Solid-state Fermentation Process for Gibberellin Production Using Enzymatic Hydrolysate Corn Stalks

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Solid-state fermentation was carried out for production of gibberellin *via* the addition of enzymatic hydrolysate from steam-exploded corn stalks during the culture period. The enzymatic hydrolysate from the steam-exploded corn stalks was added to the culture medium during the solid-state fermentation period, which improved gibberellin production. When the enzymatic hydrolysate was added into the 400 mL/kg dry basis substrate in the solid-state fermentation after 60 h, the temperature was 30 °C, the pH was 7.00, the mass ratio of solid to liquid was 1:1.1, and the fermentation period was 168 h. This led to the largest gibberellin yield (9.48 g/kg dry basis), and when compared with pre-optimization, the gibberellin yield increased by 135%. The optimum conditions to maximize the biomass for the fermentation process were obtained; the temperature was 32 °C for a gibberellin yield of 9.20 g/kg dry basis, the pH was 6.00 and the mass ratio of solid to liquid was 1:1.1 for a gibberellin yield of 9.48 g/kg dry basis, and the fermentation period was 96 h for a gibberellin yield of 6.94 g/kg dry basis. Therefore, a new alternative way for gibberellin production *via* solid-state fermentation has been demonstrated.

Keywords: Gibberellin; Solid-state fermentation; Fusarium moniliforme; Steam explosion

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INTRODUCTION

Gibberellins are tetracyclic diterpenoid acid compounds. As an important plant growth regulator, gibberellin is widely used in agriculture, nurseries, horticulture, viticulture, tea plantations, *etc*. (Martin 1983). It contributes to various efficacies, such as the promotion of stem elongation, germination, and breaking seed dormancy, as well as affecting flowering, gender performance, inhibition of leaf, and fruit aging (Loreto *et al*. 2008; Dissanayake *et al*. 2010; Mckenzie and Deyholos 2011). Therefore, the market demand for gibberellin continually increases.

Sawada (1912) pointed out that the overgrowth of rice seedlings could result from fungal infection. Subsequently, Kurosawa (1926) found that rice seedlings and other water grass elongate if the culture filtrate of the dried rice seedlings is used; pathogenic bacteria stimulate the extension of stems but inhibit the formation of chlorophyll and root growth by secreting a compound. Sun (1981) found that the rice bakanae disease was caused by the *Fusarium* infection, and Teijiro (1934) obtained the effective component from the fermentation filtrate of the pathogen. This component inhibits the growth of rice seedlings under any test concentration, and it was officially named gibberellin in 1935 (Yabuta 1935). Gibberellin produced from fungi is a class of bioactive diterpenoid plant hormones

(Tudzynski and Hölter 1998). At least 126 types of gibberellin have been identified, which come mainly from plants and microbes (Tudzynski 1999; Shukla *et al.* 2003).

Gibberellin was first produced *via* solid-state fermentation in 1988 (Durand and Chereau 1988). The initial liquid fermentation of gibberellin used a liquid surface culture, and then the commonly used process gradually evolved into a liquid medium system, which became the primary production method. Early trials for gibberellin production in Japan were based on surface cultivation with gibberellin yields of 40 mg/L to 60 mg/L. Before 1961, most submerged fermentation methods had maximum yields of 1 g/L of gibberellin (Tudzynski 1999). Lu *et al.* (1995) investigated the production of gibberellin *via* immobilized cells fermentation, which exhibited a maximum yield of 210 mg/L after 84 days and a high level of efficiency. The prototype for a traditional solid-state fermentation was the Koji fermenting process, in which fungus was cultured on grains, such as rice or soy beans (Hesseltine 1977a,b). As early as the 1950s, the solid-state fermentation process has been applied to *Fusarium moniliforme* (Focke 1967). Gibberellin production levels from solid-state fermentation were found to be 1.6 times higher than that of the deep liquid fermentation with equal amounts of carbon (Kumar and Lonsane 1987). While *Gibberella fujikuroi* produces 23 mg/mL of gibberellin in 120 h *via* liquid fermentation, a solid-state fermentation process using cassava flour shows higher production (250 mg/kg of dry solid media) in a shorter timeframe (Tomasini *et al.* 1997). Kumar and Lonsane (1987) investigated the influence of the physical and nutritional factors on gibberellic acid production and found that the gibberellin yield was increased 2.9 times *via* judicious selection of the parameters. In recent years, renewed attention has been given to gibberellins (Li and Sun 2018).

Three stages form the synthetic pathway of gibberellin: stage 1 is the synthesis of Geranylgeranyl diphosphate (GGDP). In stage 2, GGDP is transformed into ga12-aldehyde. Stage 3 involves the transformation of ga12-aldehyde into different types of GA. Different culture conditions stimulate the same strain to produce different types of Gibberellins (Hu *et al*. 2013).

Gibberellin has been produced by these methods using submerged fermentation, but some investigations indicated that a solid-state culture was still necessary, especially for the demands of sustainable development and environmental protection.

Compared with liquid fermentation, solid-state cultures have the following advantages: (1) There is less investment and the process is simpler; (2) There is a high concentration of the end product, leading to simpler and more complete extraction, and the downstream processes are easy and create less pollution in the environment with less water consumption; (3) The fermented raw materials are generally agricultural products, such as bran or maize flour, which are easy obtained; (4) The substrate of the solid culture is easy to adjust according to production demands.

