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Agricultural Wastes Potential (Pineapple Crown, Durian Peel and Sugarcane Leaves) on Reducing Sugar Production by Using Sulfuric Acid Pretreatment Following Enzymatic Hydrolysis.

Thirawan Boonwong[1],Sujinna Karnnasuta[1], Teerapatr Srinorakutara[2]*

[1]*Department of Environmental Science, Faculty of Environment, Kasetsart University*

[2]*Thailand Institute of Scientific and Technological Research (TISTR)*

**Correspondent author: dear_thirawan@hotmail.com*

Abstract

Studies are underway to use the potential of agricultural wastes (pineapple crown, durian peel and sugarcane leaves) for reducing sugar production by using sulfuric acid pretreatment following enzymatic hydrolysis. After pretreatment lignocellulosic feedstocks with 2.0%(w/v) sulfuric acid (H_2SO_4) and autoclave at 121°C pressure 15 lb/in^2 for 15 min, reducing sugar of the feedstock contained between 49.00-61.87 g/L, the pineapple crown had a high concentration (61.87±0.59 g/L), durian peel (56.83±0.36 g/L) and sugarcane leaves (49.00ฑ1.14 g/L) respectively. When hydrolyzed the sulfuric acid pretreated feedstocks with 60 FPU/g (DS) cellulase enzyme (Cellic®CTec2), the reducing sugar of pineapple crown was remained highest (113.70±0.47 g/L), the second was durian peel (100.00±0.99 g/L) and sugarcane leaves (95.09±1.54 g/L) respectively. Fermentation of reducing sugar solution of hydrolysate pineapple crown by *Saccharomyces cerevisiae* TISTR 5596 found to be maximal ethanol 33.58±1.96 g/L or 4.3%(v/v) at 72 hours, which equal 230.81 kg/ton (DW).

Keywords: agricultural wastes, sulfuric acid pretreatment, reducing sugar, ethanol

1. Introduction

Agricultural wastes were some substance from agriculture, horticulture and industry activity, which the holder discards and required to discard. This lignocellulosic is the lowest cost biomass and removal by landfill, animal feed and burning which increase CO_2 emission in the atmosphere (air pollution and global warming) and cementing agent in soil was destroyed.

However, lignocellulosic biomass remained to be potential sources of sugars of producing fuels and chemicals. In the past few years the automobile manufacturers produced E85 (85% ethanol and 15% gasoline by volume) to replace gasoline an alternative source reducing agricultural wastes (1).

Lignocellulosic materials mainly composed of cellulose, hemicellulose and lignin (2). The contents of these components maybe varied which on type of plant, species and condition of growing plant, etc. The contents of biomass showed in Table 1, express as percentage of cellulose, hemicellulose and lignin. Cellulose is a high molecular weight which can appear as a highly crystalline material. Hemicelluloses are organic compound which contain some different sugar monomer and present in a branched polysaccharides with the pentoses and the hexoses (3). And lignin is a complex phenol compound. The most commonly derived from wood and an integral part of the secondary cell walls of plant (4).

Table 1. The compositions of cellulose, hemicellulose and lignin in agricultural wastes (4, 9).

Lignocellulosic materials	Cellullose (%)	Hemicellulose (%)	Lignin (%)
Corn cobs	45	35	15
Grasses	25–40	35–50	10–30
Wheat straw	30	50	15
Nut shells	25–30	25–30	30-40
Pineapple peel	21.16	9.50	42.11

Figure 1. Material was reduced in size by wood chopper with the 2.0 mm sieve. (A) Pineapple crown, (B) durian peel and (C) sugarcane leaves about lesser than10 mm.

Pineapple (*Ananas comosus*) is the common name of a tropical fruit and bromeliad fruit in widespread cultivation. Thailand is the biggest exporter of cannery pineapple around the world (5). Of the pineapple wastes, crowns were 2–4%(w/w) of total pineapple weight. Pineapple leaf and crowns were waste product of pineapple cultivation and its fiber were multicellular and lignocellulosic (6). Pineapple crown was about 82,322 ton per year in 2013 (7).

