JAP&T
TPA-Timişoara

Available online at http://journal-of-agroalimentary.ro

Journal of Agroalimentary Processes and Technologies 2017, *23* (4), 253-259

Journal of Agroalimentary Processes and Technologies

Optimization of growing medium composition in obtaining *Saccharomyces cerevisiae* yeast

Adriana Dabija*, Ioana Rebenciuc, Silvia Mironeasa

[1]*Faculty of Food Engineering, Stefan cel Mare University,Suceava, Romania*

Received: 19 June 2017; Accepted: 17 December 2017

Abstract

Every day, the evaluation and optimization become parts of our life, while the technologies have already maximized, because the raw resources become lowly and world needs increase on. A maximized growing medium for bakery yeast multiplication really means an insurance of the bread manufacturing's future. Industrial medium supplementation with admixture stimulates the *Saccharomyces cerevisiae* growth when bakery yeast has to be produced. The stimulation is reflected by the generation time decrease and the biomass accumulation. The yeast produced on such medium present a higher fermentative capacity. The aim of this work was to optimization the growing medium used for the bakery yeast's obtaining on laboratory level using factorial design and response surface methodology. Applying these methods allows the simultaneous analysis the effect of two (molasses and wheat embryos) and, respectively three factors (complex fertilizer, ammonium sulphate and magnesium sulphate) on the biomass accumulation.

Keywords: bakery yeast, biomass accumulation, Response Surface Methodology, optimization

1. Introduction

Saccharomyces cerevisiae is used as compressed yeast or dried yeast in the baking industry as a biological and flavor enhancer in the manufacture of bread and bakery products. Saccharomyces cerevisiae strains selected for producing baker's yeast must possess the following properties: high viscosity multiplication capacity in viscous media with high osmotic pressure, superior maltase capacity, superior dough leaching ability, storage resistance, high consistency [1, 2].

For estimating the microelements necessary for yeast growth, the data presented in the literature on the nutritional needs of microorganisms can be used. Although environmental recipes are presented in the papers, use of these recipes must be done with caution, since industrial recipes are manufacturing secrets.

A very important aspect to be considered during the design and optimization process of the culture media is the technical and economic requirements, because the raw material price is 10 ÷ 60% of the production cost [3, 4].

The study of the dynamics of the cultivation process has shown that the initial conditions of growth are important. Mono and multifactorial kinetic dependencies have been established to describe the influence of the concentration of the basic components in the nutrient medium, the temperature, the pH of the culture medium, the mixing intensity and the concentration of unusable molasses components on the rate of yeast multiplication [5-7].

In order to optimize a culture medium, classical methods or more rigorous methodologies such as continuous culture techniques or statistical methods can be used. The classical method involves

Corresponding author: *e-mail:* adriana.dabija@fia.usv.ro,

changing a component of the environment, while preserving the other components at a certain level. Statistical methods are more rigorous, allowing for optimization of the environment for the accumulation of yeast biomass, the detection of interactions, and also the consideration of other process variables.

Response surface methodology (RSM) is one of the most opportune tools for studying the influence of various input variables simultaneously to an output response and the aim is to optimize the response [8]. RSM, a collection of statistical and mathematical techniques, has been increasingly used in biological, chemical process or for different phases of an optimization process [9]. By applying RSM, an experimental model could be obtained, which predict the correlation and interaction between the experimental variables used in the study and observed results, and finally gives optimized conditions [10].

In this study, response surface methodology (RSM) was used to determine the optimum value of growing medium components for maxim biomass accumulation and to better understand the interaction between molasses and wheat embryos, and between complex fertilizer, ammonium sulphate and magnesium sulphate and biomass accumulation production.

2. Materials and Methods

2.1. Materials

In the experiments a strain of *Saccharomyces cerevisiae* was used for the industrial production of bakery yeast. The basic medium subjected to optimization contained molasses as a source of carbon, complex fertilizer, ammonium sulphate and magnesium sulphate as sources of nitrogen, phosphorus and magnesium, wheat germ extract as a source of growth factors. The culture medium, after sterilization and cooling, was inoculated with 10^6 yeast cells / cm^3. Sample collection was performed at 24 hours since cultivation at 30^0C.

