



## Optimization of Bioethanol Production from Biodegradable Municipal Solid Waste using Response Surface Methodology (RSM)

Dr. Nadhim H. Hayder<sup>1</sup>, Dr. Hussain M. Flayeh<sup>2</sup>, \*Ali W. Ahmed<sup>3</sup>

- 1) Assistance Professor, Biotechnology Department, Baghdad University, Baghdad, Iraq.
- 2) Lecturer, Environmental Engineering Department, Baghdad University, Baghdad, Iraq.
- 3) B.Sc. environmental engineering department, Environmental Engineering Department, Baghdad University, Baghdad, Iraq.

**Abstract:** In this work the production of bioethanol from lignocellulosic biodegradable municipal solid waste (BMSW), was studied. The maximum yield of reducing sugar was 4102.27 mg/L with optimum conditions; initial concentration 4%, pH 6, incubation time 16 hrs., and inoculum 2%. Response surface methodology (RSM) was employed to optimize the process parameters of bioethanol production. Maximum bioethanol yield 332.9 mg/L, was practically achieved following thirty different experimental runs, as specified by 2<sup>4</sup> –full factorial central composite design (CCD). The optimum values for the aforementioned four parameters, corresponding to the maximum yield. were; initial sugar weight = 75 g/L, pH = 6, fermentation time = 39 hrs. (aerobic fermentation = 24 hrs. and anaerobic fermentation = 15 hrs.), and finally yeast inoculum = 2 mL/L. The obtained data were utilized to develop a semi-empirical model, based on a second degree polynomial, which help to predict bioethanol yield. The model was tested using ANOVA software (Design Expert 10) and the (R<sup>2</sup>= 0.9771), which is acceptable. The develop model using to generate contour plots and yield response surface. Maximum bioethanol production was observed in lab scale bioreactor reached to 492.9 mg/L within optimum conditions.

**Keywords:** Biodegradable municipal solid waste, Bioethanol and Bioreactor.

### تحديد الظروف المثلى لإنتاج الوقود الحيوي من المخلفات البلدية القابلة للتحلل باستخدام منهجية (RSM)

**الخلاصة:** هدفت الدراسة الحالية الى إنتاج الوقود الحيوي (البايو إيثانول) من مخلفات المدن الصلبة القابلة للتحلل وعلى وجه الخصوص من المخلفات السليلوزية الممزوجة مع بعضها البعض. بينت النتائج بان اعلى تركيز للسكر المختزل من المخلفات السليلوزية بلغ 4102.27 ملغم/لتر في الظروف المثلى, التركيز الابتدائي للمخلفات السليلوزية 4%, الدالة الحامضية 6, فترة الحضارة 16 ساعة وحجم اللقاح 2%. تم استخدام طريقة (Response surface methodology) لتحديد الظروف المثلى لإنتاج البايوإيثانول, اذ بلغ اعلى إنتاج 332.9 ملغم/لتر, و تم التوصل لهذه النتيجة من خلال 30 تجربة مختبرية وفق مبدأ (central composite design). بينت التجارب بأن الظروف المثلى والتي سجلت اعلى مقدار للبايوإيثانول عند التركيز السكر 75 غرام/لتر للسكر المختزل, الدالة الحامضية 6, فترة الحضارة 39 ساعة (24 ساعة للظروف الهوائية و15 ساعة للظروف اللاهوائية) وعند استخدام حجم اللقاح 2 مللتر/لتر. تم استخدام النتائج التي تم الحصول عليها في تحليل وتكوين دالة رياضية متعددة من الدرجة الثانية والتي ستساهم بدرجة كبيرة في تخمين إنتاج البايو إيثانول. تم اختبار الدالة العددية بواسطة برنامج (ANOVA) وكانت قيمة (R<sup>2</sup>) هي 0.9771 والتي تعتبر ذات مقبولية عالية جداً. كما استخدمت الدالة المتعددة في تمثيل البيانات بخطوط كنتورية وكذلك اسطح مجسمة ثلاثية الأبعاد. كما بينت النتائج بان اعلى إنتاج للبايوإيثانول بلغ 492.9 ملغم/لتر باستخدام المفاعل الحيوي المختبري عند الظروف المثلى.

\*[ali\\_17\\_11@yahoo.com](mailto:ali_17_11@yahoo.com)

## 1.Introduction

The major source of energy comes from non-renewable fossil fuel that caused global warming, environmental degradation, and human health problems [1]. The growing energy demands encourage scientists to explore low cost, environmental friendly and sustainable alternative energy sources [2]. Bioethanol is one of the promising future energy alternatives contributing to the reduction of negative environmental impacts generated by the use of fossil fuels [3]. The ethanol market is expected to reach a level equivalent to 10-20% of the gasoline consumption by 2030 [4]. The demand for ethanol is increasing nowadays, due to it is different uses, such as chemical feed stock and more majority as an alternative source of liquid fuel for automobiles.

A wide range of biomass can be used as a feedstock for ethanol production, fermentable sugary and starchy substrates to lignocellulosic biomass after some special process. The feedstock cost typically represents more than 50% of the total production cost, and it is the driving factor for researching the potentials of low-cost lignocellulosic biomass for ethanol fermentation [5]. The replacement of biomass with biodegradable municipal solid waste (BMSW) can bring environmental advantages particularly waste management, carbon dioxide cut, water quality and quantity control, land use and biodiversity [6]. For instance, Kádár *et al.* [7] examined the simultaneous saccharification and fermentation of waste cardboard and paper sludge produces ethanol in the range of 0.31-0.34 g/g waste. The bread residues can be fermented to get the ethanol yield around 0.35 g/g substrate [8]. Wilkins *et al.*[9] reported that the citrus peel waste can undergo steam explosion process and subsequently consumed by the *Saccharomyces cerevisiae* to get ethanol yield of ~ 0.33 % (v/v).

