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Bioaccumulation of Cu^{2+} ion by Saccharomyces Cerevisiae

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Abstract

Bioaccumulation, using micro-organisms such as bacteria, fungi, yeast and algae, is regarded as a cost-effective biotechnology for heavy metal removal from water bodies. Among the promising micro-organisms for removal of heavy metal ions which have been researched during the past decades, *Saccharomyces cerevisiae* has received increasing attention. This yeast is readily available and easy to cultivate in normal laboratory conditions. It is also an important micro-organism used for investigating those aspects of cell biology that occur in more complex eukaryotes. It is therefore, planned to investigate the bioaccumulation of copper by *Saccharomyces cerevisiae*. Yeast cells were grown in the presence of different concentrations of copper. Dry mass of the cells are correlated with accumulated copper concentrations. Accumulation of copper is correlated with variation in total protein contents.

Keywords: Bioaccumulation, Cu^{2+}, *saccharomyces Cerevisiae*, Total Protein Content.

Introduction

Biosorption of metals receiving a great deal of attention for it's scientific importance and application potential. The rapid development of various industries and discharge of wastes containing metals into the environment causes the environmental pollution[1-3]. Removal of heavy metals by biosorption has been investigated during last decades[4]. It is beneficial over conventional treatment methods. Yeast cells are capable of accumulation of various heavy metals[5,6]. They retain their ability to accumulate heavy metals under a wide range of ambient conditions. Baker's yeast has been found to possess two or more substrate specific transport systems for accumulating any single metal ion. They can produce or excrete extracellular polymeric substances such as polysaccharides, glucoprotein, lipopolysaccharide, soluble peptides, etc[7,8]. Metal ions can bind to active groups present on the cell surface, through chelation[9]. The adsorbed ions are transported across the membrane by the same mechanism by which metabolically important ions (K, Mg and Na) are accumulated[10]. The metals accumulated by organisms either in large amounts or in traces give active participation in important metabolic activities[11].

Copper is an essential micronutrient being a component of copper proteins. Copper proteins are involved in electron transport and redox reactions[12]. Biosorption of copper by *Chlorella vulgaris* and *Zoogloea ramigera* have been investigated. It was found that complexation between metal ion and amino group as well as carboxyl group of proteins is responsible for transport of metal ions[13]. Cox-11 is a copper-binding protein contains several conserved cysteins, methionines and histidines capable of binding copper[14]. Metallothionein like proteins appear to play an important role in mediating metal uptake and hence accumulation[15,16]. These are responsible for transport of nutrients[17,18]. In the budding yeast *Saccharomyces cerevisiae* a specific copper transport function has been identified which appears to be due to the product of CTR-1, a plasma membrane protein required for high affinity copper uptake[19].

The toxicity of metal ion is owing to their ability to bind with protein molecules and prevent replication of DNA and thus subsequent cell division[20]. The most important aspect governing the toxic or stimulating influence of copper on fungal growth is obviously its concentration. At higher concentrations, heavy metal ions form unspecific complex compounds, which lead to toxic effects[21-23]. The biochemical implications of heavy metal pollutants in micro-organisms need to be studied in details. Present study therefore will show the correlation between metal concentrations in the environment and accumulation by *Saccharomyces*

cerevisiae along with the involvement of metal binding proteins. Variation in protein contents therefore, will indicate the change in metabolic activities in-vivo.

Experimental

Biosorbent

Culture of *Saccharomyces cerevisiae* (strain 3131) obtained from NCIM Pune, India.

Maintenance of Medium

Stocks of strains were maintained on standard YEPD rich medium comprising in 1% Yeast extract, 2% Peptone, 2% Dextrose and 2% Agar-Agar.

Inoculum Preparation

A loop full of (YEPD) slant yeast cells was cultivated in 50 ml liquid synthetic growth medium (SGM) in 250ml Erlenmeyer flask at 25ºC on a horizontal shaker for 15h. Synthetic growth medium was prepared in 50ml double distilled water with 0.5% Glucose, 0.3% KH_2PO_4, 0.3% $(NH_4)_2SO_4$, 0.025% $CaCl_2$, 0.025% $MgSO_4$ and 0.001% Biotin. SGM was autoclaved at 1lb/inch^2 pressure.

