Improved Itaconic Acid Production from Undetoxified Enzymatic Hydrolysate of Steam-Exploded Corn Stover using an *Aspergillus terreus* Mutant Generated by Atmospheric and Room Temperature Plasma

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Itaconic acid production by *Aspergillus terreus* (*A. terreus*) was investigated using the undetoxified enzymatic hydrolysate of steam-exploded corn stover as the sole carbon source. The fermentation conditions for *A. terreus* were optimized based on glucose as the carbon source. Unfortunately, wild-type *A. terreus* did not grow in the undetoxified enzymatic hydrolysate. Therefore, atmospheric and room temperature plasma (ARTP) mutagenesis was applied to obtain *A. terreus* mutant AT-90. *A. terreus* mutant AT-90 grew and secreted itaconic acid in the undetoxified enzymatic hydrolysate. The highest itaconic acid concentration (19.30 g/L) with a yield of 36.01% was obtained from the undetoxified enzymatic hydrolysate of 10% (w/v) steam-exploded corn stover. This work demonstrated that the *A. terreus* mutant generated by ARTP efficiently improved itaconic acid production from lignocellulose-based carbon source.

Keywords: Itaconic acid; Aspergillus terreus; Atmospheric and room temperature plasma; Undetoxified enzymatic hydrolysate; Steam-exploded corn stover

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INTRODUCTION

Itaconic acid is an unsaturated five-carbon dicarbonic acid, also called methylene succinic acid, methylene butanedioic acid, 3-carboxy-3-butanoic acid, or propylene-dicarboxylic acid (Willke and Vorlop 2001). Due to the two carboxylic groups, itaconic acid can be used as a building block for the production of polymers and bioactive compounds in the areas of agriculture, pharmacy, *etc.* (Okabe *et al.* 2009). Generally, itaconic acid has been industrially produced by the fermentation of carbohydrates (such as glucose) by *Aspergillus terreus* (*A. terreus*). Glucose is too expensive, however, for the production of itaconic acid at the industrial scale. Therefore, researchers have paid more attention to an alternative carbon substrate to replace glucose for the microbial production of itaconic acid. Major research has focused on starch-based carbon sources, such as corn, wheat, potato, cassava, sorghum, *etc.* (Petruccioli *et al.* 1999; Okabe *et al.* 2009). Currently, production of bio-based chemicals from lignocellulose-based raw materials has received much interest in the biological area. Unlike starch-based carbon sources, lignocellulose-based raw materials are the most promising future feedstock for the sustainable production routes of bio-based chemicals because they are the most abundant

non-edible carbon source on earth (Klement and Büchs 2013). Tippkötter *et al.* (2014) reported production of itaconic acid from the enzymatic hydrolysate of beech wood. *A. terreus* NRRL 1960 from Northern Regional Research Laboratory (NRRL) could not produce itaconic acid from undetoxified beech wood hydrolysate, which meant that *A. terreus* was sensitive to some inhibitors in lignocellulosic hydrolysate. After detoxification by a series of methods, *A. terreus* NRRL 1960 fermented detoxified beech wood hydrolysate to produce itaconic acid (Tippkötter *et al.* 2014).

Microorganisms are sensitive to inhibitors in lignocellulosic hydrolysates, which are abundantly produced at severe pretreatment conditions. Improving microorganisms' tolerability to inhibitors is an effective method to enhance microbial production. The atmospheric and room temperature plasma (ARTP) mutation system has been successfully applied to achieve the mutation of more than 40 kinds of microorganisms including bacteria, fungi, and microalgae (Zhang *et al.* 2014). The ARTP mutation system could generate diverse breakages of plasmid DNA and oligonucleotides with variations in plasma dosage. Jiang *et al.* (2014) showed that a mutant *E. coli* by ARTP could ferment hydrolysate generated from the acid hydrolysis of corn stover for the production of succinic acid. Qi *et al.* (2014) reported that mutants of *Rhodosporidium toruloides* by ARTP showed strong tolerance to inhibitors and could grow in undetoxified hydrolysate generated by acid-hydrolysis of sugarcane bagasse. This research demonstrated that ARTP could efficiently improve microbial tolerability to lignocellulosic hydrolysate. The authors are unaware of any study employing lignocellulose-based carbohydrates containing inhibitors to produce itaconic acid by *A. terreus*. We applied ARTP to obtain an inhibitor-tolerated *A. terreus* mutant, and the mutant was used to produce itaconic acid from lignocellulosic materials.

