CHAPTER IV

SELECTION OF SUITABLE CARBON AND NITROGEN SOURCES FOR BIOSORPTION OF Hg^{++} BY Hg^{++} RESISTANT *Saccharomyces cerevisiae*A100

For the maintenance and sustenance of life, all living organisms require energy. On the basis of sources from which energy is derived, organisms are of two types – phototrophs and chemotrophs. Both the organisms have certain basic requirements of raw materials for their nutrition. Some of the important substances are carbon, nitrogen, hydrogen, oxygen, sulphur, phosphorous, sodium, potassium, calcium, magnesium, iron, manganese, zinc, copper, cobalt and molybdenum. Among them, carbon occupies a unique position, as compounds having carbon-carbon linkage are the characteristic features of the animal world as a whole. On contrary to the autotrophs, the non-chlorophyllous organisms including fungi entirely depend upon the autotrophs for meeting their carbon requirement as the heterotrophs are unable to utilize the inorganic sources of carbon.

Like the rate of chemical reaction, the growth rate of the microorganism depends on the concentration of chemical nutrients. Monod expressed this fact by the relationship: $\mu = \mu_m [S / (Ks + S)]$, where μ and μ_m are the specific growth rate and maximum value (h^{-1}) respectively, S is the substrate concentration and Ks is the substrate concentration at $\mu = 0.5\ \mu m$.(221). The significance of this expression is that the dependence of growth on chemical concentration can be described by two constants – the saturation constant Ks and the maximum growth rate. This is found to be true for a wide variety of nutrients (222).

A. Selection of Suitable Carbon Source For biosorption of Hg^{++} by Hg^{++} resistant *Saccharomyces cerevisiae*A100

Carbon is a component of both structural and functional constituents of cell. It comprises about fifty percent of the total mycelial dry weight in yeast. A multitude of organic constituent of yeast cell, like carbohydrates, proteins, nucleic acids, enzymes etc. are all made up of carbon. All the important components of cell wall like

cellulose, chitin and pectin substances contains carbon in varying form and concentration, and thus provides the structural frame work of the microbial cell.

As fungi exhibited carbon heterotrophy, they obtain their carbon requirement from various organic sources. There have been few reports on utilization of inorganic carbon by fungi in the form of CO_2 but they did not use it as a sole source. A variety of organic compounds are utilized by fungi and the nature of the organism largely determines the range of substrates to the large extent. A number of carbon sources including carbohydrates, organic acids and amino acids along with their derivatives as well as some polycyclic compounds and alkaloids are used by fungi. It has been reported that the monosaccharides are more easily utilizable carbon source than oligo or polysaccharides.

Monosaccharides usually are easily assimilable form of carbohydrate, among which glucose has been reported to be the most efficient source of carbon and energy for most of the fungi. Hasisa and Wolf reported that in *Aspergillus niger*, glucose is present always as a constituent of mycelium irrespective of the carbon source (223,224). According to Tandon, the comparative study with four hexoses showed that glucose and fructose are good carbon sources for most of the fungal species, galactose is after glucose and fructose but mannose is a poor carbon source (225). It has been reported that compounds having more than three carbon atoms are better nutrients for fungi (226). This is due to the fact that after one or two initial reaction, the most of those compounds are oxidized through glycolysis and TCA cycle.

Disaccharides like sucrose, maltose, cellobiose, lactose and melibiose have got greater applicability in fungal nutrition. Plant pathogenic fungi preferentially use sucrose as a carbon source among different disaccharides. Sucrose is metabolized by breaking down into glucose and fructose. Bilgrami reported that among the above two monosaccharides, the utilization of glucose fraction is comparatively rapid (227).

Generally the organic acids, like citric acid, succinic acid, fumaric acid, lactic acid, maleic acid which are products of glucose metabolism, are poor carbon sources. This phenomena is due to the impermeability of cell due to low pH level caused by organic acids.

