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Full Length Research Paper

Enhanced ethanol production from stalk juice of sweet sorghum by response surface methodology

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Sweet sorghum (sugar sorghum, *Sorghum bicolor*) is one kind of non-grain energy crops. As a novel green regenerated high-energy crop with high utility value, high yield of biomass, the sweet sorghum is widely used and developed in China. Stalk juice of sweet sorghum was used as the main substrate for ethanol production by a *Saccharomyces cerevisiae* strain because of the high content of sugar. Effects of different medium compositions, including urea, KH_2PO_4 and $MgSO_4$, on ethanol production were studied by response surface methodology in this paper. A second-order model that related the concentration of ethanol was developed and thus the optimal medium composition was obtained, which was 4.75 g l^{-1} urea, 3.58 g l^{-1} KH_2PO_4, and 0.98 g l^{-1} $MgSO_4$. Under this condition, the highest ethanol concentration reached 86.2 g l^{-1}.

Key words: Ethanol, sweet sorghum, stalk juice, medium composition, response surface methodology.

INTRODUCTION

Ethanol as an alternative energy resource has attracted more and more attention with the rising of oil price. Currently, in biotechnological ways, ethanol is produced mainly from sugar or starches by fermentation (Hu et al., 2006; Limtong et al., 2007). As part of grain has been used for ethanol production in China, it is considered that food security is threatened. It is suggested by the government that only “non-grain” materials can be used to produce ethanol in middle and long term program of renewable energy development (Zhang et al., 2010). Therefore, ethanol production from lignocellulose resources has been widely investigated (Lin et al., 2010; Tang et al., 2006; Wang et al., 2007, 2008). As a non-grain energy crop, sweet sorghum (also known as sugar sorghum) has the advantages of high biomass yield, rapid growth, clean and relatively low production cost (Zhang et al., 2010). Since stalk juice of sweet sorghum is rich in fermentative sugar, it is regarded as one of the most promising feedstock sources for bioethanol production (Laopaiboon et al., 2009; Oberoi et al., 2011; Yu et al., 2010). Conventional method changing one independent variable at a time for process optimization is laborious and time-consuming. Response surface methodology (RSM) is a powerful and useful tool in rapidly searching the key factors and the optimal conditions from a multivariable system, thus reducing the number of required experiments and workload (Myers, 1999). It has been widely applied to many biotechnological areas including bioethanol production (Lin et al., 2010; Oberoi et al., 2011; Wang et al., 2007). In this work, the stalk juice of sweet sorghum was used as main substrate for ethanol production by a *Saccharomyces cerevisiae* strain. The fermentation medium compositions were optimized by RSM, from which the yield of ethanol was enhanced.

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MATERIALS AND METHODS

Materials

Sweet sorghum was obtained from the countryside of Jinan,

Table 1. Characteristics of the stalk juice of sweet sorghum.

Parameter	Concentrations (gl^{-1})
Reducing sugar	35
Sucrose	150
Total sugar	185
Nitrogen	2.6
K^+	3.42*10^{-3}
Na^+	0.04*10^{-3}
Mg^{2+}	0.33*10^{-3}
Fe^{2+}	0.13*10^{-3}
Ca^{2+}	0.94*10^{-3}
Cu^{2+}	0.01*10^{-3}
Zn^{2+}	0.06*10^{-3}

Shandong, China. The stalk juice of sweet sorghum was prepared as described (Laopaiboon et al., 2009; Yu et al., 2010) and detailed characteristics are shown in Table 1. All chemicals were of the highest purity commercially available, which were purchased from Sinopharm Group Company Limited (Beijing, China).

Strain and fermentation

Laboratory strain of *S. cerevisiae* SEMF1 was used in this study and maintained on agar slants at 4°C. The microorganism was cultured for 24 h before used. Cultures were first incubated in seed medium (200 rpm, 33°C and 75 ml in 300-ml flask), which contained 20 g l^{-1} glucose, 5 g l^{-1} yeast extract, 5 g l^{-1} peptone and 5 g l^{-1} NaCl (pH 5.0). The mid-logarithmic-stage preculture was then inoculated (10%, v/v) into fermentation medium prepared with the stalk juice of sweet sorghum, in which urea, KH_2PO_4 and $MgSO_4$ were added. Fermentation was conducted at 200 rpm, 33°C and 150 ml medium in 500-ml flask. At regular time intervals, samples were removed to determine OD_{620nm} of the culture, and the concentrations of total sugar and ethanol.

