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DAFTAR ISI

Increase of Citric Acid Production by *Aspergillus niger* InaCC F539 in Sorghum's Juice Medium Amended with Methanol
(Peningkatan Asam Sitrat yang Diproduksi oleh *Aspergillus niger* InaCC F539 dengan Menggunakan Jus Sorgum yang Ditambah Metanol)

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ABSTRACT

Citric acid demand increases steadily, and there is a need to increase productivity through selection of suitable carbon sources, and addition of substances that increase citric acids production rate. Methanol has been suggested to increase citric acid fermentation on high carbohydrate containing substances. The objective of the study was to evaluate the suitability of sweet sorghum juice for citric acids production and to verify the effect of methanol on citric acids production using *Aspergillus niger* InaCC F539 as inoculant. Sweet sorghum juice with the total initial reducing sugar of 11.5 % (w/v) was used as the sole carbon sources. To study the effect of total initial reducing sugar on citric acid production the initial reducing sugar was adjusted to the concentration of 30 to 75 g/L. Preliminary experiment was conducted to get the optimum methanol concentration that stimulate citric acid production. The optimum methanol concentration that stimulate citric acid production was 4% (v/v). Submerged fermentation was conducted as shake culture (125 rpm at 28 °C). Citric acids production was affected by total initial reducing sugar. Higher total initial reducing sugar produced higher citric acids. Maximum citric acid production was 18.96g/L on sweet sorghum juice with 75 g/L total initial reducing sugar. Methanol 4 % (v/v) increase citric acid production by 41.35 to 65.89 %. Juice of sweet sorghum was a good medium for citric acids production, and methanol stimulate and increase citric acid production. It is a good basis for exploring efficient and cost effective industrial scale citric acid production.

Keywords: Citric acid, Methanol, Sweet sorghum, *Aspergillus niger*

ABSTRAK

Kebutuhan asam sitrat terus meningkat, oleh karena itu perlu dilakukan penelitian untuk meningkatkan produksi asam sitrat melalui penambahan substrat yang mampu meningkatkan produksi asam sitrat. Metanol dilaporkan dapat meningkatkan produksi asam sitrat pada substrat yang mengandung karbohidrat yang tinggi. Tujuan penelitian ini adalah mengevaluasi peluang penggunaan jus sorghum untuk produksi asam sitrat, menggunakan inokulan *Aspergillus niger* InaCC F539, dan mengevaluasi peran methanol dalam meningkatkan produksi asam sitrat. Jus sorgum manis dengan kandungan total gula reduksi sekitar 11,5 % (w/v) digunakan sebagai substrat utama. Pengaruh konsentrasi gula reduksi terhadap produksi asam sitrat dipelajari dengan mengatur konsentrasi gula reduksi dari 30 sampai dengan 75 g/L. Penelitian awal dilakukan untuk mengetahui kadar metanol yang optimal untuk meningkatkan produksi asam sitrat. Kadar metanol optimum untuk meningkatkan produksi asam sitrat adalah 4 % (v/v). Fermentasi asam sitrat dilakukan menggunakan sistem *submerge fermentation* (SmF). Produksi asam sitrat dipengaruhi oleh kadar gula reduksi awal pada kondisi penggoyangan (125 rpm pada suhu 28 °C). Maksimum produksi gula reduksi adalah 18,96 g/L pada jus sorgum dengan kadar gula reduksi awal 75 g/L. Penambahan metanol 4 %, meningkatkan produksi asam sitrat sekitar 41,35 sampai dengan 65,89 %. Jus sorgum merupakan media yang baik untuk produksi asam sitrat, dan penambahan metanol diperlukan untuk meningkatkan produksi asam sitrat. Hasil penelitian ini dapat digunakan sebagai basis penelitian untuk memproduksi asam sitrat yang lebih murah.

