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Research article

Ethanol production from hot-water sugar maple wood extract hydrolyzate: fermentation media optimization for *Escherichia coli* FBWHR

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Abstract: We report the first time statistical study of the optimization for ethanol production from hot-water sugar maple hemicellulosic wood hydrolyzate by *Escherichia coli* FBWHR. Response surface methodology was employed to investigate the effect of fermentation media on the ethanol production from concentrated hot-water sugar maple hemicellulosic wood extract hydrolyzate by *Escherichia coli* FBWHR. The critical media components were firstly selected according to Plackett–Burman design and further optimized by central composite design. Based on the response surface analysis, the optimum concentrations of the significant components were obtained: yeast extract, 10.19 g/L; tryptone, 14.55 g/L; $Na_2HPO_4 \cdot 7H_2O$, 23.21 g/L; KH_2PO_4, 5 g/L and NH_4Cl, 2 g/L. An ethanol concentration of 15.23 ± 0.21 g/L was achieved under the optimized media, which agreed with the predicted value. Ethanol production was enhanced to 22.18 ± 0.13 g/L by scaling up the fermentation from shaker flask to 1.3 L bioreactor.

Keywords: hot-water sugar maple wood hydrolyzate; recombinant *Escherichia coli*; ethanol fermentation; response surface methodology

1. Introduction

Development of bio-fuels has recently drawn attention due to the depletion of crude oil, increased oil prices and environmental benefits [1-4]. For example, ethanol as a renewable energy source as well as an important industrial ingredient has been produced from different kinds of raw materials such as urban waste, agricultural residue and forest materials which are counted low-cost lignocellulosic biomass [5,6]. Woody biomass, comprising about 40% hemicellulose with the major

components of hexoses (glucose, galactose, mannose, rhamnose) and pentoses (xylose and arabinose), is the most abundant organic source on Earth and has been widely studied in bio-ethanol production process [7-10]. Recently, our group has reported an ethanol batch fermentation using a new improved strain adapted from FBHW, *E. coli* FBWHR, from hot-water sugar maple wood extract hydrolyzate and its kinetic study [11].

The optimization of fermentation media is very important to the ethanol process by possibly improving the consumption of the woody biomass constituents and maximizing the ethanol production. The classical method of "one factor at a time" is laborious and time-consuming, especially when the number of variables is large. Design of experiments (DoE) is a structured and robust methodology for optimizing fermentation processes and media and well documented in the literature [12,13,14]. Plackett–Burman (PB) design, involving a two-level fractional factorial saturated design, tests the largest number of factor effects with the least number of observations and screens for significant factors [15]. It has been proved to be useful in evaluating the relative significance of variables and screening a large number of media components [16,17]. Response surface methodology (RSM) is followed for identifying the effect of individual variables and for seeking the optimal fermentation conditions for a multivariable system with minimum numbers of experiments efficiently. It has been successfully adopted to optimize the production of ethanol by different microorganisms [18-21]. Central composite design (CCD) is the most widely used response surface design that provides statistical models which help in understanding the interactions among the parameters that have been optimized [22,23]. RSM uses CCD to fit a model by the least squares technique. Three major steps are involved in RSM: performing the statistically designed experiments, estimating the coefficients in a mathematical model, and predicting the response and checking the adequacy of the model.

However, there are no literature reports on media optimization of ethanol fermentation from hot-water sugar maple hemicellulosic wood extract hydrolyzate. In this study, we report the optimization of the fermentation media specifically comprising hot-water sugar maple hemicellulosic wood extract hydrolyzate and a new improved strain adapted from FBHW, *E. coli* FBWHR by statistical design of experiments. To enhance the production of ethanol using RSM, a PB design was performed to screen for components of the production medium that had significant effects on the ethanol production. A CCD was then employed to optimize the significant factors identified by PB design to further increase ethanol production. Finally, batch fermentation was scaled up in a 1.3 L fermenter.

