Effect of Initial Fermentation Medium on Bioacetone, Biobutanol, and Bioethanol (BioABE) Production from Fermentable Sugars of *Acacia mangium* using *Clostridium acetobutylicum* YM1

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Bioacetone, biobutanol, and bioethanol (BioABE) production is dependent on the fermentable sugars produced from lignocellulosic biomass and on the composition and initial pH of the medium. Understanding these process variables and their interconnectedness could enhance the BioABE product yield. *Acacia mangium* is available abundantly and it is a potential feedstock for BioABE production. In this study, BioABE was produced from fermentable sugars of *A. mangium* using *Clostridium acetobutylicum* YM1. Alkaline treated *A. mangium* (70 °C, 3 h, 5.50 %w/v NaOH) was further hydrolyzed *via* enzymatic hydrolysis using a multi-enzyme of white rot fungi to convert it into fermentable sugars. Approximately 15 g/L of fermentable sugars was produced from *A. mangium* (100 g/L) and was used for BioABE production in comparison with glucose. Initial findings showed that only 0.94 g/L of BioABE was produced in comparison with glucose (2.86 g/L) at a pH of 6.2. Decreasing the initial pH of the medium to 4.50 increased the BioABE (2.87 g/L), and after the medium was supplemented with tryptone-yeast-acetate (TYA), the BioABE yield increased by more than 100% to 6.84 g/L. This study discovered that BioABE produced from *A. mangium* was comparable to using commercial glucose, thus offering high potential as a low-cost feedstock.

Keywords: Acacia mangium; Clostridium acetobutylicum YM1; Bioacetone-biobutanol-bioethanol (BioABE); Enzymatic hydrolysis

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INTRODUCTION

Interest in biofuels production from sustainable renewable resources has increased over the last decades as a means to overcome the depletion of fossil fuel. Hence, various actions and initiatives have been taken to identify renewable alternatives to fossil fuels (Holmgren and Sellstedt 2008; Zhao *et al*. 2013; Xue *et al.* 2017). The key factors that promote alternatives to fossil fuels are the increasing climate change issues through the release of monoxide gas either from industries or vehicles, greenhouse gas (GHG) emissions, and rising of fossil fuel prices (Lellan 2010; Cao *et al*. 2016). In addition, awareness is rising among the world community surrounding the need to protect the environment. This has attracted interest in exploring plant-based resources to replace fossil fuels and producing bio-based chemicals along with other value-added products.

Biobutanol is an environmentally friendly substitute for fossil fuels that is produced through acetone-butanol-ethanol (ABE) fermentation using *Clostridium* spp. bacteria (Al-Shorgani *et al*. 2018). Today, the method for biobutanol production from lignocellulosic biomass is very limited. Like bioethanol, biobutanol applications that serve as an alternative to fossil fuels, such as gasoline, also have a significant reduction in the impact of green gas emissions (Rass-Hansen *et al*. 2007; Baral and Li 2013; Zabed *et al.* 2014). The characteristics of biobutanol as an alternative fuel were compared to the characteristics of gasoline and bioethanol. The bioethanol-gasoline mixture has a limit of up to 15% without any modifications to the vehicle engine. By contrast, the biobutanol-gasoline mixture has no specific limits and can be mixed with gasoline at any proportion without affecting the existing engine system; the mixture does not separate due the presence of water. In addition, biobutanol has higher energy content than bioethanol (Kenneth 2010; Kaminski *et al.* 2011; Raganati *et al.* 2014; Yusoff *et al*. 2015; Cao *et al*. 2016). The production of biobutanol involves two main phases: acidogenesis and solventogenesis. During the acidogenesis phase, organic acid consisting of acetic acid and butyric acid are produced in line with the bacteria cell growth. As the acid concentrations is increased, the pH of medium decreases to below 5.0, and the phase is changed to solventogenesis, where BioABE solvents start to be produced (Raganati *et al*. 2014; Ndaba *et al*. 2015).

Some obstacles to biobutanol production are its low yield and productivity, the high cost of feedstock, and product recovery (Al-Shorgani *et al*. 2018). Although many efforts and initiatives have been taken to overcome these problems, more research is still required. Due to this, various technologies have been developed to select the lignocellulosic biomass to ensure its suitability as feedstock for biobutanol production. The need to convert this lignocellulosic biomass into biobutanol is crucial to meet current demand and it has high impact on social and economic growth. Indeed, environmental issues such as global warming and climate change can be solved through this approach (Yusoff *et al*. 2015). *Acacia mangium* is one of the short-term rotations of forest species (Fig. 1) containing low lignin and has high potential as a raw material for biofuels production (Rawat *et al*. 2013).

