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Banana frond juice as novel fermentation substrate for bioethanol production by *Saccharomyces cerevisiae*

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ABSTRACT

Bioethanol has been produced from sugars originating from starchy staple crops such as wheat, sugarcane or corn. However, these sugar- and starch-containing feedstocks are predominantly used for food and feed, which affecting their continuous supply. In Malaysia, banana residual is the second largest agricultural waste after oil palm wastes. Banana fronds need only a milling process for the extraction of sugars to fermentation medium, and ethanol can be produced directly from juice. The sugars and minerals composition of the banana frond juice (BFJ) and the effects of BFJ concentration, addition of nitrogen sources and pH were studied. The pressed juice of the banana fronds was found to contain a total sugar of 14% with the amount of glucose (18.9 g/L), sucrose (13.29 g/L) and fructose (15.63 g/L) with total volume of 0.33 L of BFJ/kg of banana frond. The BFJ at 80% (v/v) supplemented with yeast extract at 15 g/L with optimum pH at 6.8 successfully increased the bioethanol concentration to 42.47 g/L. The optimized fermentation conditions in shake flask were scaled up to 2 L bioreactor. The bioethanol concentration that obtained in the bioreactor system was 45.75 g/L, which is 0.33 g ethanol/g sugar or 65% of the theoretical yield. The results obtained from this study showed the potential of production of sugars from BFJ for the subsequent production of bioethanol and further optimization on the parameters of bioreactor is needed to improve the bioethanol production.

1. Introduction

Bioethanol is an alternative, non-petroleum-based source of energy, as a mean to enhance security of the oil supply and reduce the negative impacts of fossil fuels on the environment. Hence, global demand for bioethanol production is increasing continuously (Awasthi et al., 2015). The primary feedstock for first generation bioethanol is obtained from sugarcane followed by other starchy crops such as corn, wheat, sugar-beet and sorghum. Biofuels productions from these conventional crops are non-sustainable, uneconomical and raise ethical concerns due to primary value of food and feed (Ho et al., 2014). Thus, at present, much focus is on the bioethanol produced from biomass that possesses lignocellulosic content to ensure the production of renewable energy without compromising the need of resource or food for today and next generation (Abdullah et al., 2015). These lignocellulosic raw materials are more abundant and generally considered to be more sustainable.

Many studies and reports related to lignocellulosic bioethanol have been done in recent years. However, the lignocellulosic biomass need to be broken down (hydrolysed) into sugars (mostly pentose and hexoses) prior to production of bioethanol by fermentation. Hence, high cost of enzymes like cellulase, xylanase, mannanase and pectinase is required to release sugars in cellulose and hemicellulose hydrolysis stage, resulting overall process becomes costly (Mood et al., 2013). Therefore, there is an urgent need for an alternative approach of producing renewable sugars from the agro-wastes as fermentation feedstock, whereby the production method does not require harsh pre-treatment steps and the use of enzymes. Production of juices containing free sugar from biomass waste is therefore a good chosen. Juice extraction from biomass waste does not require time-consuming and costly steps such as pretreatment and/or hydrolysis to obtain fermentable sugars. Recently, fresh oil palm frond juice had been used as fermentation substrate for the production of bioethanol (Abdullah et al., 2015), which open a true sight of using agricultural waste juice as feedstock. Thus, this alternative fermentable substrate is considered to be more cost-effective and

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sustainable to support the biotechnology industries in the long term.

Grown extensively in tropics and developing countries, banana plantations are one of the world's most important crops. Banana is one of the highly produced fruits crops, located in Southeast Asia and extends throughout Pacific region (Padam et al., 2014). In Malaysia, banana production ranks fourth after oil palm, rubber and paddy and it is the second most widely cultivated fruit crop. Banana is the common name for the herbaceous plants of the genus Musa and is cultivated mainly for its fruit. The total planted area of banana in Malaysia reached 33,704.2 ha in 2001 (Khalil et al., 2007). Most of the edible bananas are cultivated mainly for their fruits and banana tree has the fact that it bears fruit only once in lifetime. Thus, it loses its agriculture value after the edible banana fruit has been harvested in which the whole plant has turned into underused by-products and wastes. However, the whole part of the plant biomass of the banana is highly contained with sugar (Padam et al., 2014). Therefore, the bulk amount of banana biomass containing high level of sugar can be utilized as renewable substrate in the production of bioethanol.

Several attempts have been made to utilize banana waste in the production of bioethanol. This included the exploitation of banana pseudostem as a feedstock for bioethanol production by *S. cerevisiae* NCIM 3570 under solid state fermentation, which required fungal pretreatment as well as longer fermentation time of 72 h (Ingale et al., 2014). Bioethanol fuel production from rotten banana by *S. cerevisiae* had also been reported. In this study, combination of enzyme (pectinase and cellulase) was used to hydrolyse rotten banana for production of bioethanol (Alshammari et al., 2011). Danmaliki et al. (2016) reported that high ethanol concentration was produced with acid saccharification and fermentation using waste banana peel. Their results have confirmed that the ultimate production of ethanol was pretreatment dependent.