2. Material and Methods

2.1. Sugar maple wood extract hydrolyzate, E. coli FBWHR strain and cell growth

The details of preparation of sugar maple wood extract hydrolyzate, microorganism and cell growth, strain adaptation, and analytical methods have been reported by Wang et al. [11]. The hot-water extraction was carried out in a 1.84 m^3 digester with a wood to liquor ratio of 1:4 at about 160 °C for 2 h, followed by Ultra-filtration and Nano-filtration. The concentrated extracts were hydrolyzed at 135 °C for 25 min with 1 wt% sulfuric acid added to remove lignin. Hydrolyzate was neutralized by $Ca(OH)_2$ and then fractionated by Nano-filtration. According to previous investigation, hot-water sugar maple hemicellulosic wood extracts hydrolyzate used in this study contained six

monosaccharides, primarily xylose and minor amounts of glucose, mannose, arabinose, galactose and rhamnose, other compounds such as phenolics and aromatics, and several trace metals [11]. *E. coli* FBWHR was grown in 250 mL shaker flasks containing 100 mL sterile liquid medium (10 g/L tryptone, 5 g/L yeast extract, and 5 g/L NaCl) adjusted to a pH of 7.0, and supplemented with 10 g/L xylose and 20 mg/L tetracycline. The liquid culture was incubated at 35 °C for 12–14 hours on an incubator shaker with a speed of 160 rpm. After growth, 5 mL of culture was used to inoculate 100 mL of sterile fermentation media.

2.2. Ethanol fermentation conditions

2.2.1. Ethanol fermentation in shaker flasks

Concentrated hot-water sugar maple wood extract hydrolyzate was diluted to 30% (v/v) with different concentrations of defined media solutions (100 mL), and then transferred into a 250 mL shake flask. The pH of the fermentation media was adjusted to 7.0 by addition of ammonia hydroxide. Concentrated hot-water sugar maple wood extract hydrolyzate and ammonia hydroxide were filter-sterilized. Other media solutions were autoclaved separately at 121 °C for 15 min. Fermentations studied by RSM were conducted at 35 °C with the incubator shaker at a shaking speed of 160 rpm.

2.2.2. Ethanol fermentation in bioreactor

Batch fermentation was scaled up from shaker flasks to a 1.3 L New Brunswick Bioreactor (BIOFLO 110) with a working volume of 800 mL under micro-aerobic condition. Sugar concentration of fermentation media was achieved by diluting concentrated hydrolyzate to 30% (v/v) containing 48.53 g/L total sugar. After cooling, concentrated hydrolyzate was added to the bioreactor and adjusted to pH 7.0 by ammonia hydroxide prior to inoculation. Samples (2 mL) were taken intermittently and centrifuged prior to analysis.

2.3. Analytical methods

Cell density (g/L) was estimated by using a predetermined correlation between dry weight cell concentrations (oven dry at 105 °C) versus optical density. Ethanol concentration was measured by GC using Thermo Scientific Focus GC systems equipped with a Triplus automatic sampler and a TRACE TR-WaxMS (30 m × 0.25 mm × 0.25 μm) GC column. Sugar concentrations were determined by nuclear magnetic resonance (NMR) spectroscopy using a modified two dimensional heteronuclear single quantum coherence (HSQC) experiment.

Element concentrations of the concentrated hot-water sugar maple wood extract hydrolyzate were analyzed by inductively coupled plasma (ICP) on a Perkin Elmer 3300DV Inductively Coupled Plasma Emission Spectrometer. Samples were diluted 100-fold with 2% nitric acid for analysis. The element concentrations were obtained via emissions.

2.4. Experimental design

2.4.1. Plackett–Burman (PB) design

Six medium components (yeast extract, tryptone, $Na_2HPO_4 \cdot 7H_2O$, KH_2PO_4, NH_4Cl and NaCl) were chosen in the present study for the effect on the ethanol production by hot-water sugar maple wood extract hydrolyzate. The main effect of variables was firstly determined as follows:

$$E_{(xi)} = \frac{(\sum y_{i+} - \sum y_{i-})}{N/2} \quad (1)$$

Where, $E_{(xi)}$ represents the effect of variable *i*; y_{i+} is the response of the high level of variable *i*; y_{i-} is the response of the low level of variable *i*; N stands for the total number of trials.

