Optimization of Glucose and Nitrogen Feeding Rates During *Saccharomyces cerevisiae* biomass Production Under Fed-Batch Fermentation from Grape Juice by Using The Response Surface Methodology

Sawsan Mahmood*(1), Ali Ali (1), Mohamad Ayhem Darwesh (2), Wissam Zam (3)

(1) Department of Food Technology, Faculty of Technical Engineering, Tartous University, Tartous, Syria.

(2) Faculty of Medical Engineering, Andalusia University, Tartous, Syria.

(3) Faculty of Pharmacy, AL- Wade University Homs, Syria.

(*Corresponding author: Eng. Sawsan Mahmood. E-mail address: sawsanmahmood480@gmail.com).

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Abstract:

This research was carried out in the fermentation technology laboratory in department of the Food Technology of the Faculty of Technical Engineering at Tartous University, in 2021.Optimization of fermentation processes for the production of yeast is very complex, and it is necessary to use a method to optimize the fermentation system and to operate the biological fermenter under optimal conditions in order to obtain the maximum possible amount of yeast (*Saccharomyces cerevisiae*). In this work, the Response Surface Methodology method (RSM) was used to aim optimization of the fed-batch fermentation system with the aim of achieving a higher production of yeast based on grape juice as a sole source of carbon compared with the production obtained from the batch fermentation system. The aim was to determine the optimum feeding rates for both substances glucose and nitrogen that are supplied to the fermentation medium through the fed- batch fermentation system so that the desired result is achieved, which is to obtain the maximum possible production of yeast *Saccharomyces cerevisiae*. The results demonstrated the success of using the surface response method (RSM) to determine optimal rates of glucose and nitrogen feeding during certain periods of nutrient fermentation where maximum biomass production was obtained from *Saccharomyces cerevisiae* (42.03 g /L) using glucose feed rate values of 0.0314 (L /h) and the nitrogen feed rate is 0.0274 (L /h), and the fermentation power of the yeast obtained was 480 ml.

Keywords: Response Surface Methodology, optimization, fermentation, Fed-batch fermentation, *Saccharomyces cerevisiae.*

Introduction:

Baker's yeast is a typical low value, high volume commercial product. Baker's yeast, in its final form, is mostly delivered as a solid block with about 25-29% dry weight, composed of living cells *Saccharomyces cerevisiae*, or as a dried powder (dry yeast) with about 95% dry weight (Richelle,2014).

Yeast constitutes an interesting group from a technical and industrial standpoint in the microbial world. Strains of *Saccharomyces cerevisiae*, among known genera and species of yeast, is used

commercially for baking, alcohol beverage production, foodstuffs, animal feeds and the production of biochemical (Patel, 2014).

Saccharomyces cerevisiae biomass, mainly in the form of baker's yeast, represents the largest bulk production of any single-cell microorganism in the world. Several million tons of fresh baker's yeast cells are produced for human food use (Walker, 1998). The most important requisites in the commercial production of baker's yeast are rapid growth and high biomass yield, coupled with good dough-leavening properly. These are achieved by the use of a well-established fed-batch fermentation method, with sequential stages differing in fermenter size and in the aeration and feeding conditions (Randez-Gil et al.; 1999). Biochemical processes produce high value end products like vitamins and antibiotics and bulk products such as ethanol and baker's yeast. The traditional and large scale beer and ethanol manufacturing processes based on using Saccharomyces cerevisiae (also called Baker's yeast). Novel applications of Saccharomyces cerevisiae have been developed during recent years. An example of its new application is insulin production for diabetics (Gil et al., 2009).

Molasses is the most used raw material in the production of Baker's yeast, and it may be sourced from sugar beet, or sugar cane, and it contains about 50-55% of fermentable sugars, and some vitamins and minerals that are important in cell proliferation, also any substance containing fermentable sugars can be used such as date and grape juices (Ghahremani *et al*., 2009).

