

Valorization of Banana Peel for Citric Acid Production under Solid State Fermentation with *Aspergillus niger*



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T. Chysirichote*

*Department of Food Engineering, Faculty of Engineering, King Mongkut's Institute of Technology Ladkrabang, 1 Chalongkrung 1, Chalongkrung Rd., Ladkrabang, Bangkok 10520, Thailand

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Valorization of banana peel (BP) through solid state fermentation (SSF) was aimed in this research. The appropriate conditions of citric acid (CA) production by SSF of *Aspergillus niger* were investigated. Firstly, the optimum initial pH of the BP and the aeration rate were studied by conducting SSF in a 250-mL flask and 2-L glass column, respectively. It was found that the initial pH of the BP and aeration rates affected the CA production. The results showed that the initial pH of 5.0 and 1.0 vvm aeration were appropriate for the CA production of *A. niger* using BP as a substrate. The problem of rising temperature during SSF was found when applying the optimum condition to the SSF in the 20-L packed bed bioreactor (PBB), which caused a decrease in the CA production compared to that of the glass column. The cooling air jacket constructed to the PBB to remove the heat during the SSF helped increase the CA production from that in the PBB. The maximum CA production in the 20-L air-jacketed PBB was $124.0 \pm 19.2 \text{ mg g}^{-1}_{\text{DS}}$.

Keywords:

citric acid, solid-state fermentation, packed bed bioreactor, *Aspergillus niger*, banana peel

Introduction

Banana is an important economic crop of Thailand. The manufacture of various banana products has resulted in their residue. One of the residues is banana peel (BP) which is around 40 % of whole banana¹. Generally, BP has been used as a raw material for compost² and animal feed³, biodegradable film⁴ and fertilizer⁵. As BP is high in carbohydrates^{6,7}, one interesting way to valorize it is its fermentation, because it contains abundant substrates such as carbon and nitrogen sources for microbial metabolism⁸.

Citric acid (CA) is vital and versatile in many industries. It has been applied for various functions such as a flavor enhancer, a blood preservative, and anti-foam agent in the food, medical, and chemical industries, respectively^{9,10}. Although CA can be produced by an extraction of citrus fruit and chemical synthesis, fermentation has been widely selected for commercial production of CA due to higher productivity and yields, and lower operating costs¹¹. Among three different fermentation methods, including submerged, surface, and solid-state fermentations^{12,13}, the most commercial CA has been produced by submerged fermentation of *Aspergillus niger* due to easiness of parameter control, and sup-

port to continue the process^{14–16} in spite of high costs of equipment. Many research studies have evidently shown the suitability of solid-state fermentation (SSF) in CA production over submerged fermentation^{15,17,18} and suggested that SSF was useful for CA production. Common substrates for fermentation of CA come from agricultural and agro-industrial wastes, which contain high reducing sugar substrates such as molasse^{19,20} or other carbohydrates supplemented with reducing sugars^{21,22} and minerals^{23–25}. Recently, Hou and Bao²⁶ proved that oxygen was satisfactory for the CA production by *A. niger*. This is consistent with the suggestion of Kaiser *et al.*²⁷ that oxygen availability changes the acetyl-coA pool sizes resulting in accumulation of acids including citric acid, pyruvic acid, and malic acid. The amount of supplied oxygen is therefore critical for CA production²⁸. However, Socol *et al.*²⁹ suggested that low aeration curbed the respiration activity resulting in changing the metabolism from biomass production to citric acid synthesis, while higher aeration caused the decreased accumulation of citric acid.

The purpose of this research was to study the appropriate conditions for CA production by the SSF of *A. niger* on BP, including the initial pH of substrate and the aeration rate. This research conducted the SSF in both small-scale using a 250-mL Erlenmeyer flask and a 2-L glass column, and a

*Corresponding author: E-mail address: teerin.ch@kmitl.ac.th, Tel: +66-2329-8356 Ext.12, Fax: +66-2329-8356 Ext.13

larger scale using a 20-L packed bed bioreactor (PBB), to which oxygen could be supplied by aeration. The main problem of increasing temperature from a fungal respiratory heat generation²⁹ during scale-up of SSF was challenged. Many researchers have conducted studies on reducing the increasing temperature during the SSF in PBBs such as by using an internal cooling plate³⁰, injecting cool dry air together with moisture³¹, covering the PBB with a water jacket³² and dividing the bed into multilayers³³. This research also aimed to solve this problem by running cooling air in the jacket.