This study focused on the production of itaconic acid from a lignocellulose-based carbon source by *A. terreus*. First, optimization of fermentation conditions based on glucose was carried out with wild-type *A. terreus*. However, wild-type *A. terreus* did not produce itaconic acid using the undetoxified enzymatic hydrolysate of steam-exploded corn stover (SECS) at optimized conditions. A mutant of *A. terreus* generated by ARTP did tolerate and grow in the undetoxified enzymatic hydrolysate of SECS for itaconic acid production.

EXPERIMENTAL

Materials

Corn stover and enzymes

The corn stover was a gift from Jiangsu Kangwei Biologic Co., Ltd. (Dongtai, Jiangsu Province, China). The corn stover was cut 3 to 5 cm length. The air-dried corn stover chips were stored in the sealed plastic bags at room temperature. The contents of the corn stover (in percent of the dry weight, w/w) were: 38.14% glucan, 22.68% xylan, 2.78% arabinan, and 23.34% lignin.

Cellulase from *Trichoderma reesei* (ATCC 26921) and *β*-glucosidase from *Aspergillus niger* (Novozyme 188) were purchased from Sigma-Aldrich (St. Louis, USA). The cellulase activity and *β*-glucosidase activity were measured according to the International Union of Pure and Applied Chemistry procedures (Ghose 1987).

Strain and seed medium

A. terreus CICC 2452 was purchased from the China Center of Industrial Culture Collection (CICC; Beijing, China). The seed medium consisted of (g/L): 40 glucose, 4 NH_4NO_3, 1 yeast extract, 4 $MgSO_4{\cdot}7H_2O$, 0.04 $FeSO_4{\cdot}7H_2O$, 1 K_2HPO_4, and 0.02 $ZnSO_4{\cdot}H_2O$. The pH of the seed medium was adjusted to between 4.0 and 5.0 using dilute HCl. The seed medium was sterilized at 121 °C for 30 min.

Agar slant and spore suspension

The composition of the slant medium was as follows (g/L): 30.0 sucrose, 2.0 $NaNO_3$, 0.5 $MgSO_4$, 0.5 KCl, 1.0 K_2HPO_4, 0.01 $FeSO_4$, and 20.0 agar. The inoculated slant was cultured in an incubator (BINDER, KB53, Tuttlingen, Germany) at 30 °C for 6 to 8 days and then stored at 4 °C until further use. The spores were washed from the slants with sterile water, and the spore density was adjusted to 10^7 spores per milliliter.

Fermentation medium

The fermentation medium consisted of (g/L): 80 to 120 glucose or enzymatic hydrolysate; a mixed nitrogen source (NH_4NO_3 : yeast extract = 2:1) ranged from 2.5 to 6.5; 8 $MgSO_4{\cdot}7H_2O$, 0.04 $FeSO_4{\cdot}7H_2O$; 0.1 $CaCl_2{\cdot}H_2O$, 0.02 $ZnSO_4{\cdot}H_2O$. The pH of the fermentation medium was adjusted to between 2.5 and 3.5 using dilute HCl before autoclaving. The fermentation medium was sterilized at 121 °C for 30 min.