2.2. Methods

To determine the wet biomass accumulation in the fermented plaques, a centrifugation method was used at 6000 rpm. for 10 minutes. Determination of dry matter content of wet biomass was carried out gravimetrically by drying the sample to be analyzed at 105^0C for 3 hours until it reached constant mass.

2.3. Optimization of the culture medium components using factorial design and RSM

The medium culture components, molasses and wheat embryos, along with complex fertilizer, ammonium sulphate and magnesium sulphate were significant variables for *Saccharomyces cerevisiae* yeast biomass accumulation.

First the optimum content of molasses in the medium and wheat germ extract was determined to lead to the accumulation of a maximum amount of biomass. The experiment was conducted into two factor full factorial experiment, each factor at 3 levels, as follows: molasses, 20, 30 and 40 g/cm^3 and wheat embryos, 0.5, 1.0 and 1.5 g/cm^3. Molasses and wheat embryos were chosen as independent variables and the biomass accumulation was response of the design.

Then the optimization of the environmental components aimed to determine the optimal quantity of complex fertilizer, ammonium sulphate and magnesium sulphate. The experiment was conducted into three factor full factorial experiment. The 3 levels from each factor, as independent variables, complex fertilizer (6.8, 7.8 and 8.8 g/cm^3), ammonium sulphate (6.5, 7.5 and 8.5 g/cm^3) and magnesium sulphate (0.5, 0.6 and 0.7 g/cm^3) was used in this study, while the biomass accumulation was response variable.

Multiple linear regression analysis was applied to fit the experimental results obtained by full factorial design to linear, quadratic and cubic models. The sequential F-test, coefficients of determination (R^2), adjusted coefficients of determination (Adj-R^2) and significant probabilities were used to chosen the most accurate model for biomass accumulation which characterize the *Saccharomyces cerevisiae* growth. The coefficients of model were given as coded values of the independent variables.

The combined effect of the molasses and wheat embryos factors and respectively of complex fertilizer, ammonium sulphate and magnesium sulphate factors on the response, biomass accumulation was modelled using a polynomial response surface.

The full factorial designs was designed and made using the software State-Ease Design Expert 9.0 (trial version).

The optimization of growing medium variables simultaneously is very important to predict the yeast

biomass accumulation. The desirability function approach was used to optimize the multiple factors concurrently to achieve the desired response goal, maxim biomass accumulation [11].

3. Results and Discussions

The first time, the medium culture components, molasses and wheat embryos have been optimized for maxim biomass accumulation. Table 1 shows the experimental design matrix with coded and real values of factors used.

A full factorial design gave a total of 9 experiments which are required to assess the coefficients of the model using linear regression analysis.

The analysis of variance (ANOVA) was used to estimate the significance of model coefficients and the *p* values showed the significance of each coefficient and the interaction strength between the independent variables.

The model obtained in terms of coded factors was expressed by Eq.1.

$$\text{Biomass accumulation} = 5.54 - 0.18 \times \text{Molasses} - 0.81 \times \text{Wheat embryos} + 0.18 \times \text{Molasses} \times \text{Wheat embryos} \quad (1)$$

The results revealed that the two factor interaction (2FI) model was significant ($p < 0.05$) and the coefficient of determination (R^2) was shown as 0.7713, indicating that 77.13% of the variability of the response could be explained by the model and only 22.87% of the total variations were not explained by the model. The model shows that wheat embryos shows the significant effect ($p < 0.05$) on biomass accumulation, meanwhile molasses and interaction between molasses and wheat embryos (AB) showed non-significance effect at $p < 0.05$. However, interaction term AB had positive effect on biomass accumulation (Table 2).

