Biocatalytic mineralization of the insecticide 1α, 2α, 3β, 4α, 5α, 6β- hexachlorocyclohexane (γ-HCH) in presence of auxiliary carbon sources by bacteria enriched on non-chlorinated pesticides

A Major Project dissertation submitted

In partial fulfilment of the requirement for the degree of

Master of Technology

In

Industrial Biotechnology

Submitted by

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(DTU/13/M. Tech/376**)**

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CERTIFICATE

This is to certify that the M. Tech dissertation entitled "*Biocatalytic mineralization of the insecticide 1α, 2α, 3β, 4α, 5α, 6β- hexachlorocyclohexane (γ-HCH) in presence of auxiliary carbon sources by bacteria enriched on non-chlorinated pesticides*" submitted by **Neeti (DTU/13/M. Tech/376)** in partial fulfillment of the requirement for the award of the degree of Master of Technology in Industrial Biotechnology, Delhi Technological University is an authentic record of the candidate's own work carried out by her under my guidance. The information and data enclosed in this dissertation is original and has not been submitted elsewhere for the award of any other degree or diploma in India or abroad.

Date:

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DECLARATION

I, **Neeti**, hereby declare that the Major Project 2 entitled, **"Biocatalytic mineralization of the insecticide 1α, 2α, 3β, 4α, 5α, 6β- hexachlorocyclohexane (γ-HCH) in presence of auxiliary carbon sources by bacteria enriched on non-chlorinated pesticides"**, submitted by me to Delhi Technological University, in partial fulfillment of the requirement for the award of the degree of **Master of Technology, Industrial Biotechnology** is a bonafide record of the work carried out by the me under the guidance of **Dr. Jai Gopal Sharma, Associate Professor**, Department of Biotechnology, DTU. I, further declare that the work reported in this has not been submitted, and will not be submitted, either in part or in full, for the award of any other degree or diploma of this University or of any other institute or University.

Date: 30/06/2015

NEETI

2K13/IBT/08

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Author	NEETI
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LIST OF ABBREVIATIONS

M4	Minimal media
U / ml	Units/mililitre
H	Hours
U/L	Units/litre
Gm	Grams
wt/vol	Weight/volume
v/v	volume/ volume
UV light	Ultra- violet light
°C	Degree Celsius
%	percentage
γ-HCH	Gamma-hexachlorocyclohexane
OCPs	Organochlorine pesticides
DDT	dichlorodiphenyltrichloroethane
DDE	Dichlorodiphenyldichloroethylene
HCB	hexachlorobenzene

ABSTRACT

Naturally occurring compounds have been used as pesticides since decades. That time of pesticides were most liable sulphurous rock, red pepper, extracts of tobacco, salt, and etc. Though the execution of the synthetic pesticides was a blessing for the world, yet the same has turned into a curse due to the heavy usage by people ultimately leading to their accumulation in the environment and taking its way in to the food web. We tried to investigate the negative effects of lindane on major food crop (wheat) in laboratory. We have found that mean shoot length decreased to 2.93 cm in 50 ppm lindane as compared to 10.2 cm in control. The corresponding mean root length were 0.86 cm and 10.64 cm indicating very heavy deleterious effect of increasing concentration of lindane on the development of wheat seedling even-though the percentage germination in 10, 20, and 50 ppm were very near to that of control (98%) while the percentage in 5 ppm was 84 % indicating heavy loss in the germination ability of seeds at low concentrations even though the mean root and shoot length were comparatively higher than the other concentrations. Many of the synthetic pesticides have been shown to persist in nature and their degradation is either very slow (longer half- life) or negligible. Most of the pesticide remained unused and entered into the ecosystem. These excessive pesticide residues accumulate in the biosphere and create ecological stress. Soil and water are the ultimate sinks for the excessive pesticides. Microbial degradation involves the use of microbes to detoxify and degrade environmental contaminants. The study on co-metabolic mineralization of organochlorine pesticide (lindane) was undertaken for this project. We isolated morphologically four different strains of bacteria by enrichment technique and designated them as LRN1, LRN2, LRN3 and LRN4 respectively. These four strains *viz.* LRN1, LRN2, LRN3 and LRN4 were able to individually act on lindane co-metabolically thereby reducing the initially added concentration to approximately 80% in a very short period of 5 days under aerobic conditions. Co-metabolism is the best applicable bioremediation methods and the use of consortium is a promising approach .Our 4 strains may be constituted into a defined consortium and applied to the contaminated sites for efficient treatment. Further studies need to be conducted to optimize the conditions and investigate the enhanced efficiency of these four strains individually and in combinations to develop best remediation technologies.

Chapter 1

INTRODUCTION

1.1 Pesticide:

Pesticides were essential to our modern agriculture. Pesticides were chemical compound that kill or manage the population of pests. Various type of pesticides were available in the market, out of these herbicides and insecticides were very common. They were used to kill unwanted plants and insects. Currently among the various groups of pesticides that are being used world over, from these, organophosphates form of group is most widely used in more than 35% of total world market (Pradnya *et al*., 2003).

Pesticides used for prevention or destroy or control any pest, including vectors of animal or human diseases, undesired species of plants or animals, causing alteration during or otherwise it was created problem with the production or marketing of food, agricultural artifact, wood and wood products or animal foodstuffs, etc (Vargas, 1975).

Though the implementation of the synthetic pesticides was a blessing for the world, yet the same has turned into a curse due to the heavy usage by people ultimately leading to their accumulation in the environment and taking its way in to the food web. Many of the synthetic pesticides have been shown to persist in nature and their degradation is either very slow (longer half- life) or negligible. In such cases they are termed as recalcitrant. Even though the natural degradation by photolysis, hydrolysis, microbial degradation, etc. usually took care of many pollutants, these pesticides are not easily degraded by any of the natural processes. The common type of degradation occurred by the activity of microorganisms, especially the fungi and bacteria. There was nothing mysterious about microbial degradation of pesticides. Microbes usually obtain food, essential elements, or energy to carry on their life functions from the compounds in their habitat (Bidlan, 2003).

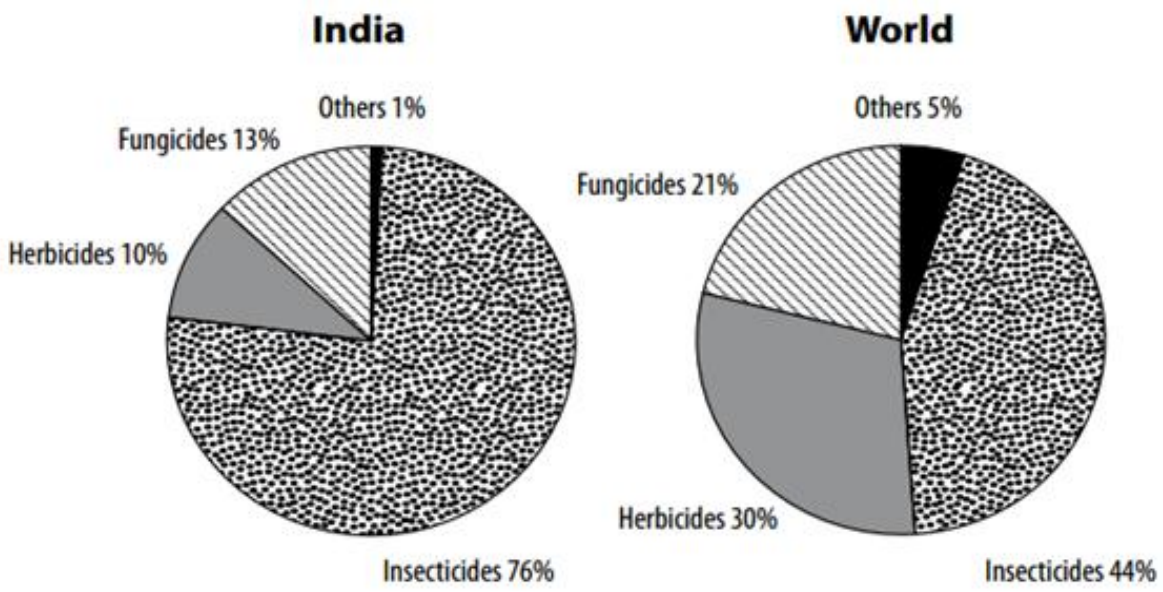

Fig 1.1 Consumption pattern of pesticides (Source: Aktar *et al.*, 2009)

1.2 Pesticides classification

Pesticides are classified according to their toxicity i.e. large amount of chemicals and pesticides combinations of compounds for use in insecticides, fungicides, nematicides, miticides, rodenticides, molluscicides and herbicides. The World Health Organization proposed classification based on their toxic behavior in rats , based on their health risk and other laboratory animals by administering dermal and oral and estimating the median lethal dose (LD50) that produces death in 50% of exposed animals (WHO 2004).

Pesticides are classified into different families according to its chemical structure, ranging from organochlorine and organophosphorus compounds to inorganic compounds.

Table 1.1 Classification of pesticides on the basis of their toxicity, implied as LD50 (mg/kg)

Class	Toxicity	Examples
Class IA	Extremely dangerous	Parathion, Dieldrin
Class IB	Highly dangerous	Eldrin, Dichlorvos
Class II	Moderately hazardous	DDT, Chlordane
Class III	Slightly hazardous	Malathion

Source: Garcia *et al.*, 2012

1.2.1 Organochlorine

In the environment, organochlorine pesticides were known to be highly exist. Organochlorines comprise with chlorine, hydrogen and carbon. Its chlorine-carbon bonds are very strong therefore they did not break down easily. They were attracted to fats and

are highly insoluble in water. This class of pesticides consider the chlorinated derivatives of dichlorodiphenyldichloroethane - DDD, and methoxychlor, DDT, such as DDE, HCB, the group of hexachlorocyclohexane (α-HCH, β-HCH, γ-HCH, δ-HCH, or lindane), and heptachlor-epoxide), and chlorinated hydrocarbons (toxaphene, dodecachlorine) and the group of cyclodiene like nonachlor, dieldrin, endrin, chlordane, heptachlor (Porto *et al*., 2011).

DDT

In 1873, DDT was synthesized by a German graduate student, and in 1938 it was rediscovered by Dr. Paul Muller while searching for a long-lasting insecticide against the clothes moth. It was very effective against flies and mosquitoes and in North America. It was used as a domestic and agricultural pesticide.

DDE

The breakdown of DDT in an organism's body made a product that is Dichloro diphenyl dichloroethylene. Most of animals's body produced it. Thinning of eggshells in fish-eating birds identified as the problem related to contaminants. In female birds, this was caused by the presence of DDE.

1.2.2 Organophosphates

They are known as a organic phosphates, phosphorus insecticides, nerve gas relatives, and phosphoric acid esters. They are chemically unstable so, they have replaced the exist organochlorine compounds.

1.2.3 Carbamates

They are derivatives of carbamic acid. They also suppress the enzyme cholinesterase. Carbamates have very less mammalian dermal toxicity and oral and they may be used for a broad spectrum of insect control.

1.2.4 Hydrocarbons

They have only carbon and hydrogen. They are divided into aromatic or aliphatic. Aromatic hydrocarbons contain elements linked together in rings. Aliphatic hydrocarbons are not linked together to form a ring. Compounds include such as alkenes, alkynes and alkanes. The substances derived from them by replacing one or more hydrogen atoms by atoms of other elements or groups of atoms.

1.3 Significance of Organochlorine pesticides:

Organochlorine pesticides (OCPs) were present on the market since 1940s and 1950s and initially played a role in the controlling of certain pests and vector's disease (WHO 1990). Organophosphorous insecticides were esters of phosphoric acid and also called

organophosphates, which involved phenyl aliphatic and heterocyclic derivatives and have one of the basic building blocks. Though organophosphates are biodegradable in nature but their residues are found in the environment. According to Girish *et al*, (2013), mainly their toxicity property, research is going on biodegradation of organophosphates all over the world.

Organochlorine pesticides moved into the environment direct runoff, through waste incinerators, released gases from manufacturing plants that produced these chemicals and disposal of contaminated wastes into landfills. Some organochlorine pesticides were volatile or stick to soil or particles in the air. The diet is the very first source of exposure to organochlorine pesticides, mainly the ingestion of fatty foods (such as dairy products, fish and milk) in the general population. The population get contaminated through the exposure of contaminated water and air. According to Jaseetha Abdul Salam (2013), toxicity of pesticides may vary pesticides to pesticides.

1.4 Lindane

Gamma-hexachlorocyclohexane (*γ*-HCH or lindane), one of the most enormously used pesticides in agriculture & medicine to world level. The use of technical mixture of eight stereoisomer was banned in several advanced countries since 1970s, some developing countries still used lindane (γ-HCH) for economic reasons (Maria *et al.*, 2010*)*. The HCH formulation consists of γ- (10-12%), α- (60-70%), β- (5-10%), δ- (6-10%) isomers and out of these only γ-HCH possesses insecticidal activity (Li *et al*., 2003). Lindane had been mainly used in agriculture; this pesticide is highly toxic. Lindane was present animal, in water, air, plants, agricultural products, foods, microbial environment, soil and many other things. *γ*-HCH or lindane was a lipophilic compound, because of this property, it inclined to increase and concentrate in the body fats of human & animals. In the environment lindane residues were present and it was migrated with air flow, accumulated in cold regions that caused widespread contamination. In the human body, the food chain affected through the entrance of the γ-HCH residues, and at each trophy level, they become biomagnified (Johri *et al.*, 2000). Pertaining to its toxicity, persistency and potential carcinogenic effect.

Hence remediation of lindane has become one of the most permeant problems in environmental management. So, it needs to remediate through the use of endemic microorganisms. It is well known through some reports that the degradation of chlorinated pesticides through microbes (microbial degradation) such as HCH is mostly degraded by using either mixed or pure culture system.

1.5 Treatments

There were three methods such as chemical treatments, physical treatments, and biological treatments (bioremediation) have been reported for the removal of lindane residue from the environment.

1.5.1 Chemical treatments- Chemical treatments included harsh chemicals like degradation induced by microwave with NaOH i.e modified sepiolite and the addition of H_2O_2 (R.

Salvador *et al*., 2002).These treatments were not found eco-friendly (U. Ahlborg *et al*., 1980).

1.5.2 Physical treatment– Physical treatments include thermal desorption and incineration provide efficient degradation and need huge infrastructure so that required high treatment costs. They generate high toxic gases like phosgene. Lindane adsorption on porous inorganic and organic material caused the problem of disposal of adsorbed lindane (K M Paknikar *et al*., 2005).

