CHAPTER – III

CHAPTER-III

OPTIMIZATION OF PHYSICAL PARAMETERS FOR BIOSORPTION OF Hg^{++} BY Hg^{++} RESISTANT *Saccharomyces cerevisiae*A100

Biosorption process is highly dependent on the charge bearing ligands present on the cell surface of the organism. The number of charged ligands increases with the increase of cell volume and cell number. Hence, optimum cell growth and multiplication greatly influence the biosorption process. Growth will be dependent on the availability and transport of necessary nutrients to the cell and subsequent uptake and on environmental parameters such as temperature, pH being optimally maintained. The important physical parameters that regulate the growth and metabolism of the organism as well as the rate of biosorption are

i)Initial metal ion concentration ii) Initial pH of the medium iii) Period of incubation iv) Volume of the medium v) Temperature of incubation vi) Age of inoculum vii) Volume of inoculum. In the following Chapter, the optimization of these physical factors are discussed in details.

The uptake rate of the metal ion will increase along with increasing the initial concentration if the amount of biomass is kept unchanged. Contrary to that, biosorptive capacity of the metal ions is inversely proportional to the initial concentration of biomass when the initial metal ion concentration is kept constant. Increase of the biomass concentration of the biosorption system could result in increasing the sorption site interactions. When the biomass concentration is low, metal ions in the solution would not only be absorbed to the surface of the biomass, but also enter into intracellular part through facilitating the concentration gradient of metal ion (183). As a matter of fact , biosorptive capacity of metal ions was reported to be related to the ratio of the concentration of initial metal ions to the concentration of biomass. Vasudevan et. al found that equilibrium uptake for cadmium (II) ion by deactivated protonated yeast was directly proportional to the ratio of the initial metal ion concentration to the sorbent biomass (184). Therefore, both aspects cannot be neglected when

assessing the influence of concentration of metal ion and biomass on biosorption process, otherwise, error would occur. (185).

Fungi generally utilize a substrate in the form of solution only if the reaction of the solution is conductive to fungal growth and metabolism. Experiments with fungi have generally indicated that they are more tolerant of acidic ions (H^+) than basic ions (OH^-). However, most fungi grow between pH 4.0 and 8.0, although there are some exceptions, which have either a narrower or wider range of tolerance (186,187). pH of the surrounding medium executes profound influence upon the availability of each metallic ions, which may at specific pH form insoluble complexes. Metals like magnesium, iron, calcium and zinc are available to the fungus at low pH, but they become insoluble at higher pH. Narasimha et.al. found that utilisation of nitrate from ammoniacal nitrogen was pH dependent in some species (188,189). pH of the substrate also affects the permeability of the cell membrane and internal pH of the mycelium. At acidic pH, the cell membrane becomes saturated with hydrogen ion, which limits the passage of essential cations.

Another aspect of hydrogen ion concentration and fungal growth is regarding their pH altering effects on the media in which they grow. Such an effect is caused due to the uptake or release of anions or cations from or to the medium. If this aspect of fungal nutrition is left unreacted for, it ultimately leads to the cessation of growth and metabolism and causes death of the fungus. Excretion of different metabolites of sugars like organic acids, amino acids, carbon dioxide etc. contribute to the pH changes of the medium, which ultimately influence product formation. Obviously, this pH alteration can be resisted by the incorporation of suitable buffer into the fermentation medium. In a fermentation strategy, the pH must be regulated to produce no cell damage, lengthening the stationary phase and resulting in a more abundant Hg^{++} biosorption(190). Thus, to get both qualitative and quantitative insight into the fermentation, pH of the same is to be monitored and controlled and it is exclusively important for industrial fermentation process (191 -194).

Activities of enzymes are also governed by the pH of the surrounding medium. Although, different enzymes have different pH optima for their activity, the general favourable range lies between pH 4 to pH 8.