Table 1. Experimental design matrix with coded and real values of factors used in full factorial design

Run	Coded value of factors		Real value of factors	
	A: Molasses	B: Wheat embryos	Molasses (g/cm^3)	Wheat embryos (g/cm^3)
1	-1.00	1.00	20.00	1.50
2	-1.00	0.00	20.00	1.00
3	0.00	0.00	30.00	1.00
4	0.00	-1.00	30.00	0.50
5	1.00	0.00	40.00	1.00
6	1.00	-1.00	40.00	0.50
7	1.00	1.00	40.00	1.50
8	0.00	1.00	30.00	1.50
9	-1.00	-1.00	20.00	0.50

Table 2. Analysis of variance (ANOVA) for the experimental result of the two factor interaction model for biomass accumulation

Source	Sum of Squares	DF	Mean Square	F Value	Prob > F	
Model	4.28	3	1.43	5.62	0.04	Significant
A: Molasses	0.21	1	0.21	0.81	0.40	
B: Wheat embryos	3.95	1	3.95	15.57	0.01	Significant
AB	0.12	1	0.12	0.48	0.51	
Residual	1.27	5	0.25			
Cor Total	5.55	8				
Std. Dev. = 0.50		Mean = 5.54		Adequate precision: 5.935		
$R^2 = 0.7713$		Adj. $R^2 = 0.63$				

The variation of biomass accumulation in function of the combined effect of molasses and wheat embryos concentrations is shows in Figure 1. It was obvious that increasing the molasses and wheat embryos concentrations leads to increase of yeast biomass accumulation.

The optimization of molasses concentration and wheat embryos concentration were made so as the

biomass accumulation to be maxim. Using numerical optimization it seems that molasses concentration of 20.00 g/cm^3 and wheat embryos concentration of 0.50 g/cm^3 are the optimum values to obtain the maximum yeast biomass accumulation (6.71 g/cm^3) at a desirability value of 0.859 (Figure 2).

Then, medium components complex fertilizer, ammonium sulphate and magnesium sulphate have been optimized for maxim biomass accumulation. Table 3 shows the full factorial design matrix and the levels of each independent variable. The twenty-seven experiments were resulted according to the experimental design.

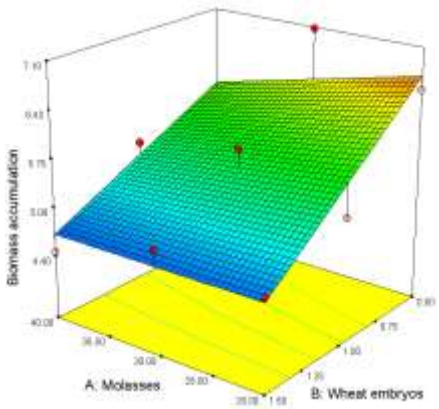


Figure 1. The response surface plots of biomass accumulation as influenced by the concentration of molasses and wheat embryos

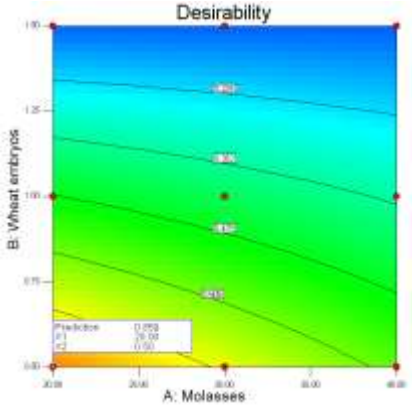


Figure 2. The contour plot of optimum values for molasses and wheat embryos concentrations

Table 3. Experimental design matrix with coded and real values of factors used in full factorial design