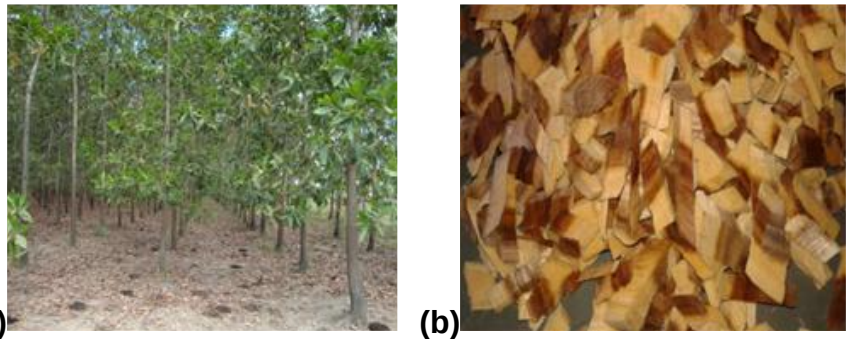

Fig. 1. Acacia mangium tree (a) and wood chips (b)

A. mangium consists of 45% to 50% cellulose, 25% to 35% hemicellulose, and 15% to 25% lignin (Yahya *et al*. 2010; Raphy *et al*. 2011; Mohd Hazim *et al*. 2017; Takazawa *et al.* 2018). Increasing the cellulose and hemicellulose content of *A. mangium* can be done through a pretreatment process to reduce the lignin content and to open up the structure for enzymatic attack during enzymatic hydrolysis (Sendelius 2005; Singh *et al*. 2011; Isikhuemhen *et al*. 2014; Xue *et al*. 2017). Various methods can be used to pretreat *A. Mangium* including chemical, physical, mechanical, or biological methods.

Alkaline pretreatment is one of the best chemical methods to modify the structure of hemicellulose and lignin in lignocellulosic biomass by breaking the cell wall and hydrogen bonds to increase surface area and pore size (Quiroz-castaneda *et al*. 2010; Singh *et al*. 2011; Nazarpour *et al*. 2013; Nur Izzati *et al*. 2013). Lignin content can be reduced to below 10% after alkaline treatment (Maeda *et al*. 2013; Sharma *et al*. 2016; Sornlake *et al*. 2017; Lukajtis *et al*. 2018).

The aim of this study was to evaluate the effectiveness of fermentable sugars produced from *A. mangium* as a feedstock for BioABE production in comparison with commercial glucose using *Clostridium acetobutylicum* YM1 and to study the effect of fermentation medium conditions (initial pH of the medium and supplementation of nutrients) on BioABE production.

EXPERIMENTAL

Raw Material Preparation

20 years old of fresh *Acacia mangium* with 30 cm diameter size (average) was obtained from Forest Research Institute Malaysia (FRIM). The top part of the *A. mangium* was taken and was cut into chips using a wood crusher (Mobark, USA). Then, the chips of *A. mangium* were dried in oven at 60 °C until the moisture content was less than 10%. After that, the *A. mangium* chips were ground using a grinder machine and the particles were sieved using a 250 μm mesh filter to obtain fine particles.

Clostridium acetobutylicum YM1 Inoculum Preparation

C. acetobutylicum YM1 used in this study was obtained from Biotechnology Laboratory, Chemical and Process Engineering Department, Universiti Kebangsaan Malaysia (UKM), Bangi, Selangor, Malaysia (Al-shorgani *et al. 2016*). It is a locally sourced stock of bacteria *C. acetobutylicum* YM1. *C. acetobutylicum* YM1 was cultured using a tryptone-yeast extract-acetate (TYA) medium with an initial pH of 6.2. The medium was sterilized at 121 °C, 15 psi for 15 min. Inoculum of *C. acetobutylicum* YM1 was prepared by transferring 1 mL of the spore suspension of *C. acetobutylicum* YM1 source stock into 9 mL of TYA medium and was incubated at 30 °C for 1 to 2 days in anaerobic conditions.

Next, 10 mL of this culture was transferred into 90 mL of TYA medium and further incubated for 18 to 20 h to be used as an inoculum source (Al-Tabib *et al*. 2017). The TYA medium consisted of the following components: 6 g/L tryptone; 2 g/L yeast extract; 3 g/L ammonium acetate; 0.5 g/L KH_2PO_4; 0.3 g/L $MgSO_4.7H_2O$; and 0.01 g/L $FeSO_4.7H_2O$. BioABE production from fermentable sugars of *A. mangium* (total sugar 15 g/L) was compared to BioABE production from TYA medium supplemented with 15 g/L of commercial glucose at same pH value (6.20).

Alkaline Pretreatment of *A. mangium*

A. mangium was treated by sodium hydroxide (5.50 %w/v) at a liquid to solid ratio of 1:20. For this study, 20 g of *A. mangium* samples were mixed with 400 mL of sodium hydroxide solution (NaOH). The treatment was carried out at a reaction temperature of 70 °C for 3 h. Subsequently, treated samples were filtered through Grade 1F filter paper with a diameter of 110 mm (Munktell & Filtrak GmbH, Barenstein, Germany) by using a vacuum pump to separate the *A. mangium* sample from the liquid medium. The treated samples were oven-dried overnight at 60 °C until constant weight (Rafidah 2019).

Enzymatic Hydrolysis of *A. mangium*

Experiments were performed using enzymatic hydrolysis. A treated *A. mangium* sample with a substrate concentration of 10 %w/v was mixed with 100 mL of 0.05 M sodium citrate buffer solution at 4.8 pH and was sterilized at 121 °C, 15 psi for 15 min. Then, multi-enzyme cocktails with a concentration of 4.28 %v/v were added to the medium. Table 1 shows the enzyme activity of multi-enzyme cocktails used in this study. Multi-enzyme cocktails were prepared with a combination of three different species of white rot fungi, which were *Pycnoporus sanguineus*, *Trametes menziesii*, and *Lentinus similis* at mixing ratio of 0.50:0.28:0.22. Crude multi-enzyme cocktail was concentrated with concentration factor of 7.40x and had increased cellulase and xylanase enzymes activity by 21.2% and 22% respectively. The experimental work was carried out at a temperature of 47 °C, 27.60 h of hydrolysis time, and an agitation speed of 112 rpm (Rafidah 2019). After the hydrolysis process was completed, the sample was centrifuged at 6000 rpm using a benchtop centrifuge machine (Sartorius 2-16P, Gottingen, Germany) to separate the supernatant and solid biomass.

