Korean J. Chem. Eng., **28**(7), 1566-1572 (2011)
DOI: 10.1007/s11814-010-0535-6

Efficient production of glutathione using hydrolyzate of banana peel as novel substrate

Xue-Dong Chen, Gong-Yuan Wei†, Jun-Li Zhang, and Ying-Ying Dong

School of Basic Medicine and Biological Science, College of Medicine, Soochow University, Suzhou 215123, P. R. China
(*Received 12 July 2010 • accepted 31 December 2010*)

Abstract−The hydrolyzate of banana peels containing abundant fermentable sugars as glucose, xylose, mannose, and arabinose was successfully used as a novel substrate for the efficient production of glutathione by *Candida utilis* SZU 07-01. Xylose was first selected as the sole carbon source for glutathione production, medium optimization for better cell growth and higher glutathione using response surface methodology consisting of PB design, the steepest ascent experiment and CCD was carried out, and the optimal combination of nutrients was obtained as follows: xylose 20 g/L, $(NH_4)_2SO_4$ 9.59 g/L, KH_2PO_4 3 g/L, L-methionine 5.72 g/L and $MgSO_4$ 0.20 g/L. The maximum dry cell weight and glutathione achieved using the optimized medium were 7.36 g/L and 154.32 mg/L, respectively. Following with the content in this medium, other sugars like glucose, mannose and arabinose were chosen as the sole carbon source and all tested available for glutathione production. Based on these results, the hydrolyzate of banana peels was selected as a novel substrate, and a high DCW of 7.68 g/L and glutathione yield of 111.33 mg/L were obtained with the initial sugar concentration of 20 g/L in the hydrolyzate of banana peels.

Key words: Glutathione, *Candida utilis*, Banana Peel, Hydrolyzate, Fermentable Sugars, Response Surface Methodology

INTRODUCTION

Glutathione, a biologically active tripeptide, is biosynthesized intracellularly in the presence of ATP and its three precursor amino acids (L-glutamate, L-cysteine and glycine) [1]. It is the most abundant intracellular thiol and can be found in most living organisms [2]. Glutathione fulfills many physiological functions in higher eukaryotic organisms, serving as antioxidant, immunity booster and detoxifier [3]. Specifically, glutathione plays an important role in maintaining the normal cellular redox environment [4], which results in its wide utilization in the medicine, food and the cosmetic industries. Therefore, the commercial demand for glutathione keeps rapidly expanding [5].

The fermentative production of glutathione using sugar materials is regarded as one of the most promising methods, and yeasts such as *S. cerevisiae* and *C. utilis* are the commonly used microorganisms [5]. Belonging to the Crabtree negative [6] and Kluyver positive yeast [7], *C. utilis* possesses very weak catabolic repression effect when sugars are used as substrate. In addition, *C. utilis* also has the ability to utilize hexose (e.g., glucose, mannose) and pentose (e.g., xylose, galactose) degraded from lignocellulosic biomass as carbon source [8]. These characteristics make *C. utilis* an ideal cell factory for glutathione production, especially on an industrial scale.

Fermentable sugars are widely used in the fermentative process to produce bulk chemicals and/or fine chemicals together with bioactive compounds. Sugar like glucose is usually derived from starch-containing agricultural crops. However, the transformation from crops to sugars is now bringing high risk to food safety, particularly in developing countries [9]. Researchers have already excitedly focused on lignocellulosic materials such as agricultural wastes and forest residues for the production of fermentable sugars. Considerable benefits have been shown in using agricultural biomass because of the unreasonable disposal of these wastes on land and into waterbodies, which results in environmental and ecological problems faced by the agro-based industry [10]. Moreover, global warming, oil reserve depletion and the high costs of fossil fuels have been the driving forces for further research on the use of lignocellulosic biomass [11]; the current emphasis is on biological conversion of agricultural wastes into value-added products. For this purpose, different agro-industrial residues such as bagasse, corn stalk, wheat straw, fruit peels and forest residues have been investigated as possible substrates [12-16]. In this study, we have taken the hydrolyzate of banana peels as a novel substrate for the fermentative production of glutathione by *C. utilis*.

Bananas are one of the most common fruits in tropical and subtropical regions. World banana production is estimated at 48.9 million tones, which is mostly contributed by China, India, Brazil, Indonesia, Philippines and Australia [17]. Banana peels, which are abundantly available in banana producing countries, appear to be favorable feedstock for biotechnological application as they are cheaply available and have high content of lignocellulose. In many districts of China, banana peels are considered as trash. However, banana peel is an organic material containing high content of carbohydrate and other basic nutrients that can be used by microorganisms. The hydrolyzate of banana peels by dilute sulfuric acid contains fermentable sugars such as glucose, xylose, mannose and arabinose (Fig. 1), which can all be used as carbon source by *C. utilis*. The content of xylose was higher than the other sugars in the hydrolyzate (data not shown). Hence, in this study, we first focused on xylose as the carbon source for glutathione production, using response surface methodology (RSM) consisting of Plackett-Burman design (PB), the steepest ascent experiment and the central composite design (CCD)