Experimental design and optimization

Central composite design (CCD) was used to optimize ethanol production from the stalk juice of sweet sorghum. Urea (X_1, g l^{-1}), KH_2PO_4 (X_2, g l^{-1}) and $MgSO_4$ (X_3, g l^{-1}) were chosen as the independent variables, as presented in Table 2. The range of values was set based on the results of preliminary studies and the codes that correspond to these parameters were resolved. A factorial 2^3 design was determined from CCD with ethanol concentration (Y, g l^{-1}) as an output that depends on three basic variables. The experiment comprised of 17 runs, which were performed under the conditions presented in Table 2. Three-replicate runs were carried out at the center point. The ethanol-producing response was estimated using the following equation (a second-order response surface model):

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_{12} X_1 X_2 + \beta_{13} X_1 X_3 + \beta_{23} X_2 X_3 + \beta_{11} X_1^2 + \beta_{22} X_2^2 + \beta_{33} X_3^2$$

Where, Y is the predicted response (ethanol concentration, g l^{-1}); β_0, β_1, β_{11}, β_{12}, β_{13}, β_2, β_{22}, β_{23}, β_3 and β_{33} were the regression coefficients; and X_1, X_2, X_3 were the coded levels of the independent variables. The results were analyzed using a Design Expert 7.0 software package (STATISTICA, Inc.). Three-dimensional plots were obtained to study the interaction of one parameter with another. The critical concentration was determined using an established regression model and was based on the hump in the three-dimensional plots.

Analytical methods

Cell growth was measured spectrophotometrically at a wavelength of 620 nm (OD_{620nm}). Total sugar was determined by the DNS (3, 5-dinitrosalicylic acid) colorimetric method. Concentration of ethanol was measured by a SBA-40E biosensor (Biology institute of Shandong Academy of Sciences, Shandong, China) after diluted with distilled water.

RESULTS AND DISCUSSION

RSM modeling of results

The design matrix of the variables and the experimental responses are shown in Table 2. By applying multiple regression analysis to the experimental data, the following second order polynomial equation giving the concentration of ethanol (Y, g l^{-1}) as a function of urea (X_1, g l^{-1}), KH_2PO_4 (X_2, g l^{-1}), $MgSO_4$ (X_3, g l^{-1}) was obtained:

$$Y = 85.37 + 2.53X_1 + 0.82X_2 + 1.01X_3 - 1.75X_1X_2 - 0.65X_1X_3 - 0.32X_2X_3 - 2.45X_1^2 - 1.42X_2^2 - 1.28X_3^2$$

Statistical testing was carried out using Fisher's test for analysis of variance (ANOVA) (Table 3). The F and P values were 12.95 and 0.0014, respectively. The test model was statistically significant at the 99% level of significance. The quality of the fit of the quadratic regression model equation is expressed by the coefficient of determination (R^2). The results show the value of R^2 was 0.9433, indicating that 94.33% of the variability in the response could be explained by the model. The value of the adjusted determination coefficient, R^2_{Adj}, was 0.8704, which was also very high. These results show that the response equation provided a suitable model for the CCD experiment. The significant levels of each variable determined by t test are shown in Table 4. The Student's t test and P values were applied to check the significance of each coefficient. Among the model terms, the linear of urea (X_1, P = 0.0002) was more significant than the other two factors, which indicate that the concentration of urea in the medium had a direct effect on ethanol production. At the same time, the concentration of urea was also very significant in the quadratic level (X_1^2, P = 0.0005). In addition, the linear of $MgSO_4$ (X_3), the quadratic of KH_2PO_4 (X_2^2) and $MgSO_4$ (X_3^2), and the interactions between urea and KH_2PO_4 (X_1X_2) were also significant model terms.

The interactions between urea and $MgSO_4$ (X_1X_3), KH_2PO_4 and $MgSO_4$ (X_2X_3), had non-significant influence on ethanol production. The three-dimensional graphs for

Table 2. Design and results of the central composition experiment.