Growth Characteristics

Optical density per hour was quantified at 570nm using Shimadzu UV–Visible 160 spectrophotometer at 25ºC for 15h. A plot, time v/s optical density was drawn (Figure 1) for the determination of the mid log phase. The metabolic activity and accumulation of nutrients by cell become maximum at mid log phase. It was obtained after 7h.

Preparation of Heavy Metal Solutions

The metal ions were added to achieve final concentrations of 1µg/ml, 2µg/ml, 5µg/ml, 7µg/ml and 10µg/ml Cu $(NO_3)_2$ in biosorption media.

Determination of Dry mass of *S. Cerevisiae*

Concentrations of Cu^{2+} ion were adjusted between 1-10µg/ml in 50ml biosorption media in 250ml Erlenmeyer flasks, inoculated, incubated for 7h and were evaluated in relation to growth. At mid log phase of growth cells were harvested, centrifuged and treated with citrate buffer (pH 4.8). The pellets were washed with distilled water 3 to 4 times and dried at room temperature. Dry mass of the cells were measured by weighing on mettler balance. The results were compared with control containing no metal at 25ºC for 7h.

Analysis of Copper Accumulated by Yeast Cells

Accumulation was studied in the presence of 1µg/ml, 2µg/ml, 5µg/ml, 7µg/ml and 10µg/ml of Cu^{2+} ions in SGM. Yeast cells were grown in SGM at different concentrations of copper at 25ºC for 7h. After 7h yeast cells were collected by centrifugation. Then the cells were harvested and washed using citrate buffer (pH 4.8). The harvested cells were dried, weighed and digested with 1% HNO_3 solution[12,24]. Accumulated Copper by yeast cells was determined by Varian Atomic Absorption Spectrophotometer 300. Results were compared with control, which show the correlation between metal concentration and accumulation of metal in µg/ml by the yeast cells.

Analysis of Total Protein Contents

Yeast cells were grown in SGM containing 1µg/ml, 2µg/ml, 5µg/ml, 7µg/ml and 10µg/ml of Cu^{2+} ions for 7h at 25ºC in aerobic conditions. After 7h cells were collected by centrifugation. Dried cells were then treated with 5ml of 10% Trichloroacetic acid (TCA) and 5ml of ethanol-ether (1:1v/v) mixture followed with addition of 10ml of Tris glycine buffer of 0.2M and pH 8.6 and then boiled for 3min. The supernatant were collected by centrifugation and used for the determination of total protein content. Total proteins were determined by Lowry's method[25] using Follin's reagent. The results were compared with control, indicating the effect of different concentrations of Cu^{2+} ions on total proteins when present inside the cells.

Results and Discussion

Effect of Copper Concentration on Growth of *S. Cerevisiae*

As it could be seen (Figure 2), the dry mass measurement indicated that selected working concentrations, were (1,2,5,7)µg/ml of Cu(II), as it moderately inhibit the growth of *saccharomyces* compared to the biomass growth, obtained under control conditions for 7h at 25^0C . Above 7µg/ml of Cu(II) increased growth of yeast cells was seen as indicated by dry mass (Figure 2). Copper bounds mainly to albumin, travels to the organs where it then re-emerges in plasma associated with ceruloplasmin. The chelated copper then travels to the various tissues where it is transferred to internal cellular processes, most probably by cell-surface receptors. Concentration of copper is most important aspect explaining the harmful influence on fungal growth. It has been investigated that before accumulating into the yeast cells, Cu(II) is first reduced by the Iron/Copper specific Reductases FRE-1p and FRE-2P to Cu(I)[26,27], which is transported into the cell by the CTR-1p[19,27] transporter, a novel protein with two related possible copper transporters (CTR-2p,CTR-3p) in yeast[28]. At 10µg/ml of copper increased growth of cells was seen which indicate that due to reduction of Cu^{2+} ions some specific system became active resulting into higher dry mass.

