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Kusmiyati Kusmiyati

*Renewable Energy Research Centre, Muhammadiyah University of Surakarta, Surakarta 57102, Indonesia,*  
kusmiyati@ums.ac.id

Ali Mahmudi

*Engineering Faculty, Chemical Engineering Department, Muhammadiyah University of Surakarta,*  
Surakarta 57172, Indonesia

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## Bioethanol Production from Iles-Iles and Sorghum Starch as Raw Materials (Effect of CaCl<sub>2</sub> Addition and Saccharification Time)

Kusmiyati<sup>1\*</sup> and Ali Mahmudi<sup>2</sup>

1. Renewable Energy Research Centre, Muhammadiyah University of Surakarta, Surakarta 57102, Indonesia
2. Engineering Faculty, Chemical Engineering Department, Muhammadiyah University of Surakarta, Surakarta 57172, Indonesia

\*e-mail: kusmiyati@ums.ac.id

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### Abstract

Bioethanol produced from agricultural sources is one alternative energy that has been developed to substitute for petroleum. However, the use of food sources such as corn and cassava to produce bioethanol still face obstacles that lead to the scarcity of food and an increase in food prices. The aim of this study was to produce bioethanol from sorghum and iles-iles, which are not used by humans as food, especially in Indonesia. For both materials, the variables studied were saccharification time (4, 8, 24, and 48 h) and concentration of CaCl<sub>2</sub> added to the liquefaction slurry (0, 100, 200, 300, and 400 mg/L). The bioethanol was produced by enzymatic hydrolysis, which consists of liquefaction using  $\alpha$ -amylase at 1.6% v/w (t = 1 h; T = 95-100 °C; pH 6) and saccharification of the liquefaction slurry by using  $\beta$ -amylase at 3.2% v/w (t = various; T = 60 °C; pH 5) and fermentation by *Saccharomyces cerevisiae* (t = 120 hours; pH 4, 5; yeast 5g). The best conditions were obtained for sorghum at saccharification t = 24 h, which resulted in the highest ethanol yield, 91.5 g/L, and for iles-iles at t = 48 h, which yielded 107.1 g/L. The optimum amount of CaCl<sub>2</sub> to be added was 200mg/L, which resulted in the highest ethanol yield, 98.5 g/L and 92.4 g/L for sorghum and iles-iles, respectively. This results shows that sorghum and iles-iles are promising raw materials for the production of bioethanol, since they produce it in large amounts.

### Abstrak

**Produksi Bioetanol Berbahan Dasar Iles-iles dan Pati Sorgum (Efek Penambahan CaCl<sub>2</sub> dan Waktu Sakarifikasi).** Bioetanol yang dihasilkan dari sumber pertanian merupakan salah satu energi alternatif yang dikembangkan untuk menggantikan minyak bumi. Namun, penggunaan bahan makanan seperti jagung dan singkong untuk memproduksi bioetanol menimbulkan kendala karena dapat menyebabkan kelangkaan pangan dan kenaikan harga pangan. Tujuan dari penelitian ini adalah untuk menghasilkan bioetanol dari sorgum dan iles-iles, dimana bahan ini tidak digunakan oleh manusia sebagai makanan, terutama di Indonesia. Pada kedua bahan baku, variabel yang diteliti adalah waktu sakarifikasi (4, 8, 24, dan 48 jam) dan konsentrasi CaCl<sub>2</sub> yang ditambahkan ke dalam larutan umpan likuifikasi pada berbagai konsentrasi (0, 100, 200, 300, dan 400 mg/L). Proses hidrolisis enzimatis terdiri dari likuifikasi menggunakan  $\alpha$ -amilase sebesar 1,6% v/w (t = 1 jam; T = 95-100 °C, pH 6) dan sakarifikasi hasil likuifikasi dengan menggunakan  $\beta$ -amilase sebesar 3,2% v/w (t = variasi, T = 60 °C, pH 5) dan fermentasi oleh *Saccharomyces cerevisiae* (t = 120 jam, pH 4, 5, ragi 5g). Kondisi terbaik diperoleh untuk sorgum yang di sakarifikasi t = 24 jam, yang menghasilkan yield etanol tertinggi, 91,5 g/L, dan untuk iles-iles pada t = 48 jam, yang menghasilkan 107,1 g/L. Kondisi optimum dari CaCl<sub>2</sub> yang n ditambahkan adalah 200mg/L, yang mengakibatkan hasil tertinggi etanol, 98,5 g/L dan 92,4 g/L untuk masing-masing sorgum dan iles-iles. Hasil ini menunjukkan bahwa sorgum dan iles-iles merupakan bahan baku yang menjanjikan untuk produksi bioetanol, karena dapat dihasilkan bioethanol kadar tinggi.

