## Contents

Section No. Acknowledgments		Page No I
Contents		V
Acronyms		Ix
List of Figure		Х
List of '	List of Table	
Chapt	er 1 Introduction and Literature review	22-35
1.1	History of Benzene	1
1.2	Natural and industrial sources of benzene	3
1.3	Exposure and toxicity of benzene into the environment	3
1.4	Health Effect	4
1.5	Bioremediation	5
1.6	Bioremediation technology	7
1.6.1	In-situ bioremediation	8
1.6.2	Types of In situ bioremediation	8
	Bioattenuation	8
	Biostimulation	8
	Bioaugmentation	8
	Bioventing	9
	Biosparging	9

1.7	Ex Situ Treatment Processes	9
	Land Treatment	9
	Composting	9
	Biopiles	9
1.8	Bioreactor used for the treatment of benzene	10
1.9	Types of the bioreactor	11
1.9.1	Fibrous bed bioreactor	11
1.9.2	Two-phase partitioning bioreactor (TPPB)	12
1.10	Packing media used in bioreactor	13
1.10.1	Polyurethane foam	14
1.10.2	PVA/Sodium alginate	14
1.11	Affect of processes Parameters like pH, temperature, dissolve	15
	oxygen and nutrients affected the bioremediation benzene in	
1.12	wastewater Literature survey on bioremediation of benzene	16
1.13	Findings of the literature survey	23
1.14	Research objective	24
Chapter 2 Materials and methods		25
2.1	Material	26
2.1.1	Chemicals and reagents	26
2.1.2	Materials and equipments	28
2.1.3	Glass wares	28
2.1.4	Packing material: PUF and alginate beads	28
2.1.5	Packed bed bioreactor (PBBR)	30

2.2	Methods	31
2.2.1	Site description and soil collection	31
2.2.2	Isolation and enrichment	32
2.2.3	Molecular characterization of bacterium isolate	34
2.2.4	Biochemical test for bacterial isolates	35
2.2.5	Batch study	36
2.2.5.1	Optimization of process parameters, bacterial growth and	36
	biodegradation of benzene in free cell	
2.2.6	Bioreactor study	37
2.2.7	Biodegradation kinetics	37
2.2.8	Continuous study	38
2.2.8.1	Optimization of process parameter in batch bioreactor	38
	immobilization experiment	
2.2.9	Packing material and adsorption studies	38
2.2.10	Continuous packed bed bioreactor: startup, operation and	39
	performance evaluation	
2.2.11	SEM, FT-IR, GC AND GC-MS ANALYSIS	43
2.2.12	Proteomic study: extraction, quantification and 2-Dgel	44
	electrophoresis	
2.2.13	Protein digestion and identification by MALDI-TOF MS/MS	45
2.2.14	In silico analysis and characterization of identified proteins	47

## Chapter 3 Results and discussion Section A: Batch Study

3.1	Isolation and characterization (morphological, biochemical and	49		
	molecular16S-rRNA)			
3.2	Optimization of process parameters and screening of potential	51		
	microbial species for biodegradation of benzene			
3.3	Comparing the performance of free cell vs. immobilized packed	52		
	(alginate and PUF) bed bioreactor for degradation of Benzene			
3.4	SEM, FT-IR and GC/MS analysis	56		
3.5	Growth and inhibition kinetics for biodegradation of benzene	60		
Section B Continuous Study				
3.6	Biochemical and molecular characterization (16s rRNA) of	66		
	bacterial isolate			
3.7	Batch bioreactor immobilization study	67		
3.8	Continuous packed bed bioreactor: performance evaluation	69		
3.9	Residual analysis: GC, FT-IR and GC-MS	73		
3.10	Proposed metabolic pathway for benzene biodegradation	78		
3.11	2-D gel electrophoresis and MALDI-TOF MS/MS analysis	80		
3.12	In silico analysis and characterization of identified protein	82		
3.13	Active site analysis and Docking	88		
3.14	Structure predictions of other identified proteins	92		
4	Conclusions	95		
	References	97		
	Appendix	116		