†To whom correspondence should be addressed.
E-mail: weigy@suda.edu.cn

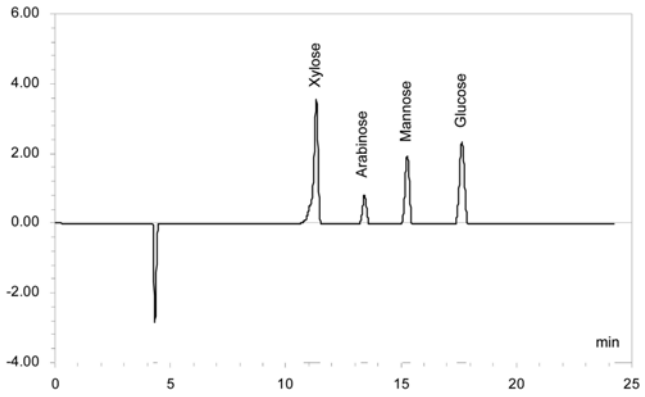

Fig. 1. HPLC analysis of the sugars in the hydrolysate of banana peels pretreated by 1% H_2SO_4 under 120 °C for 20 min, showing xylose, arabinose, mannose and glucose in the hydrolyzate.

for optimization of the medium. Based on this medium, other sugars together with the hydrolyzate of banana peels were all successfully deemed as suitable carbon source for efficient fermentative production of glutathione by *C. utilis*.

MATERIALS AND METHODS

1. Materials

Fresh bananas were purchased from a local market in Suzhou, China, and banana peels were obtained after removal of the fruit pulp. The peels were milled in a hammer mill after being dried in an oven at 70 °C for 24 h; particles ranging in size from 250 μm to 600 μm were collected for further use.

Glucose, xylose, mannose, arabinose, 5, 5′-dithio-bis (2-nitrobenzoic acid) (DTNB), NADPH, and glutathione reductase were all purchased from Sigma-Aldrich Co., St Louis, MO, USA. Other chemicals used were all of standard analytical grade, which were obtained from the Sinopharm Chemical Reagent Co., Ltd., China.

2. Preparation of the Hydrolyzate of Banana Peels

Powdered banana peels were hydrolyzed by 1% (v/v) dilute sulfuric acid at 120 °C for 20 min. The hydrolysis was performed in a 1 L stirred reactor equipped with a tetrafluoroethylene inner bladder containing 30 g peels with a reaction volume of 300 mL, and the agitation rate was set at 100 rpm. The pretreated slurry was separated by filtration to remove solid fraction, the filtrate was then adjusted to pH 7.0 and decolored by 2% (w/v) active carbon at 60 °C for 30 min. After treatment, the syrup was diluted to about 20 g/L followed with sugar assay, and was then used as the carbon source for glutathione fermentation.

3. Microorganism and Cultivation

C. utilis SZU 07-01, which can accumulate glutathione intracellularly with pentose and/or hexose as carbon source, was used throughout this study. The strain was maintained at 4 °C on the slant containing seed medium (10 g/L yeast extract, 20 g/L peptone and 20 g/L glucose, pH 6.0) and 20 g/L agar, and sub-cultured once a month.

The seed was prepared by transferring colonies from a fresh agar slant into a 50 mL seed medium in a 500 mL Erlenmeyer flask, and incubated at 30 °C for 24 h on a rotary shaker at 200 rpm. The culture was then inoculated at 10% (v/v) into the fermentation broth. The starting medium for glutathione fermentation contained (g/L): xylose 20, $(NH_4)_2SO_4$ 8, KH_2PO_4 3 and $MgSO_4$ 0.25. Batch culture was carried out in 500 mL flasks contained 50 mL fermentation media; the culture conditions consisted of 30 °C, an initial pH of 5.5 and 200 rpm in flasks for 30 h. Fermentation medium was steam-sterilized in an autoclave at 121 °C for 15 min except for sugars, which were microfiltered by a Sartorious® membrane with the pore size of 0.20 μm.

4. Analytical Methods

Chemical composition of banana peels with respect to carbohydrate, crude fat, crude fiber, crude protein, moisture and ash was determined in duplicate samples following the methods recommended by AOAC as described by Ranjhan and Krishna [18]. Individual sugar (glucose, xylose, mannose and arabinose) in the hydrolyzate of banana peel was analyzed by HPLC according to the method described before [19]. HPLC was equipped with an XBridge Amide column (4.6×250 mm, Waters Corporation) packed with 3.5 μm particle size of silica gel bonding NH_2 packing material and a refractive index detection. Acetonitrile-water solution (4 : 1, v/v) was used as the mobile phase with a flow rate of 0.8 mL/min, and the column temperature was maintained at 35 °C. Total reducing sugars present in the hydrolyzate were also estimated by the DNS method [20].

