

الاختيار الامثل للظروف التشغيلية لإنتاج الايثانول الحيوي من التمور العراقية ذات النوعية الرديئة

Optimization of Process Parameters for Bioethanol Production from Low grade Iraqi Dates

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Abstract

Box-Wilson experimental design method was employed to optimize bioethanol production from low grade, unclassified, waste Iraqi dates. The optimization process was based on four independent relevant parameters-initial sugar concentration (50-100 g/l), pH (4.5-6.5), fermentation time (48-96 hrs), and temperature (25-35°C). A maximum bioethanol yield of 33.9 g/l was practically achieved following thirty different experimental runs, as specified by 24-Central Composite Design (CCD). The optimum values for the aforementioned four parameters, corresponding to the maximum yield, were: 75g/l, pH 5.5, 72 hrs, 30°C, respectively. The obtained experimental data were utilized to develop a semi-empirical model, based on a second-degree polynomial, to predict bioethanol yield. The model was tested using ANOVA software (Design expert® 9) and found acceptable ($R^2=0.9025$). Yield response surface and contour plots were created using the developed model, which revealed the presence of high-yield plateaus whose specifications will be useful in controlling pilot-or industrial scale future units to ensure economical feasibility.

Keywords: optimization, bioethanol, Low grade Iraqi Dates, Response surface Methodology, ANOVA, Design expert® 9 software.

1. Introduction

The energy needs of the world are rapidly growing leading to the consumption of all the non-renewable energy resources in nature. When the effects of the petroleum crises in the 1970's and the gulf war in 1991 on petroleum reserves are considered, it is clear that there is no other choice for the world but to use the reserves in hand in the best way and to direct efforts towards developing new energy resources.

Renewable energy resources are considered to be "Eco-friendly" and allow countries with no fossil fuel reserves to improvement energy security and independence, [4]. Biofuel is generally viewed upon favorably as a renewable energy resource. One reason this

resource is frequently promoted is that much of the CO₂ released from its combustion is offset by the CO₂ absorbed by the plant through its life cycle to produce biofuel, [13].

One of the most immediate and important applications of biofuel energy systems is the fermentation of ethanol from organic matter (OM). The OM, in the form of carbohydrates, municipal wastes, or livestock manures, is hydrolyzed and then anaerobically fermented to produce ethanol. The ethanol fuel must subsequently be distilled and dehydrated to remove water, [14].

The three major classes of organic feedstocks for ethanol production are sugars (e.g., molasses, cane juice), starches (corn, wheat, cassava) and lignocelluloses (rice straw, wheat straw, bagasse, wood ...etc.). Starch and sugar-based ethanol is often referred to as a first-generation biofuel. Even though the production of ethanol from starch represents the most convenient and technically advanced option for bioenergy, it would result in severe competition between energy and food supplies. Lignocellulosic feedstocks can be acquired from either dedicated crops or forestry and agricultural waste, [7; 22]. The key obstacle for transitioning from starch-based to lignocellulosic biofuels is the complex structure of the cell wall. Current processes for lignocellulosic materials include pretreatment, hydrolysis and fermentation, [19; 8]. These processes are enhancement or replacement crucial for increasing efficiency and for decreasing biofuel production costs. Avoiding pretreatment, along with simultaneous hydrolysis fermentation, are two important factors that would decrease the cost of ethanol production, [19]. Ethanol production is a simple process that can be run either as a continuous process or as a batch reactor.

Fermentation processes from any material that contains sugar could obtain ethanol. Sugars (from sugar beets, sugarcanes, fruits, and molasses) can be changed into ethanol directly. Starches (from potatoes, cassava, root crops, and corn) must first be hydrolyzed to fermentable sugars by the action of enzymes from molds or malt. Cellulose (from paper mills, wood, and agricultural residues) must be converted into sugars, generally by the action of mineral acids.

Once simple sugars are made, enzymes from microorganisms can readily ferment them to ethanol, [1].

Wilkins et al., 2007 reported that ethanol production from simultaneous saccharification and fermentation of citrus peel waste by *S.cerevisiae* was greatest when the pH and fermentation temperature were adjusted to 6.0 and 37 °C, respectively. Higher ethanol yield from kitchen waste using *S. cerevisiae* KF-7 as inoculum were reported at conditions pH 5.5, and fermentation temperature of 35 °C [24].