All nutrients supplied to the medium have to be utilized for proper growth and activity of the microbe. Thus, keeping in view the need for maximum cell growth and biosorption of Hg^{++} as well, efforts to modify the chemical composition of medium were made.

Material & Methods:

For the selection of suitable carbon source, the 50 ml basal medium consisted of $NaNO_3$ – 0.2%, KH_2PO_4 – 0.1%,KCl – 0.05%, $MgSO_4$,$7H_2O$ – 0.05%, $FeSO_4$,$7H_2O$ – 0.05µg/ml. pH of the medium was adjusted to 5.0. The solution was sterilized at 121^0C for 15 minutes. Hg^{++} ion concentration in the biosorption medium is adjusted to 30ppm by adding specific volume of Hg^{++} stock solution . Biosorption experiment was carried out at $30^0C \pm 0.5^0C$ at B.O.D. incubator for 48 hrs using 48 hrs old culture with 2 ml inoculum volume having cell density 1.7×10^6 per ml.

A series of carbon sources were used in the medium to observe their effect on the cell growth of Hg^{++} resistant *S.cerevisiae*A100 and biosorption of Hg^{++} and the results are shown in Fig.18. The concentration of each carbon source was maintained as 1% (w/v) in the biosorption medium. Each carbon source was sterilized separately and added to the above mentioned basal medium aseptically. The microorganism and the method of determination of the percentage of Hg^{++} and dry cell weight were same as discussed in the earlier Chapter (Chapter 1, of the Thesis).

Result & Discussion:

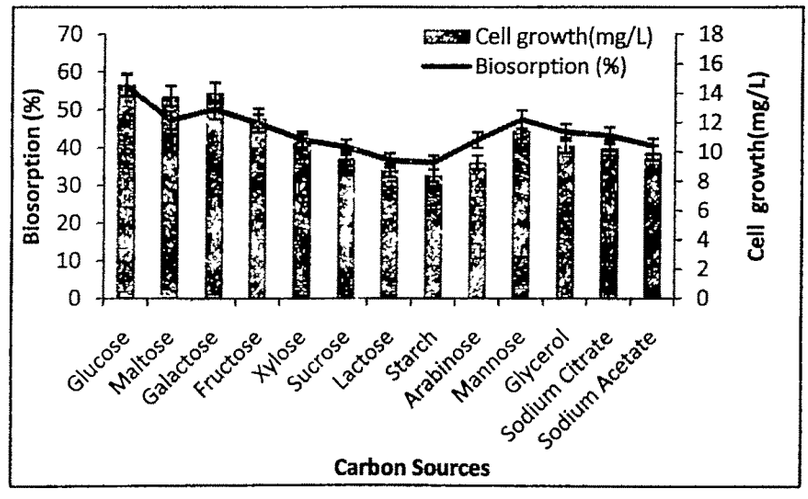

FIGURE 18: EFFECT OF DIFFERENT CARBON SOURCES ON BIOSORPTION OF Hg^{++} AND CELL GROWTH

Fig.18. indicates that glucose is the most suitable carbon source for maximum growth of the biomass and consequently it facilitates maximum biosorption of Hg^{++}.Glucose is a widely used carbon source and also acts as an important source of energy. For the biosorption process using living cells, the addition of glucose enhance the growth and the biosorption capacity of the used biomass. Stoll and Duncan stated that pretreatment of yeast biomass increased the total metal removal but addition of glucose directly into the yeast and metal contaminated solution had no significant effect on the amount of metal accumulated (117). Avery and Tobin observed that live cells of *S.cerevisiae* incubated in presence of glucose(2%,W/V), stimulates Sr^{++} uptake(228). Mapolelo and Torto reported that the pretreatment of *S.cerevisiae* using 10-20mM glucose increased the removal efficiency for Cd^{++}, Cr^{3+},Cu^{++},Pb^{++} and Zn^{++} by 30-40% but pretreatment by 60mM glucose decreased the removal of Cr^{6+} by almost 50% (199).On the contrary, Lin and Vazquez stated that *T.atroviride* was capable of removing more metal ion in absence of glucose comparing in presence of glucose (229). Chojnacka et.al. found that decrease in initial glucose concentration favoured the synthesis of cell resulting better uptake of cation by microalgae *Spirulina sp.* (230).