Materials and methods

Preparation of the banana peel

Banana peel (green with a yellow trace) supplied from Lankathitong Company, a banana supplier for the food industry, was cut into 1x1 cm² and dried at 80 °C for six hours. Its moisture content was then adjusted to 60 % w.b. by 0.1, and a 1.0 M H₂SO₄ solution at various pH values to achieve the desired pH of the substrate. The banana peel was sterilized by autoclaving at 121 °C for 20 minutes before SSF. To study the optimum initial pH of the banana peel, the pH was adjusted to 3.0, 4.0, 5.0, and 6.0, respectively. The obtained optimum pH for CA production was then used to study the effect of aeration rate on CA production. Acid degradation during thermal sterilization causing an increasing initial pH of the BP³⁴ was avoided by studying the relationship between the pH value of the H₂SO₄ solution obtained before and after the thermal sterilization at 121 °C for 20 minutes. The pH value of the prepared acid solution was calculated using equation 1 to achieve the target initial pH of the BP for the SSF.

$$\text{pH value}_{\text{after thermal sterilization}} = (1.108 \cdot \text{pH value}_{\text{before thermal sterilization}}) \quad (1)$$

Preparation of the spores of *A. niger*

A. niger ATCC 16888 was cultured on a potato dextrose agar (PDA) medium and incubated at 30 °C for three days. The spores were then collected by sterilized water. The number of spores in the suspension was counted using a hemocytometer under a microscope (40x magnification). The number of spores in the suspension was diluted to 10⁸ spores mL⁻¹ by adding sterilized water.

SSF for CA production

Prepared banana peel was mixed with the spore suspension of 1·10⁵ spores g⁻¹_{DS}. The mixture was

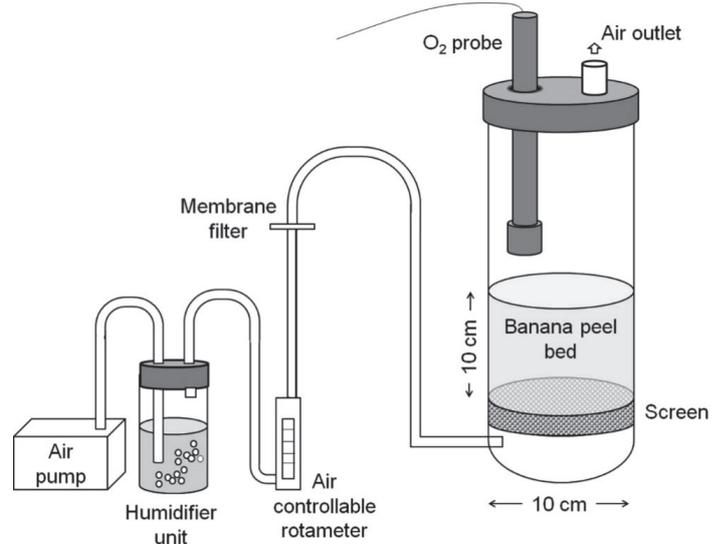


Fig. 1 – SSF in glass column

then placed in three bioreactors (250-mL Erlenmeyer flask, 2-L glass column and 20-L packed bed bioreactor) and incubated at 30 °C for five days. To conduct the SSF in the flask, a 25-g mixture was put into a 250-mL flask (five flasks for one test) and closed with cotton balls. During incubation, one flask was selected every day until the fifth day to analyze the CA production. The optimum pH of BP obtained from the SSF in the flask (pH 5.0) was then used to study the effect of aeration rate in the 2-L glass column by placing a 300-g mixture in the 10-cm diameter glass column (25 cm in height) and aerated with sterilized air at 30 °C from the bottom at 0.1, 0.5, 1.0, and 1.5 vvm (Fig. 1). The columns were placed in the incubator at 30 °C during the SSF. About 25 g of the fermented solution was collected every day until the fifth day to analyze the CA production. Furthermore, the oxygen concentration of air in the column was monitored during the SSF using the oxygen detector (Model SAO2-100, Bestone, China) set in the column to prevent any interruption during sampling. In addition, SSF was conducted in a 20-L packed bed bioreactor (PBB) to study the feasibility of applying the studied optimum initial pH of the BP and aeration rate in the larger bioreactor. A 3.5-kg mixture (pH 5.0) was placed in the bioreactor (Fig. 2), and 30 °C air was forced from the bottom at 1.0 vvm. Temperatures at the center of the bed and at the outlet of the air of both the PBB and jacketed PBB were monitored during the SSF using thermocouples type T (1.5 mm diameter) connected to the data logger (Model 34970A, Key-sight Technologies, USA). The oxygen detector (Model SAO2-100, Bestone, China) was used to measure the oxygen concentration in the bioreactors during the SSF.

