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## Effect of torrefaction pretreatment for ethanol fermentation from sugarcane bagasse

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### Abstract

Torrefaction is an appropriate pre-treatment technique to enhance the pore structure of lignocellulosic material for accelerating enzymatic reaction and subsequent ethanol production. This research aimed to study the effect of torrefaction pretreatment of sugarcane bagasse (SCB) derived from sugarcane juice squeezing on ethanol fermentation by the thermotolerant yeast *Kluyveromyces marxianus* in Semi-Simultaneous Saccharification and Fermentation (SSSF). The study examined the effect of different torrefaction times (10-60 min), temperatures (120-220°C), SCB particle sizes (0.3-2.0 mm and <0.3 mm) and initial solid loading (30 g/L and 100 g/L) to the fermentation and on the efficiency of ethanol production. The results showed that all the studied parameters affect and exhibit interaction with the ethanol yield. The higher torrefaction temperatures and time may lead to a higher ethanol production yield. However, the torrefaction at temperatures higher than 180°C and longer than 30 min, in which the inhibitors could also be produced, is not suggested due to the drop in ethanol yield observed. The highest ethanol global yield of 26.24% was obtained from the condition of 30 g/L solid loading of 0.3-2.0 mm SCB particle size torrefied at 180°C for 10 min corresponded to 23.95% ethanol global yield. The yield increased when compared with the non-torrefied SCB. This research reveals the feasibility of applying torrefaction pre-treatment to the SCB bio-refinery with the Eco-Efficiency concept.

**Keywords:** Ethanol, *Kluyveromyces marxianus*, Sugarcane Bagasse, Torrefaction

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### 1. Introduction

Sugarcane bagasse (SCB) is globally found in abundance as an agro industry processing waste. Thailand being the fourth-largest sugarcane producer with an average productivity of 100,000 metric tons per year generates about 17,500 metric tons of SCB per year [1-3]. Normally, sugarcane juice from crushing peeled sugarcane is consumed as beverage in many places in Thailand while the SCB waste after the extraction of sugar is utilized as a solid fuel for boiler to produce heat source for the sugar mills [4]. However, generally the SCB remains from sugarcane juices processing which is regarded as uncontrolled disposal waste can generate environmental problems and human health [5]. The composition of SCB varies with plant variety, cultivation conditions, harvesting practices, and processing methods. It is typically comprising of cellulose (32-45%), hemicellulose (20-32%), lignin (17-32%), ashes (1-9%) and extractive [6].

According to its composition, SCB has become an important substrate for producing bioethanol. The SCB could achieve a bioethanol (a biofuel) yield of > 0.2 g/g at optimal conditions [5]. Among the bioconversion stage of lignocellulosic biomass includes SCB, whereby pretreatment is a crucial step and also a costly process [7]. Pretreatment process is required to eliminate lignin and hemicellulose from the structure of lignocellulose, reduce cellulose crystallinity and increase the porosity, hence, improving cellulose hydrolysis. There are methods that can be used to pretreat SCB by using alkaline and alkali-acid processes that enable high glucose production from

hydrolysis [5]. However, cellulose and hemicelluloses degradation products like furfural, the hydroxymethylfurfural (HMF) generated during the process of using corrosive chemicals and severe heat may inhibit the microbial activity of the fermentation. Moreover, pH adjustment steps are needed to be applied in order to favor the fermentation. Thus, an emerging cost-effective pretreatment and environmentally friendly processes are needed [5].

Torrefaction process is one of the thermochemical decompositions, in which organic materials transform into a carbon solid and volatile matter by the absence of oxygen [8]. The torrefaction applied for biomass densification which ranges from increasing the heating value to upgrading the solid biofuel has been proven in terms of its application and practicality, but relatively not so many researchers have addressed its effect on lignocellulosic biomass structure, saccharification and bioethanol fermentations [9]. Torrefaction, aimed at producing high quality solid biomass products, is carried out at 200-300°C while for the pretreatment purposes, lower temperatures can be applied. The range for degradation temperature of hemicellulose, cellulose, and lignin are 200-400°C, 275-430°C, and 127-900°C, respectively. Therefore, the torrefaction process mainly decomposes hemicelluloses and partially degrades cellulose and lignin [10]. Sheikh et al. [11] found that after torrefaction treatment of rice straw at 220°C for 40 min the highest sugar yield was 351 mg/g which could increase yield ethanol 150 mg/g representing 60.68% and 50.67%, respectively compared to the untreated sample. Sheikh et al. [12] observed that treated waste money bills with torrefaction at 180°C for 40 min could achieve the highest glucose yield (81.59 mg/mL) and generate ethanol of 38.92 mg/mL which increases 47.92% compared to the untreated sample in the subsequent fermentation. While Chaluvadi et al. [13] reported that using torrefaction prior to size reduction of SCB and waste jute caddies (WJC) at 200°C for 20 min and at 180°C for 40 min, respectively were observed to produce highest glucose yields of 199.62 and 234.77 mg/g, respectively. However, after saccharification, ethanol yields representing 19.34% increase for SCB and 20.28% increase for WJC were noticed when compared with the untreated biomass. Obviously, this demonstrated that torrefaction pretreatment could enhance the bioethanol yield by using moderate heat, without wastewater production and chemical requirements.