In the last years, the price of molasses has increased because of their use in other industrial applications such as animal feeding or bioethanol production (Arshadm *et al*., 2018), thus rendering the evaluation of new substrates for yeast biomass propagation a trending topic for biomass producers' research. New assayed substrates include molasses mixtures with corn steep liquor (20:80), different agricultural waste products (Kopsahelis *et a*l., 2019), and other possibilities as date juice or agricultural waste sources, also called wood molasses that can be substrate only for yeast species capable of using xylose as a carbon source (Xandé *et al*., 2020).

In this research, the possibility of using grape juice to produce a good yield from the yeast was studied. Grape juice was chosen because it has a chemical composition similar to the chemical composition of molasses in terms of its good content of hexane-sugars and its richness with many important nutrients for the growth of yeast cells, in addition to the fact that grape cultivation is spread in various parts of the world, including Syria, which is one of the grape-producing countries.

During the last war period, Syria was exposed to difficult economic conditions and the suspension of the work of the only sugar factory in the country, and this was accompanied by the suspension of the yeast factory and the tendency to import yeast. So researchers went to study the possibility of an alternative or additional option for molasses that supports yeast production, and this is in line with the researchers' interest. In different parts of the world about studying the possibility of using available raw materials to support biotechnology industries and finding many options or alternatives that support any vital industry.

Syrian Arab Republic is the richest country in the Middle East in the cultivated varieties of grapes, and the number of varieties is about 100 varieties spread across the country where the most important varieties are spread, which are four varieties that represent 85 percent of the total grape production (Zaini 15%, Baladi 20% and Salti 20%, Heloani 30%), the main objective of the present work is to study the optimization of *Saccharomyces cerevisiae* biomass production, using grape juice as the only source of carbon, as grape juice is a good source of carbon and many important nutrients for the growth of yeast and it has a chemical composition close to the chemical composition of molasses (Alexeeva *et al*., 2002).

Saccharomyces cerevisiae like most other yeasts multiply by budding. Its production is carried out in a fed-batch mode in which a feed stream containing substrate and/or nutrients is fed into the fermenter during operation to gain higher performance levels, as compared to the batch mode. Numerous researches have been carried out by previous workers to develop suitable, fast and robust optimization techniques for these types of fermentation processes (Patidar *et al*., 2005).

The traditional technique used for optimizing a multivariable fermentation process is difficult and does not take the alternative effects between components into consideration (Rajendhran *et al*., 2002; Reed and Nagodawithana, 2011). Recently, many statistical experimental design methods have been employed in bioprocess optimization (Makhoul *et al*., 2018; Sayyad *et al*., 2007).

Among these, the central composite experimental design (CCD) is the most suitable for identifying the individual variables to optimize a multivariable system (Kennedy and Krouse, 1999; Chakravarti and Sahai, 2002). This method was used to optimize many fermentation process, such as acids, antibiotics, enzymes, and biomass and ethanol production by several micro-organisms types (Kar *et al*., 2002; Boudjema *et al*., 2015). Furthermore, it was used in the design, analysis, and in unit operations. The advantages of this method are the reduction of the number of experiments, reagents, time, financial input and energy (Montgomery, 2009).

Material and methods:

Commercial materials:

All materials used in these experiments are HIMEDA Company. Glucose and vitamin solutions were sterilized by filtration and added to the autoclaved medium.

Origin and Reactivation of the yeast *Saccharomyces cerevisiae*:

Dried powder yeast form of *Saccharomyces cerevisiae* (ATCC20408/S288c) used in this study has a commercial origin in fact, it is produced by the Biometric-The Biostability Company.

The yeast was reactivated on agar plates containing YPGA medium composed of yeast extract 10 g/L, peptone 10 g/L, glucose 20 g/L, agar 20 g/L with a pH 6, incubated at $30C^0$ for 24 h (Burrows, 1979).

Preparation of grape juice:

The Baladi grape (figure 1) was chosen and it is one of the varieties available in Syria. Its production reaches 20% of the grape production. It is a local variety that is distinguished by the size of its large clusters and has a single conical shape, and the grains are spherical in shape, with a large size, with a yellowish-white color, and a thin crust in a light pink color. The pulp is flaky, has a good taste, and has a distinctive flavor, one of the late ripening varieties, and it is one of the famous and luxurious table varieties, suitable for remote transportation and long winter storage.