Response surface methodology (RSM) is one such scientific approach that is useful for developing, improving and optimizing processes and is used to analyze the effects of several independent variables on the system response, main objective being the determination of optimum operational conditions within the operating specifications [10]. The main advantage of RSM is reduced number of experimental runs needed to provide sufficient information for statistically acceptable results, its suitability for multiple factor experiments and exploration of common relationship between various factors towards finding the most appropriate production conditions for the bioprocess and forecast response [2]. In this study, the response surface methodology (RSM) based on central composite design (CCD), were applied to estimate the number of runs and optimum conditions for four independent variables that effecting fermentation process. The processes will cover some lignocellulosic materials which is usually exist in the organic fraction of Iraqi municipal solid waste, and the independent variables will be initial concentration, pH, inoculum and fermentation time. In fermentation process the *S. cerevisiae* will be used as an inoculum. Fermentor used as final step. The aim of current study is bioethanol production from the cellulosic biomass under controlled optimum conditions. All the experiments done at the Environment and Biotechnology laboratory, college of science, university of Baghdad, Bagdad, Iraq, during the period September 2015 to May 2016.

## 2. Material and Methods

### 2.1 Substrate and Media Preparation

#### 2.1.1 Substrate Preparation

Sample collection starts with collecting Banana peels, Orange peels, Lemon peels, Citrus peels, yellow apple peels, red apple peels, wheat and corn residuals. These materials were dried under the sun rays for few days – approximately 3 to 4 days with temperature equal to 30 to 35°C – till it completely dried. The materials grinded separately and sieved by 200 µm sieving to get uniform particles less than or equal to previous size. The equal weight from each substance was mixed together to obtain the final biomass source and used as lignocellulosic waste.

#### 2.1.2 *Pseudomonas sp. isolates*

Sixty-five isolates of *Pseudomonas sp.* were collected and identified using EPI test. The isolates were maintained on a nutrient agar and stored at 4°C.

#### 2.1.3 Screening of cellulose degrading *Pseudomonas* isolates for cellulase production

This test was done according to Kasana *et al.* [11]. Mineral salt medium (MSM) carboxymethylcellulose agar (CMC) 0.5 g/L, NaNO<sub>3</sub> 0.1 g/L, K<sub>2</sub>HPO<sub>4</sub> 0.1 g/L, MgSO<sub>4</sub>.H<sub>2</sub>O 0.05 g/L, yeast extract 0.5 g/L, agar 15 g/L, was conducted for cellulose degradation efficiency test. The isolates were grown on CMC agar at pH= 7, were spot inoculated with pure isolates over solid medium and incubated at 30°C for 2 days, to allow for secretion of cellulase. To indicate bioconversion rate activity of the organisms, diameters of clear zone around colonies on CMC agar were measured. The cultures which gave the largest clear zone more than 10 mm were selected for further studies. Secondary screening of isolate was conducted in MSM supplemented with 1% of CMC in submerged fermentation at pH 7, Temperature 30 °C in shaker incubator (150 rpm) for 72 hrs. At the end of incubation time bioconversion rate and reducing sugar concentration were measured.

### 2.2 The effect of environmental and cultural parameters on hydrolysis of lignocellulosic waste

Sugar production depends upon the composition of the fermentation media. Medium optimization for over production of the reducing sugar is an important step that involves a number of physio-chemical parameters such as the incubation period, pH, and initial concentration. For the initial optimization of the medium, the traditional method of “one variable at a time” approach was used by changing one component at a time while keeping the others at their original level. The selected *Pseudomonas* isolate was grown in nutrient broth for 48 hr at 30 °C, this culture was used as stock culture inoculums at concentration of 2% (V/V). For optimum conditions of reducing sugar production, Rahna *et al.* [12] supplemented with 1% cellulosic waste powder as a sole source of

carbon. The pH of the medium was adjusted to 7.0. cultivation was performed in 250 ml flasks containing 50 mL medium at 30 °C and stirred in a rotary shaker incubator 150 rpm. At the end of each experiment, the reducing sugar concentration and bioconversion percentage were determined.

### *2.2.1 Effect of incubation time on reducing sugar production*

Time course (incubation period) of reducing sugar production was studied. The isolate was grown on mineral salt medium containing 1% cellulosic powder, at different incubation period (0-36) hr at pH 7 and 30°C in shaker incubator at 150 rpm.

### *2.2.2 Effect of pH on reducing sugar production*

The effect of pH on reducing sugar production was studied. MSM containing 1% cellulosic powder was adjusted to different initial pH (5, 6, 7, 8 and 9), and incubated at optimized incubation period 16 hr in shaker incubator (150 rpm) at 30 °C.

### *2.2.3 Effect of different initial concentration of cellulosic waste on reducing sugar production*

Different concentration of cellulosic waste for reducing sugar production was conducted. MSM containing different concentrations of cellulosic wastes (1, 2, 3, 4, 5 and 6%) (w/v) were used. In all experiments above fermentation was carried out for 16 hr in shaker incubator (150 rpm) at 30 °C. The cell-free culture supernatants were assayed for reducing sugar.

### *2.2.4 Effect of inoculum concentration on reducing sugar production*

Different concentrations of inoculum included (0.5, 1, 2, 3, 4, and 5%), were tested to estimate the best inoculum concentration for reducing sugar production. MSM contained 4% of cellulosic waste at pH 6 was inoculated with above concentrations of inoculums and incubated at shaker incubator for 16 hr at 150 rpm and 30 °C.

## **2.3. Measurement of reducing sugar**

The reducing sugar in fermentation broth filtrate was estimated by using suitable glucose oxidase kit, Cromatest MR 4×250 by Linear Chemicals was used, date of production 2015. The calculations conducted to manufactures procedures, which is briefly, the blank was prepared by adding 3mL from reagent (R1), and the standard was prepared by adding 30μL of glucose solution to 3mL reagent, 30 μL of each sample (supernatant) was added to 3ml of reagent (R1). These solutions were mixed well and incubated for 10 minute in room temperature, the absorbance of samples was measured at 500nm, then the reducing sugar concentration was calculated by the following equation:

$$\text{Glucose concentration mg/dL} = \frac{\text{absorbance reading for sample}}{\text{absorbance reading for standard}} \times C_{\text{standard}} \quad (1)$$

#### 2.4. Measurement of bioconversion rate of cellulosic waste

The experiment conditions were carried out as previously mentioned. After the incubation time, the culture broth was centrifuged at 8000 rpm for 15 min, the precipitate was transferred to previously weighted container. The container with the residues was dried in the oven at 70°C for a constant weight and reweighted. The difference between the initial and the final weights gave the amount of cellulosic substrate degraded by the isolate. The bioconversion rate was calculated by:

$$\text{Bioconversion percentage (\%)} = \frac{(\text{Dry weight} - \text{empty weight})}{\text{sample volume}} \times 100 \quad (2)$$

#### 2.5 Fermentation and ethanol production in a bioreactor

The influences of initial substrate concentration (*reducing sugar*), pH, inoculum dosage, and fermentation time were studied to select the optimum conditions for fermentation and ethanol production in a bioreactor.

