

CONTENTS

Cł	IAPTER	S	page no.
LI	ST OF T	ABLES	Ĭ
LI	ST OF F	IGURES	ii - iv
LI	ST OF A	BBREVIATIONS	v - vi
Ał	BSTRAC	Г	1 - 2
RI	EVIEW C	OF LITERATURE	3-46
Tł	IE PRES	ENT STUDY	47-48
1.	Chapte	er:	49-76
	Isolatic	on and identification of γ -HCH degrading bacteria	
1.1	Introdu	action	50-51
1.2	2 Materia	als and Methods	52-60
	1.2.1	Soil sampling	
	1.2.2	Chemicals and reagents	
	1.2.3	Media used for isolation, screening and analysis of γ -HG	CH degrading
		bacteria	
	1.2.4	Isolation of γ -HCH degrading bacteria from agricultural soil sa	mples
	1.2.5	Isolation of y-HCH degrading bacteria from Industrial site soil	Samples
		a. Isolation of bacteria from soil samples without any en	richment
		b. Enrichment and isolation of γ-HCH degrading	bacteria from
		industrial site soil	
	1.2.6	Preliminary screening for putative γ -HCH degrading bacteria	
	1.2.7	Identification of γ -HCH biodegrader by Biolog system	
	1.2.8	Identification of γ -HCH degrading bacteria based on 16S rDN	IA analysis
		a. Genomic DNA extraction and PCR amplification of 1	6S rRNA
		gene	
			2

b. Sequencing, identification and Phylogenetic analysis

1.3 Results and discussion

61-76

78-79

80-87

- 1.3.1 Isolation and characterization of γ -HCH degrading bacteria from the agriculture soil samples
- 1.3.2 Isolation, identification and confirmation of γ -HCH degrading bactria from the supernatant of the industrial soil samples
- 1.3.3 Isolation and confirmation of γ -HCH degrading bacteria from enrichment culture of industrial soil

 2. Chapter :
 77-102

 Characterisation of γ-hexachlorocyclohexane degrading bacterium Shewanella

 sp. CGR-L1

2.1 Introduction

2.2 Materials and methods

- 2.2.1 Soil analysis
- 2.2.2 Protein Extraction from *Shewanella* sp. CGR-L1
- 2.2.3 Dehalogenase activity assay for the isolated *Shewanella* sp. CGR-L1
- 2.2.4 Antibiotic assay
- 2.2.5 Growth kinetics study of Shewanella sp. CGR-L1
- 2.2.6 Analytic techniques
- 2.2.7 GC-MS analysis for identification of metabolites
- 2.2.8 Identification of metabolites produced in Cell-free extract assay by GC-MS
- 2.2.9 PCR amplification of the *lin*A
- 2.2.10 Plasmid curing from the Shewanella sp. CGR-L1
- 2.2.11 Southern analysis of *lin* genes