The screening medium

The screening medium using the undetoxified enzymatic hydrolysate consisted of (g/L): 54 glucose; 3 NH_4NO_3; 8 $MgSO_4{\cdot}7H_2O$, 0.04 $FeSO_4{\cdot}7H_2O$; 0.1 $CaCl_2{\cdot}H_2O$; 0.02 $ZnSO_4{\cdot}H_2O$; 1.5 yeast extract. The pH of the screening medium was adjusted to between 2.5 and 3.0 using dilute HCl. The screening medium was sterilized at 121 °C for 30 min.

Methods

Steam-exploded pretreatment of the corn stover and enzymatic hydrolysis

The corn stover was first soaked in 1% (w/w) dilute sulfuric acid solution for 2 h. The steam-exploded pretreatment of the soaked corn stover was carried out at the pressure of 1.8 MPa for 5 min. The liquid fraction was removed by filtration; the solid fraction was washed to neutralization by water with a solid/liquid ratio of 1:10 (w/v). After filtration, the solid fraction was stored in plastic bags at 4 °C until use. The solid fraction contained (in percent of the dry weight, w/w) 52.3% glucan, 7.43% xylan, 0.36% arabinan, and 34.09% lignin.

Enzymatic hydrolysis of the pretreated corn stover with a substrate loading of 10% to 15% (w/v) was carried out in a 250-mL shaker flask at 50 °C and 200 rpm for 72 h in 50 mmol/L citrate buffer (pH 4.8). The enzyme dosage was 20 FPIU cellulase and 15 IU β-glucosidase per gram of glucan. After hydrolysis, hydrolysate was centrifuged at 5000 rpm for 15 min. The supernatant was used in subsequent experiments.

Seed cultivation

The spore suspension was transferred to the seed medium and cultured in an incubator (New Brunswick Scientific, INNOVA 40R, Edison, USA) for 24 h at 30 °C and 200 rpm.

Fermentation

10% (v/v) of the seed medium was transferred into 250-mL Erlenmeyer flasks containing 50 mL of the fermentation medium. The fermentation cultivation was carried out between 30 °C and 40 °C for 72 h to 120 h in an incubator (New Brunswick Scientific, INNOVA 40R, Edison, USA) at 200 rpm.

Helium-based ARTP mutation of A. terreus

The ARTP-II mutation machine was purchased from Si Qing Yuan Biotechnology Co., Ltd. (Wuxi, China). For the mutation of *A. terreus*, 10 μL of spore suspension (10^6 to 10^7 spores/mL) was dipped onto a sterilized stainless-steel plate and dried in sterile air for 2 min. The plate was placed into the ARTP-II vessel under the nozzle. Operating conditions of ARTP-II were at a helium gas flow rate of Q_{He} = 10 slpm (standard liters per minute), RF power input of 100 W and plasma treatment time of 30 s to 90 s. After treatment, the plate was washed with 0.8 mL sterilized saline solution and shaken for 5 min. Next, the suspension was spread onto a potato-dextrose agar plate. After 5d-cultivation at 30 °C, colonies were selected and spores were collected for preparation of spore suspension. Ten percent (v/v) of the seed medium was transferred into 250-mL Erlenmeyer flasks containing 50 mL of screening medium. The screening cultivation was carried out at 40 °C for 72 h in an incubator (New Brunswick Scientific, INNOVA 40R, Edison, USA) at 200 rpm.

Analytic methods

The spore concentration was determined by counting spores on a haemocytometer under a microscope. Components of SECS and its original were analyzed according to the National Renewable Energy Laboratory's methods for the determination of structural carbohydrates and lignin in biomass (Sluiter *et al.* 2008). The itaconic acid, glucose, and xylose were determined by high performance liquid chromatography (HPLC, Agilent Technologies, 1260 Infinity, Santa Clara, USA*)* equipped with a Bio-Rad Aminex HPX-87H column at 45 °C and a refractive index detector. The mobile phase was 5 mmol/L H_2SO_4, and the flow rate was 0.6 mL/min. All the data presented in this study were the average of two experiments. The itaconic acid yield was calculated as follows:

$$\text{Itaconic acid yield (\%)} = \text{Itaconic acid (g)} \times 100 / \text{consumed glucose (g)} \quad (1)$$

