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Oil Palm Trunk of Lignocellulolytic Resources as Raw Material for Production of Fermentable Sugars *via* Enzymatic Hydrolysis Using Cellulose Thermostable

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This study aimed to explore the optimization of the concentration of oil palm trunks and thermostable cellulose enzyme preformance produce highest sugar (glucose total and reducing sugars) as well as the degree of polymerization. The design used in this study was a completely randomized design (CRD) factorial using 2 factors: factor A consists of four levels of cellulose enzyme that A1: 250 U/kg, A2: 500 U/kg, A3: 750 U/kg and A4: 1000 U/kg. Factor B is the concentration of oil palm trunk yitu B1: 20 % ; B2: 40 % and B3: 60 % were repeated 3 times. The results showed that there is a significantly effect ($P < 0.05$) in the levels of cellulose enzyme (factor A) with the concentration of oil palm trunks (factor B) and interactions of enzyme and oil palm trunk (factor AB) to reducing sugar, total sugars and degree of polymerization. This research can be concluded that the best total sugar produced was 19.68 mg/mL, the reducing sugars was 11.17 and degree of polymerization was 3.47.

Keywords: Cellulose, Glucose, Reducing sugars, Degree of polymerization.

INTRODUCTION

The oil palm industry is one of the largest industries in Indonesia, Malaysia and Nigeria[1]. The main production is the palm oil, but the oil produced only about 10 % of the total biomass from oil palm tree, meanwhile the remaining is in the form of solid biomass. The residues include oil palm trunks (OPT), oil palm fronds (OPF), oil palm empty fruit bunch (EFB), mesocarp fibres and palm kernal shells. The total biomass was estimated about 90 million tonnes per year with 8.2 million tonnes of oil palm trunk, 12.9 million tonnes of oil palm fronds and 15.8 million tonnes of oil palm empty fruit bunch[2,3]. Some of these are being used as fuel, fertilizer and animal feed[4,5].

Oil palm trunk (OPT) is available during replanting in which the economic life span of oil palm tree is declining at age about 25-30 years. Oil palm trunk consists of lignocellulosic materials that are valuable for the conversion of bioethanol. It consists of 34.5 % cellulose and 31.8 % hemicellulose, which is close association with 25.7 % lignin[6]. Oil palm trunk has high moisture content up to 80 %, it can be squeezed using squeezing machine to extract out the sap, sugar content in sap is about 8-10 %[7]. Yetti *et al.*[6] added the sugar content produced was 9.98 mL/10 g of oil palm trunk. Maximum glucose yield was at a hydrolysing acid concentration of about 1.7 %[8]. From one trunk, about 200 L of sap can be extracted and 70 L of bioethanol can be produce from this amount of sugar solution. Chin *et al.*[8] reported that oil palm trunk has ahigher glucose conversion yield than those of rubberwood sawdust and mixed hardwood sawdust. Sugar as a renewable resource, can be derived from a variety ofbiological feedstocks such as ligno-cellulosic biomass. The sugar derived from lignocellulosic biomass can be further converted to a number of high-value bio-based fuels

Due to replanting activities large quantities of oil palm trunks will be generated annually in Indonesia starting from this decade. A project was initiated to study the feasibility of converting palm trunks into glucose which could then be used to produce ethanol. The different concentration of oil palm trunks were hydrolyzed using different concentrations of cellu-lose thermostable. The present work was aimed to investigate the potential use of oil palm trunk as an alternative feedstock for lignocellulosic-glucose production and to evaluate the optimal treatment condition for these lignocellulosic bio-masses.

EXPERIMENTAL

Production of cellulose thermostable: The cellulose thermostable produced by thermophilic bacteria was performed in 5000 mL Erlenmeyer flask containing 50 g of pretreated oil palm trunk. The medium was prepared with the following composition (g/L) 10 g; urea, 0.3; peptone, 0.75; yeast extract, 0.25; $(NH_4)_2SO_4$, 1.4; KH_2PO_4, 2.0; $CaCl_2$, 0.3; $MgSO_4{\cdot}7H_2O$, 0.3 and trace elements (mg/L): $FeSO_4{\cdot}7H_2O$, 5; $MnSO_4{\cdot}4H_2O$, 1.6; $ZnSO_4{\cdot}7H_2O$, 1.4 and $CoCl_2{\cdot}6H_2O$, 20. The medium and the trace elements were autoclaved separately. The flask was cooled down at room temperature and a known amount of sterilized trace elements was added. The flasks were then inoculated with (50 mL) (1×10^6 spores/mL) of the thermophilic bacteria and incubated for 60 h at the 60 °C[9].

Preparation of oil palm trunk: Oil palm trunk (OPT) collected from field used as substrate for enzyme production, it was ground and sieved to 18-20 mesh by shredding machine. The shredded oil palm trunk was dried at 60 °C in an oven for 12 h. The dried oil palm trunk was kept ready for the further use.