1.5.3 Biological treatments – Biological treatments include bioremediation (Arisoy M & Konlankaya N.,1997), are relatively slow process requiring long period of time but are attractive and eco-friendly , low costs (5 to 10 thousand less than other non biological techniques) (Sadowsky M J.,1999) .

The work on microbial degradation of Organochlorine pesticides have been reviewed wherein the degradation is reported in plants, soils, water, animals in anaerobic cultures and by soil microorganisms (Pradnya *et al*., 2003).Therefore, it will be good to use the microbes isolated from the same site where it is being cause. This report discusses that we have developed microbial consortium to degrade pesticides very efficiently and also in very short time than others.

Chapter 2

REVIEW OF LITERATURE

The use of xenobiotics continued to be controversial because they were vital and had harmful persistence in the environment. One of the vital pollutants is aromatic compounds. So these compounds are recognized as toxic by Environment and Forests and Environmental Protection Agency of USA and therefore must be eliminated from contaminated regions (Mishra *et al*, 2001). These contaminated sites and resources which have these compounds have been utilized by humans and lead to contamination and degradation of the environment. Inorganic as well as organic compounds are different classes of contaminants. Organic contaminants have compounds which are prevalent in industries. The compounds of agricultural pesticides such as water, soil and house surroundings are deliberately applied. Moreover, there are some other compounds which get included in soil accidently for example polychlorinated dibenzo-*p*- dioxins, others, polynuclear aromatic hydrocarbons (PNH), furans and polychlorinated biphenyls (PCB). The techniques and apparatus for evaluating the organic contaminants is an expensive task.

The use of pesticides had become an vital tool in agriculture for the restriction of pests and in public health programmes for the removal of vector borne diseases. It was esteemed that nearly four million tones of pesticides were applied to the world crops annually for controlling the pests population. Applied pesticide often may reached to the target pests i.e. less than 1% (Pimentel, 1983). Therefore most of the pesticide remained unused and entered into the ecosystem. These excessive pesticide residues accumulate in the biosphere and create ecological stress. Soil and water are the ultimate sinks for the excessive pesticides.

2.1 Pesticides

A pest is any kind of living organism causing undesirable effects. Pesticides including insecticides ,fungicides ,herbicides and various other types is a kind of material or agent that have been used to kill, prevent, or mitigate pests. A report that was published in "USA Today" (Nov.29, 2000), India can afford to spray 70% of malicious areas with DDT and if it uses the next cheapest alternative, only 23% of the area could be sprayed. Similarly, the use of other chemical pesticides as well cannot be discontinued despite their persistence in nature, their tendency towards bioaccumulation and toxicity towards organisms including human beings as well as the toxicological and environmental problems of its residues (Singh *et al*, 1989). DDT was responsible for the thinning of Falcons' eggshell rendering them less supportive for the production of future generation. Therefore decontamination and detoxification of pesticide-contaminated environments has become very important so it needs to some techniques for its remediation.. Special attention has been given to the

remediation of contaminated soil and aquifers worldwide by CERCLA and SARA after the Love Canal case, in Niagara Falls, NY, USA (It is assumed that 22,000 tones of chemical waste had been disposed in this region that include PCBs, dioxins and other pesticides by Hooker Chemical Company during 1940s and 50s. This was reflected as abnormalities like miscarriages, and birth defects in the residents of the area) (Iwamoto and Nasu., 2001). In the environment most of the chemicals put usually undergo various breakdown processes. These transformations of the applied chemicals can be attributed to various factors such as chemical and physical nature of the compound that include stability of the compound, ability to bind to the soil, sediments or any other support matrix, half-life, solubility, volatility, vapor pressure, ability of the organisms to degrade or accumulate, its biocompatibility, and its effect on the surrounding environment, etc. The effect of air, light, heat, moisture, and biota on the compound, have importance on the type of transformation it undergoes. It will always be the combined effect of all these major factors that determines the transformation process in the environment Table 3.1 describes the persistence of some chlorinated organic compounds in soil.

Table 2.1 Persistence of Some Organochlorines in Soil Compound
(Source: Manonmani and Kunhi, 1999)

	Approximate duration of Persistence (years)
PCBs	>30
Chlordane	>12
HCH	>10
DDT	>10
Aldrin	>9
Heptachlor	>9
Toxaphene	>6
Dicamba	2

Many workers then started to look for various methods to lessen pesticide residue concentration in the environment. Similarly residues of many other chemicals that pose environmental threat have been studied for degradation/removal from the environment by researchers in various ways. Remediation includes photochemical remediation, phytoremediation, chemical remediation and microbial remediation.

2.2 Bioremediation: Bioremediation can broadly be classified as phytoremediation and microbial remediation.

2.2.1 Phytoremediation: In this method vegetation was used to treat contaminated sediments, water and soils .Phytoremediation is utilized at sites which contain metal pollutants,

nutrient and organics , which are accessible by the plant roots and where they were secluded, destroyed, immobilised in place. Metals and inorganics like, lead, zinc, nickel, cadmium, selenium and arsenic, are successfully remediated by plants.

Plants are advantageous due of their aesthetic assets which are cost effective, and have long-term application (Schnoor *et al*, 1995). It includes hazardous waste stations where as different mode of treatments were expensive. Drawbacks of phytoremediation were the availability of hazardous metabolites in the food chain. The time which was needed to clear up below action-level is long. Some plants were demonstrated to have the ability of withdrawing high concentrations of organic chemicals which did not show toxicity effects (Burken and Schnoor, 1998). Plants have the capability to take up and change the contaminants to lesser toxic metabolites (Newman *et al*, 1997). In reference to Burken and Schnoor, (1997), plants may initiate the destruction of organic chemicals by releasing root exudates and enzymes in the rhizosphere.

Table 2.2 Various Phytoremediation applications using plants

Application	Media	Contaminants	Typical Plants
1. Phytotransformation	Soil, Groundwater, Landfill leachate, Land application of wastewater	· Herbicides (atrazine, alachlor) · Aromatics (BTEX) · Chlorinated aliphatics (TCE) · Nutrients (NO_3^-, NH_4^+, PO_4^{3-}) · Ammunition wastes (TNT, RDX)	· Phreatophyte trees (poplar, willow, cottonwood, aspen); · Grasses (rye, Bermuda, sorghum, fescue); · Legumes (clover, alfalfa, cowpeas)
2. Rhizosphere Bioremediation	Soil, Sediments, Land application of wastewater	· Organic contaminants (pesticides, aromatics, and polynuclear aromatic hydrocarbons [PAHs])	· Phenolics releasers (mulberry, apple, osage orange); · Grasses with fibrous roots (rye, fescue, Bermuda) for contaminants 0-3 ft deep; · Phreatophyte trees for 0-10 ft; · Aquatic plants for sediments
3. Phytostabilization	Soil, Sediments	· Metals (Pb, Cd, Zn, As, Cu, Cr, Se, U) · Hydrophobic Organics (PAHs, PCBs, dioxins, furans, pentachlorophenol, DDT, dieldrin)	· Phreatophyte trees to transpire large amounts of water for hydraulic control; · Grasses with fibrous roots to stabilize soil erosion; · Dense root systems are needed to sorb/ bind contaminants
4. Phytoextraction	Soil, Brownfields, Sediments	· Metals (Pb, Cd, Zn, Ni, Cu) with EDTA addition for Pb Selenium (volatilization)	· Sunflowers · Indian mustard · Rape seed plants · Barley, Hops · Crucifers · Serpentine plants · Nettles, Dandelions
5. Rhizofiltration	Groundwater, Water and Wastewater in Lagoons or Created Wetlands	· Metals (Pb, Cd, Zn, Ni, Cu) · Radionuclides (^{137}Cs, ^{90}Sr, U) · Hydrophobic organics	· Aquatic Plants: - Emergents (bullrush, cattail, coontail, pondweed, arrowroot, duckweed); - Submergents (algae, stonewort, parrot feather, Eurasian water milfoil, Hydrilla)

2.2.2 Microbial Degradation: In the use of microbes to degrade environmental contaminants was called microbial degradation. This had been received increased attraction as effective biotechnological approach to remove a polluted environment (Iwamoto and Nasu, 2001). Microbial remediation of soils and ground water can be done by digging the materials or supplying the groundwater and handle them on-site or off-site. When the contamination of the surface is constricted to the soil in the unsaturated zone, the need for oxygen and water restricts the use of *in-situ* bioremediation to near surface contamination (Ritter and Scarborough, 1995). The microbiological approach involved increase of a contaminated site with one or more species of contaminant-specific degrading microorganisms. Two methods have been used to achieve augmentation of contaminated site with species of contaminant-specific degrading microorganisms. Such microorganisms were usually found at the contaminated sites through giving the native microflora to stress conditions of raised concentrations of the contaminant for a very long time. The second method involved in cultures selection, microorganism specific site selection, that showed desirable degradative qualities. For bacterial strains, it was very simple to sample the contaminated soil or water at a site and analysed them. These strains that showed desirable attribute for degrading specific contaminant were cultured and applied to the contaminated sites in high densities along with nutrients identified as being essential for high activity of the microbe.

2.2.3 Ex-situ bioremediation is the treatment that removes contaminants at a separate treatment facility. After remediation, the remediated soil is brought back to the site and refilled. Water can also be added to maintain the moisture content of the soil. Though there are different strategies adapted worldwide to get rid of these contaminants, the best strategy appears to be only bioremediation. This is the best way to treat pollution because it leaves less or no toxic metabolites in the environment compared to other strategies wherein the intermediary metabolites may remain in the environment that might pose more danger than the parent compound. Moreover, all the other strategies are more expensive and energy consuming. Bioremediation, on the other hand works out to be cheaper and more effective strategy to eliminate these toxic chemicals from the environment. Phytoremediation involves the cultivation of plants having an affinity towards the contaminant and that take up the maximum amount of the contaminant from the surroundings and then this flora can be taken for incineration as further treatment of the accumulated chemical. Another strategy here is that the plant itself might take the charge to mineralise the chemical into simpler forms that can be used elsewhere in the metabolism. Phytodegradation of DDT by aquatic plant *Elodea Canadensis* and a terrestrial plant *Puerariathunbergiana* was reported by Garrison *et al* (2000). The major disadvantage here appears to be the length of treatment: the site needs to be left only for such plants till the plant absorbs the complete or maximum quantity of the toxicant. This depends upon the ability of the plants to take up the chemical. Moreover, the plant needs to grow up to maximum state to absorb the chemicals from the site through roots that may take a longer time factor. With this scenario, microbial remediation appears to be the only way out for treatment of contaminated

resources in short time and with more efficiency and safety, with less investment. Many reports were available on the microbial degradation (microbial bioremediation) of environmental pollutants.

2.2.4 In Situ Bioremediation

Various types of *in situ* bioremediation were used, out of these two were very important such as: intrinsic and enhanced bioremediation. Enhance and intrinsic both were depended on natural processes to degrade contaminants. In enhance bioremediation, there were need to amendments while in intrinsic method, there were no need to amendments. From very few years, in situ bioremediation have been very populaIar for treating the contaminated soil and water as well. Rates of removal and time may vary based on the contaminant of concern and characteristics of site-specific. *In situ* bioremediation was selected as a treatment for the removal of the contaminants.

Table 2.3 Microorganisms responsible for Pesticide Degradation

Pesticide	***Microorganism***	**Reference**
2,4-D	*Alcaligeneseutrophus*	Don and Pemberton (1981)
	Alcaligenesxylosoxidans	Kilbane*et al* (1982)
	*Flavobacterium*sp. 50001	Bulinski and Nakatsu (1998)
	Pseudomonas putida	Gunulan and Fournieer (1993)
	Pseudomonas cepacia	Chaudhry and Huang(1988)
	*Comamonas*sp.	Lillis *et al* (1983)
2,4,5-T	*Pseudomonas cepacia*	Karns*et al* (1982)
DPA	*Flavobacterium*sp.	Horvath *et al* (1990)
Mecoprop	*Sphingomonasherbicidivorans MH*	Zipper *et al* (1966)
Mecocarp	*Alcaligenesdenitrificans*	Tett*et al* (1997)
DDT	*Aerobacteraerogenes*	Wedemeyer (1966)
	Alcaligeneseutrophus A5 *Agrobacterium tumefaciens*	Nadeau *et al* (1994)
		Johnson *et al* (1967)
	Serratiamarcescens DT 1P	Bidlan and Manonmani (2002)
	Pseudomonas aeroginosa DT ct 1 and DT ct2	Bidlan (2003)
Dichlorvos	*Xanthomonas*	Ning*et al.* (2010)

Cypermethrin	*Bacillus cereus ZH-3* *Streptomyces aureus HP-S-01*	Chen *et al.*(2012)
Triazophos	*Canna indicaLinn.*	Xiao *et al.* (2010)

2.3 Metabolic pathways of HCH degradation microorganisms

Isolation of Lindane-degrading microorganisms by enrichment culture has confirmed the ability of specific species of bacteria to degrade HCHs either aerobically or anaerobically. Some of these strains are able to grow on HCH as a sole carbon source. Several bacteria capable of degrading Lindane and other HCH isomers have been described.

2.3.1 Anaerobic HCH degradation in bacteria

In the absence of a bacterial model for HCH degradation, rat liver microsomes (enriched in cytochrome P-450) were initially used to study the degradation and relative rates of dechlorination of a-, c- and b-HCH (Beurskens *et al,*. 1991). Metabolic intermediates detected from microsomal degradation of a- and c-HCH, under anoxic conditions, were d-3,4,5,6-tetrachlorocyclohexene (TCCH) and monochlorobenzene (MCB). TCCH was later identified as a metabolite using soil microcosms and pure cultures under both oxic and anoxic conditions, while MCB was identified as a metabolite of HCH biodegradation in anoxic soils.HCH degradation studies in methanogenic glass columns packed with contaminated sediments revealed an anaerobic degradation pathway for the b-isomer that differed from those determined for a- and c-HCH (Middeldorp *et al.,* 1996). TCCH was identified by gas chromatographymass spectroscopy (GC-MS) as an intermediate of b-HCH degradation, and the degradation pathway was proposed to proceed via anaerobic dihalo- elimination producing TCCH, to two end products, MCB and benzene, that accumulated in the medium. A schematic diagram depicting this degradation pathway is provided in Figure 2.1.