According to Esposito et.al. pH value of the solution strongly influences the solution chemistry and the availability of the heavy metals (195). Mapolelo and Torto found that the optimal pH for Cd and Pb biosorption is 5.8 and for Cr(III) is 5.2 (196).Vianna et. al reported that optimum pH for Cu, Cd and Zn was found to be 4.5 (197). Marques et.al studied the pH effects on removal of Cu, Cd and Pb from unbuffered aqueous solution by non-viable *S.cerevisiae*. A shift from 4.5-5.0 to a final value of 7.0-8.0 range was observed (198). Ozer and Ozer found the optimal pH value for Pb(II) and Ni (II) ion uptake is 5.0 (199).

Due to rapid multiplication of microorganism, noticeable changes occur in the culture media in a short time interval. But the rate of multiplication of the organism is not remaining constant throughout because of exhaustion of the nutrients, accumulation of toxic metabolic waste products and destruction of the microorganism due to over population. The death rate increases as the culture ages. The more sensitive cells die first where as the most resistant survives till the end of the cycle. Thus, in microbial biosorption process, optimum time period of incubation is important as the organism takes a specific time for its growth and desired activity. Further incubation after the optimum time period will not be beneficial to the process. Therefore, for the biosorption of Hg^{++} from Hg^{++} amended growth medium by *Saccharomyces cerevisiae*A100, optimum time period of biosorption is quite important and hence, that has to be determined.

Water is at the center of all biotechnological process and in most cases will be the dominant component of the media in which microorganisms grow. The quality of water is highly relevant because it affects microbial growth and the production of specific by-products. It is increasingly being realized that, in respect of volume, water is one of the most important raw materials in many biotechnological processes and that its supply and use must be carefully monitored and controlled (200).

Microbial growth rate as with all chemical reactions is a function of temperature. In general, the growth of the fungi stops at temperatures above 40^0C. Below 0^0C, fungal activities stop but still they manage to survive. The general optimum temperature range for many fungi for their better propagation and activity lies between 15°C to 32°C (201-206).

The effect of temperature on the specific growth rate is described by Arrhenius equation $\lambda = Ae^{-Ea/RT}$, where A is the Arrhenius constant, Ea is the activation energy (Kcal/mole), R is the universal gas constant, T is the absolute temperature. For fungal growth, the activation energy lies between 12.8 to 17.1 Kcal. mol^{-1} whereas, the energy of activation for the death rate of fungi is in the range of 70 to 90 Kcal. mol^{-1}. Above the maximum temperature, the growth rate begins to fall. This results from increased rate of microbial death. The dependence of death rate is a strong function of temperature. The high value of Ea for microbial death means that the rate of death increases much faster with temperature, than the rate of growth (low values for Ea) (207). Temperature also affects the efficiency of the carbon energy substrate conversion to cell mass as reported in studies of the growth of yeast and population of bacteria (208). Temperature also affects a variety of metabolic processes in the cell. Macromolecular compounds especially RNA as well as growth rate are strong functions of temperature (209).Thus, in process optimization temperature plays a key role in growth rate and intracellular biosorption rate. Hence, temperature is an important factor in process optimization. This fact has also been reported by many other investigators (210-212).

For biosorption, one of the important factors is growth of the microorganism which depends on the ratio of volume of inoculum to the volume of growth medium. If this ratio is either less or more than the critical value, then, cell propagation will be severely affected. According to Wang and Staba (213), Verma and Van Huystee (214), and Veliky and Genest (215), ten to fifteen percent (Volume/ Volume) of an actively growing microorganism suspension is an effective inoculum for microbial process. The biosorption medium should have homogeneous cell suspension, as the growth rate of microorganisms depends on the number of free cells or spores. The ratio of free cells to cell aggregates also influence the growth rate (216). Also, the medium is to be conditioned by the factors released from the cells of inoculum, and growth of the microorganism resumes only after a critical concentration of these factors is attained in the medium (214). Again, if the volume of the inoculum is more than the critical value, then, the conditioning time of the medium is less but the relative growth rate is also reduced (217). On the other hand, the number of progeny microorganisms is less than required if the volume of inoculum used is less. Thus, for better biosorption of Hg^{++} from Hg^{++} containing medium an optimum inoculum

volume is to be maintained for a definite volume of biosorption medium and thus, both have to be determined.

