Research Article

Evaluation of Growth Kinetics and Biomass Yield of Baker's Yeast on Potato Flour

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Abstract: The study aims to evaluate the growth kinetics of two different Baker's yeast (*S. cerevisiae*) strains and to establish regression models for predicting and optimizing the growth conditions of the strains. Two yeast strains of *S. cerevisiae*: one was a Commercial Strain (CS) and another was isolated from traditional dry wine residue from the Madhupur region, Bangladesh (MS), were used in the study. The effects of four different factors, *viz*., time, temperature, agitation, and the concentration of potato powder were assessed. The performance of the growth of the strains was monitored using three responses, like OD at 600 nm, ethanol production, and biomass yield (g·L^{-1}). A fractional factorial design (2^{4-1}) was used to generate the experimental trials, as well as to analyze the data to design a geometrical representation. The highest value of optical density, ethanol, and biomass production for *S. cerevisiae* (CS) were 1.439, 6.56 (g·L^{-1}), and 0.39 (g·L^{-1}), respectively, and for *S. cerevisiae* (MS) were 0.645, 0.621 (g·L^{-1}), and 0.23 (g·L^{-1}), respectively. The best conditions for biomass production were at 1% potato powder concentration, a temperature of 30 °C, and agitation at 150 rpm. By using a regression model, it can be said that for two-factor interactions, potato powder concentration with time and time with agitation had the significant effects, but three-factor interactions had no significant effect on the experiment. By establishing regression models from the obtained data, prediction and optimization of the strains' growth conditions can be easily done.

Keywords: potato flour, Baker's yeast, biomass and ethanol production, factorial design

1. Introduction

 S. cerevisiae (Baker's yeast) is widely known for its ethanol and biomass production in a broad context of fermentation because it is less susceptible to infection than any other microbes like bacteria. Furthermore, its ability to tolerate ethanol is also quite better than other ethanol producing microbes [1]. Among all of the microorganisms, Saccharomyces *cerevisiae* is generally taken into consideration for its advantageous utilization as safe for human consumption. In a wide range of bread production, *S. cerevisiae* is used in the leavening of dough because it can produce ethanol and carbon dioxide by utilizing the sugar present in the dough [2]. Both active and instant dry yeast can be produced from Saccharomyces cerevisiae [3].

 The substrates recommended for biomass and ethanol production should be efficient and cheap containing a high percentage of carbon source such as molasses B [4], sugar beet pulp [5], sugarcane [6], waste produced during starch production from cassava [7], food waste [8], and waste newspapers [9]. These carbon sources are considered as high

value products of food source [10]. Potato flour used in the study, is an ideal substrate for growth of Baker's yeast. 100 g potato flour contains about 91% carbohydrate and 4% minerals with a small amount of protein and fat. Potato can be an alternative source for both biomass production of *S. cerevisiae* [11] and ethanol production because of its high percentage of carbon. For commercial production of yeast, potato flour is a good source of carbon because of its low cost and availability throughout the country. But in Bangladesh, most of the baking industries use baking powder as it is less expensive than bakers' yeast and also our country doesn't produce it. That's why it is being imported from foreign countries in a large amount every year for the baking purpose which makes it costly too [12].

In an experiment, generally the number of trials rapidly increases with the number of factors. For reducing experiment trial numbers, times, and expenses, the fractional factorial design is very useful [13]. The use of fractional factorial design in the study can have a significant effect on statistical analysis to get a better result. This research employed fractional factorial design 2^{4-1} to investigate the influence of four parameters like time, temperature, agitation and substrate concentration on the laboratory scale yeast cell growth monitoring biomass yield at OD 600 nm and weighing dry yeast cells. Ethanol production was also monitored.

2. Materials and methods

2.1 *Collection and transportation of dry wine residue sample*

Dry wine residue (Figure 1) used to isolate *Saccharomyces cerevisiae* strains were collected from traditional dry wine residue of Madhupur upazila, Mymensingh collected using sterilized enclosed polythene bags with proper labeling. These samples were carried to the laboratory and kept in normal storage condition for microbiological studies.

Figure 1. Collection of dry wine residue sample

2.2 *Isolation of strain from dry wine residue*

Isolation of yeast strain was done by using the serial dilution plate technique method. 1 g of the dry wine residue sample was taken in a 250 ml Erlenmeyer flask containing 50 ml sterilized water. The flasks were shaken using a rotary shaker for 10 min tear of the spores from the solution. Then the suspended matter was allowed to settle down and the suspension was serially diluted up to 10^{-6} times.

