Chapter

Optimization of Baker's Yeast Production on Grape Juice Using Response Surface Methodology

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Abstract

The purpose of this study is to complete as an example the fermentation conditions allowing the production of *Saccharomyces cerevisiae* yeast biomass in large quantities using the juice as the same carbon source. Determination of the best of five factors affects the production of dry biomass by baker's yeast. The optimal value of the five factors affecting the process of biomass production by the baker's sourdough was determined. The experimental design was performed using CCD (Central Composite Experimental Design), and the response surface methodology method was used to determine the best possible amount of production of yeast and has reached (41.44 g/L) after 12 hours of fermentation, under the following optimal conditions (temperature (30.11°C), pH (4.75), sugar concentration (158.36 g/L), the ratio of carbon to nitrogen (an essential nutrient for yeast growth) that is (11.9), and initial concentration of yeasts (2.5 g/L). Three kinematic models (Monod, Verhulst, and Tessier) were also selected for the purpose of studying the kinetic performance of *S. cerevisiae* yeast, and the best results were obtained based on the Verhulst model. The Leudeking Piret model has also been successfully used to estimate substrate during fermentation.

Keywords: *Saccharomyces cerevisiae*, response surface methodology, kinetic models, assumption, statistics

1. Introduction

Fermentation is one of the oldest methods used by humans since ancient times to preserve food and improve its organoleptic properties. More than 5000 fermented foods and beverages are produced worldwide, from alcohol, beer, and vinegar to cheese, yogurt, sourdough bread, olives, sausages, kimchi, and soybean paste [1].

Fermentation is simply the biochemical transformation of raw materials which is supported by the synthesis and stabilization of bacteria which convert sugars into simple acids, alcohols, and carbon dioxide to improve the flavor, texture, and aroma of processing and extend the shelf life of fermented products. Goods. During fermentation, many secondary metabolites including vitamins, antioxidants, and bioactive compounds are formed by the microbial community, contributing to the nutritional and nutraceutical value of the final product [2].

There has also been a rapid and significant development in fermentation technologies in recent years after understanding the bio-physiology of microorganisms and controlling it. Among this biology is the yeast, which has received more attention after recent developments in understanding its physiology [3].

Yeasts are micro-organisms, single-celled, unicellular eukaryotes. Their shapes and structure differ from one species to another. They are spherical or oval in shape and their dimensions range between 5 and 30 μm in length and 3–10 m in width. The yeast multiplies quickly and grows well in the contained environment. On sugars where they multiply by budding or by division [4, 5]. Yeasts play vital roles in food biotechnology, especially in fermented products [6].

S. cerevisiae yeast is the most important type of yeast due to its use in many industrial fields. It is used in the production of food, bread, pastries, ethyl alcohol, beer, wine, and as well as in the production of single-cell protein and a number of medicinal foods [7, 8].

S. cerevisiae yeast is considered to be the most important product of biotechnology due to its widespread use in the industrial field [9].

S. cerevisiae biomass is produced by using bioreactors that contribute to controlling growth conditions and the production is carried out according to batch or fed-batch fermentation system [10].

Baker's yeast industrially relies on a variety of disciplines, including variations of different generations, times and stages of aeration, differentiation of bioreactors, and control of the final stage of cultivation [11]. It is an aerobic process based on the expansion of cells from pure culture to larger bioreactors by increasing the volume at each stage of expression in the sugar medium [12].

Commercial bread yeast comes in three forms: Pressed yeast that is sold in the form of pressed briquettes or cubes wrapped with wax paper or cellophane, and its shelf life does not exceed one week from the date of its production due to the speed of its corruption. Active dry yeast is sold in airtight containers and needs to be reactivated before use, its cells are about 8–10% moisture and its shelf life ranges from six months to a year depending on the storage temperature. The instant dry yeast contains 4–5% moisture and its shelf life reaches more than a year and is added to the dough directly without the need for revitalization [13].

The global yeast market is estimated to be valued at USD 3.9 billion in 2020 and is projected to reach USD 6.1 billion by 2025 [14].

Molasses is the most used raw material in the production of Baker's yeast, it may be sourced from sugar beet or sugar cane, and contains about 50–55% of fermentable sugars, some vitamins and minerals that are important in cell proliferation, also any substance containing fermentable sugars can be used such as the date and grape juices [15].

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 [16], 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 [17], and other possibilities such 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 [18].

In this research, the possibility of using grape juice to produce a good yield from the yeast was studied in this study. 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, the 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 studying the possibility of using available raw materials to support biotechnology industries and finding many options or alternatives that support any vital industry. The 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%, Salti 20%, and Heloani 30%). The main objective of the present work is to study the optimization of *S. 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 [19].

The efforts of many researchers are directed toward improving various biological manufacturing processes [20], including fermentation processes, with the aim to determine the best conditions for the production of the required product, as well as with the aim to solve the problems that may face the required manufacturing process, reaching the highest possible production of the final product and reducing the costs of the manufacturing process as possible [21, 22].

Several statistical experimental design methods have been used to optimize biological processes [23, 24].

These methods, including the central composite experimental design (CCD), are characterized by reducing the number of experiments required, reducing financial and energy costs, reducing the time required, as well as reducing the reagents and materials required during work [25, 26].

The central composite experimental design (CCD) is one of the methods that contributed to the improvement of a number of biological processes such as the production of antibiotics, enzymes, organic acids, and ethanol [27, 28].

The study was conducted by selecting the best for five measurements (temperature, initial pH, sugar content in the juice, carbon to nitrogen ratio, and primary yeast) in order to get high yields of yeast using optimization with the surface response methodology method, we use grape juice as carbon source for cell growth and produce *S. cerevisiae* at high performance, and finally predict the biomass production process with three kinetic models.

2. Material and methods

2.1 Origin and reactivation of the yeast *S. cerevisiae*

The yeast used in this study is a commercial yeast from the sigma company, it is a dry powder form of *S. cerevisiae* (ATCC20408/S288c). This yeast needs to be reactivated before use with a suitable nutrient medium Yeast Peptone Glucose Agar

(YPGA) consisting of 20 g/L agar, 10 g/L yeast extract, 10 g/L glucose, 10 g/L peptones with a pH 6, with incubated at 30°C for 24 h.

2.2 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, 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.

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 a doubly folded cloth, then the juice was pasteurized at 85°C for 3 minutes.

2.3 Preparation of culture medium based on grape juice and inoculums

The juice resulting from the above preparation was supplemented with mineral salts: 0.44 g of magnesium sulphate, 12.70 g of urea, and 5.30 g of ammonium sulphate. Finally, the medium was placed in 250 mL deltas at a volume of 100 mL per well and sterilized at 120° C for 20 min. The preculture was obtained by inoculating two colonies of *Saccharomyces cerevisiae* yeast in 250 mL flasks containing 100 mL of juice as mentioned above. Pre-cultures were incubated at 30°C for 3 h and then used as inoculum for potassium biomass production [29].

