CHAPTER –VI

CHAPTER VI

EFFECT OF COMPLEX NUTRIENTS ON BIOSORPTION OF Hg^{++} BY *Saccharomyces cerevisiae*A100

Availability and type of nutrient can exert strong physiological control over biosorption process and cell growth. Presence of some essential growth factors in complex nutrients of cheap natural products or by products was found to have stimulatory effect on biosorption experiment. The basic nutritional requirements of micro-organisms were an energy or carbon source, an available nitrogen source, inorganic elements and trace metals and these were optimized in previous chapter. But the proposed synthetic medium is not economical and conventional for large scale biosorption process. Hence complex medium have to be employed for large scale Hg^{++} removal from waste water and industrial effluent and growth of microorganism. For this purpose, the effect of complex nutrients from various plant and animal origin like, yeast extract, beef extract, malt extract, wheat bran extract, rice bran extract, paddy soak liquor, peptone, corn steep liquor, soyabean meal were studied to explore the synergistic effect of various nutrients present in complex nutrients. These complex media also served as inexpensive nitrogen and carbon sources with several amino acids, vitamins and minor amount of trace elements.

Several investigators used complex nutrients for carrying out the various industrial processes like biosorption, enzyme productions etc. Pal and Bhattacharya studied the effect of several complex nutrients on biosorption of Cd^{++} by *Aspergillus niger*(171). Fujiwara and Yamamoto reported the maximum enzyme production using 3% soyabean meal and 1.5% bonito extract(329). Basu et. al. used complex nutrient for enhanced protease production by *Aspergillus niger*(330).

Many large scale fermentative processes utilize complex nutrients as nitrogen source. A nitrogen source, which is effectively metabolized, is corn steep liquor which is formed during starch production from corn. The concentrated extract contains numerous amino acids, viz. alanine, arginine, glutamic acid etc. The sugar present in corn steep liquor is largely converted into lactic acid (9 – 20%) by lactic acid bacteria. Yeast extracts are excellent substrates for many micro-organisms. They are

produced from baker's yeast through autolysis at 50 – 55^{0}C or through plasmolysis in the presence of high concentration of NaCl. Total nitrogen contents of yeast extract ranges from 7.4 – 8.8% and contains various other micronutrients. The variation in the composition of yeast extract is due to use of substrate for yeast cultivation to control the quality(331).

Peptones (protein hydrolysates) can be utilized by many microorganisms but they are relatively expensive for industrial applications. Sources of peptone include meat, casein, gelatin, keratin, peanut seeds, soya meal, cotton seeds and sunflower seeds. Peptone composition varies depending upon its origin, for instance peptone from gelatin is rich in proline and hydroxyproline but almost no sulphur containing amino acid. On the other hand, peptone from keratin has a larger proportion of proline and cysteine, but lacks lysine. Peptones of plant origin (soya peptone, cotton seed peptone) have large proportions of carbohydrates (332).

Soya meal, the residue from soyabeans after the extraction of soyabean oil, is a complex substrate. Analysis shows a protein content of about 42%, a carbohydrate content of about 30% (sucrose, raffinose, arabinose and acidic polysaccharides), 1% residual fat and 1.8% lecithin. Soya meal is frequently used in antibiotic fermentation, catabolic regulation does not occur because of the slow catabolism of this complex nutrient (333).

Since complex nutrients are a source of carbohydrates, nitrogen, amino acids, vitamins, and minerals, the effect of complex nutrients was studied to determine whether any of these nutrients associated with complex nutrients was involved in the growth and biosorption of Hg^{++} by *Sacharomyces cerevisiae*A100.

Material & Methods:

For the determination of the effect of different complex nutrients on biosorption of Hg^{++} by *Saccharomyces cerevisiae*A100, the biosorption experiments were carried out with biosorption medium consisting of glucose : 5%, urea : 0.15%, K_2HPO_4 : 0.15%, $MgSO_4.7H_2O$: 0.06%, KCl : 0.06%, Fe^{++}: 1μg/ml, Mn^{++}: 5μg/ml, Mo^{6+} : 10μg/ml. The pH of the biosorption medium was adjusted to 5.0.