*Saccharomyces cerevisiae* is commonly a yeast for industrial bioethanol production. Alternatively, superior microorganism, *Kluyveromyces marxianus*, has been observed currently interesting. *K. marxianus* represents several advantages over *S. cerevisiae* for example, it has the capability to consume both hexoses and pentoses, thermotolerance, high resistance of the cell membrane, ability to assimilate toxic compounds and has high growth rate [14-16]. The volumetric ethanol productivity of acid enzymatic hydrolysed SCB by *K. marxianus* SLP1 obtained ethanol production reaching 0.292 g/L·h which was about two times higher than fermentation with *S. cerevisiae* in the presence of growth inhibitors of yeast such as HMF and furfural [14].

Varieties of sugarcane plantation to produce sugarcane juices has been promoted in the south of Thailand, providing the potential raw material for ethanol production in the future. The SCB composition and sugarcane processing method for juice production are different from the typical SCB generated from sugar factory. Therefore, the performance of ethanol production from the local variety of SCB needs to be investigated. Moreover, from the literature review database of the researcher, it was found that there is no report on bioethanol production from torrefied by *K. marxianus*. Thus, the present study aimed to explore the effect of torrefaction temperature, torrefaction time, different SCB particle size, and solid loading during enzymatic hydrolysis and fermentation on the efficiency of bioethanol production by *K. marxianus*.

## 2. Materials and methods

### 2.1 Materials

The Suphanburi 50" variety SCB, was obtained from Yala Province, Thailand which is the left over residue after the sugarcane juice production stage. Prior to torrefaction, the SCB was oven-dried at a temperature of 105°C for 24 h. Then the SCB was milled and screened, the fraction remains on Mesh 10 and 50 sieve which correspond to the particle size 0.3-2.0 mm and less than 3.0 mm were chosen for the study. Then, the SCB samples were stored in a desiccator at room temperature until further use.

Cellic® CTec2 (cellulase with high beta-glucosidase activity) with 148 FPU/mL cellulase activity from Novozymes was used for the enzymatic hydrolysis. Yeast *K. marxianus*, TISTR 5925, was purchased from the Thailand Institute of Scientific and Technological Research (TISTR), Bangkok, Thailand. Before being used in the fermentation, the commercial yeast was inoculated in liquid Yeast Extract-Peptone-Dextrose (YPD) medium at 40°C and 200 rpm for 24 h and stored at temperature 4°C.

### 2.2 Pretreatment of SCB by torrefaction

Prior to saccharification and subsequent ethanol fermentation by yeast, the cellulosic material, SCB was pretreated by torrefaction. 40 g of SCB particle was placed in a covered crucible and then thermally pretreated by

torrefaction in a muffle furnace (Model FB1410M-33, Thermolyne™). Four torrefaction temperatures of 120, 150, 180, or 220°C and three residence times of 10, 30, or 60 min were carried out.

### 2.3 Semi-simultaneous saccharification and fermentation (SSSF) for ethanol production

The torrefied SCB was further used as substrate for semi-simultaneous saccharification and fermentation (SSSF). Firstly, the hydrolysis process was initiated in 250 mL Erlenmeyer flasks with the 30 g/L and 100 g/L initial solid loading of dried weight SCB, 10 mL Cellic® CTec2 while the volume was later adjusted to the working volume of 100 mL by 0.05 sodium citrate buffer pH  $4.9 \pm 0.1$  and incubated at 40°C with shaking at 150 rpm for 12 h.

Then, 10% (v/v) of *K. marxianus* and yeast extract,  $\text{KH}_2\text{PO}_4$ ,  $(\text{NH}_4)_2\text{HPO}_4$ , and  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$  in the amount of final concentration 2 g/L, 0.05%, 0.05%, and 0.15%, were added respectively [17]. The fermentation was further incubated at the same condition, while samples were withdrawn at 0, 12, 24, 48 and 72 h for the purpose of analyzing ethanol concentration. All experimental sets were carried out in triplicate and a controlled trial of Avicel® was used as reference material.

### 2.4 Analytical methods

Cellulose, hemicelluloses, and lignin contents were determined by standard method of the National Renewable Energy Laboratory (NREL) [18]. Moisture, ash, and volatile matter contents were determined by ASTM D 3173-73, ASTM D 3174-82, and ASTM D 3175-877 standards, respectively. The experiments of thermal decompositions of SCB were carried out by using thermogravimetric analysis (TGA7, Perkin Elmer, USA). Ultimate analysis was done using CHNS/O analyzer (Thermo Quest, Flash EA 1112 Series). Scanning electron microscopy (SEM) was used to observe the morphology of the SCB and torrefied SCB to evaluate the changes in the external structure caused by the pretreatments (Phillips XL-30 ESEM, USA). The ethanol concentration was measured using the gas chromatography (GC) (Agilent®) equipment with HP-INNOWax Polyethylene glycol column for separation. The flame ionization detector (FID) was used at 250°C and the oven temperature was set at 70°C. The Helium gas was used as carrier gas, with the flow rate set at 2 mL/min. Statistically significant differences ( $p \leq 0.05$ ) in the results were determined using the one-way analysis of variance (ANOVA) of SPSS v26.0 software (IBM, USA).