Where indicated, a fermentation broth of 25 mL was centrifuged at 6,000 ×g for 10 min, and after being washed twice with distilled water, the wet cells were dried at 70 °C to a constant weight for dry cell weight (DCW) determination. Glutathione was extracted from the wet cells by 40% (v/v) ethanol at 30 °C for 2 h, and centrifuged at 8,000 ×g for 15 min. The supernatant was used for glutathione assay. Glutathione concentration was determined according to the method described by Tietze [21]. Unless stated otherwise, all experiments were repeated in triplicate.

5. Response Surface Methodology

PB is a two-level fractional factorial design based on incomplete equilibrium piece principle. It can pick up the main factors from a list of candidate factors with the least number of experiments [22, 23]. The steepest ascent experiment can approach the largest response area rapidly, determine the center point of the CCD described further, and ensure the validity and correctness of the results from response surface analysis. Based on the results obtained from the PB design and the steepest ascent experiment, the CCD was conducted to gain the optimized levels of the main factors [24]. In this work, PB together with steepest ascent experiment and CCD were applied to optimize the composition of medium for glutathione production with the carbon source of xylose.

6. Software for Experimental Design and Statistical Analysis

Statistical Analysis System (SAS) Version 9.0 was used for the experimental design and statistical analysis of the experimental data. The quality of fit for the regression model equation was expressed by the coefficient of determination R^2, and its statistical significance was determined by an *F* test. The significance of the regression coefficients was checked by a *t*-test. All of the experiments were performed in triplicate, and the average value was used for SAS analysis.

RESULTS

Banana peels have a high content of carbohydrates and fibers (Table 1) that can be converted into fermentable sugars through pre-

Table 1. Chemical composition of banana peels

Composition	Value (%, w/w)
Crude protein	8.15±0.20
Crude fat	11.80±0.30
Crude ber	8.05±0.30
Carbohydrate	60.07±0.25
Moisture	2.50±0.25
Total ash	11.54±0.50

Values are mean of three determinations

treatment with dilute sulfuric acid. After being determined by HPLC method, the hydrolyzate of banana peels was found to contain glucose, mannose, xylose and arabinose (Fig. 1), which could all be used for the fermentative production of glutathione by *C. utilis* SZU 07-01 in this study.

1. Xylose as the Sole Carbon Source for Glutathione Production

It is already known by us that *C. utilis* SZU 07-01 can use hexose like glucose for efficient glutathione production [25]. How about the effects of other sugars in the hydrolyzate of banana peels on cell growth and glutathione biosynthesis? First, we should turn to xylose, a high content of pentose in the hydrolyzate, and investigate the feasibility of xylose as the sole carbon source on glutathione production. Fig. 2 shows that the yeast grew well on the medium under different initial xylose concentrations, and higher DCW was achieved according to the increase of xylose concentration. However, little enhancement of glutathione was observed when xylose concentration was higher than 20 g/L, and more residual sugar of xylose was accumulated in the fermentation broth. Hence, 20 g/L of xylose was selected in the following study, for it can provide a higher DCW together with an entire utilization of sugar and avoid sugar accumulation as much as possible. Under this initial concentration of xylose, DCW and glutathione production was obtained as 7.50 g/L and 85.69 mg/L, respectively.

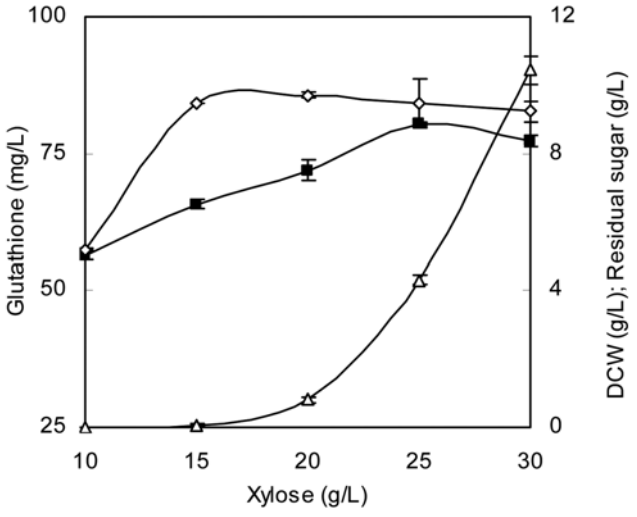

Fig. 2. Cell growth and glutathione production by *C. utilis* SZU 07-01 with xylose as the sole carbon source under diverse xylose concentrations. ■, DCW; ◇, glutathione; △, residual sugar.

