CHAPTER – I

CHAPTER 1

SCREENING OF MICROORGANISMS AND DEVELOPMENT OF Hg^{++} RESISTANT STRAIN FOR IMPROVED Hg^{++} BIOSORPTION

Bacteria, actinomycetes, cyanobacteria, algae, fungi and yeasts have been tested for metal biosorption with very encouraging results (173,174).

When choosing the biomass for metal biosorption experiments its origin is a major factor to be taken into account. Biomass can come from i) industrial waste which should be obtained free of charge ii) organisms easily available in large amounts in nature and iii) Organism of quick growth, especially cultivated or propagated for biosorption purpose (175).

As mercury itself is a highly toxic metal [permissible limit for heterotrophic organism is 0.001ppm (176)], mercury resistant bacteria (*Bacillus circulans* MTCC 3161), Yeast (*Saccharomyces cerevisiae*), Fungi (*Asperigullus niger* and *Rhizopus arrhizus*) were developed in our laboratory which facilitates more Hg^{++} tolerance compared to their parent strains.(123).

Materials and Methods:

Microorganism and Cultural Conditions: Four different microorganisms were used in the present study. *Bacillus circulans* MTCC3161 was maintained by regular transfer to nutrient agar slant test tubes containing (g/L) Dextrose 10.0, Yeast Extract 2.0, peptone 5.0, Beef Extract 1.0, Sodium Chloride 5.0 and agar 40.0. pH was adjusted to 7.0 slants were incubated at 37°C for 48 hr.

Saccharomyces cerevisiae was maintained by regular transfer to YPDA slant test tubes containing (g/L) Dextrose 10.0, Yeast Extract 5.0, Peptone 5.0 and Agar 40.0. pH was adjusted to 5.0. Slants were incubated at 30°C for 48 hrs.

Aspergillus niger was maintained by regular transfer to Czapek Dox agar slant test tubes containing (g/L) Dextrose 50.0, KH_2PO_4 1.0, KCl 0.5, $NaNO_3$ 2.0,

$MgSO_4,7H_2O$ 0.5, $FeSO_4$, $7H_2O$ Trace amount and Agar 40.0, pH was adjusted at 4.8. Slants were incubated at 30°C for 7days.

Rhizopus arrhizus was maintained regular transfer to PDA slant test tubes containing (g/L) peeled boiled Potato 250, Dextrose 20.0, Agar 40.0. pH was adjusted to 3.5. Slants were incubated at 30°C for 7days.

Preparation of Biosorbent: 10ml of sterilized demineralized double distilled water was poured into each slant containing different groups of microorganisms. Then using separate inoculation loop for different microorganisms, grown on the surface of different media containing agar slants were scraped off carefully .Each type of microorganism was harvested in separate sterilized cotton plugged conical flasks shaken properly in order to minimize clumping of microorganisms.

Preparation of Hg^{++} stock solution: Hg^{++} stock solution (1000ppm) was prepared by dissolving $HgCl_2$ in deionized double distilled sterile water. Working solutions of different concentration was prepared by adding stock solution of different volume to the biosorption medium.

Screening of microorganisms for Hg^{++} tolerance study: Before isolation of a suitable microbial strain for Hg^{++} biosorption study, it is necessary to screen microorganisms under consideration for their Hg^{++} tolerance, so that we can concentrate our study onto a specific microorganism. Cell suspension of four organisms were prepared separately. 2ml volume of each culture suspension with cell density of 1.6 X 10^7 cells/ml (*B.circulans* MTCC 3161), 1.7 X 10^7 cells/ml (*S.cerevisiae*), 2.1X 10^7 cells/ml (*A.niger*) and 2.0 X 10^7 cells/ml (*R. arrhizus*) were inoculated onto separate 150ml conical flasks containing suitable fermentation broth. These medium were amended with Hg^{++} in the form of $HgCl_2$ solution. In this tolerance study, Hg^{++}concentrations in the media was gradually increased from 5ppm to 40ppm. Effect of increasing concentration of Hg^{++} on different microorganisms under test, were understood by measuring the dry cell weight of the microorganism.