Kata Kunci: Asam Sitrat, Metanol, Sorgum manis, *Aspergillus niger*

INTRODUCTION

Citric acid ($C_6H_8O_7$, 2–hydroxy–1,2,3–propane tricarboxylic acid), a natural constituent and common metabolite of plants and microorganism. It is widely used organic acid. Citric acid is GRAS (generally recognized as safe) substances and is extensively used in food industry to adjust pH and improve flavor, account for 70% of its application. Citric acid also used in pharmaceuticals and cosmetics for acidification and metal ion chelation (Kamzolova *et al.* 2011; Najafpour

2015; Rossi *et al*. 2009). There is constant increase (3.5-4%) each year in its consumption, showing the need of finding new alternatives for its manufacture (Najafpour 2015). Citric acid production system has been developed since 1917, the first microorganism used for the submerge fermentation was *Aspergillus niger* with sugar as the main carbon sources (Ali *et al*. 2002; Najafpour 2015). Significant improvement of citric acid yield was started in the 1950s when the glycolytic pathway and the tricarboxylic acid cycle (TCA) as biochemical basis of citric acid synthesis was proposed (Akram 2014; Najafpour 2015). Complexity of citric acid synthesis and its dependency on several complex nutritional conditions for effective fermentation requires intensive biochemical and production system engineering investigations (Den Haan *et al*. 2013). The most popular conventional citric acid production is the submerged culture using high-yielding mutant strains of *Aspergillus niger*, but this system still need further investigation on manufacturing process and effective microorganism for efficient fermentation to increase yield and subsequently minimize overall operating costs (Chaturvedi 2010).

The most common substrate for citric acid production is high sugar content substrate such glucose and sucrose which are quite expensive. To reduce production cost, a variety of media have been proposed such as molasses, several starchy materials, and agricultural by product (Dhillon *et al*. 2011). There are two groups of raw materials used for citric acid production: (i) substrate with a low ash content from which the cations could be removed by standard procedures (e.g. cane or beet sugar, dextrose syrups and crystallized dextrose); (ii) raw materials with a high ash content and high amounts of other non sugar substances (e.g. cane and beet molasses, crude unfilteredhydro-lysates) (Chaudhary & Raj 2012). Earlier studies showed that the critical parameters for citric acid production on submerge culture fermentation by *A. niger* were control of high carbohydrate concentration, keeping low but finite manganese concentrations, maintaining high dissolved oxygen, provision of constant agitation, and maintainin glow pH to reduce contamination (Lotfy *et al*. 2007). These physical and chemical conditions are crucial to obtain best pellet morphology, which is also critical for effective susbtrate absorption by microorganism and stimulate citric acid production on submerge fermentation. Effective production of citric acid could be conducted in SSF (solid state fermentation) (Anastassiadis *et al*. 2008). To reduce citric acid production cost, use of waste residues and by products derived from the fruit-processing industry innoculated with *A. niger* in both SSF and submerged fermentation were proposed by Kieliszek (Kieliszek *et al*. 2017). Other works, used pineapple peel as a cheap medium to produce citric acid, resulting in a production of 60.6 mg/L of citric acid in optimized conditions. Citric acids also produced using apple pomace solid waste, citrus waste, brewery spent grain, and sphagnum peat moss as main C-sources (Rossi *et al*. 2009).

Efficient citric acid producing microbes is one of key issue on achieveing cost effective citric acids production. *Aspergillus niger* has been used during the past 50 years as a commercial producer of citric acid. Due to complexicity of citric acid production process, selection of efficient citric acid producer may not offer the only solution for cost effective citric acid production. But understanding over all interlinked factors that influence fermentation processes, which include detail citric acid synthesis and its dependency on several nutritional conditions for cell growth and citric acid synthesis are critical to obtain high yield fermentation process (Ciriminna *et al*. 2017). Although conventional citric acid production by submerged culture of high-yielding mutant strains of *Aspergillus niger* has been optimized, but there is still interest in redesigning the traditional manufacturing process to increase yield and subsequently to minimize overall operating costs.

Juice of sweet sorghum contains fermentable sugars about 11.8 %, it is higher than energy cane i.e 9.8 %. The sweet sorghum bagasse contains 45% cellulose, 27% hemicellulose, and 21% lignin (Kusumah *et al*. 2016). Due to its high fermentable sugar composition, juice of sweet sorghum could be good substrate for citric acid production (Rooney 2014).