##### 2.5.1 Statistical design of experiments

Central Composite Design (CCD), based on four independent process parameters was used to optimize the ethanol production. These important parameters, which affect ethanol fermentation, are initial substrate concentration,  $x_1$  (50-100 g/l); pH-value  $x_2$  (4-8); inoculum  $x_3$  (1-3 ml); and fermentation time  $x_4$  (24-54 hr) were conducted (Table 1) using the experimental design obtained by full factorial center compost design (CCD). The variable were coded according to the Eq.

$$X_i = \frac{x_i - x_0}{\Delta x_i} \quad (3)$$

Where,  $X_i$  is the coded value of variable  $i$ ,  $x_i$  the real value of an independent variable,  $x_0$  the midpoint value of the  $i$ 'th variable range, and  $\Delta x_i$  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,

$$X_1 = \frac{(x_1 - 75)}{25}, \quad X_2 = \frac{(x_2 - 6)}{2}, \quad X_3 = \frac{(x_3 - 2)}{1}, \quad \text{and} \quad X_4 = \frac{(x_4 - 39)}{15}$$

A complete  $2^4$ -factorial design, (ii)  $n_0$ , center point ( $n_0 > 1$ ) and (iii) two axial points on the axis of each design variable at a distance of  $\alpha = \pm\sqrt{K} = 2$ , depending on the number of variables  $K$  from the design center. Hence a total number of design points of  $N = 2^K + 2K + n_0 = n_j + n_\alpha + n_0$  and is given by the expression  $N =$  (star points;  $2^k = 16$ ) + (axial points;  $2K = 8$ ) + (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.

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	8
C-inoculum	ml	1	3
D-fermentation time	hr	24	54

Table 2. Real and coded ranges and levels values for independent variables.

Independent Parameters	Ranges and Levels					
	Coded	-2	-1	0	+1	+2
Initial concentration	Real	25	50	75	100	125
pH	Real	2	4	6	8	10
Inoculum	Real	0	1	2	3	4
Fermentation time	Real	9	24	39	54	69

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:

$$Y = \beta_0 + \sum_{i=1}^k \beta_i x_i + \sum_{i=1}^k \beta_{ii} x_i^2 + \sum_{i=1}^{k-1} \sum_{j=2}^k \beta_{ij} x_i x_j + e_i \quad (4)$$

where Y is the response,  $\beta_0$  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).

The optimum condition values were used in the LAMBDA MINIFOR bench-top laboratory fermenter to study the bioethanol production, the biomass percentage and remaining sugar with fermentation time.

### 2.5.2. *S. cerevisiae* isolates for bioethanol production

Four different types of *S. cerevisiae* isolates were collected from different markets. Two of these samples took from two different local shops producing Iraqi bread. The others samples were brought from industrial dried yeast used in bakery and pastry productions. For the *S. cerevisiae* cultivation Potato Dextrose Agar (PDA) was prepared. Using 200g of potato extract suspended in 1000mL of distilled water and boiled for 30 min.

The potato then separated by slight squeeze. The extent stored in a large glass container and then 20g agar added with 20g glucose with slow heating until the agar was dissolved. The pH was adjusted to 5.6 and the mixture autoclaved. The medium then ready to use for cultivation and maintained yeast isolates.

### 2.5.3 Fermentor uses for bioethanol production

The LAMBDA MINIFOR bench-top laboratory fermenter was used for bioethanol production in optimized conditions. The main specifications of this model are easy to use and with the capacity to measure and control all the important parameters of the biological culture. Also it had to take up minimum space on the bench but with good access to all parts. Total volum of the bioreactor was 5L with working volum 3.75L. The bioreactor is equipped with different probs for pH, Temperature, air and mixing with completely controlled system (Figure 1).



Figure 1. LAMPADA Fermentor

### 2.5.4 Fermentation conditions for bioethanol production in lab scale bioreactor

Fermentation were performed as described by Ciani and Maccarlli [13], with some modification. Pure glucose was used as a substrate instead of cellulosic waste to ferment with yeast isolate *S. cerevisiae*, due to some operational and cultural difficulties as viscosity of cellulosic waste if it's used at higher concentration. The suggested conditions which obtained from design expert 10 were used in the fermentation process in the bioreactor. The MSM medium (3.5L) was supplemented with optimum conditions of design expert, and 0.5% yeast extract. The culture was inoculated by transferring of 2 % (v/v) of activated *S. cerevisiae* containing (OD= 0.5,  $1.5 \times 10^8$  CFU/mL) yeast cells. The fermentation process left under aerobic conditions in the bioreactor for 24 hr at 30°C multiplications of cells and then under anaerobic conditions for 70hr at 30°C. During the fermentation the samples were withdrawn after 0, 20, 24, 44, 48, 67 and 70hr of incubation. The samples of different period of fermentation in duplicates were taken and centrifuged at 8000 rpm for 15min to estimate biomass, total reducing sugar and ethanol. Ethanol concentrations were determined by using HPLC (RID, shimadzu, Japan). The column used in HPLC analysis was trans genomic, USA (250×4.6 Id) with



mobile phase 10% (1% trifluoroacetic acid) and 90% acetonitrile with flow rate 1L/min keeping column oven temperature at room temperature with UV. vis at 210 nm detector.

### 3. Results and Discussion

#### 3.1 Substrate preparations

The preparations of the substrates started with cleaning the wastes from any impurities might effect negatively on the biological reactions. The wastes were cut for small pecies and subjected to sun rays. The wastes were electricaly grinded and sieved by 200  $\mu\text{m}$  cieving, to increase its susceptibility to biological reactions. Pradip Saha et al., [14] reported partcils with sizes (45-63 $\mu\text{m}$ , 63-125 $\mu\text{m}$ , and 125-250 $\mu\text{m}$ ) which produced high amount of bioethanol from pteris as a biomass.