Design and construction of air-jacketed packed bed bioreactor

The air-jacketed PBB was made of aluminium (0.3 mm thickness) due to its strength and high heat transfer coefficient. The 20-L double-jacketed PBB was designed to contain 10 L of the substrate for the SSF (Fig. 2). The height and diameter of the external jacket were 50 and 45 cm, respectively, whereas the internal ones were 30 and 30 cm, respectively. SSF was performed in the internal chamber. The temperature of the air forced for the SSF was controlled by passing the air through a 50-cm copper tube immersed in a 30 °C water bath before passing an adjustable gas rotameter (Model K-1011, Nitto Denko Corporation, Japan) and a PTFE membrane (Millex®-FG, Millipore corporation, USA) for sterilization. Additionally, the air was forced to the substrate through the cone to achieve the uniformity of the air velocity through the substrate. A polystyrene insulator was installed at the internal surface of the external jacket to avoid the heat transfer between the surroundings and air in the jacket. The running cooling air in the outer jacket of PBB was used to absorb the heat generated during the SSF in the PBB, its temperature was adjustable depending on the temperature of the exhaust air from the SSF. The temperature sensor measured the temperature of the outlet air of the PBB connected to a temperature controller (Model EW-988H, Ewelly, China), which set the point at 30 °C and commanded a heater (200 watts, Sobo, China) to heat the 25 °C air to the set temperature (30 °C) before forcing it into the jacket. The velocity of cooling air in the jacket was

controlled at 10 L min⁻¹ to ensure that the amount of cooling air was enough to exchange temperature with the increasing temperature in the PBB.

Analysis of BP compositions

BP was dried at 65 °C for 24 h. Total starch content of the dried BP was assessed by the method of AACC Method 76-13.01³⁵. Other carbohydrates, including cellulose and hemicellulose, were analyzed according to the method of Goering and Van Soest³⁶.

Analysis of the citric acid

Citric acid was extracted from the fermented BP, which was known for its moisture content by the modified method of Karthikeyan *et al.*³⁷ A 5-g sample was soaked and stirred in 50 mL of deionized water at 150 rpm at 22 °C for one hour, and analyzed using a Megazyme citric acid kit (Megazyme International, Ireland) for the citric acid quantification^{38,39}. The CA concentration was calculated and reported as milligrams in one gram of dry substrate (mg g⁻¹_{DS}). Its production rate was calculated using exponential equation $CA = e^{-rt}$, where CA is citric acid concentration in dry sample (mg g⁻¹_{DS}), r is the production rate of CA (d⁻¹), and t is time (d).

Analysis of fungal growth

Glucosamine was used as an indirect indicator of biomass dry weight²⁹. The glucosamine content was analyzed by a colorimetric method according to the method of Chysirichote⁴⁰. Briefly, 10 mg of fer-