When microorganism is inoculated into a nutrient medium, multiplication does not take place in a regular manner. It proceeds through a number of distinctive growth phases. Every microorganism has its own growth curve. For a particular organism, a definite time is needed for its optimum growth. Here, the term 'definite growth' means a specific microbial population having definite age where the desired activity is maximum. Thus, for biosorption purpose, the biosorption medium is to be inoculated by microorganism having specific age and that has to be determined.

In the present study, optimum cultural conditions with particular reference to the effect of the following factors have been observed

i. Initial metal ion concentration
ii. Initial pH of the medium
iii. Period of incubation
iv. Volume of the medium
v. Temperature of incubation
vi. Age of inoculum
vii. Volume of inoculum.

Materials and Methods

The medium used for the biosorption of Hg^{++}, consisted of Glucose 1%, $NaNO_3$ 0.2%, KH_2PO_4 0.1%, KCl 0.05%, $MgSO_4$, $7H_2O$ 0.05%, $FeSO_4,7H_2O$ 0.05µg/ml. pH was adjusted to 5.0. This solution was sterilized at 121°C for 15 minutes.

The optimum cultural conditions for the biosorption of Hg^{++} from Hg^{++} containing medium by *Saccharomyces cerevisiae*A100 were determined by keeping all the factors constant except the one which was to be optimized. The microorganism and the method of determination of % of biosorption of Hg^{++} and dry cell weight were same as discussed in the earlier Chapter (Chapter I, of the Thesis).The pH of the medium was measured by using pH-meter.

Result & Discussion :

Initial Hg^{++} ion concentration: The Hg^{++} resistant (35ppm) *S.cerevisiae*A100 was grown for 48hours in different concentration of Hg^{++} containing 50ml broth medium ranging from 5-50ppm.

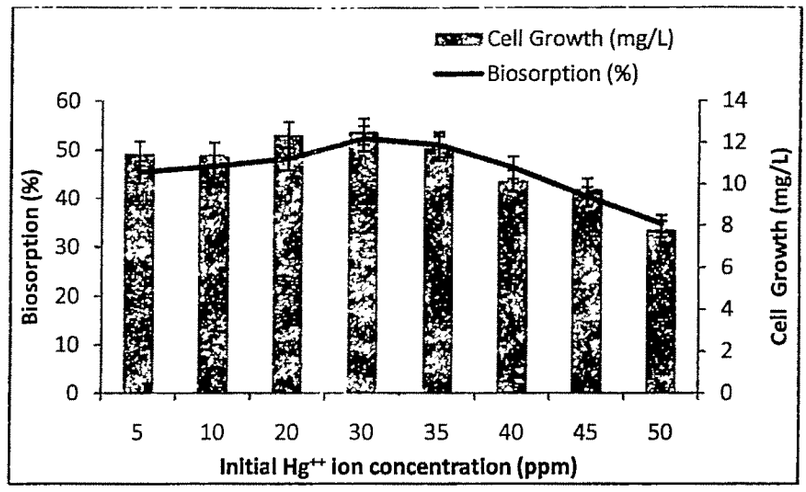

FIGURE 11: EFFECT OF INITIAL Hg^{++} ION CONCENTRATION ON BIOSORPTION OF Hg^{++} AND CELL GROWTH

Fig.11. depicted that the optimum biosorption (52.3%) was noticed at 30ppm Hg^{++} containing medium, after which the biosorption gradually decreases (123). As the organism used in this study was 35ppm Hg^{++} resistant, it can grow , multiply and survive only below 35ppm Hg^{++} concentration. As the organism cannot withstand higher than 35ppm Hg^{++} concentration, the intracellular biosorption stops and total biosorption decreases.

The increase of biosorption with the increase of metal ion concentration is probably due to higher interaction between metal ions and each of biosorbents(158).

Initial pH of the medium: For biosorption of heavy metal ions , pH is one of the most important physical parameters. To study the effect of different pH value, the Hg^{++} resistant *S.cerevisiae*A100 was inoculated at 5 different medium containing 30ppm Hg^{++} ion concentration with different initial pH value ranging from 4.0-7.0.

