Synopsis

Industrialization, urbanization and technological developments have improved the standard of living for humans on the one hand, but they also have resulted in the generation of wastes containing toxic heavy metals that are detrimental to the ecosystem on the other hand. Therefore, the treatment of waste water containing toxic heavy metals before discharging into the environment has become imperative. Though, the conventional waste water treatment methods like adsorption, electrochemical process, ion-exchange, precipitation, solvent extraction, etc. have served the purpose of removing toxic heavy metals, they have certain limitations such as formation of secondary sludge, inefficiency in removing lower metal concentration, high cost, to name a few. Thus, it becomes of interest to explore alternative cost effective methods capable of removing lower concentrations of heavy metals from waste water.

The method of bioremediation which uses microorganisms for toxic heavy metal removal has gained significance. Various combinations of microorganisms and heavy metals have been researched to assess the abilities of the selected microorganisms in removing the considered metals. Majority of the research studies have focused on the biosorption of metals by the whole cells. However, there is a paucity of research on the role played by the individual cell wall components in metal removal. In addition to remediation, the detection of heavy metals in waste waters is also of equal importance.

In the present research study, the significance of bacteria of *Pseudomonas species* namely *P. putida, P. aeruginosa* and *P. fluorescens* for lead remediation have been assessed. Further, detailed studies have been carried out to elucidate the mechanisms of lead removal through the assessment of the roles played by the individual cell wall components. The lead removal capacities of the individual EPS components purified from the *Pseudomonas sp.* have been determined. Various strategies have been adopted to enhance the lead removal capacities of the three *Pseudomonas sp.* by thermolysis. For the biosorption studies, the parameters namely pH, time of contact, biomass loading and lead concentration have been optimized to obtain the maximum lead binding. Apart from lead removal, biologically modified carbon paste electrodes (CPEs) have been developed using the *Pseudomonas sp.* cells and their EPS components.

The major objectives of this research work are enumerated as follows:

1. Study of the bioremediation of lead from aqueous solutions using cells of *P. putida*, *P. aeruginosa* and *P. fluorescens.*
2. Understanding the role of bacterial cell wall and its components in lead uptake.
3. Effect of thermolysis of the chosen bacterial cells on lead uptake
4. Determination of the lead uptake capacity of extracellular polymeric substances (EPS) of the selected *Pseudomonas sp.* namely, proteins, polysaccharides, biosurfactants and DNA.
5. Enrichment of lead binding proteins from total bacterial protein and examination of the lead binding capacity of the purified protein.
6. Comparison of the protein profiles of the three *Pseudomonas sp.* in the absence and presence of lead.
7. Detection of Pb (II) ions in aqueous solutions using carbon paste electrodes modified with *Pseudomonas sp.* cells and their EPS components using an electro-analytical technique.

The key findings of the research work are summarized below:

The fully grown cells of *Pseudomonas sp.* harvested from the nutrient medium have been used for the experiments. The lead biosorption studies using the *Pseudomonas sp.* show substantial lead biosorption by all the three bacteria chosen namely, *P. putida*, *P. aeruginosa* and *P. fluorescens* in independent studies. The three *Pseudomonas sp.* however show a variation in their lead removal capacities. The highest lead removal is obtained when *P. putida* is used as the biosorbent. The characterization studies using FTIR, EDAX and zeta potential have been carried out for the *Pseudomonas sp.* cells before and after interaction with lead. The EDAX studies confirm the presence of lead ions on the *Pseudomonas sp.* surface. Electro-kinetic studies indicate that the negatively charged bacterial surface, become less electronegative after interaction with lead. The carboxyl and phosphate groups are found to play major role in lead binding by *P. putida* and *P. fluorescens*. In addition to the carboxyl and phosphate groups, amide group also play role in lead binding on the *P. aeruginosa* cell. The lead biosorption using all the three *Pseudomonas sp.* adhere to the Langmuirian isotherm model and follow the pseudo second

order kinetics. The lead removal by the *Pseudomonas sp.* is further improved after thermolysis presumably due to the exposure of more lead binding sites. After thermolysis, the lead uptake is found to increase by about 27 % in the case of *P. putida*, about 18 % in the case of *P. aeruginosa* and about 26 % in the case of *P. fluorescens*.

Taking into consideration that the intact and thermolysed *Pseudomonas sp.* are effective in removing lead, further studies have been carried out to understand which of the individual cell wall components namely DNA, protein, polysaccharide or lipid play a role in lead uptake. The biosorption studies carried out after digesting the cell wall components one at a time using specific enzymes have shown that the lead uptake differs for each component, both in the case of the intact and the thermolysed cells. Though all of the major cell wall components are found to be responsible for lead removal, a greater reduction in lead removal is observed, when the polysaccharide component of *Pseudomonas sp.* is digested and used as a biosorbent.

When the individual cell wall components namely DNA, protein, polysaccharide and biosurfactant are studied for their lead binding capacities in their purified forms, the purified protein from all the three *Pseudomonas sp.* are found to remove a higher percentage of lead, compared to the other purified components. In the case of DNA, the lead biosorption has been studied using both ssDNA and dsDNA. Amongst the two forms of DNA, ssDNA shows a better lead uptake vis-a-vis dsDNA. This is possibly due to the exposure of more lead binding sites in the case of ssDNA which are otherwise masked in the double helical structure of dsDNA. Further, the hydrophilic part of the DNA is found to play a major role in lead binding compared to the hydrophobic part.

Recognizing that the purified total protein is found to be capable of removing more lead compared to the other components, an affinity column chromatography technique has been used to enrich the lead binding protein from the total proteins. The SDS-PAGE documentation of the total and enriched proteins has confirmed the enrichment of specific proteins. The enriched protein fractions exhibit 65 to 95 % of the lead binding capacities of their corresponding total proteins in all the three species of *Pseudomonas* studied. The SDS-PAGE analysis of protein profile in the absence and presence of lead have shown significant differences consequent to lead binding.

Extensive lead detection studies carried out using carbon paste electrode (CPE) modified with the *Pseudomonas sp*. cells and their purified EPS components have highlighted the potential of the biomass modified CPEs for lead detection. The lowest limit of detection (LLOD) for lead has been estimated for each of the modified CPEs studied. The estimated LLODs show that amongst the many biomass modified CPEs studied, the CPE modified using whole cells indicate a better detection of lead compared to the individual purified EPS components. Amongst the CPEs modified using whole cells, the one modified by blending the lyophilized cells with carbon paste is capable of detecting lower concentrations of lead than the one modified with drop coated cells.