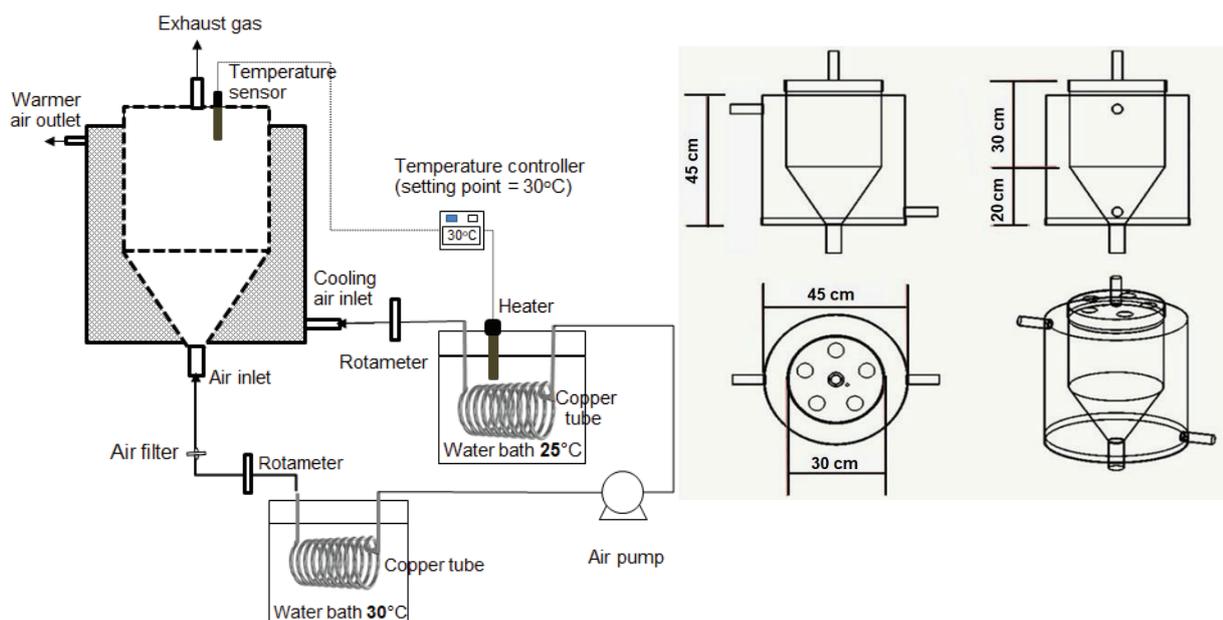


Fig. 2 – Design of air-jacketed packed bed bioreactor (a) Air-jacketed packed bed bioreactor (b) SSF system using air-jacketed packed bed bioreactor

mented sample was hydrolyzed by 60 % sulfuric acid solution for 24 h and heated at 121 °C for 1 h. The hydrolyzed solution was reacted with NaNO_2 and KHSO_4 after it was neutralized. Its color was then developed by reacting with $\text{FeCl}_3 \cdot 7\text{H}_2\text{O}$ and methyl-2-benzothiazolinone hydrazone hydrochloride. The absorbance of the solution was measured at 650 nm using a UV-Vis spectrophotometer (Model Genesys 10S, Thermo Fisher Scientific, USA), and the concentration of glucosamine calculated using the standard curve obtained from the standard solution of HCL-glucosamine solution. The obtained values of glucosamine in fermented sample was converted to the biomass dry weight⁴¹ by equation 2. The growth of *A. niger* is presented as biomass dry weight in dry fermented substrate ($\text{mg g}^{-1}_{\text{DS}}$). Biomass content was plotted with time and the specific growth rate calculated by an exponential equation in the growth phase as in equation 3.

$$X = \frac{\text{Glucosamine content} - 6.57}{222.89} \quad (2)$$

$$\frac{dX}{dt} = \mu X \quad (3)$$

where X is microbial biomass ($\text{mg g}^{-1}_{\text{DS}}$), t is time (d), and μ is the specific growth rate (d^{-1}).

Analysis of moisture content

Moisture content of the fermented sample (MC) was determined by heating ~ 1 g sample with known accurate weight in a hot air oven at 65 °C for 24 h. The weight was measured after cooling the samples to room temperature in a desiccator. The moisture content (wet basis) was calculated by

$$\text{MC} = 100 \cdot \frac{W_{\text{wet}} - W_{\text{dry}}}{W_{\text{wet}}} \quad (4)$$

where W_{wet} and W_{dry} are weight (g) of wet and dry samples, respectively.

Results and discussion

Effect of the initial banana peel pH on the citric acid production

Fig. 3(a) shows that the highest CA concentration was obtained in the SSF of the BP with the initial pH value of 5.0 at Day 3 as $34.6 \pm 2.9 \text{ mg g}^{-1}_{\text{DS}}$, and the highest rate was found as 1.19 d^{-1} shown in Table 1. The maximum concentration of CA from this research was lower than that in the work of Monroy *et al.*⁴², who obtained $96 \text{ mg g}^{-1}_{\text{DS}}$ from the SSF of banana peel with initial pH of 1.4 and supplemented with NH_4^+ , Mg^{2+} and PO_4^{3-} , while CA content from this research was very low at low pH