2. Optimization of Medium Composition for Glutathione Production with Xylose

Xylose of 20 g/L was chosen above for the sole suitable carbon source. However, the nitrogen source, inorganic salts, growth factors and culture conditions are also very essential to cell growth and metabolism of microorganisms besides the carbon source. That is, suitable concentrations of medium composition and growth conditions are very crucial to the fermentation process. In this part, "single-factor" experiments on diverse nutrients and culture conditions such as $(NH_4)_2SO_4$, KH_2PO_4, $MgSO_4$, L-methionine, temperature and volume of medium were first investigated in order to choose the more efficient nutrients and/or conditions for glutathione production by *C. utilis* SZU 07-01 with xylose as the sole carbon source. Fig. 3 illustrates the optimal nutrient concentrations and culture conditions that were determined, as follows: $(NH_4)_2SO_4$ 8 g/L, KH_2PO_4 3 g/L, $MgSO_4$ 0.2 g/L, L-methionine 4 g/L, temperature 30 °C and volume of medium 30 mL/500 mL.

The RSM design was used for further study of the influences of major factors and interaction between them on the response value. Based on the results of the single factor experiments, the levels of the variables for the PB design were selected (Table 3), and a 12-run PB experiment was chosen to pick the main factors in the production of glutathione by *C. utilis* SZU 07-01 (Table 2); the main factors were picked at the 95% confidence level. According to the *t*-test results in Table 3, $(NH_4)_2SO_4$ and L-methionine were both considered as the most significant factors in glutathione production. A first-order regression equation as follows (Eq. (1)) can be obtained by the analysis of the experimental data from PB design:

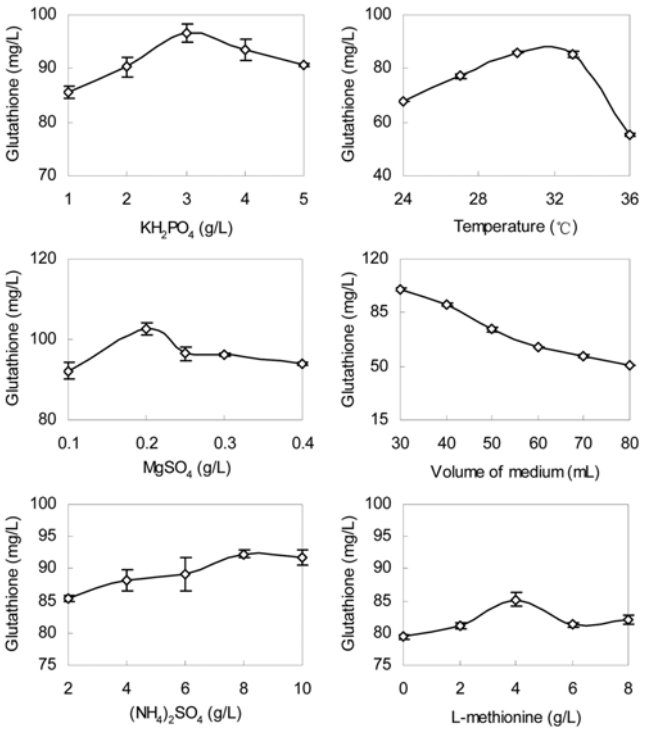

Fig. 3. Effects of nutrients and culture conditions within single-factor experiment on glutathione production with xylose as the sole carbon source.

Table 2. The experiments and results of the Plackett-Burman design

Run	Factor-coded levels									Glutathione (mg/L)
	X_1	X_2	X_3	X_4	X_5	X_6	X_7	X_8	X_9	
1	1	−1	1	−1	−1	−1	1	1	1	137.91±1.09
2	1	1	−1	1	−1	−1	−1	1	1	144.44±1.12
3	−1	1	1	−1	1	−1	−1	−1	1	123.64±0.86
4	1	−1	1	1	−1	1	−1	−1	−1	154.24±2.03
5	1	1	−1	1	1	−1	1	−1	−1	167.20±1.16
6	1	1	1	−1	1	1	−1	1	−1	147.11±0.33
7	−1	1	1	1	−1	1	1	−1	1	150.60±0.25
8	−1	−1	1	1	1	−1	1	1	−1	148.89±1.76
9	−1	−1	−1	1	1	1	−1	1	1	126.38±0.25
10	1	−1	−1	−1	1	1	1	−1	1	138.31±0.69
11	−1	1	−1	−1	−1	1	1	1	−1	123.10±0.86
12	−1	−1	−1	−1	−1	−1	−1	−1	−1	117.45±0.78

X_1-X_6 represent different assigned factors and X_6-X_8 are the dummy factors; '−1' is for low level of factors and '+1' is for high level of factors

Table 3. The factors, levels, and the regression analysis of Plackett-Burman design

Factors	Levels		Effect	t (X_i)	P value
	−1	1			
X_1 $(NH_4)_2SO_4$ (g/L)	4	8	16.525	6.992	0.0198*
X_2 KH_2PO_4 (g/L)	1	2	5.485	2.321	0.1461
X_3 $MgSO_4$ (g/L)	0.1	0.2	7.585	3.209	0.0849
X_4 L-Methionine (g/L)	2	4	17.372	7.350	0.0180*
X_5 Volume of medium (mL)	30	50	3.965	1.678	0.2354
X_6 temperature (°C)	27	30	0.035	0.015	0.9895
X_7 Dummy			8.792	3.720	0.0653
X_8 Dummy			−3.935	−1.665	0.2378
X_9 Dummy			−6.118	−2.589	0.1224

