Production of Fumaric Acid by *Rhizopus oryzae* in Simultaneous Saccharification and Fermentation using Xylo-Oligosaccharides Manufacturing Waste Residue

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Production of fumaric acid from xylo-oligosaccharides manufacturing waste residue (XOR) by *Rhizopus oryzae* CICC 40351 was investigated in a simultaneous saccharification and fermentation (SSF) process. The fermentation conditions for SSF were optimized by an orthogonal design method to maximize the fumaric acid concentration. The highest fumaric acid concentration (12.54 g/L) was reached with a substrate loading of 5% (w/v) XOR in the SSF process at 38 °C. The fumaric acid concentration of the SSF process was 1.8 times greater than that of the separate hydrolysis and fermentation (SHF) process under the same conditions. In addition, the SSF process yielded 0.34 g/g of glucose, whereas the SHF process yielded only 0.20 g/g of glucose. The results indicated that the SSF process notably improved the production of fumaric acid from lignocellulose by *R. oryzae*.

Keywords: Rhizopus oryzae; Fumaric acid; Simultaneous saccharification and fermentation; Lignocellulose

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INTRODUCTION

Fumaric acid is a four-carbon unsaturated dicarboxylic acid. It serves as an important organic material and bulk chemical. Fumaric acid has been used as a food acidulant, beverage ingredient, and antibacterial agent (Xu *et al.* 2012). Fumaric acid is also used to produce polymers and resins due to a carbon-carbon double bond and two carboxylic acid groups in its structure (Roa Engel *et al.* 2008; Xu *et al.* 2012). In the 1940s, Pfizer developed an industrial fermentative process by *Rhizopus arrhizus* for production of fumaric acid with an annual production of 4000 tons (Goldberg *et al.* 2006). Because of higher raw material cost, lower fumaric acid concentration, lower yield, and lower productivity, the fermentative production of fumaric acid did not compete economically with a chemical process (Xu *et al.* 2012). Consequently, the fermentative production of fumaric acid was stopped in the 1970s and replaced by a more economical chemical process from petrochemical feedstocks (Goldberg *et al.* 2006; Roa Engel *et al.* 2008; Xu *et al.* 2012). Fumaric acid is currently synthesized by isomerization of maleic acid with an annual production of 90,000 tons (Roa Engel *et al.* 2008); however, the chemical process causes environmental problems, including toxic airborne pollution and water pollution. With increasing concern over sustainability and consumer preference for natural products, the

discovery of an economical biotechnological route for producing fumaric acid has gained a renewed attention from researchers.

The *Rhizopus* genus, grown on simple nutrients required for fermentation, contains strains used for fumaric acid production, especially *Rhizopus oryzae* (Huang *et al.* 2010). Roa Engel *et al.* (2008) reported that fermentative production of fumaric acid was mainly from glucose and starch-based materials. However, glucose is an expensive carbon source, and starch-based materials as feedstock are inevitably facing the challenge of the increased food security (Valentine *et al.* 2012). Therefore, the cheaper, abundant, and non-edible feedstock is crucial for fermentative production of fumaric acid by *Rhizopus oryzae*. Some studies have focused on seeking a suitable feedstock, such as wheat bran (Wang *et al.* 2015), pulp and paper solid waste (Das *et al.* 2016), apple industry waste biomass (Das *et al.* 2015a), brewery wastewater (Das *et al.* 2015b), corn straw (Xu *et al.* 2010), and dairy manure hydrolysate (Liao *et al.* 2008). Lignocellulosic materials (especially wood and agricultural residues) are promising potential alternatives for biorefinery characterized by renewable, cheap, abundant, and non-edible biomass. *Rhizopus oryzae* is a suitable strain for the production of fumaric acid using lignocellulose-based carbohydrate as a carbon source. A two-stage corn straw utilization strategy was reported to convert concentrated glucose into 27.79 g/L fumaric acid, with a glucose yield of 0.35 g/g (Xu *et al.* 2010). *Rhizopus oryzae* (ATCC 20344) was added to dairy manure hydrolysate (lignocellulosic material) and pure glucose to produce 31 g/L of fumaric acid, with a yield of 31% (Liao *et al.* 2008). These experiments required the enzymatic hydrolysis of pretreated lignocellulosic materials before fermentation, which is described as a separate hydrolysis and fermentation (SHF) process. In recent literature, fermentative production of fumaric acid has been carried out mainly through submerged fermentation (Liao *et al.* 2008; Xu *et al.* 2010; Das *et al.* 2015a; Wang *et al.* 2015; Das *et al.* 2016), solid state fermentation (Das *et al.* 2015a; Das *et al.* 2016), or immobilized submerged fermentation (Das *et al.* 2015b). Different from recent studies, we developed a simultaneous saccharification and fermentation (SSF) process for production of fumaric acid from lignocellulose by *R. oryzae*. A SSF process was carried out using enzymatic hydrolysis of lignocellulose and the fermentation of releasing sugars in the same vessel. In comparison with SSF, SHF may pose several problems, such as end-product inhibition of enzymatic hydrolysis, lower hydrolysis rate, lower yield, and lower product concentration.

