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Application of a Two Stage Temperature and Aeration Control Strategy for Enhanced Diosgenin Production in an Improved Solid-State Fermentation Reactor

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ABSTRACT

A two stage temperature and aeration control strategy was proposed for the production of diosgenin from *Dioscorea zingiberensis* (DZW) with mixed culture of *Trichoderma reesei* and *Aspergillus fumigatus*. The effect of temperature on fungal growth and enzyme production was investigated. The optimum growth temperature for *T. reesei* and *A. fumigatus* was 30°C and 40°C, respectively. The influence of temperature on enzyme transformation was also studied. High temperature (55°C or 60°C) was more suitable than low temperature (30°C or 40°C). A different temperature control strategy experiment was carried out in a flask, first. 73.8±0.51μmol/g of diosgenin was obtained from DZW by setting the temperature in the first stage of 30°C for 2 days, in the second stage of 40°C for 2 days, and in the third stage of 55°C for 2 days. Scaled up experiment was carried out in a new tray bioreactor. The temperature was set at 30°C for 2 days, and increasing to 40°C for next 4 days. The aeration speed was decreased from 200 to 100 mL/min at the 5th and 6th days. Diosgenin concentration of 72.9±1.62 μmol/g was obtained.

INTRODUCTION

In China, *Dioscorea zingiberensis* (DZW) is a cash crop cultivated especially for the extraction of diosgenin, which is an important steroid in pharmaceutical industry (Wang et al. 2008, Pan et al. 2014). Diogenin is widely used in the synthesis of oral contraceptives, sex hormones and other steroids (Bertranda et al. 2009, Adham et al. 2009). Diosgenin mainly exists in plant tubers in the combined forms of saponins, in which sugar chains are linked to aglycon with C-O glycosidic bonds at C3 and C26 positions (Zhu et al. 2010a, Zhang et al. 2015). Diosgenin is released from saponins when the sugar chains at the two positions are hydrolysed. In traditional industry, H_2SO_4 is usually used for the purpose. However, this method causes many environmental problems because high concentration of chemical oxygen demand (COD; 50,000-80,000 mg/L), SO_4^{2-}(29,700-46,200 mg/L) and acid (pH 0.18-0.46) are produced in acid hydrolysis process (Zhao et al. 2008, Cheng et al. 2009).

Compared with chemical hydrolysis, microbial transformation is an economic and environmental-friendly alternative (Fernandes et al. 2003, Huang et al. 2008). Efforts have been made by many researchers to release diosgenin from saponins with fungal strains submerged fermentation (SmF) (Liu et al. 2010, Zhu et al. 2010b, Lei et al. 2012). While, the high energy requirement and low production yield prevent the scale-up of these approaches. Solid state fermentation (SSF) has advantages over SmF in the aspect of low energy requirement, low capital and operating cost, less water usage and wastewater generation (Mahadik et al. 2002, Matsumoto et al. 2004). In our previous research, SSF of DZW was carried out with mixed cultures of *Trichoderma reesei* and *Aspergillus fumigatus* (Cheng et al. 2015). In order to handle SSF in large scale, a tray bioreactor which provides better air transfer and two sporulation areas is developed in our laboratory (Cheng et al. 2016). The fermentation process was affected by various operating parameters. One of the most important factors in mixed culture fermentation is temperature since *T. reesei* is a mesophilic fungi, while *A. fumigatus* is a thermophilic fungi (Vaseghi et al. 2013, Bagagli & Sato 2013). The optimal temperatures for fungal growth, enzyme production and enzymatic transformation are different. It has been suggested that different stage temperature control strategy may have a beneficial effect on mixed culture SSF (Wu et al. 2010, Zhu et al. 2013). However, there is no study on this aspect.

In this paper, the influence of temperature on mixed culture SSF was investigated in detail. Based on our previous study, scaled up SSF was carried out in an improved tray bioreactor and an optimal two stage temperature and aeration control strategy was developed to improve the diosgenin yield in mixed culture SSF.