Table 1. Enzyme Activity of Multi Enzyme Cocktails

Enzymes	Enzyme Activity (U/mL)
Endoglucanase	22.51
Exoglucanase	22.37
β-glucosidase	0.23
Xylanase	26.27
Cellulase	45.11

Source: Rafidah (2019)

BioABE Fermentation

A process flow diagram of BioABE fermentation is shown in Fig. 2. Approximately 1 mL of *C. acetobutylicum* YM1 cells were cultured in 9 mL of TYA medium and were incubated for 1 to 2 days at 30 °C under anaerobic conditions. This culture was subcultured in 90 mL TYA medium and incubated for 18 to 20 h at 30 °C to be used as inoculum (Al-Tabib *et al.* 2017). Before the inoculation process, the nitrogen gas was purged into fermentation medium (TYA medium/fermentable sugars) to prepare the anaerobic conditions for cell growth and BioABE production.

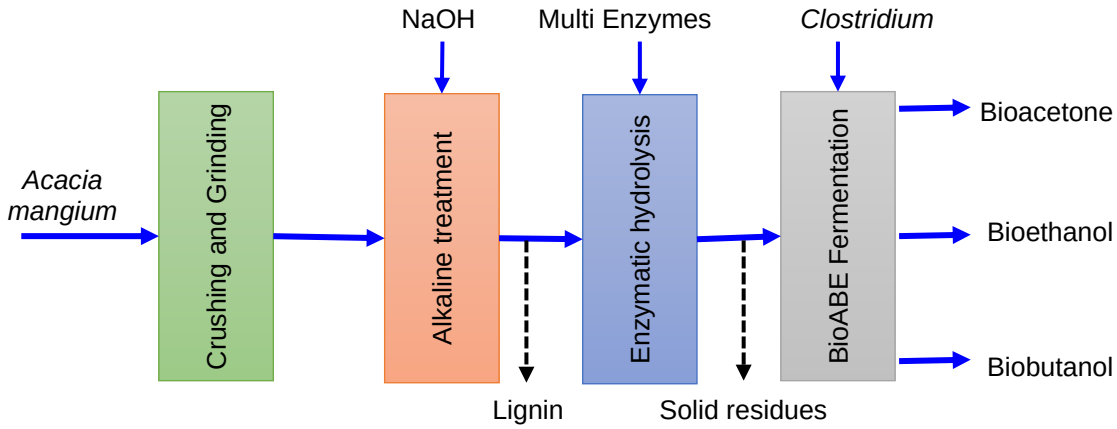

Fig. 2. BioABE production process flow diagram

After that, 10% v/v of *C. acetobutylicum* YM1 inoculum was introduced into 90 mL of TYA medium containing fermentable sugars (initial medium pH was set at

6.20) for BioABE production *via* the fermentation process. The fermentation process was carried out at a temperature of 30 °C for 84 h. Samples were withdrawn in 12 h intervals to measure optical density (OD), pH, sugars, and generated BioABE throughout the fermentation process. Based on Raganati *et al*. (2014) and Ndaba *et al.* (2015), the solventogenesis process starts to produce BioABE at a pH below 5.00. Because the pH of the fermentable sugars was 4.50, the experimental work was done without adjusting the pH, and the result obtained was compared with an experiment at initial pH medium (6.20).

Analytical Methods

The reducing sugar content was determined by heating a mixture of 0.50 mL of sugar solution with 1.50 mL of 3,5-dinitrosalicylic acid (DNS) reagent at 100 °C in a water bath for 5 min and cooled down before 10 mL of distilled water was added. The analysis was done in triplicates. Then, the absorption value (OD) was measured by using a spectrophotometer at 540 nm wavelength. The OD value obtained was compared to the standard curve of glucose to determine the reducing sugar content produced (Miller 1959). For the characterization of fermentable sugars, a total of 2 mL of samples of sugar in a vial was used for analysis using HPLC (Agilent Technologies 1200S, California, USA) equipped with a refractive index detector (RID) using Rezex ROA column. Sulfuric acid (0.005 N) was used as a moving phase at a flow rate of 0.50 mL/min and the column temperature was set at 60 °C.

For the characterization of BioABE, samples were centrifuged at a speed of 10,000 rpm for 10 min to separate sediment and supernatant. BioABE sample analysis was divided into two parts. The first part was an analysis of the growth profile of *C. acetobutylicum* YM1, pH, and reducing sugar using a spectrophotometer. Sample analysis was done every 24 h. The growth profile of *C. acetobutylicum* YM1 was measured at 600 nm wavelength. The second part was BioABE and organic acid product analysis measurement by using gas chromatographic (GC) equipment with a flame ionization detector (FID) (Agilent Technologies 7890A). The injection and detector temperatures were set at 250 °C and 280 °C respectively. Helium gas was used as a carrier gas with a flow rate of 1.50 mL/min (Al-Tabib *et al*. 2017).