RESULTS AND DISCUSSION

Itaconic Acid Production from Glucose

Effect of the initial pH on itaconic acid production

The initial pH was a key point for itaconic acid production by *A. terreus*. Several studies were performed to demonstrate the influence of pH on the cultivation of *A. terreus* in the pH range 2.0 to 5.9 (Hevekerl *et al.* 2014). The optimal initial pH values ranged from 2.5 to 3.1 and were found in relation to the used conditions and strains (Hevekerl *et al.* 2014). Figure 1 shows the effects of the initial pH on itaconic acid production by *A. terreus* CICC 2452 in the pH range 2.0 to 3.5. Increasing the initial pH resulted in an increase in itaconic acid concentration when the initial pH was lower than 2.5. Further increasing the initial pH from 2.5 to 3.5 showed a decrease in itaconic acid concentration. The maximum itaconic acid concentration and yield was obtained when the initial pH was 2.5. This data

indicated that the production of itaconic acid was best at pH 2.5 for *A. terreus* CICC 2452. Therefore, pH 2.5 was selected in the following experiments. This value was similar to the optimal pH of 2.4 found by Riscaldati *et al.* (2000). Low pH value (< 3.0) could also suppress the formation of by-products and create an auto-sterile condition for the fermentation process (Klement and Büchs 2013).

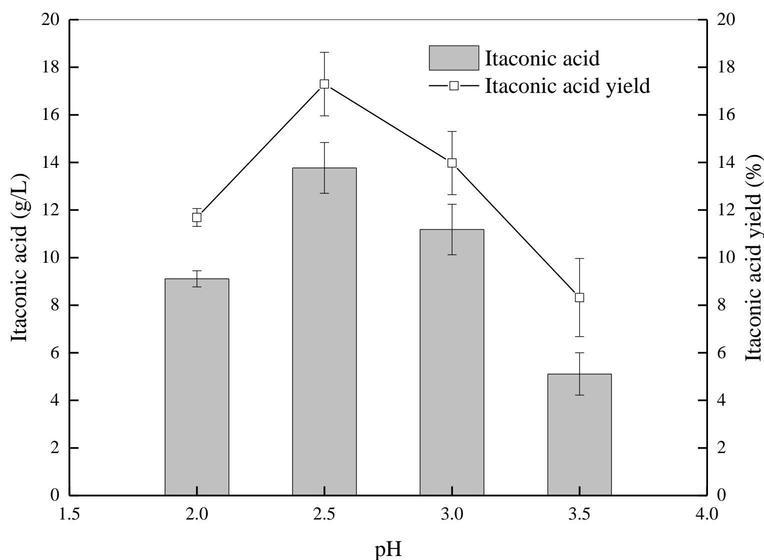

Fig. 1. Itaconic acid production from glucose by *A. terreus* CICC 2452 at different initial pH values

Effects of the initial glucose concentration and temperature on itaconic acid production

The itaconic acid fermentations were generally performed at sugar concentration in the range of 100 to 150 g/L (Willke and Vorlop 2001). Studies showed that the temperature of itaconic acid fermentation occurred in the range of 30 °C to 40 °C (Gyamerah 1995; Dwiarti *et al.* 2007). Table 1 examines the effects of initial glucose concentration and temperature on itaconic acid production by *A. terreus* CICC 2452. As shown in Table 1, the highest itaconic acid concentration (26.46 g/L) and yield (26.63%) at 100 g/L glucose were obtained at 40 °C.