Addition of other carbon sources such as methanol stimulate and increase citric acids production (Yu *et al*. 2017), which offer

possibility to increase effectiveness of sweet sorghum juice for citric acid production. The hyphothesis was also proposed based on the previous studies, which revealed that citric acid production was markedly increased when 1% methanol and 10% sweet potato vine hydrolysate were added to basal medium under standard fermentation conditions. Molasses could be used as a carbohydrate source for the production of citric acid. Strain type, addition of whey, methanol and tricalcium phosphate had a significant impact on citric acid production by *Aspergillus niger* (Yu *et al*. 2017).

Objective of research was to evaluate and to compare the ability of *Aspergillus niger* to produce citric acids from different concentration of total initial reducing sugar of Sorghum's Juice and to evaluate the effect of methanol on citric acid production in submerge fermentation.

MATERIALS AND METHODS

Aspergillus niger InaCC F539 obtained from Indonesian Culture Collection (InaCC), was used in this study. Stock cultures were stored at -80° C on 10%+5% trehalosa. *Aspergillus niger* InaCC F539 was inoculated on PDA agar and cultured at 28 °C for 120 h. Spores were eluted with 20 mL 0.1% (v/v) Tween-80, of which approximately 15 ml was filtered through lens paper, transferred to a sterilized 50-mL centrifuge tube, and then separated by centrifugation (3000×g, 7 min). The supernatant was removed and the spores re-suspended in 20 ml of 0.1% (v/v) Tween-80, and then centrifuged again. The resulting pellet was re-suspended in 3 ml sterile water and diluted to 1×10^9CFU/ml in seed broth. Fermentation experiments were performed in a 500-mL Erlenmeyer flask containing 150 ml medium and cultured at 37°C at 120 rpm for 6 days. For citric acid production by *Aspergillus niger* InaCC F539, sweet sorghum juice of super 1 (*Sorghum bicolor*) was used with different concentrations of total reducing sugar. The medium was cotton filtered to remove suspended impurities before sterilization. Initial total reducing sugar was 11.5 % (w/v). To the basal medium (g/L) NH_4NO_3, 0.5; KH_2PO_4, 0.5; $MgSO_4.7H_2O$, 0.1; peptone, 7.0; $ZnSO_4.7H_2O$,0.001; ferrous ammonium sulphate, 0.001 and $CuSO_4.5H_2O$, 0.0006 were added. For studying the effect of initial reducing sugar on citric acid production was adjusted to 30, 45, 60 and 70 g/L of total reducing sugar. Spores from 192 h old slant of *Aspergillus niger* InaCC F539 was counted using a hemocytometer and spore concentration of 1 x 10^9/ml was added directly to the production medium in all studies with this organism.

Citric acid production by *Aspergillus niger* InaCC F539 was carried out in 500 ml erlenmeyer flask with a working volume of 150 mL. An initial pH and temperature of all the runs were 5.5 and 28°C respectively. Shaker speed was 120 rpm. All experiments were carried out in triplicate. In the studies with methanol, 4 % (v/v) methanol was added at 24 h of fermentation. Preliminary experiment was conducted to obtain optimum methanol concentration to stimulate citric acid production. The 4 % (v/v) of methanol addition was the optimum concentration that stimulate citric acid production. Therefore 4 % (v/v) methanol was used for all experiment to evaluate the effect of methanol on citric acids production.

Citric acid concentration was determined with a Shimadzu Series high-performance liquid chromatography (HPLC) instrument equipped with a UV/Vis detector and Eclipse Plus C18 column (250 × 4.6 mm × 5 µm; Agilent Technologies, Santa Clara, CA, USA). A known volume of fermentation broth was removed every 24 h under aseptic condition and centrifuged at 6000 rpm. The processed broth was diluted 10-fold in the phosphate buffer (25 mM, pH 2.4), filtered through a 0.22-µm membrane, and injected into 2.0-mL auto sampler vials. Citric acid was separated with a mobile phase composed of methanol and phosphate buffer (25 mM, pH 2.4) at a 1:9 ratio (v/v) with a flow rate of 1.0 mL/min at 30 °C. The injection volume was 20 µL and we performed three replicates of each trial. Citric acid quantitation was performed at the wavelength of maximum absorbance for each analyses (λ = 210 nm; A210) obtained from UV spectrophotometer spectra determination. Citric acid was identified by comparing its retention time with that of the standard substance.