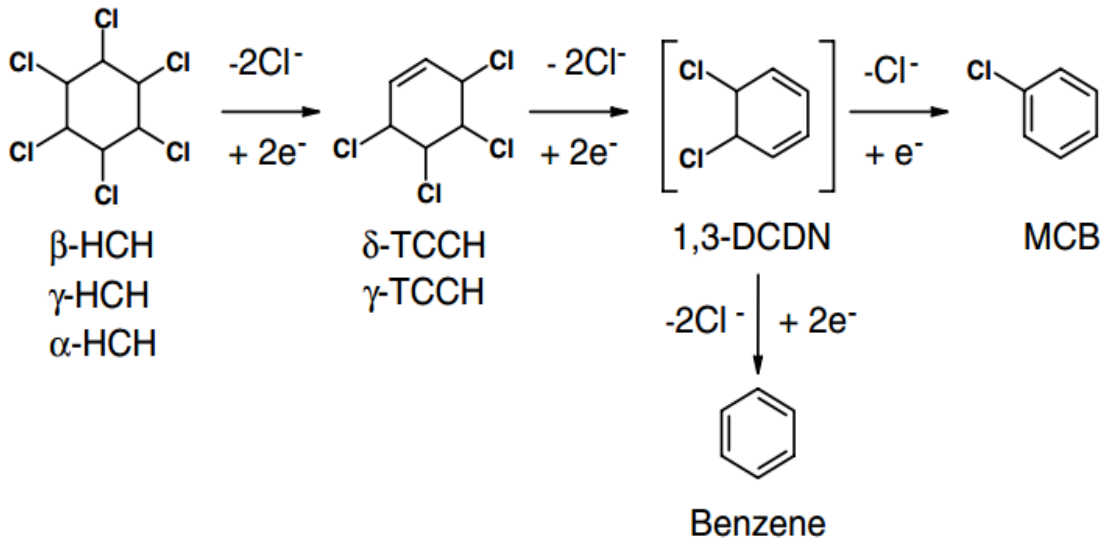

Fig 2.1: Proposed anaerobic degradation pathway of b-HCH, and conversion of a-, d-, and c-isomers to chlorobenzene

2.3.2 Aerobic HCH degradation in bacteria

The most studied pathway for aerobic bacterial degradation of HCHs appeared to be that found in Sphingomonas paucimobilis UT26 and summarized in Figure 2.2. Lindane is first initiative to dehydrochlorination to yield pentachlorocyclohexene (PCCH) in this pathway. Aerobic Lindane degradation by a Pseudomonas sp. isolated from a Canadian soil produced pentachlorobenzene, c-PCCH and a-, b-, or c-3,4,5,6- TCCH.

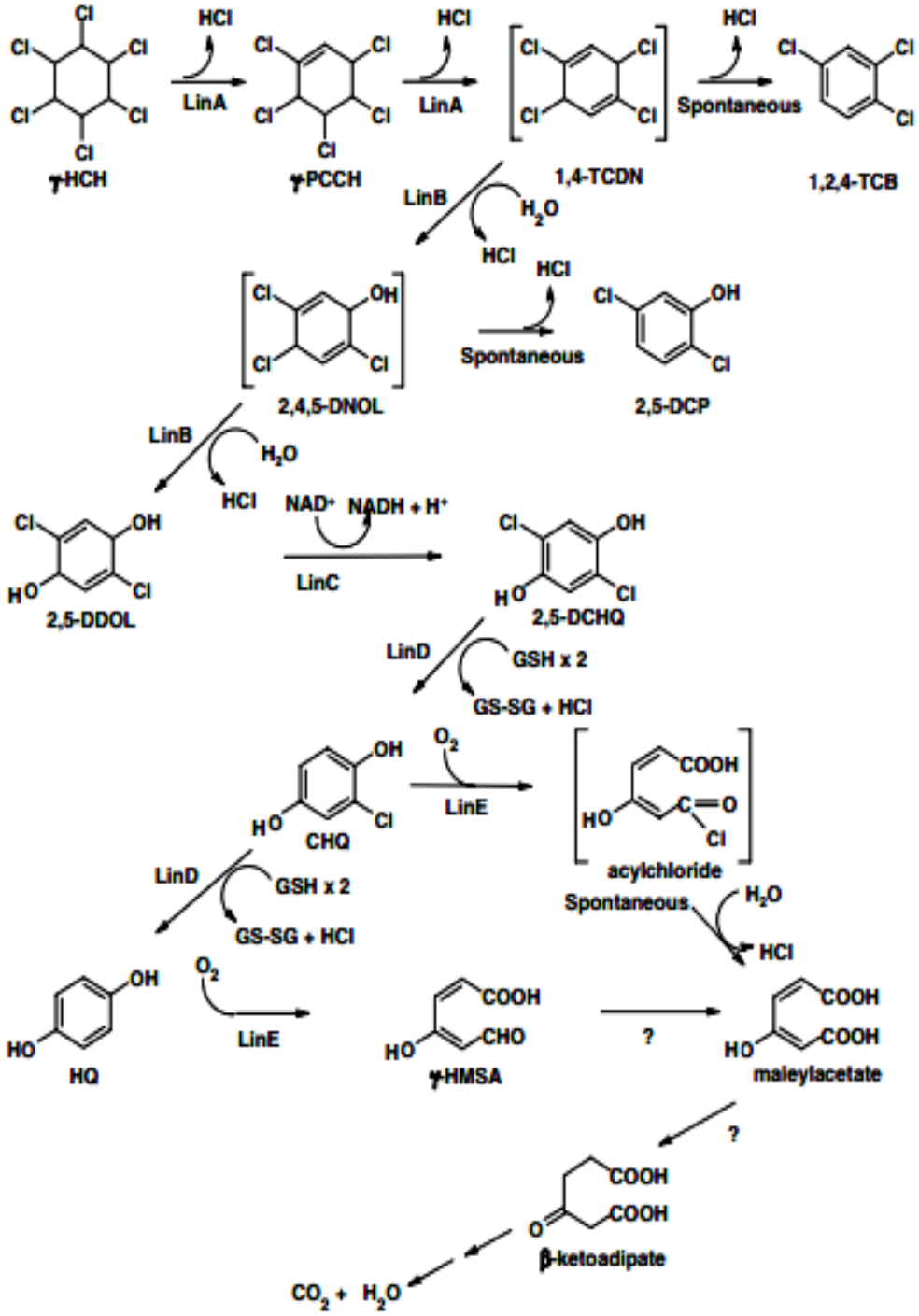

Fig 2.2 Proposed aerobic degradation pathway of c-HCH by *Sphingomonas paucimobilis* UT26.

2.4 The enzymatic basis for pesticide bioremediation

Enzymes were very important for many pesticides. They were involved in their modes of action, target species resistance's mechanisms and environmental fates. Enzymes were responsible for pesticide turnover had involved rapidly from the starting of synthetic xenobiotic pesticides, in both by chance exposed the target organisms and biota. Most of the pesticides were activated *in situ* through enzymatic action, and many pesticides involved in targeting particular enzymes with essential physiological roles. In the wider environment, enzymes were also involved in the pesticide degradation compounds through both developed intrinsic detoxification mechanisms and metabolic resistance (Scott *et al*., 2008). Enzymes have many important characteristics. In the biota, they were the main effectors of all the transformations occurring. They were catalysts with either narrow (region-, chemo- and stereo-selectivity) or broad specificity and, so, they may be applied to a large range of different compounds in mixture, as well.They may be used under extreme conditions limiting microbial activity (Rao *et al*., 2010). The transformation of different xenobiotic substances has been tested for many of these enzymes mainly under laboratory conditions (Whiteley & Lee, 2006).

Examples of hydrolases are amidases, phosphotriesterases, proteases, depolymerise, carbohydrases (cellulases and amylases).Main classes of oxidoreductase are mono- or di-oxygenases, reductases, dehalogenases, phenoloxidases (laccases, tyrosinases) and cytochrome P450 monoxygenases, peroxidases (lignin and manganese peroxidases). The breakdown of amidic, peptidic and esteric, amidases, proteases and bonds by esterases, may lead to products with less or no toxicity. For bacterial degradation of HCH, genes were encoding the enzymes responsible have been cloned.

Chapter 3

Material and methods

3.1 Material

3.1.1 Chemicals: Lindane was purchased from Sigma Aldrich Chemical Company, India. O-toludine, potassium di hydrogen phosphate, Di-sodium hydrogen phosphate, ammonium nitrate, peptone, beef/yeast extract, glucose, nutrient agar, sucrose, nutrient broth were purchased from Hi Media chemical laboratories, India. Sodium chloride, triton x-100 were purchased from Sisco Research Laboratories, India.

3.1.2 Solvent: Cyclohexane, hexane, acetone, ethanol, dichloro-methane, N, N'- dimethyl formamaide were of analytical grade and purchased from CDH, India. Silica gel was obtained from Sisco Research Laboratories, India.. All other chemical and reagents had been used in this study were of analytical grade and were purchased from standard companies.

3.1.3 Microbial Culture: The microbial culture was obtained by long term enrichment of contaminated sludge and sewage water from around the DTU campus with mixture of four different non- chlorinated pesticides in the laboratory. The four strains LRN1, LRN2, LRN3 and LRN4 were isolated from this enriched population by repeated streaking.

3.1.4 Media

3.1.4.1 Minimal Medium (M_4):

Ingredients	Gram per liter distilled water
KH2PO4	0.675 gm
Na2HPO4	5.455 gm
NH4PO3	0.25 gm

M 4 media was prepared in distilled water and was autoclaved at 15 lbs**,** 121º C for 20 minutes. pH of medium was approx 7.

3.1.4.2 Nutrient broth:

Ingredients	Gram per liter distilled water
Peptone	5 gm

Beef extract	3 gm
Sodium chloride	5 gm

All the ingredients were dissolved in distilled water. pH was adjusted to 7.2 and was autoclaved at 15 lbs**,** 121º C for 20 minutes.

3.1.4.3 Nutrient Agar:

Nutrient agar was prepared in 1 liter distilled water by adding 28 gram of nutrient agar in it. It was autoclaved at 15 lbs**,** 121º C for 20 minutes.

3.1.4.4 Stock solutions of glucose & peptone:

Glucose and peptone solutions were prepared in 100 ml distilled water by adding 20 gram of glucose and peptone respectively and were autoclaved at 15 lbs, 121º C for 20 minutes.

3.1.4.5 Stock solutions of Tween-80 and Triton X-100:

Tween 80 and triton x-100 solutions were prepared in 100 ml distilled water by adding 1 ml (v/v) of tween-80 and triton X-100 respectively and both were autoclaved at 15 lbs, 121º C for 20 minutes.

3.1.5 Gram stain:

3.1.5.1 Crystal violet

Ingredients	**Quantity**
Crystal violet	2.0 gm
Ethyl alcohol	20.0 ml
Ammonium oxalate	0.8 gm
Distilled water	80.0 ml

Crystal violet and ammonium oxalate were dissolved respectively in ethyl alcohol and distilled water and the two solutions were mixed well. The prepared stain was dribbled out and packed in a clean and dry glass bottle with stopper.

3.1.5.2 Lugol's Iodine:

Ingredients	**Quantity**
Iodine	1.0 gm
Potassium iodide	2.0 gm

Distilled water	300.0 ml

3.1.5.3 Safranin stain (Counter stain):

Ingredients	**Quantity**
Safranin	2.5 gm
Ethyl alcohol (95%)	100.0 ml

10 ml of the above stock solution was blended with 90 ml of mili pore water for use as counter stain.

3.2 Methods

3.2.1 Growth of culture:

The already established cultures were grown on nutrient agar plates by repeated streaking and incubated at room temperature (30 ^{0}C) for 48 hr incubation and these cultures also inoculated in nutrient broth and incubated at room temperature for 24-48 hours for the growth of culture.

3.2.2 Inoculum:

The cultures/consortium were inoculated to nutrient broth followed by 24 h incubation at room temperature. The culture was harvested by centrifugation washed in M4 (thrice), re-suspended in M4 and 5 ppm lindane was added and incubated for 72 hrs at room temperature induction. This induced culture was harvested by centrifugation and washed with M4 media thrice and it was used as inoculum for the further degradation studies.

3.2.3 Degradation of lindane by induced consortium

All experiments on the degradation of lindane were carried out in triplicates. The tubes covered with cotton plugs were autoclaved at 15 lbs **,** 121º C for 20 minutes.. Each tube filled with 5 ml of M4 medium with different concentration of lindane (dissolved in DMF),then consortium was added as a biotic control and then incubated it at room temperature for each sampling hours 0 h, 3 h, 6 h, 9 h, 18 h, 24 h, 36 h, 48 h, 60 h, 72 h, 96 h, 120 h, 144 h. Taking out each sample at every sampling hours accordingly. Removed all the samples from all test tubes in each sampling hour (0 h, 3 h, 6 h, 9 h, 18 h, 24 h, 36 h, 48 h, 60 h, 72 h, 96 h, 120 h, 144 h). All samples were pipette out 3ml samples from each test tubes later through mixing and take OD at 540 nm for each sample. Then removing all the samples and add 2-3 drops of concentrated HCL and mixed well for stopping the growth of microbes.

3.2.4 Co-metabolic degradation of lindane

The consequences of different carbon sources on the degradation of lindane was studied by adding 1 % carbon sources in 20ppm (20 µg/ml in DMF) lindane. It was added to sterile, dry 15ml test tubes inside a laminar hood and test tubes was already filled with M4 media and the total volume of each test tubes were 10 ml. Then different 4 bacterial strains designated as LRN1, LRN2, LRN3, LRN4 were added into test tubes and then incubated it at room temperature in a rotary shaker (180 rpm) for each sampling hours 0h,24h,48h,72h,96h,120h,144h.All the experiments were done in triplicates.