As a large agricultural country, more than 200 million tons of corn stalks are produced each year in China (Wang *et al.* 2010; Li 2012). However, corn stalks are not fully utilized; most are left idle or burned on the farmland. This process wastes the resources and pollutes the environment. There are multiple pretreatment methods for corn stalks, such as hydrogen peroxide presoaking prior to ammonia fiber expansion (Zhao *et al.* 2016). Steam explosion technology is a practical method of corn stalk pretreatment (Datar *et al.* 2007; Elander *et al.* 2009; Wyman *et al.* 2009). Corn stalks are more easily hydrolyzed after steam-explosion treatment, and the enzymatic hydrolysate are added as part of the solid-state culture media process. In this paper, the authors investigated the solid-state fermentation process for gibberellin by adding the enzymatic hydrolysate from

steam-exploded corn stalks. As no methodology had been found in other literature, this work provides a new alternative way to use corn stalks.

EXPERIMENTAL

Microorganism

Fusarium moniliforme CICC (China Center of Industrial Culture Collection) 2490 was purchased from the management center of Chinese industrial microbial strain preservation (Chaoyang District, Beijing) in 2015. *Fusarium moniliforme* was domesticated and improved to adapt to the cultural media, which was added to the enzyme hydrolysate of steam-exploded corn stalks and was named "zzushzxbjlfl50".

Table 1. Reagents Used in the Experiments

Reagent Name	Purity	Manufacturer
Potassium phosphate monobasic	AR	Tianjin Kermel Chemical Reagent Co., Ltd. (Tainjin, Hebei, China)
Magnesium sulfate heptahydrate	AR	Guangzhou Chemical Reagent Factory (Zhaoqing City, Guangdong, China)
Hydrochloric acid	AR	Luoyang Haohua Chemical Reagent Co., Ltd. (Shantou, Guangdong, China)
Ethyl acetate	AR	Sinopharm Chemical Reagent Co., Ltd. (Ningbo, Zhejiang, China)
Sulfuric acid	AR	Luoyang Haohua Chemical Reagent Co., Ltd. (Shantou, Guangdong, China)
Glucose	AR	Zhengzhou paini Chemical Reagent Factory (Zhengzhou, Henan, China)
Ammonium sulfate	AR	Tianjin Kermel Chemical Reagent Co., Ltd. (Tainjin, Hebei, China)
Sodium hydroxide	AR	Tianjin Kermel Chemical Reagent Co., Ltd. (Tainjin, Hebei, China)
3,5-Dinitrosalicylic acid	CP	Sinopharm Chemical Reagent Co., Ltd. (Ningbo, Zhejiang, China)
Sodium tartaric	AR	Tianjin Fengchuan Chemical Reagent Technology Co., Ltd. (Tainjin, Hebei, China)
Phenol	AR	Luoyang Haohua Chemical Reagent Co., Ltd. (Shantou, Guangdong, China)
Anhydrous sodium sulfate	AR	Laohekou chemical group Co., Ltd. (Xiangyang, Hubei, China)
Ethyl alcohol absolute	AR	Tianjin Fengchuan Chemical Reagent Technology Co., Ltd. (Tainjin, Hebei, China)
Sodium citrate	AR	Tianjin Kermel Chemical Reagent Co., Ltd. (Tainjin, Hebei, China)
Citric acid	AR	Suzhou Chemical Reagents Factory (Haidian, Beijing, China)
Augar	BC	Tianjin Kermel Chemical Reagent Co., Ltd. (Tainjin, Hebei, China)
Trichloroacetic acid solution	AR	Henan Sanlian science and Trade Co., Ltd. (Zhengzhou, Henan, China)
Cellulase	AR	Novozymes (China) Biotechnology Co., Ltd. (Tainjin, Hebei, China)
AR, analytical reagent; CP, chemically pure; BC, biochemical		

Corn Stalk Pretreatment

The corn stalks were sourced from Ye County, Pingdingshan City, Henan Province, China. The pre-treatment process first exploded the corn stalks with steam and then followed with enzymatic treatment. The corn stalks were cut into 3 cm to 4 cm long segments and impregnated in water for 24 h, until the mass ratio of corn stalks to water was 1:1. Then the corn stalks were placed into a vessel with a volume-filling coefficient of 30%. Steam was introduced into the vessel until the pressure reached 1.5 MPa, and temperature was maintained at 205 °C for 8 min. Subsequently, the steam was quickly released to standard atmospheric pressure. The corn stalks were broken into small debris during the process. The steam-exploded corn stalks (CSS) were dried to a constant weight at 80 °C and were stored in the storage room. One kg of corn stalks and 1.275 kg of cellulase was added into 352.941 L of citrate buffer liquid, whose pH was 4.8 ± 0.05. Since 1275 g of cellulase is equal to 42.5 mL of cellulase, the enzyme activity was 50 U/g. The hydrolysis flasks were incubated on a shaker at 50 °C and 200 rpm for 48 h. The supernatant was retained as a matrix ingredient after the enzymatic hydrolysate was centrifuged, which can be abbreviated to SEHC.

The raw materials are commercially available, except for those stated, and the reagents used in the experiments are shown in Table 1.

Culture media

Table 2 showed the experimental instruments used; vertical pressure steam sterilization pot, visible light spectrophotometer, water bath, electronic analytical balance, artificial climate incubator, incubate the shaker at constant temperature, centrifuge, and steam explosion vessel.