Table 1. Effects of Initial Glucose Concentration and Temperature on Itaconic Acid Production by *A. terreus* CICC 2452

Temperature (°C)	Initial glucose (g/L)	Residual glucose (g/L)	Itaconic acid (g/L)	Itaconic acid yield (%)
30	80.72±0.54	ND*	13.77±1.07	17.05±1.21
	100.49±0.15	16.26±0.18	11.51±1.06	13.67±1.26
	120.55±0.51	35.74±0.26	11.14±0.04	13.13±0.01
35	80.72±0.54	ND	20.30±2.09	25.16±2.76
	100.49±0.15	3.89±0.33	19.38±3.51	20.06±3.59
	120.55±0.51	23.47±5.39	17.91±0.61	18.49±1.56
40	80.72±0.54	ND	15.56±0.71	19.27±0.75
	100.49±0.15	1.14±0.22	26.46±0.88	26.63±0.99
	120.55±0.51	20.71±1.77	25.49±2.47	25.53±3.06

*ND: not detected
Values represent the mean ± standard deviation

Further increase of the temperature from 40 °C to 45 °C (data not shown) caused no detection of itaconic acid in the fermentation broth and there was little glucose consumed. Therefore, 100 g/L initial glucose concentration and 40 °C were used in the subsequent experiments because more glucose was converted into itaconic acid.

Effects of the nitrogen source concentration on itaconic acid production

The nitrogen source, one of the major culture nutrients, played a dominant role in the formation of biomass and metabolites (Casas López *et al.* 2004). The nitrogen source (a mixture of ammonia nitrate and yeast extract; ammonia nitrate: yeast extract = 2:1) ranged from 2.5 g/L to 6.5 g/L and was tested in a shake flask culture for demonstrating the effects of the nitrogen source concentration on itaconic acid production. As shown in Fig. 2, an increase in the itaconic acid concentration was linked to an increase in nitrogen source concentration (< 3.5 g/L). Further increasing the nitrogen source concentration from 3.5 g/L to 6.5 g/L resulted in a decrease in the itaconic acid concentration. The highest itaconic acid concentration was obtained when the nitrogen source concentration was 3.5 g/L, showing that a lower nitrogen source concentration enhanced *A. terreus* by converting glucose into itaconic acid. *A. terreus* cultivation with a limited nitrogen source was shown to promote the accumulation of products (Casas López *et al.* 2003; Tevž *et al.* 2010). Only a few metabolic studies, however, have shown the regulation mechanism of *A. terreus* under nitrogen-limited conditions. Klement and Büchs (2013) reported that microorganisms probably reduced their high energy level and accumulated intermediates under nitrogen-limited condition.

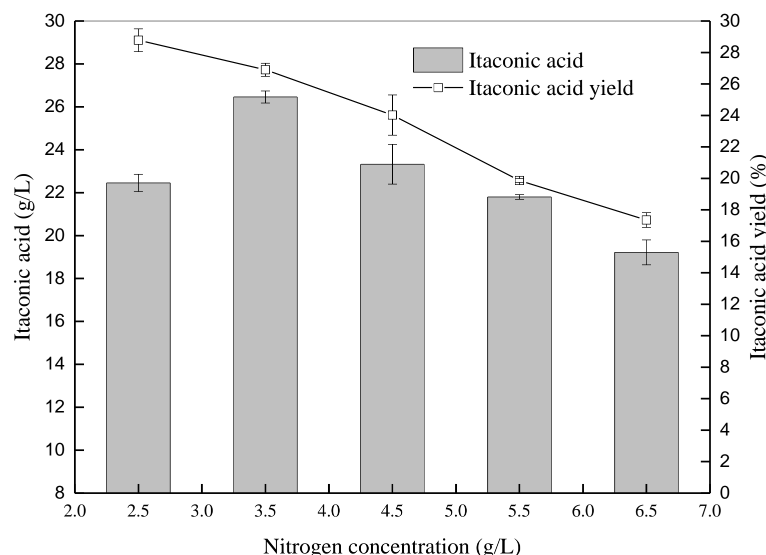

Fig. 2. Itaconic acid production from glucose by *A. terreus* CICC 2452 at different nitrogen source concentrations