A known volume of fermentation broth

was removed every 24 h under aseptic condition and centrifuged at 6000 rpm. Cells, after centrifugation, were washed with distilled water and dried at 105 °C to a constant weight for cell mass estimation. The supernatant was used for the estimation of total reducing sugar by the dinitrosalicylic method (McCleary & McGeough 2015).

The data were analyzed using SPSS 18 (SPSS, Chicago, IL, USA). The statistical significance of differences was calculated using Tukey's HSD, $P<0.005$. The primary analyses were paired comparison of citric acids production and biomass at the various initial total reducing sugar concentration at 6 day fermentation time. Data are presented as mean ± SD. or medians if the data were not normally distributed.

RESULTS

Citric acid production by *Aspergillus niger* InaCC F539

On sorghum juice, *Aspergillus niger* InaCC F539 produced maximum 18.0±1.49 g/L extracellular citric acid at the highest set up initial reducing sugar concentration (Figure 1).

The production of extracellular citric acid was much affected by initial total reducing sugar concentration (Figure 1). Citric acid production increased when initial total reducing sugar increased. There was significant increase of citric acid production rate when initial reducing sugar increase (Figure 2). Maximum citric acid production rate was 0.04 g/L/h (Figure 2). Therefore it can be assumed that sorghum juice of *Sorghum bicolor* var1 is a good medium for citric acids production.

Biomass production

On juice of sorghum, biomass growth was associated with initial total reducing sugar concentration (Figure 3). Increased initial total reducing sugar result in higher biomass production (Figure 3). Maximum biomass production was 20.14 ± 4.2 g/l (Figure 3). Biomass growth rate also associated with total initial reducing sugar concentration. Cell biomass growth rate was about0.046- 0.023 g/l.h (Figure 4).

Effect of methanol

Methanol stimulate production of citric acids. In basal medium (sweet sorghum juice) production of citric acids was around 2.86-17.52 g/L, but when methanol was added the ethanol production increase to 12.8-39.9 g/L (Figure 1 and 5). The rate of citric acids production also increase significantly when methanol was added (Figure 2 and 6).

Biomass production on methanol augmented medium

Methanol addition only slightly increase biomass production (Figure 3 and 6). Maximum biomass production (24.98 ± 4.2 g/L) was achieved on initial reducing sugar 75 g/L with addition of 4 % methanol (Figure 7). Cell biomass production rate also not much affected (Figure 4 and Figure 8).

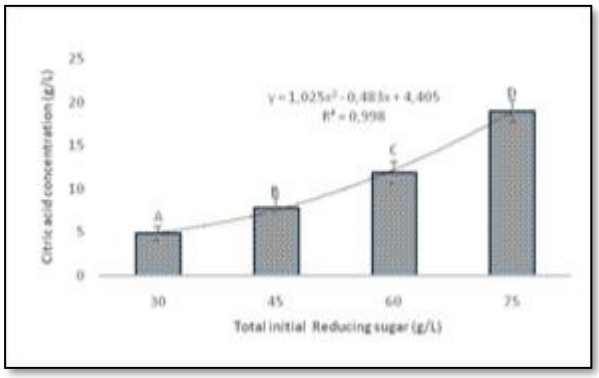

Figure 1. Citric acid production by *Aspergillus niger* InaCC F539 at various initial total reducing sugar concentration on SmF (shake culture) 125 rpm at 28°C after 6 days. Bars with different letter are significantly different (Tukey's HSD, $P<0.05$).

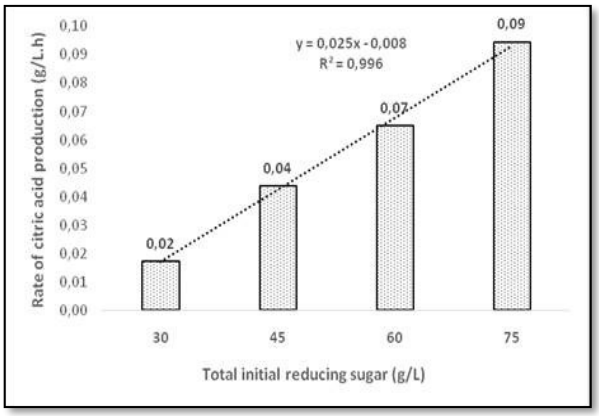

Figure 2. Rate of production of Citric acid by *Aspergillus niger* InaCC F539 at various initial total reducing sugar concentration on SmF (shake culture) 125 rpm at 28°C.

