SUMMERY OF THESIS

There has been increasing concern over dangerous levels of mercury, contaminating the aquatic environment and sources of potable water because of its known accumulation in food chain and its persistence in nature when they are discharged even in small quantity by numerous industrial activities. Remediation of mercury contaminated waste water is generally accomplished by reverse osmosis, precipitation, evaporation, ion exchange and other physical and chemical methods. The potential use of microorganisms in the treatment of mercury contaminated waste water and in the recovery of metals in mining waste or in metallurgical effluents is of special importance. Biosorption utilizes the ability of biological materials to accumulate heavy metals from waste stream by either metabolically, or purely physico-chemical pathways of uptake. The special surface properties of microorganisms enable them to adsorb metal ions from solution. In the present study *Saccharomyces cerevisiae* is the choice of biosorbent to study the biosorption of Hg^{++} because of its easy availability and large scale cultivation in inexpensive growth medium using unsophisticated cultivation technique. It can be easily genetically and morphologically modified to enhance the biosorption capacity. Permissible limit of Hg^{++} for heterotrophic organisms is 0.01ppm. According to Von Constein et.al.(1999) in Europe, Hg^{++} concentration in Chlor-alkali effluent is 16 to 76ppm. As Hg^{++} itself is a toxic metal and exhibit an inhibitory effect on living system, the objective of our present study is to develope a Hg^{++} resistant organism that can withstand the toxic effect of Hg^{++} ion and accumulate it within and on the outer surface of the cell.

In Chapter I four different organisms, *Bacillus circulans*MTCC3161, *Saccharomyces cerevisiae*, *Aspergillus niger*, *Rhizopus arrhizus* were tested to their Hg^{++} tolerance capacity. Among the four tested organism *S.cerevisiae* is found to have maximum Hg^{++} tolerance capacity (35ppm). The Hg^{++} tolerant strain of all four organisms were isolated form the different concentration of Hg^{++} containing medium. The biosorption capacity of the resistant strain of each organism were compared with their respective parent strains. It is observed that only Hg^{++} tolerant *S.cerevisiae*A100 shows higher biosorption capacity on respect to its parent strain where as in case of other organisms the biosorption capacity declines compared to their parent strain. So, Hg^{++} resistant

*S.cerevisiae*A100 was selected as the most potent biosorbent for biosorption of Hg^{++} from Hg^{++} containing medium.

The appropriate maintenance medium for the *S.cerevisiae*A100 to retain the Hg^{++} removal capacity was selected in Chapter II. The composition of the most suitable maintenance medium for *S.cerevisiae*A100 is:

Dextrose – 1%
$(NH_4)_2SO_4$ – 0.5%
KH_2PO_4 – 0.1%
$MgSO_4,7H_2O$ – 0.025%
$FeSO_4,7H_2O$ – 0.002%
Biotin – 0.5µg/ml
pH – 5.0

In Chapter III the physical parameters involve in Hg^{++} biosorption process was optimized. The optimized physical parameters for biosorption of Hg^{++} increased the biosorption potential of *S.cerevisiae*A100 from 52.3% to 56.4%. The optimized physical condition for this process is found to be:

Initial Hg^{++} ion Concentration in the biosorption medium : 30ppm

Initial pH of the biosorption medium : 5.0

Temperature of the Biosorption Experiment : 30°C

Incubation Period : 48 hours

Age of inoculum : 48 hours

Volume of biosorption medium : 50ml

Cell Density of the inoculums : 1.7×10^6 cells/ml

In Chapter IV suitable carbon and nitrogen source for the proper growth and multiplication of *S.cerevisiae*A100 was optimized. Glucose and Urea were found to be the most suitable carbon and nitrogen sources for this purpose respectively. The optimum concentration of glucose and urea were also determined in this chapter.

Optimum concentration of Glucose : 5.0%

Optimum concentration of Urea : 0.15%

Chapter V deals with the both macro and micro nutrient requirement of *S.cerevisiae*A100. Concentration of phosphase, sulphate and chloride sources were determined in this chapter.