Fig1. Baladi grape

The grape is obtained from local markets. The grape berries were removed from their clusters and cleaned and washed with warm water. The juice was extracted by breaking and pressing in doubly folded cloth, then the juice was pasteurized at $85c^0$ for 3 minutes.

Preparation of Culture Medium Based on grape juice and Inoculums:

The method cited by Kocher and Uppal (Kocher and Uppal, 2013) was used with minor modifications. The obtained grape juice from the above preparation was supplemented by mineral salts: magnesium sulfate 0.44 g, urea 12.70 g, and ammonium sulfate 5.30 g. Finally, the medium was distributed in an Erlenmeyer of 250 mL with a ratio of 100 mL per flask and sterilized at 120 c^0 for 20 min. The pre-culture was obtained by inoculating two colonies of the yeast *Saccharomyces cerevisiae* in 250 mL shake flasks containing 100 mL of grape juice, mentioned above. The pre-culture was incubated at 30 °C for 3 h, and used further as inoculums for the yeast biomass production.

Fed-batch fermentation:

The fermentation was carried out within a biological fermenter with a capacity of 6 liters with an engineering design that fits with the requirements of the fermentation processes, using an initial volume of the fermentation medium used, which is grape juice, about 3 liters. The initial conditions for the fermentation process were: temperature (30.11°C), pH (4.75), sugar concentration (158.36 g/L), the ratio of carbon to nitrogen (11.9), initial concentration of yeasts (2.5 g/L), stirrer speed (630 r.p.m), air flow (020 min/L), for a period 12 h of fermentation (Reyman, 1992).

Solutions of glucose and nitrogen are added during fermentation at certain periods of time, according to the results of using the experimental design method by the central composite experimental design (CCD) using a Minitab 19 Statistical Software (Minitab, Inc., State College, PA, USA).

The temperature of the fermentation medium is set at the required degree by using the cooling and heating coils in the biological fermenter. The pH is also adjusted by pumping appropriate quantities of 10 %(w/v) NaoH and 10% (v/v) H_2So_4 as needed into the fermentation medium.

Statistical Design of Experiments:

Factor Selection and Organization of Experiments:

The organization of the experiments was carried out using the experimental design obtained by the central composite experimental design (CCD). Two independent variables were selected (glucose feed rateF_G, nitrogen feed rateF_N). Table 1 shows the lower and upper level of studied variables.

Table (1): The lower and upper level of studied variables

variables	Lower level (-1)	Upper level (+1)
$\boldsymbol{F_G}$= Glucose feed rate (L/h)	0.01	0.05
$\boldsymbol{F_N}$=**Nitrogen feed rate** (L/h)	0.01	0.05

The design summary for factors is given in Table 2, and the Point of types are given in Table 3.

The central composite experimental design (CCD) matrix of different variables (coded levels). The CCD matrix employed for two independent variables is given in Table 4. Each column represents the different variables (factors) and each line represents the different experiments (14).

Table (2): Design Summary

Factors:	2	Replicates:	1
Base runs:	14	Total runs:	14
Base blocks:	2	Total blocks:	2

α = 1, 41421, Two-level factorial: Full factorial.

Table(3): Point Types

Cube points:	4
Center points in cube:	3
Axial points:	4
Center points in axial:	3

Table (4): The central composite experimental design matrix for different variables (coded levels)

Run	A (F_G)	B (F_N)
1	-1.00000	1.00000
2	0.00000	0.00000
3	0.00000	0.00000
4	-1.00000	-1.00000
5	1.00000	1.00000
6	0.00000	0.00000
7	1.00000	-1.00000
8	0.00000	0.00000
9	-1.41421	0.00000
10	0.00000	0.00000
11	0.00000	-1.41421
12	1.41421	0.00000
13	0.00000	1.41421
14	0.00000	0.00000

The CCD matrix is composed of a complete factorial design, four axial points and four cup points of design variable at a distance of α = 1.41421 and two-level factorial. The CCD matrix is composed of a complete factorial design, 2^5; four axial points on the axis of each design variable at a distance of α = 1.41421 from the design center and three points at the axial center.