Figure 1.
The Baladi grape.

2.4 Statistical design of experiments

2.4.1 Factor selection and organization of experiments

Five independent variables were selected (temperature, initial pH, concentration of sugars in grape juice, the ratio of carbon to nitrogen, and initial concentration of yeasts).

In a previous study, carried out by Naser and Abdelrahman [30], with the aim of determining the optimal conditions for producing baker's yeast using sugar cane molasses and achieving the best yield and lowest production cost, the best results were obtained when using the concentration of sugars within the range (14–18) %, Yeast inoculum level 2 to 3 g/L, agitation speed between 150 r.p.m. and 200 r.p.m., adding (40–50) % urea and ammonium sulfate at pH = 5.

In another study by Muhammad et al. [31], the baker's yeast production process was improved and the effect of various physical and chemical factors on the production of yeast cells was evaluated. The optimal conditions were determined to obtain the maximum possible growth of yeast cells at a concentration of sugars equal to 100 g/L, the agitation speed at 150 r.p.m., at pH = 4.5, and T = 28°C.

Optimization of baker's yeast production using date juice as the sole carbon source using the response surface methodology method has been studied by Ali et al. [32] and the study showed the success of using date juice in obtaining a good yield of the yeast biomass at the initial conditions of the fermentation process (sugar concentration 70.93 g/L, temperature 32.9°C and pH 5.35).

A study carried out by Taleb et al. [33] showed that the use of ammonium sulfate and urea as a source of nitrogen during the production of break's yeast by (50–50) % contributed to improving production yield by more than 36%, and adding thiamine vitamin at a concentration of 0.6 had a positive role in improving production by more than 6%.

A study by Sokchea et al. [34] indicated that the best amount of biomass for yeast is obtained when the ratio between the concentration of glucose and nitrogen (C/N) used during the fermentation process is equal to 10.

After reviewing previous studies, the lower and upper levels of studied variables were selected, **Table 1** shows the lower and upper levels of studied variables.

A program Minitab 19 software was used to optimize the Baker's yeast production The CCD matrix is composed of a complete factorial design, 32 cube points, eight center points in a cube, 10 axial points, and four center points in axial design variable at a distance of $\alpha = 2.366$ and two-level factorial. Each experiment was carried out twice and the average value is used.

variables	Lower level (−1)	Upper level (+1)
X1 = Temperature (°C)	25	35
X2 = Initial pH	3	6
X3 = Concentration of sugars (g/L)	100	200
X4 = The ratio of carbon to nitrogen	8:1	15:1
X5 = Initial concentration of yeasts (g/L)	2	3

Table 1.
The lower and upper levels of studied variables.

2.4.2 Effect estimation

The real values X have been calculated according to Eq. (1).

$$X = \left(x - x_{\circ}\right) / \triangle x \tag{1}$$

Where, X is the coded value for the independent variable, x, is the natural value, x_0, is the natural value at the center point, and ΔX is the step change value (the half of the interval $(-1 + 1)$).

Regression Equation in Uncoded Units:

$$\begin{aligned} Yi = {} & \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_4 X_4 + \beta_5 X_5 + \beta_{11} {X_1}^2 + \beta_{22} {X_2}^2 + \beta_{33} {X_3}^2 \\ & + \beta_{44} {X_4}^2 + \beta_{55} {X_5}^2 + \beta_{12} X_1 X_2 + \beta_{13} X_1 X_3 + \beta_{14} X_1 X_4 + \beta_{15} X_1 X_5 \\ & + \beta_{23} X_2 X_3 + \beta_{24} X_2 X_4 + \beta_{25} X_2 X_5 + \beta_{34} X_3 X_4 + \beta_{35} X_3 X_5 + \beta_{45} X_4 X_5 \end{aligned} \tag{2}$$

Yi is the predicted response (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 X were performed using a Minitab 19 Statistical Software (Minitab, Inc., State College, PA, USA) [32].

2.5 Statistical analysis

The statistical analysis was performed using (ANOVA), in order to validate the square model regression. It included the following parameters: coefficient of determination R^2; Fisher test (F); p-value and Student test (t); and the statistical significance test level was set at (probability <0.05) [32].

2.6 Validation of biomass production in optimum medium

After completing the optimization of the production of baker's yeast in grape juice, the optimum values obtained, and representative of the fermentation conditions were confirmed by conducting an experiment.

The experiment was carried out on 250 mL shake flasks and the agitation speed was 200 r.p.m. 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 the obtained value of 4.75. Shake flasks were sterilized at 120°C for 20 min and incubated at 30°C (optimum Value) for 12 h.

2.7 Analytical methods

2.7.1 Determination of total reducing sugars

1 ml of the sample is taken after filtering it and placed in a glass tube, then 98% sulfuric acid and 0.6 mL of 5% (w/v) phenol were added and mixed well after which it is left at room temperature for 30 minutes, the absorbance is measured using a spectrophotometer (Analytik Jena- specord 200uv-vis spec.) at a wavelength of 490 nm, the concentration of the reducing sugar is calculated depending on the calibration curve, which was formed between different concentrations of standard

solutions of glucose and between the absorbance values corresponding to each concentration [35].

2.7.2 Determination of biomass concentration

1 ml of the sample is taken and subjected to a centrifugation process for 5 minutes at 5000 r.p.m., after which the supernatant is collected on the surface and washed twice with water and then placed in a drying oven at 105°C, the drying continues until the weight is stable [36].

2.7.3 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) [37].

2.8 Modeling

In order to fit the experimental data, three kinetic models (Monod, Verhulst, and Tessier) were chosen.

Monod kinetic model is a substrate concentration-dependent, Verhulst kinetic model is an unstructured model that depends on biomass, and Tessier is an unstructured model for a substrate concentration-dependent [32].

The Kinetic parameters (μ_{max}, K_s, and X_m), were determined after obtaining the curve fitting method of each model performed using Excel software (2016 Microsoft Corporation), and the results showed in **Table 2**, [38].