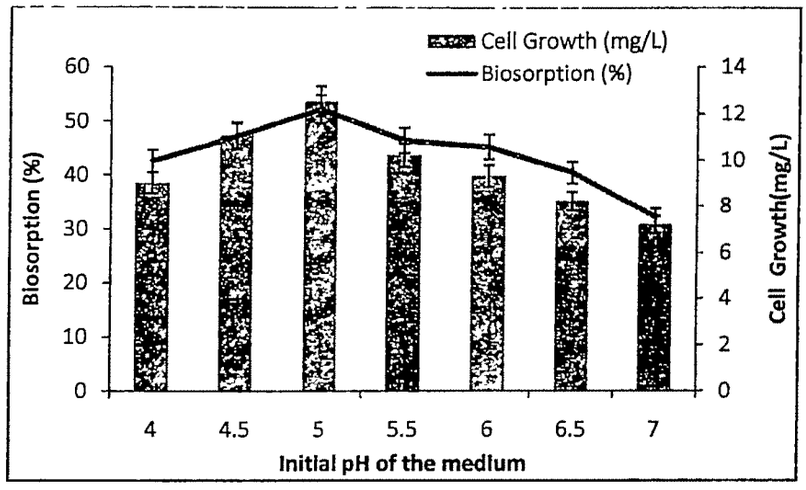

FIGURE 12: EFFECT OF INITIAL pH OF THE MEDIUM ON BIOSORPTION OF Hg^{++} AND CELL GROWTH

It is found in the Fig.12. that maximum biosorption (52.3%) occurred at pH 5.0. (123).

At lower pH, the cell surface ligands were closely associated with hydronium ions (H^3O^+) and restricted the apprcach of metal cations as a result of repulsive force, while at higher pH hydroxo species of the metals can be formed and do not bind to the adsorption sites on the surface of the biosorbent (183).

At pH 5.0 divalent positive ions are suitable to interact with negatively charged groups in biomass. On the otherhand, the outer layer of the cell wall of *S.cerevisiae* consists of a coat protein which can cause a charge through dissociation of ionisable side group of the amino acids. The ionic state of ligands such as carboxyl, phosphate, imidazole and amino groups will promote reaction with the positively charged metal ions (129,133,154,156,183). pH 5.0 was reported to be the optimum pH for biosorption of Hg^{++} by yeast previously (116,156,218).

Temperature: Biosorption of Hg^{++} by Hg^{++} resistant *S.cerevisiae*A100 was employed for biosorption at different temperatures ranging from 23-36°C.

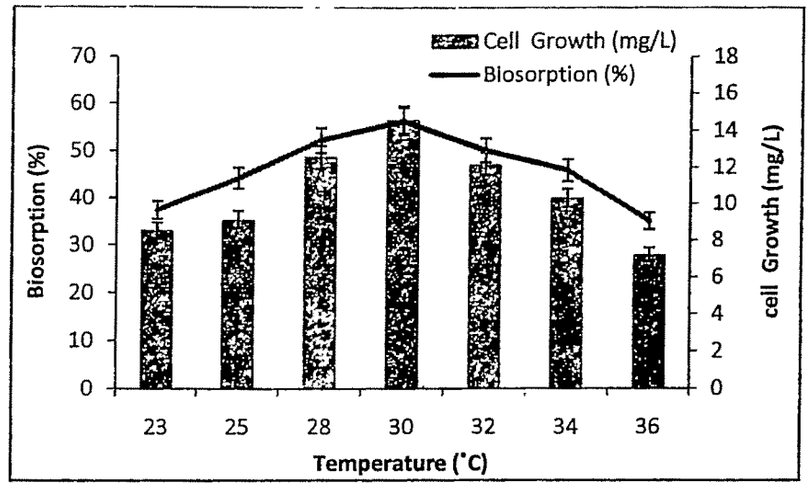

FIGURE 13: EFFECT OF TEMPERATURE (°C) ON BIOSORPTION OF Hg^{++} AND CELL GROWTH

Fig.13. shows that optimum biosorption (56.4%) was noticed at 30°C (170).