*Keywords: bioethanol, CaCl<sub>2</sub>, iles-iles, S. cerevisiae, sorghum*

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### 1. Introduction

The shortage of energy and environmental pollution are two important problems for human beings in the twenty-first century [1]. Industrial development and rapid

population growth have resulted in a 17-fold increase in world energy consumption [2]. Approximately 44% of the world's energy demand is for petroleum, which is produced from fossils [3], which is used to make gasoline, diesel, and kerosene [4]. Petroleum is a limited

energy source; therefore, it will be depleted faster if the dependence on oil is high. Moreover, the use of fossil fuels has a negative impact on the environment because it can increase greenhouse gas emissions (CO<sub>2</sub>) and air pollution [4]. Much attention has currently been focused on the development of biofuel as a renewable energy source that can be used as a substitute for petroleum [1].

Bioethanol is one biofuel that is considered a promising renewable energy source due to its potential for use as a clean fuel for transportation, replacing gasoline [5]. In Europe and America, as well as several developing countries, bioethanol is already used as a fuel [6]. Increased bioethanol production as a renewable energy source has had a positive impact on the development of agricultural products and the reduction of greenhouse gas emissions by 10-20% [7].

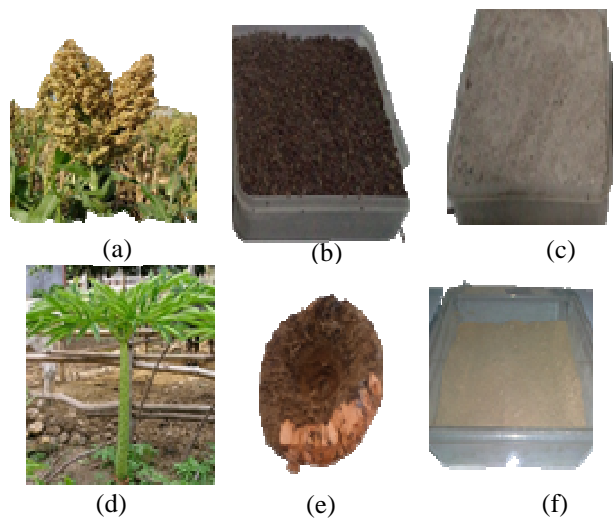
Bioethanol is produced from the fermentation of such biomass as sugar, carbohydrates, and cellulose [8]. Today, molasses, a byproduct of sugarcane, is the raw material most used in the bioethanol industry. The limited availability of molasses has led to the use of alternative raw materials to substitute for molasses. Corn and cassava have been used as raw materials, but both are staple foods for humans [5,9]. The use of foodstuffs as raw materials to produce ethanol has reduced the availability of food, so it is necessary to search for an alternative [8]. Sorghum (*Sorghum bicolor* (L) Moench) is a plant with a high sugar content [10]. Iles-iles (*Amorphophallus campanulatus*) is a tuber with a high carbohydrate content [11]. Neither of these plants is used for human food, so they can be used as alternative materials for the production of ethanol.

The aim of this study was to compare the concentration of ethanol produced from sorghum and iles-iles at various saccharification times and various concentrations of added CaCl<sub>2</sub>.