The standard error (SE) of the effect was the square root of an effect, and the significance level (*p*-value) of each variable effect was determined by *T*-test as follows:

$$t_{(xi)} = \frac{E\,(xi)}{SE} \quad (2)$$

Where, $E_{(xi)}$ represents the effect of variable *i*; SE is the standard error [17,24].

2.4.2. Central composite design (CCD)

After the critical medium components were screened by PB design, Response Surface Methodology (RSM) was adopted to optimize the concentrations of the components to maximize the production of ethanol. Three components (yeast extract, tryptone, and $Na_2HPO_4 \cdot 7H_2O$) that significantly affected the ethanol production were optimized by RSM using a 3-factor-5-level CCD with 6 replications of center points. The CCD matrix included six central points and six axial points, with an axial distance of ±1.68 to make the design orthogonal [25]. In order to develop the second-order regression model, the variables were coded to the following equation:

$$x_i = (X_i - X_0)/\Delta X_i,\ i = 1,2,\ldots,k \quad (3)$$

Where x_i and X_i are the dimensionless and the actual values of the independent variable *i*, X_0 is the actual value of the independent variable at the center point, and ΔX_i is the step change of X_i corresponding to a unit variation of the dimensionless value. In order to correlate the response variable (i.e., ethanol concnetration) to the independent variables, second-order polynomial model for predicting the optimal point was expressed as follows:

$$Y = b_0 + \sum b_i x_i + \sum b_{ii} x_i^2 + \sum b_{ij} x_i x_j,\ i = 1,2,\ldots,k;\ j = 1,2,\ldots,k,\ i \neq j \quad (4)$$

Where Y represents the predicted response, b_0 is the interception coefficient, b_i is the coefficient of the linear effect, b_{ii} is the coefficient of the quadratic effect, b_{ij} is the coefficient of the interaction effect when $i < j$, and k is the numbers of the involved variables.

2.5. Statistical analysis

The experiments were carried out in triplicate unless other specified. The average ethanol concentration was used as the dependent variable. The Design-Expert 8.0.7.1 trial software was used for multiple regression analysis of the experimental data. The analysis of variance (ANOVA) was evaluated. The statistical and regression coefficient significance was checked with F-test and t-test, respectively. The multiple coefficients of correlation (R) and the determination coefficient of correlation (R^2) were calculated to evaluate the adequacy of the model. The optimum values of the selected variables were obtained by analyzing the response surface plot and solving the regression equation.

3. Results and Discussion

3.1 Screening of significant media components that enhance ethanol production

PB design experiments were performed to select the important fermentation parameters which have significantly improved the ethanol production by *E. coli* FBWHR. An initial set of six media components with two levels of concentration was proposed with a potential effect on the ethanol fermentation. Yeast extract and tryptone are widely employed as complex sources of peptides, amino acids, vitamins, and trace elements such as magnesium for microorganisms in fermentation media [26,27]. It has been reported that cells have to synthesize amino acid and vitamin and require more energy and carbon source without the supplement of yeast extract [28]. Na_2HPO_4, KH_2PO_4 NH_4Cl and NaCl are the typical substances for *E. coli* growth. The experimental design matrix based on the Plackett–Burman design was presented in Table 1. Twelve experiments were carried out using different combinations of the variables. The results were analyzed by using the analysis of variance (ANOVA) as appropriate to the experimental design used. The sum of squares, mean squares, F-values and *p*-values were estimated. It was demonstrated that the model was highly significant with the F-value of 19.39 and a very low probability value of 0.0062 (p-value < 0.05) meaning only a 0.62% chance that a "Model F-Value" this large could occur due to noise. According to the ANOVA results (Table 2), yeast extract, tryptone and $Na_2HPO_4 \cdot 7H_2O$ had significant positive effects on the ethanol production, because the values of "Prob > F" were less than 0.05. KH_2PO_4 and NH_4Cl also had positive effects but the contributions were not significant, so their concentrations were fixed at the high level and entered to the CCD experiments. NaCl was the only media component that had an insignificantly negative impact and a low level of 0 g/L. Therefore, NaCl was removed from the media fomulation. The determination coefficient (R^2) was 0.9714, which meant that the model could explain 97.14% of the total variations in the system. The predicted determination coefficient (Pred R^2 = 0.7277) was in reasonable agreement with the adjusted determination coefficient (Adj R^2 = 0.9213). The signal to noise ratio was measured by "Adeq Precision". A ratio greater than 4 is desirable. A ratio of 12.497 indicated an adequate signal,which implied that this model could be used to navigate the design space. The optimum values of yeast extract, tryptone and $Na_2HPO_4 \cdot 7H_2O$ were further investigated by CCD.

