J Ind Microbiol Biotechnol (2010) 37:1033–1040
DOI 10.1007/s10295-010-0750-5

ORIGINAL PAPER

Development of succinic acid production from corncob hydrolysate by *Actinobacillus succinogenes*

Jie Yu · Zhimin Li · Qin Ye · Yong Yang · Shulin Chen

Received: 3 August 2009 / Accepted: 20 May 2010 / Published online: 9 June 2010

Abstract Succinic acid is one of the most important platform chemicals since it has great potential in industrial applications. In this study, corncob hydrolysate was used for succinic acid production. After diluted acid treatment, xylose was released from hemicellulose as the predominant monosaccharide in the hydrolysate, whereas glucose was released very little and most was retained as cellulose in the raw material. Without any detoxification, corncob hydrolysate was used directly as the carbon source in the fermentation. *Actinobacillus succinogenes* could utilize the sugars in the hydrolysate to produce succinic acid efficiently. Through medium optimization, yeast extract was selected as the nitrogen source and $MgCO_3$ was used to control pH. A total of 23.64 g/l of succinic acid was produced with a yield of 0.58 g/g based on consumed sugar, indicating that the waste corncob residue can be used to produce value-added chemicals practically.

Keywords *Actinobacillus succinogenes* · Succinic acid · Corncob hydrolysate · Xylose · Optimization

Introduction

Succinic acid is one of the most important platform chemicals and can be used as a precursor for many chemicals of industrial importance, such as adipic acid, 1,4-butanediol, and tetrahydrofuran [13, 17]. For sustainable development in this era of petroleum shortage, production of succinic acid by microbial conversion of renewable feedstock has attracted great interest. Among the succinic acid-producing strains, *Actinobacillus succinogenes* has high potential because it can convert different carbon sources to succinic acid efficiently [5]. Du et al. [3] developed a wheat biorefining strategy for succinic acid production using *A. succinogenes*. Liu et al. [9] utilized cane molasses as a low-cost carbon source for succinic acid production by *A. succinogenes*. In these studies, the raw materials contained starch or sucrose, which were better carbon sources for microbial fermentation, but they were still expensive. Furthermore, Zheng et al. [18] successfully used straw hydrolysates, obtained by diluted alkali pretreatment and enzymatic hydrolysis, for production of succinic acid by *A. succinogenes*. Chen et al. [1] used diluted acid hydrolysate of corn fiber as carbon source for the production of succinic acid by *A. succinogenes*. However, these materials were pretreated by enzymatic hydrolysis or detoxified additionally, which meant additional processing and cost.

Corncob is a major waste and residue in corn production, and is produced in large amounts all over the world. It can be used as animal feed, recycled fertilizer in soil, or burned as a fuel [11]. Recently, more and more attention has been paid to its potential applications because of the development of biorefinery industry. Corncob, being rich in cellulose and hemicellulose, can be applied in the production of biobased chemicals and biofuels as an inexpensive and renewable carbon source. Corncob contains 40–45% cellulose, 30–35% hemicellulose, and 10–20% lignin [7]. After chemical or physical pretreatment, the structure of raw materials will be broken down and cellulose and hemicellulose can be hydrolyzed into

J. Yu · Z. Li (✉) · Q. Ye
State Key Laboratory of Bioreactor Engineering, East China University of Science and Technology, Shanghai 200237, China
e-mail: lizm@ecust.edu.cn

J. Yu · Y. Yang · S. Chen (✉)
Institute of Microbiology, Chinese Academy of Sciences, Beijing, China

monosaccharides enzymatically. The composition of corncob hydrolysate depends on the pretreatment methodology. Alkaline pretreatment dissolves lignin and releases ferulic acid into the hydrolysate [4], whereas acid pretreatment causes hemicellulose to depolymerize to pentoses. Pentoses from diluted pretreatment are an attractive source for microbial fermentation. Many microorganisms can utilize and convert pentoses in corncob hydrolysate to value-added products. For example, corncob hydrolysate has been used for ethanol and xylitol production by *S. cerevisiae* [8] and lactic acid by *Rhizopus oryzae* [12].

In this study, *A. succinogenes* is used to produce succinic acid from corncob hydrolysates, and succinic acid production is optimized by statistical methodology. While corncob hydrolysate mainly provides the carbon source, an appropriate nitrogen source and other components are still needed and were therefore explored in this study. The developed method for succinic acid production from corncob hydrolysate will benefit the application of corncob in the biorefinery industry.

