Studies on Removal of Heavy Metals from Wastewater using Biofiltration

THESIS

Submitted in partial fulfillment
of the requirements for the degree of
DOCTOR OF PHILOSOPHY

by

SUBHAJIT MAJUMDER

Under the
Supervision of
Prof. Suresh Gupta
and
Co-Supervision of
Dr. Smita Raghuvanshi

BITS Pilani
Pilani | Dubai | Goa | Hyderabad

BIRLA INSTITUTE OF TECHNOLOGY AND SCIENCE, PILANI
2015

Studies on Removal of Heavy Metals from Wastewater using Biofiltration

THESIS

Submitted in partial fulfillment
of the requirements for the degree of

DOCTOR OF PHILOSOPHY

by

SUBHAJIT MAJUMDER

(2009PHXF440P)

Under the

Supervision of

Prof. Suresh Gupta

and

Co-Supervision of

Dr. Smita Raghuvanshi

BITS Pilani
Pilani | Dubai | Goa | Hyderabad

BIRLA INSTITUTE OF TECHNOLOGY AND SCIENCE, PILANI

2015

BIRLA INSTITUTE OF TECHNOLOGY & SCIENCE

PILANI - 333031 (RAJASTHAN) INDIA

CERTIFICATE

This is to certify that the thesis entitled **"Studies on Removal of Heavy Metals from Wastewater using Biofiltration"** and submitted by **Subhajit Majumder**, ID. No. **2009PHXF440P** for the award of PhD Degree of the Institute embodies the original work done by him under our supervision.

Signature of the Supervisor _____

Name in capital letters **PROF. SURESH GUPTA**

Designation Associate Professor and Head
Department of Chemical Engineering
BITS-Pilani, Pilani Campus, India

Date:

Signature of the Co-Supervisor _____

Name in capital letters **DR. SMITA RAGHUVANSHI**

Designation Assistant Professor
Department of Chemical Engineering
BITS-Pilani, Pilani Campus, India

Date:

DEDICATED

TO

MY PARENTS

ACKNOWLEDGEMENTS

It gives me a deep sense of gratitude and immense pleasure to sincerely thank my supervisor **Prof. Suresh Gupta**, Associate Professor and Head, Department of Chemical Engineering for taking me under his able supervision for Ph.D. I sincerely thank him for his consistent encouragement, constructive advice, scholarly inputs, innovative suggestions during the entire period of this work. A person with an amicable and positive disposition, Sir has always made himself available to clarify my doubts despite his busy schedules and I consider it as a great opportunity to do my doctoral programme under his guidance. This feat was possible only because of the unconditional support provided by Sir. I also thank him for providing the necessary supports and facilities to carry out the research work at Department of Chemical Engineering.

I would like to express my appreciation and thanks to my co-supervisor **Dr. Smita Raghuvanshi**, Assistant Professor, Department of Chemical Engineering for agreeing to co-supervise my Ph.D. work. I sincerely thank Madam for encouraging my research and allowing me to grow as a research scholar. Madam, your advices on research have been invaluable. The joy and enthusiasm she has for her research is contagious and motivational for me, even during tough times in the Ph.D. pursuit.

I would also like to thank the members of Doctoral Advisory Committee, Prof. Arvind Kumar Sharma, Associate Professor and Former Head and Dr. Hare Krishna Mohanta, Assistant Professor, Department of Chemical Engineering for their invaluable suggestions and constructive advices to carry out this work.

I thank both the examiners, Prof. Ligy Philip, IIT-Madras and Dr. Malini Balakrishnan, TERI, New Delhi for reviewing the thesis thoroughly and giving invaluable suggestions.

I extend my sincere thanks to Prof. V. S. Rao, Vice-Chancellor, BITS-Pilani and Prof. B. N. Jain, Former Vice-Chancellor, BITS-Pilani for giving me the opportunity to carry out the Ph.D. work in BITS-Pilani, Pilani Campus. My sincere thanks go to Prof. A. K. Sarkar, Director and Prof. G. Raghurama, Former Director, BITS-Pilani, Pilani Campus for providing the necessary infrastructure to carry out this research work. I am also indebted to Prof. S. K. Verma, Dean and Prof. H. R. Jadhav, Associate Dean, Academic Research (Ph. D. Programme) Division for providing the necessary research facilities required for my work. Prof. S. K. Verma was instrumental in explaining me the protocols for bio-based experimental techniques in the course Bioremediation and Biometallurgy. I also thank Prof. S. Kapur, Dean, International Programmes and Collaboration Division for helping me to understand protocols used in isolation and identification of microbial strains.

Some faculty members of the institute have been very kind enough to extend their help at various phases of this research, and I do hereby acknowledge all of them. I thank, Dr. S. Murugesan, Assistant Professor and Head, Dr. A. T. Paul, Assistant Professor, Department of Pharmacy; Prof. R. Mehrotra, Associate Professor and Head, Prof. S. Chowdhury, Associate Professor and Former Head, Department of Biological Sciences for providing me the facilities to carry out biological sample preparation in laboratories. I also thank Garima and Panchsheela, Research Scholars, Biological Sciences for their help in bio-based experiments.

I am thankful to Dr. Banasri Roy, Assistant Professor, Department of Chemical Engineering in helping me to interpret the results obtained from FTIR spectral analysis. I extend my special thanks to Dr. Pradipta Chattopadhyay, Dr. Pratik N. Sheth, Dr. Sushil Kumar, Dr. Dipaloy Dutta, Dr. Nikhil Prakash Saxena, Dr. Raman Sharma, Dr. Srinivas Appari, Dr. (Ms.) Priya C. Sande, Dr. Amit Jain, Dr. Ajaya Kumar Pani and Dr. Utkarsh Maheshwari for their support throughout the work. I also thank my other colleagues for their help and encouragement received during the entire journey of Ph.D.

I extend my sincere thanks to Dr. Sonal Mazumder, Assistant Professor, Department of Chemical Engineering for her constructive advices which helped me to focus more towards quantitative analysis for my biological samples.

I thank University Grant Commission (UGC) and Department of Science and Technology (DST) for providing necessary supports to carry out research at department laboratories.

I would also like to express my special thanks to Mr. Ashok Saini for his help and support in fabricating the in-house biofilter column and Mr. Subodh Kumar Azad, Demonstrator for his help in analyzing samples using various analytical instruments. Thanks are also due to Mr. Jangvir Sheoran, Mr. Babulal Saini, Mr. Jeevan Lal Verma, Mr. Naveen for their help and cooperation during this work.

I also thank my students Dwaipayan, Deepak, Gayathri, Giridhar, Pratyusha, Amrita, Vedansh and Kalyani for their dedication and efforts in helping me to carry out this research. I extend my special thanks to Mr. Somesh Mishra, Research Scholar, Department of Chemical Engineering for helping me in various phases of this research.

I owe a lot to my parents, who encouraged and helped me at every stage of my personal and academic life. I am fortunate to have a wonderful elder brother Dr. Abhijit Mazumdar, Senior Scientist, Birbal Sahni Institute of Paleobotany and sister-in-law Dr. Neelam Pereira Mazumdar who have always been instrumental in encouraging me to become an academician.

SUBHAJIT MAJUMDER

ABSTRACT

The present work deals with the bioremediation and biofiltration experimental studies for the removal of chromium [Cr(VI)], copper [Cu(II)] and zinc [Zn(II)] from wastewater. This study includes isolation of three distinct bacterial strains *Pseudomonas taiwanensis*, *Acinetobacter guillouiae* and *Klebsiella pneumoniae* from activated sludge of the Sewage Treatment Plant, BITS-Pilani, Pilani, Rajasthan, India. These strains are utilized to treat Cr(VI), Cu(II) and Zn(II) contaminated aqueous solutions. The extensive batch studies are performed to see the effect of initial metal concentration, initial pH, MSM dosage, Nitrogen (N)-Phosphorus (P)-Potassium (K) composition in MSM, aerobic and facultative anaerobic conditions, temperature and inoculum volume on metal removal. The maximum values of specific growth rate of *P. taiwanensis*, *A. guillouiae* and *K. pneumoniae* are obtained as 0.262 h^{-1} for 150 mg L^{-1} of Cr(VI), 0.208 h^{-1} for 80 mg L^{-1} of Cu(II) and 0.043 h^{-1} for 80 mg L^{-1} of Zn(II), respectively.

Growth kinetic models such as Monod and Monod-Inhibition are tested with the experimentally obtained bioremediation data of three bacterial strains for Cr(VI), Cu(II) and Zn(II) removals. The rate kinetic approaches (zero-order and three-half-order) are applied to experimental data obtained from bioremediation study.

A novel consortium of three bacterial species (*P. taiwanensis*, *A. guillouiae* and *K. pneumoniae*) is developed and used to remove single and multiple metals from aqueous solutions. Metal uptake capacity of consortium is compared with the uptake capacity of the individual species for single and multiple metal ions. Efficacy of consortium is also tested for the simulated industrial effluents contaminated with multiple metals at different compositions. Consortium shows maximum removal efficiency and uptake capacity for the single as well as multiple metal removal.

The continuous biofiltration studies are carried out for the removal of individual metals Cr(VI), Cu(II) and Zn(II) in an in-house fabricated biofilter column by varying initial concentration and flow rate of metals. The total operating time of biofilter column for Cr(VI), Cu(II) and Zn(II) removal are 83, 69 and 74 days, respectively including the study of shock loading. The operation of the biofilter column is divided into various phases which depends on operating conditions i.e. inlet metal concentration and flow

rate. The maximum steady state removal efficiencies are obtained as 89.4 %, 97.5 % and 91.5 % for 38-40 mg L^{-1} of Cr(VI), 17.5-20 mg L^{-1} of Cu(II) and 18.5-20 mg L^{-1} of Zn(II) concentrations, respectively. The stability (stable response) of the biofilter column is also checked for Cr(VI), Cu(II) and Zn(II) removal by providing the fluctuating input loads for 20, 14 and 14 days, respectively immediately after phase V of biofilter operation. The steady value of removal efficiency achieved for all the metals during the shock loading conditions confirms the stability of the biofilter column. Michaelis-Menten kinetic model is applied to estimate the kinetic parameters using the steady state values obtained in each phase for all the metals during biofilter operation. The values obtained for saturation constant fall in the range of the inlet concentration of all the metals which indicate that bioremediation kinetics of Cr(VI), Cu(II) and Zn(II) is better explained by zero-order kinetics with diffusion limitation. Ottengraf-van den Oever model is also fitted with the experimental data of each phase except phase I (acclimation period) during biofilter operation. The elimination capacity predicted from Ottengraf-van den Oever model is compared with the elimination capacity obtained experimentally.

Fourier transform infrared (FTIR) spectral analysis is carried out for obtained biomass of *P. taiwanensis*, *A. guillouiae*, *K. pneumoniae* and consortium for the removal of Cr(VI), Cu(II), Zn(II) and mixed metals, respectively. FTIR analysis shows the presence of various surface functional groups such as carboxyl, amino, hydroxyl, methyl, phosphate and sulphonate groups for metal complexation. Field emission scanning electron microscope coupled with energy dispersive X-ray (FESEM-EDX) analysis is also carried out for the metal unloaded and loaded biomass. This analysis reveals the change in the surface morphology of the individual bacterial strains and consortium during metal bioremediation.

Keywords: *Bioremediation; heavy metals; Monod-Inhibition model; rate kinetic models; consortium; simulated industrial effluents; biofiltration; shock loading conditions; Michaelis-Menten kinetic model; Ottengraf-van den Oever model.*

TABLE OF CONTENTS

Acknowledgments i
Abstract iii
Table of contents v
List of figures ix
List of plates xvi
List of tables xvii
Nomenclature xxv

1 Introduction 1
1.1 Water crisis 1
1.2 Heavy metals in wastewater 3
1.3 Physico-chemical treatment techniques 6
1.4 Bio-based separation techniques 9
1.4.1 Bioremediation 10
1.4.2 Biofiltration 16
1.4.3 Theoretical aspects of bioremediation and biofiltration 17
1.5 Motivation for the present study 18
1.6 Objectives 19
1.7 Organization of thesis 19

2 Literature review 21
2.1 Batch bioremediation studies 21
2.2 Continuous column and reactor studies 32
2.3 Gaps in existing research 41
2.4 Scope of the present study 42

3 Experimental studies 44
3.1 Batch bioremediation studies 44
3.1.1 Glasswares and chemicals 44
3.1.2 Preparation of reagents and media 44
3.1.3 Microorganism culture conditions 48
3.1.4 Acclimatization procedure 48
3.1.5 Isolation and identification of microbial strain 49
3.1.6 Preservation of isolated microbial strain 50
3.1.7 Batch studies of Cr(VI), Cu(II) and Zn(II) remediation 51
3.1.8 Batch studies for simultaneous remediation of Cr(VI), Cu(II) and Zn(II) 58
3.1.8.1 Selection of efficient microbial strains 58
3.1.8.2 Characterization and identification of microbial strain 59
3.1.8.3 Kinetic study 59
3.1.8.4 Development of consortium 60

3.1.8.5 Efficacy of consortium over individual strains in metal removal 62
3.1.8.6 Use of consortium for simulated industrial effluents 62
3.2 Mass balance and metal removal mechanism 63
3.3 Biofiltration studies 64
3.3.1 Biofilter column setup 64
3.3.2 Preparation of culture and development of biofilter column 64
3.3.3 Operating conditions for biofiltration 65
3.3.4 Stability of biofilter column 66
3.3.5 Adsorption capacity of coal 66
3.4 Analytical methods 71
3.4.1 Methods for bioremediation study 71
3.4.1.1 Metal analysis 71
3.4.1.2 Measurement of cell density 71
3.4.2 Methods for biofiltration study 75
3.4.2.1 Metal analysis and measurement of biomass concentration 75
3.4.2.2 Performance evaluation of biofilter operation 75
3.4.3 Fourier transform infrared (FTIR) spectroscopy 76
3.4.3.1 Preparation of microbial cultures 76
3.4.3.2 Sample preparation for FTIR spectra acquisition 77
3.4.4 Field-emission scanning electron microscopy and energy-dispersive X-ray (FESEM-EDX) analysis 77
3.4.4.1 Preparation of microbial cultures 77
3.4.4.2 FESEM sample preparation 78

4 Mathematical modeling 80
4.1 Specific growth rate 80
4.2 Growth kinetic models 81
4.2.1 Monod model 82
4.2.2 Models with growth inhibitors 83
4.3 Rate kinetic models 84
4.3.1 Zero-order rate kinetics 84
4.3.2 Three-half-order rate kinetics 85
4.4 Kinetic modeling for biofiltration 87
4.4.1 Michaelis-Menten kinetic model 87
4.4.2 Modeling with Ottengraf-van den Oever model 88

5 Results and discussion 90
5.1 Experimental studies 90
5.1.1 Batch bioremediation studies 90
5.1.1.1 Acclimatization procedure 90
5.1.1.2 Identification of isolated microbial strains 91
5.1.1.3 Metal removal study without bacterial strains 91
5.1.1.4 Effect of time on initial concentration of metals 92
5.1.1.5 Effect of initial pH 103
5.1.1.6 Effect of minimal salt media (MSM) dosage 105
5.1.1.7 Effect of macronutrients (N, P and K) 110

5.1.1.8 Effect of aerobic and facultative anaerobic conditions 114
5.1.1.9 Effect of temperature 119
5.1.1.10 Effect of inoculum volume 120
5.1.2 Specific growth rate 127
5.1.3 Growth kinetic modeling 131
5.1.3.1 Monod model 131
5.1.3.2 Monod-Inhibition model 132
5.1.4 Rate Kinetic Modeling 132
5.1.4.1 Zero-order rate kinetics 132
5.1.4.2 Three-half-order rate kinetics 132
5.1.5 Simultaneous bioremediation of multiple metals Cr(VI), Cu(II) and Zn(II) 139
5.1.5.1 Isolation and selection of microbial strains 139
5.1.5.2 Characterization and identification of selected microbial strains 140
5.1.5.3 Kinetic study 141
5.1.5.4 Development of consortium 150
5.1.5.5 Efficacy of consortium over individual strains in metal removal 150
5.1.5.6 Use of consortium for simulated industrial effluents 153
5.1.6 Mass balance and metal removal mechanism 159
5.1.6.1 Microbial mechanism of chromium, copper and zinc bioremediation 163
5.1.7 Biofiltration studies 168
5.1.7.1 Cr(VI) removal 168
5.1.7.2 Cu(II) removal 175
5.1.7.3 Zn(II) removal 182
5.1.7.4 Adsorption capacity of coal 188
5.1.7.5 Determination of Michaelis-Menten kinetic constants 188
5.1.7.6 Modeling with Ottengraf-van den Oever model 189
5.2 FTIR analysis 197
5.2.1 Biomass of *P. taiwanensis, A. guillouiae* and *K. pneumoniae* 197
5.2.2 Biomass of consortium 199
5.3 FESEM-EDX analysis 207
5.3.1 Biomass of *P. taiwanensis* 207
5.3.2 Biomass of *A. guillouiae* 208
5.3.3 Biomass of *K. pneumoniae* 208
5.3.4 Biomass of consortium 209

6 Concluding remarks 214
6.1 Summary 214
6.1.1 Introduction 214
6.1.2 Gaps in literature 216
6.1.3 Scope of the work 218
6.1.4 Experimental studies 219
6.1.5 Mathematical modeling 220
6.1.6 Results and discussion 220
6.1.6.1 Identification of isolated microbial strains 220

6.1.6.2 Batch bioremediation studies 221
6.1.6.3 Metal removal mechanism 225
6.1.6.4 Biofiltration studies 226
6.1.6.5 FTIR analysis 228
6.1.6.6 FESEM-EDX analysis 229
6.2 Conclusions 229
6.3 Major contributions 231
6.4 Future scope of research 232

References 234
List of publications 257
Biographies 260
Appendix I Given in CD ROM 263
Appendix II Given in CD ROM 289

LIST OF FIGURES

Figure No	Title	Page No
3.1	The flowchart of the consortium development (A to Q represent steps)	61
3.2	Schematic diagram of biofilter set-up	67
3.3	Calibration plot for Cr(VI) analysis in final solution	73
5.1	Phylogenetic tree of Cr(VI) resistant bacterial strain based on 16S rRNA gene sequence comparison (Query indicates isolated microbial strain)	96
5.2	Phylogenetic tree of Cu(II) resistant bacterial strain based on 16S rRNA gene sequence comparison (Query indicates isolated microbial strain)	96
5.3	Phylogenetic tree of Zn(II) resistant bacterial strain based on 16S rRNA gene sequence comparison (Query indicates isolated microbial strain)	97
5.4	Cr(VI) removal by *P. taiwanensis* at different time for various initial Cr(VI) concentrations (T = 37 ^{o}C, pH = 7.0±0.2, RPM = 150, S_o = 20-200 mg L^{-1})	97
5.5	Cu(II) removal by *A. guillouiae* at different time for various initial Cu(II) concentrations (T = 37 ^{o}C, pH = 7.0±0.2, RPM = 150, S_o = 20-200 mg L^{-1})	98
5.6	Zn(II) removal by *K. pneumoniae* at different time for various initial Zn(II) concentrations (T = 37 ^{o}C, pH = 7.0±0.2, RPM = 150, S_o = 20-200 mg L^{-1})	98
5.7	Growth of *P. taiwanensis* with time (T = 37 ^{o}C, pH = 7.0±0.2, RPM = 150, 1 % v/v inoculum)	99
5.8	Kinetics of substrate utilization by *P. taiwanensis* for different initial Cr(VI) concentration	99

(*T* = 37 ^{o}C, pH = 7.0±0.2, RPM = 150, 1 % v/v inoculum)

5.9 Growth of *A. guillouiae* with time (*T* = 37 ^{o}C, pH = 7.0±0.2, RPM = 150, 1 % v/v inoculum) 100

5.10 Kinetics of substrate utilization by *A. guillouiae* for different initial Cu(II) concentration (*T* = 37 ^{o}C, pH = 7.0±0.2, RPM = 150, 1 % v/v inoculum) 100

5.11 Growth of *K. pneumoniae* with time (*T* = 37 ^{o}C, pH = 7.0±0.2, RPM = 150, 1 % v/v inoculum) 101

5.12 Kinetics of substrate utilization by *K. pneumoniae* for different initial Zn(II) concentration (*T* = 37 ^{o}C, pH = 7.0±0.2, RPM = 150, 1 % v/v inoculum) 101

5.13 Effect of pH on growth of *P. taiwanensis* (*T* = 37 ^{o}C, RPM = 150, 1 % v/v inoculum, S_o = 150 mg L^{-1} of Cr(VI)) 107

5.14 Effect of pH on growth of *A. guillouiae* (*T* = 37 ^{o}C, RPM = 150, 1 % v/v inoculum, S_o = 80 mg L^{-1} of Cu(II)) 107

5.15 Effect of pH on growth of *K. pneumoniae* (*T* = 37 ^{o}C, RPM = 150, 1 % v/v inoculum, S_o = 40 mg L^{-1} of Zn(II)) 108

5.16 Effect of Nutrient (MSM) dosage on growth of *P. taiwanensis* (*T* = 37 ^{o}C, pH = 7.0±0.2, RPM = 150, 1 % v/v inoculum, S_o = 150 mg L^{-1} of Cr(VI)) 108

5.17 Effect of Nutrient (MSM) dosage on growth of *A. guillouiae* (*T* = 37 ^{o}C, pH = 7.0±0.2, RPM = 150, 1 % v/v inoculum, S_o = 80 mg L^{-1} of Cu(II)) 109

5.18 Effect of Nutrient (MSM) dosage on growth of *K. pneumoniae* (*T* = 37 ^{o}C, pH = 7.0±0.2, RPM = 150, 1 % v/v inoculum, S_o = 40 mg L^{-1} of Zn(II)) 109

5.19 Effect of N-P-K composition in MSM on growth of *P. taiwanensis* (*T* = 37 ^{o}C, pH = 7.0±0.2, RPM = 150, 1 % v/v inoculum, S_o = 150 mg L^{-1} of Cr(VI)) 116

5.20 Effect of N-P-K composition in MSM on growth of *A. guillouiae* (*T* = 37 ^{o}C, pH = 7.0±0.2, RPM = 150, 1 % v/v 116

inoculum, S_o = 80 mg L^{-1} of Cu(II))

5.21 Effect of N-P-K composition in MSM on growth of *K. pneumoniae* (T = 37 ^oC, pH = 7.0±0.2, RPM = 150, 1 % v/v inoculum, S_o = 40 mg L^{-1} of Zn(II)) 117

5.22 Effect of aerobic and facultative anaerobic conditions on growth of *P. taiwanensis* (T = 37 ^oC, pH = 7.0±0.2, RPM = 150, 1 % v/v inoculum, S_o = 150 mg L^{-1} of Cr(VI)) 117

5.23 Effect of aerobic and facultative anaerobic conditions on growth of *A. guillouiae* (T = 37 ^oC, pH = 7.0±0.2, RPM = 150, 1 % v/v inoculum, S_o = 80 mg L^{-1} of Cu(II)) 118

5.24 Effect of aerobic and facultative anaerobic conditions on growth of *K. pneumoniae* (T = 37 ^oC, pH = 7.0±0.2, RPM = 150, 1 % v/v inoculum, S_o = 40 mg L^{-1} of Zn(II)) 118

5.25 Effect of temperature on growth of *P. taiwanensis* (pH = 7.0±0.2, RPM = 150, 1 % v/v inoculum, S_o = 150 mg L^{-1} of Cr(VI)) 123

5.26 Effect of temperature on growth of *A. guillouiae* (pH = 7.0±0.2, RPM = 150, 1 % v/v inoculum, S_o = 80 mg L^{-1} of Cu(II)) 123

5.27 Effect of temperature on growth of *K. pneumoniae* (pH = 7.0±0.2, RPM = 150, 1 % v/v inoculum, S_o = 40 mg L^{-1} of Zn(II)) 124

5.28 Effect of inoculum volume on growth of *P. taiwanensis* (T = 37 ^oC, pH = 7.0±0.2, RPM = 150, S_o = 150 mg L^{-1} of Cr(VI)) 124

5.29 Effect of inoculum volume on growth of *A. guillouiae* (T = 37 ^oC, pH = 7.0±0.2, RPM = 150, S_o = 80 mg L^{-1} of Cu(II)) 125

5.30 Effect of inoculum volume on growth of *K. pneumoniae* (T = 37 ^oC, pH = 7.0±0.2, RPM = 150, S_o = 40 mg L^{-1} of Zn(II)) 125

5.31 Calculation of specific growth rate (μ) of *P. taiwanensis* for Cr(VI) removal 128

5.32 Calculation of specific growth rate (μ) of *A. guillouiae* for Cu(II) removal 128

5.33 Calculation of specific growth rate (μ) of *K. pneumoniae* for Zn(II) removal 129

5.34	Zero-order kinetics for Cr(VI) bioremediation at different S_o of Cr(VI)	134
5.35	Zero-order kinetics for Cu(II) bioremediation at different S_o of Cu(II)	134
5.36	Zero-order kinetics for Zn(II) bioremediation at different S_o of Zn(II)	135
5.37	Three-half-order kinetics for Cr(VI) bioremediation at different S_o of Cr(VI)	135
5.38	Three-half-order kinetics for Cu(II) bioremediation at different S_o of Cu(II)	136
5.39	Three-half-order kinetics for Zn(II) bioremediation at different S_o of Zn(II)	136
5.40	Percentage metal removal of isolated microbial strains	146
5.41	Growth of isolated bacterial strains and pH with time in biotic control (T = 37 ºC, pH = 7.0±0.2, RPM = 150)	147
5.42	Growth and metal removal by isolated bacterial strains with time in the presence of Cr(VI) (T = 37 ºC, pH = 7.0±0.2, RPM = 150, S_o = 150 mg L^{-1})	147
5.43	Growth and metal removal by isolated bacterial strains with time in the presence of Cu(II) (T = 37 ºC, pH = 7.0±0.2, RPM = 150, S_o = 150 mg L^{-1})	148
5.44	Growth and metal removal by isolated bacterial strains with time in the presence of Zn(II) (T = 37 ºC, pH = 7.0±0.2, RPM = 150, S_o = 150 mg L^{-1})	148
5.45	Relative growth of individual bacterial strain in presence of other bacterial strain (PT: *P. taiwanensis*; AG: *A. guillouiae* and KP: *K. pneumoniae*)	154
5.46	Efficiency of the consortium over individual strain in removal of Cr(VI) (T = 37 ºC, pH = 7.0±0.2, RPM = 150, S_o=150 mg L^{-1} of Cr(VI))	154
5.47	Efficiency of the consortium over individual strain in removal of	155

Cu(II) (T = 37 ºC, pH = 7.0±0.2, RPM = 150, S_o=150 mg L^{-1} of Cu(II))

5.48 Efficiency of the consortium over individual strain in removal of Zn(II) (T = 37 ºC, pH = 7.0±0.2, RPM = 150, S_o = 150 mg L^{-1} of Zn(II)) 155

5.49 Efficiency of the consortium over individual strains in removal of mixed metals (T = 37 ºC, pH = 7.0±0.2, RPM = 150, each metal 50 mg L^{-1}) 156

5.50 Efficiency of the consortium over individual strains in removal of mixed metals in terms of relative biomass growth reduction (T = 37 ºC, pH = 7.0±0.2, RPM = 150, each metal 50 mg L^{-1}) 156

5.51 Performance evaluation of the consortium in multi-metals removal in five sets of simulated industrial effluents (square dot with line represents change in biomass concentration) (T = 37 ºC, pH = 7.0±0.2, RPM = 150) 158

5.52 Mass balance and removal mechanism for Cr(VI) (T = 37ºC, pH = 7.0 ± 0.2, RPM = 150, medium MSM, S_o = 150 mg L^{-1}) (PT: *P. taiwanensis*; AG: *A. guillouiae* and KP: *K. pneumoniae*) 161

5.53 Mass balance and removal mechanism for Cu(II) (T = 37ºC, pH = 7.0 ± 0.2, RPM = 150, medium MSM, S_o = 150 mg L^{-1}) (PT: *P. taiwanensis*; AG: *A. guillouiae* and KP: *K. pneumoniae*) 161

5.54 Mass balance and removal mechanism for Zn(II) (T = 37ºC, pH = 7.0 ± 0.2, RPM = 150, medium MSM, S_o = 150 mg L^{-1}) (PT: *P. taiwanensis*; AG: *A. guillouiae* and KP: *K. pneumoniae*) 162

5.55 Plausible mechanism of Cr(VI) remediation using *P. taiwanensis* in aerobic condition. Adapted from Cheung and Gu (2007) (ChrR: Chromium reductase, SR: Soluble reductase) 166

5.56 Plausible mechanism of Cu(II) remediation using *A. guillouiae*. Adapted from Cervantes and Gutierrez-Corona (1994) 166

5.57 Plausible mechanism of Zn(II) remediation using *K. pneumoniae*. Adapted from Cervantes and Gutierrez-Corona (1994) and 167

	Nies (1999)	
5.58	Performance of biofilter with change in flow rates and inlet concentrations of Cr(VI) solution	173
5.59	Change in biomass growth with operating time at different heights of filter bed in Cr(VI) removal	173
5.60	Performance of biofilter under shock loading conditions for Cr(VI) removal (change in concentration and removal efficiency with operating time)	174
5.61	Performance of biofilter under shock loading conditions for Cr(VI) removal (change in biomass growth and pH with operating time)	174
5.62	Performance of biofilter with change in flow rates and inlet concentrations of Cu(II) solution	180
5.63	Change in biomass growth with operating time at different heights of filter bed in Cu(II) removal	180
5.64	Performance of biofilter under shock loading conditions for Cu(II) removal (change in concentration and removal efficiency with operating time)	181
5.65	Performance of biofilter under shock loading conditions for Cu(II) removal (change in biomass growth and pH with operating time)	181
5.66	Performance of biofilter with change in flow rate and inlet concentrations of Zn(II) solution	186
5.67	Change in biomass growth with operating time at different heights of filter bed in Zn(II) removal	186
5.68	Performance of biofilter under shock loading conditions for Zn(II) removal (change in concentration and removal efficiency with operating time)	187
5.69	Performance of biofilter under shock loading conditions for Zn(II) removal (change in biomass growth and pH with operating time)	187
5.70	Kinetic constants obtained for removal of metals using Michaelis-Menten kinetic model	192
5.71	Comparison of elimination capacity obtained experimentally with	194

	elimination capacity calculated from Ottengraf-van den Oever model in Cr(VI) removal for phases II to V	
5.72	Comparison of elimination capacity obtained experimentally with elimination capacity calculated from Ottengraf-van den Oever model in Cu(II) removal for phases II to V	194
5.73	Comparison of elimination capacity obtained experimentally with elimination capacity calculated from Ottengraf-van den Oever model in Zn(II) removal for phases II to V	195
5.74	FTIR spectra of *P. taiwanensis* biomass: (a) Cr(VI) unloaded, (b) Cr(VI) loaded	201
5.75	FTIR spectra of *A. guillouiae* biomass: (a) Cu(II) unloaded, (b) Cu(II) loaded	202
5.76	FTIR spectra of *K. pneumoniae* biomass: (a) Zn(II) unloaded, (b) Zn(II) loaded	203
5.77	FTIR spectra of consortium: (a) metal unloaded, (b) metal loaded	204
5.78	SEM-EDX images of (a) *P. taiwanensis* (control), (b) *P. taiwanensis* loaded with chromium	210
5.79	SEM-EDX images of (a) *A. guillouiae* (control), (b) *A. guillouiae* loaded with copper	211
5.80	SEM-EDX images of (a) *K. pneumoniae* (control), (b) *K. pneumoniae* loaded with zinc	212
5.81	SEM images of: (a) consortium (control), (b) consortium loaded with metals	213

LIST OF PLATES

Plate No	Title	Page No
3.1	Photograph of activated sludge sample	53
3.2	Photograph of centrifuge	54
3.3	Photograph of laminar air flow chamber	54
3.4	Photograph of UV-Vis spectrophotometer	55
3.5	Photograph of microbial strain grown on agar in the presence of Cr(VI)	55
3.6	Photograph of microbial strain grown on agar in the presence of Cu(II)	56
3.7	Photograph of microbial strain grown on agar in the presence of Zn(II)	56
3.8	Photograph of continuous biofiltration experimental setup	68
3.9	Photograph of atomic absorption spectrophotometer	74
3.10	Photograph of freeze dryer	79

LIST OF TABLES

Table No	Title	Page No
1.1	Selected heavy metals and their regulatory limits in surface and ground water as per USEPA	4
1.2	Microorganisms and mixed consortia with heavy metal removal capacity	13
3.1	Glasswares used	46
3.2	Chemicals used	46
3.3	Composition of mineral salts used for MSM preparation	47
3.4	Actual volume of stock solutions used and composition of heavy metals in synthetic wastewater	47
3.5	Parameters studies and their range/ composition/ mode	57
3.6	Operating conditions of biofilter operation for Cr(VI) removal (IL: Inlet loading, EBRT: Empty bed residence time)	69
3.7	Operating conditions of biofilter operation for Cu(II) removal (IL: Inlet loading, EBRT: Empty bed residence time)	69
3.8	Operating conditions of biofilter operation for Zn(II) removal (IL: Inlet loading, EBRT: Empty bed residence time)	69
3.9	Operating conditions of biofilter operation for Cr(VI) removal under shock loading (IL: Inlet loading, EBRT: Empty bed residence time)	70
3.10	Operating conditions of biofilter operation for Cu(II) removal under shock loading (IL: Inlet loading, EBRT: Empty bed residence time)	70
3.11	Operating conditions of biofilter operation for Zn(II) removal under shock loading (IL: Inlet loading, EBRT: Empty bed residence time)	70
5.1	Results obtained for effect of time on Cr(VI) removal using *P. taiwanensis*	102
5.2	Results obtained for effect of time on Cu(II) removal using *A. guillouiae*	102

5.3	Results obtained for effect of time on Zn(II) removal using *K. pneumoniae*	102
5.4	Optimum values of the parameters obtained from batch studies for the removal of Cr(VI), Cu(II) and Zn(II) using *P. taiwanensis, A. guillouiae* and *K. pneumoniae*, respectively	126
5.5	Specific growth rate of three bacterial strains used in bioremediation of Cr(VI), Cu(II) and Zn(II)	130
5.6	Growth kinetic parameters for Cr(VI) bioremediation obtained from growth models	137
5.7	Growth kinetic parameters for Cu(II) bioremediation obtained from growth models	137
5.8	Growth kinetic parameters for Zn(II) bioremediation obtained from growth models	137
5.9	Zero-order and three-half-order kinetic models parameters at different initial Cr(VI) concentrations	138
5.10	Zero-order and three-half-order kinetic models parameters at different initial Cu(II) concentrations	138
5.11	Zero-order and three-half-order kinetic models parameters at different initial Zn(II) concentrations	138
5.12	Minimum inhibitory concentration (MIC) of four isolated microbial strains in the presence of Cr(VI), Cu(II) and Zn(II)	145
5.13	Specific growth rate, percentage metal removal and maximum uptake of metals by three bacterial strains (T = 37 ºC, pH = 7.0±0.2, RPM = 150, S_o = 150 mg L^{-1})	149
5.14	Comparison between consortium and individual bacterial strains for the removal of single and multiple metals	157
5.15	Composition and percentage removal of metals in five sets of simulated industrial effluents	157
5.16	Michaelis-Menten kinetic constants calculated for Cr(VI), Cu(II) and Zn(II) removal at different operating conditions	193
5.17	Model parameters calculated using Ottengraf-van den Oever Model	196

at various operating conditions for removal of metals (bold values indicate maximum biofilm thickness)

5.18 The observed FTIR band for *P. taiwanensis* biomass in Cr(VI) unloaded (control) and loaded conditions 205

5.19 The observed FTIR band for *A. guillouiae* biomass in Cu(II) unloaded (control) and loaded conditions 205

5.20 The observed FTIR band for *K. pneumoniae* biomass in Zn(II) unloaded (control) and loaded conditions 206

5.21 The observed FTIR band for consortium biomass in metals unloaded (control) and loaded conditions 206

Given in CD ROM

A 1.1 Cr(VI) removal by *P. taiwanensis* at different time for various initial Cr(VI) concentrations (T = 37 ^{o}C, pH = 7.0±0.2, RPM = 150, S_o = 20-200 mg L^{-1}) 263

A 1.2 Cu(II) removal by *A. guillouiae* at different time for various initial Cu(II) concentrations (T = 37 ^{o}C, pH = 7.0±0.2, RPM = 150, S_o = 20-200 mg L^{-1}) 264

A 1.3 Zn(II) removal by *K. pneumoniae* at different time for various initial Zn(II) concentrations (T = 37 ^{o}C, pH = 7.0±0.2, RPM = 150, S_o = 20-200 mg L^{-1}) 265

A 1.4 Growth of *P. taiwanensis* with time in the presence of Cr(VI) (T = 37 ^{o}C, pH = 7.0±0.2, RPM = 150, 1 % v/v inoculum, S_o = 20-200 mg L^{-1}) 266

A 1.5 Growth of *A. guillouiae* with time in the presence of Cu(II) (T = 37 ^{o}C, pH = 7.0±0.2, RPM = 150, 1 % v/v inoculum, S_o = 20-200 mg L^{-1}) 267

A 1.6 Growth of *K. pneumoniae* with time in the presence of Zn(II) (T = 37 ^{o}C, pH = 7.0±0.2, RPM = 150, 1 % v/v inoculum, S_o = 20-200 mg L^{-1}) 268

A 1.7 Effect of pH on growth of *P. taiwanensis* in the presence of Cr(VI) (T = 37 ^{o}C, RPM = 150, 1 % v/v inoculum, S_o = 150 mg L^{-1} of 269

Cr(VI))

A 1.8 Effect of pH on growth of *A. guillouiae* in the presence of Cu(II) (T = 37 ^{o}C, RPM = 150, 1 % v/v inoculum, S_o = 80 mg L^{-1} of Cu(II)) 269

A 1.9 Effect of pH on growth of *K. pneumoniae* in the presence of Zn(II) (T = 37 ^{o}C, RPM = 150, 1 % v/v inoculum, S_o = 40 mg L^{-1} of Zn(II)) 269

A 1.10 Effect of Nutrient (MSM) dosage on growth of *P. taiwanensis* in the presence of Cr(VI) (T = 37 ^{o}C, pH = 7.0±0.2, RPM = 150, 1 % v/v inoculum, S_o = 150 mg L^{-1} of Cr(VI)) 270

A 1.11 Effect of Nutrient (MSM) dosage on growth of *A. guillouiae* in the presence of Cu(II) (T = 37 ^{o}C, pH = 7.0±0.2, RPM = 150, 1 % v/v inoculum, S_o = 80 mg L^{-1} of Cu(II)) 270

A 1.12 Effect of Nutrient (MSM) dosage on growth of *K. pneumoniae* in the presence of Zn(II) (T = 37 ^{o}C, pH = 7.0±0.2, RPM = 150, 1 % v/v inoculum, S_o = 40 mg L^{-1} of Zn(II)) 270

A 1.13 Effect of N-P-K composition in MSM on growth of *P. taiwanensis* (T = 37 ^{o}C, pH = 7.0±0.2, RPM = 150, 1 % v/v inoculum, S_o = 150 mg L^{-1} of Cr(VI)) 271

A 1.14 Effect of N-P-K composition in MSM on growth of *A. guillouiae* (T = 37 ^{o}C, pH = 7.0±0.2, RPM = 150, 1 % v/v inoculum, S_o = 80 mg L^{-1} of Cu(II)) 271

A 1.15 Effect of N-P-K composition in MSM on growth of *K. pneumoniae* (T = 37 ^{o}C, pH = 7.0±0.2, RPM = 150, 1 % v/v inoculum, S_o = 40 mg L^{-1} of Zn(II)) 272

A 1.16 Effect of aerobic and facultative anaerobic conditions on growth of *P. taiwanensis* in the presence of Cr(VI) (T = 37 ^{o}C, pH = 7.0±0.2, RPM = 150, 1 % v/v inoculum, S_o = 150 mg L^{-1} of Cr(VI)) 272

A 1.17 Effect of aerobic and facultative anaerobic conditions on growth of *A. guillouiae* in the presence of Cu(II) (T = 37 ^{o}C, pH = 7.0±0.2, RPM = 150, 1 % v/v inoculum, S_o = 80 mg L^{-1} of Cu(II)) 272

A 1.18 Effect of aerobic and facultative anaerobic conditions on growth of *K. pneumoniae* in the presence of Zn(II) (T = 37 ^{o}C, pH = 7.0±0.2, 272

RPM = 150, 1 % v/v inoculum, S_o = 40 mg L^{-1} of Zn(II))

A 1.19 Effect of temperature on growth of *P. taiwanensis* in the presence of Cr(VI) (pH = 7.0±0.2, RPM = 150, 1% v/v inoculum, S_o = 150 mg L^{-1} of Cr(VI)) 273

A 1.20 Effect of temperature on growth of *A. guillouiae* in the presence of Cu(II) (pH = 7.0±0.2, RPM = 150, 1 % v/v inoculum, S_o = 80 mg L^{-1} of Cu(II)) 273

A 1.21 Effect of temperature on growth of *K. pneumoniae* in the presence of Zn(II) (pH = 7.0±0.2, RPM = 150, 1 % v/v inoculum, S_o = 40 mg L^{-1} of Zn(II)) 273

A 1.22 Effect of inoculum volume on growth of *P. taiwanensis* in the presence of Cr(VI) (T = 37 ^oC, pH = 7.0±0.2, RPM = 150, S_o = 150 mg L^{-1} of Cr(VI)) 274

A 1.23 Effect of inoculum volume on growth of *A. guillouiae* in the presence of Cu(II) (T = 37 ^oC, pH = 7.0±0.2, RPM = 150, S_o = 80 mg L^{-1} of Cu(II)) 274

A 1.24 Effect of inoculum volume on growth of *K. pneumoniae* in the presence of Zn(II) (T = 37 ^oC, pH = 7.0±0.2, RPM = 150, S_o = 40 mg L^{-1} of Zn(II)) 274

A 1.25 Calculation of specific growth rate (μ) of *P. taiwanensis* for Cr(VI) removal 275

A 1.26 Calculation of specific growth rate (μ) of *A. guillouiae* for Cu(II) removal 275

A 1.27 Calculation of specific growth rate (μ) of *K. pneumoniae* for Zn(II) removal 276

A 1.28 Zero-order kinetics for Cr(VI) bioremediation at different initial concentrations of Cr(VI) 277

A 1.29 Zero-order kinetics for Cu(II) bioremediation at different initial concentrations of Cu(II) 277

A 1.30 Zero-order kinetics for Zn(II) bioremediation at different initial concentrations of Zn(II) 278

A 1.31	Three-half-order kinetics for Cr(VI) bioremediation at different initial concentrations of Cr(VI)	278
A 1.32	Three-half-order kinetics for Cu(II) bioremediation at different initial concentrations of Cu(II)	279
A 1.33	Three-half-order kinetics for Zn(II) bioremediation at different initial concentrations of Zn(II)	279
A 1.34	Growth of isolated bacterial strains and pH with time in absence of metals (T = 37 ºC, pH = 7.0±0.2, RPM = 150)	280
A 1.35	Growth and metals removal by isolated bacterial strains with time in the presence of Cr(VI) (T = 37 ºC, pH = 7.0±0.2, RPM = 150, S_o = 150 mg L^{-1})	281
A 1.36	Growth and metals removal by isolated bacterial strains with time in the presence of Cu(II) (T = 37 ºC, pH = 7.0±0.2, RPM = 150, S_o = 150 mg L^{-1})	282
A 1.37	Growth and metals removal by isolated bacterial strains with time in presence of Zn(II) (T = 37 ºC, pH = 7.0±0.2, RPM = 150, S_o = 150 mg L^{-1})	283
A 1.38	Relative growth of individual bacterial strain in presence of other bacterial strain (PT+AG indicates *P. taiwanensis* in combination with *A. guillouiae*. Similarly, other notations are used for different combinations)	284
A 1.39	Efficiency of the consortium over individual strain in removal of Cr(VI) (T = 37 ºC, pH = 7.0±0.2, RPM = 150, S_o = 150 mg L^{-1} of Cr(VI))	284
A 1.40	Efficiency of the consortium over individual strain in removal of Cu(II) (T = 37 ºC, pH = 7.0±0.2, RPM = 150, S_o = 150 mg L^{-1} of Cu(II))	285
A 1.41	Efficiency of the consortium over individual strain in removal of Zn(II) (T = 37 ºC, pH = 7.0±0.2, RPM = 150, S_o = 150 mg L^{-1} of Zn(II))	285
A 1.42	Performance evaluation of the consortium in multi-metals removal in	286

simulated industrial effluent: Set 1 (T = 37 ºC, pH = 7.0±0.2, RPM = 150)

A 1.43 Performance evaluation of the consortium in multi-metals removal in simulated industrial effluent: Set 2 (T = 37 ºC, pH = 7.0±0.2, RPM = 150) 286

A 1.44 Performance evaluation of the consortium in multi-metals removal in simulated industrial effluent: Set 3 (T = 37 ºC, pH = 7.0±0.2, RPM = 150) 287

A 1.45 Performance evaluation of the consortium in multi-metals removal in simulated industrial effluent: Set 4 (T = 37 ºC, pH = 7.0±0.2, RPM = 150) 287

A 1.46 Performance evaluation of the consortium in multi-metals removal in simulated industrial effluent: Set 5 (T = 37 ºC, pH = 7.0±0.2, RPM = 150) 288

A 2.1 Performance of biofilter with change in flow rate and inlet concentrations of Cr(VI) solution 289

A 2.2 Change in biomass growth and pH with operating time at different heights of filter bed in Cr(VI) removal 291

A 2.3 Performance of biofilter with change in flow rate and inlet concentrations of Cu(II) solution 293

A 2.4 Change in biomass growth and pH with operating time at different heights of filter bed in Cu(II) removal 295

A 2.5 Performance of biofilter with change in flow rate and inlet concentrations of Zn(II) solution 297

A 2.6 Change in biomass growth and pH with operating time at different heights of filter bed in Zn(II) removal 299

A 2.7 Performance of biofilter under shock loading conditions for Cr(VI) removal (change in concentration with operating time) 301

A 2.8 Performance of biofilter under shock loading conditions for Cr(VI) removal (change in biomass growth and pH with operating time) 302

A 2.9 Performance of biofilter under shock loading conditions for Cu(II) 303

removal (change in concentration with operating time)

A 2.10 Performance of biofilter under shock loading conditions for Cu(II) removal (change in biomass growth and pH with operating time) 303

A 2.11 Performance of biofilter under shock loading conditions for Zn(II) removal (change in concentration with operating time) 304

A 2.12 Performance of biofilter under shock loading conditions for Zn(II) removal (change in biomass growth and pH with operating time) 304

NOMENCLATURE

$C_{critical}$	Critical concentration of substrate, mg L^{-1}
C_i	Inlet metal concentration, mg L^{-1}
C_{ln}	Log mean concentration difference, mg L^{-1}
C_o	Oulet metal concentration, mg L^{-1}
$IL_{critical}$	Critical inlet loading of substrate, mg L^{-1} min^{-1}
k_o	Zero-order rate constant
k_1	First-order rate constant, s^{-1}
k_2	Second-order rate constant, s^{-2}
K	Positive constant in Edwards model
K_I	Substrate inhibition constant, mg L^{-1}
K_o	Zero-order kinetic rate constant used in Michaelis-Menten kinetics
K_1	First-order rate constant used in Michaelis-Menten kinetics, s^{-1}
K_s	Saturation constant, mg L^{-1}
m	Maintenance rate
r_{max}	Maximum bioremediation rate, mg L^{-1} min^{-1}
Q	Volumetric flow rate, mL min^{-1}
R^2	Coefficient of determination
S_o	Initial metal concentration, mg L^{-1}
S	Metal concentration at time t, mg L^{-1}
S_{crit}	Critical substrate concentration, mg L^{-1}
S_m	Maximum substrate concentration, mg L^{-1}
t	Time, min or h
T	Temperature, °C
V	Volume of the packing material, m^3
Y	Term used in Three-half-order kinetics

Greek Symbols

μ	Specific growth rate, h^{-1}
μ_m	Maximum specific growth rate, h^{-1}
δ	Thickness of biofilm, μm

Subscripts

0	Zero-order
1	First-order
2	Second-order
critical	Critical
I	Inhibitor
m	Maximum
o	Initial
s	Substrate

Abbreviations

AG	*Acinetobacter guillouiae*
ATPases	Adenosine triphosphate synthases
CFU	Colony forming unit
ChrR	Cr(VI) reductase
CPCB	Central pollution control board
CtrA	Cell cycle transcriptional regulator
EBRT	Empty bed residence time
EC	Elimination capacity
EDX	Energy-dispersive X-ray spectroscopy
FESEM	Field emission scanning electron microscopy
FTIR	Fourier transform infrared spectroscopy
IL	Inlet loading
KP	*Klebsiella pneumoniae*
MCL	Maximum contamination level
MEGA	Molecular evolutionary genetics analysis
MSM	Minimal salt medium

NADH	Nicotinamide adenine dinucleotide
NADPH	Nicotinamide adenine dinucleotide phosphate
NCBI	National Center for Biotechnology Information
PT	*Pseudomonas taiwanensis*
RE	Removal efficiency
ROS	Reactive oxygen species
SR	Soluble reductase
USEPA	US Environmental Protection Agency
YieF	Chromate reductase Class I flavoprotein
ZRT	Zinc-regulated transporter

CHAPTER 1

INTRODUCTION

1.1. Water crisis

The water, which makes up almost 70 % of the Earth's surface has been available since the age of dinosaurs. However, the fresh water availability is only 2.5 %. A report published by United Nations Development Programme (UNDP) reveals that the total amount of water on earth is about 14,000 Billion Cubic Meters (BCM) (Watkins, 2006). Much of freshwater is trapped in glaciers and snowfields. In essence, the water availability for 6.8 billon people in the world is 0.007 % of the Earth's water (Watkins, 2006). Due to geographical location, climate, regulation, and competition for resources, some regions are reserved with huge amount of freshwater, while others have limited reserve for freshwater. Humans need water to survive. Apart from that, water is also used for making food, clothing, keeping the environment healthy and many more actvities. It has been assessed that nearly 1,869 BCM of water is required in India. Out of which, the availability of utilizable water resources is 1,121 BCM (CWC, 2013). Unfortunately, humans are inefficient water users. With the advent of the industrial revolution, humans were able to enter into the cutting edge technologies of 21st century. However, industrial emanations have started adversely affecting quality and availability of clean water.

In India, the industrialization depends on availability of huge amount of fresh water throughout the year. Recent assessment indicates that India’s water requirements for industries are expected to increase from 25 BCM in 1990, to 120 BCM by the year 2025 (CWC, 2013). The increase in utilization of fresh water in industries is a serious

problem in India and all over the world. There are other reasons for the water crisis such as unbalanced population growth, desire for better living standards, improper utilization of water by humans and inefficient utilization of water in irrigation. A significant amount of fresh water is polluted due to industrialization. Thus, this part of fresh water is not available for various applications. This also leads to the water crisis. A joint report by United Nations Children's Fund (UNICEF) and World Wide Fund for Nature (WWF) published in 1998 predicted that India will be water stressed by 2017 (UNICEF, 2013).

Water pollution is attributed to industrial effluents and sludge. The environmental regulatory boards across the globe have set some norms to curb the water pollution. In India, concentration based standards are employed to quantify the pollutants. Industries can meet the required standards by diluting the effluents with clean water which leads to water pollution and water crisis. The major challenge faced by us is to effectively conserve, manage, and utilize the water. Now-a-days regulators are focusing towards the use of pollution load based standards to determine the total amount of pollutants released per unit production. This pollution load based standards use quota system to allow amount of water given to various industries. Therefore, with this standard, the amount of fresh water consumed can also be calculated along with monitoring pollution levels (Gupta and Babu, 2008). It is more realistic as it gives emphasis to the actual consumption of fresh water by industries. Efficient effluent treatment systems can be installed in industries to meet the stringent regulatory limits of various pollutants. The reclaimed wastewater after the treatment can be further reused in the same industrial processes which helps to reduce the requirement of fresh water significantly.

1.2. Heavy metals in wastewater

Water pollution is occurred due to direct release of industrial wastes into waterways and onto land. These wastes percolate through soil and contaminate the groundwater. These wastes contain various toxic heavy metals. Metals are integral part of nature and play an integral role in the life cycles of living organisms (Valls et al., 2000). Heavy metals are elements having atomic weights between 63 and 200 (Srivastava and Majumder, 2008). Some heavy metals such as chromium (Cr), copper (Cu), cobalt (Co), magnesium (Mg), manganese (Mn), nickel (Ni) and zinc (Zn) are essential up to a maximum concentration limit and serve as micronutrients. Many other metals, such as cadmium (Cd), lead (Pb), mercury (Hg), etc. have no biological role and are non-essential. These metals are potentially toxic to humans and other living organisms. At high concentration levels, both essential and non-essential metals could change enzyme activity, imbalance cellular functions and destroy cell membranes and the structure of DNA (Bruins et al., 2000). Over the last few years, the contamination level of these heavy metals in water ways and soils have increased at an alarming rate. As a consequence, concentrations of toxic metals in vegetables and grains have increased. This poses a significant threat to humans, other living organisms and the environment due to the toxicity, non-biodegradability and bioaccumulation of heavy metals (Bahadir et al., 2007; Reddad et al., 2003). In order to sustain the ecological stability (flora and fauna etc.), there is a need to treat the contaminated water up to its permissible limit before actually disposing it to the environment. The maximum contamination level (MCL) values for various heavy metals in surface and ground water as given by the US Environmental Protection Agency (USEPA) are summarized in Table 1.1.

Table 1.1. Selected heavy metals and their regulatory limits in surface and ground water as per USEPA

(USEPA, 2009)

S No	Heavy Metals	Maximum contamination level (mg L^{-1})
1	Arsenic (As)	0.010
2	Lead (Pb)	0.015
3	Mercury (Hg)	0.002
4	Chromium (Cr)	0.010
5	Cadmium (Cd)	0.005
6	Zinc (Zn)	5.000
7	Copper (Cu)	1.300
8	Manganese (Mn)	0.050
9	Silver (Ag)	0.050

Most of the sources of these heavy metal pollutants are wastewater released from mining, metal processing, tanneries, pesticides and textile industries (Das and Mishra, 2008; Ramakrishna and Philip, 2005; Srivastava and Majumder, 2008). Out of these sources, metal processing; tanneries and textile industries are the biggest generators of various toxic heavy metal ions such as Cr, Cu, Zn etc. (Ramakrishna and Philip, 2005; Savin and Butnaru, 2008). These heavy metals play a significant role in polluting the fresh water.

Chromium (Cr) is considered as most toxic and highly soluble metal pollutant. It is used in various metal processing and chemical industries (Kotaś and Stasicka, 2000). Thus, its presence is dominant in most of the industrial effluent streams (such as effluents from tanneries, cement, metal processing, paint industries, etc.) as compared to other heavy metal ions. Different diverse forms of Cr exist in the environment such as trivalent Cr(III), pentavalent Cr(V), hexavalent Cr(VI) etc., of which hexavalent chromium, Cr(VI), is a potential soil and water contaminant (Cervantes et al., 2001). It is listed as possible human carcinogen and mutagenic to living organisms (USEPA, 2009). It also affects the human skin, kidney, liver and respiratory tracts which result in various diseases such as dermatitis, renal failure, hepatitis, asthma, perforation nasal septum, pulmonary cancer etc (Baral and Engelken, 2002; Srivastava and Thakur, 2006; Venitt and Levy, 1974).

Copper (Cu) is an important trace element for most living organisms since it is the main constituent of metalloenzymes and proteins which are involved in electron transport and redox reactions. It exits in environment in two forms, monovalent Cu(I) and divalent Cu(II). Cu(I) is unstable and readily undergoes transition to form stable Cu(II). Cu(II)

compounds find applications in various industries such as floatation reagent in mining industry, fertilizers and pesticides, preparation of catalysts, petroleum refineries, textile industries, synthetic rubber manufacturing etc. The discharges of these industries contain unconverted Cu(II). High doses of Cu(II) can cause anaemia, liver & kidney damages, stomach & intestinal irritation, central nervous system irritation and Wilson's disease leading to brain and liver damage (Jaman et al., 2009; Yao et al., 2010).

Zinc (Zn) acts as a micronutrient for human body. The most stable ionic state is divalent Zn(II). It plays a very important role in maintaining the functions of living organisms such as wound healing, cell growth, immune systems etc. Zn(II) has antioxidant properties which protects skin and body muscles from aging. It finds its applications in various industrial processes such as manufacturing, paint, rubber, cosmetics etc. High dosage of Zn(II) (80 mg L^{-1}) causes malfunctioning of kidney in human beings (Johnson et al., 2007). Inhalation of freshly formed zinc oxide during galvanization process may induce "zinc chills" in humans which causes fever, nausea, headache, vomiting and joint pains. Since these heavy metals are used as valuable resources in various industries, their removal; recovery and recycling have greater significance.

1.3. Physico-chemical treatment techniques

Various environmental regulatory boards across the globe have set some norms for minimizing the levels of heavy metals such as Cr(VI), Cu(II), Zn(II) etc. in water bodies. This has created an added responsibility on industries to treat their effluents to comply with permissible limit for each of these heavy metals as reported in Table 1.1. Over the

last few decades, various physico-chemical and biological-based methods have shown to be potential candidates for the removal of heavy metals from wastewater such as ion-exchange (Abdelwahab et al., 2013; Kim et al., 1998; Sapari et al., 1996), chemical precipitation (Benefield and Morgan, 1999; Charerntanyarak, 1999; Wang et al., 2004), electrochemical precipitation (Golder et al., 2007; Kurniawan et al., 2006), coagulation-flocculation (Charerntanyarak, 1999; Semerjian and Ayoub, 2003; Shammas, 2004), membrane filtration (Ahn et al., 1999; Juang and Shiau, 2000; Saffaj et al., 2004), adsorption (Gupta and Babu, 2009; Hamadi et al., 2001; Monser and Adhoum, 2002) etc. Most of the physico-chemical methods (ion-exchange, chemical precipitation, electrochemical precipitation and adsorption) are effective, but the metal solubility is highly affected by changes in pH. These treatment methods suffer from serious limitations. Biological-based methods also suffer from limitations such as slower reaction rate as compared to physico-chemical methods and challenges in scale-up of the operation.

Ion exchange method is highly selective and specific. Ion exchange resins need to be replenished with fresh resins which incurs high operating cost. Chemical precipitation finds less application in removal of heavy metals from aqueous solution as metals do not precipitate out by simple pH adjustment. A large amount of chemical is needed in chemical precipitation to reduce concentration of metals up to acceptable levels for discharge (Juttner et al., 2000). Other drawbacks includes production of sludge, associated cost for sludge disposal, slow metal precipitation and the environmental impacts of sludge disposal (Yang et al., 2001). In electrochemical precipitation, the removal efficiency is largely affected by low pH and the presence of other ions. It

requires addition of other chemicals, which eventually leads to the generation of toxic sludge. Direct disposal of this sludge to the environment creates hazards. The major limitation associated with coagulation–flocculation is high operational cost due to excessive consumption of chemicals. Membrane filtration has a major limitation i.e. fouling of membrane. It decreases the performance of the membrane over time which eventually results in reducing the permeate flow rate. This limitation restricts its application in wastewater treatment. In membrane filtration, high energy is consumed and it also requires experienced personnel to run the process (Slater et al., 1983). Moreover, the selection of the appropriate membrane is a crucial judgement for effective removal of the pollutants. In-spite of having greater significance and technical applicability, adsorption may not be an efficient and cost effective method to treat heavy metals due to frequent regeneration of adsorbents.

High usage of chemicals and ineffective metal removal are the major drawbacks associated with conventional treatment techniques. These methods are also relatively expensive and have high environmental impacts due to disposal of secondary wastes. These disadvantages can further increase the cost of the removal process in case of contaminated ground waters and industrial wastewaters due to the presence of low metal contamination in voluminous amount of effluents (Malik, 2004). Moreover strict environmental regulations compel various industries for the development of economical, environmental friendly and efficient treatment technique for metal laden effluents (Malik, 2004).

1.4. Bio-based separation techniques

Bio-based separation techniques can be successfully applied in the removal of heavy metals from wastewater. In these techniques, living or non-living microorganisms are employed to scavenge the heavy metals such as Cr(VI), Cu(II), Zn(II) etc. from aqueous solution. Selectivity in removing the desired heavy metal ions is an added advantage of bio-based separation techniques (Mehta and Gaur, 2005). These techniques are proved to be economical and eco-friendly for the removal of heavy metal ions (Srivastava and Majumder, 2008). These factors have initiated research on the bio-based treatments for metal removal.

Various microorganisms have been identified for metal removal. These are bacteria, yeasts, fungi, mycelia and marine algae (Hartmeier and Berends, 1995; Luef et al., 1991; Volesky and Holan, 1995). Few more studies were also carried out for the removal of different heavy metals using bacteria (Aryal and Liakopoulou-Kyriakides, 2014; Barbosa et al., 2014; Batool et al., 2014; Elangovan et al., 2006; Ge et al., 2015; Islam et al., 2014; Jeyasingh et al.; 2010; Kang et al., 2014; Kilic et al., 2014; Mishra et al., 2010; Mohamed, 2015; Ramakrishna and Philip, 2005; Somasundaram et al., 2009; Yadav et al., 2013), yeasts (Amirnia et al., 2015; Ertuğrul et al., 2009; Infante J et al. 2014; Machado et al., 2010) and fungi (Abd Al Hameed et al., 2015; Mishra and Malik, 2014; Srivastava and Thakur, 2006). These microorganisms adopt various mechanisms to remove heavy metals from aqueous solution which include transport through cell membrane, biosorption to cell walls, extracellular entrapment, oxidation-reduction reactions etc. (Brady et al., 1994; Huang et al., 1990; Krauter et al., 1996; Rai et al., 1981; Veglió et al., 1997).

In 'biosorption' or 'passive uptake', the metals are entrapped in the cellular structure of the microorganisms. These metals are eventually biosorbed onto the binding sites present in the cellular structure. Cell metabolic cycle can also play a major role in transporting the heavy metals inside the cell through cell membrane. This mode of metal uptake is known as ‘active uptake’ or 'bioaccumulation'. In recent past, biosorption studies have been extensively used to treat heavy metal contaminated aqueous solution due to its low cost and ease of availability of dead biomass (Mogollón et al., 1998). However, it has been reported that live microorganisms posses better metal intake capacity than dead biomass (Kapoor et al., 1999).

Studies revealed the fact that the effectiveness and high specificity of active microbial cells in metal binding is due to physical adsorption and subsequent continuous metabolic uptake of heavy metals (Sandau et al., 1996; Wilde and Benemann, 1993). This fact led to the use of growing microbial cells in the removal of heavy metals which avoids the need of separate biomass production process (such as cultivation, harvesting, drying, storage etc.) during the metal uptake (Malik, 2004). The accumulated heavy metals inside the microbial cells can be extracted out by reactive extraction (Vaxevanidou et al., 2008) or sonication (Xing et al., 2012) methods. Biological treatment techniques such as bioremediation, biofiltration etc. employ growing cells of microorganisms to treat heavy metal contaminated wastewater.

1.4.1. Bioremediation

Bioremediation is a removal process that employs biological route to reduce the toxicity of a pollutant to an acceptable value. In this process, the microorganisms transform or degrade various pollutants in the environment. Bioremediation follows the principle of

microbial metabolism. This brings use of various biochemical reactions which help in activity, growth and reproduction of microorganisms. This microbial metabolism system allows microorganisms to regain carbon, electrons and other necessary components for their existence. During bioremediation process, heavy metals are penetrated into the cells, get attached to intracellular proteins and then are associated with vacuoles or other intracellular sites (Malik, 2004; Srivastava and Majumder, 2008). This mechanism possesses less risk of metal releasing back to the environment due to its irreversibility (Gekeler et al., 1988). Bioprocess techniques are employed to recover these metals from intracellular proteins. Microorganisms transform the non-biodegradable toxic heavy metals into non-hazardous or less hazardous biochemical species which can be safely disposed to the environment (Srivastava and Majumder, 2008).

The selection of microorganisms (bacteria, fungi, yeast etc.) and type of culture (pure or mixed strain) for the remediation of particular heavy metal plays an important role in the successful application of this process (Malik, 2004). Several studies have been carried out for the bioremediation of various heavy metals using pure strains and are listed in Table 1.2. It has been reported that the use of pure strain may lead to the formation of toxic intermediates. Moreover, pure strain gives less stability during in-situ condition. Mixed consortium of species can better survive in extreme conditions like industrial wastewater of high pH or high metal ion concentrations (Pümpel et al., 2001). During in-situ condition, the positive interaction between the species helps in survival of selective species which leads to better removal of specific heavy metals from industrial wastewater (Bradshaw et al., 1998). Few studies have been reported for the bioremediation of various heavy metals using mixed consortium of species (Table 1.2).

The bioremediation ability of the microorganisms can be increased by acclimatizing the mixed culture. Acclimatization is a process in which the growth of the mixed species is trained in a controlled environment by using nutrients and a particular substrate (Raghuvanshi and Babu, 2010). It enhances the rate of bioremediation of the particular toxic species. Considering all these characteristics, the applicability of acclimated mixed culture in bioremediation of heavy metals from industrial wastewater appears to be promising.

Table 1.2. Microorganisms and mixed consortia with heavy metal removal capacity

Microorganisms	Toxic heavy metals removed	References
Bacterial species		
Escherichia coli	Hg, Ni	Deng et al. (2003)
Pseudomonas species	Cr, As	Valls et al. (2000)
Desulfovibrio spicies	Cu, Zn, Ni, Fe, As	Jong and Parry (2003)
Leptothrixspecies	As, Mn, Fe	Katsoyiannis and Zouboulis (2004)
Thiomonas species	As, Fe	Casiot et al. (2003)
Bacillus species	Cr	Elangovan et al. (2006)
Pseudomonas aeruginosa	Cr	Batool et al. (2014)
Ochrobactrum intermedium	Cr	Batool et al. (2014)
Brevibacterium sp.	Cr	Ge et al. (2015)
Stenotrophomonas sp.	Cr	Ge et al. (2015)
Pseudomonas aeruginosa	Zn	Islam et al. (2014)
Sample E	Cr	Jeyasingh (2010)
Pseudomonas aeruginosa	Cr	Kang et al. (2014)
Fungal species		
Aspergillus niger	Ni, Cu, Pb, Cr	Dursun et al. (2003)
P.Chryogenum	Cu, Zn, As, Ni	Loukidou et al. (2003)
Trametes versicolor	Cr, Co	Blánquez et al. (2004)
Mucor rouxi	Cd, Pb, Zn, Ni	Yan and Viraraghavan (2000)
Aspergillus sp.	Cr	Srivastava and Thakur (2006)
Isolates of *Aspergillus* genus	U, Cr, Cu, Zn, Co, Pb	Abd Al Hameed et al. (2015)
Fomitopsis palustris or *Antrodia xantha*	Cu	Hattori et al. (2015)
Ustilago maydis and *U. digitariae*	Cd, Cr, Cu, Zn, Ni	Sargin et al., (2016)
Neocosmospora sp., *Trichoderma* sp., *Rhizopus* sp.	As	Srivastava et al. (2011)
Algal species		
Brown algae	Cu, Cd, Zn, Pb, Cr, Hg	Davis et al. (2003)
Green algae	Cu, Hg, Fe, Zn, Pb, Cd	Haritonidis and Malea (1999)
Nanochloropsis, Pavlova lutheri, Tetraselmis chuii and *Chaetoceros muelleri*	Zn	Richards and Mullins (2013)
Spirulina platensis	Cu	Hadiyanto et al. (2014)
Chlorella vulgaris	Cu	Hadiyanto et al. (2014)
Chlorella vulgaris	Cr	Indhumathi et al. (2014)
Spirulina platensis	Zn	Kőnig-Péte et al. (2014)

Microorganisms	Toxic heavy metals removed	References
Mixed consortia		
Sulfate-reducing bacterial consortium	Cu, Zn, Ni, As	Jong and Parry (2003)
Enterobacter sp.+ *Stenotrophomonas* sp. + *Comamonas* sp.+ *Ochrobactrum* sp.	Cu, Cd, Co, Cr	Ebtesam El. Bestawy et al. (2013)
Bacterial consortium	Cd, Cu, Cr, Ni, Pb, Zn	Singh et al. (2012)
Acidithiobacillus thiooxidans + *Acidithiobacillus ferrooxidans*	Cr, Cu, Pb, Zn	Akinci and Guven (2011)
Sulfate-reducing bacteria	Ni, Mn, Cu	Barbosa et al. (2014)
Aspergillus lentulus + *Aspergillus terreus* + *Rhizopus oryzae*	Cr, Cu	Mishra and Malik (2014)
Bacterial consortium	Cu	Mejias Carpio et al. (2014)
Sulfate-reducing bacterial consortium	Cd, Cu, Ni, Fe, Pb, Zn	Kiran et al. (2016)
Sulfate-reducing bacterial consortium	Cu, Zn, Ni, Cr	Kieu et al. (2011)
Sulfate-reducing bacterial consortium	Cr	Márquez-Reyes et al. (2013)
Sulfobacillus thermosulfidooxidan + *Thermoplasma acidophilum*	Cu, Zn, Ni, Cd, Al, Cr, Pb	Ilyas et al. (2014)
Algal-bacterial consortium	Cu, Ni, Cd, Zn	Muñoz et al. (2006)
Consortium of *Pseudomonas* sp.	Pb, Ni, Cr. Mg	Jadhav et al. (2010)
Pseudomonas sp. + *Pantoea* sp. + *Enterobacter* sp.	U	Chabalala and Chirwa (2010)
Bacterial consortium	Pb, Cd	Wong et al. (2015)

Microorganisms	**Toxic heavy metals removed**	**References**
Mixed consortia		
Sulfate-reducing bacterial consortium	Cu, Zn, Ni, As	Jong and Parry (2003)
Enterobacter sp.+ *Stenotrophomonas* sp. + *Comamonas* sp.+ *Ochrobactrum* sp.	Cu, Cd, Co, Cr	Ebtesam El. Bestawy et al. (2013)
Bacterial consortium	Cd, Cu, Cr, Ni, Pb, Zn	Singh et al. (2012)
Acidithiobacillus thiooxidans + *Acidithiobacillus ferrooxidans*	Cr, Cu, Pb, Zn	Akinci and Guven (2011)
Sulfate-reducing bacteria	Ni, Mn, Cu	Barbosa et al. (2014)
Aspergillus lentulus + *Aspergillus terreus* + *Rhizopus oryzae*	Cr, Cu	Mishra and Malik (2014)
Bacterial consortium	Cu	Mejias Carpio et al. (2014)
Sulfate-reducing bacterial consortium	Cd, Cu, Ni, Fe, Pb, Zn	Kiran et al. (2016)
Sulfate-reducing bacterial consortium	Cu, Zn, Ni, Cr	Kieu et al. (2011)
Sulfate-reducing bacterial consortium	Cr	Márquez-Reyes et al. (2013)
Sulfobacillus thermosulfidooxidan + *Thermoplasma acidophilum*	Cu, Zn, Ni, Cd, Al, Cr, Pb	Ilyas et al. (2014)
Algal-bacterial consortium	Cu, Ni, Cd, Zn	Muñoz et al. (2006)
Consortium of *Pseudomonas* sp.	Pb, Ni, Cr. Mg	Jadhav et al. (2010)
Pseudomonas sp. + *Pantoea* sp. + *Enterobacter* sp.	U	Chabalala and Chirwa (2010)
Bacterial consortium	Pb, Cd	Wong et al. (2015)

1.4.2. Biofiltration

Biofiltration technique is employed to treat large quantities of heavy metals from wastewater in continuous mode. This technique has been used since 1960. However, for the last few decades biofiltration has been emerged as a viable heavy metal separation technique in various metal processing and solvent industries. In this technique, microorganisms are attached to a porous medium which eventually forms the biofilm. This biofilm is responsible for detoxifying pollutants present in the industrial wastewater.

Biofiltration differs from other biological treatment methods as in this technique, microbial population is immobilized to the packing material (static) and the treated fluid flows through pores of packing materials (mobile). The packing medium consists of inert substances which gives large surface area for attachment of the microorganisms. The successful operation of a biofilter depends on various characteristic properties of the support medium such as porosity, water retention capabilities, degree of compaction, ability to attach microbial populations etc (Srivastava and Majumder, 2008).

In a biofilter, the heavy metals are removed due to biological remediation. Toxic heavy metals are passed into a wet biolfilm layer surrounding the support medium and are aerobically converted to less toxic chemical species, water and biomass. Biofiltration technique incorporates all the basic processes such as adsorption, absorption, bioremediation etc. For the successful operation of biofilter, a wet layer of biomass needs to be maintained on the surface of the packing medium. The choice of packing medium depends on following factors (Srivastava and Majumder, 2008): (i) high bed porosity for the uniform distribution of the pollutants throughout the packing, (ii) high attachment area for the growth of the microbial species and (iii) high water retention capacity to

sufficiently wet the packing material. Various materials are used for packing such as compost, soil, coal, sand, peat, activated carbon etc. Compost develops back pressure in the biofilter due to gradual compaction with time and it gives aging effect due to microbial mineralization. Peat and coal impose certain disadvantages which include provision for less nutrient supply and not providing a better medium for the growth of microorganisms. Hence, an indigenous packing material needs to be developed which gives better functioning over the above mentioned packing materials. The performance of biofilter also depends on the inlet loading (IL) of heavy metals, empty bed residence time (EBRT), nutrient composition and its flow rate.

1.4.3. Theoretical aspects of bioremediation and biofiltration

The modeling of biofilters dates back to early 1980s. Bioremediation and biofiltration employ complex physical, chemical and biological processes. Successful application of biofiltration technique in heavy metal removal depends on the thorough understanding of these processes which can be expressed in the form of various microbial rate kinetic models, growth kinetic models, chemical and biochemical reactions. Rate kinetic models help to describe the bioremediation of heavy metals by microbial culture using zero-order, first-order, second-order and three-half-order reactions. The determination of rate kinetic parameters is also important to understand the behavior of biofilter system. It helps in predicting the efficiency of the biolfilter column at a specified operating condition. More recently, emphasis is given to estimation of various growth kinetic constants by using kinetic models (Monod and Monod-Inhibition models). Several bio-kinetic constants are determined by using Michaelis-Menten model and Ottengraf-Van

den Oever model. These constants help to understand the behavior of microorganisms in the biofilter column (Srivastava and Majumder, 2008).

1.5. Motivation for the present study

Contamination of groundwater, surface water and soil with toxic heavy metal ions is one of the major concerns in today's world. It is owing to the uncontrolled increment in population, industrialization and urbanization. A greatest challenge for the scientists is to tackle the heavy metals contamination that jeopardizes the environment and human health. Latest advancements in science and technologies parallel to industrialization have given many solutions to this problem. In the last few decades, various physico-chemical treatment techniques have been widely exploited (ion-exchange, chemical precipitation, electrochemical precipitation, adsorption etc.) to treat the heavy metals from wastewater (Abdelwahab et al., 2013; Ahn et al., 1999; Benefield and Morgan, 1999).

However, these techniques have failed to meet the desired contamination level of heavy metals set by the various regulatory bodies. Recently, focus was moved towards the more idyllic and eco-friendly approach, "bioremediation" for the treatment of heavy metals (Ahemad and Kibret, 2013). It is becoming one of most efficient techniques for the removal of heavy metals from wastewater. Bioremediation uses relatively less expensive and eco-friendly processes which has a high public acceptance. Acceptability of this techniques also lies in the fact that the bioremediation process can be carried out on-site in continuous mode by employing a biofilter column. Most of the metal processing industries emanate large amount of mixed metal ions (such as Cr(VI), Cu(II), Zn(II), Ni(II), Cd(II), Co(II) etc.) through the effluent. These metals need to be treated

simultaneously before releasing to the environment. Biofiltration technique has emerged as a viable alternative to this problem. Motivation to carry out the present study is emphasized by the tremendous potential of bioremediation and biofilter for the removal of heavy metals from wastewater.

1.6. Objectives

Based on the background of this area and limitations of the existing research, the objectives of the present study are formulated as:

1. To isolate and identify microorganisms for bioremediation of Cr(VI), Cu(II) and Zn(II) from aqueous solution.
2. To carry out batch bioremediation studies at different operating conditions for the removal of Cr(VI), Cu(II) and Zn(II).
3. To identify appropriate growth kinetic model and rate kinetic model in the presence of Cr(VI), Cu(II) and Zn(II).
4. To carry out biofiltration studies for the selected metal ions by varying various parameters such as inlet concentration, flow rate, bed height, pH etc.
5. To carry out the kinetic modeling of biofilter column using obtained experimental results.

1.7. Organization of thesis

An exhaustive literature survey for the removal of Cr(VI), Cu(II) and Zn(II) using pure and mixed culture is carried out and is given in Chapter-2. The details on experimental setup and experimental procedures for batch (bioremediation) and continuous

(biofiltration) studies are elaborated in Chapter-3. Chapter-4 deals with growth kinetic models and rate kinetic models applied for bioremediation studies. This chapter also emphasizes the kinetic models applied for biofilter column. Results obtained from batch and continuous experimental studies are analyzed and discussed in detail in Chapter-5. This chapter also includes the validation of proposed kinetic models with the obtained experimental data and data available in the literature. The results obtained from Fourier Transform Infrared Spectroscopy (FTIR) and Field-Emission Scanning Electron Microscopy and Energy-Dispersive X-ray (FESEM-EDX) analysis are also covered in Chapter-5. Concluding chapter (Chapter-6) deals with the important conclusions drawn from the present study and summary of the work carried out.

CHAPTER 2

LITERATURE REVIEW

Various studies on bioremediation and biofiltration were carried out for the removal of Cr(VI), Cu(II) and Zn(II) from wastewater. These are discussed in sections 2.1 and 2.2., respectively.

2.1. Batch bioremediation studies

Chang et al. (1997) investigated the biosorption kinetics of Pb(II), Cu(II) and Cd(II) using growing cells of *Pseudomonas aeruginosa* PU21. Initial concentration of all the metals was taken as 1000 mg L^{-1}. Maximum uptake capacity was obtained as 110 mg Pb(II) g dry $cell^{-1}$, 58 mg Cd(II) g dry $cell^{-1}$ and 23 mg Cu(II) g dry $cell^{-1}$ for *P. aeruginosa* PU21. Uptake capacity was found to be dependent on pH and growth phase of *P. aeruginosa* PU21.