### 2.5 Calculation

The ethanol yield during fermentation with regards to the amount of ethanol produced per torrefied SCB loaded can be calculated follow Equation 1.

$$\text{Ethanol yield (\%)} = \frac{\text{Ethanol concentration (g/L)}}{\text{Solid loading (g/L)}} \times 100 \quad (1)$$

While Ethanol global yield specifically the amount of ethanol produced per raw SCB loaded can be calculated using the ethanol yield from the fermentation step and the solid recovery from the torrefaction process as shown in Equation 2.

$$\text{Ethanol global yield (\%)} = \text{Ethanol yield (\%)} \times \text{Solid recovery (\%)} / 100 \quad (2)$$

## 3. Results and discussion

### 3.1 Characteristic of sugarcane bagasse

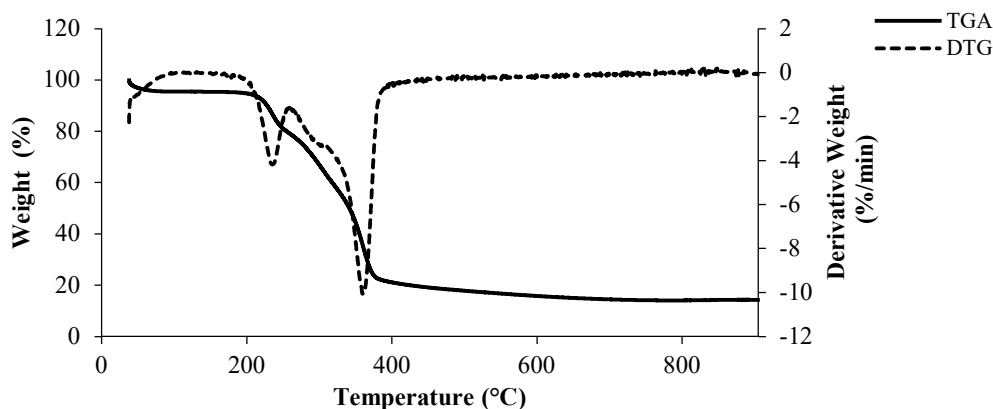
The characteristics of SCB as fiber composition (cellulose, hemicellulose, and lignin), ultimate analysis (C, H, N, S, and O contents), and proximate analysis (volatiles, ash, and fixed carbon), are presented in Table 1. The characteristics of SCB in this study are similar to those previously reports even though the SCB used in this study is taken from the variety for sugarcane juice production while the others are SCB variety for sugar production. It can be seen that SCB has high cellulose and hemicellulose which can be decomposed into fermentable sugars. However, one of the major challenges in developing technology for producing bioethanol from SCB is proper pretreatment techniques. Therefore, the torrefaction process is useful in reducing the crystallization of cellulose and optimizing the degradation of enzymes before fermentation, thus, making the process economically feasible [19].

**Table 1** Some characteristics of sugarcane bagasse.

Fiber composition (% w/w, dry basis)					
Cellulose	Hemicellulose	Lignin	Extractives	References	
41.10	24.70	22.40	6.80	This study	
32-45	20-32	17-32	2.8-8.2	[5]	
Ultimate analysis (% w/w, dry basis)					
C	H	N	O	S	References
43.16	6.07	0.24	42.10	<0.05	This study
44-48	5.4-5.8	0.06-0.4	39-49	<0.01-1	[20–23]
Proximate analysis (% w/w, dry basis)					
Moisture	Ash	Fixed carbon	Volatiles	References	
5.20	3.90	8.30	82.50	This study	
8-14	5-6	12-18	73-84	[24]	

The thermal analysis of SCB was investigated by TGA and its derivative thermal gravimetry (DTG) curve is shown in Figure 1. The SCB presents two regions of weight, the first weight loss step is from 200-259°C, and the second weight loss step ranges from 275-400°C. The maximum weight loss occurred at 361°C, indicating the loss of volatiles that was owing to the decomposition of the main components of the SCB such as cellulose, hemicellulose, and lignin [25]. These results correspond with the characteristics of SCB reported by Munir et al. [26]. The SCB weight loss processes occurred at less than 400°C, which are associated with dehydration and release of volatiles from the cellulosic structure [27].

The process of thermal decomposition of the lignocellulose material composition consists of hemicellulose, cellulose, and lignin, which decomposed at a temperature range of about 220-315, 315-400 and 160-500°C, respectively [28]. Since the preliminary pretreatment procedure of SCB is intended to increase the hydrolysis efficiency by decomposing lignin, reducing the crystallinity of cellulose, and increasing the surface area of SCB, to achieve greater exposure to enzymes, using thermal pretreatment may help in enhancing these issues, however some volatiles will also be lost. Therefore, based on the consideration of using the TGA profile and to minimize the SCB weight loss, the temperature range of 150-220°C was chosen for the torrefaction.

**Figure 1** Thermo gravimetric Analysis (TGA) of sugarcane bagasse.

### 3.2 The effect of torrefaction pretreatment on SCB characteristics

A summary of the weight loss of SCB after torrefaction at different temperatures and different torrefaction times is shown in Table 2. The weight of all samples progressively decreased when torrefaction temperature and time increased. While different SCB particle sizes in this studied did not cause any affects to the loss of their weight when torrefied for 10 min.

