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FACTORIAL DESIGN FOR SOME PARAMETERS AFFECTING ON CHROMIUM III UPTAKE BY *SACCHAROMYCES CEREVISIAE*

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ABSTRACT: The microelements play an important role in the metabolism, therefore the supplementation of theses elements are necessary. The growth of *Saccharomyces cerevisiae* under aerobic and static conditions with the addition of chromic chloride to the cultivation medium were investigated. The studies showed that the addition of $CrCl_3$ into the growth medium stimulate yeast biomass growth. The results showed that the maximum specific chromium up take was obtained under aerobic conditions 120 rpm, chromium conc. 200mg/l ,intact time 6hr, at pH 6 and temperature $35C^0$. Comparison between the aerobic and static conditions revealed that the priority of using the aerobic conditions. The tested parameters were found to be significant according to the selected design and student's t- test .

Keywords: *Saccharomyces cerevisiae*, chromium uptake, fermentation conditions

INTRODUCTION

The role of microelements in the metabolism of higher organisms and yeasts has recently, become a very interesting field of research work. Particular attention has been given to the effect of zinc, copper, iron, chromium, selenium, manganese and other trace elements in the prevention of certain diseases in humans and animals (Mertz, 1993, Jones, and Gadd,1990 ;and Anderson, *et al.,* 1998).).
Two stable oxidation states of chromium in the environment Cr^{+3} and Cr^{+5} .The trivalent chromium is essential in human nutrition especially in glucose metabolism ,most of the hexavalent compounds are toxic and cause several diseases as lung cancer (Saifuddin and Raziah,2007, Izabella, M., *et al.,* 2007). Chromium in the trivalent form is an essential nutrient in carbohydrate, lipid and nucleic acid metabolism, insufficient dietary intake of Cr^{+3} is associated with increased risk factors associated with type II diabetes mellitus and cardiovascular diseases. Chromium functions in glucose and insulin metabolism were primarily connected through its role in the regulation of insulin. Chromium deficiency in human results in symptoms comparable to those associated with diabetes (Anderson, *et al*., 1998).
It has been established that chromium, present even in the trivalent state does not readily convert into its biologically active form in mammals and microorganisms. The trivalent chromium has a very strong tendency to form octahedral complexes with biological ligands on the cell's membrane. Mannan-β-glucan is the main structural polymer of the cell wall in *S. cerevisiae*, in addition to these proteins, lipids and pigments which reflected a range of different metal-complexing sites e.g. carboxyl, phosphate, sulphate and amine groups ((Kompulainen *et al*., 1978; Rapoport and Muter, 1995). The role of Cr^{3+} ions in the metabolism of mammals and yeast is connected with the glucose tolerance factor (GTF). It is cationic Cr^{3+} complex of low molecular weight, this complex factor consists of Cr^{3+}, nicotinic acid and the amino acids (glycine, glutamic acid and cysteine), which is biologically active (Toepfer *et al*., 1977, Beran *et al*., 1995).
The biological role of the above complex in yeast cells is mainly connected with carbohydrate metabolisms (Ducros, 1992 and Helena *et al*., 2003).

As a result of the increase demand of trace element specially Cr^{3+} by human being and overcome the trace metal deficiency symptoms. Therefore, the present study aims to investigate the incorporation of optimal concentrations of Cr^{+3} ions into the baker's yeast cells during cultivation at semi-aerobic and static conditions.

MATERIALS AND METHODS

Materials

Microorganism

The yeast used in this study was baker's yeast *S. cerevisiae* NRC 10 and was obtained from Natural and Microbial Products Chemistry Department, National Research Center (NRC), Egypt.

Chemicals

The authentic trivalent chromium ($CrCl_3$ in 4.2% HCl) was obtained from Merck KGa A, 64271 Darmstadt, Germany. All the other chemicals used in the current study were fine analytical grade obtained from Sigma company.

Methods

Maintenance of the microorganism

The experimental microorganism was maintained on agar slants containing the following medium (g/l) malt extract 10, yeast extract 1, agar 20, the slants were kept at 4 °C .

Cultivation process

According to the method described by Vlatka et al. (2001) Erlenmeyer flasks 250 ml containing 100 ml of sterile cultivation medium which consists of (g/l): sucrose 50, $(NH_4)_2HPO_4$ 2, $MgSO_4$ 0.5 at pH 4.5 were inoculated by the experimental organism. Chromium solution was added as 200 mg/100 ml prior to sterilization. The flasks were incubated at static or under aerobic conditions ,where the microorganism continued to grow at 120 rpm, 30 ± 1 °C for the required time.