The above mentioned complex nutrients were sterilized separately and added to the basal medium aseptically in varying concentration (w/v). Solutions of yeast extract,

beef extract, malt extract, meat extract, peptone, were prepared by dissolving the required amount of those materials in double distilled water separately, sterilized and added to the above mentioned biosorption medium.

Other complex nutrients were prepared by the following method:-

a) Preparation of Wheat bran and Rice bran extract:

20 gms of bran (free from mud) was taken in 200 ml of hot distilled water (55^0C) in a 500 ml beaker. The suspension was kept at 55^0C for 18 hours. The extract was filtered through cotton, sterilized at 15 lbs/square inch pressure for 15 minutes and stored at 4^0C. The solid contents of wheat bran extracts and rice bran extracts were 10.50% and 2.45% respectively.

b) Preparation of Paddy soak liquor:

300 gm paddy was thoroughly washed with distilled water to remove mud and other extraneous materials adhering to paddy grains. The washed paddy was then dipped into 300 ml hot distilled water (55^0C) and was allowed to soak water for 2 hours at about 55^0C when the grains swelled completely. The soaked water was then filtered through several layer of cotton. The solution then sterilized and stored at 4^0C. The solid content of paddy soak liquor was 2.85% of the extract.

c) Preparation of Corn steep liquor:

200 gms of maize was taken in 250 ml of double distilled water containing 0.52% potassium metabisulphite in a 1000 ml beaker and heated over a controlled bath for 48 hours at a temperature of 50 – 55^0C. The corn steep solution was filtered off and then concentrated by evaporation under vacuum. The concentrated solution was sterilized at 15 lbs/square inch pressure for 15 minutes and stored at 4^0C. The solid content was 3.53% of the extract.

d) Preparation of Soyabean Meal Extract:

100 gms of soyabean were made fat free by alcohol distillation. This soyabean was taken in a 1L beaker containing 300 ml of hot (55^0C) double distilled water. This suspension was kept at 55^0C for 18 hours. The extract was then filtered through

cotton and sterilized at 15 lbs / square inch pressure for 15 minutes and stored at 4^0C. The solid content of soybean meal was 4.68% of the extract(334).

After preparation of the extracts, to investigate the effect of different complex nutrients, varying amounts of each of them were added to the fermentation medium. For this purpose 48 hours old culture of *Saccharomyces cerevisiae*A100 with 2 ml volume having spore density of 1.7 x 10^6 per ml used as an inoculum for 50 ml biosorption medium. Hg^{++} ion concentration of the medium was adjusted to 30ppm and biosorption of Hg^{++} was carried out at pH 5.0 for 48hours in 30^0C ($\pm$ 0.5^0C) at B.O.D. incubator. Other cultural conditions remained the same as before (Chapter 4, of the Thesis).

Result & Discussion:

Effect of Meat Extract on biosorption of Hg^{++} by *S.cerevisiae*A100:

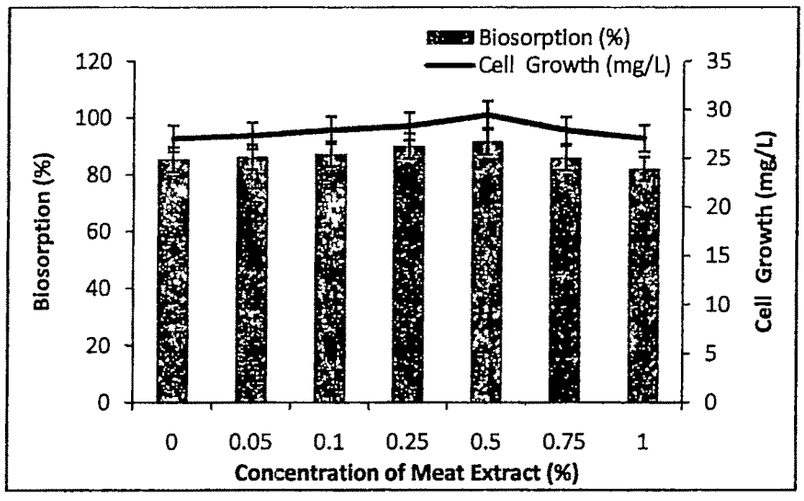

FIGURE 31: EFFECT OF DIFFERENT MEAT EXTRACT CONCENTRATION ON BIOSORPTION OF Hg^{++} AND CELL GROWTH

Fig.31 shows that Meat Extract has a little positive effect on biosorption of Hg^{++} upto 0.5% concentration. At higher concentration the biosorption drops down.