Coded level			Response
X_1 (Urea, g l^{-1})	X_2 (KH_2PO_4, g l^{-1})	X_3 ($MgSO_4$, g l^{-1})	Y (Ethanol, g l^{-1})
1 (5.0)	1 (4.0)	1 (1.2)	81.5
1 (5.0)	1 (4.0)	-1 (0.6)	81.3
1 (5.0)	-1 (3.2)	1 (1.2)	83.9
1 (5.0)	-1 (3.2)	-1 (0.6)	81.4
-1 (4.0)	1 (4.0)	1 (1.2)	81.9
-1 (4.0)	1 (4.0)	-1 (0.6)	78.1
-1 (4.0)	-1 (3.2)	1 (1.2)	76.3
-1 (4.0)	-1 (3.2)	-1 (0.6)	72.2
0 (4.5)	0 (3.6)	0 (0.9)	85.2
0 (4.5)	0 (3.6)	0 (0.9)	84.9
0 (4.5)	0 (3.6)	0 (0.9)	85.7
0 (4.5)	0 (3.6)	-1.68 (0.4)	81.7
0 (4.5)	0 (3.6)	1.68 (1.4)	83.6
0 (4.5)	-1.68 (2.9)	0 (0.9)	81.6
0 (4.5)	1.68 (4.3)	0 (0.9)	82.9
-1.68 (3.7)	0 (3.6)	0 (0.9)	74.9
1.68 (5.3)	0 (3.6)	0 (0.9)	83.8

Table 3. ANOVA for evaluation of the second-order model.

Source of variation	Degrees of freedom	Sum of squares	Mean square	*F*	*P*
Model	9	215.06	23.90	12.95	0.0014
Residual	7	12.92	1.85		
Lack of fit	5	12.59	2.52	15.42	0.0620
Pure of error	2	0.33	0.16		
Total	16	227.98			

R^2 = 0.9433, R2Adj = 0.8704.

Table 4. Coefficients and *t* values calculated from the central composition experiment.

Parameter	Regression coefficient	Stand error	*t*	*P*
Intercept	85.37	0.7828	109.06	<0.0001
X_1	2.53	0.3676	6.89	0.0002
X_2	0.82	0.3676	2.23	0.0611
X_3	1.01	0.3676	2.75	0.0286
X_1X_2	-1.75	0.4803	-3.64	0.0082
X_1X_3	-0.65	0.4803	-1.35	0.2180
X_2X_3	-0.33	0.4803	-0.68	0.5204
X_1^2	-2.45	0.4046	-6.05	0.0005
X_2^2	-1.42	0.4046	-3.52	0.0097
X_3^2	-1.28	0.4046	-3.17	0.0157

the response surface model are shown in Figure 1. It is evident that the response surfaces are convex in nature, showing that there are well-defined optimum conditions. It is obvious from the plots that ethanol production has a maximum point in the studied region. When X_1 = 0.4982, X_2 = -0.0498, and X_3 = 0.2739, ethanol yield would reach