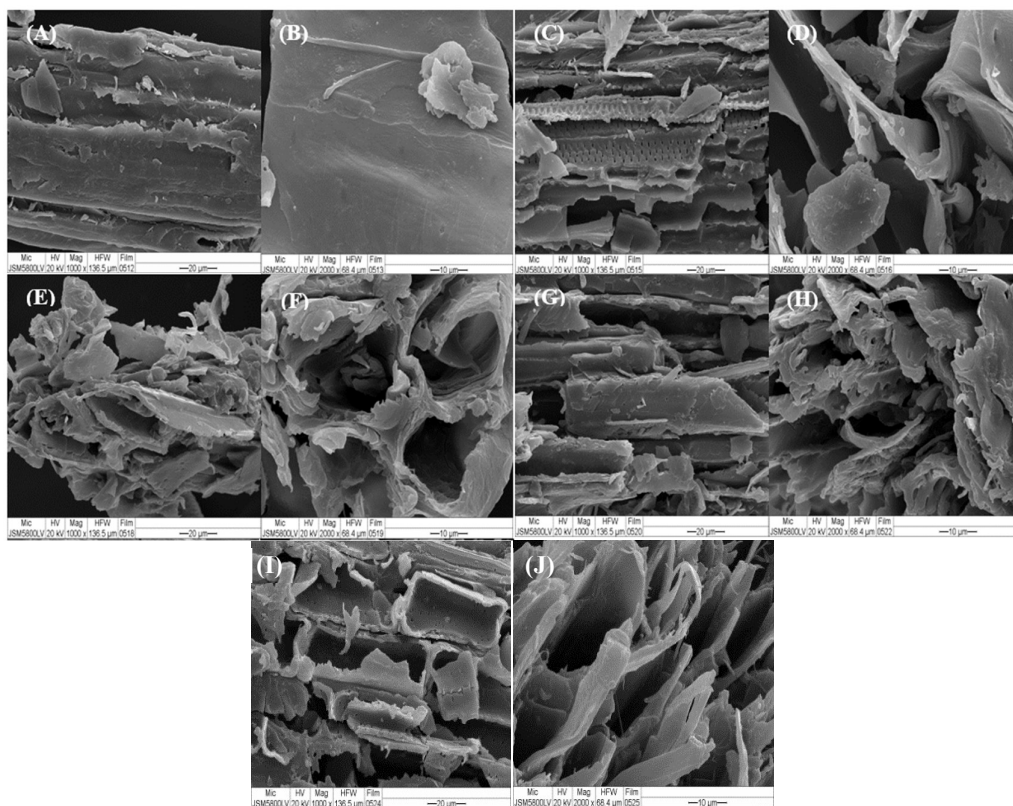
During this torrefaction process, hydroxyl ions ( $\text{OH}^-$ ) of the polymer will be broken to form  $\text{H}_2\text{O}$ , while some carbon compounds decompose to  $\text{CO}_2$  and  $\text{H}_2\text{O}$  [11]. This is in accordance with Prins et al. [29], which states that the torrefaction process draws water out of the biomass, resulting in the decomposition of hemicellulose, lignin, and some cellulose and causing the torrefied biomass to have lower hygroscopicity and higher brittleness which can easily be grounded. Hemicellulose is decomposed in a two-stage reaction. Volatile substances are first to be decomposed. Then carbon dioxide gas and carbonyl such as hydroxylacetone, methanol, propanol, acetic acid, furfural, water, lactic acid, and formic acid are formed by the thermal reactions.

**Table 2** Weight loss of sugarcane bagasse after torrefaction at different temperatures and time.

Torrefaction temperature (°C)	Weight loss (%)				
	Particle size 0.3-2.0 mm		Particle size <0.3 mm		
	Torrefaction time (min)		Torrefaction time (min)		
	10		10	30	60
120	1.04±0.23		1.02±0.31	1.33±0.24	2.01±0.23
150	2.25±0.22		2.27±0.39	2.75±0.12	3.92±0.51
180	4.13±0.88		4.12±0.51	4.71±0.83	6.21±0.78
220	11.20±1.00		11.21±1.12	13.80±1.14	15.82±1.45

Figure 2 shows the results of the morphological analysis of the raw SCB and torrefied SCB with torrefaction time of 10 min by SEM. It obviously shows that the morphologies of the torrefied SCB presented subtle differences when compared to the non-torrefied SCB. The surface of raw SCB is smooth and tightly arranged. While, as expected after heating the disorganized structure, rough surfaces of torrefied SCB were presented, hence confirming the losses of some compounds. Thermal pretreatment causes the SCB material to become more perforated, which is beneficial for enzymatic degradation in the next step.

Proximate compositions of torrefied SCB are shown in Table 3. It has been found that the amount of moisture and volatile substances decreases as the torrefaction temperature increases. Meanwhile, the amount of carbon stabilized, and ash increases as torrefaction temperatures rise. According to research conducted by Kang et al. [30], the amount of ash from biomass is not very high. However, the composition of the ashes should be considered since the amount of ash is mainly obtained from the composition of inorganic substance while high ash concentrations are unfavorable for bioethanol production. Volatile substances obtained by torrefaction in the temperature range of 180-220°C are rapidly reduced from 70.8% to 61.76% since, at low temperatures, moisture, volatile substances, and carbon in the SCB surface are quickly converted into gas and evaporated in volatile form. When the temperature rises to 220°C, volatile substances are also increasingly removed. For fixed carbon obtained from the experiment, it was found that when the temperature of torrefaction increases, the amount of carbon stabilizes increases as higher temperatures have removed moisture and volatile substances that are more constituents.