Table 2. The Instruments Used in the Experiments

Equipment Name	The Product Model and Manufacturer
Vertical pressure steam sterilizer	LDZF-30KB-III Shen An Medical (Jiading, Shanghai, China)
Visible light spectrophotometer water bath	722N Shanghai Jingke Scientific Instrument Co., Ltd. (MinHang, Shanghai, China)
Electronic analytical balance	JB/T 5374-1991 Mettler Toledo (Columbus, OH, US)
Artificial climate incubator	ZRG-130A Shanghai Binglin Electronic Technology Co., Ltd. (City, Province, Country)
Temperature-controlled incubator/shaker	ZWY-240 Shanghai Zhicheng Analytical Instruments Manufacturing Co., Ltd (Fengxian, Shanghai, China)
Centrifuge	TGL-16C Shanghai Anting Scientific Instrument Factory (Changji, Shanghai, China)
Steam explosion vessel	Custom device
Water bath	HWCL-3 Zhengzhou Great Wall Instrument Co., Ltd (Zhengzhou, Henan, China)

The slant culture media included 20.00 g of potatoes, 2.00 g of glucose, 2.00 g of agar, 100 mL of distilled water, and natural pH. The culture media was composed of 120.10 g/L of glucose, 4.40 g/L of ammonium sulfate, 2.30 g/L of potassium dihydrogen phosphate, 4.10 g/L of magnesium sulfate heptahydrate, and had a pH of 4.50. The solid-state fermentation process was performed in a 250-mL Erlenmeyer flask with a mixture of 5.00 g wheat bran and 5.00 g corn flour. This substrate was supplemented with 0.01 g of potassium dihydrogen phosphate and 0.01 g of magnesium sulfate.

Experimental procedures

The domesticated *Fusarium moniliforme* was inoculated into the slant media and cultured at 28 °C for 3 to 4 days. Then the hypha of the strain was made into a suspension and the suspension was inoculated into the seed culture medium. Finally, the strain was cultured on a shaker at 28 °C and 200 rpm for 36 h.

The fermentation conditions were investigated *via* a single factor experiment, which included the SEHC volume, SEHC adding moment, inoculation quantity, initial pH of media, mass ratio of solid to liquid, culture temperature, and culture period. 10 % (w/v) of the seed culture solution was inoculated into the prepared solid fermentation media for the experimental process of the first two factors (Qian *et al.* 1994). The cultural conditions for the first two factors included the temperature (30 °C), the initial pH (7.00), the mass ratio of solid to liquid (1:1.1), and the fermentation period (168 h). Each experiment was repeated at least three times.

Analytical Methods

Determination of glucose content

To detect the glucose content, the DNS (3,5-dinitrosalicylic acid) method was used, as shown by Wang (2002). Table 3 showed the relationship between the glucose content and the A_{540}.

Table 3. Relationship Between the Glucose Contents and the A_{540}

Glucose Content (mg/mL)	0	0.50	1.00	1.50	2.00	2.50	3.00	3.50
A_{540}	0	0.101	0.225	0.351	0.471	0.611	0.738	0.854
A_{540} is the absorbency values at 540 nm wavelength. $R^2 = 0.991$								

The glucose content after enzymatic hydrolysis was 28.00 mg/mL of SEHC, which was calculated by the glucose standard curve Eq. 1,

$$y = 0.2486x\text{-}0.0162 \quad (1)$$

where y is the absorbance, and x is the concentration.

Determination of gibberellin contents

To detect the gibberellin content, visible spectrophotography was used (Xiao and Yang 1997). Table 4 shows the relationship between the gibberellin content and the A_{412}.

Table 4. Relationship Between the Gibberellin Content and the A_{412}

Glucose Content (mg/mL)	0	0.315	6.25	25	50	100	200
A_{412}	0	0.009	0.013	0.054	0.122	0.22	0.442
A_{412} is the absorbency values at 412 nm wavelength. $R^2 = 0.992$							

The gibberellin content was calculated by the standard curve Eq. 2,

$$y = 0.0022x + 0.0017 \quad (2)$$

where y is the absorbance, and x is the content.

Determination of dry biomass contents

In this work, dry biomass referred to the weight of fermented mycelium (*Fusarium moniliforme*) after being dried. To detect the dry biomass content, the nucleic acid method was used, as shown by Wei et al. (2006). Table 5 showed the relationship between the dry biomass content and the A_{260}.

Table 5. Relationship between the Dry Biomass Content and the A_{260}

Dry Biomass Content (g)	0	0.05	0.10	0.15	0.20	0.25
A_{260}	0	0.350	0.673	0.982	1.279	1.570
A_{260} is the absorbency values at 260 nm wavelength. $R^2 = 0.998$						

The dry biomass content was calculated by the standard curve Eq. 3,

$$y = 6.255x + 0.027 \quad (3)$$

where y is the absorbance, and x is the quality.

RESULTS AND DISCUSSION

Effects of the Addition of SEHC to the Culture Media

In the following experiment, SEHC was added into the fermentation process after 60 h to accurately compare the effects of SEHC on the gibberellin yield. This time (60 h) was obtained from earlier experiments.

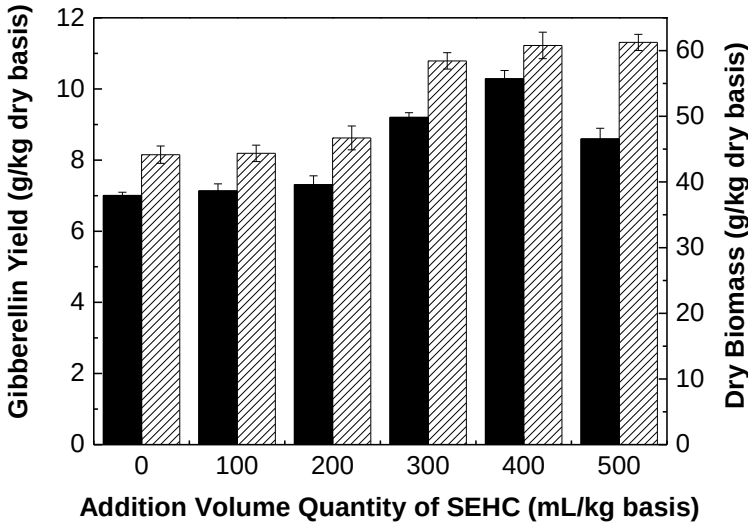

Fig. 1. The gibberellin yield (g/kg dry basis) (▉) and the total dry biomass (g/kg dry basis) (▒) during solid substrate cultivation with different volumes of SEHC