•						
	2.2.12	Tolerance of γ -HCH by iso	late <i>Shewanella</i> s	p. CGR-	L1	

2.3 Result	s and Discussion	88-102
2.3.1	Soil analysis	
2.3.2	Dehalogenase activity assay for the isolated Shewanella sp.	CGR-L1
2.3.3	Growth kinetics of Shewanella sp. CGR-L1	
2.3.4	Identification of the metabolites produced during	g the γ-HCH
	biodegradation by Shewanella sp. CGR-L1	
2.3.5	PCR amplification of <i>linA</i> and its sequence identity	
2.3.6	Localization of catabolic properties and plasmid curing f	rom Shewanella
· ·	sp.CGR-L1	
2.3.7	Tolerance of γ -HCH by isolate Shewanella CGR-L1	
3. Chapte	Δ Υ*	103-148
	duction rials and methods	104
3.2 Mater	rials and methods	104 105-113
3.2 Mater 3.2.1	rials and methods Soil analysis, Dehalogenase and antibiotic sensitivity assays	104 105-113 s
3.2 Mater3.2.13.2.2	rials and methods Soil analysis, Dehalogenase and antibiotic sensitivity assay Growth kinetics of CGR-L2 in MSM-L2 in presence of γ -H	105-113 s
 3.2 Mater 3.2.1 3.2.2 3.2.3 	rials and methods Soil analysis, Dehalogenase and antibiotic sensitivity assay Growth kinetics of CGR-L2 in MSM-L2 in presence of γ -H Analysis of γ -HCH biodegradation by CGR-L2	104 105-113 s ICH
3.2 Mater 3.2.1 3.2.2 3.2.3 3.2.4	rials and methods Soil analysis, Dehalogenase and antibiotic sensitivity assay: Growth kinetics of CGR-L2 in MSM-L2 in presence of γ -H Analysis of γ -HCH biodegradation by CGR-L2 Dechlorination assay for CI ⁻ (chloride) estimation from γ -H	104 105-113 s ICH ICH degradation
 3.2 Mater 3.2.1 3.2.2 3.2.3 	rials and methods Soil analysis, Dehalogenase and antibiotic sensitivity assay: Growth kinetics of CGR-L2 in MSM-L2 in presence of γ -H Analysis of γ -HCH biodegradation by CGR-L2 Dechlorination assay for CI ⁻ (chloride) estimation from γ -H Effect of various Parameter on γ -HCH biodegradation by I	104 105-113 s ICH ICH degradation
3.2 Mater 3.2.1 3.2.2 3.2.3 3.2.4	rials and methods Soil analysis, Dehalogenase and antibiotic sensitivity assays Growth kinetics of CGR-L2 in MSM-L2 in presence of γ -H Analysis of γ -HCH biodegradation by CGR-L2 Dechlorination assay for CI ⁻ (chloride) estimation from γ -H Effect of various Parameter on γ -HCH biodegradation by I MSM-L2	104 105-113 s ICH ICH degradation
3.2 Mater 3.2.1 3.2.2 3.2.3 3.2.4	 rials and methods Soil analysis, Dehalogenase and antibiotic sensitivity assays Growth kinetics of CGR-L2 in MSM-L2 in presence of γ-H Analysis of γ-HCH biodegradation by CGR-L2 Dechlorination assay for Cl⁻ (chloride) estimation from γ-H Effect of various Parameter on γ-HCH biodegradation by I MSM-L2 a. Effect of inoculum size 	104 105-113 s ICH ICH degradation solate CGR-L2
3.2 Mater 3.2.1 3.2.2 3.2.3 3.2.4	 rials and methods Soil analysis, Dehalogenase and antibiotic sensitivity assays Growth kinetics of CGR-L2 in MSM-L2 in presence of γ-H Analysis of γ-HCH biodegradation by CGR-L2 Dechlorination assay for Cl⁻ (chloride) estimation from γ-H Effect of various Parameter on γ-HCH biodegradation by I MSM-L2 a. Effect of inoculum size b. Carbon utilization profile of <i>Sphingobium</i> sp. CGH 	104 105-113 s ICH ICH degradation solate CGR-L2
3.2 Mater 3.2.1 3.2.2 3.2.3 3.2.4	 rials and methods Soil analysis, Dehalogenase and antibiotic sensitivity assays Growth kinetics of CGR-L2 in MSM-L2 in presence of γ-H Analysis of γ-HCH biodegradation by CGR-L2 Dechlorination assay for Cl⁻ (chloride) estimation from γ-H Effect of various Parameter on γ-HCH biodegradation by I MSM-L2 a. Effect of inoculum size b. Carbon utilization profile of <i>Sphingobium</i> sp. CGH c. Effect of supplementary carbon source (glucose) 	104 105-113 s ICH ICH degradation solate CGR-L2
3.2 Mater 3.2.1 3.2.2 3.2.3 3.2.4	 rials and methods Soil analysis, Dehalogenase and antibiotic sensitivity assay: Growth kinetics of CGR-L2 in MSM-L2 in presence of γ-H Analysis of γ-HCH biodegradation by CGR-L2 Dechlorination assay for Cl⁻ (chloride) estimation from γ-H Effect of various Parameter on γ-HCH biodegradation by I MSM-L2 a. Effect of inoculum size b. Carbon utilization profile of <i>Sphingobium</i> sp. CGH c. Effect of supplementary carbon source (glucose) d. Effect of pH 	104 105-113 s ICH ICH degradation solate CGR-L2
3.2 Mater 3.2.1 3.2.2 3.2.3 3.2.4	 rials and methods Soil analysis, Dehalogenase and antibiotic sensitivity assays Growth kinetics of CGR-L2 in MSM-L2 in presence of γ-H Analysis of γ-HCH biodegradation by CGR-L2 Dechlorination assay for Cl⁻ (chloride) estimation from γ-H Effect of various Parameter on γ-HCH biodegradation by I MSM-L2 a. Effect of inoculum size b. Carbon utilization profile of <i>Sphingobium</i> sp. CGH c. Effect of supplementary carbon source (glucose) 	104 105-113 s ICH ICH degradation solate CGR-L2

- 3.2.7 GC-MS analysis for identification of metabolites produced during growth of *Sphingobium* sp. CGR-L2
- 3.2.8 Analysis of technical HCH degradation by CGR-L2
- 3.2.9 Identification of γ -HCH tolerance level of CGR-L2
- 3.2.10 Primer designing, PCR amplification of *lin* genes and its
- 3.2.11 Southern-blot analysis
- 3.2.12 Analysis of biodegradation of Hydroquinone
- 3.2.13 Bioremediation Technical HCH degradation in soil
 - a. Microcosm studies
 - b. Analysis of residual concentration of technical HCH in microcosm study
 - c. Growth of CGR-L2 bacteria in microcosm study

3.3 Results and discussion

114-148

- 3.3.1 Soil analysis, dehalogenase and antibiotics assay
- 3.3.2 Growth curve and γ-HCH degradation analysis
- 3.3.3 Free chloride analysis
- 3.3.4 Effect of various Parameter on γ-HCH biodegradation by Isolate CGR-L2 in MSM-L2
 - a. Effect of inoculum size
 - b. Carbon utilization profile of Sphingobium sp. CGR-L2
 - c. Effect of supplementary carbon source (glucose)
 - d. Effect of pH
 - e. Effect of temperature
- 3.3.5 Optimization of γ-HCH biodegradation and GC-MS analysis for identification of metabolites
- 3.3.6 Analysis of technical HCH degradation by CGR-L2
- 3.3.7 Identification of γ -HCH tolerance level of CGR-L2 in 1/3LB
- 3.3.8 PCR amplification of *lin* genes and its homology

- 3.3.9 Confirmation of presence of *lin* genes in *Sphingobium* sp. CGR-L2 by southern analysis
- 3.3.10 Analysis of biodegradation of Hydroquinone
- 3.3.11 Bioremediation study: Technical HCH degradation studies in soil
- 3.3.12 PCR amplification of the *lin* genes from other bacterial isolates of enrichment culture

Summary

References

149-151

i-xxix