**Figure 2** SEM images: (A) SCB ( $\times 1,000$ ), (B) SCB ( $\times 2,000$ ), (C) 120°C torrefied SCB ( $\times 1,000$ ), (D) 120°C torrefied SCB ( $\times 2,000$ ), (E) 150°C torrefied SCB ( $\times 1,000$ ), (F) 150°C torrefied SCB ( $\times 2,000$ ), (G) 180°C torrefied SCB ( $\times 1,000$ ), (H) 180°C torrefied SCB ( $\times 2,000$ ), (I) 220°C torrefied SCB ( $\times 1,000$ ), and (J) 220°C torrefied SCB ( $\times 2,000$ ) (torrefaction time = 10 min).

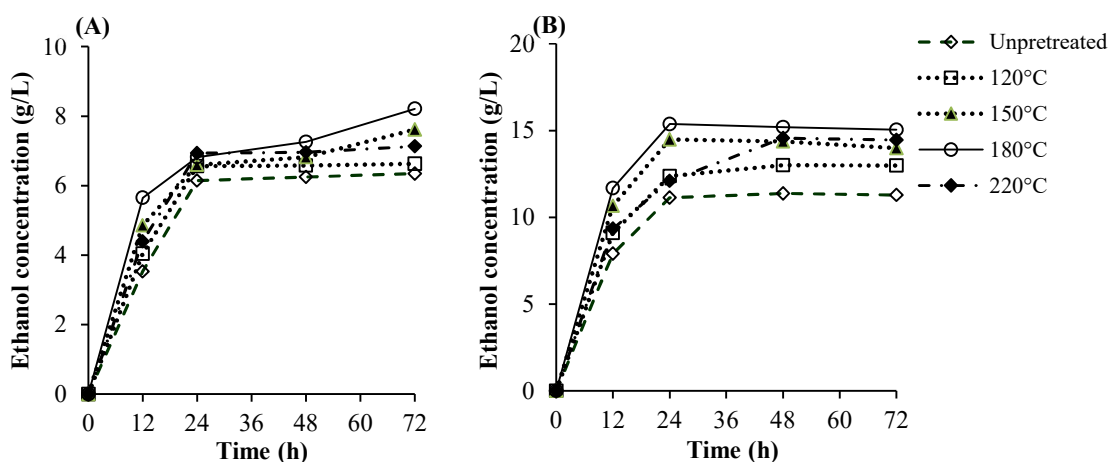
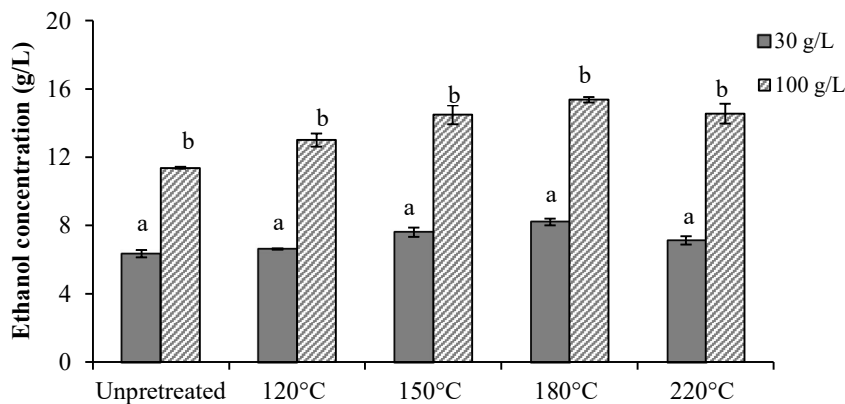
**Table 3** Proximate composition of torrefied SCB (particle size = <0.3 mm, torrefaction time = 10 min).

Torrefaction temperature (°C)	Moisture (%)	Volatile Matter (%)	Fixed Carbon (%)	Ash (%)
Non-torrefied	5.2	82.5	8.4	3.9
120	5.0	81.9	8.5	4.6
150	4.7	79.0	9.3	7.0
180	4.3	70.8	15.2	9.7
220	2.1	61.7	25.9	10.2

### 3.3 Bioethanol production

#### 3.3.1 Effect of solid loading during SSSF

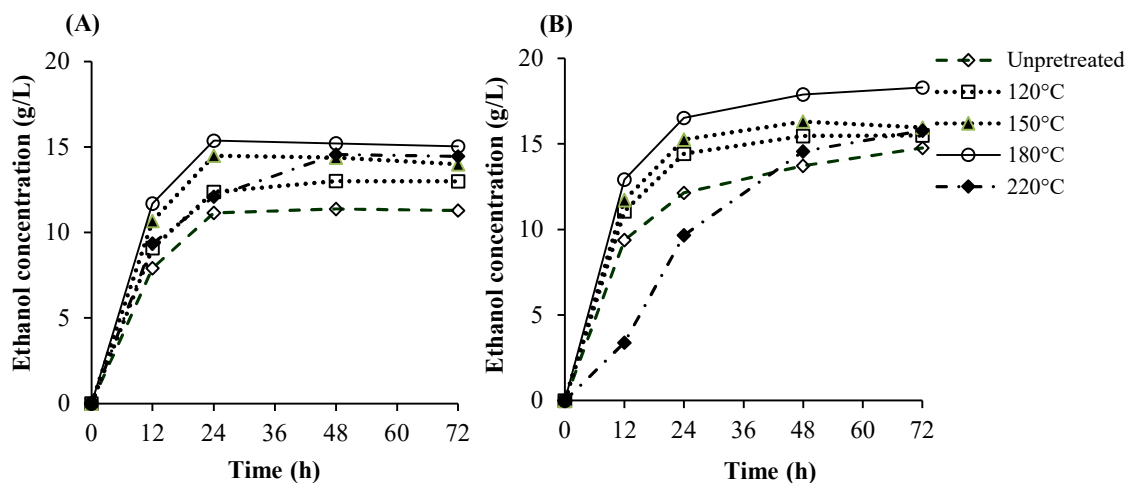
The Ethanol production at different solid loading during SSSF of SCB are presented in Figure 3 and 4. There are significant differences in the ethanol concentrations between 30 and 100 g/L as shown in Figure 4. The ethanol was rapidly produced in 12 h and trended to become constant after 24 h of fermentation. As expected, higher solid loading achieved significantly higher ethanol concentrations, and at 30 g/L solid loading, the ethanol concentration of 6.35-7.13 g/L were obtained while for 100 g/L loading, the achieved ethanol concentration was 11.38-15.37 g/L. Furthermore, the highest ethanol concentrations attained was from the temperature of 180°C.