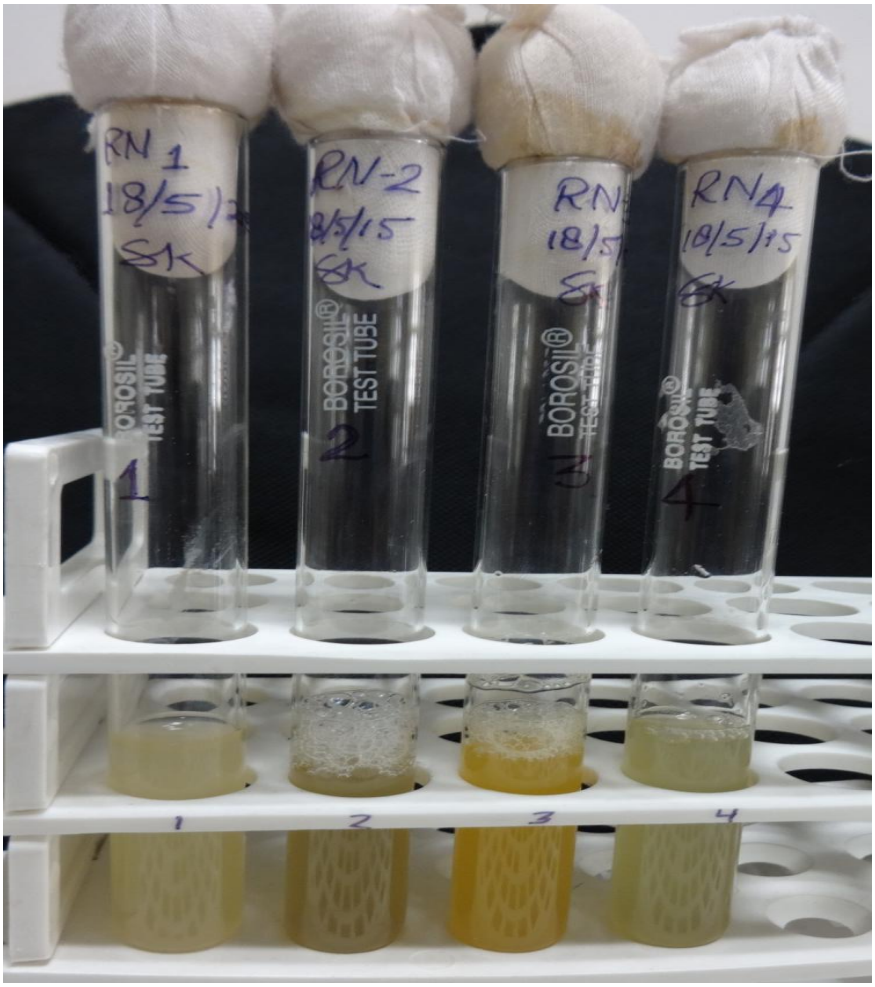

Fig. 3.1 Four different microbial strains in M4 media.

Fig. 3.2 Rotary shaker

3.2.5 Extraction of residual lindane

For residual lindane, extracted out the whole samples thrice with equal volume of dichloromethane in separating funnel. Sample and solvent were taken in separating funnel and mixed well both to each-other properly. Two layers were formed to separate out. Solvent layer were passed through anhydrous sodium sulphate (Na_2SO_4) then allowed to evaporate and then added minimum amount of acetone in each tubes.

3.2.6 Residual lindane

Residual lindane was estimated by thin layer chromatography.

3.2.6.1 Thin layer Chromatography

Thin layer Chromatography was done on silica gel plates that were made by silica gel spread over 20×20 cm^2 glass plate. These plates were left to dry after spreading at room temperature then activated in hot air oven at 105°C for 60 min. Known volume of residual extract of lindane (dissolved in acetone) were spotted on these plates. Spots were moved with solvent system (cyclohexane : hexane, 4: 1) and spotted plates were developed in solvent and the residues were detected by spraying o-toludine (2% solution in acetone) in the presence of bright sunlight. The chloro compound gave greenish blue color with this chromogen. Spots were marked with a needle and area measured. Quantity of lindane in each spot was estimated by standard graph prepared for square root of area *vs.* log (lindane concentration).

3.2.7 Gram staining

The pure culture was grown in nutrient broth for overnight. Overnight cultures have been used for the gram's staining. Smear was prepared on glass slide. It should be air dried and fixed with low heat. This was stained with crystal violet for 1 min and washed off excess of stain with water. Then Lugol's Iodine was added drop wise and left it for 1 min for reaction. After washing off excess of Lugol's Iodine with water. The smear was treated with 95% alcohol for 30 second and remove excess of crystal violet. The last step of gram's staining was addition of safranin stain used as counter stain for 30 second then washed with distilled water, dried and examine under a microscope. Gram's positive cells appeared violet and gram's negative cells appeared pink color.

3.2.8 Effect of lindane on seed germination

Germination of wheat seed on soil was performed in plastic cups that were filled with 100 gm of sterile soil. Different concentration of lindane (5ppm, 10ppm, 20ppm and 50 ppm) as a acetone solution were added in each cup and mixed well to achieve the uniform distribution of insecticide. Then water added to obtain final moisture level of 20 % atleast. The bottom and sides of the cups were pricked with a needle for aeration. 10 seeds were sown at the depth of 0.5 cm. The cups kept at room temperature (26- 30 ° C). Every alternate day, water must be added to each cup. After 6-7 days, seedlings were counted as well as their shoot and root length were measured. The seedling vigor calculated as –

$$vigor\ index = (mean\ root\ length + mean\ shoot\ length) \times (percentage\ germination)/10$$

Chapter 4

Results

4.1 Growth of culture

Cultures were grown separately on nutrient broth and nutrient agar plates for 24-48 h. The plates showed mixed population of bacteria (Fig. 4.1) while turbidity was observed in nutrient broth indicating the growth. Since the population was of mixed bacterial cultures, they were purified by picking up single colonies from the plate and streaking on fresh NA plates.

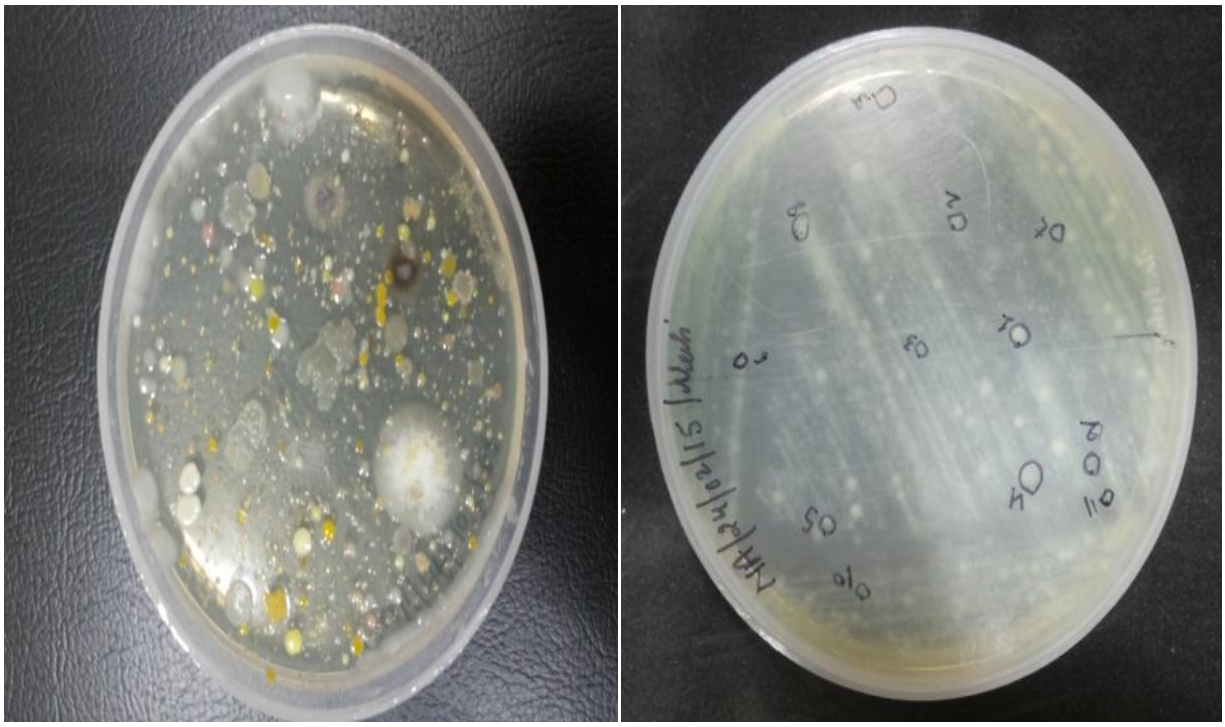

Fig. 4.1 Mixed microbial populations from the enrichment technique.

Then single colonies were picked from mixed population and streaked over nutrient agar plates followed by 24-24 h incubation. After the incubation, single axenic colonies were observed on the plates and 4 cultures were taken from them which were designated as LRN1, LRN2, L RN3 and LRN4 (Fig.4.2).

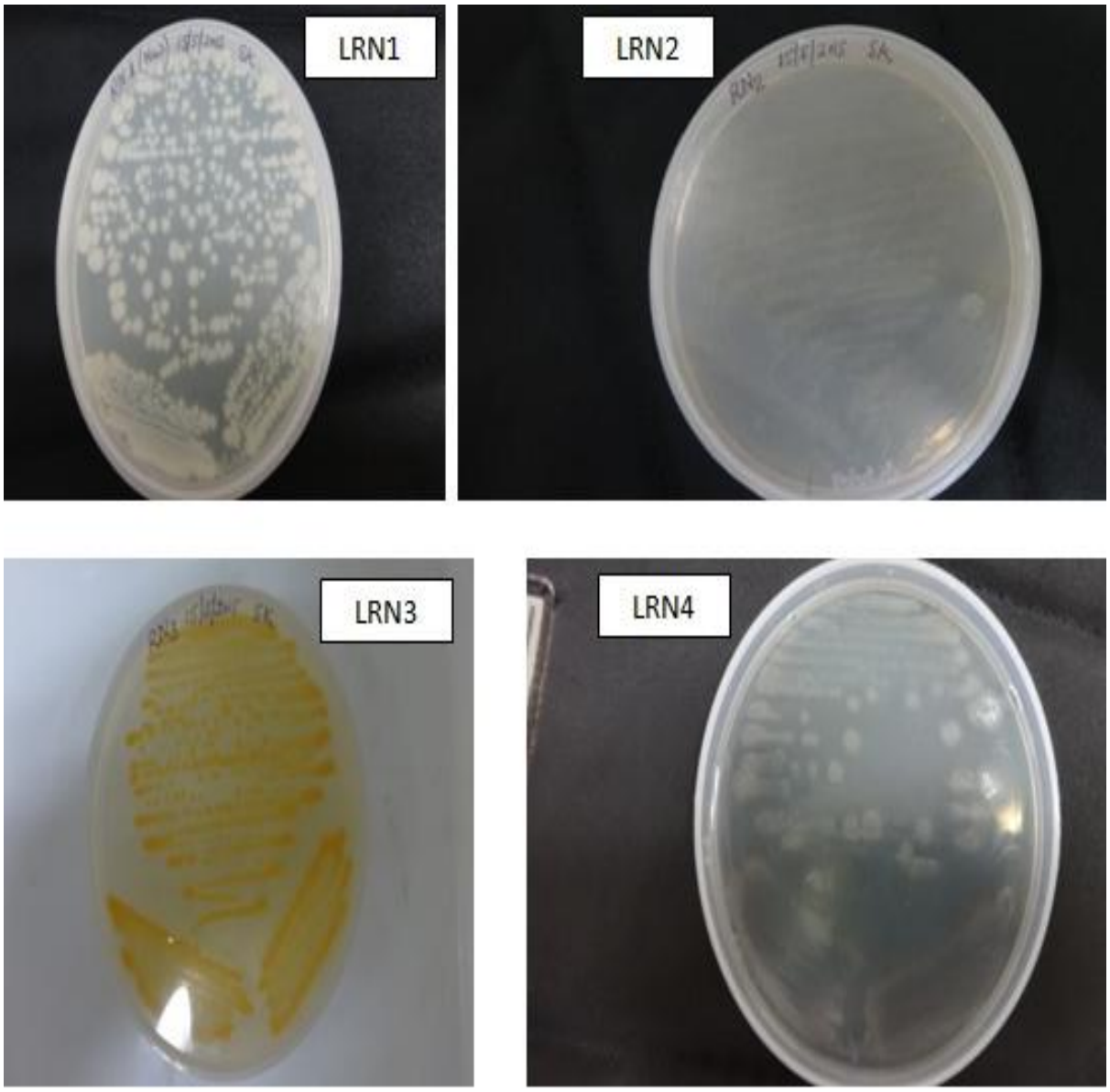

Fig. 4.2 Four bacterial strains isolated from the enriched consortium.

4.2 Growth and degradation of lindane by induced consortium-

The degradation of lindane by induced consortium was observed after the different sampling hours in shaking condition. The growth of consortium increased with time till 18 h and subsequently decreased till 36 h (Fig 4.3). and then again growth of consortium were increased till 72 hrs and again it were slow down till 120 hrs in defined zig-zag pattern. This was a clear indication that lindane was being utilized as a carbon source to help increase the biomass with incubation.