*Statistically significant at 95% of confidence level

$$Y=140+8.26*X_1+2.74*X_2+3.79*X_3+8.69*X_4+1.98*X_5 +0.02*X_6+4.40*X_7-1.97*X_8-3.06*X_9 \quad (1)$$

The fitness of the model was examined by the value of the coefficient R^2, which was found to be 98.6%, indicating that the regression equation can be fitted very well. The first-order model (Eq. (1)) obtained from the PB test determined the ascent direction and the length of ascent pace. Following that, the center point of the parameters in subsequent experiments through the steepest ascent experiment (Table 4) could be achieved, which was $(NH_4)_2SO_4$ 9.5 g/L and L-methionine 5.5 g/L.

The optimal concentration of medium components was determined by CCD with the two factors of $(NH_4)_2SO_4$ and L-methionine using the central point obtained from the steepest ascent experiment. The CCD design included a 13-run experiment; the center point experiment was repeated five times in order to make the regression model more accurately. The asterisk arm length γ=1.414 was determined by the number of significant factors. The CCD design and the results are listed in Table 5 and the statistical analysis of data by SAS 9.0 is shown in Table 6. The analysis of the CCD experiment indicated that the results could be fitted into a second-order regression model as follows (Eq. (2)):

Table 4. The design and results of the steepest ascent experiment

Run	$(NH_4)_2SO_4$ (g/L)	L-methionine (g/L)	Glutathione (mg/L)
1	8.0	4.0	127.76±2.09
2	8.5	4.5	133.28±0.74
3	9.0	5.0	152.78±0.22
4	9.5	5.5	168.44±2.32
5	10.0	6.0	155.52±0.65
6	10.5	6.5	154.23±0.62
7	11.0	7.0	158.60±0.22
8	11.5	7.5	156.64±0.05
9	12.0	8.0	151.49±1.28
10	12.5	8.5	149.27±0.15
11	13.0	9.0	146.24±1.29
12	13.5	9.5	159.60±0.28
13	14.0	10.0	146.74±0.24

Table 5. The experiment design and results of the CCD

Run	Coded factor values		Y
	X1 $(NH_4)_2SO_4$ (g/L)	X2 L-methionine (g/L)	Glutathione (mg/L)
1	−1 (8.5)	−1 (4.5)	130.81±1.11
2	−1 (8.5)	1 (6.5)	141.04±1.05
3	1 (10.5)	−1 (4.5)	144.43±1.03
4	1 (10.5)	1 (6.5)	142.43±0.43
5	−1.414 (8.1)	0 (5.5)	131.25±1.33
6	1.414 (10.9)	0 (5.5)	134.79±1.18
7	0 (9.5)	−1.414 (4.1)	137.12±0.84
8	0 (9.5)	1.414 (6.9)	147.34±0.11
9	0 (9.5)	0 (5.5)	154.95±0.83
10	0 (9.5)	0 (5.5)	155.28±1.88
11	0 (9.5)	0 (5.5)	155.16±1.35
12	0 (9.5)	0 (5.5)	155.09±0.19
13	0 (9.5)	0 (5.5)	154.47±1.30

Table 6. Coefficient estimates by the regression model

Term	Coefficient	SE Coef.	t	Pr>|t|
Constant	154.990	0.8650	179.2400	<0.0001
X1	2.5021	0.6836	3.6600	0.0081**
X2	2.8354	0.6836	4.1476	0.0043**
X1*X1	−10.4720	0.7331	−14.284	<0.0001**
X1*X2	−3.0575	0.9668	−3.1625	0.0159*
X2*X2	−5.8669	0.7331	−8.0028	<0.0001**

*Statistically significant at 95% of confidence level
**Statistically significant at 99% of confidence level

Table 7. Analysis of variance

Variance sources	DF	SS	MS	F	P
Regression	5	1058.02	211.60	56.60	<0.0001
Residual Error	7	26.17	3.74		
Total	12	1084.19			
R^2	97.6%				

F-test was used for the analysis of variance, a bigger F value and smaller *P* value represented a higher confidence level of the regression model "DF" degree of freedom; "SS" sum of square; "MS" mean square

$$Y=154.990+2.5021*X_1+2.8354*X_2-10.4720*X_1^2 -3.0575*X_1*X_2-5.8699\,X_2^2 \quad (2)$$

where, Y is the response variable indicated by the production of glutathione, X_1 and X_2 are the concentrations of $(NH_4)_2SO_4$ and L-methionine, respectively.

