CHAPTER - 6

RÈSUMÈ
The findings of the research for bioremoval of Mercury, obtained during the investigations are incorporated in this thesis. The studies are far from complete, but it does picturize the immense potential of microorganisms and their metabolic capability which has not been sapped completely till now. As a part of this thesis extensive work had been undertaken to understand the role of microbial biomass in mercury removal from waste-water.

With the micro organism isolated from various habitats, experiments were conducted to select the most suitable organism and culture it as biosorbent for mercury. Attempts were also made to standardize the waste biomass from pharmaceutical industries as mercury sorbents.

Various physical, chemical and biological parameters that influenced the bioremediation process were outlined and optimization of the process was achieved. Scale up of the process using biosorbent developed from spent mycelia and treatment of waste obtained from different industries and laboratory were also tried.

Honest attempts were made to standardize the entire process at least at laboratory level so that this thesis may provide a sound platform for further optimization and field level application of the developed process.

Ultimately the success of bioremediation process would depend on the selection of appropriate organism for the suitable metal. The working conditions should also match the chosen metal and organism. Success in developing a suitable biosorbent for treating mercury contaminated soil, ground water, surface water and industrial waste process stream would provide the world with a cost effective and environmental friendly technology which may serve as alternative or supplementary to already existing technologies for heavy metal remediation. Bioremediation of metals can offer salvation to the polluted environment.

The important conclusions derived during the entire research work are outlined below:

- Naturally occurring microorganisms in polluted environment showed considerable mercury resistance and remediation ability.
- The isolates showed wide variation in mercury removal from aqueous solutions.
Bacteria and fungi occupied first and second position amongst the group of microorganisms screened for mercury removal ability.

Actinomycetes, apart from slow growers prove poor mercury removers.

Isolate A gave maximum MIC of 18 ppm and better adaptibility to increased mercury concentration, when grown in presence of mercury.

Adapted culture of Isolate A showed better improvement in generation time as compared to the unadapted culture.

At lower mercury concentration (20 ppm) virtually no lag phase was observed for both adapted and unadapted cultures.

The growing cells of Isolate A gave 100% mercury removal at lower initial mercury concentration. The removal was slow and gradual as it employed enzymatic action.

The influence of initial mercury concentration was dictated by the type of the organism used as biosorbent. In all the cases with the increase in initial mercury concentration the % removal decreased.

In case of Gram negative bacteria the loading capacity decreased above critical concentration of mercury which was 800 µg.

In case of Gram positive bacteria, *Fusarium* and spent mycelia the loading capacity got constant above the critical initial mercury concentration.

Any change in the optimum adsorbate-biosorbent ratio resulted in decreased uptake of mercury/unit cellmass.

The loading capacity of any biosorbent was directly proportional to its surface area. Bacterial isolates gave the better mercury loading at lower metal: cellmass ratio as compared to that obtained with fungi.

The pH optima for mercury removal varied with different biosorbents

Bioremediation of mercury was not significantly dependent on pH, under the
experimental conditions.

Preservation of the laboratory grown cellmass enhanced its loading capacity for mercury giving 355μg mercury/mg cellmass.

The chemicals used for pH adjustment of the system had a considerable influence on mercury removal.

The presence of organic acids, citrate buffer and disodium hydrogen phosphate caused reduction in mercury removal.

Mercury removal process by *Fusarium* and spent mycelia was fast compared to bacterial Isolate A.

*Fusarium* biomass showed an equilibrium time of 45 minutes above which the desorption and readsorption cycle was observed.

The rate of mercury removal decreased with increase in contact time.

The Isolate 6 was found to be selective for mercury ions, not influenced much by the presence of sodium ions in the system but sodium ions had a considerable effect on mercury removal by spent mycelia and Isolate A.

Optimum reaction temperature for mercury removal was found to be 30° C.

Any type of pretreatment to the biomass of Isolate A proved detrimental. Apart from the acid treatment, all the other pretreatments to Isolate 6 proved beneficial.

All the pretreatment to spent mycelia had a positive effect on mercury removal but the removal was decreased significantly with methanol, formaldehyde and KOH treated *Fusarium* biomass.

Heat treatment gave maximum improvement in loading capacity of spent mycelia enhancing the removal by 44.78%.

Solvent treatment to the already autoclaved biomass proved futile.

The substrates used for the growth of bacterial Isolate Unk were found to influence the removal process. Sucrose facilitating the production of capsule
was found to be the best.

The optimum conditions for mercury removal by bacterial isolates were as follow:-

- Cellmass : 10mg/system
- Mercury concentration : 20ppm
- pH : 6
- Contact time : 2 hours
- Mercury : cellmass ratio : 1:5

The *Fusarium* and spent mycelia gave best removal under following conditions:

- Cellmass : 1g/system
- Mercury concentration : 200mg/l
- pH : 8
- Contact time : 40 minutes
- Mercury : Biomass ratio : 1:80

Mercury removal process with Isolate 6 and spent mycelia was found to be a function of its initial concentration following Freundlich adsorption isotherms.

Under the experimental conditions *Fusarium* and Isolate A did not followed Freundlich isotherm.

The cellfree water soluble extract of Isolate A resulted in 46.8% mercury removal confirming the role of enzymatic activity in mercury removal.

Significant mercury removal with cellfree extract of Isolate 6 and *Fusarium* was not observed indicating the passive phenomena.

Mercury removal by immobilized cells using sodium alginate and cellmass bound to PVC pieces did not gave encouraging results. Wood shavings and sand were found promising as immobilizing support for mercury removal.

Laboratory level scale up of the experiment was successful giving increase in
loading capacity despite the increase in cellmass and mercury concentration.

Of the studied scale up designs, column study provided the best results with loading capacity of 23.13 mg mercury/g cellmass.

Growing cells of Isolate A were able to remove mercury from actual waste collected from an industry in Vapi.

The bioremediation of mercury was also possible from commercial MEMC powder used as pesticide.

Complete decolourization along with mercury removal from waste benzene containing mercury-iodide-malachite green complex was obtained.

The initial mercury concentration, cellmass concentration, contact time and reactor volume influenced the waste treatment process.

Following conditions resulted in 100% mercury removal from the laboratory organic waste:

- Initial mercury concentration: 20ppm
- Cellmass concentration: 10mg
- Waste volume: 30ml
- Reactor volume: 100ml
- Contact time: 90 minutes
- Aeration speed: 125rpm