International Journal of Pharma and Bio Sciences V1(2)2010

EFFECT OF TRACE ELEMENTS ON BIOSORPTION OF Hg^{2+} BY Hg^{2+} TOLERANT *Saccharomyces cerevisiae* A100

KHUSHI MUKHERJEE* AND AJIT KUMAR BANIK

Department Of Chemical Engineering, Calcutta University, Kolkata, India

Corresponding author khushi_microbio@yahoo.co.in

ABSTRACT

Use of microorganisms for removing mercury (Hg^{++}) is an effective technology for the treatment of industrial wastewaters and can become an effective tool for the remediation of man-impacted coastal ecosystems with this metal. In our laboratory a high Hg^{++} resistant *Saccharomyces cerevisiae*A100 is already developed to facilitate Hg^{++} biosorption even from Hg^{++} rich solution. Inorganic anions and cations are essential for the growth and metabolism of *Saccharomyces cerevisiae*A100 and also play significant role on biosorption process. Experiments revealed 0.15% K_2HPO_4, 0.06% KCl , 0.06% $MgSO_4,7H_2O$ as the optimized inorganic ingredients of the nutrient medium (Glucose 5.0%, Urea 0.25%). Supplementation of nutrient broth with 1µg/ml $FeSO_4,7H_2O$, 5µg/ml $MnSO_4,H_2O$ and 10µg/ml $Na_2MoO_4,2H_2O$ exhibit marked positive effect on biosorption of Hg^{++}. Optimization of the concentration of inorganic nutrients enhances biosorption efficiency of *Saccharomyces cerevisiae*A100 from 90% to 96.6% (developed significance level $p<0.001$) . Addition of $CuSO_4,5H_2O$; $ZnSO_4,7H_2O$; $CoCl_2,6H_2O$; $NiSO_4,7H_2O$; NH_4VO_3 to the biosorption medium was found to play inhibitory role on the growth and biosorption of Hg^{++} by *Saccharomyces cerevisiae*A100.

KEYWORDS

Biosorption, Mercury, *Saccharomyces cerevisiae*, Trace element

EFFECT OF TRACE ELEMENTS ON BIOSORPTION OF Hg^{2+} BY Hg^{2+} TOLERANT *Saccharomyces cerevisiae* A100

INTRODUCTION

Mercury pollution and removal have been the subject of extensive research in the past few decades and optimal solution have yet to materealize[1-4]. Metal sequestering properties of certain types of microbial biomass after considerable promise in removing heavy metals from environment including waste water and industrial effluents economically, effectively and safely[5]. *Saccharomyces cerevisiae* is the choice of biosorbent in the present study due to its easy availability, easy and economic cultivation method and it can be easily manipulated genetically and morphologically that is necessary to optimize the biosorption capacity[6].Microorganism exhibit metabolism-dependent and metabolism-independent mechanism for uptake and accumulation of heavy metals[7]. Metabolism-independent mechanism of biosorption depends on the ligands present on the cell surface of the organism[6]. Increase in cell growth or cell number ultimately increase ligands availability to the Hg^{++} present in the surrounding solution. Requirement of mineral element for cell growth is an essential factor[7].In the present study the effect of various ions, such as phosphate, chloride, magnesium, ferrous, manganese and molybdenum on biosorption of Hg^{++} by *Saccharomyces cerevisiae* A100 were studied. The optimum concentration of growth promoting minerals were also determined.

MATERIALS AND METHODS

(i)Culture preparation:

Saccharomyces cerevisiae culture was collected from the Department of Chemical Engg., Calcutta University ,India. The biomass was maintained in YPDA (Yeast Extract-0.5%, peptone-0.5%, Dextrose-1% ,Agar-4%) medium, pH-5.0. After 48 hours growth at 30˚C temperature culture was stored at 4˚C temperature for biosorption of Hg^{++} ion from surrounding broth.