The gibberellin yield was investigated using six different addition volumes; 0 mL/kg, 100 mL/kg, 200 mL/kg, 300 mL/kg, 400 mL/kg, and 500 mL/kg of SEHC (dry basis). Figure 1 showed the gibberellin yield and the total dry biomass during solid substrate cultivation with different volumes of SEHC.

As shown in Fig. 1, the gibberellin yield was no more than 8.00 g/kg (dry basis) when the volume of the added SEHC was less than 200 kg/mL (dry basis). The gibberellin yield increased and then decreased as the volume of SEHC added increased. The lowest gibberellin yield was 7.01 g/kg (dry basis) without additional SEHC, and the highest was 9.48 g/kg (dry basis) when the added volume of SEHC was 400 kg/mL (dry basis). The total dry biomass gradually increased when the volume of the added SEHC increased. Carbon sources were no longer the dominant factor when the volume of the added SEHC was 300 kg/mL (dry basis).

The experiment indicated that the addition of small amounts of SEHC cannot meet the demand for carbon sources by the mycelium growth and fermentation process, and excess amounts of SEHC had an inhibitory action on the gibberellin fermentation process.

The Effects of Adding SEHC to the Culture Media at Different Timepoints

The volume of the added SEHC was 400 kg/mL (dry basis) in order to accurately compare different timepoints for the addition of SEHC on the gibberellin yield. The volume of the added SEHC was obtained through earlier experiments.

The gibberellin yield was investigated using five different timepoints to add the SEHC; at 30 h, 45 h, 60 h, 75 h, and 90 h during the fermentation process. Figure 2 showed the gibberellin yield and the total dry biomass results from the addition of SEHC to the solid substrate cultivation process at different timepoints. It was shown that the gibberellin yield increased and then decreased when the addition of SEHC was delayed. The lowest gibberellin yield was 6.65 g/kg (dry basis) at 30 h, and it reached a peak value of 9.48 g/kg (dry basis) at 60 h. The total dry biomass gradually increased when the addition of SEHC was delayed. The amount of carbon source available had little effect on the growth of mycelium.

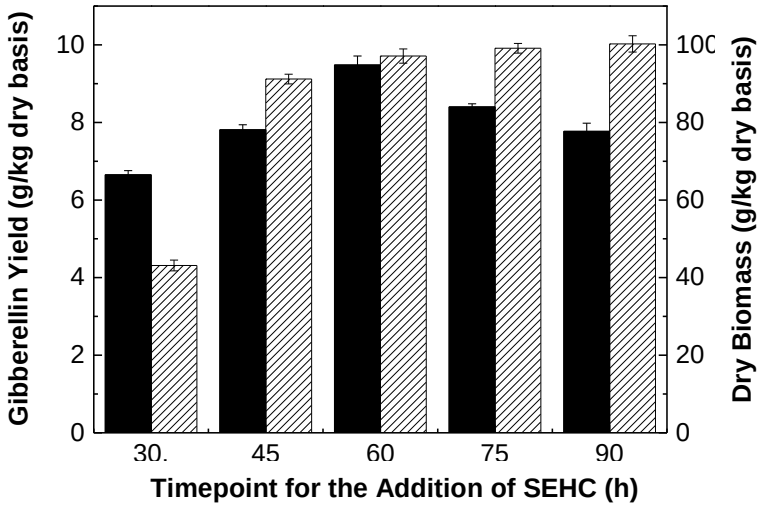

Fig. 2. The gibberellin yield (g/kg dry basis) (▉) and total dry biomass (g/kg dry basis) (▒) during solid substrate cultivation at different timepoints for the addition of SEHC

The experimental phenomena can be explained by Borrow's theory (Borrow *et al.* 1964). The gibberellin fermentation process generally experiences five stages, which include the adjustment period, logarithmic growth stage, storage period stage, stable stage, and the last stage. Jiang and Feng (2001) pointed out that the gibberellin yield could be improved when extra carbon sources were added to the culture media in the storage period stage. During this experiment, the 60 h timepoint happened during the storage period stage. Mao (2017) and Lei *et al.* (2015) studied the addition of vegetable oil to the gibberellin fermentation process. The results showed that adding vegetable oil after 60 h could increase the gibberellin yields.

The Effects of the Initial pH

The effects of the initial pH on the gibberellin yield were investigated by adjusting the starting pH to 3.00, 4.00, 5.00, 6.00, 7.00, 8.00, or 9.00 for the solid-state fermentation media. The pH was adjusted through the addition of hydrochloric acid or sodium hydroxide. The conditions for the culture were as follows; the inoculum concentration was 10%, the temperature was 30 °C, the solid to liquid mass ratio was 1:1.1, the fermentation period was 168 h, and the enzyme solution was added to the 400 kg/mL (dry basis) substrate during the solid-state fermentation process at 60 h.