RESULTS AND DISCUSSION

BioABE Production from Glucose at 6.2 pH

A preliminary study was carried out to produce BioABE using commercial glucose as a carbon source at 6.20 pH. The obtained result from this study will be used as a baseline for BioABE production from fermentable sugars of *A. mangium*. Figure 3 shows the BioABE production using 15 g/L glucose as the carbon source in TYA medium for a duration of 84 h fermentation time. The experimental results showed that the production of BioABE was interrelated with the growth of *C. acetobutylicum* YM1 cells in the fermentation medium. As shown in Fig. 3, *C. acetobutylicum* YM1 cells underwent a short lag phase (less than 12 h) before entering the exponential phase and then entering the stationary phase between 36 to 48 h, during which there was no considerable increase in OD values before they slowly decreased. During this phase, the growth rate and death rate of cells are equal. The number of new cells created is limited by the growth factor and as a result the rate of cell growth matches the rate of cell death. At 72 h, the cells already entering the death phase and the OD started to decrease (5.5%) from 2.00 until 1.89 at 84 h. The cells consumed the sugar to convert it into organic acid (acetic acid and butyric acid) during acidogenesis phase. The highest

amount of organic acid started to be produced after 24 hours fermentation time (1.48 g/L) which was during the exponential phase of cells growth rate, showing the high ability of the cells to consume the carbon source. Simultaneously, BioABE concentrations were low in the first 24 h and began to rise sharply in the next 36 h with the amount of acids, increasing drastically within the first 24 h of fermentation and accompanied with pH decrease from 6.20 to 4.80. After 24 h, the conversion from the acidic phase to solventogenesis occurred with a slight increase in pH value from 4.80 to 4.97.

In the solventogenesis phase, 2.86 g/L BioABE was produced at 30 °C after 72 h of fermentation. Al-Tabib *et al*. (2017) reported that 10.27 g/L BioABE was produced from 30 g/L glucose after 54 h of fermentation. Besides that, this finding showed a relationship between the production of solvents and pH in which the pH of medium decreased during the acidogenesis process. The total organic acid (acid in the figure refers to acetic acid and butyric acid) accumulation was converted into BioABE products by *Clostridia* bacteria during the solventogenesis phase where the formation of BioABE products was initiated while bacterial cells were in static conditions (Ndaba *et al*. 2015; Roth and Tippkotter 2016). According to Ibrahim *et al*. (2015), the best pH value for the phase change from acidogenesis to solventogenesis is within the range of 4.80 to 6.20, depending on the type of culture medium and bacterial species of *Clostridia*.

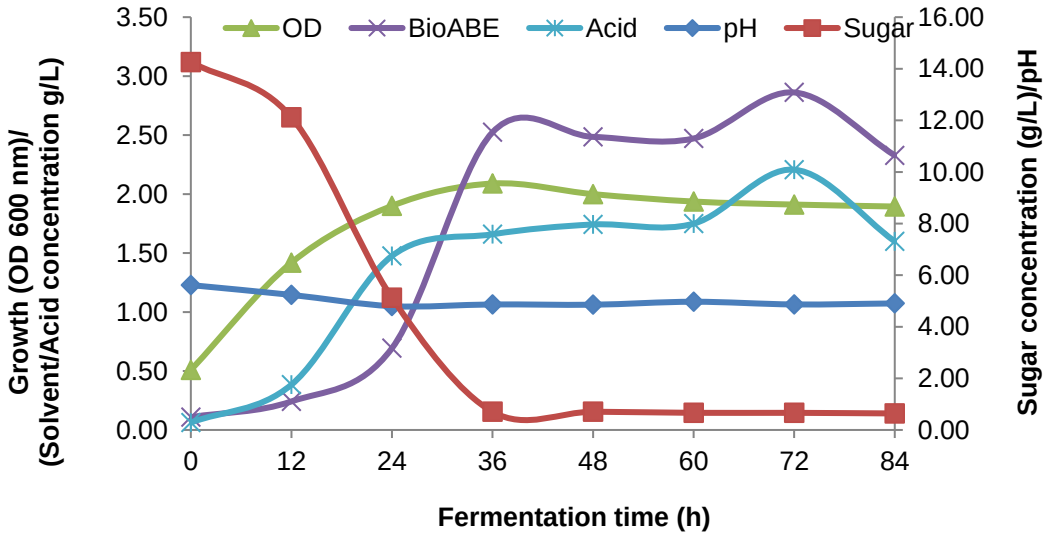

Fig. 3. Profile of BioABE productions from glucose

BioABE Production from Fermentable Sugars of *Acacia mangium* at pH 6.2

BioABE fermentation was carried out by using fermentable sugars released from *A. mangium* using the same experimental condition of glucose with the pH set at 6.20. Figure 4 shows the BioABE production using 15 g/L fermentable sugars for a duration of 84 h fermentation time. The relationship of *C. acetobutylicum* YM1 cell growth in the new fermentation medium on BioABE production was measured. It was found that *C. acetobutylicum* YM1 was able to convert fermentable sugars of *A. mangium* into BioABE.