2.9 Profile prediction of biomass and substrate concentration

The integration of the Verhulst model was used (Eq. (3)), in order to predict the experimental profile of biomass of *S. cerevisiae* during time [32].

$$X = \left(x_0 * exp^{\mu_m * t}\right) / \left(1 - \left(x_0 / x_m\right) * \left(1 - exp^{\mu_m * t}\right)\right) \quad (3)$$

Kinetic Models	Equations	Linearized form	Symbols
Monod model	$\mu = \mu_{max} * (s/(s + k_s))$	$(1/\mu) = (k_s/\mu_{max}) + (1/s) + (1/\mu_{max})$	μ: is the specific growth rate (h^{-1}). μ_{max}: is the maximum specific growth rate (h^{-1}). K_S: is the half-saturation constant (g/L). S: is the concentration in limiting substrate (g/L). X: is the biomass concentration (g/L). X_m: is the Maximum biomass concentration (g/L).
Verhulst model	$\mu = \mu_{max} * (1\text{-}x/x_m)$	$\mu = \mu_{max} - (\mu_{max}/x_m) * x$	
Tessier model	$\mu = \mu_{max} * (1\text{-}exp^{-k_s * s})$	$ln(\mu) = (1/k_s) * s + ln(\mu_{max})$	

Table 2.
Unstructured kinetic models to determine the kinetic parameters. [32].

The substrate model (Leudeking Piret) as described below (Eq. (4)) was also applied to predict an experimental profile for total reducing sugars consumption by *S. cerevisiae* during the time fermentation.

$$-ds/dt = p^{*}(dx/dt) + q^{*}x \tag{4}$$

Where ($p = 1/y_{x/s)}$ and q is a maintenance coefficient ($q = \mu_{max}/y_{x/x0}$.) Eq. (4) is rearranged as follows:

$$-ds = p^{*}\,dx + q\int x_{(t)}{}^{*}\,dt \tag{5}$$

Substituting Eq. (3) in Eq. (5) and integrating with initial conditions ($S = S_0$; $t = 0$) give the following Equation:

$$\begin{aligned} S = s_0 - p\,x_0\left\{{}_{\exp}{}^{\mu}{}_{m}{}^{*t}/1 - (x_0/x_m)*(1 - exp^{\mu_m * t})\right\} \\ -q*(x_m/\mu_m)*\ln\,(1 - x_0/x_m)*(1 - exp^{\mu_m * t} \end{aligned} \tag{6}$$

3. Results and discussion

The improvement of dry yeast biomass production was studied by determining the optimum values of the following factors (temperature, initial pH, concentration of sugars in grape juice, the ratio of carbon to nitrogen, and initial concentration of yeasts) that have their influence on the production process using the central composite experimental design, and the central composite design for biomass production in **Table 3**.

Ammonium sulfate and urea were added as a source of nitrogen in a ratio of (50–50) %, taking into account the achievement of the specified ratio between carbon and nitrogen for each experiment, and the agitation speed used during fermentation was 200 r.p.m.

Using the results obtained in diverse experiments, the correlation gives the influence of temperature (x_1), initial pH ($_{X2}$), total sugar concentration ($_{X3}$), the ratio of carbon to nitrogen (x_4), and initial concentration of yeasts (x_5) on the response. This correlation is obtained by Minitab 19 software and expressed by the following second-order polynomial (Eq. (7)).

$$\begin{aligned} Y = {} & -261.1 + 8.96\,T + 16.10\,pH + 0.353\,C + 6.55\,C/N + 49.8\,X \\ & - 0.1527\,T^{*}T - 1.769\,pH^{*}\,pH - 0.001414\,C^{*}C \\ & - 0.3025\,C/N^{*}C/N - 9.30\,X^{*}X + 0.0316\,T^{*}\,pH + 0.00096\,T^{*}C \\ & + 0.0206\,T^{*}C/N - 0.117\,T^{*}X + 0.00414\,pH^{*}C - 0.0390\,pH^{*}C/N \\ & - 0.165\,pH^{*}X + 0.00163\,C^{*}C/N + 0.0096\,C^{*}X - 0.016\,C/N^{*}X \end{aligned} \tag{7}$$

Table 4 shows the coefficient regression corresponding with t and p-values for all the linear and the analysis of variance (ANOVA), quadratic, and interaction effects of 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 [39].

Run	Actual Values					(Yi): Biomass (g/L)	
	Temperature (°C)	Initial pH	Concentration of sugars (g/L)	The ratio of carbon to nitrogen	Initial concentration of yeasts (g/L)	experimental Value	Predicted Value
01	35.00	6.000	200.0	8.000	2.000	22.41	23.0429
02	25.00	6.000	200.0	15.000	2.000	20.81	23.1708
03	25.00	3.000	100.0	15.000	2.000	20.01	19.6875
04	25.00	6.000	200.0	15.000	3.000	21.54	24.1742
05	25.00	3.000	200.0	8.000	2.000	19.18	18.5480
06	35.00	6.000	200.0	15.000	3.000	23.02	25.4896
07	25.00	6.000	200.0	8.000	2.000	20.02	21.9975
08	25.00	3.000	100.0	15.000	3.000	19.91	20.2285
09	30.00	4.500	150.0	11.500	2.500	40.45	38.5060
10	30.00	4.500	150.0	11.500	2.500	40.45	38.5060
11	35.00	3.000	200.0	8.000	3.000	18.91	19.0818
12	25.00	3.000	200.0	15.000	2.000	19.71	20.5400
13	35.00	3.000	100.0	15.000	2.000	18.84	20.2692
14	35.00	3.000	100.0	8.000	3.000	17.73	17.4531
15	30.00	4.500	150.0	11.500	2.500	40.45	38.5060
16	35.00	6.000	100.0	15.000	3.000	18.76	21.4784
17	35.00	3.000	200.0	8.000	2.000	17.79	18.6446
18	25.00	6.000	100.0	15.000	2.000	20.11	21.0771
19	35.00	6.000	100.0	8.000	2.000	20.23	21.1317
20	25.00	6.000	100.0	15.000	3.000	20.81	21.1218
21	35.00	6.000	100.0	8.000	3.000	20.91	20.1139
22	35.00	3.000	200.0	15.000	2.000	22.07	22.0803
23	25.00	6.000	100.0	8.000	2.000	21.06	21.0451
24	25.00	6.000	200.0	8.000	3.000	21.92	23.1122
25	35.00	6.000	200.0	15.000	2.000	23.07	25.6599
26	25.00	6.000	100.0	8.000	3.000	20.61	21.2010
27	35.00	3.000	200.0	15.000	3.000	21.41	22.4063
28	25.00	3.000	200.0	15.000	3.000	20.67	22.0397
29	30.00	4.500	150.0	11.500	2.500	40.45	38.5060
30	30.00	4.500	150.0	11.500	2.500	40.45	38.5060
31	30.00	4.500	150.0	11.500	2.500	40.45	38.5060
32	35.00	6.000	100.0	15.000	2.000	23.00	22.6074
33	35.00	3.000	100.0	8.000	2.000	21.11	17.9747
34	35.00	3.000	100.0	15.000	3.000	21.93	19.6364