Durian (*Durio zibethinus* Murray) is an economically important plant of Thailand. Thailand harvested 569,238 ton per year in 2013 (7). Durian Peels were 67.4% (w/w) of total durian weight, about 48,739.9 ton per year. Durian Peel eliminated difficulty and required a long time to decompose.

Sugarcane (*Saccharum officnarum*), Thailand is the 4th exporter country of the world that can export sugarcane in term of sugar and by-product (molasses) (8). Sugarcane leaves were 24% (w/w) of total sugarcane production weight, about 24,022,939 ton per year in 2013 (7).

So, Three lignocellulosic biomass; pineapple crown, durian peel and sugarcane leaves can hydrolyzed to reducing sugar. Biomass feedstocks can comminute by combination chipping, grinding and milling to reduction cellulose crystallinity (9). This the literature has almost focused on dilute sulfuric acid pretreatment. The dilute sulfuric acid pretreatment can make high reaction rates and significantly improve cellulose hydrolysis (10). Dilute acid pretreatment is the most studies process to treat of lignocellulosic materials such as H_2SO_4 and HCl. Sulfuric acid pretreatment substantially solubilized lignin in feedstock and temperature had the most significant effect on the lignin reduction, xylan and glucan were solubilized during dilute acid pretreatment (11). This procedure can be a simple single-stage process in which biomass treated with dilute sulfuric acid at suitable of concentrations, temperatures and time (12). Hemicellulose and lignin will solubility to make the cellulose better accessible to enzymes.

Following, enzymatic hydrolysis of cellulose carried out by cellulase enzymes which were highly specific and under mild conditions. Enzymatic hydrolysis involved cleaving the polymers of cellulose and hemicellulose using enzymes. Consequently, the main hydrolysis product of cellulose is glucose because the hemicellulose gives rise to several pentoses and hexoses (12). An acid treatment at 121°C for 15 min was an effective pretreatment method for converting lignocellulosic (rice straw) to sugar up to 21.45% (w/w), sugar could measure after acid treatment (13). During the enzymatic hydrolysis, cellulose degraded by the cellulase to reducing sugar (9), which would as a suitable substrate for ethanol production.

In this work, the first method pretreatment the feedstocks (durian peel, pineapple crown and sugarcane leaves) with sulfuric acid to make this structure suitable to convert in hydrolysis processes and adjusted pH to proper enzyme working. Focus on hydrolysis process by studying effectiveness of agricultural wastes and effect of enzyme dosage converting holocellulose to reducing sugar. And, select the suitable material for ethanol production.

2. Material and method

2.1 Lignocelluloses

Three agricultural wastes, durian peels and pineapple crown collected from Nakhon Si Thammarat province, and sugarcane leaves collected from Nakhon Ratchasima province, Thailand. These materials chipped and screened by wood chopper with the 2.0 mm sieve. The material sizes were about lesser than 10 mm, and stored in sealed plastic bags at room temperature until used for pretreatment and enzymatic hydrolysis processes.

2.2 Pretreatments

Sulfuric acids (H_2SO_4) at concentrations 2.0% (w/v) used to pretreat lignocellulosic feedstocks with solid loading of 20g/100ml and autoclave at 121°C, pressure 15 lb/in^2 (103.4 kPa) for 15 min. After that the sample cooled, and adjusted pH to 5.0 with 80% (w/v) NaOH.

2.3 Enzyme hydrolysis

The pretreatment samples were added cellulase enzyme (Cellic®CTec2) (The enzyme activities were 864.15 FPU/mL, measured by Filter Paper Activity at pH 4.8, temperature 50 °C) with 20, 30, 40, 60 and 80 FPU/g (DS) and incubated at 50 °C with shaking rate 200 rpm for 72 hours. The samples were periodically taken at 0, 6, 12, 24, 48 and 72 hours, centrifuged and measured reducing sugar.

2.4 Ethanol production

For ethanol fermentation, choose the material that produces reducing sugar was high dosage and then was filtered the hydrolyzed solution through a filter paper No.2 by vacuum filtration. Fermentation performed by *Saccharomyces cerevisiae* TISTR 5596, inoculated at 1x10^7 cell/mL, while fermentation condition was 30 °C, under limitation of oxygenat shaking rate 130 rpm for 72 hours. The samples, 500 ฅl derived from triplicate experiment.

