

Contents

List of Abbreviations.....	vii
List of Figures.....	xi
List of Tables.....	xiii
ABSTRACT.....	xv
Chapter I: INTRODUCTION	1
1.1 General	3
1.2 Arsenic.....	3
1.3 Sources and distribution	4
1.4 Mobility of arsenic	5
1.5 Health effects of arsenic	6
1.6 Current state of arsenic contamination.....	8
1.6.1 Global scenario	8
1.6.2 National scenario for India	9
1.7 Technologies for arsenic removal.....	10
1.8 Co-contamination of arsenic and chromium	13
1.9 Bioremediation	17
1.10 Factors affecting bioremediation process.....	19
1.10.1 pH	20
1.10.2 Temperature	21
1.10.3 Metal(loid)s and toxic compounds	21
1.10.4 Water content and geological characteristics.....	21
1.10.5 Nutrient availability.....	22
1.10.6 External electron availability.....	22
1.10.7 Bioavailability of pollutants	22
1.10.8 Co-metabolism	22

1.10.9 Gene expression.....	22
1.11 Organization of the Thesis.....	23
Chapter II: LITERATURE REVIEW	25
2.1 General	27
2.2 Bioremediation of arsenic	27
2.2.1 Algae.....	27
2.2.2 Bacteria.....	29
2.2.3 Fungi and yeast.....	29
2.2.4 Phytoremediation.....	29
2.3 Arsenic removal using bacteria	31
2.3.1 Arsenic uptake and efflux pathways.....	31
2.3.2 Genes involved in arsenic metabolism.....	33
2.3.3 Arsenic metabolism	34
2.3.3.1 Oxidation	36
2.3.3.2 Reduction	37
2.3.3.3 Methylation and demethylation	38
2.3.4 Genetically modified bacteria.....	39
2.4 Use of immobilized bacteria for arsenic removal	40
2.5 Bacterial removal of arsenic from soil	44
2.6 Research gaps	45
2.7 Aims and objectives	45
Chapter III: MATERIALS AND METHODS.....	47
3.1 Chemicals and media	49
3.1.1 Chemicals	49
3.1.2 Media.....	49
3.1.2.1 Luria Bertani media	49
3.1.2.2 Luria Bertani agar media	49

3.2 Analytical techniques and instruments	49
3.2.1 Determination of arsenic	49
3.2.1.1 Molybdenum blue method	49
3.2.1.2 Arsenator	50
3.2.2 Determination of chromium	50
3.2.2.1 Diphenylcarbazide method	50
3.2.3 UV-visible spectrophotometry	51
3.2.4 Preparation of calibration curve	51
3.2.4.1 Arsenic	51
3.2.4.2 Chromium	51
3.2.5 Polymerase chain reaction	52
3.2.6 Characterization	52
3.3 Methods	53
3.3.1 Sample collection	53
3.3.2 Enrichment and isolation of arsenic-resistant bacteria	54
3.3.3 Identification of the isolated bacterial strains and phylogenetic analysis	54
3.3.4 Determination of minimum inhibitory concentration	55
3.3.5 Parameter optimization for bacterial growth	56
3.3.5.1 Effect of pH and temperature	56
3.3.5.2 Effect of initial arsenic concentration and inoculum dose	56
3.3.6 Determination of biotransformation ability of bacteria	56
3.3.7 Resistance of bacteria against other heavy metal(loid)s	57
3.3.8 Phytotoxicity assessment	57
3.3.9 Recirculating packed bed bioreactor	58
3.3.10 Immobilization of bacteria and operating conditions of RPBBR	58
3.3.11 Bacterial toxicity assessment	59
3.3.12 Soil sample preparation for the study	59

3.3.13 Extraction of arsenic and chromium from soil	60
3.3.14 Extraction of arsenic and chromium from plant	61
3.3.15 Plant growth and chlorophyll assessment	61
3.3.16 Preparation and modification of peanut shell biochar	62
3.3.17 Effects of parameters on arsenic removal efficiency of the biochar	62
3.3.18 Adsorption isotherms and kinetic studies	63
3.3.19 Immobilization of bacteria on biochar and preparation of beads	64
3.3.20 Effect of parameters on arsenic removal efficiency of beads	64
3.3.21 Water absorption and mechanical strength of beads	64
3.3.22 Arsenic removal in a recirculating packed bed bioreactor using beads	65
Chapter IV: ISOLATION AND IDENTIFICATION OF ARSENIC RESISTANT BACTERIA	67
4.1 General	69
4.2 16S rDNA amplification and phylogenetic analysis	69
4.3 Bacterial resistance against arsenic	70
4.4 Effect of initial arsenic concentration and inoculum dose on bacterial growth	72
4.5 Effect of pH and temperature on bacterial growth	72
4.6 Biotransformation and bioaccumulation ability of bacteria and genes involved in biotransformation of arsenic	74
4.7 Resistance of bacteria against other heavy metal(loid)s	76
4.8 Phytotoxicity assessment	77
4.9 Summary	78
Chapter V: BIOREMEDIATION OF ARSENIC IN PACKED BED BIOREACTOR	79
5.1 General	81
5.2 Working conditions of the reactor	81
5.3 Effect of flow rate and initial arsenic concentration on removal efficiency	82
5.4 Biotransformation of arsenic by bacteria	83

5.5 Characterization	83
5.6 Batch and column study: Comparison of arsenic removal efficiency	88
5.7 Toxicity assessment.....	90
5.8 Disposal of immobilized arsenic	90
5.9 Summary	92
Chapter VI: SIMULTANEOUS BIOREMEDIATION OF ARSENIC AND CHROMIUM(VI) FROM CONTAMINATED SOIL	93
6.1 General	95
6.2 Effect of initial concentration on removal efficiency.....	95
6.3 Effect of chromium on arsenic removal efficiency	98
6.4 Effect of extraction medium on removal of arsenic and chromium.....	99
6.5 Accumulation of arsenic and chromium in plants	100
6.6 Plant growth and chlorophyll assessment	101
6.7 Summary	105
Chapter VII: ARSENIC REMOVAL USING IMMOBILIZED AND ENCAPSULATED BACTERIA	107
7.1 General	109
7.2 Characterization of biochar	109
7.3 Thermogravimetric analysis.....	110
7.4 Effect of operating parameters on removal efficiency of biochar	111
7.5 Adsorption isotherm	113
7.6 Kinetic modeling	114
7.7 Thermodynamic study	115
7.8 Adsorption mechanism of arsenic on biochar	117
7.9 Regeneration of biochar	118
7.10 Disposal of spent biochar	119
7.11 Effect of PVA, SA and CaCl ₂ content on bead formation and arsenic removal efficiency.....	119

7.12 Effect of temperature and pH on efficiency of arsenic removal	120
7.13 Effect of initial arsenic concentration	123
7.14 Comparison of removal efficiencies of various combinations	123
7.15 Characterization of beads	125
7.16 Water absorption capacity and mechanical strength of beads	126
7.17 Reusability of the beads	126
7.18 Arsenic removal in packed bed bioreactor using beads	128
7.19 Summary	131
Chapter VIII: CONCLUSIONS AND SUGGESTIONS	133
8.1 Concluding remarks	135
8.2 Limitations of the present work	136
8.3 Scope for further work	136
REFERENCES.....	139
Appendix A.....	187
Appendix B.....	190
Appendix C.....	198
Appendix D.....	200
Appendix E.....	206
PUBLICATIONS	209