Wang and Shen (1997) studied the reduction of Cr(VI) from wastewater using three bacterial species (*Escherichia coli* ATCC 33456, *Bacillus* sp. and *Pseudomonas fluorescens* LB300). Batch experimental data were analyzed using an enzyme based kinetic model. The model was developed by considering enzyme kinetics and finite reduction capacity of Cr(VI) to investigate the toxic effect of Cr(VI) on the bacterial species. It was observed that Cr(VI) reduction decreased when the maximum capacity of Cr(VI) was reached.

A copper ion tolerant bacteria *Thiobacillus ferrooxidans* DSM 583 was isolated by Boyer et al. (1998) after repeated subculturing method on ferrous sulphate oxidation

and protein. It was determined that *T. ferrooxidans* could uptake 700 mg Cu(II) g dry cell^{-1} for 1000 mg L^{-1} initial Cu(II) concentration.

Chen and Hao (1998) carried out a comprehensive review on the microbial reduction of Cr(VI) from industrial wastewater which was either plasmid or membrane-associated phenomena. It has been postulated that detoxification of Cr(VI) in a biological system generally occurs within the neutral pH range and Cr(III) co-precipitates with biosolids. This study emphasized the use of mixed microbial cultures for effective Cr(VI) reduction during in-situ operation.

Hassen et al. (1998) studied the biosorption of Cr(VI) and Cu(II) by *Pseudomonas aeruginosa* and *Bacillus thuringiensis*, respectively. The effects of these metals on growth of the bacteria, quantity of dry cells and protein synthesis were investigated. Uptake capacity was determined as 40.4 mg Cr(VI) g dry cell^{-1} and 1.8 mg Cu(II) g dry cell^{-1} for *P. aeruginosa* and *B. thuringiensis*, respectively.

Valls et al. (2000) studied the metal adsorbing phenomena of metallothioneins (MT) fused gram negative bacteria to treat heavy metals [such as Cr(VI) and Zn(II)] pollution in industrial sewage. In this work, they engineered *Pseudomonas putida*, a highly robust microorganism able to grow in polluted habitats in order to further increase its metal-chelating ability.

A detailed review on interaction of chromium with microorganisms was presented by Cervantes et al. (2001). Effect of chromium toxicity in microbial growth was discussed in detail. It was reported that chromate crosses the biological membranes of various bacterial species with the help of sulfate uptake pathway. The efficiency of the passage of Cr(VI) through cell membranes is very low due to the formation of insoluble

compounds with Cr(VI). Inside the cell, Cr(VI) is reduced to Cr(III) by various enzymatic or non-enzymatic reactions. The generated Cr(III) can be toxic for the cytoplasm of the microbial cell.

Srinath et al. (2002) carried out batch experiments for the removal of Cr(VI) from tannery effluent using two strains, *Bacillus circulans* and *Bacillus megaterium* isolated from same tannery effluent. Separate studies were conducted to compare the bioaccumulation capacity using living and dead cells of the two strains. It was observed that *B. circulans* and *B. megaterium* showed bioaccumulation of 34.5 and 32.0 mg Cr(VI) g dry cell^{-1}, respectively for 50 mg L^{-1} of initial Cr(VI) concentration. Biosorption by the dead cells was higher than the living cells due to pH conditioning of the dead cells.

The effect of initial pH and initial metal ion concentration on the removal of Cu(II), Pb(II) and Cr(VI) from aqueous solution using *Aspergillus niger* was investigated by Dursun et al. (2003). The optimum values of pH were determined as 5.0, 4.5 and 3.5 for Cu(II), Pb(II) and Cr(VI), respectively. Inhibition effect was observed by all the metals on the growth of *A. niger*. Maximum specific uptake capacity was determined as 15.6 mg Cu(II) g dry cell^{-1} and 34.4 mg Pb(II) g dry cell^{-1} at 100 mg dm^{-3} initial concentration of Cu(II) and Pb(II). Growth of *A. niger* was inhibited by 75 mg dm^{-3} initial concentration of Cr(VI).

Copper biosorption using *Pseudomonas cepacia, P. aeruginosa, P. fluorescens* and *P. testosteroni* were investigated by Savvaidis et al. (2003). Biosorption of copper by *P. cepacia* was significantly affected by the solution pH. It showed maximum biosorption (65.3 mg Cu(II) g dry cell^{-1}) at neutral pH. Results also suggested that copper uptake by

P. cepacia was solely due to surface binding and not due to intracellular accumulation by active transport.

Viamajala et al. (2003) reported significant reduction of Cr(VI) using stationary phase cultures of *Shewanella oneidensis* MR-1 grown in nitrate and fumarate. It was observed that initially *S. oneidensis* MR-1 reduced the Cr(VI) concentration rapidly, which was followed by a slower and steady decrease of Cr(VI) concentration. A model was developed to describe the kinetics of Cr(VI) reduction by two parallel mechanisms: (i) faster Cr(VI) reduction mechanism that was depleted quickly and (ii) slower mechanism sustained for longer period. Bio-kinetic parameters such as maximum specific reduction rate, initial Cr(VI) reduction rate due to the deactivating enzyme, coefficient analogous to a first-order deactivation were determined by fitting the developed model with the experimental data.

Katsoyiannis and Zouboulis (2004) used biological-oxidation process for the removal of As(III) and Cr(VI) from groundwater. They have examined the metal removing capacity of iron oxidizing bacteria. In addition, the oxidation of trivalent arsenic was found to be catalyzed by bacteria, leading to enhanced removal of arsenic. This was attributed to the fact that arsenic in the form of arsenites, cannot be efficiently adsorbed onto iron oxides.

Pal and Paul (2004) isolated 34 chromium-resistant bacterial species from serpentine soil of Andaman (India) to investigate the Cr(VI) removal efficiency under aerobic conditions. Out of 34 isolates, *Bacillus sphaericus* gave maximum Cr(VI) removal (>80 %) for 800 mg L^{-1} initial concentration of Cr(VI). Removal of Cr(VI) was affected by the addition of glucose and yeast extract as carbon sources. Optimum pH and

temperature for reduction of Cr(VI) were obtained as 6.0 and 25 °C, respectively. It was observed that Cr(VI) removal increased with increase in cell density and initial Cr(VI) concentration.

Chen et al. (2005) investigated the removal of Cu(II) and Zn(II) from aqueous solution using living and nonliving *Pseudomonas putida* CZ1 as functions of initial pH of the solution, initial metal concentration and reaction time. Optimum pH for Zn(II) removal by living and nonliving cells was found to be 5.0. For Cu(II) removal, optimum pH was determined as 5.0 and 4.5 by living and nonliving cells, respectively. The biosorption capacities of metals were found to be 27.6 and 13.2 mg Cu(II) g dry cell^{-1} for living and nonliving cells, respectively while it was 24.4 and 14.4 mg Zn(II) g dry cell^{-1} for living and nonliving cells, respectively. Results clearly showed that living cells of *P. putida* gave better removal over nonliving cells.

Lu et al. (2006) studied the removal of Pb(II), Cu(II) and Cd(II) from aqueous solution using *Enterobacter* sp. J1 isolated from wastewater of a local industry. Regeneration of biomass was also carried out. Experiments showed that *Enterobacter* sp. could uptake over 50 mg Pb(II) g dry cell^{-1}, 32.5 mg Cu(II) g dry cell^{-1} and 46.2 mg Cd(II) g dry cell^{-1} from 100 mg L^{-1} initial concentration of metals.

Tunali et al. (2006) investigated the biosorption of Pb(II) and Cu(II) ions from aqueous solution using *Bacillus* sp. isolated from metal polluted soil. The optimum values of initial metal concentrations, initial pH, contact time at constant temperature (25 ºC) for biosorption of metals were determined. *Bacillus* sp. showed maximum biosorption capacities of 92.27 mg Pb(II) g dry cell^{-1} and 16.25 mg Cu(II) g dry cell^{-1} at 250 mg L^{-1} and 200 mg L^{-1} initial concentration of Pb(II) and Cu(II), respectively.

The mechanism of Cr(VI) detoxification by microorganisms was presented by Cheung and Gu (2007). This study reported that in the presence of oxygen, Cr(VI) is catalyzed by soluble enzymes [such as Cr(VI) reductase (ChrR) and chromate reductase Class I flavoprotein (YieF)]. ChrR reduces Cr(VI) to Cr(IV) and Cr(III) via one electron shuttle followed by two electron shuttle. YieF does four electron transfer that reduces Cr(VI) directly to Cr(III).

Preetha and Viruthagiri (2007) studied the metal resistant capacity and metal ion accumulation capacity of *Rhizopus arrhizus*. It was reported that *R. arrhizus* could uptake 5.36 mg Cr(VI) g dry cell^{-1} in presence of Cr(VI), Cu(II) and Ni(II). Inhibition kinetics of bioaccumulation of Cr(VI), Cu(II) and Ni(II) using *R. arrhizus* were also investigated using Lineweaver–Burk plot, Aiba model and Bazua and Wilke models. Different model parameters were evaluated using experimental data. The Inhibition was found to be competitive.

Kaushik et al. (2008) demonstrated the application of pure culture *Azotobacter* sp. and two more bacterial isolates (B1 and B2) for removal of Cr(VI) from aqueous solution. Separate experimental studies were conducted using live and dead cells of three strains to investigate the effects of initial Cr(VI) concentration, biomass dose and contact time on biosorption and bioaccumulation of Cr(VI). It was observed that dead bacterial isolate B2 showed maximum Cr(VI) removal capacity (102 mg Cr(VI) g dry cell^{-1}) at 150 mg L^{-1} initial Cr(VI) concentration over other isolates. Live bacterial isolate B2 showed maximum bioaccumulation of Cr(VI) (106 mg Cr(VI) g dry cell^{-1}) over other isolates at initial Cr(VI) concentration of 150 mg L^{-1} and pH 4.0.

Lee et al. (2008) developed a consortium of various microorganisms for the removal of heavy metals such as Cu, Pb, Cr, Ni, Zn etc. from heavy metal-contaminated water. For all the metals, approximately 97~100 % of metal removal efficiency was obtained for an average concentration of 200 mg L^{-1} of each heavy metal.

Jeyasingh et al. (2010) carried out bioremediation studies for the treatment of Cr(VI) using indigenous microorganisms isolated from Cr contaminated site. Effects of different operating parameters such as moisture content, initial substrate and biomass concentrations on the bioremediation of Cr(VI) were investigated by conducting batch and constinuous studies. The minimum moisture content, initial substrate concentration and biomass concentration were determined as 40 %, 50 mg g^{-1} and 15 mg g^{-1}, respectively.

Bioremediation of Zn(II) using living, dead and dried biomass of *Fusarium* sp. was investigated by Velmurugan et al. (2010). The initial Zn(II) concentration was taken in the range of 10-320 mg L^{-1}. Removal efficiency was influenced by initial Zn(II) concentration, pH, temperature, agitation and inoculum volume.

Mangaiyarkarasi et al. (2011) carried out batch experimental studies to detoxify Cr(VI) under alkaline pH condition using gram-positive bacteria *Bacillus subtilis* isolated from tannery effluent contaminated soil. Removal efficiency of upto 100 % for 50 mg L^{-1} initial Cr(VI) concentration was reported at pH 9.0. Removal efficiency was decreased with decline in pH at acidic range. The kinetics of Cr(VI) reduction were investigated using Monod kinetic model. Saturation constant (K_s) was obtained as 0.00032 g L^{-1} which indicated high affinity of the organism to the metal.

Cerino-Cordova et al. (2012) applied an experimental design methodology to investigate the effects of temperature, pH, stirring speed and biomass dose on Cu(II) removal from aqueous solution using *Aspergillus terreus*. Optimization was carried out to find out the effect of main factors and their interaction on Cu(II) removal efficiency. The optimum operating conditions for Cu(II) removal were obtained as temperature of 50 ºC, pH 6.0, stirring speed of 50 min^{-1} and biomass dose of 0.175 g.

Copper tolerant bacteria were isolated from soil samples located near metal industries (Ölmezoğlu et al., 2012). Two isolates (N1c and N5a) with relatively high removal efficiency were selected for Cu(II) removal from wastewater. Experiments were conducted to determine the effects of initial Cu(II) concentration, pH, and temperature on the bioremoval efficiency of the growing isolates. Maximal bioremoval efficiency was obtained as 82 % and 75 % for N1c and N5a, respectively in 20 mg L^{-1} of Cu(II) containing medium at pH 6.8 and 30 °C.

Samuel et al. (2012) isolated three indigenous strains from the wastewaters of chromite mining sites at Sukinda Valley, Orissa, India. These strains were identified as *Bacillus subtilis*, *Acinetobacter junii* and *Escherichia coli*. The three isolates showed a high tolerance to chromate at 500-1000 mg L^{-1} of Cr(VI) concentration. Reduction rate of Cr(VI) was increased from 0.199-0.477 mg L^{-1} h^{-1} to 0.5-1.16 mg L^{-1} h^{-1} at 5-20 mg L^{-1} of initial concentration of Cr(VI).

Ghosh and Saha (2013) studied the bioremediation of Cu(II) from aqueous solution using *Stenotrophonas maltophilia* PD2. The influence of different process parameters such as intial Cu(II) concentration, initial pH, contact time was investigated. The process parameters were optimized using response surface methodology which

resulted 50 mg Cu(II) L^{-1} as initial concentration, pH 5.5, contact time 26 h and removal 90 %.

Kumar and Thatheyus (2013) investigated the removal of Zn(II), Ni(II) and Cr(VI) from wastewater of electroplating industry using *Escherichia coli*. Bacterial strain was subjected to 15, 20 and 25 % of metals concentration in the effluent for 20 days. The initial concentrations of Zn(II), Ni(II) and Cr(VI) were 739, 0.184 and 0.089 mg L^{-1}, respectively. Maximum removal efficiency was obtained as 100 % for Ni(II). Results also showed that approximately 69 % of Zn(II) was removed from the effluent.

Márquez-Reyes et al. (2013) investigated the ability of a sulfate-reducing microbial consortium for the removal of Cr(VI) and Pb(II) in aqueous solution using peat moss as carbon source. The batch study was conducted at different pH values to evaluate the sulfate-reducing activity and metal tolerance. Consortium was shown to be resistant to 2.6 mg Cr(VI) L^{-1} and 500 mg Pb(II) L^{-1}. The substrate affinity constants K_s and the ratio COD/SO_4^{-2} was determined as 740 mg COD L^{-1} and 0.71, respectively.

Panneerselvam et al. (2013) examined the efficiency of a novel bacterial consortium on the reduction of Cr(VI) to Cr(III) in minimal salt medium and storm water. Three Cr(VI) resistant bacteria *Bacillus endophyticus*, *Microbacterium paraoxydans* and *Bacillus simplex* were isolated from a tannery waste disposal site located at Mount Barker, South Australia. The three bacterial strains were tested both as an individual and as a consortia for the reduction of 100 mg Cr(VI) L^{-1}. The rate of Cr(VI) reduction was found to be 84.1-92.6 % and 31.3-38.3 % higher in minimal salt medium and storm water, respectively, compared to individual bacterial strain.

Singh et al. (2013) investigated the role of Mn(II), Fe(II) and Cr(VI) on Cd(II) removal in a multi metals simulated effluent using *Pseudomonas aeruginosa*. Overlay plots of multi response surface methodology was explored for simulated eflluent treatment potential. The results showed that about 80-90 % Cd, 85- 90% Mn, 50-55 % Fe and 70-75 % Cr removal could be obtained by fixing the pH, oxidation-reduction potential (mV) and one of the constituents in the simulated effluent.

Barbosa et al. (2014) investigated the efficiency of a mixed culture of sulfate reducing bacteria (SRB) for the simultaneous removal of Ni(II), Mn(II) and Cu(II) from aqueous solution. The mixed culture was cultivated at different lactate concentrations and at pH 7.0. Three different sets of synthetic wastewater were prepared by varying the initial concentration of Ni(II) (4, 20 and 50 mg L^{-1}), Mn(II) (1.5, 10 and 25 mg L^{-1}) and Cu(II) (1.5, 10 and 25 mg L^{-1}). The maximum sulfate removal efficiency was obtained as 98 % at a ratio of 2 for the chemical oxygen demand (COD) to sulfate.

Batool et al. (2014) studied the stress effect of variable chromate concentrations, pHs and temperatures on biokinetic parameters of two Cr(VI)-resistant bacterial strains *Pseudomonas aeruginosa* Rb-1 and *Ochrobactrum intermedium* Rb-2. Significant enhancement of the biokinetic parameters was observed at initial stress of 1000 µgmL^{-1}, pH of 7.0 and temperature of 37 °C. Transmission electron microscopy (TEM) of the cells revealed the non uniform distribution of Cr precipitates in the cytoplasm and outer membrane. Fourier transform infrared spectroscopy (FTIR) revealed the possible linkage of carboxyl, amino, sulpohonate and hydroxyl groups present on the bacterial cell surface with Cr(VI) ions.

Islam et al. (2014) isolated plant growth promoting bacterial strain *Pseudomonas aeruginosa* from arable land irrigated with industrial wastewater and utilized for the removal of Zn(II) from wastewater. In this study the biomass production, nutrient uptake and oxidative stress tolerance in relation to the activities of the antioxidant enzymes were determined. Investigations revealed the fact that *P. aeruginosa* is a potential candidate for bioremediation of Zn(II) and wheat growth promotion against Zn-induced oxidative stress.

Mishra et al. (2014) conducted batch studies to investigate the removal of Zn(II) from industrial effluent by Zn(II) removing bacterium *VMSDCM*. Various biosorption models were found unsatisfactory after validating with the obtained experimental data. Based on the results, a new model was proposed to interpret the biosorption of Zn(II) on the surface of bacterium. The uptake capacity of Zn(II) was obtained as 431.5×10^3 mg g dry cell^{-1}.

Ge et al. (2015) investigated the Cr(VI) removal ability of two bacterial strains *Brevibacterium* sp. K1 and *Stenotrophomonas* sp. D6 isolated from soil samples. These two bacterial strains showed complete reduction of Cr(VI) in Luria-Broth medium at 200 mg Cr(VI) L^{-1} within 72 h. Analyses revealed the fact that D6 cells reduced Cr(VI) more effectively than K1. It was concluded that D6 was the more effective bacterium for the removal of Cr(VI) from industrial effluents.

Mohamed (2015) carried out the biosorption experiments for the removal of Zn(II) and Cd(II) using *Klebsiella pneumoniae* KM609983. Effects of operating parameters such as solution pH and contact time on biosorption of metals were investigated at 20 and 40 mg L^{-1} initial concentrations of each metals. Maximum

biosorption capacity of biomass was obtained as 243.9 and 227.3 mg g^{-1} for Zn(II) and Cd(II), respectively.

Wong et al. (2015) reported the biodegradation of phenol and bioremediation of Pb(II) & Cd(II) from a mixed -pollutant medium using a bacterial consortium. This consortium displayed specific growth rate of 0.04 h^{-1}, phenol degradation rate of 6.11 mg L^{-1} h^{-1} and biomass of 8.45 ± 0.35 (log_{10} CFU mL^{-1}) at the highest phenol concentration of 1200 mg L^{-1}. The highest metal removal was obtained in 0.1 mg Pb(II) L^{-1} (44.9 %) and 0.01 mg Cd(II) L^{-1} (45.3 %). These findings confirmed the dual function of the consortium for the degradation of phenol and the removal of heavy metals.

Kiran et al. (2016) investigated the combined effect of Cd(II), Cu(II), Ni(II), Fe(III), Pb(II) and Zn(II) on each other removal by sulfate reducing bacterial biomass. The maximum removal of metal is obtained in the following order Cu(II) (98.9 %) > Ni(II) (97 %) > Cd(II) (94.8 %) > Zn(II) (94.6 %) > Pb(II) (94.4 %) > Fe(III) (93.9 %). Analysis of variance (ANOVA) of the sulfate and chemical oxygen demand (COD) reduction showed that the effect due to copper on sulfate and COD removal was highly significant.

2.2. Continuous column and reactor studies

After development and selection of suitable strain, the next step is to develop a continuous biofilter column for treating voluminous amount of heavy metals [i.e. Cr(VI, Cu(II) and Zn(II)] present in the wastewater. A set of studies have reported improvement in the metal removal by immobilization of bacterial cells on solid surfaces (Ghozlan et al., 1999; Yusef, 1997).

Chirwa and Wang (1997) demonstrated the potential for fixed-film bioreactor for the detoxification of Cr(VI) using *Bacillus* sp. The bioreactor was operated under a range of Cr(VI) concentrations (10-200 mg L^{-1}) and hydraulic detention times (6-24 h). Complete removal of Cr(VI) was achieved at the outlet of the bioreactor. It was observed that total biomass in the bioreactor decreased with increasing Cr(VI) loading. It was stabilized after a loading limit reached to 1016 mg of Cr(VI) L^{-1} day^{-1} under 24 h hydraulic detention time.

Jong and Parry (2003) conducted continuous experimental studies in a bench scale bioreactor using coarse pool filters sand as adsorbent for the removal of Cu, Zn and Ni, As, Fe, Mg and Al from aqueous solution. Mixed population of sulfate reducing bacteria (SRB) was used to develop the biolayer on the adsorbent. Initial metal concentrations were 5, 10, 50, 10, 20, 20 and 20 mg L^{-1} for Cu, Zn, Ni, As, Fe, Mg and Al, respectively. Removal efficiencies for various metals were determined as: 97.5 % for Cu, Zn and Ni, more than 77.5 % for As, 82 % for Fe and no removal of Mg and Al.

Willow and Cohen (2003) investigated the effects of the pH on the removal efficiency of heavy metals from acid-rock drainage in an anaerobic bioreactor supplemented with sulfate reducing bacteria (SRB). Two conditions were considered to investigate the metal removal efficiency and hydraulic residence time required for the reactors: (i) near neutral pH, metal laden influent and (ii) low pH influent. The effects of oxygen content of feed water on metal removal were also investigated. The rate of bacterial remediation of metals was modeled with the zero-order kinetics. Less microbial sulfate reduction was observed in the reactor receiving low-pH drainage water with short residence time. It was attributed to the fact that the growth of sulfate-reducing bacteria

might have been inhibited by the high flow rates of low-pH water. Dissolved oxygen content of the feed waters did not have significant effect on metal removal capacity and sulfate reduction.

Dermou et al. (2005) constructed a pilot-scale trickling filter for the removal of Cr(VI) from industrial wastewater using mixed culture of microorganisms which was isolated from industrial sludge. Three different operating conditions (i.e. batch, continuous and Sequencing Batch Reactor (SBR) with recirculation) were considered to examine the optimal performance and efficiency of the filter. It was found that SBR gave better removal of Cr(VI) (530 g m^{-2} day^{-1}) than other two conditions. It gave an insight into a technologically viable alternative for the biological removal of Cr(VI) from wastewater.

Ekenberg et al. (2005) investigated the use of trickling biofilter packed with plastic carriers for the removal of Cr(VI) in leachate originating from ferrochromium slags. Column system was operated in upward flow mode and under anaerobic condition. Acetic acid was added as a carbon source. Reduction of Cr(VI) obtained was from 20 mg L^{-1} to below 0.03 mg L^{-1} at a hydraulic retention time of 11 h. It was observed that decrease in water temperature has significant effect on removal efficiency of Cr(VI).

Ramakrishna and Philip (2005) developed a biological system comprising of a leaching column followed by an immobolized bioreactor and a desorption unit for the bioremediation of Cr(VI) from contaminated soil. Two bacterial strains were isolated from chromium-contaminated soils and were used as potential agents for Cr(VI) removal in aerobic and facultative anaerobic conditions. The percentage removal of Cr(VI) was obtained as more than 80 % for 50 mg L^{-1} of initial Cr(VI) concentration within 8 h.

Adsorption column was able to remove significant amount of Cr(III) from the effluent of the biorector. The developed biosystem proved to be a viable alternative for the bioremediation of Cr(VI) contaminated soils.

Brunet et al. (2006) designed a pilot biofilter column of 200 m^3 volume to treat Cr(VI) polluted groundwater. The column was filled with pozzolona and supplemented with sulfate-reducing microorganism *Desulfomicrobium norvegicum*. H_2 + CO_2 gas mixture was passed through the column to maintain the pH in the range of 7.5-8.5. Experiments were carried out for 3 months using synthetic solution followed by real polluted groundwater containing 15 mg dm^{-3} Cr(VI). The residence time was determined as 7 h. The Cr(VI) concentration in the outflow obtained was lower than 0.2 mg dm^{-3}.

Dermou and Vayenas (2007) investigated removal of Cr(VI) from aqueous solution in two pilot-scale trickling filters using mixed aerobically grown indigenous culture. Two different filter media, plastic media and calcitic gravel were used. Feed concentrations of Cr(VI) in the influent were maintained at 5, 10, 20, 30, 50 and 100 mg L^{-1}. The concentration of organic carbon was maintained at 400 mg L^{-1} in the bulk liquid. Maximum reduction rate was obtained as 4.8 g Cr(VI) day^{-1} for Cr(VI) feed concentration of 5 mg L^{-1} using the filter with the plastic support material. For the filter with the gravel media, maximum reduction rate was found to be 4.7 g Cr(VI) day^{-1} for Cr(VI) feed concentration of 5 mg L^{-1}.

Zakaria et al. (2007) designed a biofilter column packed with wood-husk as support material for biofilm. Continuous experiments were carried out in the biofilter to detoxify Cr(VI) to Cr(III) using *Acinetobacter haemolyticus*. Initial concentration and the flow rate of Cr(VI) in the influent to the biofilter were maintained at 15 mg L^{-1} and 8 mL

min^{-1}, respectively. Around 97 % removal of the Cr(VI) was obtained. Final wastewater was disinfected using 0.1 % (v/v) formaldehyde which inhibited the growth of *A. haemolyticus*. This finding gave an insight into the possible solution in the direct introduction of exogenous bacterial species into the environment.

Tziotzios et al. (2008) investigated the efficiency of a packed bed bioreactor for combined removal of phenol and Cr(VI) from wastewater using mixed culture. Phenol was used as an electron donor which facilitates the reduction of Cr(VI) and removal of phenol itself. Bioreactor was operated in aerobic condition and under draw-fill mode with circulation. Continuous experiments were performed at 5.5 mg L^{-1} of Cr(VI) concentration and phenol concentration in the range of 350-1500 mg L^{-1}. Experiments revealed the fact that the reduction of Cr(VI) was inhibited by higher concentration of phenol. The maximum reduction rate of Cr(VI) obtained was 0.062 g Cr(VI) L^{-1} day^{-1} for 500 mg L^{-1} of phenol concentration while it was 3.574 g phenol L^{-1} day^{-1} for 500 mg L^{-1} of phenol concentration.

Zakaria et al. (2008) used a two-stage Cr(VI) removal system consisting of an adsorption unit by untreated rubber wood sawdust (URWS) and a bioremediation unit by bacterial species immobilized on URWS. Experiments were carried out using Cr(VI) concentrations in the range of 200-350 mg L^{-1} and using brown sugar as carbon source. It was reported that the two-stage Cr(VI) removal system gave 99.5-100 % removal of Cr(VI) for 236.5–320.4 mg L^{-1} of Cr(VI) concentration when operated continuously for 5 days. It was also observed that the URWS showed 16.7-67.3 % removal of Cr(VI) while remaining Cr(VI) was reduced to Cr(III) using immobilized bacterial species.

Three viable process alternatives i.e. aerobic suspended, aerobic attached and anoxic attached growth system had been reported by Elangovan and Philip (2009) for the biological removal of Cr(VI) from aqueous, synthetic and actual wastewater. *Arthobacter rhombi* RE (MTCC 7048) was isolated from Cr(VI) contaminated soil which was further enriched and used in all the bioreactors. Various bio-kinetic parameters were evaluated for aerobic and anoxic conditions in batch mode. The values obtained for aerobic system were: μ_{max} = 2.34 day^{-1}, K_s = 190 mg L^{-1}, K_i = 3.8 mg L^{-1} of Cr(VI) and Y_T = 0.377 while values for anoxic system were μ_{max} = 0.57 day^{-1}, K_s = 710 mg L^{-1}, K_i = 8.77 mg L^{-1} of Cr(VI) and Y_T = 0.13. It was found that aerobic attached growth system performed better (99.8 % removal for 20 mg L^{-1} of initial Cr(VI) concentration) than aerobic suspended and the anoxic attached growth system under identical conditions.

The enzymatic reduction of Cr(VI) to Cr(III) using *Acinetobacter haemolyticus* followed by chemical precipitation was investigated by Ahmad et al. (2010). Neutralized electroplating wastewater containing Cr(VI) (in the range of 17-81 mg L^{-1}) was used as influent to a biorector supplemented with culture of *A. haemolyticus*. The entire system produced 0.02 mg L^{-1} of Cr(VI) at the outlet. It was also investigated that the performance of the bioreactor was not affected by fluctuations in pH, inlet Cr(VI) concentration, nutrient and temperature.

Christian et al. (2010) examined the operations of a field-scale permeable bioreactor and a laboratory-scale bioreactor for the removal of Zn, Al, As, Cd, Fe, Ni and Pb from mine runoff waters. Both the reactors contained mixed compost, straw and gravel as medium for the development of the biofilm of sulfate reducing bacteria. Field-

scale bioreactors showed better removal for all the metals due to bacterial sulphate reduction and formation of metal sulphide complexes.

Jeyasingh et al. (2010) carried out batch and continuous studies for the treatment of Cr(VI) contaminated sludge using indigenous microorganisms. After conducting the batch studies, the performance of the treament process was evaluated in a pilot scale system (soil reactor). An attempt was made to develop a mathematical model for simulating the overall treatment process in continuous mode. The results were embedded into a management model using a simulation-optimization framework. The optimum values of initial soil amount, initial biomass, COD were obtained as 3189 kg, 25 mg g^{-1} and 69.5 mg g^{-1}, respectively.

Dalcin et al. (2011) carried out continuous studies in a tubular reactor supplemented with mixed culture immobilized on a polymeric support for the removal of Cr(VI). Response surface methodology was employed to optimize pH, initial Cr(VI) concentration and to determine their interaction on metal removal. The surface response showed optimal behavior at 3.94 mg L^{-1} of initial Cr(VI) concentration and at pH 6.2. The kinetics of Cr(VI) removal was described by the Michaelis-Menten kinetic model.

Kieu et al. (2011) investigated the removal of metals [Cu(II), Zn(II), Ni(II) and Cr(VI)] using a consortium of sulfate-reducing bacteria in five parallel anaerobic semi-continuous stirred tank reactors (R1-R5). The concentrations of each metal ion were maintained at 30, 60, 90, 120 and 150 mg L^{-1}. The reactors were operated with a hydraulic retention time of 20 days for 12 weeks. The loading rates of each metal in R1-R5 were 1.5, 3, 4.5, 6, and 7.5 mg L^{-1} d^{-1}, respectively. The results displayed heavy metal removal efficiencies in the range of 94-100 % for all the metals. It was also

observed that the growth of sulfate-reducing bacteria was not inhibited due to the presence of metals.

Rezić et al. (2011) carried out continuous study in a horizontal rotating tubular bioreactor (HRTB) for the removal of multiple metals Fe(III), Ni(II), Cu(II) and Zn(II) using mixed microbial culture. The experiments were conducted at different medium inflow rates (0.5, 1.0 and 2.0 L h^{-1}) and reactor rotation speed (5, 15 and 30 min^{-1}). The inlet glucose concentration in the medium and the inlet concentration of each metal were maintained at 10 g L^{-1} and 0.125 g L^{-1}, respectively. The metal removal efficiency was obtained in the range of 38.1-95.5 % at all combinations of operating conditions.

Li et al. (2013) evaluated the effectiveness of Cr(VI) bioremediation from soil in a bench-scale column using an indigenous bacteria. Effects of initial Cr(VI) concentration, particle size, circulation mode, spray intensity and the depth of soil on bioremediation of Cr(VI) were studied. The initial content of water soluble Cr(VI) was 1520.54 mg kg^{-1} of soil. Results showed that the final content of water soluble Cr(VI) in the soil is 0.68 mg kg^{-1} after 6 days of operation with spray intensity in the range of 29.6-59.2 mL min^{-1}.

Márquez-Reyes et al. (2013) investigated the effect of pre-treated peat moss (carbon source) on the removal of Cr(VI) and Pb(II) from aqueous solution in a up-flowpacked bed using consortium of sulfate-reducing microorganisms. The bed was operated at a flow rate of 8.3 mL min^{-1} for 180 h. The initial concentration of Cr(VI) and Pb(II) was 10 and 50 mg L^{-1}, respectively. The removal efficiency was obtained as 65 % and 90 % for Cr(VI) and Pb(II), respectively.

Altun et al. (2014) studied the removal of As(V) from synthetic acidic wastewater contaminated with As(V), Fe(II) and sulfate. The removal study was carried out in an

anaerobic up-flow fixed bed column at constant hydraulic rentention time (HRT) of 9.6 h. The entire experiment was operated for a period of 245 days under eight different operating periods. The concentration of As(V) and Fe(II) were maintained in the range of 0.5-20 mg L^{-1} and 100-200 mg L^{-1}, respectively. The concentration of sulfate in the influent was 2000 mg L^{-1}. Maximum removal of As(V) was obtained as 96 % by maintaining 200 mg L^{-1} of Fe(II) concentration in the influent.

Mejias Carpio et al. (2014) studied Cu(II) removal ability of a bacterial consortium in a fixed-bed column. The copper removal capacity of the live consortium and the effects of copper on the formation of consortium biofilm were also investigated. The bed was packed with granular activated carbon (GAC) which supports the consortium biofilm. Results showed that the biofilm could retain 45 % of Cu(II) mass present in the influent as compared to 17 % in the coulmn that contained GAC only.

Janyasuthiwong et al. (2015) evaluated the performance of two sulfate-reducing inversed fluidized bed bioreactors (IFBs) for the removal of Cu(II), Ni(II) and Zn(II) from a synthetic acid mine drainage. The effect of pH on metal removal was investigated at pH 7.0 and 5.0. The removal efficiency for Cu(II) and Zn(II) was obtained more than 90 % (initial metal concentration: 25 mg L^{-1}) at both pH values. It was observed that high concentrations of Ni(II) (25 mg L^{-1}) inhibited the sulfate reducing activities.

Lim et al. (2015) compared the heavy metal [Cu(II), Zn(II), Cd(II) and Pb(II)] removal ability of five different packing materials (potting soil, compost, coconut coir, sludge a commercial mix) using laboratory columns. It was observed that potting soil and commercial mix displayed best metal metal uptake at low [Cu(II): 44.78 µg L^{-1}, Zn(II): 436.4 µg L^{-1}, Cd(II): 1.82 µg L^{-1}, Pb(II): 51.31 µg L^{-1}] and high concentrations of metals

[Cu(II): 241 µg L^{-1}, Zn(II): 1127 µg L^{-1}, Cd(II): 4.57 µg L^{-1}, Pb(II): 90.25 µg L^{-1}]. Compost and sludge also displayed high metal removal efficiency (>90 %).

2.3. Gaps in existing research

From the above literature survey, it is evident that very few studies are reported for the removal of heavy metals from wastewater using biofiltration techniques. Toxicity study of wastewater samples collected from Taloja industrial belt, Mumbai, India revealed the fact that paper, textile, tanneries, fine chemicals, dyes and paint industries are the biggest generators of Cr(VI), Cu(II) and Zn(II) in mixed form (Lokhande et al., 2011). However, limited studies were conducted for simultaneous removal of Cr(VI), Cu(II) and Zn(II) from wastewater.

The literature on bioremediation and biofiltration experimental studies revealed that very few researchers have carried out the bioremediation and biofiltration for the removal of Cr(VI), Cu(II) and Zn(II) using acclimated mixed culture. Most of the studies are limited to the use of pure strain for the removal of heavy metals from wastewater. Pure strain has its own advantages as far as small scale systems or batch reactors are concerned. However, for actual industrial-scale applications, acclimated mixed cultures are preferred over the culture trained from pure strain. The mechanism of metal intake by microorganisms is still not well understood. Limited studies are reported on the mechanism of bioremediation of heavy metals. This requires bioremediation and biofiltration experiments to be carried out extensively. Various growth kinetic models and rate kinetic models are fitted with the data obtained from experiments to determine

the kinetic parameters. These parameters are also helpful in understanding the mechanism of biological transformation of heavy metals.

Past studies are mainly focused on the experimental work. Less attempts have been made for the kinetic modeling of biofilter column. Bioremediation and biofiltration experimental data are required to validate the available kinetic models, which is very useful for the designing of biofiltration column in industrial scale. Moreover, effects of different bioremediation process parameters (such as incubation time, temperature, initial metal ion concentration, pH etc.) on metal removal need to be investigated.

In summary, some major limitations are outlined below:

1. Bioremediation experimental studies for simultaneous removal of Cr(VI), Cu(II) and Zn(II) using a bacterial consortium are scarce.
2. Reported studies in the literature are mainly concerned with the use of microbial culture trained from pure strains.
3. The kinetics and mechanism of metal bio-transformation needs to be understood both with respect to experimentation and validation with the available kinetic models.
4. Effects of various process parameters (such as incubation time, temperature, initial metal ion concentration, pH etc.) for bioremediation of metals need to be investigated.

2.4. Scope of the present study

There is a need to carry out batch studies for the bioremediation of Cr(VI**),** Cu(II) and Zn(II) in order to understand the mechanism of transformation of these metals. In the

present study, batch bioremediation experiments are required to be performed using acclimated mixed culture. Growth kinetic models, Monod and Monod-Inhibition are to be fitted with the obtained experimental data. It helps in understanding the mechanism of microbial transformation of the metals. Final biomass is to be analyzed using Fourier Transform Infrared (FTIR) spectroscopy and Scanning Electron Microscopy (SEM) to investigate the role of various functional groups in metal binding and changes in morphology of the bacterial cell surface during metal sequestration, respectively.

There is scope to carry out the biofiltration (continuous column) studies for the removal of Cr(VI), Cu(II) and Zn(II) by varying different operating conditions. It helps to understand the adaptability of microorganisms in the changed operating conditions and during shock loading conditions. Biofiltration studies will help in estimating the desired parameters needed for designing the biofilter column on bench scale and during scale-up to industrial level. Kinetic aspects of bioremediation process during the biofiltration will also be understood by fitting the experimental data with the Michaelis-Menten kinetic model and Ottengraf-van-den Oever model.

CHAPTER 3

EXPERIMENTAL STUDIES

The present chapter provides the details of experimental studies carried out for the batch bioremediation and biofiltration of hexavalent chromium [Cr(VI)], divalent copper [Cu(II)] and divalent zinc [Zn(II)] from aqueous solution.

3.1. Batch bioremediation studies

The batch bioremediation studies are carried out for Cr(VI), Cu(II), and Zn(II) in 250 mL conical flask in a Biochemical Oxygen Demand (BOD) Incubator and Shaker (MSW-132, Macro Scientific Works, India) at 150 rpm and 37 °C.

3.1.1. Glasswares and chemicals

All glasswares (Conical flasks, Beakers, Petriplates, Test tubes, Measuring cylinders etc.) used in the present study are listed in Table 3.1. Pure and analytical grade chemicals used throughout the experiments including media preparation are listed in Table 3.2.

3.1.2. Preparation of reagents and media

In all experiments and analysis, de-ionized water (Milli-Q Millipore 15.2 MΩ cm^{-1}) is used. Aqueous solutions of Cr(VI), Cu(II) and Zn(II) (1000 mg L^{-1} each) are prepared by dissolving 2.828 g of anhydrous potassium di-chromate ($K_2Cr_2O_7$), 3.92 g of copper sulfate ($CuSO_4$. $5H_2O$) and 4.4 g of zinc sulfate ($ZnSO_4$. $7H_2O$), respectively in distillated water and the volume of the solutions are made up to 1000 mL. These stock solutions are further used to prepare different concentrations of aqueous Cr(VI), Cu(II) and Zn(II) solutions. One liter of minimal salt media (MSM) solution is prepared using mineral salts

in specific amount as given in Table 3.3. This composition of MSM is considered as standard composition in all experimental studies. A 10 g of D-glucose is dissolved in 100 mL of distilled water to prepare 10,000 mg L^{-1} of stock glucose solution. The nutrient agar media used for microbial growth are prepared by dissolving 5 g peptone, 1.5 g beef extract, 1.5 g yeast extract, 15 g agar and 5 g sodium chloride in 1 L of distilled water. The nutrient broth is prepared by using same components mentioned above except agar. The pH of all media is adjusted at 7.0±0.2 using 1 M HCl or 1 M NaOH. All solutions are sterilized in a Vertical Autoclave (MSW-101, Macro Scientific Works, India) at 15 kPa and 120 ^{o}C for 30 min before using for the experimental studies. The operating conditions for sterilization are maintained same throughout the experimental studies unless otherwise specified.

Five sets of simulated industrial effluent of tannery industries are prepared by varying the concentrations of Cr(VI), Cu(II) and Zn(II) for the bioremediation studies of mixed metals (Lokhande et al., 2011). The compositions of five sets of simulated industrial effluent are given in Table 3.4. The set 1 is prepared by adding 15.2, 23.85 and 15.25 mL of stock Cr(VI), Cu(II) and Zn(II) solutions, respectively in a volumetric flask and then the volume is made upto 500 mL. Similarly, other sets are prepared. The bioremediation study for all the sets is conducted in the presence of 100 mL of sterilized MSM and 400 mg L^{-1} of glucose.

Table 3.1. Glasswares used

Glasswares	**Make**
Conical flasks (250 mL)	Rankem®
Conical flasks (500 and 1000 mL)	Borosil®
Conical flasks (2000 mL)	Rankem®
Beakers (100 and 500 mL)	Borosil®
Petriplates	Borosil®
Test tubes (10 and 20 mL)	Borosil®
Measuring cylinders (100 and 1000 mL)	Borosil®

Table 3.2. Chemicals used

Chemicals	**Make**	**Use**
Potassium di-chromate ($K_2Cr_2O_7$, $14H_2O$)	Rankem®	Preparation of Cr(VI) stock solution
Copper sulfate ($CuSO_4$. $5H_2O$)	Rankem®	Preparation of Cu(II) stock solution
Zinc sulfate ($ZnSO_4$. $7H_2O$)	Rankem®	Preparation of Zn(II) stock solution
Di-potassium hydrogen phosphate (K_2HPO_4)	Rankem®	Preparation of MSM used as nutrient medium for microbial growth
Potassium di-hydrogen phosphate (KH_2PO_4)	Rankem®	-do-
Magnesium sulfate ($MgSO_4$. $7H_2O$)	Rankem®	-do-
Calcium sulfate ($CaSO_4$. $2H_2O$)	Rankem®	-do-
Ammonium sulfate [$(NH_4)_2SO_4$]	Rankem®	-do-
Ferrous sulfate ($FeSO_4$)	Rankem®	-do-
D-glucose	Avra	-do-
Peptone	CDH	Preparation of nutrient medium and broth
Beef extract	Rankem®	Preparation of nutrient medium and broth
Yeast extract	Rankem®	Preparation of nutrient medium and broth
Agar	Rankem®	Preparation of nutrient medium
Sodium chloride (NaCl)	SRL	Preparation of nutrient medium and broth
Hydrochloric acid (HCl)	CDH	pH adjustment
Sodium hydroxide (NaOH)	CDH	pH adjustment
Glutaraldehyde	Sigma-Aldrich	Staining for FESEM analysis
Osmium tetroxide	Fluka	Staining for FESEM analysis

Table 3.3. Composition of mineral salts used for MSM preparation

Mineral salts	**Amount (g L^{-1})**
Di-potassium hydrogen phosphate (K_2HPO_4)	0.8
Potassium di-hydrogen phosphate (KH_2PO_4)	0.2
Magnesium sulfate ($MgSO_4 \cdot 7H_2O$)	0.5
Calcium sulfate ($CaSO_4 \cdot 2H_2O$)	0.05
Ammonium sulfate [$(NH_4)_2SO_4$]	1.0
Ferrous sulfate ($FeSO_4$)	0.01

Table 3.4. Actual volume of stock solutions used and composition of heavy metals in synthetic wastewater

Set No	**Actual volume of stock solution (mL)**			**Composition in simulated industrial effluent (mg L^{-1})**		
	Cr(VI)	**Cu(II)**	**Zn(II)**	**Cr(VI)**	**Cu(II)**	**Zn(II)**
1	15.2	23.85	15.25	30.4	47.7	30.5
2	15.2	10.75	7.9	30.4	21.5	15.8
3	9.6	23.85	15.25	19.2	47.7	30.5
4	9.6	10.75	7.9	19.2	21.5	15.8
5	12.75	17.75	10.75	25.5	35.5	21.5

3.1.3. Microorganism culture conditions

An activated sludge consisting of aerobic mixed microbial culture is obtained from the Municipal Sewage Treatment Plant of Birla Institute of Technology & Science (BITS), Pilani, Pilani campus, India. One such sample is shown in Plate 3.1. The sludge is then added with distilled water and mixed thoroughly. It is kept for 3 h at room temperature in order to separate the dissolved impurities present in the supernatant. The supernatant is discarded and the sludge is retained as it contains the microbial culture. Ten grams of the sludge is taken and again thoroughly mixed with 100 mL of distilled water in a beaker. After gentle shaking, the sludge is allowed to settle for a short time (1 min) in order to screen out the settled impurities from the mixture. The supernatant is collected which contains microorganisms.

Two milliliter of the supernatant is taken in three centrifuge tubes. The centrifugation is carried out in a Centrifuge (CPR-24, Remi Cooling Centrifuge, India) for 2 min at 10,000 rpm and 4 °C. The centrifuge used in the present work is shown in Plate 3.2. After centrifugation, clear pellets are obtained in all the three centrifugation tubes. The top portion of the liquid is removed carefully from the centrifuge tubes without disturbing the pellets. Then the pellets are taken out with the help of loops and transferred in three 250 mL conical flasks inside a Laminar Airflow Chamber (MSW-101, Macro Scientific Works, India). The laminar airflow chamber used in the present work is shown in Plate 3.3.

3.1.4. Acclimatization procedure

Acclimatization of the culture is carried out in three conical flasks over a period of 6 days for Cr(VI), Cu(II) and Zn(II). One hundred milliliter of sterilized MSM is taken in three

250 mL conical flasks. Each conical flask is added with a pellet obtained after centrifugation process. The samples are supplemented with 1 mL of stock glucose solution to maintain the concentration at 1000 mg L^{-1}. These samples are then kept in a BOD incubator and shaker at 37 ^{o}C and at 150 rpm for 24 h to maintain proper aseptic and aerobic environment required for the growth of the culture. After 24 h, the samples are withdrawn and analyzed using UV-Vis Spectrophotometer to determine the growth of microbial culture. The UV-Vis Spectrophotometer used in the present work is shown in Plate 3.4. The samples obtained after 24 h are centrifuged to get pellets. The obtained pellets are taken out and added to three 250 mL conical flasks containing 100 mL sterlized MSM. These flasks are used for the acclimatization of culture over a period of 6 days for Cr(VI), Cu(II) and Zn(II) removal. The concentration of glucose is maintained at 800 mg L^{-1} by supplementing the solution in each flask with 0.8 mL stock glucose solution. A 0.2 mL of stock Cr(VI), Cu(II) and Zn(II) solutions are also added in three different flasks to make the concentration of metals to 2 mg L^{-1} in each flask. This procedure is continued till the end of 6th day by reducing the amount of glucose to 0 mg L^{-1} with a decrement of 200 mg L^{-1} each day and increasing the concentration of metals to 20 mg L^{-1}. Each day, the solutions are kept in a BOD incubator and shaker at 37 ^{o}C and at 150 rpm for 24 h. The final acclimated cultures obtained from three flasks contain 20 mg L^{-1} of metals which are then used for the isolation study.

3.1.5. Isolation and identification of microbial strain

The isolation of Cr(VI), Cu(II) and Zn(II) tolerant microbial strains is carried out by spreading 1 mL of each acclimated culture on three PYE medium (peptone and yeast extract) agar plates (Greenberg et al., 1985). The PYE medium agar plates contain 100 µg

of each metal per mL of samples. The medium for agar plates is prepared in a 250 mL flasks by dissolving 1 g peptone, 0.5 g yeast extract and 1 g NaCl in 100 mL of distilled water. The pH of the solutions are adjusted at 7.0±0.2. The solutions are mixed with 1.5 g of agar and sterilized. The growth of the microorganisms in the form of colonies are observed after 24 h of incubation at 37 ^{o}C and are shown in Plates 3.5, 3.6 and 3.7 for Cr(VI), Cu(II) and Zn(II) removal strains, respectively. Single colonies are picked up with sterilized loop and then streaked on PYE medium agar plate. The plates are incubated at 37 ^{o}C for 24 h. This process is repeated with 500 µg of each metal per mL of samples. A single microbial strain capable of growing at higher concentration of each metals is isolated and sent for identification. The identifications of three microbial strains are carried out at Genei, Merck Millipore Laboratory, India and Xcelris Laboratory, India. For all the three microbial strains, gene sequence analysis is carried out using consensus primers (518F and 800R) and 16S rRNA. Sequence data obtained is aligned using Molecular Evolutionary Genetic Analysis (MEGA) software version 5.0 (Tamura et al., 2011). It is analyzed by constructing a phylogenetic tree and the closest homologous microbe is identified.

3.1.6. Preservation of isolated microbial strain

The selected metal removal microbial strains are transferred from agar plates to three 250 mL conical flasks containing 100 mL sterlized nutrient broth. The cells grown in the liquid nutrient broths are centrifuged which results in pellets. The obtained pellets are washed with deionized water and suspended in phosphate buffer (1/15 mol L^{-1} NaH_2PO_4, 1/15 mol L^{-1} Na_2HPO_4, pH 7.0) for preserving the strains. These preserved microbial strains are used for further experimental studies.

3.1.7. Batch studies of Cr(VI), Cu(II) and Zn(II) remediation

The bioremediation of heavy metals Cr(VI), Cu(II) and Zn(II) is studied for initial metals concentrations ranging from 20-200 mg L^{-1} in different sets of 250 mL conical flasks. The parameters studied and their ranges are reported in Table 3.5. Experiments are carried out to determine the effect of time on the bioremediation of metals at different initial concentrations. In these experiments, 100 mL of MSM is sterilized and amended with 400 mg L^{-1} of glucose. The concentration of glucose is taken same (400 mg L^{-1}) in all the batch experiments unless otherwise specified. The sample is inoculated with 1 % (v/v) microbial inoculum obtained from the acclimatization procedure. A known amount of stock metal solution is also amended with the sample to maintain the required concentration in the range of 20-200 mg L^{-1}. In order to maintain aerobic conditions, the flasks are sealed with cotton and wrapped with soft paper. The samples are kept in a BOD incubator and shaker at 37 ^{o}C and 150 rpm. The incubation and shaking conditions (temperature and rotational speed) are maintained same (37 ^{o}C and 150 rpm) throughout the experiments unless otherwise specified. The samples are withdrawn at regular intervals of time based on visual observation (turbidity).

The effect of different parameters such as initial pH of the solution, dosage of MSM, macronutrients amount, aerobic and facultative anaerobic conditions, incubation temperature and inoculum volume are studied at optimum concentration (150, 80 and 40 mg L^{-1}) of respective metals obtained from kinetic studies. The effect of initial pH on the percentage removal of metals is also studied. This study is performed for pH values in the range of 2.0-8.0. The pH of the solution is maintained by using 1 M NaOH and 1 M HCl solutions. Experiments are also conducted to determine the optimum dosage of MSM for

the bioremediation of metals. This study is performed by varying the composition of standard MSM. The composition of various salts used in preparing the standard MSM solution is halved, doubled and tripled. The single composition of the salts in MSM is considered as standard composition. In all the above mentioned studies, final samples are analyzed to find out biomass concentration in the solution. Effects of macronutrients (N, P and K) on bioremediation of metals are also studied by varying the amounts of the respective elements (half, standard, double and triple) in the standard MSM dosage. The optimum values of N, P and K obtained from their individual studies are utilized to carry out another batch study to corroborate the findings.

Effects of aerobic and facultative anaerobic conditions on bioremediation of metals are also investigated by purging oxygen (O_2) and nitrogen (N_2) in two different samples, respectively. Effect of temperature on the bioremediation of metals is investigated by varying the operating temperature in the range 25-45 ^{o}C. Inoculum volume of isolated microbial strain are varied (i.e. 0.5 %, 1 %, 1.5 % and 2 %) to investigate the effects of inoculum volume on bioremediation of metals. All the experiments are performed in triplicate to determine the biomass concentration as discussed in section 3.4.1.2. Appropriate controls are also run to examine the removal of respective metals under biotic and abiotic conditions. Biotic controls are prepared with MSM and isolated microbial strains except metals while abiotic control consist of MSM and respective metals without isolated microbial strains. For biotic control, final samples are analyzed to find out biomass concentration in the solution. The initial and final metal ions concentrations are analyzed for abiotic controls.

Plate-3.1. Photograph of activated sludge sample

Plate-3.2. Photograph of centrifuge

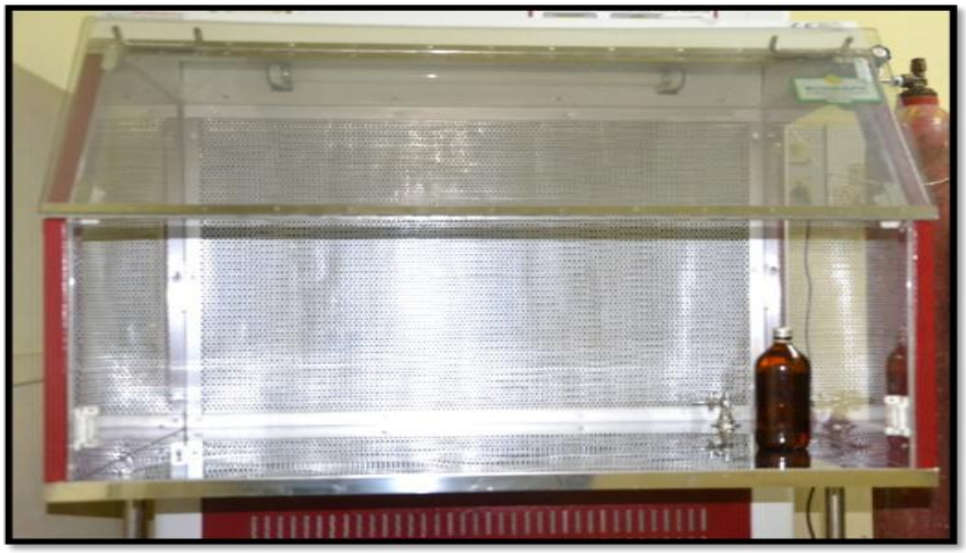


Plate-3.3. Photograph of laminar air flow chamber

Plate-3.4. Photograph of UV-Vis spectrophotometer

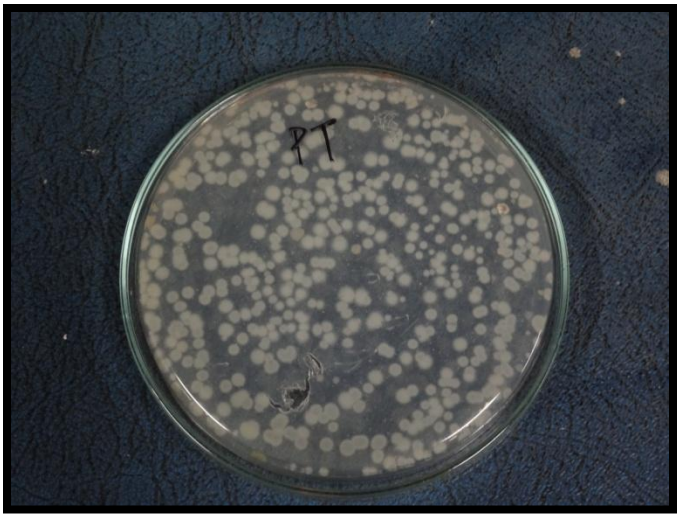


Plate-3.5. Photograph of microbial strain grown on agar in the presence of Cr(VI)

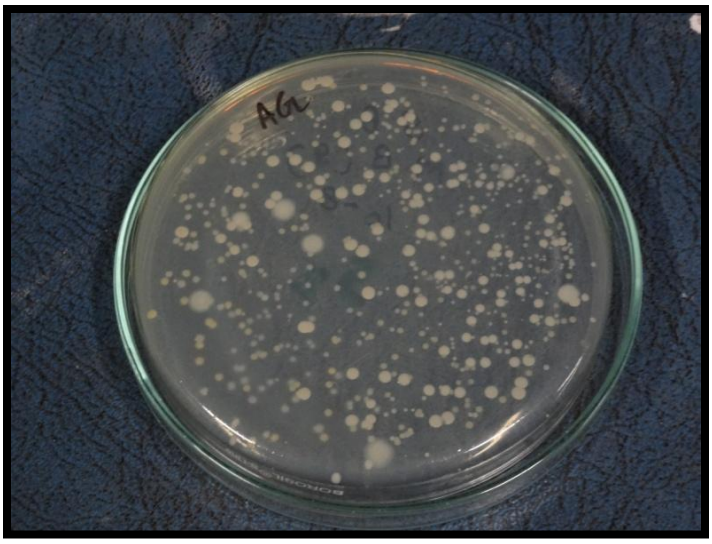


Plate-3.6. Photograph of microbial strain grown on agar in the presence of Cu(II)

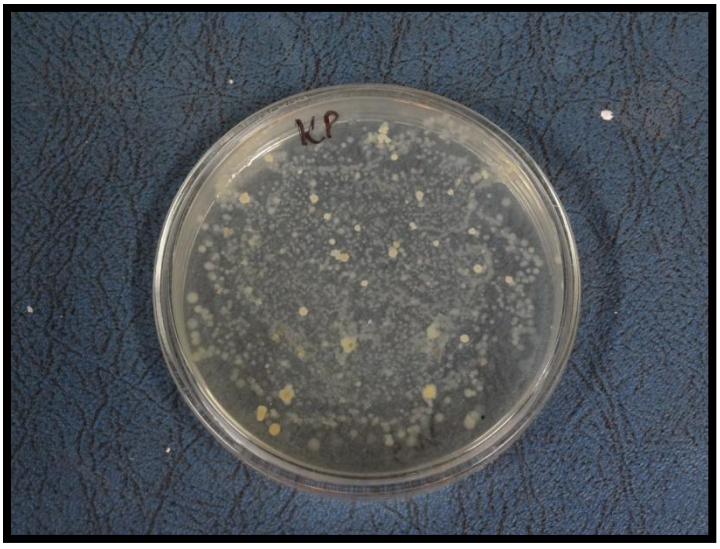


Plate-3.7. Photograph of microbial strain grown on agar in the presence of Zn(II)

Table 3.5. Parameters studies and their range/ composition/ mode

S No	Parameters studied	Range of value/ composition/ mode
1	Initial concentration of metals	20-200 mg L^{-1}
2	pH	2.0-8.0
3	MSM dosage	Half, double and triple the standard composition of MSM dosage
4	N-P-K	Half, standard, double and triple in standard MSM dosage
5	Conditions	Aerobic and facultative anaerobic
6	Temperature	25-45 ºC
7	Inoculum volume	0.5 % -2 %

3.1.8. Batch studies for simultaneous remediation of Cr(VI), Cu(II) and Zn(II)

In this study, a consortium is developed using microbial strains isolated from aerobic mixed culture of Municipal Sewage Treatment Plant, BITS-Pilani, Pilani Campus. The acclimatization and isolation of the microbial strains are carried out as mentioned in sections 3.1.3. and 3.1.4. The following sections describe the performance evaluation of consortium over individual strains for the removal of single and multiple metals [Cr(VI), Cu(II) and Zn(II)].

3.1.8.1. Selection of efficient microbial strains

Four isolates (B1 to B4) obtained from isolation studies are subjected to further screening and selection based on their metal tolerance and metal removal efficiency. Metal tolerance is determined in terms of minimum inhibitory concentration (MIC) exhibited by isolates (Mishra and Malik, 2014). Estimation of MIC is carried out using a solution of nutrient broth (NB), MSM and glucose with a concentration of 400 mg L^{-1}. One hundred milliliters of this solution are taken in 60 conical flasks of 250 mL which are further amended with either Cr(VI) or Cu(II) or Zn(II) metal ions by maintaining initial concentration in the range of 50 to 1000 mg L^{-1}. The solubility of each metal are 50, 100, 150, 200, 250, 300, 350, 400, 450, 500, 550, 600, 650, 700, 750, 800, 850, 900, 950 and 1000 mg L^{-1}. The pH of all media is adjusted at 7.0±0.2 using 1 M HCl or 1 M NaOH. After sterilization, the samples for each metal ions are inoculated with 1 % (v/v) microbial inoculum (B1 to B4) and then incubated at 37 ^{o}C and 150 rpm in a BOD incubator and shaker. In the next 4 days, daily monitoring is carried out to check the growth of isolated microbial strains. Abiotic controls consisting of NB, MSM and

respective metals are also considered to check the reduction of metals in the presence of NB and MSM.

3.1.8.2. Characterization and identification of microbial strain

Out of four isolated microbial strains, three microbial strains (B1, B2 and B3) are selected based on the results obtained from MIC study. The microbial strains B1 and B2 are sent to Genei, Merck Millipore Laboratory, India for characterization and identification. B3 is characterized and identified at Xcelris Laboratory, India. The identifications are carried out in the similar ways as mentioned in section 3.1.5.

3.1.8.3. Kinetic study

Batch bioremediation study of metals are conducted using identified microbial strains. This study is conducted in 250 mL conical flasks containing 100 mL MSM. The glucose concentration is maintained at 400 mg L^{-1} in each solution. In this study, the growth rate and metal removal efficiency of the microbial strains are determined by keeping each metal ion concentration as 150 mg L^{-1}. The samples are sterilized and then inoculated with 1 % (v/v) inoculum of respective microbial strains. Incubation of samples are carried out at 150 rpm and 37 ^{o}C. Samples are withdrawn at regular time intervals (4, 8, 12, 16, 20, 24, 28, 32, 36 and 40 h) based on visual observation (turbidity) and analyzed for biomass concentration and amount of metal removal as discussed in section 3.4.1. Appropriate controls are also taken to examine the removal of metals under abiotic and biotic conditions. Biotic control is prepared with MSM and microbial strain except metal while abiotic control consists of MSM and metal without microbial strain. All the batch experiments are conducted three times and the average values are considered.

3.1.8.4. Development of consortium

To develop microbial consortium successfully, microbial strains must be mutually compatible with each other in order to remove metals. This study is aimed to assess the mutual compatibility between three identified microbial strains during metal removal as mentioned by Mishra et al. (2014). Flask containing 100 mL sterilized MSM and 400 mg L^{-1} glucose is inoculated with 1 % (v/v) inoculum of B1. It is incubated at 37 ^{o}C and at 150 rpm for 120 h to ensure the production of secondary metabolites. Biomass obtained is filtered through filter paper (Whatman No. 1). Spent filtrate is retained and amended with 100 mL sterilized MSM and 400 mg L^{-1} of glucose. The sample is sterilized and the sterilized sample is inoculated with 1 % (v/v) inoculum of B2. It is incubated at 37 ^{o}C and 150 rpm for 120 h. This sample contains B1 and B2. Similar procedure is adopted to prepare five more combinations viz. B1+B3, B2+B3, B2+B1, B3+B1 and B3+B2. Biomass growth in spent filtrate is measured and compared with the growth of individual microbial strain in terms of percentage relative growth as discussed in section 3.4.1. A consortium consisting of three identified microbial strains (B1, B2 and B3) is developed based on results obtained from mutual compatibility study. The flowchart of the consortium development procedure is shown in Fig. 3.1.

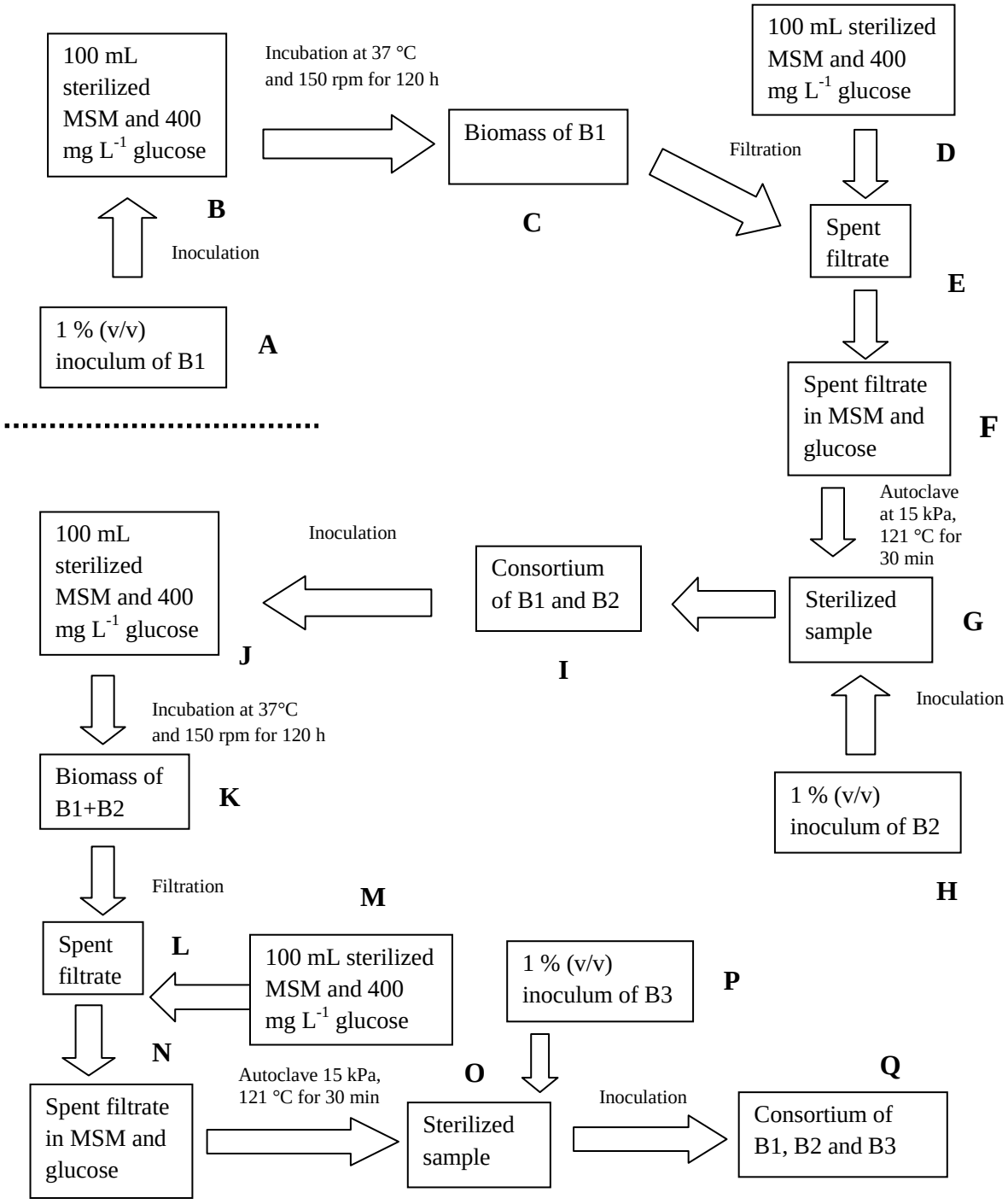


Fig. 3.1. The flowchart of the consortium development (A to Q represent steps)

3.1.8.5. Efficacy of consortium over individual strains in metal removal

Batch experiments are conducted to check the efficacy of the consortium over individual strains in the removal of single metal as well as in the presence of mixed metals (interference).

Different sets of conical flasks containing 100 mL MSM, 400 mg L^{-1} glucose and 150 mg L^{-1} initial concentration of respective metals are taken. The samples are sterilized and then inoculated with either 1 % (v/v) inoculum of individual microbial strains or with the developed microbial consortium. Flasks are incubated at 37 ^{o}C and 150 rpm. After 32 h, samples are withdrawn and analyzed for biomass concentration and amount of metal removal as discussed in section 3.4.1.

The multiple metals removal efficiency of individual strains and consortium is investigated in different sets of 250 mL conical flasks containing 100 mL MSM and 400 mg L^{-1} concentration of glucose. The samples are supplemented with equal initial concentration (50 mg L^{-1}) of Cr(VI), Cu(II) and Zn(II) and then sterilized. Flasks are inoculated with 1 % (v/v) inoculum of individual microbial strains or with the developed microbial consortium. All the flasks are incubated at 37 ^{o}C and 150 rpm for 32 h. At the end of incubation, samples are withdrawn and analyzed for biomass concentration and amount of metal removal.

3.1.8.6. Use of consortium for simulated industrial effluents

To evaluate the performance of the consortium in multiple metal exposure, a separate kinetic study is conducted using simulated industrial effluent of a textile industry (Lokhande et al., 2011). The details for the preparation of different sets of simulated industrial effluent is given in section 3.1.2.

Five sets (set 1-5) of 250 mL flasks containing 100 mL MSM and 400 mg L^{-1} concentration of glucose are taken. Samples are amended with specific proportions of respective stock solution of metals to maintain the metals concentration in the solution as per simulated industrial effluents (Table 3.4). The samples are sterilized and then are inoculated with 1 % (v/v) of the developed microbial consortium. The samples are incubated at 150 rpm and 37 °C. Samples are withdrawn at regular intervals (4, 8, 12, 16, 20, 24, 28, 32, 36 and 40 h) and are analyzed for biomass growth and metal removal as discussed in section 3.4.1.

3.2. Mass balance and metal removal mechanism

The metal ion removal mechanism is assessed by conducting a separate batch study in various sets of 250 mL flasks containing sterilized MSM solution supplemented with inidividual metal [Cr(VI) or Cu(II) or Zn(II): 150 mg L^{-1} initial concentration] and 400 mg L^{-1} of glucose. Each flask is inoculated with 24 h old individual bacterial strain and incubated. Samples are withdrawn after a specific time period (48 h) and filtered. The filtrates are analyzed for metal removal as discussed in section 3.4.1. To understand the metal biosorption mechanism of isolated bacterial strain, desorption of metals from biomass is carried out by suspending it in 100 mL 0.1 M NaOH solution (Mishra and Malik, 2014). The samples are incubated at 37°C and 150 rpm for 30 min. The solutions are filtered and analyzed for metal concentration. The biomass samples obtained after desorption study are amended with concentrated HNO_3 and the final concentration of metal ions is determined (Mishra and Malik, 2014).

3.3. Biofiltration studies

3.3.1. Biofilter column setup

In the present study, three different biofilter columns are used for the removal of Cr(VI), Cu(II) and Zn(II) separately. The biofilter column is made using perspex tube of 5 cm inner diameter and 1 m length (Fig. 3.2). The photograph of the experimental setup is shown in Plate 3.8. The biofilter column is packed with packing material and it is supported on two stainless steel mesh fixed at the top and bottom of the column. The packing height is maintained as 70 cm and a mixture of matured compost and coal (1:1 w/w) is used as packing material. The aqueous solution of metal is passed from overhead tank into the biofilter column in down flow mode of operation. MSM solution is sprinkled periodically from top of the column to provide necessary nutrient supply for the growth of the microorganism. Four sampling ports (10, 30, 50 and 70 cm from top of the column) are made in the column to collect the samples from different heights of the column. Flow rates of aqueous solution of metal and MSM are maintained using liquid rotameters.

3.3.2. Preparation of culture and development of biofilter column

The three isolated and identified microbial strains obtained from isolation studies (section 3.1.5) are grown in bulk. These microbial strains are used to conduct biofiltration experiments for the removal of respective metals.

One of the packing materials, coal is procured from local market of Pilani, India. It is crushed and sieved through 8-10 mm mesh screen. The final coal size is obtained as 2.36 mm. The coal is washed with distilled water in order to remove impurities. It is then dried in a Hot Air Oven at 100 °C for 1 day. The matured compost is obtained from The

Dairy, Birla Education Trust, Pilani, India. It is derived from cow dung. The moisture content of the packing material was analyzed as 62.5 % on wet weight basis. The biofilter column is packed with this mixture. The total amount of coal and compost used in the present study is 800 and 200 g, respectively. The packing material is then mixed with 200 mL inoculum of microbial strain as reported in earlier study (Raghuvanshi and Babu, 2009). The leachate collected at the bottom during each transfer of microbial strain, is also transferred to the packed column (Raghuvanshi and Babu, 2009) in order to maintain proper wetting of the packing material with the microbial strain. Compost contains macronutrients (N, P and K) and organic matters (C, H and O) which are required for the growth of the microorganisms (Delhoménie and Heitz, 2005). The mixture of coal and compost also provides an indigenous ecosystem for the growth of the microorganisms (Raghuvanshi and Babu, 2009). Due to these reasons, compost along with coal are used as a support medium in the biofilter column for the growth of the microbial strain.

3.3.3. Operating conditions for biofiltration

Three separate biofiltration studies for the removal of Cr(VI), Cu(II) and Zn(II) are conducted by varying the flow rates (5-40 mL min^{-1}) and inlet concentration of metals (10-50 mg L^{-1}). Entire biofilter operation is divided into 5 phases (Phase I to Phase V) based on the flow rates and inlet concentration of metals. The operating conditions of the biofiltration experiments are given in Tables 3.6-3.8 for the removal of Cr(VI), Cu(II) and Zn(II), respectively. All the continuous experiments are conducted twice and the average values are reported.

3.3.4. Stability of biofilter column

Biofilter columns are subjected to shock loading conditions for a period of two weeks. Shock loading experiments are carried out immediately after the 63, 55, 60 days biofilter operations for Cr(VI), Cu(II) and Zn(II), respectively. The operating conditions for the shock loading experiments are reported in Tables 3.9-3.11. The Inlet Loading (IL) of metal in each phase is calculated using Eq. 4.31 by considering $C_{critical}$ as C_i. The Empty Bed Residence Time (EBRT) is the time required to flow the metal solution from top to bottom of the column when the bed is free from microorganism.

3.3.5. Adsorption capacity of coal

In order to evaluate the maximum adsorption capacity of the coal in metal removal, separate batch studies (adsorption isotherm studies) are conducted in 100 mL conical flasks. Each metal solution of 20 mL (initial concentration: 10, 20, 30, 40, 50, 60, 80, 100 and 150 mg L^{-1}) is amended with 0.5 g of coal. Flasks are kept in a water bath-cum-mechanical shaker at 30°C. Flasks are removed at regular intervals and filtered. Filtrates were analyzed for residual metal concentration. Langmuir isotherm is used to calculate the maximum adsorption capacity of the coal.

