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Optimization of selenium enriched *Saccharomyces cerevisiae* by Response Surface Methodology (RSM)

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Abstract: Three parameters including initial sodium selenite (Na_2SeO_3) concentration, initial pH and incubation temperature were studied for the optimization of selenium enriched yeast production in molasses medium (12%) by *Saccharomyces cerevisiae* and using Response Surface Methodology (RSM) as statistical analysis. The optimum conditions for the highest biomass and Se yield were (Na2SeO3) concentration 22.5 µg/mL, pH=4 and incubation temperature=31.5˚Cwhich generated6.69g/L of biomass and 3766.07ppm of total Se yield and 3756.89ppm of organic selenium, and made the yeast *Saccharomyces cerevisiae* a promise organism for industrial selenium enriched yeast production, and the RSM a good tool for the optimization of selenium production.
Key words: Selenium, *Saccharomyces cerevisiae*. RSM, sodium selenite, yeast.

1. Introduction

The trace element Selenium (Se) is an essential nutrient for human and animals[14]. This element is a component of some important selenoproteins and enzymes required for main functions in organisms as antioxidant defense, reduction of inflammation, thyroid hormone[9] and killing cancer cells (by reducing the blood supply to them) [3].

In some parts of the world where sea is insufficiently available to plants, se-deficiency diseases have been identified, such as Keshan disease, an endemic cardiomyopathy found in the North East of China , that formerly caused many deaths. Supplementation with Se has greatly reduced the incidence of the condition Supply of the Se enriched food, especially, Se enriched biomass (yeast or bacterial) with organic forms of this mineral is one efficient way to overcome Se deficiency[1].

Yeasts contain high amount of protein and they can incorporate Se by replacement of sulfur in proteins. During fermentation, the yeast cells can accumulate large amounts of Se and incorporate them into organic Se-containing compounds, mainly Se-Met[2]. Na_2SeO_3can be bio-transformed to organic form and being absorbed by the yeast[13].By this process the inorganic selenite as a low bioavailable toxic component can be converted to safer highly bioactive species with improved nutritional properties.

Saccharomyces cerevisiae is only yeast strain that has been used by manufacturers for production of Se-enriched yeast[4].The growth of yeast rely on physico-chemical factors such as incubation temperature, pH and minerals concentrations, which may influence their metabolism, cell morphology and reproduction[10] .Several culture conditions of Se enriched yeast production including Se, concentration, pH, and temperature, on yield of biomass production have been reported[12,13].

Response surface methodology (RSM) is a collection of mathematical and statistical techniques useful for designing experiments, establishing models, and analyzing the effects of several independent factors .The main advantage of RSM is the reduced number of experimental trials needed to evaluate multiple factors and their interactions. Also, study of the individual and interactive effects of these factors will be helpful in efforts to find the target value ،RSM can be employed to optimize the process[7].

In this study, We identified the optimum fermentation conditions (initial sodium selenite (Na_2SeO_3) concentration, initial pH and temperature) from *Saccharomyces cerevisiae* for maximal total Se yield of selenium-enriched yeast.

2. Materials and Methods

2.1. Microorganism:

The active dry baker's yeast*, Saccharomyces cerevisiae*(China)was bought from a supper market.0.5 g of this yeast were suspended in 50 ml distilled water. The yeast was grown in PDA(Yeast potato Dextrose Agar), and incubated at 25^0C for 48h. The yeast was maintained in PDA slant at 4 °C for further study.

2.2. Inoculum preparation

The loops from YPD slant was transferred to100 ml PDB (Potato dextrose broth) medium containing(3g/L)yeast extract, (5g/L)Peptone, (2g/L)Glucose, (2.4g/L)$Na_2HPO_4.12H_2$, (0.075g/L) $MgSO_4.7H_2O$ and(5.1g/L) $(NH4)_2SO_4$and incubated at 30˚C, 200 rpm, for 24 h.