Materials and methods

Microorganism

Actinobacillus succinogenes purchased from the China Center of Industrial Culture Collection (CICC 11014) was used for all succinic acid fermentations in this study.

Preparation of corncob hydrolysate

Dry corncob was obtained from the suburbs of Beijing, P.R. China. Prior to diluted acid pretreatment, corncob was ground by using a commercial plant grinder (Rong Tsong Precision Technology Co.) without mesh screening. The dry corncob particles were mixed with diluted sulfuric acid aqueous solution (0.8%) at a ratio of 1:4 (w/v). The suspension was hydrolyzed in an autoclave at 110°C for 2 h. The raw hydrolysate was neutralized to pH 6.5 with solid $Ca(OH)_2$, and then was filtrated with filter paper to remove the solid materials. Table 1 shows the major composition of corncob hydrolysate. The filtrate is the corncob hydrolysate employed in all succinic acid fermentation in this study.

Table 1 The major composition of corncob hydrolysate

	Concentration (g/l)
Xylose	37.27
Glucose	1.34
Arabinose	6.20
Cellobiose	3.39
Fufural	0.59

Media and cultivation

Inoculum medium contained 15 g/l tryptone, 5 g/l soy peptone, and 5 g/l NaCl. Corncob hydrolysate was diluted to contain 30 g/l of xylose, then inorganic salts were added to make the production medium contain (g/l): NaH_2PO_4 1.5, Na_2HPO_4 1.5, NaCl 1.0, $MgCl_2$ 0.2, and $CaCl_2$ 0.2. The concentrations of xylose, nitrogen source, and pH regulator were optimized in this study. Six nitrogen sources, namely, wheat bran, beef extract, soy peptone, soy bean powder, yeast extract, and corn steep liquor (CSL), were evaluated separately. The concentration of each organic nitrogen source was set at 20 g/l. $MgCO_3$, $NaHCO_3$, and Na_2CO_3 acted as pH regulators to be screened and the concentration of each was 40 g/l.

For inoculum cultivation, *A. succinogenes* was incubated in a 250-ml flask containing 100 ml of the inoculum medium aerobically at 37°C for 11 h. For succinic acid production, the inoculum was transferred (6%, v/v) into the production medium in 100-ml anaerobic bottles containing 25 ml medium with the headspace filled with CO_2 gas. The cultures were grown in a rotary shaker at 37°C and 150 rpm for 48 h. All experiments were performed in triplicate.

Analytical methods

Culture supernatant was collected by centrifuging the fermentation broth at 10,000*g* for 10 min. The concentrations of products (succinic acid, acetic acid, formic acid, and lactic acid) and substrates (glucose, xylose, arabinose, cellobiose) were analyzed by HPLC (Waters, Milford, MA, USA) equipped with a cation-exclusion column (Waters IC-Pak^{TM} ion exclusion column) and a refractive index detector (Waters). The mobile phase was 2 mM H_2SO_4 solution at a flow rate of 0.5 ml/min, and the column was operated at 45°C.

Experimental design and analysis

Plackett–Burman design and analysis

The Plackett–Burman design [10] was used to screen the factors having significant effects on succinic acid production from the corncob hydrolysate. The variables evaluated are listed in Table 2, including corncob hydrolysate, yeast extract, $MgCO_3$, and inorganic salts. Each independent variable was investigated at a high and a low level. Eight variables and four dummy variables (D1–D4) were evaluated in 12 experiments (Table 3). The effect of each

Table 2 Levels of the variables and statistical analysis of Plackett–Burman design

Number	Variable	Code	Level	
			−1	1
1	Xylose/g l^{-1}	X_1	15.88	31.75
2	Yeast extract/g l^{-1}	X_2	10	20
3	Na_2HPO_4/g l^{-1}	X_3	1.5	3
4	NaH_2PO_4/g l^{-1}	X_4	1.5	3
5	NaCl/g l^{-1}	X_5	1	3
6	$MgCl_2$/g l^{-1}	X_6	0	0.4
7	$CaCl_2$/g l^{-1}	X_7	0.2	0.4
8	$MgCO_3$/g l^{-1}	X_8	40	60