Referring to Fig. 4, *C. acetobutylicum* YM1 cell underwent a long lag phase before entering the exponential phase and then entered the stationary phase between 60 and 72 h before decreasing slowly. This is due the different sugar composition in fermentable sugars that was obtained after enzymatic hydrolysis (Noomtim and

Cheirsilp 2011). At 84 hours, the cells already were entering the death phase, and the OD started to decrease (3.7%) from 1.62 until 1.56. Besides that, the BioABE concentrations were very low during the first 48 h and began to rise sharply at 60 h with a drastic increase in the amount of acid within 60 h of fermentation time and reached the maximum condition at 72 h. The long lag phase at the early stages was related to the fermentable sugars used in this study, in which bacterial cells took a longer time to adjust to growing in a new medium containing mainly glucose. Nur Syazana *et al*. (2016) suggested maximizing the inoculum size of *C. acetobutylicum* to increase the yield of BioABE and reduce long phases of lagging. Ndaba *et al*. (2017) have studied the biobutanol production using various sizes of inoculum, finding that 6.49 g/L of biobutanol has been produced from sweet sorghum juice using 10% v/v *C. acetobutylicum* compared to 1.90 g/L biobutanol when using 5% v/v of inoculum. Therefore, it is important to study the size of inoculum when using different media.

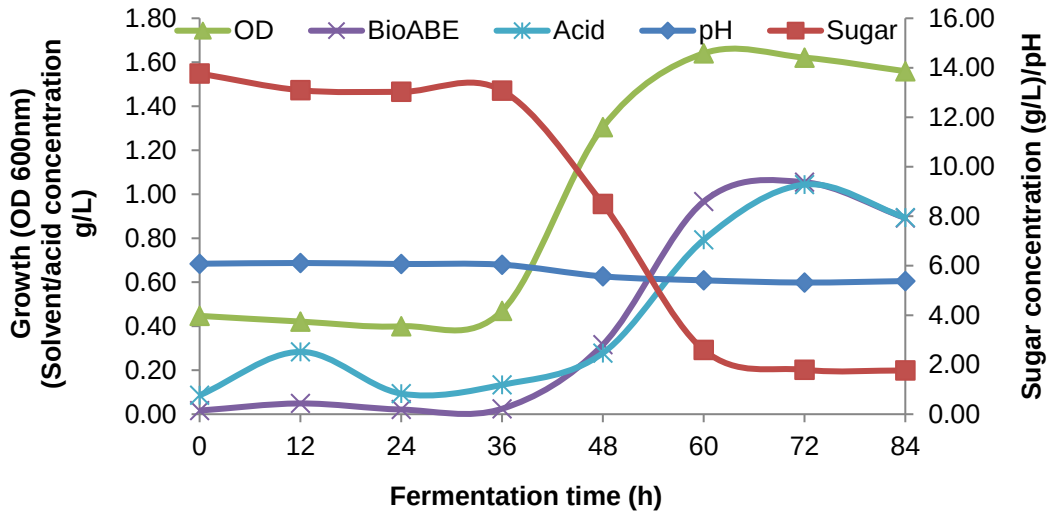

Fig. 4. Profile of BioABE productions from fermentable sugars of *A. mangium*

Moreover, the transition from the acidogenesis to the solventogenesis phase did not occur completely when the pH only decreased to 5.32 with an acid concentration of 1.05 g/L, and as a result, only 0.94 g/L of BioABE was produced at 72 h of fermentation time. In general, the phase change of acidogenesis to solventogenesis only occurred when the medium pH was less than 5.0, where the pH is at the trigger point to the phase change (Ibrahim *et al.* 2015; Xue *et al*. 2017). Besides that, the amount of acids present (1.05 g/L) did not reach the minimum amount (1.50 g/L) required to start the BioABE production (Yang *et al.* 2013). In addition, the presence of a buffer solution affected the production of BioABE by regulating the pH of the medium. A fermentable sugar in this study was produced by using a sodium citrate buffer solution. When the initial pH of the medium was set at 6.2, the presence of the buffer controlled the pH of the medium, which resulted in a pH no less than 5.0 and a small yield of biobutanol (0.77 g/L). Ibrahim *et al.* (2015) reported that BioABE fermentation using 20 g/L glucose with the presence of buffer and pH 5.0 increased the yield of biobutanol by 201% from 0.70 g/L (without buffer) to 2.11 g/L. As compared to the present study, about 4.20 g/L of biobutanol has been produced using 15 g/L fermentable sugars which is much higher (>100%) with 25% lower of glucose.

Comparison of BioABE Production from Fermentable Sugars (*A. mangium*) with Glucose

A summary of BioABE production from two different sources of glucose with an initial pH medium of 6.20 is shown in Table 2. It was found that the BioABE yield produced from fermentable sugars was 67.1% lower than BioABE produced from glucose with only 0.06 g/g BioABE yield. According to Al-Tabib *et al*. (2017), glucose is the most important sugar for the production of biobutanol, followed by mannose.

