ISSN 0975 - 6299 Vol 2 / Issue 3 / Jul - Sept 2011

International Journal of Pharma and Bio Sciences

RESEARCH ARTICLE

BIOTECHNOLOGY

ROLE OF COMPLEX NUTRIENTS, VITAMINS AND AMINO ACIDS ON BIOSORPTION OF Hg^{++} BY Hg^{++} RESISTANT *Saccharomyces cerevisiae*A100

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ABSTRACT

Remediation of Hg^{++} contaminated wastewater can be accomplished by Hg^{++} resistant *Saccharomyces cerevisiae*A100. The physico-chemical mode of biosorption process is largely dependent on the charge bearing cell surface ligands. Optimum cell growth and multiplication provides the exposure of adequate amount of charged ligands on the cell surface that ultimately facilitate greater rate of biosorption of Hg^{++}. Hence, The growth promoting factors like vitamins , amino acids indirectly play a significant role in biosorption process. Thiamine-HCl (2.0µg/ml) and biotin (1.0µg/ml) increases the biosorption upto 95.67% and 96.78% respectively. L(-)Tryptophan (0.75mg/ml) promotes the biosorption upto 90.24%. Complex nutrients are the cheap sources of vitamins, amino acids, metals in trace amounts etc. Among 10 different tested complex nutrients, meat extract and soya been meal show some positive effect where as peptone and rice bran extract significantly declines the biosorption potential of the used biomass.

KEY WORDS

Biosorption, Mercury, *Saccharomyces cerevisiae*, Complex Nutrients, Vitamins, Amino Acids.

INTRODUCTION

Mercury poisoning in the aquatic system has been of great concern due to their toxicity even at lower concentration [1].The major sources of mercury pollution in the aquatic environment are chlor-alkali, paint, pulp and paper, oil refining, electrical, pharmaceutical and battery manufacturing industries [2]. Metal sequestering properties of certain microbial biomass offers considerable promise in removing heavy metals from environment, including waste water and industrial effluents economically, effectively and safely [3]. Metabolism-independent mode of biosorption depends on the charge bearing ligands present on the cell surface of the organism. Increase in the cell growth and cell number ultimately enhance the ligands availability to the Hg^{++} ion present in the surrounding solution [4]. Along with essential nutritional requirements, some growth promoting factors like vitamins and amino acids play significant role in the cell growth. Complex nutrients are the cheap sources of many nutrients like vitamins, amino acids, metal ions even sometimes carbon and nitrogen sources. The nutrients present in complex nutrient may exert growth promoting or growth inhibitory effect on the microbial biomass [5].

The present study was designed to investigate the impact of some growth enhancing factors like vitamins and amino acids on cell growth as well as biosorption capacity of the Hg^{++} resistant *Saccharomyces cerevisiae* A100 already developed in our laboratory [6]. The synergistic effect of many nutrients some times exhibit different impact on biosorption in reference to individual nutritional factors. Effect of some vitamins, amino acids and complex nutrients were extensively studied to find out their influence on cell growth and biosorption of Hg^{++}.

MATERIAL & METHODS

Microorganisms: 35ppm Hg^{++} resistant strain was developed and maintained in synthetic medium. 48 hrs old culture was stored at 4˚C for biosorption experiment [6].

Preparation of Hg^{++} stock solution: Hg^{++} stock solution (1000ppm) was prepared by dissolving $HgCl_2$ in deionized double distilled sterile water. Working solutions of various aliquots were prepared by adding stock solution of different volume to the biosorption medium [4].

Biosorption Medium and Cultural Condition: Chemical composition of synthetic biosorption media is Glucose- 5%,Urea-0.15%, K_2HPO_4-0.15%, KCl-0.06%, $MgSO_4$,$7H_2O$-0.06%, Fe^{++} -1µg/ml, Mn^{++} - 5µg/ml, Mo^{6+} - 10µg/ml. pH of the media was adjusted to 5.0 . 2ml of 48hrs old inoculums (cell density 1.7X 10^6 cells/ml) was added to 50ml of biosorption medium of 30ppm Hg^{++} concentration contained in a 150ml conical flask. The biosorption process was carried out at 30˚C temperature for 48hrs [4].

***Preparation of Complex nutrients stock solution*:** 10 different complex nutrients (Yeast Extract, Meat Extract, Beef Extract, Paddy Soak Liquor, Peptone, Malt Extract, Rice Bran Extract, Wheat Bran Extract, Soya Been Meal, Corn Steep Liquor) were supplemented to the biosorption medium in a concentration of 0.05, 0.1, 0.25, 0.5 percent based on their solid content to observe their influence on biosorption of Hg^{++} using the Hg^{++} resistant *S.cerevisiae*A100.

Natural and commercially unavailable complex nutrients were prepared by soaking the nutrient source in hot water for adequate time period

and filtered through cotton and evaporated to dryness under vacuum to recover the solid material [5].