(ii)Preparation of Hg^{++} stock solution:

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

(iii)Developement of Hg^{++} resistant strain:

High Hg^{++} resistant strain of *Saccharomyces cerevisiae* was isolated from 35ppm Hg^{++} containing medium by pour plate method[6]. The resistant strain was maintained in Dextrose-1%, $(NH_4)_2SO_4$-0.5%, K_2HPO_4-0.1%, $MgSO_4,7H_2O$-0.025, $FeSO_4,7H_2O$-0.002%, Biotin-0.5µg/ml broth, pH-5.0. After 48 hours growth the culture was stored at 4˚C.

(iv)Analysis of Hg^{++}:

After the biosorption experiment the biosorption medium was filtered through Whatman No. 1 filter paper and the filter was analysed to measure the remaining Hg^{++} concentration in the solution, by Mercury Analyser MA5840 [8] (Electronic Corporation of India Limited, Hyderabad).

EFFECT OF TRACE ELEMENTS ON BIOSORPTION OF Hg^{2+} BY Hg^{2+} TOLERANT *Saccharomyces cerevisiae* A100

(v)Measurement of cell growth :

While filtration biomass was entraped in preweighed filter paper. The filter papers were dried to a constant weight at 105˚C temperature for 6 hours and the dry weight of the biomass was calculated.

(vi)Preparation of trace metal stock solution:

Stock solution of trace elements were prepared by dissolving the metal salt in sterilized triple distilled water.

(vii)Preparation of media for biosorption:

The sterilized biosorption medium for biosorption consist of glucose-5%,Urea-0.25%, KH_2PO_4- 0.1%, KCl-0.05%, $MgSO_4$,$7H_2O$-0.05%, pH was adjusted to 5.0. 2 different PO_4^{3-} salts and 3 different Chloride salts were tested independently and separately as the nutrient supplement to the biosorption medium and the optimum concentration of most suitable minerals were determined.

To study the effect of trace metal ions all the media components were made free of mineral impurities by the process of solvent extraction where chloroform was used as the solvent[9]. And 8-hydroxyquinoline as the chelating agent. Thereafter, biosorption process were performed with different trace concentrations of metal ions.

2 ml of inoculums (cell density 1.7 X 10^6 cells/ml) were added to 50 ml of biosorption medium of 30ppm Hg^{++} concentration. The pH of the biosorption medium was adjusted to 5.0. The biosorption process was carried out at 30˚C for 48 hours.

(viii)Statistical analysis:

Statistical analyses of all data were performed according to student's t distribution[10]. The level of significance for two-tail test was determined from the table with critical values of t. Error bars of each data are shown in the figure.

RESULT AND DISCUSSION

P plays essential role for the growth of yeast, and among other functions this element plays important role in carbohydrate metabolism[11]. Between KH_2PO_4 and K_2HPO_4, K_2HPO_4 exhibit remarkable positive effect on cell growth of the organism and biosorption of Hg^{++} as well. At lower concentration differences between two salts are not prominent, but as the concentration increases K_2HPO_4 is proved to be more suitable PO_4^{3-} source for the organism. K_2HPO_4 helps to maintain the pH of the biosorption medium at a more suitable range for biosorption of Hg^{++}. The acidic nature of KH_2PO_4 drops the pH down resulting declination in biosorption rate . The optimum concentration of K_2HPO_4 for biosorption is found to be 0.15% (Table.1.).

EFFECT OF TRACE ELEMENTS ON BIOSORPTION OF Hg^{2+} BY Hg^{2+} TOLERANT *Saccharomyces cerevisiae* A100

Table 1

Effect of Different concentration of K_2HPO_4 and KH_2PO_4 on cell growth of S.cerevisiae A100 and Hg^{2+} biosorption

Salt Concentration(%)	K_2HPO_4		KH_2PO_4	
	Cell Weight*(mg/L)	Biosorption*(%)	Cell Weight*(mg/L)	Biosorption*(%)
0.05	22.94 ± .50	90.47 ± 1.05	22.53 ± .97	75.30 ± 1.17
0.075	23.80 ± .32	91.46 ± .85	23.36 ± .58	81.30 ± 1.26
0.10	24.97 ± .11	92.03 ± .45	24.37 ± .53	87.60 ± 1.11
0.15	25.36 ± .46	92.46 ± .85	21.19 ± .68	83.30 ± .70
0.20	25.18 ± .37	91.53 ± 1.00	20.40 ± .87	81.36 ± 1.30
0.25	24.43 ± .31	89.63 ± .85	19.65 ± .71	76.33 ± 1.22
0.30	21.47 ± .57	87.60 ± .1.11	17.40 ± .59	73.50 ± 1.16