Table 3 Plackett–Burman experimental design matrix

Run	X_1	X_2	X_3	X_4	X_5	X_6	X_7	X_8	Succinic acid/g l^{-1}
1	1	−1	1	−1	−1	−1	1	1	19.11
2	1	1	−1	1	−1	−1	−1	1	16.85
3	−1	1	1	−1	1	−1	−1	−1	8.21
4	1	−1	1	1	−1	1	−1	−1	21.98
5	1	1	−1	1	1	−1	1	−1	19.34
6	1	1	1	−1	1	1	−1	1	17.94
7	−1	1	1	1	−1	1	1	−1	8.28
8	−1	−1	1	1	1	−1	1	1	7.68
9	−1	−1	−1	1	1	1	−1	1	8.67
10	1	−1	−1	−1	1	1	1	−1	18.10
11	−1	1	−1	−1	−1	1	1	1	7.69
12	−1	−1	−1	−1	−1	−1	−1	−1	8.76

The variables in this table have been coded in Table 2

variable (E_i) on the response was determined by subtracting the average response at the low level from that at the high level. The data of dummy variables reflect the standard error of the experiments, and can be used to estimate the significance of the variable examined. In this work, the statistical software package SAS version 9.0 (USA) was used for calculating the P value through the F test to analyze the significance.

Box–Behnken design and analysis

Once the significant factors were identified in the experiments using the Plackett–Burman design, a Box–Behnken design [6] was used to optimize the levels. The levels of the variables and the experimental design are shown in Table 3. Based on the experimental results shown in Table 3, the response (succinic acid yield) was correlated to the variables by a second-order polynomial equation:

$$Y = \beta_0 + \sum \beta_i x_i + \sum \beta_i x_i^2 + \sum \beta_{ij} x_i x_j$$

where Y is the predicted response, β is the coefficient of the equation, and x_i and x_j are the coded levels of variables i and j, respectively. The SAS software was used for estimation of the coefficients through nonlinear regression. The F test was used to evaluate the significance of the models.

Results and discussion

Screening of nitrogen sources

Carbon and nitrogen sources are significant factors affecting cell growth and succinic acid production. In this study, xylose was the major monosaccharide in the corncob hydrolysate (Table 1). Six substrates, namely, wheat bran, beef extract, soy peptone, soy bean powder, yeast extract, and corn steep liquor, were evaluated as nitrogen source separately. It has been reported that inorganic nitrogen sources are not good for cell growth of *A. succinogenes*. A chemically defined medium needs amino acids and vitamins for cell growth [9, 16]. Therefore, the effects of inorganic nitrogen sources were not considered. According to Yang's paper [16], the concentration of the nitrogen source was set at 20 g/l initially. The results are shown in Fig. 1.

Among the complex nitrogen sources evaluated, yeast extract gave the best results for both succinic acid production and the ratio of succinic acid to other by-products. When yeast extract and corncob hydrolysate were applied in the fermentation of *A. succinogenes*, succinic acid was produced at 22.09 g/l and the ratio of succinic acid to acetic acid was 5.26, higher than those results in

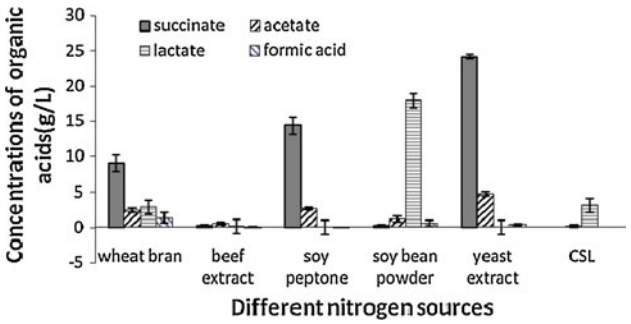

Fig. 1 Production of organic acid including succinate, acetate, lactate, and formic acid on different nitrogen sources (wheat bran, beef extract, soy bean peptone, yeast extract, and CSL). Cells were grown in anaerobic bottles for 48 h with different nitrogen sources (20 g/l). Symbols are the succinate (*shaded bar*), acetate (*striped bar*), lactate (*horizontal striped bar*), formic acid (*backward striped bar*). All experiments were performed in triplicate

fermentation with soy peptone. It was interesting that not only succinic acid production but also the ratio of organic acids was changed when using different nitrogen sources. Succinate was the major product when wheat bran, soy peptone, and yeast extract were used as the nitrogen source. Lactate was the main product when soy bean powder was applied, and acetate was the dominant product when beef extract medium was used. CSL has been applied to succinic acid production from whey and cane molasses by *A. succinogenes* [9, 15]. However, our results showed that using CSL as the nitrogen source produced very little succinic acid and more acetate and lactate. This phenomenon was different from the results using whey and cane molasses as carbon sources. It has been reported that the yeast extract and bioTrypcase affect lactic acid formation and the metabolic profiles of *Bacillus thermoamylovorans* [2]. The complicated composition of the organic nitrogen source makes it difficult to explain which component has a significant effect on metabolite production. The literature and our result indicate that the interaction between the carbon and nitrogen sources contributes significantly to succinate production and metabolite profile.