***Measurement of Cell Growth*:** While filtration after biosorption experiment, biomass was entrapped in preweighed filter paper. The filter papers were dried to a constant weight at 105˚C temperature for 6hrs and dry weight of the biomass was calculated.

Analysis of Hg^{++}: After completion of the biosorption experiment, the medium was filtered through Whattman No. 1 filter paper and the filtrate was analysed to measure the remaining Hg^{++} ion concentration in the solution by Mercury Analyser MA 5840 [4].

Statistical Analysis: Statistical analysis of all data were performed according to student's t distribution [7]. The level of significance was determined from the table with critical value of t. Error bars of each data are shown in the figure.

RESULT & DISCUSSION

Effect of Complex Nutrients: Among the 10 tested complex nutrients, only meat extract (0.5%) (Fig.1.) and Soya Been meal (0.25%) (Fig.2.) show significant improvement in cell growth and biosorption where as Peptone (Fig.3.) and Rice Bran Extract (Fig.4.) cause remarkable declination in biosorption.

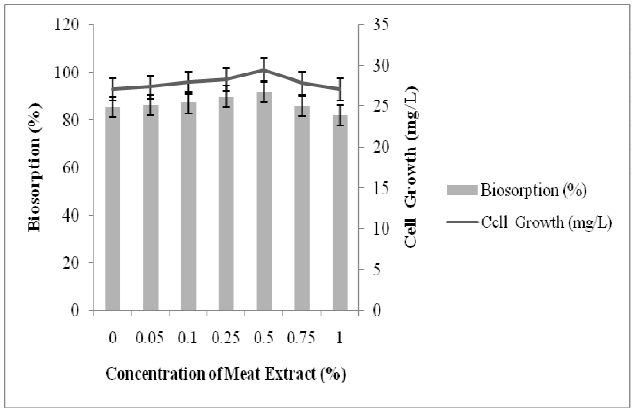

Fig.1
Effect of different Meat Extract concentration on biosorption of Hg^{++} and cell growth

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 [5].

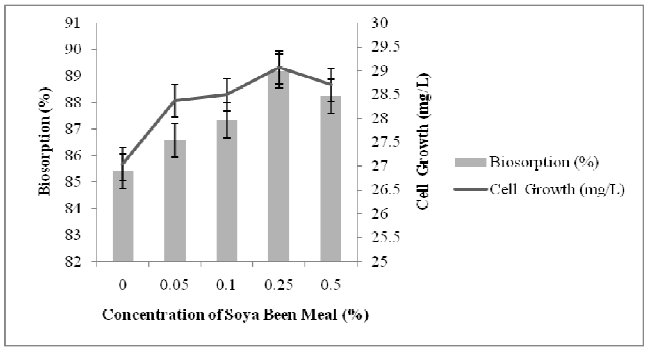

Fig.2
Effect of different concentration of Soyabeen Meal on biosorption of Hg^{++} and cell growth

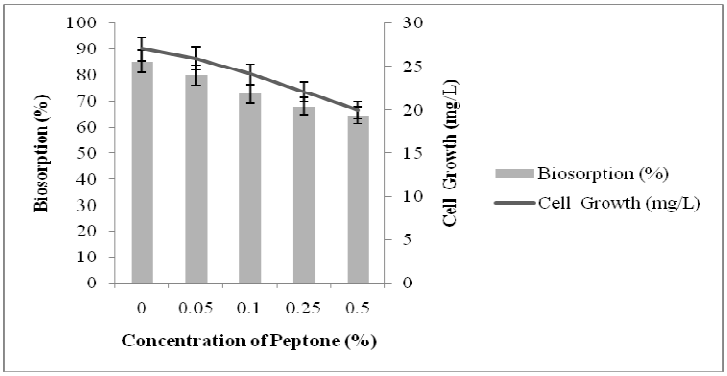

Fig.3

Effect of different concentration of Peptone on biosorption of Hg^{++} and cell growth

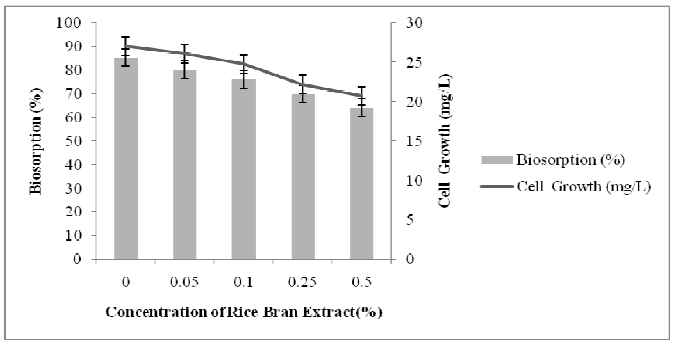

Fig.4

Effect of different Rice Bran Extract concentration on biosorption of Hg^{++} and cell growth

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 [8,9]. Soyabeen meal was also found to be a cheap and suitable nitrogen source (due to its high protein content) during feather degradation by bacteria [10]. Rice bran extract is reported to have growth inhibitory effect on *Aspergillus niger* [11].