In addition, the BioABE ratio obtained in this study showed that biobutanol yield using glucose was 15 times higher than bioethanol yield compared to 11 times higher when using 15 g/L fermentable sugars. The ratio of BioABE products reported by Kaminski *et al*. (2011) is 3:6:1, which implies that biobutanol production was six times higher than bioethanol. Liew *et al*. (2006) also reported that the BioABE ratio produced from 30 g/L sago is 4.5:7:1 with biobutanol yield seven times higher than bioethanol. Therefore, the results of this study using fermentable sugars showed a higher BioABE ratio even though a lower concentration of glucose was used. Based on the discussion from the previous section, the lowest production of BioABE from fermentable sugars was influenced by the initial pH of the medium solutions. Al-Shorgani *et al.* (2018) agreed that bacterial growth and BioABE production was affected by the initial pH of the fermentation medium. Due to that, the initial pH was studied to compare the BioABE yield.

Table 2. BioABE using Glucose and Fermentable Sugars from *A. mangium*

Fermentation Characteristics	Glucose	Fermentable Sugars
Glucose (g/L)	15.00	15.00
Biobutanol concentration (g/L)	1.61	0.77
Bioacetone concentration (g/L)	1.15	0.07
Bioethanol concentration (g/L)	0.11	0.10
BioABE concentration (g/L)	2.86	0.94
Total acid (g/L)	2.21	1.16
BioABE yield (g/g)	0.19	0.06
Biobutanol yield (g/g)	0.11	0.05
BioABE productivity (g/(L×h))	0.04	0.01
Biobutanol productivity (g/(L×h))	0.02	0.01
BioABE ratio	11:15:1	1:11:1

Effects of Initial pH on BioABE Production from Fermentable Sugars (*A. mangium*)

The initial pH of the medium significantly affected the production of biobutanol from fermentable sugars. According to Al-Tabib *et al.* (2017) and Al-Shorgani *et al*. (2018), the best pH for biobutanol production was 6.20 using TYA-glucose as the growth medium of *C. acetobutylicum* YM1. The results obtained showed that only 0.77 g/L of biobutanol was produced by 15 g/L of fermentable sugars with the initial pH value of medium 6.20, and the minimum pH achieved was 5.31. Since a pH of less than 5.0 is necessary for the production of biobutanol, the study continued to determine the effect of lowering the pH value. The fermentable sugars obtained from *A. mangium* after the enzymatic hydrolysis process was a fermentable sugars solution consisting of 0.05 M sodium citrate buffer solution. The final pH value of the fermentation sugar solution was 4.50, where it is then continuously fermented into BioABE without adjusting it. The production of BioABE using fermentable sugars at pH 4.50 and 6.20 was studied, and the findings are shown in Fig. 5. It was found that, at lower pH (4.50), BioABE, as well as its individual products: biobutanol, bioethanol, and bioacetone, production was increased by more than 100 %.

Tsai *et al*. (2014) reported that at a lower pH value (4.50), higher biobutanol yields of 11.10 g/L (compared to 5.4 g/L at pH 5.5) were obtained when pH 4.50 was the best parameter condition to increase biobutanol yield. The results of this study also got the same results as when the pH value decreased from 6.20 to 4.50, where the BioABE yield increased by 205% from 0.94 g/L to 2.87 g/L. Yang *et al*. (2013) also noted that it is important to maintain a pH value of around 4.70 to increase the production of biobutanol.

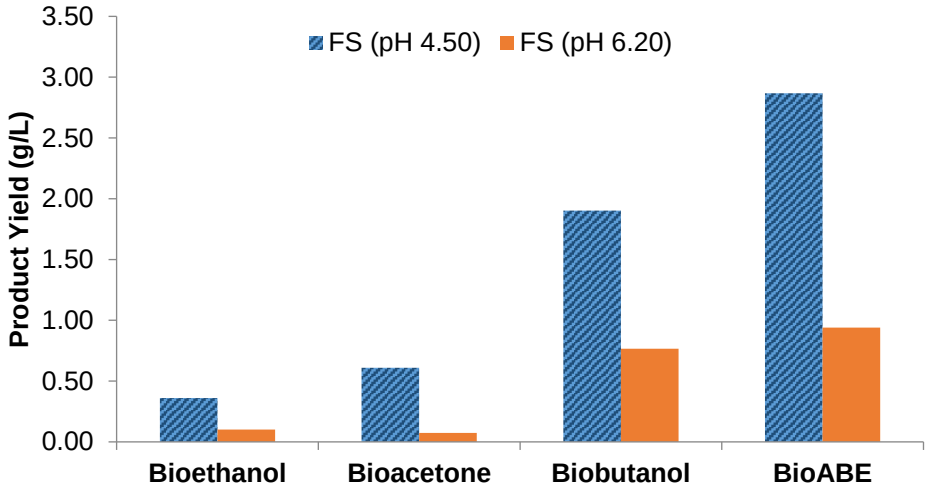

Fig. 5. BioABE productions from fermentable sugars of *A. mangium*

Improvement of BioABE using FSTYA (pH 4.5) was attributed to the addition of TYA to existing medium containing buffer solution. This has further enhanced the capacity of the buffering effect to enhance the cell growth, conversion of substrates to organic acids and solvent production (BioABE) (Ibrahim *et al.* 2015). This buffer solution serves to regulate the pH value, which in turn helps the cell growth to reach a maximum of 60 h at a minimum pH of 4.48 (Yang *et al.* 2013). Tsai *et al.* (2014) stated that pH 4.5 is the best condition for increasing the rate and yield of biobutanol.