In spite of the above reports there is no information available to date on the direct use of juice extracted from banana waste biomass as the source of fermentable sugars for production of bioethanol. In our previous study, abundant fermentable sugar has been found in the part of banana frond, also known as banana petiole. The frond part of banana itself is soft and easily squished by pressing to extract the juice containing immense of sugar. Hence, the potential of using fermentable sugar in BFJ for direct bioethanol production by *Saccharomyces cerevisiae* was investigated in this study. This study focuses on the characterization of BFJ in terms of sugar composition and minerals content, effect of fermentation parameters for the production of bioethanol in shake flask and bioreactor.

2. Materials and methods

2.1. Source of chemicals and reagents

Yeast extract, peptone, and ammonium sulphate, $(NH_4)_2SO_4$ were purchased from HiMedia (India). Glucose and sodium hydroxide (NaOH) were supplied by QRëC, Malaysia. The yeast strain, *Saccharomyces cerevisiae* Thermosacc® Dry was purchased from Lalleman, USA.

2.2. Sample sources and preparation

The frond part of banana plant (*Musa sp.*) was collected from a local banana plantation located at Bukit Gambir, Penang, Malaysia. The leaves of the fronds were cut and removed. The fronds were cleaned and washed with tap water to remove soils and dirt on the outer surface. The fronds were cut into short segments equivalently prior to juice extraction.

2.3. Extraction of juice from banana fronds

The banana frond segments were weighted and the BFJ was extracted by pressing the fronds using sugarcane juice machine (KT-160 A, 750 W, 50 Hz). All fronds were pressed through the machine for three passes to standardize the juice extraction. The extracted BFJ was then centrifuged at 10,000×*g* for 15 min at 4 °C using centrifuge machine (Kubota 6500, Japan). The resultant supernatant was filtered using filter Whatman Grade 1 filter paper (11μm) to discard the remaining undesired substances such as fiber debris and solid particles contained in the supernatant. The filtered BFJ was stored at −4 °C for subsequent experiment use. The juice was extracted within a day after the fronds had been harvested to obtain fresh juice feedstock.

2.4. Stock culture and inoculum culture preparation

Stock culture was prepared by adding 0.1 g of *S. cerevisiae* Thermosacc into 50 mL autoclaved Yeast Extract Peptone-Dextrose (YPD) broth containing peptic digest of animal tissue 20 g/L, yeast extract 10 g/L and dextrose 20 g/L with pH 6.5 ± 0.2. The culture was incubated in incubator shaker (Infors HT Ecotron, Switzerland) at 30 °C, 150 rpm for 4 h where the optical density (OD) reading at 600 nm reached about 0.4 using spectrophotometer (Hitachi U-1900, Japan). Subsequently, 0.5 ml stock culture was transferred to cryovial and maintained in 50% (v/v) glycerol aseptically at −20 °C. The inoculum culture was prepared by inoculating 2% (v/v) of stock culture into 50 mL YPD broth in a 250 ml shake flask. The culture was incubated in incubator shaker (Infors HT Ecotron, Switzerland) at 150 rpm, 30 °C for 16 h prior to inoculation to the production medium.

2.5. Bioethanol production by S. cerevisiae using BFJ in shake flask

To evaluate the feasibility of using BFJ as substrate for bioethanol production by *S. cerevisiae*, medium formulations comprised of different concentration of BFJ (%, v/v), types of nitrogen sources, concentrations of nitrogen sources (g/L) and initial pH were studied. Five concentrations of BFJ (20%, 40%, 60%, 80% and 100%) were chosen in this study as production medium. Different concentrations of BFJ were prepared by adding distilled water with BFJ in the shake flask with respective volume to obtain desired concentration. Three types of nitrogen sources (peptone, yeast extract and ammonium sulphate) at the range from 3 to 15 g/L were then investigated. The initial pH of the BFJ media was adjusted to 4, 5, 6, 7, 8 and 9 by 0.1 M NaOH and 0.1 M H_2SO_4. In all experiments, 50 mL of production medium in 250 mL shake flask was inoculated with 10% (v/v) inoculum and cultivated in incubator shaker at 30 °C, 150 rpm. During the fermentation, 200 μL of sample was withdrawn at time intervals for OD reading using microplate reader, and 1.7 mL of sample was taken for the determination of ethanol concentration. All samples were stored at -4 °C before analysis. All experiments in this study were performed in triplicates.

2.6. Bioethanol production by S. cerevisiae using formulated BFJ in 2-L bioreactor

A standard 10% (v/v) of inoculum was inoculated into the BFJ which had been formulated in shake flask and cultivated in 2-L bench-top Minifors Bioreactor with total working volume of 1 L. The bioreactor was equipped with a pH, temperature and dissolved oxygen monitoring and control system. The fermentation process was agitated at 100 rpm (impeller tip speed (πND) = 0.3 m/s) with air flow rate of 1 vvm and initial O_2 concentration at 30% (Lee and Halim, 2014). The temperature and culture pH within the bioreactor vessel was controlled at 30 °C and 6.8 throughout the fermentation of 57 h. During the fermentation process, 2 mL of sample was taken at different time intervals for analysis of the growth of *S. cerevisiae* and bioethanol production. Samples were stored at -4 °C if not used immediately.