Stastical design

A complete first order design (Adinarayana and Ellaiah,(2002) Beudeker, *et al.*1990*),* based on two levels and three variables was used to study the influence of the three factors tested (intact time , chromium concentration and pH) at the optimum temperature 35 C^0 0n the biomass and the specific chromium uptake under the stirred conditions (aerobic conditions).The biomass was smoothed by generalized logistic equation

$$CDW_{(t)} = k/(1+ Exp(C- \mu t) \quad (1)$$

Where, CDW cell dry weight (the biomass g/l)
K, C are general parameters of the model
μ the specific growth rate h^{-1}
t is the time in hr

Analysis

At the end of the growth period, the cells were harvested by centrifugation at 3500 g for 10 min. Dry matter of yeast biomass(CDW) was determined by drying yeast biomass at 105 °C to constant weight. Chromium ion concentration in the supernatant was analyzed by using "Varian" spectra AA300 atomic absorption spectrophotometer using an air acetylene flame. Chromium concentration was determined by reference to an appropriate standard metal solution (Vlatka *et al.,* 2001). The specific chromium concentration was calculated by dividing the up take Cr^{+3} concentration by the yeast biomass yield

RESULTS AND DISCUSSION

Intact time effects

The effect of incubation time on the chromium III uptake by *S. cerevisiae* was investigated. The results in fig (1) showed that incubation time clearly affected on the amount of Cr^{3+} up take under both static and aerobic conditions. The best specific uptake of Cr^{3+} (27.4 mg/g biomass) was obtained at 6 hr on using the aerobic conditions while, low level (18.2mg/g) was obtained by using the static conditions at the same time. The results also showed that at the incubation period more or less 6 hr, the chromium uptake activity has been reduced.

The accumulation of Cr^{+3} ions into yeast cells was increased and completed at the exponential growth phase as stated by Vlatka *et al.* (2001). This explained the fact that chromium accelerate the metabolism of carbohydrate, catalyzed the phosphoglucomutase reaction and stimulate other enzyme systems such as the succinate-cytochrome reductase (Moore and Pettigrew, 1990). Consequently, high uptake activity has been noticed during the early growth stage. On the other hand, the reduction in Cr^{3+} uptake at the longer incubation period has been detected to be reduced Saifuddin and Raziah,(2007).

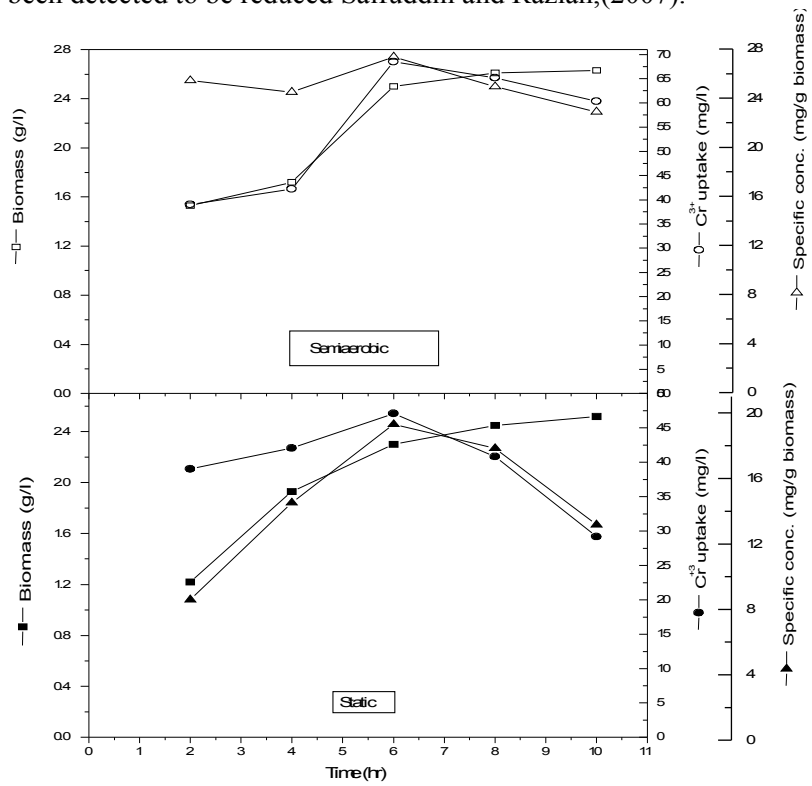

Fig 1. Effect of incubation time on the chromium uptake by *S. cerevisiae*

Chromium concentration

The chromium levels presented the fermentation medium affect on the amount of Cr^{3+} uptake by the yeast cells The data showed in fig (2) clearly indicated that , since the amount of Cr^{3+} up take by the cells, increased as the Cr^{3+} concentration increase. The maximum specific Cr^{3+} uptake (31.8 mg/g biomass) was obtained at 200 mg/l. At the higher concentrations above (200 mg/l), a constant specific up take was noticed ,since the uptake process depend up on the cell activities .