Table 1. The PB design for screening variables in ethanol production from 30% (v/v) of hot-water sugar maple hemicellulosic wood hydrolysate by *E. coli* FBWHR.

Run	Levels	Yeast Extract (X_1), g/L	Tryptone (X_2), g/L	$Na_2HPO_4 \cdot 7H_2O$ (X_3), g/L	KH_2PO_4 (X_4), g/L	NH_4Cl (X_5), g/L	NaCl (X_6), g/L	Ethanol concentration, g/L (n = 3)
	−1	1.00	1.00	1.00	1.00	0.50	0.00	
	+1	10.00	10.00	20.00	5.00	2.00	10.00	
1		1.00	10.00	1.00	5.00	2.00	0.00	1.56 ± 0.10
2		1.00	10.00	20.00	5.00	0.50	0.00	5.88 ± 0.24
3		1.00	1.00	20.00	1.00	2.00	10.00	1.31 ± 0.15
4		10.00	10.00	1.00	5.00	2.00	10.00	1.09 ± 0.12
5		1.00	10.00	20.00	1.00	2.00	10.00	6.79 ± 0.18
6		10.00	1.00	20.00	5.00	0.50	10.00	2.67 ± 0.08
7		1.00	1.00	1.00	1.00	0.50	0.00	0.28 ± 0.10
8		10.00	1.00	1.00	1.00	2.00	0.00	3.74 ± 0.05
9		10.00	1.00	20.00	5.00	2.00	0.00	3.88 ± 0.15
10		1.00	1.00	1.00	5.00	0.50	10.00	0.1± 0.05
11		10.00	10.00	20.00	1.00	0.50	0.00	7.01± 0.20
12		10.00	10.00	1.00	1.00	0.50	10.00	0.8 ± 0.06

Table 2. The ANOVA analysis for PB design.

Source	Coefficient	Sum of squares	df	Mean square	F-value	p-value Probe > F
Model		67.34	7	9.62	19.39	0.0062
X_1	0.82	5.47	1	5.47	11.03	0.0294
X_2	0.95	8.74	1	8.74	17.61	0.0137
X_3	1.59	26.71	1	26.71	53.83	0.0018
X_4	0.089	0.051	1	0.051	0.1	0.7648
X_5	0.8	3.64	1	3.64	7.33	0.0537
X_6	−0.33	1.1	1	1.1	2.22	0.2104
$X_2 \times X_3$	1.64	13.09	1	13.09	26.39	0.0068
Residual		1.98	4	0.5		
Cor Total		69.33	11			
R^2 = 0.9714; Pred R^2 = 0.7277; Adj R^2 = 0.9213; Adeq Precision = 12.497.						

3.2. Optimization of media components for ethanol fermentation

PB design experiments were performed to select the critical fermentation parameters which have significant effects on ethanol production with *E. coli* FBWHR. Yeast extract, tryptone, and $Na_2HPO_4 \cdot 7H_2O$ screened out by PB design were further studied by a five-level three-factor CCD

with six replications of center points to enhance the ethanol production. The design matrix of CCD, the variables and the corresponding results were shown in Table 3. The results of the second-order response surface model fitting in the ANOVA were presented in Table 4. The interactive model term yeast extract × tryptone was insignificant, because the *p*-value was greater than 0.05. All the other model terms were significant, because low *p*-values ($p < 0.05$) were obtained. Of the modeled terms, yeast extract, tryptone, $Na_2HPO_4 \cdot 7H_2O$, yeast extract × $Na_2HPO_4 \cdot 7H_2O$ and tryptone × $Na_2HPO_4 \cdot 7H_2O$ positively affected the ethanol production, while (yeast extract)^2, (tryptone)^2, and $(Na_2HPO_4 \cdot 7H_2O)^2$ negatively affected the ethanol production. The coefficient of multiple determination (R^2) was found to be 0.9898, which means that the model could explain 98.98% of the total variations in the system. The high value of the adjusted R^2 (0.9824) further supported the accuracy of the model. The predicted R^2 of 0.9517 was in the reasonable agreement with the adjusted R^2 value. Therefore, the results in terms of the production of ethanol can be illustrated by the following quadratic regression equation:

$$Y = 13.62 + 0.77\,X_1 + 1.71\,X_2 + 1.88\,X_3 + 1.23\;X_1 \times X_3 + 0.75\;X_2 \times X_3 - 2.88\,X_1^2 - 1.18\,X_2^2 - 1.53\,X_3^2 \quad (5)$$

Where, the production of ethanol as Y is a multiple function of yeast extract, trypone and $Na_2HPO_4 \cdot 7H_2O$ concentrations.

Table 3. The CCD design for three significant variables screened by PB design in ethanol production from 30% (v/v) of hot-water sugar maple hemicellulosic wood hydrolysate by *E. coli* FBWHR.

Run	Levels	X_1, g/L	X_2, g/L	X_3, g/L	Ethanol concentration, g/L (n = 3)
	1.682	14.20	1.59	11.59	
	1.00	12.50	5.00	15.00	
	0.00	10.00	10.00	20.00	
	−1.00	7.50	15.00	25.00	
	−1.682	5.80	18.41	28.41	
1		7.50	5.00	15.00	5.95 ± 0.23
2		12.50	5.00	15.00	4.58 ± 0.16
3		7.50	15.00	15.00	7.7 ± 0.28
4		12.50	15.00	15.00	6.16 ± 0.11
5		7.50	5.00	25.00	5.68 ± 0.07
6		12.50	5.00	25.00	9.7 ± 0.30
7		7.50	15.00	25.00	10.88 ± 0.17
8		12.50	15.00	25.00	13.8 ± 0.12
9		5.80	10.00	20.00	3.52 ± 0.25
10		14.20	10.00	20.00	7.35 ± 0.08
11		10.00	1.59	20.00	7.04 ± 0.14
12		10.00	18.41	20.00	13.42 ± 0.12
13		10.00	10.00	11.59	6.29 ± 0.26
14		10.00	10.00	28.41	12.21 ± 0.14
15		10.00	10.00	20.00	13.25 ± 0.08

16	10.00	10.00	20.00	13.64 ± 0.23
17	10.00	10.00	20.00	14.05 ± 0.05
18	10.00	10.00	20.00	13.28 ± 0.07
19	10.00	10.00	20.00	13.52 ± 0.13
20	10.00	10.00	20.00	13.98 ± 0.10

Table 4. The ANOVA analysis and variance analysis for the quadratic response surface model using CCD design.

Parameter	Coefficient	Sum of squares	df	Mean square	F-value	*p*-value Probe > F
Intercept	13.62					
X_1	0.77	8.03	1	8.03	32.05	0.0002
X_2	1.71	39.96	1	39.96	159.53	< 0.0001
X_3	1.88	48.09	1	48.09	191.98	< 0.0001
$X_1 \times X_2$	−0.16	0.2	1	0.2	0.8	0.3907
$X_1 \times X_3$	1.23	12.13	1	12.13	48.42	< 0.0001
$X_2 \times X_3$	0.75	4.46	1	4.46	17.79	0.0018
X_1^2	−2.88	119.39	1	119.39	476.67	< 0.0001
X_2^2	−1.18	20.17	1	20.17	80.52	< 0.0001
X_3^2	−1.53	33.71	1	33.71	134.6	< 0.0001
Model		263.6	8	32.95	133.93	< 0.0001
Residual		2.71	11	0.25		
Lack of fit		2.13	6	0.35	3.07	0.1193
Pure error		0.58	5	0.12		
Cor Total		266.3	19			
CV = 5.06; R^2 = 0.9898; Pred R^2 = 0.9517; Adj R^2 = 0.9824; Adeq Precision = 30.568.						