Figure 2, Substrate preparations; cleaning, cutting drying and grinding

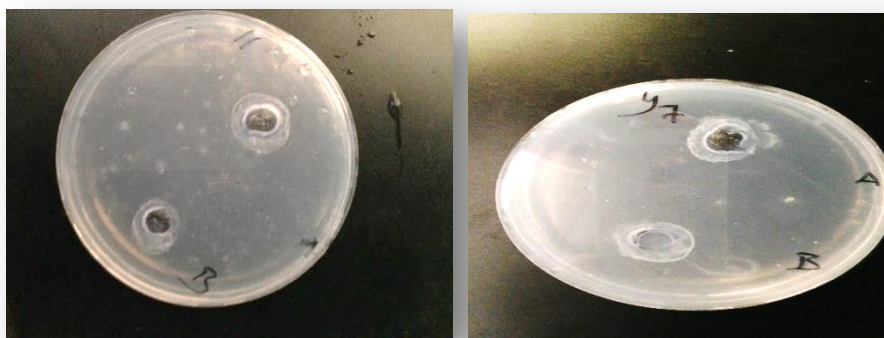
#### 3.2 Screening of *Pseudomonas sp.* isolates for cellulosic degradation

Sixty five isolates were collected from different places Industrial, electrical generator, contaminated dyes, agricultural and garden soil. A suitable test was done according to Kasana *et al.*[11], method, and modified cellulose agar replacing CMC agar for cellulose degrading efficiency test. The isolates were spot inoculated with pure isolates over CMC agar, and incubated at 30°C for 5 days, to allow for secretion of cellulase. To indicate the cellulase activity of the organisms, diameters of clear zone around colonies on agar were measured. After 2 days, 11 isolates of *Pesudeomonas sp.* were showed an activate degrader on CMC in solid media (table 3). The isolates gave higher growth more than (10 mm) were selected for screening liquid media. Out of eleven isolates tested, the isolate K16 gave high bioconversion and reducing sugar production reached to (63%) and mg/L respectively. Therefor, the isolate K16 was selected for further haydrolysis process (Table 3).



Table 3, Screening of isolates for cellulose degradation and cellulase activity

No. of isolates	Isolate name	Growth zone (mm)	Bioconversion rate (%)	Reducing sugar (mg/L)		
1	K13	16.45	55	1659.09		
2	K23	16.67	57	1668.18		
3	P13	13.94	40	1245.45		
4	P14	13.46	50	1502.23		
5	K16	17.05	63	1708		
6	B11	10.1	38	1231.18		
7	K4	16.76	60	1679.55		
8	K17	13.86	52	1518.18		
9	K11	12.73	38	1229.55		
10	B1100	10.74	32	1218.18		
	11		Y5	12.31	30	1209.09

Figure 3, Some isolates of *Pseudomonas sp.* showing clear zones of cellulose degradation

### 3.3. The effect of environmental and cultural parameters for hydrolysis of lignocellulosic waste to reducing sugar

*Pseudomonas species* have always been a source of thousands of bioactive compounds. Enzymes considered as one of the important products of this unusual group of bacteria. In the current study *Pseudomonas sp.* K16 with potential cellulolytic activity was subjected to produce reducing sugar in submerged culture. The culture with isolate K16 subjected to different conditions to select optimum conditions for hydrolysis of lignocellulosic waste to sugar.

#### 3.3.1 The effect of incubation time

The figure 4 showed that the highest concentration of reducing sugar observed after 16 hrs. reached 1759.045 mg/L. Also it showed that after 24 hrs. the sugar concentration was depleted until 36 hrs which reached zero level. The bioconversion rate increased from the beginning to approximately 16 hrs. which it reached a highest rate (50 %), then it decreased until reached 3% after 36 hrs.

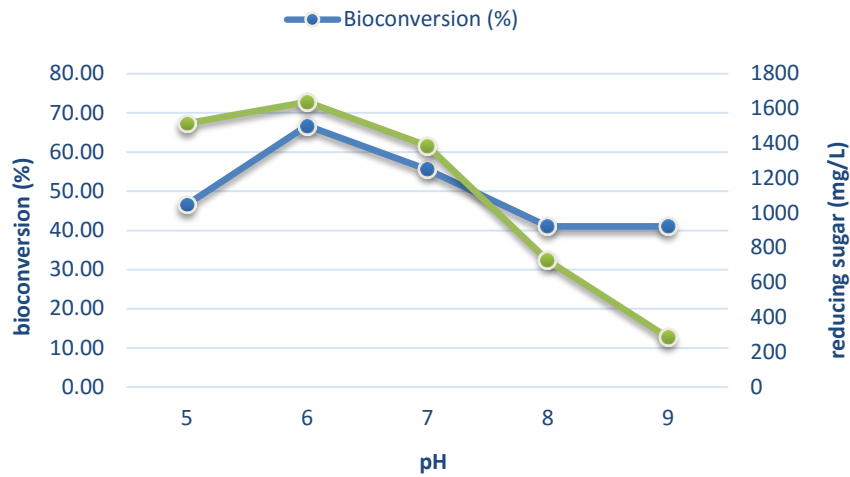


Figure 4. Effect of incubation time on hydrolysis of lignocellulosic waste by *Pseudomonas* K16 in shaker incubator (150 rpm) with pH 6 at time 36 hr.

### 3.3.2 Effect of pH

The modified cellulose broth medium was prepared with initial pH range (5 – 9). The best reducing sugar concentration was obtained at pH 6 at 30 °C. Also the results showed that the highest bioconversion rate and reducing sugar calculated were 1636.36 mg/L and 22% respectively, (Figure 5). This result was approximately in correlation with the findings of many other workers, Bakare *et al.* [15] found pH 6.5-7.0 optimum for the bioconversion of CMC as a substrate by *P. flourescens*. Shankar and Isaiarasu [16] and Ariffin *et al.* [17] revealed that *Bacillus pumillus* produced maximum reducing sugar at pH 6.0.