The temperature of the biosorption process is important for energy dependent mechanism in metal biosorption by microorganisms(133). The decrease of the biosorption capacity after 30°C may be due to the damage of active binding sites in the biomass(183).

Incubation period: Biosorption process using *S.cerevisiae*A100 was carried out at different incubation periods ranging from 24hours to 96hours. It is observed that Hg^{++} biosorption by *S.cerevisiae*A100 was gradually increased and reached maximum at 48hours,after which it declined. Results are shown in Fig.14

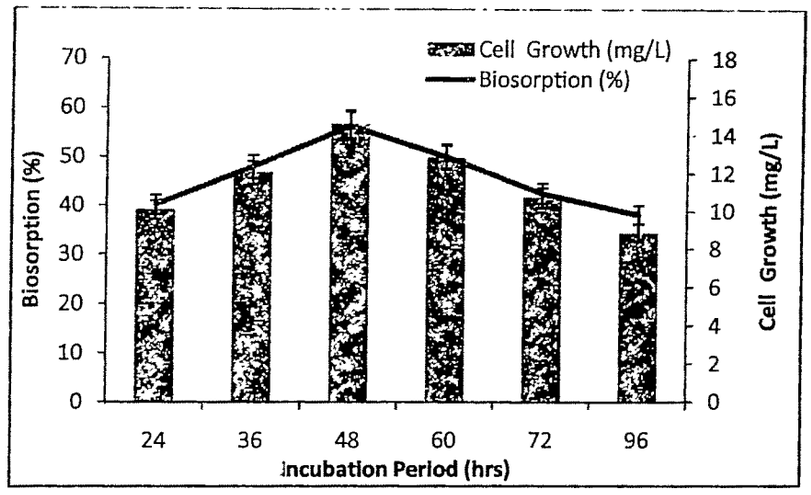

FIGURE 14: EFFECT OF INCUBATION PERIOD ON BIOSORPTION OF Hg^{++} AND CELL GROWTH

At lower time period, biosorption at cell surface was completed but intra-cellular accumulation was in progress, so the biosorption capacity observed was increasing in nature. After 48hours cells enter the death phase and intracellular accumulation stops, so total biosorption decreases. During biosorption process when equilibrium reaches, some cations are desorbed from the cell surface, so the total biosorption of the metal falls down(219, 220).

Volume of the media: *Saccharomyces cerevisiaea*A100 was inoculated at different volume of biosorption media ranging from 30ml to75ml. Maximum biosorption was observed at 50ml media. Results are shown in Fig.15.

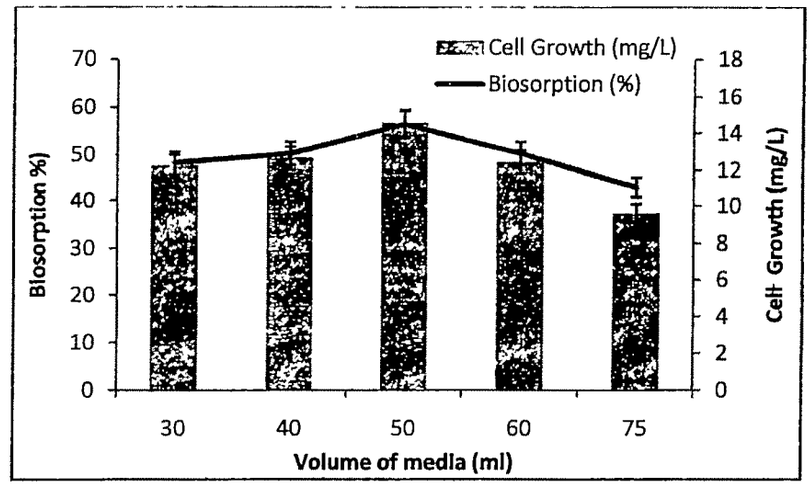

FIGURE 15: EFFECT OF VOLUME OF BIOSORPTION MEDIA ON BIOSORPTION OF Hg^{++} AND CELL GROWTH

At lower media volume nutrient was not sufficient for optimum growth and multiplication of the organism. So biosorption rate decreases. At adequate nutrient availability biosorption was maximum. At more than 50ml media volume , the excess nutrient acts as interfering element and decrease the rate of biosorption (183).