Measurement of Dry Cell Weight: After biosorption experiment microorganisms were separated from broth by filtration using Whatmann filter paper. Biomass was dried in hot air oven for 6 hrs at 115°C to remove the total water content of the cell.

Isolation of Hg^{++} resistant colonies of different microorganisms : From the Hg^{++} tolerance study it was evident that microorganisms can resist different concentrations of Hg^{++}, which is assumed by comparing the dry cell weight of the experiment of different microorganisms. Each organism was found to be resistant upto a certain concentration of Hg^{++}. Firstly each of the microorganisms was inoculated into corresponding fermentation broth media amended with maximum Hg^{++} concentration which they can resist. After proper incubation period, the metal resistant microorganisms are isolated by pour plate method. Larger identical colonies from each plate for each microorganisms were isolated. They were transferred to suitable agar slant test tubes and after adequate incubation period they were stored at 4°C.

Comparative study of biosorption potency of isolated Hg^{++} resistant strain: Each of the high Hg^{++} concentration resistant colony of each microorganisms were tested for their biosorption potency from the suitable biosorptiontion broth amended with increasing Hg^{++} ion concentration. After biosorption experiment, the biomass was separated by filtration with Whatman filter paper. The filtrate was subjected to residual Hg^{++} analysis to determine the biosorption potency of the organism. Biosorption potency of the parent strain were also determined in the same manner.

Analysis of Hg^{++}: The residual Hg^{++} ion concentration in the biosorption broth after biosorption experiment was measured by Mercury Analyser MA5840 (155,177).

Percentage of Hg^{++} biosorbed can be calculated by :

$$\% \text{ of } Hg^{++} \text{ biosorption} = [(C_i - C_f)/C_i] \times 100$$

Where, Ci = Initial Hg^{++} concentration present in the biosorptiontion media.

C_f = Residual Hg^{++} concentration in the filtrate.

Statistical Analysis:

Statistical analysis of all data were performed according to student's 't' distribution (178). The level of significance for two tail test was determined from the table with critical values of t.

Result and Disscussion:

Screening of microorganism for Hg^{++} tolerance study: Effect of increasing concentration ranging from 5ppm to 36ppm of Hg^{++} in the biosorption media on *B.circulans* MTCC 3161, *S.cerevisiae*, *A niger* and *R.arrhizus* are given in Fig. 3-6.