**Figure 3** Ethanol production-time profile at solid loading during SSSF (A) 30 g/L and (B) 100 g/L of torrefied SCB (particle size = 0.3-2.0 mm, torrefaction time = 10 min).**Figure 4** Maximum ethanol at different solid loading during SSSF of torrefied SCB (particle size = 0.3-2.0 mm, torrefaction time = 10 min) (<sup>a</sup> and <sup>b</sup> are the statistically significant differences ( $p < 0.05$ ))

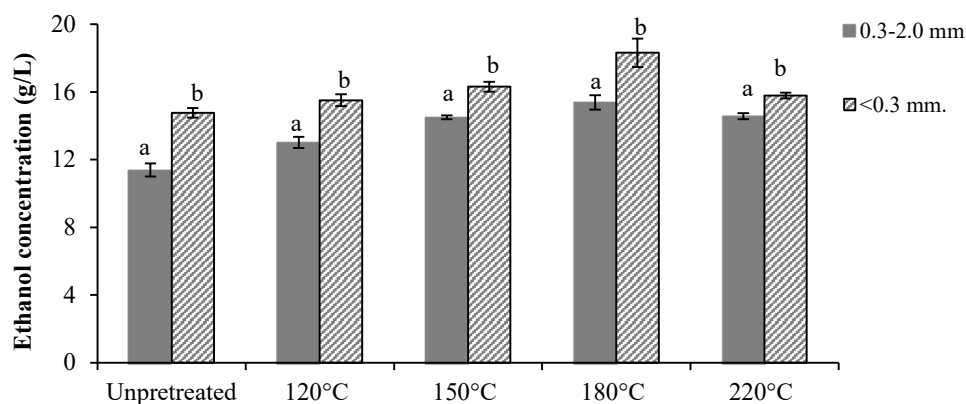
#### 3.3.2 Effect of SCB particle size

The effect of SCB particle size on ethanol productions is shown in Figure 5 and 6. The smaller particles which lead to the higher surface resulted in a more effective reaction not only in the thermal decomposition but also the biochemical reactions in the fermentation. In the lower torrefaction temperature range, higher ethanol production

rate occurred in the smaller SCB size while slower rate happens in biodegradation experiment at higher temperature. Moreover, at 180°C of torrefaction temperature, both SCB particle sizes received maximum ethanol concentration. This reaction can be explained as follows whereby at 180°C, which is the optimum temperature, the degradation process is more rapid; hence, more compound can be degraded especially the hemicellulose and cellulose which are the main substrate for bioethanol production.



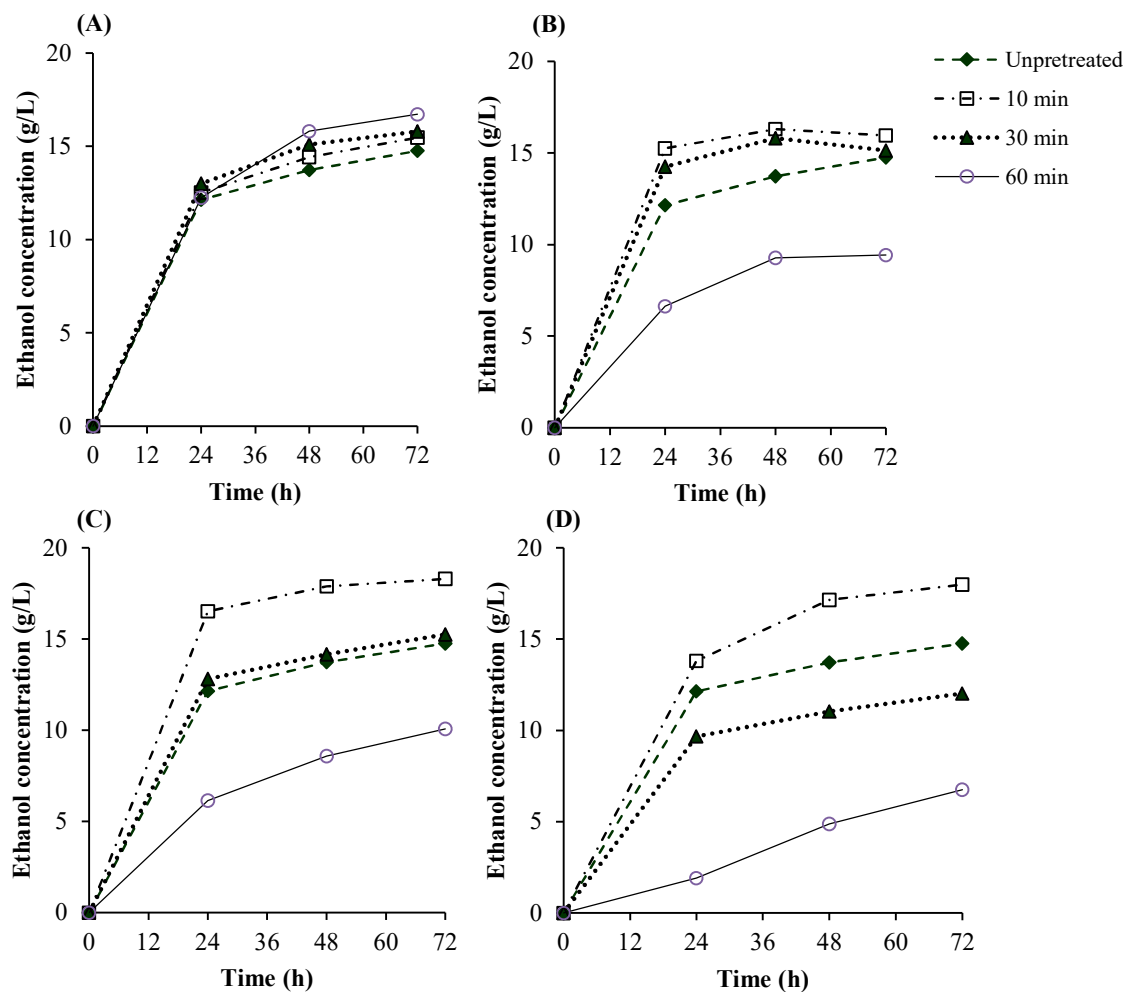
**Figure 5** Ethanol production-time profile at SCB particle size (A) 0.3-2.0 mm and (B) <0.3 mm of torrefied SCB (solid loading = 100 g/L, torrefaction time = 10 min).