2.7. Determination of sugar composition of BFJ

Four main types of fermentable sugars in BFJ content such as glucose, fructose, sucrose and xylose were determined by high

performance liquid chromatography (HPLC) (Shimadzu LC, Japan) equipped with RI detector using APS-Hypersil column (diameter of 250 mm × 46 mm) (Lee and Halim, 2014). The mobile phase used was 70% HPLC-grade Acetonitrile (Lichrosolv, Merck). The temperature of the oven was set at 40 °C. The pump flow rate was set constant at 0.6 mL/min and the maximum pressure was at 10MPa.

2.8. Determination of mineral content using Inductively Coupled Plasma Optical Emission Spectrometer (ICP-OES)

Inductively Coupled Plasma equipped with Optical Emission Spectrometer was used to analyse mineral content in fresh BFJ (Akpinar-Bayizit et al., 2010). The minerals to be analysed were silver(Ag), aluminium(Al), boron(B), copper(Cu), iron(Fe), potassium(K), lead(Pb), sodium(Na), magnesium(Mg), and zinc(Zn). The concentration of the minerals element was analysed in mg/L. A standard curve was prepared using multi-elements solution containing the desired elements specifically for ICP-OES/AAS by diluting it into 5, 10, 15, 20, 25 ppm with ultra-pure water. The sample was diluted using ultra-pure water at 100 dilution factor. Before the sample was injected into ICP-OES/AAS, the wavelength of all element of mineral had to be determined and set in the system.

2.9. Determination of ethanol using gas chromatography system

Gas Chromatography (GC) (Shimadzu, Japan) equipped with flame ionization detector (FID) was used to analyse all samples taken from fermentation for determination of ethanol percentage (%, v/v) (Lee and Halim, 2014). The column used was RT-Q-BOND (inner diameter of 0.32 mm). Helium (He) was used as the carrier gas. The temperature of column oven was set at 200 °C. The total flow rate was 21.9 mL/min and the operating pressure was 71.1 kPa. The holding time for ethanol determination for each sample was set at 5 min.

3. Result and discussion

3.1. Extraction of BFJ from banana fronds

From juice extraction, approximately 33% (v/w) filtered BFJ was obtained (Table 1). Total 37.5% (w/v) of insoluble content was removed by filtration. Compared to oil palm frond juice, amount of BFJ recovered is 13.4% lower than oil palm frond juice (OPFJ) recovered which was 46.4% (v/w). There is no report so far on the amount of extracted juice from banana frond. The advantage of using juice extracted from banana frond, oil palm frond or other secondary generation feedstock as fermentation substrate is that the pre-treatment on the lignocellulosic part such as grinding, heating, acid/alkaline hydrolysis or enzymatic hydrolysis could be eliminated. The BFJ obtained after pressing was light green colour, considered being chloroplast content. The BFJ formed two layers after stored for 2 h as the insoluble particles and debris were sediment (Fig. 1A). A cloudy BFJ could be observed before as compared to BFJ after filtration as shown in Fig. 1B. The colour of BFJ after filtration showed yellowish which could be due to the degradation of chlorophyll or removal of chloroplast content.

Table 1
Material balance of BFJ extraction from banana fronds.

Material balance	Weight
Total wet weight of banana fronds before pressed, (kg)	4.50
Total wet weight of banana fronds after pressed, (kg)	2.1
Total volume of fresh BFJ, (L)	1.50
Residues (Losses after centrifugation and filtration), (kg)	0.90
Amount of BFJ recovered per kg banana frond, (L)	0.33