According to Ibrahim *et al.* (2015), in the metabolic pathway of *Clostridium acetobutylicum* YM1, acetate in the form of acetic acid is required to produce higher acetone and ethanol. By contrast, butyrate in the form of butyric acid is highly important for the production of butanol. Due to that, it is important to determine the suitable buffer concentration in the medium depending on which BioABE products that we want to produce. In this study, fermentable sugars were produced using sodium citrate buffer with pH 0.05 M, which has a tendency to produce more butanol rather than acetone and ethanol. This finding in line with the results obtained by Ibrahim *et al.* (2015) where buffer concentration more than 0.40 M produced higher acetone and ethanol compared to butanol.

Effects of Additional of TYA Medium on BioABE Production from Fermentable Sugars (*A. mangium*)

Referring to Table 3, when the initial pH of fermentation medium was 4.50, 2.87 g/L BioABE and 1.90 g/L biobutanol were produced. In the present work, 2.86 g/L of BioABE were produced by using 15 g/L glucose with supplementation of TYA medium. Another experimental work was carried out by adding TYA medium components into fermentable sugars medium with pH 4.50. It was found that the concentration of BioABE was increased by 138.3% from 2.87 g/L to 6.84 g/L at 84 h of fermentation time.

Table 3. Comparison of BioABE Yield from Fermentable Sugars of *A. mangium*

Fermentation Characteristics	FS (pH 4.5)	FSTYA (pH 4.5)
Glucose (g/L)	15.00	15.00
Biobutanol concentration (g/L)	1.90	4.20
Bioacetone concentration (g/L)	0.61	2.22
Bioethanol concentration (g/L)	0.36	0.42
BioABE concentration (g/L)	2.87	6.84
Total acid (g/L)	3.45	5.26
BioABE yield (g/g)	0.19	0.46
Biobutanol yield (g/g)	0.13	0.28
BioABE productivity (g/(L×h)	0.03	0.08
Biobutanol productivity (g/(L×h))	0.02	0.05
BioABE ratio	2:5:1	5:10:1

Previously, the fermentable sugars supplemented with TYA and pH set at 6.20 was also studied (Rafidah 2019). It was found that only 0.57 g/L of BioABE was produced. This shows that the pH highly affects the production of BioABE, where the pH reduction from 6.20 to 4.50 increased the concentration of BioABE from 0.57 g/L to 6.84 g/L. This is because at pH values below 5.0, the changing from acidogenesis to solventogenesis phase occurs, which acts to convert organic acid into BioABE products (Ibrahim *et al.* 2015). The effect of external pH (pH of the medium) is one of the key factors involved in triggering the switch from acidogenesis to solventogenesis phase by *Clostridium* (Gheshlaghi *et al*. 2009; Ranjan and Moholkar 2011).

Figure 6 shows the effect of TYA supplementation in fermentable sugars medium on BioABE production. Comparisons were made based on the presence of the TYA medium, where TYA is a nitrogen source to increase the growth rate of *C. acetobutylicum* YM1. It was found that the fermentable sugars with an initial medium pH of 4.50 and in the presence of TYA produced the highest BioABE (6.84 g/L) with 4.20 g/L biobutanol.

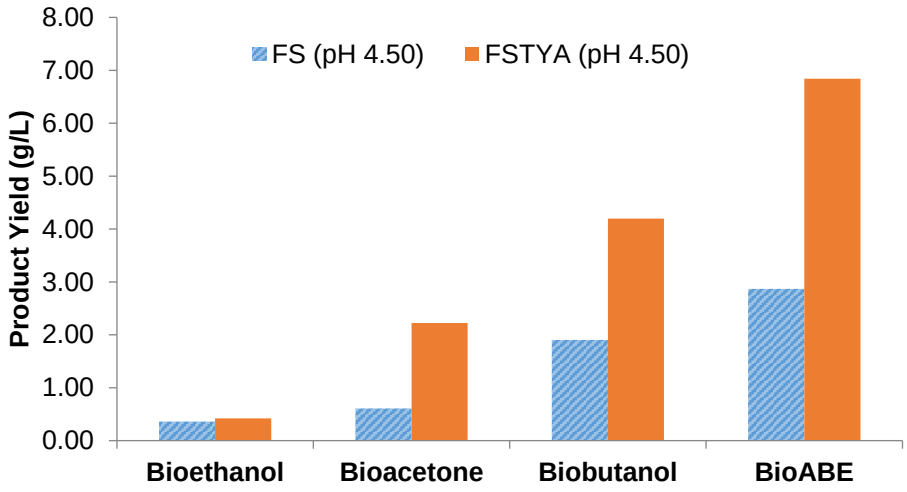

Fig. 6. Effects of supplementation of TYA into fermentable sugars medium

The previous study reported that 0.74 g/L biobutanol was produced from EHPKC (palm oil cottage hydrolysate) after 72 hours of fermentation using 11 g/L of sugar concentration at 6.2 pH (Al-Tabib *et al*. 2017). In contrast, 12.30 g/L biobutanol was produced from 20 g/L glucose after 72 hours of fermentation time with pH value

of 4.40 (Yang *et al.* 2013). In addition, fermentation from rice straw with 10 g/L of glucose concentration produced 1.62 g/L biobutanol after 72 hours with pH 5.5. It was found that the BioABE and biobutanol yield obtained was influenced by the type of medium, initial pH of the medium and glucose content.