The present study investigated the production of fumaric acid using SSF of xylo-oligosaccharides manufacturing waste residue (XOR) with *R. oryzae* (CICC 40351). The XOR was a cellulose-rich solid residue resulted from the alkali-pretreatment of corncobs. Optimization of key factors using SSF was carried out according to an orthogonal design. A commercial cellulase with supplemental *β*-glucosidase was used for the enzymatic hydrolysis of cellulose.

EXPERIMENTAL

Materials

Strains

R. oryzae CICC 40351 was purchased from the China Center of Industrial Culture Collection (CICC; Beijing, China).

Xylo-oligosaccharides manufacturing waste residue

XOR was a gift from the Jiangsu Kangwei Biologic Co., Ltd. (Dongtai, Jiangsu Province, China). The XOR was a cellulose-rich solid residue from the alkali-pretreatment of corncobs. The corncobs were pretreated in 7% (w/v) sodium hydroxide solution for 1 h at 85 to 90 °C. After solid/liquid separation by filtration, the liquid fraction containing hemicellulose was used for the production of xylo-oligosaccharides. The solid fraction was soaked in water at a solid-liquid ratio of 1:10 (w/v) and then neutralized by 72% (w/w) sulfuric acid to a pH of 4.8 to 5.0. The mixture was filtered to remove the liquid fraction to obtain the solid fraction (named XOR). And the XOR was stored in plastic bags at 4 °C.

Enzymes

Cellulase and *β*-glucosidase were purchased from Sigma Aldrich (Novozymes, Bagsvaerd, Denmark) and used without further purification.

Agar slant

The composition of the slant medium was as follows: 10.0 g/L glucose, 3.0 g/L yeast extract, 3.0 g/L malt extract, 5.0 g/L peptone, and 20.0 g/L agar. The inoculated slant was cultured in an incubator at 30 °C for one week and then stored at 4 °C until further use.

Seed medium

The seed medium was composed of the following: 40 g/L glucose, 4.4 g/L $(NH_4)_2SO_4$, 0.5 g/L $MgSO_4 \cdot 7H_2O$, 0.6 g/L KH_2PO_4, 0.0176 g/L $ZnSO_4 \cdot 7H_2O$, and 0.000498 g/L $FeSO_4 \cdot 7H_2O$.

The spores were washed from the slant with sterile water, and the spore density was adjusted to10^7 spores per milliliter.

Fermentation medium

The SSF medium consisted of the following: 5% (w/v) XOR, 0.71 g/L $(NH_4)_2SO_4$, 0.5 g/L $MgSO_4 \cdot 7H_2O$, 0.6 g/L KH_2PO_4, 0.01 g/L $ZnSO_4 \cdot 7H_2O$, 0.0004 g/L $FeSO_4 \cdot 7H_2O$, and 30 g/L $CaCO_3$ in 0.05 mol/L sodium acetate-acetic acid (NaOAc-AcH) buffer (pH of 4.8).

The SHF medium consisted of the following: enzymatic hydrolysate at 5% (w/v) XOR, 0.71 g/L $(NH_4)_2SO_4$, 0.5 g/L $MgSO_4 \cdot 7H_2O$, 0.6 g/L KH_2PO_4, 0.01 g/L $ZnSO_4 \cdot 7H_2O$, 0.0004 g/L $FeSO_4 \cdot 7H_2O$, and 30 g/L $CaCO_3$.

All chemicals used in the present work were analytical grade without further purification.