S.cerevisiae is reported to grow well within the temperature range from 28 to 40 °C, Rivera et al., 2006. The increase in temperature go faster the inhibition effect of ethanol on the cell activities, thereby lowering both ethanol yields and cell, [18]. The deleterious effect of higher temperatures on ethanol produce can be attributed to the denaturation of ribosomes and enzymes and problems associated with the membranes fluidity, [15].

The inhibitory effect of high pH on the bioethanol produce could be due to the lower ATP production during the metabolic changes in *S.cerevisiae*, [17]. Through the investigation of bioethanol production from several waste resources (viz., bread residue, kitchen garbage, citrus peel, and pineapple cannery waste) *S.cerevisiae* was found to grow well within the range of pH 4 – 6, [16; 11; 28; 27].

The date palm (*Phoenix dactylifera*) is a palm of the genus *Phoenix*, extensively cultivated for its edible fruit. Its sap is highly sugar content which varies from (60-70%), and has high nutritive. Limited countries are actively involved in producing biofuel from date palms at a very large scale by setting up various companies aimed at ethanol yield. "Plans Large Refinery, Oman Green Energy Company Makes Ethanol from Date Palm, 100 Ethanol Pumps By 2010", reads a news account, [12]. In Algeria, production of bioethanol from dates by Algerian Biotech Company, [5]

If we compare the sugar content per tonne of crop on dry basis, dates are by far in an advantageous position with 65% compared to sugar cane or beet with 13% and 18%, respectively. Meanwhile, palm trees, sugar beet and sugar cane have different crop densities, the first being a primary crop whilst the other two are secondary crops. As temporary cultivation crops, sugar cane (90t/ha) and sugar beet (60t/ha) are by far the crops which offer the highest possible density. The date palm is a permanent cultivation plant and offers poor density (100–150 date palms per hectare, 22.5 tonnes of dates per hectare).

Palm wine fermentation is always alcoholic-lactic-acetic acid fermentation, including mainly lactic acid bacteria and yeasts.

In the fermenting sap, *S.cerevisiae* is invariably present but lactic acid bacteria such as mesenteroides, *Lactobacillus plantarum* L., or other species of bacteria like *Acetobacter* spp and *Zymomonas mobilis*. Vary, [2]. The other yeast types contain *S. exiguous*, *Saccharomyces chevalieri*, *Schizosaccharomyces pombe*, *Candida* spp.; *Saccharomyces ludwigii*, *S. pombe*, *S.cerevisiae*, and *Hanseniaspora occidentalis* which are described as maximum ethanol makers in toddy, [6]. The yeasts, especially *Saccharomyces* spp., are mostly responsible for the characteristic aroma of palm wine, [26]. Through fermentation, there is continuous effervescence as a result of the making of carbon dioxide. A yeasty odor develops and after a couple of hours yeasts form sediment at the bottom of the container.

The aim of this study is to adopt Box-Wilson design to optimize the process parameters- initial substrate concentration, pH value, fermentation time, and temperature for maximum bioethanol production from low grade, unclassified dates, which are considered as wastes, by *Saccharomyces cerevisiae*. CCD design was applied to reveal the effect and relation among process parameters to attain the aforementioned goal.

2. Materials and Methods

2.1 Material

2.1.1 Media Preparation

Saccharomyces cerevisiae was used for ethanol fermentation. 10 g of sugar (Dextrose), 0.2 g of yeast extract, 1 g of urea, and 1g of Mg SO₄.7H₂O were mixed in 100 ml of makeup water. 1 g of glucose selective yeast, *S. cerevisiae*, was added to the previous mixture in a 250 ml Erlenmeyer flask and properly covered with aluminum foil. The conical flask was then placed in a shaking incubator (Model: SI-600R Lab. Companlon, Korea), at 30°C and 150 rpm for 24 hours. This final mixture was taken as an inoculum for fermentation process.

2.1.2 Substrate

Low grade, unclassified dates, usually used as animal fodder, were selected from the local market in Bagdad-Iraq. The dates were washed using deionized water. Sugar in the dates was extracted by using deionized water with a ratio of 2:5 (w/w sample: water) at 50 °C for two hours to prepare the substrate for the fermentation. Fibers, and suspended solid (S.S) in date extract were removed by centrifugation at 4000 rpm in a centrifuge (Model: Centrifuge PLC Series) for five minutes and then filtered through a Whatmann filter paper. Clear date extract was decanted and collected in 2L plastic bottles.

Different initial sugar concentrations according to experimental design were prepared

and sterilized in an autoclave (Autoclave Model: Sturdy SA-260MA) at 121 °C for 15 minutes.