Run	Actual Values					(Yi): Biomass (g/L)	
	Temperature (°C)	Initial pH	Concentration of sugars (g/L)	The ratio of carbon to nitrogen	Initial concentration of yeasts (g/L)	experimental Value	Predicted Value
35	25.00	3.000	200.0	8.000	3.000	20.27	20.1589
36	30.00	4.500	150.0	11.500	2.500	40.45	38.5060
37	30.00	4.500	150.0	11.500	2.500	40.45	38.5060
38	35.00	6.000	200.0	8.000	3.000	22.03	22.9839
39	25.00	3.000	100.0	8.000	2.000	20.17	18.8368
40	25.00	3.000	100.0	8.000	3.000	20.91	19.4890
41	30.00	4.500	150.0	11.500	2.500	40.45	43.9227
42	41.83	4.500	150.0	11.500	2.500	23.81	22.8190
43	30.00	4.500	150.0	11.500	3.683	33.02	31.1860
44	30.00	4.500	268.3	11.500	2.500	32.17	26.3327
45	30.00	4.500	150.0	11.500	1.317	31.56	30.6159
46	18.17	4.500	150.0	11.500	2.500	24.07	22.2828
47	30.00	4.500	150.0	11.500	2.500	40.45	43.9227
48	30.00	4.500	150.0	11.500	2.500	40.45	43.9227
49	30.00	8.049	150.0	11.500	2.500	30.94	24.7659
50	30.00	0.951	150.0	11.500	2.500	15.11	18.5059
51	30.00	4.500	150.0	11.500	2.500	40.45	43.9227
52	30.00	4.500	31.7	11.500	2.500	18.87	21.9291
53	30.00	4.500	150.0	3.219	2.500	19.11	21.1956
54	30.00	4.500	150.0	19.781	2.500	30.03	25.1663

Table 3.
The central composite design for biomass production.

3.1 Model summary

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 4** shows that all coefficient regression of the quadratic terms are statistically significant $p \leq 0.05$ and negatively affect the biomass production (**Figure 2**). In contrast, the interaction terms (T, C/N, X, T* pH, T*C, T*C/N, T*X, pH *C, pH *C/N, pH *X, C*C/N, C*X, C/N*X) are statistically not significant $p > 0.05$, and the interaction terms (pH, C, T*T, pH * pH, C*C, C/N*C/N, X*X) are significant with $p < 0.05$ and have a synergistic effect on the response.

It is known that the F-value with a low probability p-value indicates a high significance of the regression model [40].

Looking at the analysis of variance (ANOVA), the study shows that the model is important as the F-value had a low probability p-value ($p = 0.000$), and the resulting value of R^2 was equal to 92.9% and this indicates that only 7.1% of the variance is not explained by the model and therefore there is a good agreement between the model and the experimental data [41]. **Figure 3** shows the fit between the model and experimental data of cell growth.

By reviewing previous studies, Bennamoun et al. [42] used response surface methodology in order to improve and optimization of the medium components,

Term	DF	Adj SS	Adj MS	Coef	SE Coef	T-Value	P-Value	VIF	P-Value
T	1	0.55	0.555	0.113	0.447	0.25	0.802	1.00	0.802
pH	1	75.60	75.595	1.323	0.447	2.96	0.006	1.00	0.006
C	1	37.41	37.408	0.931	0.447	2.08	0.045	1.00	0.045
C/N	1	30.42	30.415	0.839	0.447	1.88	0.070	1.00	0.070
X	1	0.63	0.627	0.120	0.447	0.27	0.789	1.00	0.789
T*T	1	869.04	869.040	−3.818	0.381	−10.03	0.000	1.01	0.000
pH * pH	1	945.05	945.046	−3.981	0.381	−10.46	0.000	1.01	0.000
C*C	1	745.29	745.295	−3.536	0.381	−9.29	0.000	1.01	0.000
C/N*C/N	1	818.56	818.560	−3.705	0.381	−9.74	0.000	1.01	0.000
X*X	1	322.63	322.625	−2.326	0.381	−6.11	0,000	1.01	0.000
T* pH	1	1.80	1.800	0.237	0.519	0.46	0.651	1.00	0.651
T*C	1	1.84	1.838	0.240	0.519	0.46	0.648	1.00	0.648
T*C/N	1	4.17	4.169	0.361	0.519	0.69	0.492	1.00	0.492
T*X	1	2.76	2.755	−0.293	0.519	−0.56	0.576	1.00	0.576
pH *C	1	3.08	3.081	0.310	0.519	0.60	0.554	1.00	0.554
pH *C/N	1	1.34	1.341	−0.205	0.519	−0.39	0.696	1.00	0.696
pH *X	1	0.49	0.493	−0.124	0.519	−0.24	0.813	1.00	0.813
C*C/N	1	2.60	2.605	0.285	0.519	0.55	0.587	1.00	0.587
C*X	1	1.84	1.838	0.240	0.519	0.46	0.648	1.00	0.648
C/N*X	1	0.02	0.025	−0.028	0.519	−0.05	0.958	1.00	0.958
S			**R-sq**		**R-sq(adj)**			**R-sq(pred)**	
2.93825			92.85%		88.16%			69.22%	

Table 4.
Estimated regression coefficients of t and p and analysis of variance (ANOVA).