MATERIALS AND METHODS

Materials: DZW tubers were purchased from Yunxi city, Hubei Province, China. The raw material was washed, dried and hydrolysed with cellulase and amylase by the method described previously to recover cellulose and starch from DZW (Zheng et al. 2014). The residue was pretreated DZW (PDZW) and used as the substrate for fermentation. With pretreatment, about 25 g dried PDZW was obtained from 100 g dried DZW. Cellulase (10,000 IU/g) and α-amylase (2000 IU/g) were supplied by Shandong Longda Bio-product Co., Ltd., Shangdong, China. p-Nitrophenyl-β-D-glucopyranoside (pNPG), p-nitrophenyl-α-L-rhamnopyranoside (pNPR) and standard of diosgenin were procured from Sigma Company, Shanghai, China.

Microorganisms: *T. reesei* (ACCC 30597) was stored in the Agriculture Culture Collection of China (Beijing China). *A. fumigatus* was isolated from DZW and identified by Sichuan Microbiological Resources Infrastructure and Culture Collection Center, China (Lei et al. 2012). The two strains were cultured on potato dextrose agar slant.

Preparation of medium: The inoculum medium is composed of (in grams per litre) 30 sucrose, 3 $NaNO_3$, 1 K_2HPO_4, 0.01 $FeSO_4$, and 0.1 Tween 80. The SSF medium was prepared using PDZW as substrate. The moisture content of 75% was adjusted using medium of 2.67% peptone, 0.27% K_2HPO_4 and 1.3% Tween 80.

Experimental design: The temperature optimization experiment was conducted in 50 mL shake flask with 2 g PDZW as substrate. In the first stage, *T. Reesei* and *A. fumigatus* spores were inoculated in the flasks respectively. The effects of different temperatures (25-45°C for *T. reesei*, 35-55°C for *A. fumigatus*) on the fungal growth, enzyme production (α-rhamnase and β-glucosidase) and diosgenin yield were studied. In the second stage, the influence of different temperatures (45-70°C) on enzyme activities was investigated. In the third stage, based on the above results, five different temperature control strategies were carried out in flask to obtain the highest diosgenin yield.

Fermentation in the bioreactor: To estimate the bioprocess of diosgenin production on a large scale and to verify the results obtained in flask level, SSF was conducted in an improved tray bioreactor. The tray bioreactor with dimensions of 2m × 1.5m × 1m was made of plexiglass. It contained three aluminium shelves with length (55 cm), width (35 cm) and height (1 m) (Fig. 1a). As shown in Fig. 1b, the shelf was divided into four layers. Each layer contained perforated stainless steel pipes that support the tray and through which forced aeration is provided with an air pump to increase the accessibility of O_2. The air flow was passed

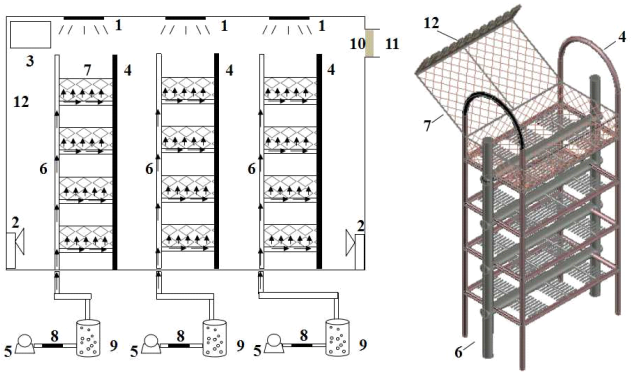

Fig. 1: Diagram of the new solid-fermentation bioreactor. 1: UV light; 2: wet conditioner; 3: air conditioner; 4: aluminum shelves; 5*: air pump; 6: perforated pipes; 7: tray; 8*: sterilized filter; 9*: water container; 10*: eight pieces of gauze 11: gas exit; 12: rake. *: The units improved from bioreactor we reported previously

through eight pieces of gauze to prevent bacterial contamination. The fermentation tray (50 cm×30 cm×15 cm), fitted with a lid was fabricated from stainless steel wire-mesh frame. The inside of tray was covered with eight pieces of gauze to prevent contamination. One rake was fixed inside the lid for uniform distributions of mass and heat transfer during the fermentation process. The humidity and temperature were auto-controlled by the controllers installed inside the bioreactor.

Solid substrate was prepared and placed in each tray. The tray was autoclaved at 121°C for 20 min and inoculated with 7.5×10^6 *T. reesei* spores/g PDZW. Fermentation was carried out at 200 mL/min of air flow rate with temperature optimized in the flask-level experiment. After 48 h, 100 mL medium containing 1.5×10^7 *A. fumigatus* spores/g PDZW was added. The fermentation was carried out for 6 days, three mixings were carried out at 1st, 3rd and 5th day. Samples of ten gram were harvested from center and four sides of the bed every day; the fungal biomass, α-rhamnase, β-glucosidase activities and diosgenin concentration were detected (Huang et al. 2008).