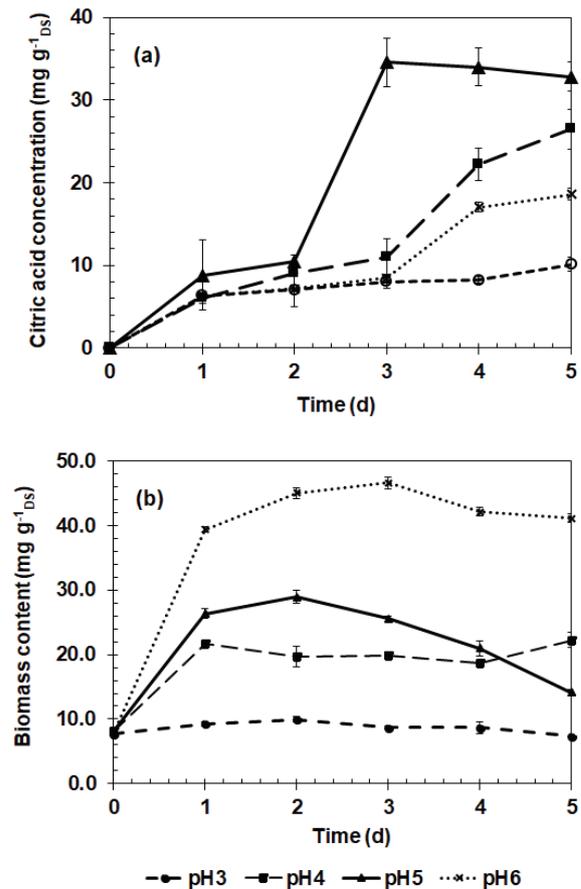


Fig. 3 – Citric acid production from the SSF at different initial banana peel pH (a) Citric acid concentration (b) Biomass content

(pH 3.0). Karthikeyan and Sivakumar³⁷ also produced up to 18 % of CA by the SSF of *A. niger* with the optimum initial pH of 3.0, despite the reports of Abdel-Rahim and Arbab⁴³ that the fungal spore germination was suppressed at a pH lower than 3.5. From this research, both the citric acid production rate and the growth rate (Fig. 3(a), (b) and Table 1) were lowest when the initial pH of the BP was lower (pH 3.0) as 0.11 d^{-1} and 0.18 d^{-1} , respectively; in contrast to the high CA and microbial growth obtained from the submerged fermentation⁴⁴ using sugar as a carbon source. However, Lingappa *et al.*⁴⁵ suggested that the initial pH of the carob pod extract for CA production in submerged fermentation was 5.5, and the production decreased as the initial pH of the medium decreased. The different effect of initial pH in SSF and submerged fermentation might have been due to the presence of monosaccharides, which was a carbon source for fungal metabolism.

Since most carbon sources in the BP were polysaccharides consisting of 21.9 % starch, 7.6 % hemicellulose, and 5.0 % cellulose, the specific hydrolytic enzymes including amylase/glucosidase,

Table 1 – Parameters of growth and citric acid production under solid-state fermentation of banana peel by *A. niger* in different conditions

Conditions of SSF		Specific growth rate (d ⁻¹)	Citric acid production rate (d ⁻¹)	Maximum yield (mg g ⁻¹ _{DS})
pH	3.0	0.18	0.11	10.1 ± 0.8
	4.0	0.98	0.41	26.5 ± 2.5
	5.0	1.18	1.19	34.6 ± 2.3
	6.0	1.63	0.70	17.0 ± 0.6
Aeration (vvm) at pH 5.0	0.1	0.25	1.11	32.8 ± 1.2
	0.5	0.32	1.45	120.0 ± 12.4
	1.0	0.51	1.58	133.1 ± 11.8
	1.5	0.20	0.51	53.2 ± 6.0
Configuration of PBB	Control	1.11	0.37	54.9 ± 7.1
	Air jacketed PBB	0.52	1.59	124.0 ± 19.2

xylanase, and cellulase were essential for secreting monosaccharides from the BP in SSF. The highest citric acid productivity was obtained from the SSF with the initial pH 5.0 because it was the optimum initial pH to produce β -glucosidase⁴⁶, xylanase, and cellulase⁴⁷ by *Aspergillus*. In addition, these enzymes worked well at the optimum pH 5.0^{31,47–49}. The importance of the glucose supply rate was also mentioned in the study of Wang *et al.*⁵⁰ because it involved the formation of gluconic and oxalic acids and the increase of citric acid⁵¹. In contrast, the initial pH 6.0 enhanced the fungal growth, but the CA production was quite low because this level of pH was suitable for TCA resulting in increased biomass production, but CA accumulation was low due to an affinity of tricarboxylate transporter to citric acid⁵². Moreover, the pH of 5.0 was appropriate for *A. niger* germination⁵³ which released protons resulting in the enhancement of citric acid production. In addition, this was the reason why the lowest CA production was found when the initial pH of BP was equal to 3.0. The optimum initial pH of BP (pH 5.0) obtained from this experiment was used to study the effect of aeration rate on the CA production in a 2-L glass column.