The determining coefficient of R^2 was 97.6% (Table 7), which indicated that this model congressed well with the results of experiments and also could be used to predict the yield of glutathione in fermentation. The effects of $(NH_4)_2SO_4$ and L-methionine concentrations on glutathione production are shown in Fig. 4. The shapes of the response surface plot showed that they also had great interaction with each other. The extreme coordinate (0.0711, 0.2192) of the model can be obtained, and the corresponding concentrations of $(NH_4)_2SO_4$ and L-methionine were 9.59 g/L and 5.72 g/L, respectively. The maximum yield of glutathione can be predicted by the model as 155.42 mg/L according to the concentrations of significant factors. Accordingly, the optimal medium compositions for glutathione production with xylose as the sole carbon source by *C. utilis* SZU 07-01 could be summarized as follows (g/L): xylose 20, $(NH_4)_2SO_4$ 9.59, KH_2PO_4 3, L-methionine 5.72 and $MgSO_4$ 0.20. To validate the prediction of the model, additional triplicate experiments were performed in shake flasks, and the mean value of the glutathione was 154.32 mg/L (Fig. 5), which agreed well with the predicted value (155.42 mg/L), and improved by 80.09% compared to the yield of glutathione on the starting medium (85.69 mg/L).

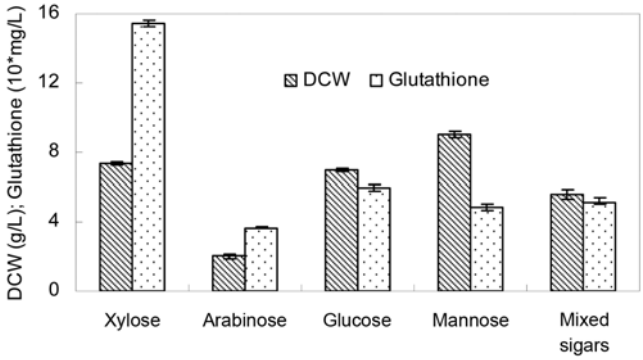

Fig. 5. Comparison of cell growth and glutathione production with the sole carbon source of xylose, glucose, mannose, arabinose and mixed sugars at an initial concentration of 20 g/L.

3. Glutathione Production with Other Sugars as Carbon Source

Besides xylose, other sugars in the hydrolyzate of banana peels such as glucose, mannose and arabinose were further investigated as the sole carbon source based on the medium compositions obtained with xylose. The effects of glucose, mannose and arabinose on cell growth and glutathione production are illustrated in Fig. 5. It was shown that *C. utilis* SZU 07-01 could also grow well on the medium with the sole carbon source of glucose and mannose at 20 g/L, together with about 50 mg/L of glutathione production. The yeast cell cannot grow well on the medium containing arabinose, and only 2.03 g/L of DCW was obtained after batch cultivation for 30 h. Moreover, improvement of DCW and glutathione was found with glucose if the concentration of $(NH_4)_2SO_4$ and L-methionine was increased. But for mannose and arabinose, little change in DCW and glutathione was observed, followed with the increase of $(NH_4)_2SO_4$ and L-methionine (data not shown). Therefore, glucose and mannose can serve as a suitable carbon source for the cell growth of *C. utilis* SZU 07-01 and glutathione production. Arabinose can also be utilized by the yeast for glutathione production, although fewer DCW were achieved compared to other sugars.

Mixed sugars of xylose, glucose, mannose and arabinose followed with the sugar content in the hydrolyzate of banana peels

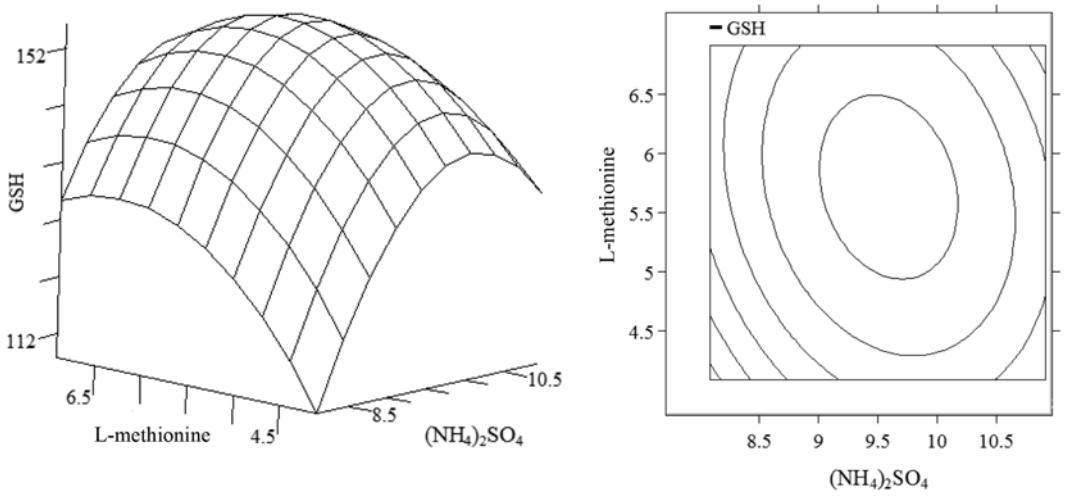

Fig. 4. Response surface plot for the effects of $(NH_4)_2SO_4$ and L-methionine on glutathione production.