2.3. Molasses

Mixture of beet and cane molasses with the ratio of (1:1) were used as a fermentation medium with the following specifications : soluble solids content, (77%), Water content (23%), Total sugar, (48.9%), pH(6.4), Ash (8.3%), Density(g/cm3) (1.36), Ca (6%), Na (0.74%), Cl (1.065%), K(K2O) (1.5%), P (P2O5) (0.85 %).

2.4. Fermentation conditions

Fermentations were carried out in 250 ml flasks using 125ml of molasses adjusted to (12%)) included (2%Peptone, 1% yeast extract, 0.05%$MgSO_4.7H_2O$, 0.3%KH_2PO_4, 0.5%$(NH4)_2SO_4$, 0.014%Urea, 0.013% $CaCl_2$) and sterilized at 121ºC for 15 min. Sodium selenite (Na_2SeO_3) solution was added in different concentration according to the RSM experimental design of three parameters. The flasks were inoculated with2% (v/v) of pre-inoculum. The cultures were incubated in a rotary shaker incubator for 24h at 200 rpm. The levels of initial pH and temperature used in the optimization studies by RSM are given in Table 1.

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2.5. Determination of dry cell weight (DCW)

The yeast cells were separated after cultivation by centrifugation (3000 rpm, 5min) and washed three times by deionized water to remove adsorbed Se on the cell surface. The cells were dried at 40˚C to a constant weight.

2.6. Determination of Se Content in Yeast:

The Se determination was carried out according to the hydride generation atomic fluorescence spectrometry (HG-AAS) method described by[5] with some modifications. 0.1 g dried samples were digested) (120°C, 20 min) with 10 ml of concentrated HNO_3.Then the solution was cooled and for Experimental design and data analysis 2ml of concentrated HCl (80°C, 10 min). After cooling, the solution was filtered and made a constant volume with ultra-pure water. The digested product was used for total Se determination. For measurement of inorganic Se, the suspension of biomass in ultra-pure water was extracted in boiling bath for 1 h and made a constant volume. Then the mixture was centrifuged at 8300 ×g for 15 min and the supernatant liquor was filtrated. Organic Se yield was calculated from the difference between the total and inorganic Se yield.

Experimental design and data analysis

The statistical analysis of the data was performed using Minitab Statistical Software (13.2). The levels of factors used in the experimental design are listed in Table1.

The data of the factors were selected to cover a wide range of values which have not been studied before. Response surface model was fitted to the response variable, namely specific yeast biomass (g/l). The second order response function for the five quantitative factors is given by Equation [1]:

$$Y=a+bX_1+cX_2+dX_3+eX^2_1+fX^2_2+gX^2_2+hX_1X_2+iX_1X_3+jX_2X$$

where

Y: response. /a: constant/ b, c, d: linear coefficient./e, f ,g: square coefficient./h, i , j: interaction coefficient.

Result and Discussion

In our study, the level of three factors (initial sodium selenite (Na_2SeO_3) concentration, initial pH and incubation temperature) were applied in the optimization of selenium enriched yeast production by *Saccharomyces cerevisiae* using RSM were determined in a wide range of values(Table 1).The effect of the three previously mentioned variables, each at three levels, and their interactions on biomass and selenium yield were determined by carrying out thirty experiments given by the model (Table 2).A central composite design was used to determine the optimum levels of these parameters leading to a maximum biomass and selenium synthesis. In order to determine the maximum total and organic selenium corresponding to the optimum levels of initial sodium selenite (Na_2SeO_3) concentration, initial pH and incubation temperature second order polynomial model was used to calculate the values of these variables

Table (1):Levels of factors used in the experimental.

Variables	Levels		
	+1	0	-1
sodium selenite (µg/ml)	30	22.5	15
pH value	5	4	3
Temperature	33	31.5	30

Table (2): **The results of three process variables on total, organic and biomass yield**.