Effect of Accumulated Copper on *S. Cerevisiae*

Baker's yeast has been found to possess two or more substrate specific transport systems for accumulating any single metal ion. They can produce or excrete extracellular polymeric substances such as polysaccharides, glucoprotein, lipopolysaccharide, soluble peptides, etc[7,8]. The adsorbed ions are transported across the membrane by the same mechanism by which metabolically important ions (K, Mg and Na) are accumulated[10]. Cu^{2+} is accumulated by yeast cells as free ions and uncomplexed to any potential ligand present in the periplasm[27]. Accumulation of copper increased with increasing concentrations of Cu^{2+} ions (Table 1). Copper increases the free radical attack on the extra cellular matrix of unicellular organisms. Accumulation of Cu^{2+} ions leads to the production of free radicals in tissues. In the presence of copper, production of peroxide radicals and interaction with the cell membrane causes cell poisoning.

Effect on Total Protein Contents of *S. Cerevisiae*

Total protein contents obtained in the presence of 1µg/ml, 2µg/ml, 5µg/ml and 7µg/ml copper are 28µg/ml, 23.6µg/ml, 18µg/ml and 9.6µg/ml

respectively (Table 2). Decreased total proteins with increasing concentration of Cu^{2+} ions indicate that proteins may start degenerating or are involved in other metabolic reactions leading to the efflux of accumulated Cu^{2+} ions. Role of protein molecules therefore become important. Copper is bound to proteins in the body either as metalloproteins or as enzymes. The periplasmic Cop-A Protein shows conservation of copper binding sites. The Cop-C and Cop-D Proteins seem to catalyze copper uptake into the cytoplasm. Copper proteins are involved in electron transport and redox reactions[12]. It has demonstrated that copper-bound Prion Protein (a cell surface glycoprotein) is fully able to accept electrons and donate electron cyclically[29,30]. It has shown that copper is reduced from Cu^{2+} to Cu^{+} on binding to PrP[31]. Protein is able to reduce the amount of hydroxyl radicals present in a Cu^{2+}/Ascorbate/Oxygen system without affecting hydrogen peroxide levels[32]. When cells were grown in the presence of 7µg/ml and 10µg/ml Cu^{2+}, lesser accumulation in terms of µg/ng of total proteins was observed at 10µg/ml concentration (Table 3). In the presence of 1µg/ml, 2µg/ml, 5µg/ml and 7µg/ml Cu^{2+}, the Cu-binding proteins may get involve in metabolic reactions increasing its accumulation. At 10µg/ml concentration of Cu^{2+} highest total protein content was observed. Its concentration influences various biomolecules. It has been proposed that copper co-ordinates with two imidazole nitrogens and two glycine nitrogens inside the cell[33]. De novo synthesis of proteins may help the cells for initiating the efflux of copper ions accumulated at higher concentration exposure. Total proteins decreased with increasing concentrations of copper, which reflects the harmful effect of copper.

Conclusion

Harmful effects of metal can be observed as inhibition of growth or metabolic activities in metal-treated micro-organisms. Hazardous heavy metals when enter in living cells go for the binding sites among biomolecules and get complexed. A protein may have separate binding sites for several different ligands. These specific molecular interactions are crucial in maintaining the high degree of order in a living system. Those proteins have high affinity for the Cu^{2+} ions, which have been associated with copper transport. Cu-binding proteins may get involve in metabolic reactions at lower concentrations, therefore increasing its accumulation at 1µg/ml to 7µg/ml of copper. It is now specifically clear that heavy metals when get accumulated by target protein molecules in the cell, these proteins may be in need of those heavy metals or are getting associated due to competitive accumulation. These heavy metals may also get bonded with the newly synthesized protein molecules which have the specific function of effluxing unwanted heavy metals from the cytoplasm.