2.5 Analysis methods

The extractives and lignocelluloses contents were determined by the method described by the Technical Association of Pulp and Paper Industry (TAPPI) (14-18). Analyze concentration of reducing sugar in the pretreatment and hydrolysate lignocelluloses by DNS method (19). Calculate %holocellulose conversion as follows:

$$\%\text{Holocellulose conversion} = \frac{\text{Total reducing sugar(g)}}{\text{Dry material(g)}} 100$$

Ethanol production was analyzed by Gas Chromatography (GC) (Agilent Technologies 6890N Network System) with headspace of Agilent Technologies G1888 Network Headspace Sampler. The Column and injection temperature were 120°C and 220°C, respectively. Flame-ionized detector and carrier gas was helium (flow rate at 40 mL/min). All experiments were carried out in triplicate and the data provided were average values ± standard deviation (S.D.).

Table 2. Chemical compositionof lignocelluloses in pineapple crown, durian peel and sugarcane leaves.

Chemical composition	Raw material			Standard method	Ref.
	Pineapple crown	Durian peel	Sugarcane leaves		
1. Extractives (%)	36.47	44.53	20.70		-
1.1 Alcohol-benzene solubility (%)	15.65	21.57	8.56	TAPPI-T204-om-97	14
1.2 Alcohol solubility (%)	4.89	2.97	3.19	TAPPI-T264-om-97	15
1.3 Hot water solubility (%)	15.93	19.69	8.95	TAPPI-T207-om-93	16
2. Lignin (%)	8.89	9.69	13.21	TAPPI-T222-om-98	17
3. Holocellulose (%)	42.17	36.20	61.29	Acid chloride Method of Browning	-
4. Alpha-cellulose (%)	22.30	20.57	36.64	TAPPI-T203-om-93	18
5. Moisture content (%)	13.34	8.62	9.00	Moisture analyzer (AMD MX-50)	-

3. Result and discussion

3.1 Raw material

Pineapple crown, durian peel and sugarcane leaves were chipped and screened approximately lesser than 10 mm that increased of the accessible surface area and the size of pore of cellulose and the decrease of its crystallinity and its polymerization degree, maybe suitable substrate for reducing sugar production, show in Figure1. Size of materials will be relative with reduce viscosity of slurry that increase enzyme hydrolysis to high reducing sugar. However, small size can help to viscosity of high solid loading in enzyme hydrolysis and high sugar concentration in the hydrolysate.

The chemical compositions of material listed in Table 2. The extractive, non-cell wall components include resin fats ligans flavonoids tannin etc., the content of extractive vary among different wood species and also within the different parts of the same tree (20). The composition was 44.53% (w/w) in durian peel, 36.47% (w/w) in pineapple crown and 20.70%(w/w) in sugarcane leaves. So the alcohol-benzene solubility have a definite influence on the swelling of the cell wall structure and later affect the material swelling in pretreatment process, alcohol solubility and hot water solubility accounted for only a small part of the biomass. Lignocellulosic, cell wall components are the primary building block of plant cell walls. Plant biomass is mainly composed of cellulose, hemicellulose, and lignin. Cellulose is the important component in feedstocks can used as precursor in ethanol production, the other component are the inhibitors of the reaction between cellulose and enzyme in enzymatic hydrolysis, while lignin is extremely resistant to chemical and enzymatic degradation. Each material was had composition very different, sugarcane leaves have the major constituent in holocellulose and lignin. So lignin in sugarcane leaves cause be difficult to swell and resistant in pretreatment and hydrolysis process. Pineapple crown will be suitable material to ethanol production.