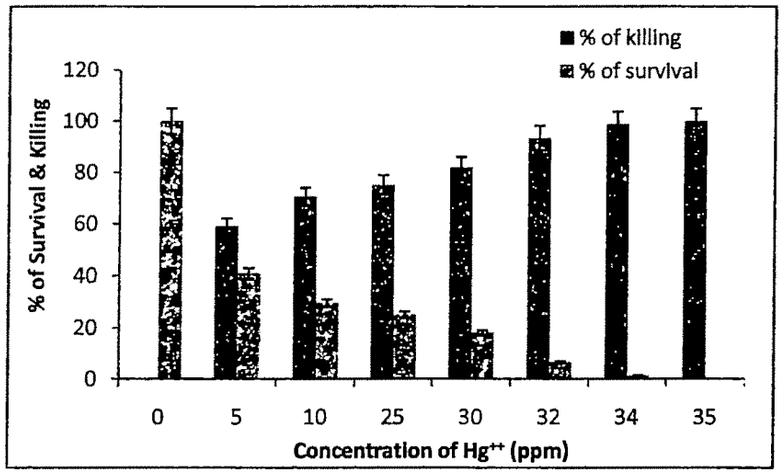

FIGURE 3: Hg^{++} TOLERANCE STUDY OF *Bacillus circulans* MTCC 3161

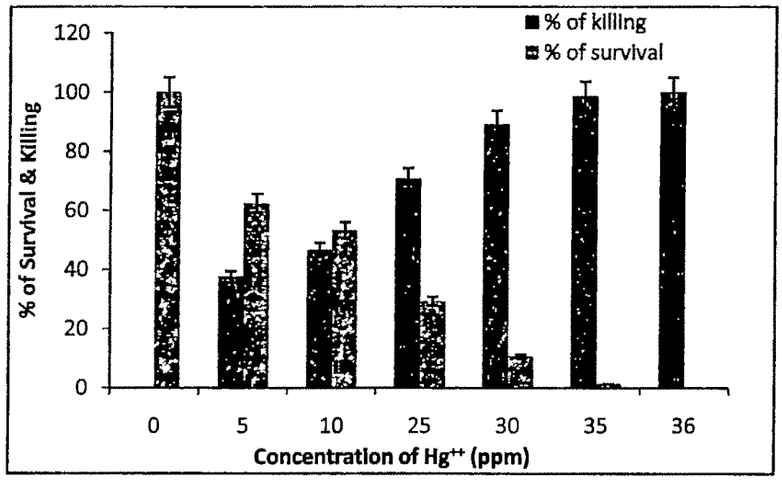

FIGURE 4: Hg^{++} TOLERANCE STUDY OF *Saccharomyces cerevisiae*

R.arrhizus from 30ppm Hg^{++} containing medium can remove 12.8% and 9.4% Hg^{++} respectively. But Hg^{++} resistant *S.cerevisiae* can biosorb much higher percentage of Hg^{++} from 35ppm Hg^{++} containing medium (52.1%).

Comperative study of Hg^{++} resistant microorganisms isolated from Hg^{++} containing biosorption medium and their respective parent strains are given in Fig. 7-10. It is observed that only in case of *S.cerevisiae* the resistant strain shows promising improvement in biosorption capacity.

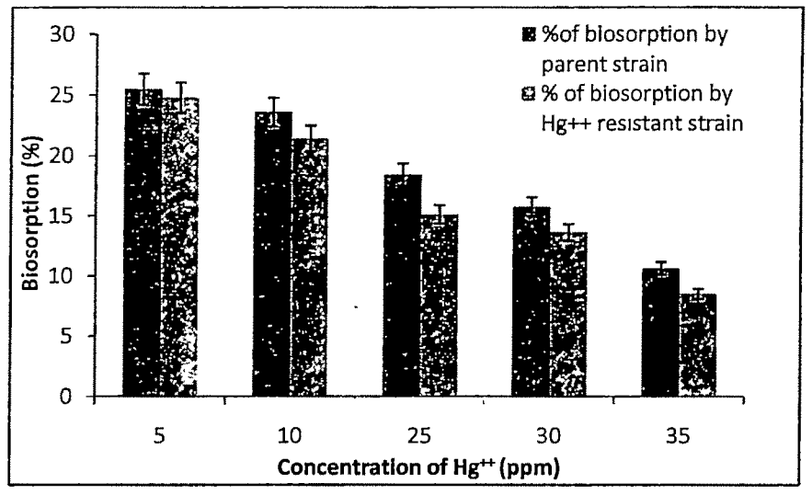

FIGURE 7: COMPARATIVE Hg^{++} BIOSORPTION STUDY BY PARENT & Hg^{++} RESISTANT *Bacillus circulans* MTCC 3161

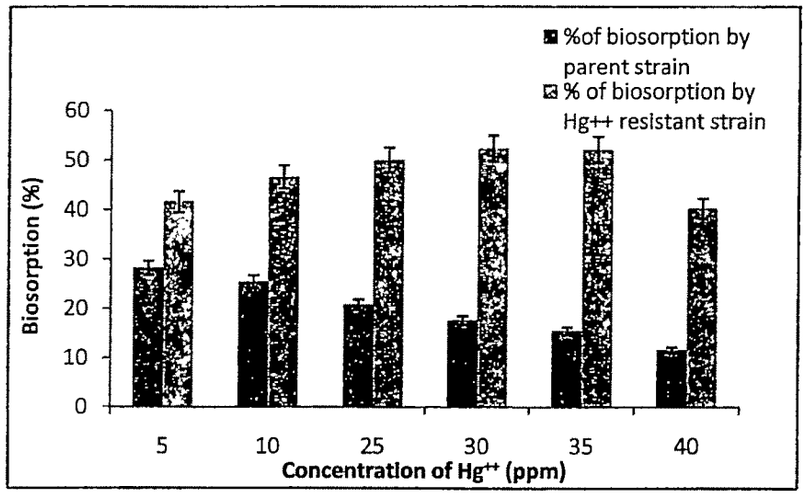

FIGURE 8: COMPARATIVE Hg^{++} BIOSORPTION STUDY BY PARENT & Hg^{++} RESISTANT *Saccharomyces cerevisiae*