Effect of different pH regulators on production of succinic acid

The effect of different pH regulators on succinic acid formation was examined. As shown in Fig. 2, $MgCO_3$ gave higher succinic acid production and less by-product formation compared with $NaHCO_3$ and Na_2CO_3. During the progress of fermentation, more and more organic acids are accumulated to make the pH decrease. In order to control pH, some regulators are needed. Carbonate can neutralize the formed acid and provide CO_2 simultaneously. CO_2 can be bound to phosphoenolpyruvate (PEP) to form succinic acid in an anaplerotic reaction catalyzed by PEP carboxylase. In succinic acid production by *E. coli*, $NaHCO_3$ and Na_2CO_3 are used as a neutralizer and donor of CO_2 [13]. Mg^{2+} has several effects on cellular metabolism. $MgCO_3$ can not only control pH and provide CO_2, but also supply the cofactor Mg^{2+} to PEP carboxykinase which is the key enzyme used to synthesize succinate during the fermentation [14]. Thus, $MgCO_3$ was selected as pH regulator in this study.

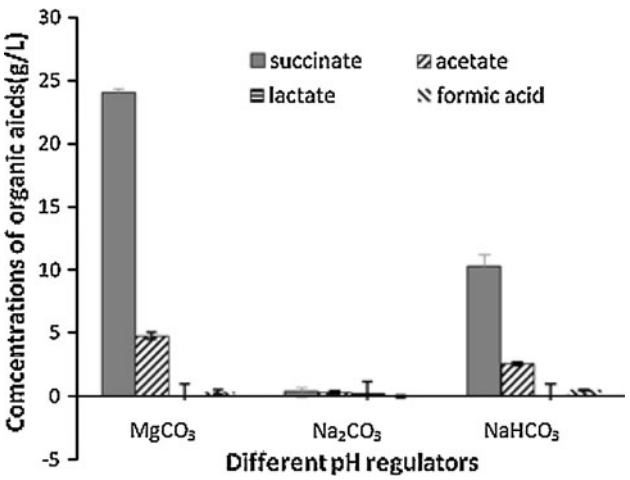

Fig. 2 Production of organic acid including succinate, acetate, lactate, and formic acid with different pH regulators. Cells were grown in anaerobic bottles for 48 h with different pH regulators. Symbols are the succinate (*shaded bar*), acetate (*striped bar*), lactate (*horizontal striped bar*), formic acid (*backward striped bar*). All experiments were performed in triplicate

Plackett–Burman design for screening of variables

The screening of factors having significant effects on succinic acid production was conducted according to the Plackett–Burman design. *A. succinogenes* was cultivated under 12 different conditions, with succinic acid production as the response (Table 3). The effects of the variables on succinate production, and their associated *P* values were calculated. Here, a variable with P value < 0.10 was considered as significant. In this study, corncob was pretreated with mild diluted acid, and the major component in the hydrolysate was xylose, which accounted for 77% of the total monosaccharides (Table 1). Harmful by-products usually formed during diluted acid pretreatment were detected at very low level in the present study. Thus, only the concentration of xylose in the hydrolysate was considered as a factor. It was found that among the eight factors investigated, only xylose had significant effect on succinic acid formation according to the *P* value (Table 2). Initial sugar concentration is reported to be important in succinic acid production; higher initial sugar benefits succinic acid production and microbial metabolism, but more than 80 g/l of reducing sugar would inhibit cell growth and succinic acid formation [18]. However, in this paper the available xylose in the corncob hydrolysate was limited, which resulted in lower yield. Yeast extract is relatively expensive compared with other medium components; therefore, it was selected as a factor to be optimized furthermore. Moreover, $MgCO_3$ was used in a large amount and also taken into consideration for further study.