Itaconic Acid Production from the Undetoxified Enzymatic Hydrolysate of Steam-Exploded Corn Stover by Wild-Type *A. terreus*

Most studies have paid more attention to itaconic acid production from glucose- or starch-based carbohydrates (Willke and Vorlop 2001; Okabe *et al.* 2009). Few studies have focused on itaconic acid production from lignocellulose-based raw material. Tippkötter *et*

al. (2014) reported 7.2 g/L itaconic acid was observed using detoxified beech wood hydrolysate by *A. terreus*. In this article, the undetoxified enzymatic hydrolysate of SECS was used for itaconic acid production. As shown in Table 2, the 72 h enzymatic hydrolysis of 10% and 15% (w/v) SECS produced 54.01 g/L and 73.45 g/L glucose, respectively. However, *A. terreus* CICC 2452 did not grow in the undetoxified enzymatic hydrolysate from 15% (w/v) SECS, and no itaconic acid was detected in the fermentation broth. Although *A. terreus* CICC 2452 could tolerate the undetoxified enzymatic hydrolysate from 10% (w/v) SECS, little itaconic acid was found in the fermentation broth. The undetoxified enzymatic hydrolysate either inhibited *A. terreus* growth or adjusted the biosynthesis of itaconic acid.

Table 2. Production of Itaconic Acid from the Undetoxified Enzymatic Hydrolysate of Steam-Exploded Corn Stover by *A. terreus* CICC 2452 after 72 h Fermentation

Substrate loading	Initial glucose (g/L)	Residual glucose (g/L)	Itaconic acid (g/L)	Itaconic acid yield (%)
15% (w/v) SECS	73.45±0.56	76.24±2.01	ND*	NA**
10% (w/v) SECS	54.01±0.34	26.03±0.57	0.54±0.08	1.89±0.32

*ND not detected
**NA not available
Values represent the mean ± standard deviation

Table 3 shows the contents in the enzymatic hydrolysate of SECS. Glucose (54.01 g/L) was the major sugar in the enzymatic hydrolysate. Xylose was at a low level (< 5 g/L) in the enzymatic hydrolysate and was not considered in this work. The enzymatic hydrolysate also contained formic acid, acetic acid, levulinic acid, furfural, and 5-hydromethyl furaldehyde. Obviously, glucose was the major carbon source released from SECS, and acetic acid accounted for the major inhibitor in enzymatic hydrolysate (shown in Table 3).

Table 3. Contents in Enzymatic Hydrolysate of Steam-Exploded Corn Stover (g/L)

Substrate loading	10% (w/v)	15% (w/v)
Glucose	54.01±0.34	73.45±0.56
Xylose	3.17±0.63	4.12±0.52
Cellobiose	2.01±0.13	3.55±0.17
Formic acid	0.21±0.04	0.32±0.07
Acetic acid	1.45±0.18	3.17±0.22
Levulinic acid	<0.01	<0.01
Furfural	<0.01	<0.01
5-Hydromethyl furaldehyde	<0.01	<0.01

Values represent the mean ± standard deviation

Acetic acid was formed from the hydrolysis of acetyl groups of hemicellulose in lignocellulose, and the pKa value of acetic acid was 4.76 (Jönsson *et al.* 2013). In this study, acetic acid was maintained in the undissociated form due to the initial pH 2.5 for *A. terreus* CICC 2452. Undissociated weak acid was liposoluble and diffused into cells across the plasma membrane (Palmqvist and Hahn-Hägerdal 2000). Acetic acid could then

dissociate due to the neutral intracellular pH, decreasing the intracellular pH (Palmqvist *et al.* 1999). A decrease in intracellular pH could cause cell death (Jönsson *et al.* 2013). A neutral intracellular pH was vital to cell growth and survival. The cell replicative activity decreased linearly with decreasing intracellular pH (Palmqvist and Hahn-Hägerdal 2000). Therefore, 3.17 g/L acetic acid in enzymatic hydrolysate from 15% (w/v) SECS caused no growth of *A. terreus* CICC 2452, which led to no itaconic acid found in the fermentation broth.