2.3 *Inoculation and incubation of the samples*

Spread plate technique was used throughout this study. 1 ml of diluted samples prepared from wine residue was transferred on MEAB plates using sterile pipette and spreaded with the help of sterile glass rod. All of the MEAB plates were then incubated in an incubator at 30 ± 2 °C for 48 hours. Inoculum prepared from yeast samples was streaked onto MEA medium and incubated at 37 °C for 24 hours. After incubation the colony morphology and color were recorded to identify the *Saccharomyces spp*. Colony of *Saccharomyce*s were sub-cultured onto Malt Extract Agar (MEA). A small

lump of yeast mycelia was picked up by sterile tooth prick and directly placed on MEA and incubated at 37 °C for 24 hours to get pure cultures.

2.4 Sub-merge cultivation of Saccharomyces cerevisiae

For sub-merge cultivation of *Saccharomyces cerevisiae*, potato flour solution was used. For the preparation of potato flour solution, 3 g and 5 g flour was dissolved in 100 ml (3%) and 500 ml (1%) distilled water respectively. The media was then sterilized by autoclaving at 121 °C and 15 psi for 15 min. The medium was sterilized by incubating overnight at 37 °C. The incubated medium was used to evaluate cultural characterization of the strain or stored at 4 °C for future use. Inoculation of inoculum on potato flour media was done at 30 °C and 40 °C, at 100 and 150 rpm for 25 and 50 hours [14].

2.5 Proximate analysis of potato flour

The proximate composition of potato flour was analysed for moisture, crude protein, crude fat, and ash content as per the methods of [15]. The total carbohydrate content of the sample was calculated by subtracting the measured crude protein, crude fat, ash and moisture content from 100 [16].

3. Study design

The influence of four factors at two different levels on the optical density, alcohol and biomass production during growth of *Saccharomyces cerevisiae* was assessed. The design matrix is given in Table 1.

Table 1. Design matrix

Label	Factors	Levels		
		-1	Central	+ 1
A	Potato powder concentration (%)	1	2	3
B	Time (min)	25	37.5	50
C	Temperature (°C)	30	35	40
D	Agitation (rpm)	100	125	150

Total trials for the above design matrix (Table 1) were including three trials at the central point. Table 2 indicates total trials with their coded value.

It was employed that the 2^{4-1} design defining the relation $I = ABCD$, where I is the identity column. Because of that choice of generator will result highest possible resolution (IV) in the design. Each main effect was evaluated with another three-factor interaction: $A = BCD$, $B = ACD$, $C = ABD$ and $D = ABC$. Also every two-factor interaction was analyzed with another two-factor interaction and that was $AB = CD$, $AC = BD$ and $DA = BC$. All experiments were made in triplicate to estimate the experimental errors, and another experiment was carried out in triplicate at the central point. Furthermore, all 54 experiments were done in the study. The ranges were chosen for factors such as potato powder concentration (A), time (B), temperature (C) and agitation (D) were 1%-3%, 25 hours-50 hours, 30 °C-40 °C and 100 rpm-150 rpm, respectively. Following the usual convention, the two extreme levels are denoted by minus one (lower level) and plus one (higher level). This leads to a convenient algorithm to analyze the experimental results. The studied dependent variables were: optical density, ethanol production (%) and biomass production (g·L^{-1}).

Table 2. Total trial with coded value

Trial	Factor levels			
	A	B	C	D
1	-1	-1	-1	-1
2	+ 1	-1	-1	+ 1
3	-1	+ 1	-1	+ 1
4	+ 1	+ 1	-1	-1
5	-1	-1	+ 1	+ 1
6	+ 1	-1	+ 1	-1
7	-1	+ 1	+ 1	-1
8	+ 1	+ 1	+ 1	+ 1
9	0	0	0	0

3.1 *Measurement of ethanol production*

The amount of ethanol was measured in a glass tube containing a scale calibrated with the reading on its level. The surface of the liquid in which the hydrometer is floating indicates the number of times heavier or lighter than water, i.e., the specific gravity of the liquid. The hydrometer was based on Archimedes› principle. The formula for converting specific gravity to alcohol was [17],

$$ABV = (OG - FG) * 131.25 \qquad (1)$$

Where OG = original gravity and FG = final gravity.