Enzyme assay: Crude enzyme from the cultured media was extracted by an addition of 10 times the volume of water (*w/v*). Contents were filtered with filter paper and filtrate obtained was centrifuged at 10000 rpm and 4°C for 15 min. The supernatant was analysed for α-rhamnase and β-glucosidase activity. The residue was dried at 60°C and weighed. α-rhamnase or β-glucosidase activity was detected using 5 mM pNPR or pNPG as substrate. 1 mL of raw enzyme solution was mixed with 1 mL of substrate in 0.1 M citrate buffer (pH 4.8) at 50°C for 10 min. The reaction was stopped by adding 1 mL of 1 M Na_2CO_3. The generated p-nitrophenol was detected at 420 nm. One unit (IU) of α-

rhamnase (or β-glucosidase) activity was defined as the amount of enzyme that released 1 μmol of p-nitrophenol in 1 min. Enzyme activity (IU/g PDZW) was calculated with the Eq (1):

$$E = \frac{Ce \times V}{m} \quad ...(1)$$

Where, *Ce* (IU/mL) is enzyme activity detected in solution, *V* (mL) is volume of supernatant and *m* (g) is weight of PDZW.

Diosgenin and diosgenin-glucoside concentrations: The substrate sample was dried at 60°C and weighted, then extracted by addition of 5 times the volume of $CHCl_3$ (*w*/*v*) and ultrasonic treating for 30 min. Contents were filtrated using 0.45 μm membrane and filtrate obtained was concentrated to dryness. The dried residue was then dissolved in 1 mL (*V*) of methanol and quantitatively determined by HPLC (Huang et al. 2008). The diosgenin (or diosgenin-glucoside) concentration (*D*, μmol/g DZW) was computed with the Eq (2):

$$D = \frac{C \times V}{(m / 0.25)} \quad ...(2)$$

Where, *C* (μmol/mL) is the diosgenin (diosgenin-glucose) concentration detected in methanol solution, *V* (mL) is volume of methanol solution, *m* (g) is weight of PDZW and 0.25 means that 25 g PDZW was obtained from 100 g DZW.

RESULTS

Effect of temperature on fungal growth and enzyme production in shake flask: The effect of temperature on cell growth and enzyme production of *T. reesei* and *A. fumigatus* was studied and shown in Fig. 2. *T. reesei* has a preferred temperature range of 25 to 35°C with which their enzymes function best. Fig. 2a shows that the optimal temperature for *T. reesei* growth was 30°C, the biomass began to decline after 3 days of fermentation. Therefore, the first 3 days were regarded as the growth stage for *T. reesei*. At 25°C, 35°C and 40°C, the biomass in each system did not show a significant difference from that obtained at 30°C. However, *T. reesei* did not grew well at 45°C. 30°C was optimal for *T. reesei* to produce α-rhamnase (Fig. 2b). The highest activity of α-rhamnase (30.2 IU/g PDZW) was obtained at this temperature after 4 days of incubation. It also can be seen that activity of α-rhamnase decreased rapidly when incubation temperature was 25°C or 45°C. The optimum temperature for β-glucosidase production was 30°C, too. The highest activity of 81.7 IU/g was obtained after 4 days incubation at this temperature (Fig. 2c). So 30°C was chosen for the *T. reesei* as the fermentation temperature. Fig. 2d showed that the optimal temperature for *A. fumigatus* growth was between 40°C and 55°C, and the growth stage of *A. fumigatus* was the first 3 days. The range of 40°C to 55°C was also found to be optimal for enzyme production (Fig. 2e, 2f). The α-rhamnase activities of 17.5- 20.3 IU/g and β-glucosidase activities of 73.9-74.7 IU/g were achieved in the range. Because 45°C was too high for the growth of *T. reesei*, 40°C was chosen for the *A. fumigatus* incubation temperature of the second stage.