Among the micro nutrients, Fe^{++}, Mn^{++} and Mo^{6+} increase the growth of *S.cerevisiae* and stimulates the biosorption rate. Cu^{++}, Ni^{++} V^{5+} shows negative effect on cell growth and biosorption process. At higher concentration Zn^{++}, Co^{++} exhibit negative influence on biosorption process.The composition of the synthetic medium for biosorption of Hg^{++} by *S.cerevisiae*A100 is as follows:

Glucose : 5.0%

Urea : 0.15%

K_2HPO_4 : 0.15%

$MgSO_4,7H_2O$: 0.06%

KCl : 0.06%

FeSO4,7H2O : 1µg/ml

MnSO4, H2O : 5µg/ml

$Na_2MoO_4,2H_2O$: 10µg/ml

Effect of Complex nutrient on biosorption of Hg^{++} was described in Chapter VI. To commercialize the process of Hg^{++} biosorption, providing all the mineral nutrients separately is not economic. Complex nutrients are the cheap source of many mutrients along with vitamins, amino acids etc growth promoting factors. 10 different natural and commercially available complex nutrient from plant and animal origin were added to the biosorption medium to observe their effect on biosorption of Hg^{++}. Among all complex nutrients meat extract, soyabean meal and paddy soak liquor shows positive influence on biosorption of Hg^{++}. Peptone, rice bran extract, Yeast

Extract, Beef Extract, Malt Extract, Wheat Bran Extract and Corn steep Liquor declines the biosorption rate.

Chapter VII deals with the effect of amino acids, vitamins, metabolic inhibitors and antibiotics on cell growth of *S.cerevisiae*A100 and biosorption of Hg^{++}.

L-Tryptophan has positive effect on biosorption of Hg^{++},while L(-)Arginine HCl, L(-)Threonine, L(-)Lysine, L(-)Valine, L(-)Alanine, L(-)Proline, L(-)Cysteine, L(-) Leucine shows negative effect on cell growth and Hg^{++} biosorption at higher concentration. L(-)Phenylalanine, L(-)Histidine, L(-)Metheonine, L(-)Serine, L (-) Glutamic Acid has no effect on biosorption of Hg^{++}.

Thiamine-HCl and Biotin, has a significant positive effect on biosorption of Hg^{++},while Inositol,Vit.B_{12}, Folic Acid, Riboflavin, Nicotinic Acid, Pyridoxin-HCl and Ca-Pantothanate shows negative effect on cell growth and Hg^{++} biosorption at higher concentration. *p*-Amino Benzoic Acid has no effect on biosorption of Hg^{++}. Thiamine-HCl (in presence of biotin at 1.0µg/ml concentration) accelerate the cell growth as well as biosorption of Hg^{++} significantly. At 1.0µg/ml concentration it shows 98% biosorption.

Addition of 2,4-dinitrophenol and 6-marcaptopurine inhibit the biosorption of Hg^{++} to a greater extend.

Among all tested antibiotics streptomycin sulphate and Chloramphenicol exhibit maximum negative effect on cell growth of *S.cerevisiae*A100 and consequently biosorption of Hg^{++}. On the other hand Tetracycline-HCl, Potassium salt of Penicillin are found to have little inhibitory effect on cell growth and biosorption of Hg^{++} by *S.cerevisiae*A100 at higher concentration when added to the biosorption medium at initial hour. Though Chlotrimazole is a fungal antibiotic, it does not exhibit any drastic declination on cell growth or biosorption process. At higher concentration it shows little negative effect on cell growth and biosorption of Hg^{++} as well.

Chapter VIII describes the effect of surface active agents on biosorption of Hg^{++} by *S.cerevisiae*A100. Tween 80 and sodium lauryl sulface declines the biosorption process significantly. Tween-20 also inhibits the growth and biosorption capacity of *S.cerevisiae*A100. But compare to Tween-80 and sodium lauryl sulphate, tween-20

has minor effect on biosorption and cell growth of the organism. It is also evident that the effect of the surface active agent increases with the increase of concentration. The effect is also dependent with the time of addition of the agent to the biosorption medium.

Chapter IX explains the biochemical changes takes place in the biosorption medium and the biomass in presence and in absence of Hg^{++}.