3. Experimental Design and Data

Analysis

Central Composite Design (CCD), based on four independent process parameters was used to optimize of ethanol production. These important parameters, which affect ethanol fermentation, are initial sugar concentration, x_1 (50-100 g/l); pH, x_2 (4.5-6.5); time, x_3 (48-96 hrs.); and temperature, x_4 (25-35°C). They were select as independent parameters in the experimental design shown in Table (1). Ethanol production (Y) was the dependent output variable.

For statistical calculations all independent variables were coded to four levels as X_i according to Equation 1.

$$X_i = \frac{x_i - \bar{x}_i}{\frac{1}{2}\Delta x_i} \quad (1)$$

where X_i stands for the coded value of the i 'th independent variable, x_i stands for the value of the i 'th variable, \bar{x}_i stands for the midpoint value of the i 'th variable range, and Δx_i stands for the difference of the limiting two values of the i 'th variable. The half value of the difference is the step size. Hence,

$$X_1 = (x_1 - 75) / 25, \quad X_2 = (x_2 - 5.5) / 1, \quad X_3 = (x_3 - 72) / 24, \quad \text{and} \quad X_4 = (x_4 - 30) / 5$$

Central Composite Design is made rotatable by the choice of value for readability ($\alpha = \pm\sqrt{K}$) depending on the number of variables K , Horitsu et al., 1992.

The total number of points N of rotatable design is obtained from $N = 2K + 2K + n_0 = n_j + n_\alpha + n_0$ and is given by the expression $N = (\text{star points}; 2k = 16) + (\text{axial points}; 2K = 8) + (\text{center points } 6)$. The names and levels of the four independent process parameters, upon which thirty experiments of the CCD matrixes were based, are shown in Table 1. Whereas the real and coded range and level values of these variables are given in Table 2.

A second degree polynomial was fitted to the experimental data shown in Table 3 using the statistical software package Design expert® 9 to predict the response of the dependent variable and the optimum values of the four independent variables in the fermentation process. The proposed second-degree polynomial is expressed as follows:

$$Y = \beta_0 + \sum_{i=1}^k \beta_i x_i + \sum_{i=1}^k \beta_{ii} x_i^2 + \sum_{i=1}^{k-1} \sum_{j=i+2}^k \beta_{ij} x_i x_j + e_i \quad (2)$$

where Y is the response, β_0 is the intercept (offset) term, $\beta_i, \beta_{ii}, \beta_{ij}$ are the first-order, quadratic, and interaction effects, respectively; i and j are the index numbers for a parameter; and

e_i is the residual error. This polynomial leads to the graphical representation known as Response Surface Method (RSM).

Glucose concentration: Different known masses of glucose from 0 to 150 mg were measured, introduced into 1000ml of water and mixed thoroughly to obtain various glucose concentrations. Fig. 1 shows the absorbance of each concentration was measured using a spectrophotometer and a calibration line was produced by plotting absorbance verses concentration. The absorbance of the experimental samples were measured and the corresponding mass concentrations of glucose in the samples found from the calibration line.

Ethanol production concentration: Various volumes of standard ethanol (98%) were measured and their corresponding masses found. These volumes were each introduced into 1000 ml of water, mixed thoroughly and their absorbance measured using a spectrophotometer. Fig. 2 a calibration line was subsequently formed from a plot of absorbance against mass concentration of ethanol.

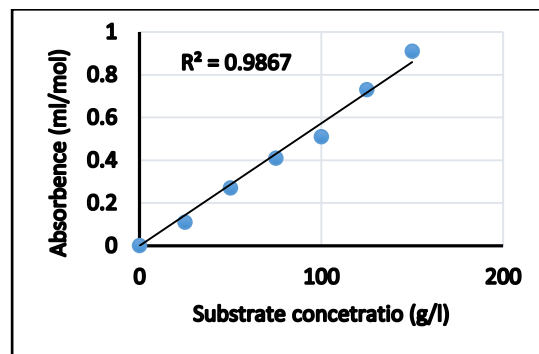


Figure 1: Calibration line for glucose concentration

The absorbance of experimental samples were measured and the corresponding mass concentrations of ethanol in these samples were obtained from the calibration curve.