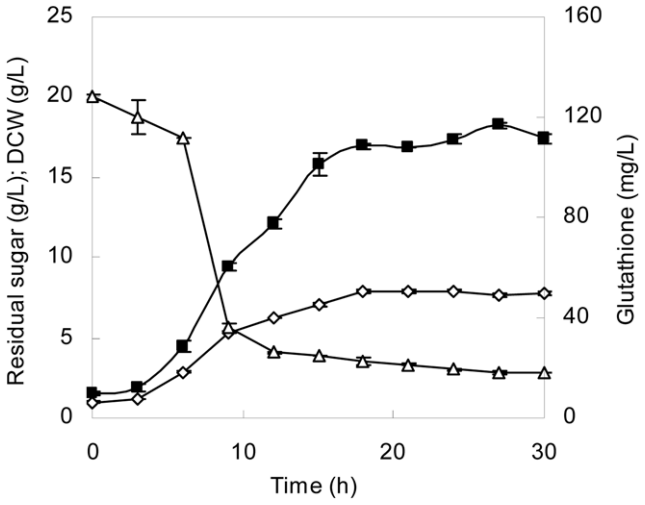

Fig. 6. Time-course of cell growth and glutathione production using the hydrolyzate of banana peels as a novel carbon source. ■, Glutathione; ◇, DCW; △, Residual sugar.

were used as the carbon source for glutathione production, and the initial sugar concentration was also set at 20 g/L. It was found that the maximum DCW and glutathione concentration was 5.6 g/L and 51.61 mg/L, respectively (Fig. 5), which was much similar to the results obtained from glucose and mannose as the sole carbon source, while much lower than those from xylose. Therefore, high cell growth and glutathione production could not be obtained with the mixed sugars that just contained pure xylose, glucose, mannose and arabinose.

4. Glutathione Production with the Hydrolyzate of Banana Peels as a Novel Carbon Source

Based on the above results on the sole carbon source of xylose, glucose, mannose and arabinose, the hydrolyzate of banana peels diluted to the initial concentration of about 20 g/L was used as a novel carbon source for cell growth and glutathione production by *C. utilis* SZU 07-01. Fig. 6 shows that the yeast could successfully consume the mixed sugars and grow well on the hydrolyzate. Although cultivation was carried out for 30 h, residual sugars of 2.83 g/L still existed in the broth, which contained mostly arabinose and few xylose after determination using HPLC method. DCW and glutathione increased gradually followed by the mixed sugars consumption; the maximum DCW and glutathione were achieved at 7.92 g/L and 116.88 mg/L, respectively. On the whole, glutathione production with the hydrolyzate of banana peels was superior to the pure mannose, glucose, arabinose and mixed sugars, which demonstrated that the hydrolyzate of banana peels was suitable to serve as the carbon source for cell growth and glutathione production by *C. utilis* SZU 07-01.

DISCUSSION

Environmental problems and the shortage of food are the two top risks at present in the world. Grain-based feedstock is widely used for the traditional fermentation, which may aggravate the crisis of agricultural grain on food and feed. Therefore, many technologies of fermentation based on non-food crops are being developed instead of starch-containing agricultural crops [26]. Lignocellulosic biomass is the most abundant and renewable resource, such as corn stover, corncobs, wheat straw, rice straw and fruit peels. The biomass may also bring serious environmental problems unless suitable disposals are carried out. These dilemmas can be solved if lignocellulosic materials are used as the potential and feasible feedstock for the production of useful compounds by fermentation with microorganisms.

In this paper, banana peel was selected as the feedstock to investigate the effect of the hydrolyzate of banana peels on the cell growth and glutathione production by *C. utilis* SZU 07-01. The successful utilization of glucose, mannose, xylose and arabinose brings the feasibility of the utilization of hydrolyzate of banana peels. Xylose is abundant in certain lignocellulosic biomass, including waste crop residues such as corn stover [27] and forest industry wastes [28]. Xylose is also one of the main pentoses in the hydrolyzate of banana peels. Successful utilization of xylose would not only help in fermentation on hydrolyzate of banana peels, but also contribute to driving the process study of other agricultural wastes for fermentation. Based on this consideration, xylose was investigated first to study the cell growth and glutathione production. Arabinose is another pentose in the hydrolyzate of banana peels, but the utilization of arabinose was unsatisfactory compared to xylose (Fig. 5). Even though, the pentose can be used as the carbon source in this study, particularly xylose was the most suitable substrate for glutathione production.