DISCUSSION

Sweet sorghum juice contain about 11.8 % *w/w) total reducing sugar, sucrose 7.6 %, glucose 2.6 % and fructose 1.6 % (w/w) (Kim & Day 2011). Those fermentable substrate are easily converted into citric acids (Geanta *et al*. 2013; Papanikolaou *et al*. 2008). Sweet sorghum also contain of protein and lipid, which are required for new cell synthesis. Sweet sorghum

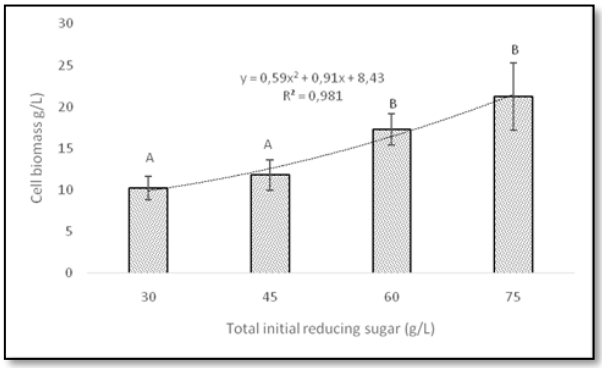

Figure 3. Biomass production by *Aspergillus niger* InaCC F539 at various initial total reducing sugar concentration on SmF (shake culture)

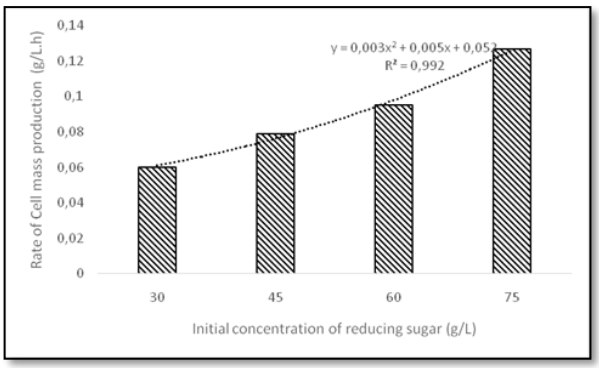

Figure 4. Rate of biomass productionof *Aspergillus niger* InaCC F539 at various initial total reducing sugar concentration on SmF (shake culture) 125 rpm at 28°C.

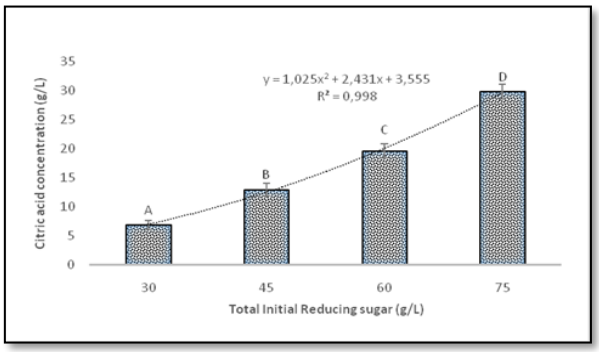

Figure 5. Citric acid production by *Aspergillus niger* InaCC F539 at various initial total reducing sugar concentration with addition of 4 % of methanol on SmF (shake culture) 125 rpm at 28°C after 6 days. Bars with different letter are significantly different (Tukey's HSD, P <0.05).