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

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

Chloride ion is essential for fluid and electrolyte balance in the cell. Different concentration of KCl were tested as chloride source for *Saccharomyces cerevisiae*A100. 0.06% KCl was found to be optimum for maximum cell growth and biosorption as well (Table 2). NaCl and $CaCl_2,2H_2O$ were also tested as an associate chloride source along with the optimized concentration of KCl. Neither NaCl nor $CaCl_2,2H_2O$ shows any promising result on biosorption experiments (Table.2.).

EFFECT OF TRACE ELEMENTS ON BIOSORPTION OF Hg^{2+} BY Hg^{2+} TOLERANT *Saccharomyces cerevisiae* A100

Table 2

Effect of Different Concentration of KCl, NaCl and $CaCl_2$,2H_2O on cell growth of S.cerevisiaeA100 and Hg^{2+} biosorption

Salt Concentration (%)	KCl		NaCl		$CaCl_2$, 2H_2O	
	Cell Weight* (mg/L)	Biosorption* (%)	Cell Weight* (mg/L)	Biosorption* (%)	Cell Weight* (mg/L)	Biosorption* (%)
0.0	7.51 ± .32	75.40 ± .36	25.89 ± .22	93.3± .36	25.89 ± .22	93.3± .36
0.02	16.15 ± .54	89.40 ± .61	23.76 ± .52	92.5± .70	22.46 ± .20	91.4± 1.70
0.03	18.22 ± .44	90 ± .87	21.59± .46	91.6± 1.09	20.73 ± .18	90 ± 1.00
0.04	21.57 ± 1.23	91.5 ± 1.05	20.75± .58	90.06± 1.22	19.09 ± .23	88.5 ± 1.05
0.05	24.38 ± .46	92.4 ± .85	19.36± .57	88.7± 1.11	18.11 ± .18	86.7 ± 1.09
0.06	25.91 ± .43	93.4 ± .96	18.85± .19	87.5 ± .61	17.35 ± .12	85 ± 1.26
0.07	24.47 ± .72	91.36 ± 1.26	17.63± .85	86.3± 1.26	16.62 ± .23	83.4 ± .85
0.08	22.58 ± .67	90.13 ± 1.40	17.05± .66	85.4 ± 1.02	15.87 ± .25	81.3 ± 1.22

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

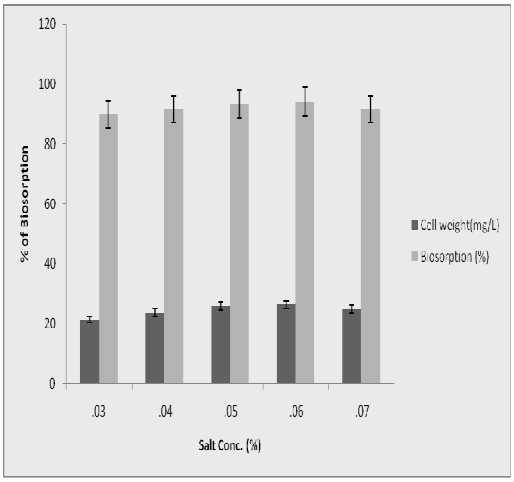
All values of cell growth and biosorption are biologically significant ($p < 0.001$).

The possible reason for such results is potassium seems to be essential for yeast growth while Na and Ca do not appear to be very important in this purpose [11].Role of magnesium in yeast metabolism is chiefly through its activation influence over various enzyme system, including those of fermentation. Magnesium ion is a very useful co-factor in kinase reaction [7]. The optimum concentration of $MgSO_4$, $7H_2O$ for Hg^{++} biosorption is found to be 0.06% (Graph 1).