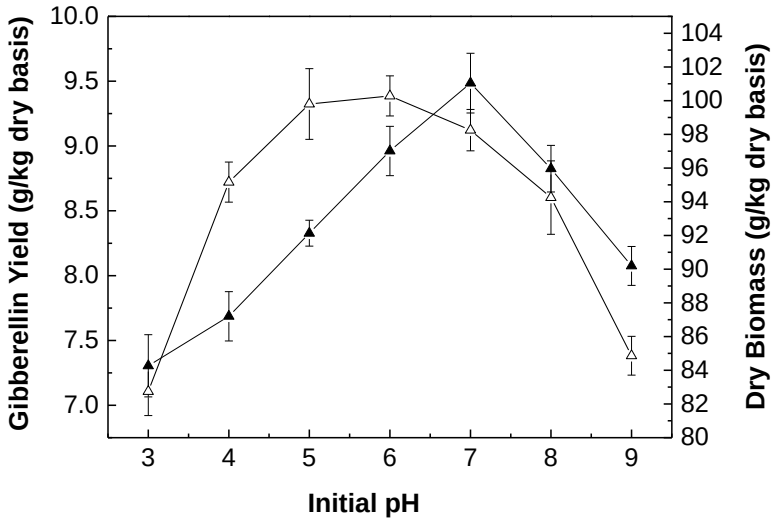

Fig. 3. The gibberellin yield (g/kg dry basis) (▲) and the total dry biomass (g/kg dry basis) (Δ) from the solid substrate cultivation process using culture media at different initial pHs

The gibberellin yield and the total dry biomass from the solid substrate cultivation process using culture media at different initial pHs are shown in Fig. 3. It was shown that the gibberellin yield gradually increased and then declined when the pH was increased from 3.00 to 9.00. The highest gibberellin yield was 9.48 g/kg (dry basis), when the initial pH was 7.00, and the lowest gibberellin yield was 7.30 g/kg (dry basis), when the initial pH was 3.00. The high gibberellin yields were obtained when the initial pH was between 6.00 and 8.00.

The total dry biomass gradually increased and then declined when the pH was increased from 3.00 to 9.00. With an initial pH of 6.00, the total dry biomass was the highest, 100.3 g/kg (dry basis), and the gibberellin yield was 8.96 g/kg (dry basis). When

the initial pH was 3.00, the total dry biomass was the lowest, 82.7 g/kg (dry basis), and the gibberellin yield was also the lowest. The experimental phenomena can be explained by the fact that the activity of many enzymes associated with microbial cell metabolism are often affected by the pH level. A high or a low pH value made it difficult for the fungus to grow, therefore, a pH of 7.00 was the most appropriate.

The Effects of the Solid to Liquid Ratio

Different solid to liquid mass ratios for the culture media were investigated: 1:0.7, 1:0.9, 1:1.1, 1:1.3, and 1:1.5. The conditions for the culture were as follows; the inoculum concentration was 10%, the temperature was 30 °C, the initial pH was 7.00, the fermentation period was 168 h, and the enzyme solution was added to the 400 kg/mL (dry basis) substrate during the solid-state fermentation process at 60 h. It was found that the gibberellin yield increased and then decreased as the mass ratio increased, as shown in Fig. 4. The highest gibberellin yield was 9.48 g/kg (dry basis), when the solid to liquid mass ratio was 1:1.1, and the lowest gibberellin yield was 2.72 g/kg, when the solid to liquid mass ratio was 1:0.7

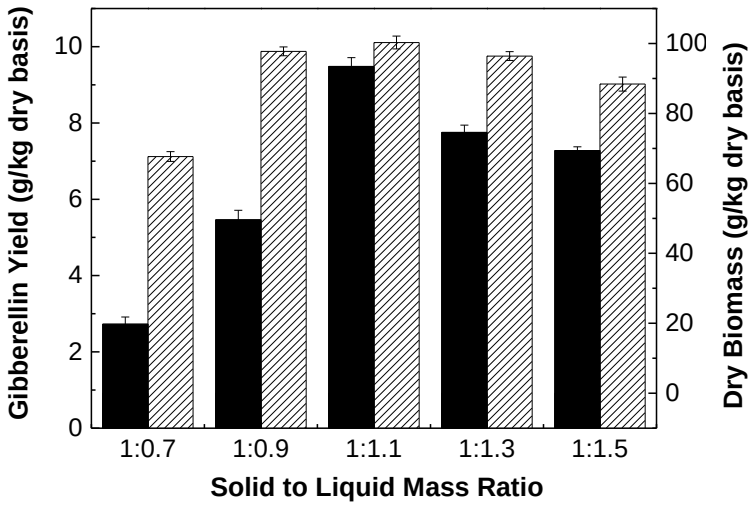

Fig. 4. The gibberellin yield (g/kg dry basis) (▉) and the total dry biomass (g/kg dry basis) (▒) from the solid substrate cultivation process at different solid to liquid mass ratios

The Effects of the Culture Temperature

The effects of the temperature during the solid-state fermentation process were investigated by varying the fermentation temperature to 26 °C, 28 °C, 30 °C, 32 °C, and 34 °C. The conditions for the culture were as follows; the inoculum concentration was 10%, the solid to liquid mass ratio was 1:1.1, the initial pH was 7.00, the fermentation period was 168 h, and the enzyme solution was added to the 400 kg/mL (dry basis) substrate during the solid-state fermentation process at 60 h. Figure 5 shows the gibberellin yield and the total dry biomass during the solid substrate cultivation process at different culture temperatures. The gibberellin yield increased and then decreased as the culture temperature increased. The highest gibberellin yield was 9.48 g/kg (dry basis), when the culture temperature was 30 °C, and the lowest gibberellin yield was 8.03 g/kg (dry basis), when the culture temperature was 26 °C.