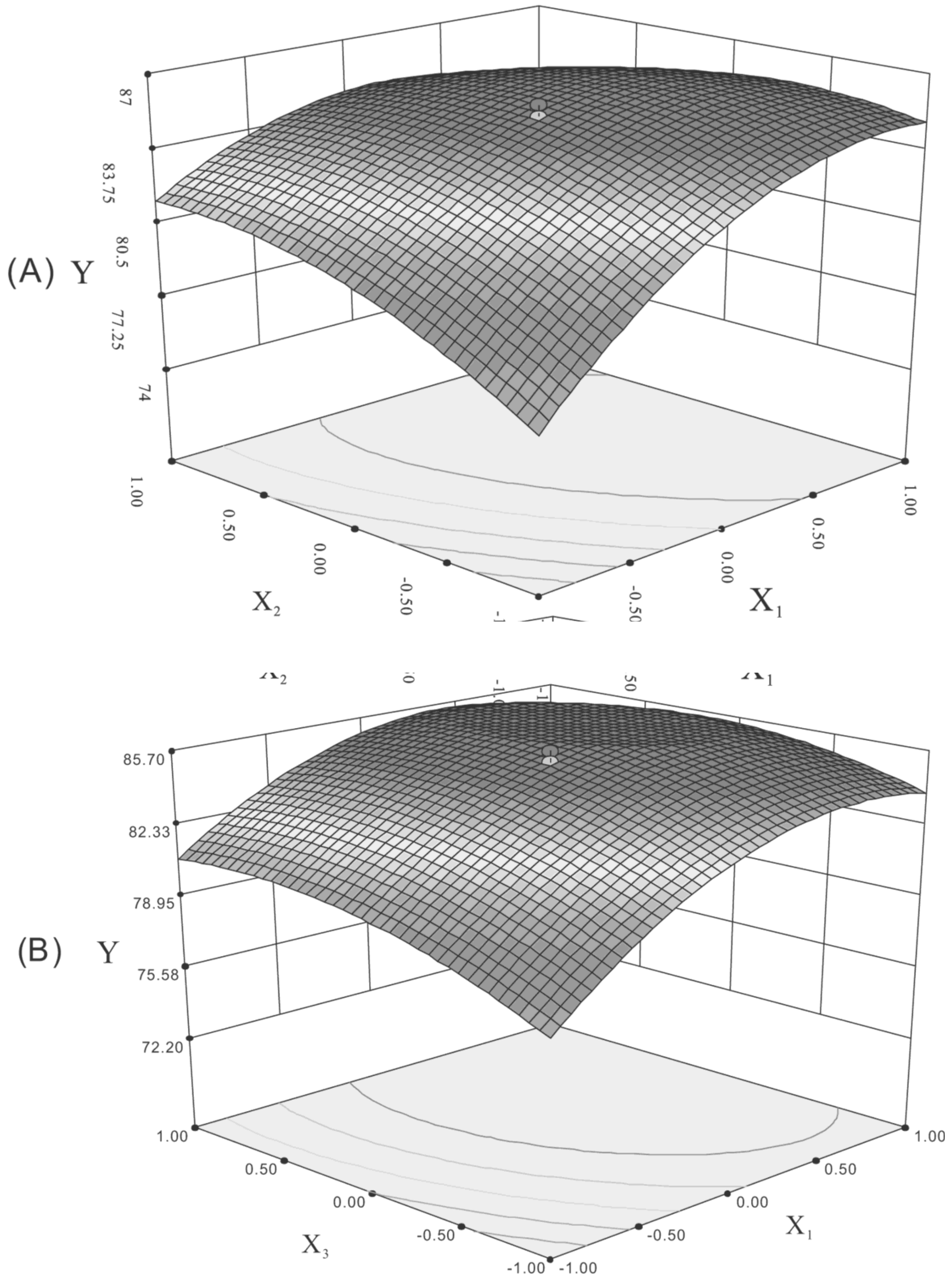

Figure 1. The response surface plots of ethanol production as a function of urea (X1), KH2PO4 (X2), and MgSO4 (X3). (A), the three-dimensional plot of ethanol concentrations (Y) vs. urea (X1) and KH2PO4 (X2). (B), the three-dimensional plot of ethanol concentrations (Y) vs. urea (X1) and MgSO4 (X3). (C), the three-dimensional plot of ethanol concentrations (Y) vs. KH2PO4 (X2) and MgSO4 (X3). When X1 = 0.4982 (urea, g l-1), X2 = –0.0498 (KH2PO4, g l-1), and X2 = 0.2739 (MgSO4, g l-1), Y will arrive at the maximum point (ethanol, 86.1 g l-1).

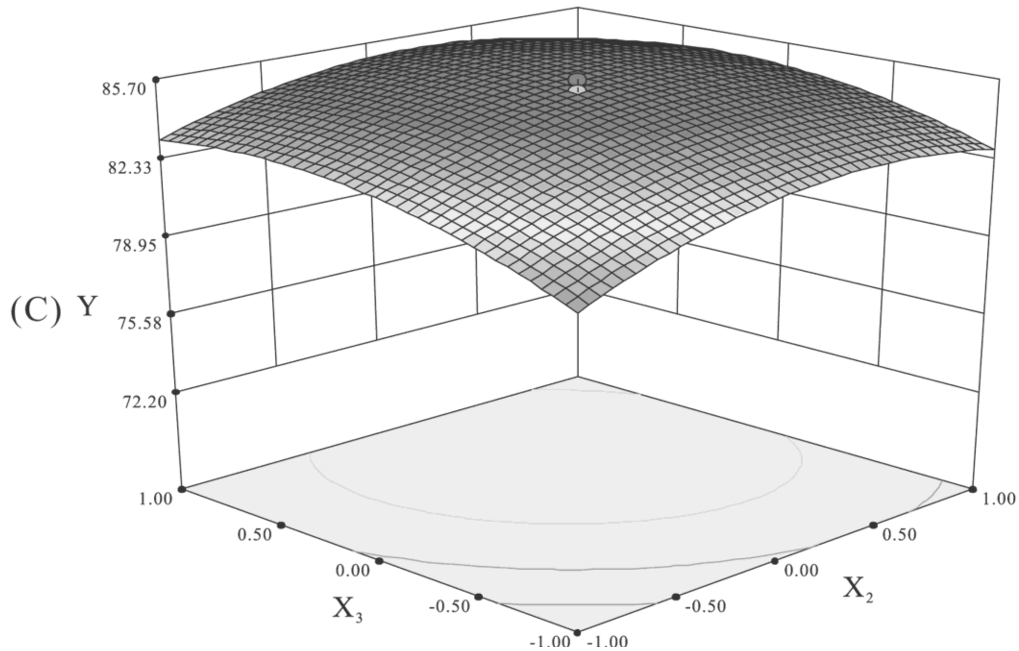

Figure 1. Contd.