Effect of Amino Acids: Among 14 amino acids examined in the present study, only L-Proline, L-Cysteine, L-Leucine and L-Tryptophan exert remarkable effect on biosorption of Hg^{++} from aqueous solution (Table.1.).

Table.1

Effect of amino acids on cell growth and biosorption of Hg^{++} by S.cerevisiaeA100

Concentration (mg/ml)	L(-)Proline		L(-)Cysteine		L(-)Leucine		L(-)Tryptophan	
	Cell Growth (mg/L)	Biosorption* (%)	Cell Growth (mg/L)	Biosorption* (%)	Cell Growth (mg/L)	Biosorption* (%)	Cell Growth (mg/L)	Biosorption* (%)
Control (00)	27.04	85.40±0.29	27.04	85.04±0.29	27.04	85.40±0.29	27.04	85.04±0.29
0.50	26.02	83.22±0.42	21.18	70.10±0.47	26.20	81.22±0.73	27.82	86.20±0.38
0.75	25.84	81.60±0.31	20.20	64.20±0.58	25.10	77.68±0.57	28.09	90.24±0.45
1.0	24.05	79.10±0.68	17.50	60.33±0.76	22.00	72.32±0.53	27.97	88.33±0.51

**= Values are expressed as mean ± Standard Deviation*

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

It is noticed that L-Tryptophan (0.75mg/ml) is fairly stimulatory for cell growth and biosorption in this study. The aromatic amino acid participates in coenzyme Q biosynthesis, one of the essential components for energy producing reaction in mitochondria [5].Sulpher rich amino acids are generally poor source of nutrients to microorganisms. Some essential amino acids are often act as growth inhibitor. This is often link to transport systems within the cell, since most of the share a permease with resulting antagonism uptake [12,13].

Effect of Vitamins: Among 10 tested vitamins in the present study, Thiamine-HCl (2.0 µg/ml) and biotin (2.0 µg/ml) fairly increase the biosortion. On the contrary mesoinositol and vit. B_{12} show significant decrease in cell growth and biosorption (Table.2.).

Table.2

Effect of vitamins on cell growth and biosorption of Hg^{++} by S.cerevisiaeA100

Concentration (µg/ml)	L(-)Proline		L(-)Cysteine		L(-)Leucine		L(-)Tryptophan	
	Cell Growth (mg/L)	Biosorption* (%)	Cell Growth (mg/L)	Biosorption*(%)	Cell Growth (mg/L)	Biosorption* (%)	Cell Growth (mg/L)	Biosorption (%)
Control (00)	27.04	85.40±0.29	27.04	85.04±0.29	27.04	85.40±0.29	27.04	85.04±0.29
0.20	27.87	87.10±0.41	28.60	87.88±0.33	26.68	84.20±0.22	26.55	83.23±0.44
0.50	28.39	89.76±0.28	29.19	92.98±0.40	26.00	81.22±0.53	25.46	80.40±0.51
1.0	29.58	93.22±0.33	29.40	96.78±0.54	25.19	79.60±0.45	24.83	76.20±0.63
2.0	29.88	95.67±0.53	28.43	95.20±0.42	24.83	76.32±0.60	24.62	74.22±0.23
3.0	26.33	93.10±0.62	28.27	92.26±0.35	22.16	70.70±0.32	22.95	72.00±0.30

**= Values are expressed as mean ± Standard Deviation*

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

Thiamine is required by the largest number of fungi. It is present in the form of Thiamine Pyrophosphate (TPP) and performs the functions of coenzyme catalyzing, the decarboxylation of α-keto acids like pyruvic acid, α-ketoglutaric acid etc [14,15].

Growth promoting activity of biotin was reported by many workers. Biotin is generally associated with reactions involving fixation of CO_2 into large organic molecules and carbohydrate metabolism in *Saccharomyces cerevisiae* [14,16].

CONCLUSION

Though Thiamnine-HCl and biotin individually promotes the biosorption potential of the organism to its maximum (above 95%), it is not economical to supply these nutrients individualy to the organism during large scale Hg^{++} removal from industrial effluent. Soyabeen meal- the most promising complex nutrient found in this study, shows less than 5% difference in biosorption on reference to the mentioned vitamins.

Thus, the results from the present study ensure the possibility of using complex nutrients as the composite and cheap sources of growth promoting factors during biosorption of Hg^{++} from Hg^{++} contaminated aqueous system by Hg^{++} resistant *Saccharomyces cerevisiae* A100.

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