In addition, microorganisms survived at a low concentration of weak acids. *A. terreus* CICC 2452 conducted the glucose uptake in enzymatic hydrolysate from 10% (w/v) SECS containing 1.45 g/L acetic acid. However, little itaconic acid was found in the fermentation broth. The production of itaconic acid by *A. terreus* was mainly dependent on glycolysis and the tricarboxylic acid cycle (Willke and Vorlop 2001). The decrease in intracellular pH caused by the inflow of weak acid was neutralized by pumping out protons through the plasma membrane ATPase at the expense of ATP hydrolysis. Additional ATP must be generated to maintain the neutral intracellular pH (Palmqvist and Hahn-Hägerdal 2000). Therefore, the carbon flux in *A. terreus* might be redirected toward ATP generation for cell survival, not for itaconic acid production.

Improved Itaconic Acid Production by ARTP Mutant from the Undetoxified Enzymatic Hydrolysate of Steam-Exploded Corn Stover

Screening of A. terreus mutants by ARTP

ARTP mutation is a rapid and diverse microbial mutation tool for strain modification. The ARTP mutation system produced the helium radio-frequency atmospheric-pressure glow discharge (RF APGD) plasma jet, which could break plasmid DNA and form DNA fragments (Zhang *et al.* 2014). This system has been successfully used for the mutation of fungi to generate diverse fungal mutants. Qi *et al.* (2014) showed that *Rhodosporidium toruloides* mutants by helium ARTP could notably tolerate inhibitors in sugarcane bagasse hydrolysate without detoxification.

In this study, three mutants of *A. terreus* (AT-30, AT-60, and AT-90) were selected that could grow on the screening medium containing 100% undetoxified enzymatic hydrolysate of SECS (Table 4). Although *A. terreus* mutants AT-30 and AT-60 consumed over 70% more glucose in the medium than did *A. terreus* CICC 2452, little itaconic acid was detected in the fermentation broth. *A. terreus* mutant AT-90 not only consumed over 80% glucose in the medium, but it also produced 6.17 g/L itaconic acid with a 13.77% itaconic acid yield. *A. terreus* mutant AT-90 showed a better capacity to produce itaconic acid from lignocellulosic hydrolysate than did *A. terreus* CICC 2452.

Table 4. Itaconic Acid Production from the Undetoxified Enzymatic Hydrolysate of Steam-Exploded Corn Stover by ARTP Mutants from *A. terreus* after 72 h Fermentation

Strains	Residual glucose (g/L)	Glucose consumption (%)	Itaconic acid (g/L)	Itaconic acid yield (%)
CICC 2452	26.12±1.54	53.28±0.24	0.64±0.16	2.33±0.69
Mutant AT-30	14.57±3.08	72.90±5.73	0.04±0.02	0.12±0.06
Mutant AT-60	14.62±0.81	72.81±1.50	0.06±0.01	0.15±0.04
Mutant AT-90	8.89±0.62	83.47±1.16	6.17±0.76	13.77±1.88

Values represent the mean ± standard deviation

Itaconic acid production by A. terreus mutant AT-90 from lignocellulosic hydrolysate

