ABSTRACT

Heavy metals are non-biodegradable and have bioaccumulating properties, which makes them potentially hazardous. They also have high toxicity and lead to adverse effects on human and environmental health and are a cause of global concern. Arsenic and chromium are such toxic metal(loid)s. Arsenic ranks number one on the United States Agency for Toxic Substances and Disease Registry Priority List. Arsenic and chromium can result from both natural and anthropogenic sources, including weathering of rocks, soil erosion, volcanic eruption, industrial emissions, wood preservatives, mining activities, etc. They are carcinogen and can also affect the nervous system, skin, lungs, heart, kidneys and pancreas. Conventional methods of treatment, such as adsorption, chemical precipitation, electrokinetic techniques, membrane technologies, etc., suffer from certain limitations, such as selective removal, generation of toxic sludge, high cost, low removal efficiency under natural conditions, low regeneration and reusability, etc. Biological remediation (also known as bioremediation) has been preferred over conventional techniques due to its benefits, such as cost-effectiveness and environment-friendly nature.

The main objective of this study is to investigate the efficacy of bioremediation in removal of arsenic from water and arsenic and chromium from soil. The study aims to assess the impact of varying operating conditions on the bioremediation process and to determine the effectiveness of this approach in removing arsenic from different media. The research provides valuable insights into the potential application of bioremediation as a sustainable solution for arsenic and chromium contamination.

This Thesis is divided into eight chapters, excluding the references. A brief description of each of these chapters is presented below.

Chapter 1: Introduction – This chapter discusses the natural and anthropogenic sources of arsenic, its release mechanisms in the environment, and its effects on human and environmental health. It also elucidates the problem of arsenic in the global and Indian context. It discusses about the co-contamination of arsenic and chromium in the environment. The conventional methods employed for the treatment of arsenic and also the bioremediation approach have been discussed in this section. The chapter culminates with a description of structure of the Thesis.

Chapter 2: Literature Review – This chapter presents a brief account of the efforts made so far for the remediation of arsenic using various microorganisms and plants. The use of bacteria for arsenic remediation has been discussed in detail, including the uptake and efflux pathways, genes involved in biotransformation of arsenic and metabolism of arsenic in bacteria. Use of immobilized bacteria and use of bacteria for removal of arsenic from soil media have also been discussed. The chapter concludes with the enumeration of research gaps and objectives of this study.

Chapter 3: Materials and Methods – This chapter gives a detailed description of the materials and methods used in the experimental studies. It covers information about various chemicals and media used in the study, as well as the analytical techniques employed. Further, the chapter discusses the isolation and identification of arsenic-resistant bacteria, batch studies for arsenic removal and optimization of operating parameters. Arsenic remediation in recirculating packed bed bioreactor, phytotoxicity, acute and chronic toxicity using luminescent bacteria, removal of arsenic and chromium from soil and chlorophyll assessment have also been discussed. In addition, the chapter also elucidates the preparation of biochar, its optimization, isotherm and kinetic study, immobilization of bacteria on biochar, its encapsulation in polyvinyl alcohol-sodium alginate beads and its performance evaluation for removal of arsenic.

Chapter 4: Isolation and Identification of Arsenic Resistant Bacteria – It details the phylogenetic analysis of the isolated bacteria. It also elaborates on the influence of several factors, such as pH, temperature and initial arsenic concentration, on the growth of bacteria, resistance of bacteria against arsenic and other heavy elements and the ability of bacteria to oxidize or reduce arsenic. Phytotoxicity assessment using chickpea and Vigna radiata seeds was also performed. Arsenic-resistant bacteria were isolated from Ratanpur Village of Varanasi District, Uttar Pradesh, by enrichment technique. The isolates, *Proteus alimentorum* strain TY6 and *Pseudomonas aeruginosa* strain K7Pb showed high resistance to As(V) with a minimum inhibitory concentration of 610 mM and 350 mM, respectively, and 10 mM and 8 mM for As(III), respectively. TY6 could remove 96±0.82% As(V) and 66±0.47% As(III), whereas K7Pb could remove 98±0.94% As(V) and 78±1.25% As(III).

Chapter 5: Bioremediation of Arsenic in Packed Bed Bioreactor – Arsenic removal using bacteria immobilized on a polyurethane foam (used as a packing material) was performed in this study. It investigates the removal of arsenic at different flow rates and concentrations in the reactor. The study concluded with the assessment of acute and chronic toxicity of arsenic using bioluminescent bacteria. It was observed that the removal efficiency of TY6 varied from 0.45% to 99.50% for As(III) and 0.75% to 98.40% for As(V). For K7Pb, the efficiency ranged from 0.79% to 99.30% for As(III) and 0.80% to 99.40% for As(V)

Chapter 6: Simultaneous Bioremediation of Arsenic and Chromium(VI) Contaminated Soil – This chapter investigates the removal of arsenic and chromium(VI) from soil using bacteria. It explores the effect of concentration and different extraction agents on removal of arsenic and chromium(VI). The ability of chickpeas to accumulate arsenic and chromium(VI) was also assessed. Plant growth and chlorophyll assessment was also carried out to evaluate the plant growth-promoting trait of the bacteria. It was found that, for arsenic, $CaCl_2$ was the most effective extraction agent with a maximum efficiency of 61.82±2.47% for As(III) and 64.18±3.21% for As(V), and NaOH for Cr(VI) with a maximum removal efficiency of 54.54±2.73% and 56.06±2.80% with TY6 and K7Pb, respectively after 15 days.

Chapter 7: Arsenic Removal Using Immobilized and Encapsulated Bacteria – This chapter focused on encapsulation of bacteria immobilized on peanut shell biochar in polyvinyl alcohol (PVA)-sodium alginate (SA) beads. A thorough investigation was conducted for the evaluation of different parameters that affect the adsorption capacity of on peanut shell biochar and on modified peanut shell biochar for arsenic. In addition, this study elucidates the effect of various factors such as pH, temperature and initial arsenic concentration on removal efficiency. Water absorption capacity and reusability of beads were also carried out. The chapter concludes with a study on removal of arsenic using beads in a recirculating packed bed bioreactor. The optimum parameters were found to be PVA – 5% (w/v), SA – 2% (w/v) and $CaCl_2$ – 1% (w/v) for the most efficient beads. As(III) and As(V) removal efficiency varied between 77% - 94% for different combinations of bacteria. It was observed that beads with individual strains could remove up to 35% arsenic, and those of bacterial consortium (TY6+K7Pb) removed up to 80% of arsenic even at a concentration of 100 mg/L (100,000 μg/L).

Chapter 8: Conclusions and Suggestions – The final chapter summarizes the thesis and draws major conclusions from the research. In addition, limitations of the present work and potential areas for future research have also been suggested.

The research work explores how operating conditions such as pH and temperature affect bioremediation of arsenic using these bacteria and aims at providing insights into their potential as a prospective sustainable solution for bioremediation of arsenic and chromium contaminated soils.