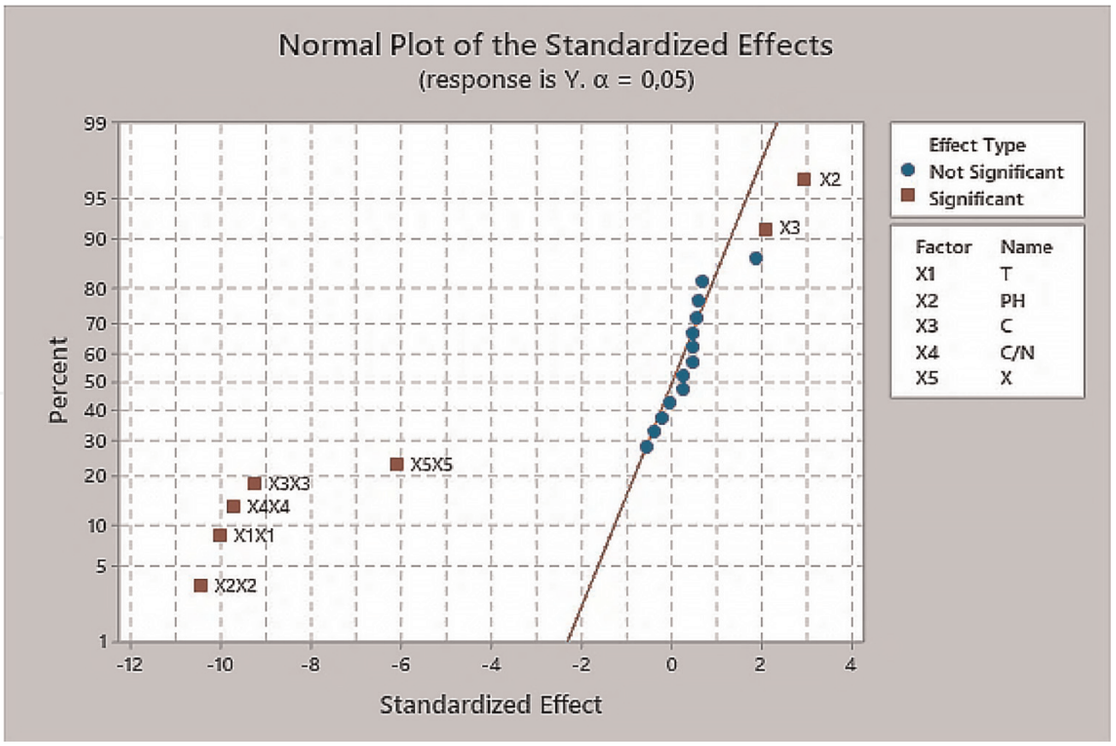

Figure 2.
Variable effect signification on biomass production.

which enhance the polygalacturonase activity of the strain Aureobasidium pullulans, and they got good results (a very low p-value (0.001) and a high coefficient of determination (R^2 = 0.9421), the results confirm the importance and success of using this method.

A previous study by Boudjema, Fazouane-Naimi, and HellaL [27] showed the success of using the experimental design method in the study of the production of *Saccharomyces cerevisiae* DIV13-Z087°CVS using sweet cheese serum, as it confirmed a high significance of the regression model, and the results showed a good agreement with experimental data (a low probability p-value ≤0.000 and a good correlation coefficient (R^2 = 0.914%).

The optimization of the response Yi (Biomass production) and the prediction of the optimum levels of (temperature, initial pH, concentration of sugars in grape juice, the ratio of carbon to nitrogen, and initial concentration of yeasts) were obtained. This optimization resulted in surface plots (**Figure 4**), the figure shows 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 5**.

Figure 5 shows the maximum biomass production by *Saccharomyces cerevisiae* (41.444 g/L) corresponding to values of temperature (30.11°C), pH (4.75), sugar concentration (158.36 g/L), the ratio of carbon to nitrogen (11.9), initial yeast concentration (2.5 g/L). The amount of urea was 6.65 g/L and the amount of ammonium sulfate used was 6.65 g/L, so that the concentration of added urea and ammonium sulfate was (50–50)% and the required C/N ratio was achieved, and the stirring speed was equal to 200 r.p.m. during the fermentation process. Jiménez-Islas et al. [36] obtained the highest cell concentration of *S. cerevisiae* ATCC 9763 (7.9 g/L) after 26 h

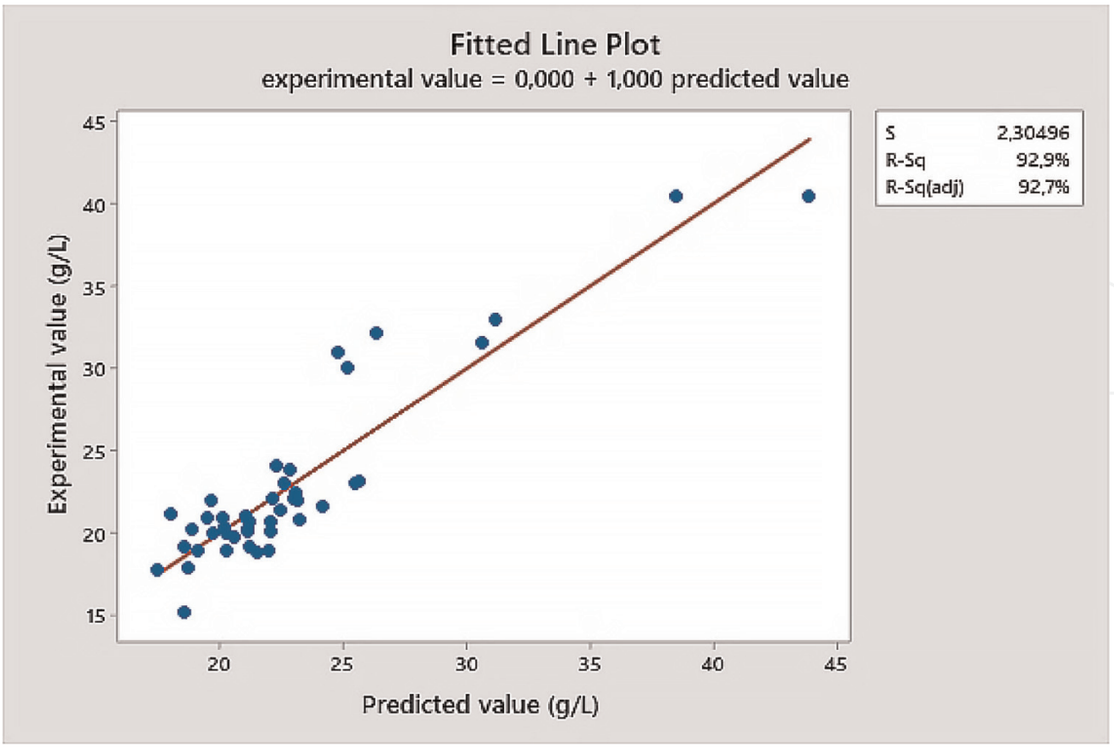

Figure 3.
The fit between the model and experimental data of cell growth.

when the strain grew at 30°C and pH 5.5, so we note that our study gave a good result in achieving the greatest possible production of baker's yeast.

The validation of the baker's yeast biomass concentration and total reducing sugar consumption, over time fermentation, at optimized conditions, are presented in **Figure 6**.

At the beginning of the fermentation process, the concentration of the resulting biomass increases and is associated with the consumption of sugar. After 12 hours of the fermentation process, the sugar concentration has reached a very low level, and this is associated with a decrease in yeast production.

The same results were obtained by Ali et al. [32] where they study the optimization of Baker's Yeast production on Date extract using Response Surface Methodology (RSM), and the resulting yeast was equal to 40 g/L.

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.

Depending on the Monod model, the curve fitting of cell growth is formed (1/μ versus 1/S) and shown in **Figure 7**. **Figure 8** shows the resulting graph according to the Verhulst model (μ versus X), and in **Figure 9** the graphical curve is formed according to the growth of the Tessier model (μmax and Ks).

The kinetic parameters of growth of *Saccharomyces cerevisiae* using different kinetic models according to the curve fitting method are presented in **Table 5**.