## 2. Methods

**Materials preparation.** Sorghum grain was obtained from a traditional market in Solo, Kleco. Sorghum grain was washed, dried, and ground to flour. Sorghum contains 70.9% starches and 32.8% crude fiber [12]. Iles-iles was obtained from Wonogiri Central, Java, peeled, and then washed and cut into pieces approximately 5 × 5 × 0.1 cm. The pieces were sun dried until the moisture content was less than 10% and then ground into flour. Iles-iles contains 78.7% starch and 30.9% crude fiber.

**Hydrolysis.** The enzymatic hydrolysis process consisted of two stages: liquefaction using α-amylase (Spectra Genencor, USA) and saccharification using β-amylase (Spectra Genencor, USA).



**Figure 1.** (a) Sorghum Plant, (b) Sorghum Grain, (c) Sorghum Flour, (d) Iles-iles Plant, (e) Iles-iles Tuber, (f) Iles-iles Flour

In the liquefaction process, flour was dissolved in distilled water to obtain slurry with a flour:water ratio of 1:4 (w/w). The slurry was conditioned to pH 6 and then α-amylase was added at a concentration of 0.32% (v/v). CaCl<sub>2</sub> at concentrations of 0, 100, 200, 300 and 400 mg/L were then added to the flour slurry. The liquefaction process was done at 95-100 °C using a water bath in an oven (Memmert UNE 500, [USA]). The slurry was stirred with a digital stirrer (IKA RW 20D, [USA]) at 350 rpm for 1 hr. The resulting liquid was cooled to 60 °C and conditioned to pH 5. A solution of β-amylase 0.64% (v/v) was then added.

The saccharification process was done at 60 °C using a water bath in an oven (Memmert UNE 500, [USA]). The slurry was stirred with a digital stirrer (IKA RW 20D, [USA]) at 350 rpm for times of 4, 8, 24, and 48 h.

**Preparation of *Saccharomyces cerevisiae*.** *Saccharomyces cerevisiae* was obtained from the Chemical Laboratory UMS in the form of dry yeast (Mauripan, [Indonesia]). It was cultivated in the starter media of 0.5 g KH<sub>2</sub>PO<sub>4</sub>, 0.5 g yeast extract, 0.5 g peptone water, 0.5 g (NH<sub>4</sub>)<sub>2</sub>HPO<sub>4</sub>(DAP), 0.1 g MgSO<sub>4</sub>, and 2 g glucose dissolved in 100 mL aquadest, then incubated for 24 hours at 30 °C.

**Fermentation.** The result of the hydrolysis was conditioned to pH 4.5, after which 1 g urea, 0.5 g DAP, and a solution of 10% (v/v) starter yeast was added. This mixture was then allowed to ferment for 120 hr.

**Analysis.** Measuring the reduction of sugar content was carried out using a photometer (Boehringer 1040, [USA]) at a wave length of 546 nm. Of the result 0.01 mL (10 μL) was put into reaction tube. A reagent of

glucose, 1000 µL/mL, was added, and the result was incubated for 10 min in the water bath at 37 °C. The content of ethanol produced by the fermentation was determined using a GC (Agilent 6890N) equipped with an HP-INNOWax column (30 m × 320µm × 0.5µm) and an FID detector.

### 3. Results and Discussion

**Effect of CaCl<sub>2</sub> on reducing sugar content.** It can be seen in Figure 2 that the highest reduction of sugar from both sorghum and iles-iles occurred when CaCl<sub>2</sub> was added at a concentration 200 mg/L. This resulted in a sugar concentration of 249.7 g/L for sorghum and 248.6 g/L for iles-iles. Previous studies reported that enzyme activity was affected by some factors, one of which was metal ions, especially Ca<sup>+</sup> [13]. A Ca<sup>+</sup> ion is usually gained from the molecule CaCl<sub>2</sub>·2H<sub>2</sub>O. The cofactor for the enzyme, CaCl<sub>2</sub>, can reactivate the enzyme [14], though the addition of too much cofactor would slow down the work of enzyme, as shown in Figure 2. The addition of low concentrations of Ca<sup>+</sup>, 100 mg/L and 200 mg/L, resulted in an increased sugar content, but at higher concentrations of Ca<sup>+</sup>, 300 mg/L and 400 mg/L, the yield of sugar decreased [13]. Shewale et al. (2009) found that the maximal sugar reduction was obtained with the addition CaCl<sub>2</sub> concentration of 200 ppm [15].