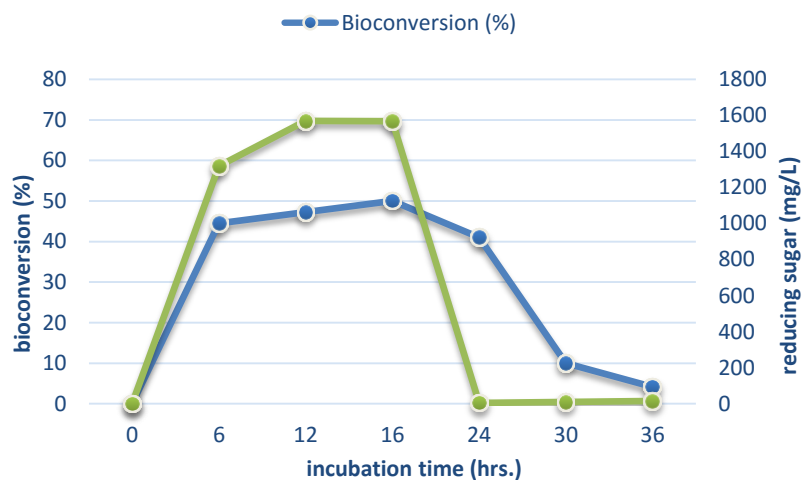


Figure 5. Effects of pH values of hydrolysis of lignocellulosic waste by *Pseudomonas* K16 in shaker incubator (150 rpm) at 30 °C after 16 hrs.

### 3.3.3 The effect of initial concentration of the substrate

Mineral salt medium (50 ml) was dispensed in to 250 ml flasks in duplicate and (1, 2, 3, 4, 5, and 6%) of mixed cellulosic waste were added as substrate source in each flask. The flasks were autoclaved and inoculated with the isolate *Pseudomonas sp.* K 16 and kept in the shaker incubator for a period of 16 h, pH 6 and at 30 °C. Figure 6, showed that the best bioconversion rate (74.5%) appeared with initial concentration of the substrate equal 4%, with reducing sugar concentration 3506.82 mg/L. The result was agreed with that reported by Harchand and Singh [18] which investigated that *S. albaduncus* showed highest level of cellulase activity with 3% concentration of cotton used as a substrate.

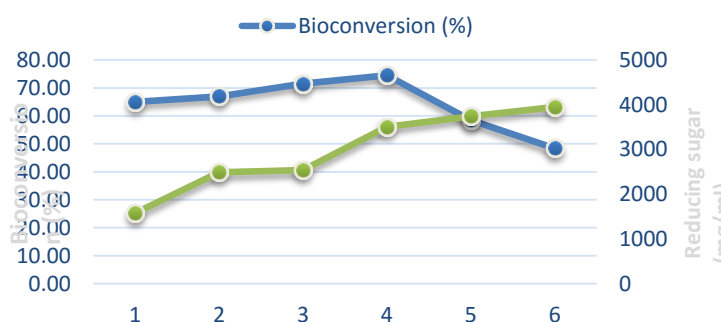


Figure 6. Effects of different concentration of lignocellulosic waste on hydrolysis process by *Pseudomonas* K16 in shaker incubation (150 rpm) at pH 6, temperature 30 °C after 16 hrs.

### 3.3.4 Effect of inoculum

The initial inoculum level in the media is a critical factor in fermentation process Shankar and Isaiarasu [16]. The effect of various inoculum size of (0.25, 0.5, 1, 1.5, 2, and 2.5) were tested in MSM at pH 6 and 4% of initial concentration of cellulosic after 16 hrs. From figure 7 it can be seen that the maximum bioconversion rate and reducing sugar concentration were (77%) and 4102.2727 mg/L respectively, were

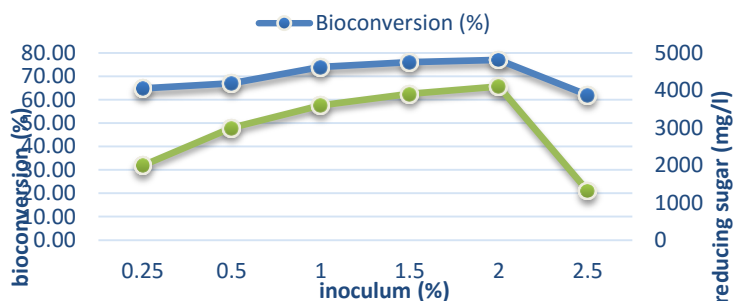


Figure 7. Effect of inoculum size on hydrolysis process of lignocellulosic waste by *Pseudomonas* K16 in shaker incubator (150 rpm) at pH 6, Temperature 30 °C after 16 hrs.

observed at 2% v/v of inoculum size. Shankar and Isaiarasu [16] found 2% inoculum size optimum for the cellulase activity when CMC used as a substrate by *Bacillus pumillus*.

#### 4. Statistical Analysis

According to  $2^4 - \text{CCD}$ , thirty trial runs were adjusted as in table 3. It is obvious that the table included two readings for the bioethanol; experimental and predicted bioethanol. The experimental bioethanol readings come from the experimental work according to suggested conditions by design expert @10 to independent variables. While the predicted bioethanol readings come from analysis of data by using the ANOVA. After the assessing of data, the fitting equation was obtained:

$$\begin{aligned} \text{Predicted bioethanol yield (Y)} = & 332.78 + 3.87 \times A - 12.86 \times B + 14.01 \times C + 16.78 \times D \\ & + 13.11 \times A \times B - 5.24 \times A \times C - 17.52 \times A \times D - 3.55 \times B \times C - \\ & 2.79 \times B \times D + 7.94 \times C \times D - 44.66 \times A^2 - 61.36 \times B^2 - \\ & 59.66 \times C^2 - 57.91 \times D^2 \end{aligned} \quad (5)$$