Effect of temperature on enzyme activity in shake flask: The characteristics of enzymes at different temperatures were different. The effects of various temperatures on enzyme activity were also studied and given in Table 1. The optimal temperatures for α-rhamnase from *T. reesei* were 50°C and 55°C. The enzyme activities of 2.58 and 2.74 IU/mL were detected at the two temperatures. The highest value of 7.93 IU/mL for β-glucosidase from *T. reesei* was also obtained at 55°C. The optimum temperature range for α-rhamnase produced from *A. fumigatus* was between 55°C and 65°C, while the β-glucosidase activity reached highest value at the temperature range of 50°C to 65°C.

Effect of two temperature strategy on diosgenin production in shake flask: As the temperature needed for fungal growth, enzyme production and enzyme reaction were different, a stage temperature control method was developed. Five temperature control strategies were investigated (Fig. 3). Because *T. reesei* was added at the start of 2 days, the temperature of this stage was controlled at 30°C for fungal growth. After 2 days, *A. fumigatus* was added. Two temperature control strategies were carried out. In strategy 1, the temperature was controlled at 40°C from 3rd to 6th day. In strategy 2, the temperature was controlled at 40°C from 4th to 6th day. The results showed that strategy 1 was more suitable for diosgenin production. Diosgenin achieved from strategy 1 was significantly more than that from strategy 2. Based on the strategy 1, three other strategies were developed. In strategy 3, the temperature was controlled at 50°C during the last two days. In strategy 4, the temperature was controlled at 55°C. In strategy 5, the temperature was controlled at 60°C. It can be seen that strategy 4 led to production of maximum diosgenin concentration of 74.2±0.6 μmol/g DZW after 6 days. The diosgenin concentration of 67.5±0.65 μmol/g was achieved under traditional temperature control strategy (the temperature was controlled at 35°C in the whole fermentation process).

Effect of two-temperature strategies on diosgenin production in tray bioreactor : The *T. reesei* spores were inoculated in the substrate and mixed with the rake in the tray lid and fermented at 30°C for two days. Then *A. fumigatus* spores were inoculated in each tray, mixed and fermented at 40°C for two days. At the fifth day, the substrate was mixed

Table 1: The effect of temperature on α-rhamnase and β-glucosidase activities from *T. reesei* and *A. fumigatus*.

Fungal	Enzyme	Temperature (°C)					
		45	50	55	60	65	70
T. reesei	α-rhamnase	1.74±0.12^{b}	2.58±0.11^{a}	2.74±0.23^{a}	2.02±0.31^{b}	1.27±0.22^{c}	-
	β-glucosidase	5.29±0.43^{c}	6.23±0.48^{bc}	7.93±0.55^{a}	6.45±0.38^{b}	3.23±0.19^{d}	-
A. fumigatus	α-rhamnase	0.88±0.13^{b}	1.02±0.32^{b}	1.77±0.15^{a}	1.43±0.26^{a}	1.62±0.53^{a}	0.88±0.14^{b}
	β-glucosidase	4.32±0.26^{b}	5.97±0.67^{a}	6.09±0.49^{a}	6.73±0.22^{a}	6.24±0.37^{a}	4.51±0.33^{b}

Note: a-d: Indication letters. Values in the same column not sharing the same letters are significantly different (one-way ANOVA with SNK method, $p < 0.05$).

Table 2: The effect of tray bed depth and aeration speed on diosgenin concentration obtained from DZW in new tray bioreactor.

Tray bed depth (cm)	Aeration (mL/min)	Diosgenin concentration (μmol/g)
1.5	0	68.1±0.45^{b}
	100	70.1±1.13^{d}
	200	70.8±0.85^{de}
2	0	70.2±1.09^{de}
	100	73.8±0.51^{f}
	200	71.4±1.05^{e}
2.5	0	64.3±1.14^{a}
	100	66.5±0.55^{b}
	200	66.6±0.89^{b}

Note: a-e: Indication letters. Values in the same column not sharing the same letters are significantly different (one-way ANOVA with SNK method, $p < 0.05$).

Table 3: The fermentation parameters of new tray bioreactor with two stage temperature control strategy.