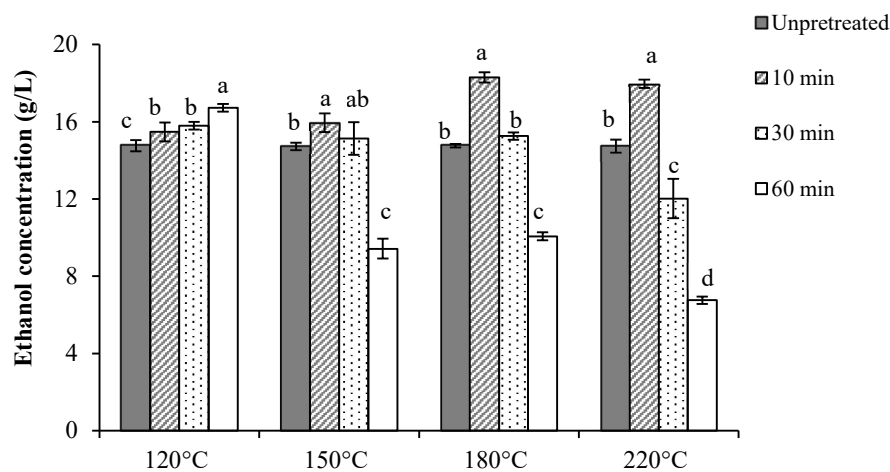
**Figure 6** Maximum ethanol concentration at different SCB particle size of torrefied SCB (solid loading = 100 g/L, torrefaction time = 10 min) (<sup>a</sup> and <sup>b</sup> are the statistically significant differences ( $p < 0.05$ )).

### 3.3.3 Effect of torrefaction temperature and time

Figures 7 and 8 show the ethanol production at different torrefaction times and temperature. It is clearly observed that the fermentation of torrefied SCB achieved significantly higher ethanol production. At low temperature of 120°C, the longer torrefaction time of SCB, the higher ethanol was obtained. At higher temperatures, overtime torrefaction is not suggested, in which 10 min of torrefied pretreatment has shown superior achievement whereas the 60 min long torrefaction did not obtain more ethanol production than the non-torrefied. In addition, 180°C of torrefaction temperatures gave the maximum ethanol concentration at 10 min of torrefied pretreatment, which was higher than at 150 and 220°C. Once again, it can be explained that the torrefied temperatures and time has interacted significantly and influenced the production of ethanol. The longer torrefaction and higher temperature have led to the loss of volatile compounds which are easily hydrolyzed. Moreover, compounds such as carboxylic acid, HMF, or furfural, inhibitors to microorganisms are also produced and accumulated into the torrefied biomass and retarding the biochemical activities [31,32].



**Figure 7** Ethanol production-time profile at different torrefaction time and temperature (A) 120°C, (B) 150°C, (C) 180°C and (D) 220°C of torrefied SCB (solid loading = 100 g/L, particle size <0.3 mm).



**Figure 8** Maximum ethanol at different torrefaction time and temperature (solid loading = 100 g/L, particle size <0.3 mm) (<sup>a-d</sup> are the statistically significant differences ( $p < 0.05$ )).