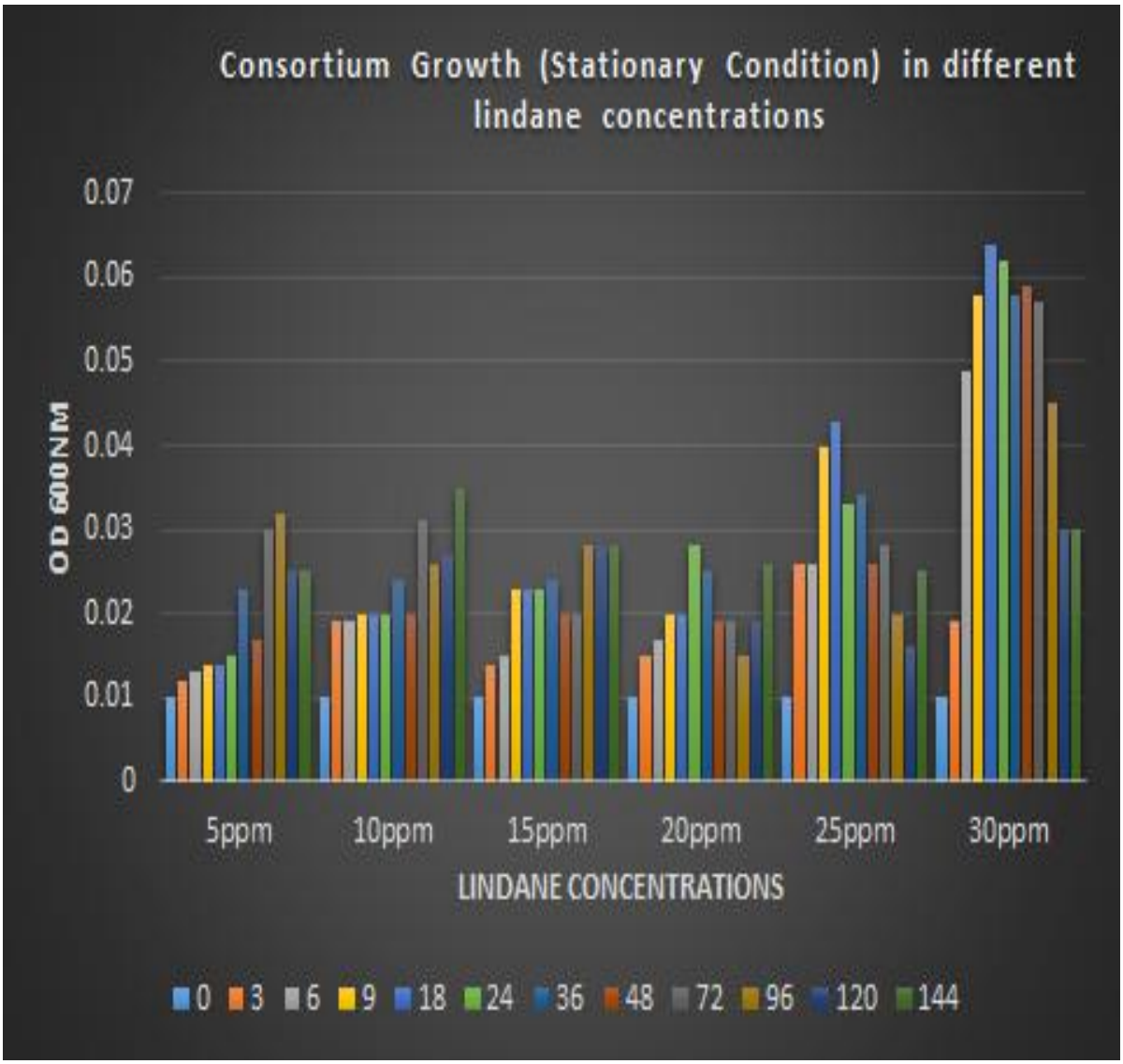

Fig. 4.3 Degradation of lindane under stationary conditions. Each cluster represents residual lindane of a single initial concentration at different incubation periods.

Fig 4.4 implies that degradation of different lindane concentration by consortium increased with time till 144 h. In 30 ppm, 25 ppm, 20 ppm, 15ppm, 10ppm, 5 ppm of lindane concentration degraded 70 % , 72 %, 68 %, 70 %, 70 %, 82 % respectively.

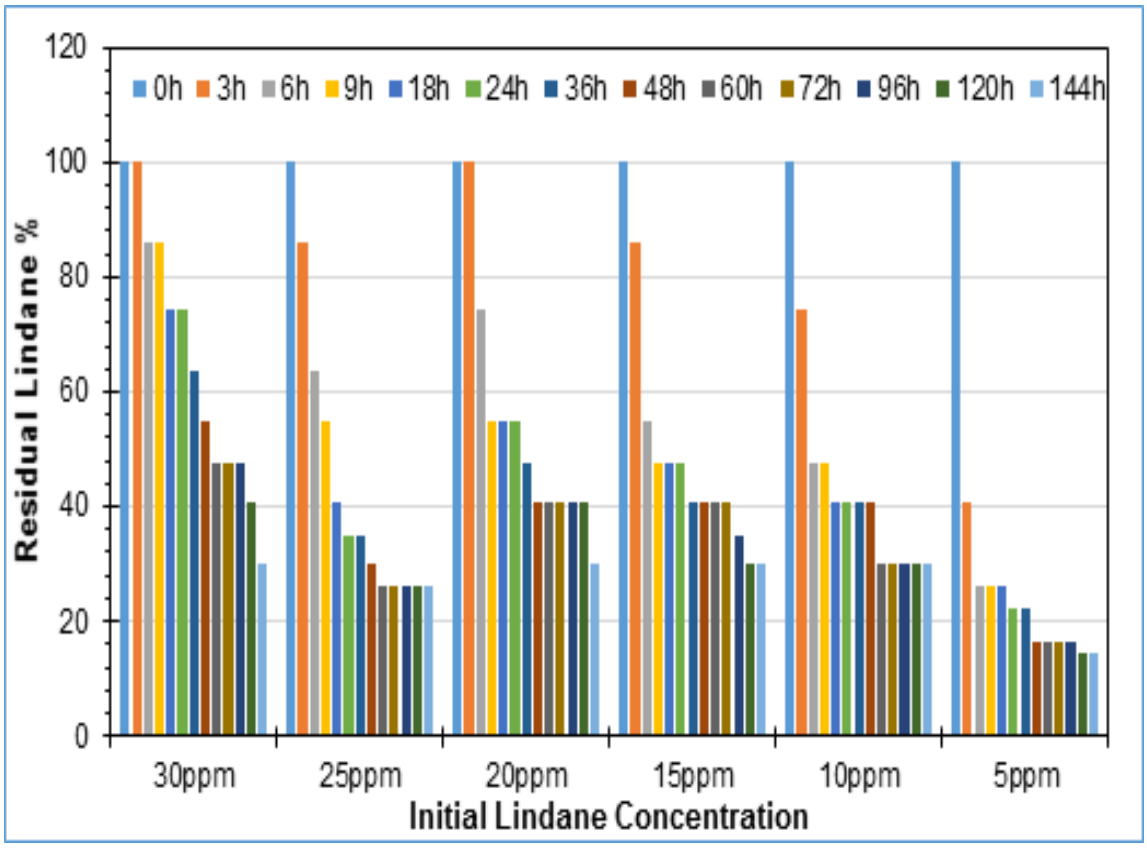

Fig. 4.4 Degradation of different concentrations of lindane by consortium

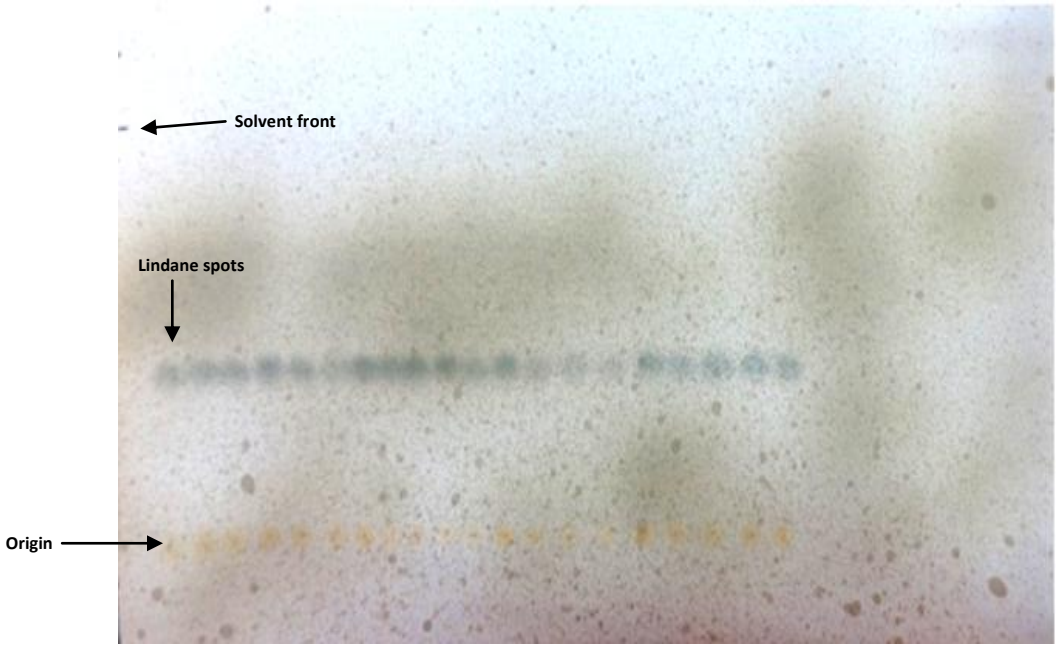

Fig. 4.5 TLC plate of degradation of lindane by consortium

4.3 Degradation of lindane by individual strains

The four strains were used individually to inoculate 20ppm lindane in minimal medium. The results at different periods of incubation are depicted in Fig.4.6. 38.9%, 27.46%, 28.44% and 44.6% of the initially added lindane were degraded by LRN1, LRN2, LRN3 and LRN4 respectively by the end of 5 days of incubation. LRN2 and LRN3 showed similar degradation rate while LRN1 and LRN4 were slightly similar with LRN4 being the best with 44.6% degradation among all the four strains in the 5 days incubation.

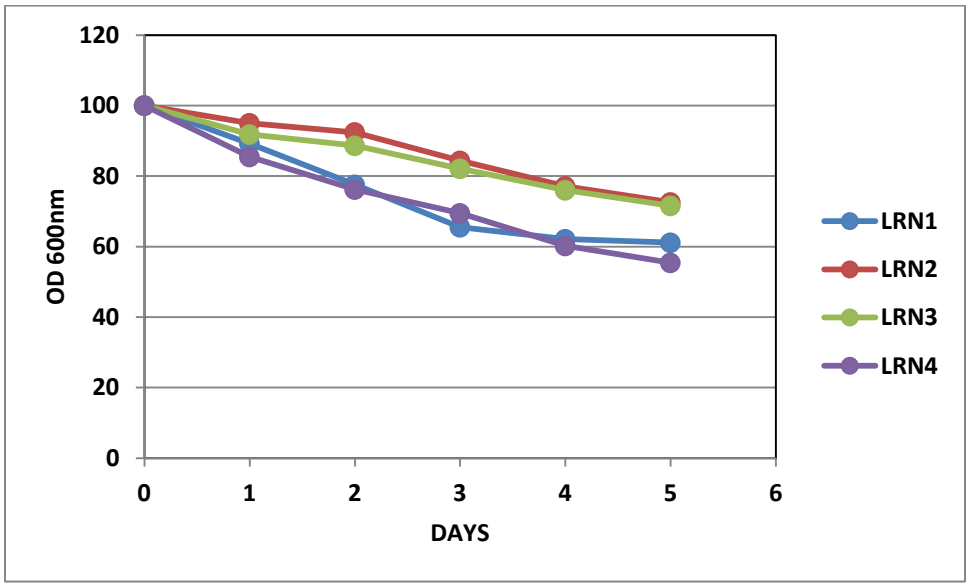

Fig. 4.6 Lindane degradation by individual strains

4.3 Growth and degradation of lindane in presence of different carbon sources by four bacterial strains

4.3.1 Glucose

In presence of glucose, growth of different strains were observed in 20 ppm of lindane that the growth of LRN1, LRN2, LRN3 and LRN4 strain increased with time till 48 h than it was decreased for 24 h while again it has increased for 120 h (Fig. 4.7). The growth pattern of all four strains was same to each-other. Maximum growth was observed in LRN3 bacterial strain than other strains. So this was a clear indication that glucose was being utilized as a good carbon source for the growth of all four strains.

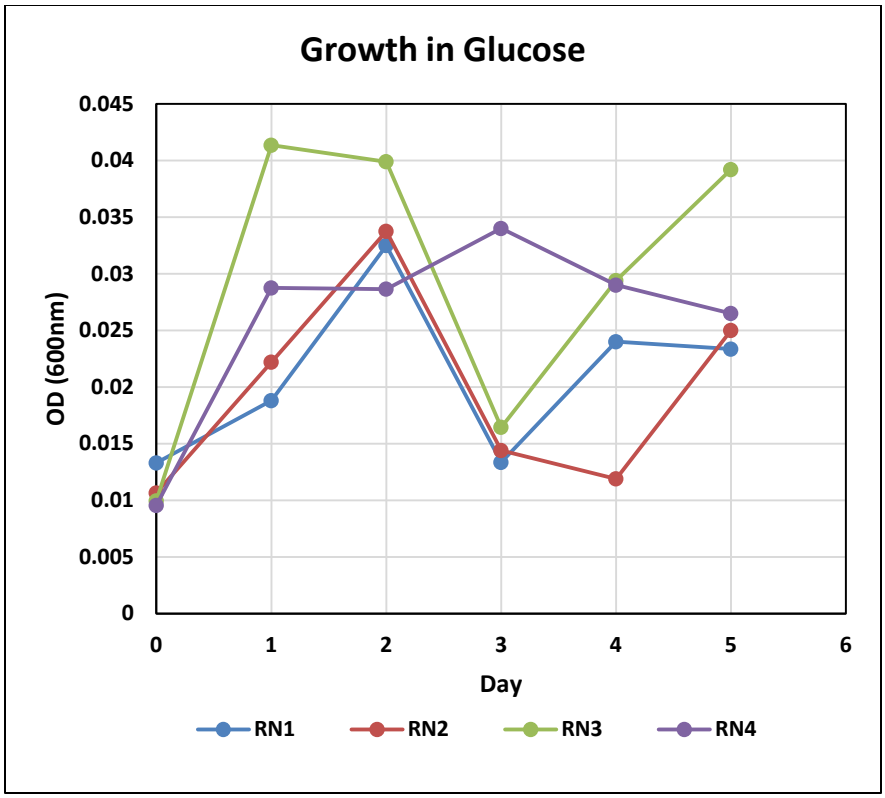

Fig. 4.7 Effect of glucose on four strains

Fig. 4.8 implies that the effect of glucose on lindane degradation , initially supplied 20 ppm lindane was degraded by 48.99 %, 77.61 %, 83.40 %, 83.40 % respectively by the strains LRN1, LRN2, LRN3 and LNR4 by the end of 5th day of incubation . LRN3 and LRN4 showed the maximum degradation of lindane in presence of glucose than others.