Methods

Enzymatic hydrolysis

Enzymatic hydrolysis of 5% (w/v) XOR was conducted in an incubator at 50 °C and 150 rpm. The dosages of cellulase and *β*-glucosidase were 25 FPIU/(g cellulose) and 20 IU/(g cellulose), respectively. The glucose yield was calculated as follows:

$$\text{Glucose yield (\%)} = \text{Glucose (g)} \times 0.9 \times 100 / \text{initial [cellulose] in substrate (g)} \quad (1)$$

Seed culture

The seed suspension was transferred to the medium and cultured in an incubator (New Brunswick Scientific, INNOVA 40R, USA) for 24 h at 35 °C and 200 rpm.

Simultaneous saccharification and fermentation (SSF)

The SSF was conducted using free enzymes (cellulase and *β*-glucosidase) at 38 °C and 220 rpm in fermentation medium (50 mL) with a 10% (v/v) inoculum.

Separated hydrolysis and fermentation (SHF)

Enzymatic hydrolysis of 5% (w/v) XOR was conducted using a cellulase cocktail at 50 °C and 150 rpm. The supernatant resulting from enzymatic hydrolysate was obtained by centrifugation at 5000 rpm for 10 min. The supernatant and other fermentation medium components comprised the SHF medium. Fermentation of the SHF medium was carried out in an incubator at 38 °C and 220 rpm.

Analytical method

The fumaric acid, ethanol, and glucose concentrations were determined by high performance liquid chromatography (HPLC, Agilent Technologies, 1260 Infinity, USA) equipped with a Bio-Rad Aminex HPX-87H column and a refractive index detector. The mobile phase was 5 mM H_2SO_4, and the flow rate was maintained at 0.6 mL/min.

Because of the low solubility of fumaric acid at room temperature (Lange and Sinks 1930), the final culture broth was treated with 25% (w/w) NaOH solution to convert fumaric acid into soluble sodium fumarate. The excess NaOH was neutralized with 36% (w/w) H_2SO_4 solution before the samples were tested.

The analysis of chemical composition of XOR was carried out according to the National Renewable Energy Laboratory standard method for the determination of structural carbohydrates and lignin in biomass (Sluiter *et al*. 2008). Filter paper and *β*-glucosidase activities were determined according to the International Union of Pure and Applied Chemistry procedures (Ghose 1987). One FPIU was defined as the amount of enzyme needed to release 1 μmol of glucose equivalent from Whatman No.1 filter paper per min. One unit of *β*-glucosidase was defined as the amount of enzyme needed to convert 1μmol of cellobiose to 2 μmol of glucose per min.

Mycelial biomass was washed with deionized water after neutralization of residual $CaCO_3$ in fermentation medium by 6 M hydrochloric acid. Then, the biomass was dried at 65 °C until a constant weight was obtained.

All the data are presented as the mean of two experiments. All the experiments were carried out in 250-mL flasks containing 50 mL of medium.

RESULTS AND DISCUSSION

Xylo-Oligosaccharides Manufacturing Waste Residue (XOR) and Enzymatic Hydrolysis

After the corncob was treated with sodium hydroxide, the compositional comparison of raw material and XOR was obtained (Table 1). The corncob contained (% dry wt.) 36.01% cellulose, 36.84% hemicellulose, 17.43% lignin (including acid-soluble lignin and acid-insoluble lignin), and 3.43% ash. After the alkali pretreatment, XOR contained 66.95% cellulose, 21.87% hemicellulose, 5.92% lignin (including acid-soluble lignin and acid-insoluble lignin), and 1.20% ash. The percentage of glucan after the pretreatment increased from 36.01% to 66.95% because of the solubilization of xylan. The alkali pretreatment effectively decreased the hemicellulose and lignin fractions of the lignocellulosic materials (Kim and Holtzapple 2006; Qin *et al.* 2010).

The 48-h enzymatic hydrolysis of 5% (w/v) XOR released 34.97 g/L of glucose (Fig. 1). The glucose yield after the hydrolysis of XOR was greater than 94%. The alkali pretreatment removed most of the hemicellulose and a portion of lignin. The removal of hemicellulose and lignin was beneficial for improving the glucose yield due to the strong structural modification of lignocellulosic material caused by pretreatment (Mussatto *et al.* 2008).

Table 1. Comparison of Corncob and Xylo-Oligosaccharides Manufacturing Waste Residue (XOR) (% dry wt.)