**The effect of saccharification time on sugar content.** Figure 3 shows that the highest rate of sugar reduction, 236 g/L, occurred after 48 hours of saccharification. A saccharification time of 48 hours also produced a reduction of sugar in iles-iles, 254 g/L. The sugar content of iles-iles was higher than that of sorghum because the carbohydrate content of iles-iles flour is higher than that of sorghum. Based on the raw material analysis, the starch content of iles-iles and sorghum was 78.9% and 70.7%, respectively.

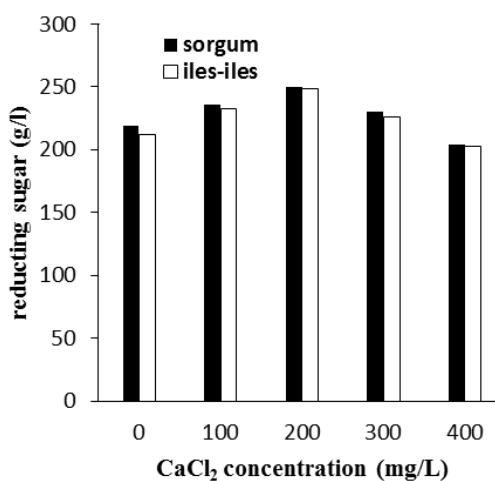


Figure 2. The Reduction of Sugar (g/L) from Addition of CaCl<sub>2</sub> (mg/L)

Aggarwal et al. (2001) reported that when sorghum was used as the raw material, the saccharification time had a considerable effect on the sugar content: the highest sugar content was obtained after 24 hr of saccharification, with about 90% conversion [16].

**Influence of CaCl<sub>2</sub> on the content of ethanol.** The typical chromatogram of the ethanol concentration (% v/v) obtained by GC analysis are shown in Figure 4 (A) for sorghum and (B) for iles-iles.

Without added CaCl<sub>2</sub>, the ethanol concentration obtained from sorghum and iles-iles were 87.9 g/L and 77.5 g/L, respectively. The maximum ethanol concentration was reached when the addition of CaCl<sub>2</sub> was 100 mg/L; this resulted in ethanol concentrations for sorghum and iles-iles of 99.9 g/L and 95.4 g/L, respectively. This indicated that adding CaCl<sub>2</sub> increased ethanol production by both sorghum and iles-iles. However, ethanol concentration decreased when the addition of CaCl<sub>2</sub> increased from 200 to 400 mg/L.

Figure 5 shows that the lowest ethanol concentration was obtained with the addition of 400 mg/L of CaCl<sub>2</sub>, which produced ethanol at 78.6 g/L and 71.2 g/L for sorghum and iles-iles, respectively. The addition of 200 mg/L of CaCl<sub>2</sub> produced a concentration of ethanol of 98.5 g/L and 92.4 g/L for sorghum and iles-iles, respectively. These results indicate that ethanol production was less with the addition of 200 mg/L of CaCl<sub>2</sub> than it was with an addition of 100 mg/L. Previous studies reported by Zeng et al. [13] suggested that maximum ethanol production was obtained with the addition of CaCl<sub>2</sub> 1.6 mg/L. When more CaCl<sub>2</sub> was added, ethanol production decreased.