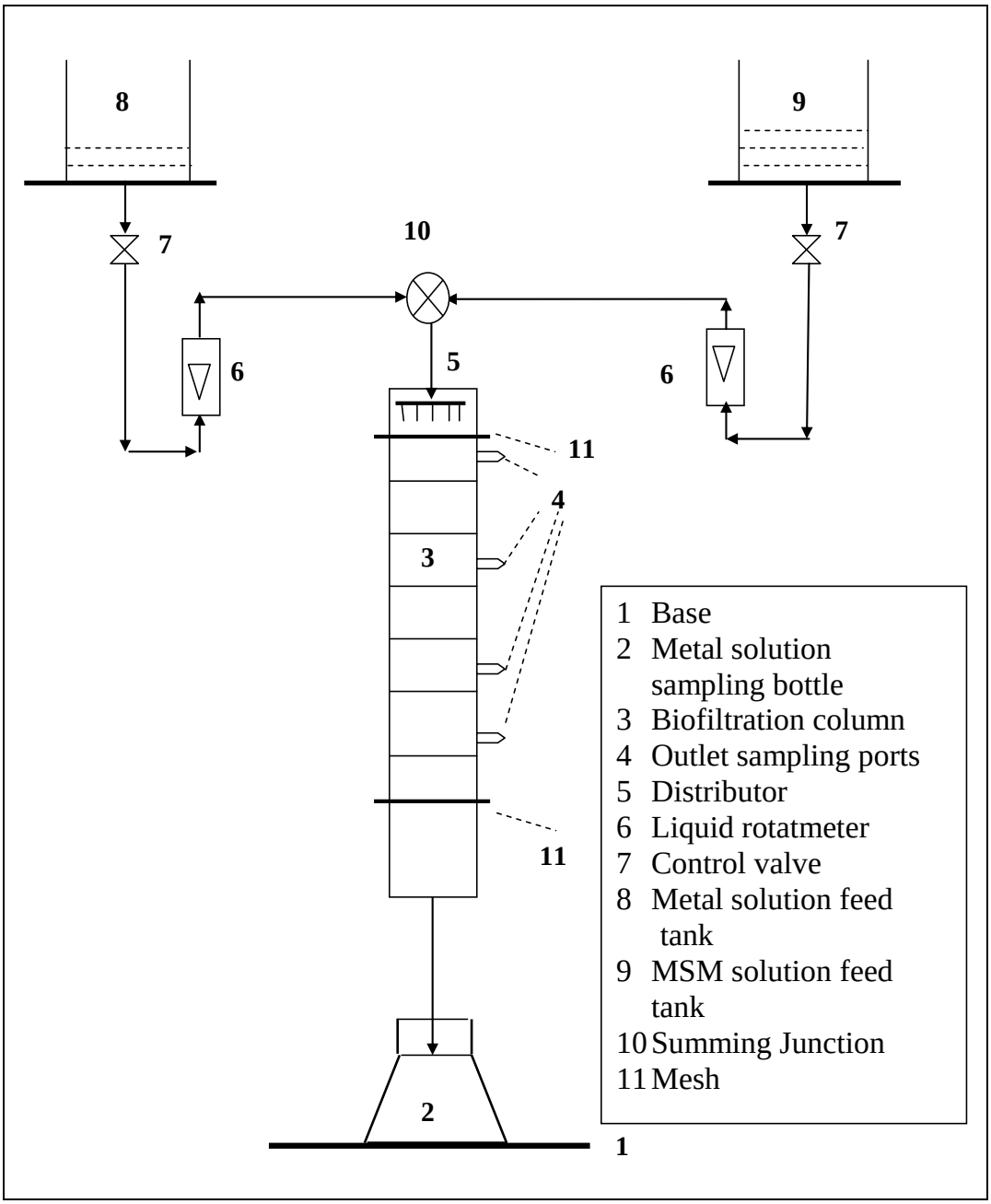


Fig. 3.2. Schematic diagram of biofilter set-up

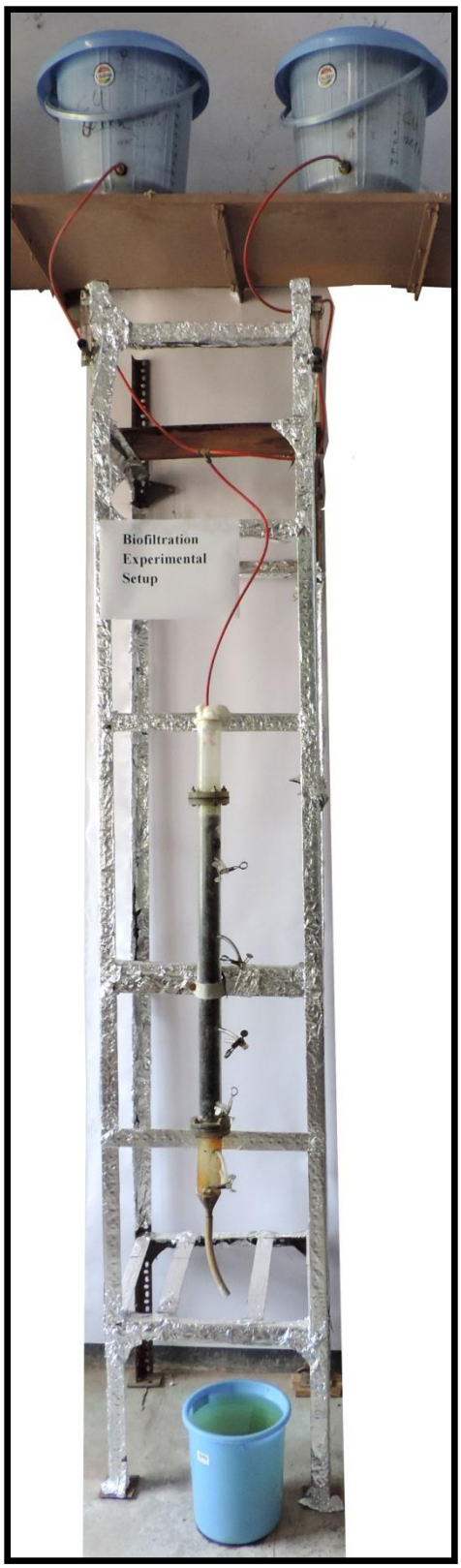


Plate-3.8. Photograph of continuous biofiltration experimental setup

Table 3.6. Operating conditions of biofilter operation for Cr(VI) removal

(IL: Inlet loading, EBRT: Empty bed residence time)

Phase	Operating time (day)	Flow rate (mL min^{-1})	Range of C_i (mg L^{-1})	IL (mg L^{-1} min^{-1})	EBRT (min)
I (acclimation)	20	5	8.75-10	0.0318-0.0364	274.88
II	10	10	18.50-20	0.1346-0.1455	137.44
III	12	20	28.50-30	0.4147-0.4366	68.72
IV	10	40	48-50	1.3970-1.4552	34.36
V	11	30	38-40	0.8295-0.8731	45.81

Table 3.7. Operating conditions of biofilter operation for Cu(II) removal

(IL: Inlet loading, EBRT: Empty bed residence time)

Phase	Operating time (day)	Flow rate (mL min^{-1})	Range of C_i (mg L^{-1})	IL (mg L^{-1} min^{-1})	EBRT (min)
I (acclimation)	15	5	8.45-10	0.0307-0.0364	274.90
II	10	10	17.50-20	0.1273-0.1455	137.40
III	10	20	27.50-30	0.4000-0.4366	68.70
IV	10	40	47-50	1.3679-1.4552	34.40
V	10	30	37-40	0.8076-0.8731	45.80

Table 3.8. Operating conditions of biofilter operation for Zn(II) removal

(IL: Inlet loading, EBRT: Empty bed residence time)

Phase	Operating time (day)	Flow rate (mL min^{-1})	Range of C_i (mg L^{-1})	IL (mg L^{-1} min^{-1})	EBRT (min)
I (acclimation)	20	5	8.50-10	0.0307-0.0364	274.90
II	10	10	18.50-20	0.1273-0.1455	137.40
III	10	20	28.50-30	0.4000-0.4366	68.70
IV	10	40	48-50	1.3679-1.4552	34.40
V	10	30	38.50-40	0.8076-0.8731	45.80

Table 3.9. Operating conditions of biofilter operation for Cr(VI) removal under shock loading (IL: Inlet loading, EBRT: Empty bed residence time)

Operating time (day)	Flow rate (mL min^{-1})	Range of C_i (mg L^{-1})	IL (mg L^{-1} min^{-1})	EBRT (min)
1-4	35	28.50-30	0.7258-0.7640	39.27
5-8	35	38-40	0.9677-1.0186	68.72
9-14	20	28-30	0.4075-0.4366	68.72
15-20	20	48.50-50	0.7058-0.7276	68.72

Table 3.10. Operating conditions of biofilter operation for Cu(II) removal under shock loading (IL: Inlet loading, EBRT: Empty bed residence time)

Operating time (day)	Flow rate (mL min^{-1})	Range of C_i (mg L^{-1})	IL (mg L^{-1} min^{-1})	EBRT (min)
1-4	32	28.50-30	0.6636-0.6984	42.95
5-8	32	38-40	0.8847-0.9313	42.95
9-14	20	30-33	0.4366-0.4802	68.72

Table 3.11. Operating conditions of biofilter operation for Zn(II) removal under shock loading (IL: Inlet loading, EBRT: Empty bed residence time)

Operating time (day)	Flow rate (mL min^{-1})	Range of C_i (mg L^{-1})	IL (mg L^{-1} min^{-1})	EBRT (min)
1-4	32	28.50-30	0.6636-0.6984	42.95
5-8	32	38-40	0.8847-0.9313	42.95
9-14	20	30-33	0.4366-0.4802	68.72

3.4. Analytical methods

3.4.1. Methods for bioremediation study

3.4.1.1. Metal analysis

Final concentration of the metals [Cu(II) and Zn(II)] and total concentration of Cr is measured using Atomic Absorption Spectrophotometer (AAS, AA-7000, Shimadzu, Japan) (Plate 3.9). The concentration of free Cr(VI) is analyzed using UV-Vis Spectrophotometer (Evolution-201, Thermo Scientific, India) at 540 nm by forming a purple-violet color with 1,5-diphenyl carbazide in acidic solution as a complexing agent (APHA, 2005). The analysis of Cr(VI) in final solution is carried out using the calibration curve (Fig. 3.3).

Percentage removal of metal is calculated using Eq. 3.1:

$$\text{Percentage Removal} = \frac{M_o - M_t}{M_o} \times 100 \qquad (3.1)$$

where, M_o is the initial metal concentration at time t = 0 and M_t is the final metal concentration at time t h.

3.4.1.2. Measurement of cell density

The optical densities (OD) of the isolated microbial strains are determined by using UV-Vis Spectrophotometer at 600 nm and correlated to dry weight of the biomass (Bae et al. 2000). In all the bioremediation experiments, 2 mL inoculum of isolated microbial strain is centrifuged and the cell pellet is obtained. The cell pellets are washed with physiological saline water thrice, resuspended in saline water and then homogenized. Homogenized samples are used as stock solutions for the preparation of different dilutions. Known volumes of these solutions are filtered through 0.8 µm filter paper

(Whatman, USA) to find out dry weight of cells. Corresponding absorbance values are measured using UV-Vis Spectrophotometer at 600 nm. Absorbance versus dry weight relationships are obtained for biomass grown on Cr(VI), Cu(II) and Zn(II) by plotting biomass concentration (dry weight) vs absorbance graph. The relationships are given by Eqs. (3.2), (3.3) and (3.4) for biomass grown on Cr(VI), Cu(II) and Zn(II), respectively. For unknown biomass samples, the absorbance values are measured at 600 nm and these are converted to to biomass concentration (dry weight) using the above mentioned equations (Bae et al., 2000).

$$\text{Biomass concentration (or dry weight)} = 0.20758 \times \text{Absorbance} \tag{3.2}$$

$$\text{Biomass concentration (or dry weight)} = 0.04653 \times \text{Absorbance} \tag{3.3}$$

$$\text{Biomass concentration (or dry weight)} = 0.01566 \times \text{Absorbance} \tag{3.4}$$

Percentage relative growth is determined by Eq. 3.5:

$$\text{Percentage relative growth} = \frac{X_2 - X_1}{X_1} \times 100 \tag{3.5}$$

where, X_1 is the growth of biomass of individual bacterial strain and X_2 is biomass growth of bacterial strain in residual filtrate after 120 h.

Relative biomass growth reduction (%) is determined using Eq. 3.6:

$$\text{Relative biomass growth reduction (\%)} = \frac{\left(\text{Biomass without metals} - \text{Biomass in the presence of metals}\right)}{\text{Biomass without metals}} \times 100 \tag{3.6}$$

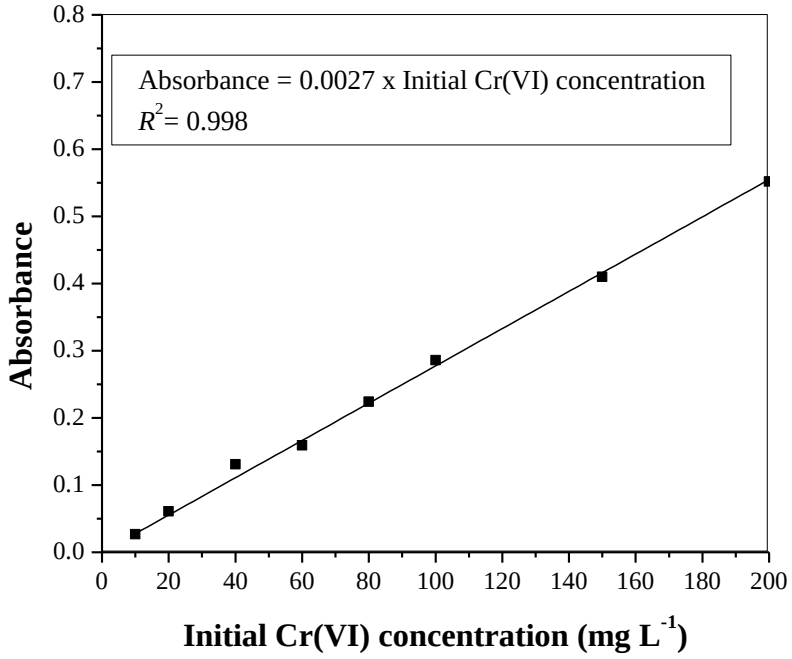


Fig. 3.3. Calibration plot for Cr(VI) analysis in final solution

Plate-3.9. Photograph of atomic absorption spectrophotometer

3.4.2. Methods for biofiltration study

3.4.2.1. Metal analysis and measurement of biomass concentration

Final heavy metal concentrations are measured using Atomic Absorption Spectrophotometer. Biomass concentration is measured in terms of colony forming units CFU g^{-1} of packing material. At the end of acclimation period (i.e. 20, 15 and 20 days for biofilter operation of Cr(VI), Cu(II) and Zn(II), respectively), 1 g of moist packing material is collected from the first sampling port (10 cm from top of the column) with the help of a sterile loop. The sample is immediately transferred into a sterilized conical flask and sealed. Similarly, at the end of entire biofilter operation same amount of moist packing material is collected from different sampling ports (i.e. 10, 30, 50, 70 cm from top of column). In each conical flasks, the moist packing material is added with 0.8 % of NaCl solution and kept in a mechanical shaker for 15 min. The sample is serially diluted with sterilized water to isolate the microbial strain from the packing material. Spread plating technique is used to estimate the CFU values (CFU g^{-1} of packing material) (Raghuvanshi and Babu, 2009).

3.4.2.2. Performance evaluation of biofilter operation

The performances of biofilter columns for the removal of heavy metals are evaluated in terms of the percentage removal efficiency (RE) and the elimination capacity (EC) and are given by Eqs. 3.7 and 3.8, respectively.

$$RE(\%) = \frac{C_i - C_O}{C_i} \times 100 \tag{3.7}$$

$$EC\,(\text{mg L}^{-1}\ \text{min}^{-1}) = \frac{Q(C_i - C_O)}{V} \tag{3.8}$$

where C_i and C_o are the inlet and outlet heavy metal concentration (mg L^{-1}), respectively; Q is the inlet flow rate of heavy metal solution (mL min^{-1}) and V is the volume of the packing (m^3).

3.4.3. Fourier transform infrared (FTIR) spectroscopy

Infrared spectroscopy helps to obtain qualitative information about molecules. Dipole moment (polarity) of the molecule is changed during the absorption in the infrared region. Absorbing groups in the infrared region are absorbed within a certain wavelength region. In infrared spectroscopy, some absorption bands for similar compounds occur at a definite wavelength with intensities proportional to the concentration of absorbing species. It gives a quantitative analysis of complex mixture of similar compounds.

3.4.3.1. Preparation of microbial cultures

In order to investigate the role of various functional groups in the bioremediation of metals, FTIR spectral analysis of metal unloaded dried biomass (control) and metal loaded [Cr(VI), Cu(II) and Zn(II) of initial concentration 80 mg L^{-1}] dried biomass of individual microbial strains are carried out.

Different sets of 250 mL conical flasks containing 100 mL of sterilzed MSM are amended with 80 mg L^{-1} of each metal in the respective flasks. The flasks are inoculated with active microbial cultures of three different strains (10 % v/v) and incubated at 37 °C and 150 rpm. Controls containing only active microbial cultures of three strains (10 % v/v) are also considered in the experiment. After 24 h of incubation, controls and metal loaded microbial cells are centrifuged which result in pellets. The pellets are washed three times with 0.85 % sodium chloride (NaCl) solution and rinsed with double distilled (DDI) water. The pellets are lyophilized using Freeze Dryer (Labconco, US) which result

in dried and powdered biomass. The Freeze Dryer used in the present study is shown in Plate 3.10.

3.4.3.2. Sample preparation for FTIR spectra acquisition

One milligram of finely crushed biomass is mixed with 400 mg of potassium bromide (KBr) and ground into fine powder. The powder is used to prepare a translucent disk by applying a pressure of 100 kg cm^{-2} for 10 min in a manual hydraulic press. The disk is then attached in a FTIR Spectrometer (Frontier, Perkin Elmer). FTIR spectrum of metal unloaded biomass and metal loaded biomass are obtained in mid-infra red region (400-4000 cm^{-1}) at a resolution of 4 cm^{-1}. Background correction for atmospheric air is conducted for each spectrum.

3.4.4. Field-emission scanning electron microscopy and energy-dispersive X-ray (FESEM-EDX) analysis

Electron microscopy uses the electrons to illuminate a specimen and thereby creates an enlarged image. In field emission (FE) scanning electron microscopy, large number of electrons are emitted in a relatively small beam spot. The resolution of the image is enhanced due to smaller beam spot diameter. This is attributed to the fact that a field emission source produces an electron beam containing many electrons with the smaller beam spot diameter. It has sufficient electrons to form an image (Lee, 1993).

3.4.4.1. Preparation of microbial cultures

FESEM is employed to observe the effect of metals on the morphology of isolated microbial strains. EDX is carried out to confirm the presence of metals on the outer layer of isolated microbial strains.

Active microbial cultures of three different strains (10 % v/v) are inoculated into three sets of conical flasks containing 25 mL of nutrient broth (NB). The samples are incubated at 37 °C and 150 rpm for 5 h. The mid-exponential phase cultures (0.1 mL) are then spread over the surface of three NB agar plates supplemented with 80 mg L^{-1} of metals [Cr(VI), Cu(II) and Zn(II)] using a sterile glass spreader at dilutions of 10^{-6} and 10^{-7}. Control plates consist with three different microbial strains on NB agar. All the plates are incubated at 37 °C for 24 h prior to FESEM sample preparation.

3.4.4.2. FESEM sample preparation

The cells prepared (as described in section 3.4.4.1) are picked up from NB agar plate and washed with 0.15 M phosphate buffer saline (pH 6.8). The samples are then fixed in 2.5 % glutaraldehyde and kept for 1-2 h at room temperature. After fixation, cells are washed with fresh phosphate buffer (pH 6.8) and post-fixed with 2 % osmium tetroxide for 1 h. The cells are sequentially dehydrated in 10 %, 30 %, 50 %, 70 % and absolute ethanol for 5 min each. The samples are left to dry for 12 h in a desiccator. The dried dehydrated samples are mounted onto aluminum stubs and further gold coated by cathodic spraying prior to viewing in a Field-Emission Scanning Electron Microscope (FEI Quanta 200FEI) fitted with EDX (Oxford IE250 X-MAX 80).

Plate-3.10. Photograph of freeze dryer

CHAPTER 4

MATHEMATICAL MODELING

The present chapter deals with the various growth and rate kinetic models for the batch bioremediation of hexavalent chromium [Cr(VI)], divalent copper [Cu(II)] and divalent zinc [Zn(II)] from aqueous solution. This chapter also discusses the kinetic modeling of biofilter column for the removal of individual metals Cr(VI), Cu(II) and Zn(II) from aqueous solution. Michaelis Menten and Ottengraf-van den Oever model are considered for explaining the kinetic behaviour of the biofilter column.

4.1. Specific growth rate

Metal bioremediation occurs due to the microbial activity which leads to the formation of biomass. During the log phase, the amount of biomass increases exponentially with respect to time which is attributed to the depletion of substrate concentration. The kinetics of metal bioremediation is related to the specific growth rate of microorganisms. In the log phase, the growth rate of the microorganisms at a given time is proportional to the number of microorganisms present at that time. During kinetic studies, the experimental data obtained for biomass concentration of the exponential growth phase (log phase) are used to determine the specific growth rate (μ) as given by Eq. 4.1:

$$\mu = \frac{1}{x}\frac{dx}{dt} \tag{4.1}$$

where x is the biomass concentration (g L^{-1}) at time, t (h) and dt is the change in time (h). After integrating, Eq. 4.1 becomes Eq. 4.2:

$$\ln x = \ln x_o + \mu t \quad (4.2)$$

where x_o is the biomass concentration (g L^{-1}) at t = 0. A plot of ln x vs t gives a straight line with ln x_o as its intercept and μ as the slope. There is no growth of biomass observed in other phases such as lag, stationary and death. Due to this reason, the data for these phases are not considered in the present study. In general, the obtained values of specific growth rates in batch bioremediation study are used to fit the growth kinetic models reported in literature to estimate the respective model parameters.

4.2. Growth kinetic models

Bioremediation is occurred due to the microbial enzymatic activity. Metal is utilized by the microorganisms under proper environmental conditions in the presence of carbon and energy sources. Substrate utilization and product formation can be described using various growth kinetic models which relate the substrate concentration (S) to the specific growth rate (μ) of microorganisms. This relationship is described by considering a set of theoretical kinetic models. These models help to understand the mechanism of metal bioremediation. Growth kinetic models reported in the literature such as Monod model (Monod, 1949) and Monod-Inhibition model (Elangovan and Philip, 2009) are briefly described in the following sections. Heavy metals significantly inhibit the specific growth rate and lag time of microorganisms during the substrate utilization (Şengör et al., 2009). Due to this reason, Monod model is not widely exploited in metal bioremediation study (Okpokwasili and Nweke, 2005). Present study discusses the microbial growth kinetics for the removal of three metals [Cr(VI), Cu(II) and Zn(II)] using two growth kinetics models mentioned above.

4.2.1. Monod model

Monod model (Monod, 1949) is relatively simple and used for explaining the microbial growth kinetics in VOCs and organics removal. Metal toxicity inhibits the growth of the microorganisms, impacting their ecology and thus reducing the rate of bioremediation. Due to this reason, Monod model is not widely used in explaining the microbial growth kinetics in metal removal. Monod model gives the relationship between growth rate (μ) and concentration of the single growth-controlling substrate as given by Eq. (4.3):

$$\mu = \mu_m \frac{S}{K_s + S} \tag{4.3}$$

where, μ is the specific growth rate (h^{-1}), S is the substrate concentration (mg L^{-1}), μ_m is the maximum specific growth rate (h^{-1}) and K_S is the saturation constant (mg L^{-1}). Linearization of Eq. (4.3) yields Eq. (4.4):

$$\frac{1}{\mu} = \frac{K_S}{\mu_m}\left(\frac{1}{S}\right) + \frac{1}{\mu_m} \tag{4.4}$$

A graph can be drawn between (1/μ) and (1/S) using log phase data of microbial growth which gives a straight line. The value of μ_m and K_S can be determined from the intercept and the slope of the graph, respectively.

Monod model does not take into account the fact that cells may synthesize product during their inactive stage of growth. It is not applicable for substrates which may limit the growth of the cells at low substrate concentration. Self inhibitory effects of substrate on the growth of microorganisms is ignored in this model. The classical difficulty of Monod model lies in its application in metal bioremediation where toxicity of metals inhibits the growth of microorganisms.

4.2.2. Models with growth inhibitors

At high substrate concentration or in the presence of toxic substances in the medium (e.g. metal ions) growth of microorganisms is inhibited. Product can also inhibit the growth of microorganisms. The inhibition pattern of microbial growth is similar to enzyme inhibition and the growth rate depends on inhibitor concentration (Shuler and Kargi, 2002). Various substrate and product-inhibition patterns are given in Eq. (4.5-4.8):

Noncompetitive substrate inhibition: $$\mu = \frac{\mu_m}{1+\frac{K_S}{S} \; . \; 1+\frac{S}{K_I}} \tag{4.5}$$

Competitive substrate inhibition: $$\mu = \frac{\mu_m S}{K_S . \; 1+\frac{S}{K_I} \; + S} \tag{4.6}$$

where K_I is the substrate inhibition constant (mg L^{-1}).

Noncompetitive product inhibition: $$\mu = \frac{\mu_m}{1+\frac{K_S}{S} \; . \; 1+\frac{P}{K_P}} \tag{4.7}$$

Competitive product inhibition: $$\mu = \frac{\mu_m S}{K_S . \; 1+\frac{P}{K_P} \; + S} \tag{4.8}$$

where, P and K_P are product concentration (mg L^{-1}) and product inhibition constant (mg L^{-1}), respectively.

The growth rate expressions for noncompetitive, competitive and uncompetitive inhibitions are given in Eq. (4.9-4.11):

Noncompetitive inhibition: $$\mu = \frac{\mu_m}{1+\frac{K_S}{S} \; . \; 1+\frac{I}{K_I}} \tag{4.9}$$

Competitive inhibition: $\mu = \dfrac{\mu_m S}{K_S .\ 1 + \dfrac{I}{K_I} + S}$ (4.10)

Uncompetitive inhibition: $\mu = \dfrac{\mu_m S}{\dfrac{K_S}{1 + \dfrac{I}{K_I}} + S\ .\ 1 + \dfrac{I}{K_I}}$ (4.11)

where, I and K_I are inhibitor concentration and inhibitor constant (mg L^{-1}), respectively. Eqs. (4.9-4.11) are known as Monod's equations with inhibition.

4.3. Rate kinetic models

In the batch bioremediation study, the amount of biomass increases exponentially with respect to time during the log phase. The increase in biomass concentration depends on the depletion of substrate concentration with respect to time. In bioremediation, several kinetic approaches are used to describe the transformation of metal ions into other ionic states. The change in substrate concentration can be described by zero-order and three-half-order rate kinetics.

4.3.1. Zero-order rate kinetics

The differential form of Monod model equation can be represented by Eq. (4.12):

$$\frac{dS}{dt} = -\mu_m \frac{S}{K_s + S} \quad (4.12)$$

where, dS/dt is the rate of substrate utilization.

It describes the utilization of a single rate-limiting substrate which results in the microbial growth. When S is several times greater than K_s ($S >> K_s$), Eq. 4.12 can be approximated by a zero-order rate equation (Eq. 4.13):

$$\frac{dS}{dt} = -k_o \tag{4.13}$$

where,

$$k_o = \mu_m = \text{zero - order rate constant} \tag{4.14}$$

On integration, Eq. 4.13 yields Eq. 4.15.

$$S = S_o - k_o t \tag{4.15}$$

where, S_o is the substrate concentration at time, $t = 0$.

4.3.2. Three-half-order rate kinetics

The conceptual and mathematical difficulties associated with first-order and second-order kinetics led to the formulation of a single deterministic model known as three-half-order kinetic model (Brunner and Focht, 1984; Xie et al., 1996). It includes the term to explain the biomass formation which was not incorporated in first-order and second-order kinetic models. The biomass formation can be measured in terms of the rate of product formation. The three-half-order model is developed by incorporating an additional term in the first-order kinetic model which explains the detailed kinetics of biomass formation. It is also based on the assumption of the first-order model. The modified first-order model is given by Eq. (4.16):

$$\frac{dS}{dt} = -k_1 S - aES \tag{4.16}$$

where, k_1 is the proportionality constant (time^{-1}), E is the cell concentration (g L^{-1}) and a is the proportionality constant (biomass concentration^{-1} time^{-1}).

The term, E can be assumed to vary linearly with time (t) as given in Eq. (4.17):

$$aE = k_2 t \tag{4.17}$$

where, k_2 is a constant (time^2)$^{-1}$. Substituting the value of aE from Eq. (4.17) into Eq. (4.16) gives Eq. (4.18).

$$\frac{dS}{dt} = -k_1 S - k_2 St \tag{4.18}$$

On integration, Eq. (4.18) yields Eq. (4.19).

$$S = S_o e^{-k_1 t - \left(k_2 t^2\right)/2} \tag{4.19}$$

The rate of product formation in three-half-order model is expressed by Eq. (4.20).

$$\frac{dP}{dt} = -\frac{dS}{dt} + k_o \tag{4.20}$$

Eq. (4.20) can be integrated to give Eq. (4.21) which is known as three-half-order model.

$$P = S_o - S + k_o t \tag{4.21}$$

Substituting the value of S from Eq. (4.19) into Eq. (4.21), Eq. (4.22) is obtained.

$$P = S_o \left(1 - e^{-k_1 t - \left(k_2 t^2\right)/2}\right) + k_o t \tag{4.22}$$

Eq. (4.22) can be simplified and rearranged to give Eq. (4.23).

$$Y = -k_1 - \frac{k_2 t}{2} \tag{4.23}$$

where,

$$k_2 = aE / t \tag{4.24}$$

$$Y = \frac{1}{t}\left[\ln\left(S_o - P + k_o t\right) / S_o\right] \tag{4.25}$$

where, P is the rate of product formation which is directly related to the change in biomass concentration. The unknowns, k_o and S_o are determined by Eqs. (4.15). In Eq. (4.23), k_1 and k_2 are determined by plotting Y vs t graph.

4.4. Kinetic modeling for biofiltration

In biofiltration of metals, kinetic parameters are important to understand the behavior of the column. In the present study, biofiltration operation are modeled using Ottengraf-van den Oever model and the kinetic constants are determined using Michaelis-Menten kinetic model.

4.4.1. Michaelis-Menten kinetic model

Determination of kinetic parameters is important to understand the kinetic behavior of a continuous biofilter column for metal removal. In the present study, Michaelis-Menten kinetic model is used to determine the kinetic constants for the biofilter operation. In this model, it is assumed that at steady state, the growth rate of microorganisms is balanced

by its own decay rate. It maintains a biological equilibrium in the system. The kinetic constants are determined using Eq. (4.26).

$$\frac{V/Q}{C_i - C_O} = \frac{K_s}{r_{\max}} \frac{1}{C_{\ln}} + \frac{1}{r_{\max}} \tag{4.26}$$

where, C_i and C_o are the inlet and outlet metal concentration (mg L^{-1}), Q is the inlet flow rate of metal solution (mL min^{-1}), V is the volume of the packing (L), K_s is the saturation constant (mg L^{-1}), r_{max} is the maximum bioremediation rate per unit packing volume (mg L^{-1} min^{-1}) and C_{ln} is the log mean concentration difference as calculated using Eq. (4.27).

$$C_{\ln} = \frac{C_i - C_o}{\ln\left(\frac{C_i}{C_o}\right)} \tag{4.27}$$

4.4.2. Modeling with Ottengraf-van den Oever model

The diffusion limitation occurs in the wet biofilm (Ottengraf and van den Oever, 1983). The biofilm is not fully active and the depth of penetration in the biofilm is smaller than the thickness of the biofilm (δ). Thus the bioremediation is controlled by the diffusion. Ottengraf-Van den Oever (1983) proposed the zero-order kinetics with diffusion limitation as given in Eq. (4.28).

$$\mathrm{EC} = \mathrm{IL}\left(1 - \left(1 - K_1\sqrt{\frac{\mathrm{EBRT}}{\mathrm{IL}}}\right)^2\right) \tag{4.28}$$

$$\text{where, } K_1 = \sqrt{\frac{K_o D_e a}{2m\delta}} \tag{4.29}$$

In Eq. (4.29), K_o is the zero-order kinetic constant; D_e is the effective diffusivity of metal in the biofilm (m^2 h^{-1}); m is Henry's constant for gaseous pollutants in water; a is the interfacial area per unit volume (m^{-1}) and δ is the biofilm thickness (μm). In the present study, metal ion is diffusing through aqueous system. Hence, partition coefficient (m) is considered in place of Henry's constant. K_1 is calculated using Eq. (4.30).

$$\frac{C_o}{C_i} = \left[1 - \text{EBRT}\frac{K_1}{\sqrt{C_i}}\right]^2 \tag{4.30}$$

The zero-order kinetics with reaction limitation is given by Eq. (4.31).

$$\text{EC} = K_o \tag{4.31}$$

The critical inlet concentration (C_{critical}) is defined as the inlet concentration at which bioremediation mechanism shifts from diffusion-limited to reaction-rate controlled (Ottengraf and van den Oever, 1983). It can be calculated using Eq. (4.32).

$$C_{critical} = \frac{1}{4}\left(\frac{K_o}{K_1} + K_1 \times \text{EBRT}\right)^2 \tag{4.32}$$

The critical inlet load (IL_{critical}) can be determined using Eq. (4.33).

$$\text{IL}_{\text{critical}} = \frac{C_{\text{critical}} \times Q}{V} \tag{4.33}$$

where, *EBRT* is the empty bed residence time (min^{-1}).

CHAPTER 5

RESULTS AND DISCUSSION

The present chapter deals with the results obtained on batch bioremediation and biofiltration studies for the removal of metals [Cr(VI), Cu(II) and Zn(II)] from aqueous solution. An attempt has been made to investigate the mechanism of bioremediation of different metals with the help of growth kinetic models and rate kinetics. To validate the proposed mechanism of metal bioremediation, FTIR and FESEM-EDX analyses of metal loaded biomass are also carried out. These results are presented in subsequent sections.

5.1. Experimental studies

5.1.1. Batch bioremediation studies

The batch bioremediation studies are carried out for the removal of Cr(VI), Cu(II) and Zn(II) from aqueous solution. The effect of various important parameters such as initial metal concentration, initial pH, MSM dosage, amount of macronutrients (N-P-K), aerobic & facultative anaerobic conditions, temperature and inoculum volume are studied. Various growth kinetic and rate kinetic models are used to obtain the growth and rate kinetic parameters which are helpful in the design of biofilter column. Results obtained from these models help to understand the mechanism of bioremediation of metals from aqueous solution.

5.1.1.1. Acclimatization procedure

Acclimatization of the mixed microbial culture is carried out over a period of 6 days. The obtained biomass concentrations for bioremediation of Cr(VI), Cu(II) and Zn(II) are

0.3464, 0.3565 and 0.4523 g L^{-1}, respectively at the end of the 1^{st} day. At the end of 6^{th} day, the biomass concentrations for Cr(VI), Cu(II) and Zn(II) bioremediation are estimated to be 0.0187, 0.0115 and 0.0359 g L^{-1}, respectively. Different microbial species are present in the activated sludge collected from sewage treatment plant. In the presence of metals, some of the species survive at the end of 6^{th} day. These species are capable of removing metals. They are further used for identification and bioremediation studies.

5.1.1.2. Identification of isolated microbial strains

Identification of isolated microbial strains are carried out at Genei, Merck Millipore Laboratory, India and Xcelris Laboratory, India. Using consensus primers (518F & 800R) and 16S rRNA, gene sequence analyses are carried out. Sequence data obtained are aligned using Molecular Evolutionary Genetic Analysis (MEGA) software version 5.0 (Tamura et al., 2011). The strains are analyzed with the help of amplified gene sequence and are compared with sequence of known strains. Phylogenetic trees are prepared (Figs. 5.1, 5.2 and 5.3) which show maximum homology with three strains *Pseudomonas taiwanensis* (NCBI GenBank Accession Number**:** KT070310), *Acinetobacter guillouiae* (NCBI GenBank Accession Number: KT070311) and *Klebsiella pneumoniae* (NCBI GenBank Accession Number: KT070312). Hence, the three microbial strains which are capable of removing Cr(VI), Cu(II) and Zn(II) are identified as *P. taiwanensis, A. guillouiae* and *K. pneumoniae*, respectively.

5.1.1.3. Metal removal study without bacterial strains

In the batch studies, the concentration of metals present in the respective abiotic controls are analyzed. The change in the concentration and precipitation of metals are not

observed. It indicates that the reduction of respective metals is not occurred due to the presence of MSM.

5.1.1.4. Effect of time on initial concentration of metals

The removal of Cr(VI), Cu(II) and Zn(II) by the three isolated bacterial strains for various initial concentrations of the metals against the time are shown in Figs. 5.4-5.6, respectively. The growth of *P. taiwanensis, A. guillouiae* and *K. pneumoniae* in biotic control (i.e. in absence of metals) and in the presence of metals are shown in Figs. 5.7, 5.9 and 5.11, respectively. The kinetic of substrate utilization by *P. taiwanensis*, *A. guillouiae* and *K. pneumoniae* in biotic control and in the presence of metals are shown in Figs. 5.8, 5.10 and 5.12, respectively.

For the removal of Cr(VI), the time required by *P. taiwanensis* is obtained as 20, 22, 28, 38, 40, 44 and 46 h for 20, 40, 60, 80, 100, 150 and 200 mg L^{-1} of initial Cr(VI) concentration, respectively (Fig. 5.4). The maximum removal of Cr(VI) is obtained as 108.3 mg L^{-1} for 150 mg L^{-1} of initial Cr(VI) concentration. The maximum biomass concentration (3.57 g L^{-1}) and shorter lag phase (4 h) are observed in biotic control (Fig. 5.7). In the presence of Cr(VI), the lag phase is obtained as 2, 6, 8, 12, 10, 14 and 14 h for 20, 40, 60, 80, 100, 150 and 200 mg L^{-1} of initial Cr(VI) concentration, respectively. The maximum biomass concentrations are obtained as 0.178, 0.215, 0.223, 0.252, 0.253, 0.258 and 0.259 g L^{-1} for initial Cr(VI) concentrations of 20, 40, 60, 80, 100, 150 and 200 mg L^{-1}, respectively. Growth becomes stable after 12, 14, 24, 36, 38, 40 and 42 h for initial Cr(VI) concentrations of 20, 40, 60, 80, 100, 150 and 200 mg L^{-1}, respectively. Complete substrate utilization is observed for *P. taiwanensis* in biotic control (Fig. 5.8). The maximum substrate utilization is obtained as 0.14, 0.21, 0.257, 0.272, 0.28, 0.286

and 0.3 g L^{-1} for initial Cr(VI) concentrations of 20, 40, 60, 80, 100, 150 and 200 mg L^{-1}, respectively.

The removal of Cu(II) is achieved at 18, 20, 24, 24, 24, 28 and 28 h for 20, 40, 60, 80, 100, 150 and 200 mg L^{-1} of initial Cu(II) concentration, respectively (Fig. 5.5). Maximum percentage removal of Cu(II) is achieved as 98.7 % for 80 mg L^{-1} of initial concentration of Cu(II). In biotic control, the lag phase is found to be very short (2 h) for *A. guillouiae*. In biotic control, the maximum biomass concentration is obtained as 2.75 g L^{-1} (Fig. 5.9). For the initial Cu(II) concentrations of 20, 40, 60, 80, 100, 150 and 200 mg L^{-1}, the maximum biomass concentrations are obtained as 0.125, 0.178, 0.260, 0.352, 0.318, 0.220 and 0.189 g L^{-1}, respectively. The lag phase is obtained as 4, 4, 6, 8, 8, 10 and 10 h for 20, 40, 60, 80, 100, 150 and 200 mg L^{-1} of initial concentration of Cu(II), respectively. After 16, 18, 20, 24, 28, 28 and 28 h, the growth is found to be stable for 20, 40, 60, 80, 100, 150 and 200 mg L^{-1} of initial Cu(II) concentrations, respectively. For *A. guillouiae*, almost complete substrate utilization is observed in biotic control (Fig. 5.10). The maximum substrate utilization is obtained as 0.09, 0.117, 0.129, 0.169, 0.205, 0.26 and 0.263 g L^{-1} for 20, 40, 60, 80, 100, 150 and 200 mg L^{-1} initial Cu(II) concentrations, respectively.

The time required for Zn(II) removal at 20, 40, 60, 80, 100, 150 and 200 mg L^{-1} of initial concentration of Zn(II) are 24, 24, 24, 24, 28, 28 and 28 h, respectively (Fig. 5.6). Maximum removal of Zn(II) is achieved as 98.8 % for 40 mg L^{-1} of initial concentration of Zn(II). It is observed that the lag phase is very short (2 h) for *K. pneumoniae* in biotic control. The maximum biomass concentration is obtained as 3.31 g L^{-1} in biotic control (Fig. 5.11). The lag phase is found to be 4, 8, 6, 6, 10, 14 and 14 h for 20, 40, 60, 80, 100,

150 and 200 mg L^{-1} of initial concentration of Zn(II), respectively. The maximum biomass concentrations are obtained as 0.286, 0.293, 0.220, 0.245, 0.261, 0.273 and 0.281 g L^{-1} for 20, 40, 60, 80, 100, 150 and 200 mg L^{-1} of initial Zn(II) concentrations, respectively. Growth becomes stable after 24, 28, 28, 28, 24, 28 and 32 h for 20, 40, 60, 80, 100, 150 and 200 mg L^{-1} of initial Zn(II) concentrations, respectively. For *K. pneumoniae,* almost complete substrate utilization is observed in biotic control (Fig. 5.12). The maximum substrate utilization is obtained as 0.091, 0.122, 0.14, 0.154, 0.181, 0.303 and 0.319 g L^{-1} for 20, 40, 60, 80, 100, 150 and 200 mg L^{-1} initial Zn(II) concentrations, respectively.

A newly inoculated bacterial strain does not begin to grow immediately (Malik, 2004; Srivastava and Majumder, 2008). Initially, it takes some time to get acclimatized with new environment which results lag phase. Then the sudden increase in the growth was observed during the log phase (Figs. 5.7, 5.9 and 5.11). Log phase is a consequence of each cell's multiplication. The rate of multiplication is influenced by the various process parameters such as temperature, aeration and composition of nutrient media. For all three strains, the maximum biomass concentration is observed in biotic control. The higher value of maximum biomass concentration may be due to the presence of mineral salts in MSM which eventually precipitate out. In the presence of metals, the maximum biomass concentrations are found to be less as compared to values obtained in biotic control. It may be due to the toxicity of metal for the growth of microorganisms (Malik, 2004). Growths of *P. taiwanensis*, *A. guillouiae* and *K. pneumoniae* become stable after a certain period of time. It is attributed to the fact that the microbial cell's multiplication rate is reached to a stable state. Growths are ceased after this and decay is observed.

For *P. taiwanensis*, *A. guillouiae* and *K. pneumoniae*, results obtained are presented in Tables 5.1, 5.2 and 5.3, respectively. In the present study, 108.3 mg L^{-1} of Cr(VI) (initial concentration: 150 mg L^{-1}); 78.96 mg L^{-1} of Cu(II) (initial concentration: 80 mg L^{-1}) and 39.52 mg L^{-1} of Zn(II) (initial concentration: 40 mg L^{-1}) removals are obtained using *P. taiwanensis*, *A. guillouiae* and *K. pneumoniae*, respectively. The initial concentration of the three metals [150 mg L^{-1} of Cr(VI), 80 mg L^{-1} of Cu(II) and 40 mg L^{-1} of Zn(II)] are considered as optimum concentration in rest of the batch studies unless otherwise specified. Earlier study reported 4.2 mg L^{-1} of Cr(VI) removal (initial Cr(VI) concentration: 5 mg L^{-1}) using *Escherichia coli* 33456 (Lin et al., 2011). In a separate study, the removal of Cu(II) was calculated as 190 mg L^{-1} (initial Cu(II) concentration: 500 mg L^{-1}) using *Acinetobacter* sp. (Andreazza et al., 2012) after 36 h. Present study shows better removal of Cu(II) using *Acinetobacter* sp. Shamim and Rehman (2012) reported 368, 544, 664 and 744 mg L^{-1} of Zn(II) removal (initial Zn(II) concentration: 800 mg L^{-1}) from a multiple metals system [Cd(II), Cr(VI), Cu(II), Pb(II), Zn(II) and Ni(II)] using *K. pneumoniae* after 24, 48, 73 and 96 h, respectively. The results obtained in the present study shows significant removal of Zn(II) using *K. pneumoniae* within less time.

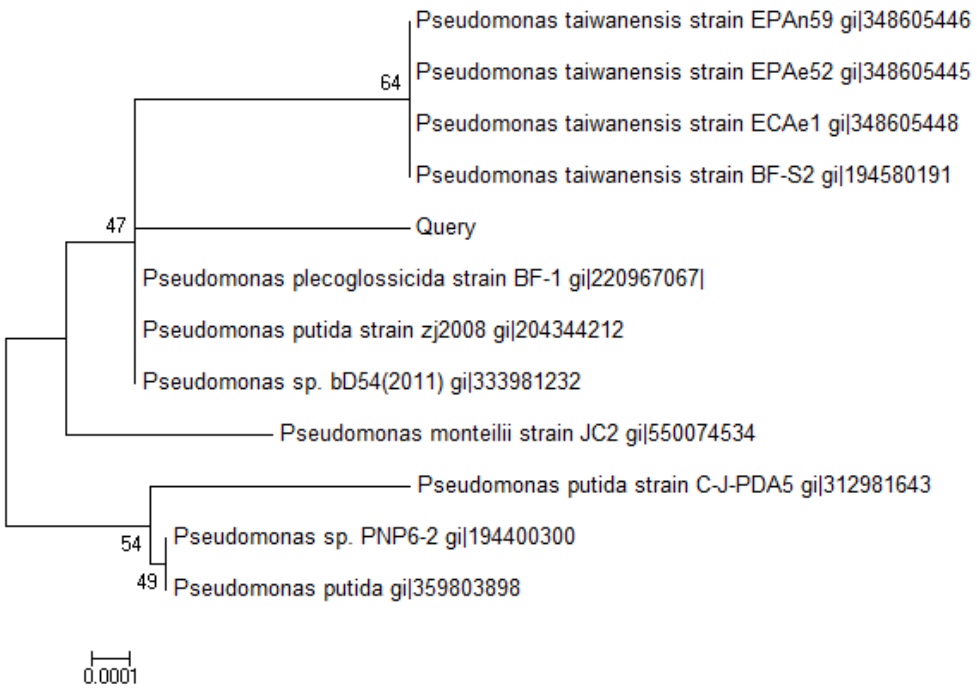


Fig. 5.1. Phylogenetic tree of Cr(VI) resistant bacterial strain based on 16S rRNA gene sequence comparison (Query indicates isolated microbial strain)

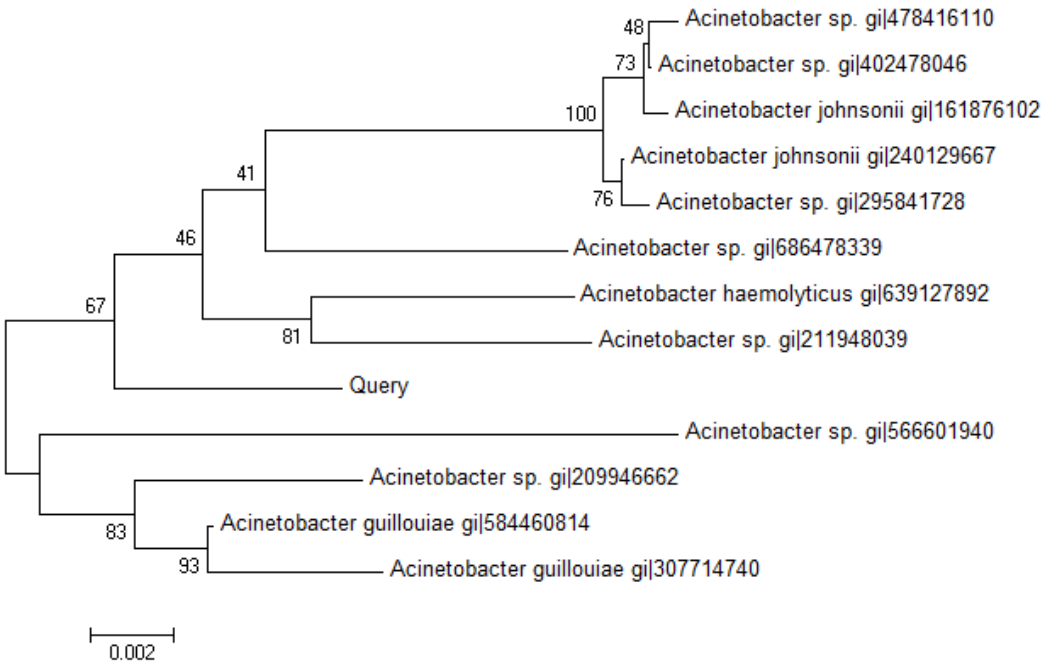


Fig. 5.2. Phylogenetic tree of Cu(II) resistant bacterial strain based on 16S rRNA gene sequence comparison (Query indicates isolated microbial strain)

Fig. 5.3. Phylogenetic tree of Zn(II) resistant bacterial strain based on 16S rRNA gene sequence comparison (Query indicates isolated microbial strain)

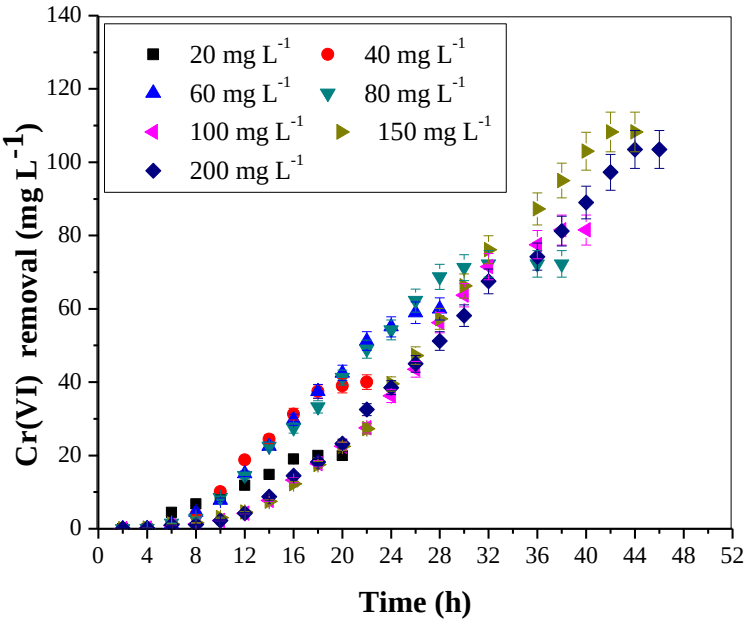


Fig. 5.4. Cr(VI) removal by *P. taiwanensis* at different time for various initial Cr(VI) concentrations (T = 37 ^{o}C, pH = 7.0±0.2, RPM = 150, S_o = 20-200 mg L^{-1})

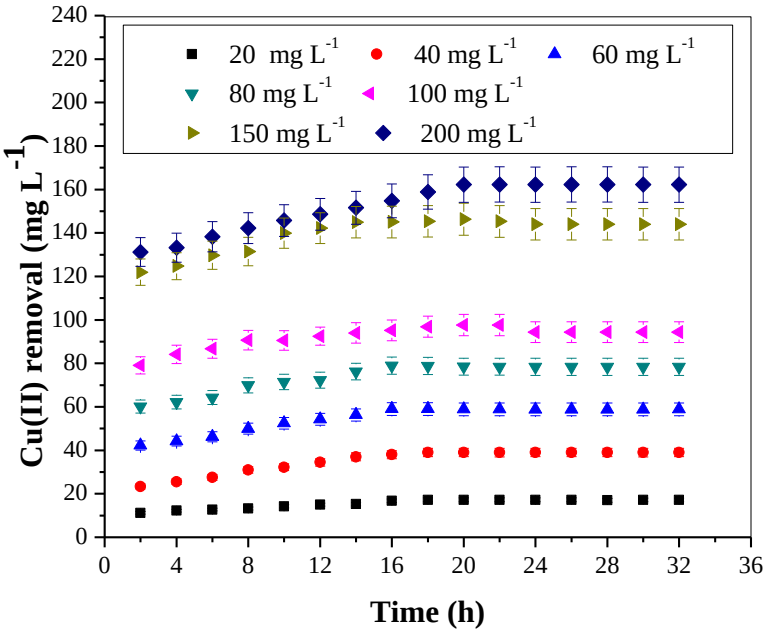


Fig. 5.5. Cu(II) removal by *A. guillouiae* at different time for various initial Cu(II) concentrations (T = 37 °C, pH = 7.0±0.2, RPM = 150, S_o = 20-200 mg L^{-1})

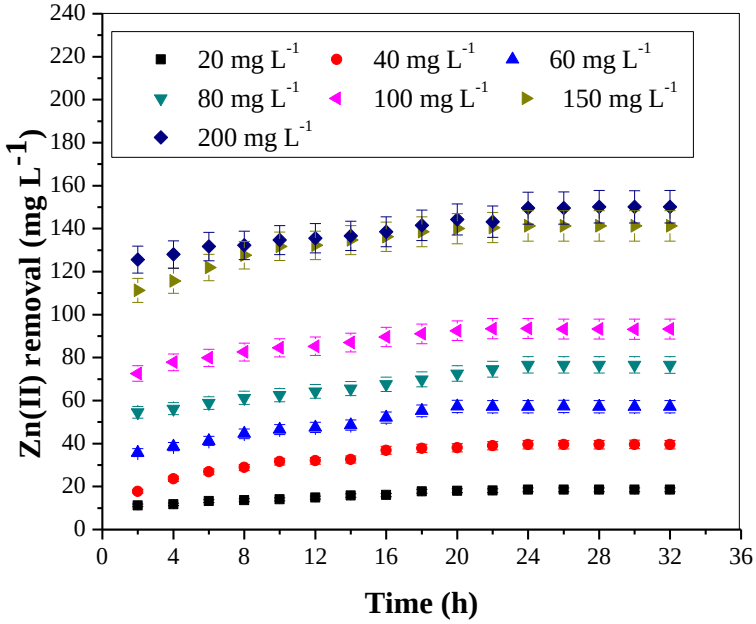


Fig. 5.6. Zn(II) removal by *K. pneumoniae* at different time for various initial Zn(II) concentrations (T = 37 °C, pH = 7.0±0.2, RPM = 150, S_o = 20-200 mg L^{-1})

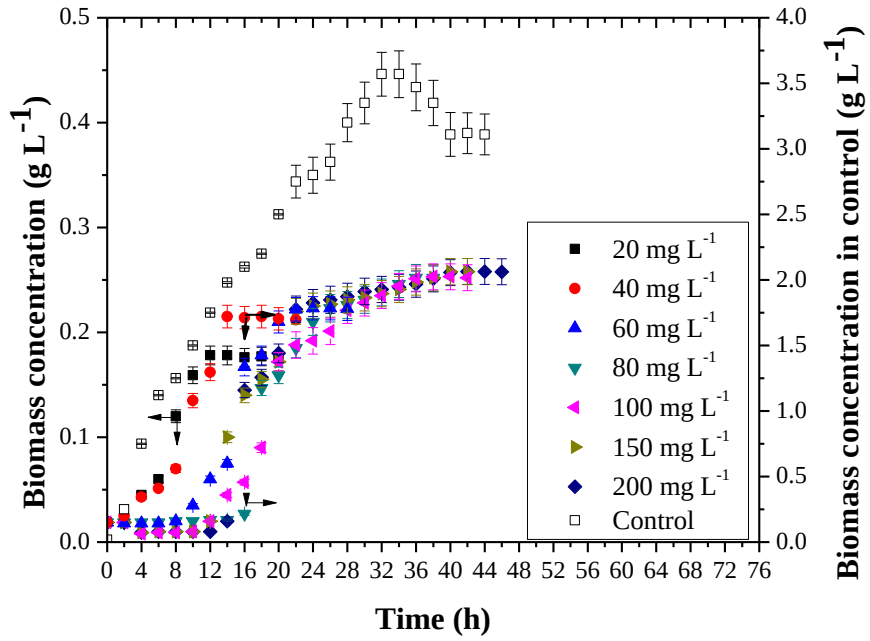


Fig. 5.7. Growth of *P. taiwanensis* with time
(*T* = 37 °C, pH = 7.0±0.2, RPM = 150, 1 % v/v inoculum)

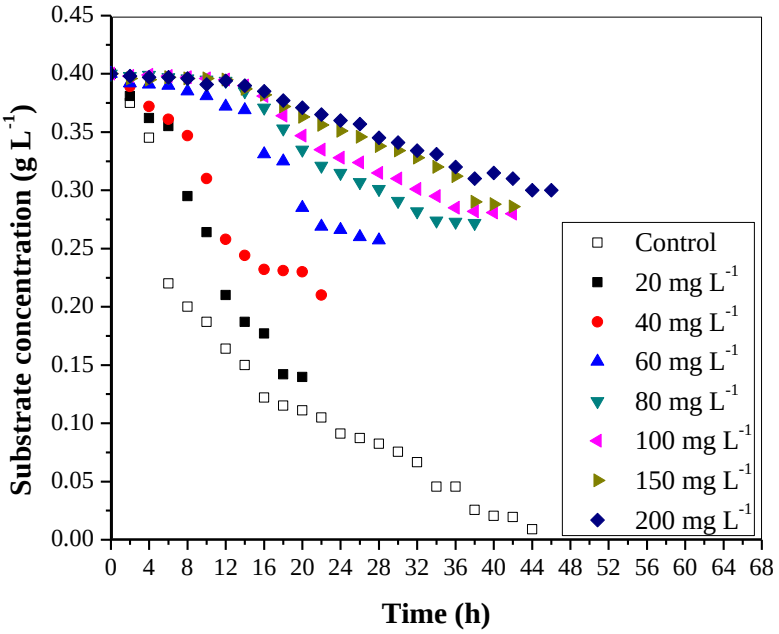


Fig. 5.8. Kinetics of substrate utilization by *P. taiwanensis* for different initial Cr(VI) concentration (*T* = 37 °C, pH = 7.0±0.2, RPM = 150, 1 % v/v inoculum)

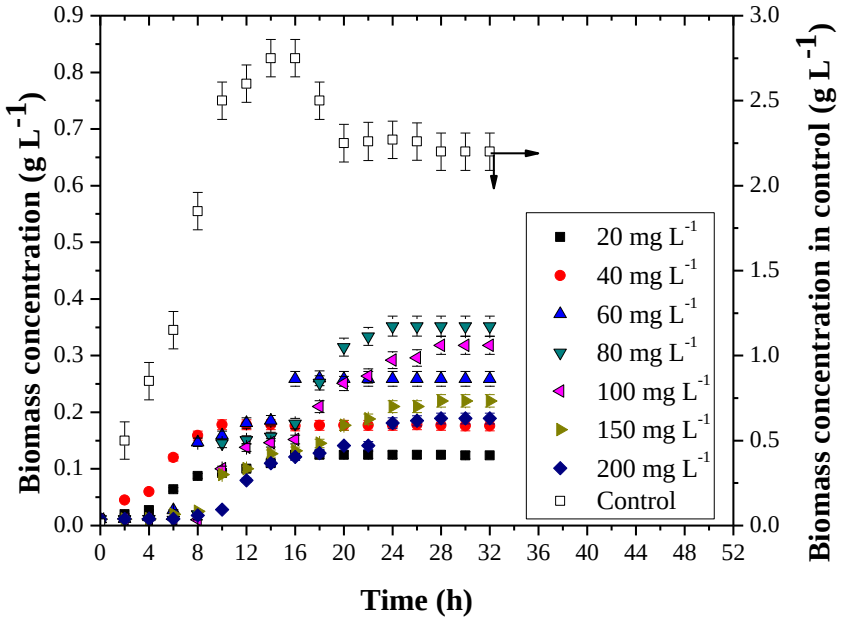


Fig. 5.9. Growth of *A. guillouiae* with time
(*T* = 37 °C, pH = 7.0±0.2, RPM = 150, 1 % v/v inoculum)

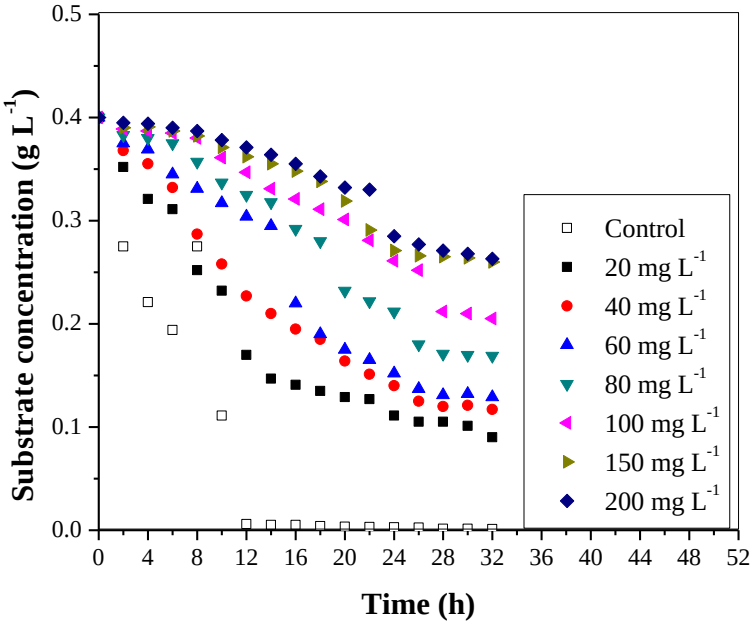


Fig. 5.10. Kinetics of substrate utilization by *A. guillouiae* for different initial Cu(II) concentration (*T* = 37 °C, pH = 7.0±0.2, RPM = 150, 1 % v/v inoculum)

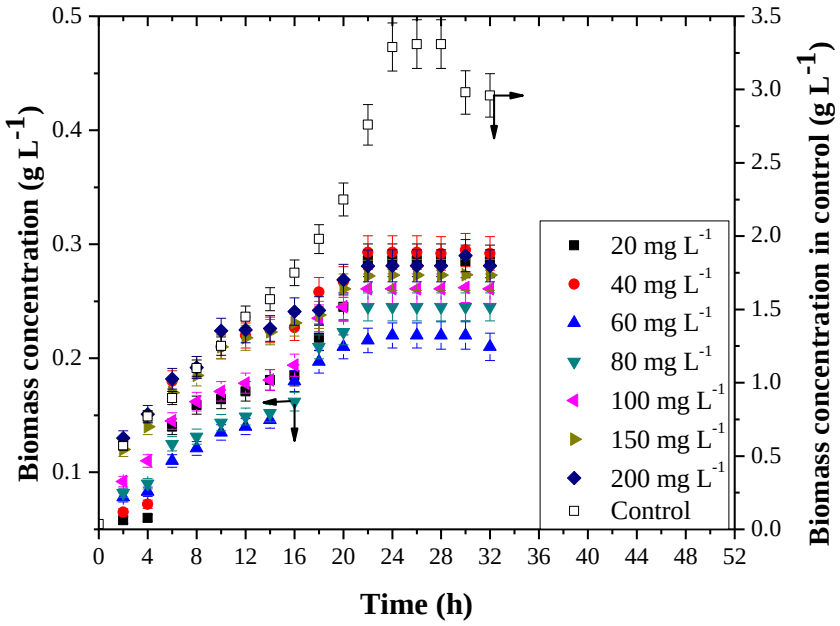


Fig. 5.11. Growth of *K. pneumoniae* with time
(*T* = 37 °C, pH = 7.0±0.2, RPM = 150, 1 % v/v inoculum)

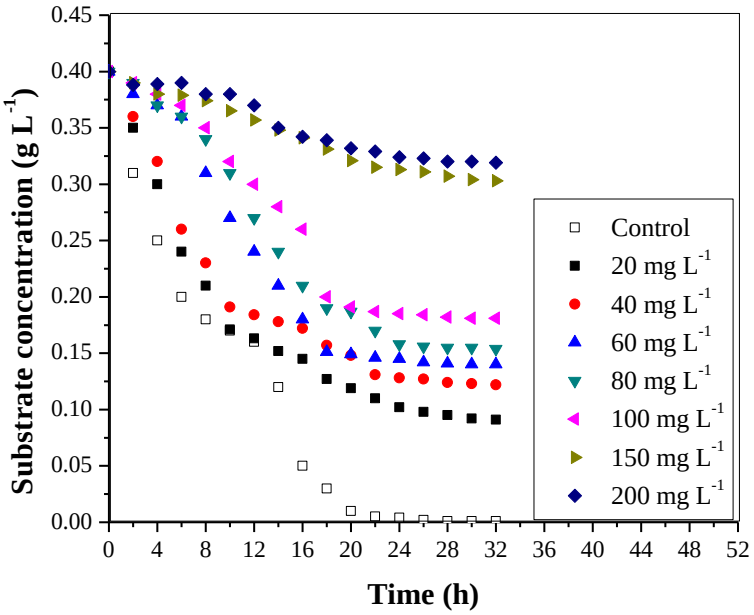


Fig. 5.12. Kinetics of substrate utilization by *K. pneumoniae* for different initial Zn(II) concentration (*T* = 37 °C, pH = 7.0±0.2, RPM = 150, 1 % v/v inoculum)

Table 5.1. Results obtained for effect of time on Cr(VI) removal using *P. taiwanensis*

Initial Cr(VI) concentration (mg L^{-1})	**Lag phase (h)**	**Maximum biomass concentration (g L^{-1})**	**Maximum Cr(VI) removal (%)**
0 (control)	2	3.57	-
20	2	0.258 (for 150 mg L^{-1})	72.2 (for 150 mg L^{-1})
40	6		
60	8		
80	12		
100	10		
150	14		
200	14		

Table 5.2. Results obtained for effect of time on Cu(II) removal using *A. guillouiae*

Initial Cu(II) concentration (mg L^{-1})	**Lag phase (h)**	**Maximum biomass concentration (g L^{-1})**	**Maximum Cu(II) removal (%)**
0 (control)	2	2.75	-
20	4	0.352 (for 80 mg L^{-1})	98.7 (for 80 mg L^{-1})
40	4		
60	6		
80	8		
100	8		
150	10		
200	10		

Table 5.3. Results obtained for effect of time on Zn(II) removal using *K. pneumoniae*

Initial Zn(II) concentration (mg L^{-1})	**Lag phase (h)**	**Maximum biomass concentration (g L^{-1})**	**Maximum Zn(II) removal (%)**
0 (control)	2	3.31	-
20	4	0.293 (for 40 mg L^{-1})	98.8 (for 40 mg L^{-1})
40	8		
60	6		
80	6		
100	10		
150	14		
200	14		

5.1.1.5. Effect of initial pH

The effects of initial pH on the growth of *P. taiwanensis, A. guillouiae* and *K. pneumoniae* are investigated in terms of biomass concentration (g L^{-1}). The results are shown in Figs. 5.13-5.15. The initial metal concentration of Cr(VI), Cu(II) and Zn(II) are maintained as 150, 80 and 40 mg L^{-1}, respectively in separate flasks. These initial concentrations of three metals are taken based on the results obtained in section 5.1.1.4.

In biotic control, the biomass concentrations of *P. taiwanensis* are obtained as 0.5, 1.2, 1.9, 2.45 and 1.7 g L^{-1} at pH 2.0, 4.0, 6.0, 7.0 and 8.0, respectively (Fig. 5.13). The maximum biomass concentration (2.45 g L^{-1}) is obtained at pH 7.0 in biotic control. In the presence of Cr(VI), the biomass concentrations are obtained as 0.0125, 0.015, 0.018, 0.023 and 0.018 g L^{-1} at pH 2.0, 4.0, 6.0, 7.0 and 8.0, respectively (Fig. 5.13). The maximum biomass concentration (0.023 g L^{-1}) is obatined at pH 7.0.

It is observed that the extreme pH values (2.0 and 8.0) inhibit the growth of *Pseudomonas* sp. and result in less Cr(VI) removal (McLean and Beveridge, 2001). It is attributed to the fact that the variation in pH affects the degree of ionization of enzymes by changing the conformation of protein structures present in the microorganism. Cr(VI) in the solution usually exists in the form of hydrogenchromate ($HCrO_4^-$) and chromate anions (CrO_4^{2-}) (Wang et al., 2002). The chromate ion is the predominant species in alkaline media while hydrogenchromate is the predominant ion in acidic media (McLean et al., 1996). The surface of *Pseudomonas* sp. is negatively charged due to the ionization of the functional groups which contributes to the metal binding (McLean and Beveridge, 2001). For *Pseudomonas* sp., the uptake capacity of Cr(VI) is less at low pH values (below 7) due to the presence of $HCrO_4^-$. Low pH values restricts $HCrO_4^-$ to get sorped

on the surface of the *Pseudomonas* sp. At high pH values (greater than 7), *Pseudomonas* sp. shows less uptake of Cr(VI) due to the interference of negatively charged microbial surface with CrO_4^{2-} (McLean et al., 1996). Donati et al. (2003) reported that at pH 7.0, *Thiobacillus thioparus* has the highest free bacterial populations and the highest chromium reduction values.

In biotic control, the biomass concentrations of *A. guillouiae* are obtained in the range of 0.89-2.75 g L^{-1} for pH in the range of 2.0-7.0, respectively (Fig. 5.14). In biotic control, the maximum biomass concentration (3.2 g L^{-1}) of *A. guillouiae* is obtained at pH 8.0. In the presence of Cu(II), the biomass concentrations are found to be 0.004, 0.011, 0.018, 0.021 and 0.029 g L^{-1} at pH 2.0, 4.0, 6.0, 7.0 and 8.0, respectively (Fig. 5.14). In presence of Cu(II), *A. guillouiae* shows maximum biomass concentration (0.029 g L^{-1}) at pH 8.0. Various co-enzymes or complex Cu-protein molecules may be formed in presence of Cu. Due to this reason, the enzymatic activity of the microbial cells is changed. In a similar study, it has been reported that growth of *A. guillouiae* decreases with pH below 6.0 and above 8.0 (Kratochvil and Volesky, 1998).

In biotic control, *K. pneumoniae* gives biomass concentrations in the range of 0.89-2.21 g L^{-1} for pH in the range of 2.0-6.0, respectively (Fig. 5.15). The biomass concentrations is obtained as 1.92 g L^{-1} for pH 8.0. The maximum biomass concentration (3.29 g L^{-1}) of *K. pneumoniae* is obtained at pH 7.0. In the presence of Zn(II), *K. pneumoniae* gives 0.0158, 0.0397, 0.151, 0.2157 and 0.1889 g L^{-1} of biomass concentrations at pH 2.0, 4.0, 6.0, 7.0 and 8.0, respectively (Fig. 5.15). The maximum biomass concentration is obtained as 0.2157 g L^{-1} at pH 7.0.

5.1.1.6. Effect of minimal salt media (MSM) dosage

The effects of MSM dosage on the growth of three bacterial strains are determined in terms of biomass concentration (g L^{-1}) in biotic control and in the presence of metals (Figs. 5.16-5.18).

In biotic control, the biomass concentrations of *P. taiwanensis* are obtained as 1.1, 1.9 and 2.1 g L^{-1} for half, single and triple the standard composition of MSM dosage, respectively (Fig. 5.16). It is observed that *P. taiwanensis* shows maximum biomass concentration (2.75 g L^{-1}) in biotic control at double the standard composition of MSM dosage. In the presence of Cr(VI), *P. taiwanensis* gives 0.004, 0.008 and 0.023 g L^{-1} of biomass concentration for half, single and triple the standard composition of MSM dosage, respectively while it givess 0.025 g L^{-1} of maximum biomass concentration at double the standard composition of MSM dosage (Fig. 5.16).

The biomass concentrations of *A. guillouiae* are obtained as 1.5, 2.75, 3.25 and 2.9 for half, single, double and triple the standard composition of MSM dosage, respectively in biotic control (Fig. 5.17). In the presence of Cu(II), the biomass concentrations of *A. guillouiae* are decreased to 0.005, 0.009, 0.031 and 0.029 g L^{-1} for half, single, double and triple the standard composition of MSM dosage, respectively. The obtained results for this study indicates that the double the standard composition of MSM dosage gives the maximum biomass concentration in biotic control and in the presence of Cu(II) for *A. guillouiae*.

The biomass concentration for *K. pneumoniae* under biotic control is obtained as 3.75 g L^{-1} at double the standard composition of MSM dosage (Fig. 5.18) while the values are obtained as 1.789, 3.29 and 1.66 for half, single and triple the standard

composition of MSM dosage, respectively. In the presence of Zn(II), *K. pneumoniae* shows 0.157, 0.216 and 0.162 g L^{-1} of biomass concentration for half, single and triple the standard composition of MSM dosage, respectively (Fig. 5.18). However, the maximum biomass concentration of *K. pneumoniae* is obtained as 0.354 g L^{-1} for double the standard composition of MSM.

Mineral salts present in the nutrient (MSM) help in the growth of the bacterial strain by promoting ribosome synthesis. However, excess amount of mineral salts present in the MSM increase translation rates which leads to increased concentration of inhibitory protein (cell cycle transcriptional regulator, CtrA). It decreases the growth of the cell and any further increase in the concentration of this protein leads to the decay of the cell (Morgan 2007). Excess mineral salts may also decrease the enzyme activity of the cell beyond a threshold value. A slight decrease in biomass concentration is observed when the composition of mineral salts is tripled the standard MSM dosage for all the three strains. This indicates that the threshold amount of nutrient for the cell growth is lying between the double and triple the standard compositions of MSM dosage. The biomass concentration is decreased when the composition of mineral salts are taken as single and half the standard composition in MSM dosage. This is attributed to the fact that presence of less mineral salts in MSM restricts the growth of the three bacterial strains during the removal of metals.

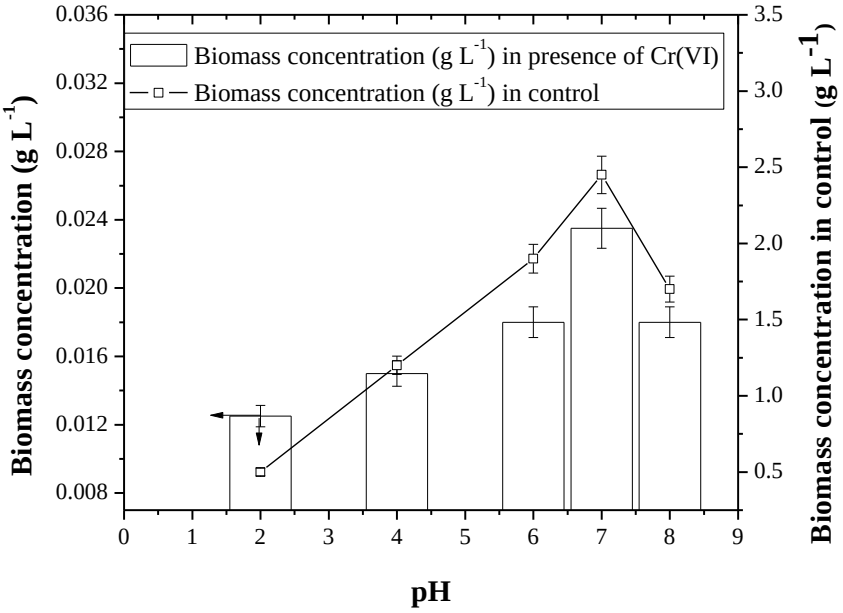


Fig. 5.13. Effect of pH on growth of *P. taiwanensis*
(*T* = 37 °C, RPM = 150, 1 % v/v inoculum, S_o = 150 mg L^{-1} of Cr(VI))

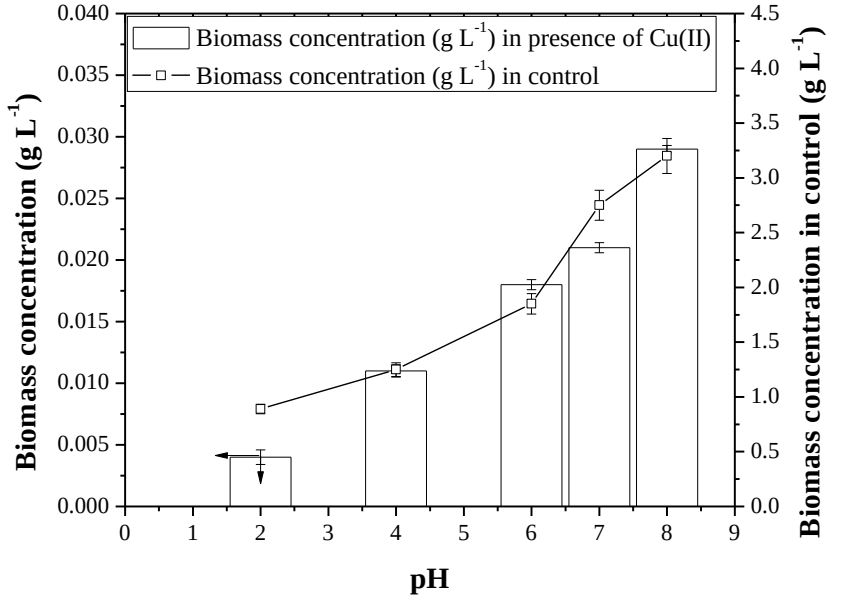


Fig. 5.14. Effect of pH on growth of *A. guillouiae*
(*T* = 37 °C, RPM = 150, 1 % v/v inoculum, S_o = 80 mg L^{-1} of Cu(II))

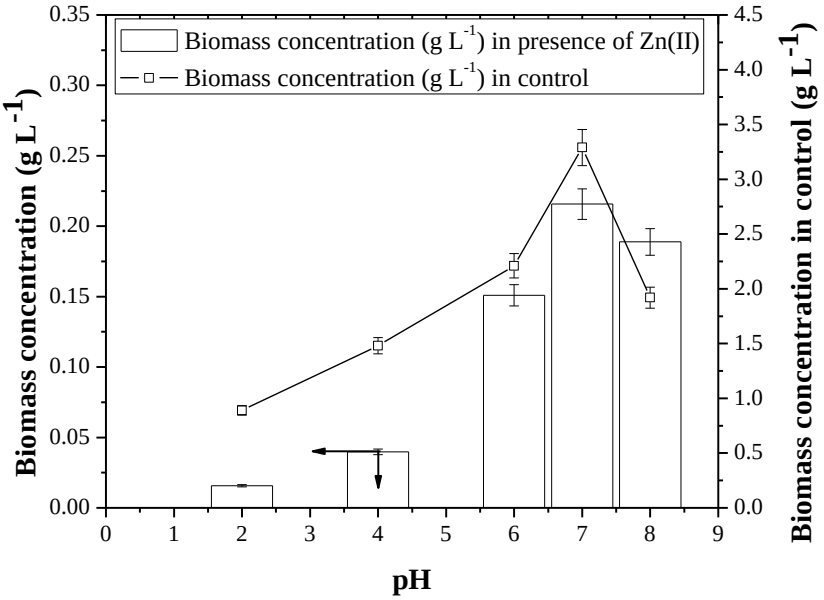


Fig. 5.15. Effect of pH on growth of *K. pneumoniae*
(*T* = 37 °C, RPM = 150, 1 % v/v inoculum, S_o = 40 mg L^{-1} of Zn(II))

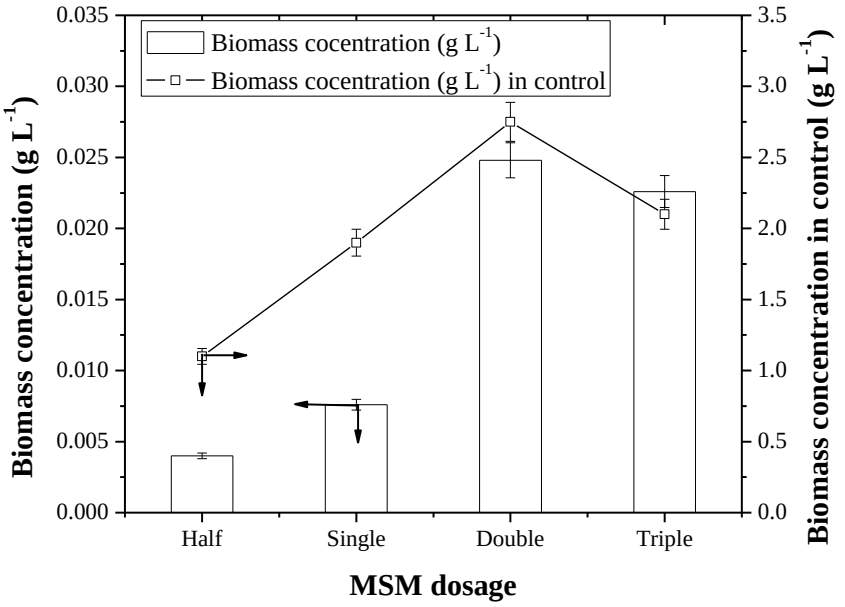


Fig. 5.16. Effect of Nutrient (MSM) dosage on growth of *P. taiwanensis*
(*T* = 37 °C, pH = 7.0±0.2, RPM = 150, 1 % v/v inoculum, S_o = 150 mg L^{-1} of Cr(VI))

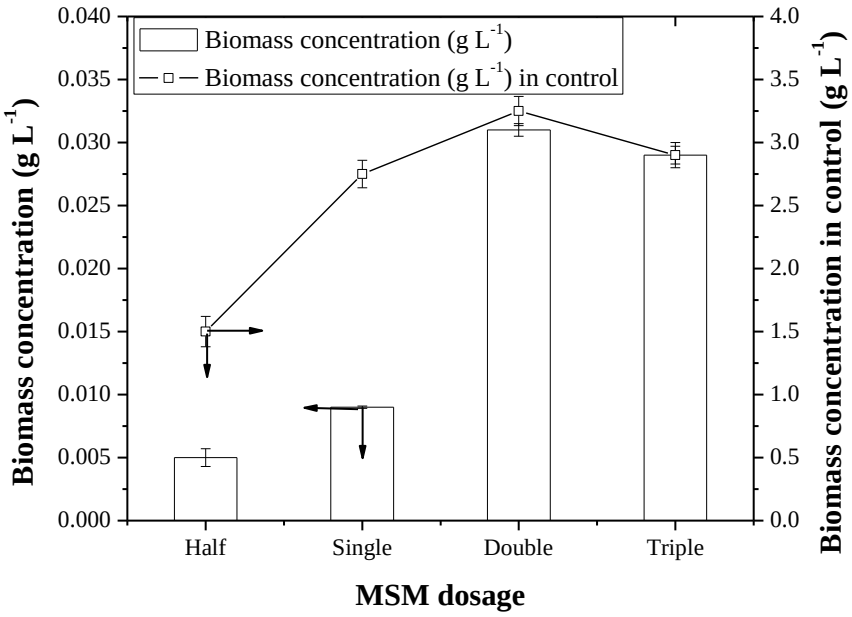


Fig. 5.17. Effect of Nutrient (MSM) dosage on growth of *A. guillouiae*
(*T* = 37 ^{o}C, pH = 7.0±0.2, RPM = 150, 1 % v/v inoculum, S_o = 80 mg L^{-1} of Cu(II))

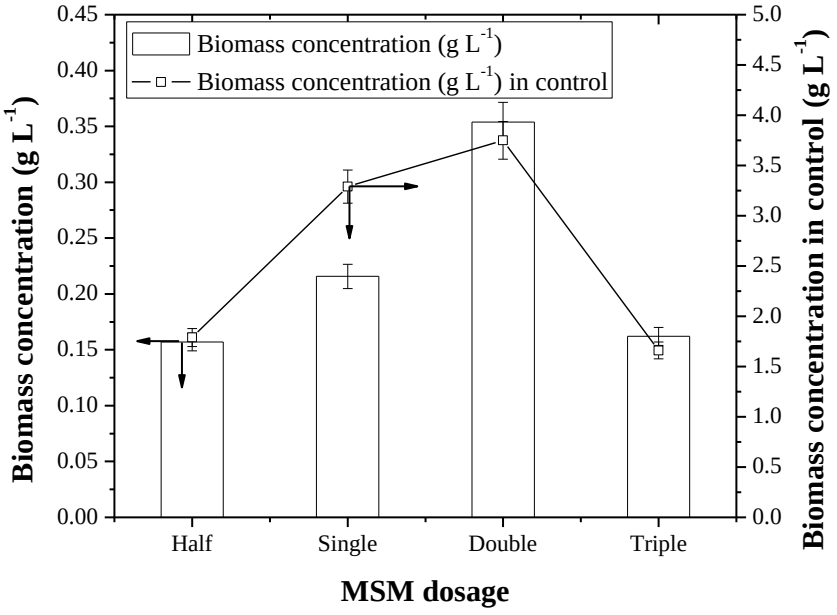


Fig. 5.18. Effect of Nutrient (MSM) dosage on growth of *K. pneumoniae*
(*T* = 37 ^{o}C, pH = 7.0±0.2, RPM = 150, 1 % v/v inoculum, S_o = 40 mg L^{-1} of Zn(II))

5.1.1.7. Effect of macronutrients (N, P and K)

The effects of macronutrients (N, P and K) compositions (in MSM dosage) on the growth of the three bacterial strains are studied in terms of biomass concentration (g L^{-1}) and are shown in Figs. 5.19-5.21.