The actual experimental values corresponding to the coded levels used for the creation of the experiment matrix are presented below (Table 5).

Table (5): Actual values for the three independent variables

Experiments	Actual values	
	F_G	F_N
01	0.0100000	0.0500000
02	0.0300000	0.0300000
03	0.0300000	0.0300000
04	0.0100000	0.0100000
05	0.0500000	0.0500000
06	0.0300000	0.0300000
07	0.0500000	0.0100000
08	0.0300000	0.0300000
09	0.0017157	0.0300000
10	0.0300000	0.0300000
11	0.0300000	0.0017157
12	0.0582843	0.0300000
13	0.0300000	0.0582843
14	0.0300000	0.0300000

Effect Estimation:

The real values F have been calculated according to Equation [1].

$$F_{IN} = \frac{F - F_O}{\triangle F} \quad [1]$$

Where F, is the coded value for the independent variable, F, is the natural value, x_0, is the natural value at the center point and ΔF, is the step change value (the half of the interval (−1 +1)). The mathematical model describing the relation between dependent and independent variables for this process has the quadratic form for the experimental design used:

Regression Equation in uncoded units:

$$Yi = \beta_0 + \beta_1 A + \beta_2 B + \beta_{11} A^2 + \beta_{22} B^2 + \beta_{12} AB \quad [2]$$

Yi, is the predicted response (in our case, the Biomass production (g/L)). The calculation of the effect of each variable and the establishment of a correlation between the response Yi and the variables, were performed using a Minitab 19 Statistical Software (Minitab, Inc., State College, PA, USA).

Statistical Analysis:

The statistical analysis was performed using analysis of variance (ANOVA), in order to validate the square model regression. It included the following parameters: Student test (t) and p-value. In our study, the statistical significance test level was set at 5% (probability (p) < 0.05).

Validation of Biomass Production in Optimum glucose and nitrogen feed rates using fed-batch fermentation system:

In order to confirm the optimized conditions obtained by the central composite design, an Experiment was carried out on 250 mL shake flasks. To do this, 100 mL of grape juice was seeded with 11 mL of the yeast pre-culture and the pH of the medium was adjusted to 4.75. Shake flasks were sterilized at 120 °C for 20 min, incubated at 30 °C, values of glucose feed rate 0.0314 (L/h) and nitrogen feed rate 0.0274 (L/h), for 12 h fermentation (Reyman, 1992).

Determination of Biomass Concentration:

The measurement of biomass was followed by estimation of cell dry weight, expressed in g/L. One ml of yeast culture was centrifuged at 5000 rpm for 5 min. The supernatant obtained was washed twice with water and dried by incubation at 105 °C until at a constant weight (Jiménez-Islas *et al*., 2014).

Determine the fermentation power of the obtained yeast:

6.75 g of the sugar-phosphate mixture was mixed with 75 ml of calcium sulfate solution in the beaker. Then add 0.893 g of dry baker's yeast. Stir well to disperse the yeast. Then the fermentation power was measured using fermentometer (RHEO FERMENTOMETER F4) (COFALEC**,** 2012).

Results and discussion:

In the present study (glucose feed rate F_G, nitrogen feed rate F_N) were supposed to optimize the biomass production of *Saccharomyces cerevisiae* using the central composite experimental design. The biomass concentration over 12h of fermentation varied with the change in glucose feed rate F_G, nitrogen feed rate F_N. (Table 6).