3.2 *Measurement of optical density by spectrophotometer*

The yeast cell in the broth is directly proportional to the absorbance at 600 nm. Based on this principle, yeast cell growth was monitored by measuring optical density of the yeast broth using a spectrophotometer at 600 nm wavelength [18].

3.3 *Measurement of cell growth by dry cell weight methods*

Dry cell weight was measured by oven drying method as described by Reis [19] with slight modification. In this method, about 10 mL broth was taken in a pre-weighed centrifuge tube (w_1) and centrifuged at 6,000 rpm. After centrifugation of the cell broth, the supernatant was discarded and the filtrated remained in the tube was placed in an air oven for drying at 65 °C for 1 hour. After drying, weight (W_2) was taken. Then the biomass yield or dry weight was measured by using the formula,

$$Drywt\left(\frac{g}{L}\right) = \frac{w_2 - w_1}{5} \times 1,000 \qquad (2)$$

4. Statistical analysis

The estimates of the effects obtained from the experimental "2^{4-1}" design. All effects were estimated as differences between two averages and each average containing half of the experimental responses. The intermediate trials were ignored at that stage, and used only to investigate possible curvatures. The interaction effects are linear combinations of the form [13],

$$\frac{1}{4}\sum_{i}^{n} a_i y_i \qquad (3)$$

Where, y_i was the average response in trial i and the coefficient a_i was set equal to plus or minus one, depending on the sign of the product of the columns of the factors involved. The calculations were done using statistica (version 5.1) and MATLAB (version 4.2 c.1) software's.

5. Results and discussion

5.1 *Proximate analysis of potato flour*

Potatoes can be an appropriate alternate substrate for biomass yield and bioethanol production due to their plenty of availability, low cost, and easy processing. The major part of potato was carbohydrate as starch followed by ash. The dominancy of carbohydrate constituents of potato flour was also revealed from its analysis on the basis of dry basis. From the proximate analysis of potato flour it was found that the amount of carbohydrates was 90.71% which were similar to the findings of [20] who observed 90.6% carbohydrate in potato flour. The fat content was found 0.99% which was closed to the findings (1.02%) as reported by [21]. Boni et al. [22] reported the similar percentages of moisture, protein and ash content in the potato flour with that of found in this study. The nutritional composition of 100 g potato flour is shown in Table 3.

Table 3. Proximate analysis of potato flour

Components	Content (%) (wb)	Content (%) (db)
Protein	0.90 ± 0.07	0.93 ± 0.03
Fat	0.96 ± 0.05	0.99 ± 0.06
Carbohydrates	90.71 ± 0.13	93.91 ± 0.19
Ash	4.02 ± 0.02	4.16 ± 0.09
Moisture	3.41 ± 0.07	3.53 ± 0.08

5.2 *Effect of experimental condition on the growth of yeast*

Effects of time, temperature and agitation on the growth and biomass yield of *S. cerevisiae* of both CS and MS. These parameters were also observed for their effect on ethanol production and optical density measurement of cell growth. The inoculated yeast with potato flour solution was incubated at 30 °C and 40 °C at 100 and 150 rpm for 25 hours and 50 hours according to the experimental design. The obtained results for Optical Density (OD), % alcohol and dry weight were given in Table 4. The experimental factors like temperature range and pH were selected based on a performance study carried out with unhydrolyzed potato waste to produce ethanol using *S. cerevisiae* at a temperature range of 20 °C to 50 °C and at pH range of 4 to 7 for 7 days and the optimum pH and temperature for the fermentation of waste potatoes was reported to be 6 and 30 °C, respectively [23]. Based on this study, experimental factors were selected as temperature ranged between 30 °C and 40 °C, speed 100 and 150 rpm and time ranged between 25 hours and 50 hours to the experimental design.

5.3 *Biomass production*

In the study, all *S. cerevisiae* strain showed better biomass production about two to three fold in shaking at 150 rpm than 100 rpm culture [24]. From the observation of biomass production of *Saccharomyces cerevisiae* (CS), the highest yield was 0.39 g·L^{-1} and 0.23 g·L^{-1} (Table 4) for *Saccharomyces cerevisiae* MS which corresponded to the experiment at trial of number 3 for both of the strains. The conditions of trial no. 3 were 1% potato powder concentration for 50 hours' at 30 °C and at speed of 150 rpm.