In biotic control, the biomass concentrations of *P. taiwanensis* are found to be 1.89, 2.25 and 0.85 g L^{-1} at half, double and triple the standard compositions of N in MSM dosage, respectively while it is 1.95 g L^{-1} at standard composition of N (Fig. 5.19). *P. taiwanensis* shows 1.5, 2.5 and 0.9 g L^{-1} of biomass concentration at half, double and triple the standard compositions of P, respectively. The standard composition of P gives 1.75 g L^{-1} of biomass concentration. For K, the standard composition gives 2.2 g L^{-1} of biomass concentration while half, double and triple the standard compositions give 1.95, 1.65 and 0.8 g L^{-1} of biomass concentration, respectively. In biotic control, *P. taiwanensis* shows maximum biomass concentrations of 2.25, 2.5 and 2.2 g L^{-1} at double the standard composition of N, P and standard composition of K, respectively. In the presence of Cr(VI), The biomass concentrations of *P. taiwanensis* are obtained as 0.015, 0.083 and 0.019 g L^{-1} at half, double and triple the standard compositions of N while the value is obtained as 0.025 g L^{-1} in standard composition of N (Fig. 5.19). The biomass concentrations are obtained as 0.019, 0.095 and 0.021 g L^{-1} for half, double and triple the standard compositions of P, respectively. *P. taiwanensis* gives 0.035 g L^{-1} of biomass concentration at standard composition of P. The biomass concentrations are found to be 0.069, 0.046 and 0.011 g L^{-1} at half, double and triple the standard composition of K. The standard composition of K gives 0.189 g L^{-1} of biomass concentration.

The biomass concentration of *A. guillouiae* under biotic control is obtained as 1.85 g L^{-1} at standard composition of N while the values are 1.75, 2.25 and 1.1 g L^{-1} at

half, double and triple the standard composition of N, respectively (Fig. 5.20). For P, the biomass concentrations are found to be 1.5, 1.75 and 1.125 g L^{-1} at half, double and triple the standard composition of P, respectively while the value is 1.7 g L^{-1} at standard composition of P. *A. guillouiae* shows 0.25, 0.5 and 0.11 g L^{-1} of biomass concentration at half, double and triple the standard composition of K, respectively. Standard composition of K in MSM shows 0.82 g L^{-1} of biomass concentration. In biotic control, the maximum biomass concentration of *A. guillouiae* is found to be 2.25, 1.75 and 0.82 g L^{-1} at double the standard composition of N and P and standard composition of K, respectively. In the presence of Cu(II), *A. guillouiae* shows 0.01, 0.052 and 0.01 g L^{-1} of biomass concentration at half, double and triple the standard composition of N, respectively while it shows 0.012 g L^{-1} of biomass concentration at standard composition of N. The biomass concentrations of *A. guillouiae* are obtained as 0.013, 0.023 and 0.019 g L^{-1} at half the standard composition, standard and triple the standard composition of P, respectively. The maximum biomass concentration is obtained as 0.042 g L^{-1} at double the standard composition of P. The standard composition of K shows 0.106 g L^{-1} of biomass concentration while half, double and triple the standard composition of K show 0.058, 0.022 and 0.012 g L^{-1} of biomass concentration, respectively.

In biotic control, *K. pneumoniae* shows 2.1, 1.28, 3.21 and 0.72 g L^{-1} of biomass concentration at standard composition, half, double and triple the standard composition of N, respectively while it shows 2.75, 1.21, 3.45 and 1 g L^{-1} of biomass concentration at standard composition, half, double and triple the standard composition of P (Fig. 5.21). The biomass concentrations are obtained as 1.86, 1.48 and 0.91 g L^{-1} at half, double and triple the standard compositions of K while the value is obtained as 3.24 g L^{-1} at standard

composition of K. In biotic control, the maximum biomass concentration of *K. pneumoniae* are obtained as 3.21, 3.45 and 3.24 g L^{-1} at double the standard composition of N & P and standard composition of K, respectively. In the presence of Zn(II), standard composition of N gives 0.086 g L^{-1} of biomass concentration. *K. pneumoniae* shows 0.019, 0.091 and 0.013 g L^{-1} of biomass concentration at half, double and triple the standard composition of N, respectively. The biomass concentration of *K. pneumoniae* is found to be 0.078 g L^{-1} at standard composition of P while the value is 0.045, 0.099 and 0.028 g L^{-1} at half, double and triple the standard composition of P, respectively. Standard composition of K shows 0.278 g L^{-1} of biomass concentration for *K. pneumoniae*. The biomass concentrations are obtained as 0.125, 0.035 and 0.026 g L^{-1} at half, double and triple the standard composition of K.

In the presence of metals, the maximum biomass concentration of all the three bacterial strains are obtained at double the standard compositions of N & P and standard composition of K. For Cr(VI) bioremediation, the maximum biomass concentrations are 0.083, 0.095 and 0.189 g L^{-1} using *P. taiwanensis* at respective compositions of N, P and K mentioned above. *A. guillouiae* gives 0.052, 0.042 and 0.106 g L^{-1} of maximum biomass concentrations in the presence of Cu(II) while *K. pneumoniae* shows maximum growth of 0.091, 0.099 and 0.278 g L^{-1} in the presence of Zn(II) at respective compositions of N, P and K mentioned above.

Macronutrients (N, P and K) are considered as primary nutrients as they are the essential elements for the growth and development of the microbial cells. Syntheses of amino acids, DNA and RNA are majorly governed by nitrogen. It is the main constituent of amino acids, nucleic acids, nucleotides and coenzymes (El-Sharkawi, 2012; Kisand et

al., 2001). Phosphorus is considered to be an essential element for nucleic acid synthesis and for the construction of phospholipids (Kisand et al., 2001; Vidal et al., 2011). Potassium has many functions in growth such as smoothening the progress of cell division and growth. It also acts as a key component to activate enzymes for metabolization of carbohydrates. It helps in the manufacturing of amino acids and proteins (Epstein, 2003). In the present study, the growths of *P. taiwanensis*, *A. guillouiae* and *K. pneumoniae* in biotic control are found in decreasing order beyond the double the standard composition of N and P in MSM. The excess mineral elements hinder the growth of the microbial cells (Epstein, 2003). Similar trend is observed at half and standard compositions of N and P. It is attributed to the fact that limited supply of N and P restricts the growth and development of microbial cells (Epstein, 2003). Excess composition of K in MSM dosage can be toxic for the growth of microbial strain. Similar results are reported for the degradation of crude oil using marine bacteria (Kanaly et al., 2002). The decreasing trend in biomass concentration with increase in the N, P and K compositions beyond certain limit may be due to the presence of multiple metals in the solution which may hinder the transfer of the N, P and K inside the bacterial strains.

A separate batch study is conducted in three 250 mL conical flasks to validate the obtained optimum values of the N, P and K for the growth of *P. taiwanensis*, *A. guillouiae* and *K. pneumoniae*. The concentration of the three metals is maintained same as mentioned in above study. A stock 1 L MSM solution is prepared by adding specific amounts of salts to maintain the optimum composition of N, P and K. One hundred milliliter of sterilzed MSM is taken in each 250 mL of conical flask. Each sample is amended with respective concentrations of metals and then inoculated with 1% (v/v) of

individual bacterial strains. The samples are incubated for 24 h. The growth of *P. taiwanensis, A. guillouiae* and *K. pneumoniae* is found to be 0.3, 0.17 and 0.41 g L^{-1}, respectively. The obtained values are higher as compared to the values obtained at any other composition of N, P and K in the above study. No earlier reports have been found on assessing the individual effects of macronutrients (N, P and K) in MSM dosage for the growth of metal removal bacteria *P. taiwanensis, A. guillouiae* and *K. pneumoniae*.

5.1.1.8. Effect of aerobic and facultative anaerobic conditions

This study is conducted to determine the effect of oxygen (aerobic) and nitrogen (facultative anaerobic) on the growth of *P. taiwanensis, A. guillouiae* and *K. pneumoniae* in terms of biomass concentration (g L^{-1}). Figs. 5.22-5.24 show the growth of three bacterial strains in biotic control and in the presence of metals under aerobic & facultative anaerobic conditions.

In biotic control, the biomass concentrations of *P. taiwanensis* are obtained as 2.42 and 1.25 g L^{-1} for aerobic and facultative anaerobic conditions, respectively (Fig. 5.22). In the presence of Cr(VI), *P. taiwanensis* gives 0.252 g L^{-1} of maximum biomass concentration under aerobic condition which is decreased to 0.095 g L^{-1} in facultative anaerobic condition. Earlier study reported 83.4 % removal of Cr(VI) for an initial concentration of 360 mg L^{-1} using *Brevibacterium casei* under aerobic condition (Ng et al., 2010).

In biotic control, *A. guillouiae* shows the maximum biomass concentration (1.75 g L^{-1}) for facultative anaerobic condition while the value for aerobic condition is obtained as 0.85 g L^{-1} (Fig. 5.23). In the presence of Cu(II), the maximum biomass concentration of *A. guillouiae* is found to be 0.077 g L^{-1} for facultative anaerobic condition as compared

to biomass concentration of 0.034 g L^{-1} for aerobic condition. Results corroborate the fact that *A. guillouiae* shows better growth in the facultative anaerobic condition. It has been reported that facultative anaerobic condition favors the growth of metal-respiring bacteria (i.e. *Acinetobacter* sp.) for the removal metal(loids) as they possess specialized dissimilatory metabolic pathways to uptake metals (Csotonyi et al., 2006; Shen and Wang, 1994).

In biotic control, *K. pneumoniae* shows 3.287 g L^{-1} of maximum biomass concentration for aerobic condition as compared to 1.1 g L^{-1} of biomass concentration obtained for facultative anaerobic condition (Fig. 5.24). In the presence of Zn(II), the biomass concentrations of *K. pneumoniae* are obtained as 0.216 and 0.121 g L^{-1} for aerobic and facultative anaerobic conditions, respectively. Earlier study reported 744 mg L^{-1} of Zn(II) removal using *K. pneumoniae* under biotic control (Shamim and Rehman, 2012).

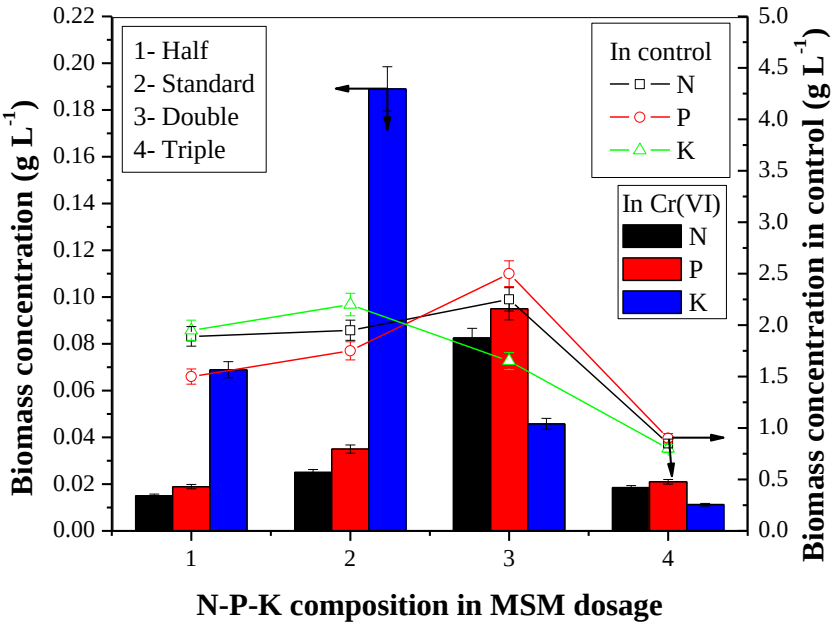


Fig. 5.19. Effect of N-P-K composition in MSM on growth of *P. taiwanensis* (*T* = 37 °C, pH = 7.0±0.2, RPM = 150, 1 % v/v inoculum, S_o = 150 mg L^{-1} of Cr(VI))

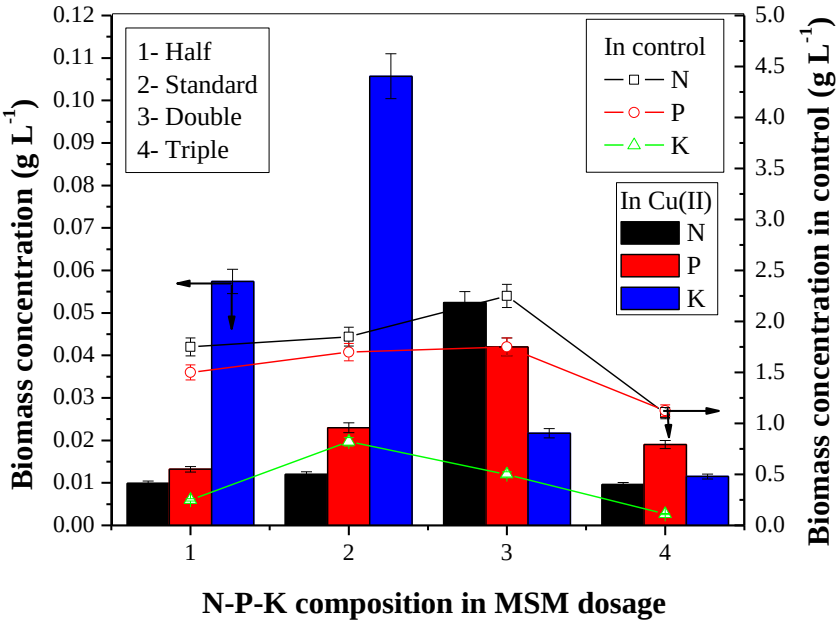


Fig. 5.20. Effect of N-P-K composition in MSM on growth of *A. guillouiae* (*T* = 37 °C, pH = 7.0±0.2, RPM = 150, 1 % v/v inoculum, S_o = 80 mg L^{-1} of Cu(II))

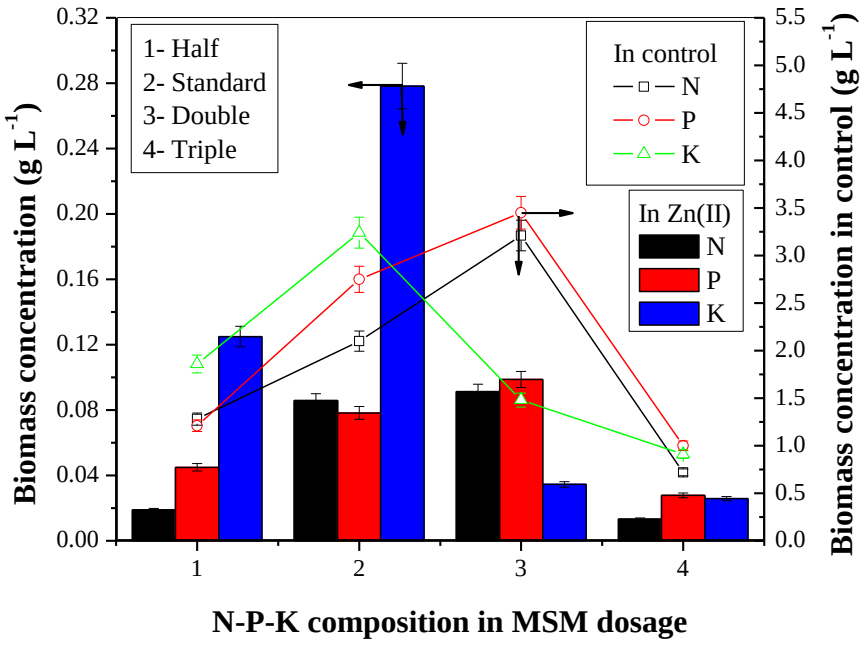


Fig. 5.21. Effect of N-P-K composition in MSM on growth of *K. pneumoniae* (*T* = 37 °C, pH = 7.0±0.2, RPM = 150, 1 % v/v inoculum, S_o = 40 mg L^{-1} of Zn(II))

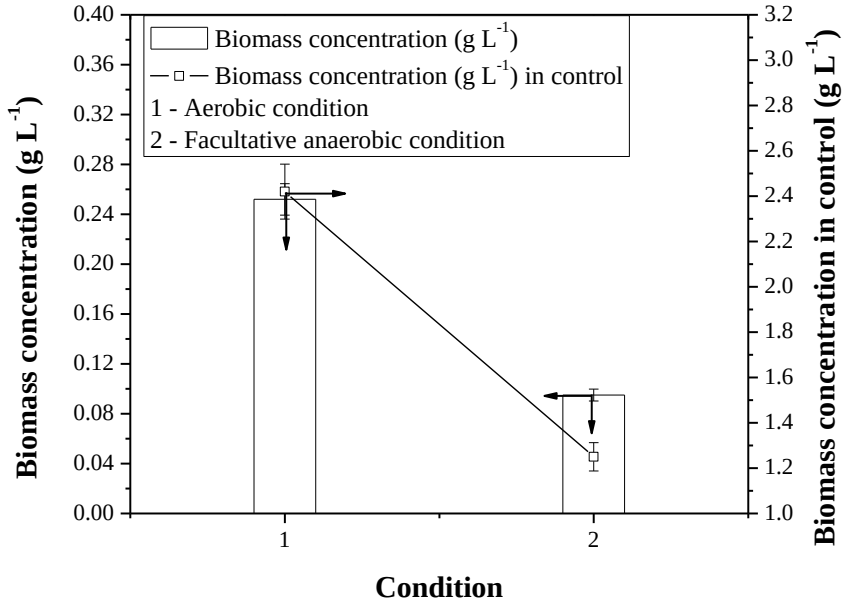


Fig. 5.22. Effect of aerobic and facultative anaerobic conditions on growth of *P. taiwanensis* (*T* = 37 °C, pH = 7.0±0.2, RPM = 150, 1 % v/v inoculum, S_o = 150 mg L^{-1} of Cr(VI))

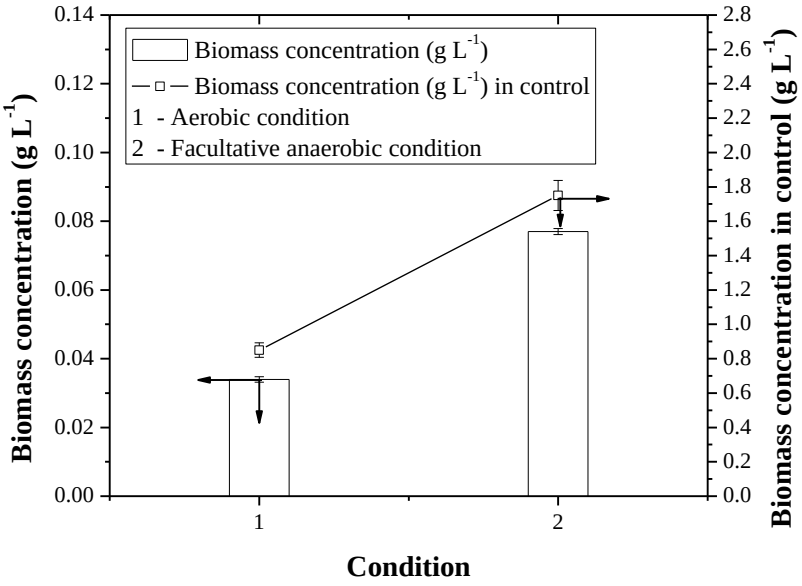


Fig. 5.23. Effect of aerobic and facultative anaerobic conditions on growth of *A. guillouiae* (*T* = 37 °C, pH = 7.0±0.2, RPM = 150, 1 % v/v inoculum, S_o = 80 mg L^{-1} of Cu(II))

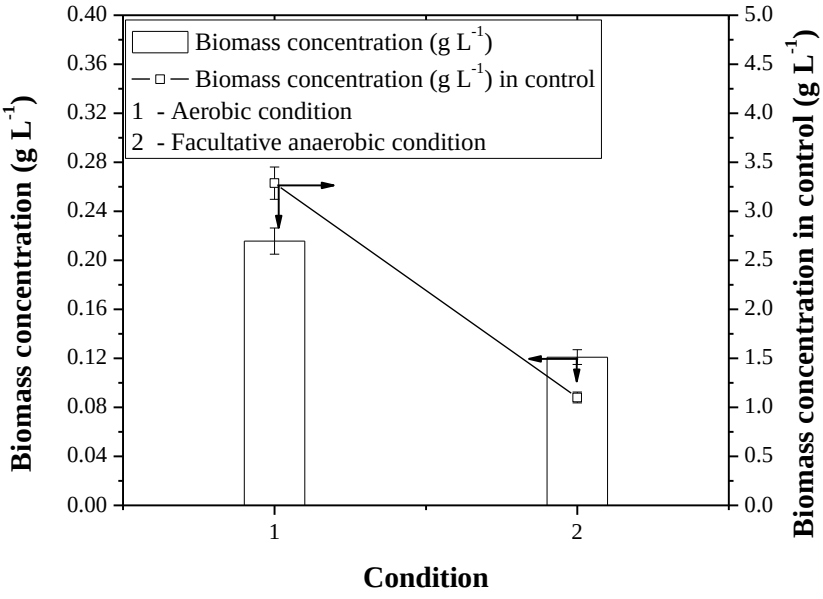


Fig. 5.24. Effect of aerobic and facultative anaerobic conditions on growth of *K. pneumoniae* (*T* = 37 °C, pH = 7.0±0.2, RPM = 150, 1 % v/v inoculum, S_o = 40 mg L^{-1} of Zn(II))

5.1.1.9. Effect of temperature

In the present study, effect of temperature on the growth of *P. taiwanensis, A. guillouiae* and *K. pneumoniae* is investigated in terms of biomass concentration (g L^{-1}). Figs. 5.25-5.27 show the growth of three bacterial strains at different temperatures in biotic control and in the presence of metals.

In biotic control, *P. taiwanensis* gives 0.125, 0.579, 1.87, 2.89, 2.45 and 0.115 g L^{-1} of biomass concentration at temperature 25, 30, 35, 37, 40 and 45 °C, respectively (Fig. 5.25). *P. taiwanensis* gives 2.89 g L^{-1} of maximum biomass concentration at 37 °C. The biomass concentrations are decreased beyond temperature 37 °C. In the presence of Cr(VI), *P. taiwanensis* gives 0.0098, 0.0115 and 0.0211 g L^{-1} of biomass concentration at temperature 25, 30 and 35 °C, respectively. The biomass concentration is further increased and reached to maximum value of 0.0237 g L^{-1} at temperature 37 °C. The biomass concentration of *P. taiwanensis* is decreased beyond temperature 37 °C. The biomass concentrations are obtained as 0.0234 and 0.0087 g L^{-1} at temperature 40 and 45 °C, respectively.

In biotic control, the biomass concentrations of *A. guillouiae* are found in the range of 0.098-2.75 g L^{-1} for temperatures in the range of 25-37 °C, respectively (Fig. 5.26). The maximum biomass concentration is obtained as 3.24 g L^{-1} at temperature 40 ^{o}C. *A. guillouiae* shows decrease in biomass concentration (0.087 g L^{-1}) at temperature 45 ^{o}C. In presence of Cu(II), the biomass concentrations of *A. guillouiae* are obtained in the range of 0.072-0.163 g L^{-1} for temperatures in the range of 25-37 °C, respectively. *A. guillouiae* gives 0.164 g L^{-1} of maximum biomass concentration at temperature 40 ^{o}C. The biomass concentration is decreased to 0.068 g L^{-1} at temperature 45 ^{o}C.

In biotic control, the biomass concentrations of *K. pneumoniae* are obtained as 0.224, 0.678 and 1.975 g L^{-1} at temperatures 25, 30 and 35 °C, respectively (Fig. 5.27). The maximum biomass concentration is obtained as 3.29 g L^{-1} at temperature 37 °C. *K. pneumoniae* shows decline in biomass concentration at temperatures beyond 37 °C. The biomass concentrations are obtained as 3.21 and 0.175 g L^{-1} at temperatures 40 and 45 ^{o}C, respectively. In presence of Zn(II), the biomass concentrations of *K. pneumoniae* are found in the range of 0.013-0.138 g L^{-1} for temperatures in the range of 25-35°C, respectively. The maximum biomass concentration is obtained as 0.216 g L^{-1} at 37 °C. The biomass concentrations are decreased to 0.158 and 0.026 g L^{-1} at 40 and 45 °C, respectively.

In biotic control and in the presence of metals, all the three bacterial strains show less growth at 25 ^{o}C and 45 ^{o}C. This is attributed to the fact that the lipids of the membrane of the bacterial cells stiffen below 30 ^{o}C (Nedwell, 1999). The efficiency of transport proteins embedded in the membrane are decreased which restricts the supply of the substrate i.e. metals to the microbial cells (Nedwell, 1999). It leads to less growth of bacterial strains The membrane of the bacterial cells becomes fragile above 40 ^{o}C which leads to the rupture of the cell membrane (Nedwell, 1999).

5.1.1.10. Effect of inoculum volume

Effects of inoculum volume (0.5 %, 1 %, 1.5 % and 2 % v/v) on the growth of *P. taiwanensis, A. guillouiae* and *K. pneumoniae* are studied in terms of biomass concentrations (g L^{-1}). Figs. 5.28-5.30 show the growth of three bacterial strains at different inoculum volumes in biotic control and in the presence of metals.

In biotic control, the biomass concentrations of *P. taiwanensis* are obtained as 1.25, 1.58 and 1.15 g L^{-1} for 0.5 %, 1.5 % and 2 % of inoculum volumes, respectively

(Fig. 5.28). The maximum biomass concentration is observed as 2.46 g L^{-1} for 1 % of inoculum volume. In the presence of Cr(VI), *P. taiwanensis* gives 0.016, 0.019 and 0.013 g L^{-1} of biomass concentrations for 0.5 %, 1.5 % and 2 % of inoculum volumes, respectively while the maximum biomass concentration is found to be 0.024 g L^{-1} for 1 % of inoculum volume.

In biotic control, *A. guillouiae* gives 1.15 g L^{-1} of biomass concentration for 0.5 % inoculum volume while the maximum biomass concentration is obtained as 3.2 g L^{-1} for 1 % inoculum volume (Fig. 5.29). *A. guillouiae* shows decrease in biomass concentration for inoculum volume more than 1 % (Fig. 5.26). In the presence of Cu(II), the biomass concentration of *A. guillouiae* are obtained as 0.008, 0.009 and 0.008 g L^{-1} for 0.5 %, 1.5 % and 2 % of inoculum volumes, respectively. The maximum biomass concentration of 0.012 g L^{-1} is obtained for 1 % of inoculum volume.

The biomass concentrations of *K. pneumoniae* are found to be 0.9, 2.1 and 1.87 g L^{-1} for 0.5 %, 1.5 % and 2 % of inoculum volumes, respectively in biotic control (Fig. 5.30). *K. pneumoniae* gives 3.29 g L^{-1} of maximum biomass concentration for 1 % of inoculum volume. In the presence of Zn(II), the biomass concentration of *K. pneumoniae* is obtained as 0.097, 0.12 and 0.089 for 0.5 %, 1.5 % and 2 % of inoculum volumes, respectively while the maximum biomass concentration is obtained as 0.216 g L^{-1} for 1 % of inoculum volume.

The maximum growths of *P. taiwanensis, A. guillouiae* and *K. pneumoniae* are obtained for 1 % inoculum volume under biotic control and in the presence of the metals. The higher surface area to volume ratio increases the production of enzyme which leads to better metal uptake (Rahman et al., 2005). Increase in inoculum volume (i.e. for 1.5 %

and 2 %) results in the decrease of growth of the bacterial strains. The controlled volume of a culture medium contains limited amount of nutrients for the growth of bacteria. The consumption of the nutrients depends on the population of bacteria. A higher inoculum volume may result in the lack of oxygen and faster depletion of nutrients in the culture media (Abusham et al., 2009; Rahman et al., 2005). The growth obtained is less with 0.5 % inoculum volume for all the three bacterial strains. It may be due to the fact that small inoculum volume produces insufficient number of bacterial population (Abusham et al., 2009).

The optimum value of the parameters obtained from batch studies are presented in Table 5.4 for the removal of Cr(VI), Cu(II) and Zn(II) using *P. taiwanensis*, *A. guillouiae* and *K. pneumoniae*, respectively.

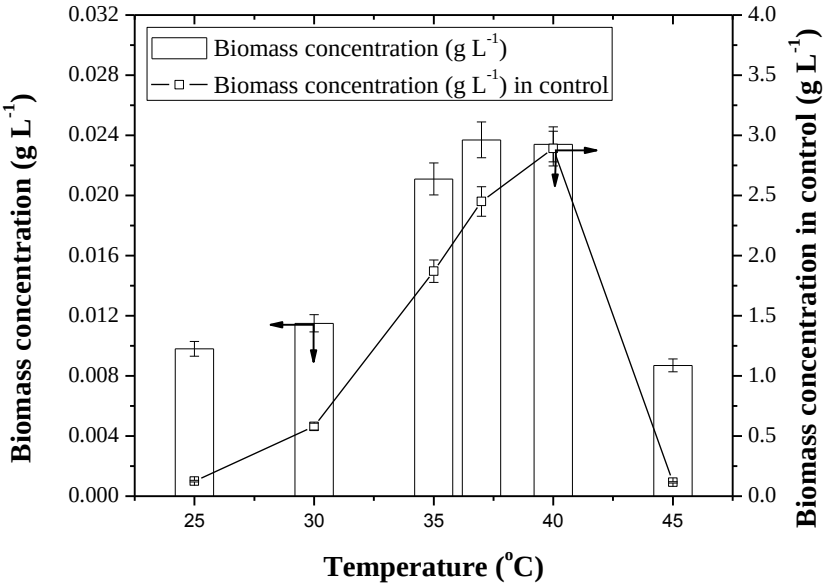


Fig. 5.25. Effect of temperature on growth of *P. taiwanensis*
(pH = 7.0±0.2, RPM = 150, 1 % v/v inoculum, S_o = 150 mg L^{-1} of Cr(VI))

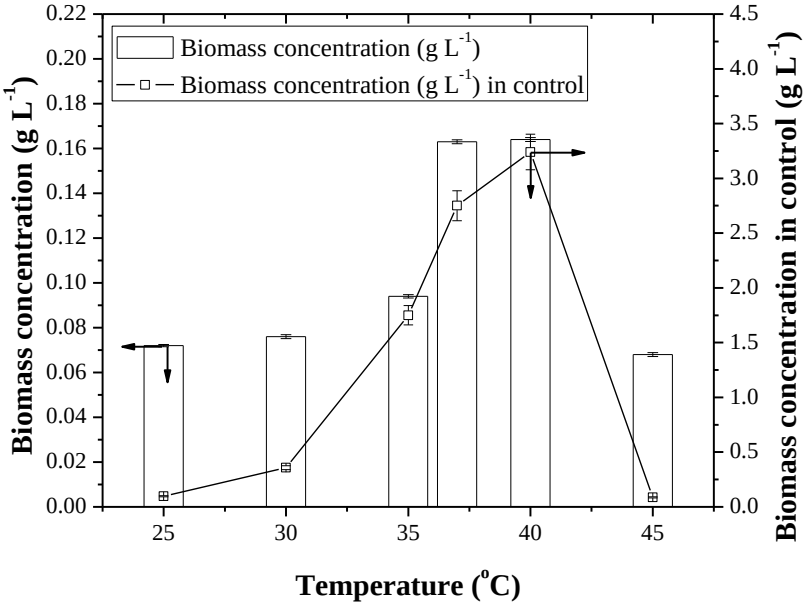


Fig. 5.26. Effect of temperature on growth of *A. guillouiae*
(pH = 7.0±0.2, RPM = 150, 1 % v/v inoculum, S_o = 80 mg L^{-1} of Cu(II))

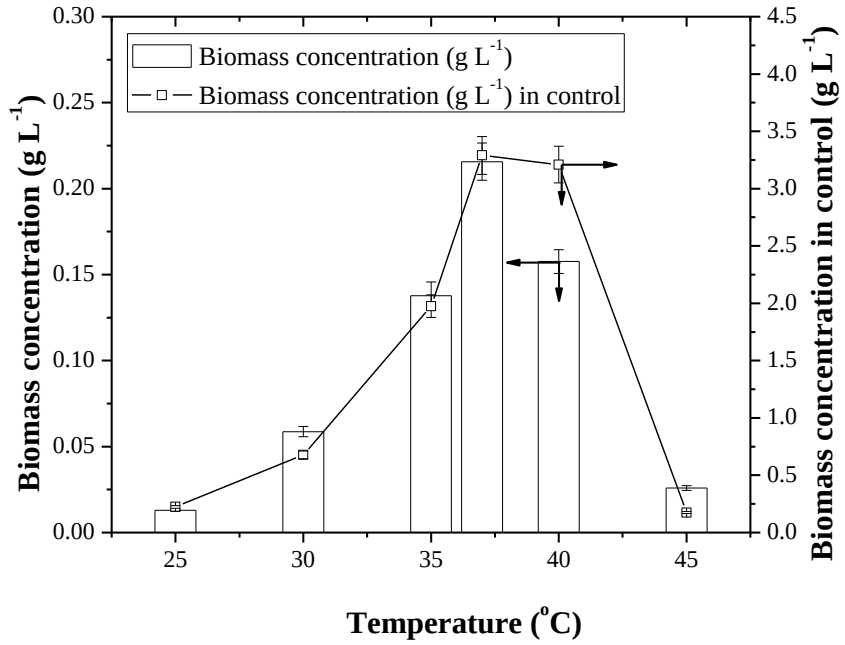


Fig. 5.27. Effect of temperature on growth of *K. pneumoniae* (pH = 7.0±0.2, RPM = 150, 1 % v/v inoculum, S_o = 40 mg L^{-1} of Zn(II))

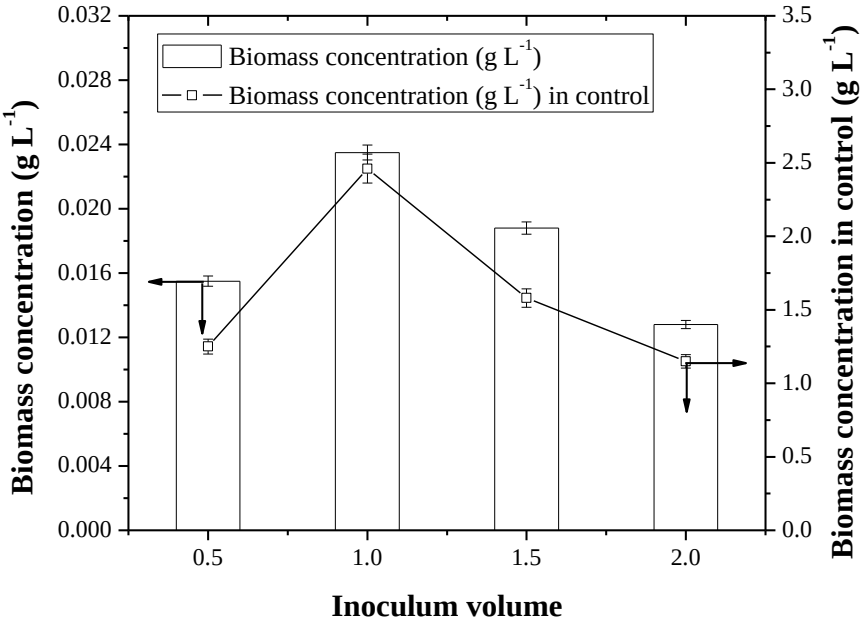


Fig. 5.28. Effect of inoculum volume on growth of *P. taiwanensis* (T = 37 °C, pH = 7.0±0.2, RPM = 150, S_o = 150 mg L^{-1} of Cr(VI))

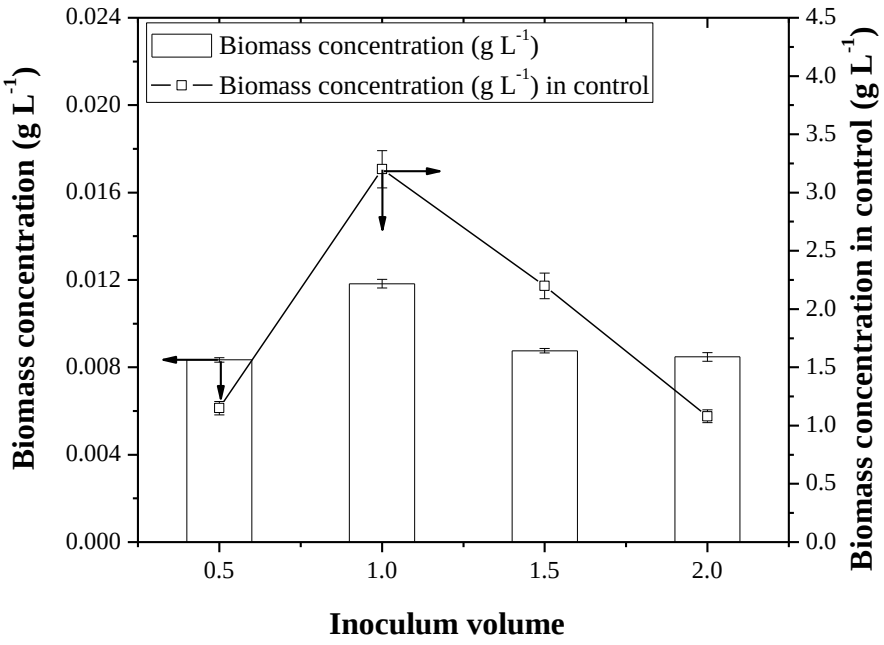


Fig. 5.29. Effect of inoculum volume on growth of *A. guillouiae* (*T* = 37 °C, pH = 7.0±0.2, RPM = 150, S_o = 80 mg L^{-1} of Cu(II))

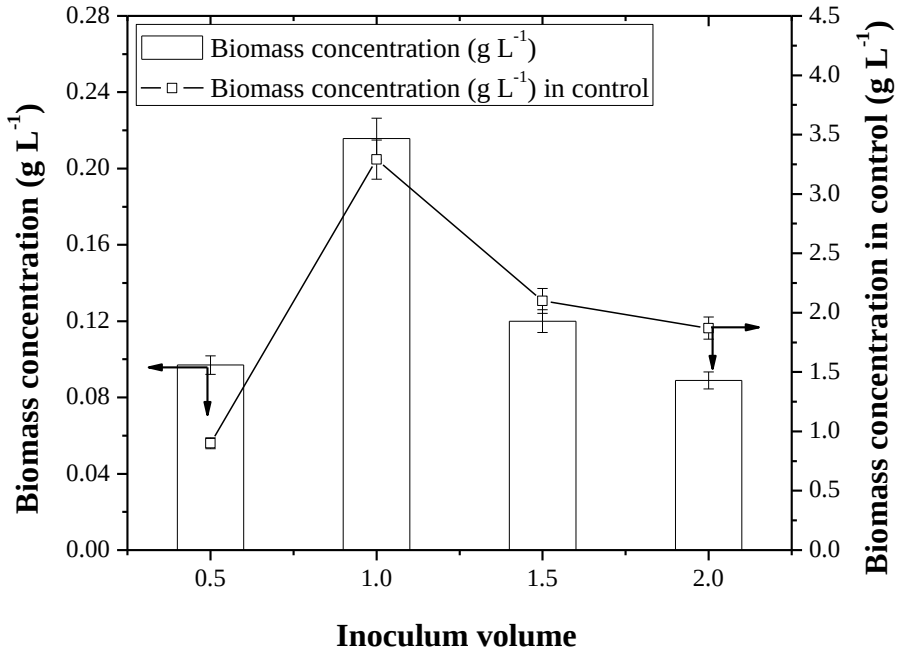


Fig. 5.30. Effect of inoculum volume on growth of *K. pneumoniae* (*T* = 37 °C, pH = 7.0±0.2, RPM = 150, S_o = 40 mg L^{-1} of Zn(II))

Table 5.4. Optimum values of the parameters obtained from batch studies for the removal of Cr(VI), Cu(II) and Zn(II) using *P. taiwanensis, A. guillouiae* and *K. pneumoniae*, respectively

S No	Parameters	*P. taiwanensis*	*A. guillouiae*	*K. pneumoniae*
1	Initial metal concentration (mg L^{-1})	150	80	40
	Biomass concentration (g L^{-1})	0.258	0.352	0.293
2	pH	7.0	8.0	7.0
	Biomass concentration (g L^{-1})	0.023	0.029	0.216
3	MSM composition of standard	double	double	double
	Biomass concentration (g L^{-1})	0.025	0.031	0.354
4	N-P-K composition	double N & P, standard K	double N & P, standard K	double N & P, standard K
	Biomass concentration (g L^{-1})	N: 0.083 P: 0.095 K: 0.189	N: 0.052 P: 0.042 K: 0.106	N: 0.091 P: 0.099 K: 0.278
5	Condition	Aerobic	Facultative anaerobic	Aerobic
	Biomass concentration (g L^{-1})	0.252	0.077	0.216
6	Temperature (ºC)	37	40	37
	Biomass concentration (g L^{-1})	0.024	0.164	0.216
7	Inoculum volume (% v/v)	1	1	1
	Biomass concentration (g L^{-1})	0.024	0.012	0.216

5.1.2. Specific growth rate

The specific growth rates (μ) of *P. taiwanensis*, *A. guillouiae* and *K. pneumoniae* are calculated for different initial concentration (20-200 mg L^{-1}) of Cr(VI), Cu(II) and Zn(II), respectively using log phase data as discussed in section 4.1. Figs. 5.31-5.33 show the plots of ln$x$ vs t for different initial concentrations of Cr(VI), Cu(II) and Zn(II), respectively. Log phase data are considered due to the growth of bacterial strains in this phase. The values of specific growth rates of three bacterial strains for the removals of Cr(VI), Cu(II) and Zn(II) are reported in Table 5.5. The values of μ are found to be 0.099, 0.102, 0.180, 0.210, 0.256, 0.262 and 0.233 h^{-1} for 20, 40, 60, 80, 100, 150 and 200 mg L^{-1} of initial concentrations of Cr(VI), respectively. The maximum value of μ for Cr(VI) bioremediation is obtained as 0.262 h^{-1} at 150 mg L^{-1} of initial Cr(VI) concentration. For different initial concentrations of Cu(II), the values of μ are obtained in the range 0.090-0.208 h^{-1}. The maximum value of μ for Cu(II) bioremediation is obtained as 0.208 h^{-1} at 80 mg L^{-1} of initial Cu(II) concentration. At 20, 40, 60, 100, 150 and 200 mg L^{-1} of initial Zn(II) concentrations, the values of μ are obtained as 0.027, 0.035, 0.041, 0.038, 0.032 and 0.026 h^{-1}, respectively while the maximum value of μ is obtained as 0.043 h^{-1} at 80 mg L^{-1} of initial Zn(II) concentration. The values of μ obtained at different initial metal concentrations are used in growth kinetic models to determine different kinetic parameters for bioremediation of Cr(VI), Cu(II) and Zn(II).

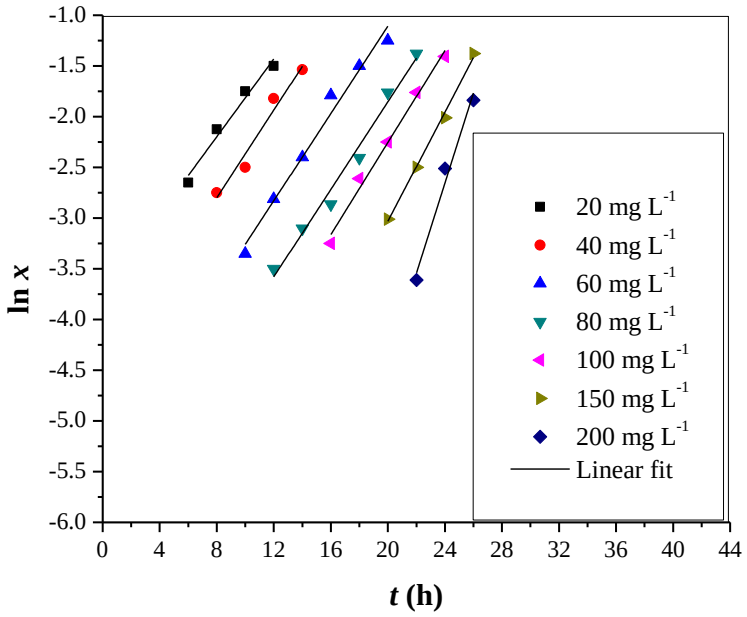


Fig. 5.31. Calculation of specific growth rate (μ) of *P. taiwanensis* for Cr(VI) removal

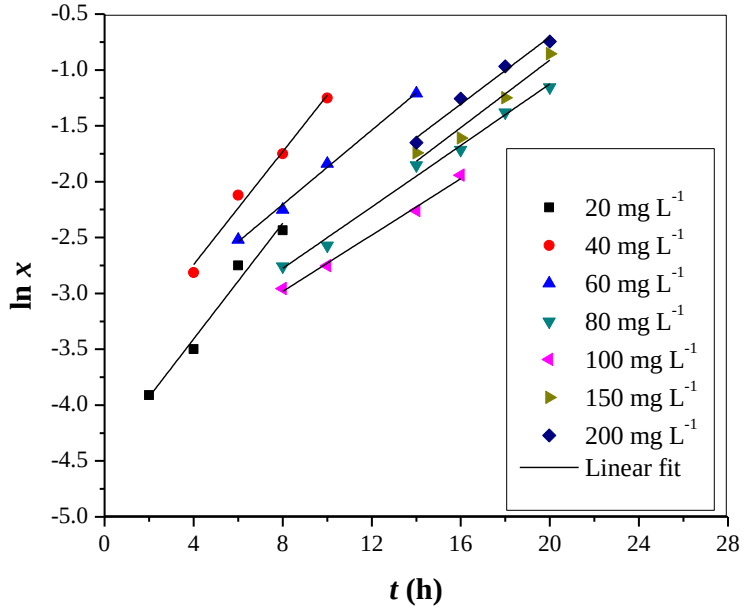


Fig. 5.32. Calculation of specific growth rate (μ) of *A. guillouiae* for Cu(II) removal

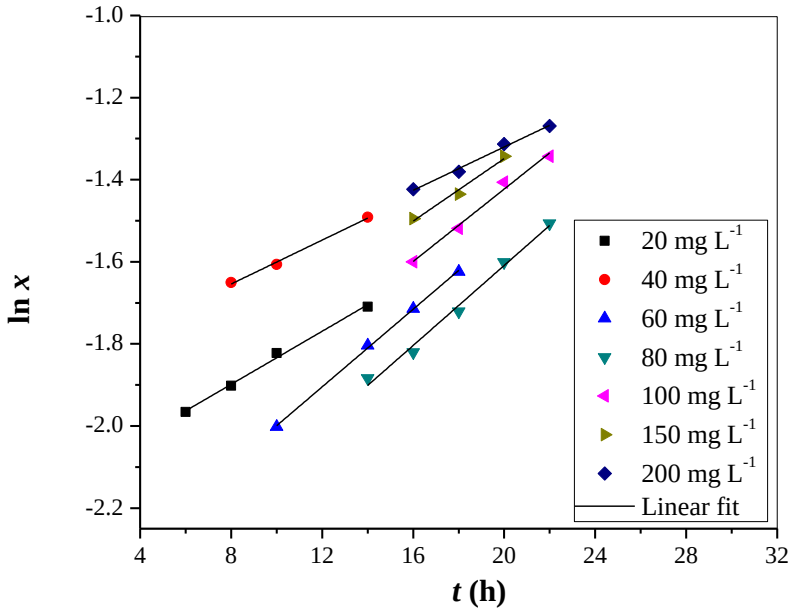


Fig. 5.33. Calculation of specific growth rate (μ) of *K. pneumoniae* for Zn(II) removal

Table 5.5. Specific growth rate of three bacterial strains used in bioremediation of Cr(VI), Cu(II) and Zn(II)

S No	S_o (mg L^{-1})	Specific growth rate, μ (h^{-1})		
		P. taiwanensis **in Cr(VI) removal**	***A. guillouiae*** **in Cu(II) removal**	***K. pneumoniae*** **in Zn(II) removal**
1	20	0.099	0.102	0.027
2	40	0.102	0.176	0.035
3	60	0.180	0.191	0.041
4	80	0.210	0.208	0.043
5	100	0.256	0.178	0.038
6	150	0.262	0.120	0.032
7	200	0.233	0.090	0.026

5.1.3. Growth kinetic modeling

Various growth kinetic models are tested with the obtained bioremediation experimental data of three bacterial strains for the removal of metals and results are shown in Figs. 5.31-5.34.

5.1.3.1. Monod model

The log phase experimental data for bioremediation of each metal is considered in plotting the graph between ($1/\mu$) and ($1/S$) which gives a straight line. The details are discussed in section 4.2.1. The values of maximum specific growth rate (μ_m) are obtained as 0.336, 0.122 and 0.039 h^{-1} for Cr(VI), Cu(II) and Zn(II) bioremediation, respectively while the values of substrate affinity constant (K_s) are found as 54.356, 11.726 and 5.720 mg L^{-1} for respective metal bioremediation. The coefficient of determination (R^2) values are obtained as 0.739, 0.325 and 0.076 for Cr(VI), Cu(II) and Zn(II) bioremediation, respectively.

Monod model does not fit the obtained experimental data adequately (less R^2 values) which is attributed to the fact that self-inhibition effects during the bioremediation process at higher initial concentrations of substrate are not considered in this model. It was reported in earlier study that substrate inhibition models give more adequate fitting than the Monod model when the substrate concentration (S) is much larger than the substrate affinity constant values (K_s) (Dapena-Mora et al., 2007). The specific growth rates are decreased above 150, 80 and 80 mg L^{-1} of initial concentrations of Cr(VI), Cu(II) and Zn(II), respectively which is an indication of self-inhibition effect caused by metals.

5.1.3.2. Monod-Inhibition model

The values of μ_m and K_s are used in Eq. 4.5 to find out the inhibition constant (K_I) for the removal of Cr(VI), Cu(II) and Zn(II) using the three bacterial strains. The estimated growth kinetic parameters are presented in Tables 5.6-5.8 for Cr(VI), Cu(II) and Zn(II) bioremediation, respectively.

5.1.4. Rate Kinetic Modeling

In this study, zero-order and three-half-order kinetic models are utilized to fit the experimental data and the details are discussed in the subsequent sections.

5.1.4.1. Zero-order rate kinetics

Figs 5.34-5.36 show the plots between S and t for different initial concentrations of metals (Eq. 4.13). The details are discussed in section 4.3.1. The slope and intercept give zero-order constants, k_o and S_o, respectively. The zero-order kinetic constants for Cr(VI), Cu(II) and Zn(II) bioremediation are listed in Tables 5.9-5.11. The values of R^2 for zero-order kinetics are determined in the range of 0.934-0.999, 0.912-0.973 and 0.930-0.997 for various initial concentrations of Cr(VI), Cu(II) and Zn(II), respectively.

5.1.4.2. Three-half-order rate kinetics

Three-half-order rate constants (k_1 and k_2) are estimated by plotting Y vs t for different initial concentrations of metals as discussed in section 4.3.2 (Figs. 5.37-5.39). The kinetic constants, k_1 and k_2 for bioremediation of three metals for different initial concentrations are found in the range of 0.245-3.500 h^{-1} and 0.133-4.900 h^{-2} for Cr(VI), 1.690-6.173 h^{-1} and 0.616-8.520 h^{-2} for Cu(II) and 0.005-0.052 h^{-1} and 0.141-3.000 h^{-2} for Zn(II). These values are reported in Tables 5.9-5.11. The values of k_1 and k_2 are decreased with an increase in the initial concentration of three metals. The values of R^2 are obtained in the

range of 0.959-0.990, 0.973-0.991 and 0.977-0.994 for Cr(VI), Cu(II) and Zn(II) bioremediation, respectively. Results reveal the fact that the three-half-order kinetic model is suitable to explain the bioremediation rate kinetics of Cr(VI), Cu(II) and Zn(II) using *P. taiwanensis*, *A. guillouiae* and *K. pneumoniae*, respectively as it incorportes the biomass formation term.

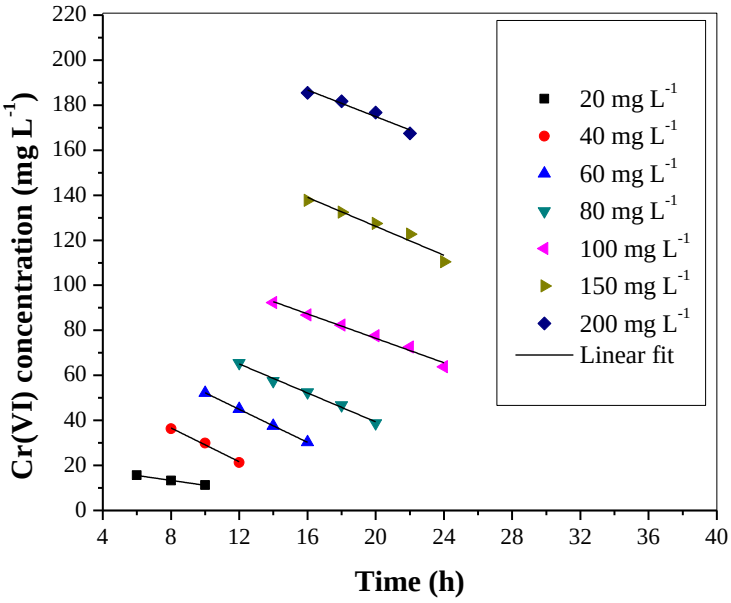


Fig. 5.34. Zero-order kinetics

for Cr(VI) bioremediation at different S_o of Cr(VI)

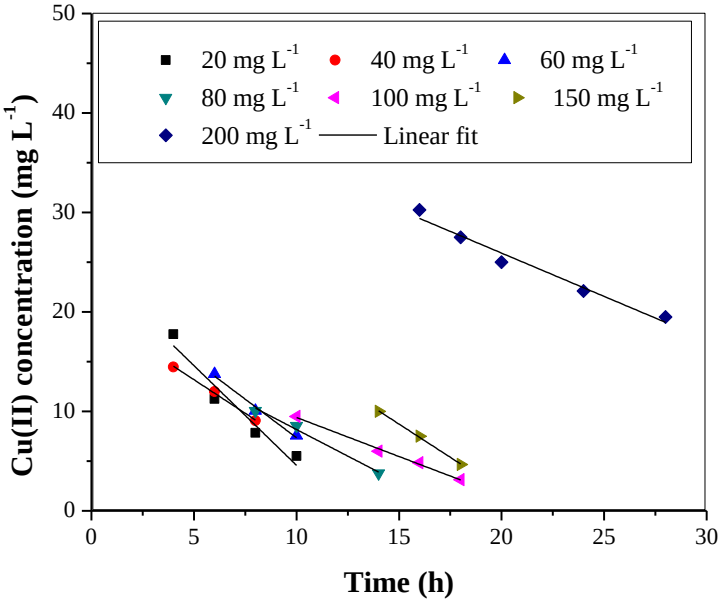


Fig. 5.35. Zero-order kinetics

for Cu(II) bioremediation at different S_o of Cu(II)

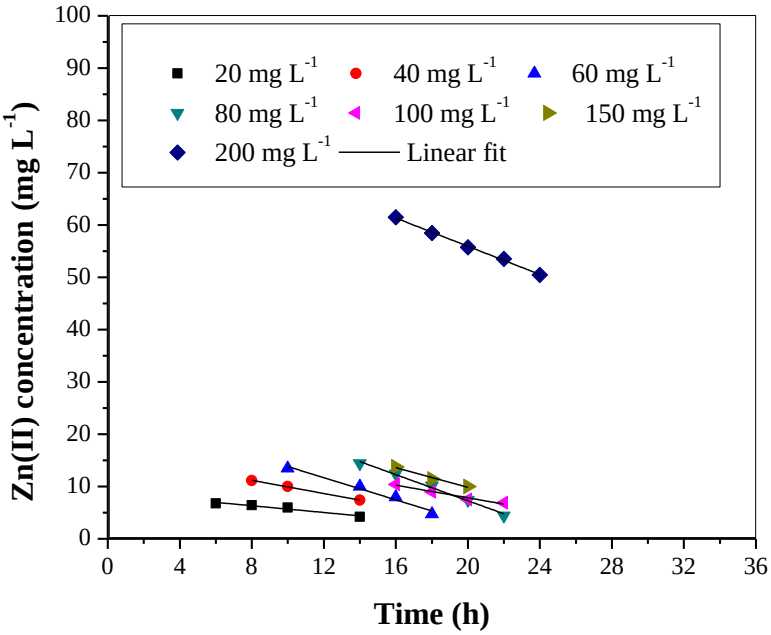


Fig. 5.36. Zero-order kinetics

for Zn(II) bioremediation at different S_o of Zn(II)

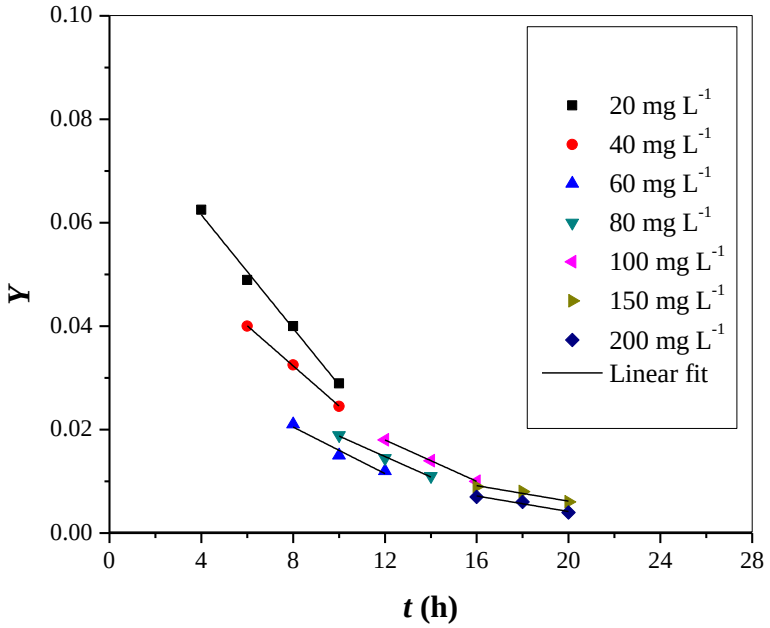


Fig. 5.37. Three-half-order kinetics

for Cr(VI) bioremediation at different S_o of Cr(VI)

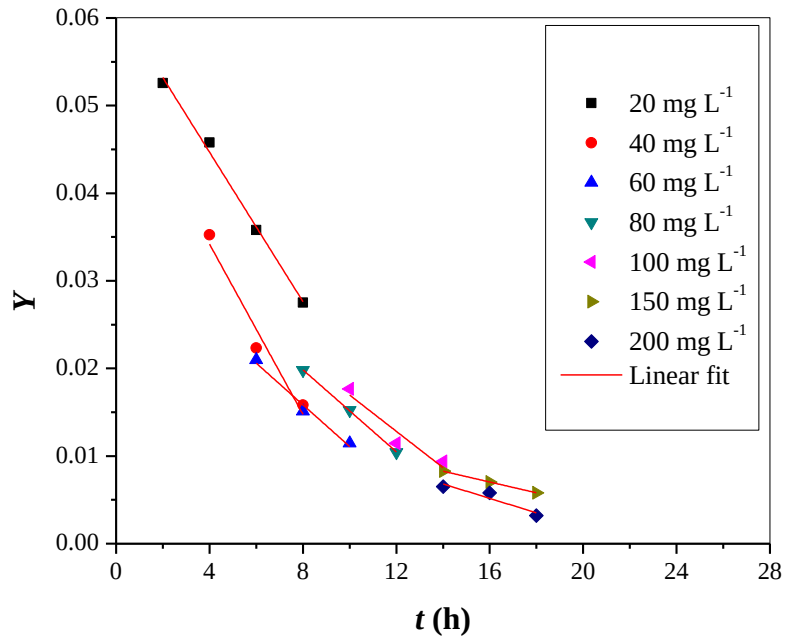


Fig. 5.38. Three-half-order kinetics for Cu(II) bioremediation at different S_o of Cu(II)

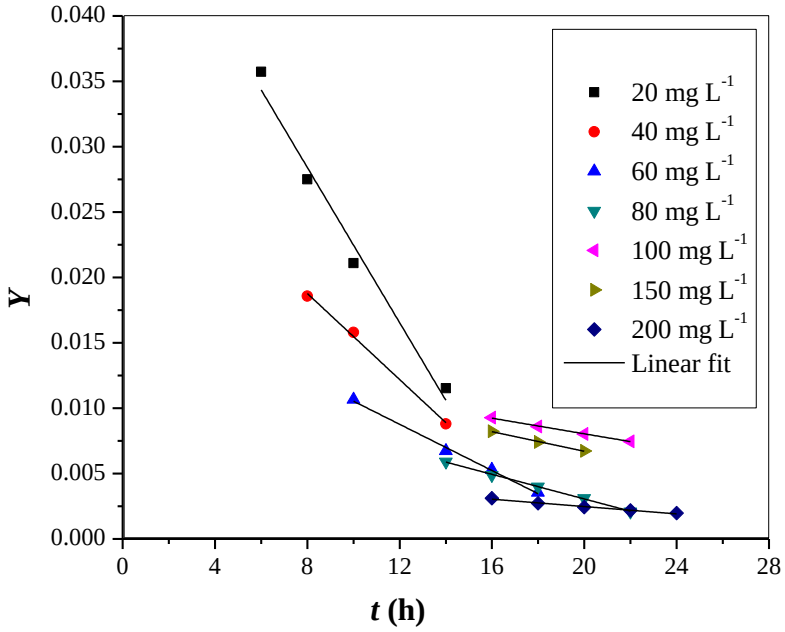


Fig. 5.39. Three-half-order kinetics for Zn(II) bioremediation at different S_o of Zn(II)

Table 5.6. Growth kinetic parameters for Cr(VI) bioremediation obtained from growth models

S No	Model	μ_m (h^{-1})	K_s (g L^{-1})	K_I (g L^{-1})	R^2
1	Monod	0.1614	0.0791	-	0.989
2	Monod-Inhibition	-	-	0.035	0.982

Table 5.7. Growth kinetic parameters for Cu(II) bioremediation obtained from growth models

S No	Model	μ_m (h^{-1})	K_s (g L^{-1})	K_I (g L^{-1})	R^2
1	Monod	0.225	0.02	-	0.991
2	Monod-Inhibition	-	-	0.016	0.978

Table 5.8. Growth kinetic parameters for Zn(II) bioremediation obtained from growth models

S No	Model	μ_m (h^{-1})	K_s (g L^{-1})	K_I (g L^{-1})	R^2
1	Monod	0.287	0.035	-	0.986
2	Monod-Inhibition	-	-	0.022	0.982

Table 5.9. Zero-order and three-half-order kinetic models parameters at different initial Cr(VI) concentrations

S No	S_o (mg L^{-1})	Zero order kinetics			Three half order kinetics		
		k_o	S_o	R^2	$k_1 \times 10^4$	$k_2 \times 10^3$	R^2
1	20	1.090	22.060	0.996	3.500	4.900	0.959
2	40	3.740	66.620	0.985	1.250	1.460	0.989
3	60	3.676	88.922	0.999	0.776	0.680	0.978
4	80	3.210	103.630	0.990	0.562	0.392	0.975
5	100	2.710	130.727	0.982	0.397	0.225	0.975
6	150	3.210	190.457	0.937	0.275	0.145	0.986
7	200	2.952	233.931	0.934	0.245	0.133	0.990

Table 5.10. Zero-order and three-half-order kinetic models parameters at different initial Cu(II) concentrations

S No	S_o (mg L^{-1})	Zero order kinetics			Three half order kinetics		
		k_o	S_o	R^2	$k_1 \times 10^4$	$k_2 \times 10^3$	R^2
1	20	2.000	24.620	0.922	6.173	8.520	0.990
2	40	1.351	19.940	0.960	5.361	4.860	0.981
3	60	1.550	22.850	0.957	3.487	2.380	0.973
4	80	1.069	18.870	0.912	3.857	2.340	0.990
5	100	0.785	17.240	0.973	3.766	2.070	0.970
6	150	1.336	28.760	0.957	1.690	0.616	0.991
7	200	0.871	43.330	0.960	1.840	0.825	0.978

Table 5.11. Zero-order and three-half-order kinetic models parameters at different initial Zn(II) concentrations

S No	S_o (mg L^{-1})	Zero order kinetics			Three half order kinetics		
		k_o	S_o	R^2	$k_1 \times 10^4$	$k_2 \times 10^3$	R^2
1	20	0.323	8.910	0.930	0.052	3.000	0.977
2	40	0.631	16.241	0.997	0.031	1.650	0.992
3	60	1.061	24.428	0.970	0.019	0.884	0.990
4	80	1.248	32.247	0.993	0.012	0.470	0.994
5	100	0.602	19.903	0.957	0.014	0.299	0.990
6	150	0.942	28.694	0.972	0.014	0.376	0.991
7	200	1.348	82.897	0.990	0.005	0.141	0.980

5.1.5. Simultaneous bioremediation of multiple metals Cr(VI), Cu(II) and Zn(II)

5.1.5.1. Isolation and selection of microbial strains

This study is aimed to isolate the suitable microbial strains from aerobic mixed culture for metal removal [Cr(VI), Cu(II) and Zn(II)]. The minimum inhibitory concentration (MIC) values of four microbial strains (B1, B2, B3 and B4) for the removal of Cr(VI), Cu(II) and Zn(II) are reported in Table 5.12. It is observed that B1 shows highest tolerance to Cr(VI) (650 mg L^{-1}) followed by B3 (400 mg L^{-1}) , B2 (300 mg L^{-1}) and B4 (100 mg L^{-1}). The tolerance for Cu(II) by all four microbial strains is obtained in the order of B2 (900 mg L^{-1}) > B1 (400 mg L^{-1}) > B3 (250 mg L^{-1}) > B4 (200 mg L^{-1}). Highest MIC for Zn(II) is obtained as 950 mg L^{-1} for B3. For Zn(II), 500 and 450 mg L^{-1} of MIC values are obtained for B1 and B2, respectively which are significantly higher. However, the lowest MIC value for Zn(II) removal is obtained for B4 (200 mg L^{-1}).

Same fact can also be confirmed by the metal removal efficiency of four microbial isolates (Fig. 5.40). Three microbial strains (B1, B2 and B3) show higher metal removal as compared to B4 for all three metals. It is observed that B1 shows higher removal of Cr(VI) (96.5 %) followed by B3 (86.5 %) and B2 (77.5 %). Highest copper removal is achieved by using B2 (98.5 %). B3 exhibits greater removal of 99.25 % for 958 mg L^{-1} of initial Zn(II) concentration. B4 shows 40.25 % (Cr(VI) initial concentration: 250 mg L^{-1}), 45.5 % (Cu(II) initial concentration: 440 mg L^{-1}) and 47.5 % (Zn(II) initial concentration: 420 mg L^{-1}) removal for respective metals which are lowest as compared to values obtained for other three microbial strains.

Earlier study reported that *Pseudomonas aeruginosa* could tolerate up to 400 mg L^{-1} of Cr(VI) and 100 mg L^{-1} of Cu(II), respectively in nutrient broth medium (Kang et

al., 2014). Another study reported that *P. aeruginosa* has MIC of 1500 mg L^{-1} for Zn(II) (Islam et al., 2014). In other studies, it was reported that *Acinetobacter* sp. could tolerate up to 70 mg L^{-1} of Cr(VI), 639 mg L^{-1} of Cu(II) and 200 mg L^{-1} of Zn(II) (Yadav et al., 2013; Zakaria et al., 2007). There are no significant reports available on the MIC values of Cr(VI), Cu(II) and Zn(II) using *Klebsiella* sp. In the present study, it is observed that B2 and B3 have more MIC values against Cr(VI), Cu(II) and Zn(II) while B1 has more MIC values against Cr(VI) and Cu(II) as compared to the MIC values mentioned in previous studies. Based on the results discussed above, out of four microbial strains B1, B2 and B3 are selected for further studies.

5.1.5.2. Characterization and identification of selected microbial strains

Identification and characterization are carried out for three selected microbial strains (B1, B2 and B3). Gene sequencing of B1 and B2 are carried out at Genei, Merck Millipore Laboratory, India while for B3, it is done at Xcelris Laboratory, India. Results obtained from the gene sequencing of three bacterial strains are compared with the sequence of already available strains in the National Center for Biotechnology Information (NCBI) repository and three phylogenetic trees are constructed with the help of Molecular Evolutionary Genetics Analysis (MEGA) software (ver 5.0). B1, B2 and B3 are identified based on NCBI BLAST search (Figs. 5.1-5.3). B1 shows maximum similarity with *Pseudomonas taiwanensis* (NCBI GenBank Accession Number**:** KT070310) while B2 shows maximum homology with *Acinetobacter guillouiae* (NCBI GenBank Accession Number: KT070311). B3 is identified as *Klebsiella pneumoniae* (NCBI GenBank Accession Number: KT070312).

5.1.5.3. Kinetic study

In this study, the growth rate and metal removal efficiency of the bacterial strains are determined by keeping each metal ion concentration as 150 mg L^{-1}. In biotic control (absence of metals), the change in biomass concentration and pH with time is shown in Fig. 5.41. It is observed that all three bacterial strains (*P. taiwanensis*, *A. guillouiae* and *K. pneumoniae*) exhibit shorter lag phase (4-8 h) in biotic control. Significant change in pH is not observed throughout the batch study. In the presence of Cr(VI), the lag phase of *P. taiwanensis* and *A. guillouiae* are extended up to 16 h while it is found to be 24 h for *K. pneumoniae* (Fig. 5.42). Higher lag phase time is observed as 20 h for *P. taiwanensis* and *K. pneumoniae* in the presence of Cu(II) while it is reduced to 16 h for *A. guillouiae* (Fig. 5.43). In the presence of Zn(II), the duration of lag phase for *K. pneumoniae* remains same (i.e. 4 h) as biotic control while higher duration of lag phase is observed for *P. taiwanensis* and *A. guillouiae* (16 h) (Fig. 5.44).

The lag phase of respective bacterial strains is increased in the presence of metals except for *K. pneumoniae* in the presence of Zn(II). Biomass concentration is found to be maximum in the range of 1.8-3.25 g L^{-1} in biotic control. The biomass concentration is less in the presence of metals and is obtained in the range of 1.1-2.4 g L^{-1}, 1.7-2.2 g L^{-1} and 1.25-2.12 g L^{-1} for Cr(VI), Cu(II) and Zn(II), respectively. In biotic control, all three bacterial strains exhibit shorter lag phase which may be due to the faster adaptibility with the new environment. However, different microorganisms have different levels of resistance to metals. In the presence of metals, all three bacterial strains show different and higher lag phase as they need more time to get adapted with the new environment.

To evaluate the efficiency of each bacterial strains for metal removal, specific growth rate; percentage removal of metals; and uptake capacity of each metals are calculated. The obtained values are shown in Table 5.13. It is observed that *P. taiwanensis* exhibits higher specific growth rate in biotic control (0.22 h^{-1}). It is reduced to 0.137, 0.06 and 0.099 h^{-1} in the presence of Cr(VI), Cu(II) and Zn(II), respectively. The specific growth rate of *A. guillouiae* is reduced from 0.119 g L^{-1} under biotic control to 0.033, 0.099 and 0.073 h^{-1} in the presence of Cr(VI), Cu(II) and Zn(II), respectively. *K. pneumoniae* shows maximum specific growth rate of 0.145 h^{-1} under biotic control while it is declined to 0.038, 0.048 and 0.099 h^{-1} in the presence of Cr(VI), Cu(II) and Zn(II), respectively. The relationship between specific growth rate (μ) and metal pollutant concentration (x) helps in understanding the growth of microorganisms. In the present study, it is observed that all the bacterial strains show higher specific growth rate in biotic control. The results also indicate that Cr(VI) is more toxic than Cu(II) and Zn(II) for all the bacterial strains except for *P. taiwanensis* (Table 5.13). Earlier study reported the similar trend of higher tolerance to Cr(VI) by a group of *Pseudomonas* sp. (Hussein et al., 2005). Yadav et al. (2013) reported higher tolerance of Cu(II) by *Acinetobacter junii* BB1A.

P. taiwanensis is able to remove 94.8 % Cr(VI) within 32 h while *A. guillouiae* and *K. pneumoniae* take 36 h to remove 76.8 % and 43.67 % Cr(VI), respectively. In the present study, about 90.33 % and 74.83 % Cu(II) removal are achieved after 36 h using *A. guillouiae* and *K. pneumoniae*, respectively while 66.83 % Cu(II) removal is obtained using *P.taiwanensis*. For Zn(II) removal, about 82.33 % removal is achieved after 40 h using *K. pneumoniae* as compared to 74.83 % and 63.67 % after 36 h using *P.*

taiwanensis and *A. guillouiae*, respectively. The results reveals the fact that *A. guillouiae* and *P. taiwanensis* are able to remove all the metals in less time (32-36 h) as compared to metal removal using *K. pneumoniae* (36-40 h). The removal preference of Cr(VI) is given in the order: *P. taiwanensis* (94.8 %) > *A. guillouiae* (76.8 %) > *K. pneumoniae* (43.67 %). The specific Cr(VI) uptake capacities are found almost same as 59.25 and 59.55 mg g dry cell^{-1} for *P. taiwanensis* and *K. pneumoniae*, respectively. For *A. guillouiae*, the specific Cr(VI) uptake capacity is 60.63 mg g dry cell^{-1} which is approximately same to the uptake capacity calculated for other two bacterial strains. For Cu(II), the removal preference is given in the following order: *A. guillouiae* (90.33 %) > *K. pneumoniae* (74.83 %) > *P.taiwanensis* (66.83 %). *A. guillouiae* shows 61.60 mg g dry cell^{-1} uptake capacity of Cu(II) which is maximum as compared to 60.03 and 58.62 mg g dry cell^{-1} uptake capacity obtained for *K. pneumoniae* and *P.taiwanensis*, respectively.

Maximum Zn(II) removal is achieved using *K. pneumoniae* (82.33 %) followed by *P. taiwanensis* (74.83 %) and *A. guillouiae* (63.67%). *K. pneumoniae* shows maximum uptake (68.80 mg g dry cell^{-1}) of Zn(II) followed by *P. taiwanensis* (52.94 mg g dry cell^{-1}) and *A. guillouiae* (45.48 mg g dry cell^{-1}). Earlier study reported about 7-7.5 mg L^{-1} of Cr(VI) removal (initial concentration 10 mg L^{-1}) using *Pseudomonas* sp. in a multi metals ions system (Singh et al., 2013). Zahoor and Rehman (2009) reported 8.3 and 7.0 mg Cr(VI) g dry cell^{-1} uptake in 96 h from a medium containing multiple metals [Cr(VI), Cu(II), Cd(II), Hg(II), Pb(II) and Ni(II)] using *Bacillus* sp. JDM-2-1 and *Staphyllococcus capitis*, respectively. In a separate study, 70.53 mg Cr(VI) g dry cell^{-1} uptake was reported using *Acinetobacter clcoaceticus* (Mishra et al., 2010). A group of researchers showed approximately 16.25 mg Cu(II) g dry cell^{-1} uptake using *Bacillus* sp.

(Tunali et al., 2006). The present study shows that most of the metal uptake is achieved in less times (Table 5.12) as compared to values reported in earlier studies (Singh et al., 2013; Zahoor and Rehman, 2009). The results of metal uptake capacity and specific growth rate for *A. guillouiae* and *P. taiwanensis* indicate that these bacterial strains are potential candidates for bioremediation of metals at higher concentration range.