Hexose in the hydrolyzate of banana peels such as glucose and mannose were effective in use because of the higher DCW and higher concentrations of glutathione. Mixed sugars of pure glucose, xylose, mannose and arabinose can also be utilized for glutathione production by *C. utilis* SZU 07-01. However, it was inferior to the results from the pure single sugar as carbon source. Based on these results, the feasibility of the hydrolyzate of banana peels as carbon source for glutathione production was tested accordingly. It was shown that the hydrolyzate of banana peels can well be used by the yeast, and no inhibition was found by the hydrolyzate on the cell growth and glutathione production, despite an incomplete utilization of residual sugars existed in the broth. In addition, it is also obvious that cell growth and glutathione production with the hydrolyzate of banana peels is better than that with mixed sugars of pure glucose, xylose, mannose and arabinose. This should contribute to other compositions in the hydrolyzate of banana peels, including amino acids, fatty acids, ions, *etc.*, which may have positive effects on cell growth and glutathione production. The mechanism of the promotion should be further studied in the future.

ACKNOWLEDGEMENT

This research was financially supported by the National Natural Science Foundation of China (No. 20906065), together with the Natural Science Project for Universities in Jiangsu Province (No. 09KJB530009).

REFERENCES

1. A. Meister and M. E. Anderson, *Annu. Rev. Biochem.*, **52**, 711 (1983).

2. M. J. Penninckx, *FEMS Yeast Res.*, **2**, 295 (2002).
3. A. Pastore, G. Federici, E. Bertini and F. Piemonte, *Clin. Chim. Acta*, **333**, 19 (2003).
4. H. Sies, *Free Radical Bio. Med.*, **27**, 916 (1999).
5. M. Nie, G. Wei, N. Shao and X. Ge, *Korean J. Chem. Eng.*, **27**, 1246 (2010).
6. H. van Urk, W. S. L. Voll, W. A. Sheffers and J. P. van Dijken, *Appl. Environ. Microb.*, **56**, 281 (1990).
7. J. I. Castrillo, J. Kaliterna, R. A. Weusthuis, J. P. van Dijken and J. T. Pron, *Biotechnol. Bioeng.*, **49**, 621 (1996).
8. T. W. Jeffries and Y. S. Jin, *Appl. Microbiol. Biot.*, **63**, 495 (2004).
9. S. Brethauer and E. W. Charles, *Bioresour. Technol.*, **101**, 4862 (2010).
10. J. E. Smith, J. G. Anderson, E. Senior and K. Aiddo, *Philos. T. R. So. A*, **321**, 507 (1987).
11. H. I. Velásquez-Arredondo, A. A. Ruiz-Colorado and S. De Oliveira Junior, *Energy*, **12**, 1 (2010).
12. C. A. Cardona, J. A. Quintero and I. C. Paz, *Bioresour. Technol.*, **101**, 4754 (2010).
13. C. Fei and H. Chen, *Bioresour. Technol.*, **101**, 1315 (2009).
14. F. Talebnia, D. Karakashev and I. Angelidaki, *Bioresour. Technol.*, **101**, 4744 (2010).
15. E. S. Pinheiro, I. M. Silva, L. V. Gonzaga, E. R. Amante, R. F. Teófilo, M. M. Ferreira and R. D. Amboni, *Bioresour. Technol.*, **99**, 5561 (2008).
16. M. Junginger, A. Faaij and R. van den Broek, *Biomass Bioenerg.*, **21**, 259 (2001).
17. A. Karthikeyan and N. Sivakumar, *Bioresour. Technol.*, **101**, 5552 (2010).
18. S. K. Ranjhan and G. Krishna, *Laboratory manual for nutrition research*, Vikas Publishing House, New Delhi (1981).
19. M. A. N. Benhura and C. Chidewe, *Food Chem.*, **76**, 343 (2002).
20. G. L. Miller, *Anal. Chem.*, **31**, 426 (1959).
21. F. Tietze, *Anal. Biochem.*, **27**, 502 (1969).
22. R. L. Plackett and J. P. Burman, *Biometrika*, **33**, 305 (1946).
23. S. J. Kalil, F. Maugeri and M. I. Rodrigues, *Process Biochem.*, **35**, 539 (2000).
24. J. X. Heck, S. H. Flores, P. F. Hertzm and M. A. Z. Ayub, *Process Biochem.*, **40**, 107 (2005).
25. G. Wei, Y. Li, G. Du and J. Chen, *Chin. J. Appl. Environ. Biol.*, **9**, 642 (2003).
26. Y. Sun and J. Cheng, *Bioresour. Technol.*, **83**, 1 (2002).
27. D. J. Schell, C. J. Riley, N. Dowe, J. Farmer, K. N. Ibsen, M. F. Ruth, S. T. Toon and R. E. Lumpkin, *Bioresour. Technol.*, **91**, 179 (2004).
28. H. K. Sreenath and T. W. Jeffries, *Bioresour. Technol.*, **72**, 253 (2000).