The results obtained from the modeling process appear as follows: the Monod model gave a good value for the parameter R^2 equal to 0.94, which indicates that it is an acceptable model for studying the kinetic performance of a strain *S. cerevisiae,* and

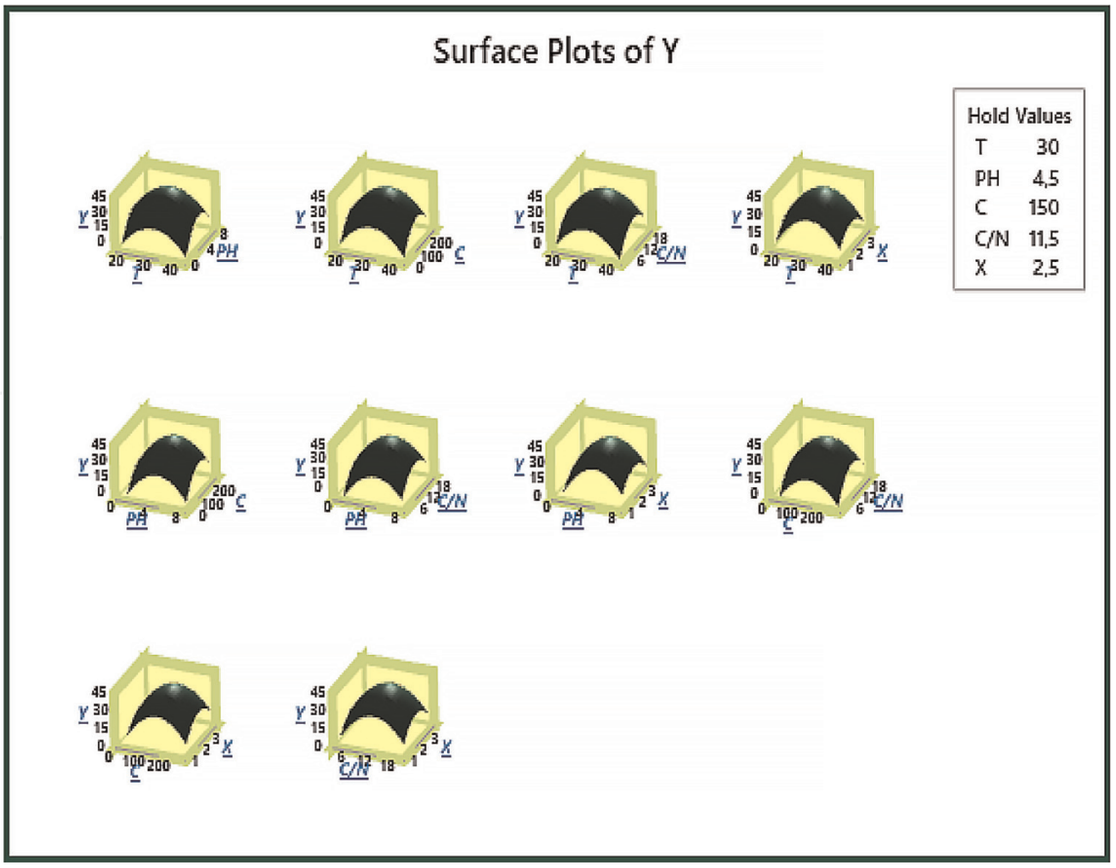

Figure 4.
Surface plot for the effect of different parameters on biomass production.

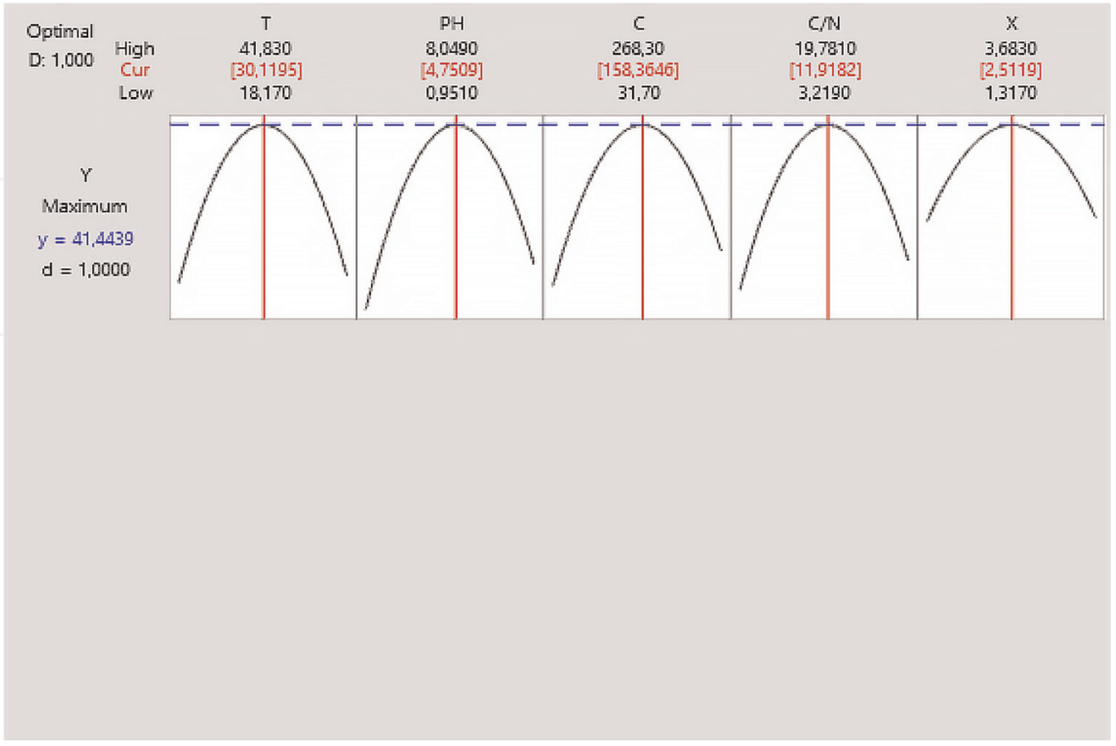

Figure 5.
Values of optimal conditions on biomass production.

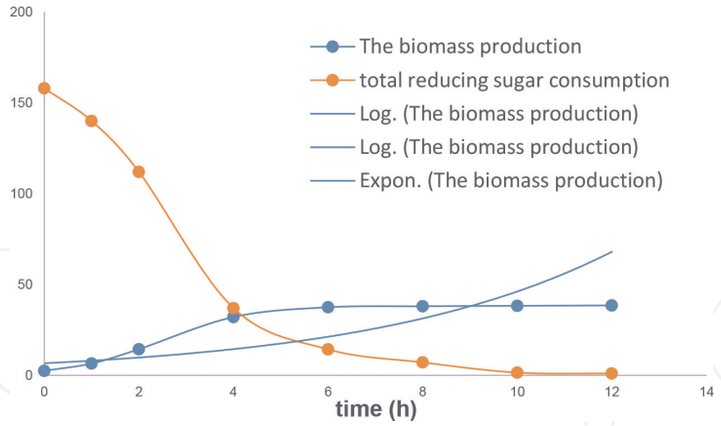

Figure 6.
The biomass production, and total reducing sugar consumption over time at optimized conditions.