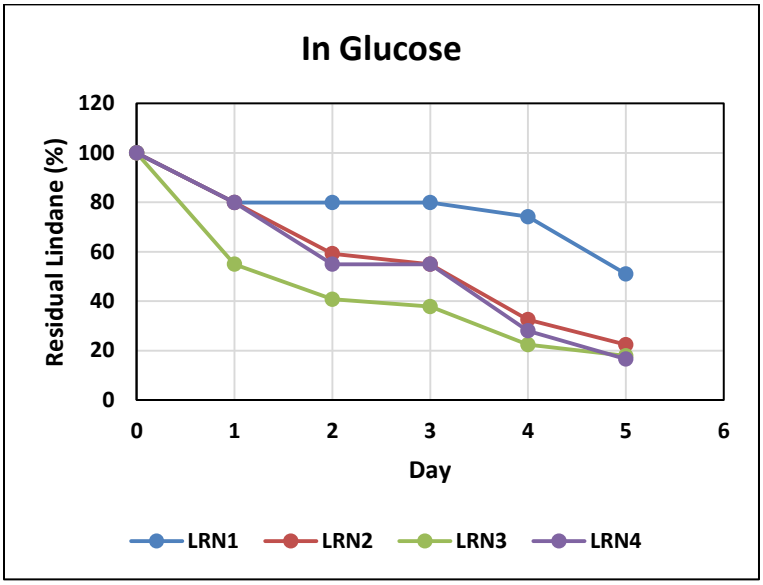

Fig. 4.8 Co-metabolism of lindane in presence of glucose by four stains

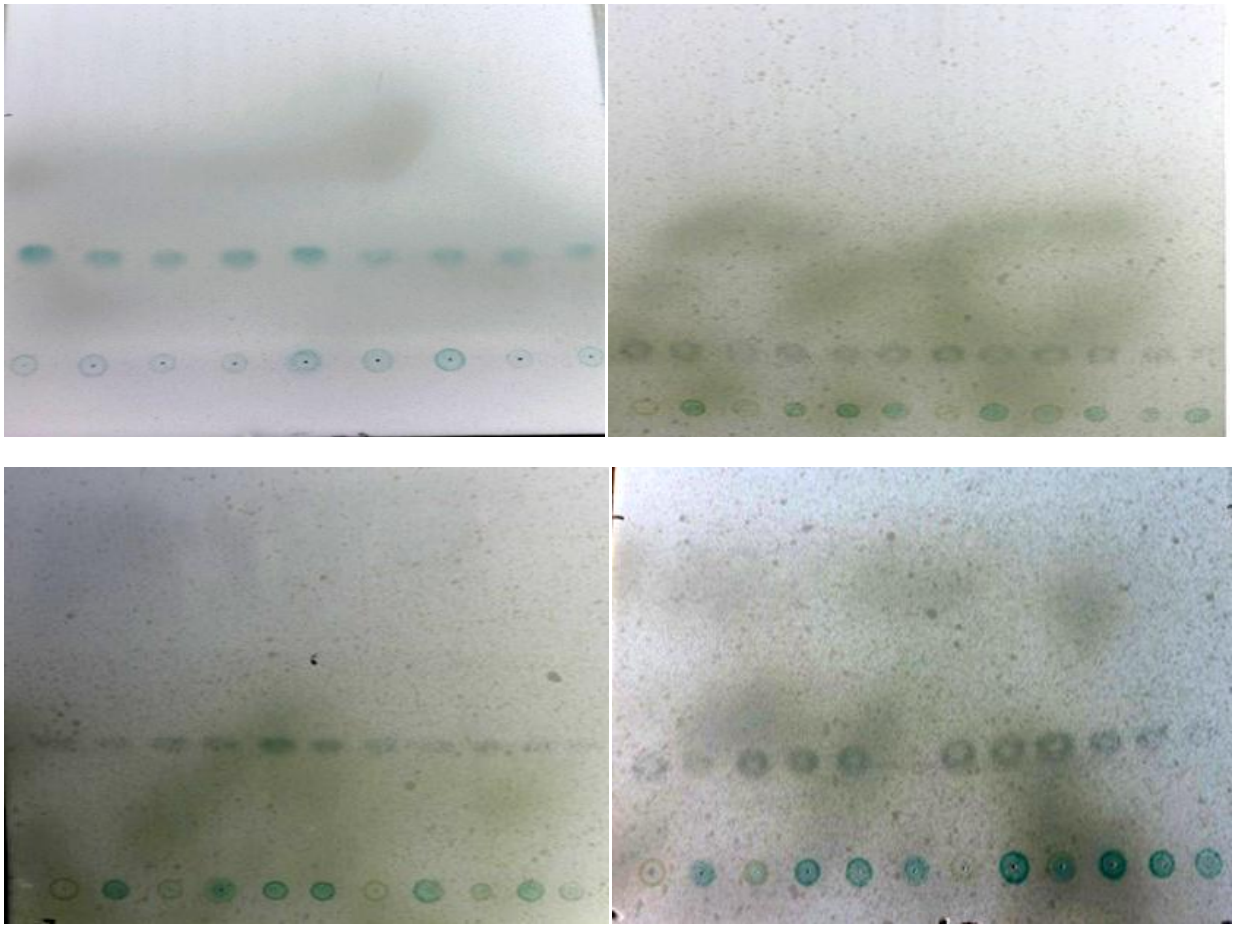

Fig. 4.9 TLC plates of LRN1, LRN2, LRN3 and LRN4 respectively.

4.3.2 Peptone

In presence of peptone, growth of different strains were observed in 20 ppm of lindane that the growth of LRN1, LRN2, LRN3 and LRN4 strain increased with time till 48 h than it was decreased for 24 h while again it has increased for 120 h (Fig. 4.10). The growth pattern of all four strains was same to each-other. Maximum growth was observed in LRN3 bacterial strain than other strains. So this was a clear indication that peptone was being utilized as a carbon source for the growth of all four strains.

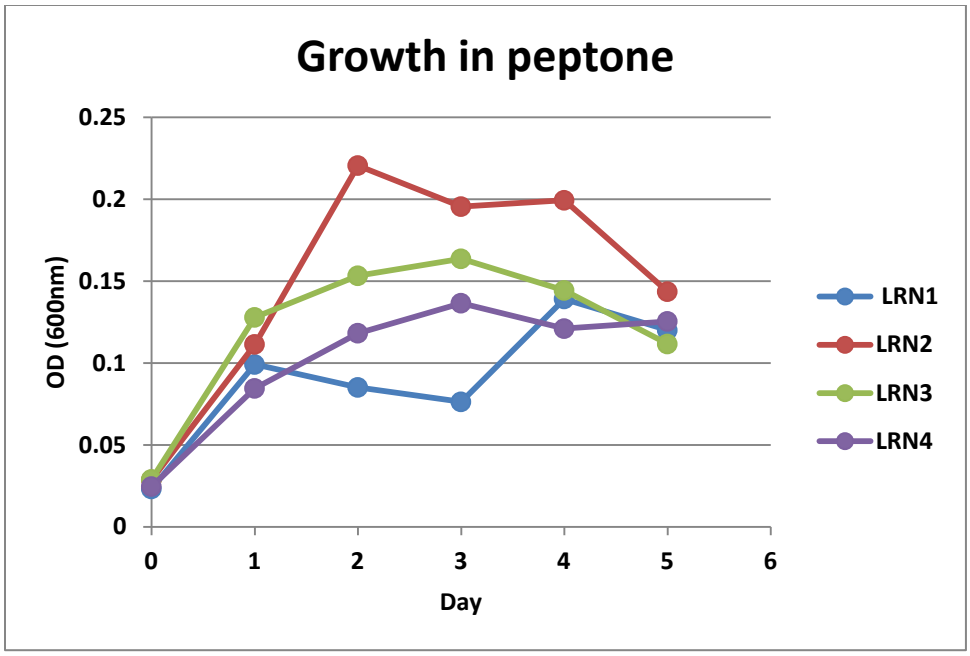

Fig. 4.10 Effect of peptone on four strains

Fig. 4.11 implies that the effect of peptone on lindane degradation, initially supplied 20 ppm lindane was degraded by 31.21 %, 20.11%, 52.68 % and 31.21 % respectively by the strains LRN1, LRN2, LRN3 and LNR4 by the end of 5th day of incubation. LRN3 strains showed maximum degradation of lindane than other strains.

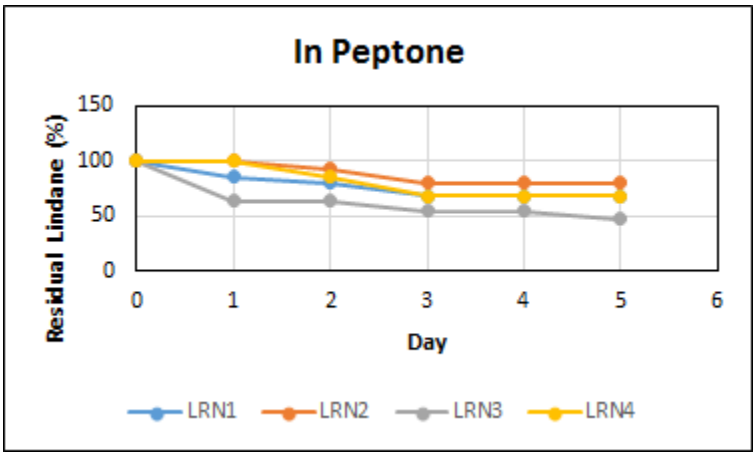

Fig. 4.11 Co-metabolism of lindane in presence of peptone by four stains

4.4 Effect of surfactant on lindane degradation

4.4.1 Triton X-100

In presence of triton X-100, growth of different strains were observed in 20 ppm of lindane that the growth of LRN1, LRN2, LRN3 and LRN4 strain increased with time till 48 h than it was decreased for 24 h while again it has increased for 120 h (Fig. 4.12). The growth pattern of all four strains was same to each-other. Maximum growth was observed in LRN3 bacterial strain than other strains. So this was a clear indication that triton X-100 was being utilized as a carbon source for the growth of all four strains.

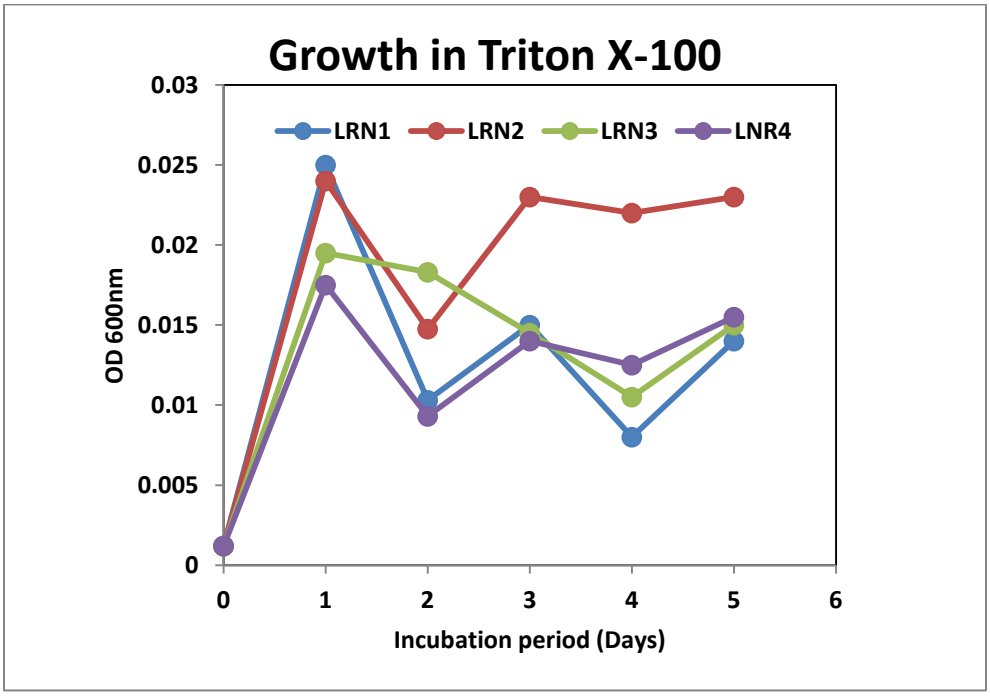

Fig. 4.12 Effect of Triton X-100 on four strains

In presence of triton X-100, initially supplied 20 ppm lindane was degraded by 67.4 %, 64.9 %, 77.6 %, 56.1 % respectively by the strains LRN1, LRN2, LRN3 and LNR4 by the end of 5th day of incubation (Fig. 4.13). It showed that maximum degradation of lindane in presence of triton X-100 surfactant observed in LRN3 bacterial strain than other strains.

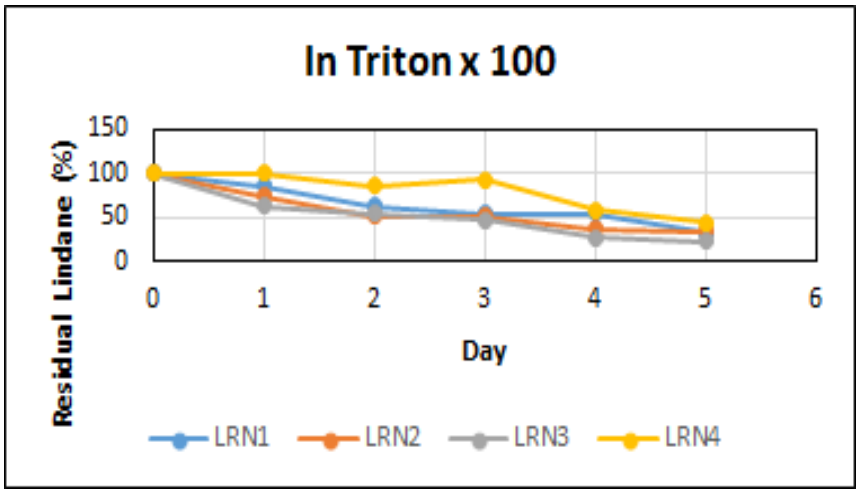

Fig 4.13 Co-metabolism of lindane in presence of Triton X-100 by four stains

4.4.2 Tween-80

In presence of tween-80, growth of different strains LRN1, LRN2, LRN3, LRN4 increased with time for 72 h and then decreased till 6th day of incubation while LRN1, LRN3 and LRN4 have increased end of the 7th day of incubation but LRN2 has decreased end of the 7th day of incubation at room temperature in shaking condition (Fig.4.14)

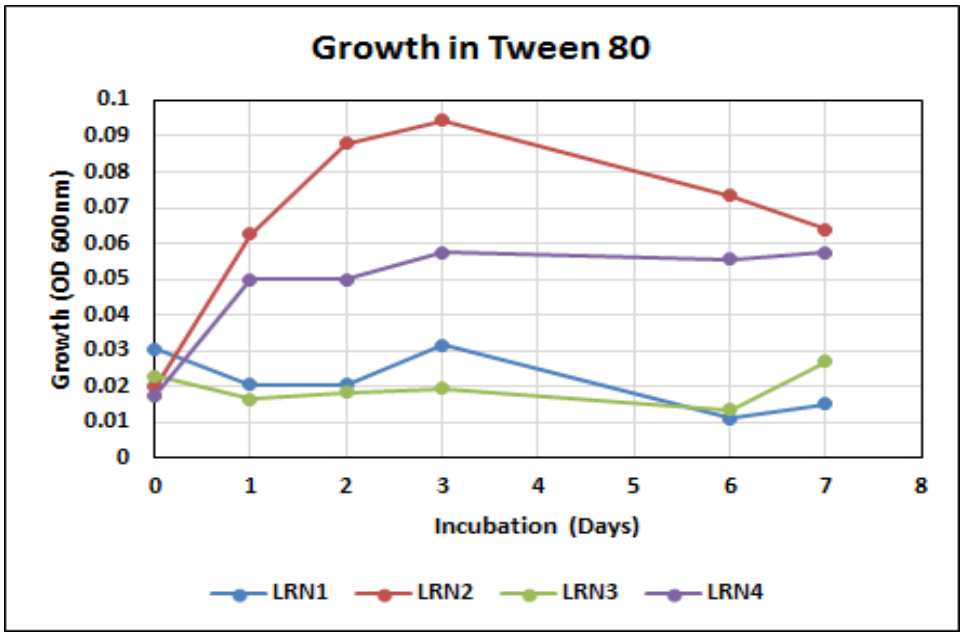

Fig. 4.14 Effect of Tween 80 on four strains

Fig. 4.15 implies that Tween-80 supplemented medium showed the degradation of lindane by 25.9 %, 25.9 %, 52.7 % and 36.2 % by the strains LRN1, LRN2, LRN3 and LNR4 by 7 days of incubation. Maximum degradation of lindane observed in LNR3 strain than others in presence of tween-80.