5.4 *Ethanol production*

Considering ethanol as the final product of fermentation, the best production of alcohol was observed as 0.656% (6.566 g/L⁻¹) for *S. cerevisiae* CS and 0.621% (6.21 g/L⁻¹) (Table 4) for *Saccharomyces cerevisiae* MS which corresponded to the experiment trial number 8 for both of the strains. The conditions of trial no. 8 were 3% potato powder concentration for 50 hours' time at 40 °C temperature and agitation at 150 rpm. The maximum ethanol production was 27.7 g/L obtained at controlled pH and inoculum size as 3% [25]. Temperature between 25-30 °C was commonly found optimum for thermophilic *S. cerevisiae* strain for ethanol production in various substrates such as apple pomace, sweet sorghum, etc. [26].

5.5 *Optical density*

Both *Saccharomyces cerevisiae* CS and MS showed higher optical density than others at trial number 3. The conditions of trial no. 3 were 1% potato powder concentration for 50 hours time at 30 °C temperature and agitation at 150 rpm. Commercial yeast showed higher optical density 1.439 at 1% potato powder concentration for 50 hours at 30 °C and at a speed of 150 rpm. MS yeast showed higher optical density 0.645 also at 1% potato powder concentration for 50 hours at 30 °C and a speed of 150 rpm (Table 4). In the study, it was found that the best output obtained at 30 °C. For maximum growth and ethanol production, 30 °C was the most appropriate temperature for *S. cerevisiae* y-1646 was reported by scientists. At 30 °C temperature, biomass was estimated as 2.47 (OD 500 nm) but the production of ethanol by *S. cerevisiae* y-1646 was favored by temperature of 35 °C and reached its maximum (5.29 g·L⁻¹) after 36 h [14].

Table 4. Total trial with actual value for both *S. cerevisiae* CS and *S. cerevisiae* MS

Potato powder conc. (%)	Time (min)	Temp. (°C)	Agitation (rpm)	Optical Density (OD) CS	Optical Density (OD) MS	% ethanol CS	% ethanol MS	Dry wt. (g·L⁻¹) CS	Dry wt. (g·L⁻¹) MS
1	25	30	100	0.927	0.309	0.196	0.131	0.16	0.130
3	25	30	150	1.209	0.209	0.393	0.135	0.22	0.090
1	50	30	150	1.439	0.645	0.315	0.433	0.39	0.230
3	50	30	100	1.344	0.621	0.525	0.513	0.18	0.150
1	25	40	150	1.014	0.242	0.262	0.196	0.19	0.120
3	25	40	100	1.036	0.252	0.433	0.261	0.23	0.110
1	50	40	100	1.135	0.381	0.371	0.315	0.21	0.14
3	50	40	150	1.379	0.631	0.656	0.621	0.23	0.210

5.6 *Average results for biomass, ethanol production and optical density*

Data in Table 5 showed the average results of the production of biomass, optical density and ethanol, using the experimental design from Table 1.

The estimates of the effects obtained from the 2^{4-1} design to get the main effects one thus refers to the responses in Table 6, the signs of the corresponding columns in Table 1, performs the algebraic sum, and divides the result by four.

To illustrate the calculations, the linear combination of observations associated with the effect A in optical density for *Saccharomyces cerevisiae* CS, changes is

$$I_A = (1/4) [- (0.16) + (0.22) - (0.39) + (0.18) - (0.19) + (0.23) - (0.21) + (0.23) = 0.0225] \rightarrow A + BCD.$$

For *Saccharomyces cerevisiae* MS, changes is

$$I_A = (1/4) [- (0.13) + (0.09) - (0.23) + (0.15) - (0.12) + (0.11) - (0.14) + (0.21) = - 0.015] \rightarrow A + BCD.$$

The interaction effects are linear combinations of the form [13].

$$\frac{1}{4}\sum_{i}^{n} a_i y_i$$

Where, y_i was the average response in trial i and the coefficient a_i was set equal to plus or minus one, depending on the sign of the product of the columns of the factors involved. For example, to calculate the AB (= CD) interaction for *Saccharomyces cerevisiae* CS,

$I_{AB} = (1/4) [+ (0.16) - (0.22) - (0.39) + (0.18) + (0.19) - (0.23) - (0.21) + (0.23) = - 0.0725 \rightarrow AB + CD.$