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Figure 1

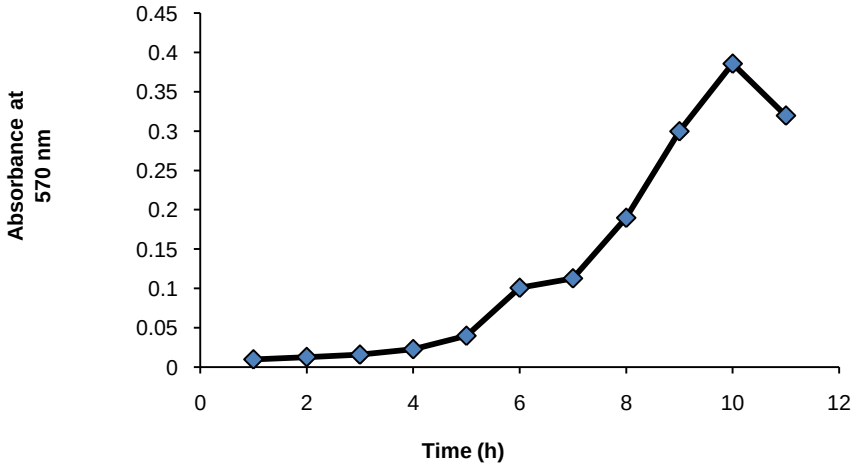

Figure 2

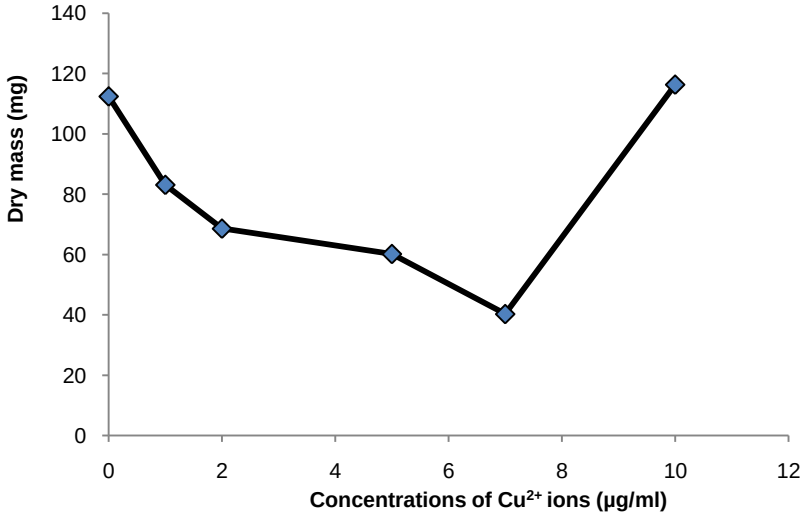

Table-1
Accumulation of Cu^{2+} by *Saccharomyces Cerevisiae*

S.N	Concentration of Cu (µg/ml)	Absorbance at 324.7 nm	Concentrations of Cu on AAS (µg/ml)	Accumulated Copper (µg/ml)
1	Control	0.0001	0.000	0.000
2	01.0	0.0424	0.273	0.273
3	02.0	0.0666	0.425	0.425
4	05.0	0.1416	0.884	0.884
5	07.0	0.2452	1.552	1.552
6	10.0	0.5688	3.699	3.699

Table-2
Total Proteins in *S. Cerevisiae* when Grown with Different Concentrations of Cu^{2+} ions

S. N.	Concentration of Cu (µg/ml)	Absorbance at 600 nm	Total Proteins (µg/ml)
1	Control	0.17	38.0
2	01	0.14	28.0
3	02	0.13	23.6
4	05	0.12	18.0
5	07	0.10	09.6
6	10	0.19	48.0

Table-3
Accumulation of Cu^{2+} ions by yeast *Saccharomyces Cerevisiae* in µg/ng of Total Proteins

S. N.	Total Cu concentration supplemented (µg/ml)	Dry Mass (mg)	Total Protein (ng/mg of dry mass)	Absorbance on A.A.S.	Accumulated Cu (µg/mg of dry mass)	Accumulated Cu (µg/ng of proteins)
1	Control	112.4	2704.62	0.0001	0.0000	0.000
2	01	083.1	2595.66	0.0424	0.1642	0.06328×10^{-3}
3	02	068.6	2587.46	0.0666	0.3097	0.11972×10^{-3}
4	05	060.2	2511.62	0.1416	0.7342	0.29233×10^{-3}
5	07	040.3	1799.00	0.2452	1.9255	1.07034×10^{-3}
6	10	116.3	3136.71	0.5688	1.5902	0.50699×10^{-3}

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