Run number	Na2SeO3 (µg/ml)	pH	Temperature C°	Biomass (g/l)	total Se ppm	organic Se ppm
1	30	5	33	3.84	15600	15596.85
2	15	3	30	2.49	8940	8939.73
3	30	5	30	9.44	3710	3708.835
4	30	3	33	0.81	29800	29795.28
5	22.5	4	33	4.66	8030	8022.485
6	15	3	33	3.33	4430	4424.4
7	30	3	30	0	0	0
8	15	5	30	6.01	446	444.22
9	22.5	5	31.5	7.13	27400	27382.07
10	15	4	31.5	7.11	1770	1767.78
11	30	3	30	0	0	0
12	30	5	30	9.93	9990	9985.36
13	30	3	33	0.67	29900	29896.78
14	15	4	31.5	4.42	1110	1107.8
15	22.5	5	31.5	8.25	3000	2985.57
16	22.5	4	33	5.44	1560	1555.885
17	15	5	33	8.28	3140	3135.4

18	30	3	33	0.80	26520	26516.78
19	30	5	30	8.95	9900	9895.455
20	15	3	30	2.75	2660	2656.23
21	30	4	31.5	5.56	8740	8732.6
22	22.5	3	31.5	5.88	3550	3537.465
23	22.5	4	30	4.81	1480	1477.62
24	15	5	33	8.23	11040	11038.875
25	15	5	30	7.12	1074	1068.72
26	15	3	33	3.51	11090	11087.875
27	30	5	33	5.92	9320	9318.725
28	30	4	31.5	7.15	2460	2458.035
29	22.5	3	31.5	5.74	6690	6680.965
30	22.5	4	30	5.75	880	877.6

Y=-378487X1-8328X2+25758X3-4X21+6394X22-346X23-69X1X2 +294X1X3 -1865X2X3.

Y=-376193-8332X1+8615X2+25613X3-4X21+6390X22-344X23-69X1X2 +294X1X3 -1865X2X3.

And to determine the maximum biomass production corresponding to the optimum levels of variables which studied second order polynomial model was used to calculate the values of these variables

$Y=-774.153+2.401X_1+13.8945X_2+45.949X_3-0.011X^2_1+0.062X^2_2$

$-0.677X^23+0.084X_1X_2 -0.073X_1X_3 -0.439X_2X_3.$

Estimated regression coefficients for total and organic selenium (Table 3) of the experimental data showed that initial sodium selenite (Na2SeO3) concentration, initial pH and incubation temperature demonstrated insignificant ($P > 0.05$), but $(pH)^2$had an significant effect. On the other hand, initial sodium selenite concentration and incubation temperature demonstrated significant positive linear effects on biomass yield ($P< 0.05$), and $(pH)^2$ had significant effect(Table4) . These results are not agreed with the result of[5] who reported that Se concentration had not any significant effect on biomass yeast yield.The effect of interrelated factors on total and organic Se accumulation formation production was observed in table (3) where the correlation between Se concentration and temperature was significant ($P<0.05$),and the R^2 =62.6%, for the regression equation of the three factors affected change 62.6% in selenium production. while relationships between Se concentration - temperature ,Se concentration –pH and pH-temperature were significant for total biomass as shown in table(4) and R^2 89.5% indicating that the model as fitted explained 89.5% of the variability in specific yeast biomass.

Table (3): Regression Coefficients for totals and organic selenium

Total selenium					**Organic selenium**				
P	**SE Coef**	**T**	**Coef**		**P**	**SE Coef**	**T**	**Coef**	Term
0.782	1351695	-0.280	-378487		0.784	1351549	-0.278	-376193	**Constant**
0.136	5345	-1.558	-8328		0.135	5344	-1.559	-8332	**Se**
0.844	43023	0.200	8600		0.843	43019	0.2	8615	**pH**
0.769	86320	0.298	25758		0.77	86311	0.297	25613	**T**
0.937	55	-0.080	-4		0.938	55	-0.078	-4	**Se * Se**
0.041	3072	2.081	6394		**0.041**	3072	2.08	6390	**pH*pH**
0.803	1366	-0.253	-346		0.804	1365	-0.252	-344	**T*T**
0.757	218	-0.314	-69		0.757	218	-0.314	-69	**Se *pH**
0.047	145	2.023	294		**0.047**	145	2.023	294	**Se *T**
0.103	1089	-1.713	-1865		0.103	1089	-1.713	-1865	**pH*T**