The theory of metal up take had explained to be dependent on many factors, it is dependent on the initial biosorption step of metal ions which is rapid. Consequently, it rapidly accumulated on the surface (Batic and Raspar, 1998).

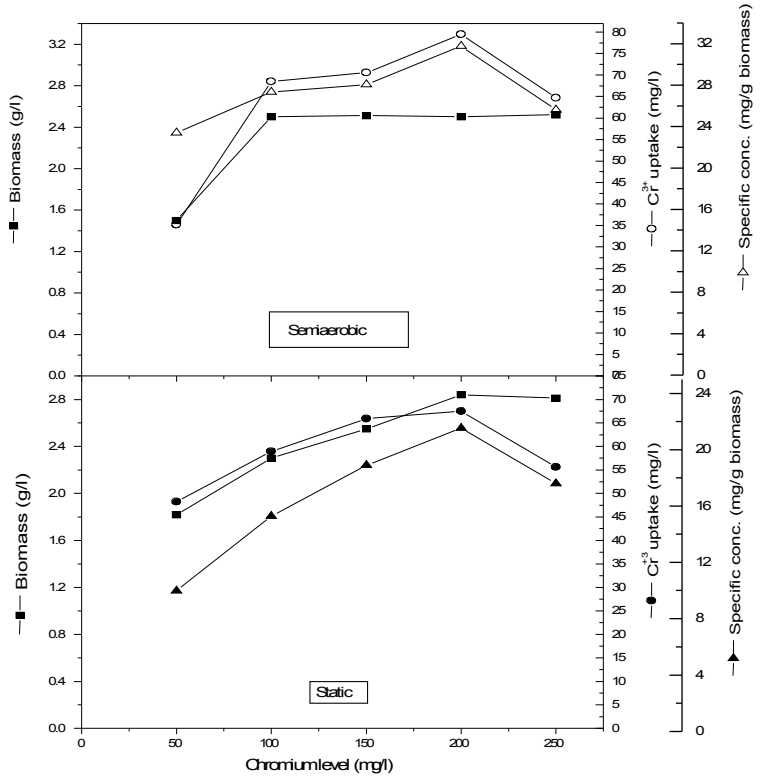

Fig 2. Uptake of Cr^{+3} by *S. cerevisiae* under static and aerobic conditions at different concentrations.

The chromium uptake from the cell surface to inside the cells is enzymatic ally controlled and depends mainly on the cell wall components (White and Gad, 1987, Brady *et al*., 1994 and Helena *et al*., 2003). Therefore, as more Cr^{3+} presented in the medium, the more ions attached to the cell surface which is accompanied by more Cr^{3+} incorporated into the cells till an equilibrium conditions which has been achieved at 200 mg/l in our experimental studies.

pH relation

The investigation of the Cr^{3+} uptake at different pH values of the cultivation medium was tested. The results in fig (3) revealed that the maximum specific Cr^{3+} uptake (39.15 mg/g biomass) was obtained at pH 5.5. But, reduction in Cr^{+3} concentration has been noticed as the pH value shifted to neutral and alkaline values

Vlatka *et al* (2001) showed that the best Cr^{3+} uptake has been obtained at pH 4.5. , generally, the lower pH values increases the availability of metal ions, but the high pH value decrease this availability (Gadd and Grifiths, 1978). In acidic pH medium metals exist as free ionic cat ion, the alkaline pH medium the ionic cat ion precipitate as insoluble hydroxides or oxides and most of the heavy metal hydroxides are insoluble (Izabella, M., *et al.,* 2007).

Effect of temperature

The temperature at which the fermentation process achieved was found to affect on the activity of the tested microorganism, since most of the metabolic reactions are enzymatically controlled and the enzymes are sensitive to temperature changes.. The results presented in table (2) showed that the Cr^{+3} uptake by yeast cells clearly affected by the temperature used.(25, 30, 35, 40, 45 C^0).The data indicated that the best Cr^{+3} up take (95.6 mg/l) was obtained at 35C^0 using the aerobic conditions. However, at higher temperature (40and 45 C^0) lower uptake chromium noticed, since, high temp. affects reversely on the metal up take (Saifuddin and Raziah, 2007).