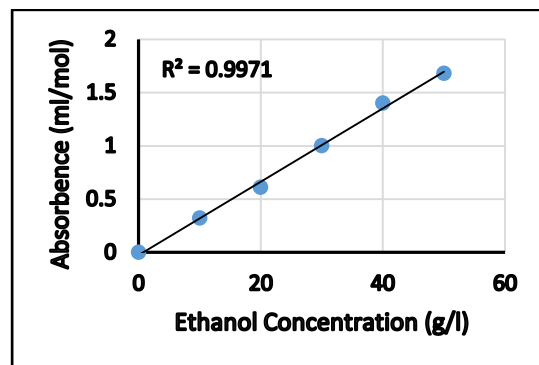


Figure 2: Calibration line for ethanol concentration.

4. Result and Discussion

4.1 Statistical Analysis

The thirty experimental trials, as specified by 24 – CCD, along with their results are shown in Table 3.

Comparison of these values specifies that there is good agreement between predicted and experimental values over the considered range.

The predicted values of Table 3 were obtained from the aforementioned second-degree polynomial utilizing the experimental values and using multiple-linear regressions. Analysis of variance, using the software ANOVA, was carried out to assess the significance of fit of the second-degree polynomial. Results of this analysis are shown in Table 4.

A model F-value of 3163.32 (Table 4) and a very low probability value [(Probability > F) less than 0.001] indicate significant model fit. In addition, the model did not show lack of fit and presented a high determination coefficient R2 equal to 0.9025 (a value > 0.75 indicates aptness of the model, Chauhan et al., 2004). This indicates that 90.25% of the variability is explained by the model. Hence, the predicted values of bioethanol yield were obtained by using Equation (3)

$$\text{Ethanol Production (y)} = 33.68 + 1.02 x_1 - 1.15 x_2 + 0.69 x_3 + 1.30 x_4 + 1.66 x_1 x_2 - 1.28 x_1 x_3 + 0.031 x_1 x_4 - 1.47 x_2 x_3 - 1.28 x_2 x_4 - 0.34 x_3 x_4 - 3.72 x_1^2 - 8.54 x_2^2 - 5.35 x_3^2 - 1.87 x_4^2 \quad \text{-----(3)}$$

Also, values of "Probability> F" less than 0.05 specify that the model terms are significant. This means that the offset term, the linear coefficients of xi, the quadratic coefficients of xixj, and the coefficients of xi2 in Eq. (3) are all significant with a probability of 95%. Furthermore, the significance of the x1x2 term indicates interaction between the initial sugar concentration and the pH value. The model's statistical significance explains the good agreement between experimental and predicted values of the yield in all thirty experiments as shown in Table 3.

The results also show that when the values of the process parameters increased from the lower limits of their ranges, the bioethanol yield also increased reaching a maximum at their ranges' midpoints. Thereafter, the yield decreased with increasing values of the parameters. This is due to the growth-inhibiting effect of the high sugar concentration as well as product-formation which possibly distorts microorganisms' metabolism-poisonous effect, [25]. Additionally, although bioethanol yield is significantly affected by all four parameters, the initial sugar

concentration and pH value had a greater impact than fermentation time and temperature.

4.2 Three-dimensional graphical representation of bioethanol production optimization

To elucidate the optimum conditions for bioethanol production, the second-degree polynomial model was used to generate response surface plots by RSM (response surface method). The three-dimensional plots were created by keeping two of the variables constant at their midpoints and changing the other two variables within their experimental range. The resulting response surfaces showed the effect of initial substrate (glucose) concentration, pH value, fermentation time, and temperature on bioethanol yield. Fig. 3 shows the corresponding contour and response surface plot for bioethanol yield as a function of initial substrate concentration and pH value. It can be seen that a high-yield plateau exists in the surface over the initial substrate concentration and pH ranges of 70-80 g/l and 5.25-5.75, respectively.

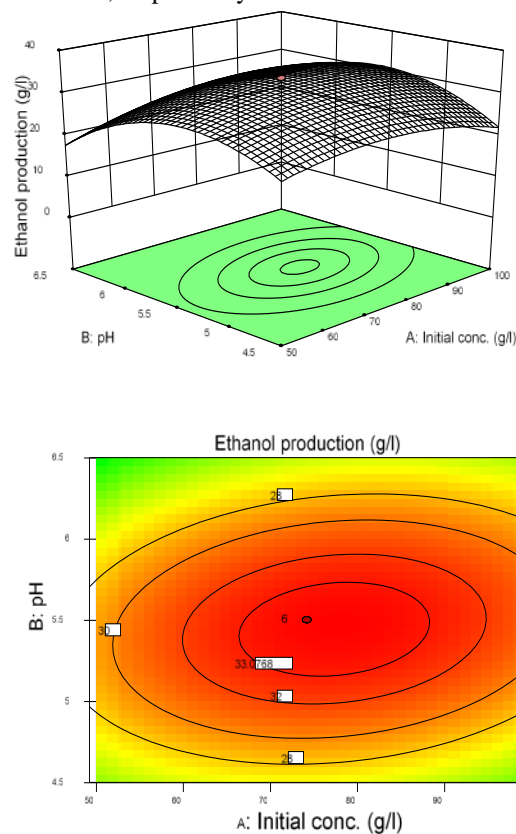


Figure 3: Interaction effects of initial concentration and pH on ethanol concentration: surface plot; contour plot.