Table 5.12. Minimum inhibitory concentration (MIC) of four isolated microbial strains in the presence of Cr(VI), Cu(II) and Zn(II)

Bacterial strains	Minimum inhibitory concentration (mg L^{-1})		
	Cr(VI)	Cu(II)	Zn(II)
B1	650	400	500
B2	300	900	450
B3	400	250	950
B4	100	200	200

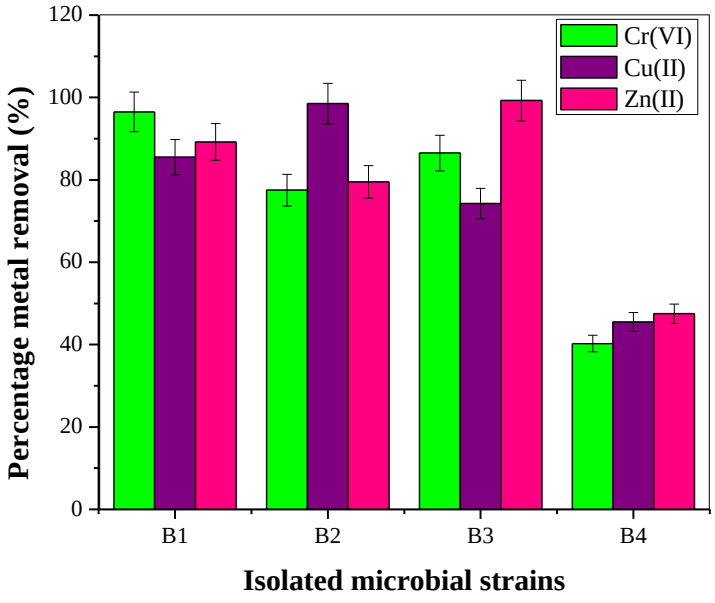


Fig. 5.40. Percentage metal removal of isolated microbial strains

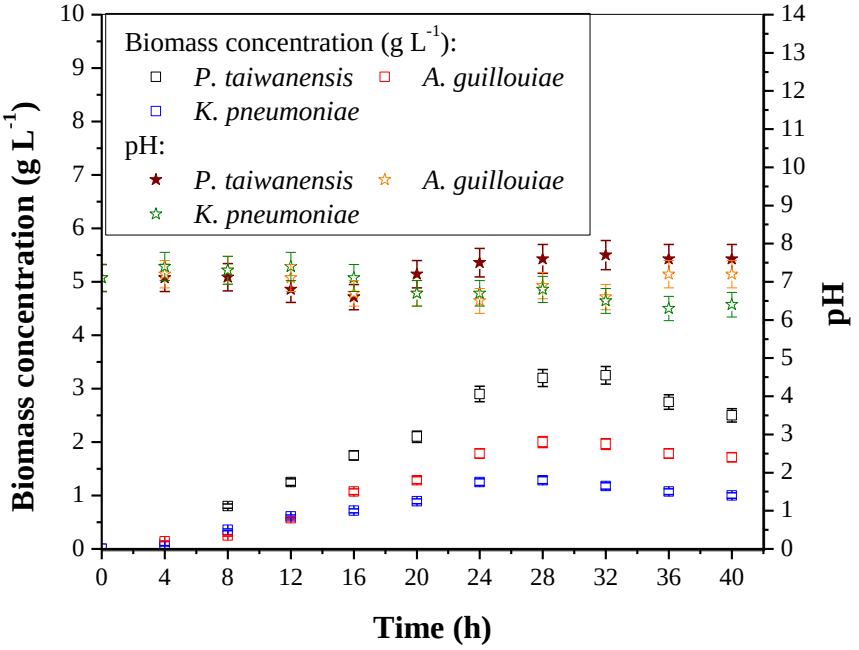


Fig. 5.41. Growth of isolated bacterial strains and pH with time in biotic control (*T* = 37 ºC, pH = 7.0±0.2, RPM = 150)

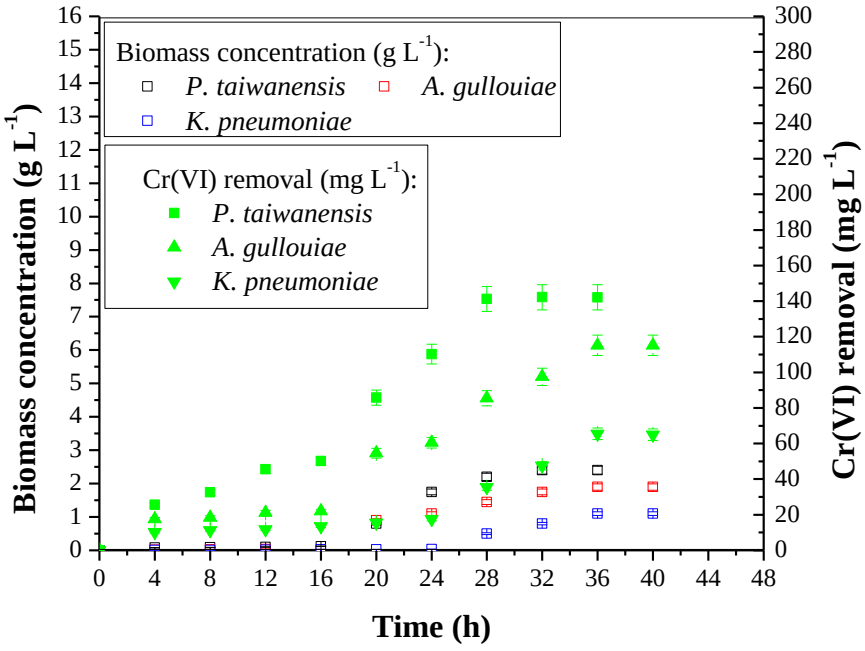


Fig. 5.42. Growth and metal removal by isolated bacterial strains with time in the presence of Cr(VI) (*T* = 37 ºC, pH = 7.0±0.2, RPM = 150, S_o = 150 mg L^{-1})

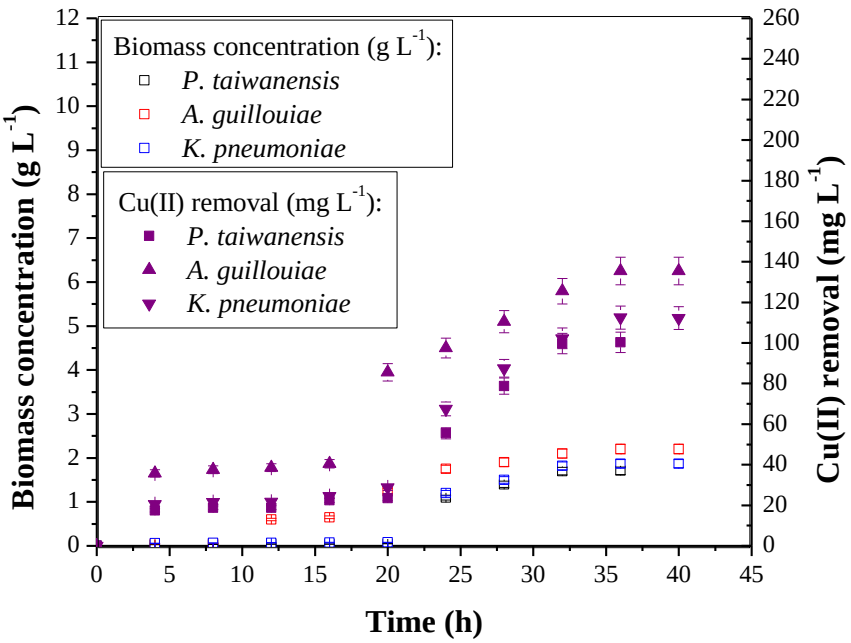


Fig. 5.43. Growth and metal removal by isolated bacterial strains with time in the presence of Cu(II) (T = 37 ºC, pH = 7.0±0.2, RPM = 150, S_o = 150 mg L^{-1})

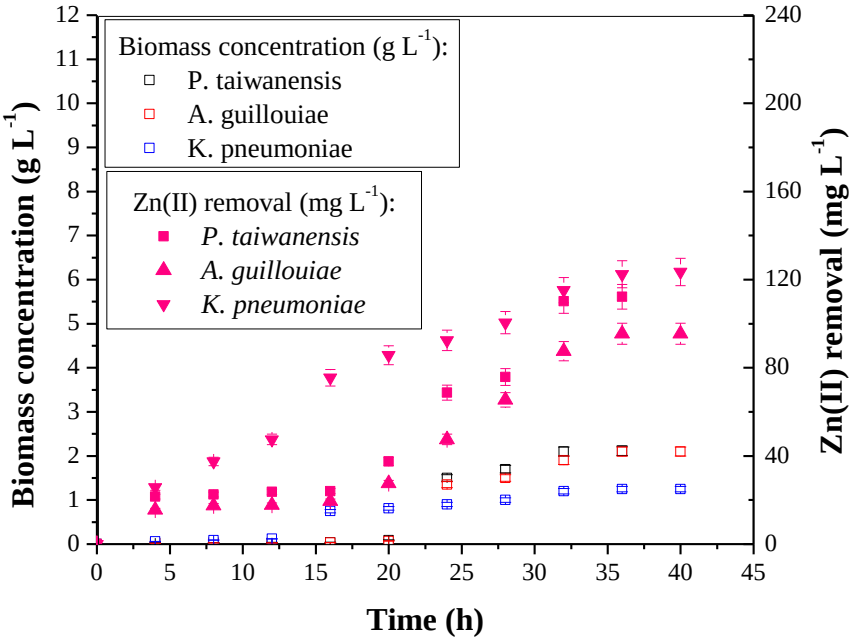


Fig. 5.44. Growth and metal removal by isolated bacterial strains with time in the presence of Zn(II) (T = 37 ºC, pH = 7.0±0.2, RPM = 150, S_o = 150 mg L^{-1})

Table 5.13. Specific growth rate, percentage metal removal and maximum uptake of metals by three bacterial strains

(T = 37 ºC, pH = 7.0±0.2, RPM = 150, S_o = 150 mg L^{-1})

Bacterial strains	**Biotic control/ Metals**	**Specific growth rate μ (h^{-1})**	**Percentage metal removal (%)**	**Maximum metal uptake capacity (mg g dry $cell^{-1}$)**
P. taiwanensis	Control	0.2204	-	-
	Cr(VI)	0.1373	94.80	59.25
	Cu(II)	0.0596	66.83	58.62
	Zn(II)	0.0986	74.83	52.94
A. guillouiae	Control	0.1186	-	-
	Cr(VI)	0.0332	76.80	60.63
	Cu(II)	0.0982	90.33	61.60
	Zn(II)	0.0725	63.67	45.48
K. pneumoniae	Control	0.1445	-	-
	Cr(VI)	0.0381	43.67	59.55
	Cu(II)	0.0476	74.83	60.03
	Zn(II)	0.0986	82.33	68.80

5.1.5.4. Development of consortium

A consortium consisting of three bacterial strains, *P. taiwanensis* (PT), *A. guillouiae* (AG) and *K. pneumoniae* (KP) is developed based on the mutual comptability study. Six combinations (PT+AG, PT+KP, AG+KP, AG+PT, KP+PT and KP+AG) are prepared as mentioned in section 3.1.8.4. The mutual compatibility between the two bacterial strains in each combination is determined in terms of percentage relative growth (Eq. 3.5) and is shown in Fig. 5.45. This study is aimed to investigate the growth of individual bacterial strain in the presence of metabolites of another bacterial strain in biotic control. The growth of *P. taiwanensis*, *A. guillouiae* and *K. pneumoniae* is obtained as 2.3, 2 and 1.34 g L^{-1}, respectively after 120 h of incubation. The growth of *P. taiwanensis* is decreased by 2.5 % and 6.2 % in the presence of *A. guillouiae* and *K. pneumoniae*, respectively. However, the growth of *A. guillouiae* is increased by 4.2 % in the presence of *P. taiwanensis* while growth is reduced by 8.5 % in the presence of *K. pneumoniae*. Growth of *K. pneumoniae* is enhanced by 7.5 % and 25.5 % in the presence of *P. taiwanensis* and *A. guillouiae*, respectively. Above results indicate that *P. taiwanensis* exhibits least growth while growth of *K. pneumoniae* is more in the presence of other two bacterial strains. The results obtained in the above study indicate a positive mutual compatibility between three bacterial strains. Based on the result, a consortium consisting of the three bacterial strains is developed and utilized to treat the single and multiple metals.

5.1.5.5. Efficacy of consortium over individual strains in metal removal

The efficacy of the developed consortium over individual strains for the single metal as well as mixed metal removal is evaluated. The concentrations of all three metals are maintained at 150 mg L^{-1} to investigate the removal of single metal using consortium.

Figs. 5.46-5.48 show the efficiency of consortium over individual bacterial strains in the individual metal removal. No lag phase is observed during the removal of metals using consortium. It may be attributed to the faster metal removal ability of the consortium owing to the toxicity response and improved metal sequestration mechanism. However, individual strains show significant lag phase (4-24 h). The use of consortium shows approximately 99 %, 94.5 % and 96.5 % removal in 36, 32 and 36 h for Cr(VI), Cu(II) and Zn(II), respectively. The obtained values of percentage removal for all the metal ions are higher for consortium over individual bacterial strains (Figs. 5.46-5.48). For *P. taiwanensis, A. guillouiae* and *K. pneumoniae*, the maximum values of uptake capacity are obtained as 59.25, 60.63 and 59.55 mg Cr(VI) g dry cell^{-1}, respectively while these values are 58.62, 61.60 and 60.03 mg of Cu(II) g dry cell^{-1}, respectively. The maximum values of Zn(II) uptake capacity are obtained as 52.94, 45.48 and 68.80 mg of Zn(II) g dry cell^{-1} for *P. taiwanensis, A. guillouiae* and *K. pneumoniae*, respectively. The use of consortium gives maximum uptake of Cr(VI), Cu(II) and Zn(II) as 91.20, 89.20 and 88.78 mg metal g dry cell^{-1}, respectively. The metal uptake values of Zn(II) obtained in the present study are high as compared to value (42.5 mg Zn(II) g dry cell^{-1} for Zn(II) removal in 20 h using *Acinetobacter* sp.) reported in earlier study (Bejestani et al., 2013). Results indicates that the developed consortium shows better removal of metals [Cr(VI), Cu(II) and Zn(II)] as compared to individual strains.

The efficacy of the developed consortium over individual strains for mixed metal removal is evaluated by maintaining 50 mg L^{-1} initial concentration of each metals. Fig. 5.49 shows the performance of consortium over individual strains in individual as well as mixed metal removal. *P. taiwanensis* shows a decline in maximum uptake capacity of

Cr(VI) from 59.25 to 38.22 mg Cr(VI) g dry cell^{-1} in the presence of mixed metals. Similarly, the maximum uptake capacities of Cu(II) and Zn(II) are decreased from 61.60 to 58.27 mg metal g dry cell^{-1} and 68.80 to 66.52 mg metal g dry cell^{-1} using *A. guillouiae* and *K. pneumoniae*, respectively. Earlier study reported that mixed metals show greater toxicity as compared to individual metals towards microorganisms (Malik, 2004). The similar phenomena is observed in the present study.

The comparison between the consortium and individual bacterial strains in single and multiple metals removal is reported in Table 5.14. In the multiple metal system using consortium, the maximum uptake capacities are obtained as 92.05, 89.00 and 89.20 mg metal g dry cell^{-1} for Cr(VI), Cu(II) and Zn(II) removal, respectively which are higher than the values obtained by individual strains. Results clearly indicate that the developed consortium is superior in terms of individual and mixed metals removal as compared to individual bacterial strains. It may be attributed to the different metal tolerance capacity of the individual bacterial strains present in the consortium and the presence of growth inhibitors.

Relative biomass growth reduction in metal laden biomass is considered as the key parameter to understand the reason behind the improved performance of consortium over individual strains. This parameter is calculated by Eq. 3.6. It is observed that consortium produces 2.27 g L^{-1} of biomass while *P. taiwanensis*, *A. guillouiae* and *K. pneumoniae* produce 3.25, 2.75 and 1.65 g L^{-1} of biomass, respectively in biotic control. Fig. 5.55 shows the relative biomass growth reduction of consortium and individual strains in the presence of metals. The relative biomass growth reduction of consortium is in the range of 9.7-11.9 % as compared to 20-47.4 % for all individual strains. This also

confirms the absence of lag phase in the single metal removal using consortium (Figs. 5.42-5.44). Earlier report showed similar results for a novel consortium developed using three different fungal strains in the presence of Cr(VI) and Cu(II) (Mishra and Malik, 2014).

In the presence of mixed metals, the values of percentage relative growth reduction are obtained as 35.4 %, 34.5 % and 39.4 % for *P. taiwanensis, A. guillouiae* and *K. penumoniae*, respectively. The growth of consortium is decreased by 20.7 % in the presence of mixed metals in comparison to biotic control. Results corroborate the fact that the consortium gives improved performance as compared to individual bacterial strains in single metal as well as mixed metals environments. Earlier studies reported similar results for the removal of metals (Zn, Cd and Hg) using seven different consortia (Sprocati et al., 2006). Results obtained in the present study give a possible solution for the removal of multiple metals from industrial discharges using the novel consortium.

5.1.5.6. Use of consortium for simulated industrial effluents

The performance of the developed consortium in multiple metal exposure is investigated using five sets of simulated industrial effluent. The different compositions of Cr(VI), Cu(II) and Zn(II) used to prepare the simulated industrial effluents are given in Table. 3.4. The values of percentage removal of three metals in respective sets are given in Table 5.15. The change in biomass concentration and metal removal with time in multiple metal exposure of consortium is shown in Fig. 5.51. For simulated industrial effluents, significant removals of Cr(VI) (97.6 % - 99 %), Cu(II) (95.6 % - 99.2 %) and Zn(II) (97.1 % - 99.4 %) are achieved. Results may help to facilitate the industrial application of the developed consortium in multiple metal removal from wastewater.

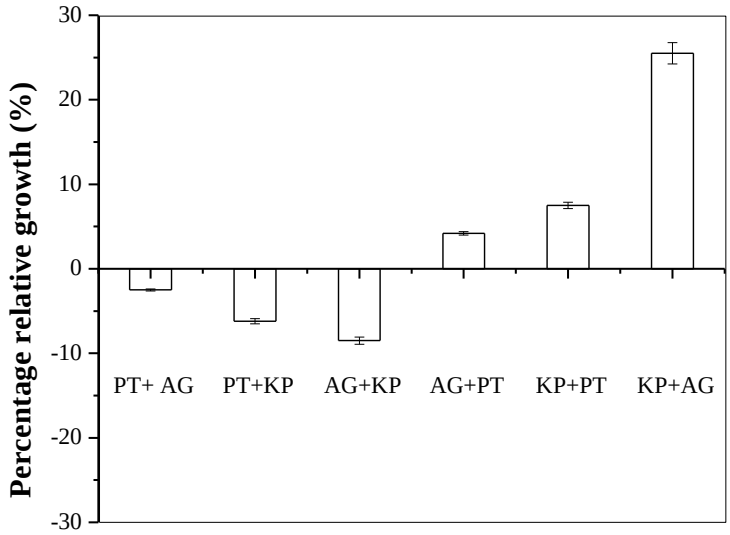


Fig. 5.45. Relative growth of individual bacterial strain in presence of other bacterial strain (PT: *P. taiwanensis*, AG: *A. guillouiae* and KP: *K. pneumoniae*)

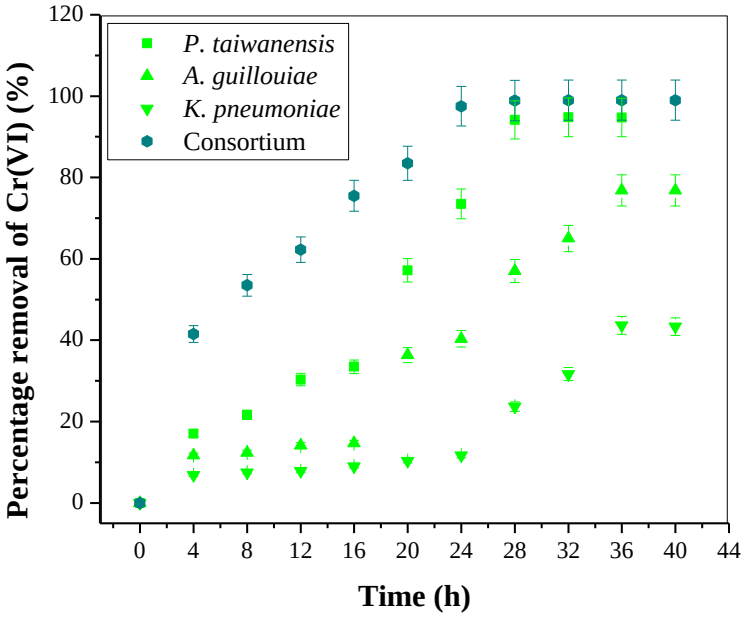


Fig. 5.46. Efficiency of the consortium over individual strain in removal of Cr(VI) (T = 37 ºC, pH = 7.0±0.2, RPM = 150, S_o = 150 mg L^{-1} of Cr(VI))

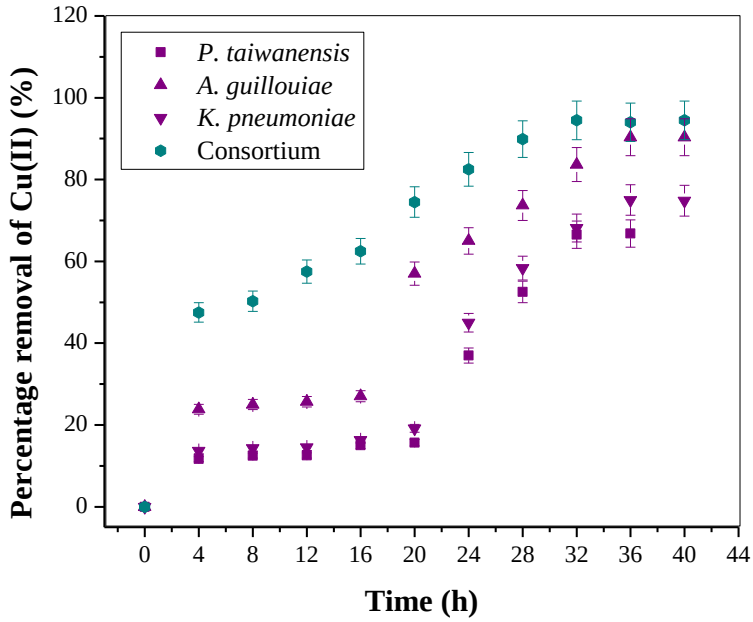


Fig. 5.47. Efficiency of the consortium over individual strain in removal of Cu(II) (T = 37 ºC, pH = 7.0±0.2, RPM = 150, S_o = 150 mg L^{-1} of Cu(II))

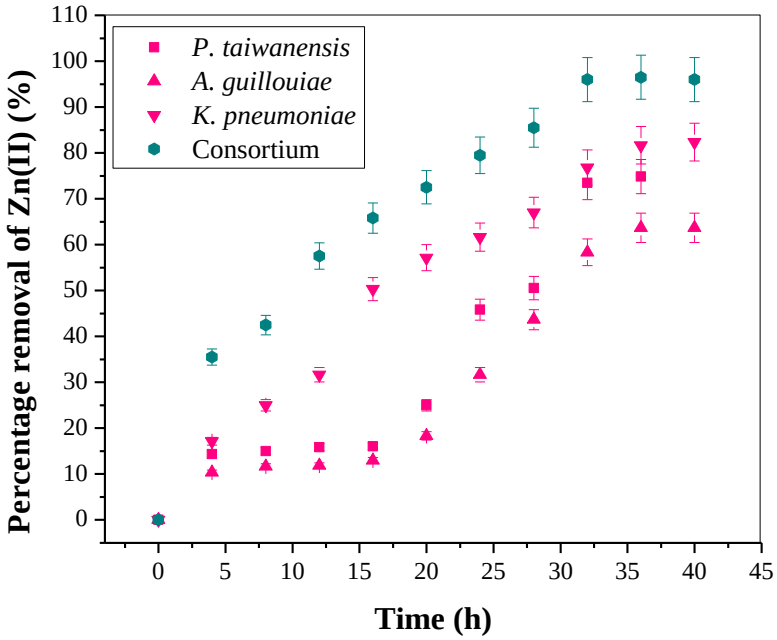


Fig. 5.48. Efficiency of the consortium over individual strain in removal of Zn(II) (T = 37 ºC, pH = 7.0±0.2, RPM = 150, S_o = 150 mg L^{-1} of Zn(II))

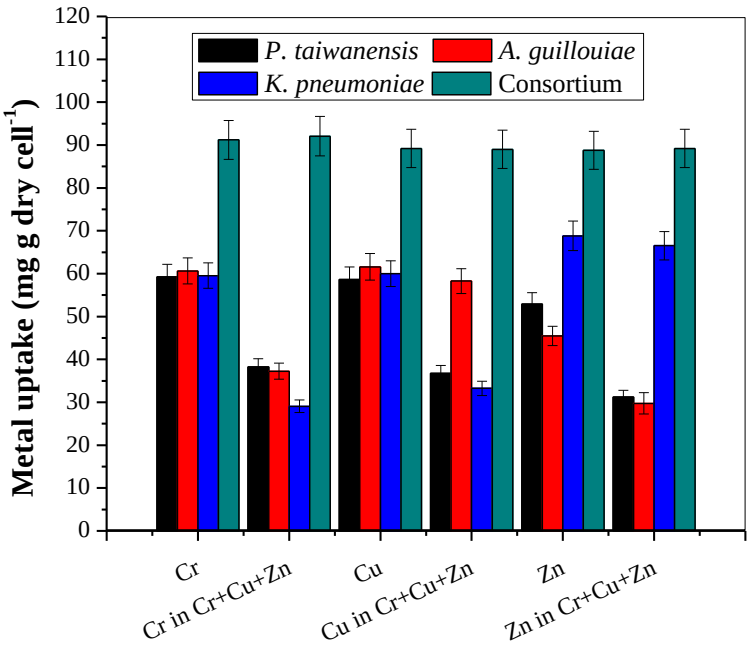


Fig. 5.49. Efficiency of the consortium over individual strains in removal of mixed metals (*T* = 37 ºC, pH = 7.0±0.2, RPM = 150, each metal 50 mg L^{-1})

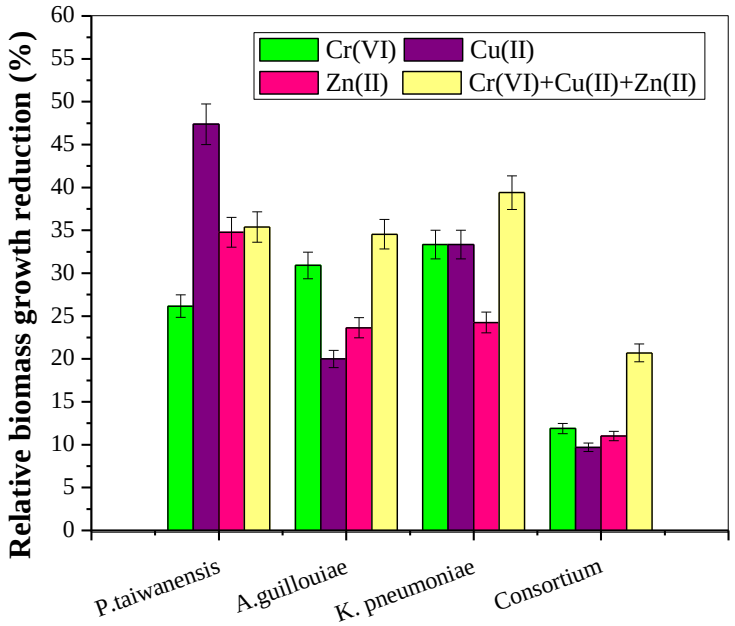


Fig. 5.50. Efficiency of the consortium over individual strains in removal of mixed metals in terms of relative biomass growth reduction (*T* = 37 ºC, pH = 7.0±0.2, RPM = 150, each metal 50 mg L^{-1})

Table 5.14. Comparison between consortium and individual bacterial strains for the removal of single and multiple metals

Metals	Bacterial strains/ Consortium	Percentage metal removal (%)		Maximum metal uptake capacity (mg metal g dry cell^{-1})	
		Single metal	Multiple metals	Single metal	Multiple metals
Cr(VI)	*P. taiwanensis*	94.8	62.50	59.25	38.22
	A. guillouiae	76.8	60.01	60.03	37.25
	K. pneumoniae	43.7	38.42	59.55	29.06
	Consortium	99.0	99.01	91.20	92.05
Cu(II)	*P. taiwanensis*	66.8	58.03	58.62	36.76
	A. guillouiae	90.3	79.90	61.60	58.27
	K. pneumoniae	75.0	59.86	60.03	33.26
	Consortium	94.5	94.00	89.20	89.00
Zn(II)	*P. taiwanensis*	74.8	59.50	52.94	31.25
	A. guillouiae	63.7	38.42	45.48	29.76
	K. pneumoniae	82.3	77.70	68.80	66.52
	Consortium	96.5	97.80	88.78	89.20

Table 5.15. Composition and percentage removal of metals in five sets of simulated industrial effluents

Set No	Composition in simulated industrial effluent (mg L^{-1})			Percentage removal of metals (%)		
	Cr(VI)	Cu(II)	Zn(II)	Cr(VI)	Cu(II)	Zn(II)
1	30.40	47.70	30.50	99.00	95.60	98.04
2	30.40	21.50	15.80	98.02	98.61	99.06
3	19.20	47.70	30.50	98.75	95.60	97.06
4	19.20	21.50	15.80	97.66	99.17	98.11
5	25.50	35.50	21.50	97.67	96.27	99.44

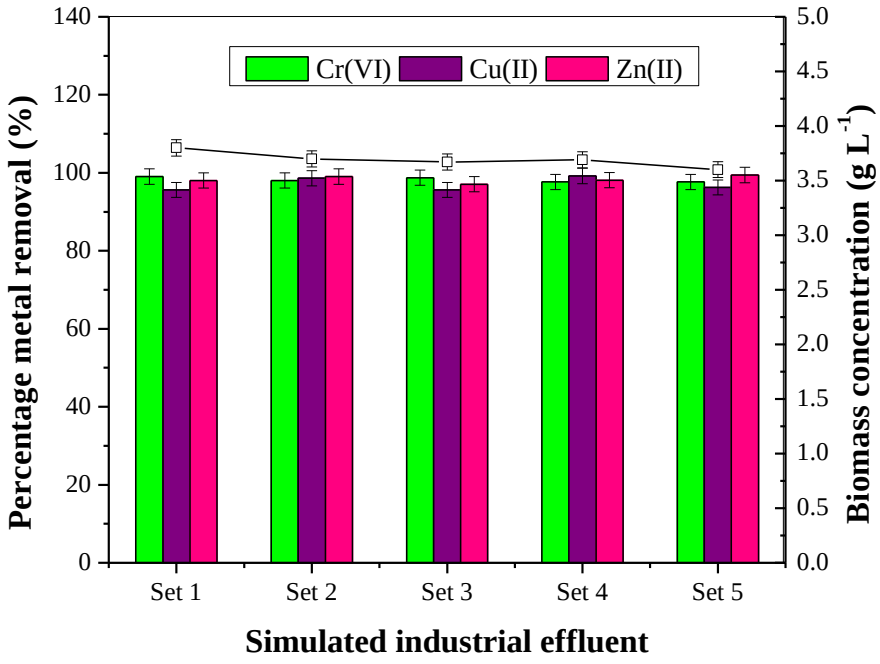


Fig. 5.51. Performance evaluation of the consortium in multi-metals removal in five sets of simulated industrial effluents (square dot with line represents change in biomass concentration) (*T* = 37 °C, pH = 7.0±0.2, RPM = 150)

5.1.6. Mass balance and metal removal mechanism

In the present study, an attempt is made to investigate the removal mechanisms of Cr(VI), Cu(II) and Zn(II) by *P. taiwanensis*, *A. guillouiae* and *K. pneumoniae*, respectively. The initial concentration of each metal is maintained as 150 mg L^{-1} in each 250 mL conical flasks as mentioned in section 3.2. The concentration of each metal ion present in each filtrate and biomass is analyzed to quantify the amount of metals. The mass balance for Cr(VI), Cu(II) and Zn(II) along with plausible removal mechanism are shown in Figs. 5.52, 5.53 and 5.54, respectively. The results obtained in section 5.1.5.3 show 94.8 % Cr(VI) removal using *P. taiwanensis* (Table 5.13). However, about 31 % total Cr (46.5 mg L^{-1}) is still present in the filtrate (Fig. 5.52). It corroborates the fact that in the filtrate, Cr(VI) may have reduced to Cr(III) by *P. taiwanensis*. Earlier studies reported similar type of reduction mechanism for the removal of Cr(VI) using bacterial strains (Dhal et al., 2013; Ramakrishna and Philip, 2005). The maximum sequestration of total Cr is obtained for *P. taiwanensis* biomass (69 %) followed by *A. guillouiae* (≈ 64.3 %). *K. pneumoniae* biomass is able to sequester 41.5 % of total Cr. It is noticed that *P. taiwanensis* accumulates about 60 % of total Cr inside the cell. *A. guillouiae* stores about 70.5 % of total Cr on the cell surface. *K. pneumoniae* stores about 75.1 % of total Cr on the cell surface.

It is observed that in control, *P. taiwanensis* gives better biomass production as compared to other two bacterial strains during the log phase (Fig. 5.41). However, in the presence of Cr, *P. taiwanensis* gives more biomass (Fig. 5.42) as compared to *A. guillouiae* and *K. pneumoniae*. It indicates that *P. taiwanensis* possess robust mechanism to withstand the toxicity due to Cr. This robust mechanism helps to sequester more Cr

and accumulate inside the cells of *P. taiwanensis*. On the contrary, *A. guillouiae* and *K. pneumoniae* are sensitive to Cr toxicity. They possess mechanism to reduce Cr through biosorption (Ahmad et al., 2010; Bejestani et al., 2013).

In case of Cu(II), *A. guillouiae* and *K. pneumoniae* show similar trends for metal uptake capacity and removal kinetics (Fig. 5.53). However, all the three bacterial strains display higher values (≈ 80 %) for distribution of metal inside the cell as compared to values obtained for distribution of metal ion outside the cell surface. It indicates that for all the three bacterial strains, bioaccumulation is the mechanism for sequestration of Cu(II) (Cervantes and Gutierrez-Corona, 1994; Nies, 1999).

P. taiwanensis, *A. guillouiae* and *K. pneumoniae* display almost similar distribution of metal inside the cells (86.5 % - 89.4 %) for Zn(II) removal (Fig. 5.54). However, the distribution of metal outside the cell surface seems to be less for all the three bacterial strains (10.6 % - 13.5 %). The results reveal the fact that the removal of Zn(II) is occured due to the transfer and accumulation of metal inside the cells.

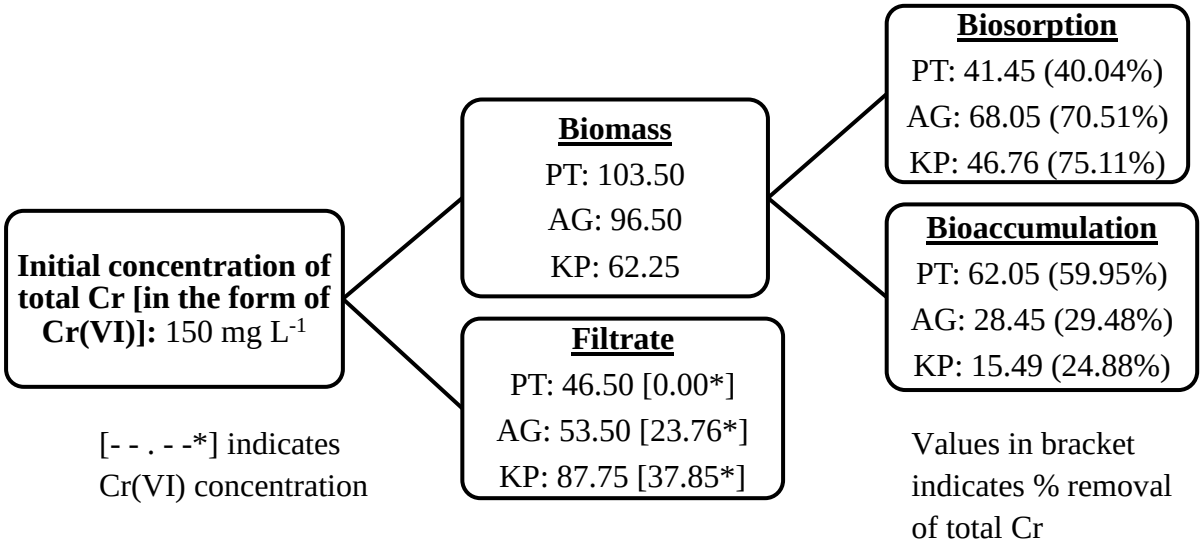


Fig. 5.52. Mass balance and removal mechanism for Cr(VI)

(T = 37°C, pH = 7.0 ± 0.2, RPM = 150, medium MSM, S_o = 150 mg L^{-1})

(PT: *P. taiwanensis*; AG: *A. guillouiae* and KP: *K. pneumoniae*)

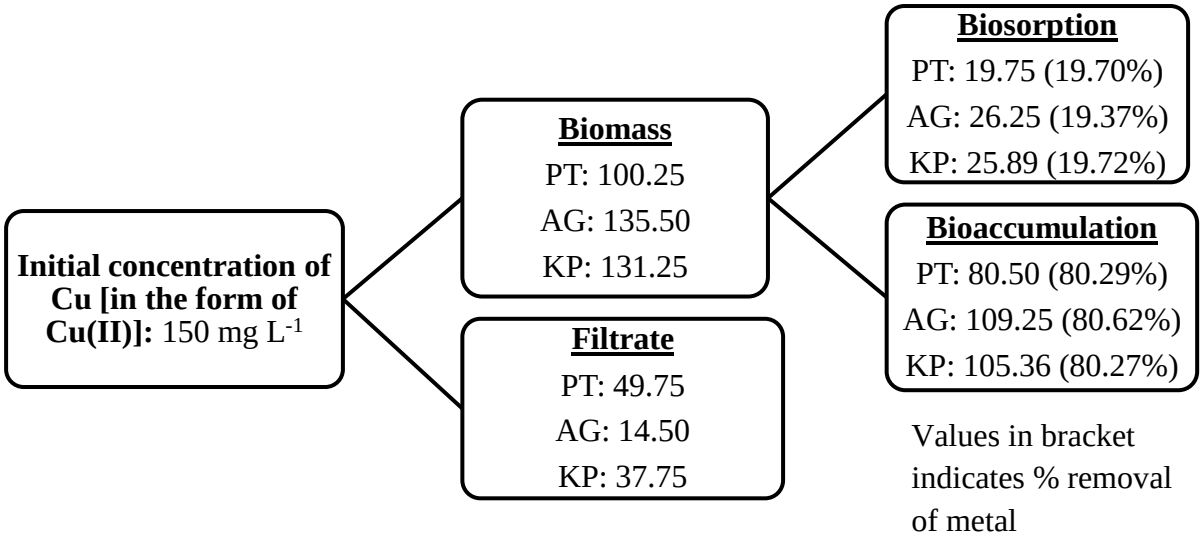


Fig. 5.53. Mass balance and removal mechanism for Cu(II)

(T = 37°C, pH = 7.0 ± 0.2, RPM = 150, medium MSM, S_o = 150 mg L^{-1})

(PT: *P. taiwanensis*; AG: *A. guillouiae* and KP: *K. pneumoniae*)

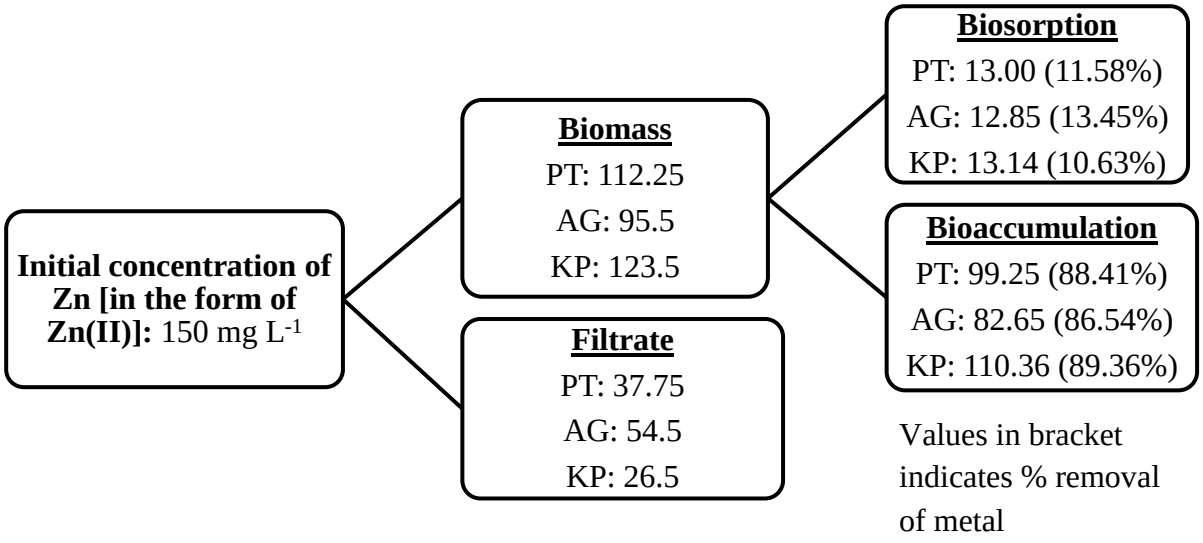


Fig. 5.54. Mass balance and removal mechanism for Zn(II)

(*T* = 37°C, pH = 7.0 ± 0.2, RPM = 150, medium MSM, S_o = 150 mg L^{-1})

(PT: *P. taiwanensis*; AG: *A. guillouiae* and KP: *K. pneumoniae*)

5.1.6.1. Microbial mechanism of chromium, copper and zinc bioremediation

The different mechanisms proposed for heavy metal remediation in bacteria and other microorganisms are as follows (Choudhury and Srivastava, 2001; Diels et al., 1995; Ji and Silver, 1995; Nies, 1992; Nies, 2000; Rouch et al., 1995; Srivastava et al., 1998):

1. Metal exclusion by a permeable barrier.
2. Active export exclusion of the metal by a permeability barrier.
3. Physical sequestration of the metal by forming linkage with intracellular proteins or other ligands.
4. Extracellular sequestration of metal.
5. Transformation and detoxification of metal.

The non-biodegradable water soluble Cr(VI) ions are either oxidized or reduced by the microorganisms. Microbial activity produces less soluble species (Cr(III) compounds). It has been reported that chromate actively crosses biological membranes of various bacterial species with the help of sulfate uptake pathway (Cervantes and Campos-Garcia, 2007). The efficiency of this movement is very low as Cr(VI) forms insoluble compounds (Cary, 1982). Inside the cell, Cr(VI) is reduced to Cr(III) by various enzymatic or non-enzymatic reactions. The Cr(III) generated may then adversely affect the activity of the cytoplasm inside the microbial cell (Cervantes et al., 2001).

Several *Pseudomonas* strains are capable of removing Cr(VI). A group of researchers concluded that the basis of plasmid mediated chromate resistance in *Pseudomonas* is the reduction in chromate uptake by the plasmid bearing strain (Ohtake et al., 1987). The chromate resistance level of *Pseudomonas* strains is greatly affected by the sulfur source used for the cell growth. In *P. aeruginosa*, the rate of chromate uptake

was also regulated by the sulfur source (Ohtake et al., 1987). In the present study, Cr(VI) remediation by *P. taiwanensis* may occur through a two-or-three-step process. In the Cr(VI) remediation process, Nicotinamide adenine dinucleotide (NADH), Nicotinamide adenine dinucleotide phosphate (NADPH) and an electron from the endogenous reserve are used as electron donors (Appenroth et al., 2000). In aerobic condition, Cr(VI) is reduced to form the short-lived intermediate Cr(V) using the Cr(VI) reductase (ChrR). Finally, Cr(V) was reduced to the thermodynamically stable end product Cr(III) with a two-electron transfer with the help of soluble reductase (SR) (Cheung and Gu, 2007; Mishra et al., 2012). It is also reported that, some proportion of Cr(V) intermediate is oxidized to produce reactive oxygen species (ROS). In order to reduce the harmful effects of the produced ROS on bacterial cells, ChrR spontaneously catalyzes the reduction of ROS by two-electron shuttle mechanism (Cheung and Gu, 2007). A plausible mechanism for Cr(VI) remediation using *P. taiwanensis* is shown in Fig. 5.55.

In the present study, *A. guillouiae* may possess a robust copper removal mechanism to overcome the toxicity of copper by transporting and accumulating the copper inside the cells. A chromosomal system works at low copper concentration and a plasmid system functions at high copper concentrations to maintain the copper concentration inside the bacterial cells. In chromosomal system, two mechanisms may occur to ensure transport and accumulation of copper (Cervantes and Gutierrez-Corona, 1994; Nies, 1999). These two system are designated as CutA and CutB. Inside the bacterial cells, Cu(II) is reduced to toxic Cu(I) by glutathione which is harmful for the cells (Brown et al., 1993). Rouch et al. (1985) reported the presence of two intracellular copper storage or carrier proteins (CutE and CutF) which protect the cells from the toxic

effects of Cu(I). These two proteins are also responsible for transferring copper to the protein synthesis sites (Brown et al., 1993; Rouch et al., 1985). The two proteins, CutC and CutD are known as copper efflux Adenosine triphosphate synthases (ATPases) which help to efflux the Cu(I) from the bacterial cells. Fig. 5.56 shows the plausible mechanism for Cu(II) remediation using *A. guillouiae*.

Various microbial mechanisms are prevalent in zinc removal. Resistance to toxic level of zinc may be due to extracellular accumulation, sequestration by metallothioneins (MT) and efflux-based sequestration or intracellular sequestration (Choudhury and Srivastava, 2001; Morby et al., 1993; Nies, 1999; Olafson et al., 1988). Generally, two separate systems of zinc uptake can be seen in yeast, *Saccharomyces cerevisiae*. It includes high substrate affinity which is induced in cells with zinc deficiency and low susbtrate affinity which is controlled by zinc concentration. Two Zinc-regulated transporter (*ZRT*) proteins, high affinity (Zrt1p) and low-affinity (Zrt2p) are coded by *ZRT1* and *ZRT2* gene, respectively (Zhao and Eide, 1996a; Zhao and Eide, 1996b). In an earlier study, it is reported that *Pseudomas stutzeri* RS34 isolated from the polluted soil in New Delhi shows resistance to Zn(II) by accumulating high levels of zinc on its outer membrane (Bhagat and Srivastava, 1994). One plausible mechanism of Zn(II) remediation by *K. pneumoniae* is shown in Fig. 5.57.

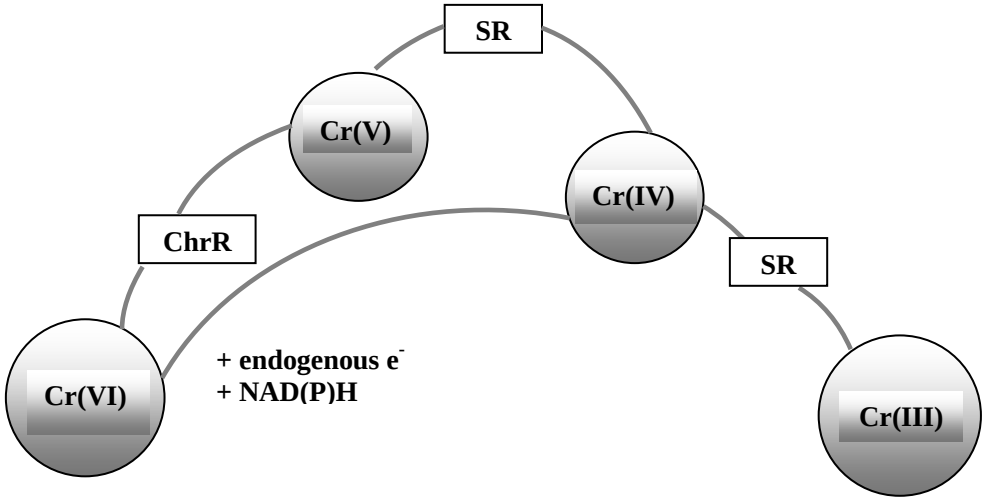


Fig. 5.55. Plausible mechanism of Cr(VI) remediation using *P. taiwanensis* in aerobic condition. Adapted from Cheung and Gu (2007) (ChrR: Chromium reductase, SR: Soluble reductase)

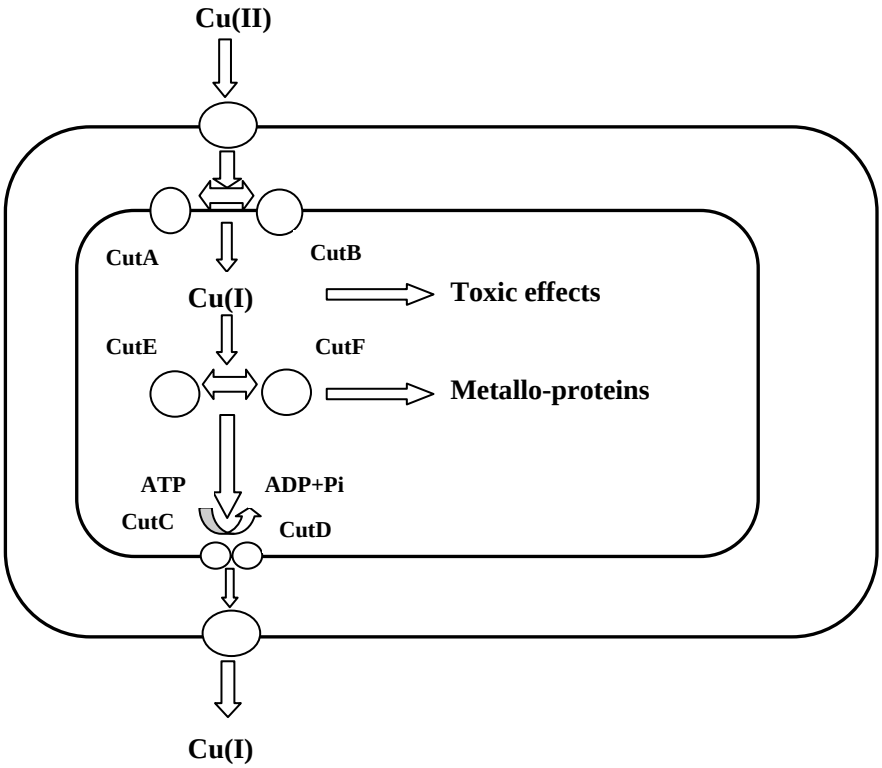


Fig. 5.56. Plausible mechanism of Cu(II) remediation using *A. guillouiae*. Adapted from Cervantes and Gutierrez-Corona (1994)

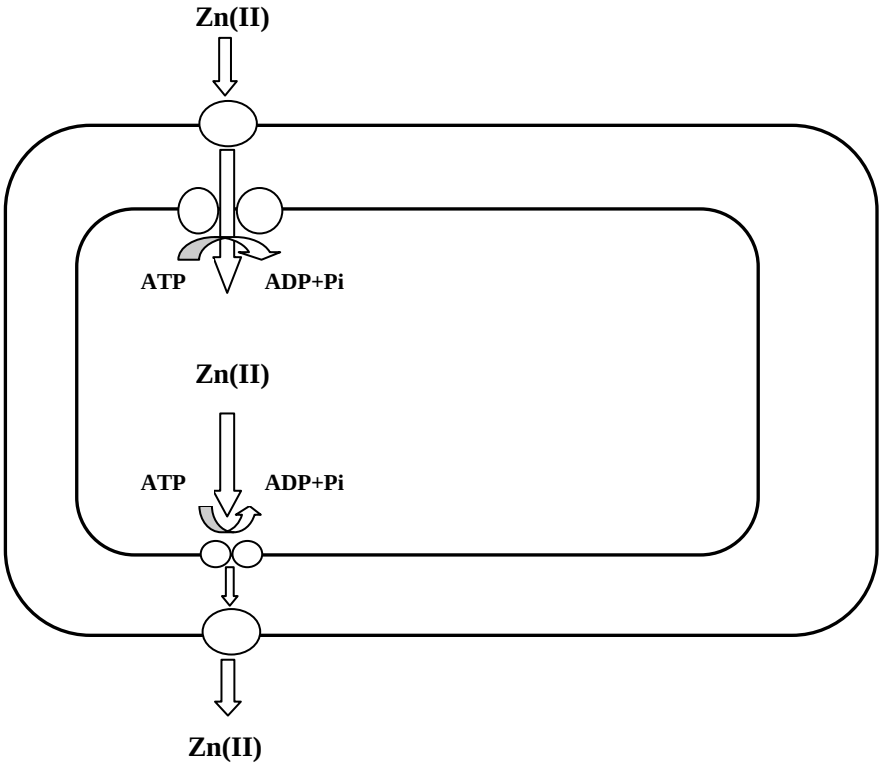


Fig. 5.57. Plausible mechanism of Zn(II) remediation using *K. pneumoniae*. Adapted from Cervantes and Gutierrez-Corona (1994) and Nies (1999)

5.1.7. Biofiltration studies

In the present study, the removal of Cr(VI), Cu(II) and Zn(II) from aqueous solution is investigated using biofilter column packed with a mixture of coal and matured compost. The acclimation periods for three bacterial strains in three metals removal are estimated. The effects of time on the performance of biofilter column for different operating conditions such as inlet metal concentration, flow rate and shock loading are studied. The performance of the biofilter is investigated in terms of the removal efficiency.

5.1.7.1. Cr(VI) removal

The profiles of inlet concentration, outlet concentration and removal efficiency of Cr(VI) at various flow rates during 63 days of biofilter operation are shown in Fig. 5.58. The biomass growth in terms of colony forming unit CFU g^{-1} of packing material is also calculated at different heights of the filter bed (10, 30, 50 and 70 cm from top of the column) in each day and is shown in Fig. 5.69.

In phase I (acclimation period), removal efficiency is reported as 50 % at the end of 3^{rd} day. It is increased with increase in number of operating days and reached to 94.8 % at the end of phase I (20^{th} day). It may be due to the fact that microorganism takes some time to get acclimated with the operating conditions rendered in the biofilter column. Later stages of acclimation show significant increase in removal efficiency of Cr(VI) as the significant growth of *P. taiwanensis* is observed in the biofilter column. At the end of phase I, the growth of *P. taiwanensis* is confirmed by the obtained value of CFU as 3×10^6, 2.9×10^6, 2.62×10^6 and 2.45×10^6 CFU g^{-1} of packing material for 10, 30, 50, 70 cm heights of the filter bed, respectively. In phase II, the inlet concentration of Cr(VI) is increased from 10 to 20 mg L^{-1} and the flow rate is increased from 5 to 10 mL

min^{-1}. Due to this increase, the removal efficiency is decreased from 94.8 % which is achieved at the end of phase I to 62.5 % on 21^{st} day. It is further increased with increase in operating time and reached to approximately constant values of 84.6 % (steady state) at the end of phase II (30^{th} day). The sudden decrease in the removal efficiency may be due to the decrease in the growth of *P. taiwanensis* at new operating conditions (Fig. 5.59) as it needs some time to get adapted with the new conditions. The growth of *P. taiwanensis* is increased with operating time from 1.17×10^7 - 0.78×10^7 g^{-1} of packing material on 22^{nd} day to 2.4×10^7 - 1.32×10^7 CFU g^{-1} of packing material on 30^{th} day at respective heights of the column. The removal efficiency is increased continuously and reached to constant value at the end of 29^{th} day.

In phase III, the inlet concentration of Cr(VI) and flow rate are increased from 20 mg L^{-1} to 30 mg L^{-1} and 10 to 20 mL min^{-1}, respectively. A sudden decrease in removal efficiency is observed from 84.2 % to 39.6 % at the end of 31^{st} day due to the increase in the flow rate. This is also supported by the fact that the Empty Bed Residence Time (EBRT) value is decreased from 137.44 min at phase II to 68.72 min at phase III (Table 3.6). Due to this reason, *P. taiwanensis* gets less time to adjust with the new operating conditions at the start of phase III. The biomass growth of *P. taiwanensis* is obtained as 1.37×10^7, 1.17×10^7, 1.01×10^7 and 0.87×10^7 CFU g^{-1} of packing material on 31^{st} day at 10, 30, 50, 70 cm heights of the column, respectively. Removal efficiency is then increased gradually and reached to a steady state value of 76.3 % at the end of phase III (42^{nd} day). The growth of the *P. taiwanenesis* is obtained as 3.12×10^7 CFU g^{-1} of packing material and 2.25×10^7 CFU g^{-1} of packing material at 10 and 70 cm heights of the column, respectively on 42^{nd} day. In phase IV, the flow rate and inlet concentration of

Cr(VI) solution are increased from 20 to 40 mL min^{-1} and 30 to 50 mg L^{-1}, respectively. The removal efficiency is decreased from 76.3 % on 42^{nd} day to 45% on 43^{rd} day. It is increased to 79 % at the end of phase IV (52^{nd} day). Although the inlet concentration and flow rate are more in phase IV as compared to phase III, the steady state value of the removal efficiency is higher in phase IV. This may be due to the increase in biomass growth from 1.86×10^7 to 4.5×10^7 CFU g^{-1} of packing material (on 43^{rd} day) and 1.05×10^7 to 2.18×10^7 CFU g^{-1} of packing material (on 52^{nd} day) at 10 and 70 cm heights of the column, respectively. This also supports the more stable behaviour of the biofilter column at different operating conditions. In phase V (53-63 days), 40 mg L^{-1} of inlet Cr(VI) concentration and 30 mL min^{-1} of flow rate of Cr(VI) solution are maintained. The removal efficiency is decreased from 79 % on 52^{nd} day to 75.7 % on 53^{rd} day. There is no significant change in removal efficiency is observed till the end of 63^{rd} day which also confirms the stable behaviour of the biofilter column at the end of phase V. Final value of removal efficiency is obtained as 89.4 % at the end of phase V. The slight increase in the removal effciency at the end of phase V may be due to the increase in the biomass growth of *P. taiwanensis* in the range of 3.75×10^7 - 1.21×10^7 CFU g^{-1} of packing material on 53^{rd} day to 5.3×10^7 - 1.77×10^7 CFU g^{-1} of packing material on 63^{rd} day at different heights of the column.

Final value of removal efficiency is obtained as 89.4 % at the end of phase V (i.e. on 63^{rd} day) for 40 mg L^{-1} of inlet Cr(VI) concentration. This is attributed to the fact that increase in EBRT from 34.4 min (phase IV) to 45.8 min (phase V) gives more contact time between the population of *P. taiwanensis* and Cr(VI) which further enhances the remediation of Cr(VI).

Response to sudden change in inlet conditions is represented in terms of removal efficiency and biomass growth in the biofilter column. In the present study, the fluctuating inputs of Cr(VI) solution (shock loadings) is provided for 20 days immediately after 63 days of biofilter operation and shown in Fig. 5.60. The profile for biomass growth with operating time is given in Fig. 5.61.

During the first 4 days of biofilter operation, the removal efficiency is obtained in the range of 83.5 to 88 % when the inlet concentrations and flow rate of Cr(VI) solution are maintained at 30 mg L^{-1} and 35 mL min^{-1}, respectively. Higher values of removal efficiency (83.5-88 %) are obtained which may be due to the fact that that biofilter column is already acclimated with *P. taiwanensis*. During this period, high bioremediation rate is obtained by *P. taiwanensis* even at lower inlet concentration of Cr(VI) (28.5-30 mg L^{-1}) as compared to the inlet concentration of Cr(VI) (38-40 mg L^{-1}) maintained in phase V of bioflter operation. In the next phase (5-8 days) of shock loading operation, inlet concentration of Cr(VI) is increased from 30 to 40 mg L^{-1} by maintaining the flow rate at 35 mL min^{-1}. The variation in the inlet concentration is measured in the range of 38-40 mg L^{-1}. The removal efficiency is obtained in the range of 86.8-88 %. This result shows that the obtained removal efficiency is fairly comparable to the removal efficiency (83.5-88 %) obtained in previous phase of shock loading. It is attributed to the fact that the biofilter column is already adapted to the fluctuating input conditions.

For the next 6 days of operation (9-14 days), inlet concentration of Cr(VI) is maintained as 30 mg L^{-1} and flow rate is decreased from 35 mL min^{-1} to 20 mL min^{-1}. The removal efficiency is obtained in the range of 83.2-84.7 %. The biofilter column shows stable operation at lower inlet concentration (28-30 mg L^{-1}) and lower flow rate

(20 mL min^{-1}) of Cr(VI). In the last phase of shock loading operation (15-20 days), inlet concentration of Cr(VI) is increased from 30 to 50 mg L^{-1} and the flow rate is maintained same (20 mL min^{-1}) as previous phase. The variation in the inlet concentration is measured in the range of 48.5-50 mg L^{-1}. The removal efficiency obtained is in the range of 89.7-90 %. It clearly reveals the fact that the biofilter column can also be operated at higher inlet concentration of Cr(VI) (48.5-50 mg L^{-1}) and lower flow rate (20 mL min^{-1}).

At the end of 1st day, the growth *P. taiwanensis* is obtained as 5.3×10^7, 4.2×10^7, 3.42×10^7 and 1.7×10^7 CFU g^{-1} of packing material at 10, 30, 50 and 70 cm heights of the column, respectively (Fig. 5.62). At 10, 30, 50 and 70 cm heights of the column, the growths are obtained as 5.32×10^7, 4.2×10^7, 3.38×10^7 and 1.65×10^7 CFU g^{-1} of packing material, respectively at the end of 20th day. The obtained results of shock loading conditions indicate that bioflim developed in the column is quite stable.

It is also observed that pH remains fairly stable (6.5-7.1) throughout the shock loading study (Fig. 5.61). Removal efficiency of Cr(VI) is almost same in all the phases which shows greater stability of the biofilter column. Based on this study, it can be concluded that the biofilter column can be used for the industrial application where inlet concentration of pollutants vary from lower to higher values at a lower to moderate flow rate.

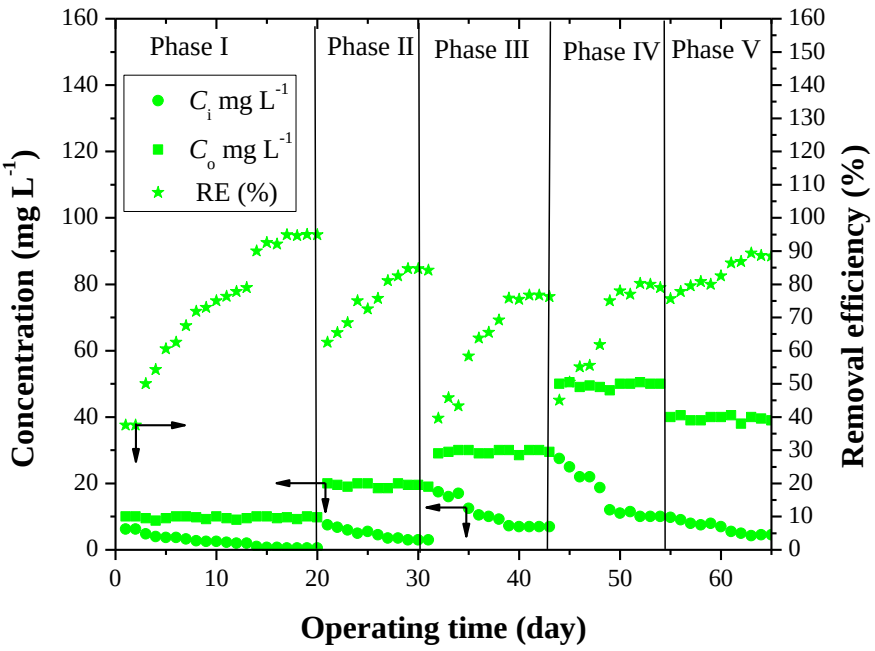


Fig. 5.58. Performance of biofilter with change in flow rate and inlet concentrations of Cr(VI) solution

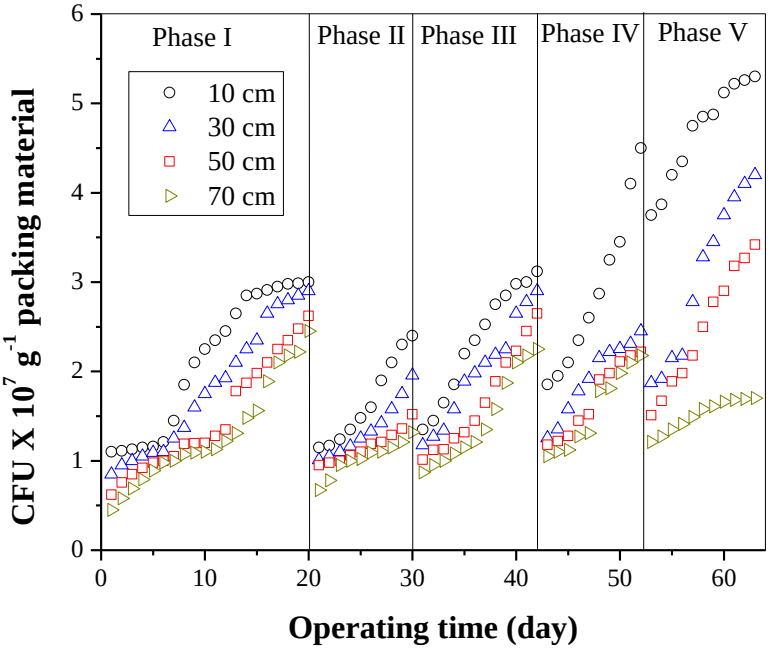


Fig. 5.59. Change in biomass growth with operating time at different height of filter bed for Cr(VI) removal

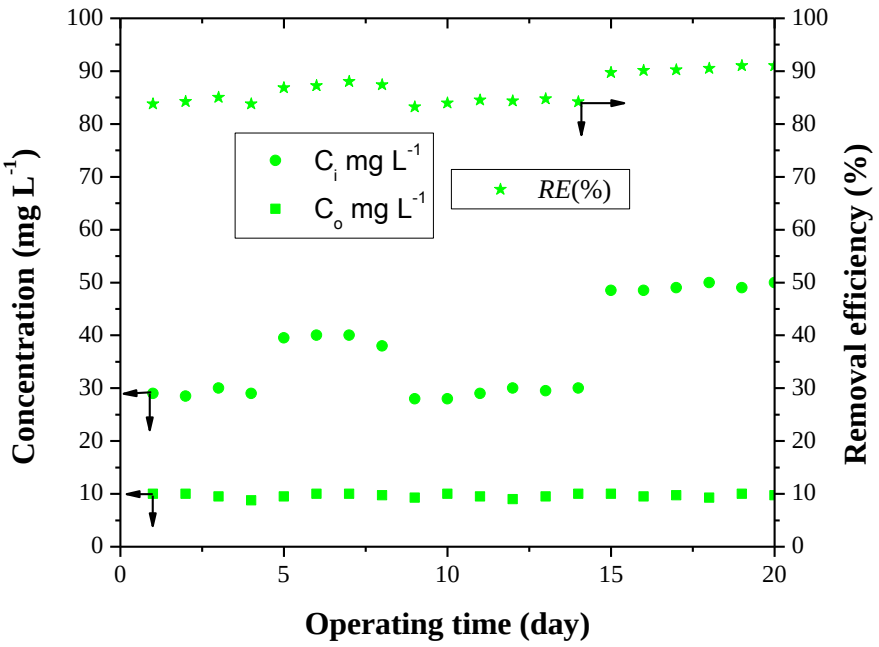


Fig. 5.60. Performance of biofilter under shock loading conditions for Cr(VI) removal (change in concentration and removal efficiency with operating time)

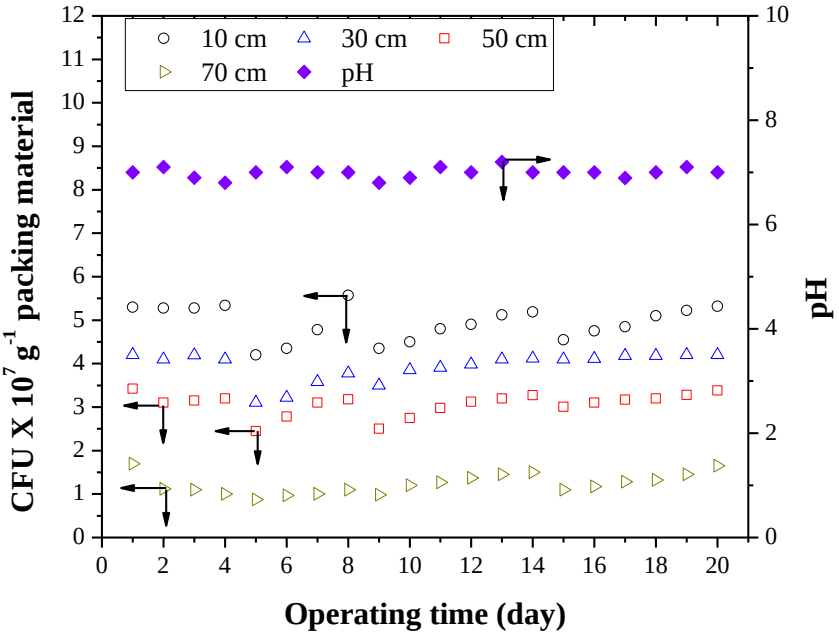


Fig. 5.61. Performance of biofilter under shock loading conditions for Cr(VI) removal (change in biomass growth and pH with operating time)

5.1.7.2. Cu(II) removal

The effects of operating conditions on outlet concentration and removal efficiency are shown in Fig. 5.62 for Cu(II) removal. The biomass growth in terms of CFU g^{-1} of packing material is determined at different heights of the filter bed (10, 30, 50 and 70 cm from top of the column) in each day and is shown in Fig. 5.63. In phase I, the removal efficiency is obtained as 50 % at the end of 7^{th} day. It is increased with operating days and reached to 95 % at the end of phase I (on 15^{th} day). During the early days of acclimation period, *A. guillouiae* takes some time to get adapted with the operating conditions in the biofilter column. At the later stages of acclimation, the removal efficiency is found to be increased as the significant growth of *A. guillouiae* is achieved in the biofilter. At the end of phase I, significant biomass growth is obtained as 4.01×10^7 and 1.82×10^7 CFU g^{-1} of packing material at 10 and 70 cm heights of the column, respectively.

The concentration and flow rate of Cu(II) solution are increased from 10 to 20 mg L^{-1} and from 5 to 10 mL min^{-1}, respectively in phase II. It is observed that, the removal efficiency decreased from 95 % on 15^{th} day to 77.5 % on 16^{th} day. The sudden decrease in the removal efficiency is attributed to the decrease in EBRT value from 274.88 to 137.44 min. Due to this reason, *A. guillouiae* gets less time to acquire the new operating conditions. During the transition from phase I to phase II, the growths of *A. guillouiae* are obtained as 4.01×10^7 to 3.2×10^7 CFU g^{-1} of packing material and 1.82×10^7 to 1.5×10^7 CFU g^{-1} of packing material for 10 and 70 cm heights of the column, respectively. Population of *A. guillouiae* is increased with operating time which results in further increase in the removal efficiency after 19^{th} day. It shows increasing trend till the 25^{th}

day. With increase in operating time, the removal efficiency is increased and reached to almost constant value of 97.5 % on 25^{th} day (at the end of phase II). This increase in the removal efficiency is due to the increase in the growth of *A. guillouiae* with operating time. The growth of *A. guillouiae* is determined as 4.96×10^7, 3.91×10^7, 2.91×10^7 and 2.47×10^7 CFU g^{-1} of packing material at 10, 30, 50 and 70 cm heights of the column, respectively. The increase in the biomass growth results in the increase of the removal efficiency.

In phase III (26^{th}-35^{th} day), inlet Cu(II) concentration is increased from 20 mg L^{-1} to 30 mg L^{-1}. Flow rate of Cu(II) solution is increased to 20 mL min^{-1}. The removal efficiency is suddenly decreased from 97.5 % to 64.7 % on 26^{th} day. This can be attributed to the decrease in EBRT from 137.44 to 68.7 min. The growths of *A. guillouiae* are obtained as 3.75×10^7, 3.1×10^7, 2.5×10^7 and 1.9×10^7 CFU g^{-1} of packing material at respective heights of the column on 26^{th} day. Removal efficiency is increased gradually and reached to 76 % at the end of phase III (35^{th} day). In phase IV, the flow rate of Cu(II) solution is increased from 20 to 40 mL min^{-1}. Inlet Cu(II) concentration is increased from 30 to 50 mg L^{-1}. The removal efficiency is decreased from 76 % on 35^{th} day to 55 % on the 36^{th} day. It is increased to 64 % at the end of phase IV (45^{th} day). In phase IV, sudden decrement in the removal efficiency is observed as compared to phase III. Moreover, the removal efficiency obtained in phase IV is less as compared to values obtained in other phases. It may be due to the decrease in EBRT from 68.7 to 34.4 min with sudden increase in the flow rate from 20 to 40 mL min^{-1}. Microbial population is exposed to a high concentration of Cu(II) which leads to the self inhibition effect. It further affects the growth of the *A. guillouiae*. Less EBRT value (34.4 min) also indicates

that *A. guillouiae* gets less contact time for the bioremediation of Cu(II). For 10, 30, 50 and 70 cm heights of the column the growths of *A. guillouiae* are obtained as 4.56×10^7, 3.96×10^7, 3.37×10^7 and 3.05×10^7 CFU g^{-1} of packing material, respectively.

In phase V, the biofilter column is operated for 10 days (46-55 days). In this phase, inlet Cu(II) concentration is decreased from 50 to 40 mg L^{-1}. The flow rate of Cu(II) solution is decreased from 40 to 30 mL min^{-1}. The removal efficiency is increased from 64 % (on 45^{th} day) to 75 % (on 46^{th} day). The removal efficiency is increased with increase in operating time and reached to 90.6 % at the end of phase V (on 55^{th} day). The increase in EBRT from 34.4 min (phase IV) to 45.8 min (phase V) increases the contact time between *A. guillouiae* and Cu(II) which further enhances the bioremediation of Cu(II). The growths of *A. guillouiae* are obtained as 6×10^7, 5.8×10^7, 5.6×10^7 and 4.9×10^7 CFU g^{-1} of packing material at 10, 30, 50 and 70 cm heights of the column, respectively on 55^{th} day.

In the present study, 97.5 % of Cu(II) removal is achieved at the end of phase II by using combination of coal and matured compost as packing material for 20 mg L^{-1} of inlet Cu(II) concentration. It is observed that, the time taken to achieve this removal efficiency is less (25 days) as compared to other biofilter systems reported in earlier studies (Holmes et al., 1994; Srivastava and Majumder, 2008). It is also found that, present biofilter column gives 97.5 % Cu(II) removal (end of phase II) at lower inlet concentration and low flow rate of Cu(II) solution. Earlier study showed similar value of percentage removal (97.5 %) of Cu(II) in a bench scale biofilter column packed with silica sand and enriched with mixed population of sulfate-reducing bacteria (SRB) (Jong and Parry, 2003).

Stability of the column for Cu(II) removal is checked under fluctuating input conditions for a period of 2 weeks immediately after the 55 days of biofilter operation and reported in Fig. 5.64. During the period of 14 days, the biomass growth of *A. guillouiae* is determined in terms of CFU g^{-1} of packing material in each day and is shown in Fig. 5.65. This study is carried out to understand the behavior of column during sudden changes in flow rate and concentration of Cu(II) solution so that it can be used in industrial practice.

In the first 4 days of biofilter operation under shock loading conditions, the removal efficiency is obtained in the range of 85.2-87 % by keeping inlet concentration and flow rate of Cu(II) solution as 30 mg L^{-1} and 32 mL min^{-1}, respectively (Fig. 5.64). The high values of removal efficiency (85.2-87 %) indicates that biofilter column is acclimated with *A. guillouiae* and higher accumulation is achieved in less time by *A. guillouiae* as compared to phase V of biofilter operation. During the next phase (5-8 days) of shock loading operation, inlet concentration of Cu(II) is increased from 30 to 40 mg L^{-1} by maintaining the same flow rate (32 mL min^{-1}). The variation in the inlet Cu(II) concentration is observed in the range of 38-40 mg L^{-1} and the removal efficiency is obtained in the range of 81.1-81.75 %. It is observed that the change in the removal efficiency of this phase is not significant as compared to previous phase. For the next 4 days, inlet concentration of Cu(II) is maintained as 30 mg L^{-1} and flow rate is decreased from 32 mL min^{-1} to 20 mL min^{-1}. The variation in the inlet Cu(II) concentration is observed in the range 30-33 mg L^{-1} and the removal efficiency is obtained in the range of 86-87 %. Obtained results clearly indicate that biofilter column is quite stable and it gives better removal for lower to medium range of inlet Cu(II) concentration and at lower flow

rate. Due to the higher values of EBRT (68.72 min), microbial population gets more contact time for the remediation of Cu(II).

The growths of *A. guillouiae* are obtained as 6×10^7, 5.8×10^7, 5.6×10^7, and 4.9×10^7 CFU g^{-1} of packing material at 10, 30, 50 and 70 cm heights of the column, respectively on 1^{st} day (Fig. 5.65). At the end of 14^{th} day, the biomass growths are obtained as 5.9×10^7, 5.8×10^7, 5.6×10^7 and 4.85×10^7 CFU g^{-1} of packing material at different heights of the column. The results obtained for biomass growth under shock loading conditions indicate that biofilm developed in the column is stable (Fig. 5.65). It is also observed that pH value remains constant throughout the shock loading study (Fig. 5.65). Removal efficiency of Cu(II) is comparable in all the phases which shows stability of the biofilter column.