Table (6): The central composite design for biomass production

Experiments	Actual values		(Yi): Biomass (g/L)	
	F_G	F_N	**experimental Value**	**Predicted Value**
01	0.0100000	0.0500000	39.0	38.5895
02	0.0300000	0.0300000	42.0	42.3857
03	0.0300000	0.0300000	42.0	42.3857
04	0.0100000	0.0100000	41.0	41.0302
05	0.0500000	0.0500000	41.5	40.8913
06	0.0300000	0.0300000	42.0	42.3857
07	0.0500000	0.0100000	40.0	39.8319
08	0.0300000	0.0300000	42.0	41.6143

09	0.0017157	0.0300000	38.7	38.8491
10	0.0300000	0.0300000	42.0	41.6143
11	0.0300000	0.0017157	39.9	39.8777
12	0.0582843	0.0300000	39.2	39.6295
13	0.0300000	0.0582843	38.3	38.9009
14	0.0300000	0.0300000	42.0	41.6143

Using the results obtained in diverse experiments, the correlation gives the influence of glucose feed rate, and nitrogen feed rate on the response. This correlation is obtained by Minitab 19 software and expressed by the following second order polynomial (Equation [3])

Regression Equation in Uncoded Units:

Y = 38.898+ 126.3 FG+ 84.0 FN- 2969 FG*FG- 2781 FN*FN+ 2188 FG*FN [3]

Table 7 shows the coefficient regression corresponding to t and p-values for all the linear, quadratic and interaction effects of the parameters tested. A positive sign in the t-value indicates a synergistic effect, while a negative sign represents an antagonistic effect of the parameters on the biomass concentration (Leman *et al*., 2010).

Table (7): Estimated regression coefficients to t and p-values of the model

Term	Coef	SE Coef	T-Value	P-Value	VIF
Constant	42.000	0.220	191.08	0.000	
1	0.386	0.144	2.68	0.032	1.00
FG	0.276	0.190	1.45	0.191	1.00
FN	-0.345	0.190	-1.81	0.113	1.00
FG*FG	-1.187	0.198	-5.99	0.001	1.01
FN*FN	-1.113	0.198	-5.62	0.001	1.01
FG*FN	0.875	0.269	3.25	0.014	1.00

Model Summary:

S	R-sq	R-sq(adj)	R-sq(pred)
0.538402	92.46%	85.99%	48.43%

S: represents the standard deviation of the distance between the data values and the fitted values, the lower the value of S, the better the model describes the response.

R-sq (R^2): is the percentage of variation in the response that is explained by the model, the higher the R^2 value, the better the model fits your data. R^2 is always between 0% and 100%.

R-sq (adj): Adjusted R2 is the percentage of the variation in the response that is explained by the model.

R-sq (pred): Predicted R^2 is calculated with a formula that is equivalent to systematically removing each observation from the data set, estimating the regression equation, and determining how well the model predicts the removed observation. The value of the predicted R^2 ranges between 0% and 100%. By referring to the values obtained in the current study for these parameters, we find that the current study model is acceptable.

The examination of Table 7 shows that all coefficient regression of the quadratic terms are statistically significant $p \leq 0.05$ and negatively affect the biomass production.

In contrast the interaction terms (F_G, F_N) are statistically not significant $p > 0.005$, and the interaction terms (F_G*F_G, F_N*F_N, F_G*F_N) are significant with $p < 0.05$ and have a synergistic effect on the response Figure 2.