Figure 5 shows an increase in ethanol concentration when fermentation time is 120 hr, while the concentration of sugar decreased. These results indicate

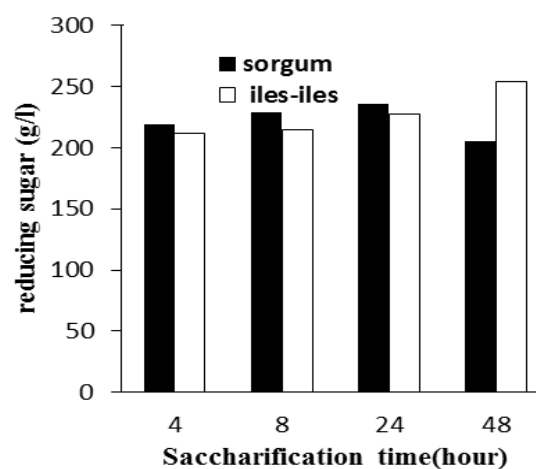
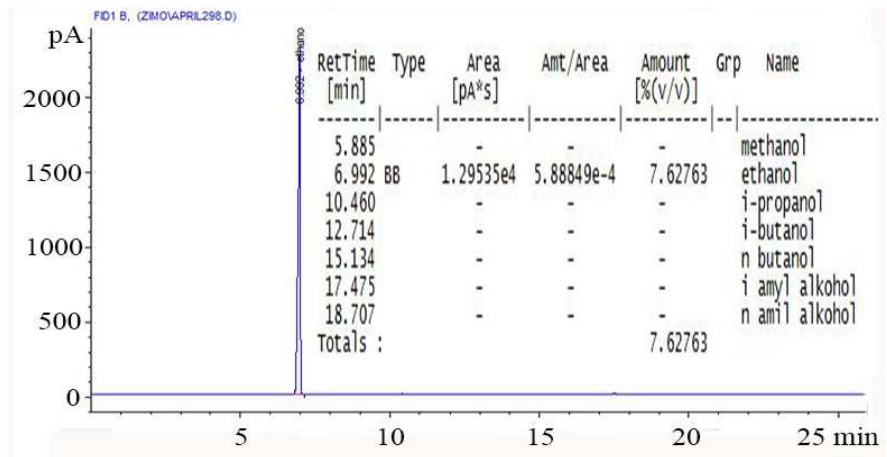
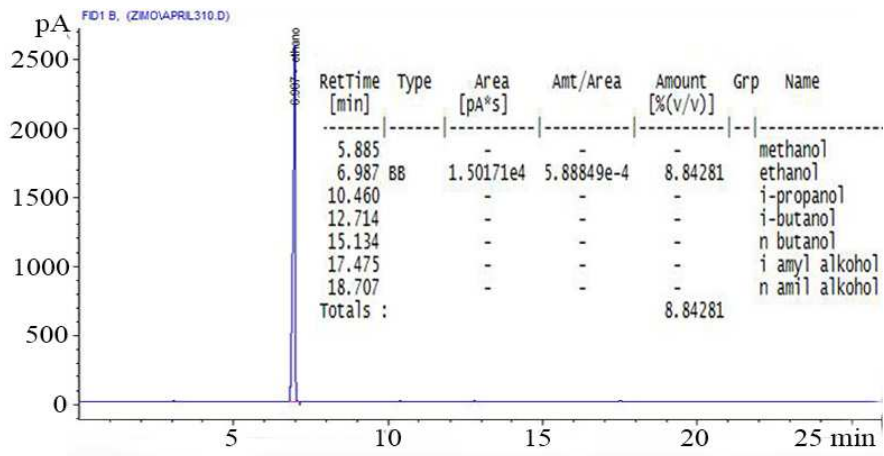