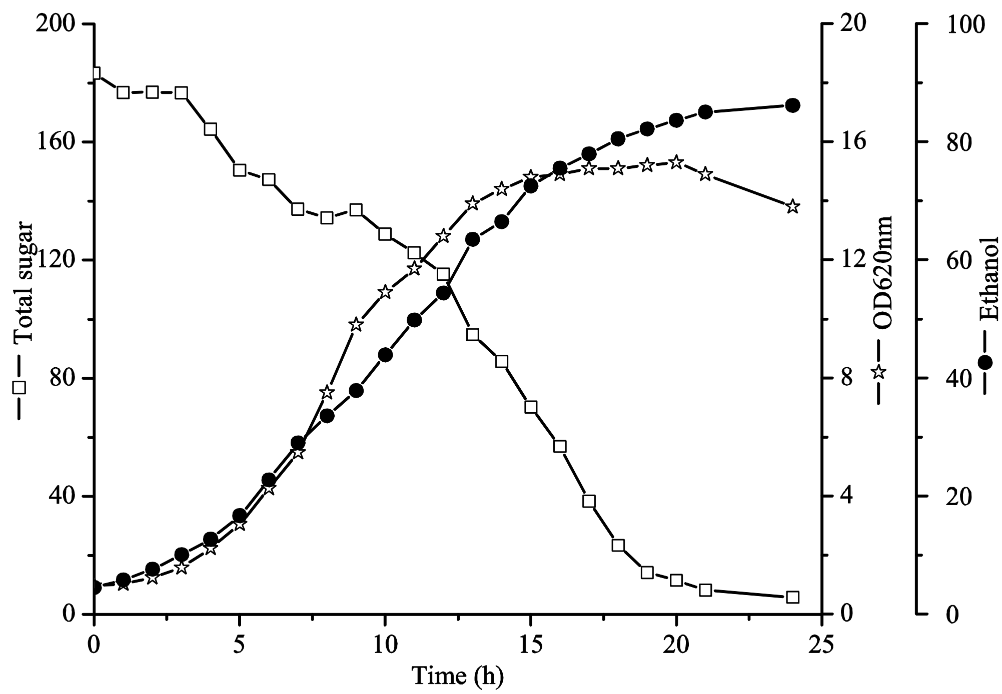

Figure 2. Time courses of ethanol production. Symbols represent:□, total sugar; ◊, OD620nm; ●, ethanol.

the point of 86.1 g l^{-1}. Therefore, the optimum concentrations of the three medium compositions added into the stalk juice of sweet sorghum for ethanol production by *S.cerevisiae* are as follows: 4.75 g l^{-1} urea, 3.58 g l^{-1} KH_2PO_4, and 0.98 g l^{-1} $MgSO_4$.

Verification experiment

To confirm the second-order model for predicting maximum ethanol production, verification experiment was performed using the optimal medium. Figure 2 shows the time course of ethanol production, from which we could clearly see the changes of OD_{620nm} and total sugar. The highest concentration of ethanol reached 86.2 g l^{-1} at 24 h, which was closely consistent with the model predicted value of 86.1 g l^{-1}. The residue sugar was about 5 g l^{-1} and the yield of ethanol was 91.9%. The good correlation justified the existence of an optimum point and showed that the established ethanol production model reliable.

Conclusion

In this study, the stalk juice of sweet sorghum was used as main substrate for ethanol production, and it was very suitable for ethanol fermentation because of the high content of fermentable sugars. The medium composition was optimized by RSM and a second-order model that related ethanol production was developed. The optimal medium composition was thus obtained, which was 4.75 g l^{-1} urea, 3.58 g l^{-1} KH_2PO_4, and 0.98 g l^{-1} $MgSO_4$, under these conditions, the ethanol producion reached 86.2 g l^{-1}. The present study suggests that stalk juice of sweet sorghum can be used as an alternative substrate for ethanol production by *S. cerevisiae*. In the experiment, we also found that the stalk juice very unstable, which seriously influenced the fermentation process and the final ethanol production. Therefore, if the storage method of the sweet sorghum juice was developed, the ethanol yield would be higher in the mass production in the future.

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