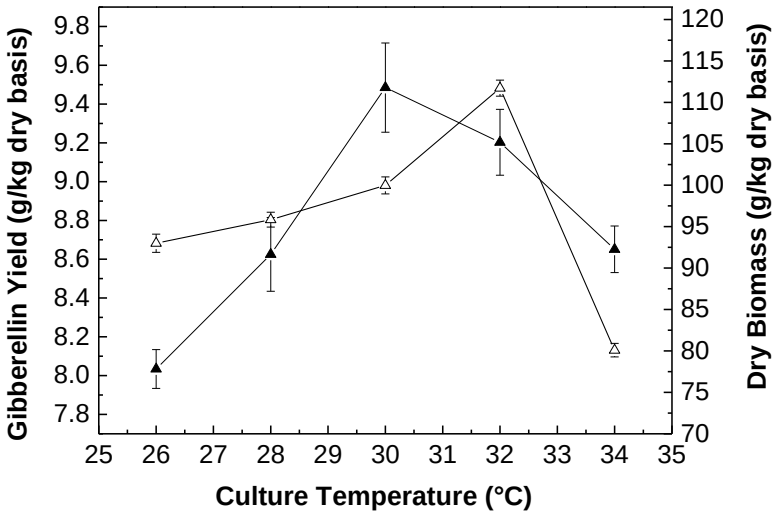

Fig. 5. The gibberellin yield (g/kg dry basis) (▲) and the total dry biomass (g/kg dry basis) (Δ) during the solid substrate cultivation process at different culture temperatures.

The total dry biomass gradually increased and then started to decline when the culture temperature was increased. When the culture temperature was 32 °C, the largest total dry biomass was 111.7 g/kg (dry basis) and the gibberellin yield was 9.20 g/kg (dry basis). When the culture temperature was 26 °C, the lowest total dry biomass was 93.0 g/kg (dry basis) and the gibberellin yield was 8.65 g/kg (dry basis).

A culture temperature between 28 °C to 32 °C benefited mycelium growth and gibberellin fermentation. The lag period stage is lengthened when the temperature was low, and therefore, the gibberellin yield decreased due to the length of the hyphal growth stage. The lag period stage length decreased, as well as the stable stage length, when the temperature was high, which led to a decrease in gibberellin yield (Wang *et al.* 2017).

The Investigation of the Optimal Gibberellin Culture Period

In this work, the optimal total length for the gibberellin culture process was investigated. The conditions for the culture were as follows; the inoculum concentration was 10%, the solid to liquid mass ratio was 1:1.1, the initial pH was 7.00, the temperature was 30 °C, and the enzyme solution was added to the substrate of 400 kg/mL (dry basis) substrate during the solid-state fermentation process at 60 h. The gibberellin yield gradually increased at first and then sharply increased before plateauing as the culture time increased, as shown in Fig. 6. The peak gibberellin yield was 9.48 g/kg (dry basis) after 168 h and maintained as the culture time increased. The total dry biomass sharply increased before plateauing, and then slightly decreasing as the culture temperature increased. When the total culture time was 96 h, the largest total dry biomass was 100.3 g/kg (dry basis) and the gibberellin yield was 6.94 g/kg (dry basis). The mycelium primarily grew when the total culture time was between 0 h to 48 h. The gibberellin was primarily produced by *Fusarium moniliforme* when the culture time was between 48 h to 150 h. The nutrients had been consumed by the mycelium and then broke into smaller parts, or melted, when the mycelium grew until 168 h.

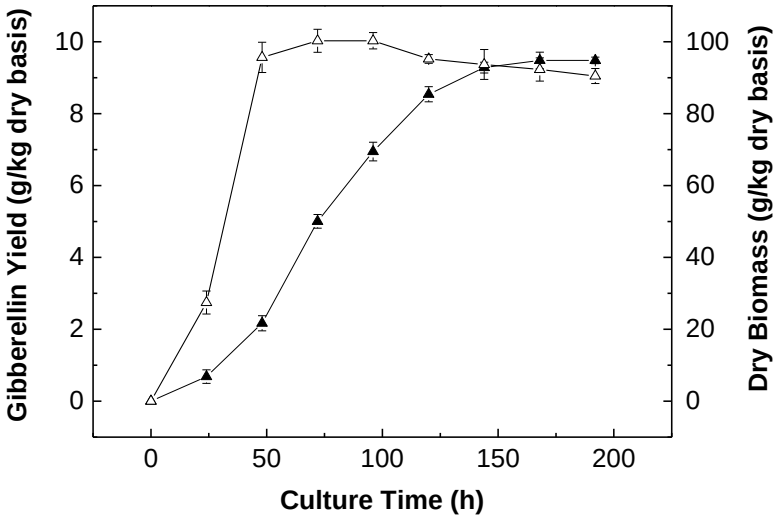

Fig. 6. The gibberellin yield (g/kg dry basis) (▲) and the total dry biomass (g/kg dry basis) (Δ) during the solid substrate cultivation process at different culture times

Comparison with the Experimental Results

To verify the feasibility of the experiment, a large amount of literature was read, and relatively high gibberellin yields were selected to compare. The experimental results of this study and other literatures are shown in Table 6. The data indicate that the peak gibberellin yield was slightly less than documented by Yang (1994) at optimum conditions; however, it was higher than submerged fermentation. The peak gibberellin yield was not the highest one when compared to the literature; but it provided a new alternative method for gibberellin production *via* solid-state fermentation with the utilization of biomass considered as well.