Day	1	2	3	4	5	6
Fungal	*T. reesei*	-	*A. fumigatus*	-	-	-
Mixing	mixing	-	mixing	-	mixing	-
Temperature (°C)	30	30	40	40	40	40
Aeration (mL/min)	200	200	200	200	100	100
Tray bed depth (cm)	2	2	2	2	2	2
Initial moisture (%)	75	75	75	75	75	75

again and the whole SSF was carried out for six days. Tray bed depths of 1.5, 2, 2.5 cm were studied, and the aeration speed during the last two days was decreased to 0, 100, 200 mL/min, respectively. The results in Table 2 show that the tray bed depth of 2 cm and aeration speed of 100 mL/min during last two days were selected as the operational parameters of the tray bioreactor. Under the conditions, diosgenin concentration of 73.8±0.51 μmol/g was detected. The operational parameters of the two stage temperature control strategy are listed in Table 3.

Comparison of new strategy and traditional technique: The changes of fungal growth, enzyme production, diosgenin-glucose and diosgenin concentrations in the new and traditional SSF process were investigated. The biomass analyses of the substrate obtained with the different temperature strategies, both demonstrated a gradual increase from 0 to the highest value (Fig. 4a). *T. reesei* and *A. fumigatus* grew better at the two stage temperature because 30°C and 40°C were suitable for fungal growth, respectively. The highest activity of α-rhamnase obtained from the new technique was 33.4 IU/g, which is significantly higher than that achieved from the traditional one (Fig. 4b). The new technique also led to the production of more β-glucosidase, with a highest value of 94.3 IU/g (Fig. 4c).

Diosgenin-glucose is the main by-product in the biotransformation system. The diosgenin-glucose and diosgenin contents can reflect the substrate consumption and product accumulation. In this study, we determined the diosgenin-glucose and diosgenin contents to investigate the effect of changing temperature on the biotransformation efficiency. Fig. 5a shows the changes in the diosgenin-glucose content during the two fermentation processes. It was found that there was an increase in the diosgenin contents during the initial 96 h and then a gradual decrease with time

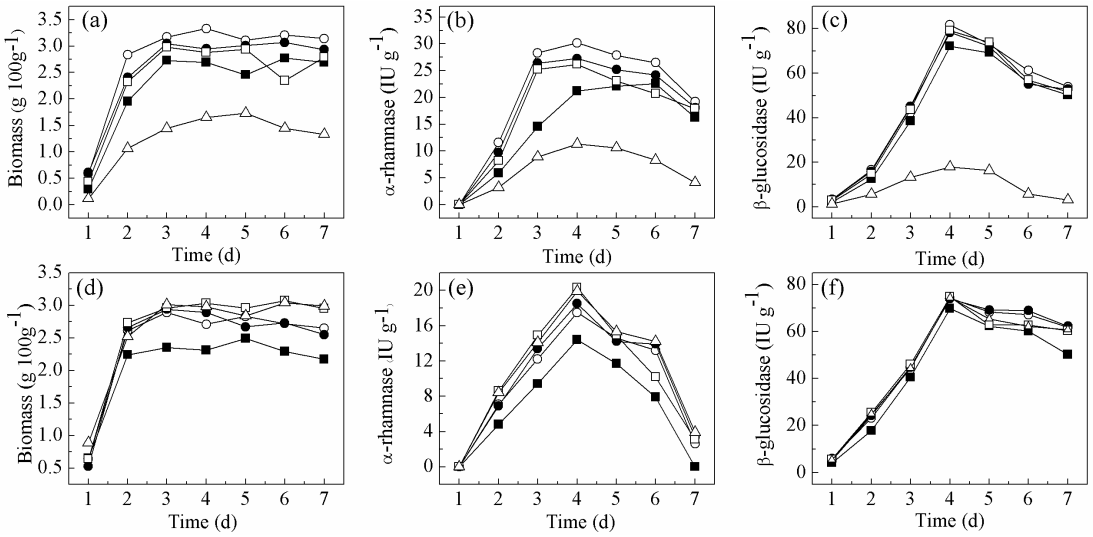

Fig. 2 (a-c): The effect of temperature on fungal growth (a), α-rhamnase (b) and β-glucosidase (c) activities of *T. reesei* during fermentation. (■: 25°C; ○: 30°C; ●: 35 °C; □: 40 °C; △: 45 °C). (d-f): The effect of temperature on fungal growth (d), α-rhamnase (e) and β-glucosidase (f) activities of *A. fumigatus* during fermentation. (■: 25°C; ○: 30°C; ●: 35 °C; □: 40 °C; △: 45 °C) Data were expressed as mean value. The standard deviations were less than 10%.

