) Determine the role of biosurfactant in enhancing the degradation of HCH.

5) Assess the composition of microbial communities in hexachlorocyclohexane (HCH) contaminated soils from various locations in India.

6) Explore the presence of γ-HCH degradation genes in pesticide contaminated soil samples.