Materials	Cellulose (%)	Hemicellulose (%)	Lignin (%)	Ash (%)
Corncob	36.01 ± 0.07	36.84 ± 0.03	17.43 ± 0.16	3.43 ± 0.20
XOR	66.95 ± 0.15	21.87 ± 0.06	5.92 ± 0.12	1.20 ± 0.11

Values represent the mean ± standard deviation

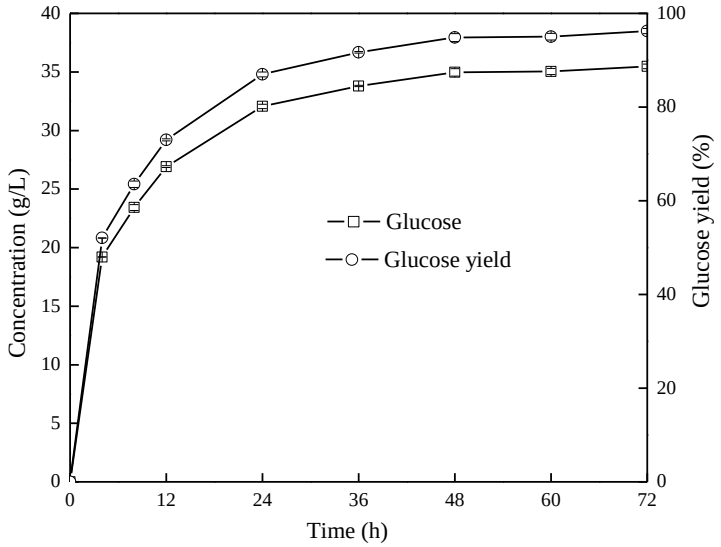

Fig. 1. Time-course of glucose production during enzymatic hydrolysis at 50 °C

Selection of Temperatures for SSF Processing

The processing temperature is a key factor for both cellulase hydrolysis and fermentation of SSF (Narra *et al.* 2015). Generally, the optimum temperature of cellulase hydrolysis ranged from 48 °C to 50 °C (Xu *et al.* 2009; Rodríguez-López *et al.* 2012), and the optimum temperature of fermentation for *R. oryzae* ranged from 30 °C to 35 °C (Liao *et al.* 2008; Kang *et al.* 2010; Ding *et al.* 2011; Deng *et al.* 2012). No substantial growth of *R. oryzae* was observed over 40 °C (Gao *et al.* 2011). Therefore, it was crucial for SSF with *R. oryzae* to select a proper temperature closer to the optimal temperature of cellulase hydrolysis.

Figure 2 and Table 2 show the effect of *R. oryzae* on glucose utilization at different temperatures. As shown in Fig. 2, *R. oryzae* consumed glucose at 35 and 38 °C after 24 h of fermentation; however, 10 g/L glucose was observed in the broth after 60 h of fermentation when the temperature was increased to 41 °C. Increasing the temperature

beyond a certain point decreased the consumption of glucose and limited the fermentation capacity of *R. oryzae*. Table 2 shows a decrease in fumaric acid concentration with increasing temperature within the range of 35 to 41 °C. *R. oryzae* produced 7.56 g/L of fumaric acid, with a yield of 0.19 g/g glucose and a productivity of 0.13 g/(L·h) at 38 °C, which was similar to that at 35 °C. The biomass of *R. oryzae* was maintained at a relatively constant level (Zhou *et al.* 2011).

Table 2. Product Concentrations after 60 h of Fermentation

Temperature (°C)	Biomass (g/L)	Fumaric acid (g/L)	Ethanol (g/L)	Yield* (g/g)	Productivity (g/(L·h))
35	2.49 ± 0.02	8.75 ± 1.11	ND	0.22	0.15
38	2.33 ± 0.09	7.56 ± 2.08	ND	0.19	0.13
41	2.51 ± 0.18	6.56 ± 1.66	3.30 ± 0.73	0.16	0.11

*g fumaric acid/g glucose
ND: not detected
Values represent the mean ± standard deviation