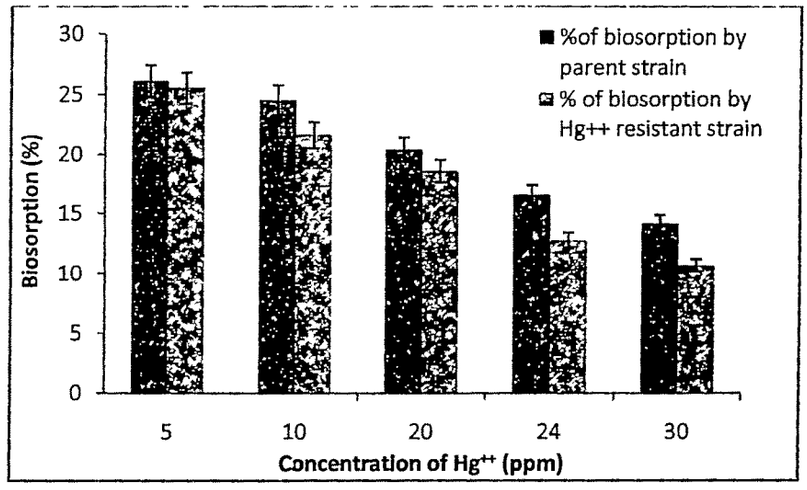

FIGURE 9: COMPARATIVE Hg^{++} BIOSORPTION STUDY BY PARENT & Hg^{++} RESISTANT *Aspergillus niger*

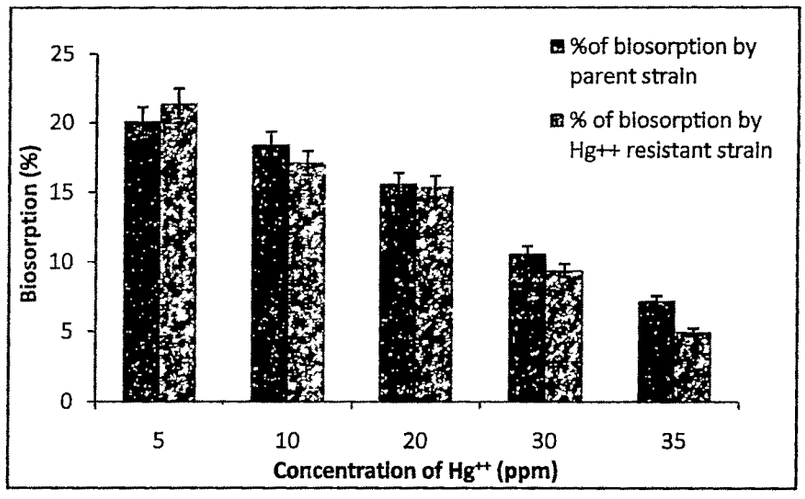

FIGURE 10: COMPARATIVE Hg^{++} BIOSORPTION STUDY BY PARENT & Hg^{++} RESISTANT *Rhizopus arrhizus*

Metal resistance may often be associated with decreased uptake or impermeability. In addition, those external factors which reduce uptake often result in reduced toxicity (179,73). In contrast, a Mn^{++} resistant strain of *S.cerevisiae* is reported to exhibit more Mn^{++} uptake capacity than sensitive parental strain (180). *Aspergillus niger* is previously reported as not a potent organism for biosorption of metal (181,182).

Saccharomyces cerevisiae is easily available from food, beverage and fermentation industries. It can be easily cultivated in large scale in economical and simple growth medium. The organism is safe to handle (183).

Based on all these views Hg^{++} resistant *S.cerevisiae* is considered to be the most suitable biosorbent compared to the bacterial and fungal species.