Effect of the aeration rate on citric acid production in the 2-L glass column

Fig. 4(a) shows the highest CA concentration in the SSF with 1.0 vvm aeration at Day 3, as 133.1±11.8 mg g⁻¹_{DS} which was slightly higher than that of 1.0 vvm (120.0±7.4 mg g⁻¹_{DS}), followed by that of 1.5 vvm (53.2±6.0 mg g⁻¹_{DS}) and 0.1 vvm (32.7±1.2 mg g⁻¹_{DS}), while the fungal growth of the SSF with 0.1 and 0.5 were quite similar and higher than that with 1.5 vvm, as presented in Fig. 4(b). The lowest growth was found in the SSF with 1.5

vvm as 0.20 d⁻¹ as shown in Table 1, whereas the highest oxygen was supplied by aeration because the higher aeration caused more evaporation leading to a reduction in bed moisture to around 55 % from Day 1 (Fig. 4(c)), which was suitable for fungal growth but inappropriate for CA production⁸. The lowest CA production in the SSF with high aeration (1.5 vvm) was also consistent with the research of Soccol *et al.*²⁸ who reported that stronger aeration decreased the accumulation of citric acid.

The results of CA production of the SSF in 0.1 and 0.5 vvm were quite different although the biomass growths were similar, since the measured biomass contents included both aerial and substrate mycelium. Aerial mycelium was produced extensively in conditions of limited oxygen availability^{54,55}. The higher content of aerial mycelium (as detected by white color of mycelium) found in the SSF with 0.1 vvm aeration than that with 0.5 and 1.0 vvm aerations, implied that oxygen availability in the SSF with 0.1 vvm aeration was lower than that in 0.5 and 1.0 vvm aeration, which agreed well with the results in Fig. 4(d). Hence, the reason that the lowest CA production found in the SSF with 0.1 vvm was not only a low growth but also an insufficient oxygen supply for the tricarboxylic acid cycle⁵⁶.

Scale-up of the CA production in a 20-L packed bed bioreactor

The optimum initial pH of 5.0 and aeration rate of 1.0 vvm from the SSF in the Erlenmeyer flask and the glass column were applied for CA production of *A. niger* in the 20-L packed bed bioreactor (PBB). Figs. 5(a) and (b) indicate that although the aeration, the oxygen concentration in the PBB, and moisture content of bed during the SSF were the