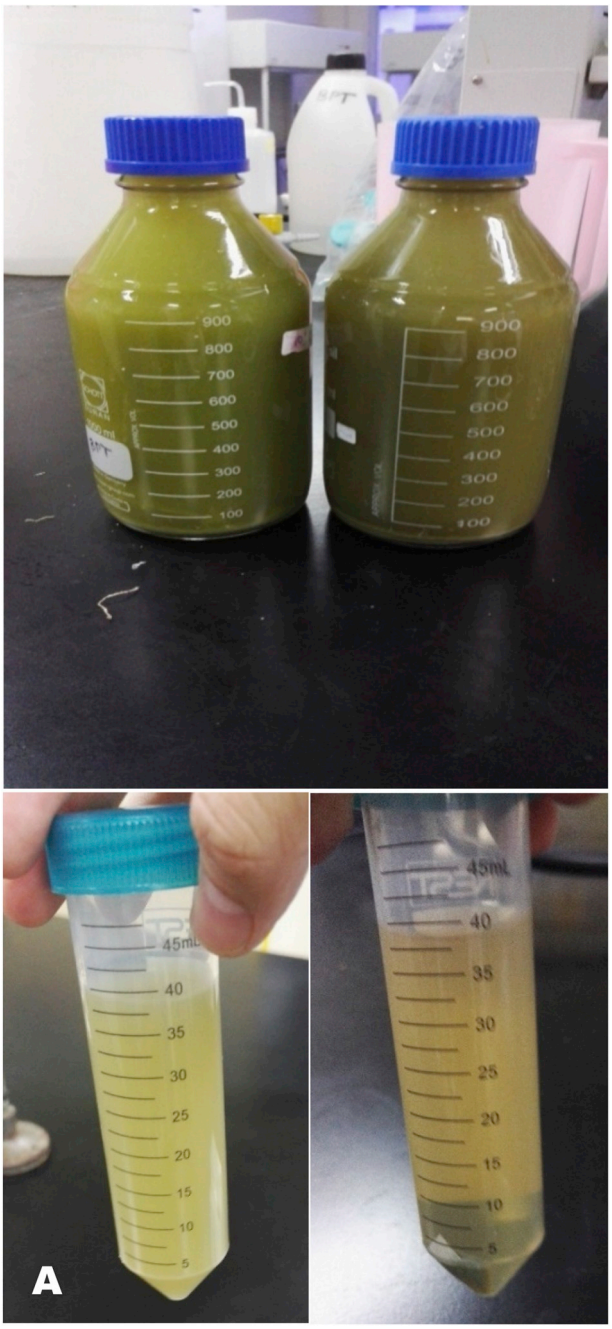


Fig. 1. Banana frond juice obtained directly after pressing. The BFJ condition before (A) and after (B) filtered.

3.2. Total fermentable sugar content and minerals content in BFJ

In Table 2, three main types of fermentable sugars was identified in the BFJ with their respective concentrations using HPLC. Three sugar monomers that found in BFJ were monosaccharide (glucose and fructose) and disaccharide (sucrose). These are the sugars that commonly used as the substrate in the fermentation which favourable in the production of bioethanol by yeast. Apart from the monosaccharide, yeast also has been studied to have the ability on utilizing disaccharide sugars such as sucrose which also found in BFJ. From the sugar composition analysis, glucose concentration was found to be the highest (39.51%) in the BFJ, followed by fructose (32.69%) and then sucrose (27.80%). However, other types of sugar such as xylose, arabinose, rhamnose and mannose were not identified in this study due to some constraints.

Table 2
Sugar composition of fresh BFJ, OPFJ and sugarcane juice.

	BFJ	OPFJ	Sugarcane juice
	Sugar concentration (g/L)		
Glucose	18.90	41.78	34.24
Sucrose	13.29	9.85	75.26
Fructose	15.63	3.27	25.98

The sugar composition of OPFJ and sugarcane juice was adapted from Abdullah et al. (2015).

As comparison to OPFJ and sugarcane juice which reported by Abdullah et al. (Abdullah et al., 2015), the glucose (18.90 g/L) in BFJ in this study was 2.2-fold and 1.8-fold lower than OPFJ (41.78 g/L) and sugarcane juice (34.24 g/L), respectively. Fructose showed higher concentration (15.63 g/L) in BFJ compared to OPFJ (3.27 g/L) while the sucrose in BFJ was 35% higher than OPFJ. The result is in agreement with the research founded by Mohapatra et al. (2010) which stated that glucose in banana frond or petiole contained the highest concentration compared to other sugars. The total amount of free sugars in BFJ indicated that it could be a promising feedstock as it contained crucial sources of carbohydrates that can be utilised in yeast fermentation to contribute in the bioethanol production.

Minerals content in the BFJ were analysed using ICP-OES as shown in Table 3. BFJ contained significant amount of macroelements (K, Na and Mg) and trace amount of microelements (Fe, Cu, Zn and B). The presence of these macroelements (K, Na and Mg), microelements (Fe, Cu, Zn and B) in the growth media was considered to be crucial in microbial fermentation as they have a significant effect to the growth rate of yeast, substrate consumption and the product yield formation (Walker and Stewart, 2016). Potassium containing salts are less inhibitory effect to yeast than the salts with sodium or ammonium. Under normal growth conditions yeast maintain a low intracellular sodium concentration. However, potassium is needed for many different physiological functions. Therefore, yeast maintained high intracellular potassium content. At potassium concentration of 0.1M (3909 mg/L), the productivity of bioethanol was about 1.5 g/L/h, which was ~20% higher than other salts. At a potassium concentration of 0.5M, the productivity was reduced by nearly 50% on average when compared to the control (no salt addition) (Casey et al., 2013). Our result showed that BFJ had substantial amount of potassium (4114 mg/L) compared to other elements (<100 mg/L) but the concentration of potassium did not exceed the toxicity level. In addition, the bioethanol yield in BFJ was comparable to OPFJ and sugarcane juice. Hence, BFJ is a potential fermentation substrate for the production bioethanol by *S. cerevisiae* (see Table 4).