Itaconic acid was one of the top value-added chemicals from starch, cellulose, and hemicellulose reported by the US Department of Energy (Jäger and Büchs 2012). Production of itaconic acid from lignocellulosic materials gained the most interest in the cited study. Figure 3 shows the fermentation time course of *A. terreus* mutant AT-90 using the undetoxified enzymatic hydrolysate from 10% (w/v) SECS supplemented with other nutrients. In the first 48 h of fermentation, little itaconic acid was found in the fermentation broth while *A. terreus* mutant AT-90 consumed over 50% glucose. Subsequently, itaconic acid concentration and yield increased with time in the following fermentation. The highest itaconic acid concentration (19.30 g/L) with a better itaconic acid yield of 36.01% was found at the end of fermentation. These results indicated that *A. terreus* mutant AT-90 tolerated the undetoxified enzymatic hydrolysate and efficiently converted the glucose of the undetoxified enzymatic hydrolysate into itaconic acid, while *A. terreus* CICC 2452 did not grow in the undetoxified enzymatic hydrolysate. The results showed that the *A. terreus* mutant generated by ARTP had a notable ability to produce itaconic acid from the undetoxified enzymatic hydrolysate of SECS.

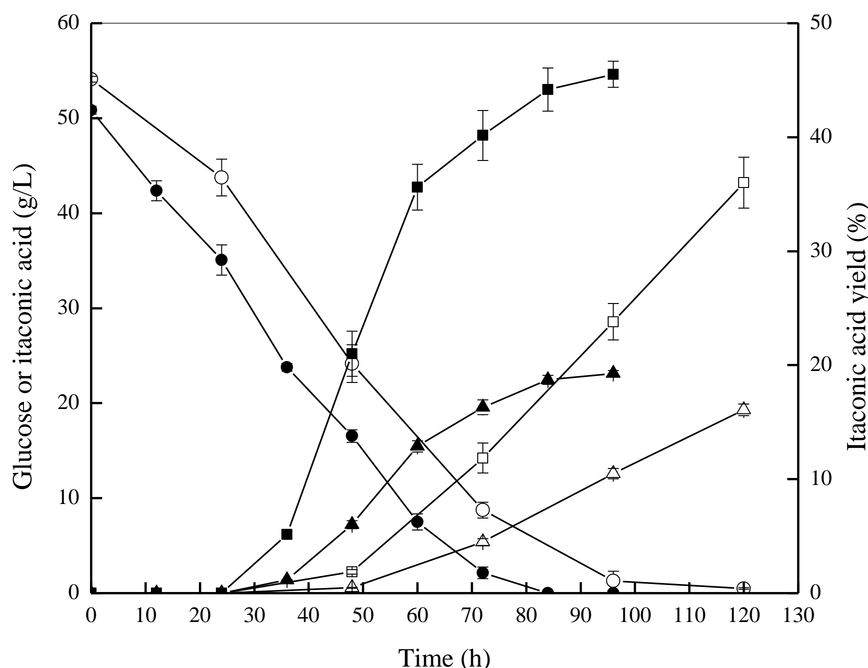

Fig. 3. Production of itaconic acid over fermentation time by *A. terreus* mutant AT-90 generated by atmospheric and room temperature plasma. Carbon sources are the undetoxified enzymatic hydrolysate of steam-exploded corn stover (Open) and pure glucose (Solid), respectively. (○) Glucose in the undetoxified enzymatic hydrolysate; (●) Pure glucose; (△) Itaconic acid from the undetoxified enzymatic hydrolysate; (▲) Itaconic acid from pure glucose; (□) Itaconic acid yield from undetoxified enzymatic hydrolysate; (■) Itaconic acid yield from pure glucose.

CONCLUSIONS

1. Itaconic acid production by *Aspergillus terreus* was carried out using the undetoxified enzymatic hydrolysate SECS.
2. *A. terreus* mutant generated by atmospheric and room temperature plasma (ARTP) showed a better tolerance to the undetoxified enzymatic hydrolysate.
3. The highest itaconic acid concentration (19.30 g/L) with an itaconic acid yield of 36.01% was obtained from the undetoxified enzymatic hydrolysate of 10% (w/v) SECS.
4. ARTP mutation notably improved itaconic acid production from undetoxified lignocellulosic hydrolysate by *A. terreus*.

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