3.2 Effect of dilute sulfuric acid pretreatment

When autoclave pineapple crown, durian peel and sugarcane leaves at 121°C, pressure 15 lb/in^2 (103.4 kPa) for 15 min with sulfuric acid (H_2SO_4) at concentrations 2.0% (w/v). After pretreatment process, the structure of

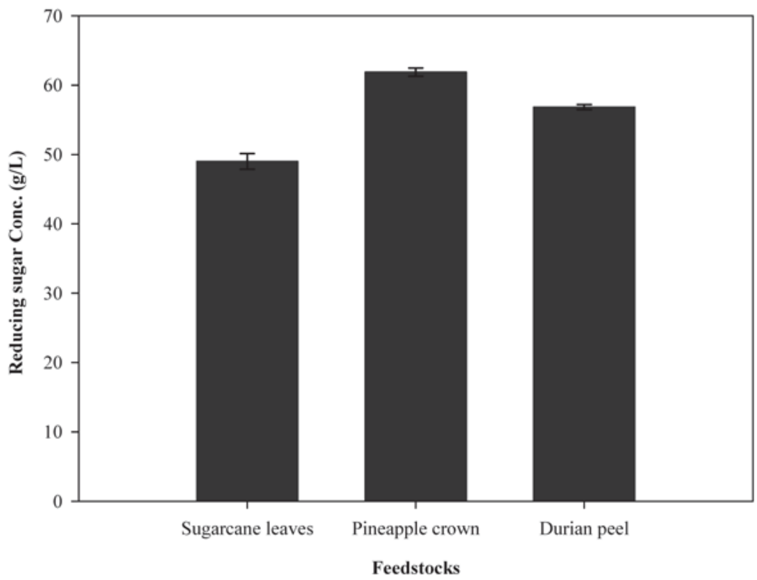

Figure 2. Effect of sulfuric acid pretreatmenton reducing sugar concentration in each feedstocks.

materials converted to a non-packed and more accessible structure in treated. The reducing sugar of the feedstocks contained between 49.00-61.87 g/L, the pineapple crown had a high content (61.87±0.59 g/L), durian peel (56.83±0.36 g/L) and sugarcane leaves (49.00±1.14 g/L), respectively. (Figure2) The lignin was solubilized during dilute acid pretreatment, which the pretreatment methods predominantly affect hemicellulose with impact on lignin degradation (21).That meant to degrade lignin so that the polysaccharide can available for enzymatic actions. It could improve the rate of enzyme hydrolysis and increase yields of intended products.

3.3 Effect of enzyme hydrolysis process

Dose of cellulose enzyme (Cellic^{1}CTec2) on reducing sugar concentration were proven with dilute acid pretreatment showed in Figure3. Five different dosage of cellulose (Cellic®CTec2) were used in hydrolysis process, which 20, 30, 40, 60 and 80 FPU/g (DS). After hydrolysis of pretreatment feedstocks, the reducing sugar yields in feedstocks with 40, 60 and 80 FPU/g (DS) were increased as the saccharification time increased, from 0 to 24 hour and stable after 24 hours. The concentrate of reducing sugar in case of cellulase dosage 40, 60 and 80 FPU/g (DS) were different with 20 and 30 FPU/g (DS), which related to low concentration and specific activity of enzyme. The highest concentration of total reducing sugar in each feedstock was hydrolyzed at 60 FPU/g (DS). Due to the dosage of cellulase has a high impact on converse the cellulose. Structures, material size and lignin content in lignocellulosic biomass were difficult to break and influence on digestibility of the material. During enzymatic hydrolysis, cellulase tended to irreversibly bind to lignin through hydrophobic interactions that cause loss in enzyme activity. Total reducing sugar of pineapple crown is highest (113.70ฑ0.47 g/L) and the second is durian peel (100.00ฑ0.99 g/L). Which the sugarcane leaves (95.09ฑ1.54 g/L) had the lowest reducing sugar yield was partly caused by the low lignin removal during pretreatment (11) because the lignin content in sugarcane leaves were highest (13.21% w/w) including long and branches of hemicelluloses.