Increasing of BioABE production using fermentable sugars supplemented with TYA (FSTYA) (pH 4.50) was due to the addition of TYA to the existing medium containing buffer solution. The addition of this nutrient into the existing medium improved its buffer capacity, which caused an increase in cell growth, substrate exchange to organic acids, and subsequent solvent production (BioABE) (Ibrahim *et al.* 2015). This buffer solution works to control the pH value in which this condition helps the cell growth to reach the maximum value at 60 hours at a minimum pH of 4.48 (Yang *et al*. 2013). Tsai *et al*. (2014) stated that pH 4.50 is the best condition for increasing biobutanol levels and results. The results obtained in this study were much better than biobutanol produced from sago (30 g/L) of 5.09 g/L (Liew *et al*. 2006) compared to 4.20 g/L biobutanol using FSTYA (15 g/L) with BioABE yield 0.30 g/g (sago) and 0.28 g/g (this study) respectively. This may be influenced by the pH factor, where the initial pH of the medium used was 6.00 compared to 4.50 in this study. This confirms that pH significantly affects the production of biobutanol from fermentable sugars. It is also influenced by sodium citrate buffer (0.05 M) solution used during the *A. mangium* enzymatic hydrolysis process using multi enzymes cocktails to produce fermentable sugars.

According to Xue *et al*. (2017), 0.06 M sodium citrate buffer solution produced the highest biobutanol yield of 11.2 g/L compared to 4.00 g/L (0.02 M) and 7.40 g/L (0.04 M). However, the biobutanol yield decreased to 10.10 g/L when 0.08 M buffer solution was used as it inhibited the growth of *Clostridia's* bacterial cells. This buffer solution was also important in controlling the pH changes during the BioABE fermentation process that could improve the use of glucose by bacterial cells and promote conversion from acidogenesis to solventogenesis phase. However, the concentration of buffer solution affected the production of biobutanol in which the concentration of phosphate buffer solution exceeded 0.20 M, where no biobutanol was produced because bacterial cells were unable to maintain their metabolism at a high concentration of buffer solution (Ibrahim *et al.* 2015).

Instead of TYA supplementation into fermentable sugars medium, the addition of glucose could help to increase the biobutanol yield as well as other BioABE products. BioABE products increased from 3.44 g/L to 9.89 g/L when glucose was increased from 3 to 5 %v/v into POME with a glucose concentration of 10 g/L. However, BioABE yield decreased drastically to 2.82 g/L when 5 %v/v of 15 g/L glucose was added into POME (Azima Syafaini *et al*. 2017).

Biobutanol Yield from Fermentable Sugars of *Acacia mangium*

Among the three BioABE products, biobutanol and bioethanol have higher demand as alternative fuels from lignocellulosic biomass. However, the characteristics of biobutanol are similar to that of gasoline due to its high energy content compared to bioethanol. The octane number of biobutanol is in the range of octane number for petrol and blending of biobutanol with petrol not required engine modification. The comparisons of biobutanol yields obtained in this study with other researchers are shown in Table 4.

A total of 0.28 g of biobutanol/g-fermentable sugars of *A. mangium* was produced. These findings are almost the same with the study conducted by Rahnama *et al*. (2014), which produced 0.27 g of biobutanol/g-glucose using *C. acetobutylicum* and rice straw as a substrate. This indicates that a fermentable sugar of *A. mangium* has the

potential to become the carbon source for biobutanol production. Based on the comparison made in Table 4, the highest biobutanol yield was obtained by using *A. mangium* followed by rice straw, and oil palm frond juice as a feedstock.

Table 4. Comparison of Biobutanol Yield

Substrate	Biobutanol Yield (g/g glucose)	References
Acacia mangium	0.28	This study
Glucose	0.18	Tsai *et al*. 2014
Glucose	0.24	Raganati *et al*. 2014
Rice straw	0.27	Rahnama *et al*. 2014
Oil palm frond juice	0.24	Nu Syazana *et al*. 2016
Palm kernel cake (PKC)	0.20	Al-Tabib *et al*. 2017
Reed	0.21	Zhu *et al*. 2015
Empty fruit bunches	0.10	Nur Atheera Aiza *et al*. 2018
Glucose	0.27	Al-Shorgani *et al.* 2018
Switch grass	0.25	Wang *et al*. 2019
Jerusalem artichoke	0.25	Xue *et al*. 2017

CONCLUSIONS

1. Enzymatic hydrolysis of alkaline treated *A. mangium* produced 15 g/L of fermentable sugars including 12 g/L glucose and 3 g/L mannose.
2. *C. acetobutylicum* YM1 utilized fermentable sugars from *A. mangium* and commercial glucose in TYA medium, and it produced different amounts of BioABE solvents. Fermentation of 15 g/L of fermentable sugars resulted in production of 0.94 g/L of BioABE, while fermentation 15 g/L of commercial glucose resulted in the production of 2.86 g/L of BioABE.
3. BioABE yield from *A. mangium* fermentable sugars increased by 205 % from 0.94 g/L to 2.87 g/L when the initial pH value was decreased from 6.20 to 4.50.
4. Nutrients supplementation to the alkaline treated *A. mangium* increased the BioABE and biobutanol yield.

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