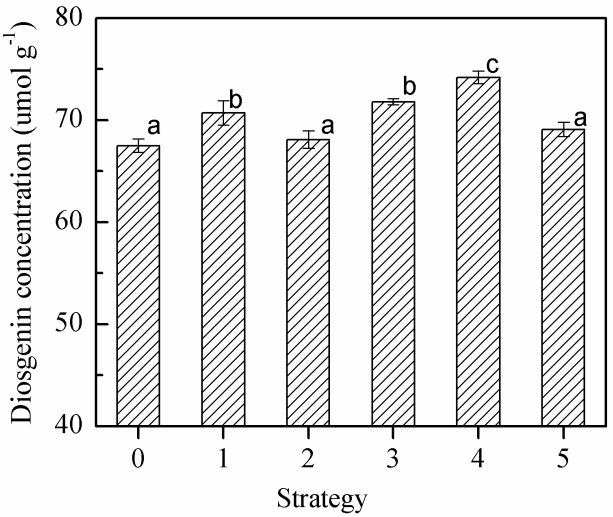

Fig. 3: Diosgenin concentration obtained from DZW with different temperature control strategies in flask. 0: 35°C for 6 days; 1: 30°C for first two days, 40°C for next four days; 2: 30°C for first three days, 40°C for next three days; 3: 30°C for first two days, 40°C for second two days, 50°C for next two days; 4: 30°C for first two days, 40°C for second two days, 55°C for next two days; 5: 30°C for first two days, 40°C for second two days, 60°C for next two days. Values not sharing same letters are significantly different (one-way ANOVA with SNK method, $p<0.05$).

after that point. While the change in the new process was more significant than that in the traditional process, Fig. 5b showed an apparent increase in diosgenin in the two process. However, the diosgenin contents achieved from 3-6 days using the variable technique was higher than that obtained with the isothermal strategy.

DISCUSSION

T. reesei is a mesophilic fungi, while *A. fumigatus* is a thermophilic fungi (Latifian et al. 2007, Gurpreet et al. 2011). So temperature plays an important role in the mixed culture fermentation. In addition, the thermostable of the glucosidase produced by the two fungi are different (Liu et al. 2011, Saqib et al. 2010). There are very few reports available on this aspect. Results from the two temperature strategy experiments described here showed that temperature is an important factor influencing the fungal growth, enzyme activity and biotransformation efficiency of mixed culture fermentation. To some extent, the temperature of a fermentation system is a measure of the molecular kinetic energy (Laidler & Peterman 1979). The kinetic energy of enzyme and substrate increased with increasing temperature (Cay & Van der Stede 2010, Zhang et al. 2009). Most microorganisms have a suitable temperature ranges in which the function of enzymes is best. The optimal temperature for the growth of *T. reesei* and *A. fumigatus* are significantly different. *T. reesei* preferred low temperatures (30-40°C), while the thermophilic fungi, *A. fumigatus* grew better at higher temperatures (40-55°C).

As α-rhamnase and β-glucosidase are the leading enzymes needed for the efficient hydrolysis of saponins (Lei et al. 2012). Optimization was carried out with respect to α-rhamnase and β-glucosidase in the study (Zhu et al. 2010c, Zhu et al. 2014). The preferred temperature ranges for the two enzymes were different, but both of the two enzymes could function well at 50-60°C. Therefore, the best tem-