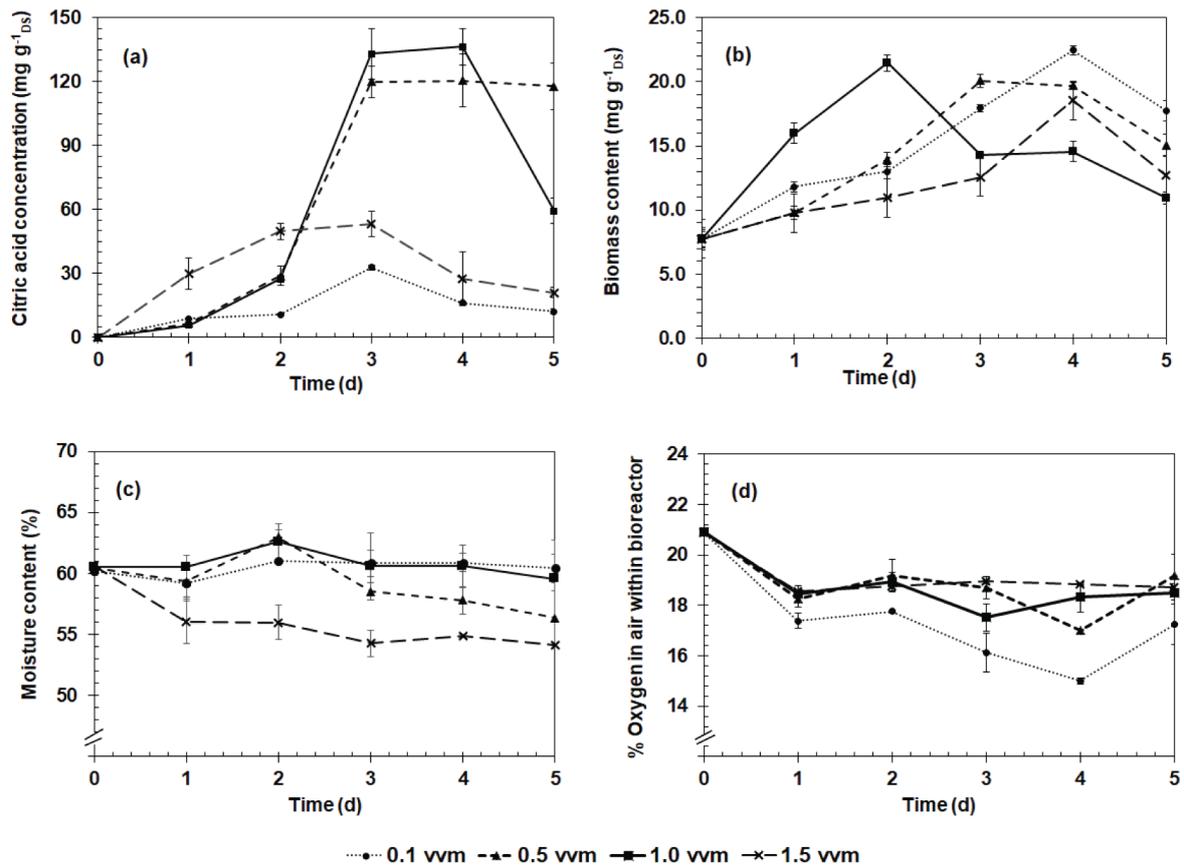


Fig. 4 – SSF of the banana peel in the 1-L glass column at different aeration rates (a) Citric acid concentration (b) Biomass content (c) Moisture content of beds (d) Oxygen concentration

same as shown in Figs. 5(c) and 5(e), the CA production was different. Lower CA production in the PBB was found compared to the result in the glass column (Fig. 4(a)). This occurred due to the increase in bed temperature during the SSF (Fig. 5(d)) presenting the temperature of the bed at 38 °C at Day 1 and 42 °C at Day 2 or an increase of 8 °C and 12 °C from the optimum temperature of 30 °C, respectively. Installing the air jacket around the exterior of the PBB (air-jacketed PBB), using running cooling air as a heat exchanging medium, enabled the decrease in rising temperature. Consequently, the maximum CA concentration during the SSF in the air-jacketed PBB ($124.0 \pm 19.2 \text{ mg g}^{-1}_{\text{DS}}$) on Day 2 was much higher than that in the PBB ($54.9 \pm 7.1 \text{ mg g}^{-1}_{\text{DS}}$) on Day 3. The rate of CA production also increased up to 4.3 times (from 0.37 d^{-1} to 1.59 d^{-1} in Table 1).

Noticeably, Table 1 shows similarities between the CA production in the jacketed PBB and that in the glass column since the SSF was conducted with the same conditions (initial pH of 5.0; 1.0 vvm). At another point, the maximum citric acid concentrations were found in the SSF in both PBB and air-jacketed PBB on Day 2, than that in the glass column on Day 3 because the substrate bed in the

PBBs (15 cm) was thicker than that in a glass column (10 cm), causing more bed compaction and a greater CO_2 accumulation inside the bed, and consequently higher CA production⁵⁷. On the other hand, the enhanced fungal growth, as shown in Fig. 5(b), was found in the PBB (1.10 d^{-1} , 1 d) compared to that in the air-jacketed PBB (0.52 d^{-1} , 2 d), although a rise in temperature had occurred. This result agreed with the explanation of Bonner⁵⁸, suggesting that the spore germination of *A. niger* increased when the temperature increased together with lower humidity. The results indicated that the optimum initial pH of BP and aeration rate were suitable for CA production of *A. niger* on the BP on a larger scale provided that the bed temperature could be controlled. The air-jacket construction was an alternative way to increase heat removal from the bed.