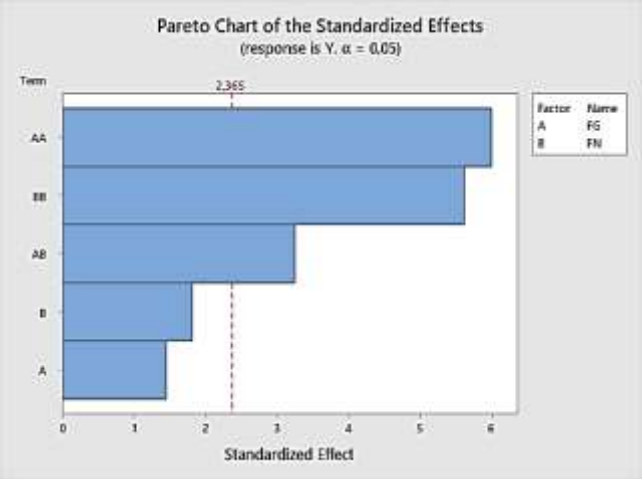


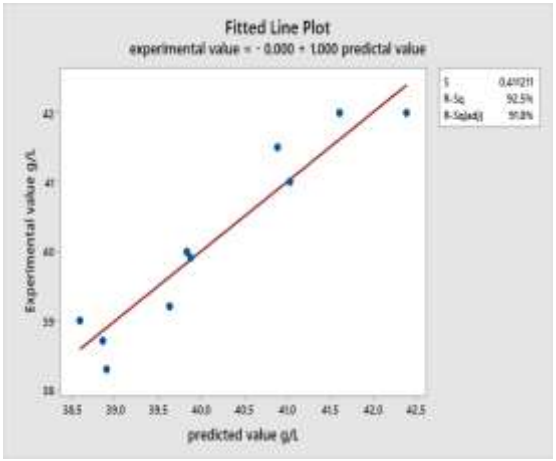
Fig (2): Variable effect signification on a biomass production

The analysis of variance (ANOVA) of the coefficient regression for the cell growth production (Table 8) demonstrates that the model is significant due to the F-value of 14.30 and the low probability p value ($p = 0.001$).

Generally, the F-value with a low probability p-value indicates a high significance of the regression model (Rene *et al*., 2007). Moreover, the coefficient of determination (R^2) measures the fit between the model and experimental data. Figure 3 was also determined to evaluate the regression model. In this study, the obtained value of R^2 is 0.925 approximate to 1, which justifies an excellent consistency of the model (Annuar *et al*., 2008). On the other hand, the obtained R^2 implies that 92.5% of the sample variation in the cell growth is attributed to the independent variables. This value indicates also that only 7.5 % of the variation is not explained by the mode.

Table(8): Analysis of variance (ANOVA)

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Model	6	24.8680	4.1447	14.30	0,001
Blocks	1	2.0829	2.0829	7.19	0,032
Linear	2	1.5630	0.7815	2.70	0,135
FG	1	0.6089	0.6089	2.10	0,191
FN	1	0.9541	0.9541	3.29	0,113
Square	2	18.1596	9.0798	31.32	0,000
FG*FG	1	10.4135	10.4135	35.92	0,001
FN*FN	1	9.1396	9.1396	31.53	0,001
2-Way Interaction	1	3.0625	3.0625	10.56	0.014
FG*FN	1	3.0625	3.0625	10.56	0.014
Error	7	2.0291	0.2899		
Lack-of-Fit	3	2.0291	0.6764	*	*
Pure Error	4	0.0000	0.0000		
Total	13	26.8971			



Fig(3): The fit between the model and experimental data of cell growth

The optimization of the response Yi (Biomass production) and the prediction of the optimum levels of (glucose feed rate F_G, nitrogen feed rateF_N) were obtained. This optimization resulted in surface plots (Figure 4) and an isoresponse contour plot (Figure 5).

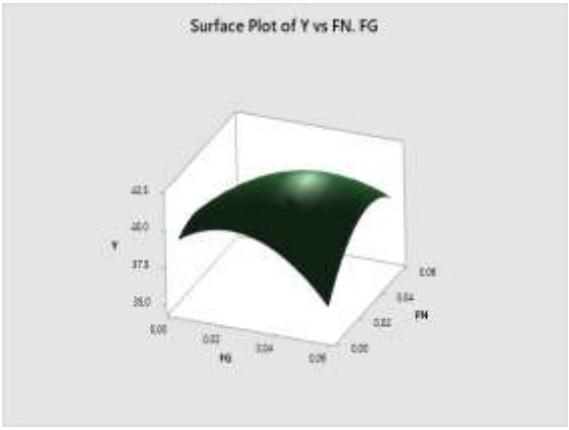
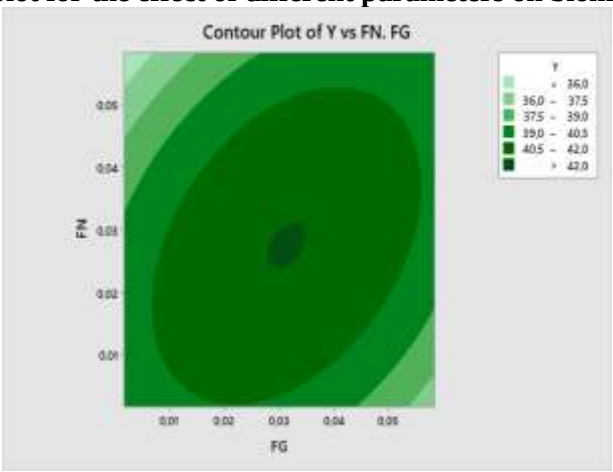