Run	Coded value of factors			Real value of factors		
	A: Complex fertilizer	B: Ammonium sulphate	C: Magnesium sulphate	Complex fertilizer (g/cm^3)	Ammonium sulphate (g/cm^3)	Magnesium sulphate (g/cm^3)
1	-1.00	-1.00	-1.00	6.80	6.50	0.50
2	0.00	-1.00	-1.00	8.80	6.50	0.50
3	1.00	-1.00	-1.00	10.80	6.50	0.50
4	-1.00	0.00	-1.00	6.80	7.50	0.50
5	0.00	0.00	-1.00	8.80	7.50	0.50
6	1.00	0.00	-1.00	10.80	7.50	0.50
7	-1.00	1.00	-1.00	6.80	8.50	0.50
8	0.00	1.00	-1.00	8.80	8.50	0.50
9	1.00	1.00	-1.00	10.80	8.50	0.50
10	-1.00	-1.00	0.00	6.80	6.50	0.60
11	0.00	-1.00	0.00	8.80	6.50	0.60
12	1.00	-1.00	0.00	10.80	6.50	0.60
13	-1.00	0.00	0.00	6.80	7.50	0.60
14	0.00	0.00	0.00	8.80	7.50	0.60
15	1.00	0.00	0.00	10.80	7.50	0.60
16	-1.00	1.00	0.00	6.80	8.50	0.60
17	0.00	1.00	0.00	8.80	8.50	0.60
18	1.00	1.00	0.00	10.80	8.50	0.60
19	-1.00	-1.00	1.00	6.80	6.50	0.70
20	0.00	-1.00	1.00	8.80	6.50	0.70
21	1.00	-1.00	1.00	10.80	6.50	0.70
22	-1.00	0.00	1.00	6.80	7.50	0.70
23	0.00	0.00	1.00	8.80	7.50	0.70
24	1.00	0.00	1.00	10.80	7.50	0.70
25	-1.00	1.00	1.00	6.80	8.50	0.70
26	0.00	1.00	1.00	8.80	8.50	0.70
27	1.00	1.00	1.00	10.80	8.50	0.70

Table 4. Analysis of variance (ANOVA) for the experimental result of the quadratic model for biomass accumulation

Source	Sum of Squares	DF	Mean Square	F Value	Prob > F	
Model	21.39	9	2.38	22.32	< 0.0001	Significant
A: Complex fertilizer	2.29	1	2.29	21.51	< 0.001	Significant
B: Ammonium sulphate	0.030	1	0.030	0.29	0.5999	
C: Magnesium sulphate	0.15	1	0.15	1.40	0.2524	
AB	7.01	1	7.01	65.82	< 0.0001	Significant
AC	0.49	1	0.49	4.62	0.0462	Significant
BC	0.19	1	0.19	1.74	0.2049	
A^2	11.06	1	11.06	103.91	< 0.0001	Significant
B^2	0.14	1	0.14	1.29	0.2723	
C^2	0.033	1	0.033	0.31	0.5835	
Residual	1.81	17	0.11			
Cor Total	23.20	26				
Std. Dev. = 0.33		Mean = 6.19		Adequate precision: 14.349		
$R^2 = 0.9220$		Adj. $R^2 = 0.88$				

The model in terms of coded variables and the biomass accumulation as the predicted response was given by Eq.2.

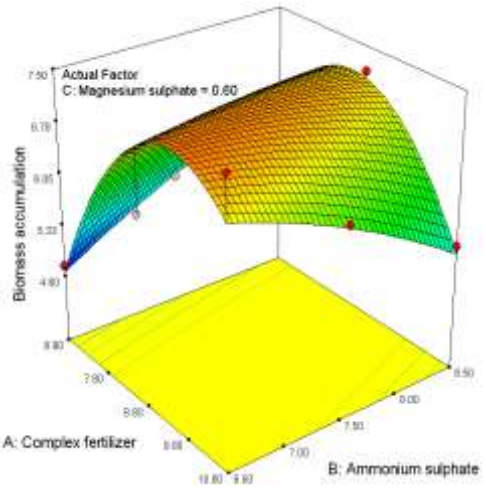
$$\text{Biomass accumulation} = 7.25 + 0.36 \times \text{Complex fertilizer} - 0.041 \times \text{x Ammonium sulphate} + 0.0091 \times \text{Magnesium sulphate} - 0.76 \times \text{x Complex fertilizer x Ammonium sulphate} - 0.20 \times \text{Complex fertilizer x x Magnesium sulphate} + 0.12 \times \text{Ammonium sulphate x Magnesium sulphate} - - 1.36 \times \text{Complex fertilizer}^2 - 0.15 \times \text{Ammonium sulphate}^2 - 0.074 \text{ Magnesium sulphate}^2 \quad (2)$$

The experiment are required to determine the combined effect of three independent variables, complex fertilizer, ammonium sulphate and magnesium sulphate on biomass accumulation at each experimental point.