Table 3. Number of runs, experimental and predictable values of bioethanol

Run	Coded values				Actual values				Response 1	
	X <sub>1</sub>	X <sub>2</sub>	X <sub>3</sub>	X <sub>4</sub>	Initial Conc. g/l	pH	Inoculum mL	Time hr.	Exp.Bioeth mg/L	Pre.Bioethm g/L
1	-1	+1	+1	+1	50	8	3	54	135.2	134.5
2	-1	-1	+1	+1	50	4	3	54	212.6	199.12
3	0	0	0	-2	75	6	2	9	48.8	67.58
4	0	0	0	0	75	6	2	39	332.6	332.78
5	-1	-1	+1	-1	50	4	3	24	110.5	109.06
6	-1	-1	-1	-1	50	4	1	24	95.2	79.34
7	+2	0	0	0	125	6	2	39	145	161.88
8	+1	-1	-1	+1	100	4	1	54	120.1	94.6
9	0	0	0	0	75	6	2	39	332.9	332.78
10	-1	+1	-1	-1	50	8	1	24	65	40.08
11	+1	+1	+1	-1	100	8	3	24	115.8	114.12
12	0	0	0	0	75	6	2	39	332.8	332.78
13	0	0	0	0	75	6	2	39	332.9	332.78
14	0	0	0	0	75	6	2	39	332.6	332.78
15	+1	+1	-1	+1	100	8	1	54	110	96.62
16	+1	-1	+1	-1	100	4	3	24	130	115.14
17	-2	0	0	0	25	6	2	39	130	146.4
18	0	0	0	0	75	6	2	39	332.9	332.78
19	+1	+1	-1	-1	100	8	1	24	124	119.56
20	0	0	0	+2	75	6	2	69	120.2	134.7
21	0	0	+2	0	75	6	4	39	130	122.16
22	0	0	-2	0	75	6	0	39	25	66.12
23	-1	+1	-1	+1	50	8	1	54	90.3	87.22
24	-1	-1	-1	+1	50	4	1	54	150.8	137.64
25	0	+2	0	0	75	10	2	39	56.4	61.62
26	-1	+1	+1	-1	50	8	3	24	48	55.6
27	+1	-1	+1	+1	100	4	3	54	125	135.12
28	0	-2	0	0	75	2	2	39	85	113.06
29	+1	-1	-1	-1	100	4	1	24	120.5	106.38
30	+1	+1	+1	+1	100	8	3	54	125	122.94

Comparison the observed results between experimental and predicted readings shows a good matching in between over the defined range. ANOVA analysis showed magnitude of F-value (144.9) and the low probability value ( $< 0.0001$ ), proof the significant model fit. Furthermore, the model didn't have any lack of fit and its  $R^2 = 0.9771$ , which is acceptable.  $R^2 > 0.75$  indicates aptness of the model, Chauhan et al. [19]. Also most values of (Probability  $> F$ ) less than 0.05, which confirms that the model terms are significant.

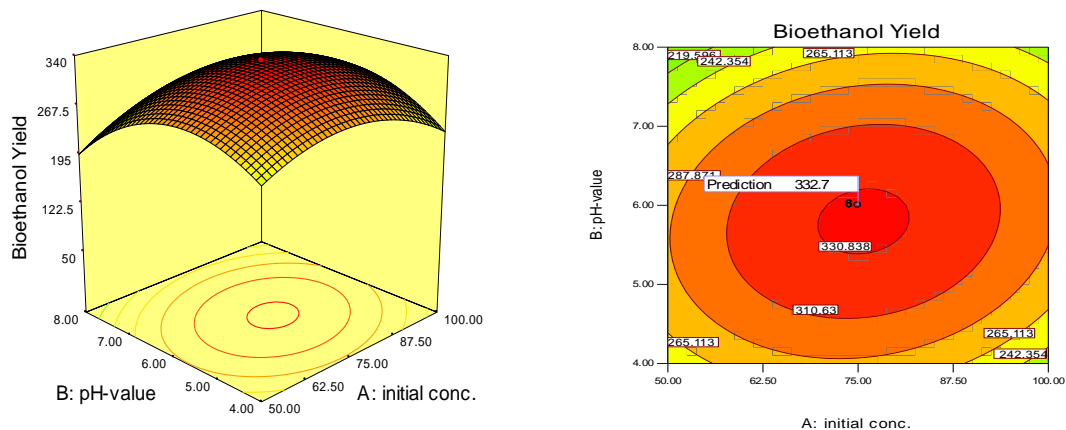
A good explain for that is the linear coefficient  $x_i$ , the quadratic coefficients  $x_i x_j$ , and the coefficient of  $x_i^2$  in equation (3) are all significant with a probability of 95%. The results values behave logically, when the parameters value increased from the lower limits to higher, the bioethanol yield also increased reaching the maximum yield at the midpoint of variable ranges.

After the midpoint range the bioethanol yield decreased in spite of variable increasing. This is due to growth-inhabiting effect of high sugar concentration, as well as product-formation which possibly distorts microorganisms' metabolism-poisonous effect, Thatipamala et al. [20]. Finally, the bioethanol production is affected by all independent variables at the fixed temperature.

#### 4.1. Graphical analysis

For more understanding to bioethanol production under optimum conditions and interactions between independent variables range, the second degree polynomial model used to build a response surface plots by RSM (response surface method). The three-dimensional plots were built by fixed two of independent variables in their midpoint value and changing the other two variables over their experimental range.

The resulting graphics gave an excellent clarification for the effects of initial concentration, pH value, inoculum and fermentation time on bioethanol yield. The effect of initial sugar concentration and pH-value on the bioethanol yield, as a response surface and contour plot, is presented by Fig. 8. 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 pH-value range of 5.4-6.3. The plot can easily show that the peak production of

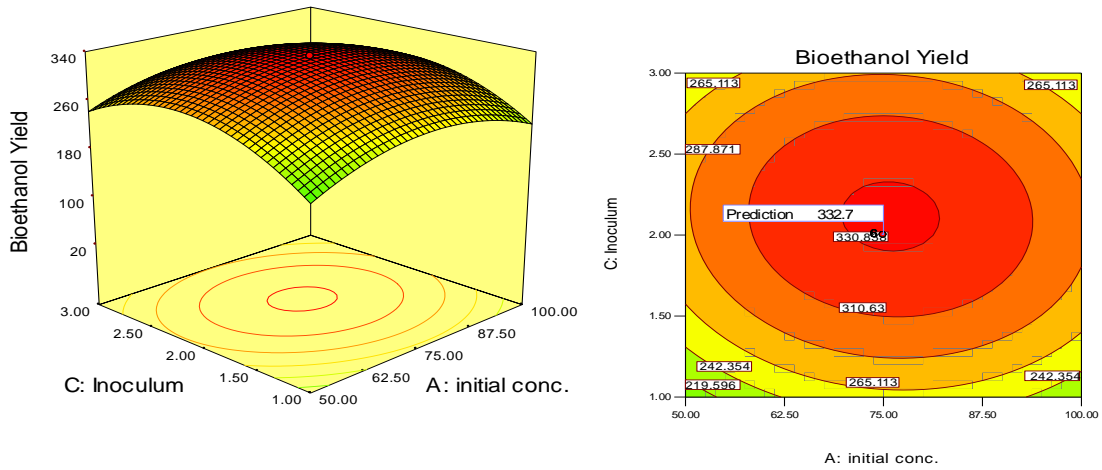


Figures 8. The effect of pH and initial substrat concentration on bioethanol production.

bioethanol appears at 332 mg/L which is compatible with Sinclair and Kristiansen [21].