At the end of 96 hours of biosorption, 95.4% glucose was utilized in absence of Hg^{++} but 96.4% glucose was utilized in presence of Hg^{++}.

On progress with biosorption experiment total urea nitrogen in the biosorption medium was decreasing while the amino nitrogen, ammonia nitrogen and cellular nitrogen was increasing. Upto 48 hours amount of cell nitrogen increased and upto 72 hours ammonical nitrogen in the broth increased. After 48 hours the cell started to enter the death phase causing a declination in cell nitrogen. After 66 hours when cell started to decay, the nitrogenous material in the cell comes out to the broth resulting the increase of amount of amino nitrogen in the broth. After 72 hours amount of the ammonical nitrogen also declines. At the end of 96 hours 70% of the total urea nitrogen is converted to amino nitrogen.

66.32% of total biosorbed Hg^{++} is bound in the cell surface and 33.68% Hg^{++} is accumulated inside the cell of *S.cerevisiae*A100.

maximum Hg^{++} was leached out by EDTA extraction from the organism at 48 hours as maximum Hg^{++} was biosorbed at that time.

Changes on the intracellular and extracellular enzyme activity also takes place due to the Hg^{++} biosorption.In this chapter extracellular and intracellular enzyme activity of acid phosphatase and polyphenol oxidase were measured.

In presence and absence of Hg^{++} mycelia Acid phosphatase activity is greater than the extracellular enzyme activity. With the increase of time both enzyme activities were increased upto the end of the exponential growth phase (48hours).

Again, during biosorption experiment acid phosphatase activity in parent strain is found to be higher than the Hg^{++} resistant *S.cerevisiae*A100.

Same results were found in case of polyphenol oxidase also.

Non-living cells of Hg^{++} resistant *Saccharomyces cerevisiae*A100 is also proved to be an efficient Hg^{++} biosorbent. Use of non-living cells in biosorption process has some advantages as low maintenance cost, no nutritional requirements, chances of contamination is less and reuse of cells after recovering the organisms if possible.

Chapter X describes the biosorption of Hg^{++} using non-living *S.cerevisiae*A100 which found to be more efficient and user friendly method for removing Hg^{++} from Hg^{++} contaminated aqueous solution.The process is temperature independent.The other physical parameters influencing the biosorption of Hg^{++} using non-living biomass were optimized. The optimum Physical conditions are as follows:

Initial Concentration of Hg^{++} in the biosorption medium : 30ppm

Initial pH of the biosorption medium : 5.0

Incubation Period : 48 hours

Cell Density : 0.456mg/ml

In Chapter XI regeneration of Hg^{++} from the *S.cerevisiae*A100 cells after biosorption was described. It is found that at 19 hours shaking 0.1M HCl is capable of regenerate 80% of biosorbed Hg^{++} from the Hg^{++} loaded biomass.

Chapter XII represents the instrumental evidences of biosorption of Hg^{++} by living and non-living *S.cerevisiae*A100 biomass.

The FTIR spectra of parent *S.cerevisiae*, Hg^{++} resistant *S.cerevisiae*A100 and Dead *S.cerevisiae*A100 determines the involvement of the various charge bearing ligands present on the cell surface. The major shift of the many functional groups after biosorption of Hg^{++} indicates the significant binding of the negative charge bearing functional groups with positively charged Hg^{++} ion present on the biosorption medium.

The differences in the surface morphology of parent *S.cerevisiae*, Hg^{++} resistant *S.cerevisiae*A100 and Dead *S.cerevisiae*A100 reveals the cause behind the different biosorption capacity of the three mentioned organism. The surface morphology differs

before and after biosorption process also. The surface morphology was studies using Scanning Electron Microscope.

EDAX Spectra of the living and non-living *S.cerevisiae*A100 strongly evident the presence of Hg^{++} ion on the cell surface only after the biosorption but not before the biosorption process.

From the above study it can be concluded that Hg^{++} resistant *Saccharomyces cerevisiae*A100 can be a potent and efficient biosorbent for removal of Hg^{++} from Hg^{++} contaminated liquid medium.