This plateau is represented by the innermost contour. The plateau's peak corresponds to an ethanol yield of 33.9 g/l. This is in conformity with the findings [23].

The effect of initial sugar concentration and temperature on the bioethanol yield, as a response surface and contour plot, is presented by Fig. 4. It can be observed that a high-yield plateau exists in the surface over an initial sugar concentration range of 70-85 g/l and a temperature range of 29 - 32 °C.

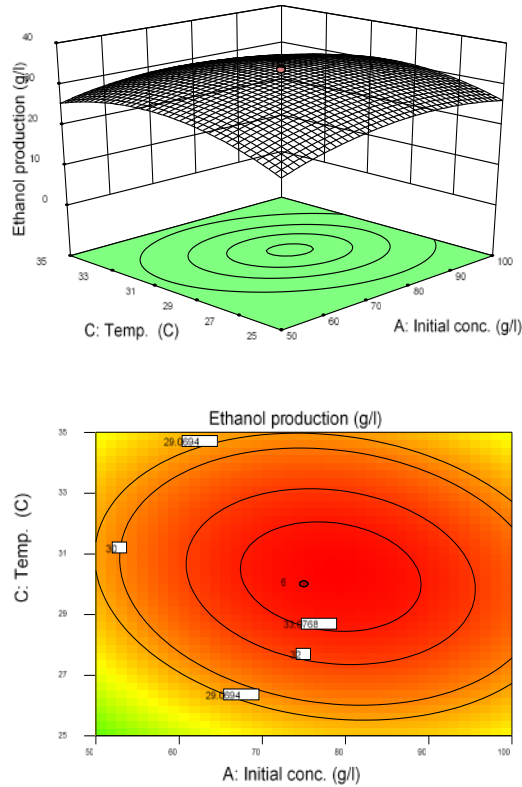


Figure 4: Interaction effects of initial concentration and temperature on ethanol concentration: surface plot; contour plot.

Figure 5 shows the response surface representing the interaction between initial sugar concentration and fermentation time on bioethanol production. A plateau exists over an initial sugar concentration and time ranges of 75-82 g/l and 72-85 hr, respectively. Max. bioethanol yield was obtained for an initial sugar concentration 79 g/l and a fermentation time of 80 hours. This is in agreement with the work of [21].

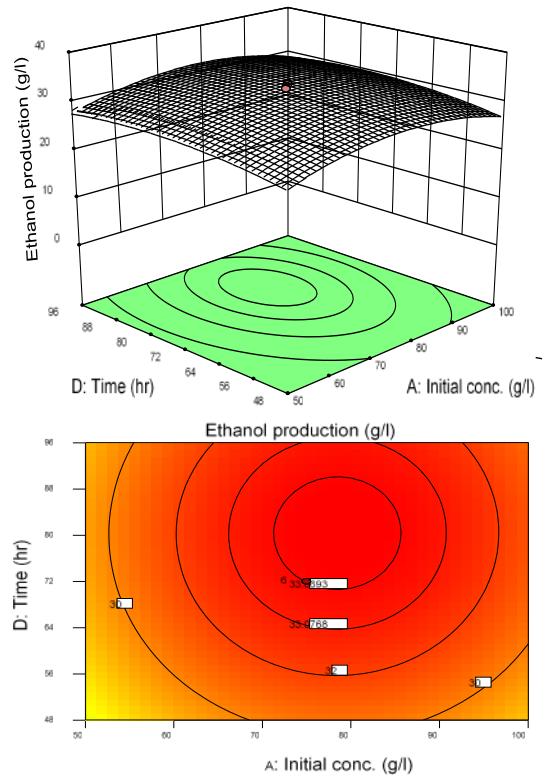
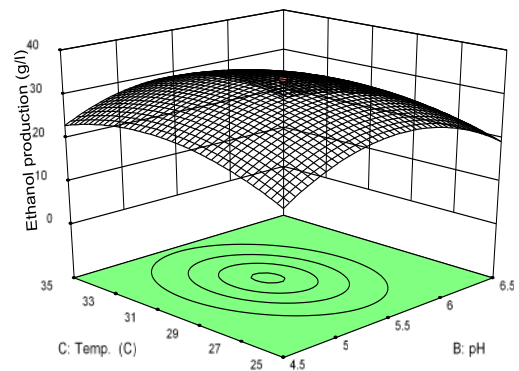


Figure 5. Interaction effects of initial concentration and fermentation time on ethanol concentration: surface plot; contour plot.