Table 3
The concentration of minerals content in BFJ.

Minerals Element	Concentration (mg/L)
K	4114.00
Na	34.50
Mg	80.00
B	3.40
Cu	0.59
Fe	3.30
Zn	0.27
Al	11.70
Pb	0.32
Ag	0.70

Table 4
Bioethanol yield of fresh BFJ, OPFJ and sugarcane juice.

	Bioethanol yield per consumed sugars (g/g)
BFJ	0.33 ± 0.02
OPFJ	0.38 ± 0.03
Sugarcane juice	0.38 ± 0.01

The sugar composition of OPFJ and sugarcane juice was adapted from Abdullah et al. (2015).

3.3. Effect of BFJ concentration on bioethanol production by S. cerevisiae in shake flask

Five different BFJ concentrations (20%, 40%, 60%, 80% and 100%) were used in this study. Fig. 2 reveals that the growth of *S. cerevisiae* and bioethanol production increased with the increasing concentration of BFJ from 20% (v/v) and reached maximum bioethanol production (35.32 g/L) at 80% (v/v). Further increase in the concentration of BFJ (>80%) resulted in 54% decrease in bioethanol production (19 g/L). The maximum growth (7.08 g/L) was obtained at 80% (v/v) BJF and decrease to 5.16 g/L when BFJ concentration more than 80% (v/v). This could be due to several factors, for example the presence of heavy metals in BFJ such as aluminium as shown in Table 3, giving detrimental effect to the growth of yeast. The presence of aluminium in sugar cane juice, reduced the ability of yeast to ferment leading to a lower production of bioethanol (Basso et al., 2011). In addition, the banana frond juice might contain the commonly exist lignocellulose-derived inhibitors including phenolic carboxylates, phenolic amides (for ammonia-pretreated biomass), phenolic aldehydes, and furfurals, which can have a negative influence on ethanol production. Inhibitors create severe environments, seriously weakening fermentative microbes and lower volumetric ethanol productivity (Sims et al., 2010). However, there was a study showed that no clear relationship between ethanol content and the concentration of heavy metals present in sugarcane juice containing heavy metals. This suggested that the heavy metals contained in the juices did not adversely affect the production of ethanol (Xie et al., 2014). Another study by Fakruddin et al. (2012) showed that the highest concentration of ethanol (86.90 g/L) was obtained with initial reducing sugar concentration 55 g/L. The increase or decrease of initial reducing sugar concentration from 55 g/L results a decrease of ethanol concentration. Hence, the initial sugar content was an important factor that influenced the bioethanol production. This could explain the optimum concentration of sugar content is needed for the bioethanol production by *S. cerevisiae*. An increase or decrease of initial sugar concentration could result a decrease of bioethanol production. The importance of the optimum concentration of BFJ in providing a favourable environment for the growth of yeast could be observed. A comparison study using a commercial medium, YPD broth which is a ready-to-use liquid growth media used for yeast culture was also conducted. The growth of yeast (5.15 g/L) and bioethanol production (28.8 g/L) when cultivated in YPD medium was about 1.6-fold lower compared to BFJ at 80%. The BFJ contained many important minerals (macronutrients and micronutrients) for the growth of yeast which unlikely present in YPD medium. These results are in accordance with the study of Palukurty et al. (2008), who also reported the addition of trace amounts of metals into the fermentation medium significantly increased the ethanol production.

3.4. Effect of addition of nitrogen sources on bioethanol production by S. cerevisiae in shake flask

The effect of addition of nitrogen sources to the BFJ was investigated (Fig. 3). The selected types of nitrogen sources namely ammonium sulphate, yeast extract and peptone were added in the BFJ of 80% and the concentration of nitrogen sources was varied from 3 g/L to 15 g/L. Addition of peptone at concentration of 15 g/L gave the highest value on the growth of yeast (12.12 g/L). By comparing different yeast extract concentrations on the bioethanol production, this study found that *S. cerevisiae* can grow effectively (10.61 g/L) with a minimum concentration of yeast extract of about 3 g/L. On the other hand, addition of ammonium sulphate had the least impact to the growth of yeast compared to yeast extract and peptone. Fig. 3 shows addition of yeast