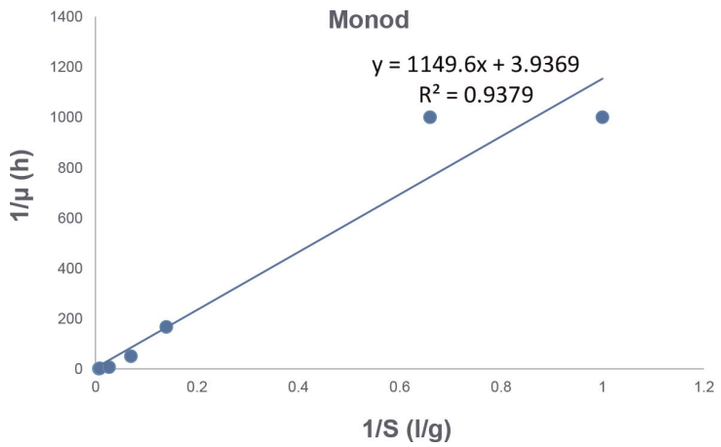

Figure 7.
The line weaver Burk linear plot fitting the experimental data using the Monod kinetic model.

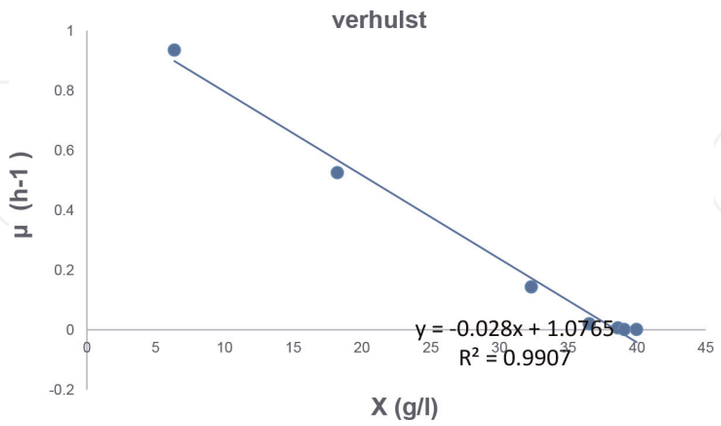

Figure 8.
A plot fitting the experimental data using the Verhulst kinetic model.

the values of each of the maximum specific growth rate (μmax) and is the half-saturation constant (Ks) were evaluated as 0.254 h^{-1} and 291.99 g/L, respectively, which are good values indicating rapid growth of cells Yeast. Tessier's model gave the lowest value for R^2 compared to the Monod and Verhulst models, where it was 0.81. Whereas the Verhulst model gave the highest value for the parameter R^2 which

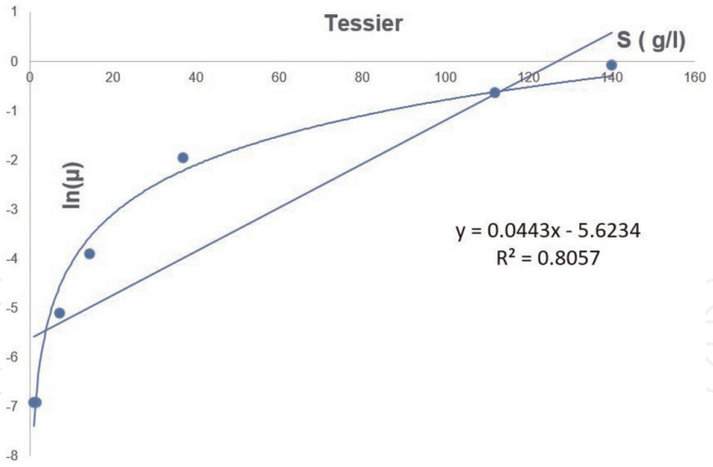

Figure 9.
A plot fitting the experimental data using the Tessier kinetic model.

reached 0.99, also gave a high value for the maximum specified growth rate reached 1.0765 h^{-1}, and the highest possible amount was obtained from the concentration of yeast according to the Tessier model reached 38.26 g/L. As a result, the Verhulst model is the best model for studying and controlling the kinetic behavior of a yeast strain *S. cerevisiae*.

A residual plot is a chart used to assess the quality of a regression fit. Examination of the remaining squares will help determine if the least-squares assumptions are ever met. When these assumptions are met, least squares regression typically yields an inaccurate estimation coefficient with minimal variance. The 4-in-1 residual plot displays four residual plots in a graph window. This configuration can be useful for comparing plans to determine if the Verhulst model meets the criteria for analysis. The remaining sections of the figure are:

- Histogram - indicates if the data is biased or outliers are contained in the data.
- Normal probability plot - indicates whether the data conforms to the normal distribution, whether other changes are affecting the response, or whether the content of the data.
- Residuals versus fitted values - indicates if the difference is continuous, if there is a nonlinear relationship, or if outliers are present in the data.
- -Residuals versus order of the data - indicates whether there is an impact on data due to the time or order of data collection.

Kinetic models	**Parameters of estimation**			
	R^2	K_S (g/L)	μ_{max} (h^{-1})	X_m
Monod	0.94	291.99	0.254	—
Verhulst	0.99	—	1.0765	38.26
Tessier	0.81	22.7	0.0036	—

Table 5.
Kinetic parameters of Saccharomyces cerevisiae *growth and substrate utilization using unstructured models.*

Minitab provides the following residual plots in **Figure 10**.

Examination of the remains indicates that there is nothing to complain about. The normal performance of the remaining sections does not seem to have much difference. There is nothing surprising here and it seems acceptable.

The kinematic models describe the growth rate of microorganisms based on biomass and substrate concentration and are useful because they help engineers design and control biological processes, including the Verhulst model which describes the experimental data obtained on the growth rate of yeast cells, where it describes the logarithmic growth of cells and shows that the first six hours of fermentation were during the initial cell growth phase, then the logarithmic growth phase began, which is characterized by a doubling of the number of yeast cells and an increase in the growth rate.

A profile of biomass and total reducing sugar concentration during fermentation time is compared to the values predicted by the equations model obtained in **Figures 11** and **12**.

During the fermentation, values of biomass between predicted and experimental data were approximately the same. And for total reducing sugar concentration, the values obtained by the Leudeking Piret model were identical to the predicted values, where the values ($p = 1/y_{x/s}$, $q = \mu/y_{x/x0}$) were 3.81 g/g and 0.065 1/h, respectively.