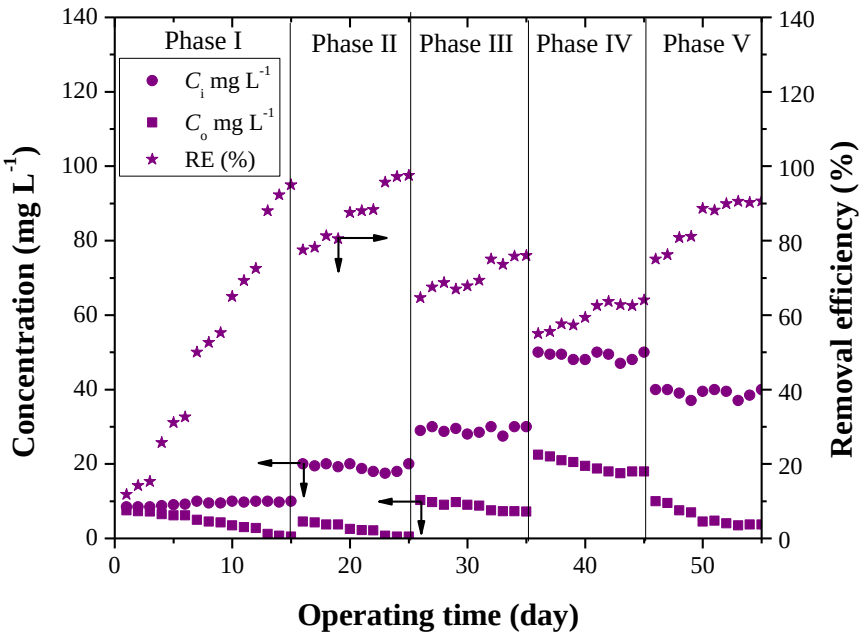


Fig. 5.62. Performance of biofilter with change in flow rate and inlet concentrations of Cu(II) solution

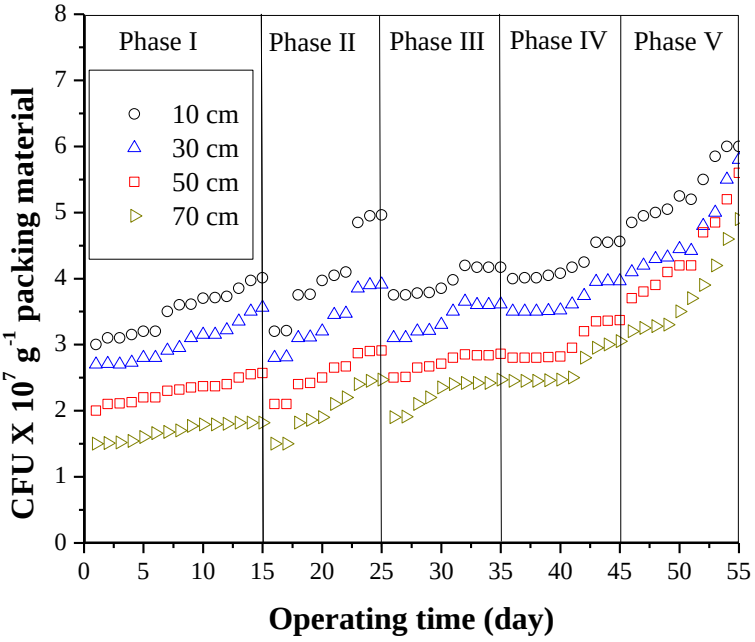


Fig. 5.63. Change in biomass growth with operating time at different height of filter bed in Cu(II) removal

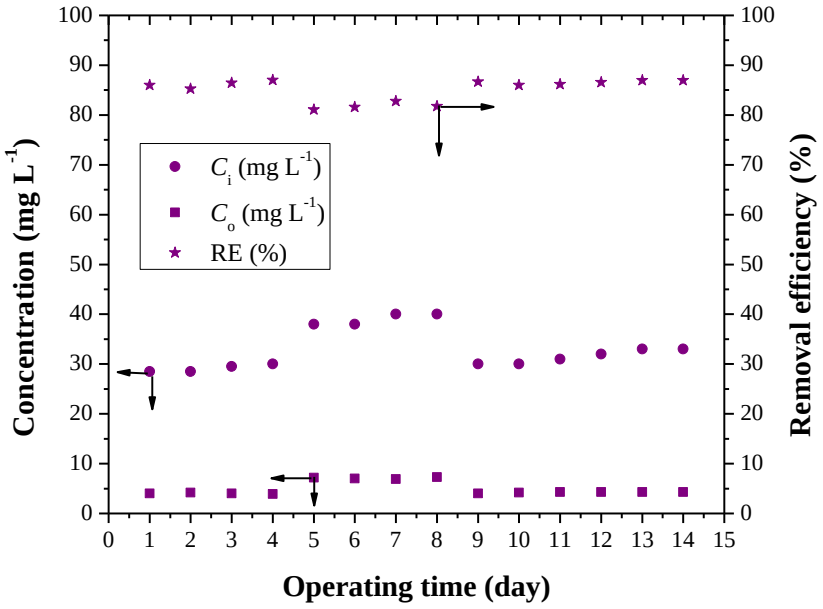


Fig. 5.64. Performance of biofilter under shock loading conditions for Cu(II) removal (change in concentration and removal efficiency with operating time)

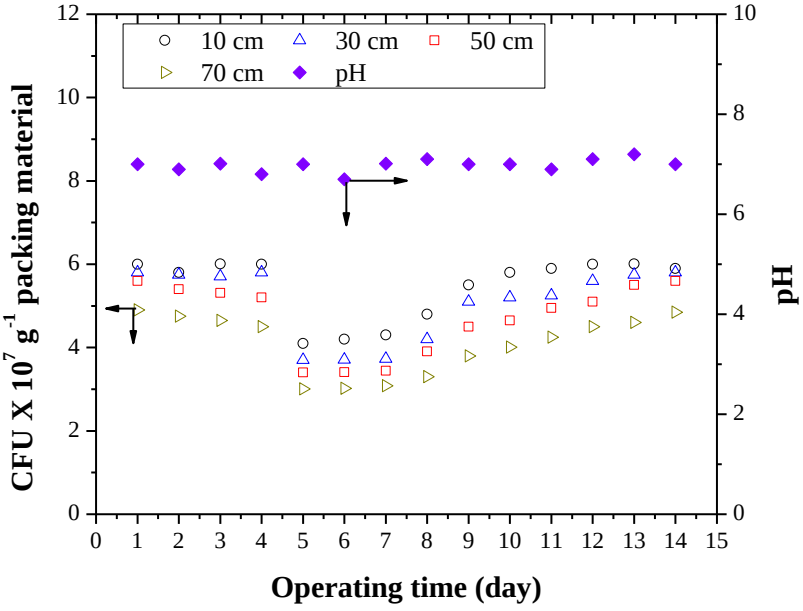


Fig. 5.65. Performance of biofilter under shock loading conditions for Cu(II) removal (change in biomass growth and pH with operating time)

5.1.7.3. Zn(II) removal

For Zn(II) removal, the effects of operating conditions on outlet concentration and removal efficiency are investigated for a period of 60 days and shown in Fig. 5.66. The change in biomass growth (CFU g^{-1} of packing material) at different heights of the filter bed (10, 30, 50 and 70 cm from top of the column) is calculated in each day (Fig. 5.67). During the acclimation period (phase I) the removal efficiency is obtained as 31.6 % at the end of 7^{th} day (Fig. 5.66). At the end of phase I (at 20^{th} day), the removal efficiency is increased to 73.7 %. It may be due to the fact that *K. pneumoniae* gets slowly acclimated with the operating conditions of biofilter column. At the end of phase I, the growth of *K. pneumoniae* is obtained in the range of 5.1×10^7- 4.5×10^7 CFU g^{-1} of packing material for 10-70 cm heights of the column, respectively (Fig. 5.67).

In phase II, the inlet concentration of Zn(II) is increased from 10 to 20 mg L^{-1} and flow rate is increased from 5 to 10 mL min^{-1}. The removal efficiency is decreased from 73.7 % on 20^{th} day to 52.8 % on 21^{st} day. The decrease in removal efficiency at the start of phase II may be attributed to the sudden increase in the Zn(II) concentration at the inlet. The EBRT value is decreased from 274.48 min to 137.44 min due to the increase in the inlet flow rate. Due to this reason, *K. pneumoniae* gets less time to adjust to the new operating conditions. With increase in operating time, the removal efficiency is further increased and reached to a steady state (91.5 %) at the end of phase II (30^{th} day). Growth of *K. pneumoniae* is increased with operating time which results in further increase in the removal efficiency after 22^{nd} day. It shows increasing trend till the 30^{th} day. At the end of 30^{th} day the growth of *K. pneumoniae* is obtained as 4.75×10^7, 3.75×10^7, 2.65×10^7

and 2.3×10^7 CFU g^{-1} of packing material at 10, 30, 50 and 70 cm heights of the column, respectively.

In phase III (31^{st}-40^{th} day), inlet Zn(II) concentration was increased from 20 mg L^{-1} to 30 mg L^{-1}. The flow rate of Zn(II) solution is maintained at 20 mL min^{-1}. The removal efficiency is decreased suddenly from 91.5 % on 30^{th} day to 55 % on 31^{st} day. It confirms that microorganism still needs more time to adapt to the new environment. It is supported by the decrease in EBRT from 137.4 to 68.7 min with sudden increase in the flow rate from 10 to 20 mL min^{-1}. The removal efficiency is then increased gradually and reached to 86.7 % at the end of phase III (40^{th} day). The growth of *K. pneumoniae* is obtained in the range 5.2×10^7 - 2.47×10^7 CFU g^{-1} of packing material at different heights of the column on 40^{th} day. In phase IV, the flow rate of Zn(II) solution is increased from 20 to 40 mL min^{-1}. Inlet Zn(II) concentration is increased from 30 to 50 mg L^{-1}. In phase IV, the removal efficiency is decreased suddenly from 86.7 % to 56.6 %. This is due to the decrease in EBRT from 68.7 to 34.4 min with sudden increase in the flow rate from 20 to 40 mL min^{-1}. Microbial population is exposed to a high concentration of Zn(II) which leads to the self inhibition effect. The growth of the *K. pneumoniae* is affected by the self inhibition effect. Less EBRT value (34.4 min) also indicates that *K. pneumoniae* gets less contact time for the bioremediation of Zn(II). The removal efficiency is gradually increased to 82 % at the end of phase IV (50^{th} day). The growth of *K. pneumoniae* is obtained in the range 5.15×10^7 - 2.5×10^7 CFU g^{-1} of packing material at 10-70 cm heights of the column, respectively. In phase V, the biofilter column is operated for 10 days (51^{st}-60^{th} day). In this phase, inlet Zn(II) concentration is decreased from 50 to 40 mg L^{-1}. The flow rate of Zn(II) solution is also

decreased from 40 to 30 mL min^{-1}. The removal efficiency is decreased from 82 % on 50^{th} day to 77.3 % on 51^{st} day. The removal efficiency is increased gradually with increase in operating time and reached to 90 % at the end of phase V (60^{th} day). The increase in EBRT from 34.4 min (phase IV) to 45.8 min (phase V) gives more contact time between *K. pneumoniae* and Zn(II) which further enhances the bioremediation of Zn(II). The growths of *K. pneumoniae* are obtained as 7.11×10^7, 6.13×10^7, 4.2×10^7 and 3.51×10^7 CFU g^{-1} of packing material at 10, 30, 50 and 70 cm from top of the column, respectively at the end of phase V.

In the present study, around 91.5 % Zn(II) removal is obtained during phase II for inlet Zn(II) concentration in the range of 18.5-20 mg L^{-1}. The removal efficiency value obtained in the present study is comparable with the reported value of removal efficiency (88.1 %) in a multi-metal [Fe(II), Cu(II), Ni(II) and Zn(II)] removal system consisting of horizontal rotating tubular bioreactor (HRBT) enriched with mixed microbial culture (Rezić et al., 2011). It is also found that, present biofilter column gives 91.5 % Zn(II) removal (end of phase II) at lower inlet concentration and low flow rate of Zn(II) solution.

The stability of the biofilter column for Zn(II) removal is checked for 14 days immediately after the 60 days of biofilter operation and shown in Fig. 5.68. The change in the growth of *K. pneumoniae* under fluctuating input conditions for 14 days is shown in Fig. 5.69. The removal efficiency is obtained in the range of 87.9-89.2 % for first 4 days by keeping inlet concentration and flow rate of Zn(II) solution as 30 mg L^{-1} and 32 mL min^{-1}, respectively (Fig. 5.68). The variation in the inlet Zn(II) concentration is observed in the range of 28.5-30 mg L^{-1}. The high values of removal efficiency (87.9-

89.2 %) indicate that biofilter column is already acclimated with the *K. pneumoniae* biomass. Due to this reason, high bioremediation of Zn(II) by *K. pneumoniae* is obtained in less time as compared to phase V of biofilter operation. During the next phase (5-8 days) of shock loading operation, 40 mg L^{-1} of Zn(II) concentration and 32 mL min^{-1} of flow rate of Zn(II) solution are maintained. The removal efficiency is obtained in the range of 87.7-89.3 % which is comparable to the removal efficiency (85.2-87 %) obtained in previous phase of shock loading. It indicates that the biofilter column is already adapted with the fluctuating input conditions. For the next 6 days, inlet concentration of Zn(II) is maintained at 30 mg L^{-1} and flow rate is decreased from 32 mL min^{-1} to 20 mL min^{-1}. The variation in the inlet Zn(II) concentration is observed in the range of 30-33 mg L^{-1}. The removal efficiency is obtained in the range of 85.7-90.3 %. Obtained results clearly indicate that biofilter column is quite stable and it gives better removal for lower to medium range of inlet Zn(II) concentration and at lower flow rate. Due to higher values of EBRT (68.72 min), microbial population gets more contact time for the bioremediation of Zn(II).

The growth of *K. pneumoniae* are obtained as 7.11×10^7, 6.13×10^7, 4.2×10^7, and 3.51×10^7 CFU g^{-1} of packing material at 10, 30, 50 and 70 cm heights of the column, respectively on 1^{st} day of shock loading operation (Fig. 5.69). At the end of 14^{th} day, the growths of *K. pneumoniae* are obtained as to 6.8×10^7, 6.1×10^7, 3.9×10^7 and 3.2×10^7 CFU g^{-1} of packing material at different heights of the column. The constant pH values are obtained throughout the shock loading study (Fig. 5.69). The values of Zn(II) removal efficiency are almost same in all the phases which indicate the stability of the biofilter column.

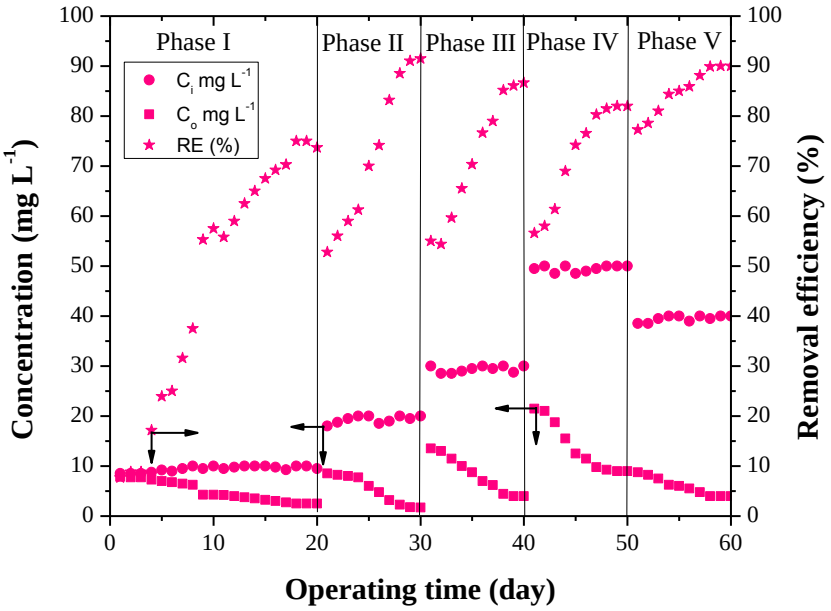


Fig. 5.66. Performance of biofilter with change in flow rate and inlet concentrations of Zn(II) solution

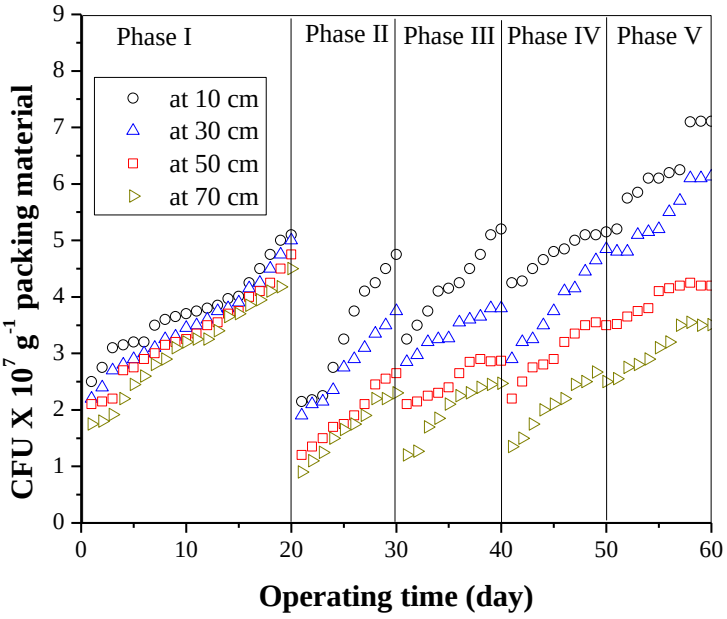


Fig. 5.67. Change in biomass growth with operating time at different heights of filter bed in Zn(II) removal

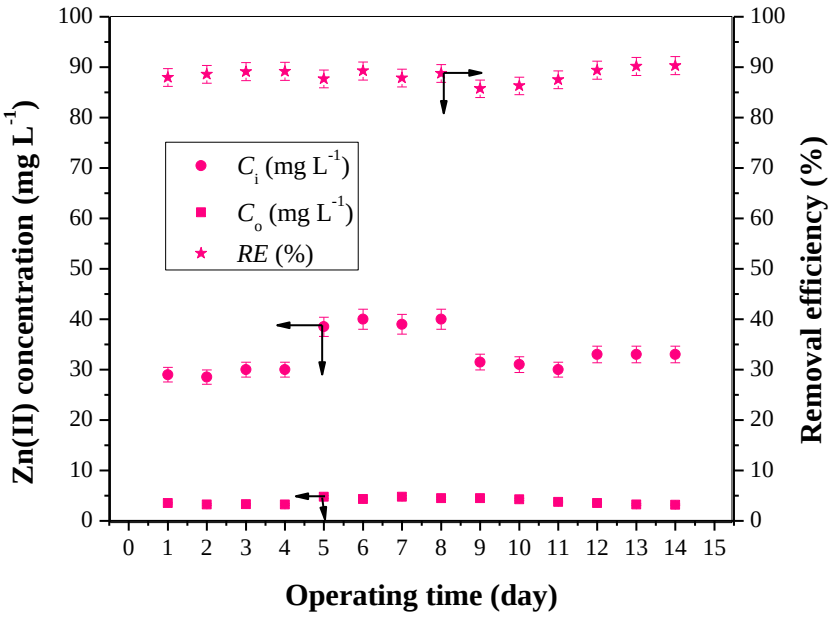


Fig. 5.68. Performance of biofilter under shock loading conditions for Zn(II) removal (change in concentration and removal efficiency with operating time)

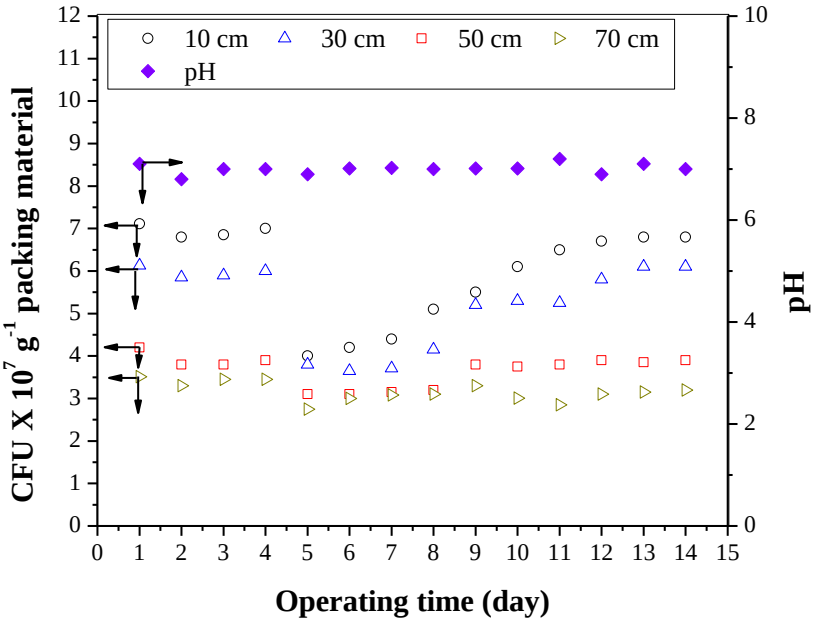


Fig. 5.69. Performance of biofilter under shock loading conditions for Zn(II) removal (change in biomass growth and pH with operating time)

5.1.7.4. Adsorption capacity of coal

In order to confirm the removal of Cr(VI), Cu(II) and Zn(II) due to bioremediation, separate adsorption experiments are performed. The maximum adsorption capacity of the coal for the adsorption of Cr(VI), Cu(II) and Zn(II) are calculated as 4.22, 5.69 and 3.25 mg g adsorbent^{-1}, respectively using Langmuir adsorption isotherm. Higher values of coefficient of determination (Cr(VI): R^2= 0.997; Cu(II): R^2= 0.993 and Zn(II): R^2= 0.989) indicate adequate match between the experimental values and adsorption isotherm parameters. It also indicates that monolayer adsorption of Cr(VI), Cu(II) and Zn(II) prevails onto the coal surface. However, the obtained maximum adsorption capacities obtained in the present study are much less as compared to maximum adsorption capacity of any other low cost adsorbents reported in the literature (Bhattacharya et al., 2008). In the biofiltration study, the filter bed is acclimated for a period of 20, 15 and 20 days for Cr(VI), Cu(II) and Zn(II) removal, respectively. The coal is saturated with metals at the end of acclimation period and acted as an inert material after that. Chaudhary et al. (2003) reported similar phenomena in a biofilter column packed with granular activated carbon (GAC) used for the treatment of wastewater.

5.1.7.5. Determination of Michaelis-Menten kinetic constants

In the present study, kinetic parameters (bioremediation rate and saturation constant) are estimated using Michaelis-Menten kinetic model (Eq. 4.26). The maximum bioremediation rate (r_{max}) and saturation constant (K_s) are obtained by plotting [$(V/Q)(C_i - C_o)$] vs. ($1/C_{ln}$) which results a straight line and is shown in Fig 5.70 for the removal of three metals. Steady state values of all the phases are considered in determining the kinetic constants. The intercepts of the straight lines give the maximum bioremediation

rate (r_{max}) as 0.256, 0.409 and 0.224 mg L^{-1} min^{-1} and the slopes give saturation constant (K_s) as 20.44, 33.62 and 24.87 mg L^{-1} for the removal of Cr(VI), Cu(II) and Zn(II), respectively. These values are also listed in Table 5.16. The coefficient of determination (R^2) are obtained as 0.996, 0.989 and 0.990 for Cr(VI), Cu(II) and Zn(II), respectively.

The Michaelis-Menten kinetics can be simplified to first-order or zero-order kinetics based on the values of initial inlet concentration of metals (C_i) and saturation constant (K_s). The obtained value of K_s falls in the range of the inlet concentration (10-50 mg L^{-1}) of metals which indicates that bioremediation kinetics of Cr(VI), Cu(II) and Zn(II) cannot be explained either by first-order or zero-order kinetics (Ottengraf and van den Oever, 1983). In the present study, Ottengraf-van den Oever model with zero-order kinetics (diffusion limitation) is used to understand the kinetic behavior of biofilter for Cr(VI), Cu(II) and Zn(II) removal.

5.1.7.6. Modeling with Ottengraf-van den Oever model

Biofiltration experimental data for Cr(VI), Cu(II) and Zn(II) are used to validate Ottengraf-van den Oever model (Eq. 4.28). Steady state data for all the phases (II to V) except for acclimation phase (phase I) of Cr(VI), Cu(II) and Zn(II) removal are considered to calculate the model parameters. For phase II, linear regression of $1 - (C_o/C_i)^{0.5}$ vs. $1/(C_i)^{0.5}$ gives K_1. Eq. 4.31 is used to calculate K_o which corresponds to the maximum elimination capacity of that phase. The values of K_o and K_1 for different phases of biofilter operation of Cr(VI), Cu(II) and Zn(II) are listed in Table 5.17. The critical inlet concentration ($C_{critical}$) and critical inlet loading ($IL_{critical}$) obtained at different phase for Cr(VI), Cu(II) and Zn(II) removal are also reported in Table 5.17. It is observed that, obtained $C_{critical}$ values are slightly higher than the inlet Cr(VI) concentrations in all the

phases except phase II. It corroborates the fact that Cr(VI) removal mechanism in biofilter can be better understood by zero-order rate kinetics with diffusion limitation for all the phase except phase II.

For the removal of Cu(II) and Zn(II), the obtained $C_{critical}$ values are comparable with the inlet Cu(II) and Zn(II) concentrations in all the phases. It confirms the fact that Cu(II) and Zn(II) removal mechanism in biofilter can be better understood by zero-order rate kinetics with diffusion limitation for all the phases. Elimination capacity for different phases (phase II to V) is predicted using Ottengraf-van den Oever model (Eq. 4.28) at different initial loading conditions for different metals using the obtained parameters K_1 and K_o. The predicted values of elimination capacity are plotted with the values of elimination capacity obtained experimentally at different phases (phase II to V) (Figs. 5.71-5.73). For Cr(VI) removal, the model predicts the performance of the biofilter well for phase III, IV and V with coefficient of determination (R^2) values 0.970, 0.990 and 0.984, respectively (Fig. 5.71). The coefficient of determination (R^2) obtained for phase II is 0.826 which corroborated the fact that model does not adequately fit the experimental data. The obtained values of R^2 for Cu(II) removal are 0.984, 0.999 and 0.964 for phase II, III and IV, respectively. However, the model does not fit well to the experimental data for phase V (R^2 = 0.876). For Zn(II) removal, the values R^2 are obtained as 0.999, 0.986 and 0.988 for phase II, III and V, respectively. It indicates that the model predicts the performance of the biofilter well for phase II, III and V. However, it does not fit well to the experimental data of phase IV for Zn(II) removal.

The biofilm thickness (δ) is also determined for different phases using Eq. 4.29. The values of effective diffusivity (D_e) and partition coefficient (m) for Cr(VI), Cu(II)

and Zn(II) in aqueous solution are considered as follows : $D_e = 4 \times 10^{-11}$ cm^2/s and m = 4.5, $D_e = 5.23 \times 10^{-6}$ cm^2/s and m = 3.981, $D_e = 1.1 \times 10^{-6}$ and m = 4.9, respectively (Akiyama et al., 2003; Alguacil et al., 2004; Leyva-Ramos et al., 2005). The estimated values of biolfilm thickness are reported in Table 5.17. The maximum biofilm thickness for Cr(VI), Cu(II) and Zn(II) removal is obtained as 287.56 μm in phase V, 119 μm in phase III and 278.25 μm in phase II, respectively. Results obtained above may be due to the fact that the Ottengraf-van den Oever model does fit the experimental data well in phase V, III and II for Cr(VI), Cu(II) and Zn(II) removal, respectively. Higher values of biofilm thickness contributes towards significant removal of metals from aqueous solution. Due to this fact, biofilter column performed well for lower to medium inlet concentration of metals.

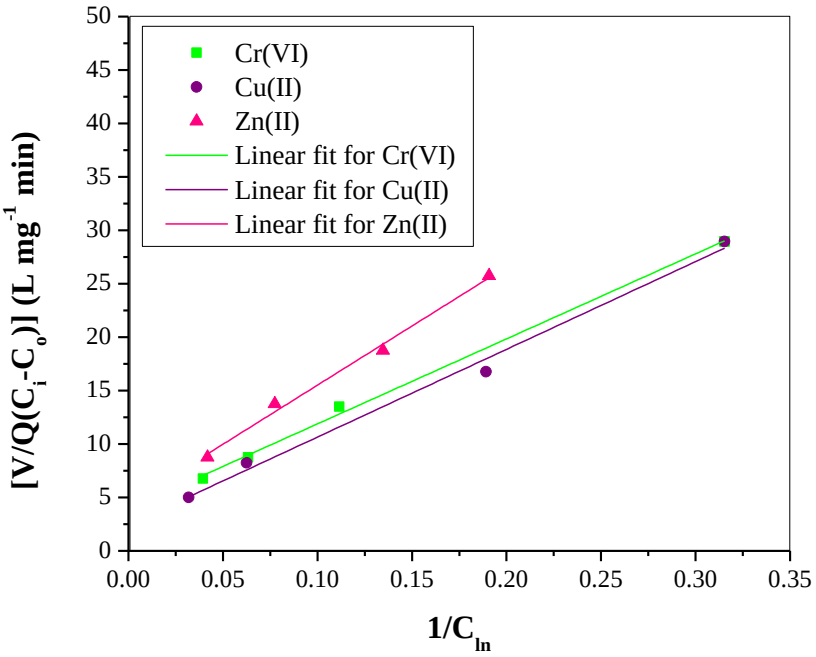


Fig. 5.70. Kinetic constants obtained for removal of metals using Michaelis-Menten kinetic model

Table 5.16. Michaelis-Menten kinetic constants calculated for Cr(VI), Cu(II) and Zn(II) removal at different operating conditions

S No	Metal	Michaelis-Menten constants		Coefficient of determination (R^2)
		r_{max} (mg L^{-1} min^{-1})	K_s (mg L^{-1})	
1	Cr(VI)	0.256	20.44	0.996
2	Cu(II)	0.409	33.62	0.989
3	Zn(II)	0.224	24.87	0.990

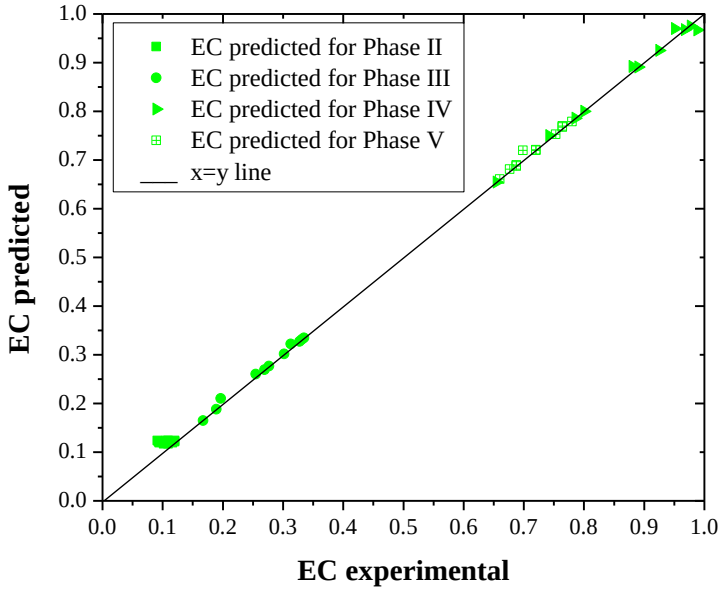


Fig. 5.71. Comparison of elimination capacity obtained experimentally with elimination capacity calculated from Ottengraf-van den Oever model in Cr(VI) removal for phases II to V

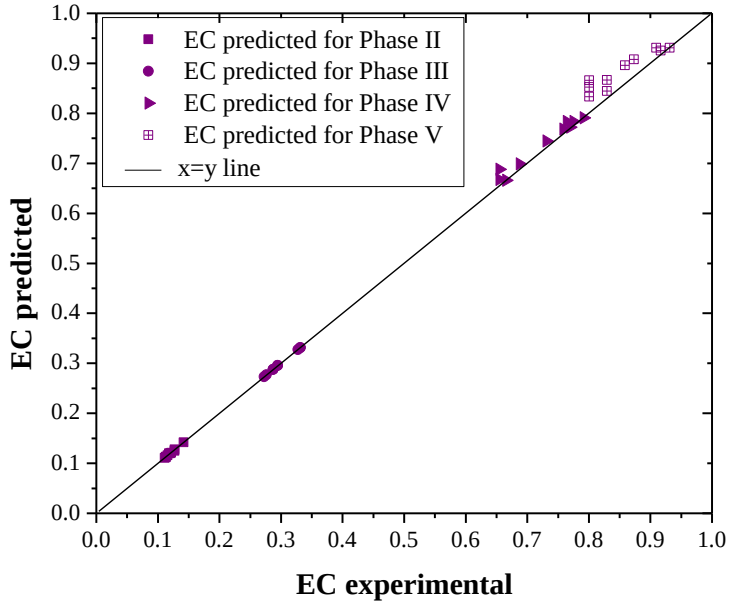


Fig. 5.72. Comparison of elimination capacity obtained experimentally with elimination capacity calculated from Ottengraf-van den Oever model in Cu(II) removal for phases II to V

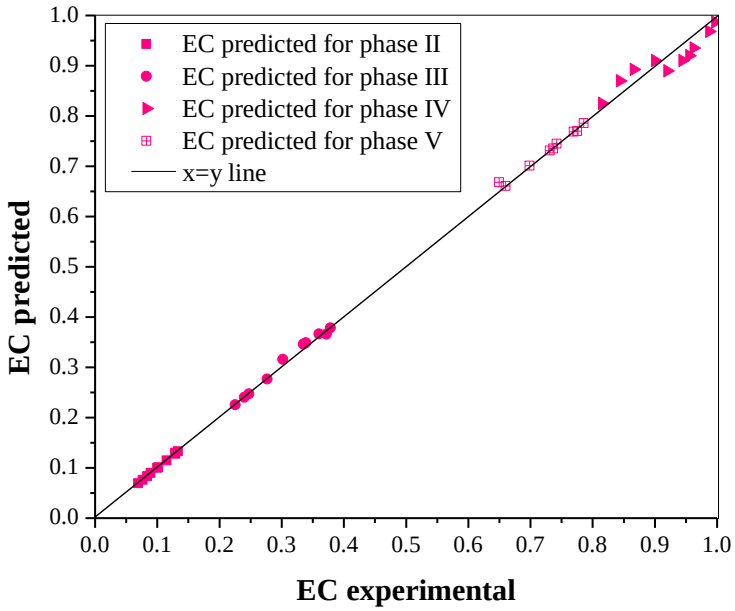


Fig. 5.73. Comparison of elimination capacity obtained experimentally with elimination capacity calculated from Ottengraf-van den Oever model in Zn(II) removal for phases II to V

Table 5.17. Model parameters calculated using Ottengraf-van den Oever Model at various operating conditions for removal of metals (bold values indicate maximum biofilm thickness)

Metal	**Phase**	C_i **(mg L^{-1})**	**Inlet loading (mg L^{-1} min^{-1})**	K_o **(mg L^{-1} min^{-1})**	K_1 **($mg^{-0.5}L^{-1.5}min^{-1}$)**	$C_{critical}$ **(mg L^{-1})**	$IL_{critical}$ **(mg L^{-1} min^{-1})**	δ **(μm)**
Cr(VI)	II	18.5-20	0.135-0.146	0.120	0.020	19.192	0.140	212.58
	III	28.5-30	0.415-0.437	0.334	0.041	30.01	0.437	178.25
	IV	48-50	1.4-1.456	1.179	0.114	50.909	1.482	201.45
	V	38-40	0.830-0.873	0.780	0.092	40.378	0.881	**287.56**
Cu(II)	II	17.5-20	0.127-0.146	0.142	0.028	20.02	0.146	111.65
	III	27.5-30	0.4-0.437	0.332	0.041	30.041	0.437	**119**
	IV	47-50	1.368-1.456	0.931	0.082	50.012	1.446	81.29
	V	37-40	0.808-0.873	0.791	0.096	40.02	0.874	51.07
Zn(II)	II	18.5-20	0.1273-0.1455	0.133	0.023	20	0.146	**278.25**
	III	28.5-30	0.4-0.4366	0.378	0.051	30	0.437	220.74
	IV	48-50	1.3679-1.4552	1.193	0.118	50	1.455	178.98
	V	38.5-40	0.8076-0.8731	0.786	0.094	40	0.873	267.66

5.2. FTIR analysis

In order to determine the functional groups involved in the metal binding, FTIR analysis of metal loaded and unloaded biomass (control) are carried out in the range of 400-4000 cm^{-1} and shown in Figs. 5.74-5.77.

5.2.1. Biomass of *P. taiwanensis*, *A. guillouiae* and *K. pneumoniae*

The FTIR spectra of *P. taiwanensis* biomass with Cr(VI) unloaded and loaded are shown in Fig. 5.74(a) and (b), respectively. The broad absorption peak in the range of 3400-3290 cm^{-1} represents -OH group of glucose and -NH stretching of protein (Bai and Abraham, 2002). The spectra of control shows different absorption band at 3284.57, 2920.11, 1646.26, 1542.93, 1236.85, 1074.87 and 532.92 cm^{-1} which are shifted to 3282.72, 2900.45, 1644.92, 1538.92, 1232.57, 1076.35 and 532.56 cm^{-1}, respectively in Cr(VI) loaded biomass. In control, another peak is obtained at 1382.64 cm^{-1} which is completely disappeared in Cr(VI) biomass.

The shift in absorption band from 3284.57 to 3282.72 cm^{-1} may be due to the complexation of -NH groups with Cr(VI) (Bai and Abraham, 2002; Gardea-Torresdey et al., 2000; Mohan, 2005; Mungasavalli et al., 2007; Park et al., 2005). The next shifts in absorption bands from 2920.11 to 2900.45 cm^{-1}, 1646.26 to 1644.92 cm^{-1} and 1542.93 to 1538.92 indicate the complexation of $-CH_3$ or -SH with Cr(VI) (Coates, 1996; Wang et al., 1989), carboxylic group with Cr(VI) (Basha et al., 2008) and primary and secondary amide bands of protein peptide bonds with Cr(VI) (Lameiras et al., 2008; Mungasavalli et al., 2007), respectively. In control, the absorption peak observed at 1382.64 cm^{-1} represents the presence of $-COO^-$ functional groups of amino acids and fatty acid derivates (Lameiras et al., 2008; Naumann, 2001). Absence of this peak in metal loaded

biomass indicates that there is no complexation of -COO^- functional groups of amino acids and fatty acid derivates with Cr(VI). The next shifts in absorption band from 1236.85 to 1232.57 cm^{-1} and 1074.87 to 1076.35 cm^{-1} represent the bonding of -SO_3 group with Cr(VI) and nitrogen from amino group with Cr(VI) (Lameiras et al., 2008), respectively. The weak peak observed at 535.92 cm^{-1} in control is shifted to 532.56 cm^{-1} in Cr(VI) loaded biomass which indicates the formation of δ(M-O) and δ(O-M-O) linkages (M is metal ion) (Kazy et al., 2009). A comparison between metal unloaded and metal loaded biomass of *P. taiwanensis* is reported in Table 5.18. It is observed that the contribution of amino groups towards metal complexation is significant. It is attributed to the presence of amino, carboxyl, nitrogen and oxygen of the peptide bonds for the complexation with Cr(VI) (Bueno et al., 2008; Pandi et al., 2009; Park et al., 2005).

The FTIR spectra of Cu(II) unloaded (control) and loaded biomass of *A. guillouiae* are shown in Fig. 5.75(a) and (b), respectively. For *K. pnuemoniae*, the FTIR spectra of Zn(II) unloaded (control) and loaded biomass are shown in Fig. 5.76(a) and (b), respectively. Comparison between control and metal loaded biomass of *A. guillouiae* and *K. pnuemoniae* are reported in Tables 5.19 and 5.20, respectively. The shifts in absorption peaks from 1072.39 cm^{-1} in Cu(II) unloaded to 1059.90 cm^{-1} in Cu(II) loaded biomass of *A. guillouiae* and 1058.66 cm^{-1} in Zn(II) unloaded to 1068.30 cm^{-1} Zn(II) loaded biomass of *K. pneumoniae* are indicative of the complexation of phosphate group with metals (Kazy et al., 2002). Other spectra observed for metal unloaded and loaded biomass of *A. guillouiae* and *K. pnuemoniae* in the region of 400-4000 cm^{-1} are similar as obtained for *P. taiwanensis* (Tables 5.18, 5.19, 5.20).

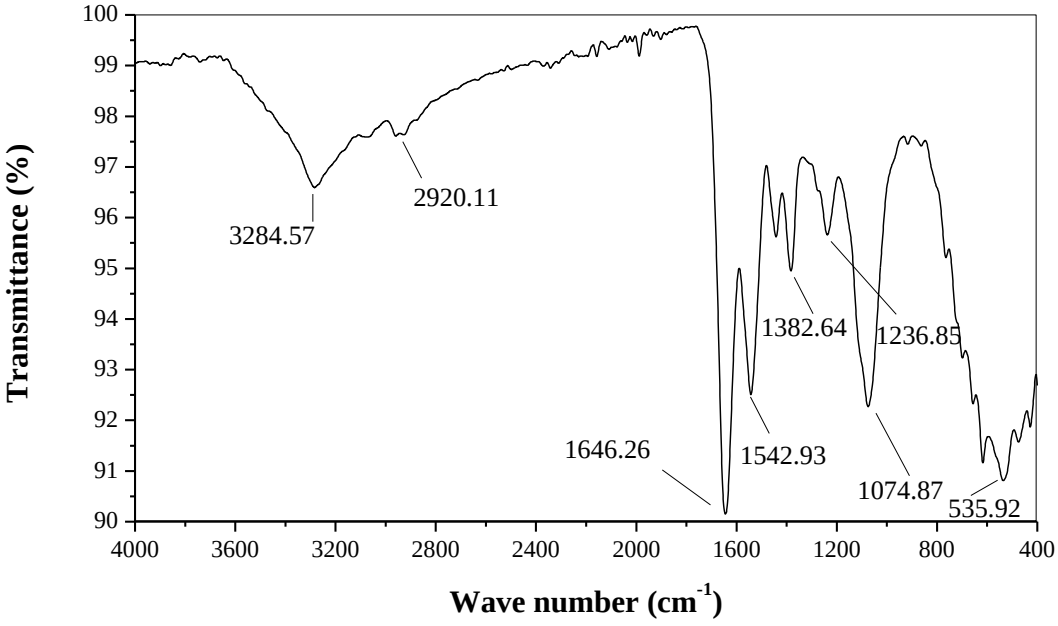
5.2.2. Biomass of consortium

The FTIR spectra of metals unloaded (control) and loaded consortium are shown in Fig. 5.77(a) and (b), respectively. A new peak is observed at 3840.49 cm^{-1} in metal loaded cells which is not present in control. It may be due to the involvement of -OH group in metal binding (specially with Cr(VI)) (Mohan, 2005). The broad absorption band obtained around 3422.10 cm^{-1} in control is indicative of the presence of -OH and -NH stretching (Mungasavalli et al., 2007; Park et al., 2005; Schulz and Baranska, 2007). This band is shifted to lower frequency (3399.92 cm^{-1}) due to complexation of -OH groups with metals (Kang et al., 2007; Mungasavalli et al., 2007). The next peak is observed at 2924.88 cm^{-1} in control which is shifted to 2925.92 cm^{-1} in metal loaded cells. Two new peaks appear at 2960.07 and 2854.36 cm^{-1} in metal loaded cells. All these peaks are appeared due to the interaction of metals with methyl groups of proteins and fatty acids (phospholipids) (Beech et al., 1999; Wang et al., 1989). It is attributed to the involvement of -NH and -CH of phospholipids and amino acid side-chain vibrations during the absorption process.

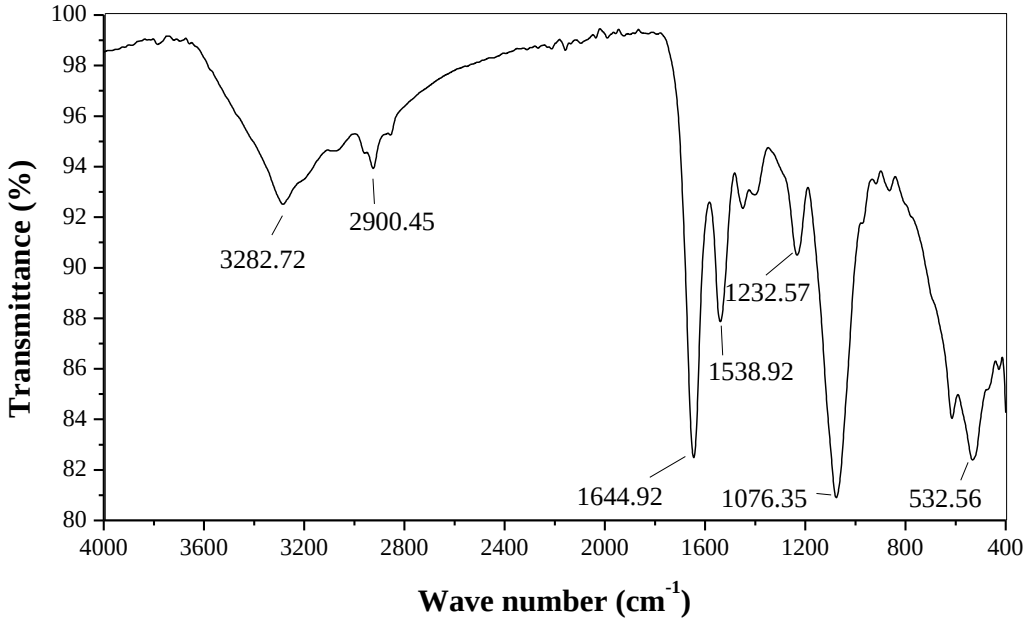
The next shift is observed at 1648.18 cm^{-1} (in control) which is shifted to 1649.07 cm^{-1}. It indicates the presence of $\gamma C=O$ of amide I and $\delta NH/\gamma C=O$ combination of the amide II bonds with metals (Beech et al., 1999; Mungasavalli et al., 2007; Paluszkiewicz and Kwiatek, 2001). The next shift appeared at 1544.83 cm^{-1} in control changes insignificantly to 1544.75 cm^{-1} in metal loaded cells. It indicates the presence of -NH stretching and -CN bending vibration in metal linkage (Lameiras et al., 2008; Park et al., 2005). The new peak appeared at 1451.70 cm^{-1} in metal loaded cells is due to the metal interactions with asymmetric bending of methyl group (Park et al, 2005). The next peak

observed at 1384.59 cm^{-1} in control is shifted to 1384.69 cm^{-1} in metal loaded cells which may be due to the interaction of symmetric stretching vibrations of $-COO^-$ functional groups of amino acids and fatty acid derivates with metals (Lameiras et al., 2008; Naumann, 2001).

The next absorption peak at 1239.62 cm^{-1} is shifted to lower frequency 1237.90 cm^{-1} due to the interaction of metals with carboxyl group (Kazy et al., 2002; Mungasavalli et al., 2007; Naumann, 2001). Another absorption peak observed at 1082.81 cm^{-1} in control is shifted to 1081.36 cm^{-1} in metal loaded cells. It is attributed to the presence of phosphodiester group of nucleic acid, membrane phospholipids and amide-III which contribute to metal binding (Kang et al., 2007). The characteristics bands observed in the region of 500-1000 cm^{-1} (539.03 and 618.56 cm^{-1}) show the existence of S = O and -C-C- stretching in metal loaded cells (Batool et al., 2014). The band observed at 400-800 cm^{-1} region may be due to the formation of δ(M-O) and δ(O-M-O) linkages (M is metal ion) (Kazy et al., 2009). A comparison between control and metal loaded biomass of consortium is reported in Table 5.21. Results indicate the presence of carboxyl, amino, hydroxyl, methyl, phosphate and sulphonate groups. These functional groups are responsible for metal binding on the surface of the microbial cells (Bueno et al., 2008). Earlier study reported the presence of carboxyl groups in Cr(VI) binding in cyanobacteria (Choudhary and Sar, 2009; Pandi et al., 2009). Carbohydrates, protein and lipids are the integral parts of bacterial cell wall. These components are indicative of the presence of above said functional groups in the bacterial outer cell wall which are responsible for sequestration of metals (Lameiras et al., 2008).

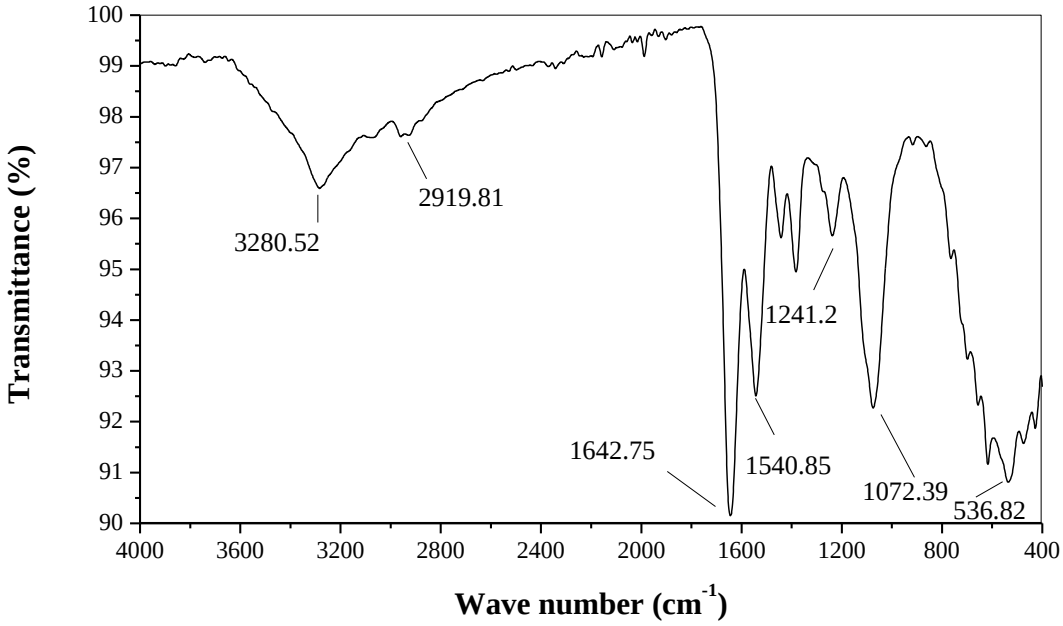


(a)

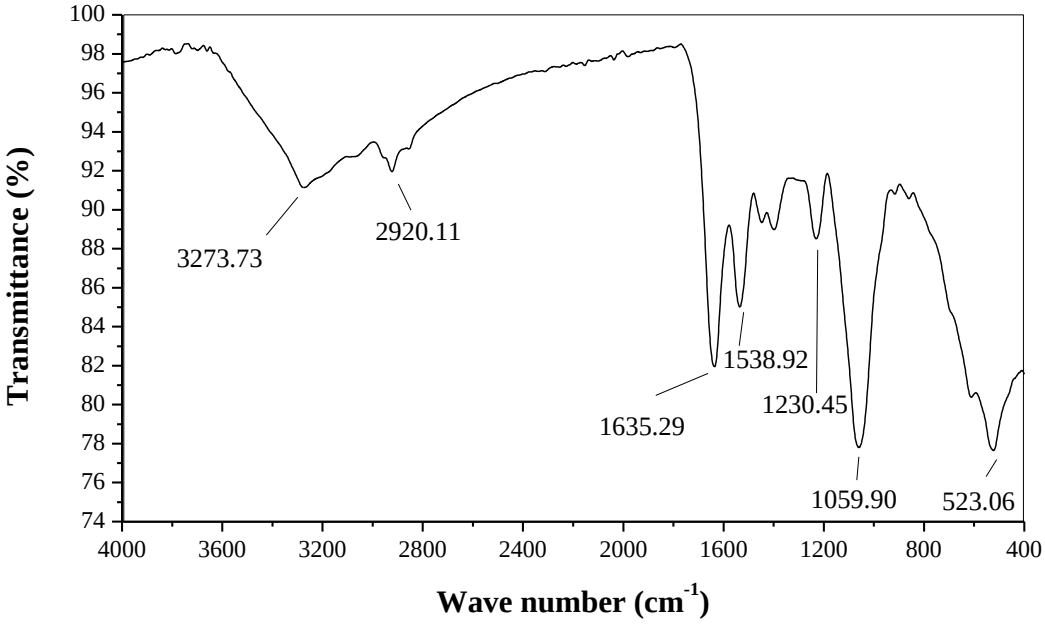


(b)

Fig. 5.74. FTIR spectra of *P. taiwanensis* biomass:
(a) Cr(VI) unloaded, (b) Cr(VI) loaded



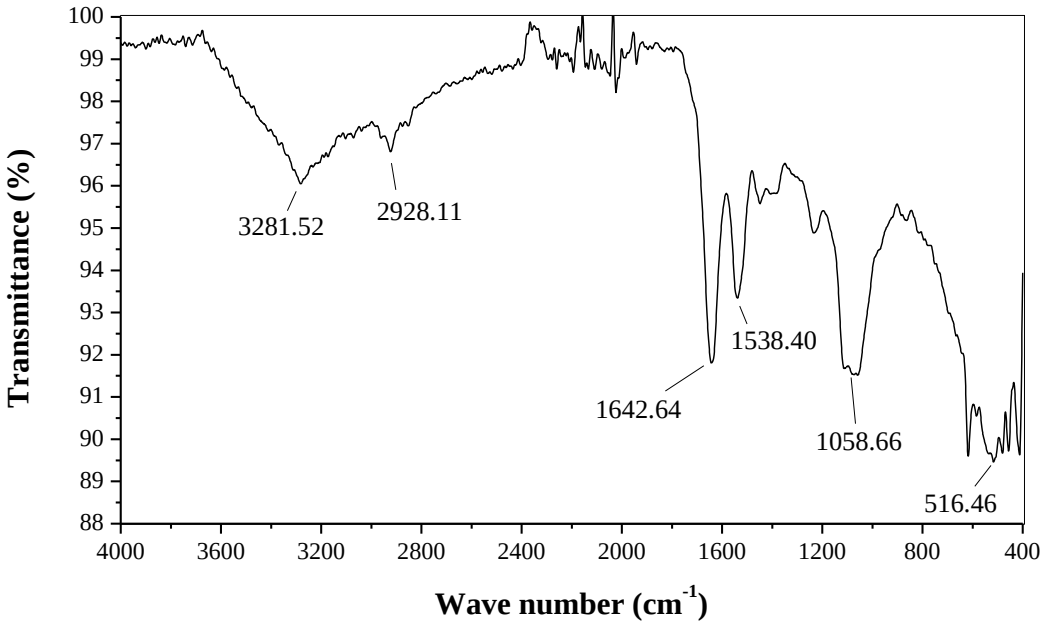
(a)



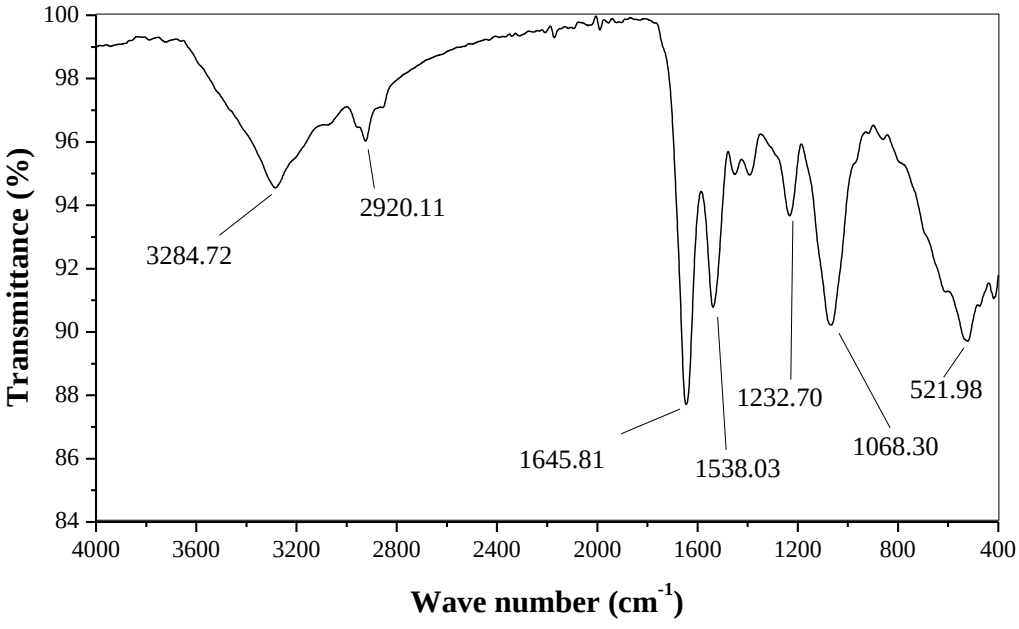
(b)

Fig. 5.75. FTIR spectra of *A. guillouiae* biomass:

(a) Cu(II) unloaded, (b) Cu(II) loaded



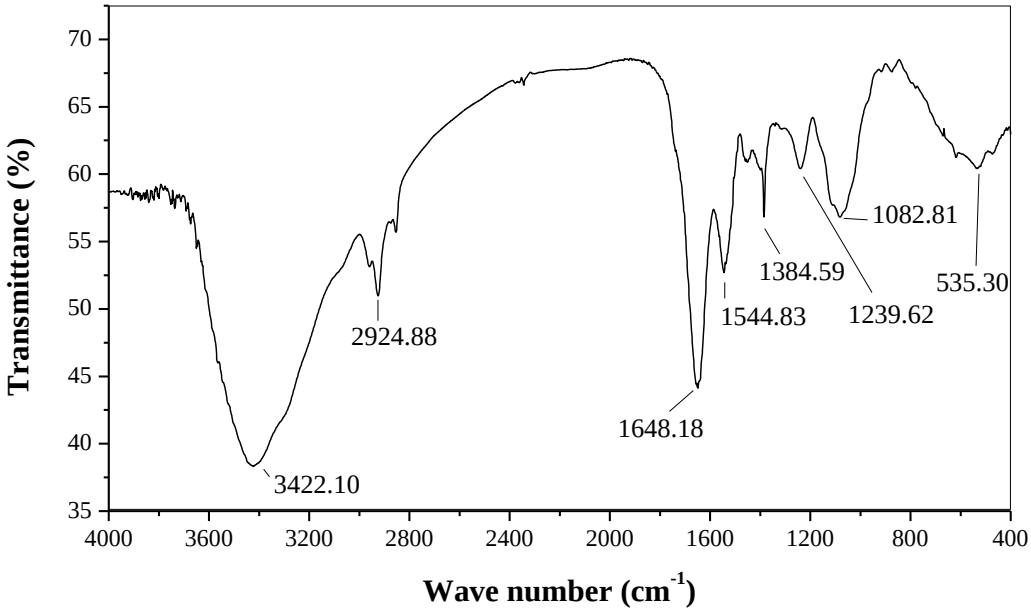
(a)



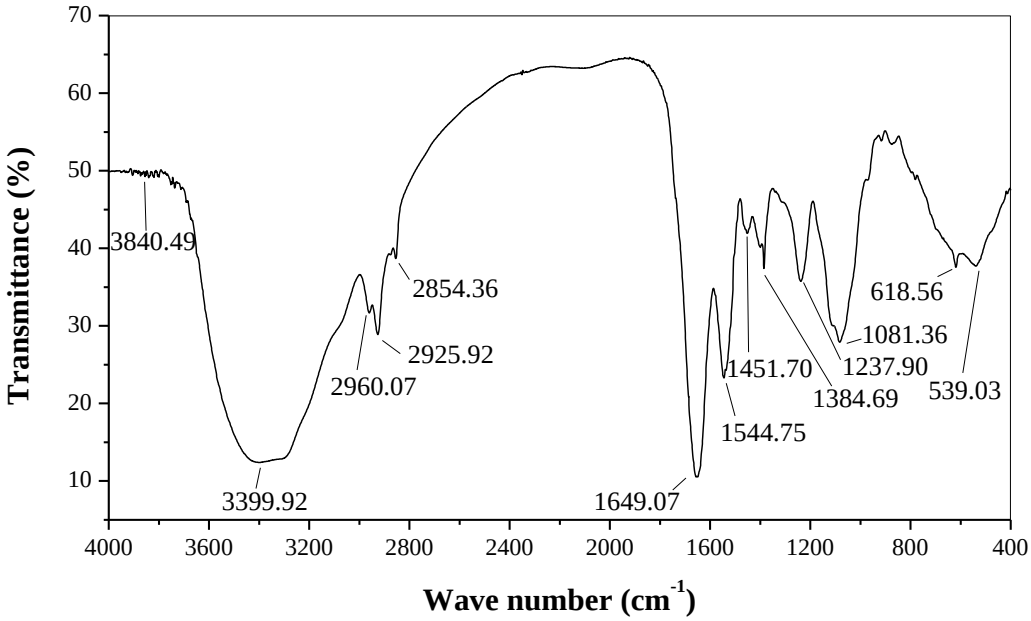
(b)

Fig. 5.76. FTIR spectra of *K. pneumoniae* biomass:

(a) Zn(II) unloaded, (b) Zn(II) loaded



(a)



(b)

Fig. 5.77. FTIR spectra of consortium:

(a) metal unloaded, (b) metal loaded

Table 5.18. The observed FTIR band for *P. taiwanensis* biomass in Cr(VI) unloaded (control) and loaded conditions

Wave number (cm^{-1})		Association of functional groups
Control	**Biomass with Cr(VI) loaded**	
3284.57	3282.72	Bonded -OH group; Primary and secondary amines and amides stretching (N–H stretching)
2920.11	2900.45	-CH_3 group of proteins or -SH group
1646.26	1644.92	C=O group
1542.93	1538.92	Primary and secondary amide bands of protein peptide bonds
1382.64	-	-COO^- functional groups of amino acids and fatty acid derivates
1236.85	1232.57	Bonded -SO_3 group
1074.87	1076.35	Nitrogen from amino group
535.92	532.56	δ(M-O) and δ(O-M-O) linkages

Table 5.19. The observed FTIR band for *A. guillouiae* biomass in Cu(II) unloaded (control) and loaded conditions

Wave number (cm^{-1})		Association of functional groups
Control	**Biomass with Cu(II) loaded**	
3280.52	3273.73	-OH group of glucose or -NH groups of amino groups
2919.81	2920.11	γC-H bond of -CH_2 groups combined with CH_3 groups
1642.75	1635.29	γC=O of amide I bond
1540.85	1538.92	δNH/γC=O combination of the amide II bond
1241.22	1230.45	Carboxyl group
1072.39	1059.90	Phosphate group
536.82	523.06	δ(M-O) and δ(O-M-O) linkages

Table 5.20. The observed FTIR band for *K. pneumoniae* biomass in Zn(II) unloaded (control) and loaded conditions

Wave number (cm^{-1})		Association of functional groups
Control	**Biomass with Zn(II) loaded**	
3281.52	3284.72	-OH group of glucose or -NH groups of amino groups
2928.11	2920.11	γC-H bond of $-CH_2$ groups combined with CH_3 groups
1642.64	1645.81	γC=O of amide I bond
1538.40	1538.03	δNH/γC=O combination of the amide II bond
-	1232.70	Carboxyl group
1058.66	1068.30	Phosphate group
516.46	521.98	δ(M-O) and δ(O-M-O) linkages

Table 5.21. The observed FTIR band for consortium biomass in metals unloaded (control) and loaded conditions

Wave number (cm^{-1})		Association of functional groups
Control	**Biomass with metals loaded**	
-	3840.49	-OH group
3422.10	3399.92	-OH group of glucose or -NH groups of amino groups
-	2960.07	γC-H bond of $-CH_2$ groups combined with CH_3 groups
2924.88	2925.90	γC-H bond of $-CH_2$ groups combined with CH_3 groups
-	2854.36	γC-H bond of $-CH_2$ groups combined with CH_3 groups
1648.18	1649.07	γC=O of amide I bond
1544.83	1544.75	δNH/γC=O combination of the amide II bond
-	1451.70	Asymmetric bending of $-CH_3$ group
1384.59	1384.69	$-COO^-$ functional groups of amino acids and fatty acid derivates
1239.62	1237.90	Carboxyl group
1082.81	1081.36	Phosphodiester and free phosphate ions and amide III groups
-	618.56	S = O and -C-C- stretching and δ(M-O) and δ(O-M-O) linkages
535.30	539.03	δ(M-O) and δ(O-M-O) linkages

5.3. FESEM-EDX analysis

FESEM is employed to investigate the effect of metals on the morphology of bacterial surface for metal unloaded (control) and loaded bacterial cells. EDX is performed to further confirm the presence of metal on bacterial surface. FESEM and EDX results are shown in Figs. 5.78-5.80 for *P. taiwanensis, A. guillouiae, K. penumoniae* biomass, respectively. FESEM result for consortium biomass is shown in Fig. 5.81.

5.3.1. Biomass of *P. taiwanensis*

The surface morphology of Cr(VI) unloaded and loaded *P. taiwanensis* biomass are presented in Fig. 5.78(a) and (b), respectively. Fig. 5.78(a) displays the rod-like shape with smooth surface of *P. taiwanensis* cells (Choudhary and Sar, 2009; Du et al., 2012). After the growth in presence of chromium, the morphology of *P. taiwanensis* cells appear to be irregular and wrinkled (Fig. 5.78b). To determine the presence of chromium on the bacterial cell surface for unloaded and metal loaded conditions, elemental analysis using EDX spectroscopy is carried out and shown in Figs. 5.78(a) and (b). Chromium and its precipitates are not detected on the surface of *P. taiwanensis* cell grown in presence of chromium. This may be due to the fact that dichromate [$(Cr_2O_7)^{2-}$] anion cannot link to the electronegative surface functional groups present on the envelop of gram-negative bacteria *Pseudomonas sp.* (McLean and Beveridge, 2001). Other elements (carbon, oxygen, nitrogen, phosphorus, sulphur, magnesium and pottasium) are localized with chromium as they are considered to be major elements required for the growth of bacterial cell (van Niftrik et al., 2008).

5.3.2. Biomass of *A. guillouiae*

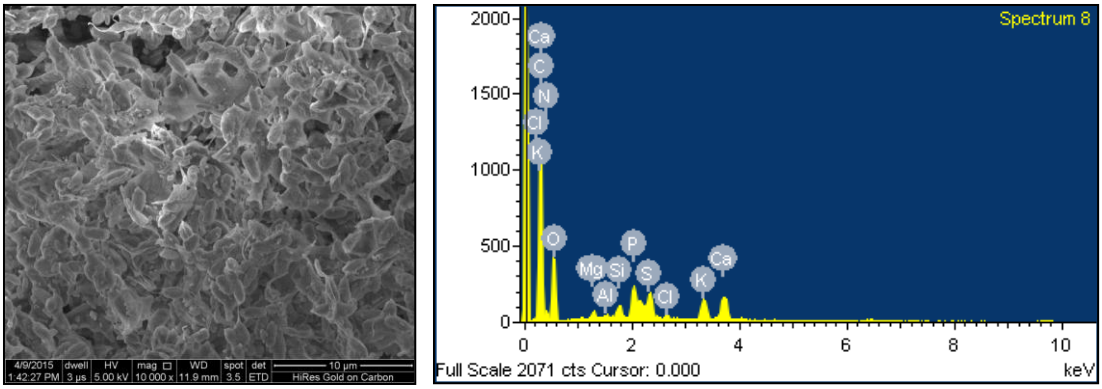
The surface morphology of Cu(II) unloaded (control) and loaded *A. guillouiae* biomass are presented in Fig. 5.79(a) and (b), respectively. FESEM micrograph shows rough surface with coccobacilli shape for *A. guillouiae* (Fig. 5.79a) (Srivastava and Srivastava, 2005). After sorption with copper, the surface of *A. guillouiae* is changed. Wrinkles are developed on the surface. The surface is covered with Cu(II) ions which is further confirmed by EDX analysis (Fig. 5.79b). In control, the peak observed between 3 and 4 keV corresponds to Ca(II) (Fig. 5.79a). It disappears after Cu(II) sorption which indicates that metal bioremediation process involves ion-exchange mechanism for both Ca(II) and Cu(II) by *A. guillouiae* (Tunali et al., 2006) (Fig. 5.80b).

5.3.3. Biomass of *K. pneumoniae*

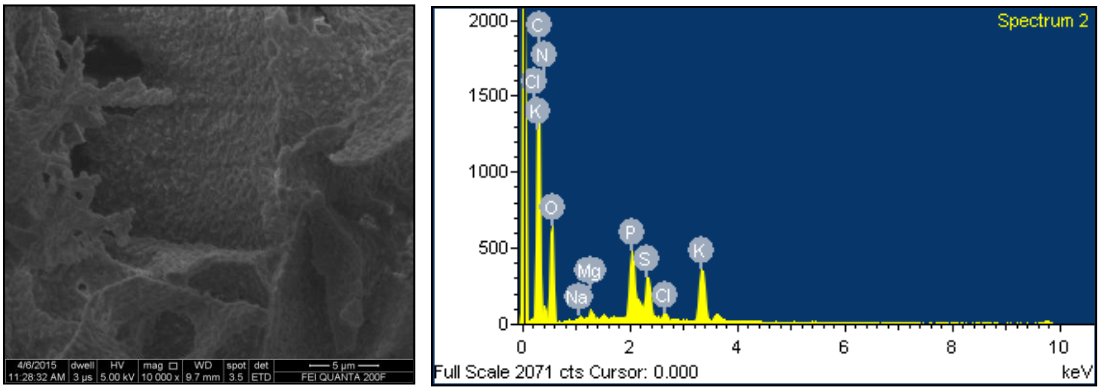
The surface morphology of Zn(II) unloaded (control) and loaded *K. pneumoniae* biomass are shown in Fig. 5.80(a) and (b), respectively. Biomass of control shows smaller size rod-shaped structure (Du et al., 2012) (Fig. 5.80a). The surface morphology of *K. pneumoniae* biomass is changed to rough texture after zinc uptake (Fig. 5.80b). Lump-like and twist-like deposits are observed on the surface of biomass (Putra et al., 2014). Few grooves are also noticed on the surface of the biomass after zinc uptake. EDX analysis of metal loaded biomass shows a new peak at 1.011 keV which represents the features of Zn(II) (Fig 5.80b). In control, the peak observed at 1.012 keV corresponding to Na(I) disappears after Zn(II) sorption which indicates that metal bioremediation process involves ion-exchange mechanism for both the ions by *K. pneumoniae* (Figs 5.80 a and b).

5.3.4. Biomass of consortium

In metals unloaded consortium, the positive interaction between three bacterial strains (*P. taiwanensis, A. guillouiae* and *K. pneumoniae*) in consortium is observed under scanning electron microscope (Fig. 5.81a). The three bacterial strains in the consortium grow smoothly. The morphological changes of the three bacterial strains in the consortium are more pronounced after loaded with metals (Fig. 5.81b). Cell surface of the gram-negative bacteria in consortium are strongly controlled by surface proteins and lipopolysaccharides (Kazy et al., 2002). Complexation of such macromolecules with three metal ions [Cr(VI), Cu(II) and Zn(II)] changes the surface architecture of the consortium which is reflected by an increase in surface roughness (Kazy et al., 2002).

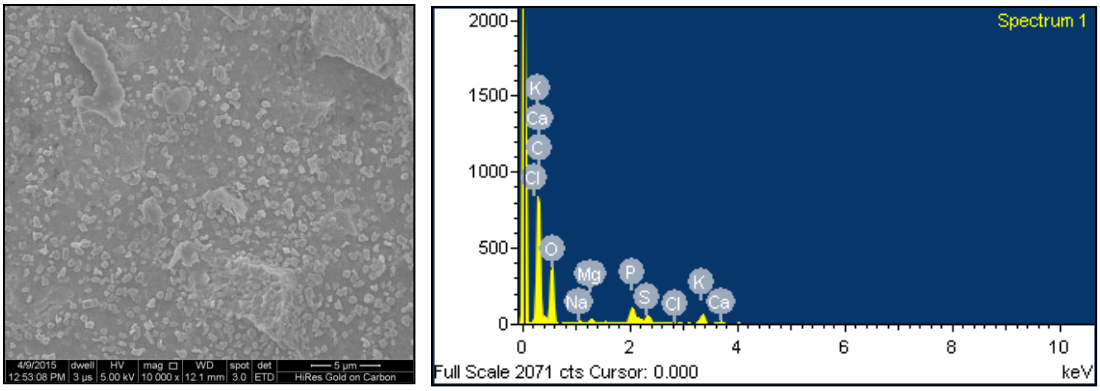


(a)

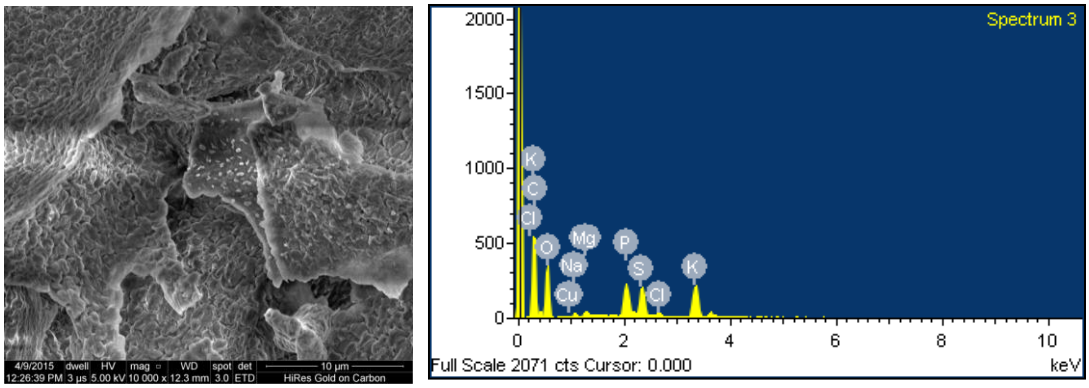


(b)

Fig. 5.78. SEX-EDX images of (a) *P. taiwanensis* (control), (b) *P. taiwanensis* loaded with chromium

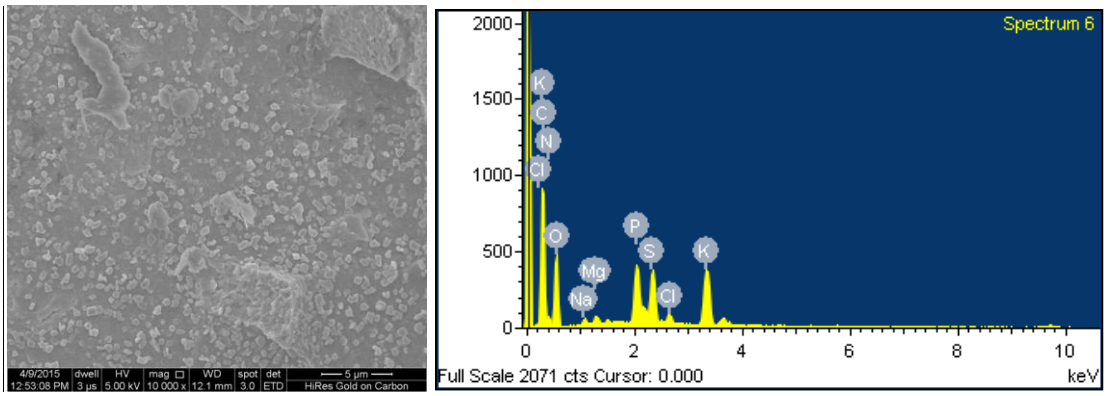


(a)

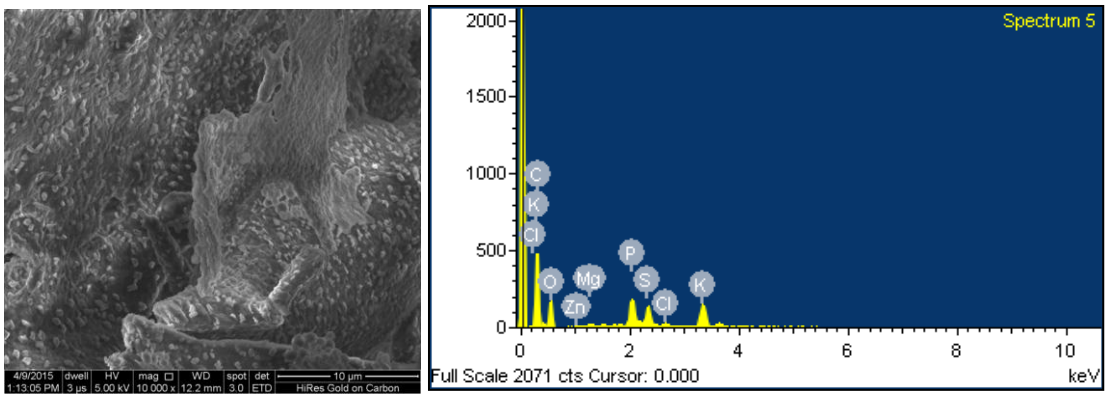


(b)

Fig. 5.79. SEM-EDX images of (a) *A. guillouiae* (control), (b) *A. guillouiae* loaded with copper

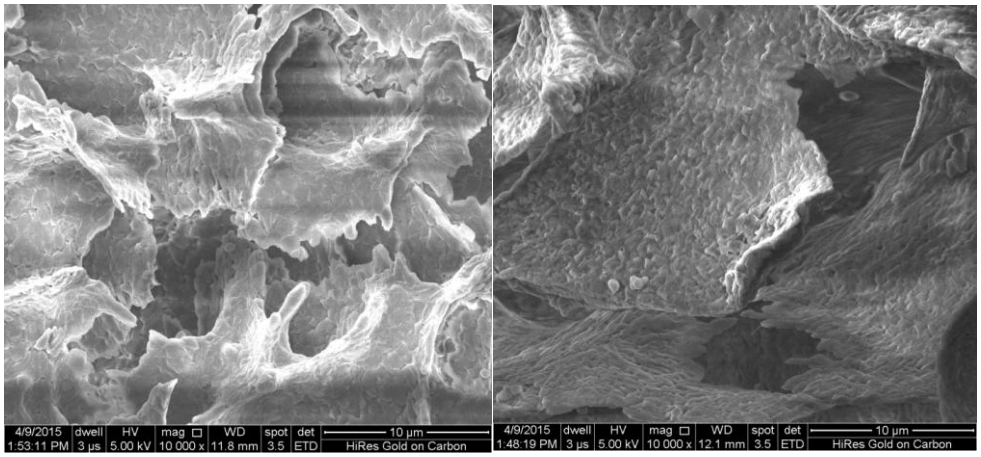


(a)



(b)

Fig. 5.80. SEM-EDX images of (a) *K. pneumoniae* (control), (b) *K. pneumoniae* loaded with zinc



(a) (b)

Fig. 5.81. SEM images of: (a) consortium (control), (b) consortium loaded with metals

CHAPTER 6

CONCLUDING REMARKS

In the present study, three different bacterial strains *Pseudomonas taiwanensis*, *Acinetobactor guillouiae* and *Klebsiella pneumoniae* are isolated from activated sludge and used for the bioremediation of three different metals Cr(VI), Cu(II) and Zn(II), respectively from aqueous solution. To monitor the removal of three metals, batch and continuous studies are conducted by varying different parameters in order to ascertain the applicability of biological treatment method in industrial practice. Experimental data obtained from batch and continuous studies are utilized to fit various growth and kinetic models. In the present study, a novel bacterial consortium is developed from the three bacterial strains mentioned above and used to evaluate its performance over individual strains for the simultaneous removal of multiple metals [Cr(VI), Cu(II) and Zn(II)] present in mixed form. This chapter delineates a brief overview of the present work followed by conclusions, major contributions and future scope for research.