B. Determination of the Optimum Concentration of Glucose for the biosorption of Hg^{++} by *S.cerevisiae*A100:

To facilitate maximum biosorption of Hg^{++}, the optimum concentration of the glucose should be maintained. If the concentration of carbon source is more than the optimum value, then, it will cause a decrease in growth rate. This phenomenon is known as "substrate inhibition effect". This is because of high osmotic pressure of the solution which causes partial dehydration of the cell. It has been observed that bacteria are more sensitive to osmotic pressure effect than are the molds and yeasts. Again, if the concentration of the carbon source is less than the optimum level, then, the rate of availability of the agents (charge bearing ligands present on the cell surface) which are responsible for the biosorption of Hg^{++} is also less. Since, glucose is the best carbon source for the biosorption of Hg^{++} by *S.cerevisiae*A100, different concentrations of glucose were tested to determine its optimum concentration for the same. The results are depicted in Fig.19.

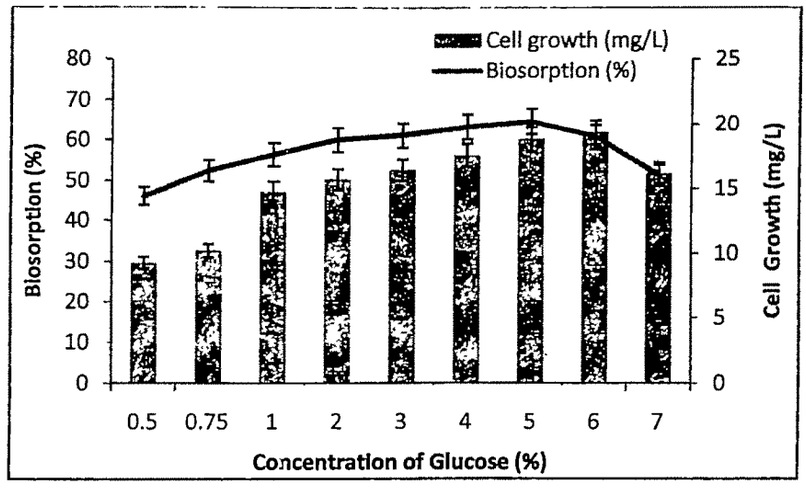

FIGURE 19: EFFECT OF DIFFERENT GLUCOSE CONCENTRATION ON BIOSORPTION OF Hg^{++} AND CELL GROWTH

Fig.19 shows that at 5% glucose concentration maximum cell growth and biosorption is achieved. Alteration in the optimum concentration declines the biosorption efficiency of the *S.cerevisiae*A100.

Different workers found various concentration of glucose to be optimum for the biosorption process depending upon the biomass used and the target metal in the solution. Adamis et.al. reported 2% glucose concentration to be optimum for Cd^{++} biosorption using *S.cerevisiae* (140). Yalcinkaya et.al. stated that 10% glucose is optimum for biosorption of Cd^{++} and Hg^{++} by immobilized *Pleurotus sapidus* (134). Awofolu et.al. reported citric acid as the suitable carbon source for *Aspergillus niger* for Pb^{++} removal (152).