On the basis of these results, good correlation coefficients showed that the proposed Verhulst model and the Luedeking Piret model were adequate to explain the development of the biomass production process in grape juice.

This study confirmed that the Logistic equation for the growth and the Leudeking Piret kinetic model for substrate utilization were able to fit the experimental data, and

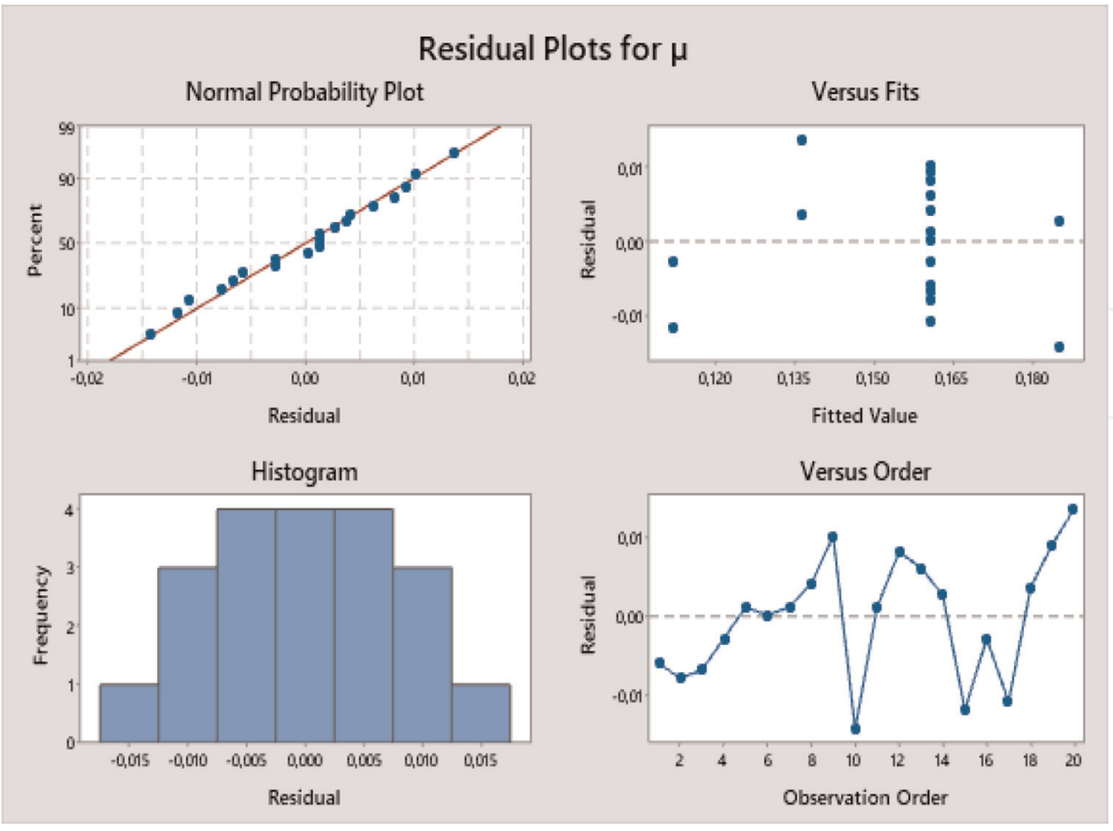

Figure 10.
Residual plots for response.

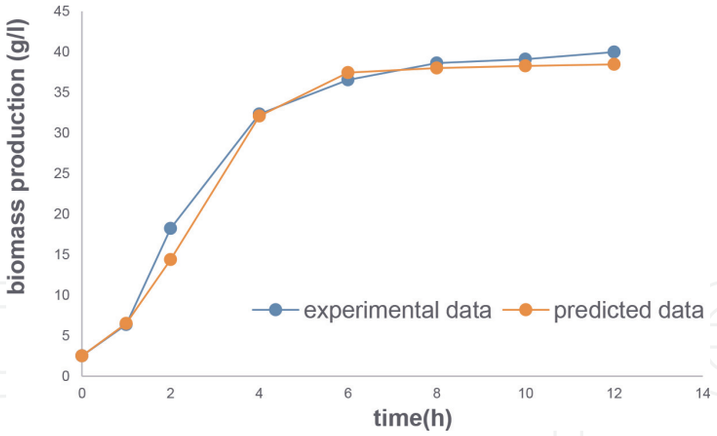

Figure 11.
The comparison between predicted and experimental data for biomass production of baker's yeast.

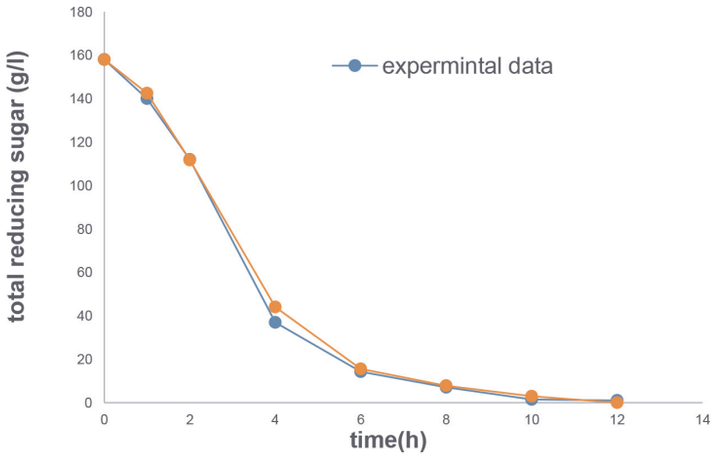

Figure 12.
The comparison between predicted and experimental data for total reducing sugar consumption.

the same result was obtained by Kara Ali et al. [43] Where they used the logistic empirical kinetic model and Leudeking Piret model and they obtained good agreement with the experimental data.

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. The work has been done with a lot of numerical and experimental analysis.

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.

4. Conclusion

The central composite design (CCD) proposed in this study seems pertinent to describe the optimum biomass production of *Saccharomyces cerevisiae*. A second-order

polynomial model was developed to evaluate the quantitative effects of temperature, initial pH, and concentration of sugars in grape juice, the ratio of carbon to nitrogen, initial concentration of yeasts in order to discover the optimum conditions for the biomass production from grape juice. According to the experimental results, a maximum biomass concentration of (41.444 g/L) corresponding to values of 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), the amount of urea was 6.65 g/L and the amount of ammonium sulfate used was 6.65 g/L, so that the concentration of added urea and ammonium sulfate was (50–50)%, and the used agitation speed was equal to 200 r.p.m. during the fermentation process. The fermenter power of the obtained yeast was 470 ml. In addition, among three unstructured kinetic models, the Verhulst model was the most suitable model to signify the baker's yeast production on grape juice medium.

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Conflicts of interest

The authors declare no conflict of interest.

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