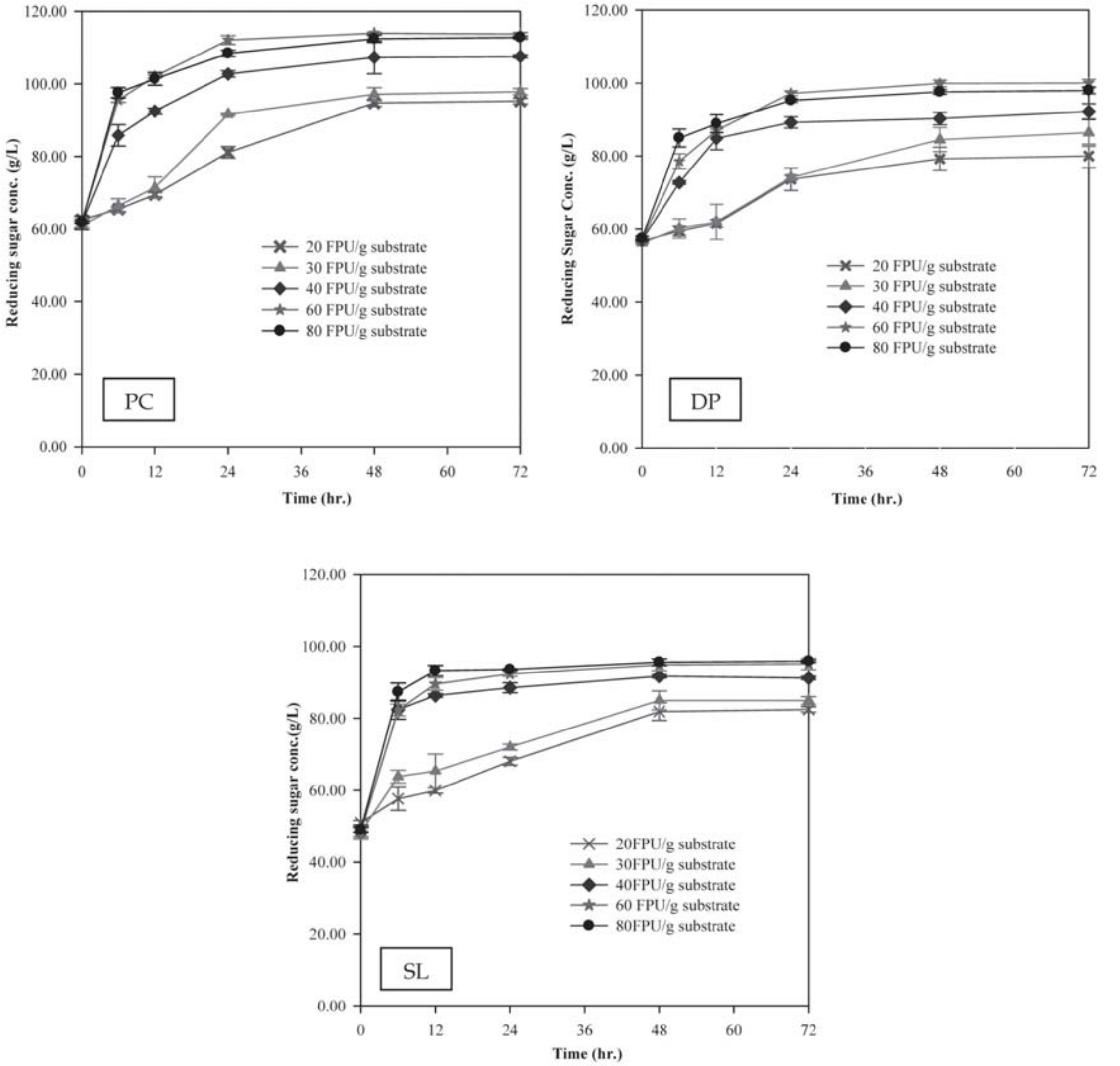

Figure 3. Effect of cellulase (Cellic®CTec2) enzyme dose on reducing sugar concentration was hydrolysis (PC) pineapple crown, (DP) durian peel and (SL) sugarcane leaves.