Figure 6 shows the convex response surface of temperature and pH interaction on bioethanol yield. Its plateau is over a temp. range of 29-31°C and pH range of 5.25-5.75 with a peak at 30°C and pH of 5.5 corresponding to max. bioethanol yield. This is in agreement with [28] and [24]. The yield decreased with increasing temperature and pH. The inhibition effect of ethanol on cell activities accelerates with the increase in temperature, thereby lowering both ethanol yields and, cell [18].



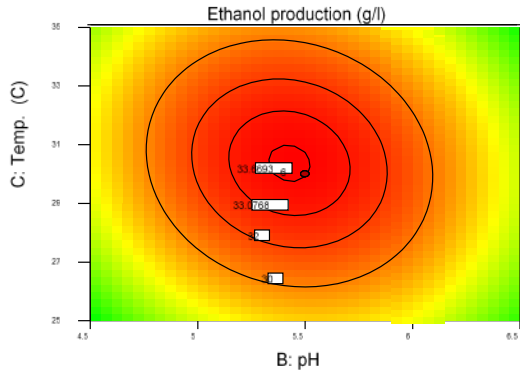


Figure 6. Interaction effects of pH and temperature on ethanol concentration: surface plot; contour plot.

The inhibitory effect of high pH level on bioethanol produce could be due to lower ATP production during metabolic variations in *S.cerevisiae*, [17].

The interaction of fermentation time and pH value on bioethanol yield is shown, as response surface and contour plot, in Fig. 7. The high-yield plateau corresponds to a pH range of 5.25-5.75 and fermentation time range of 72-88 hours.

The interaction of fermentation time and temperature on bioethanol yield Fig. 8 shows that the fermentation process should be carried out at a temperature in the range of 29 to 31°C for a time duration of 72 to 88 h to achieve high yield of bioethanol. This is in agreement with the work of [10].

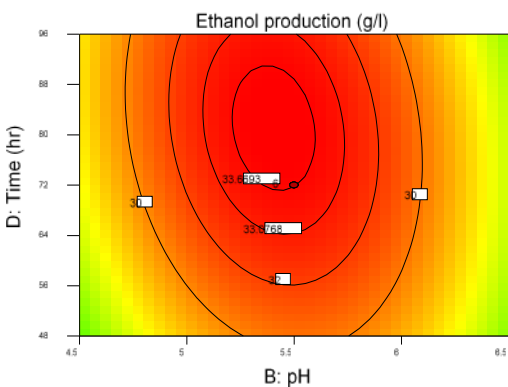
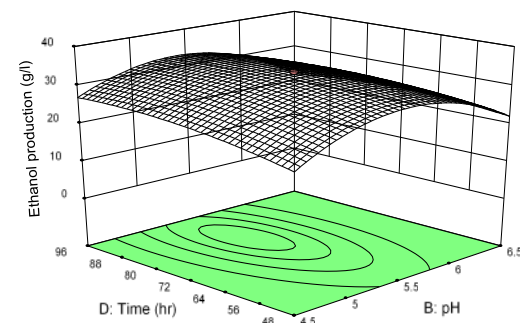


Figure 7. Interaction effects of pH and time on ethanol concentration: surface plot; contour plot.

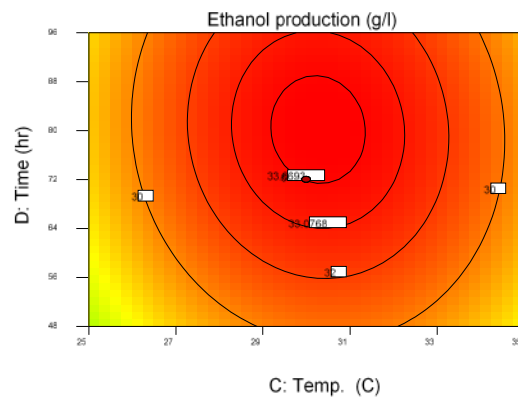
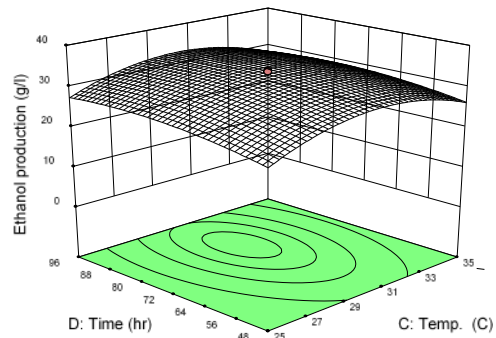