6.1. Summary

6.1.1. Introduction

Various industries such as mining, milling, metal cutting, surface finishing etc. are the biggest generators of various toxic heavy metal ions such as Cr(VI), Cu(II) and Zn(II). These metals are very harmful for humans as they adversely affect the skin, brain, liver, kidney and respiratory tracts. In the last few decades, the levels of these metals in

drinking water have increased at an alarming rate. Thus the removal, recovery and recycling of these metals have greater significance.

Various physico-chemical techniques such as ion-exchange, chemical precipitation, electrochemical precipitation, coagulation-flocculation, membrane filtration, adsorption etc. have been used to treat aqueous solution contaminated with these heavy metals. However, these treatment methods suffer from serious drawbacks. In various countries, strict environmental regulations enforce various industries to comply with the stringent rules. It causes a shift to the development of economical, environmental friendly and efficient treatment technique for metal contaminated effluents.

Recently, intention has moved towards the more eco-friendly and cost-effective approach, "bioremediation" for pollution abatement which can meet the stringent regulatory limits of various heavy metals such as Cr(VI), Cu(II), Zn(II) etc. Bioremediation process uses living microorganisms for the sequestration of metals. Different microorganisms have various toxicity level to metals. One specific microorganism may not be capable of removing all the metals present in the industrial effluents. It imposes a great challenge to tackle water pollution through biological route in case of industrial effluents contaminated with multiple metals. Therefore, there is a need to remove mixture of different metals and organic wastes using consortia of diverse microorganisms.

The performance of any microorganism in metal removal can be well understood by evaluating the various kinetic parameters in batch and continuous studies. Batch bioremediation studies are needed to investigate the effect of initial metal concentration,

initial pH, MSM dosage, N-P-K composition in MSM, aerobic and facultative anaerobic conditions, temperature and inoculum volume on biomass growth. These studies are important to estimate the kinetic parameters such as specific growth rate, saturation constant, maintenance rate, substrate inhibition constant, critical inhibitory concentration etc. which help to understand the mechanisms of the metal bioremediation process. Various kinetic parameters can be estimated using growth kinetic models such as Monod and Monod-Inhibition to understand the behaviour of microorganisms in metal removal. Rate-kinetic approaches such as zero-order model and three-half-order model can be used to describe the metal bioremediation process.

A continuous biofilter column study is necessary in order to investigate the applicability of the bioremediation process in industrial practice. So it is required to design a biofilter column for the removal of heavy metals Cr(VI), Cu(II) and Zn(II) from wastewater streams. While designing a biofilter column, various important parameters such as selection of microbial culture, inlet metal concentration, inlet flow rate, pH of the metal solution, packing material, empty bed residence time (EBRT), temperature, etc. need to be considered. The behaviour of the biofilter column in metal removal can be well explained by estimating various kinetic parameters. These parameters can be determined by using kinetic models such as Michaelis-Menten model and Ottengraf-van den Oever model.

6.1.2. Gaps in literature

The reported studies for the removal of heavy metals from wastewater using biofiltration techniques are limited. Moreover, limited studies have been conducted for simultaneous removal of Cr(VI), Cu(II) and Zn(II) using bacterial consortium from wastewater. Very

few researches have been carried out on bioremediation and biofiltration for the removal of Cr(VI), Cu(II) and Zn(II) using acclimated mixed culture. Most of the studies are limited to the use of pure strain for the removal of heavy metals from wastewater. Pure strain has its own advantages as far as small scale systems or batch reactors are concerned. However, for actual industrial applications, acclimated mixed cultures are preferred over the culture trained from pure strain. The mechanism of metal intake by microorganisms is still not well understood. Limited studies are reported on the mechanism of bioremediation of heavy metals. This requires bioremediation and biofiltration experiments to be carried out extensively. Data obtained from experiments are fitted with various growth kinetic models and rate kinetic models to obtain the kinetic parameters. These parameters are also helpful in understanding the mechanism of biological transformation of heavy metals.

Past studies are mainly focused on the experimental work. Less attempts have been made for the kinetic modeling of biofilter column. Bioremediation and biofiltration experimental data are required to validate the available kinetic models, which are very useful for designing the biofiltration column in industrial scale. The effect of shock loading conditions (sudden change in inlet metal concentration and flow rate) on metal removal are necessary for designing the biofilter column in pilot scale as well as industrial practice. This important aspect has been completely ignored in earlier studies. The effect of biofilm thickness on metal removal has not been incorporated in earlier studies.

While carrying out the literature survey, it is also observed that very few researchers have worked in the area of simultaneous removal of Cr(VI), Cu(II) and Zn(II)

using a bacterial consortium. Different microorganisms have diverse toxicity level to metals. One specific microorganism may not be capable of removing all the metals present in the industrial effluents. Industrial effluents contain voluminous amounts of various heavy metals and organic compounds. Thus, it imposes a challenge to tackle water pollution through biological route in case of industrial effluents contaminated with multiple metals. Therefore, it is necessary to remove mixture of different metals and organic wastes using consortia of diverse microbial strains.

6.1.3. Scope of the work

There is a need to carry out batch studies for the bioremediation of Cr(VI**),** Cu(II) and Zn(II) in order to understand the mechanism of transformation of these metals. In the present study, batch bioremediation experiments are performed using bacterial strains isolated from acclimated mixed culture. Growth kinetics models such as Monod and Monod-Inhibition are fitted with the obtained experimental data. It helps to understand the mechanism of microbial transformation of the metals. Final biomass is analyzed using Fourier transform infrared (FTIR) spectroscopy and scanning electron microscopy (SEM) to investigate the role of various functional groups in metal binding and changes in morphology of the bacterial cell surface during metal sequestration, respectively.

There is scope to carry out the biofiltration (continuous column) studies for the removal of Cr(VI), Cu(II) and Zn(II) by varying different operating conditions. It helps to understand the adaptability of microorganisms in different operating conditions and during shock loading conditions. Biofiltration studies will help in estimating the desired parameters needed for designing the biofilter column on bench scale and during scale-up to industrial level. Kinetic aspects of bioremediation process during the biofiltration will

also be understood by fitting the experimental data with the Michaelis-Menten kinetic model and Ottengraf-van den Oever model.

6.1.4. Experimental studies

The three bacterial strains *Pseudomonas taiwanesis*, *Acinetobacter guillouiae* and *Klebsiella pneumoniae* are isolated from acclimated mixed culture. These bacterial strains are used to remove three metals Cr(VI), Cu(II) and Zn(II) from aqueous solution. The batch bioremediation experiments are performed in a BOD incubator and shaker at different operating conditions such as initial metal concentration, initial pH, MSM dosage, N-P-K composition in MSM, aerobic and facultative anaerobic conditions, temperature and inoculum volume etc. A novel bacterial strain is developed from the three bacterial strains and used to evaluate its performance over individual strains for the simultaneous removal of multiple metals [Cr(VI), Cu(II) and Zn(II)] present in mixed form in aqueous solution.

An in-house biofilter column is fabricated to perform continuous biofiltration experiments for the removal of Cr(VI), Cu(II) and Zn(II). This study is conducted to investigate the performance and stability of the biofilter column under varying operating conditions. The final concentration of metals [Cu(II) and Zn(II)] and the total concentration of Cr are analyzed using Atomic Absorption Spectrophotometer (AAS). The final concentration of Cr(VI) is analyzed using UV-Vis spectrophometer by forming a purple-violet colour with 1,5-diphenyl carbazide in acidic solution as a complexing agent.

6.1.5. Mathematical modeling

The behaviour of the microorganisms in metal bioremediation process is explained with the help of growth kinetic models such as Monod and Monod-Inhibition. In the present study, the rate kinetics for the bioremediation of metals is described using zero-order and three-half-order kinetic models. These approaches help to investigate the role of growth rate, substrate utilization, inhibitory concentration and product formation on metal removal. In biofiltration study, various kinetic parameters are determined using Michaelis-Menten model and Ottengraf-van den Oever model which relate the kinetic behaviour of the column to the bioremediation rate kinetics. The effects of biofilm thickness is also investigated in order to ascertain the role of microorganisms in metal removal during continuous column studies.

6.1.6. Results and discussion

In this section, the results obtained in batch bioremediation and continuous biofiltration studies are presented. It also discusses the results obtained in rate kinetic modeling and growth modeling of batch bioremediation studies.

6.1.6.1. Identification of isolated microbial strains

Based on gene sequence analysis, the three strains which are capable of removing Cr(VI), Cu(II) and Zn(II) are identified as *Pseudomonas taiwanensis* (NCBI GenBank Accession Number**:** KT070310), *Acinetobactor guillouiae* (NCBI GenBank Accession Number: KT070311) and *Klebsiella pneumoniae* (NCBI GenBank Accession Number: KT070312), respectively.

6.1.6.2. Batch bioremediation studies

The bioremediation studies of heavy metals are performed for initial Cr(VI), Cu(II) and Zn(II) concentrations ranging from 20-200 mg L^{-1}. The effect of various operating conditions such as initial metal concentration, initial pH, MSM dosage, N-P-K composition in MSM, aerobic and facultative anaerobic conditions, temperature and inoculum volume on metal removal are investigated. The effect of time plays a significant role in metal removal. In the present study, it is observed that the concentration of metals decreases with time. It indicates that metal is removed by the microbes. It is also found that increase in metal concentration increases the lag phase of microbes which further increases the total time for bioremediation process. For the complete removal of Cr(VI) by *P. taiwanensis*, the required time is obtained as 20, 22, 28, 38, 40, 44 and 46 h for 20, 40, 60, 80, 100, 150 and 200 mg L^{-1} of initial concentration of Cr(VI), respectively. For Cu(II) , the complete removal is achieved at 18, 20, 24, 24, 24, 28 and 28 h for 20, 40, 60, 80, 100 and 150 mg L^{-1} initial Cu(II) concentration, respectively. While for Zn(II) , these values are 24, 24, 24, 24, 28, 28 and 28 h for 20, 40, 60, 80, 100, 150 and 200 mg L^{-1} initial Zn(II) concentration, respectively.

It is observed that the biomass growth increases with time. In the presence of Cr(VI), the maximum biomass concentrations are obtained 0.178, 0.215, 0.223, 0.252, 0.253, 0.258 and 0.259 g L^{-1} for initial Cr(VI) concentrations of 20, 40, 60, 80, 100, 150 and 200 mg L^{-1} respectively at pH 7.0. In the presence of Cu(II), the maximum biomass concentrations are obtained 0.125, 0.178, 0.260, 0.352, 0.318, 0.220 and 0.189 g L^{-1} for 20, 40, 60, 80, 100, 150 and 200 mg L^{-1} initial Cu(II) concentrations, respectively at pH 8.0. In the presence of Zn(II), the maximum biomass concentrations are obtained 0.286,

0.293, 0.220, 0.245, 0.261, 0.273 and 0.281 g L^{-1} for 20, 40, 60, 80, 100, 150 and 200 mg L^{-1} initial Zn(II) concentrations, respectively at pH 7.0. The growth curves obtained for all metals clearly show the presence of lag, log, stationary and death phases. It is observed that the extreme pH values (2 and 8) inhibit growth of *P. taiwanensis* and Cr(VI) removal. It is found that *P. taiwanensis* removes Cr(VI) 72.2 % (initial concentration: 150 mg L^{-1}) at pH 7.0 which is high as compared to any other values at different pH. For Cu(II) removal, *A. guillouiae* shows maximum removal (98.7 %) at pH 8.0 for 80 mg L^{-1} initial concentration of Cu(II). The maximum removal of Zn(II) by *K. pneumoniae* is found to be 98.8 % (initial concentration: 40 mg L^{-1}) at pH 7.0. It is also noticed that the *P. taiwanensis* and *A. guillouiae* give 0.025 and 0.031 g L^{-1} of maximum biomass concentration, respectively at double the standard composition of MSM. In presence of Zn(II), the maximum growth of *K. pneumoniae* is found to be 0.354 g L^{-1} at double the standard composition of MSM. It is found that beyond a threshold value, Cr(VI), Cu(II) and Zn(II) along with other mineral salts present in MSM cause strong inhibition effect on the growth of the microorganisms.

The study on the effect of macronutrients (N, P and K) composition on bacterial growth shows the maximum biomass concentrations of *P. taiwanensis* for Cr(VI) bioremediation as 0.083, 0.095 and 0.189 g L^{-1} at double the standard compositions of N & P and standard composition of K, respectively in MSM dosage. In the presence of Cu(II), the maximum biomass growths of *A. guillouiae* are obtained as 0.052, 0.042 and 0.106 g L^{-1} for double the standard compositions of N & P and standard composition of K, respectively in MSM dosage. In the presence of Zn(II), the maximum growths of *K. pneumoniae* are obtained as 0.091, 0.099 and 0.278 g L^{-1} for double the standard

compositions of N & P and standard composition of K, respectively in MSM dosage. For all the three bacterial strains, the growth is found in decreasing order beyond the double composition of N and P in MSM dosage as excess mineral elements hinder the growth of the microbial cells. Similar trend is observed at half and standard compositions of N and P in MSM dosage. It is due to the fact that limited supply of N and P restricts the growth and development of microbial cells. Excess composition of K in MSM dosage is toxic for the growth of isolated microbial strain. The effect of oxygen and nitrogen on growth of the bacterial strains shows that the maximum growth (0.252 g L^{-1}) of *P. taiwanensis* is observed in aerobic condition for Cr(VI) removal. In the presence of Cu(II), the maximum growth of *A. guillouiae* is obtained (0.077 g L^{-1}) in facultative anaerobic condition while for Zn(II), the maximum biomass growth is obtained (0.216 g L^{-1}) in aerobic condition. For *A. guillouiae*, facultative anaerobic condition favours the growth during removal of metal(loids) as it possesses specialized dissimilatory metabolic pathways to uptake metals.

It is observed that *P. taiwanensis* gives maximum biomass growth (0.0237 g L^{-1}) at 37 ^{o}C while for *A. guillouiae*, the maximum biomass growth is 0.164 g L^{-1} at 40 ^{o}C in the presence of metals. *K. pneumoniae* shows maximum biomass growth (0.2157 g L^{-1}) at 37 ^{o}C. For all the three bacterial strains, less biomass growth is obtained at temperature below 30 ^{o}C. This is due to the fact that the lipids of the membrane stiffen below the optimum temperature (below 30 ^{o}C). The efficiency of transport proteins embedded in the membrane is decreased which restricts the supply of the substrate i.e. metals to the microbial cells. It leads to less growth of the bacterial strains. Results reveal the fact that the growths of all three bacterial strains are more in the temperature range of 37-40 ^{o}C.

The effect of inoculum volume on the growth of the bacterial strains reveals that the maximum growths are obtained for 1 % (v/v) inoculum volume. It is due to the fact that higher surface area to volume ratio increases the production of enzyme which leads to better metal uptake.

The specific growth rate of three bacterial strains for different initial concentration of Cr(VI), Cu(II) and Zn(II) is determined using the experimental data obtained during log phase. The maximum values of specific growth rate are obtained as 0.262 h^{-1} for 150 mg L^{-1} of Cr(VI), 0.208 h^{-1} for 80 mg L^{-1} of Cu(II) and 0.043 h^{-1} for 80 mg L^{-1} of Zn(II). Growth kinetic models, Monod and Monod-Inhibition are tested with the obtained bioremediation experimental data of three bacterial strains for Cr(VI), Cu(II) and Zn(II) removals. It is necessary to find out the exact model which defines the relationship between the specific growth rate and substrate concentration. The metal inhibition effect during the metal bioremediation process should be incorporated in the model.

The rate kinetic approaches (zero-order and three-half-order) are applied to experimental data obtained from bioremediation study. The regression coefficients of determination obtained for zero-order and three-half-order kinetics are found to be in the range of 0.912-0.999 and 0.959-0.994, respectively for removal of metals. It confirms the better fitting of three-half-order kinetic model for bioremediation of all the three metals. It is due to the fact that three-half-order model includes an additional term of biomass formation in explaining the bioremediation of metals.

In the mixed metal system using consortium, the maximum uptake capacities are obtained as 92.05, 89.00 and 89.20 mg metal g dry $cell^{-1}$ for Cr(VI), Cu(II) and Zn(II)

removal, respectively which are higher than the values obtained by individual strains. Consortium shows higher removal of Cr(VI), Cu(II) and Zn(II) in simulated industrial effluents contaminated with multiple metals.

6.1.6.3. Metal removal mechanism

The removal mechanisms of Cr(VI), Cu(II) and Zn(II) are investigated for *P. taiwanensis, A. guillouiae* and *K. pneumoniae*, respectively by maintaining 150 mg L^{-1} of each metal concentration. Results reveal the fact that out of 150 mg L^{-1} of initial Cr(VI) concentration, almost 41.45, 68.05 and 46.76 mg L^{-1} of Cr(VI) is biosorbed on the outer layer of *P. taiwanensis, A. guillouiae* and *K. pneumoniae*, respectively. It is also observed that 62.05, 28.45 and 15.49 mg L^{-1} of Cr(VI) is accumulated inside the cells of *P. taiwanensis, A. guillouiae* and *K. pneumoniae*, respectively. Similarly, almost 19.75, 26.25 and 25.89 mg L^{-1} of Cu(II) is biosorbed on the outer layer of *P. taiwanensis, A. guillouiae* and *K. pneumoniae*, respectively. For *P. taiwanensis, A. guillouiae* and *K. pneumoniae*, almost 80.50, 109.25 and 105.36 mg L^{-1} of Cu(II) is accumulated inside the cells, respectively. Results also reveal that out of 150 mg L^{-1} of initial Zn(II) concentration, almost 13.00, 12.85 and 13.14 mg L^{-1} of Zn(II) is biosorbed on the outer layer of *P. taiwanensis, A. guillouiae* and *K. pneumoniae*, respectively. It is also observed that 99.25, 82.65 and 110.36 mg L^{-1} of Zn(II) is accumulated inside the cells of *P. taiwanensis, A. guillouiae* and *K. pneumoniae*, respectively.

Plausible mechanisms of Cr(VI), Cu(II) and Zn(II) remediation by *P. taiwanensis, A. guillouiae* and *K. pneumoniae*, respectively are adapted from literatures and presented in the study. Cr(VI) remediation by *P. taiwanensis* may occur through a two-or-three-step process. In the Cr(VI) remediation process, NADH; NADPH and an electron from the

endogenous reserve are used as electron donors. In aerobic condition, the Cr(VI) reductase ChrR reduces Cr(VI) to form the short-lived intermediate Cr(V). Finally, Cr(V) is reduced to produce the thermodynamically stable end product Cr(III) with the help of soluble reductase (SR). *A. guillouiae* may possess a robust copper removal mechanism to overcome the toxicity of copper by transporting and accumulating the copper inside the cells. A chromosomal system works at low copper concentration and a plasmid system functions at high copper concentrations to maintain the copper concentration inside the bacterial cells. In chromosomal system, two mechanisms (CutA and CutB) may occur to ensure transport and accumulation of Cu(II). In next step, Cu(II) is reduced to Cu(I) inside the cells of *A. guillouiae*. CutC and CutD help to efflux the Cu(I) from the cells. Resistance to toxic level of zinc inside *K. pneumoniae* may be due to extracellular accumulation, sequestration by metallothioneins (MT) and efflux-based sequestration or intracellular sequestration.

6.1.6.4. Biofiltration studies

The biofiltration studies are conducted using matured compost and coal as the packing material along with the bacterial strains as seeding culture for the removal of Cr(VI), Cu(II) and Zn(II). The column is operated for 63, 55 and 60 days for Cr(VI), Cu(II) and Zn(II) removal, respectively. The inlet concentration and flow rate of metals are varied during the entire biofiltration operation. It is observed that the removal efficiency is low during the acclimation period for all the three metals. It increases with operating days and reaches to 94.8 %, 95 % and 73.7 % at the end of acclimation period for Cr(VI), Cu(II) and Zn(II) removal, respectively. The sudden increase in the inlet concentration and flow rate of metal decrease the removal efficiency. The removal efficiency is further increased

with operating days with the same operating conditions. It reaches to a steady state value at the end of the phase. The maximum steady state removal efficiencies are obtained as 89.4 % of Cr(VI), 97.5 % of Cu(II) and 91.5 % of Zn(II) for the inlet concentrations of respective metals in the range of 38-40, 17.5-20 and 18.5-20 mg L^{-1}.

In industries, concentration and flow rate of pollutants vary on daily basis. Stability check of the biofilter column is necessary to monitor the behaviour of the column and its adaptability in industrial purpose. Response to sudden change in inlet loads is calculated in terms of removal efficiency of the biofilter column. In the present study, for the fluctuating inputs (initial concentration and flow rate) of metal solution, the stable response of the biofilter column is predicted for a period of 20 days for Cr(VI), 14 days for Cu(II) and 14 days for Zn(II) immediately after 63 days of biofilter operation for Cr(VI), 55 days of biofilter operation for Cu(II) and 60 days of biofilter operation for Zn(II), respectively. The removal efficiency obtained for all the metals during the shock loading conditions confirms the stability of the biofilter column. It gives an insight that biofilter column can be used for the industrial purpose where inlet concentration of pollutants vary from lower to higher values at a lower to moderate flow rate.

In the present study, kinetic parameters (bioremediation rate and saturation constant) are estimated using Michaelis-Menten kinetic model. The obtained values of bioremediation rate (r_{max}) are 0.256, 0.409 and 0.224 mg L^{-1} min^{-1} for Cr(VI), Cu(II) and Zn(II), respectively. The saturation constant (K_s) is calculated as 20.44, 33.62 and 24.87 mg L^{-1} for Cr(VI), Cu(II) and Zn(II), respectively. It is observed that, obtained values of K_s fall in the range of the inlet concentration (10-50 mg L^{-1}) of metals which indicates

that bioremediation kinetics of Cr(VI), Cu(II) and Zn(II) is better explained by zero-order kinetics with diffusion limitation (Ottengraf-van den Oever model).

Biofiltration experimental data for Cr(VI), Cu(II) and Zn(II) are used to validate Ottengraf-van den Oever model. Validation of model is carried out using all the experimental data obtained during phase II to V of steady state biofilter operation. The predicted values of elimination capacity are plotted with the experimental values of elimination capacity at different phases (phase II to V) for different inlet loadings of metals. The coefficients of determination (R^2) are determined in the range of 0.826-0.990 for Cr(VI), 0.912-0.996 for Cu(II) and 0.878-0.998 for Zn(II). It confirms the suitability of Ottengraf-van den Oever model in explaining the kinetic behaviour of the biofilter column. The biofilm thickness (δ) is determined using kinetic parameter values for different phases. This values are in the range of 178.25-287.56 μm for Cr(VI), 51.07-119 μm for Cu(II) and 178.98-278.25 μm for Zn(II).

6.1.6.5. FTIR analysis

Fourier transform infrared (FTIR) spectral analysis shows the peaks at 3282.72, 2900.45, 1644.92, 1538.92, 1232.57, 1076.35 and 532.56 cm^{-1} for Cr(VI) laden biomass of *P. taiwanesis*. For Cu(II) loaded biomass of *A. guillouiae*, the absorption peaks are observed at 3273.73, 2920.11, 1635.29, 1538.92, 1230.45, 1059.90 and 523.06 cm^{-1} while for Zn(II) loaded biomass of *K. pneumoniae*, the absorption peaks are observed at 3284.72, 2920.11, 1645.81, 1538.03, 1232.70, 1068.30 and 521.98 cm^{-1}. FTIR analysis of consortium shows the peaks at 3840.49, 3399.92, 2960.07, 2925.90, 2854.36, 1649.07, 1544.75, 1451.70, 1384.69, 1237.90, 1081.36, 618.56 and 539.03 cm^{-1}. The observed peaks for the three bacterial strains and consortium reveal the possible involvement of

carboxyl, amino, hydroxyl, methyl, phosphate and sulphonate groups in metal sequestration using three bacterial strains and consortium.

6.1.6.6. FESEM-EDX analysis

Field emission scanning electron microscope coupled with energy dispersive X-ray (FESEM-EDX) analysis reveals the change in the surface morphology of the individual bacterial strains in metal bioremediation. FESEM analysis also shows the positive interaction between the three bacterial strains in consortium. EDX analysis does not detect chromium on the surface of *P. taiwanensis* cell grown in presence of chromium. FESEM analysis shows the formation of wrinkles in the surface of *A. guillouiae* after Cu(II) sorption. The presence of Cu(II) ions on the surface is further confirmed by EDX analysis. FESEM analysis reveals the rough texture of *K. pneumoniae* surface after zinc uptake. Lump-like and twist-like deposits are observed on the surface of biomass. Few grooves are also noticed on the surface of the biomass after zinc uptake. EDX spectroscopy shows the presence of zinc at 1.011 keV. FESEM analysis shows that the greater adaptability of consortium in multiple metal mixture which indicates its robust growth mechanism over individual bacterial strains.

6.2. Conclusions

The following conclusions are drawn based on the results obtained in the present study:

1. Three metal resistant microbial strains are isolated and identified as *P. taiwanensis, A. guillouiae* and *K. pneumoniae* for Cr(VI), Cu(II) and Zn(II) bioremediation, respectively.

2. The batch bioremediation studies are successfully conducted to understand the effects of various operating parameters for the removal of Cr(VI), Cu(II) and Zn(II) from aqueous solutions.
3. Metal inhibition effect is found significant with increase in initial concentration of metals and it subsequently decreases the biomass growth.
4. The optimum solution pH values for the growth of *P.taiwanensis*, *A. guillouiae*, and *K. pneumoniae* are found as 7, 8 and 7 for the Cr(VI), Cu(II) and Zn(II) bioremediation, respectively.
5. The maximum biomass growth for all the three species are obtained for double the standard dosage of MSM and 1% (v/v) inoculum volume.
6. The maximum growths of *P. taiwanensis*, *A. guillouiae* and *K. pneumoniae* are obtained for N & P at double the standard compositions and for K at standard composition of MSM dosage.
7. The growth of *P. taiwanensis* and *K. pneumoniae* are found better in aerobic condition while it is better in facultative anaerobic conditions for *A. guillouiae*.
8. The optimum temperature is found in the range of 37-40 °C for the maximum biomass growth for all three species.
9. The maximum specific growth rates of *P. taiwanensis*, *A. guillouiae* and *K. pneumoniae* are obtained at 150, 80 and 80 mg L^{-1} for Cr(VI), Cu(II) and Zn(II) initial concentration, respectively.
10. The three-half-order kinetic model suitably describes the bioremediation kinetics of all the three metals.

11. Consortium shows maximum uptake of all the three metals than that for individual strains for the single & the multiple metals and simulated industrial effluents.
12. FTIR spectral analysis of biomass shows the possible complexation of carboxyl, amino, hydroxyl, methyl, phosphate and sulphonate groups with metals in metal bioremediation.
13. Surface morphology obtained from FESEM-EDX analysis of metal unloaded and loaded biomass confirms the presence of metals on the surface in metal bioremediation.
14. The exhaustive biofiltration experiments reveal that the performance of the biofilter column for metals removal is affected by the inlet metal concentration and flow rate.
15. The maximum steady state removal efficiencies are obtained as 89.4 %, 97.5 % and 91.5 % for 38-40 mg L^{-1} of Cr(VI), 17.5-20 mg L^{-1} of Cu(II) and 18.5-20 mg L^{-1} of Zn(II) concentrations, respectively.
16. The biofilter column is found stable at fluctuating inlet conditions which shows the applicability of biofilter column for the industrial purpose.
17. Ottengraf-van den Oever model adequately fits the biofiltration experimental data for Cr(VI), Cu(II) and Zn(II) removal and the bioremediation kinetics for metal removal is better explained by three-half-order kinetics.

6.3. Major contributions

1. Three indigenous bacterial strains *P. taiwanensis*, *A. guillouiae* and *K. pneumoniae* are isolated from activated sludge and utilized for the removal of Cr(VI), Cu(II) and Zn(II) from aqueous solution.

2. The performance of three bacterial strains are extensively analyzed in terms of biomass growth and percentage removal of three metals by conducting exhaustive batch bioremediation studies.
3. The experimental data obtained from bioremediation studies are fitted with Monod-Inhibition model and rate kinetic models (zero-order and three-half-order).
4. Exhaustive biofiltration studies are performed using a mixed packing material for the removal of Cr(VI), Cu(II) and Zn(II) under different operating conditions.
5. The stability of biofilter column is checked by subjecting the column to shock loading conditions.
6. Present study is the first attempt towards the development of an indigenous bacterial consortium from three bacterial strains and towards investigating its efficacy in the simultatneous removal of Cr(VI), Cu(II) and Zn(II) from aqueous solutions.

6.4. Future scope of research

The future scopes of the present work are listed below:

1. The batch bioremediation studies can be performed for other heavy metals such as cadmium, nickel, lead, arsenic etc. and for mixed wastes streams containing organic compounds, metals and dyes.
2. Biofiltration study can be extended to treat simulated industrial effluents contaminated with multiple heavy metals.
3. The biofiltration experiment can be performed to study the effect of various packing materials and their particle size.

4. The results obtained in batch bioremediation and biofiltration experimental studies can help to design the biofilter column for pilot plant studies and also for industrial practice.

REFERENCES

Abd El Hameed, A.H., Eweda, W.E., Abou-Taleb, K.A.A., Mira, H.I., 2015. Biosorption of uranium and heavy metals using some local fungi isolated from phosphatic fertilizers. Annals of Agricultural Sciences 60, 345-351.

Abdelwahab, O., Amin, N.K., El-Ashtoukhy, E.S.Z., 2013. Removal of Zinc Ions from Aqueous Solution using A Cation Exchange Resin. Chemical Engineering Research and Design 91, 165-173.

Abusham, R.A., Rahman, R.N.Z.R., Salleh, A.B., Basri, M., 2009. Optimization of Physical Factors Affecting the Production of Thermo-stable Organic Solvent-tolerant Protease from a Newly Isolated Halo Tolerant *Bacillus subtilis* Strain Rand. Microbial Cell Factories 8, 20-28.

Ahemad, M., Kibret, M., 2013. Recent Trends in Microbial Biosorption of Heavy Metals: A Review. Biochemistry & Molecular Biology 1, 19-26.

Ahmad, W.A., Zakaria, Z.A., Khasim, A.R., Alias, M.A., Ismail, S.M.H.S., 2010. Pilot-scale Removal of Chromium from Industrial Wastewater using the ChromeBac™ System. Bioresource Technology 101, 4371-4378.

Ahn, K.-H., Song, K.-G., Cha, H.-Y., Yeom, I.-T., 1999. Removal of Ions in Nickel Electroplating Rinse Water using Low-pressure Nanofiltration. Desalination 122, 77-84.

Akinci, G., Guven, D.E., 2011. Bioleaching of Heavy Metals Contaminated Sediment by Pure and Mixed Cultures of *Acidithiobacillus* spp. Desalination 268, 221-226.

Akiyama, E., Markworth, A.J., McCoy, J.K., Frankel, G.S., Xia, L., McCreery, R.L., 2003. Storage and Release of Soluble Hexavalent Chromium from Chromate Conversion Coatings on Al Alloys. Journal of the Electrochemical Society 150, B83-B91.

Alguacil, F.J., Alonso Gámez, M., López-Delgado, A., Navarro, P., 2004. Modelling Copper (II) Liquid-liquid Extraction: The System Acorga M5640-Exxsol D100-$CuSO_4$-H_2SO_4. Journal of Chemical Research 2004, 196-197.

Altun, M., Sahinkaya, E., Durukan, I., Bektas, S., Komnitsas, K., 2014. Arsenic removal in a sulfidogenic fixed-bed column bioreactor. Journal of Hazardous Materials 269, 31-37.

Andreazza, R., Okeke, B., Pieniz, S., Camargo, F.O., 2012. Characterization of Copper-Resistant Rhizosphere Bacteria from *Avena sativa* and *Plantago lanceolata* for Copper Bioreduction and Biosorption. Biological Trace Element Research 146, 107-115.

APHA, AWWA, WEF, 2005. Standard methods for the examination of water and wastewater, 21st ed., Washington, DC, 7-15.

Appenroth, K.J., Bischoff, M., Gabryś, H., Stoeckel, J., Swartz, H.M., Walczak, T., Winnefeld, K., 2000. Kinetics of Chromium(V) Formation and Reduction in Fronds of the Duckweed *Spirodela polyrhiza* — A Low Frequency EPR Study. Journal of Inorganic Biochemistry 78, 235-242.

Bae, W.C., Kang, T.G., Kang, I.K., Won, Y.J., Jeong, B.C., 2000. Reduction of Hexavalent Chromium by *Escherichia coli* ATCC 33456 in Batch and Continuous Cultures. The Journal of Microbiology 38, 36-39.

Bahadir, T., Bakan, G., Altas, L., Buyukgungor, H., 2007. The Investigation of Lead Removal by Biosorption: An Application at Storage Battery Industry Wastewaters. Enzyme and Microbial Technology 41, 98-102.

Bai, R.S., Abraham, T.E., 2002. Studies on enhancement of Cr(VI) biosorption by chemically modified biomass of Rhizopus nigricans. Water Research 36, 1224-1236.

Baral, A., Engelken, R.D., 2002. Chromium-based Regulations and Greening in Metal Finishing Industries in the USA. Environmental Science & Policy 5, 121-133.

Barbosa, L.P., Costa, P.F., Bertolino, S.M., Silva, J.C.C., Guerra-Sá, R., Leão, V.A., Teixeira, M.C., 2014. Nickel, Manganese and Copper Removal by A Mixed Consortium of Sulfate Reducing Bacteria at A High COD/sulfate Ratio. World Journal of Microbiology and Biotechnology, 1-10.

Basha, S., Murthy, Z.V.P., Jha, B., 2008. Biosorption of Hexavalent Chromium by Chemically Modified Seaweed, *Cystoseira indica*. Chemical Engineering Journal 137, 480-488.

Batool, R., Yrjälä, K., Hasnain, S., 2014. Impact of Environmental Stress on Biochemical Parameters of Bacteria Reducing Chromium. Brazilian Journal of Microbiology 45, 573-583.

Battaglia-Brunet, F., Touzé, S., Michel, C., Ignatiadis, I., 2006. Treatment of Chromate-Polluted Groundwater in A 200 dm^3 Pilot Bioreactor Fed with Hydrogen. Journal of Chemical Technology & Biotechnology 81, 1506-1513.

Beech, I., Hanjagsit, L., Kalaji, M., Neal, A.L., Zinkevich, V., 1999. Chemical and Structural Characterization of Exopolymers Produced by *Pseudomonas* sp. NCIMB 2021 in Continuous Culture. Microbiology 145, 1491-1497.

Bejestani, F.B., Ghane, M., Mirhosseininia, M., Bejestani, O.B., 2013. Isolation and Phylogenetic Analysis of Zinc Resistant *Acinetobacter* sp. and Its Potential for Bioremediation. African Journal of Biotechnology 12, 4123-4128.

Benefield, L.D., Morgan, J.M., 1999. Chemical Precipitation, in: Letterman (Ed.), Water Quality and Treatment. McGraw-Hill Inc., New York, 10.11-10.57.

Bhagat, R., Srivastava, S., 1994. Effect of Zinc on Morphology and Ultrastructure of *Pseudomonas stutseri* RS34. Journal of General and Applied Microbiology 40, 265-270.

Bhattacharya, A.K., Naiya, T.K., Mandal, S.N., Das, S.K., 2008. Adsorption, Kinetics and Equilibrium Studies on Removal of Cr(VI) from Aqueous Solutions using Different Low-cost Adsorbents. Chemical Engineering Journal 137, 529-541.

Blánquez, P., Casas, N., Font, X., Gabarrell, X., Sarrà, M., Caminal, G., Vicent, T., 2004. Mechanism of Textile Metal Dye Biotransformation by *Trametes versicolor*. Water Research 38, 2166-2172.

Boyer, A., Magnin, J.-P., Ozil, P., 1998. Copper Ion Removal by *Thiobacillus ferrooxidans* Biomass. Biotechnology Letters 20, 187-190.

Bradshaw, D.J., Marsh, P.D., Watson, G.K., Allison, C., 1998. Role of *Fusobacterium nucleatum* and Coaggregation in Anaerobe Survival in Planktonic and Biofilm Oral Microbial Communities during Aeration. Infection and Immunity 66, 4729-4732.

Brady, D., Stoll, A.D., Starke, L., Duncan, J.R., 1994. Chemical and Enzymatic Extraction of Heavy Metal Binding Polymers from Isolated Cell walls of *Saccharomyces cerevisiae*. Biotechnology and Bioengineering 44, 297-302.

Brown, N.L., Lee, B.T.O., Silver, S., 1993. Bacterial Transport and Resistance to Copper, Metal Ions in Biological Systems. Marcel Dekker, New York, 405-435.

Bruins, M.R., Kapil, S., Oehme, F.W., 2000. Microbial Resistance to Metals in The Environment. Ecotoxicology and Environmental Safety 45, 198-207.

Brunner, W., Focht, D.D., 1984. Deterministic Three-Half-Order Kinetic Model for Microbial Degradation of Added Carbon Substrates in Soil. Applied and Environmental Microbiology 47, 167-172.

Bueno, B.Y.M., Torem, M.L., Molina, F., Mesquita, L.M.S.d., 2008. Biosorption of Lead(II), Chromium(III) and Copper(II) by *Rhodococcus opacus*: Equilibrium and Kinetic Studies. Minerals Engineering 21, 65-75.

Cary, E.E., 1982. Chromium in Air, Soil and Natural Waters, in: Langard S. (Ed.), Biological and Environmental Aspects of Chromium. Elsevier, New York, 49-63.

Casiot, C., Morin, G., Juillot, F., Bruneel, O., Personné, J.-C., Leblanc, M., Duquesne, K., Bonnefoy, V., Elbaz-Poulichet, F., 2003. Bacterial Immobilization and Oxidation of Arsenic in Acid Mine Drainage (Carnoulès creek, France). Water Research 37, 2929-2936.

Cerino-Córdova, F.J., García-León, A.M., Soto-Regalado, E., Sánchez-González, M.N., Lozano-Ramírez, T., García-Avalos, B.C., Loredo-Medrano, J.A., 2012. Experimental Design for The Optimization of Copper Biosorption from Aqueous Solution by *Aspergillus terreus*. Journal of Environmental Management 95, Supplement, S77-S82.

Cervantes, C., Campos-Garcia, J., 2007. Reduction and Efflux of Chromate by Bacteria, in: Nies D.H., Silver S. (Eds.), Springer-Verlag, Berlin, 407-420.

Cervantes, C., Campos-García, J., Devars, S., Gutiérrez-Corona, F., Loza-Tavera, H., Torres-Guzmán, J.C., Moreno-Sánchez, R., 2001. Interactions of Chromium with Microorganisms and Plants. FEMS Microbiology Reviews 25, 335-347.

Cervantes, C., Gutierrez-Corona, F., 1994. Copper Resistance Mechanisms in Bacteria and Fungi. FEMS Microbiology Reviews 14, 121-138.

Chabalala, S., Chirwa, E.M.N., 2010. Removal of uranium(VI) under aerobic and anaerobic conditions using an indigenous mine consortium. Minerals Engineering 23, 526-531.

Chang, J.S., Law, R., Chang, C.C., 1997. Biosorption of Lead, Copper and Cadmium by Biomass of *Pseudomonas aeruginosa* PU21. Water Research 31, 1651-1658.

Charerntanyarak, L., 1999. Heavy Metals Removal by Chemical Coagulation and Precipitation. Water Science and Technology 39, 135-138.

Chaudhary, D.S., Vigneswaran, S., Ngo, H.H., Shim, W.G., Moon, H., 2003. Biofilter in Water and Wastewater Treatment. Korean Journal of Chemical Engineering 20, 1054-1065.

Chen, J.M., Hao, O.J., 1998. Microbial Chromium (VI) Reduction. Critical Reviews in Environmental Science and Technology 28, 219-251.

Chen, X.C., Wang, Y.P., Lin, Q., Shi, J.Y., Wu, W.X., Chen, Y.X., 2005. Biosorption of Copper (II) and Zinc (II) from Aqueous Solution by *Pseudomonas putida* CZ1. Colloids and Surfaces B: Biointerfaces 46, 101-107.

Cheung, K.H., Gu, J.D., 2007. Mechanism of Hexavalent Chromium Detoxification by Microorganisms and Bioremediation Application potential: A Review. International Biodeterioration & Biodegradation 59, 8-15.

Chirwa, E.M.N., Wang, Y.-T., 1997. Hexavalent Chromium Reduction by *Bacillus sp*. in A Packed-Bed Bioreactor. Environmental Science & Technology 31, 1446-1451.

Chirwa, E.M.N., Wang, Y.-T., 2001. Simultaneous Chromium(VI) Reduction and Phenol Degradation in A Fixed-Film Coculture Bioreactor: Reactor Performance. Water Research 35, 1921-1932.

Choudhary, S., Sar, P., 2009. Characterization of A Metal Resistant *Pseudomonas sp*. Isolated from Uranium Mine for Its Potential in Heavy Metal (Ni^{2}+, Co^{2+}, Cu^{2+}, and Cd^{2+}) Sequestration. Bioresource Technology 100, 2482-2492.

Choudhury, R., Srivastava, S., 2001. Zinc Resistance Mechanisms in Bacteria. Current Science 81, 768-775.

Christian, D., Wong, E., Crawford, R.L., Cheng, I.F., Hess, T.F., 2010. Heavy Metals Removal from Mine Runoff using Compost Bioreactors. Environmental Technology 31, 1533-1546.

Coates, J.P., 1996. The Interpretation of Infrared Spectra: Published Reference Sources. Applied Spectroscopy Reviews 31, 179-192.

Costa, F., Quintelas, C., Tavares, T., 2012. Kinetics of Biodegradation of Diethylketone by *Arthrobacter viscosus*. Biodegradation 23, 81-92.

Csotonyi, J.T., Stackebrandt, E., Yurkov, V., 2006. Anaerobic Respiration on Tellurate and Other Metalloids in Bacteria from Hydrothermal Vent Fields in the Eastern Pacific Ocean. Applied and Environmental Microbiology 72, 4950-4956.

CWC, 2013. Water and Related Statistics-2013. Central Water Commission, India.

Dalcin, M.G., Pirete, M.M., Lemos, D.A., Ribeiro, E.J., Cardoso, V.L., de Resende, M.M., 2011. Evaluation of Hexavalent Chromium Removal in A Continuous Biological Filter with The use of Central Composite Design (CCD). Journal of Environmental Management 92, 1165-1173.

Dapena-Mora, A., Fernández, I., Campos, J.L., Mosquera-Corral, A., Méndez, R., Jetten, M.S.M., 2007. Evaluation of Activity and Inhibition Effects on Anammox Process by Batch Tests Based on The Nitrogen Gas Production. Enzyme and Microbial Technology 40, 859-865.

Das, A.P., Mishra, S., 2008. Hexavalent Chromium (VI): Environment Pollutant and Health Hazard. Journal of Environmental Research And Development 2, 386-392.

Davis, T.A., Volesky, B., Mucci, A., 2003. A Review of The Biochemistry of Heavy Metal Biosorption by Brown Algae. Water Research 37, 4311-4330.

Delhoménie, M.-C., Heitz, M., 2005. Biofiltration of Air: A Review. Critical Reviews in Biotechnology 25, 53-72.

Deng, X., Li, Q.B., Lu, Y.H., Sun, D.H., Huang, Y.L., Chen, X.R., 2003. Bioaccumulation of Nickel from Aqueous Solutions by Genetically Engineered *Escherichia coli*. Water Research 37, 2505-2511.

Dermou, E., Vayenas, D.V., 2007. A Kinetic Study of Biological Cr(VI) Reduction in Trickling Filters with Different Filter Media Types. Journal of Hazardous Materials 145, 256-262.

Dermou, E., Velissariou, A., Xenos, D., Vayenas, D.V., 2005. Biological Chromium(VI) Reduction using A Trickling Filter. Journal of Hazardous Materials 126, 78-85.

Deshusses, M.A., Hamer, G., Dunn, I.J., 1995. Behavior of Biofilters for Waste Air Biotreatment. 1. Dynamic Model Development. Environmental Science & Technology 29, 1048-1058.

Diels, L., Dong, Q., van der Lelie, D., Baeyens, W., Mergeay, M., 1995. The*czc* Operon of *Alcaligenes eutrophus* CH34: from Resistance Mechanism to The Removal of Heavy Metals. Journal of Industrial Microbiology 14, 142-153.

Du, L.-N., Wang, B., Li, G., Wang, S., Crowley, D.E., Zhao, Y.-H., 2012. Biosorption of the Metal-complex Dye Acid Black 172 by Live and Heat-treated Biomass of *Pseudomonas* sp. strain DY1: Kinetics and Sorption Mechanisms. Journal of Hazardous Materials 205–206, 47-54.

Dursun, A.Y., Uslu, G., Cuci, Y., Aksu, Z., 2003. Bioaccumulation of Copper (II), Lead(II) and Chromium(VI) by Growing *Aspergillus niger*. Process Biochemistry 38, 1647-1651.

Bestawy, E., Helmy, S., Hussien, H., Fahmy, M., Amer, R., 2013. Bioremediation of Heavy Metal-Contaminated Effluent using Optimized Activated Sludge Bacteria. Applied Water Science 3, 181-192.

Edwards, V.H., 1970. The Influence of High Substrate Concentrations on Microbial Kinetics. Biotechnology and Bioengineering 12, 679-712.

Ekenberg, M., Martander, H., Welander, T., 2005. Biological Reduction of Hexavalent Chromium - A Field Study. Water Environment Research 77, 425-428.

El-Sharkawi, H.M., 2012. Effect of Nitrogen Sources on Microbial Biomass Nitrogen under Different Soil Types. ISRN Soil Science 2012, 7.

Elangovan, R., Philip, L., 2009. Performance Evaluation of Various Bioreactors for The Removal of Cr(VI) and Organic Matter from Industrial Effluent. Biochemical Engineering Journal 44, 174-186.

Epstein, W., 2003. The Roles and Regulation of Potassium in Bacteria. Progress in Nucleic Acid Research and Molecular Biology 75, 293-320.

Gardea-Torresdey, J.L., Tiemann, K.J., Armendariz, V., Bess-Oberto, L., Chianelli, R.R., Rios, J., Parsons, J.G., Gamez, G., 2000. Characterization of Cr(VI) Binding and

Reduction to Cr(III) by The Agricultural Byproducts of *Avena monida* (Oat) Biomass. Journal of Hazardous Materials 80, 175-188.

Ge, S., Ge, S., Zhou, M., Dong, X., 2015. Bioremediation of hexavalent chromate using permeabilized *Brevibacterium* sp. and *Stenotrophomonas* sp. cells. Journal of Environmental Management 157, 54-59.

Gekeler, W., Grill, E., Winacker, E.L., Zenk, M.H., 1988. Algae Sequester Heavy Metals via Synthesis of Phytochelatin Complex. Archives of Microbiology 50, 197-202.

Ghosh, A., Saha, P.D., 2013. Optimization of copper bioremediation by *Stenotrophomonas maltophilia* PD2. Journal of Environmental Chemical Engineering 1, 159-163.

Ghozlan, H.A., Sabry, S.A., Amer, R.A., 1999. Bioaccumulation of Nickel, Cobalt, and Cadmium by Free and Immobilized Cells of *Pseudomonas* spp. Fresenius Environmental Bulletin 8, 428-435.

Golder, A.K., Samanta, A.N., Ray, S., 2007. Removal of Trivalent Chromium by Electrocoagulation. Separation and Purification Technology 53, 33-41.

Greenberg, A.E., Trussell, R.R., Clesceri, L.S., 1985. Standard Methods for The Examination of Water and Wastewater, 16th ed., APHA, New York.

Gupta, S., Babu, B.V., 2008. Removal of Heavy Metal Pollutants from Wastewater Using Low Cost Adsorbents, in: Gupta K. R. (Ed.), Water Crisis in India, New Delhi, 79-123.

Gupta, S., Babu, B.V., 2009. Removal of Toxic Metal Cr(VI) from Aqueous Solutions using Sawdust as Adsorbent: Equilibrium, Kinetics and Regeneration Studies. Chemical Engineering Journal 150, 352-365.

Hadiyanto, A.G., Pradana, L., Buchori, C., Budiyati, S., 2014. Biosorption of heavy metal Cu^{2+} and Cr^{2+} in textile wastewater by using immobilized algae. Research Journal of Applied Sciences, Engineering and Technology 17, 3539-3543.

Haldane, J.B.S., 1965. Enzymes. MIT Press, 1st ed., Cambridge, MA.

Hamadi, N.K., Chen, X.D., Farid, M.M., Lu, M.G.Q., 2001. Adsorption Kinetics for The Removal of Chromium(VI) from Aqueous Solution by Adsorbents Derived from used Tyres and Sawdust. Chemical Engineering Journal 84, 95-105.

Haritonidis, S., Malea, P., 1999. Bioaccumulation of Metals by The Green Alga *Ulva rigida* from Thermaikos Gulf, Greece. Environmental Pollution 104, 365-372.

Hartmeier, W., Berends, A., 1995. Biosorption of Heavy Metals by *Bacillus amyloliquefaciens*: Contribution of Cell Wall Components. Mededelingen Faculteit Landbouwkundige en Toegepaste Biologische Wetenschappen Universiteit Gent 60, 2585-2588.

Hassen, A., Saidi, N., Cherif, M., Boudabous, A., 1998. Effects of Heavy Metals on *Pseudomonas aeruginosa* and *Bacillus thuringiensis*. Bioresource Technology 65, 73-82.

Hattori, T., Hisamori, H., Suzuki, S., Umezawa, T., Yoshimura, T., Sakai, H., 2015. Rapid copper transfer and precipitation by wood-rotting fungi can effect copper removal from copper sulfate-treated wood blocks during solid-state fungal treatment. International Biodeterioration & Biodegradation 97, 195-201.

Holmes, D.S., Dubey, S.K., Gangolli, S., 1994. Development of Biosensors for The Detection of Mercury and Copper Ions. Environmental Geochemistry and Health 16, 229–233.

Huang, C.-p., Huang, C.-p., Morehart, A.L., 1990. The Removal of Cu(II) from Dilute Aqueous Solutions by *Saccharomyces cerevisiae*. Water Research 24, 433-439.

Hussein, H., Farag, S., Kandil, K., Moawad, H., 2005. Tolerance and Uptake of Heavy Metals by *Pseudomonads*. Process Biochemistry 40, 955-961.

Ilyas, S., Lee, J.-c., Kim, B.-s., 2014. Bioremoval of heavy metals from recycling industry electronic waste by a consortium of moderate thermophiles: process development and optimization. Journal of Cleaner Production 70, 194-202.

Indhumathi, P., Syed Shabudeen, P.S., Shoba, U.S., Saraswathy, C.P., 2014. The removal of chromium from aqueous solution by using green micro algae. Journal of Chemical and Pharmaceutical Research 6, 799-808.

Islam, F., Yasmeen, T., Ali, Q., Ali, S., Arif, M.S., Hussain, S., Rizvi, H., 2014. Influence of *Pseudomonas aeruginosa* as PGPR on Oxidative Stress Tolerance in Wheat under Zn Stress. Ecotoxicology and Environmental Safety 104, 285-293.

Jadhav, J.P., Kalyani, D.C., Telke, A.A., Phugare, S.S., Govindwar, S.P., 2010. Evaluation of the efficacy of a bacterial consortium for the removal of color,

reduction of heavy metals, and toxicity from textile dye effluent. Bioresource Technology 101, 165-173.

Jaman, H., Chakraborty, D., Saha, P., 2009. A Study of The Thermodynamics and Kinetics of Copper Adsorption using Chemically Modified Rice Husk. CLEAN – Soil, Air, Water 37, 704-711.

Janyasuthiwong, S., Rene, E.R., Esposito, G., Lens, P.N.L., 2015. Effect of pH on Cu, Ni and Zn removal by biogenic sulfide precipitation in an inversed fluidized bed bioreactor. Hydrometallurgy 158, 94-100.

Jeyasingh, J., Somasundaram, V., Philip, L., Bhallamudi, S.M., 2010. Bioremediation of Cr(VI) contaminated soil/sludge: Experimental studies and development of a management model. Chemical Engineering Journal 160, 556-564.

Ji, G., Silver, S., 1995. Bacterial Resistance Mechanisms for Heavy Metals of Environmental Concern. Journal of Industrial Microbiology 14, 61-75.

Johnson, A.R., Munoz, A., Gottlieb, J.L., Jarrard, D.F., 2007. High Dose Zinc Increases Hospital Admissions due to Genitourinary Complications. The Journal of Urology 177, 639-643.

Jong, T., Parry, D.L., 2003. Removal of Sulfate and Heavy Metals by Sulfate Reducing Bacteria in Short-Term Bench Scale Upflow Anaerobic Packed Bed Reactor Runs. Water Research 37, 3379-3389.

Juang, R.-S., Shiau, R.-C., 2000. Metal Removal from Aqueous Solutions using Chitosan-Enhanced Membrane Filtration. Journal of Membrane Science 165, 159-167.

Juttner, K., Galla, U., Schmieder, H., 2000. Electrochemical Approaches to Environmental Problems in the Process Industry. Electrochimica Acta 45, 2575-2594.

Kanaly, R.A., Harayama, S., Watanabe, K., 2002. *Rhodanobacter* sp. Strain BPC1 in a Benzo[a]pyrene-Mineralizing Bacterial Consortium. Applied and Environmental Microbiology 68, 5826-5833.

Kang, C., Wu, P., Li, Y., Ruan, B., Zhu, N., Dang, Z., 2014. Estimates of Heavy Metal Tolerance and Chromium(VI) Reducing Ability of *Pseudomonas aeruginosa*

CCTCC AB93066: Chromium(VI) Toxicity and Environmental Parameters Optimization. World Journal of Microbiology and Biotechnology 30, 2733-2746.

Kang, S.-Y., Lee, J.-U., Kim, K.-W., 2007. Biosorption of Cr(III) and Cr(VI) onto The Cell Surface of *Pseudomonas aeruginosa*. Biochemical Engineering Journal 36, 54-58.

Kapoor, A., Viraraghavan, T., Cullimore, D.R., 1999. Removal of Heavy Metals using the Fungus *Aspergillus niger*. Bioresource Technology 70, 95-104.

Katsoyiannis, I.A., Zouboulis, A.I., 2004. Application of Biological Processes for The Removal of Arsenic from Groundwaters. Water Research 38, 17-26.

Kaushik, S., Juwarkar, A., Malik, A., Satya, S., 2008. Biological Removal of Cr (VI) by Bacterial Isolates Obtained from Metal Contaminated Sites. Journal of Environmental Science and Health, Part A 43, 419-423.

Kazy, S., Sar, P., Singh, S.P., Sen, A., D'Souza, S.F., 2002. Extracellular Polysaccharides of a Copper-Sensitive and a Copper-Resistant *Pseudomonas aeruginosa* Strain: Synthesis, Chemical Nature and Copper Binding. World Journal of Microbiology and Biotechnology 18, 583-588.

Kazy, S.K., D'Souza, S.F., Sar, P., 2009. Uranium and Thorium Sequestration by A *Pseudomonas sp.*: Mechanism and Chemical Characterization. Journal of Hazardous Materials 163, 65-72.

Kieu, H.T.Q., Müller, E., Horn, H., 2011. Heavy metal removal in anaerobic semi-continuous stirred tank reactors by a consortium of sulfate-reducing bacteria. Water Research 45, 3863-3870.

Kim, S., Park, Y., Moon, H., 1998. Removal of Copper Ions by A Cation-Exchange Resin in A Semifluidized Bed. Korean Journal of Chemical Engineering 15, 417-422.

Kiran, M.G., Pakshirajan, K., Das, G., 2016. Heavy metal removal from multicomponent system by sulfate reducing bacteria: Mechanism and cell surface characterization. Journal of Hazardous Materials (In press).

Kisand, V., Tuvikene, L., Nõges, T., 2001. Role of Phosphorus and Nitrogen for Bacteria and Phytopankton Development in A Large Shallow Lake. Hydrobiologia 457, 187-197.

Kőnig-Péter, A., Csudai, C., Felinger, A., Kilár, F., Pernyeszi, T., 2014. Potential of various biosorbents for Zn(II) removal. Water, Air, & Soil Pollution 225, 2089-2097.

Kotaś, J., Stasicka, Z., 2000. Chromium Occurrence in The Environment and Methods of Its Speciation. Environmental Pollution 107, 263-283.

Kratochvil, D., Volesky, B., 1998. Advances in The Biosorption of Heavy Metals. Trends in Biotechnology 16, 291-300.

Krauter, P., Martinelli, R., Williams, K., Martins, S., 1996. Removal of Cr(VI) from Ground Water by *Saccharomyces cerevisiae*. Biodegradation 7, 277-286.

Kumar, G.S., Thatheyus, A.J., 2013. Bioremediation of Chromium, Nickel and Zinc in Electroplating Effluent by *Escherichia coli*. Annual Review & Research in Biology 3, 913-920.

Kurniawan, T.A., Chan, G.Y.S., Lo, W.-H., Babel, S., 2006. Physico–Chemical Treatment Techniques for Wastewater Laden with Heavy Metals. Chemical Engineering Journal 118, 83-98.

Lameiras, S., Quintelas, C., Tavares, T., 2008. Biosorption of Cr (VI) using A Bacterial Biofilm Supported on Granular Activated Carbon and on Zeolite. Bioresource Technology 99, 801-806.

Lee, E., Lim, J., Oh, K., Lee, J., Kim, S., Lee, Y., Kim, K., 2008. Removal of Heavy Metals by An Enriched Consortium. The Journal of Microbiology 46, 23-28.

Lee, R.E., 1993. Scanning Electron Microscopy and X-Ray Microanalysis. 1st ed., Prentice-Hall, Englewood Cliffs, New Jersey.

Leyva-Ramos, R., Rangel-Mendez, J., Bernal-Jacome, L., Mendoza, M.B., 2005. Intraparticle Diffusion of Cadmium and Zinc Ions during Adsorption from Aqueous Solution on Activated Carbon. Journal of Chemical Technology and Biotechnology 80, 924-933.

Li, Q., Yang, Z.-h., Chai, L.-y., Wang, B., Xiong, S., Liao, Y.-p., Zhang, S.-j., 2013. Optimization of Cr(VI) bioremediation in contaminated soil using indigenous bacteria. Journal of Central South University 20, 480-487.

Lim, H.S., Lim, W., Hu, J.Y., Ziegler, A., Ong, S.L., 2015. Comparison of filter media materials for heavy metal removal from urban stormwater runoff using biofiltration systems. Journal of Environmental Management 147, 24-33.

Lin, Y.-H., Wu, C.-L., Li, H.-L., Hsu, C.-H., 2011. Verification of Model for Adsorption and Reduction of Chromium(VI) by *Escherichia coli* 33456 using Chitosan Bead as A Supporting Medium. Applied Mathematical Modelling 35, 2736-2751.

Lokhande, R.S., Singare, P.U., Pimple, D.S., 2011. Toxicity Study of Heavy Metals Pollutants in Waste Water Effluent Samples Collected from Taloja Industrial Estate of Mumbai, India. Resources and Environment 1, 13-19.

Loukidou, M.X., Matis, K.A., Zouboulis, A.I., Liakopoulou-Kyriakidou, M., 2003. Removal of As(V) from Wastewaters by Chemically Modified Fungal Biomass. Water Research 37, 4544-4552.

Lu, W.-B., Shi, J.-J., Wang, C.-H., Chang, J.-S., 2006. Biosorption of Lead, Copper and Cadmium by An Indigenous Isolate *Enterobacter* sp. J1 Possessing High Heavy-Metal Resistance. Journal of Hazardous Materials 134, 80-86.

Luef, E., Prey, T., Kubicek, C., 1991. Biosorption of Zinc by Fungal Mycelial Wastes. Applied Microbiology and Biotechnology 34, 688-692.

Luong, J.H.T., 1986. Generalization of Monod Kinetics for Analysis of Growth Data with Substrate Inhibition. Biotechnology and Bioengineering 29, 242-248.

Malik, A., 2004. Metal Bioremediation through Growing Cells. Environment International 30, 261-278.

Mangaiyarkarasi, M.S.M., Vincent, S., Janarthanan, S., Rao, T.S., Tata, B.V.R., 2011. Bioreduction of Cr(VI) by Alkaliphilic *Bacillus subtilis* and Interaction of The Membrane Groups. Saudi Journal of Biological Sciences 18, 157-167.

Márquez-Reyes, J.M., López-Chuken, U.J., Valdez-González, A., Luna-Olvera, H.A., 2013. Removal of chromium and lead by a sulfate-reducing consortium using peat moss as carbon source. Bioresource Technology 144, 128-134.

McLean, J., Beveridge, T.J., 2001. Chromate Reduction by a *Pseudomonad* Isolated from A Site Contaminated with Chromated Copper Arsenate. Applied and Environmental Microbiology 67, 1076-1084.

McLean, R.J.C., Fortin, D., Brown, D.A., 1996. Microbial Metal-Binding Mechanisms and Their Relation to Nuclear Waste Disposal. Canadian Journal of Microbiology 42, 392-400.

Mehta, S.K., Gaur, J.P., 2005. Use of Algae for Removing Heavy Metal Ions From Wastewater: Progress and Prospects. Critical Reviews in Biotechnology 25, 113-152.

Mejias Carpio, I.E., Machado-Santelli, G., Kazumi Sakata, S., Ferreira Filho, S.S., Rodrigues, D.F., 2014. Copper removal using a heavy-metal resistant microbial consortium in a fixed-bed reactor. Water Research 62, 156-166.

Mishra, A., Malik, A., 2014. Novel Fungal Consortium for Bioremediation of Metals and Dyes from Mixed Waste Stream. Bioresource Technology 171, 217-226.

Mishra, R., Sinha, V., Kannan, A., Upreti, R.K., 2012. Reduction of Chromium-VI by Chromium Resistant *Lactobacilli*: A Prospective Bacterium for Bioremediation. Toxicology International 19, 25-30.

Mishra, V., Balomajumder, C., Agarwal, V., 2014. Biological Removal of Heavy Metal Zinc from Industrial Effluent by *Zinc Sequestering Bacterium VMSDCM*. Clean Technologies and Environmental Policy 16, 555-568.

Mishra, V., Samantaray, D.P., Dash, S.K., Mishra, B.B., Swain, R.K., 2010. Study on Hexavalent Chromium Reduction by Chromium Resistant Bacterial Isolates of Sukinda Mining Area. Our Nature 8, 63-71.

Mogollón, L., Rodríguez, R., Larrota, W., Ramirez, N., Torres, R., 1998. Biosorption of Nickel using Filamentous Fungi. Applied Biochemistry and Biotechnology 70-72, 593-601.

Mohamed, R.M., 2015. Biosorption of zinc and cadmium by *Klebsiella pneumonia* KM609983 isolated from Sohag, Egypt. Global Advanced Research Journal of Microbiology 4, 018-026.

Mohan, J., 2005. Organic Spectroscopy Principles and Applications, 2nd ed., Narosa Publishing House, New Delhi.

Monod, J., 1949. The growth of bacterial cultures. Annual Review of Microbiology 3, 371-394.

Monser, L., Adhoum, N., 2002. Modified Activated Carbon for The Removal of Copper, Zinc, Chromium and Cyanide from Wastewater. Separation and Purification Technology 26, 137-146.

Monteiro, Á.A.M.G., Boaventura, R.A.R., Rodrigues, A.r.E., 2000. Phenol Biodegradation by *Pseudomonas putida* DSM 548 in A Batch Reactor. Biochemical Engineering Journal 6, 45-49.

Morby, A.P., Turner, J.S., Huckle, J.W., Robinson, N.J., 1993. SmtB is a Metal-Dependent Repressor of The Cyanobacterial Metallothionein Gene smtA: Identification of A Zn Inhibited DNA-Protein Complex. Nucleic Acids Research 21, 921-925.

Morgan , D., 2007. The Cell Cycle: Principles of Control, 1st ed., Sinauer Associates Inc. Publishers, Sunderland, MA.

Mungasavalli, D.P., Viraraghavan, T., Jin, Y.-C., 2007. Biosorption of Chromium from Aqueous Solutions by Pretreated *Aspergillus niger*: Batch and Column Studies. Colloids and Surfaces A Physicochemical and Engineering Aspects 301, 214-223.

Muñoz, R., Alvarez, M.T., Muñoz, A., Terrazas, E., Guieysse, B., Mattiasson, B., 2006. Sequential removal of heavy metals ions and organic pollutants using an algal-bacterial consortium. Chemosphere 63, 903-911.

Naumann, D., 2001. FT-Infrared and FT-Raman Spectroscopy in Biomedical Research. Applied Spectroscopy Reviews 36, 239-298.

Nedwell, D.B., 1999. Effect of Low Temperature on Microbial Growth: Lowered Affinity for Substrates Limits Growth at Low Temperature. FEMS Microbiology Ecology 30, 101-111.

Ng, T.W., Cai, Q., Wong, C.-K., Chow, A.T., Wong, P.-K., 2010. Simultaneous Chromate Reduction and Azo Dye Decolourization by *Brevibacterium casei*: Azo Dye as Electron Donor for Chromate Reduction. Journal of Hazardous Materials 182, 792-800.

Nies, D.H., 1992. Resistance to Cadmium, Cobalt, Zinc, and Nickel in Microbes. Plasmid 27, 17-28.

Nies, D.H., 1999. Microbial Heavy-Metal Resistance. Applied Microbiology and Biotechnology 51, 730-750.

Nies, D.H., 2000. Heavy Metal-Resistant Bacteria as Extremophiles: Molecular Physiology and Biotechnological use of *Ralstonia* sp. CH34. Extremophiles 4, 77-82.

Ohtake, H., Cervantes, C., Silver, S., 1987. Decreased Chromate Uptake in *Pseudomonas fluorescens* carrying A Chromate Resistance Plasmid. Journal of Bacteriology 169, 3853-3856.

Okpokwasili, G.C., Nweke, C.O., 2005. Microbial Growth and Substrate Utilization Kinetics. African Journal of Biotechnology 5, 205-317.

Olafson, R.W., McCubbin, W.D., Kay, C.M., 1988. Primary- and Secondary-Structural Analysis of A Unique Prokaryotic Metallothionein from a *Synechococcus* sp. Cyanobacterium. Biochemical Journal 251, 691-699.

Ölmezoğlu, E., Herand, B.K., Öncel, M.S., Tunç, K., Özkan, M., 2012. Copper Bioremoval by Novel Bacterial Isolates and Their Identification by 16S rRNA Gene Sequence Analysis. Turkish Journal of Biology 36, 469-476.

Othman, A.R., Bakar, N.A., Halmi, M.I.E., Johari, W.L.W., Ahmad, S.A., Jirangon, H., Syed, M.A., Shukor, M.Y., 2013. Kinetics of Molybdenum Reduction to Molybdenum Blue by *Bacillus* sp. Strain A.rzi. BioMed Research International 2013, 9.

Ottengraf, S.P.P., van den Oever, A.H.C., 1983. Kinetics of Organic Compound Removal from Waste Gases with A Biological Filter. Biotechnology and Bioengineering 25, 3089-3102.

Pal, A., Paul, A.K., 2004. Aerobic Chromate Reduction by Chromium-Resistant Bacteria Isolated from Serpentine Soil. Microbiological Research 159, 347-354.

Paluszkiewicz, C., Kwiatek, W.M., 2001. Analysis of Human Cancer Prostate Tissues using FTIR Microspectroscopy and SRIXE Techniques. Journal of Molecular Structure 565–566, 329-334.

Pandi, M., Shashirekha, V., Swamy, M., 2009. Bioabsorption of Chromium from Retan Chrome Liquor by *Cyanobacteria*. Microbiological Research 164, 420-428.

Panneerselvam, P., Choppala, G., Kunhikrishnan, A., Bolan, N., 2013. Potential of Novel Bacterial Consortium for the Remediation of Chromium Contamination. Water, Air, & Soil Pollution 224, 1-11.

Park, D., Yun, Y.-S., Park, J.M., 2005. Studies on Hexavalent Chromium Biosorption by Chemically-Treated Biomass of *Ecklonia sp*. Chemosphere 60, 1356-1364.

Powell, E.O., 1967. The Growth Rate of Microorganisms as Function of Substrate Concentration, in: Evans, C., Strange, R. (Eds.), Microbiology physiology and continuous culture. HMSO, 34-56.

Preetha, B., Viruthagiri, T., 2007. Bioaccumulation of Chromium (VI), Copper (II) And Nickel (II) Ions by Growing *Rhizopus arrhizus*. Biochemical Engineering Journal 34, 131-135.

Pümpel, T., Ebner, C., Pernfuß, B., Schinner, F., Diels, L., Keszthelyi, Z., Stankovic, A., Finlay, J.A., Macaskie, L.E., Tsezos, M., Wouters, H., 2001. Treatment of Rinsing Water from Electroless Nickel Plating with A Biologically Active Moving-Bed Sand Filter. Hydrometallurgy 59, 383-393.

Putra, W.P., Kamari, A., Yusoff, S.N.M., Ishak, C.F., Mohamed, A., Hashim, N., Isa, I.M., 2014. Biosorption of Cu(II), Pb(II) and Zn(II) Ions from Aqueous Solutions using Selected Waste Materials: Adsorption and Characterisation Studies. Journal of Encapsulation and Adsorption Sciences 4, 25-35.

Quesnel, D., Nakhla, G., 2006. Removal Kinetics of Acetone and MIBK from A Complex Industrial Wastewater by An Acclimatized Activated Sludge. Journal of Hazardous Materials 132, 253-260.

Raghuvanshi, S., Babu, B.V., 2009. Experimental Studies and Kinetic Modeling for Removal of Methyl Ethyl Ketone using Biofiltration. Bioresource Technology 100, 3855-3861.

Raghuvanshi, S., Babu, B.V., 2010. Biodegradation Kinetics of Methyl Iso-Butyl Ketone by Acclimated Mixed Culture. Biodegradation 21, 31-42.

Rahman, R.N.Z.A., Geok, L.P., Basri, M., Salleh, A.B., 2005. Physical Factors Affecting the Production of Organic Solvent-Tolerant Protease by *Pseudomonas aeruginosa* Strain K. Bioresource Technology 96, 429-436.

Rai, L.C., Gaur, J.P., Kumar, H.D., 1981. Phycology and Heavy-Metal Pollution. Biological Reviews 56, 99-151.

Ramakrishna, K., Philip, L., 2005. Bioremediation of Cr(VI) in Contaminated Soils. Journal of Hazardous Materials 121, 109-117.

Reddad, Z., Gérente, C., Andrès, Y., Thibault, J.-F., Le Cloirec, P., 2003. Cadmium and Lead Adsorption by A Natural Polysaccharide in MF Membrane Reactor: Experimental Analysis and Modelling. Water Research 37, 3983-3991.

Rezić, T., Zeiner, M., Šantek, B., Novak, S., 2011. Mathematical Modeling of Fe(II), Cu(II), Ni(II) and Zn(II) Removal in A Horizontal Rotating Tubular Bioreactor. Bioprocess and Biosystems Engineering 34, 1067-1080.

Richards, R.G., Mullins, B.J., 2013. Using microalgae for combined lipid production and heavy metal removal from leachate. Ecological Modelling 249, 59-67.

Rouch, D., Camakaris, J., Lee, B.T.O., Luke, R.K.J., 1985. Inducible Plasmid-Mediated Copper Resistance in *Eseherichia coli*. Journal of General Microbiology 131, 939-943.

Rouch, D.A., Lee, B.T., Morby, A.P., 1995. Understanding Cellular Responses to Toxic Agents: A Model for Mechanism-Choice in Bacterial Metal Resistance. Journal of Industrial Microbiology 14, 132-141.

Saffaj, N., Loukili, H., Younssi, S.A., Albizane, A., Bouhria, M., Persin, M., Larbot, A., 2004. Filtration of Solution Containing Heavy Metals and Dyes by Means of Ultrafiltration Membranes Deposited on Support Made of Moroccan Clay. Desalination 168, 301-306.

Samuel, J., Paul, M.L., Pulimi, M., Nirmala, M.J., Chandrasekaran, N., Mukherjee, A., 2012. Hexavalent Chromium Bioremoval through Adaptation and Consortia Development from Sukinda Chromite Mine Isolates. Industrial & Engineering Chemistry Research 51, 3740-3749.

Sandau, E., Sandau, P., Pulz, O., 1996. Heavy Metal Sorption by Microalgae. Acta Biotechnologica 16, 227-235.

Sapari, N., Idris, A., Hamid, N.H.A., 1996. Total Removal of Heavy Metal from Mixed Plating Rinse Wastewater. Desalination 106, 419-422.

Sargın, İ., Arslan, G., Kaya, M., 2016. Microfungal spores (*Ustilago maydis* and *U. digitariae*) immobilised chitosan microcapsules for heavy metal removal. Carbohydrate Polymers 138, 201-209.

Savin, I.-I., Butnaru, R., 2008. Wastewater Characteristics in Textile Finishing Mills. Environmental Engineering and Management Journal 7, 859-864.

Savvaidis, I., Hughes, M., Poole, R., 2003. Copper Biosorption by *Pseudomonas cepacia* and Other Strains. World Journal of Microbiology and Biotechnology 19, 117-121.

Schulz, H., Baranska, M., 2007. Identification and Quantification of Valuable Plant Substances by IR and Raman Spectroscopy. Vibrational Spectroscopy 43, 13-25.

Semerjian, L., Ayoub, G.M., 2003. High-pH-Magnesium Coagulation-Flocculation in Wastewater Treatment. Advances in Environmental Research 7, 389-403.

Şengör, S.S., Barua, S., Gikas, P., Ginn, T.R., Peyton, B., Sani, R.K., Spycher, N.F., 2009. Influence of Heavy Metals on Microbial Growth Kinetics Including Lag Time: Mathematical Modeling and Experimental Verification. Environmental Toxicology and Chemistry 28, 2020-2029.

Shamim, S., Rehman, A., 2012. Cadmium Resistance and Accumulation Potential of *Klebsiella pneumoniae* Strain CBL-1 Isolated from Industrial Wastewater. Pakistan Journal of Zoology 44, 203-208.

Shammas, N.K., 2004. Coagulation and flocculation, in: Wang L.K., Hung Y.T., Shammas N.K. (Eds.), Physicochemical Treatment Processes. Humana Press, New Jersey, 103-140.

Shen, H., Wang, Y., 1994. Biological Reduction of Chromium by *E. coli*. Journal of Enviromental Engineering 120, 560-572.

Shuler, M.L., Kargi, F., 2002. Bioprocess Engineering: Basic Concepts, 2 ed. Prentice Hall PTR, New Jersey.

Singh, P.K., Singh, A.L., Kumar, A., Singh, M.P., 2012. Mixed Bacterial Consortium as An Emerging Tool to Remove Hazardous Trace Metals from Coal. Fuel 102, 227-230.

Singh, R., Bishnoi, N.R., Kirrolia, A., 2013. Evaluation of *Pseudomonas aeruginosa* An Innovative Bioremediation Tool in Multi Metals Ions from Simulated System using Multi Response Methodology. Bioresource Technology 138, 222-234.

Slater, C.S., Ahlert, R.C., Uchrin, C.G., 1983. Applications of Reverse Osmosis to Complex Industrial Wastewater Treatment. Desalination 48, 171-187.

Sprocati, A.R., Alisi, C., Segre, L., Tasso, F., Galletti, M., Cremisini, C., 2006. Investigating Heavy Metal Resistance, Bioaccumulation and Metabolic Profile of

A Metallophile Microbial Consortium Native to An Abandoned Mine. Science of The Total Environment 366, 649-658.

Srinath, T., Verma, T., Ramteke, P.W., Garg, S.K., 2002. Chromium (VI) Biosorption and Bioaccumulation by Chromate Resistant Bacteria. Chemosphere 48, 427-435.

Srivastava, N.K., Majumder, C.B., 2008. Novel Biofiltration Methods for The Treatment of Heavy Metals from Industrial Wastewater. Journal of Hazardous Materials 151, 1- 8.

Srivastava, P.K., Vaish, A., Dwivedi, S., Chakrabarty, D., Singh, N., Tripathi, R.D., 2011. Biological removal of arsenic pollution by soil fungi. Science of The Total Environment 409, 2430-2442.

Srivastava, S., Mago, R., Bhagat, R., 1998. Microbes: For Health, Wealth and Sustainable Environment. 1st ed., Malhotra Publishing House, New Delhi.

Srivastava, S., Srivastava, A.K., 2005. Studies on Phosphate Uptake by *Acinetobacter calcoaceticus* under Aerobic Conditions. Enzyme and Microbial Technology 36, 362-368.

Srivastava, S., Thakur, I.S., 2006. Isolation and Process Parameter Optimization of *Aspergillus* sp. for Removal of Chromium from Tannery Effluent. Bioresource Technology 97, 1167-1173.

Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M., Kumar, S., 2011. MEGA5: Molecular Evolutionary Genetics Analysis using Maximum Likelihood, Evolutionary Distance, and Maximum Parsimony Methods. Molecular Biology and Evolution 28, 2731-2739.

Tunali, S., Çabuk, A., Akar, T., 2006. Removal of Lead and Copper Ions from Aqueous Solutions by Bacterial Strain Isolated from Soil. Chemical Engineering Journal 115, 203-211.

Tziotzios, G., Dermou, E., Politi, D., Vayenas, D.V., 2008. Simultaneous Phenol Removal and Biological Reduction of Hexavalent Chromium in A Packed-Bed Reactor. Journal of Chemical Technology & Biotechnology 83, 829-835.

UNICEF, 2013. Water in India: Situation and Prospects 11.

USEPA, 2009. National Primary Drinking Water Regulations. U.S. Environmental Protection Agency.

Valls, M., de Lorenzo, V., Gonzàlez-Duarte, R., S, A., 2000. Engineering Outer Membrane Proteins in *Pseudomonas putida* for Enhanced Heavy-Metal Bioadsorption. Journal of Inorganic Biochemistry 79, 219-223.

Van Niftrik, L., Geerts, W.J.C., van Donselaar, E.G., Humbel, B.M., Yakushevska, A., Verkleij, A.J., Jetten, M.S.M., Strous, M., 2008. Combined Structural and Chemical Analysis of The Anammoxosome: A Membrane-Bounded Intracytoplasmic Compartment In Anammox Bacteria. Journal of Structural Biology 161, 401-410.