In calculate the percentage of holocellulose conversion (Figure 4), the conversion percentage of holocellulose of pineapple crown was more than durian peel and sugarcane leaves respectively, following the same trend of reducing sugar concentration. The limitation of cellulase dosage 40, 60 and 80 FPU/g (DS) on holocellulose conversions were set at 24 hours, 98.68%±0.41 in pineapple crown, 94.66%ฑ0.94 in durian peel and 57.31%±0.34 in sugarcane leaves. Percentage of holocellulose conversion in sugarcane leaves was the lowest due to its complex nature, high lignin and ash content. It may be explained by mass transfer limitations or unproductive adsorption of enzymes. (11)

However, cellulase enzyme (Cellic®CTec2) at 60 FPU/g (DS) was suitable for cellulosic hydrolysis and ethanol productions.

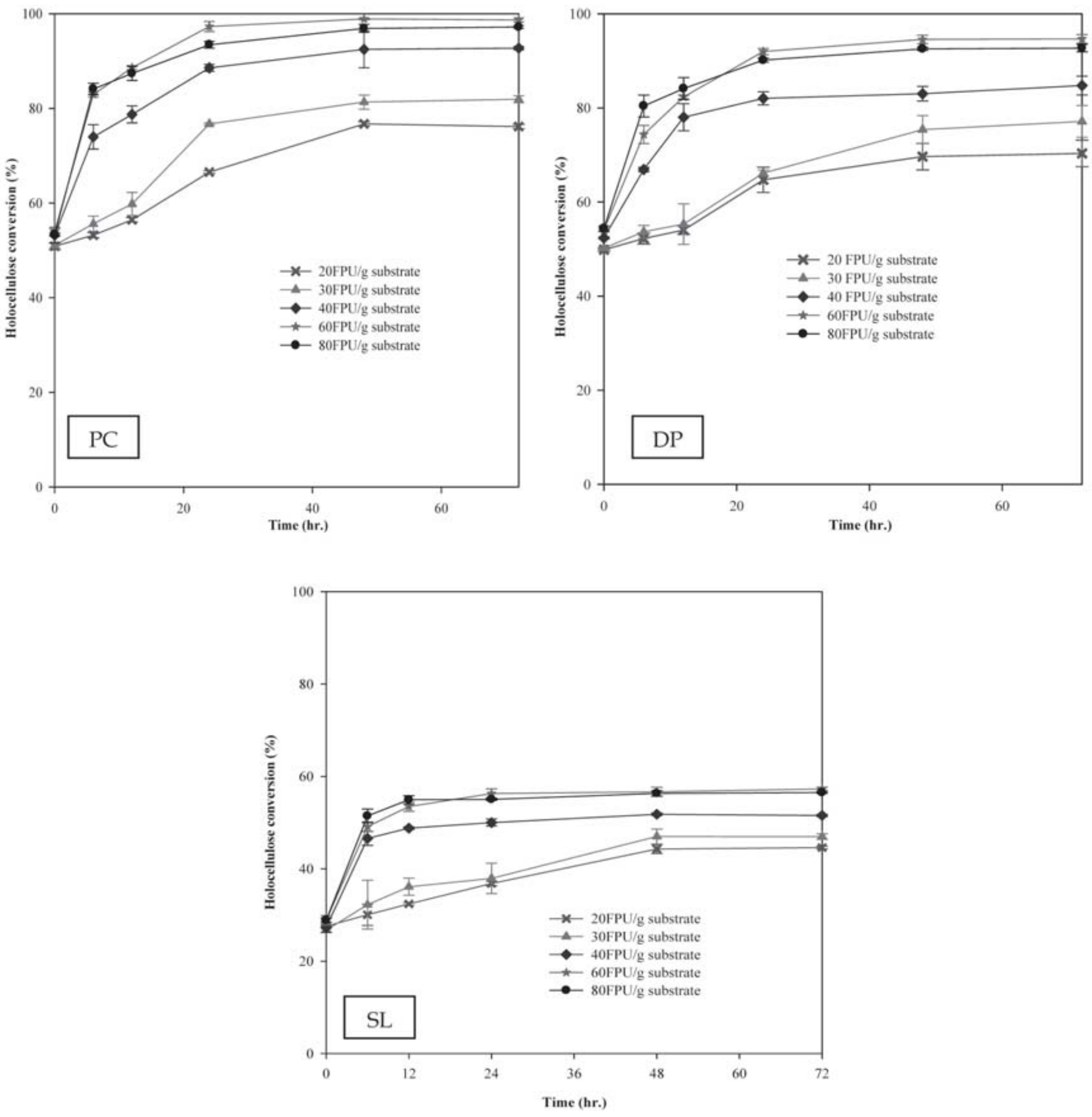

Figure 4. Percentage of holocellulose conversion by cellulase (Cellic®CTec2) enzyme in (PC) pineapple crown, (DP) durian peel and (SL) sugarcane leaves.