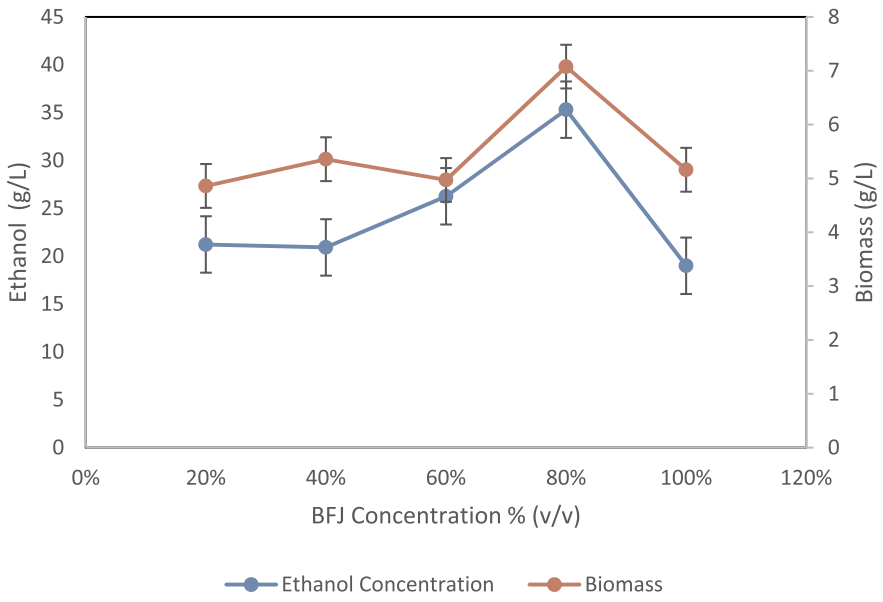


Fig. 2. Production of bioethanol by *S. cerevisiae* at different concentration of BFJ. Error bars represent standard deviation.

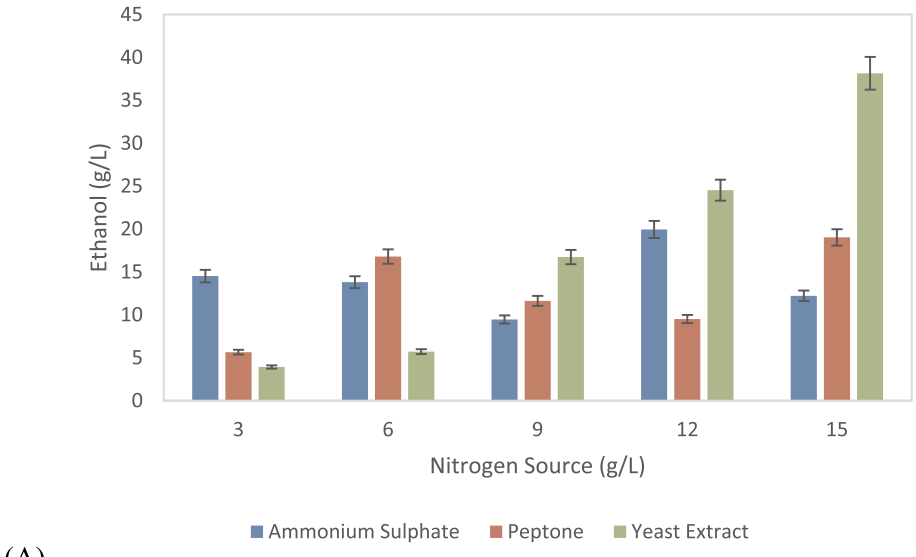
extract in BFJ increased the bioethanol production with increasing concentration of yeast extract. At 15 g/L of yeast extract, the highest bioethanol concentration (42.47 g/L) was produced by *S. cerevisiae*. It was about 3.8-fold higher than bioethanol concentration produced in BFJ with ammonium sulphate (19.96 g/L) and peptone (19.02 g/L). In this study, we could observe that peptone is favourable for the growth of yeast while the presence of yeast extract increased both growth of yeast and production of bioethanol. On the contrary, ammonium sulphate supplementation did not enhance ethanol production by the yeast. One reason for enhanced ethanol production with yeast extract supplementation was the presence of important cofactors like biotin and riboflavin (Ortiz-Muñiz et al., 2010). The addition of yeast extract in the fermentation medium greatly increased the production of ethanol (2.17%) by *S. cerevisiae* at minimum concentration of yeast extract of about 2 g/L as described by Sheikh et al. (2016). Our result also in accordance to the study of Akaracharanya et al. (2011) in which the ammonium sulphate supplementation to the hydrolysate of cassava pulp (a waste from cassava starch production) did not improve the ethanol production by the yeast. Hence, yeast extract was added as the nitrogen source in BFJ at concentration of 15 g/L in further experiment.