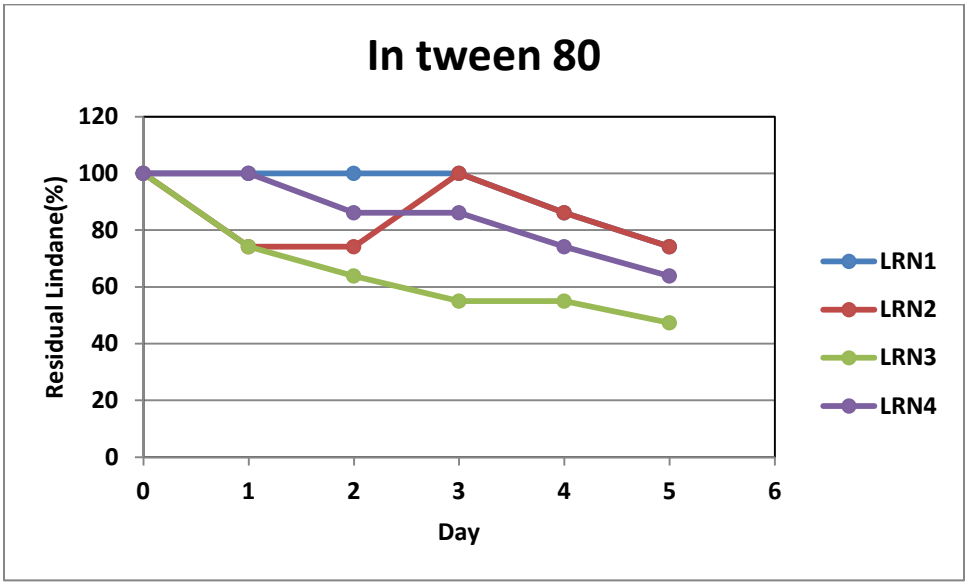

Fig 4.15 Co-metabolism of lindane in presence of Tween 80 by four stains

4.5 Gram staining

In Gram staining, observed that LRN1 showed the violet color cells in short rod shape arranging 2 cells together in parallel form and spores also present near cells. LRN2 showed pink color cells arrange in very short chains like rods (bacilli). LRN3 also showed pink color and shape was cocci. LRN4 also showed pink color and arrange in cocci form means round shape. LRN1 was gram positive cells while other three LRN2, LRN3 and LRN4 were gram negative cells.

Table 4.1 Represents Gram staining observation

Strains	Colors	Shape
LRN1	Voilet / purple	Short rods
LRN2	Pink	Very short rods
LRN3	Pink	cocci
LRN4	Pink	cocci

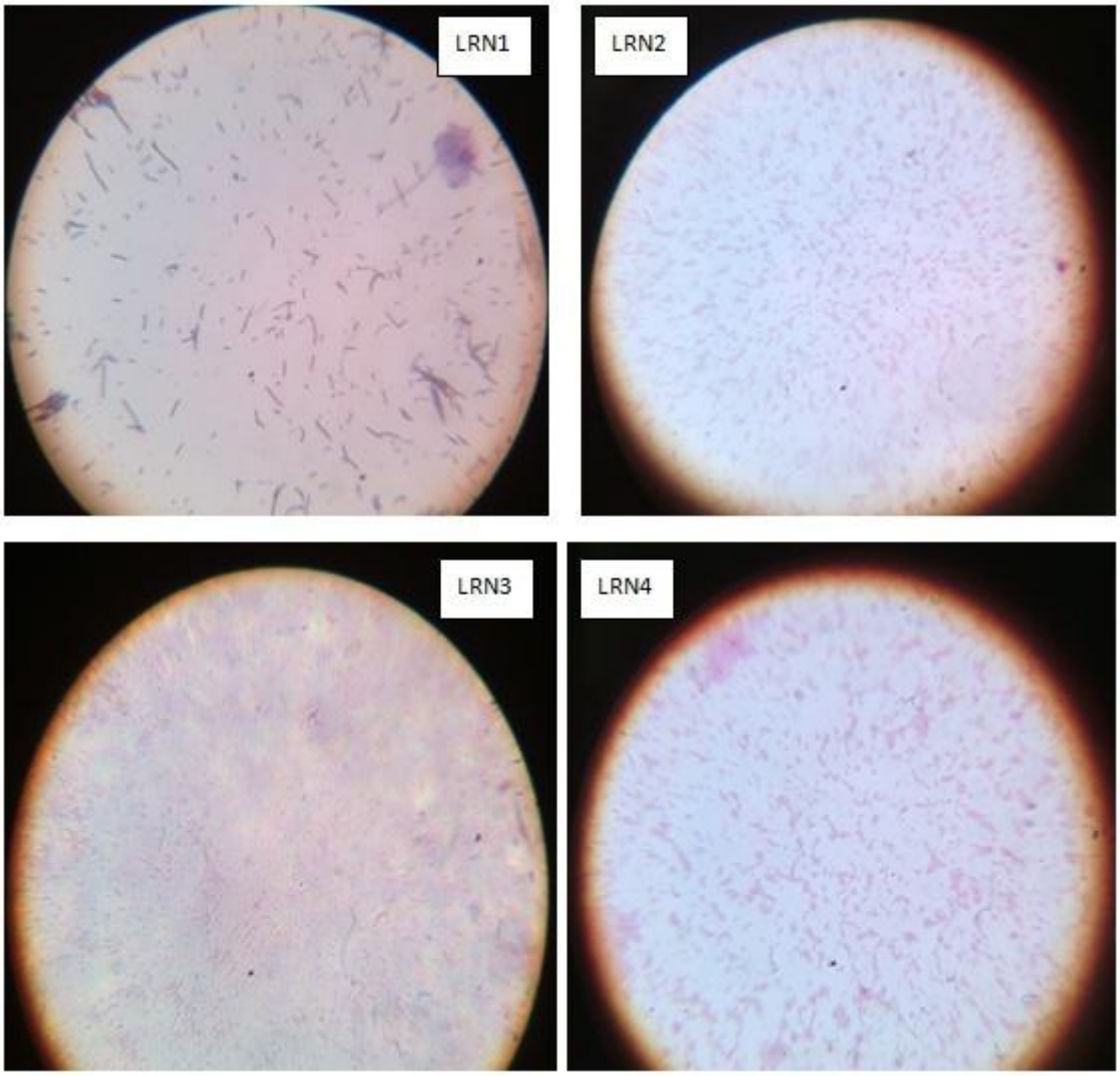

Fig. 4.16 Gram staining: Field view of various strains

4.6 Effect of different concentration of lindane on seed germination

Effect of different concentration of lindane on seed germination and development were studied. The lindane concentration is inversely propotional to development and germination of seed. With increasing concentration of lindane in soil mean root and shoot length of seedlings was reduced by 43.6 % , 29.6 %, 21.7 % and 18.1 % with respect to control in 5 ppm, 10ppm , 20ppm and 50 ppm respectively and shown in Fig. 4.17.

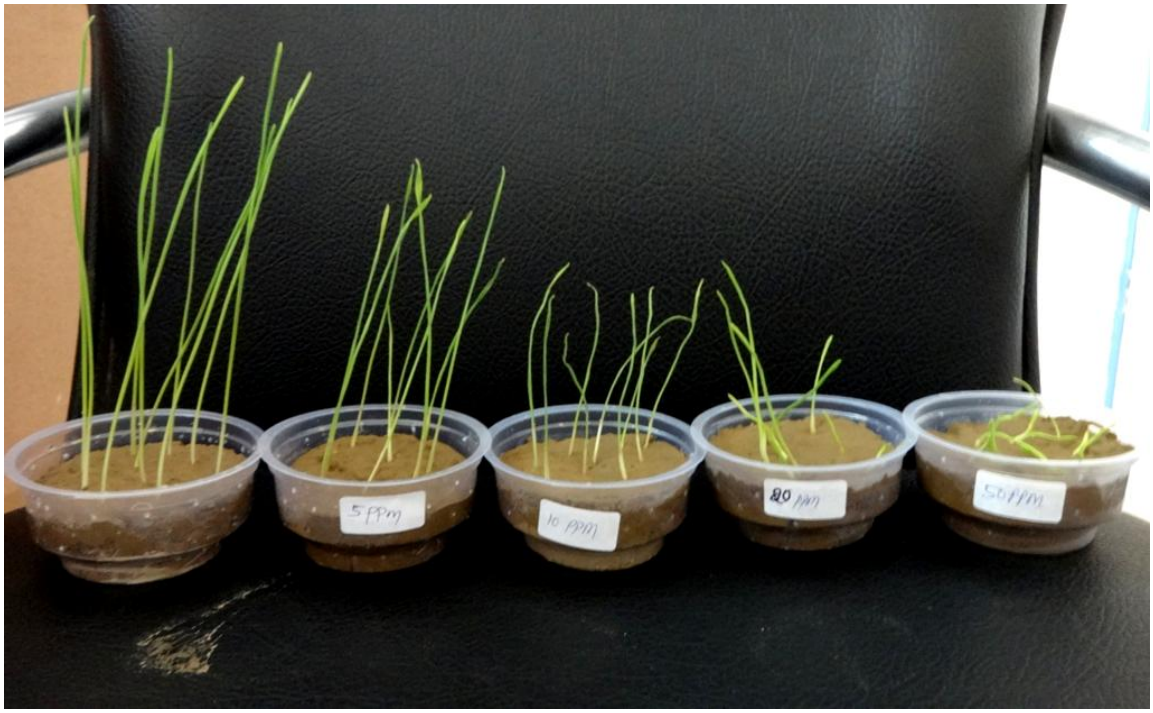

Fig 4.17 Effect of different concentration of lindane on seed development

Table 4.2 Effect of different concentration of lindane on seed germination (wheat seeds)

Concentration of Lindane (ppm)	Vigor Index (%)
Control	204.0095
5	43.57914
10	29.75478
20	21.72803
50	18.05827

Chapter 5

Discussion and future perspectives

Many studies revealed the health hazards of pesticides (Ming *et al*., 2013; Jensen and Slorach, 1991). We tried to investigate the negative effects of lindane on major food crop (wheat) in laboratory. We have found that mean shoot length decreased to 2.93 cm in 50 ppm lindane as compared to 10.2 cm in control. The corresponding mean root length were 0.86 cm and 10.64 cm indicating very heavy deleterious effect of increasing concentration of lindane on the development of wheat seedling even-though the percentage germination in 10, 20, and 50 ppm were very near to that of control (98%) while the percentage in 5 ppm was 84 % indicating heavy loss in the germination ability of seeds at low concentrations even though the mean root and shoot length were comparatively higher than the other concentrations. The vigor index reduced to 43.6 % , 29.6 %, 21.7 % and 18.1 % with respect to control in 5 ppm, 10ppm , 20ppm and 50 ppm respectively. Bidlan *et al*, (2004) have shown similar results in other food crops radish and green gram. Deepthi *et al*, (2005) have shown the toxic effect of DDT on certain crop seeds. Mitra and Raghu (1989) studied oil rich seed plants and showed the inhibitory effects on the germination of oil rich plants than cereals. This makes it imperative to develop strategies to combat the high levels of lindane through bioremediation. Usually the naturally occurring population of microorganisms cannot act on the synthetic compounds as they are not exposed to them earlier. Therefore the synthetic pesticides (including lindane) are persistent in the environment. The natural scavengers (microbes) with continuous exposure to a compound have the ability to change their genetic makeup and synthesise the required enzymes that can act upon the xenobiotic compounds thereby utilize it as a carbon source. This is usually done in the laboratory by classical enrichment technique. We have carried out the enrichment in the lab by exposing the natural population present in the sewage sludge to various commercial available synthetic pesticides with gradual increase in concentration over many months. Even though most of the organochlorine including HCH have been banned for agricultural use, their applications in past and in public health programmes at present makes these compounds easily accessible by bio-systems. The presence of high levels of lindane and other organochlorine pesticides in cow and buffalo milk have been detected in a research survey by Aslam *et al.* (2013). The presence of OCPs in the environment even after 28 yrs of ban in India is indicative of either very heavy use in the past or the illegal use by farmers and other stock bearers. Other pesticide classes are be used in different fields thereby giving good opportunity to the native microflora to degrade this new biodegradable pesticides leaving behind the already present organochlorine pesticides in the environment. We, with this view point, explode to enrich a population that is tolerant to very high concentrations of non-organochlorine pesticides and extended our