Table 6. Comparison of Experimental Results and Previous Reports

Peak Gibberellin Yield	Fermentation Mode	Raw Data of Peak Gibberellin Yield	Reference
9.48 g/kg (dry basis)	Solid-state fermentation	9.48 g/kg (dry basis)	This study
9.69 g/kg (dry basis)	Solid-state fermentation	9693 μg/g (dry basis)	(Yang Jiahua 1994)
5.80 g/kg (dry basis)	Solid-state fermentation	5.8 g/kg (dry basis)	(Rodrigues *et al.* 2009)
7.80 g/kg (dry basis)	Solid-state fermentation	7.8 mg/g (dry basis)	(Satpute *et al.* 2010)
1.48 g/L	Submerged fermentation	1480 μg/mL	(Zhuang *et al.* 2008)
0.38 g/L	Submerged fermentation	380 mg/L	(Rios-Iribe *et al.* 2011)

CONCLUSIONS

1. In this work, the solid-state fermentation process for gibberellin *via* the addition of enzymatic hydrolysate from steam-exploded corn stalks was investigated.
2. The optimum conditions for the culture were as follows; a temperature of 30 °C, an initial pH of 7.00, a solid to liquid mass ratio of 1:1.1, a fermentation period of 168 h, and an enzyme solution of 400 kg/mL (dry basis) was added to the substrate during the solid-state fermentation process at 60 h.
3. The optimum conditions for the fermentation process were as follows; a temperature of 32 °C had a gibberellin yield of 9.20 g/kg (dry basis), a pH of 6.00 had a gibberellin yield of 8.96 g/kg (dry basis), a solid to liquid mass ratio of 1:1.1 had a gibberellin yield of 9.48 g/kg (dry basis), and a fermentation period of 96 h had a gibberellin yield of 6.94 g/kg (dry basis).
4. In contrast with previous reports, the peak gibberellin yield in this work was close to the maximum value in the previous literatures. It provided a new alternative method for gibberellin production *via* solid-state fermentation in addition to considering the application of corn stalks.

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REFERENCES CITED

Borrow, A., Brown, S., Jefferys, E. G., Kessell, R. H. J., Lloyd, E. C., Lloyd, P. B., Rothwell, A., Rothwell, B., and Swait, J. C. (1964). "The kinetics of metabolism of *Gibberella fujikuroi* in stirred culture," *Canadian Journal of Microbiology* 10, 445. DOI: 10.1139/m64-055

Datar, R., Huang, J., Maness, P. C., Mohagheghi, A., Czernik, S., and Chornet, E. (2007). "Hydrogen production from the fermentation of corn stover biomass pretreated with a steam-explosion process," *International Journal of Hydrogen Energy* 32, 932-939. DOI: 10.1016/j.ijhydene.2006.09.027

Dissanayake, P., George, D. L., and Gupta, M. L. (2010). "Effect of light, gibberellic acid and abscisic acid on germination of guayule (*Parthenium argentatum* gray) seed," *Industrial Crops and Products* 32(2), 111-117. DOI: 10.1016/j.indcrop.2010.03.012

Durand, A., and Chereau, D. (1988). "A new pilot reactor for solid-state fermentation: Application to the protein enrichment of sugar beet pulp," *Biotechnology and Bioengineering* 31, 476-86. DOI: 10.1002/bit.260310513

Elander, R. T., Dale, B. E., Holtzapple, M., Ladisch, M. R., Lee, Y. Y., Mitchinson, C., Saddler, J. N., and Wyman, C. E. (2009). "Summary of findings from the biomass refining consortium for applied fundamentals and innovation (CAFI): Corn stover pretreatment," *Cellulose* 16, 649-659. DOI: 10.1007/s10570-009-9308-y

Focke, I. S. G. S. (1967). "Der Einflu komplexer Nahrsubstrate auf die Gibberellin Bildung von *Fusarium moniliforme* [The influence of complex food substrates on the gibberellin formation of *Fusarium moniliforme*]," *Sheld Biol. Zentralbl* 86, 509.

Hesseltine, C. W. (1977a). "Solid state fermentation," *Process Biochemistry* 12, 24-27.

Hesseltine, C. W. (1977b). "Solid state fermentation," *Process Biochemistry* 12, 29-32.

Jiang, L., and Feng, Y. (2001). "Research on the separation and extraction of the solid state of gibberellin," *Journal of Southwest Normal University (Natural Science)* 26, 323-328.

Kumar, P. K., and Lonsane, B. K. (1987). "Gibberellic acid by solid state fermentation: Consistent and improved yields," *Biotechnology and Bioengineering* 30, 267. DOI: 10.1002/bit.260300217

Kurosawa, E. (1926). "Experimental studies on the nature of the substance secreted by the bakanae fungus," *Natural History Society of Formosa*, pp. 213-227.

Lei, W., Liang, Q., and Liao, H. (2015). "Effects of gibberellin 978 strains on the fermentation of GA3," *Agriculture and Technology* 35, 2.

Li, A., and Sun, L. (2018). "From gibberellin to farm chemical 920: The advancement of a plant hormone in China during 1950-1970s," *Ancient and Modern Agriculture* 1, 15-22.

Loreto, P., Claudia, B., and Thomas, F. (2008). "Effect of plant growth regulators on floral differentiation and seed production in jojoba (*Simmondsia chinensis* (Link) Schneider)," *Industrial Crops and Products* 27(1), 44-49. DOI: 10.1016/j.indcrop.2007.07.001

Lu, Z. X., Xie, Z. C., and Kumakura, M. (1995). "Production of gibberellic acid in *Gibberella fujikuroi* adhered onto polymeric fibrous carriers," *Process Biochemistry* 30, 661-665. DOI: 10.1016/0032-9592(94)00042-5

Mao, Y. (2017). "Analysis of the effect of vegetable oil on the fermentation of gibberellin GA_3," *Exchange Field* 48, 193.