Box–Behnken design

Based on the results of Plackett–Burman experiments, xylose, yeast extract, and $MgCO_3$ were considered to be optimized further. A three-variable Box–Behnken design [5] with three replicates at the center point was applied. The levels of the variables and the experimental design are shown in Table 4. Table 5 shows the experimental matrix. The concentrations of the nonsignificant medium

Table 4 The Box–Behnken experimental design with three independent variables

Code	Xylose/g l^{-1} X_1	Yeast extract/g l^{-1} X_2	$MgCO_3$/g l^{-1} X_3
−1	28.8	5	30
0	36	10	40
1	43.6	15	50

Table 5 The Box–Behnken experimental design and results

Run	Xylose	Yeast extract	$MgCO_3$	Succinic acid/g l^{-1}
1	−1	−1	0	18.10
2	−1	1	0	15.18
3	1	−1	0	19.58
4	1	1	0	22.18
5	0	−1	−1	21.68
6	0	−1	1	18.56
7	0	1	−1	20.87
8	0	1	1	17.65
9	−1	0	−1	18.16
10	1	0	−1	21.77
11	−1	0	1	16.29
12	1	0	1	23.28
13	0	0	0	23.85
14	0	0	0	21.36
15	0	0	0	21.84

components were set at the lower levels (Table 2). Experiments 1–12 were performed with different combinations of substrates and experiments 13–15 were performed under standard conditions. By applying multiple regression analysis to the experimental data, the following second-order polynomial equation (Eq. 1) was obtained to predict the succinic acid production using the software SAS 9.0:

$$Y = 22.35 + 2.385X_1 - 0.255X_2 - 0.8375X_3 - 1.7025X_1^2 + 1.38X_1X_2 + 0.845X_1X_3 - 1.8875X_2^2 - 0.025X_2X_3 - 0.7725X_3^2 \quad (1)$$

where Y is the predicted succinate production and X_1, X_2, and X_3 are the coded values of xylose, yeast extract, and $MgCO_3$, respectively. The analysis of variance (ANOVA) was performed to identify the effect of each factor on succinate production (Table 6).

The value of LOF (lack of fit) indicates the probability of unfitness between the model predicted and actual values. According to the result, the P value of LOF was 0.53308, i.e., greater than 0.05, indicating that the model was appropriate. The value of adjusted R^2, a measure of fitness

Table 6 Variance analysis of regression equation

Source	Degree of freedom	Sum of squares	F value	P value
X_1	1	45.50	25.95	0.0038
X_2	1	0.52	0.30	0.6094
X_3	1	5.61	3.20	0.1336
X_1 X_1	1	10.70	6.10	0.0565
X_1 X_2	1	7.62	4.34	0.0916
X_1 X_3	1	2.86	1.63	0.2579
X_2 X_2	1	13.15	7.50	0.0408
X_2 X_3	1	0.0025	0.0014	0.9713
X_3 X_3	1	2.20	1.26	0.3132
Model	9	85.27	5.40	0.03892
Lack of fit	3	5.28	1.01	0.5330