To calculate the AB (= CD) interaction *Saccharomyces cerevisiae* MS,

$I_{AB} = (1/4) [+ (0.13) - (0.09) - (0.23) + (0.15) + (0.14) - (0.11) - (0.14) + (0.21) = 0.01 \rightarrow AB + CD.$

Table 5. Average values of optical density, ethanol and biomass production followed by the conditions defined in Table 1

Trial	OD CS	OD MS	Ethanol (%) CS	Ethanol (%) MS	Biomass (g/L) CS	Biomass (g/L) MS
1	0.927	0.309	0.196	0.131	0.160	0.130
2	1.209	0.209	0.393	0.135	0.220	0.090
3	1.439	0.645	0.315	0.433	0.390	0.230
4	1.344	0.621	0.525	0.513	0.180	0.150
5	1.014	0.242	0.262	0.196	0.190	0.120
6	1.036	0.252	0.433	0.261	0.230	0.110
7	1.135	0.381	0.371	0.315	0.210	0.14
8	1.379	0.631	0.656	0.621	0.230	0.210
9	1.365	0.550	0.359	0.453	0.206	0.130

The average results for biomass production, optical density and ethanol were obtained by using 2^{4-1} design. From Table 6 and 7, for biomass production, it is reasonable to conclude that the main effects A, B and D had large values and A, B and D were the most important effects, then it was logical to conclude that the interaction alias chain $AC = BD$ had a large effect because BD was also significant [27].

Table 6. Estimates of effects, aliases for the design 2^{4-1} (Table 1) calculated based on the results presented in Table 2 (*Saccharomyces cerevisiae* CS and MS)

Effects	Estimated (OD) CS	Estimated (OD) MS	Estimated ethanol (%) CS	Estimated ethanol (%) MS	Estimated biomass production (g/L) CS	Estimated biomass production (g/L) MS
$I_A \rightarrow A + BCD$	0.113	0.034	0.216	0.114	0.023	-0.015
$I_B \rightarrow B + ACD$	0.278	0.317	0.583	0.290	0.053	0.070
$I_C \rightarrow C + ABD$	-0.089	-0.070	0.073	-0.045	-0.023	-0.005
$I_D \rightarrow D + ABC$	0.141	0.041	0.025	0.041	0.063	0.030
$I_{AB} \rightarrow AB + CD$	-0.039	0.079	0.032	0.079	-0.073	0.010
$I_{AC} \rightarrow AC + BD$	0.012	0.096	0.012	0.072	0.053	0.045
$I_{AD} \rightarrow AD + BC$	-0.046	-0.058	0.020	-0.050	-0.043	-0.010

Effects and interactions of theoretical 2^{4-1} in yeast growth.

Effects of interactions of theoretical 2^{4-1} in yeast growth and biomass production of *Saccharomyces cerevisiae* for both strains are shown in Table 7. The principle effects of the experiment are potato powder concentration, time and agitation. Among them, time and agitation had a significant effect for both strains. For two factor interaction, potato powder concentration with time and time with agitation had a significant effect for *Saccharomyces cerevisiae* CS. For *Saccharomyces cerevisiae* MS, only time with agitation had a significant effect. Three factor interactions had no significant effect on the experiment.

Table 7. Results of principle effects and interactions of theoretical 2^{4-1} in yeast growth and biomass production

	Estimated (*S. cerevisiae* CS)	Estimated (*S. cerevisiae* MS)
Principal effects		
Potato powder concentration (*A*)	0.023	-0.015
Time (*B*)	0.053	0.07
Agitation (*D*)	0.063	0.03
Two factor interaction:		
$AB \rightarrow$	-0.073	0.01
$AD \rightarrow$	-0.043	-0.01
$BD \rightarrow$	0.053	0.045
Three factor interaction:		
$ABD \rightarrow$	-0.023	-0.005

5.7 *Geometrical representation of biomass production*

From inception of the information in Table 6, it was reasonable to conclude that the main effect *B* and *D* were the large and that the I_{AB} and I_{AC} were also significant. This agrees with the conclusion from the analysis of the complete 2^{4-1} design.

Since factor *A* and *C* were not significant, so we may drop them from consideration. Consequently we may project this 2^{4-1} design into a single replicate of the 2^{4-1} design in factors *B* and *D* (Figure 2 and Figure 3). Visual examination of this cube plot made us more comfortable with the conclusion reached above. It was noticed that if the time (*B*) and agitation (*D*) was at high level, the production of biomass had a large positive effect. And where the time and agitation at low level, the biomass production had a very small effect which was likely due to the *BD* interactions. Furthermore, if the time (*B*) was at high level, the concentration (*A*) was negligible.