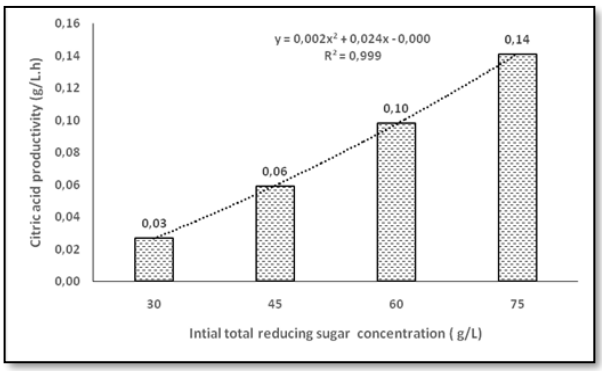

Figure 6. Rate of production of Citric acid by *Aspergillus niger* InaCC F539 at various initial total reducing sugar with addition of 4 % of methanol concentration on SmF

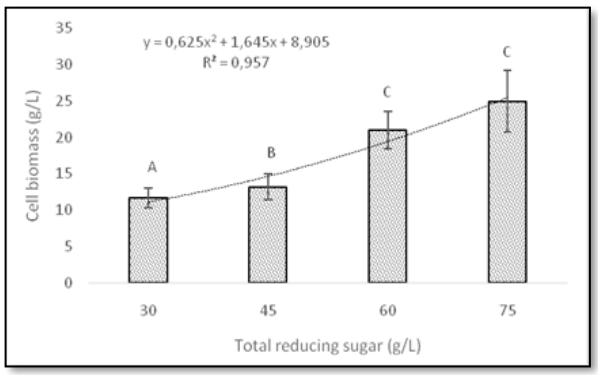

Figure 7. Biomass production by *Aspergillus niger* InaCC F539 at various intial total reducing sugar concentration with addition of 4 % of methanol on SmF (shake culture) 125 rpm at 28°C. Bars with different letter are significantly different (Tukey's HSD, P <0.05).

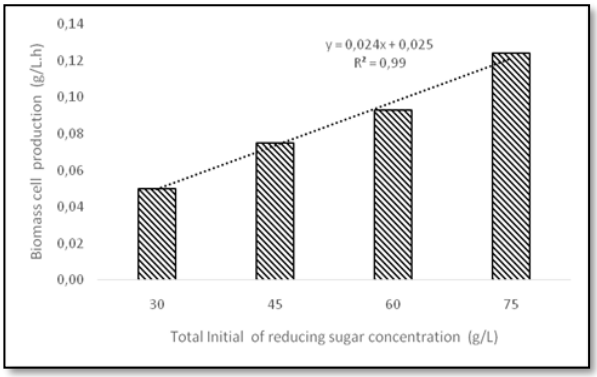

Figure 8. Rate of biomass production of *Aspergillus niger* InaCC F539 at various intial total reducing sugar concentration with addition of 4 % of methanol on SmF (shake culture) 125 rpm at 28°C.

Table 1. Citric acid production by *Aspergillus niger* InaCC F539 at optimum concentration of initial total reducing and methanol.

Organism	Substrate	Initial substrate concentration (g/L)	Methanol added after 24 h (%) (v/v)	Citric acid (g/L)	$^{+}$YP/X (g/g)	Product/ Biomas after 6 days (g/g)	$^{++}$QP (g/(L.h) Product rate
Aspergillus niger InaCC F539	Sorghum	75	-	18.96	21.24	0.89	0.09
		75	4	29.82	24.98	1.19	0.23
		60	-	11.92	17.28	0.68	0.07
		60	4	19.65	20.98	0.93	0.15
		45	-	7.80	11.81	0.66	0.04
		45	4	12.94	13.18	0.98	0.06
		30	-	4.86	10.21	0.47	0.02
		30	4	6.87	11.68	0.58	0.04

$^{+}$YP/X product per biomass, $^{++}$ QP product production rate

also rich in micronutrient i.e Fe, Cu, K, Na, Fe, Mn which are needed for enzyme activities on citric acid synthesis and cell division (Ferreyra *et al.* 2002; Sanchez-Marroquin *et al* 1970). We found that sweet sorghum produced about 18.96 g/l citric acids and 21.24 g/L cell biomass at 75 g/l initial total reducing sugar concentration (Figure 1-3). This confirmed that sweet sorghum juice is potential feeding materials for citric acids production. Methanol is clearly stimulate citric acid and cell biomass production (Figure 1- 8). The increase of citric acid production due to methanol addition was from 41 to 65 % (Figure 1-5).