EFFECT OF TRACE ELEMENTS ON BIOSORPTION OF Hg^{2+} BY Hg^{2+} TOLERANT *Saccharomyces cerevisiae* A100

Graph 1

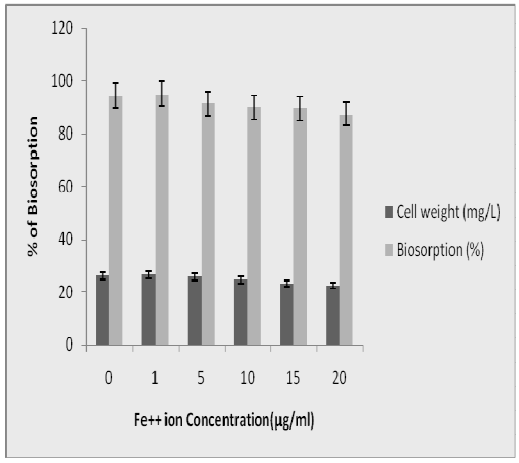
Effect of different concentrations of $MgSO_4$, $7H_2O$ on Cell Growth and Biosorption of Hg^{++} by S.cerevisiae A100



Iron is essential constituent of yeast protoplasm. Association of iron with various enzymes including cytochrome oxidase, catalase and others have lent much support to the possible role of iron[11]. In our experiment Fe^{++} ion had a positive effect on biosorption of Hg^{++} and cell growth of yeast at concentration of 1µg/ml (Graph 2).

Graph 2

Effect of different concentration of Fe^{++} ion on Cell growth and Biosorption of Hg^{++} by S.cerevisiaeA100

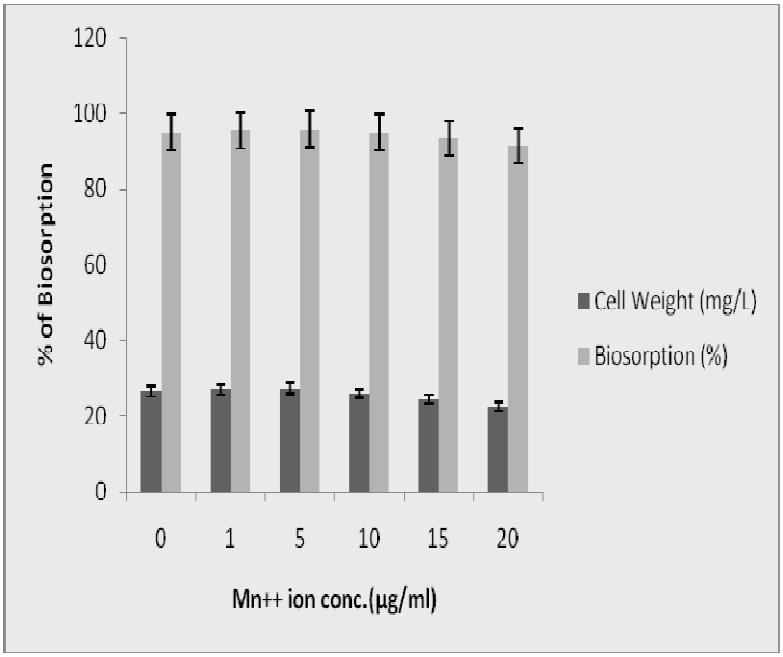


Manganese is known to effect the cellular concentration as well as activity of various enzymes. It is essential for various reductase enzymes as a co-factor[11]. Mn^{++} ion had a positive effect upto a concentration of 5µg/ml on Hg^{++} biosorption and cell growth (Graph 3).

EFFECT OF TRACE ELEMENTS ON BIOSORPTION OF Hg^{2+} BY Hg^{2+} TOLERANT *Saccharomyces cerevisiae* A100

Graph 3

Effect of different concentration of Mn^{++} ion on cell growth and Biosorption of Hg^{++} by S.cerevisiaeA100



Trace metals like Ni^{++}, Co^{++}, Cu^{++}, V^{+++} have toxic effect on the organism used causing the declination on cell growth and biosorption as well (Table 3).