The variables in this table have been coded in Table 4

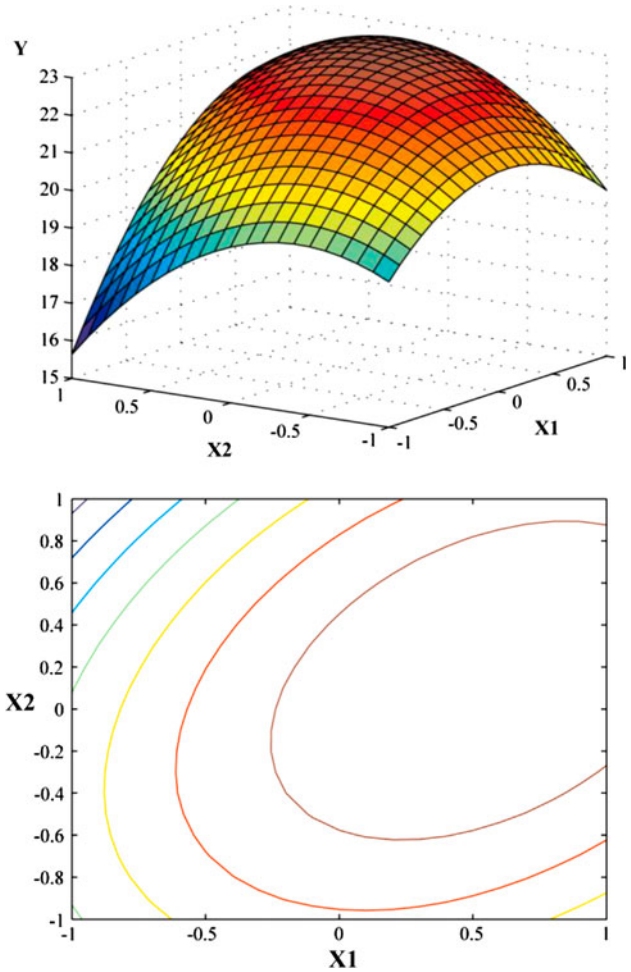

Fig. 3 The response surface plot and corresponding contour plot showing the effects of xylose (X_1), yeast extract (X_2), and their mutual interaction on succinate production, with optimum level of $MgCO_3$ (38.66 g/l)

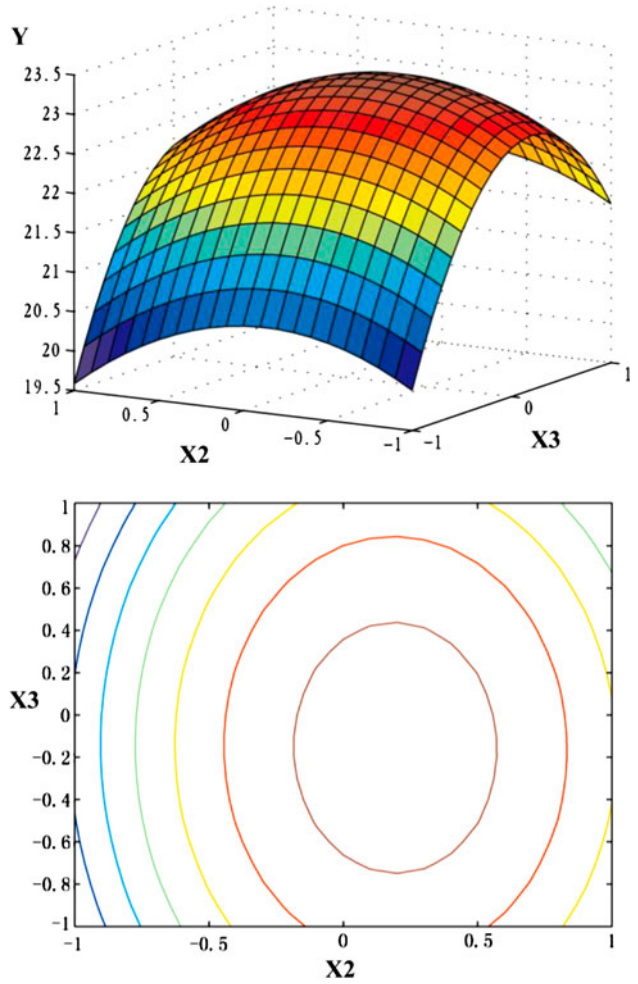

Fig. 4 The response surface plot and corresponding contour plot showing the effects of yeast extract (X_2), $MgCO_3$ (X_3), and their mutual interaction on succinate production, with optimum level of xylose (41.41 g/l)