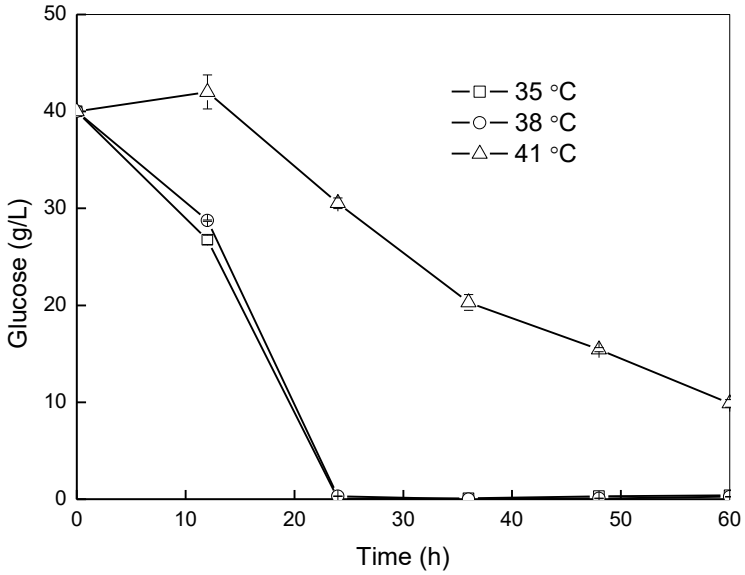

Fig. 2. Glucose consumption by *R. oryzae* over time

Enzymatic hydrolysis of XOR at 38 °C was investigated at the same cellulase concentration. A comparison of enzymatic hydrolysis at 5% (w/v) XOR at 38 and 50 °C is shown in Table 3. A slightly lower glucose concentration (32.23 g/L) at 38 °C was observed compared to 34.97 g/L at 50 °C. A more accumulated cellobiose was found at 38 °C because of the lower *β*-glucosidase activity at 38 °C. The glucose yield at 38 °C was 9.91% lower than that at 50 °C. Kaar and Holtzapple (2000) found that there was a minimal difference in the enzymatic hydrolysis of alkali-treated corn stover between 40 and 50 °C (Kaar and Holtzapple 2000). Cellulase hydrolysis showed a better glucose yield at a lower temperature. Therefore, 38 °C was used for SSF in the following experiments.

Table 3. Effect of Temperature on Enzymatic Hydrolysis Products

Temperature (°C)	Glucose (g/L)	Cellobiose (g/L)	Glucose yield (%)
38	31.35 ± 0.06	3.55 ± 0.03	84.29 ± 0.16
50	34.97 ± 0.31	2.47 ± 0.07	94.02 ± 0.83

Values represent the mean ± standard deviation

Orthogonal Design for Optimizing Fermentation Conditions of SSF

Nitrogen limitation influences the growth of *R. oryzae* when cells secrete fumaric acid (Gao *et al.* 2011). Calcium carbonate and inoculum have an effect on the growth of *R. oryzae* mycelia (Liao *et al.* 2007). The dosage of cellulase is related to the economic efficiency of the process (Wahono *et al.* 2014). Therefore, ammonium sulfate (A), calcium carbonate (B), dosage of cellulase (C) and inoculum (D) were taken into account for production of fumaric acid during the SSF process by *R. oryzae*. The orthogonal design of the SSF conditions and the analysis of results are shown in Tables 4 and 5, respectively.

Table 4. Factors and Levels of the Orthogonal Design L_9 (3^4) for SSF

Level	$(NH_4)_2SO_4$ (g/L)	$CaCO_3$ (g/L)	Cellulase (FPIU/g cellulose)	Inoculum (% (v/v))
1	0.71	20	20	5
2	0.51	30	25	10
3	0.31	40	30	15

Table 5. Results of the Orthogonal Design

Number	$(NH_4)_2SO_4$ (A) (g/L)	$CaCO_3$ (B) (g/L)	Cellulase (C) (FPIU/g cellulose)	Inoculum (D) (% (v/v))	Fumaric acid (g/L)
1	1	1	1	1	7.53
2	1	2	2	2	10.78
3	1	3	3	3	11.58
4	2	1	2	3	9.21
5	2	2	3	1	7.93
6	2	3	1	2	8.77
7	3	1	3	2	7.91
8	3	2	1	3	9.24
9	3	3	2	1	7.50
K_1[a]	29.86	24.62	25.51	22.93	
K_2[a]	25.91	27.95	27.49	27.46	
K_3[a]	24.65	27.85	27.42	30.03	
k_1[b]	9.95	8.21	8.50	7.64	
k_2[b]	8.64	9.32	9.16	9.15	
k_3[b]	8.22	9.28	9.14	10.01	
RangR[c]	1.73	1.11	0.66	2.37	

Order: D > A > B > C; Optimal scheme: $D_3A_1B_2C_2$

a: the sum of fumaric acid concentration for each factor at different levels

b: the means of fumaric acid concentration for each factor at different levels

c: the ranges of fumaric acid concentration for each factor at different levels

The effects of SSF conditions on the production of fumaric acid decreased in the order, D > A > B > C, based on the RangR values (Table 5). According to the fumaric acid concentration for each factor level, the maximum fumaric acid concentration (12.54 g/L) was obtained at 0.71 g/L ammonium sulfate, 30 g/L calcium carbonate, 25 FPIU cellulase/g cellulose, and 15% (v/v) inoculum.