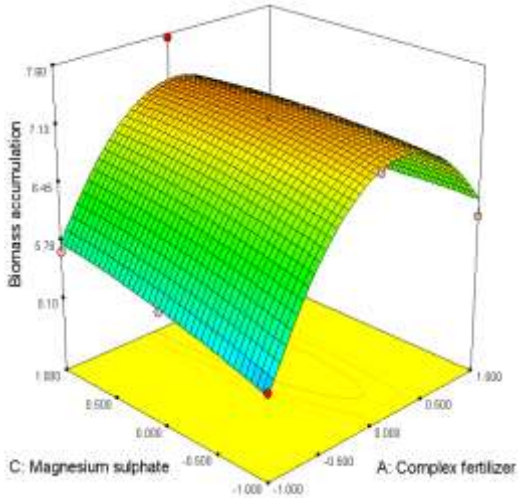
The ANOVA results of response surface quadratic model for biomass accumulation production are shows in Table 4. The model was highly significant ($p < 0.001$), with a high coefficients of determination (R^2 = 0.9220) which indicated that about 92% of the variability in the response could be explained by the model and only les than 8% of the total variations were not explained by the model.

The model revealed that complex fertilizer (A) term, the interaction term between complex fertilizer and ammonium sulphate (AB) and the interaction term between complex fertilizer and magnesium sulphate (AC) had a significant effect on biomass accumulation production (g/cm^3). Positive coefficient of A and C indicated a linear effect to increase the production of biomass accumulation, while the negative coefficient of B showed negative effect. The results indicated that all quadratic terms (A^2, B^2 and C^2) have negative effects on increases the biomass accumulation.

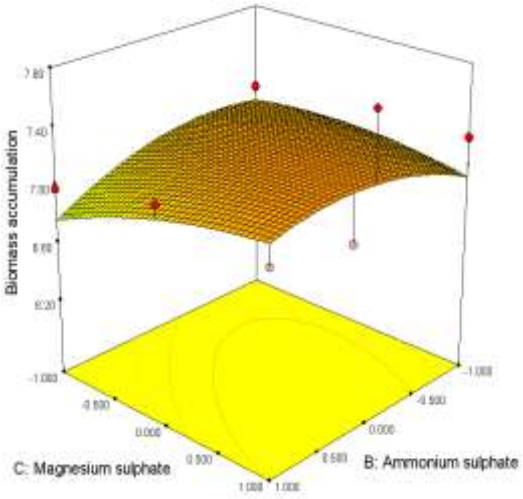
The combined effect of complex fertilizer, ammonium sulphate and magnesium sulphate on the biomass accumulation production is showed in Figure 3.



(a)



(b)



(c)

Figure 3. Response surface curve of biomass accumulation showing the interaction between (a) complex fertilizer and ammonium sulphate, (b) complex fertilizer and magnesium sulphate, (c) ammonium sulphate and magnesium sulphate

The optimization of complex fertilizer, ammonium sulphate and magnesium sulphate concentrations were made so as the biomass accumulation to be maxim.

Applying numerical optimization the result showed that complex fertilizer concentration of 9.75 g/cm^3, ammonium sulphate concentration of 6.50 g/cm^3 and magnesium sulphate concentration of 0.51 g/cm^3 are the optimum values to obtain the maximum biomass accumulation (7.42 g/cm^3) at a desirability value of 0.899 (Figure 4).

