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Asian Resonance

Bio-Accumulation of Cr^{3+} Ions by Saccharomyces Cerevisiae

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Abstract

The use of microbial cells for heavy metal removal from the water bodies offers an inexpensive alternative compared to conventional methods. Yeast being a typical eukaryote, has many essential features similar to higher eukaryotes. Bio-accumulation of Cr^{3+} ion by *Saccharomyces cerevisiae* was studied. Yeast cells were grown in the presence of different concentrations of Cr^{3+} for 7h. Variation in total protein content is correlated with accumulation of Cr^{3+}.

Keywords : Saccharomyces Cerevisiae, Bio-Accumulation, Cr^{3+} ion.

Introduction

Bio-accumulation 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 Bio-accumulation has been investigated during last decades[4]. Yeast cells are capable of accumulation of various heavy metals[5,6]. Baker's yeast has been found to possess two or more substrate specific transport systems for accumulating any single metal ion. Some heavy elements like Pb, Cd and Hg are harmful to all living organisms at all concentrations while some exhibit toxic effects when present in higher concentrations[7]. These heavy metals compete with essential metals and occupy metal binding sites in plasma membrane. Carriers and permeases, which are found in the plasma membrane of living organisms are helpful in the accumulation[8,9]. The protein molecules are responsible for transport of nutrients[10,11]. Micro-organisms and various plants growing in water bodies are exposed to these pollutants and accumulate them leading to various biochemical effects[12].

Mining of Chromium alloys and extraction leads to the emission of chromium in the environment. It is widely used in tanning, wood preservatives and dyes for plastics, paint and textiles. Chromium is an essential trace metal found in mammals. At higher concentration it is nonessential and harmful. It is a soft metal and forms stable bonds with nitrogen or sulfur containing ligands such as CN^-, RS^-, SH^-, NH^{2-} and imidazole. When it reacts with these residues in proteins, it frequently inhibits catalytic or biological activity, producing the biological effects which are harmful[13-15].

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 $Cr(NO_3)_3$ in biosorption media.

Determination of Dry mass of *S.cerevisiae*

Concentrations of Cr^{3+} 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 Cr^{3+} Accumulated by Yeast Cells

Accumulation was studied in the presence of 1µg/ml, 2µg/ml, 5µg/ml, 7µg/ml and 10µg/ml of Cr^{3+} ions in SGM. Yeast cells were grown in SGM at different concentrations of chromium 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[16,17]. Accumulated Cr^{3+} ion by yeast cells was determined by Varian Atomic Absorption Spectrophotometer 300. Results were compared with control, which show the correlation between Cr^{3+} concentration and accumulation of Cr^{3+} 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 Cr^{3+} 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[18] using Follin's reagent. The results were compared with control, indicating the effect of different concentrations of Cr^{3+} ions on total proteins when present inside the cells.

Results and Discussion

It is well known that carriers and permeases which are responsible for the transport of nutrients in living organisms are protein molecules, which are embedded in the plasma membrane either as intrinsic or extrinsic proteins. They are highly selective and specific and become activated according to the metabolic needs. Heavy metals enter into the living cells by binding with biomolecules or getting complexed. The results show that protein content is greater than the control in the presence of 1µg/ml Cr^{3+} (Figure-2). It is known that Chromium is an essential element at lower concentrations. Chromium is necessary for living organisms in traces but becomes hazardous at higher concentrations. Decreasing protein content with increasing concentration of Cr^{3+} indicates that existing proteins may get involved in efflux mechanism for accumulated Cr^{3+} ions leading to the decrease in protein contents. Chromium can bind to proteins resulting into protein degradation[19,20]. Cr^{3+} is a hard acid which binds with $-NH_2$ groups of proteins. It forms many complexes with N-ligand and chelating groups. Cr^{3+} ion when linked to amino acids or other biomolecules is more readily taken up by diffusion across the plasma membranes. Chromium shows highest number of oxidation states behind Manganese in the first transition series. The affinity of Cr^{3+} for transferrin is close to that of Fe^{3+} and therefore it competes with iron for the same binding sites. It might get involved in important metabolic reactions replacing iron from the important biomolecules and hence induce de-naturation of proteins as evidenced by the low total protein contents in *S.cerevisiae* cells. From the results we see that there is a good correlation between accumulated Cr^{3+} and decrease in protein content (Table-1).

Harmful effects of metals may be observed as inhibition of growth or metabolic activity in metal-treated micro-organisms. Dry mass values throws light on other metabolic reactions which are affected by the increased Cr^{3+} leading to the decreased growth (Table-2). Accumulation of Cr^{3+}increased with increasing concentrations of Cr^{3+}ions (Table-3). At higher concentration Cr^{3+} becomes harmful and so lesser accumulation is favoured or the existing proteins get involved to efflux out the accumulated Cr^{3+} leading to the decrease in protein contents. It has been shown that Cr^{3+} is reduced in cytoplasm resulting in the production of free radicals. These free radicals suppress the metabolic activities. These results clearly indicate that increased Cr^{3+} concentrations hinder the normal growth of cells and start to show biochemical effects.