Age of inoculum: Hg^{++} biosorption is observed to be maximum by 48hours grown *Saccharomyces cerevisiaea*A100, where the cell surface and protein content is maximum than the cell of lag phase. As the negatively charged legands of the cell surface takes part in biosorption, the mature cell surface and amino acid content of the cell wall found to be more potent than the growing cells of lag phase . Again the cell of death phase (after 48 hours) exhibit lower biosorption capacity due to the unavailability of intracellular accumulation and multiplication capacity (183). Results are shown in Fig.16.

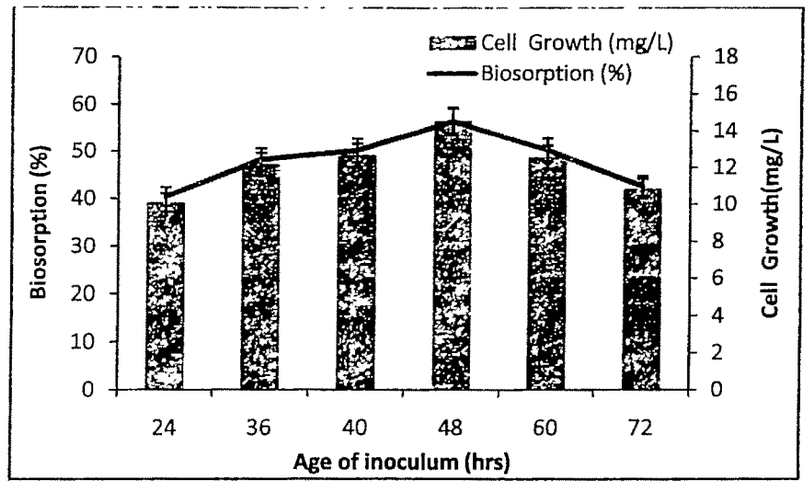

FIGURE 16: EFFECT OF AGE OF INOCULUM ON BIOSORPTION MEDIA ON BIOSORPTION OF Hg^{++} AND CELL GROWTH

Cell Density: From Fig.17 optimum cell density of the inoculums for biosorption of Hg^{++} by *S.cerevisiae*A100 is 1.7 X 10^6 cell/ml (V/V).

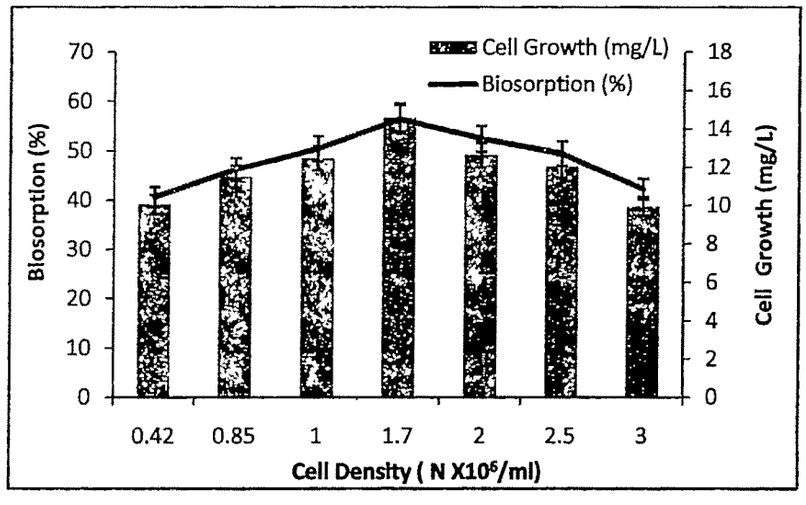

FIGURE 17: EFFECT OF CELL DENSITY OF INOCULUM ON BIOSORPTION OF Hg^{++} AND CELL GROWTH

At lower cell density the available binding sites for metal ions decreases causing the declination in biosorption .When the cell density increases above the optimum level, the nutritional requirement is not enough, resulting the cell death and biosorption rate falls down (183).

CHAPTER – IV