Martin, C. G. (1983). "The biochemistry and physiology of gibberellins," in: *Gibberellins*, A. Crozier (ed.), Praeger Publishers, New York, NY, pp. 395-411.

McKenzie, R. R., and Deyholos, M. K. (2011). "Effects of plant growth regulator treatments on stem vascular tissue development in linseed (*Linum usitatissimum* L.)," *Industrial Crops and Products* 34(1), 1119-1127. DOI:10.1016/j.indcrop.2011.03.028

Qian, X. M., Du, P. J., and Kilian, S. G. (1994). "Factors affecting gibberellic acid production by *Fusarium moniliforme* in solid-state cultivation on starch," *World Journal of Microbiology and Biotechnology* 10, 93-99. DOI: 10.1007/BF00357571

Rios-Iribe, E. Y., Flores-Cotera, L. B., Chávira, M. M. G., González-Alatorre, G., and Escamilla-Silva, E. M. (2011). "Inductive effect produced by a mixture of carbon source in the production of gibberellic acid by *Gibberella fujikuroi*," *World Journal of Microbiology and Biotechnology* 27, 1499-1505. DOI:10.1007/s11274-010-0603-4

Rodrigues, C., Vandenberghe, L. P. D. S., Teodoro, J., Oss, J. F., Pandey, A., and Soccol, C. R. (2009). "A new alternative to produce gibberellic acid by solid state fermentation," *Brazilian Archives of Biology and Technology* 52, 181-188. DOI: 10.1590/S1516-89132009000700023

Satpute, D., Sharma, V., Murarkar, K., Bhotmange, M., Dharmadhikari, D., Kumar, S., Mukherjee, S., and Fan, M. H. (2010). "Solid-state fermentation for production of gibberellic acid using agricultural residues," *International Journal of Environment and Pollution* 43, 201-213. DOI: 10.1504/IJEP.2010.035924

Sawada, K. (1912). "Diseases of agricultural products in Japan," *Formosan Agricultural Reviews* 63, 10-16.

Shukla, R., Srivastava, A. K., and Chand, S. (2003). "Bioprocess strategies and recovery processes in gibberellic acid fermentation," *Biotechnology and Bioprocess Engineering* 8, 269-278. DOI: 10.1007/BF02949216

Sun, S. K. S. W. (1981). "The bakanae disease of the rice plant," in: *Fusarium Disease*, B. A. Torry, P. E. Nelson, T. A. Toussoun, and R. J. Cook (eds.), The Pennsylvania State University Press, University Park, PA, pp. 104-113.

Tomasini, A., Fajardo, C., and Barrios-Gonza Lez, J. (1997). "Gibberellic acid production using different solid-state fermentation systems," *World Journal of Microbiology and Biotechnology* 13, 203-206.

Tudzynski, B. (1999). "Biosynthesis of gibberellins in *Gibberella fujikuroi*: Biomolecular aspects," *Applied Microbiology and Biotechnology* 52, 298-310. DOI: 10.1007/s002530051524

Tudzynski, B., and Hölter, K. (1998). "Gibberellin biosynthetic pathway in *Gibberella fujikuroi*: Evidence for a gene cluster," *Fungal Genetics and Biology* 25, 157-170. DOI: 10.1006/fgbi.1998.1095

Wang, X. (2002). *Principles and Methods of Biochemical Experiment*, China Agricultural Press, Beijing, China.

Wang, P., Li, X., Yuan, H., and Pang, Y. (2010). "Anaerobic biogasification performance of corn stalk pretreated by a combination of green oxygen and NaOH," *Journal of Beijing University of Chemical Technology* 37, 115-118.

Wang, W., Wu, Y., Li, J., and Yao, Y. (2017). "Effects of temperature on the fermentation of gibberellin and optimization strategy," *Chinese Journal of Applied and Environmental Biology* 23, 432-436.

Wei, P., Cen, P., and Sheng, C. (2006). "Comparison of three methods for the determination of biomass in solid state fermentation," *Journal of Food and Biotechnology* 25, 60-64.

Wyman, C. E., Dale, B. E., Elander, R. T., Holtzapple, M., Ladisch, M. R., Lee, Y. Y., Mitchinson, C., and Saddler, J. N. (2009). "Comparative sugar recovery and fermentation data following pretreatment of poplar wood by leading technologies," *Biotechnology Progress* 25, 333. DOI: 10.1002/btpr.142

Xiao, Z., and Yang, J. (1997). "Spectrophotometric determination of gibberellin," *Journal of Yantai University (Natural Science and Engineering)* 10, 266-268. DOI:

Yabuta, T. (1935). "Biochemistry of the "bakanae" fungus of rice," *Biological Agriculture & Horticulture* 10, 17-22.

Yang, J., and Xiao, Z. (1994). "The solid fermentation nutrition model of gibberellin," *Journal of Yantai University (Natural Science and Engineering)* 27-28.

Zhao, C., Shao, Q., Ma, Z., Li, B., and Zhao, X. (2016). "Physical and chemical characterizations of corn stalk resulting from hydrogen peroxide presoaking prior to

ammonia fiber expansion pretreatment," *Industrial Crops and Products* 83(2), 86-93. DOI: 10.1016/j.indcrop.2015.12.018

Zhuang, M., Wang, J., Ji, Z., and Chen, S. (2008). "The effect and analysis of vegetable oil on the fermentation of gibberellic acid," *Journal of the Chinese Cereals and Oils Association* 23, 77-80.

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