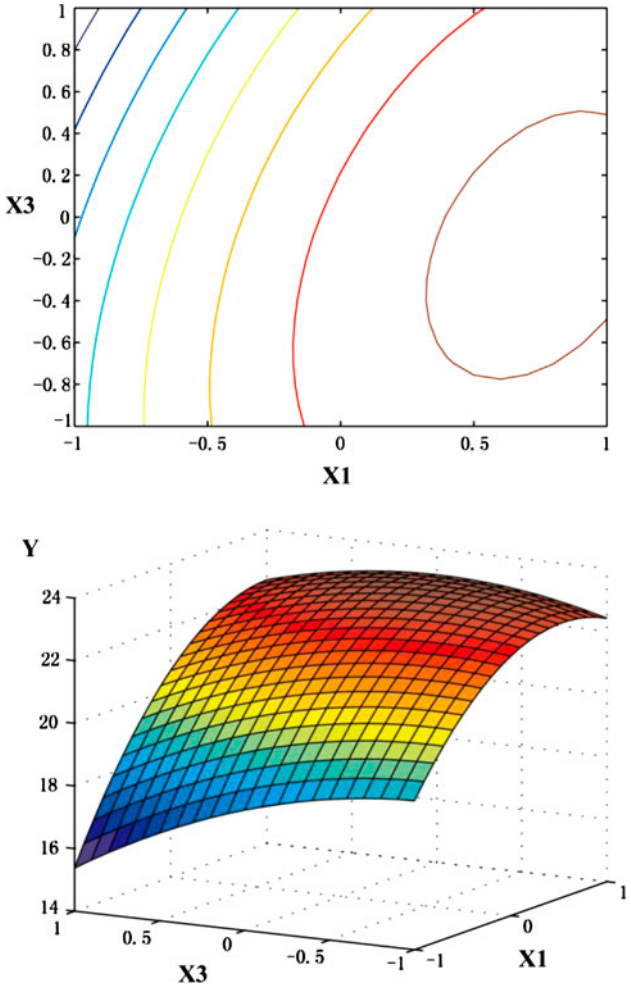

Fig. 5 The response surface plot and corresponding contour plot showing the effects of xylose (X_1), $MgCO_3$ (X_3), and their mutual interaction on succinate production, with optimum level of yeast extract (11.04 g/l)

of the regression model, was 0.9068, i.e., greater than 0.9, which suggested that the experimental data were in agreement with predicted values. From the differentiated form of Eq. 1, the optimal coded values of X_1, X_2, and X_3 were found to be 0.7514, 0.2080, and −0.1345, respectively. Correspondingly, we obtained the optimal point of the model at 41.41 g/l of xylose, 11.04 g/l of yeast extract, and 38.66 g/l of $MgCO_3$. Under such conditions, a predicted maximum of succinate production was 23.28 g/l.

Using the SAS 9.0 software, we calclulated the response surface curves and the corresponding contour curves described by the regression model, which are shown in Figs. 3, 4, and 5. Here, each response surface plot represents the effects of two independent variables at an optimal level of the third variable. The shape of the corresponding contour plots indicates whether the mutual interaction between the independent variables is significant. As shown in Figs. 3, 4, and 5, the response surface of succinate concentration shows a clear peak, indicating that the optimal conditions fall inside the design boundary. Figure 3 shows the response surface plot and corresponding contour curves based on the independent variables xylose (X_1) and yeast extract (X_2), while the third independent variable, $MgCO_3$ (X_3), was kept at an optimal level. In Figs. 4 and 5, the concentrations of xylose and yeast were varied.

Within the scopes of the variables investigated in uniform design, an additional series of fermentation trials were carried out to assess the validity of these findings. Simultaneously, the initial medium before optimization was used as control. The results of the confirmatory trials are listed in Table 7, which suggests that there is a high degree of fit between the observed values in the experiments and the values predicted by Eq. 1. The xylose concentration was not consistent with the data mentioned because the corncob hydrolysate cannot be identical between batches due to changes in the quality of feedstock and processing. In this

Table 7 The arrangement and results of confirmatory trials

Trials	Change of medium Ingredients (g/l)								Concentration of succinic acid (g/l)
	Xylose[a]	Yeast extract	$MgCO_3$	Total sugar[a]	Yield (%)[b]	Xylose[c]	Total sugar[c]	Yield (%)[d]	
Initial medium	30.52	15	40	38.70	53.60	0	5.65	49.50	16.36 ± 0.68
Optimum medium	38.07	11	38	50.59	62.10	0	9.80	57.96	23.64 ± 1.27

All experiments were performed in triplicate

[a] Initial concentration

[b] Yield based on xylose consumption

[c] Final concentration

[d] Yield based on total sugar consumption

study, the succinic acid production was increased from 16.36 to 23.64 g/l, an improvement of 44.50%. The yield was enhanced, too, either based on consumed xylose or total sugar. Additionally, as we can see from the major composition of corncob hydrolysate, C5 sugars including xylose and arabinose account for 90% of monosaccharides and glucose only 3%. Thus, nearly all the succinic acid was transformed from C5 sugars.