3.4 Ethanol production

When comparing among types of feedstocks, pineapple crown had several characteristics that make it a potential feedstock for reducing sugar and ethanol productions. When the fermentation period increased to decrease the amount of reducing sugar, at the end of fermentation in 72 hours remaining sugar in pineapple crown 44.44±1.65 g/L and found maximal ethanol 33.58±1.96 g/L, or 4.3% (v/v). So pineapple crown had a potential in ethanol production 230.81 kg/ton (DW) (Figure 5). Ethanol production yield from pineapple crown was higher than sugarcane bagasse (180 kg/ton) (22), water hyacinth leaves (140 kg/ton) (23), switch grass (170 kg/ton) (24) and equal to using molasses in industry (238 kg/ton) (25).

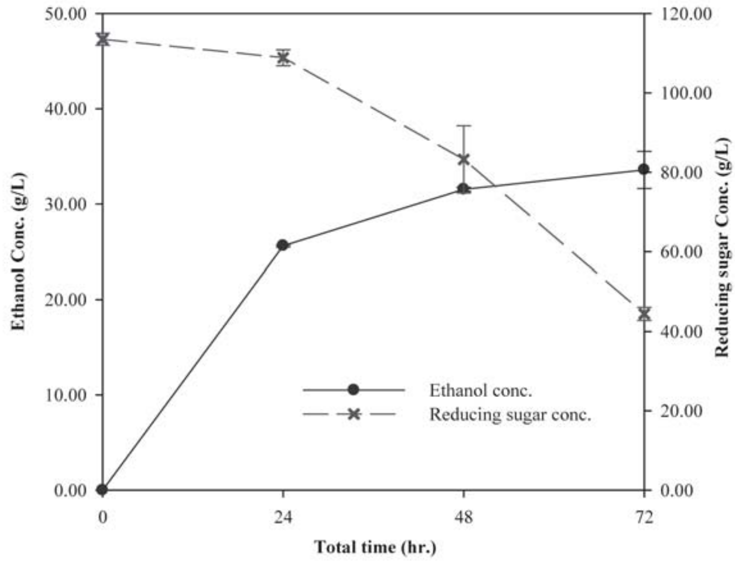

Figure 5. Ethanol production from pineapple crown loading at 20g/100ml which pretreatment with 2.0% (w/v) sulfuric acid (H2SO4) and hydrolyzed with cellulase (Cellic®CTec2) enzyme loading was 60 FPU/g (DS).

4. Conclusions

Agricultural wastes (pineapple crown, durian peel and sugarcane leaves) were pretreated with sulfuric acid (H_2SO_4), the structure of material converted to a non-packed and more accessible structure in treated. The reducing sugar of the feedstocks contained 49.00-61.87 g/L, the pineapple crown had the highest content (61.87 g/L). The highest concentration of total reducing sugar in feedstock was hydrolyzed at 60 FPU/g (DS). The total reducing sugar of pineapple crown was highest (113.70 g/L) with 98.68%±0.41 holocellulose conversion. The biomass composition indicated on a major role in determining the effectiveness of pretreatment and enzymatic hydrolysis. The fermentation with *Saccharomyces cerevisiae* TISTR 5596 at 30 ^{o}C was conducted under oxygen limited conditions at shaking rate 130 rpm and found maximal ethanol at 33.58±1.96 g/L, 4.3% (v/v) or 230.81 kg/ton (DW)at 72 hours, which has a high possibility to develop into the industrial manufacturing.

5. Acknowledgement

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