Figure 8. Interaction effects of temperature and time on ethanol concentration: surface plot; contour plot.

The results of the influence and interaction of the initial substrate concentration, pH-value, fermentation time, and temperature using CCD indicate that the highest yield could be achieved near the ranges' midpoints as shown on the contour pots **Figs. 3-8**. The optimum condition values were 75g/l, pH 5.5, 72hr, and 30°C. This showed that the model, Eq. (3), is useful. The results obtained from three replications demonstrated that the maximum experimental bioethanol yield (33.9 g/L) was close to the predicted value (33.68 g/L). This result indicates the validity of the model.

An additional advantage of the response surfaces and contour plots, particularly the high-yield plateaus and their sizes, would be the specifications for a pilot or an industrial-scale unit control loop(s) to ensure high-yield ethanol production at all times.

5. Conclusion

- 1- Experimental design Box-Wilson method was seen to be applicable and appropriate for optimizing the yield of bioethanol from Low grade Iraqi dates by fermentation.
- 2- The optimization process, based on four independent relevant parameters- initial sugar concentration, pH, fermentation time,

and temperature, resulted in an actual maximum bioethanol yield of 33.9 g/l. A semi-empirical bioethanol yield predictive model, based on a second-degree polynomial, was developed and successfully tested (via ANOVA software) against experimental result obtained from thirty experiments specified by 2⁴-CCD (R²=0.9025).

- 3- The optimum conditions, resulting in maximum experimental bioethanol yield of 33.9 g/l (predicted value 33.68 g/l) were: initial sugar concentration Of 75 g/l, pH of 5.5, fermentation time of 72 hrs, and 30°C temperature.
- 4- The predictive model was utilized to generate yield response surfaces and contour plots, by the RSM method, which revealed the presence of high-yield plateaus whose specifications will be useful in controlling pilot-or industrial scale future units to ensure economically worthy yields at all times.

5. References

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Table 1: Names and levels of process factors (parameter).

Factor	Units	Low Level (-1)	High Level (+1)
A-Initial sugar concentration	g/l	50	100
B-pH	----	4.5	6.5
C-Time	h	48	96
D-Temperature	°C	25	35

Table 2: Real and coded range and level values for the four independent variables.

Independent Parameters	Range and level					
	Coded	-2	-1	0	+1	+2
Real						
Initial sugar conc.		25	50	75	100	125
pH		3.5	4.5	5.5	6.5	7.5
Time		24	48	72	96	120
Temperature		20	25	30	35	40

Table 3: Experimental and predicted values of bioethanol production based on four-parameter CCD.

Run No.	Parameters								Yield	
	Coded values				Actual values				Bioethanol production g/l	
	X ₁	X ₂	X ₃	X ₄	Initial Conc.g/l	pH	Temp. °C	Time hrs.	Experimental	Predicted
1	-1	-1	1	1	50	4.5	35	96	22	23.67
2	1	1	-1	1	100	6.5	25	96	15	18.18
3	0	0	0	0	75	5.5	30	72	33.6	33.68
4	-1	1	1	1	50	6.5	35	96	8	10.51
5	0	0	0	0	75	5.5	30	72	33.8	33.68
6	0	0	0	0	75	5.5	30	72	33.6	33.68
7	1	1	-1	-1	100	6.5	25	48	12	17.39
8	0	-2	0	0	75	3.5	30	72	4.5	1.82
9	0	0	0	-2	75	5.5	30	24	27	21
10	1	-1	-1	1	100	4.5	25	96	15	16.78
11	0	-2	0	0	75	3.5	30	72	3	1.82
12	-1	-1	-1	-1	50	4.5	25	48	6	9.66