3.5. Effect of initial pH on bioethanol production in shake flask

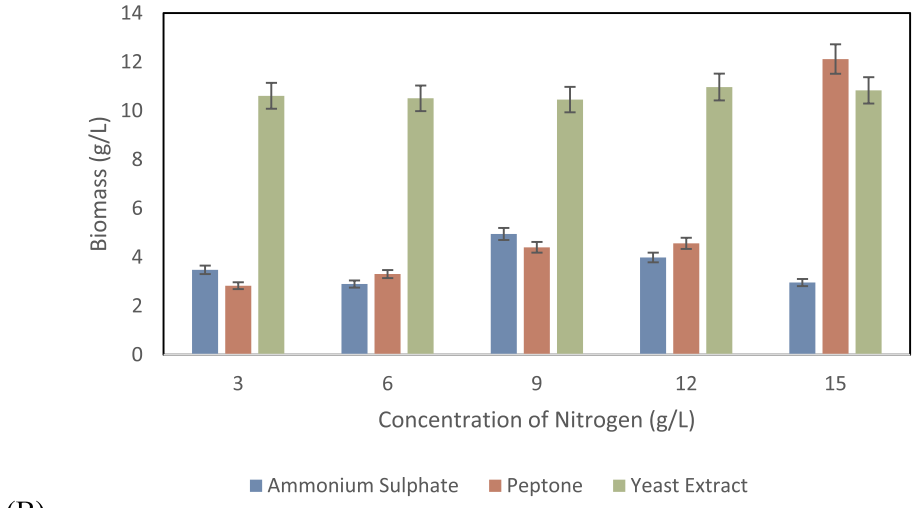
By using the 80% (v/v) BFJ and 15 g/L of yeast extract, the effect of initial pH on bioethanol production was investigated. BFJ has a pH at 6.8 and the pH of BFJ was adjusted ranging from 4, 5, 6, 7, 8, and 9. In Fig. 4, the highest of growth of yeast and ethanol production were obtained at BFJ without adjusting pH (pH 6.8), which were 9.58 g/L and 42.47 g/L, respectively. The bioethanol production was increase from pH 4 to pH 6.8 and decrease at pH 8 (27.37 g/L). Further increase in pH from 8 to 9 resulted in substantially decrease in the production of bioethanol (20.13 g/L) as well as the growth of yeast. This result is in accordance with the study of Tsunatu et al. (2017) in which the yield of bioethanol was decrease when the initial pH increased to 7. At higher pH such as pH 8 and pH 11, the bioethanol production was decreased dramatically (Togarepi et al., 2012). The inhibitory effect of pH (at the high level) on the ethanol yield could be due to the low ATP production during the metabolic changes in *S. cerevisiae* as the enzymes in yeast cells are more active in mildly acidic medium (Tsunatu et al., 2017). Our result also showed the pH of BFJ at 4 had the lowest bioethanol production (13.67 g/L) presumably because of low pH encourages the production of acid instead of alcohol. This could be due to the formation of acetic acid which inhibit the production of ethanol by yeast was enhanced with the decrease of pH. In addition, the hydrogen ion concentration in fermentation broth can change the total charge of plasma membrane affecting the permeability of some essential nutrients into the cell (Lin et al., 2012). Most of the studies revealed that the optimum pH range used in fermentation for ethanol production by *S. cerevisiae* is 4.0–5.5 (Tsunatu et al., 2017; Le, 2014; Nuanpeng et al., 2016). Different pH also had been reported by Fakruddin et al. (2012) in which pH 6.0 was selected as optimum pH for the production of ethanol by *S. cerevisiae* IFST-072011. However, their study did not further investigate the pH beyond 6 on the production of ethanol. In some cases, maximum ethanol production could be achieved at pH close to 7. For example, Udhayaraja and Sriman (2012) reported maximum ethanol yield was achieved at pH 6.5 using sorghum stovar by *S. cerevisiae*. Gabriela Bonassa et al. (2015) also revealed that the industrial yeast capable to tolerate pH at range of 4 to close to 7. This is in agreement to our result in which the growth of yeast was increased from pH 4 to 6.8.

3.6. Bioethanol production by S. cerevisiae in 2L bioreactor

A preliminary study on the bioethanol production by *S. cerevisiae* in 2L bioreactor was conducted using optimized parameters obtained from shake flask which comprises of 80% of BFJ concentration and 15 g/L of yeast extract at pH 6.8. Fig. 5 shows that the highest bioethanol production (45.75 g/L) was at 15 h while the highest growth of yeast (5.95 g/L) was at 42 h. A lag phase was occurred in the growth of *S. cerevisiae* from 0 h to 15 h, followed by exponential growth phase of *S. cerevisiae* from 15 h to 42 h of fermentation time. The lag-phase, which is typical for all newly inoculated cultures, is a stress-induced adaptation period. This could be due to the new culture medium results in a change in osmotic pressure and also new nutrient balance (Bauer and Pretorius, 2000). At exponential phase, the reproduction and metabolism of yeast were at higher rate. For bioethanol production, it was increased from 0 h until 15 h of fermentation time and decreased to 23.59 g/L at 57 h of fermentation time. The profile showed that the growth of *S. cerevisiae* continued to increase after the glucose was depleted. The could be due to the switching utilization of *S. cerevisiae* from glucose to other sugars in BFJ as the glucose in BFJ was completely depleted at 18 h. *S. cerevisiae* has a natural preference for glucose as a carbon source. The utilization of other carbon and energy sources is strongly repressed as long as there is glucose present (Conrad et al., 2014; Carlson, 1999). This becomes a problem in second generation bioethanol production, since



(A)



(B)

Fig. 3. Production of bioethanol by *S. cerevisiae* using BFJ with addition of nitrogen sources (g/L) at different concentrations. Error bars represent standard deviation.