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Figure 1

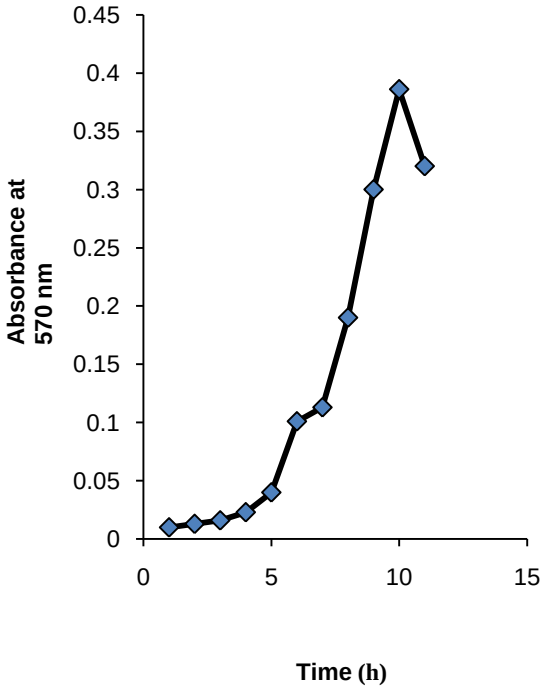

Fig 1: Growth Curve for Saccharomyces Cerevisiae At Room Temperature.

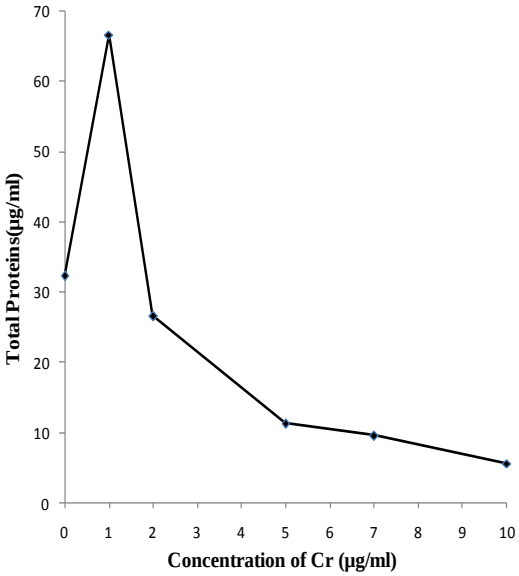

Fig. 2: Total proteins determined in *S.cerevisiae* when grown with Cr^{3+} ions.

Table: 1 Accumulation of Cr^{3+}ions by Yeast *S.cerevisiae* in µg/ng of Total Proteins.

S.N	Total Cr^{3+} concentration supplemented (µg/ml)	Dry Mass (mg)	Total Protein (ng/mg of dry mass)	Absorbance on A.A.S.	Accumulated Cr^{3+}(µg/mg of dry mass)	Accumulated Cr^{3+}(µg/ng of proteins)
1	Control	090.3	5487.26	0.0002	00.000	0.000
2	01	112.2	9199.64	0.0064	0.0557	0.0061×10^{-3}
3	02	070.2	4205.98	0.0175	0.2492	0.0592×10^{-3}
4	05	053.4	2971.16	0.0199	0.3745	0.1260×10^{-3}
5	07	044.4	3162.83	0.0696	1.5698	0.4963×10^{-3}
6	10	037.9	2119.52	0.0775	2.0474	0.9660×10^{-3}

Table: 2 Dry Mass of *S.cerevisiae* Grown with Different Concentrations of Cr^{3+} ions

S.N	Concentration of Cr^{3+} (µg/ml)	Dry Mass of yeast cells (gm)	Dry Mass of yeast cells (mg)
1	Control	0.0903	090.3
2	01	0.1122	112.2
3	02	0.0702	070.2
4	05	0.0534	053.4
5	07	0.0444	044.4
6	10	0.0379	037.9

Table: 3 Accumulation of Cr^{3+} by *Saccharomyces cerevisiae*

S.N	Concentration of Cr^{3+}(µg/ml)	Absorbance at 357.9 nm	Concentration of Cr^{3+} onAAS(µg/ml)	Accumulated Cr^{3+} (µg/ml)
1	Control	0.0002	0.000	0.000
2	01	0.0060	0.118	0.118
3	02	0.0170	0.340	0.340
4	05	0.0208	0.418	0.418
5	07	0.0712	1.426	1.426
6	10	0.0772	1.546	1.546

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