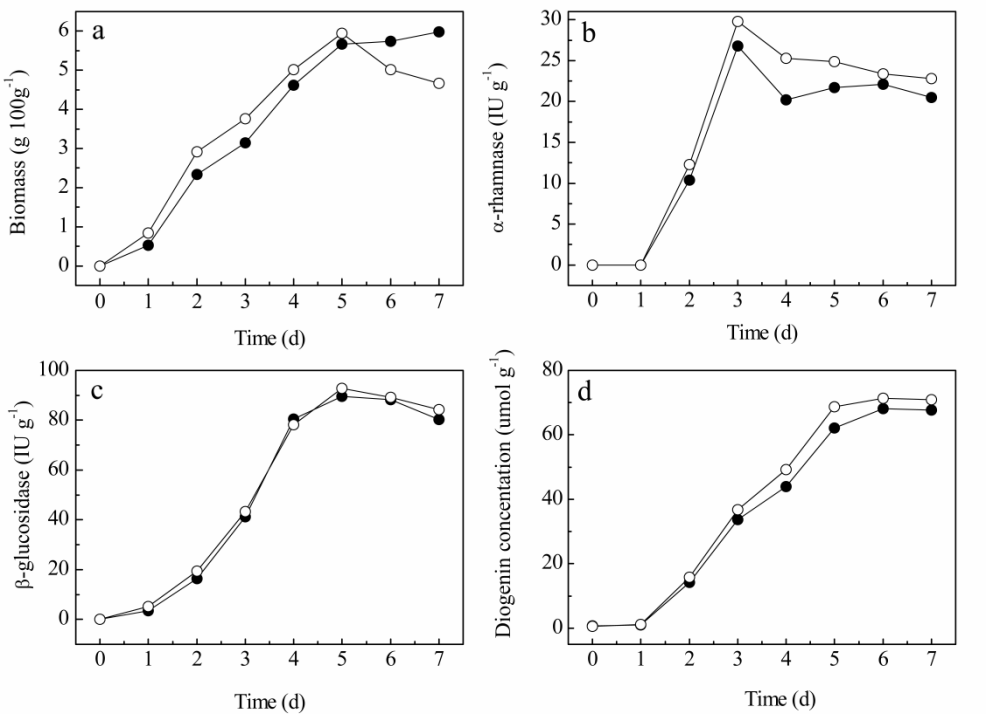

Fig. 4: Time course of biomass (a), α-rhamnase (b), β-glucosidase (c) and activities and diosgenin concentration (d) during SSF in new tray bioreactor under different temperature control strategies. ○: traditional strategy; ●: two stage temperature control strategy. The standard deviations were less than 10%.

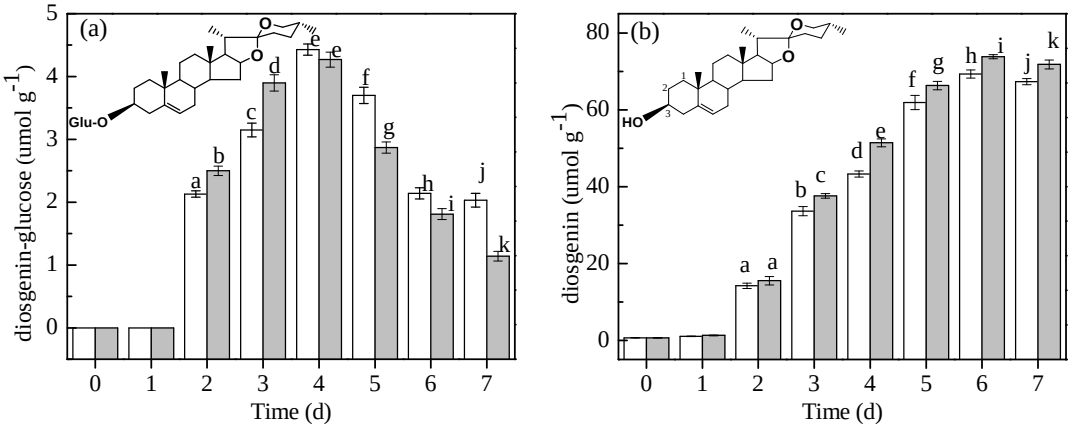

Fig. 5: Changes of the contents of diosgenin-glucose (a) and diosgenin (b) during SSF in new tray bioreactor under different temperature control strategies. Blank: traditional strategy; Gray: two stage temperature control strategy. Values not sharing same letters are significantly different (one-way ANOVA with SNK method, $p<0.05$)

perature leading to enhance cell growth were different from those resulting in increased enzyme activity. It was assumed that using 30°C for *T. reesei* growth, 40°C for *A. fumigatus* growth and 50-60°C for enzyme transformation would be better. The results confirmed our assumption. Under the temperature control strategy 4, highest diosgenin yield was obtained. In the first stage, 30°C was an optimal temperature for *T. reesei* growth. *T. reesei* grew well and showed enhanced enzyme productivity. *A. fumigatus* was added in the second stage and incubated at 40°C. On the one hand, *T. reesei* can grow at 40°C and enzymes produced performed well under the condition. On the other hand, *A. fumigatus* grew well for two days at 40°C to produce glucosidase. In the third stage, higher temperatures were suitable for optimum activity of enzymes. At 55°C, the biotransformation efficiency was increased.