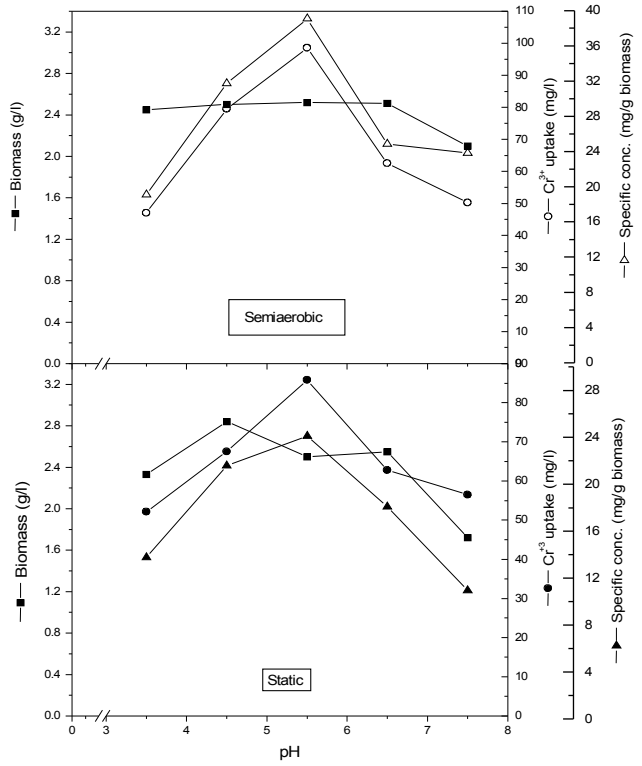

Fig 3. Effect of pH value of the cultivation medium on Cr^{3+} uptake by *S. cerevisiae*.

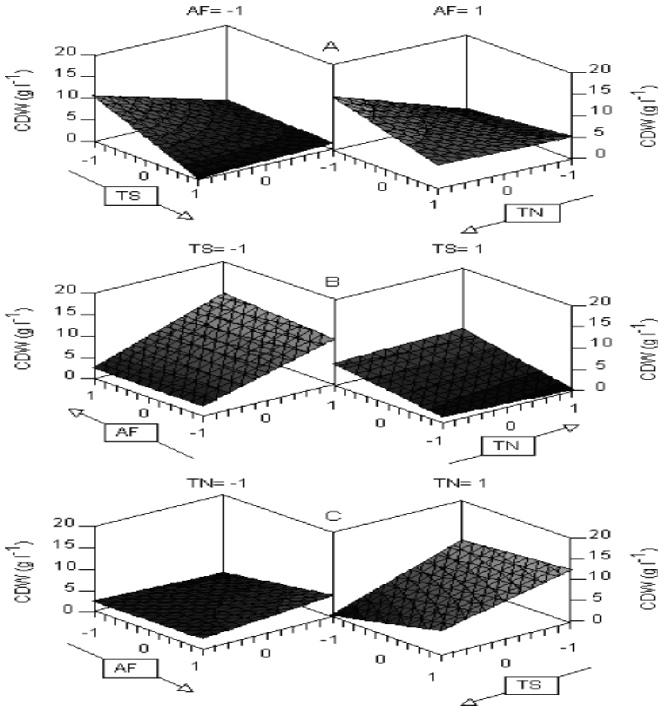

Fig.(4) The response of the tested variables according to the design

A multi-factorial composite orthogonal design(Adinaryana ,and Ellaiah,.(2002) based on five levels and three variable range was used to study the effect of intact time (TS) ,concentration of Cr^{+3} (TN) and pH value (AF) of the surrounding medium.
The results of the above experiment showed that the coefficients as well as the empirical model obtained were found to be significant according to the student's *t-test* (- ≤ 0.05) the following equation applied
Specific up take = 7.62+ 1.24 AF+ 2.23TN- 1.72TS + 0.53AF TN + 1.13 AFTS – 2.31 TNTS

Isolation of glucose tolerance Factor (GTF)
The yeast biomass was extracted with 0.1mol/L NH_4OH assuming that the extracted samples had GTF according to the method described by **(Mirsky,1993)**.Fractionation of the extracted samples were performed on a gel filtration column using Sephadex 75 **(Beran and Stahl,1995).** Collected fractions were detected spectrophotometrically at 260 nm. The results presented in Fig. (5) showed the behavior of the elution of the extract from the yeast biomass.