The results in figure 9, shows the response surface representing the interaction between initial sugar concentration and inoculum volume on bioethanol production. The maximum yield appears over an initial substrate concentration 70-85 g/L and inoculum size 1.8-2.3.

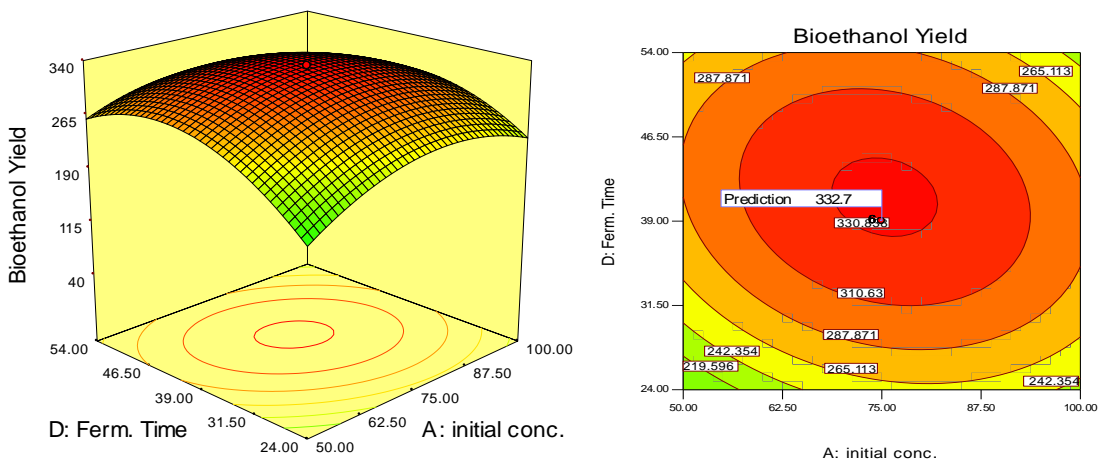
The rate of ethanol production is related to the available sugar concentration. At very low substrate concentration, the yeast starved and productivity decreases [22].



Figures 9. The effect of initial substrat concentration and inoculum size on bioethanol production.

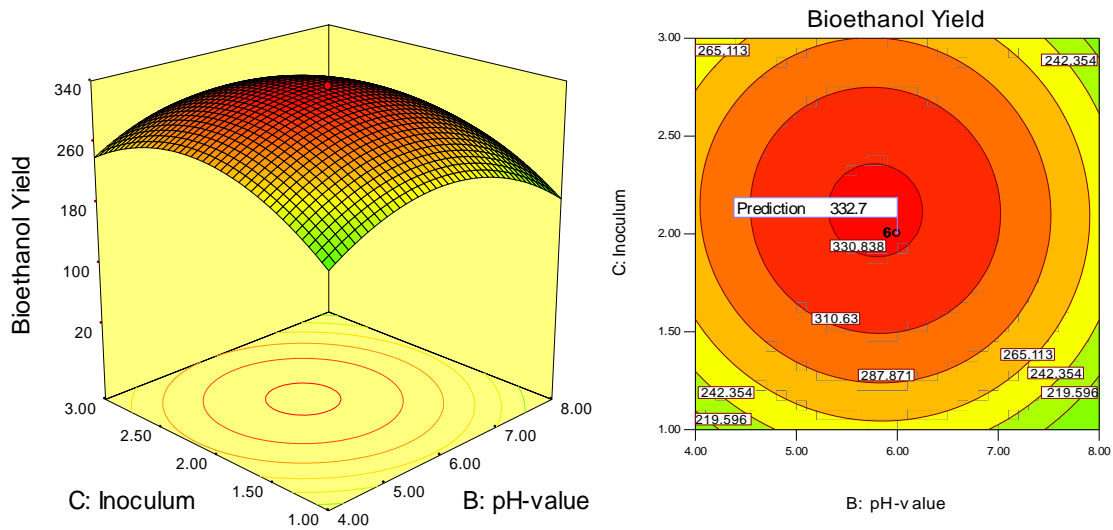
Also the result in figure 10, shows the corresponding contour and response surface plot for bioethanol yield as a function of initial substrate concentration and fermentation time.

It can be seen that a high-yield (maximum bioethanol production) appears at initial substrat concentration and the fermentation time ranges (70-86 g/L) and (37-45 hrs.), respectively.



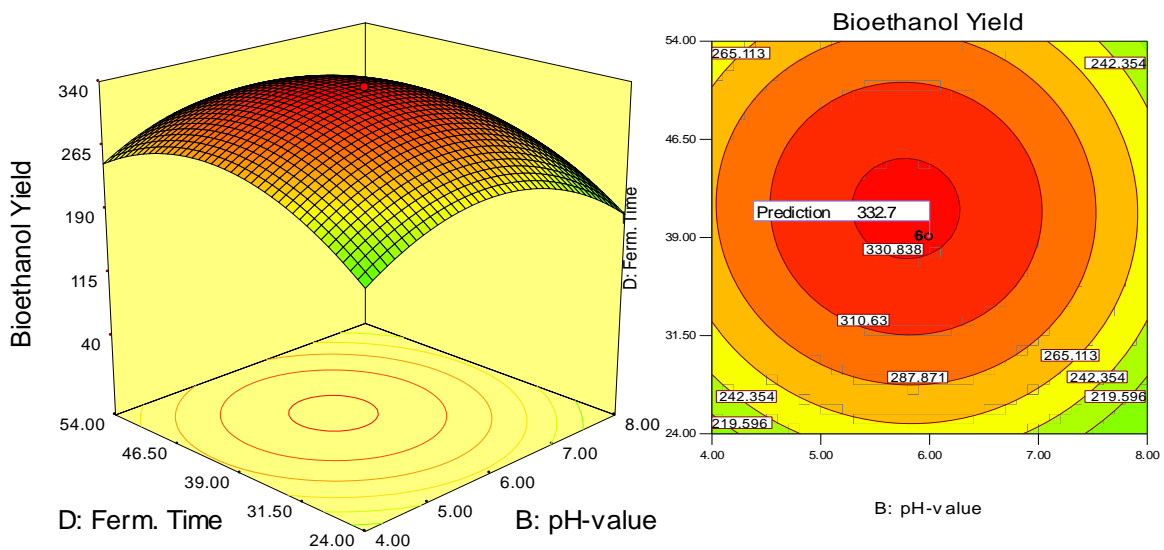
Figures 10. The effect of initial substrat concentration and fermentation time on bioethanol production.