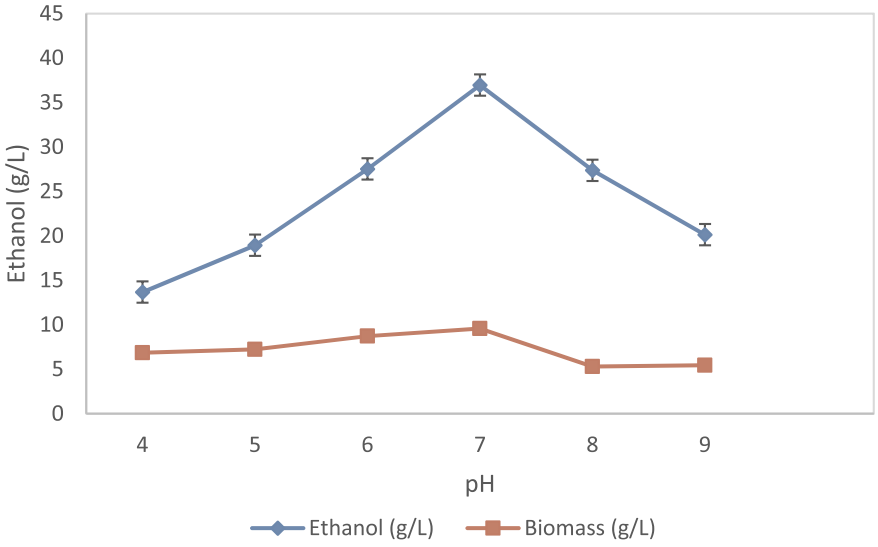


Fig. 4. Production of bioethanol by *S. cerevisiae* at different initial pH of BFJ. Error bars represent standard deviation.

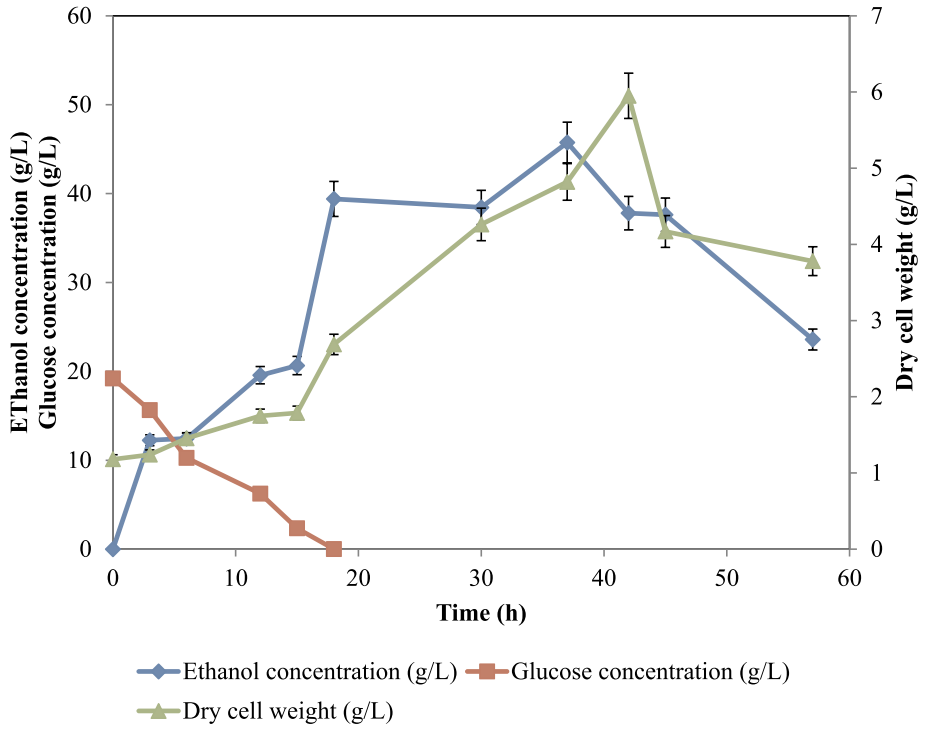


Fig. 5. The profiles of bioethanol production (g/L) and growth of *S. cerevisiae* in bioreactor system. Error bars represent standard deviation.

lignocellulosic hydrolysates typically contain several sugars. Other sugars are not utilized until most of the glucose is consumed, leading to long fermentation times (Krahulec et al., 2010).

BFJ was proven to contain desirable amount of total fermentable sugar (14%); glucose, fructose, and sucrose. The BFJ had been successfully optimized and formulated for the maximum production of bioethanol *S. cerevisiae* in shake flask system. The formulated BFJ was 80% (v/v) BFJ concentration with 15 g/L of yeast extract at pH 6.8. The *S. cerevisiae* was found to have high capability in producing ethanol (45.75 g/L) in bioreactor using formulated condition of the BFJ. Based on the research done, the potential of BFJ to be used as a cheap and non-food fermentation feedstock can be further studied for the improvement of bioethanol concentration and other biotechnological products.

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