Scaled-up SSF was conducted in a new tray bioreactor. Compared with traditional tray fermentor, the new bioreactor has three advantages. (1) The new reactor is ventilated both at the top and the bottom, while air in traditional one only penetrates from the top. (2) Because the air was saturated before it enters the cabinet and the existence of membrane in the outlet, the atmosphere in the bioreactor is near-saturation which results in low moisture loss during the fermentation. (3) The layer in the bioreactor was made of perforated stainless steel pipes, with which the accessibility of O_2 to the tray was increased and the metabolic heat accumulated in the tray bed was released.

In our previous work, it was found that the initial moisture content of 75 %, bioreactor temperature of 35°C, solid bed depth of 1.5 cm, three mixings carried out at 1st, 3rd and 5th day led to maximum diosgenin yield (Cheng et al. 2016). Under the optimized fermentation conditions, after 6 days incubation, maximum diosgenin concentration of 68.2 μmol/g was detected in DZW. In this study, based on the results obtained from shake flask, the two temperature fermentation strategy was applied in the tray bioreactor. Besides the temperature controlled conditioner, the solid bed depth and the aeration speed will affect the temperature of substrate.

The flask level temperature optimization experiment showed that strategy 4 was suitable for diosgenin production. While the temperatures of 30 and 40°C were easily controlled in the bioreactor. Temperature of 55°C was too high to control. However, the metabolic heat accumulated in the tray bed will lead to an increase in temperature. If the temperature in the substrate bed can reach 55°C in enzyme transformation stage, the diosgenin yield will increase. Aeration, tray bed depth and mixing times are three factors which affect the metabolic heat accumulation through out the process. Our previous study showed that mixings carried out at first, third and fifth day was effective and economic. Therefore, the mixing strategy was employed, the tray depth and aeration speed in enzyme transformation stage (the 5th and 6th day) were optimized to obtain higher diosgenin yield (Bagagli & Sato 2013).

It was showed that tray bed depth of 2 cm was optimal condition. In traditional one with temperature fermentation strategy 35°C, the optimal tray bed depth is 1.5 cm (Cheng et al. 2016). This is because the optimum temperature of *T. reesei* is 30°C. Deeper tray bed depth resulted in more metabolic heat accumulating, which would have negative effects on the growth of *T. reesei*. In two temperature fermentation strategy, the initial temperature is 30°C, and the aeration speed of 200 mL/min was set, the metabolic heat accumulation was slow. So the optimal tray bed depth was increased to 2.0 cm. Aeration speed reducing to 100 mL/min at 5th and 6th day was suitable for biotransformation. The aeration at first four days could offer oxygen to the fungi and release the accumulated metabolic heat. At last two days, the aeration speed was reduced to accumulate heat for enzyme transformation. The temperature in tray bed was increased to 51.4°C (data not shown). Analysis of diosgenin-glucose and diosgenin in the substrate from new and traditional processes showed that change curves of the two saponins were similar. However, the values demonstrated significant differences. The differences in the saponins change were caused by higher temperature, which increase the microbial enzyme activities and thereby increase the saponins utilization and transformation rates.

As compared the new strategy to traditional one, it was found that with the new strategy more substrate was treated and less energy was consumed. So it was more economic and effective. With respect to applications, SSF is a more effective technique to SMF for production of value-added products (Zhu et al. 2013). The bioprocess of diosgenin production from DZW by SSF in a new tray bioreactor was described in this research, which can offer a theoretical basis for scale up industrial applications. Although some problems exist in using the tray bioreactor, the fermentor is a simple reactor to enhance diosgenin production in large scale.

CONCLUSIONS

In this study, it was found that changing the temperature during the mixed culture fermentation stage in the improved tray bioreactor was an efficient way to improve diosgenin production in SSF. *T. reesei* and *A. fumigatus* grew better at 30 and 40°C respectively, resulted in higher α-rhamnase and β-glucosidase activities. With tray bed depth of 2 cm and aeration speed changing from 200 to 100 mL/min at 5th and 6th day, temperature in substrate increased to 51.4°C which was suitable for enzyme reaction. The diosgenin concentration of 73.8±0.51 μmol/g was obtained, which increased about 6.5% compared to the constant-temperature fermentation. The new strategy will have positive repercussions on economy of diosgenin production. Future researches can focus on the scale up operation of tray bioreactor for diosgenin production from DZW, which will be helpful in commercialization of the biotransformation.

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