C. Selection of Suitable Nitrogen Source for biosorption of Hg^{++} by *S.cerevisiae*A100:

Next to carbon source, nitrogen source is the most important substance of the growth medium. A few organisms utilize nitrogen source as energy source also. Nitrogen is also utilized both for functional as well as structural purposes by fungi. Different forms of nitrogen have a profound effect on the metabolism of fungi. Generally, the nitrogen content of fungi is about 14% of its dry weight. Fungi meet nitrogen requirement from nitrates, ammonium sources and organic sources specially the amino acids. Unlike the carbon sources, the nitrogen sources are good for both growth and reproduction. Nitrogen is assimilated in

the cell as glutamate and glutamine.These two compounds are responsible for the synthesis of many nitrogen containing molecules like asparagine, histidine, tryptophan and purine nucleotides. Some researchers demonstrated that nitrogen and carbon contents of the medium cause pronounced qualitative and quantitative variations in the amino acid contents of the mycelium. (188, 189, 231).

Many algae and fungi use ammonium nitrate and sodium nitrate as nitrogen sources, however, yeasts and bacteria have problems utilizing nitrogen in this form. Few organisms are able to assimilate nitrates. Organic sources of nitrogen in synthetic media are specific amino acids, purines, pyrimidines, and urea. Urea depending upon the buffer capacity of the system will raise the pH value of the medium. Organic urea is also formed from urea cycle reaction, starting with ammonia (232).

$$NH_3 + ATP + CO_2 \rightarrow H_2NCOOP + ADP$$

Ammonium sulphate produces acidic conditions because the ammonia is rapidly utilized and free acid is then liberated. Many studies suggest that the ammonium nitrogen for many fungi becomes unutilizable due to acidification of culture medium (232).

Since the composition of the biosorption medium largely influences the growth of *S.cerevsiae*A100 and thus biosorption of Hg^{++}, it is necessary to search for a suitable nitrogen sources for the same. In this Chapter, earlier we mentioned that 5% glucose is the better carbon source than the other for the biosorption of Hg^{++} from Hg^{++} containing growth medium. Here, we studied the effect of several nitrogen sources for the same.

Material & Methods:

For the selection of suitable nitrogen source, the 50 ml basal medium consisted of Glucose – 5%, KH_2PO_4 – 0.1%,KCl – 0.05%, $MgSO_4$,$7H_2O$ – 0.05%, $FeSO_4$,$7H_2O$ – 0.05µg/ml. pH of the medium was adjusted to 5.0. The solution was sterilized at 121^0C for 15 minutes. The amount of nitrogen contained 0.032% in all nitrogenous salts were sterilized separately and added to the basal medium aseptically. Hg^{++} ion concentration in the biosorption medium is adjusted to 30ppm by adding specific

volume of Hg^{++} stock solution . Biosorption experiment was carried out at $30^0C \pm 0.5^0C$ at B.O.D. incubator for 48 hrs using 48 hrs old culture with 2 ml inoculum volume having cell density 1.7 x 10^6 per ml. The results are shown in the following Fig.20.

Result and Discussion:

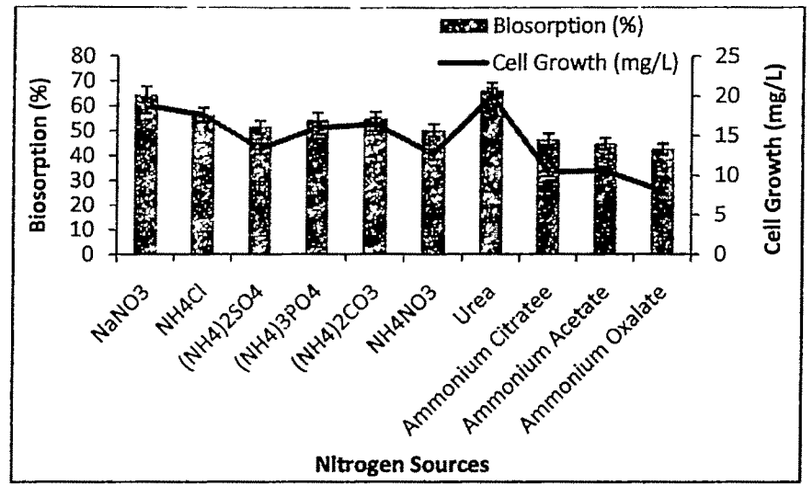

FIGURE 20: EFFECT OF DIFFERENT NITROGEN SOURCES ON BIOSORPTION OF Hg^{++} AND CELL GROWTH

Fig.20. exhibits that urea is the most suitable nitrogen source for the optimum growth and biosorption of Hg^{++} using Hg^{++} resistant *Saccharomyces cerevisiae*A100. Duru et. al. reported that urea was the best nitrogen source among $(NH_4)_2SO_4$, NH_4NO_3, NH_4Cl and urea for mutant *Aspergillus oryzae.*(233). $(NH_4)_2SO_4$ is reported to be the most suitable nitrogen source for yeast and *A.niger* during Cr^{6+} and Pb^{++} biosorption(234,152). Suh et.al. stated NH_4Cl is the most suitable nitrogen source for *S.cerevisiae* while used for Pb^{++} removal(125). During Hg^{++} biosorption study by *Pseudomonas fluorescens* BM07 $(NH_4)_2SO_4$ is found to be the most suitable nitrogen source(158).

D. Determination of Optimum Concentration of Urea for biosorption of Hg^{++} by *S.cerevisiae*A100:

The optimum concentration of nitrogenous substance is important for optimum growth and reproduction of the cell. Besides, accumulation of toxic metabolites, the pH changes in the medium due to its nitrogen content are much sharper than due to carbon sources and this may also be a reason for limiting good sporulation to a

narrower range of nitrogen concentration. As urea is found to be the most suitable nitrogen source for the growth of *S.cerevisiae*A100, it is important to optimize the suitable concentration of urea to achieve maximum growth of the biomass and consequently the maximum biosortion of Hg^{++}. Different concentration of urea were used for the biosorption experiment to determine the optimum concentration for maximum cell growth and biosorption of Hg^{++}. The result are shown in Fig.21.

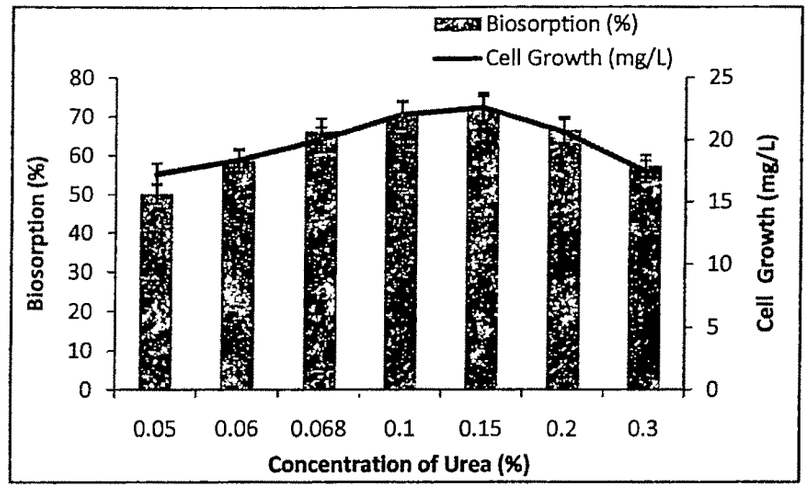

FIGURE 21: EFFECT OF DIFFERENT NITROGEN CONCENTRATION ON BIOSORPTION OF Hg^{++} AND CELL GROWTH

It was observed from the Fig.21 that maximum cell growth and biosorption of Hg^{++} is achieved at 0.15% urea nitrogen. With the higher or lower concentration of urea, the rate of cell growth and biosorption significantly declines.

Thus from the present investigation it can be concluded that:

Most suitable carbon source	: Glucose
Most suitable nitrogen source	: Urea
Concentration of glucose	: 5%
Concentration of urea	: 0.15%