efforts to further screening of enriched populations for lindane tolerant strains. This double enrichment makes the possibilities of using our strains in the present scenario where the non-organochlorine pesticides exist in high concentrations due to their continuous application. We isolated morphologically four different strains of bacteria by enrichment technique and designated them as LRN1, LRN2, LRN3 and LRN4 respectively that could degrade 38.9 %, 27.5 %, 28.4 % and 44.6 % respectively the initially added 20 ppm lindane. RP-1 , RP-3, RP-9 strains isolated by enrichment of agricultural soil were shown to degrade around 70%, 65 % and 62% of 100ppm lindane in 10 days (Pannu and Kumar, 2014). Similarly microbes capable of degrading other insecticides like DDT have been enriched by other groups (Bidlan and Manonmani, 2002; Francis *et al* ., 1976 ; Johnson *et al*., 1967 ;Bumpus and Aust, 1987 ; Langlois *et al*., 1970 ; Chacko *et al*.,1966). Macksongsee and Guthrie (1965) isolated three strains of aerobactor aerogenes , baccilus serius and baccilus magatelium while Bachman *et al*., 1988 ; Shahu *et al*., 1990 isolated different pseudomonas species capable of degrading lindane. Bidlan *et al*. (2004) described 10 different bacteria that constituted a consortium that had efficient ability to degrade various isomer of HCH in soil. We screened our consortium for its ability to degrade lindane and observed that 68 – 82 % of the supplied lindane was degraded by end of 6^{th} days. 70% of 30 ppm lindane degradation by the consortium indicates the promise of its application at higher concentrations for treating contaminated water resources. Manonmani *et al*, (2000) described isolation of consortium to degrade α- HCH aerobically. Bidlan and Manonmani (2002) discussed the significance of induction before the treatment of contaminated water. We have also induced the consortium as well as the individual strains with 5ppm lindane for 72 h before using then as inocula. Acclimatization seems to play vital role in bioremediation of xenobiotics (Bidlan and Manonnmani,2002 ; Bidlan *et al*., 2004 ; Manonmani *et al*.,2000). Bidlan (2003) described co-metabolic degradation of DDT in shake flasks wherein four different strains of bacteria were studied for DDT degradation in presence of 9 different co-substrates. In our laboratory we have studied the effect of 4 different substrates on the degradation of lindane by individual strains LRN1, LRN2, LRN3 and LRN4. LRN1 degraded 48.99 % , 31.21 %, 67.4 % and 25.9 % degradation of 20 ppm lindane in 5 days in presence of glucose, peptone, triton X-100 and tween-80 (Fig. 5.1). LRN2 degraded 77.61 %, 20.11%, 64.9 % and 25.9 % degradation of 20 ppm lindane in 5 days in presence of glucose, peptone, triton X-100 and tween-80 (Fig. 5.2). LRN3 degraded 83.4 %, 52.68 %, 77.6 % and 52.7 % degradation of 20 ppm lindane in 5 days in presence of glucose, peptone, triton X-100 and tween-80 (Fig. 5.3). LRN4 degraded 83.4 % , 31.1 %, 56.1 % and 36.2 % degradation of 20 ppm lindane in presence of glucose, peptone, triton X 100 and tween-80 (Fig. 5.4).

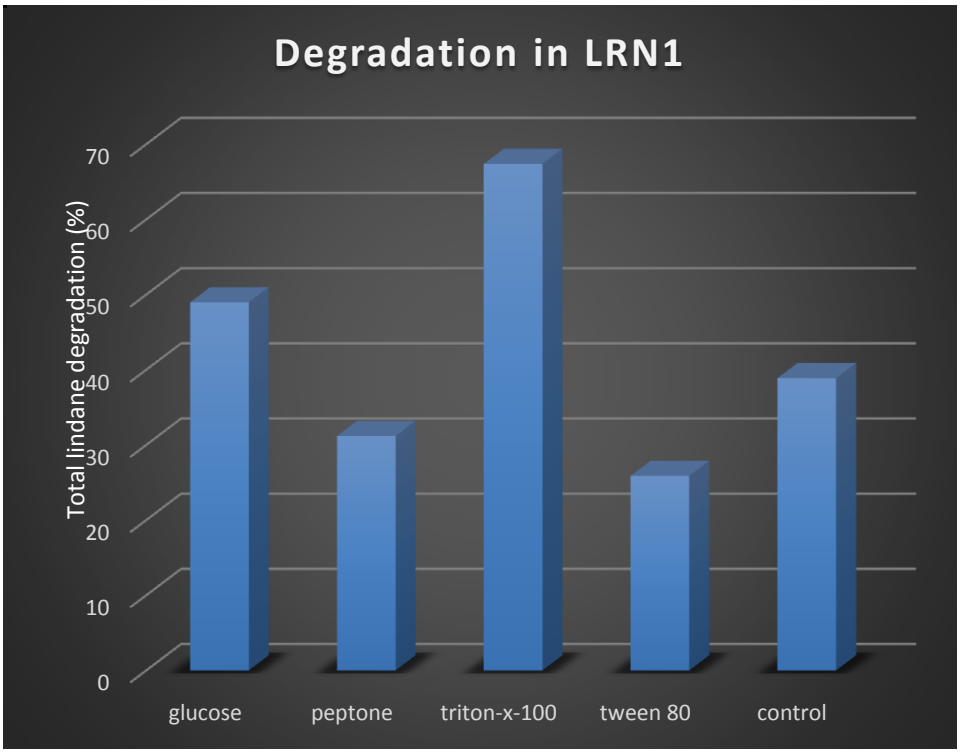

Fig 5.1 Comparitive chart of lindane degradation by different substrates for LRN1 strain

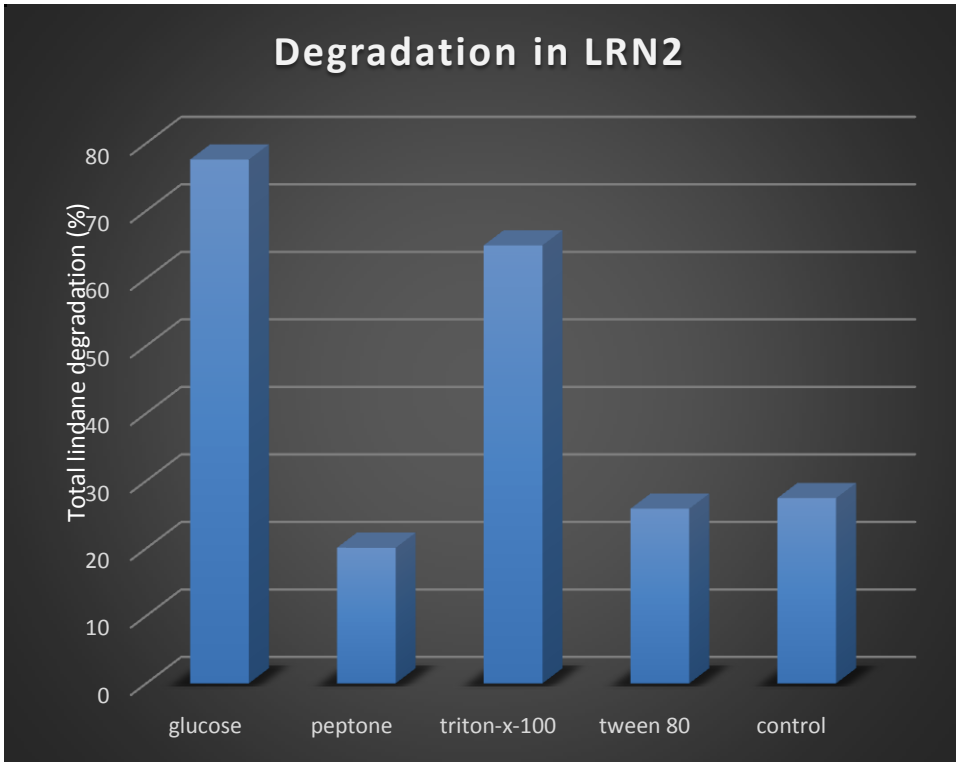

Fig 5.2 Comparitive chart of lindane degradation by different substrates for LRN2 strain

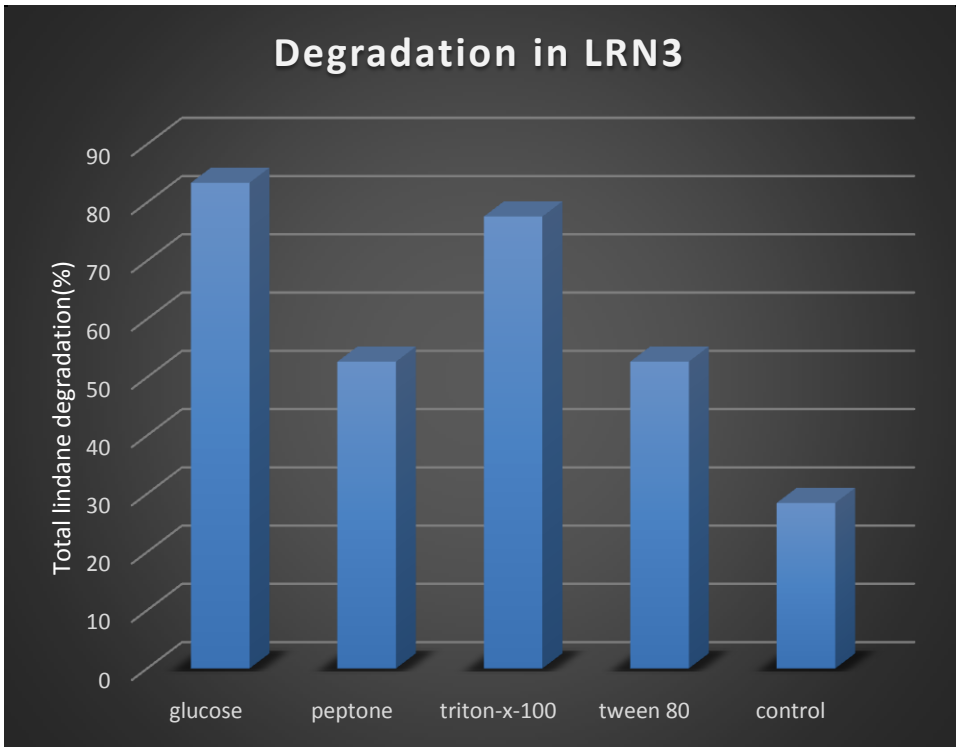

Fig 5.3 Comparitive chart of lindane degradation by different substrates for LRN3 strain

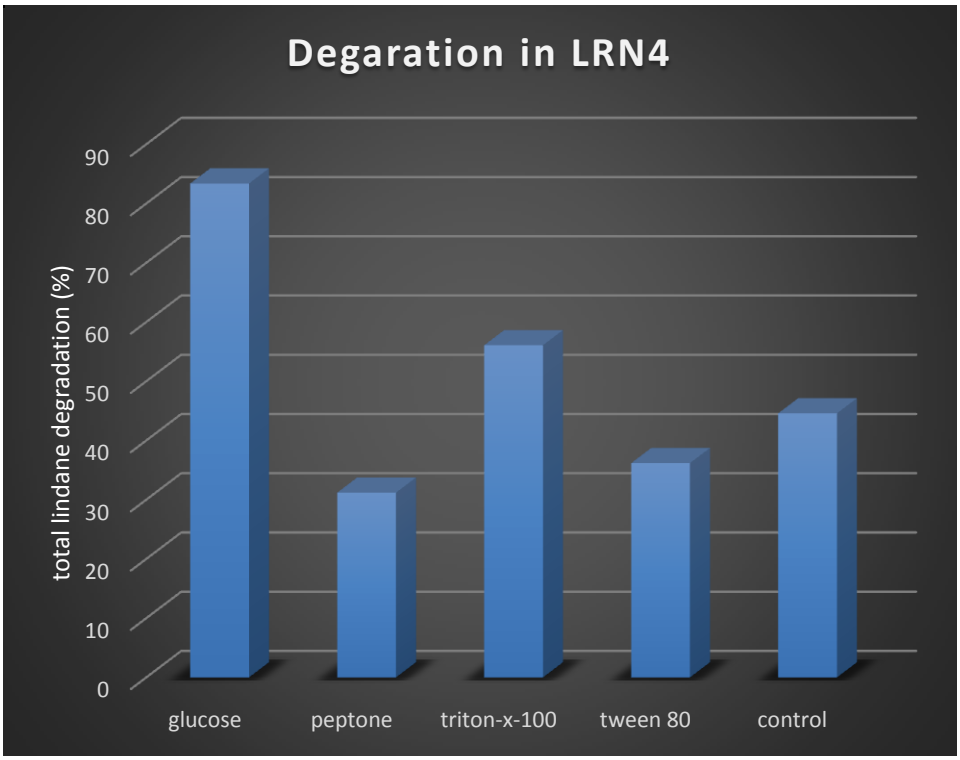

Fig 5.4 Comparitive chart of lindane degradation by different substrates for LRN4 strain

We found that peptone, though a very rich source of nutrients, could not contribute towards degradation of lindane as compare to glucose. LRN3 and LRN4 showed very high co-metabolic activity in presence of glucose while it was comparable with LRN2. Triton X-100 was the most efficient co-metabolic degrader of lindane amongst all the substrate used irrespective of the strains. Peptone and twee-80 reduced the apparent degradation percentages in LRN1, LRN2 and LRN4 while LRN3 showed enhanced degradation in presence of all the co-substrate study. Bidlan (2003) has also observed similar results with different substrates with DDT. Maria *et al*, (2011) observed that three or four strains constituted as a consortium that gave best result 60-80 % degradation of lindane while more than four strains used as a consortium shown poor efficiency than three to four strains. So we can say that we can use our four strains constituted as a consortium for better efficiency of the degradation of the lindane in future.

Chapter 6

Conclusion

Every problem always comes with a solution in nature. Lindane, being very effective pesticide, has brought lot of environmental and health concerns. Through our study, we can conclude that the consortium with ability to degrade lindane can be used for bioremediation applications. The four strains *viz.* LRN1, LRN2, LRN3 and LRN4 were able to individually act on lindane co-metabolically thereby reducing the initially added concentration to approximately 80% in a very short period of 5 days under aerobic conditions. Co-metabolism is the best applicable bioremediation methods and the use of consortium is a promising approach .Our 4 strains may be constituted into a defined consortium and applied to the contaminated sites for efficient treatment. Further studies need to be conducted to optimize the conditions and investigate the enhanced efficiency of these four strains individually and in combinations to develop best remediation technologies.

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