As shown in Table 4, the second-order regression model was statistically valid given an F-value with a low probability value ($p_{model} < 0.0001$). The lack-of-fit value ($p = 0.1193$) implied the Lack of Fit was not significant relative to the pure error. The low coefficient of variation (CV = 5.06%) demonstrated that the model was precise and reliable.

The regression equations were represented graphically by three dimensional response surface plots, which were generally used to demonstrate relationships between the response and experimental levels of each variable. Therefore, the maximum production of microbial metabolites can be found by visualization of the optimum levels of each variable through the surface plots [29,30]. Figure 1 shows a response surface plot of the effect of adding yeast extract and $Na_2HPO_4 \cdot 7H_2O$ on the production of ethanol with a tryptone concentration of 10 g/L. It can be observed that the ethanol concentration increased at first and then decreased with the concentration increase of yeast extract when the concentration of $Na_2HPO_4 \cdot 7H_2O$ varied from 15 g/L to 25 g/L. The similar trend was found as the concentration of $Na_2HPO_4 \cdot 7H_2O$ increased when the concentration of yeast extract changed from 7.5 g/L to 12.5 g/L.

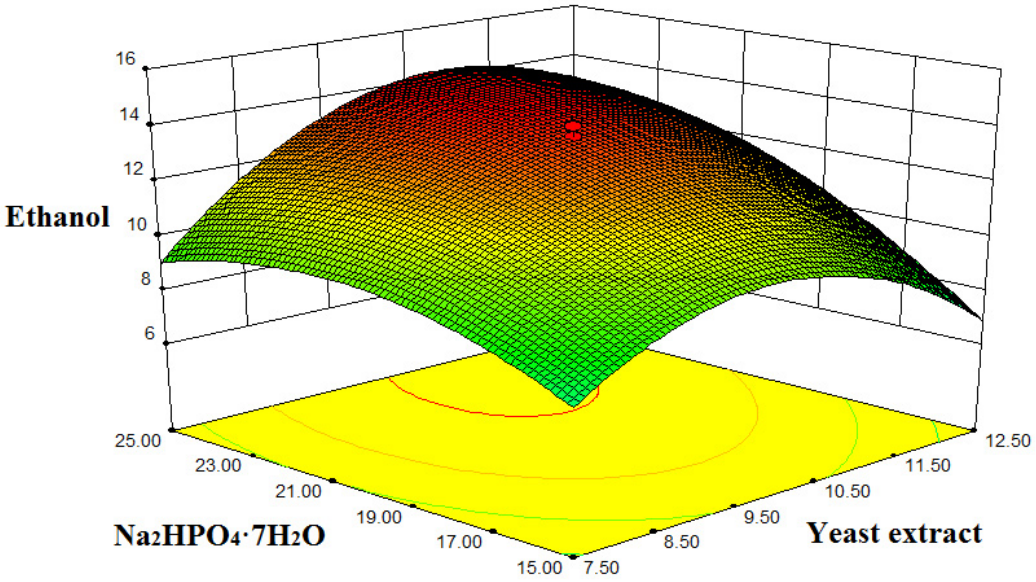

Figure 1. Response surface plot for the interaction between yeast extract and $Na_2HPO_4 \cdot 7H_2O$ on the production of ethanol with a tryptone concentration of 10 g/L.

A response surface plot that indicated the interaction between tryptone and $Na_2HPO_4 \cdot 7H_2O$ at a yeast extract concentration of 10 g/L which influenced the ethanol production was presented in Figure 2. The ethanol concentration inceased when increasing the tryptone concentration and slightly decreased at a even higher concentration level of tryptone. The ethanol production varied in a similar way as the concentration of $Na_2HPO_4 \cdot 7H_2O$ increased when the tryptone concentration varied from 5 g/L to 15 g/L.