Fig4. Surface plot for the effect of different parameters on biomass production



Fig(5): Isoresponse contour plot for the effect of the studied variables on biomass production

These figures show that there is an optimum, located at the center of the field of study.

In addition, the use of the Minitab optimizer will give exact values of the optimum operating conditions of the process figure (6).

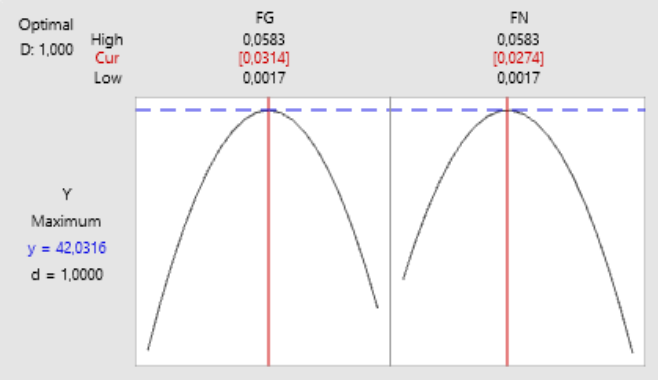


Fig (6): Values of optimal conditions on biomass production

Figure 6 shows the maximum biomass production by *Saccharomyces cerevisiae* (42.03g/L) corresponding to values of glucose feed rate 0.0314 (L/h) and nitrogen feed rate 0.0274 (L/h), and when applying the Experiment using the previous fermentation conditions and the optimum feed rates resulting from the (RMS) method used, the resulting yeast volume was 41.3g /L, it was found two results are almost identical which confirms the validity of Biomass Production in Optimum glucose and nitrogen feeding rates using fed-batch fermentation system , and it is higher than the amount of yeast obtained when applying the same conditions of fermentation, but according to the batch fermentation system (35g /L), which corresponds to many researches and interests that direct efforts and support for optimization of the fed-batch fermentation process, as it gives a higher yield than batch fermentation.

Also by comparing the amount of yeast resulting from the practical experience according to the optimal conditions obtained, which equal to (41.3 g / L) with the amount is obtained when applying practical Experiments according to the (Richelle *et al*., 2014) , which amounted to (32 g /L), we conclude that the result of our study is better, and this indicates the success of using Response Surface Methodology (RSM) method in optimization of a fed-batch fermentation process to produce biomass of baker`s yeast based on grape juice.

Also, by comparing the amount of yeast obtained in this study depending on the grape juice, it was found the same results by (Nancib *et al*., 1997), where the production of biomass from baker's yeast *Saccharomyces cerevisiae* on a medium containing date byproducts was 40 g/L. (Khan *et al*., 2017) used six different strains of *Saccharomyces cerevisiae* in fermentation medium containing date extract (with 60% sugar), In addition to 2 g/L ammonium sulfate and 50 mg/L biotin. Their results showed that the theoretical yields were about 42.8%. In addition, (Alobaidi *et al*., 1986) studied two substrates, date syrup and molasses for the propagation of baker's yeast strain *Saccharomyces cerevisiae* on a pilot plant scale. The results showed that higher productivity of baker's yeast was observed when date extract was used. Other results were obtained in several studies using an alternative substrate of fermentation. In fact, the optimal biomass production (6.3 g/L) was depicted at 24 h using *Saccharomyces cerevisiae* DIV13-Z087C0VS on a medium containing sweet cheese as a sole carbon source (Boudjema *et al*., 2015).

On the other hand, the production of baker's yeast from apple pomace gives a yield of 0.48 g/g (Bhushan and Joshi, 2006).