These metals are already reported as toxic metals [12].Zn^{++} ion also shows negative effect but not as adverse as toxic metals. Hence the declination of cell growth and biosorption is not drastic (Table 3).

EFFECT OF TRACE ELEMENTS ON BIOSORPTION OF Hg^{2+} BY Hg^{2+} TOLERANT *Saccharomyces cerevisiae* A100

Table 3

Effect of Zn^{2+},Cu^{2+},Ni^{2+},Co^{2+},V^{3+} ion on cell growth of S.cerevisiaeA100 and Hg^{2+} biosorption

Metal ion Conc. (µg/ml)	$ZnSO_4,7H_2O$		$CuSO_4,5H_2O$		$NiSO_4,7H_2O$		$CoCl_2,6H_2O$		NH_4VO_3	
	Cell Weight* (mg/ml)	Biosorption * (%)	Cell Weight* (mg/ml)	Biosorption * (%)	Cell Weight* (mg/ml)	Biosorption * (%)	Cell Weight* (mg/ml)	Biosorption * (%)	Cell Weight* (mg/ml)	Biosorption * (%)
0.0	27.60±.50	96.6±.85	27.60±.50	96.6±.85	27.60±.50	96.6±.85	27.60±.50	96.6±.85	27.60±.50	96.6±.85
1.0	25.63±.30	90.3±1.17	16.66±.26	83.4±1.05	19.91±.15	89.6±.85	20.23±.20	91.4±1.31	21.66±.42	89.8±.91
5.0	22.45±.38	86.6±1.15	14.49±.26	80.3±1.17	17.64±.30	86.3±1.26	19.18±.23	89.4±.60	20.09±.37	87.5±1.15
10.0	19.72±.46	85.3±1.09	12.18±.24	75.3±1.13	15.51±.36	84.1±.85	17.68±.40	87.26±.70	18.20±.56	85.26±1.12
15.0	17.26±.29	83.4±1.00	11.35±.29	71.3±1.26	13.20±.36	80.3±1.13	15.64±.20	85.26±1.22	16.54±.37	83.3±.85
20.0	16.63±.24	81.3±1.22	10.55±.23	68.5±1.11	11.54±.27	78.5±1.12	14.69±.33	83.3±.85	14.44±.28	81.3±1.22

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

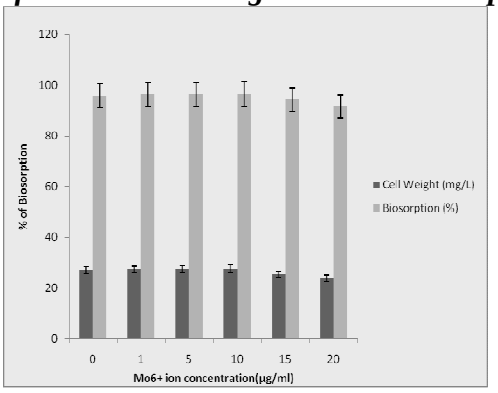
All values of cell growth and biosorption are biologically significant ($p < 0.001$).

Molybdenum acts as co-factor in some oxido-reductase enzymes and takes part in electron transfer[7]. The positive effect of Mo^{6+} upto a concentration of 10µg/ml on Hg^{++} biosorption and cell growth is revealed in our experiments (Graph 4.).

EFFECT OF TRACE ELEMENTS ON BIOSORPTION OF Hg^{2+} BY Hg^{2+} TOLERANT *Saccharomyces cerevisiae* A100

Graph 4

Effect of different concentration of Mo^{6+} ion on cell growth and Biosorption of Hg^{++} by S.cerevisiaeA100



When other positive charge bearing metals are present in the biosorption medium, behaves as competitive ion with the major metal contaminant resulting the declination of biosorption [13].

On the other hand, sometimes binding of a metal to the cell surface exposes the other metal binding sites which enhances the biosorption of the major metal contaminant [1].

Thus, from the above results, we can divide both macro and micro elements into three groups:

(i)Elements which stimulate the biosorption of Hg^{++} by *Saccharomyces cerevisiae* A100 such as phosphorous in the form of K_2HPO_4(0.15%), Cl^- in the form of KCl (0.06%), $MgSO_4$, $7H_2O$ (0.06%), Fe^{++}(1µg/ml), Mn^{++}(5µg/ml) and Mo^{6+}(10µg/ml) ions.