Comparison of SSF and SHF

The process of SSF was applied for the production of fumaric acid with lignocellulose by *R. oryzae*. Fumaric acid was produced from 5% (w/v) XOR during the SSF process. The SSF was compared with SHF at a 5% (w/v) substrate loading rate. The substrate loading rate of 5% (w/v) was chosen to avoid the propensity for viscous conditions resulting from high substrate loading (Tomás-Pejó *et al.* 2008). In SHF, hydrolysate containing 33.67 g/L glucose was used as the sole carbon source, which resulted from the enzymatic hydrolysis of 5% (w/v) XOR.

Figure 3 shows the sugar consumption from SSF and SHF. The SHF process showed a 24-h lag phase at the beginning of fermentation, and the glucose concentration remained relatively constant during the first 24 h of fermentation. This indicated that the *R. oryzae* required 24 h of adaption to lignocellulosic hydrolysate during SHF. Fortunately, SSF reduced the lag phase and enhanced *R. oryzae* tolerance to lignocellulosic hydrolysate of XOR. Enzymatic hydrolysis of XOR for 24 h released greater than 30 g/L of glucose (Fig. 1), while SSF, at the same dosage of cellulose, released less than 20 g/L of glucose (Fig. 3). Consequently, *R. oryzae* consumed approximately 30% of the glucose released from the enzymatic hydrolysis of XOR during the first 24 h of SSF. Obviously, the strain easily adapted to the environmental conditions of the SSF process (Pietrzak and Kawa-Rygielska 2015).

After 24 h of fermentation, the *R. oryzae* in SHF consumed sugars faster than SSF. Glucose was exhausted at 60 h for both processes. Table 6 shows that fumaric acid reached 12.54 g/L after 60 h of SSF, while only 6.76 g/L was obtained by SHF. The SSF process increased 85.5% of the fumaric acid concentration and demonstrated a better yield of fumaric acid (0.34 g/g) overall. The SSF process notably increased the fumaric acid yield from 0.20 to 0.34 g/g using XOR as carbon source.

Several studies on the production of fumaric acid from lignocellulosic materials have been reported and are summarized in Table 7. Xu *et al*. (2010) reported a two-stage process for fumaric acid production from corn straw, with a fumaric acid yield of 0.35 g/g. *R. oryzae* initially grew in the xylose-rich hydrolysate from acid hydrolysis of corn straw, and then was transferred into the glucose-rich hydrolysate from enzymatic hydrolysis of the acid-pretreated corn straw. The whole process required over 182 h, including the processes of enzymatic hydrolysis and concentration (Xu *et al.* 2010).

The SSF process reported herein provided a one-pot process for fumaric acid production with lignocellulosic material and achieved a fumaric acid yield of 0.34 g/g. Liao *et al*. (2008) showed that *R. oryzae* ATCC 20344 yielded fumaric acid with a fumaric acid yield of 0.31 g/g from dairy manure hydrolysate with the addition of pure glucose. However, the hydrolysate only contained approximately 20% to 26% glucose from dairy manure, and 74% to 80% glucose was contributed from pure glucose (Liao *et al.* 2008). In this article, XOR was directly sourced as the sole carbon input for fumaric acid production. The fumaric acid concentration from the SSF process was greater than that from the SHF process under the same conditions.

During fermentative production of fumaric acid, ethanol is the primary by-product in the metabolic pathways of *R. oryzae*, and the formation of ethanol reduces the carbon flux to fumaric acid (Xu *et al.* 2012). As shown in Table 6, the ethanol concentrations in SSF and SHF were 2.64 g/L and 3.20 g/L, respectively. The difference in ethanol concentration was at a low level between SSF and SHF. The SSF process showed a slight effect on carbon flux toward ethanol formation. Therefore, the results show that SSF was a better process for the production of fumaric acid using lignocellulose as the raw material. However, the production of fumaric acid by SSF is still facing some problems, such as the low fumaric acid concentration obtained in the present work.