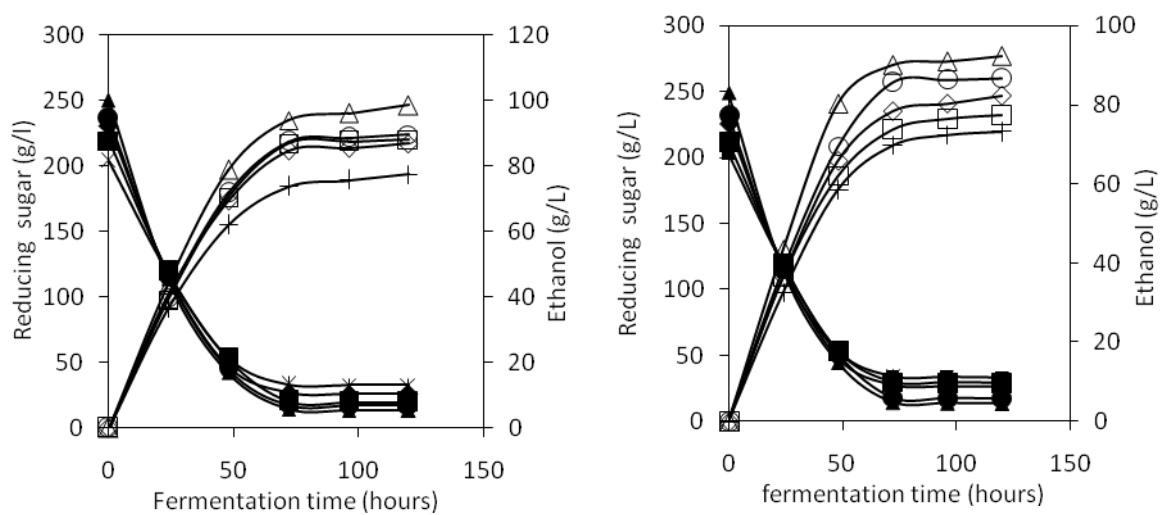
Figure 3. The Influence of Saccharification Time on Sugar Reduction (g/L)



(a)



(b)



**Figure 5. The Effect of the Addition of CaCl<sub>2</sub> on Sugar Reduction in the Liquefaction and Ethanol Production for [A] Sorghum and [B] Iles-iles as Raw Materials. Fermentation Took Place with 5 g/L Yeast at 30 °C. Reducing Sugar at Various Concentration of CaCl<sub>2</sub> Addition: [■] 0 mg/L, [▲] 100 mg/L, [●] 200 mg/L, [◆] 300 mg/L, [\*] 400 mg/L. Ethanol at Various Concentration of CaCl<sub>2</sub> Addition: [□] 0 mg/L, [△] 100 mg/L, [○] 200 mg/L, [◇] 300 mg/L, [+ ] 400 mg/L**

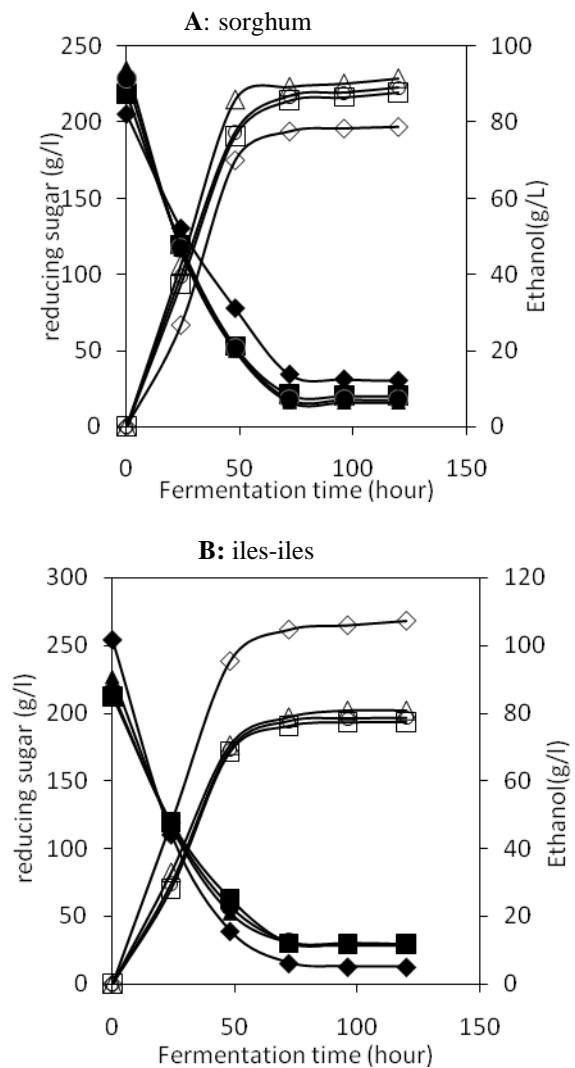
that the consumption of sugar by yeast corresponds to the conversion of sugar to ethanol [10]. Similar results were obtained by Rani et al. (2010) [17], who found that the highest ethanol level corresponded to the lowest sugar content in the fermentation.