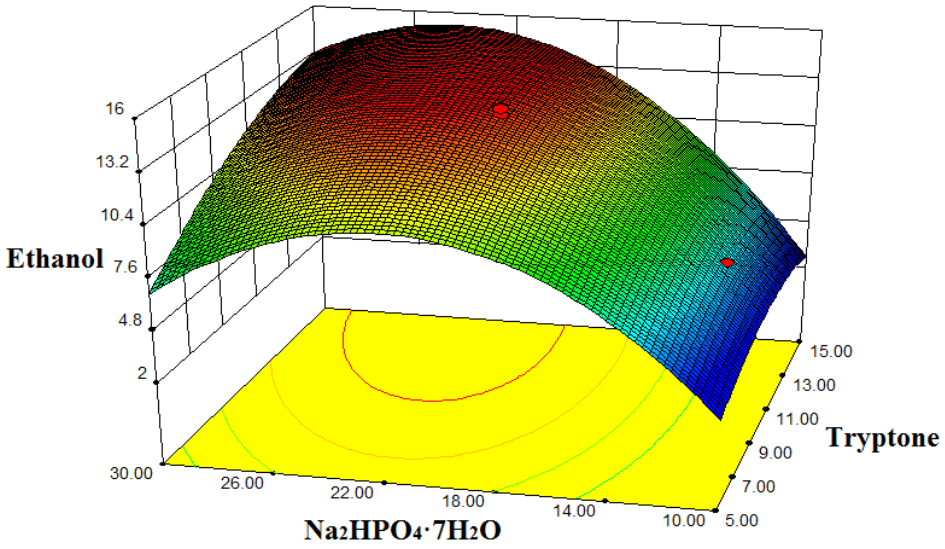

Figure 2. Response surface plot for the interaction between tryptone and $Na_2HPO_4 \cdot 7H_2O$ on the production of ethanol with a yeast extract concentration of 10 g/L.

Based on Equation 5, the optimal concentrations of yeast extract, tryptone, $Na_2HPO_4 \cdot 7H_2O$, KH_2PO_4 and NH_4Cl for achieving the maximum ethanol production from 30% (v/v) of concentrated hot-water sugar maple wood extract hydrolyzate by *E. coli* FBWHR were 10.19 g/L, 14.55 g/L, 23.21 g/L, 5 g/L and 2 g/L, respectively. The predicted maximum ethanol concentration was 15.31 g/L and the actual concentration of 15.23 ± 0.21 g/L was obtained with the optimal fermentation media, which was in close agreement to the model prediction.

3.3. Batch ethanol fermentation in 1.3 L bioreactor

Batch fermentation was conducted in a 1.3 L bioreactor for ethanol production. Utilization of individual and total sugars, biomass growth and ethanol production by recombinant *E. coli* FBWHR at 30% (v/v) of concentrated hot-water wood extract hemicellulosic hydrolyzate were depicted in Figure 3.