Conclusion

The optimum initial pH of substrate and aeration rate for citric acid (CA) production of *A. niger* by solid state fermentation (SSF) using banana peel as a substrate were pH 5.0 and 1.0 vvm aeration, which were studied using the 250-mL Erlenmeyer

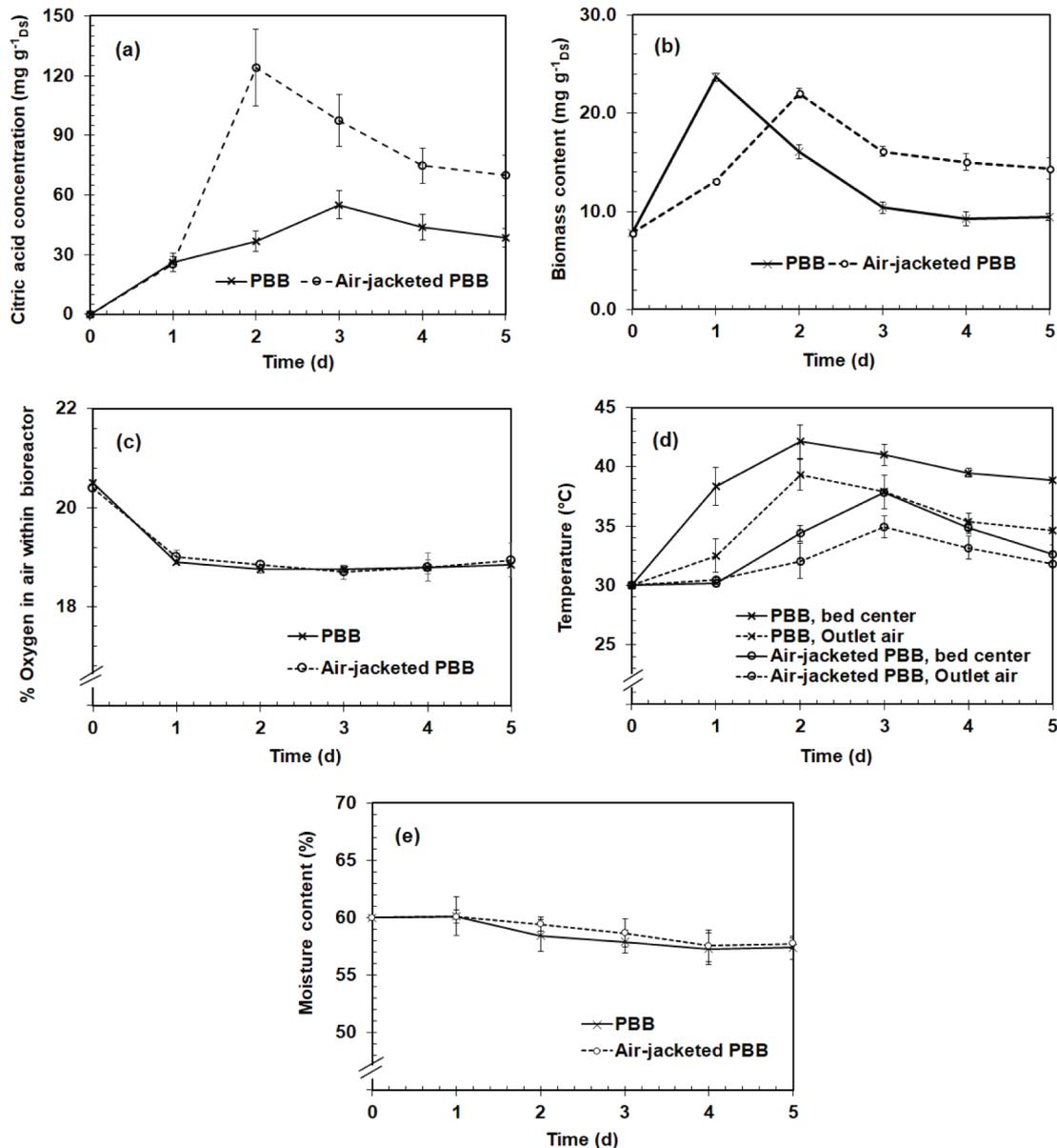


Fig. 5 – SSF of the banana peel in the 20-L packed bed bioreactors at 1.0 vvm aeration (a) Citric acid concentration (b) Biomass content (c) Oxygen concentration in the air inside the bioreactor (d) Temperatures of the bed and outlet air in the bioreactors (e) Moisture content of beds. PBB represents the packed bed bioreactor

flask and the 2-L glass column, respectively. The initial pH of banana peel and aeration rate obtained in this research were used to scale-up the CA production in the temperature controllable PBB.

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