Aspergillus niger InaCC F539 utilized 95% of initial total reducing sugar and the growth yield obtained was 24.5% (Table 1). Addition of methanol did not shift the optimum initial total reducing sugar concentration. At all concentrations studied, methanol addition increased citric acid production and cell growth (Figure 5-8). Addition of methanol (1±4% v/v) was reported to increase citric acid production in many other fungal fermentation. Increase in citric acid production was reported using glucose (Navaratnam *et al*. 1998), sucrose (Förster *et al*. 2007), apple pomace (Kumar *et al*. 2010), soyawhey (Rossi *et al*. 2009), date syrup (Saad 2006), and galactose (Maddox *et al*. 1986) as the substrate. The exact reason behind this increase due to methanol addition is not known but, there are reports suggesting some explanation to this phenomenon.

Previous study showed that addition of methanol results in retardation of growth, delays sporulation (Leßmeier & Wendisch 2015), therefore methanol was added after 24 hours of fermentation course. It is also suggested that the presence of methanol may increase the permeability of the cell to citrate, and the cell responds to the diminished intracellular citric acid level by increasing production via repression of 2-oxoglutarate dehydrogenase (Maddox *et al*. 1986; Yu *et al*. 2017). In this study, a similar phenomenon was observed for enhanced production of citric acid when methanol was added.

The increase in citric acid production was more rapid from 1.12 to 6.8 g/L without methanol addition and from 4.86 to 6.87 g/L with methanol at 30 to 75 g/L respectively. Cell production was higher in the presence of methanol at all initial total reducing sugar concentration examined (Figure 7-8). Maximum cell production was 21.24 g/L in the absence of methanol and 24.98 g/L when methanol was added, hence the biomass increase was about 20-25 %. As methanol addition increased cell concentration it may be suggested that methanol not only increases the permeability, but also increases the activity of some key enzymes involved in the basic metabolic cycle of *Aspergillus niger*. Methanol addition increased citric acid productivity up to 65 %. Hence, in both cases, in the presence and in the absence of methanol, citric acid productivity was more sensitive to substrate inhibition than cell production. As maximum cell productivity and maximum citric acid productivity were obtained at different initial total reducing sugar

concentration (Figure 3,4, 7,8), it might suggest that citric acid production by *Aspergillus niger* InaCC F539 is growth associated.

Methanol is known to boost citric acid production by *Aspergillus niger* (Navaratnam *et al*. 1998; Yu *et al*. 2017), likely by stimulating its excretion by increasing cell membrane permeability, which can reduce the mass transfer resistance of the membrane and strengthen the catalysis of the cell, without damaging intracellular organic structures or causing cell lysis. Moreover, 2-oxoglutarate dehydrogenase activity was low, whereas that of pyruvate carboxylase was high in the presence of methanol. There was strong correlation between citric acid production and the activities of these two enzymes. In the present study, the maximum citric acid production was obtained with 4 % methanol (Figure 5). Strong relationships were observed between citric acid production and the activities of the enzymes 2-oxoglutarate dehydrogenase and pyruvate carboxylase in cell-free extracts.

The rate of citric acids production is affected by the total initial reducing sugar concentration (Figure 2), and addition of methanol increase citric acids production rate (Figure 6). Biomass production rate also increased when initial total reducing sugar increased, and addition of methanol (Figure 6 - 8). There is no straight forward explanation on how methanol increase citric acid production and cell biomass production, earlier study showed that during citric acid production, in the presence of methanol, the activity of 2-oxoglutarate dehydrogenase was low and that of pyruvate carboxylase high. In the absence of methanol, where little citric acid was produced, the reverse was true. It is suggested that the presence of methanol may increase the permeability of the cell to citrate, and the cell responds to the diminished intracellular level by increasing production via repression of 2-oxoglutarate dehydrogenase (Navaratnam *et al*. 1998; Yu *et al*. 2017).