Increased solids loading led to higher potential product concentration, reducing equipment's size, energy consumption, and the burden of the downstream processing (Romaní *et al.* 2012). From the economic point of view, higher fumaric acid concentration is beneficial for the large-scale production of fumaric acid. Further study would focus on fermentative production of fumaric acid by SSF at high solid loading to achieve higher fumaric acid concentration.

Table 6. Comparison of Fumaric Acid Yield and Productivity

	Fumaric acid (g/L)	Ethanol (g/L)	Yield* (g/g)	Productivity (g/(L·h))
SSF	12.54±0.56	2.64±0.98	0.34±0.02	0.21±0.01
SHF	6.76±0.78	3.20±1.93	0.20±0.02	0.11±0.01

*g fumaric acid/g glucose
SSF: Simultaneous saccharification and fermentation
SHF: Separate hydrolysis and fermentation

Table 7. Fumaric Acid Production from Lignocellulosic Materials by *Rhizopus oryzae*

Strain	Raw material	Fumaric acid (g/L)	Yield (g/g)	References
SHF				
R. oryzae ME-F12	Corn straw	27.79	0.35	Xu *et al.* 2010
R. oryzae ATCC 20344	Dairy manure	31.00	0.31	Liao *et al.* 2008
R. oryzae NRRL 1526	Pulp and paper solid waste	23.47	0.57	Das *et al.* 2016
R. oryzae wild 1.22	Wheat bran	20.2	-	Wang *et al.* 2015
R. oryzae CICC 40351	XOR	6.76	0.20	This study
SSF				
R. oryzae CICC 40351	XOR	12.54	0.34	This study

SSF: Simultaneous saccharification and fermentation
SHF: Separate hydrolysis and fermentation
XOR: Xylo-oligosaccharides manufacturing waste residue

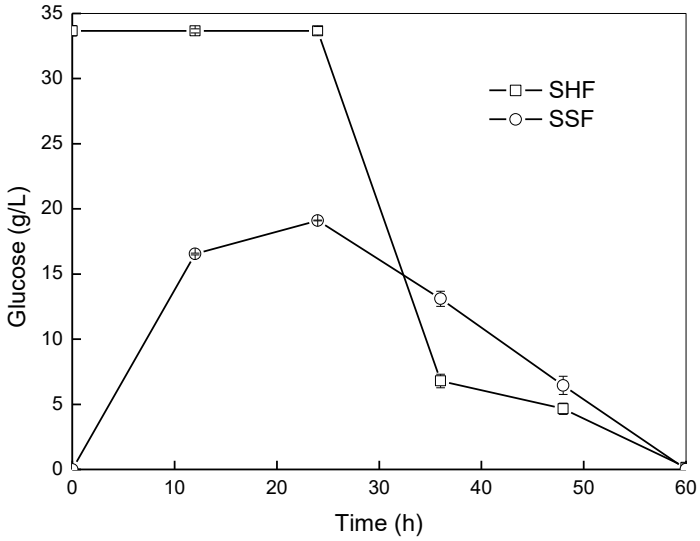

Fig. 3. A comparison of glucose consumption between SSF and SHF over time. SSF: Simultaneous saccharification and fermentation; SHF: Separate hydrolysis and fermentation.

CONCLUSIONS

1. A SSF process was developed for production of fumaric acid from lignocellulose by *R. oryzae*.
2. Optimization of SSF conditions was carried out by an orthogonal design. The processes of SSF and SHF were carried out for the production of fumaric acid from low-cost lignocellulose by *R. oryzae*. The SSF at 38 °C obtained the greatest fumaric acid concentration (12.54 g/L) from 5% (w/v) XOR compared with 6.76 g/L fumaric acid from the SHF process during the same conditions.
3. The SSF process notably improved the production of fumaric acid from XOR by *R. oryzae*.

ACKNOWLEDGMENTS

The authors are grateful for support from the Major Program of the Natural Science Foundation of Jiangsu Higher Education (14KJA220003), the Natural Science Foundation of Jiangsu Province (Grant No. BK20131426), the Key Research and Development Program of Jiangsu Province (BF2015007), and the Priority Academic Program Development of the Jiangsu Higher Education Institutions (PAPD).

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Article submitted: May 13, 2016; Peer review completed: July 11, 2016; Revised version received and accepted: August 16, 2016; Published: August 31, 2016.
DOI: 10.15376/biores.11.4.8831-8843