**The effect of saccharification time on ethanol content.** The effect of various saccharification times (4, 8, 24, and 48 hr) on liquefied flour slurry was tested under identical fermentation conditions, and the results are shown in Figure 6. For both iles-iles (Figure 6A) and sorghum flour (Figure 6B) the optimum time for fermentation was found to be 120 hr. The optimum time for the saccharification of liquefied flour slurry was found to be 24 and 48 hr for sorghum and iles-iles, respectively. For iles-iles flour there was a significant increase in the ethanol concentration at a saccharification time of 48 hr, while a saccharification time of less than 48 hr resulted in a sharp decrease in the ethanol concentration. For sorghum flour, there was a sharp decrease in the ethanol concentration at 48 hr saccharification time, but little variation of ethanol concentration was observed at saccharification times of 4, 8, and 24 hr. A maximum ethanol concentration of 107.1 g/L was obtained at a saccharification time of 48 hr after a fermentation time of 120 hr for the iles-iles flour substrate, while the highest ethanol level for sorghum flour was obtained at 91.5 g/L from a saccharification time of 24 hr and a fermentation time of 120 hr.

The results indicate that the highest ethanol concentration (Figure 6) was due to the highest reduction of sugar concentration in the saccharification of the flour substrate (Figure 3). Increasing the fermentation time of an enzymatic hydrolysate of iles-iles and sorghum flour from 1 hr to 120 hr resulted in a significant increase in ethanol concentration up to hour 60, then a lag phase until hour 120. Conversely, sugar consumption decreased significantly from hour 0 to hour 60, then decreased slightly from hour 24 until hour 60. This was due to the sugar in the substrate being consumed by the yeast at the beginning of fermentation until the yeast died due to the high ethanol concentration. Similar results were reported by Guo et al. [2], who found a significant increase in ethanol production from 24 to 60 hours fermentation time.

**Comparison of ethanol from Sorghum and Iles-Iles with different amounts of added CaCl<sub>2</sub>.** Figure 7 shows the effect of the addition of different amounts of CaCl<sub>2</sub> (0–400 mg/L) in the liquefaction step under similar conditions of saccharification and fermentation. Figure 7 shows that both sorghum and iles-iles produced the highest ethanol content when 200 mg/L CaCl<sub>2</sub> was added to the liquefaction slurry, which corresponds to ethanol 98.5 g/L and 92.4 g/L for sorghum and iles-iles flour, respectively. Sorghum produced a slightly higher ethanol level than that of iles-iles in the fermentation

process, although both substrates resulted in similar amounts of sugar reduction in the saccharification process. This indicates that the effective conversion of sugar to ethanol was also dependent on the yeast in fermentation, although CaCl<sub>2</sub> had an effect on the saccharification step. Based on Figure 7, additional CaCl<sub>2</sub> did not increase ethanol production. As can be seen at 300 and 400 mg/L CaCl<sub>2</sub>, the concentrations of ethanol were similar without additional CaCl<sub>2</sub>. The ethanol concentrations were 86.7 g/L for sorghum and 82.2 g/L for iles-iles with the addition of 300 mg/L CaCl<sub>2</sub>, and 77.4 g/L for sorghum and 73.2 g/L for iles-