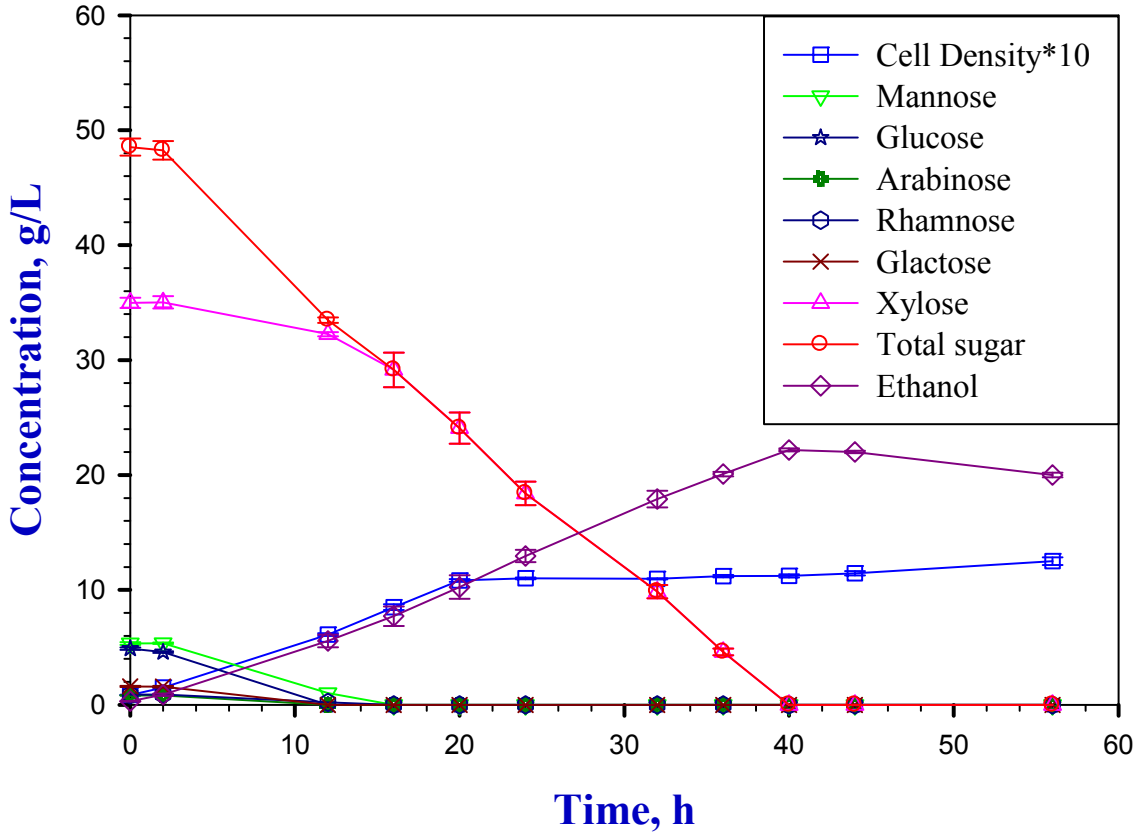

Figure 3. Utilization of individual and total sugars, biomass growth and ethanol production by recombinant *E. coli* FBWHR at 30% (v/v) of concentrated hot-water wood extract hemicellulosic hydrolyzate using optimized media in 1.3 L bioreactor.

The total sugar concentration of 48.53 ± 0.75 g/L was detected in 30% (v/v) of concentrated hot-water wood extract hydrolyzate. Under the optimal fermentation media conditions, the maximum ethanol concentration of 22.18 ± 0.13 g/L was achieved in 40 hours with a complete consumption of all the sugars. Table 5 shows the comparison of ethanol fermentation in the optimized medium to the previous study [11] (30% (v/v) of concentrated hot-water wood extract hydrolysate, recombinant *E.*

coli FBWHR). In the same fermentation process (pH, 7.0; Temperature, 35 °C, agitation, 200 rpm; air flow rate, 0.031 vvm), the maximum ethanol concentration of 22.18 ± 0.13 g/L was obtained with the optimized fermentation media, which was 1.22 times higher than that by using 20 g/L LB broth [11]. Meanwhile, the fermentation time reaching the maximum ethanol concentration was reduced from 46 hours to 40 hours. The ethanol yield and productivity increased by 1.20-fold and 1.41-fold, respectively, to the previous reported data for the same fermentation process. Owing to the sugar utilization for cell growth, cell maintenance and by-products production, the theoretical maximum ethanol yield of 0.51 g ethanol/g total sugar was not achieved.

Table 5. A comparison of ethanol fermentation by recombinant *E. coli* FBWHR using 30% (v/v) of concentrated hot-water wood extract hydrolyzate.

	This study	Wang et al. [10]
Fermentation time, hours	40	46
Ethanol concentration, g/L (n = 3)	22.18 ± 0.13	18.19
Ethanol yield ($Y_{p/s}$), g ethanol/g total sugar (n = 3)	0.46 ± 0.01	0.38
Ethanol productivity, g/(L·h) (n = 3)	0.56 ± 0.03	0.40

4. Conclusion

Optimization of fermentation media for ethanol production from concentrated hot-water hemicellulosic wood extract hydrolyzate by recombinant *E. coli* FBWHR has been successfully investigated combining PB design as well as CCD response surface methodology. A maximum ethanol concentration obtained in the optimized media agreed with the result of the model prediction. Batch scale-up fermentation with optimized media further enhanced the ethanol production. The fermentation time was shortened while the ethanol yield and productivity were improved. The optimal conditions studied in this work could contribute to the development of future pilot-scale ethanol production from hemicellulosic wood hydrolyzate.

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Conflict of Interest

All authors declare no conflicts of interest in this paper.

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