R^2 = 62.6%

Table (4):Regression Coefficients for yield :

Term	Coef	SE Coef	T	P
Constant	-774.153	225.193	-3.438	0.003
Se	2.401	0.89	2.696	0.014
pH	13.894	7.168	1.938	0.068
T	45.949	14.381	3.195	0.005
Se*Se	-0.011	0.009	-1.229	0.234
pH*pH	0.062	0.512	0.121	0.905
T*T	-0.677	0.227	-2.978	0.008
Si*pH	0.084	0.036	2.318	0.032
Si*T	-0.073	0.024	-3.006	0.007
pH*T	-0.439	0.181	-2.421	0.026

R^2= 89.5%

Selenium concentration, pH and incubation temperature are important factors and have insightful influence on selenium enriched yeast production. [11] found that the optimal condition for yeast enriched with high Se content were pH=5 and temperature =30°C. [5] illustrated that the optimum values of selenium concentration, pH and temperature were 25 µg/ml 5.8 and 28°C respectively the highest concentration of selenium accumulation from *Saccharomyces cerevisiae*. [8]demonstrated that *S. cerevisiae* 6M produced the maximum yields of selenium when the medium pH and the concentration of inorganic Se were 6 and 125 ppm. [6] indicated an optimum temperature of 28°Cfor the production of selenium –enriched yeast biomass by *Candida utilis*.

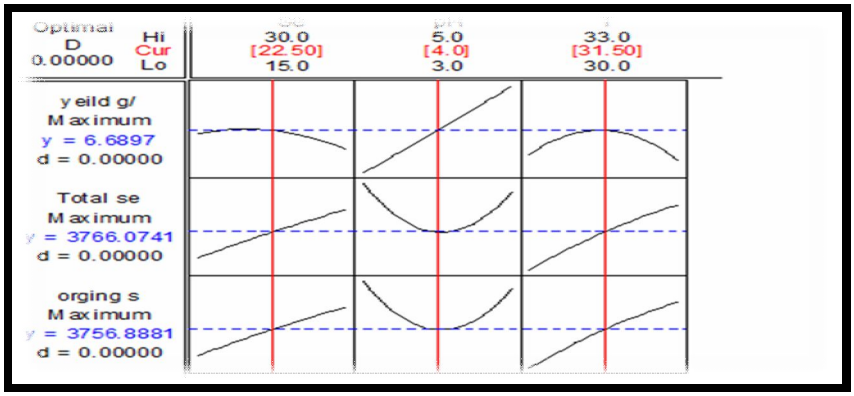

Figure (1):Optimum values of the culture parameters for yield, total and organic selenium yield using statistical program RSM.

In our study in order to determine the maximum biomass and total and organic selenium the response optimization choice from Minitab program showed the optimum values of the tested variables (Figure 1). The fitting of the experimental data to Equation [2] allowed to determine the level of pH(X_2=4) , indicated that very low and high degrees were unfavorable for the tested yeast. The optimal initial selenium concentration was(22.5µg/ml) incubation temperature(31.5°C).Differences between published results are due to the different medium components used, different strains of yeast employed and also to differing cultivation condition. A final fermentation experiment was performed at the optimal values to experiment was performed at the optimal values to optimize biomass and selenium accumulation production from molasses medium (12%) by *Saccharomyces cerevisiae* yeast in shake flask culture, and the maximum biomass, total and organic selenium were (6.69g/l, 3766.07ppm and 3756.89ppm) respectively.

4. Conclusion

RSM was used to determine the effects of three important factors (Na2SeO3 concentration, pH and temperature) on selenium enriched yeast. *Saccharomyces cerevisiae* was relatively able to accumulation high level of organic selenium and the optimum conditions for production of selenium enriched yeast were achieved on molasses medium (12°Brix) containing 22.5 µg/mL(Na_2SeO_3) with an initial pH of 4 at 31.5°C , these

optimum conditions generated 3766.07ppm of total Se , 3756.89ppm of organic selenium and 6.69g/l of the biomass.

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