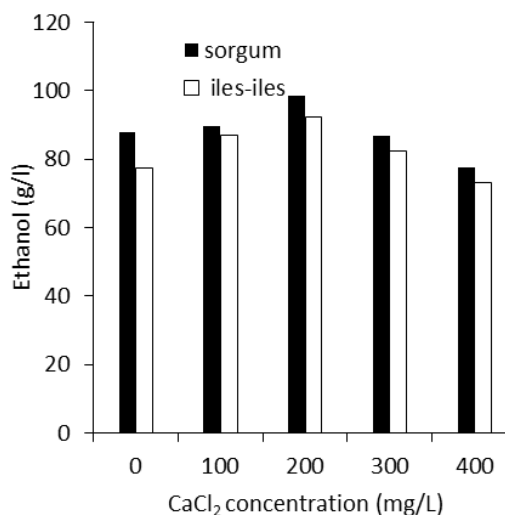
**Figure 6. The Effect of Saccharification Time on Ethanol Production from [A] Sorghum: Reducing Sugar at Various Saccharification Time: [■] 4 h, [▲] 8 h, [●] 24 h, [◆] 48 h. Ethanol at Various Saccharification Time: [□] 4 h, [△] 8 h, [○] 24 h, [◇] 48 h and [B] Iles-iles: Reducing Sugar at Various Saccharification Time : [●] 4 h, [▲] 8 h, [■] 24 h, [◆] 48 h. Ethanol at Various Saccharification Time: [○] 4 h, [△] 8 h, [□] 24 h, [◇] 48 h. Fermentation Took Place with 5 g/L Yeast at 30 °C**

iles with the addition of 400 mg/L  $\text{CaCl}_2$ . With no  $\text{CaCl}_2$  addition, the ethanol concentrations were 84.2 g/L and 79.1 g/L for sorghum and iles-iles, respectively.

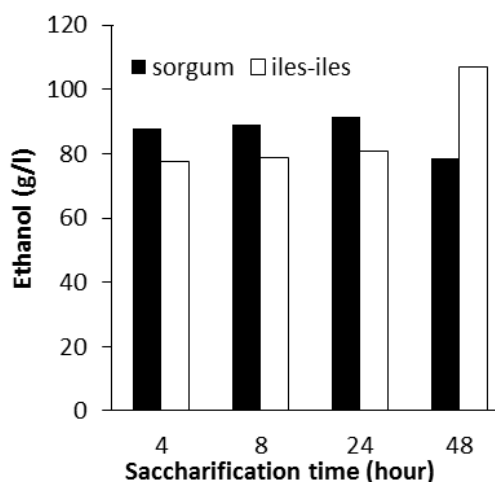
The content of ethanol from sorghum and iles-iles increased as the amount of  $\text{CaCl}_2$  added increased from 100 to 200 mg/L. From this we find that the optimum concentration of  $\text{CaCl}_2$  for producing ethanol from sorghum and iles-iles is 200mg/L.

#### Effect of saccharification time on ethanol content.

Figure 8 shows that the highest ethanol yield from sorghum (91.5 g/L) was obtained from fermented substrate at a saccharification time of 24 hr. This indicates that the ethanol content of iles-iles was higher than that of sorghum, which at the saccharification time of 48 hr was measured at 107.1 g/L ethanol.



**Figure 7. Effects of  $\text{CaCl}_2$  Concentration and Type of Flour Substrate on Ethanol Production in Fermentation**



**Figure 8. Effect of Saccharification Time on Ethanol Production**

The ethanol content resulting from fermentation of the substrate varied for different saccharification times, always higher for sorghum than for iles-iles. At the saccharification times of 4, 8, and 24 hr the ethanol content for sorghum was 87.9 g/L, 89.1 g/L, and 91.5 g/L, respectively; for iles-iles it was 77.5 g/L, 78.6 g/L, and 80.8 g/L, respectively.

## 4. Conclusions

It can be concluded that sorghum and iles-iles are promising raw materials that produce high ethanol concentrations. The results show that adding  $\text{CaCl}_2$  at 200 mg/L is optimum, producing ethanol concentrations of 98.5 g/L and 92.4 g/L for sorghum and iles-iles, respectively. The optimum saccharification time for ethanol production from sorghum (91.5 g/L) is 24 hr, while for iles-iles (107.1 g/L) it is 48 hr.

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