Therefore, it was concluded from these studies that the medium containing the grape juice as a sole carbon source is an excellent fermentation medium for baker's yeast production.

The measured fermentation power of the yeast obtained in this study from grape juice was 480 ml, so this is considered to have good fermentation capacity and is suitable for industrial use. The acceptable fermentation strength of yeast is not less than 350 ml according to the (COFALEC,2012): General characteristics of dry baker's yeast.

Finally, what distinguishes this study from previous studies is the dependence on grape juice as a source of carbon with the aim of producing biomass from dry yeast, which researchers had not previously studied.

This study will present an additional successful option for the production of yeast that commonly uses molasses. The improvement of the initial conditions of fermentation also contributed to the highest possible yield of yeast and good economic value. The fermentation power of the yeast was also good, so this study can be practically applied with the aim of producing a good mass of baker's yeast and using this yeast in various industrial and food fields.

Conclusions:

Microbial fermentation is complex and it is quite difficult to understand its complete details process. The results show that the feeding rates for both glucose and nitrogen can be determined during fed-batch fermentation at optimum values using Response Surface Methodology (RSM) method and to achieve the highest possible yield of baker`s yeast, and show that the medium containing the grape juice as a sole carbon source is an excellent fermentation medium for baker's yeast production.

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أمثلة معدلات التغذية بالغلوكوز والنيتروجين خلال إنتاج كتلة حيوية من سكارومايسس سيرفيسيا تحت التخمر شبه المتقطع من عصير العنب باستخدام منهجية سطح الاستجابة

سوسن محمود* [1] وعلي علي [1] ومحمد أيهم درويش[2] ووسام زم[3]

(1) قسم هندسة تقانة الأغذية، كلية الهندسة التقنية، جامعة طرطوس، طرطوس، سورية.

(2) كلية الهندسة الطبية، جامعة الأندلس، طرطوس، سورية.

(3) كلية الصيدلة، حمص، سورية.

(* للمراسلة: م. سوسن محمود، البريد الإلكتروني: sawsanmahmood480@gmail.com)

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الملخص:

نفذ هذا البحث في مخبر تكنولوجيا التخمير في قسم هندسة تقانة الأغذية بكلية الهندسة التقنية بجامعة طرطوس في عام 2021. تعد أمثلة عمليات التخمير لإنتاج الخميرة أمراً معقداً للغاية، ومن الضروري استخدام طريقة لتحسين نظام التخمير وتشغيل المخمر الحيوي في ظل الظروف المثلى من أجل الحصول على أكبر قدر ممكن من الخميرة(*Saccharomyces cerevisiae*) . في هذا العمل، تم استخدام طريقة منهجية سطح الاستجابة (RSM) لتحسين نظام التخمير شبه المتقطع بهدف تحقيق أعلى إنتاج من الخميرة باستخدام عصير العنب كمصدر وحيد للكربون مقارنة بالإنتاج الذي يتم الحصول عليه من نظام التخمير المتقطع. كان الهدف هو تحديد معدلات التغذية المثلى لكل من مواد الغلوكوز والنيتروجين التي يتم توفيرها لوسط التخمير خلال نظام التخمير على دفعات بحيث يتم تحقيق النتيجة المطلوبة وهي الحصول على أقصى إنتاج ممكن من خميرة *Saccharomyces cerevisiae*. أظهرت النتائج نجاح استخدام طريقة الاستجابة السطحية (RSM) لتحديد المعدلات المثلى للتغذية بالغلوكوز والنيتروجين خلال فترات معينة من التخمير حيث تم الحصول على أقصى إنتاج للكتلة الحيوية من خميرة *Saccharomyces cerevisiae* (42.03 غرام/ لتر) باستخدام قيم

معدل تغذية الغلوكوز 0.0314 (لتر/ ساعة) ومعدل تغذية النيتروجين 0.0274 (لتر/ ساعة)، وكانت قوة التخمير للخميرة التي تم الحصول عليها 480 مل.

الكلمات المفتاحية: منهجية سطح الاستجابة، الأمثلة، التخمير، التخمير شبه المتقطع، سكارومايسس سيرفيسيا.