Table (1) Effect of temperature on the chromium up take by *S .cerevisiae*

Temp.C0	Stirred			static		
	Biomass g/l	Cr+3up take mg/l	Specific mg/g	Biomass g/l	Cr+3up take mg/l	Specific mg/g
25	0.4	65.2	46.4	0.5	61.5	41.1
30	0.6	86.7	54.1	0.6	71.5	44.6
35	1.1	95.6	86.3	1.8	74.3	35
40	1.7	74.3	61.6	1.6	55	34.3
45	1.8	71.5	39.7	1.4	52	37.6

Table(2) The experimental codification of the variables used in the factorial design

Codified value	time hr (T)	concentration mg/l (C)	pH value (P)
-1	**4**	**150**	**4.5**
0	**6**	**200**	**5.5**
+1	**8**	**250**	**6.5**

Table (3) Comparison between static and stirred conditions on Cr^{+3} uptake using *S. cerevisiae*

.Activity	Control	Static conditions	aerobic conditions
Maximum Cr^{3+} uptake (mg/l)	-	74.50	95.6
Maximum biomass yield (g/l)	1.37	2.50	2.52
Maximum specific chromium uptake (mg/ g biomass)	-	35	86.3
Optimum intact time (hr)	6.00	6.0	6.0
Optimum pH	5.50	5.5	5.5
chromium concentration (mg/l)	-	200	200
Required temp.C^0	-	35	35

Control .without addition

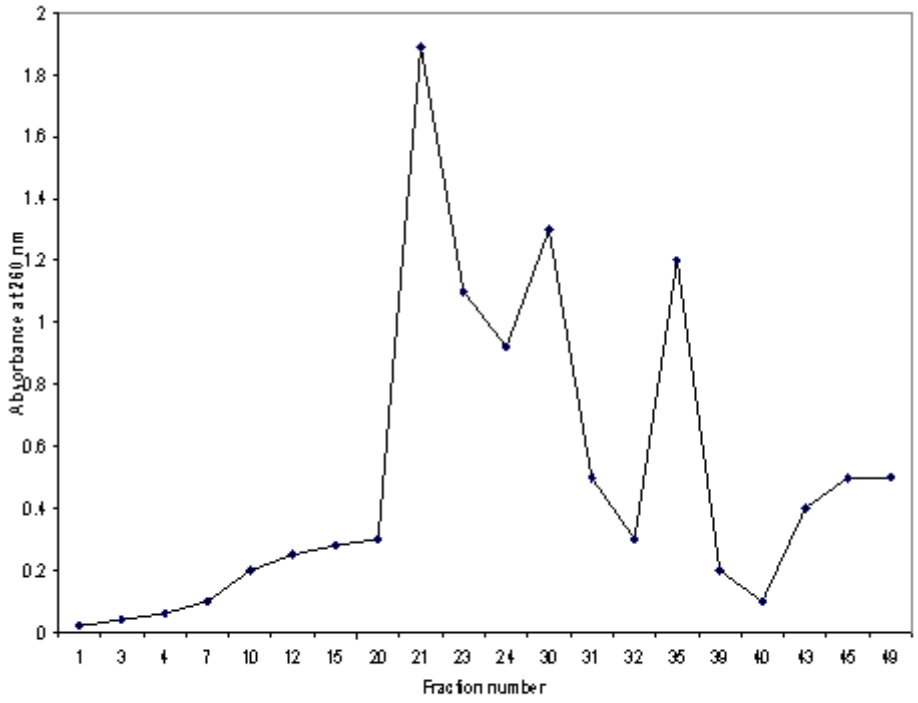

Fig (5.) fractionation of the extracted sample using Sephdex 75

Conclusion

The results obtained from the previous experiments clearly indicated that the semi-aerobic (stirred) conditions is considered to be more efficient for Cr^{3+} uptake by *S. cerevisiae*, where the maximum specific Cr^{3+} uptake (86.3 mg/g biomass) was obtained after 6 hr incubation, pH 5.5 at Cr^{3+} concentration 200 mg/l at temperature $35C^{0}$.

The present investigations gave high lights on providing strain of *S. cerevisiae* enriched with Cr^{3+} to substitute the deficiency of trace metals specially, Cr^{3+} and prevent the symptoms of trace metal deficiency diseases in human being.

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