**Table 4** Summary of ethanol yield at different torrefaction conditions

Particle size (mm)	Torrefaction		Solid recovery (%)	Solid loading (g/L)	Ethanol Production			Ethanol global yield (%)	Global yield Increase from non-torrefied (%)
	Time (min)	Temp. (°C)			Ethanol concentration (g/L)	Ethanol yield (%)	Yield increase from non-torrefied (%)		
0.3-2.0	0	Non-torrefied	-	30	6.35 <sup>a</sup>	21.17	-	21.17 <sup>a</sup>	-
		120	98.96		6.63 <sup>ab</sup>	22.10	4.41	21.87 <sup>a</sup>	3.32
		150	97.75		7.61 <sup>c</sup>	25.37	19.84	24.80 <sup>b</sup>	17.15
		180	95.87		8.21 <sup>d</sup>	27.37	29.29	26.24 <sup>b</sup>	23.95
		220	88.80		7.13 <sup>b,c</sup>	23.77	12.28	21.10 <sup>a</sup>	-0.29
0.3-2.0	10	Non-torrefied	-	100	11.38 <sup>a</sup>	11.38	-	11.38 <sup>a</sup>	-
		120	98.96		13.01 <sup>b</sup>	13.01	14.32	12.87 <sup>b</sup>	13.13
		150	97.75		14.49 <sup>c</sup>	14.49	27.33	14.16 <sup>c</sup>	24.43
		180	95.87		15.37 <sup>c</sup>	15.37	35.06	14.74 <sup>c</sup>	29.49
		220	88.80		14.56 <sup>c</sup>	14.56	27.94	12.93 <sup>b</sup>	13.61
<0.3	0	Non-torrefied	-	100	14.76 <sup>a</sup>	14.76	-	14.76 <sup>ab</sup>	-
		120	98.98		15.50 <sup>ab</sup>	15.50	5.01	15.34 <sup>b</sup>	3.94
		150	97.73		16.30 <sup>b</sup>	16.30	10.43	15.93 <sup>b</sup>	7.93
		180	95.88		18.30 <sup>c</sup>	18.30	23.98	17.55 <sup>c</sup>	18.88
		220	88.79		15.78 <sup>ab</sup>	15.78	6.91	14.01 <sup>a</sup>	-5.07
<0.3	30	Non-torrefied	-	100	14.76 <sup>b</sup>	14.76	-	14.76 <sup>b</sup>	-
		120	98.67		15.80 <sup>c</sup>	15.80	7.05	15.59 <sup>c</sup>	5.62
		150	97.25		15.81 <sup>c</sup>	15.81	7.11	15.38 <sup>c</sup>	4.17
		180	95.29		15.26 <sup>b,c</sup>	15.26	3.39	14.54 <sup>b</sup>	-1.48
		220	86.20		12.02 <sup>a</sup>	12.02	-18.56	10.36 <sup>a</sup>	-29.80
<0.3	60	Non-torrefied	-	100	14.76 <sup>c</sup>	14.76	-	14.76 <sup>c</sup>	-
		120	97.99		16.72 <sup>d</sup>	16.72	13.28	16.39 <sup>d</sup>	11.00
		150	96.08		9.42 <sup>b</sup>	9.42	-36.18	9.05 <sup>b</sup>	-38.68
		180	93.79		10.06 <sup>b</sup>	10.06	-31.84	9.44 <sup>b</sup>	-36.08
		220	84.18		6.76 <sup>a</sup>	6.76	-54.20	5.69 <sup>a</sup>	-61.45

<sup>a-d</sup> are the statistically significant difference ( $p \leq 0.05$ ) of ethanol concentration and ethanol global yield.

Ethanol yields at different torrefaction conditions are summarized in Table 4. The significantly different ( $P$ -value  $\leq 0.05$ ) of Ethanol yield (g/g torrefied SCB\*100) and Ethanol global yield (g/g raw SCB\*100) of different temperature at each solid loading, SCB particle size, and torrefaction time were analyzed and presented as a set of superscript letters. As mentioned in the previous section, when the temperature increased, the ethanol concentration also increased. However, the ethanol concentration might drop if too high a temperature was applied. The torrefaction at 30 min/220°C and 60 min/150°C achieved the lower ethanol concentration. In considering the ethanol concentration, the highest concentration was obtained at the condition of 100 g/L solid loading, size<0.03 mm, 10 min, and 180°C. On the other hand, the highest ethanol yield of 27.37% and global ethanol yield of 26.24% was obtained from the condition of 30 g/L solid loading, size 0.3-2.0 mm, 10 min, 180°C. The ethanol yield is equal  $27.37/70.8 \times 100 = 38.66\%$  of volatile solid, which is lower than the theoretical yield of bioethanol produces from glucose due to lignin and some non-fermentable hemicellulose also contained in the volatile solid. Similar research of Chaluvadi et al. [13] reported that the torrefied SCB (1 mm particle size) at 200°C for 20 min under anaerobic fermentation with supplementation of cysteine hydrochloride by *S.Cerevisiae* were noticed to produce ethanol yields of 81.85 mg/g (about 8% yield) represent 19.34% increase for SCB. The ethanol yield of this mentioned research is lower than the highest ethanol yield obtained in our study due to the more solid loss of SCB that could occur when the higher temperatures and longer torrefaction time were applied.

#### 4. Conclusion

This study demonstrates the potential application of torrefaction to pretreat the SCB remained from juice squeezing activities for bioethanol production. The result showed that the ethanol yields received from torrefied SCB were varied from (-61)-30% higher than the non-torrefied. The ethanol global yield of 23.62% was obtained from the condition of 30 g/L solid loading, size 0.3-2.0 mm, 10 min, 180°C. Hence, for industrial application, waste heat might be applied for heating up SCB, since the heat can prevent fungi growth, and there is no generation of wastewater. Therefore, this research concluded that torrefaction is a promising method.

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