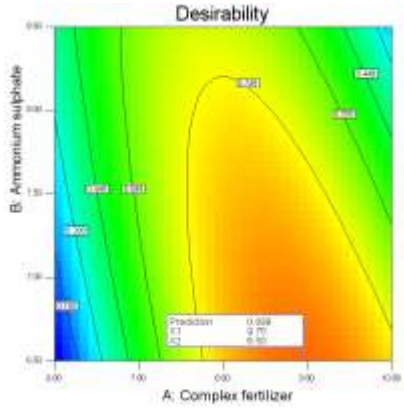


Figure 4. The contour plot of optimum values for fertilizer concentrations

4. Conclusions

The main purpose of the experiments was to study the growing conditions leading to a faster growth of *Saccharomyces cerevisiae* yeast by enriching the nutrient medium currently used to obtain yeast and by increasing fermentative capacity to adapt to existing conditions in the bakery biotechnology.

The optimization of the culture medium used for the production of *Saccharomyces cerevisiae* yeast under laboratory conditions has the role of checking and modifying, if necessary, of the nature and concentration of the components.

Maximum production of biomass accumulation was obtained under optimized growing medium components for obtaining *Saccharomyces cerevisiae* yeast. The optimized values for maxim biomass accumulation was molasses concentration 20.00 g/cm^3 and wheat embryos concentration 0.50 g/cm^3; complex fertilizer concentration 9.75 g/cm^3, ammonium sulphate concentration 6.50 g/cm^3 and magnesium sulphate concentration of 0.51 g/cm^3, respectively.

Compliance with Ethics Requirements. Authors declare that they respect the journal's ethics requirements. Authors declare that they have no conflict of interest and all procedures involving human / or animal subjects (if exist) respect the specific regulation and standards.

References

1. E. Johnson, Biotechnology of non-Saccharomyces yeasts – the ascomycetes, *Applied Microbiology and Biotechnology* **2013,** 97: 503-517
2. M. Rollini; E. Casiraghi; M.A.Pagani; M. Manzoni, Technological performances of commercial yeast strains (*Saccharomyces cerevisae*) in different complex dough formulations, *European Food Research and Technology* **2007,** 226: 19-24

3. A. Dabija, *Biotechnology for the industrial production of the bakery yeast having a high enzyme activity*, Doctorate thesis, University of Galati, 2000
4. A. Dabija, *Bakery yeast. Application – perspectives*, Tehnica-INFO Publishers, Chișinău, 2001
5. J. de Winde, et al., *Functional Genetics of Industrial Yeasts*, Springer-Verlag Berlin Heidelberg Punblishers, 2003, pp.57-85
6. S. Acourene, S.; A. Kh. Khalid, A. Kh.; Bacha, A.; Tama, M.; Taleb, B., Optimization of Bakery Yeast Production Cultivated on Musts of Dates, *Journal of Applied Sciences Research* **2007**, 3(10): 964-971
7. V. Giannone; C. Longo; A. Damigella; D. Raspagliesi; A. Spina; M. Palumbo, Technological properties of bakers' yeasts in durum wheat semolina dough, *Journal of Industrial Microbiology and Biotechnology* **2010,** 37: 371-379
8. D. Baș; I.H. Boyaci, Modeling and optimization I: Usability of response surface methodology. *Journal of Food Engineering* **2007**, 78: 836-845
9. H.H. Kim; J.G. Na; Y.K. Chang; G.T. Chun; S.J. Lee; Y.H. Jeong, Optimization of submerged culture conditions for mycelial growth and exopolysaccharides production by Agaricusblazei, *Journal of Microbiology and Biotechnology* **2004**, 14: 944-951
10. M. Arulkumar; P. Sathishkumar; T. Palvannan, Optimization of Orange G dye adsorption by activated carbon of Thespesiapopulnea pods using response methodology, *Journal of Hazardous Materials* **2001**, 186: 827- 834
11. D.C. Montgomery, *Design and analysis of experiments*, 6th ed. Hoboken: Wiley, 2005