Based on the above analysis, we obtained a model to predict biomass yield over the experimental region. The model was

$$Y = \beta_0 + \beta_2 \cdot X_2 + \beta_4 \cdot X_4 + \beta_{12} \cdot X_1X_2 + \beta_{13} \cdot X_1X_3$$

Where X_1, X_2, X_3 and X_4 were coded variables that represents *B*, *C* and *D* and the β's were regression coefficient that obtained from the effects estimate as we did previously. Therefore, the prediction equation was for *Saccharomyces cerevisiae* CS,

$$Y = 0.2262 + (0.053/2) \cdot X_2 + (0.063/2) \cdot X_4 + (0.073/2) \cdot X_1X_2 + (0.053/2) \cdot X_1X_3$$

And for *Saccharomyces cerevisiae* MS,

$$Y = 0.1475 + (0.070/2) \cdot X_2 + (0.030/2) \cdot X_4 + (0.045/2) \cdot X_1X_3$$

Remembering that the intercept β_0 is the average of all responses at the eight trials in the design [13].

The model was presented with the geometric presentation and resented in Figure 2 and Figure 3. The geometric presentation helped us to identify the best condition for the maximum cell production. According to Figure 2, the best biomass production of *S. cerevisiae* (CS) (0.39 g·L^{-1}) was obtained using 1% potato powder concentration, orbital agitation at 150 rpm for 25 hrs. According to the Figure 3, the best biomass production of *S. cerevisiae* (MS) (0.23 g·L^{-1})

was obtained using 1% potato powder concentration, orbital agitation at 150 rpm for 25 hrs.
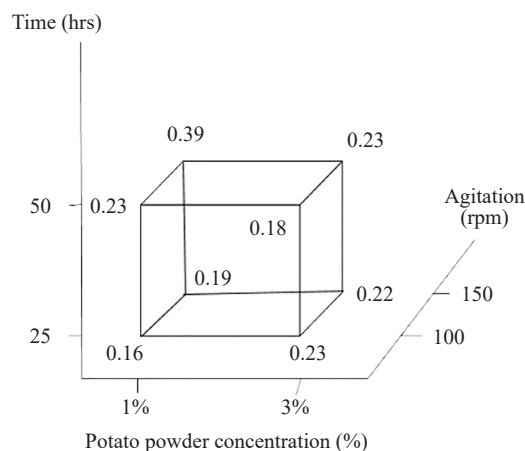

Figure 2. Geometrical representation of the results from the 2^{4-1} design of biomass production of *S. cerevisiae* (CS)
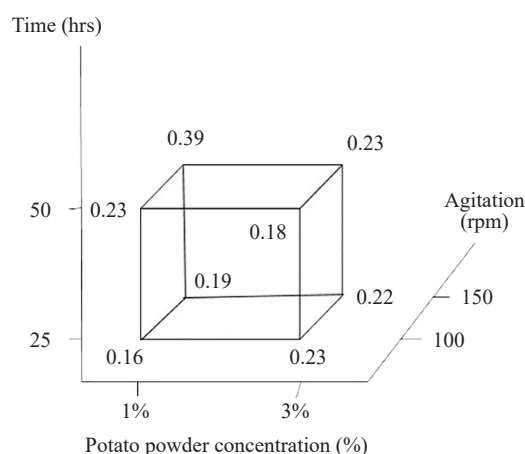

Figure 3. Geometrical representation of the results from the 2^{4-1} design of biomass production of *S. cerevisiae* (MS)

6. Conclusion

The study concludes that both strains' biomass yield and ethanol production were significantly affected by potato flour as a substrate. Four factors of biomass production were analyzed and large effects estimated for developing a model to determine the most suitable condition (at 1% of potato powder concentration, temperature at 30 °C and agitation at 150 rpm) for yeast growth on potato flour. Four variables with the two-level fractional factorial design were used to determine the effects of potato powder concentration, time, temperature, agitation on biomass and ethanol production from two types of yeast strains. The predictive model was developed to describe the relationship between the response and the variables. It allowed the identification of statistically significant variables and the quantitative evaluation of the effect of each variable on biomass production and the interactions between two variables.

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Conflict of interests

The authors declare no competing financial interest.

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