Effect of Soyabeen Meal on biosorption of Hg^{++} by *S.cerevisiae*A100:

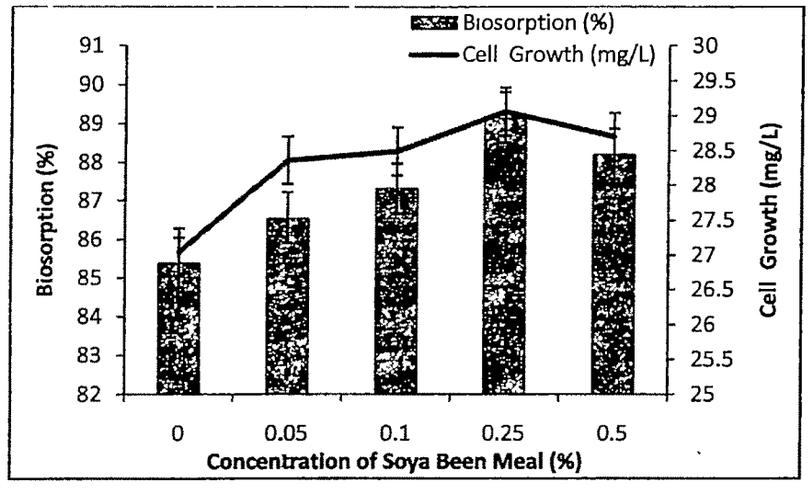

FIGURE 32: EFFECT OF DIFFERENT CONCENTRATION OF SOYABEEN MEAL ON BIOSORPTION OF Hg^{++}AND CELL GROWTH

Fig.32 indicates that Soyabeen Meal has a significant positive effect on cell growth and biosorption of Hg^{++} .

Effect of Peptone on biosorption of Hg^{++} by *S.cerevisiae*A100 :

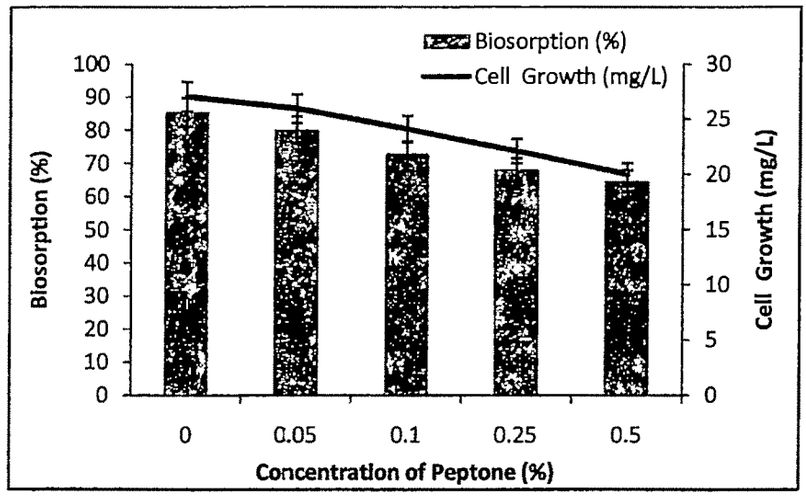

FIGURE 33: EFFECT OF DIFFERENT CONCENTRATION OF PEPTONE ON BIOSORPTION OF Hg^{++}AND CELL GROWTH

Fig.33 shows that peptone has an effective declination in cell growth and biosorption of Hg^{++} .

Effect of Rice Bran Extract on biosorption of Hg^{++} by *S.cerevisiae*A100 :

