Hydrolysis of alkaline pretreated banana peel

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Abstract. Banana peel is one of food wastes that are rich in carbohydrate. This shows its potential as fermentation substrate including bio-ethanol. This paper presented banana peel alkaline pretreatment and enzymatic hydrolysis. The pretreatment was intended to prepare banana peel in order to increase hydrolysis performance. The alkaline pretreatment used 10, 20, and 30% w/v NaOH solution and was done at 60, 70 and 80°C for 1 hour. The hydrolysis reaction was conducted using two commercial cellulose enzymes. The reaction time was varied for 3, 5, and 7 days. The best condition for pretreatment process was one conducted using 30% NaOH solution and at 80°C. This condition resulted in cellulose content of 90.27% and acid insoluble lignin content of 2.88%. Seven-day hydrolysis time had exhibited the highest reducing sugar concentration, which was 7.2869 g/L.

1. Introduction
The environmental damage and fossil fuel reserve decline issues have led to the quest for renewable and environmentally friendly fuel. Bio-ethanol is one of both renewable and environmentally more-friendly fuel compared to fossil fuel. Many carbohydrate-rich agricultural and food waste can be converted biochemically into more economic valuable products through fermentation including bio-ethanol.

Naturally abundant carbohydrate-rich wastes are grouped into lignocellulosic materials as they contain lignin, cellulose and hemicelluloses. These materials are difficult to degrade naturally as they have a strong structure and therefore compose the outer parts of plants and plant products[1]. Nevertheless, with proper processing, lignocellulosic materials are useful to produce fuels, enzymes, organic acids, bio-sorbents, bio-composites, feeds, and medicines [2]. Many researchers have currently focused on the utilization of lignocellulosic waste to produce bio-ethanol as renewable fuel. Banana peel is one of lignocellulosic agricultural food waste and therefore can be used as fermentation substrate including bio-ethanol. This waste is produced by several Banana flour industries in Indonesia as well as other banana processing small business. However, current banana peel utilization in Indonesia seems only limited for livestock feeds.

Bioconversion of lignocellulosic material into bio-ethanol is a three-step process. The steps are pretreatment, hydrolysis and fermentation. The pretreatment process is conducted to disrupt lignin-carbohydrate bond, lower cellulose degree of crystallinity, improve cellulose surface and therefore increase the enzyme access to cellulose [3,4]. Alkaline and acid pretreatment are widely used method to treat lignocellulosic material prior to hydrolysis [5,6]. Alkaline pretreatments results in less hemicelluloses solubilisation and inhibitory compounds formation compared to acid pretreatment [7]. Coconut coir alkaline pretreatment had increase cellulose content from 41.7 to 48.75% [8].
The hydrolysis step breaks β-1,4 glycosidic bonds of cellulose to produce glucose. This process requires the work of cellulose enzymes produced by cellulose-degrading microorganisms, mainly mold. Cellulose is a group of enzymes consisting of exo-1,4-β-d-glucanases or celllobiohydrolases (CBH) (EC 3.2.1.91), which moves along the cellulose chain and cuts cellobiose units from the ends; endo-1,4-β-β-d-glucanases (EG) (EC 3.2.1.4), which hydrolyses internal β-1,4-glucosidic bonds randomly within the cellulose chain; and 1,4-β-d-glucosidases (EC 3.2.1.21), which hydrolyses cellobiose to glucose [9]. Some cellulose enzymes produced by various microorganisms have been available commercially.

This research investigated the effect pretreatment condition as well as the hydrolysis reaction time on the reducing sugar production from local banana peel in Indonesia. The pretreatment condition varied was alkaline solution concentration and pretreatment temperature.

2. Materials and methods

2.1. Materials
Banana peels used in this research were steam-cooked “Raja Nangka (Musa paradica)” banana peels, obtained from local banana flour processing industry. Two commercial enzymes were used in hydrolysis process. The first enzyme was celluclast® 1.5L (EC 3.2.1.4, Sigma Aldrich), an endoglucanase produced by Trichoderma reesei with activity ≥ 700 units/g. The second enzyme was Novozyme 188 (EC 3.2.1.21, Sigma Aldrich), a β-D-glucosidases produced by Aspergillus niger with activity ≥ 250 units/g.

2.2. Banana peel powder preparation
Banana peels were sundried for six hours prior to grinding. The dry banana peels were then cut into ± 5 cm wide chips. The chips were ground using FFC 23A disc mill machine and then screened to obtain 70 mesh particles.

2.3. Alkaline pretreatment
The alkaline solution used was NaOH solution. The concentration of sodium hydroxide solution was varied into 10, 20, and 30% w/v while banana peel powder slurry concentration was constant at 7.5% w/v. The pretreatment was conducted for one hour and at varied temperature of 60, 70, and 80°C. After the pretreatment process, the powder was filtered and washed until neutral pH of filtrate was obtained.