Due to its superiority, *Aspergillus niger* is popular microbes used to produce citric acid. For instance the study of Saad using two strains of *Aspergillus niger* (ATCC 6275 and 9642) were grown in media containing different concentrations of date extract or molasses fortified with whey, methanol and tricalcium phosphate. The fermentation experiments were conducted at 25° C for 12 days and samples were withdrawn at different time intervals and analyzed for their citric acid content. Results showed that a high level of citric acid (32.4g/L was produced by *A. niger* ATCC 6275 in 20% molasses in whey. When methanol and tricalcium phosphate were added, a significant increase in citric acid production was recorded ($P < 0.05$). Citric acid concentrations were 38.4 and 42.4 g/ L, in media fortified with methanol and tricalcium phosphate, respectively (Saad 2006). This result is slightly higher than that of InaCC F539 which might suggest that citric acid production capacity is strain and substrate dependent.

In addition to *Aspergillus niger*, there are numerous of microorganisms used for citric acids production,,which include *A. aculeatus*, *A. awamori*, *A. carbonarius*, *A. wentii*, *Penicillium janthinelum*. Recently several yeasts have been proposed i.e. *Saccaharomicopsis lipolytica*, *Candida tropicalis*, *C. oleophila*, *C. guilliermondii*, *C. parapsilosis, C. citroformans,* and *Hansenula anamola.* A number of bench scale citric acid production processes by yeast have been described using raw materials such as hydrocarbons, carbohydrates, plant oils, ethanol and glycerol. *Yarrowia lipolytica* has high ability for the overproduction of extracellular citric acid and isocitric acid from glucose. The advantage with this strain is that less isocitric acid is obtained as a byproduct from glucose medium (Rywińska *et al*. 2010; Rywińska *et al.* 2009). Up to now, there is no information on how to regulate the proportion of citric acids to isocitric acids produced during fermentation (Levinson *et al*. 2007). But up to now more fungi are used for citric acid production due to rapid product synthesis, less contamination, and easy to control (Anastassiadis *et al.* 2008). The research will provide a preliminary theoretical basis for utilization of sorghum juice for citric acid production in industrial scale.

CONCLUSION

The results from the present work demonstrate that sweet sorghum is a good medium for citric acids production. Initial fermentable sugar concentration affect citric acids production, and

maximum citric acid production on sweet sorghum reach 18.96 g/L at 75 g/L initial total reducing sugar concentration. Addition of 4% of methanol increases citric acid production by 65 %. The physiological mechanism by which methanol stimulate citric acid and biomass production need further verification.

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PANDUAN PENULIS

Naskah dapat ditulis dalam bahasa Indonesia atau bahasa Inggris. Naskah disusun dengan urutan: JUDUL (bahasa Indonesia dan Inggris), NAMA PENULIS (yang disertai dengan alamat Lembaga/ Instansi), ABSTRAK (bahasa Inggris, dan Indonesia maksimal 250 kata), KATA KUNCI (maksimal 6 kata), PENDAHULUAN, BAHAN DAN CARA KERJA, HASIL, PEMBAHASAN, UCAPAN TERIMA KASIH (jika diperlukan) dan DAFTAR PUSTAKA. Penulisan Tabel dan Gambar ditulis di lembar terpisah dari teks.

Naskah diketik dengan spasi ganda pada kertas HVS A4 maksimum 15 halaman termasuk gambar, foto, dan tabel disertai CD atau dikirim melalui email redaksi/ web JBI. Batas dari tepi kiri 3 cm, kanan, atas, dan bawah masing-masing 2,5 cm dengan program pengolah kata *Microsoft Word* dan tipe huruf *Times New Roman* berukuran 12 point. Setiap halaman diberi nomor halaman secara berurutan. Gambar dalam bentuk grafik/diagram harus asli (bukan fotokopi) dan foto (dicetak di kertas licin atau di scan). Gambar dan Tabel di tulis dan ditempatkan di halaman terpisah di akhir naskah. Penulisan simbol a, b, c, dan lain-lain dimasukkan melalui fasilitas insert, tanpa mengubah jenis huruf. Kata dalam bahasa asing dicetak miring. Naskah dikirimkan ke alamat Redaksi sebanyak 3 eksemplar (2 eksemplar tanpa nama dan lembaga penulis).

Penggunaan nama suatu tumbuhan atau hewan dalam bahasa Indonesia/Daerah harus diikuti nama ilmiahnya (cetak miring) beserta Authornya pada pengungkapan pertama kali.

Pustaka didalam teks ditulis secara abjad.

Contoh penulisan Daftar Pustaka sebagai berikut :

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