13	-1	1	1	-1	50	6.5	35	48	8	11.22
14	0	0	0	0	75	5.5	30	72	33.9	33.68
15	1	-1	1	-1	100	4.5	35	48	12	15.87
16	1	-1	-1	-1	100	4.5	25	48	10	10.88
17	2	0	0	0	125	5.5	30	72	28	20.84
18	-1	1	-1	1	50	6.5	25	96	6.5	12.14
19	1	1	1	1	100	6.5	35	96	12	13.38
20	1	1	1	-1	100	6.5	35	48	11	13.95
21	0	0	0	0	75	5.5	30	72	33.7	33.68
22	-1	-1	1	-1	50	4.5	35	48	17	17.22
23	-1	-1	-1	1	50	4.5	25	96	15	12.83
24	0	0	0	0	75	5.5	30	72	33.6	33.68
25	-2	0	0	0	25	5.5	30	72	18	16.76
26	1	-1	1	1	100	4.5	35	96	12	18.17
27	0	0	-2	0	75	5.5	20	72	15	10.9
28	0	0	0	2	75	5.5	30	120	33.8	33.68
29	-1	1	-1	-1	50	6.5	25	48	12	9.54
30	0	0	2	0	75	5.5	40	72	18	13.66

Table 4. ANOVA results for employed quadratic model.

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F
Model	2829.87	14	202.13	9.91	< 0.0001
<i>A-in. conc</i>	25.01	1	25.01	1.23	0.2855
<i>. B-pH</i>	31.51	1	31.51	1.55	0.2329
<i>C-Temp</i>	11.34	1	11.34	0.56	0.4673
<i>. D-Time</i>	40.30	1	40.30	1.98	0.1801
<i>AB</i>	43.89	1	43.89	2.15	0.1630
<i>AC</i>	26.27	1	26.27	1.29	0.2742
<i>AD</i>	0.016	1	0.016	7.663E-004	0.9783
<i>BC</i>	34.52	1	34.52	1.69	0.2129
<i>BD</i>	26.27	1	26.27	1.29	0.2742
<i>CD</i>	1.89	1	1.89	0.093	0.7649
<i>A^2</i>	380.38	1	380.38	18.66	0.0006
<i>B^2</i>	1998.75	1	1998.75	98.03	< 0.0001
<i>C^2</i>	784.77	1	784.77	38.49	< 0.0001
<i>D^2</i>	96.32	1	96.32	4.72	0.0462
Residual	305.84	15	20.39		
<i>Lack of Fit</i>	305.79	10	30.58	3163.32	< 0.0001
<i>Pure Error</i>	0.048	5		9.667E-003	
Cor Total	3135.71			29	
Std. Dev.	4.52		R-Squared		0.9025
Mean	18.10		Adj R-Squared		0.8114
C.V. %	24.95		Adeq Precision		11.412
PRESS				1761.41	

الاختيار الامثل للظروف التشغيلية لانتاج الايثانول الحيوي من التمور العراقية ذات النوعية الرديئة

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الخلاصة

طريقة بوكس ولسن لتصميم التجارب استخدمت لايجاد الظروف المثلى لانتاج الايثانول الحيوي من التمور العراقية رديئة النوعية الغير مصنفة (تعتبر نفايات). عملية الاختيار الامثل لاربع متغيرات –التركيز الابتدائي للسكر(50-100 غم/لتر)، الدالة الحامضية (4.5-6.5)، زمن التخمير(48-96 ساعة)، ودرجة الحرارة(25-35 مئوية). اقصى انتاج للايثانول الحيوي 33.9 غم/لتر تم الحصول عليه من خلال ثلاثون تجربة مختبرية، المعرفة ب 2⁴ التصميم الدوار المركب المركزي (CCD). القيم المثلى للمتغيرات المذكورة أعلاه، اعتمادا على اقصى انتاجية: 75 غم/لتر، الدالة الحامضية 5.5، 72 ساعة، 30 درجة مئوية على التوالي. البيانات التجريبية الت تم الحصول عليها استخدمت لتطوير نموذج تطبيقي، معادلة متعددة الحدود من الدرجة الثانية للمتغيرات الاربعه، للتنبؤ بانتاجية الايثانول الحيوي. هذا النموذج تم اختباره باستخدام تحليل التباين (ANOVA) وباستخدام Design expert® 9 software ووجد انه مقبول ب(R²=0.9025). الاستجابة السطحية والاشكال الكنتورية التي تم الحصول عليها باستخدام النموذج، والتي تبين اعلى انتاجية بالهضبة الموجودة والتي يمكن الاستفادة منها واستخدامها للسيطرة على المنظومات الريادية والوحدات الصناعية المصنعة لاحقا ولضمان الجدوى الاقتصادية.