2.4. Enzymatic hydrolysis
Hydrolysis was conducted in 250 mL flask with total working volume of 150 mL containing 3 g banana powder. The pH was maintained 4.8 by using sterile citrate buffer solution. The reaction was conducted at constant temperature of 50°C with varied reaction time of 3, 5 and 7 days. An Enzyme mixture consisting of 0.495 mL celluclast® and 0.495 mL Novozyme 188 was used. In order to inhibit microbial growth, 40 µg/mL tetracycline was added to the mixture. To ensure homogenous condition, hydrolysis was done on an orbital incubator shaker with rotation speed of 150 rpm. At the end of hydrolysis reaction, the flask was immersed in 95-100°C hot water bath for 10 minutes to inactivate the enzymes.

2.5. Chemical analysis
The cellulose and lignin content of banana peel before and after pretreatment were determined using modified method of NREL/TP-510-42618[10], developed by US National Renewable Energy Laboratory (NREL). The modification was in the omission of HPLC analysis to determine total carbohydrate content. Instead, total carbohydrate content was obtained from the difference weight of initial sample from oven dried powder sample after sulfuric acid treatment. The cellulose content was determined using the same method except that the samples were treated first using an amylase enzyme.
prior to total carbohydrate and lignin analysis using NREL method. Reducing sugar concentrations measurement after enzymatic hydrolysis was done using Dinitrosalicylic Acid reagent [11].

3. Results and discussion

3.1. Banana peel composition
The dried and milled banana peel powder had been analyzed for its content of lignocellulosic compounds. The percentage of these compounds is presented in Figure 1.

Figure 1 shows that carbohydrate is the biggest component in banana peels. Chantawongsa (2013) also reported nearly the same content of total carbohydrate and lignin in banana peel [12]. This high carbohydrate content shows banana promising potential to be used for fermentation substrate for valuable chemical production.

3.2. Alkaline pretreatment and hydrolysis
Pretreatment process is known to have effects on the enzymatic hydrolysis of lignocellulosic materials. The effects of alkaline pretreatment on the lignocellulosic components of Raja Nangka banana peel are shown in Figure 2, Figure 3 and Figure 4. According to Figure 2, the highest final cellulose content is obtained at 30%w/v and 80°C pretreatment, which resulted in cellulose content of 90.266%. At higher temperature (70 and 80°C), cellulose concentration tends to increase with alkaline concentration. However, the result at low temperature (60°C) exhibit speaks cellulose content at 20%w/v alkaline concentration. This might be caused by degradation of carbohydrate at higher alkaline concentration.

Figure 3 shows total carbohydrate content after pretreatment using NaOH solution at various temperatures and alkaline solution concentrations. The figure shows that the higher pretreatment temperature the higher total carbohydrate content obtained. In line with the result of cellulose content, total carbohydrate tends to increase with alkaline concentration at higher temperature while at lower one it shows peak at 20%w/v alkaline concentration. The highest total carbohydrate content obtained is 96.87% after pretreatment at 30% w/v and 80°C.
Figure 4 shows that lignin content has been reduced after alkaline pretreatment. The result also shows that highest reduction is achieved at 30% w/v and 80°C pretreatment condition. This lowest lignin content is 2.884%. Results shown in figure 2-4 shows that alkaline pretreatment had successfully broken lignin carbohydrate linkage in banana peels as other reported results [13,14]. Enzymatic hydrolysis of alkaline pretreated banana peel result is presented in Figure 5. The pretreatment condition was chosen at 80°C and 30% w/v alkaline concentration which resulted in the highest total carbohydrate and cellulose content and hence the lowest lignin content. The figure shows that alkaline pretreatment had increased hydrolysis result compared to unpretreated banana peel. According to the figure, the longer hydrolysis reaction time, the higher reducing sugar concentration produced in the reaction. The highest reducing sugar obtained is 5.9974 g/L after 5 day hydrolysis.

![Cellulose content of alkaline-pretreated banana peel.](image-url)
Figure 3. Total carbohydrate content of alkaline-pretreated banana peel.

Figure 4. Lignin content of alkaline-pretreated banana peel.
Figure 5. Effect of hydrolysis reaction time on the reducing sugar production.

4. Conclusions
Alkaline pretreatment of banana peel using NaOH solution had been done and resulted in the increase of total carbohydrate and cellulose content of banana peel and the decrease of lignin content. Pretreatment at temperature of 80°C and alkaline concentration of 30%w/v was the best condition in increasing cellulose content up to 90.266%. Pretreated banana peel at the best pretreatment condition was successfully hydrolyzed using two commercial enzymes which yield the highest reducing sugar content 5.9974 g/L after 5 day hydrolysis.

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