(ii) Elements which does not effect adversely the biosorption of Hg^{++} by *Saccharomyces cerevisiae* A100 , as Zn^{++} ion.

(iii) Elements which exhibit inhibitory or drastic negative effect on Hg^{++} biosorption by *Saccharomyces cerevisiae* A100.The example of such elements include Cu^{++}, Ni^{++}, Co^{++} and V^{+++}.

CONCLUSION

Present study reveals that addition of some metal ions like Fe^{++}, Mn^{++}, Mo^{6+} enhances the biosorption rate where as, presence of metal ions like Zn^{++},Cu^{++}, Co^{++}, Ni^{++}, V^{+++} brings down the biosorption of Hg^{++} . So the most suitable synthetic nutrient medium formulated for *Saccharomyces cerevisiae* A100 to facilitate optimum growth and biosorption activity is

Glucose- 5%, Urea-0.25%, K_2HPO_4-0.15%, KCl-0.06%, $MgSO_4$,$7H_2O$-0.06%, Fe^{++}-1µg/ml, Mn^{++}-5µg/ml, Mo^{6+}-10µg/ml

EFFECT OF TRACE ELEMENTS ON BIOSORPTION OF Hg^{2+} BY Hg^{2+} TOLERANT *Saccharomyces cerevisiae* A100

ACKNOWLEDGEMENT

Thanks are due to Department of Food Processing Industries, Govt. of West Bengal, India for financial support.

REFERENCE

1. Cain A, Vannela R & Woo K L, Cyanobacteria as a biosorbent for mercuric ion. Bioresour Technol, 99: 6578-6586, (2008).
2. Say R, Yilmaz N & Denizli A, Biosorption of Cadmium, Lead, Mercury and Arsenic ions by fungus *Penicillium purpurogenum*. Sep Sci Technol, 38**:** 2039-2053, (2003).
3. Ruiz G C, Mercury (II) removal from aqueous solution by nonviable *Bacillus Sp*. From a tropical estuary. Bioresour Technol, 97: 1907-1911, (2006).
4. Kambiz A N, Hossain S Z, Abbas S L, Jamshid R, Sima N & Sung C Y, Mercury absorption by *Pseudomonas fluorescence* BM07 grown at two different temperatures. Pol J Microbiol, 56: 111-117, (2007).
5. Zhang L, Zhao L, Yaoting Y & Chen C, Removal of lead from aqueous solution by non-living *Rhizopus nigricans*.Water Res, 32: 1437-1444, (1998).
6. Mukherjee K & Banik A K, Studies on biosorption of Hg^{++} by a Hg^{++} resistant living and non-living *Saccharomyces cerevisiae* 100 : characterization of some physical parameters and spectroscopic studies. J Ind Chem Soc, 86: 849-856, (2009).
7. Gadd G M, Heavy metal accumulation by bacteria and other microorganism. Experientia, 46: 834-840, (1990).
8. Razaee A, Ramavandi B & Ganati F, Equilibrium and studies on biosorption of mercury by algae biomass.Pak J Biol Sci, 9: 777-782, (2006).
9. Basu B & Banik A K, Production of protein rich organic fertilizer from fish scale by a mutant *Aspergillus niger* AB_{100} – A media optimization study, J Sci Ind Res, 64:, 293-298, (2005).
10. Fisher R A & Yates F, Statistical tables for biological, agricultural and medical research, in Statistics in Biology and Physiology, by Debojyoti Das (Academic Publishers, Calcutta) (1993).
11. Cook A H, The Chemistry and Biology of Yeasts, (Academic Press, New York),297-300, (1958).
12. Volesky B, Sorption and Biosorption, BV Sorbex, Inc. Publication, Canada, 64-65, (2003).
13. Wang J & Chen C, Biosorption of heavy metals by *Saccharomyces cerevisiae*: A review, Biotechnol adv 24: 427-457, (2006).