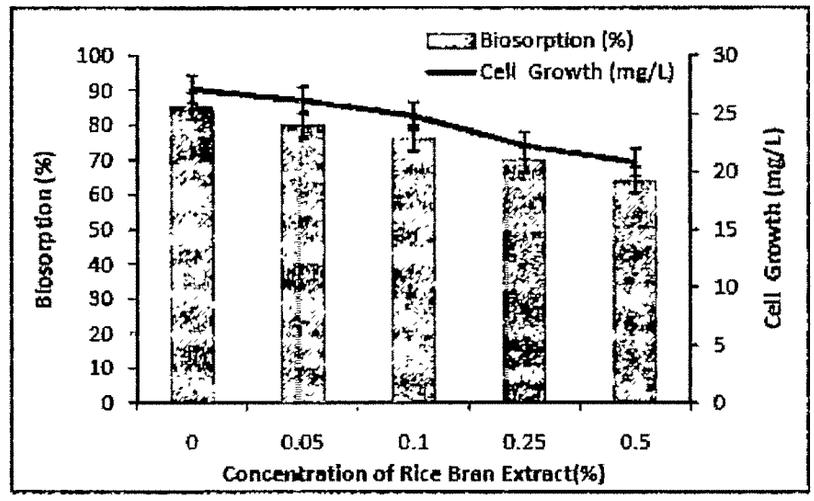

FIGURE 34: EFFECT OF DIFFERENT RICE BRAN EXTRACT CONCENTRATION ON BIOSORPTION OF Hg^{++} AND CELL GROWTH

Fig.34 depicted that Rice Bran Extract has a negative influence on biosorption of Hg^{++} at higher concentration.

Peptone and Rice bran extract are complex nutrients, i.e. they constitute of various nutrient in random proportion. The synergistic effect of the complex nutrient may prove beneficial or harmful to the cell growth. In case of peptone and rice bran extract, their constituents all together exhibit a negative impact on the cell growth.The results are shown in Fig.33 & Fig.34.

Effect of some other Complex Nutrients on Cell growth and Biosorption of Hg^{++} :

TABLE 9: EFFECT OF DIFFERENT COMPLEX NUTRIENTS ON BIOSORPTION OF Hg^{++}AND CELL GROWTH

Name of the Complex Nutrients	Concentration of the complex Nutrients	Cell Growth (mg/L)	Biosorption (%)
Control	00	27.04	85.40 ± 0.25
Yeast Extract	0.05	26.90	83.24 ± 0.34
	0.10	25.73	80.10 ± 0 45
	0.25	24.37	77.32 ± 0 27
	0.5	23.10	72.20 ± 0.44
Beef Extract	0.05	24.82	81.20 ± 0.63
	0.10	23.51	78.60 ± 0.45
	0.25	22.72	74.51 ± 0.55
	0.5	21.63	70.30 ± 0.46
Paddy Soak Liquor	0.05	28.10	86.22 ± 0.37
	0.10	28.69	87.80 ± 0.28
	0 25	27.36	85.28 ± 0.40
	0.5	27.07	84.70 ± 0 52

Control	00	27.04	85.40 ± 0.25
Malt Extract	0.05	24.07	80.72 ± 0.30
	0.10	23.52	76.22 ± 0.36
	0.25	21.78	70.70 ± 0.31
	0.5	20.00	67.20 ± 0.29
Wheat Bran Extract	0.05	26.66	81.60 ± 0.40
	0.10	24.84	76.21 ± 0.44
	0.25	23.70	73.50 ± 0.32
	0.5	23.00	67.10 ± 0.35
Corn Steep Liquor	0.05	25.72	81.22 ± 0.57
	0.10	24.82	78.50 ± 0.49
	0.25	23.70	74.16 ± 0.50
	0.5	22.60	68.07 ± 0.52

Biosorption Values are expressed as mean ± Standard Deviation

All values of cell growth and biosorption are biologically significant ($p < 0.001$).

Table.9. indicates that Paddy Soak Liquor has a little positive effect at initial concentrations, where as Yeast Extract, Beef Extract, Malt Extract, Wheat Bran Extract and Corn steep Liquor shows negative effect on cell growth and Biosorption of Hg^{++}.

It is observed that out of 3 complex nutrients from animal origin only meat extract shows promising acceleration in biosorption rate. Stimulatory role of meat extract in biosorption process was also reported previously (171).

Among 6 complex nutrients from plant source only Soyabeen meal are capable to stimulate the biosorption capacity of the organism used. Positive influence of a few non-commercial complex nutrients on filamentous fungi was observed by earlier workers (335, 336). Soyabeen meal was also found to be a cheap and suitable nitrogen source (due to its high protein content) during feather degradation by bacteria (337).Rice bran extract is reported to have growth inhibitory effect on *Aspergillus niger* (330).