Vaxevanidou, K., Papassiopi, N., Ioannis Paspaliaris, 2008. Removal of heavy metals and arsenic from contaminated soils using bioremediation and chelant extraction techniques. Chemosphere 70, 1329-1337.

Veglió, F., Beolchini, F., Gasbarro, A., 1997. Biosorption of Toxic Metals: An Equilibrium Study using Free Cells of *Arthrobacter* sp. Process Biochemistry 32, 99-105.

Velmurugan, P., Shim, J., You, Y., Choi, S., Kamala-Kannan, S., Lee, K.-J., Kim, H.J., Oh, B.-T., 2010. Removal of Zinc by Live, Dead, and Dried Biomass of *Fusarium* spp. Isolated from The Abandoned-Metal Mine in South Korea and Its Perspective of Producing Nanocrystals. Journal of Hazardous Materials 182, 317-324.

Venitt, S., Levy, L.S., 1974. Mutagenicity of Chromates in Bacteria and Its Relevance to Chromate Carcinogenesis. Nature 250, 493-495.

Viamajala, S., Peyton, B.M., Petersen, J.N., 2003. Modeling Chromate Reduction in *Shewanella oneidensis* MR-1: Development of A Novel Dual-Enzyme Kinetic Model. Biotechnology and Bioengineering 83, 790-797.

Vidal, L.O., Granéli, W., Daniel, C.B., Heiberg, L., Roland, F., 2011. Carbon and Phosphorus Regulating Bacterial Metabolism in Oligotrophic Boreal Lakes. Journal of Plankton Research 33, 1747-1756.

Volesky, B., Holan, Z.R., 1995. Biosorption of Heavy Metals. Biotechnology Progress 11, 235-250.

Wang, C.H., Huang, C.P., Sanders, P.F., 2002. Transport of Cr(VI) in Soils Contaminated with Chromite Ore Processing Residue (COPR). Practice Periodical of Hazardous Toxic and Radioactive Waste Management 6, 6-13.

Wang, L.K., Vaccari, D.A., Li, Y., Shammas, N.K., 2004. Chemical Precipitation, in: L.K. Wang, Y.T. Hung, N.K. Shammas (Eds.), Physicochemical Treatment Processes. Humana Press, 141-198.

Wang, P.-C., Mori, T., Komori, K., Sasatsu, M., Toda, K., Ohtake, H., 1989. Isolation and Characterization of an *Enterobacter cloacae* Strain that Reduces Hexavalent Chromium under Anaerobic Conditions. Applied and Environmental Microbiology 55, 1665-1669.

Wang, Y.-T., Shen, H., 1997. Modelling Cr(VI) Reduction by Pure Bacterial Cultures. Water Research 31, 727-732.

Watkins, K., 2006. Human Development Report 2006-Beyond Scarcity: Power, Poverty and The Global Water Crisis. UNDP Human Development Reports (2006).

Wilde, E.W., Benemann, J.R., 1993. Bioremoval of Heavy Metals by The use of Microalgae. Biotechnology Advances 11, 781-812.

Willow, M.A., Cohen, R.R.H., 2003. pH, Dissolved Oxygen, and Adsorption Effects on Metal Removal in Anaerobic Bioreactors. Journal of Environmental Quality 32, 1212-1221.

Wong, K.K., Quilty, B., Hamzah, A., Surif, S., 2015. Phenol Biodegradation and Metal Removal by a Mixed Bacterial Consortium. Bioremediation Journal 19, 104-112.

Xie, J.Z., Chang, H.-L., Kilbane Ii, J.J., 1996. Removal and Recovery of Metal Ions from Wastewater using Biosorbents and Chemically Modified Biosorbents. Bioresource Technology 57, 127-136.

Xing, Y., Peng, H.-y., Li, X., Zhang, M.-x., Gao, L.-l., Yang, X.-e., 2012. Extraction and isolation of the salidroside-type metabolite from zinc (Zn) and cadmium (Cd) hyperaccumulator Sedum alfredii Hance. Journal of Zhejiang University. Science. B 13, 839-845.

Yadav, K., Mandal, A., Chakraborty, R., 2013. Copper Susceptibility in *Acinetobacter junii* BB1A is Related to The Production of Extracellular Polymeric Substances. Antonie van Leeuwenhoek 104, 261-269.

Yan, G., Viraraghavan, T., 2001. Heavy Metal Removal in A Biosorption Column by Immobilized *M. rouxii* Biomass. Bioresource Technology 78, 243-249.

Yang, X.J., Fane, A.G., MacNaughton, S., 2001. Removal and Recovery of Heavy Metals from Wastewater by Supported Liquid Membranes. Water Science and Technology 43, 341-348.

Yao, Z.Y., Qi, J.H., Wang, L.H., 2010. Equilibrium, Kinetic And Thermodynamic Studies on The Biosorption of Cu(II) onto Chestnut Shell. Journal of Hazardous Materials 174, 137-143.

Yusef, H.H., 1997. Bioaccumulation of Metal Cations by Free and Immobilized Cells of *Kluyveromyces marxianus*. Advances in Food Sciences 19, 120-123.

Zahoor, A., Rehman, A., 2009. Isolation of Cr(VI) Reducing Bacteria from Industrial Effluents and Their Potential Use in Bioremediation of Chromium Containing Wastewater. Journal of Environmental Sciences 21, 814-820.

Zakaria, Z.A., Suratman, M., Mohammed, N., Ahmad, W.A., 2008. Two-Stage Biological Removal of Chromium (VI) from Solution. Journal of Biotechnology 136, Supplement, S684.

Zakaria, Z.A., Zakaria, Z., Surif, S., Ahmad, W.A., 2007. Biological Detoxification of Cr(VI) using Wood-Husk Immobilized *Acinetobacter haemolyticus*. Journal of Hazardous Materials 148, 164-171.

Zhao, H., Eide, D., 1996a. The Yeast ZRT1 Gene Encodes the Zinc Transporter Protein of A High-Affinity Uptake System Induced by Zinc Limitation. Proceedings of The National Academy of Sciences 93, 2454-2458.

Zhao, H., Eide, D., 1996b. The ZRT2 Gene Encodes The Low Affinity Zinc Transporter in *Saccharomyces cerevisiae*. Journal of Biological Chemistry 271, 23203-23210.

LIST OF PUBLICATIONS

International Journals

1. Majumder, S., Raghuvanshi, S., Gupta, S., 2011. Biodegradation Kinetics of Cr(VI) by Acclimated Mixed Culture. Research Journal of Chemistry and Environment 15, 181-184.
2. Majumder, S., Raghuvanshi, S., Gupta, S., 2014. Estimation of Kinetic Parameters for Bioremediation of Cr(VI) from Wastewater using *Pseudomonas taiwanensis* an Isolated Strain from Enriched Mixed Culture. Bioremediation Journal 18, 236-247.
3. Majumder, S., Gangadhar, G., Raghuvanshi, S., Gupta, S., 2015. Biofilter Column for Removal of Divalent Copper from Aqueous Solutions: Performance Evaluation and Kinetic Modeling. Journal of Water Process Engineering 6, 136-143.
4. Majumder, S., Gangadhar, G., Raghuvanshi, S., Gupta, S., 2015. A Comprehensive Study on the Behavior of a Novel Bacterial Strain *Acinetobacter guillouiae* for Bioremediation of Divalent Copper. Bioprocess and Biosystems Engineering 38, 1749-1760.
5. Majumder, S., Raghuvanshi, S., Gupta, S., 2016. Application of a Hybrid Biofilter Column for the Removal of Cr(VI) from Aqueous Solution using an Indigenous Bacterial Strain *Pseudomonas taiwanensis*. Bioremediation Journal 20, 10-23.
6. Majumder, S., Gupta, V., Raghuvanshi, S., Gupta, S. Simultaneous Sequestration of Cr(VI), Cu(II) and Zn(II) Metal ions from Simulated Industrial Effluents by an Indigenous Bacterial Consortium. (Under Revision)
7. Majumder, S., Raghuvanshi, S., Gupta, S. A Comprehensive Study on the Bioremediation and Biofiltration of Zn(II) from Aqueous Solution using *Klebsiella pneumoniae.* (Under Review)

International Conference Proceedings

1. Majumder, S., Gupta, S., 2010. Biofiltration for Heavy Metals Removal: A State-of-the-art Review. Proceedings of International Symposium & 63rd Annual Session of IIChE in association with International Partners (CHEMCON-2010), Chidambaram, India, December 27-29, 2010.
2. Majumder, S., Raghuvanshi, S., Gupta, S. Biodegradation Kinetics of Cr (VI) by Acclimated Mixed Culture. Proceedings of 5th International Congress of Chemistry and Environment (ICCE-2011), Glory Beach Resort, Port Dickson, Negeri Senbilan, Malaysia, May 27-29, 2011.
3. Majumder, S., Dutta Biswas, D., Raghuvanshi, S., Gupta, S., 2011. Biodegradation Kinetics of Zn(II) Using Acclimated Mixed Culture. Proceedings of International Symposium & 64th Annual Session of IIChE in association with International Partners (CHEMCON-2011), Bangalore, India, December 27-29, 2011.
4. Majumder, S., Raghuvanshi, S., Gupta, S., 2012. Bioremediation of Cr(VI) by Acclimated Mixed Culture: Kinetic Study. Proceedings of 3rd World Congress on Biotechnology, Hyderabad, India, September 13-15, 2012.
5. Majumder, S., Gangadhar, G., Raghuvanshi, S., Gupta, S., 2013. Bioremediation of Cu(II) Contaminated Aqueous Solution Using *Acinetobactor* sp. Proceedings of 2013 AIChE Annual Meeting, San Francisco, USA, November 3-8, 2013.
6. Majumder, S., Singh, A., Raghuvanshi, S., Gupta, S., 2013. Bioremediation of Cr(VI) Contaminated Aqueous Solution using *Pseudomonas* sp. Proceedings of 66th Annual Session of Indian Institute of Chemical Engineers and Joint Indo North American Symposium (CHEMCON-2013), Mumbai, India, December 27-30, 2013.
7. Majumder, S., Singh, A., Raghuvanshi, S., Gupta, S., 2014. Bioremediation Growth Kinetics of Cr(VI) Contaminated Aqueous Solution using *Pseudomonas* sp., an Indigenous Microbial Strain Isolated from Aerobic Mixed Culture. Proceedings of 2014 International Congress on Environmental, Biotechnology, and Chemistry Engineering, Pune, India, February 21-23, 2014.
8. Majumder, S., Naini, P., Raghuvanshi, S., Gupta, S., 2014. Optimization of Process Parameters in Bioremediation of Cr(VI) Contaminated Aqueous Solution through

Response Surface Methodology. Proceedings of 2014 AIChE Annual Meeting, Atlanta, USA, November 16-21, 2014.

9. Majumder, S., Gangadhar, G., Raghuvanshi, S., Gupta, S., 2014. Optimization of Divalent Copper Bioremediation by *Acinetobacter guillouiae* using Response Surface Methodology. Proceedings of 67th Annual Session of Indian Institute of Chemical Engineers (CHEMCON-2014), Chandigarh, India, December 27-30, 2014.

National Conference Proceedings

1. Gangadhar, G., Majumder, S., Raghuvanshi, S., Gupta, S., 2012. Bioremediation Kinetics of Cu(II) Using Acclimated Mixed Culture. Proceedings of Technological Advancements in Chemical and Environmental Engineering (TACEE-2012), Pilani, India, May 23-24, 2012.

Chapters in Book

1. Majumder, S., Raghuvanshi, S., Gupta, S., 2014. Removal of dissolved metals by Bio-remediation. In 'Heavy Metals in Water', Edited by S. Sharma, RSC Publishing, UK, pp. 44-56. ISBN (online): 978-1-84973-885-9 (September 2014).
2. Raghuvanshi, S., Majumder, S., Gupta, S., 2012. Biofiltration: Essentials, Research and Applications. In 'Bioremediation and Sustainability: Research and Applications', Edited by R. Mohee and A. Mudhoo, Wiley-Scrivener Publishing LLC., USA, pp. 285-315. ISBN (online): 9781118371220 (April 2012).

BIOGRAPHIES

Biography of the Candidate

Subhajit Majumder did his B.E. degree in Chemical Engineering from University of Pune in 2003. He completed his M.E. degree in Chemical Engineering from BITS-Pilani, Pilani Campus in 2007. He is having 3 years work experience in both healthcare science and process industry. He joined BITS-Pilani, Pilani campus in August, 2009 as an Assistant Lecturer. Currently, he is working as a Lecturer in this institute. He has 6 years of teaching experience. He has guided 3 M.E. Dissertation students, 3 Professional Practice I & II students, 3 B.E. Thesis students and 20 Project students. He has taught courses such as Process Design Principles, Biochemical Engineering, Modeling & Simulation in Chemical Engineering, Heat Transfer, Fluid Mechanics, Chemical Process Calculations and Process Equipment Design. He was involved in the tutorials of Selected Chemical Engineering Operations, Separation Processes, Process Control, Kinetic Reactor & Design and Engineering Thermodynamics. He received UGC Minor Research Project in 2013. His research interests include environmental engineering (bio-based separation techniques), nano-biotechnology, process design and retrofitting, process intensification (mass and heat integration).

Biography of Supervisor

Prof. Suresh Gupta is an Associate Professor in Chemical Engineering Department at BITS-Pilani, Pilani Campus. He completed his PhD from BITS-Pilani, Pilani Campus. He is involved in various academic and administrative committees at BITS-Pilani. He has been heading the Chemical Engineering Department since September, 2012. He is the coordinator for UGC Assistance to the Department of Chemical Engineering of DRS under Special Assistance Programme. He is the member of Implementation Committee of DST-FIST Support for Chemical Engineering Department. He is the chairperson of Department Committee on Academics (DCA). He is the Vice-Chariman of Pilani Regional Centre of Indian Institute of Chemical Engineers (IIChE). He is also involved in Departmental Research Committee of Chemical Engineering Department as the chairperson. He is one of the members of Library Committee, Research Board and Standing Committee of Student Discipline in BITS-Pilani, Pilani Campus. He was also actively involved in Instruction Division as Nucleus Member. He has 13 years of Teaching and Research experience. He also has 8 years of Administrative experience. He has guided 9 M.E. Dissertation students. Currently, he is guiding 6 Ph.D. students. He currently has 4 research and sponsored projects from UGC, DST and BITS-Pilani. He successfully completed 3 research and consultancy projects of MHRD, UGC and Birla Cellulosic. He is the PhD Examiner

of 1 candidate. He was also an External Examiner for BTech (Chemical Engg.) students at Banasthali University

His research interests include Environmental Engineering & Separation Processes, Bioleaching of Metal Ions from Industrial Waste, Mathematical Modeling and Simulation of Chemical Processes, Computational Transport Phenomena, Environmental Management Systems (LCA, EIA) and Energy Integration. He has around 80 research publications including International & National Journals and Conference Proceedings to his credit. He has also published 7 chapters and invited articles in various books, lecture notes, national and international journals. He was invited as distinguished speaker in 3 national universities Prof. Gupta was invited as a member in a workshop to review the existing scheme of the Engineering Services Examination conducted by the Commission organized by UPSC at the International Management Development Centre (IMDC), Indian Institute of Management, Vastrapur, Ahmedabad. He is an External Academic Expert Member in Board of Studies (BoS) Constitution for Chemical Engineering, Galgotia University, Noida. He visited various industries such as Ultratech Cement, Reddipalay. m Cement Works, IndoGulf Fertilizer, Birla Cellulosic Kharach, Bharat Aluminum Company Limited and Hindustan Zinc Limited for discussions on BITS Collaborative Programme on Academic Development Programme in Basic Process Engineering for their Employees. He also served as a resource faculty in Practice School Division, BITS-Pilani.

He is the reviewer of 16 International Repute Journals. He is a Life Member of Indian Institute of Chemical Engineers (IIChE), fellow member of International Congress of Chemistry and Environment (FICCE). He is also a members of American Institute of Chemical Engineers (AIChE), International Association of Engineers (IAENG) and Institute of Engineers (India) (IEI).

Biography of Co-Supervisor

Dr. Smita Raghuvanshi is working as an Assistant Professor in Chemical Engineering Department, BITS-Pilani, Pilani Campus. She did her Ph.D. from BITS-Pilani, Pilani Campus. She is the Nucleus Member of Practice School Division, BITS-Pilani since 2003. She is involved in various academic committees such as Departmental Research, Library, Ph.D. Qualifying Examinations, Doctoral Advisory and many more. She is having 12 years of Teaching and Research experience. Currently, she is guiding 2 Ph.D. students and 4 M.E. Dissertation students. She has guided 10 students in Professional Practice I & II, 3 students in B.E. Thesis and 1 student in M.E. Dissertation.

She is having vast expertise in Environmental Engineering & Separation Processes (biofiltration for removal of VOCs and metals; CO_2 mitigation using bio-based techniques; design of bioreactor), Mathematical Modeling and Simulation

(generalized modeling for bio-based processes; kinetic modeling of biodegradation and biofiltration process) and Environmental Management System (environmental impact assessment of various processes and life cycle assessment of industrial processes). She has two ongoing sponsored projects from DST and UGC and one completed research project from BITS-Pilani.

She has around 40 research publications in International & National Journals and Conferences. She also published 4 chapters and invited articles in various books and technical notes. She was awarded First Prize in the Paper Presentation on "Removal of Methyl Ethyl Ketone (MEK) using Biofiltration" in National Conference on Environmental Conservation (NCEC-2006), held at BITS-Pilani, September 1-3, 2006. She is the reviewer of 4 international journals Journal of Physical Sciences, Biodegradation, Journal of Food Processing & Technology and Nanoscience & Nanotechnology. She has shown exemplary acumen in application of environmental impact assessment method for integrated solid waste management activities and Physico-Chemical Analysis & Environmental Impact Assessment of Textile Industry Effluent. She is a Life Member of Indian Institute of Chemical Engineers (IIChE) & fellow member of International Congress of Chemistry and Environment (ICCE). She is also a member of American Institute of Chemical Engineers (AIChE), International Association of Engineers (IAENG) and Institute of Engineers (India) (IEI).

APPENDIX I

Raw data for batch experimental studies conducted

Table -A1.1. Cr(VI) removal by *P. taiwanensis* at different time for various initial Cr(VI) concentrations

(T = 37 ^{o}C, pH = 7.0±0.2, RPM = 150, S_o = 20-200 mg L^{-1})

S No	Time (h)	Final Cr(VI) concentration (mg L^{-1})						
		20 mg L^{-1}	40 mg L^{-1}	60 mg L^{-1}	80 mg L^{-1}	100 mg L^{-1}	150 mg L^{-1}	200 mg L^{-1}
1	2	0.001	0.014	0.018	0.019	0.022	0.035	0.057
2	4	0.002	0.102	0.115	0.115	0.125	0.120	0.150
3	6	4.418	0.985	1.200	1.500	0.900	1.250	1.000
4	8	6.775	3.750	4.500	2.850	1.250	1.600	1.150
5	10	8.778	10.115	7.800	8.500	2.220	3.000	2.250
6	12	11.842	18.745	15.000	14.500	4.250	4.500	4.250
7	14	14.775	24.438	22.500	22.500	7.750	7.500	8.750
8	16	18.977	31.258	29.750	27.500	13.250	12.250	14.500
9	18	19.994	37.445	37.500	33.250	17.750	17.50	18.250
10	20	19.997	38.985	42.500	41.250	22.500	22.500	23.250
11	22	-	39.992	51.250	49.000	27.500	27.250	32.500
12	24	-	-	55.105	54.250	36.250	39.500	38.500
13	26	-	-	58.975	62.250	43.500	47.250	45.000
14	28	-	-	59.988	68.750	56.250	57.250	51.250
15	30	-	-	-	71.250	63.750	66.250	58.150
16	32	-	-	-	72.250	71.500	76.150	67.500
17	36	-	-	-	72.250	77.500	87.250	74.250
18	38	-	-	-	72.250	81.500	95.000	81.250
19	40	-	-	-	-	81.500	103.000	89.000
20	42	-	-	-	-	-	108.250	97.250
21	44	-	-	-	-	-	108.250	103.500
22	46	-	-	-	-	-	-	103.500

Table -A1.2. Cu(II) removal by *A. guillouiae* at different time for various initial Cu(II) concentrations

(T = 37 ^{o}C, pH = 7.0±0.2, RPM = 150, S_o = 20-200 mg L^{-1})

S No	Time (h)	Final Cu(II) concentration (mg L^{-1})						
		20 mg L^{-1}	40 mg L^{-1}	60 mg L^{-1}	80 mg L^{-1}	100 mg L^{-1}	150 mg L^{-1}	200 mg L^{-1}
1	2	11.250	23.350	42.250	60.137	79.040	121.976	131.250
2	4	12.250	25.548	44.224	62.224	84.168	124.720	133.250
3	6	12.750	27.500	46.250	64.250	86.750	129.750	138.250
4	8	13.250	30.950	49.950	69.932	90.664	131.448	142.250
5	10	14.250	32.250	52.450	71.448	90.520	139.920	145.750
6	12	15.000	34.450	54.250	72.230	92.500	142.250	148.570
7	14	15.250	36.980	56.280	76.224	94.013	144.912	151.580
8	16	16.750	37.980	58.990	78.936	95.168	145.035	154.750
9	18	17.250	38.990	58.980	78.768	96.880	145.344	158.850
10	20	17.250	38.980	58.850	78.480	97.632	146.322	162.250
11	24	17.250	38.980	58.840	78.400	94.368	144.024	162.250
12	28	17.000	38.980	58.830	78.400	94.368	144.024	162.300
13	32	17.200	38.980	58.850	78.400	94.368	144.024	162.250

Table -A1.3. Zn(II) removal by *K. pneumoniae* at different time for various initial Zn(II) concentrations (T = 37 ^{o}C, pH = 7.0±0.2, RPM = 150, S_o = 20-200 mg L^{-1})

S No	Time (h)	Final Zn(II) concentration (mg L^{-1})						
		20 mg L^{-1}	40 mg L^{-1}	60 mg L^{-1}	80 mg L^{-1}	100 mg L^{-1}	150 mg L^{-1}	200 mg L^{-1}
1	2	11.250	17.750	35.780	54.500	72.589	111.250	125.560
2	4	11.750	23.580	38.580	56.250	77.856	115.693	127.980
3	6	13.247	26.890	41.250	58.850	79.850	121.890	131.660
4	8	13.588	28.850	44.562	61.250	82.580	127.650	132.220
5	10	14.012	31.580	46.532	62.530	84.560	131.780	134.702
6	12	14.870	31.980	47.500	64.250	85.250	132.220	135.580
7	14	15.789	32.620	48.590	65.580	86.950	134.680	136.630
8	16	16.025	36.850	52.068	67.580	89.580	136.250	138.540
9	18	17.750	37.780	55.259	69.850	91.025	138.560	141.520
10	20	18.025	38.025	57.250	72.560	92.520	140.020	144.250
11	22	18.110	39.000	57.200	74.580	93.450	140.510	143.250
12	24	18.500	39.520	57.200	76.580	93.560	141.250	149.530
13	26	18.490	39.500	57.220	76.590	93.260	141.300	149.600
14	28	18.500	39.520	57.200	76.580	93.250	141.300	150.200
15	32	18.500	39.520	57.200	76.575	93.250	141.300	150.200

Table -A1.4. Growth of *P. taiwanensis* with time in the presence of Cr(VI)

(T = 37 ^{o}C, pH = 7.0±0.2, RPM = 150, 1 % v/v inoculum, S_o = 20-200 mg L^{-1})

S No	Time (h)	Biomass growth (g L^{-1})						
		20 mg L^{-1}	40 mg L^{-1}	60 mg L^{-1}	80 mg L^{-1}	100 mg L^{-1}	150 mg L^{-1}	200 mg L^{-1}
1	2	0.021	-	-	-	-	-	-
2	4	0.045	0.000	0.000	0.000	0.000	0.000	0.000
3	6	0.060	0.000	0.000	0.000	0.000	0.000	0.000
4	8	0.120	0.000	0.000	0.000	0.000	0.010	0.070
5	10	0.159	0.000	0.000	0.010	0.010	0.035	0.135
6	12	0.178	0.000	0.010	0.020	0.020	0.060	0.162
7	16	0.178	0.015	0.020	0.100	0.045	0.075	0.215
8	18	0.176	0.027	0.145	0.140	0.057	0.167	0.214
9	20	0.177	0.147	0.157	0.155	0.090	0.178	0.215
10	22	-	0.159	0.180	0.172	0.172	0.210	0.213
11	24	-	0.185	0.252	0.245	0.252	0.222	-
12	28	-	0.259	0.257	0.251	0.252	0.223	-
13	30	-	0.260	0.258	0.250	0.251	0.223	-
14	32	-	0.259	0.252	0.252	0.247	-	-
15	34	-	0.257	0.251	0.249	-	-	-
16	36	-	0.258	0.248	-	-	-	-

Table -A1.5. Growth of *A. guillouiae* with time in the presence of Cu(II)

(T = 37 ^{o}C, pH = 7.0±0.2, RPM = 150, 1 % v/v inoculum, S_o = 20-200 mg L^{-1})

S No	Time (h)	Biomass growth (g L^{-1})						
		20 mg L^{-1}	40 mg L^{-1}	60 mg L^{-1}	80 mg L^{-1}	100 mg L^{-1}	150 mg L^{-1}	200 mg L^{-1}
1	2	0.045	0.000	0.000	0.000	0.000	0.020	0.000
2	4	0.060	0.015	0.000	0.000	0.000	0.027	0.000
3	6	0.120	0.027	0.010	0.000	0.000	0.064	0.000
4	8	0.159	0.147	0.020	0.010	0.000	0.088	0.000
5	10	0.178	0.159	0.145	0.100	0.090	0.093	0.000
6	12	0.178	0.181	0.151	0.138	0.100	0.100	0.080
7	14	0.178	0.185	0.157	0.146	0.127	0.110	0.110
8	16	0.176	0.259	0.180	0.152	0.132	0.125	0.121
9	18	0.177	0.260	0.252	0.210	0.145	0.125	0.128
10	20	0.177	0.259	0.315	0.251	0.177	0.125	0.141
11	24	0.177	0.259	0.352	0.292	0.210	-	0.181
12	28	-	0.259	0.352	0.318	0.220	-	0.189
13	32	-	-	0.352	0.318	0.220	-	0.189

Table -A1.6. Growth of *K. pneumoniae* with time in the presence of Zn(II)

(T = 37 ^{o}C, pH = 7.0±0.2, RPM = 150, 1 % v/v inoculum, S_o = 20-200 mg L^{-1})

S No	Time (h)	Biomass growth (g L^{-1})						
		20 mg L^{-1}	40 mg L^{-1}	60 mg L^{-1}	80 mg L^{-1}	100 mg L^{-1}	150 mg L^{-1}	200 mg L^{-1}
1	2	0.058	0.065	0.078	0.082	0.092	0.120	0.130
2	4	0.060	0.072	0.083	0.090	0.110	0.140	0.151
3	6	0.140	0.180	0.110	0.125	0.145	0.170	0.182
4	8	0.159	0.192	0.121	0.131	0.162	0.185	0.192
5	10	0.164	0.210	0.135	0.144	0.171	0.210	0.224
6	12	0.171	0.220	0.140	0.149	0.178	0.218	0.225
7	14	0.181	0.225	0.146	0.152	0.181	0.223	0.226
8	16	0.185	0.227	0.180	0.162	0.194	0.231	0.241
9	18	0.218	0.258	0.197	0.210	0.235	0.238	0.242
10	20	0.245	0.267	0.210	0.223	0.245	0.261	0.269
11	22	0.286	0.293	0.216	0.245	0.261	0.272	0.281
12	24	0.286	0.293	0.220	0.245	0.261	0.273	0.281
13	26	0.286	0.293	0.220	0.245	0.261	0.273	0.281

Table -A1.7. Effect of pH on growth of *P. taiwanensis* in the presence of Cr(VI)

(T = 37 ^{o}C, RPM = 150, 1 % v/v inoculum, S_o = 150 mg L^{-1} of Cr(VI))

S No	pH	Biomass growth (g L^{-1})
1	2	0.013
2	4	0.015
3	6	0.018
4	7	0.024
5	8	0.018
6	10	0.013
7	12	0.009

Table -A1.8. Effect of pH on growth of *A. guillouiae* in the presence of Cu(II)

(T = 37 ^{o}C, RPM = 150, 1 % v/v inoculum, S_o = 80 mg L^{-1} of Cu(II))

S No	pH	Biomass growth (g L^{-1})
1	2	0.004
2	4	0.011
3	6	0.018
4	7	0.021
5	8	0.029
6	10	0.017
7	12	0.003

Table -A1.9. Effect of pH on growth of *K. pneumoniae* in the presence of Zn(II)

(T = 37 ^{o}C, RPM = 150, 1 % v/v inoculum, S_o = 40 mg L^{-1} of Zn(II))

S No	pH	Biomass growth (g L^{-1})
1	2	0.016
2	4	0.040
3	5	0.081
4	6	0.151
5	7	0.216
6	8	0.189
7	10	0.043

Table -A1.10. Effect of Nutrient (MSM) dosage on growth of *P. taiwanensis* in the presence of Cr(VI)

(T = 37 ^{o}C, pH = 7.0±0.2, RPM = 150, 1 % v/v inoculum, S_o = 150 mg L^{-1} of Cr(VI))

S No	MSM dosage	Biomass growth (g L^{-1})
1	Half	0.004
2	Single	0.008
3	Double	0.025
4	Triple	0.023

Table -A1.11. Effect of Nutrient (MSM) dosage on growth of *A. guillouiae* in the presence of Cu(II)

(T = 37 ^{o}C, pH = 7.0±0.2, RPM = 150, 1 % v/v inoculum, S_o = 80 mg L^{-1} of Cu(II))

S No	MSM dosage	Biomass growth (g L^{-1})
1	Half	0.004
2	Single	0.008
3	Double	0.025
4	Triple	0.023

Table -A1.12. Effect of Nutrient (MSM) dosage on growth of *K. pneumoniae* in the presence of Zn(II)

(T = 37 ^{o}C, pH = 7.0±0.2, RPM = 150, 1 % v/v inoculum, S_o = 40 mg L^{-1} of Zn(II))

S No	MSM dosage	Biomass growth (g L^{-1})
1	Half	0.157
2	Single	0.216
3	Double	0.354
4	Triple	0.162

Table -A1.13. Effect of N-P-K composition in MSM on growth of *P. taiwanensis* (T = 37 ^{o}C, pH = 7.0±0.2, RPM = 150, 1 % v/v inoculum, S_o = 150 mg L^{-1} of Cr(VI)) (1- half the standard composition in MSM dosage, 2- standard composition in MSM dosage, 3- double composition in MSM dosage, 4- triple composition in MSM dosage)

S No	N-P-K Composition	Biomass growth (g L^{-1})		
		N	P	K
1	1	0.015	0.019	0.069
2	2	0.025	0.035	0.189
3	3	0.083	0.095	0.046
4	4	0.019	0.021	0.012

Table -A1.14. Effect of N-P-K composition in MSM on growth of *A. guillouiae* (T = 37 ^{o}C, pH = 7.0±0.2, RPM = 150, 1 % v/v inoculum, S_o = 80 mg L^{-1} of Cu(II)) (1- half the standard composition in MSM dosage, 2- standard composition in MSM dosage, 3- double composition in MSM dosage, 4- triple composition in MSM dosage)

S No	N-P-K Composition	Biomass growth (g L^{-1})		
		N	P	K
1	1	0.010	0.013	0.057
2	2	0.012	0.023	0.106
3	3	0.053	0.042	0.022
4	4	0.010	0.019	0.012

Table -A1.15. Effect of N-P-K composition in MSM on growth of *K. pneumoniae* (T = 37 ^{o}C, pH = 7.0±0.2, RPM = 150, 1 % v/v inoculum, S_o = 40 mg L^{-1} of Zn(II)) (1- half the standard composition in MSM dosage, 2- standard composition in MSM dosage, 3- double composition in MSM dosage, 4- triple composition in MSM dosage)

S No	N-P-K Composition	Biomass growth (g L^{-1})		
		N	P	K
1	1	0.019	0.045	0.125
2	2	0.086	0.078	0.278
3	3	0.091	0.099	0.035
4	4	0.013	0.028	0.026

Table -A1.16. Effect of aerobic and facultative anaerobic conditions on growth of *P. taiwanensis* in the presence of Cr(VI) (T = 37 ^{o}C, pH = 7.0±0.2, RPM = 150, 1 % v/v inoculum, S_o = 150 mg L^{-1} of Cr(VI))

S No	Condition	Biomass growth (g L^{-1})
1	Aerobic	0.252
2	Facultative anaerobic	0.095

Table -A1.17. Effect of aerobic and facultative anaerobic conditions on growth of *A. guillouiae* in the presence of Cu(II) (T = 37 ^{o}C, pH = 7.0±0.2, RPM = 150, 1 % v/v inoculum, S_o = 80 mg L^{-1} of Cu(II))

S No	Condition	Biomass growth (g L^{-1})
1	Aerobic	0.034
2	Facultative anaerobic	0.077

Table -A1.18. Effect of aerobic and facultative anaerobic conditions on growth of *K. pneumoniae* in the presence of Zn(II) (T = 37 ^{o}C, pH = 7.0±0.2, RPM = 150, 1 % v/v inoculum, S_o = 40 mg L^{-1} of Zn(II))

S No	Condition	Biomass growth (g L^{-1})
1	Aerobic	0.216
2	Facultative anaerobic	0.121

Table -A1.19. Effect of temperature on growth of *P. taiwanensis* in the presence of Cr(VI) (pH = 7.0±0.2, RPM = 150, 1 % v/v inoculum, S_o = 150 mg L^{-1} of Cr(VI))

S No	Temperature (^oC)	Biomass growth (g L^{-1})
1	25	0.010
2	30	0.012
3	35	0.021
4	37	0.024
5	40	0.023
6	45	0.009

Table -A1.20. Effect of temperature on growth of *A. guillouiae* in the presence of Cu(II) (pH = 7.0±0.2, RPM = 150, 1 % v/v inoculum, S_o = 80 mg L^{-1} of Cu(II))

S No	Temperature (^oC)	Biomass growth (g L^{-1})
1	25	0.072
2	30	0.076
3	35	0.094
4	37	0.163
5	40	0.164
6	45	0.068

Table -A1.21. Effect of temperature on growth of *K. pneumoniae* in the presence of Zn(II) (pH = 7.0±0.2, RPM = 150, 1 % v/v inoculum, S_o = 40 mg L^{-1} of Zn(II))

S No	Temperature (^oC)	Biomass growth (g L^{-1})
1	25	0.013
2	30	0.056
3	35	0.138
4	37	0.216
5	40	0.158
6	45	0.026

Table -A1.22. Effect of inoculum volume on growth of *P. taiwanensis* in the presence of Cr(VI) (T = 37 ^{o}C, pH = 7.0±0.2, RPM = 150, S_o = 150 mg L^{-1} of Cr(VI))

S No	Inoculum volume (% v/v)	Biomass growth (g L^{-1})
1	0.5	0.016
2	1.0	0.024
3	1.5	0.019
4	2.0	0.013

Table -A1.23. Effect of inoculum volume on growth of *A. guillouiae* in the presence of Cu(II) (T = 37 ^{o}C, pH = 7.0±0.2, RPM = 150, S_o = 80 mg L^{-1} of Cu(II))

S No	Inoculum volume (% v/v)	Biomass growth (g L^{-1})
1	0.5	0.008
2	1.0	0.012
3	1.5	0.009
4	2.0	0.009

Table -A1.24. Effect of inoculum volume on growth of *K. pneumoniae* in the presence of Zn(II) (T = 37 ^{o}C, pH = 7.0±0.2, RPM = 150, S_o = 40 mg L^{-1} of Zn(II))

S No	Inoculum volume (% v/v)	Biomass growth (g L^{-1})
1	0.5	0.097
2	1.0	0.216
3	1.5	0.120
4	2.0	0.089

Table -A1.25. Calculation of specific growth rate (μ) of *P. taiwanensis* for Cr(VI) removal

S No	Time (h)	ln *x*						
		20 mg L^{-1}	40 mg L^{-1}	60 mg L^{-1}	80 mg L^{-1}	100 mg L^{-1}	150 mg L^{-1}	200 mg L^{-1}
1	6	-2.65	-	-	-	-	-	-
2	8	-2.125	-2.75	-	-	-	-	-
3	10	-1.75	-2.5	-3.352	-	-	-	-
4	12	-1.5	-1.82	-2.813	-3.5	-	-	-
5	14	-	-1.537	-2.4	-3.101	-	-	-
6	16	-	-	-1.789	-2.864	-3.25	-	-
7	18	-	-	-1.5	-2.407	-2.61	-	-
8	20	-	-	-1.25	-1.76	-2.25	-3.01	-
9	22	-	-		-1.378	-1.76	-2.5	-3.611
10	24	-	-		-	-1.406	-2.01	-2.51
11	26	-	-		-		-1.378	-1.838

Table -A1.26. Calculation of specific growth rate (μ) of *A. guillouiae* for Cu(II) removal

S No	Time (h)	ln *x*						
		20 mg L^{-1}	40 mg L^{-1}	60 mg L^{-1}	80 mg L^{-1}	100 mg L^{-1}	150 mg L^{-1}	200 mg L^{-1}
1	2	-3.91202	-	-	-	-	-	-
2	4	-3.5	-2.81341	-	-	-	-	-
3	6	-2.74887	-2.12026	-2.5188	-	-	-	-
4	8	-2.43612	-1.751	-2.251	-2.758	-2.95651	-	-
5	10	-	-1.25127	-1.83885	-2.572	-2.75259	-	-
6	14	-	-	-1.211	-1.85151	-2.2599	-1.74297	-1.65026
7	16	-	-	-	-1.7148	-1.9421	-1.60944	-1.258
8	18	-	-	-	-1.37833	-	-1.24982	-0.96758
9	20	-	-	-	-1.15518	-	-0.85567	-0.74444

Table -A1.27. Calculation of specific growth rate (μ) of *K. pneumoniae* for Zn(II) removal

S No	Time (h)	ln *x*						
		20 mg L^{-1}	**40 mg L^{-1}**	**60 mg L^{-1}**	**80 mg L^{-1}**	**100 mg L^{-1}**	**150 mg L^{-1}**	**200 mg L^{-1}**
1	6	-1.96611	-	-	-	-	-	-
2	8	-1.90189	-1.65026	-	-	-	-	-
3	10	-1.82279	-1.60648	-2.00248	-	-	-	-
4	14	-1.70926	-1.49165	-1.80415	-1.88387	-	-	-
5	16	-	-	-1.7148	-1.82016	-1.5999	-1.49534	-1.42296
6	18	-	-	-1.62455	-1.72065	-1.51817	-1.43548	-1.38082
7	20	-	-	-	-1.60106	-1.4065	-1.34323	-1.31342
8	22	-	-	-	-1.50609	-1.34323	-	-1.2694
9	24	-	-	-	-	-	-	-
10	26	-	-	-	-	-	-	-
11	28	-	-	-	-	-	-	-
12	30	-	-	-	-	-	-	-
13	32	-	-	-	-	-	-	-

Table -A1.28. Zero-order kinetics for Cr(VI) bioremediation at different initial concentrations of Cr(VI)

S No	Time (h)	Cr(VI) concentration obtained during log phase (mg L^{-1})						
		20 mg L^{-1}	40 mg L^{-1}	60 mg L^{-1}	80 mg L^{-1}	100 mg L^{-1}	150 mg L^{-1}	200 mg L^{-1}
1	4	-	-	-	-	-	-	-
2	6	15.582	-	-	-	-	-	-
3	8	13.225	36.25	-	-	-	-	-
4	10	11.222	29.885	52.2	-	-	-	-
5	12	-	21.255	45	65.5	-	-	-
6	14	-	-	37.5	57.5	92.25	-	-
7	16	-	-	30.25	52.5	86.75	137.75	185.5
8	18	-	-	-	46.75	82.25	132.5	181.75
9	20	-	-	-	38.75	77.5	127.5	176.75
10	22	-	-	-	-	72.5	122.75	167.5
11	24	-		-	-	63.75	110.5	-

Table -A1.29. Zero-order kinetics for Cu(II) bioremediation at different initial concentrations of Cu(II)

S No	Time (h)	Cu(II) concentration obtained during log phase (mg L^{-1})						
		20 mg L^{-1}	40 mg L^{-1}	60 mg L^{-1}	80 mg L^{-1}	100 mg L^{-1}	150 mg L^{-1}	200 mg L^{-1}
1	4	17.75	14.452	-	-	-	-	-
2	6	11.25	12	-	-	-	-	-
3	8	7.85	9.05	-	10.068	-	-	-
4	10	5.52	-	-	8.552	9.48	-	-
5	14	-	-	-	3.776	5.987	10	-
6	16	-	-	13.75	-	4.832	7.5	-
7	18	-	-	10.05	-	3.12	4.656	30.25
8	20	-	-	7.55	-	-	-	27.5
9	24	-	-	13.75	-	-	-	25
10	28	-	-	10.05	-	-	-	22.1
11	32	-	-	-	-	-	-	19.5

Table -A1.30. Zero-order kinetics for Zn(II) bioremediation at different initial concentrations of Zn(II)

S No	Time (h)	Cu(II) concentration obtained during log phase (mg L^{-1})						
		20 mg L^{-1}	40 mg L^{-1}	60 mg L^{-1}	80 mg L^{-1}	100 mg L^{-1}	150 mg L^{-1}	200 mg L^{-1}
1	6	13.247	-	-	-	-	-	-
2	8	13.588	28.85	46.532	-	-	-	-
3	10	14.012	31.58	48.59	-	-	-	-
4	14	15.789	32.62	52.068	65.58	-	-	-
5	16	-	-	55.259	67.58	89.58	136.25	138.54
6	18	-	-	46.532	69.85	91.025	138.56	141.52
7	20	-	-	-	72.56	92.52	140.02	144.25
8	22	-	-	-	75.57	93.1	-	146.5
9	24	-	-	-	-	-	-	149.53

Table -A1.31. Three-half-order kinetics for Cr(VI) bioremediation at different initial concentrations of Cr(VI)

S No	Time (h)	*Y*						
		20 mg L^{-1}	40 mg L^{-1}	60 mg L^{-1}	80 mg L^{-1}	100 mg L^{-1}	150 mg L^{-1}	200 mg L^{-1}
1	4	0.0625	-	-	-	-	-	-
2	6	0.0489	0.04	-	-	-	-	-
3	8	0.04	0.0325	0.021	-	-	-	-
4	10	0.0289	0.0245	0.015	0.0189	-	-	-
5	12	-	-	0.012	0.0145	0.018	-	-
6	14	-	-	-	0.011	0.014	-	-
7	16	-	-	-	-	0.01	0.009	0.007
8	18	-	-	-	-	-	0.008	0.006
9	20	-	-	-	-	-	0.006	0.004

Table -A1.32. Three-half-order kinetics for Cu(II) bioremediation at different initial concentrations of Cu(II)

S No	Time (h)	*Y*						
		20 mg L^{-1}	**40 mg L^{-1}**	**60 mg L^{-1}**	**80 mg L^{-1}**	**100 mg L^{-1}**	**150 mg L^{-1}**	**200 mg L^{-1}**
1	2	0.05258	-	-	-	-	-	-
2	4	0.0458	0.03526	-	-	-	-	-
3	6	0.03578	0.02232	0.02098		-	-	-
4	8	0.0275	0.01583	0.01509	0.0198	-	-	-
5	10	-	-	0.01147	0.01525	0.01765	-	-
6	12	-	-	-	0.01044	0.01144	-	-
7	14	-	-	-	-	0.00937	0.00828	0.0065
8	16	-	-	-	-	-	0.00702	0.0058
9	18	-	-	-	-	-	0.00581	0.0032

Table -A1.33. Three-half-order kinetics for Zn(II) bioremediation at different initial concentrations of Zn(II)

S No	Time (h)	*Y*						
		20 mg L^{-1}	**40 mg L^{-1}**	**60 mg L^{-1}**	**80 mg L^{-1}**	**100 mg L^{-1}**	**150 mg L^{-1}**	**200 mg L^{-1}**
1	2	-	-	-	-	-	-	-
2	4	-	-	-	-	-	-	-
3	6	0.03573	-	-	-	-	-	-
4	8	0.0275	0.01856	-	-	-	-	-
5	10	0.02109	0.0158	0.01065	-	-	-	-
6	14	0.01153	0.00879	0.00674	0.00591	-	-	-
7	16	-	-	0.0053	0.00488	0.00927	0.00823	0.00311
8	18	-	-	0.00354	0.00399	0.0086	0.0074	0.00273
9	20	-	-	-	0.00311	0.00803	0.00673	0.00243
10	22	-	-	-	0.0021	0.00746	-	0.00218
11	24	-	-	-	-	-	-	0.00197

Table -A1.34. Growth of isolated bacterial strains and pH with time in absence of metals

(*T* = 37 ºC, pH = 7.0±0.2, RPM = 150)

S No	Time (h)	*P. taiwanensis*		*A. guillouiae*		*K. pneumoniae*	
		Biomass growth (g L^{-1})	pH	Biomass growth (g L^{-1})	pH	Biomass growth (g L^{-1})	pH
1	0	0	7.1	0	7.1	0	7.1
2	4	0.1	7.1	0.2	7.2	0.08	7.4
3	8	0.8	7.12	0.35	7.3	0.5	7.3
4	12	1.25	6.8	0.8	7.1	0.85	7.4
5	16	1.75	6.6	1.5	6.7	1	7.1
6	20	2.1	7.2	1.8	6.7	1.25	6.7
7	24	2.9	7.5	2.5	6.5	1.75	6.7
8	28	3.2	7.6	2.8	6.9	1.8	6.8
9	32	3.25	7.7	2.75	6.6	1.65	6.5
10	36	2.75	7.6	2.5	7.2	1.5	6.3
11	40	2.5	7.6	2.4	7.2	1.4	6.4

Table -A1.35. Growth and metals removal by isolated bacterial strains with time in the presence of Cr(VI)

(T = 37 ºC, pH = 7.0±0.2, RPM = 150, S_o = 150 mg L^{-1})

S No	Time (h)	*P. taiwanensis*		*A. guillouiae*		*K. pneumoniae*	
		Biomass growth (g L^{-1})	Cr(VI) concentration (mg L^{-1})	Biomass growth (g L^{-1})	Cr(VI) concentration (mg L^{-1})	Biomass growth (g L^{-1})	Cr(VI) concentration (mg L^{-1})
1	0	0	150	0	150	0	150
2	4	0.08	124.5	0.02	132.5	0.01	139.75
3	8	0.09	117.5	0.025	131.5	0.01	138.75
4	12	0.1	104.5	0.035	128.8	0.02	138.25
5	16	0.12	99.8	0.03	128	0.025	136.5
6	20	0.8	64.25	0.9	95.5	0.03	134.5
7	24	1.75	39.75	1.1	89.5	0.032	132.5
8	28	2.2	8.8	1.45	64.5	0.5	114.5
9	32	2.4	7.8	1.75	52.5	0.8	102.5
10	36	2.4	7.9	1.9	34.8	1.1	84.5
11	40	2.4	150	1.9	34.8	1.1	85

Table -A1.36. Growth and metals removal by isolated bacterial strains with time in the presence of Cu(II)

(T = 37 ºC, pH = 7.0±0.2, RPM = 150, S_o = 150 mg L^{-1})

S No	Time (h)	*P. taiwanensis*		*A. guillouiae*		*K. pneumoniae*	
		Biomass growth (g L^{-1})	Cu(II) concentration (mg L^{-1})	Biomass growth (g L^{-1})	Cu(II) concentration (mg L^{-1})	Biomass growth (g L^{-1})	Cu(II) concentration (mg L^{-1})
1	0	0	150	0	150	0	150
2	4	0.03	132.5	0.05	114.25	0.06	129.5
3	8	0.035	131.25	0.055	112.5	0.065	128.5
4	12	0.04	131.2	0.6	111.5	0.07	128.25
5	16	0.042	127.5	0.65	109.5	0.075	125.5
6	20	0.05	126.5	1.25	64.5	0.08	121.25
7	24	1.1	94.5	1.75	52.5	1.2	82.5
8	28	1.4	71.25	1.9	39.5	1.5	62.5
9	32	1.7	50.25	2.1	24.5	1.82	47.75
10	36	1.71	49.75	2.2	14.5	1.87	37.5
11	40	1.71	49.75	2.2	14.5	1.87	37.75

Table -A1.37. Growth and metals removal by isolated bacterial strains with time in presence of Zn(II)

(T = 37 ºC, pH = 7.0±0.2, RPM = 150, S_o = 150 mg L^{-1})

S No	Time (h)	*P. taiwanensis*		*A. guillouiae*		*K. pneumoniae*	
		Biomass growth (g L^{-1})	Zn(II) concentration (mg L^{-1})	Biomass growth (g L^{-1})	Zn(II) concentration (mg L^{-1})	Biomass growth (g L^{-1})	Zn(II) concentration (mg L^{-1})
1	0	0	150	0	150	0	150
2	4	0.03	128.5	0.02	134.5	0.06	124.25
3	8	0.035	127.5	0.027	132.5	0.09	112.5
4	12	0.036	126.25	0.028	132.25	0.12	102.5
5	16	0.04	125.95	0.035	130.5	0.75	74.5
6	20	0.09	112.5	0.07	122.5	0.81	64.25
7	24	1.5	81.25	1.35	102.5	0.9	57.5
8	28	1.7	74.2	1.5	84.5	1	49.5
9	32	2.1	39.75	1.9	62.5	1.2	34.8
10	36	2.12	37.75	2.1	54.5	1.25	27.5
11	40	2.12	37.75	2.1	54.5	1.25	26.5

Table -A1.38. Relative growth of individual bacterial strain in presence of other bacterial strain (PT+AG indicates *P. taiwanensis* in combination with *A. guillouiae*. Similarly, other notations are used for different combinations)

S No	Bacterial Combination	Percentage relative growth (%)
1	PT+KP	-6.2
2	AG+KP	-8.5
3	AG+PT	4.2
4	KP+PT	7.5
5	KP+AG	25.5

Table -A1.39. Efficiency of the consortium over individual strain in removal of Cr(VI) (T = 37 ºC, pH = 7.0±0.2, RPM = 150, S_o = 150 mg L^{-1} of Cr(VI))

S No	Time (h)	Cr(VI) concentration (mg L^{-1})			
		P. taiwanensis	*A. guillouiae*	*K. pneumoniae*	Consortium
1	0	150	150	150	150
2	4	124.5	132.5	139.75	87.75
3	8	117.5	131.5	138.75	69.75
4	12	104.5	128.8	138.25	56.625
5	16	99.8	128	136.5	36.75
6	20	64.25	95.5	134.5	24.75
7	24	39.75	89.5	132.5	3.75
8	28	8.8	64.5	114.5	1.65
9	32	7.8	52.5	102.5	1.5
10	36	7.9	34.8	84.5	1.5
11	40	7.9	34.8	85	1.5

Table -A1.40. Efficiency of the consortium over individual strain in removal of Cu(II)

(T = 37 ºC, pH = 7.0±0.2, RPM = 150, S_o = 150 mg L^{-1} of Cu(II))

S No	Time (h)	Cu(II) concentration (mg L^{-1})			
		P. taiwanensis	***A. guillouiae***	***K. pneumoniae***	**Consortium**
1	0	150	150	150	150
2	4	132.5	114.25	129.5	78.75
3	8	131.25	112.5	128.5	74.625
4	12	131.2	111.5	128.25	63.75
5	16	127.5	109.5	125.5	56.25
6	20	126.5	64.5	121.25	38.25
7	24	94.5	52.5	82.5	26.25
8	28	71.25	39.5	62.5	15.15
9	32	50.25	24.5	47.75	8.25
10	36	49.75	14.5	37.5	9
11	40	49.75	14.5	37.75	8.25

Table -A1.41. Efficiency of the consortium over individual strain in removal of Zn(II)

(T = 37 ºC, pH = 7.0±0.2, RPM = 150, S_o = 150 mg L^{-1} of Zn(II))

S No	Time (h)	Zn(II) concentration (mg L^{-1})			
		P. taiwanensis	***A. guillouiae***	***K. pneumoniae***	**Consortium**
1	0	150	150	150	150
2	4	128.5	134.5	124.25	96.75
3	8	127.5	132.5	112.5	86.25
4	12	126.25	132.25	102.5	63.75
5	16	125.95	130.5	74.5	51.3
6	20	112.5	122.5	64.25	41.25
7	24	81.25	102.5	57.5	30.75
8	28	74.2	84.5	49.5	21.75
9	32	39.75	62.5	34.8	6
10	36	37.75	54.5	27.5	5.25
11	40	37.75	54.5	26.5	6

Table -A1.42. Performance evaluation of the consortium in multi-metals removal in simulated industrial effluent: Set 1 (*T* = 37 ºC, pH = 7.0±0.2, RPM = 150)

S No	Time (h)	Consortium			
		Biomass growth ($g\ L^{-1}$)	Cr(VI) concentration ($mg\ L^{-1}$)	Cu(II) concentration ($mg\ L^{-1}$)	Zn(II) concentration ($mg\ L^{-1}$)
1	0	0	10.1	15.9	10.2
2	4	0.8	8.6	13.6	8.8
3	8	1.1	7.4	11.4	7.85
4	12	1.5	6.6	10.7	7.1
5	16	1.78	5.9	9.15	6.3
6	20	2.1	5.3	8.4	5.55
7	24	2.58	4.3	7.15	4.5
8	28	3.1	3.35	4.65	3.45
9	32	3.8	0.6	2.4	1.1
10	36	3.8	0.1	0.7	0.3
11	40	3.8	0.1	0.7	0.2

Table -A1.43. Performance evaluation of the consortium in multi-metals removal in simulated industrial effluent: Set 2 (*T* = 37 ºC, pH = 7.0±0.2, RPM = 150)

S No	Time (h)	Consortium			
		Biomass growth ($g\ L^{-1}$)	Cr(VI) concentration ($mg\ L^{-1}$)	Cu(II) concentration ($mg\ L^{-1}$)	Zn(II) concentration ($mg\ L^{-1}$)
1	0	0	10.1	7.2	5.3
2	4	0.75	8.7	5.6	3.6
3	8	1.3	7.6	4.45	2.45
4	12	1.8	6.5	3.35	1.35
5	16	2.1	6.15	3	0.85
6	20	2.58	5.4	2.25	0.3
7	24	2.9	4.45	1.35	0.15
8	28	3.4	3.6	1.1	0.1
9	32	3.75	0.9	0.3	0.09
10	36	3.8	0.2	0.1	0.05
11	40	3.75	0.25	0.1	0.05

Table -A1.44. Performance evaluation of the consortium in multi-metals removal in simulated industrial effluent: Set 3 (*T* = 37 ºC, pH = 7.0±0.2, RPM = 150)

S No	Time (h)	Consortium			
		Biomass growth (g L^{-1})	Cr(VI) concentration (mg L^{-1})	Cu(II) concentration (mg L^{-1})	Zn(II) concentration (mg L^{-1})
1	0	0	6.4	15.9	10.2
2	4	0.75	5	13.6	8.85
3	8	1.3	4.2	11.4	7.95
4	12	1.8	3.45	10.7	7.3
5	16	2.1	2.5	9.15	6.35
6	20	2.58	1.85	8.4	5.6
7	24	2.9	0.8	7.15	4.7
8	28	3.4	0.5	4.65	3.7
9	32	3.75	0.15	2.4	1.25
10	36	3.8	0.1	0.7	0.35
11	40	3.75	0.08	0.7	0.3

Table -A1.45. Performance evaluation of the consortium in multi-metals removal in simulated industrial effluent: Set 4 (*T* = 37 ºC, pH = 7.0±0.2, RPM = 150)

S No	Time (h)	Consortium			
		Biomass growth (g L^{-1})	Cr(VI) concentration (mg L^{-1})	Cu(II) concentration (mg L^{-1})	Zn(II) concentration (mg L^{-1})
1	0	0	6.4	7.2	5.3
2	4	0.9	4.6	5.3	3.9
3	8	1.4	4.3	4.8	3.4
4	12	1.78	3.44	3.55	3.1
5	16	2.4	2.1	2.84	2.4
6	20	2.78	1.6	2.1	1.8
7	24	3.1	1.2	1.45	1.4
8	28	3.6	0.65	0.9	1.1
9	32	3.7	0.3	0.4	0.5
10	36	3.7	0.15	0.05	0.1
11	40	3.69	0.15	0.06	0.1

Table -A1.46. Performance evaluation of the consortium in multi-metals removal in simulated industrial effluent: Set 5 (*T* = 37 ºC, pH = 7.0±0.2, RPM = 150)

S No	Time (h)	Consortium			
		Biomass growth (g L^{-1})	Cr(VI) concentration (mg L^{-1})	Cu(II) concentration (mg L^{-1})	Zn(II) concentration (mg L^{-1})
1	0	0	8.5	11.8	7.2
2	4	0.65	7.25	9.3	5.4
3	8	1.2	5.7	8.05	4.8
4	12	1.65	5.3	6.95	4.25
5	16	2.35	4.3	6	3.05
6	20	2.7	3.3	4.85	2.1
7	24	3.12	2.6	4	1.45
8	28	3.55	1.65	2.85	1.15
9	32	3.8	0.63	1.95	0.4
10	36	3.8	0.25	0.45	0.04
11	40	3.79	0.2	0.44	0.04

APPENDIX II

Raw data for continuous experimental studies conducted

Table -A2.1. Performance of biofilter with change in flow rate and inlet concentrations of Cr(VI) solution

S No	Phase	Operating time (day)	Cr(VI) concentration (mg L^{-1})	
			Inlet, C_i	Oulet, C_o
1	I	1	10	6.25
2		2	10	6.25
3		3	9.5	4.75
4		4	8.75	4
5		5	9.5	3.75
6		6	10	3.75
7		7	10	3.25
8		8	9.75	2.75
9		9	9.25	2.5
10		10	10	2.5
11		11	9.5	2.25
12		12	9	2
13		13	9.5	2
14		14	10	1
15		15	10	0.75
16		16	9.5	0.75
17		17	9.75	0.5
18		18	9.25	0.5
19		19	10	0.5
20		20	9.75	0.5
21	II	21	20	7.5
22		22	19.5	6.75
23		23	19	6
24		24	20	5
25		25	20	5.5
26		26	18.5	4.5
27		27	18.5	3.5
28		28	20	3.5
29		29	19.5	3
30		30	19.5	3
31		31	19	3
32	III	32	29	17.5
33		33	29.5	16
34		34	30	17

35		35	30	12.5
36		36	29	10.5
37		37	29	10
38		38	30	9.25
39		39	30	7.25
40		40	28.5	7
41		41	30	7
42		42	30	7
43		43	29.5	7
44	IV	44	50	27.5
45		45	50.5	25
46		46	49	22
47		47	49.5	22
48		48	49	18.75
49		49	48	12
50		50	50	11
51		51	50	11.5
52		52	50.5	10
53		53	50	10
54		54	50	10
55	V	55	40	9.75
56		56	40.5	9
57		57	39	8
58		58	39	7.5
59		59	40	8
60		60	40	7
61		61	40.5	5.5
62		62	38	5
63		63	40	4.25
64		64	39.5	4.5
65		65	39	4.5

Table -A2.2. Change in biomass growth and pH with operating time at different heights of filter bed in Cr(VI) removal

S No	Operating time (day)	Biomass growth (CFU g^{-1} of packing material)				pH
		10 cm	30 cm	50 cm	70 cm	
1	1	1.1	0.85	0.62	0.45	6.9
2	2	1.11	0.95	0.756	0.58	6.2
3	3	1.13	1	0.85	0.69	6.58
4	4	1.15	1.05	0.92	0.79	6.7
5	5	1.16	1.09	0.98	0.89	7.1
6	6	1.21	1.1	1	0.98	7.12
7	7	1.45	1.25	1.05	1	7.2
8	8	1.85	1.37	1.189	1.07	6.98
9	9	2.1	1.6	1.196	1.09	6.8
10	10	2.25	1.75	1.2	1.1	6.85
11	11	2.35	1.875	1.28	1.13	7.2
12	12	2.45	1.925	1.35	1.22	7.1
13	13	2.65	2.1	1.78	1.31	6.9
14	14	2.85	2.25	1.87	1.48	7
15	15	2.87	2.35	1.98	1.56	7.1
16	16	2.91	2.65	2.1	1.89	7.2
17	17	2.95	2.756	2.25	2.1	7
18	18	2.98	2.8	2.35	2.18	7
19	19	2.985	2.85	2.48	2.22	6.9
20	20	3	2.9	2.62	2.45	7.1
21	21	1.15	1.01	0.95	0.67	7.1
22	22	1.17	1.05	0.98	0.78	7.1
23	23	1.24	1.1	1	0.95	6.85
24	24	1.35	1.162	1.06	1	6.9
25	25	1.48	1.25	1.1	1.02	7.1
26	26	1.6	1.33	1.189	1.09	7.2
27	27	1.9	1.42	1.21	1.1	6.9
28	28	2.1	1.58	1.29	1.15	7.1
29	29	2.3	1.75	1.36	1.21	6.9
30	30	2.4	1.957	1.52	1.32	6.95
31	31	1.35	1.176	1.012	0.87	7.1
32	32	1.45	1.27	1.12	0.95	7.2
33	33	1.65	1.34	1.128	1	7.1
34	34	1.86	1.58	1.25	1.08	6.9
35	35	2.2	1.89	1.32	1.15	7.1
36	36	2.35	1.98	1.45	1.21	6.9
37	37	2.525	2.1	1.65	1.35	6.8
38	38	2.75	2.19	1.89	1.58	7.2
39	39	2.85	2.25	2.1	1.87	7.3
40	40	2.98	2.65	2.228	2.1	6.9

41	41	3	2.78	2.45	2.18	7.1
42	42	3.12	2.9	2.65	2.25	6.85
43	43	1.86	1.253	1.18	1.05	7.1
44	44	1.95	1.352	1.22	1.1	7.2
45	45	2.1	1.58	1.278	1.12	6.9
46	46	2.35	1.78	1.45	1.27	6.85
47	47	2.6	1.92	1.52	1.31	7.1
48	48	2.87	2.15	1.91	1.78	7
49	49	3.25	2.22	1.98	1.81	7
50	50	3.45	2.254	2.11	1.98	7
51	51	4.1	2.31	2.18	2.1	7.1
52	52	4.5	2.45	2.22	2.18	7.2
53	53	3.75	1.87	1.51	1.21	7.2
54	54	3.87	1.92	1.67	1.27	7.1
55	55	4.2	2.15	1.89	1.35	7.2
56	56	4.35	2.18	1.98	1.41	7.14
57	57	4.75	2.78	2.18	1.49	7.1
58	58	4.85	3.28	2.5	1.58	6.9
59	59	4.87	3.45	2.78	1.61	6.8
60	60	5.12	3.75	2.9	1.66	6.94
61	61	5.22	3.95	3.18	1.68	7
62	62	5.26	4.1	3.27	1.69	7
63	63	5.3	4.2	3.42	1.7	7
64	64	5.3	4.2	3.41	1.7	7
65	65	5.3	4.2	3.42	1.7	6.99

Table -A2.3. Performance of biofilter with change in flow rate and inlet concentrations of Cu(II) solution

S No	Phase	Operating time (day)	Cu(II) concentration (mg L^{-1})	
			Inlet, C_i	Oulet, C_o
1	I	1	8.5	7.5
2		2	8.45	7.25
3		3	8.5	7.2
4		4	8.75	6.5
5		5	9	6.2
6		6	9.2	6.2
7		7	10	5
8		8	9.5	4.5
9		9	9.5	4.25
10		10	10	3.5
11		11	9.75	3
12		12	10	2.75
13		13	10	1.2
14		14	9.75	0.75
15		15	10	0.5
16	II	16	20	4.5
17		17	19.5	4.25
18		18	20	3.75
19		19	19.25	3.75
20		20	20	2.5
21		21	18.75	2.25
22		22	18	2.1
23		23	17.5	0.75
24		24	18	0.5
25		25	20	0.5
26	III	26	29	10.25
27		27	30	9.75
28		28	28.75	9
29		29	29.5	9.75
30		30	28	9
31		31	28.5	8.75
32		32	30	7.5
33		33	27.5	7.25
34		34	30	7.25
35		35	30	7.2
36	IV	36	50	22.5
37		37	49.5	22
38		38	49.5	21
39		39	48	20.5
40		40	48	19.5

41		41	50	18.75
42		42	49.5	18
43		43	47	17.5
44		44	48	18
45		45	50	18
46	V	46	40	10
47		47	40	9.5
48		48	39	7.5
49		49	37	7
50		50	39.5	4.5
51		51	40	4.75
52		52	39.5	4
53		53	37	3.5
54		54	38.5	3.75
55		55	40	3.75

Table -A2.4. Change in biomass growth and pH with operating time at different heights of filter bed in Cu(II) removal

S No	Operating time (day)	Biomass growth (CFU g^{-1} of packing material)				pH
		10 cm	30 cm	50 cm	70 cm	
1	1	3	2.7	2	1.5	6.9
2	2	3.1	2.71	2.1	1.51	6.7
3	3	3.1	2.7	2.11	1.52	6.9
4	4	3.15	2.73	2.13	1.54	7
5	5	3.2	2.8	2.2	1.6	7
6	6	3.2	2.8	2.2	1.66	6.9
7	7	3.5	2.91	2.3	1.68	7
8	8	3.6	2.95	2.32	1.7	6.9
9	9	3.61	3.1	2.35	1.77	7.1
10	10	3.7	3.15	2.37	1.79	6.9
11	11	3.71	3.15	2.37	1.79	7
12	12	3.73	3.22	2.4	1.8	6.9
13	13	3.85	3.35	2.5	1.82	6.9
14	14	3.97	3.5	2.55	1.82	7
15	15	4.01	3.56	2.57	1.82	7
16	16	3.2	2.8	2.1	1.5	6.5
17	17	3.21	2.81	2.1	1.5	6.7
18	18	3.75	3.1	2.4	1.82	6.8
19	19	3.76	3.11	2.42	1.86	6.8
20	20	3.97	3.2	2.5	1.9	6.9
21	21	4.05	3.45	2.65	2.1	7.1
22	22	4.1	3.47	2.67	2.2	7.1
23	23	4.85	3.85	2.87	2.4	7
24	24	4.95	3.9	2.9	2.45	7.1
25	25	4.96	3.91	2.91	2.47	7
26	26	3.75	3.1	2.5	1.9	7.3
27	27	3.75	3.1	2.51	1.91	7.2
28	28	3.78	3.2	2.65	2.1	7
29	29	3.79	3.21	2.67	2.2	6.9
30	30	3.85	3.3	2.71	2.35	7
31	31	3.98	3.5	2.8	2.4	6.8
32	32	4.2	3.65	2.85	2.42	6.7
33	33	4.17	3.6	2.84	2.42	6.9
34	34	4.17	3.6	2.84	2.42	6.9
35	35	4.17	3.61	2.86	2.47	6.9
36	36	4	3.5	2.8	2.45	7.1
37	37	4.01	3.5	2.8	2.45	7.2
38	38	4.01	3.5	2.8	2.45	7.1
39	39	4.05	3.51	2.81	2.46	6.9
40	40	4.08	3.52	2.82	2.47	6.8

41	41	4.17	3.61	2.95	2.5	6.9
42	42	4.25	3.74	3.2	2.8	7
43	43	4.55	3.95	3.35	2.95	7.1
44	44	4.55	3.96	3.36	3	7
45	45	4.56	3.96	3.37	3.05	7
46	46	4.85	4.1	3.7	3.2	6.7
47	47	4.95	4.2	3.8	3.25	6.8
48	48	5	4.3	3.9	3.28	6.9
49	49	5.05	4.32	4.1	3.3	7
50	50	5.25	4.45	4.2	3.5	7
51	51	5.2	4.42	4.2	3.7	6.9
52	52	5.5	4.8	4.7	3.9	6.7
53	53	5.85	5	4.85	4.2	6.9
54	54	6	5.5	5.2	4.6	6.9
55	55	6	5.8	5.6	4.9	7.1

Table -A2.5. Performance of biofilter with change in flow rate and inlet concentrations of Zn(II) solution

S No	Phase	Operating time (day)	Zn(II) concentration (mg L^{-1})	
			Inlet, C_i	Oulet, C_o
1	I	1	8.5	7.85
2		2	8.5	7.75
3		3	8.5	7.75
4		4	8.75	7.25
5		5	9.2	7
6		6	9	6.75
7		7	9.5	6.5
8		8	10	6.25
9		9	9.5	4.25
10		10	10	4.25
11		11	9.5	4.2
12		12	9.75	4
13		13	10	3.75
14		14	10	3.5
15		15	10	3.25
16		16	9.75	3
17		17	9.25	2.75
18		18	10	2.5
19		19	10	2.5
20		20	9.5	2.5
21	II	21	18	8.5
22		22	18.75	8.25
23		23	19.5	8
24		24	20	7.75
25		25	20	6
26		26	18.5	4.78
27		27	19	3.2
28		28	20	2.3
29		29	19.5	1.75
30		30	20	1.7
31	III	31	30	13.5
32		32	28.5	13
33		33	28.5	11.5
34		34	29	10
35		35	29.5	8.75
36		36	30	7
37		37	29.5	6.2
38		38	30	4.45
39		39	28.75	4
40		40	30	4

41	IV	41	49.5	21.5
42		42	50	21
43		43	48.5	18.75
44		44	50	15.5
45		45	48.5	12.5
46		46	49	11.5
47		47	49.5	9.75
48		48	50	9.25
49		49	50	9
50		50	50	9
51	V	51	38.5	8.75
52		52	38.5	8.25
53		53	39.5	7.5
54		54	40	6.25
55		55	40	6
56		56	39	5.5
57		57	40	4.75
58		58	39.5	4
59		59	40	4
60		60	40	4

Table -A2.6. Change in biomass growth and pH with operating time at different heights of filter bed in Zn(II) removal

S No	Operating time (day)	Biomass growth (CFU g^{-1} of packing material)				pH
		10 cm	30 cm	50 cm	70 cm	
1	1	2.5	2.2	2.1	1.75	6.7
2	2	2.75	2.4	2.15	1.8	7
3	3	3.1	2.7	2.2	1.92	6.9
4	4	3.15	2.8	2.7	2.2	7
5	5	3.2	2.9	2.75	2.45	7.1
6	6	3.2	3	2.9	2.6	7
7	7	3.5	3.1	3	2.8	6.8
8	8	3.6	3.25	3.15	2.9	6.9
9	9	3.65	3.3	3.2	3.1	7.1
10	10	3.7	3.45	3.25	3.2	6.9
11	11	3.75	3.5	3.35	3.25	6.8
12	12	3.8	3.6	3.5	3.25	6.9
13	13	3.85	3.75	3.55	3.4	6.9
14	14	3.97	3.8	3.7	3.65	7.1
15	15	4.01	3.9	3.75	3.7	7
16	16	4.25	4.15	4	3.85	6.5
17	17	4.5	4.25	4.1	3.95	6.7
18	18	4.75	4.5	4.25	4.1	6.8
19	19	5	4.75	4.5	4.18	6.8
20	20	5.1	5	4.75	4.5	6.8
21	21	2.15	1.9	1.2	0.9	7.1
22	22	2.18	2.1	1.35	1.1	7.1
23	23	2.25	2.15	1.5	1.25	7
24	24	2.75	2.35	1.7	1.5	7.1
25	25	3.25	2.75	1.75	1.65	7
26	26	3.75	2.9	1.9	1.75	7.3
27	27	4.1	3.1	2.1	1.9	6.8
28	28	4.25	3.35	2.45	2.2	7
29	29	4.5	3.5	2.55	2.2	6.9
30	30	4.75	3.75	2.65	2.3	6.8
31	31	3.25	2.85	2.1	1.2	6.8
32	32	3.5	2.97	2.15	1.27	7.1
33	33	3.75	3.2	2.25	1.7	6.9
34	34	4.1	3.25	2.3	1.85	7
35	35	4.15	3.27	2.4	2.1	6.9
36	36	4.25	3.55	2.65	2.25	7.1
37	37	4.5	3.6	2.85	2.3	7.2
38	38	4.75	3.65	2.9	2.4	7.1
39	39	5.1	3.8	2.86	2.45	6.8
40	40	5.2	3.8	2.87	2.47	6.8

41	41	4.25	2.9	2.2	1.35	6.7
42	42	4.28	3.2	2.5	1.5	7
43	43	4.5	3.25	2.75	1.75	7.1
44	44	4.66	3.5	2.8	2	7.1
45	45	4.8	3.75	2.9	2.1	7
46	46	4.85	4.1	3.2	2.2	6.9
47	47	5	4.15	3.35	2.45	6.8
48	48	5.1	4.45	3.5	2.5	7.1
49	49	5.1	4.65	3.55	2.67	7
50	50	5.15	4.85	3.5	2.5	7.2
51	51	5.2	4.8	3.52	2.55	6.9
52	52	5.75	4.8	3.65	2.75	7.1
53	53	5.85	5.1	3.75	2.8	7.2
54	54	6.1	5.15	3.8	2.9	7.1
55	55	6.1	5.2	4.1	3.1	7
56	56	6.2	5.5	4.15	3.2	7
57	57	6.25	5.7	4.2	3.5	7.1
58	58	7.1	6.1	4.25	3.55	6.9
59	59	7.11	6.1	4.2	3.5	7
60	60	7.11	6.13	4.2	3.51	7

Table -A2.7. Performance of biofilter under shock loading conditions for Cr(VI) removal (change in concentration with operating time)

S No	Operating time (day)	Cr(VI) concentration (mg L^{-1})	
		Inlet, C_i	Oulet, C_o
1	1	30	4.75
2	2	30.5	4.2
3	3	29.5	3.9
4	4	29.5	4.2
5	5	30	4.2
6	6	40	5.2
7	7	38.5	4.75
8	8	40	4.5
9	9	40	4.5
10	10	38.5	4.5
11	11	30	4.25
12	12	30.5	4.2
13	13	30	4.25
14	14	30	4.25
15	15	29.5	4.25

Table -A2.8. Performance of biofilter under shock loading conditions for Cr(VI) removal (change in biomass growth and pH with operating time)

S No	Operating time (day)	Biomass growth (CFU g^{-1} of packing material)				pH
		10 cm	30 cm	50 cm	70 cm	
1	1	5.3	4.2	3.42	1.7	7
2	2	5.28	4.1	3.1	1.12	7.1
3	3	5.278	4.195	3.15	1.1	6.9
4	4	5.34	4.1	3.2	1	6.8
5	5	4.2	3.1	2.45	0.87	7
6	6	4.35	3.22	2.78	0.97	7.1
7	7	4.78	3.58	3.1	1	7
8	8	5.57	3.78	3.18	1.1	7
9	9	4.35	3.5	2.5	0.98	6.8
10	10	4.5	3.85	2.75	1.2	6.9
11	11	4.8	3.91	2.98	1.27	7.1
12	12	4.9	3.98	3.125	1.37	7
13	13	5.12	4.1	3.2	1.45	7.2
14	14	5.19	4.12	3.27	1.5	7
15	15	4.55	4.1	3.01	1.1	7
16	16	4.75	4.11	3.1	1.17	7
17	17	4.85	4.18	3.17	1.28	6.89
18	18	5.1	4.178	3.2	1.32	7
19	19	5.22	4.2	3.28	1.45	7.1
20	20	5.32	4.2	3.38	1.65	7

Table -A2.9. Performance of biofilter under shock loading conditions for Cu(II) removal (change in concentration with operating time)

S No	Operating time (day)	Cu(II) concentration (mg L^{-1})	
		Inlet, C_i	Oulet, C_o
1	1	28.5	4
2	2	28.5	4.2
3	3	29.5	4
4	4	30	3.9
5	5	38	7.2
6	6	38	7
7	7	40	6.9
8	8	40	7.3
9	9	30	4
10	10	30	4.2
11	11	31	4.3
12	12	32	4.3
13	13	33	4.3
14	14	33	4.3

Table -A2.10. Performance of biofilter under shock loading conditions for Cu(II) removal (change in biomass growth and pH with operating time)

S No	Operating time (day)	Biomass growth (CFU g^{-1} of packing material)				pH
		10 cm	30 cm	50 cm	70 cm	
1	1	6	5.8	5.6	4.9	7
2	2	5.8	5.75	5.4	4.75	6.9
3	3	6.01	5.71	5.31	4.65	7.01
4	4	6	5.8	5.2	4.5	6.8
5	5	4.1	3.7	3.4	3.01	7
6	6	4.2	3.71	3.41	3.02	6.7
7	7	4.3	3.73	3.44	3.08	7.01
8	8	4.8	4.2	3.91	3.3	7.1
9	9	5.5	5.1	4.5	3.8	7
10	10	5.8	5.2	4.65	4.01	7
11	11	5.9	5.25	4.95	4.25	6.9
12	12	6	5.6	5.1	4.5	7.1
13	13	6.01	5.75	5.5	4.6	7.2
14	14	5.9	5.8	5.6	4.85	7

Table -A2.11. Performance of biofilter under shock loading conditions for Zn(II) removal (change in concentration with operating time)

S No	Operating time (day)	Zn(II) concentration (mg L^{-1})	
		Inlet, C_i	Oulet, C_o
1	1	29	3.5
2	2	28.5	3.25
3	3	30	3.27
4	4	30	3.25
5	5	38.5	4.75
6	6	40	4.3
7	7	39	4.75
8	8	40	4.5
9	9	31.5	4.5
10	10	31	4.25
11	11	30	3.75
12	12	33	3.5
13	13	33	3.25
14	14	33	3.2

Table -A2.12. Performance of biofilter under shock loading conditions for Zn(II) removal (change in biomass growth and pH with operating time)

S No	Operating time (day)	Biomass growth (CFU g^{-1} of packing material)				pH
		10 cm	30 cm	50 cm	70 cm	
1	1	7.11	6.13	4.2	3.51	7.1
2	2	6.8	5.85	3.8	3.3	6.8
3	3	6.85	5.9	3.8	3.45	7
4	4	7	6	3.9	3.45	7
5	5	4	3.8	3.1	2.75	6.9
6	6	4.2	3.65	3.1	3	7.01
7	7	4.4	3.71	3.15	3.08	7.02
8	8	5.1	4.15	3.2	3.1	7
9	9	5.5	5.2	3.8	3.3	7.01
10	10	6.1	5.3	3.75	3.01	7.01
11	11	6.5	5.25	3.8	2.85	7.2
12	12	6.7	5.8	3.9	3.1	6.9
13	13	6.8	6.1	3.85	3.15	7.1
14	14	6.8	6.1	3.9	3.2	7