In succinic acid production, the cost of raw materials is an important factor. In this work, an economic process for succinic acid production from corncob hydrolysates by *A. succinogenes* was developed. Corncob hydrolysates prepared by diluted acid pretreatment without detoxification was the carbon source. After the medium optimization, the titer and yield of succinic acid were 23.64 g/l and 57.96% at 48 h. The diluted acid treatment applied to corncob in this study was mild and only released pentoses from the hemicellulose of the corncob, whereas a large amount of glucose was still retained in the form of cellulose. This indicates that corncobs could be used in two separate processes. In the first stage, pentoses are released and utilized to produce succinic acid, which has always been considered as a waste in cellulosic biomass degradation. Then in the second stage, glucose is formed and converted to other value-added chemicals. This strategy may improve the efficiency of each conversion and prevent problems in the co-degradation and metabolism of C5 and C6 sugars. Although the productivity and yield of succinic acid in this study were not high enough compared with the literature data, succinic acid can be transformed from corncob hydrolysate effectively after the optimization presented in this paper. To our knowledge, this was the first effort to develop a process for succinic acid production from pentoses in corncob, and should be useful to help the full utilization of corncob. Further optimizations are being conducted to improve the process efficiency and make this process more advantageous.

Acknowledgments This study was supported by the National Basic Research Program of China-'973' Program 2007CB714304. We thank the Institute of Microbiology, Chinese Academy of Sciences, for helping with this experiment.

References

1. Chen KQ, Jiang M, Wei P, Yao J, Wu H (2010) Succinic acid production from acid hydrolysate of corn fiber by *Actinobacillus succinogenes*. Appl Biochem Biotechnol 160:477–485
2. Combet-Blanc Y, Dieng MC, Kergoat PY (1995) Effect of organic complex compounds on *Bacillus thermoamylovorans* growth and glucose fermentation. Appl Environ Microbiol 65:4582–4585
3. Du CY, Lin SKC, Koutinas A, Wang RH, Dorado P, Webb C (2008) A wheat biorefining strategy based on solid-state fermentation for fermentative production of succinic acid. Bioresour Technol 17:8310–8315
4. Du Preez JC, Bosch M, Prior BA (1986) The fermentation of hexose and pentose sugars by *Candida shehatae* and *Pichia stipitis*. Appl Microbiol Biotechnol 23:228–233
5. Guettler MV, Rumler D, Jain MK (1999) *Actinobacillus succinogenes* sp. nov., a novel succinic-acid-producing strain from the bovine rumen. Int J Syst Evol Microbiol 1:207–216
6. Kennedy M, Krouse D (1999) Strategies for improving fermentation medium performance: a review. J Ind Microbiol Biotechnol 23:456–475
7. Larsson S, Reimann A, Nilvebrant NO, Jonsson LJ (1999) Comparison of different methods for the detoxification of lignocellulose hydrolyzates of spruce. Appl Biochem Biotechnol 77:91–103
8. Latif F, Rajoka MI (2001) Production of ethanol and xylitol from corn cobs by yeasts. Bioresour Technol 77:57–63
9. Liu YP, Zheng P, Sun ZH, Ni Y, Dong JJ, Zhu LL (2008) Economical succinic acid production from cane molasses by *Actinobacillus succinogenes*. Bioresour Technol 99:1736–1742
10. Plackett RL, Burman JP (1946) The design of optimum multifactorial experiments. Biometrika 33:305–325
11. Shi YF, Dai HX (1995) The comprehensive utilization of corncob. China West Cereals Oils Technol 20:53–55
12. Miura S, Arimura T, Itoda N, Dwiarti L, Jin BF, Cui HB, Okabe M (1997) Production of L-lactic acid from corncob. J Biosci Bioeng 97:153–157

13. Song H, Lee SY (2006) Production of succinic acid by bacterial fermentation. Enzyme Microb Technol 39:352–361
14. Van der Werf MJV, Guettler MV, Jain MK, Zeikus HG (1997) Environmental and physiological factors affecting the succinate product ratio during carbohydrate fermentation by *Actinobacillus* sp. 130Z. Arch Microbiol 167:332–342
15. Wan CX, Li YB, Shahbazi A, Xiu SN (2008) Succinic acid production from Cheese whey using *Actinobacillus succinogenes* 130 Z. Appl Biochem Biotechnol 145:111–119
16. Yang K, Zhan XB, Chen Y, Zhang LM (2008) Optimization of fermentation medium producing succinate by response surface method. J Anhui Agri Sci 36:6611–6614
17. Zeikus JG, Jain MK, Elankovan P (1999) Biotechnology of succinic acid production and markets for derived industrial products. Appl Microbiol Biotechnol 51:545–552
18. Zheng P, Dong JJ, Sun ZH (2009) Fermentative production of succinic acid from straw hydrolysate by *Actinobacillus succinogenes*. Bioresour Technol 100:2425–2429