The result in figure 11, illustrate the combined interaction of pH values and inoculum size directly on bioethanol production. The maximum yield will appear at pH ranges equal to (5.4-6.4) and the inoculum size ranges varies (1.8-2.3 mL).



Figures 11. The effect of pH values and inoculum size interaction on bioethanol production.

Also the result in figures 12 represent, the maximum bioethanol yield achieved when pH value changed (5.4-6.4) and fermentation time various (37-45 hr.). With a further increase the ethanol production was decreased because the yeast produces acid rather than alcohol and the groth of harmful bacteria is restarted by acidic solution [23].



Figures 12. The Effect of pH and fermentation time on bioethanol production.

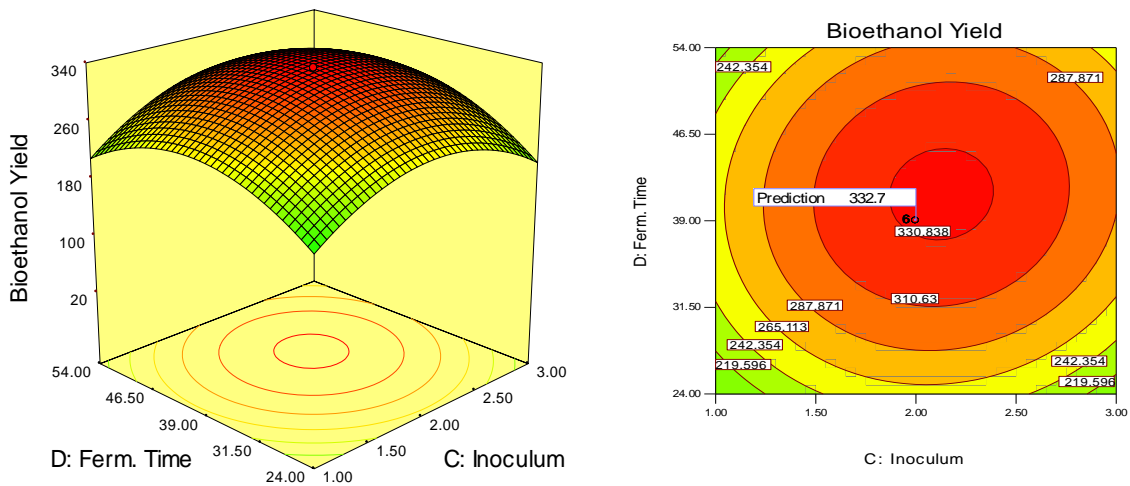
The result in figures 13 revealed the effects of inoculum size and fermentation time on bioethanol production. The amount of ethtanol production increased with the increase in the inoculum size. bioethanol appears at (1.8-2.3 mL) of inoculum dosage, and fermentation time varies (37-45 hr.) . Further increase in inoculum volume did not result



in the significant enhancement of ethanol production. This finding is in agreement with other worker [24, and 25].

#### 4.2. Optimization conditions of bioethanol production in shaking flasks and bioreactor by *Sacchromyces cerevisiae*

As it is obvious from the observed results above, the maximum bioethanol production (332.9 mg/L) in shake flask was obtained at initial substrat (Glucose), pH,



Figures 13. Inoculum and fermentation time effect on bioethanol production.

inoculum size and fermentation time equal to 75 g/L, 6, 2%, and after 39 hrs. respectively. In comparison the bioethanol production in the lab scale bioreactor, the results showed higher bioethanol production in the bioreactor than the shake flasks and reached to 492.9 mg/L (figure 14). The main reasons of increase in bioethanol production in bioreactor are controlling of different parameters in the bioreactor such as pH, Temperature and foaming as well as homogenizing the culture materials in the bioreactor by suitable mixing of all layers. The results in figure (14) explain the production of bioethanol, remaining sugar and biomass production versus the fermentation time.

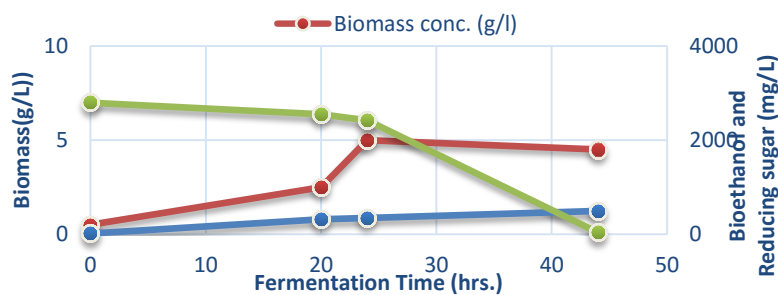


Fig 15: Analysis of residual sugar concentration during ethanol production by *Saccharomyces cerevisiae*. Fermentation conditions: initial sugar concentration, pH, inoculum dosage, and fermentation time, 75 g/l, 6, 2 ml, and 39 hr respectively.

## 5. Conclusions

1. Experimental design Box-wilson method, consider applicable and appropriate for optimizing the bioethanol yield from some of traditional components of Iraqi biodegradable municipal solid waste.
2. Main four independent variables initial concentration, pH, inoculum and fermentation time optimized at a fixed temperature. Maximum bioethanol production based on experimental work was 332.9 mg/L, while the predicted value according to the second degree polynomial developed by ANOVA software was 332.78 mg/L, which shows a good degree of matching.
3. Maximum bioethanol yield in the bioreactor was observed at optimized conditions: initial concentration equal to (75 g/L), pH (6), inoculum (2%) and fermentation time equal (39 hr.).

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