Enzymatic hydrolysis of oil plam trunk: Enzymatic hydrolysis of oil palm trunk was carried out in reaction mixture containing differents concentration of cellulose thermostable and different concentration of oil palm trunk. The reaction mixtures were incubated on a water bath rotary shaker adjusted to 60 °C and 75 rpm for 2 h[9]. The total glucose, reducing sugars and degree of polymerization were determination by reported methods[10,11]. The glucose yield was calculated as percentage of theoretical conversion rate from cellulose to glucose.

Research methods: This study used a completely randomized design (CRD) factorial using 2 factors: factor A is composed of cellulose enzyme units: A1 = 250 U/kg; A2 = 500 U/kg; A3 = 750 U/kg; A4 = 1000 U/kg. Factor B is a palm trunk level, namely: B1: 20 %; B2: 40 % B3: 60 %, which was repeated 3 times.

Data analysis: All data were analyzed using analysis of variance (ANOVA) completely randomized design (CRD) factorial and differences treatments were tested by Duncans Multiple Range Test (DMRT) according to Steel and Torrie[12].

RESULTS AND DISCUSSION

Effect of total sugar treatment: The averages of total sugars of hydrolysis product used cellulose thermostable can be seen in Table-1. The variance analysis showed that there was a significant effect ($P < 0.05$) to the level of cellulase enzymes used (factor A), the level of palm trunks (factor B) and the interaction between the enzyme levels with palm trunks. After further testing using DMRT between factor A and factor B (interaction) showed that the levels of cellulase enzyme 500 U/kg and 60 % palm trunk level (A2B3 treatment) resulted in the highest total sugars with total sugar 19.68 mg/mL.

Anindyawati[13] reported that cellulose is one of the main components of lignocellulose is composed of D-glucose monomer units attached to the 1.4-glycosidic bond. Tend to form cellulose microfibrils through inter and intra-molecular bonds that provide soluble structure. Cellulose microfibrils consist of two types, namely crystalline and amorphous. Fengel and Wegener[14] added that the cellulose is hydrolyzed called amorphous part of cellulose and cellulose generally containing 15 and 85 % amorphous part and crystalline respectively. Akahiko *et al.*[15] found that sugars contained in the sap of oil palm trunk were glucose, sucrose, fructose and galactose, all of which are fermentable by ordinary industrial yeast strains. The results strongly indicate that old oil palm trunk becomes a promising source of sugars by proper aging after logging and, thus, its sap can be a good feedstock of bioethanol and bio plastics by biomass refiner.

Effect of reducing sugar treatment: The average of reducing sugar produced after oil palm trunks treated with thermostable cellulase enzymes at various concentrations and various levels of palm trunks are given in Table-2.

The results of variance analysis showed that there was a significant effect of ($P < 0.05$) to the level of cellulase enzymes used (factor A), the level of palm trunks (factor B) and the interaction between the enzyme levels with palm trunks. After further testing using DMRT between factor A and factor B (interaction) showed that the levels of cellulase enzyme 500 U/kg and 20 % palm trunk level (A2B1 treatment) resulted in

TABLE-1
AVERAGE TOTAL SUGAR PRODUCTS HYDROLYSIS OF OIL PALM TRUNKS (mg/mL)

B [oil palm trunk (%)]	A [Cellulose (U/kg)]				Average
	A1	A2	A3	A4	
B1	19.02^{b}	19.35^{ab}	19.40^{ab}	18.13^{c}	18.98^{b}
B2	19.45^{ab}	19.56^{a}	19.51^{a}	19.48^{a}	19.50^{a}
B3	19.57^{a}	19.68^{a}	19.48^{a}	19.45^{ab}	19.54^{a}
Average	19.35^{a}	19.53^{a}	19.47^{a}	19.02^{b}	

Note: Different superscript lowercase letters indicate highly significant effect ($P < 0.05$).

TABLE-2
AVERAGE REDUCING SUGAR PRODUCTS HYDROLYSIS OF OIL PALM TRUNKS (mg/mL)

B [oil palm trunk (%)]	A [Cellulose (U/kg)]				Average
	A1	A2	A3	A4	
B1	6.67^{f}	11.17^{a}	10.43^{ab}	5.05^{g}	8.33^{b}
B2	8.41^{de}	9.61^{bcd}	8.92^{cde}	8.05^{e}	8.75^{b}
B3	7.70^{ef}	9.44^{bcd}	9.70^{bc}	10.68^{ab}	9.38^{a}
Average	7.59^{b}	10.07^{a}	9.69^{a}	7.93^{b}	

Note: Different superscript lowercase letters indicate highly significant effect ($P < 0.05$).

TABLE-3
AVERAGE DEGREE OF POLYMERIZATION PRODUCTS HYDROLYSIS OF OIL PALM TRUNKS

B [oil palm trunk (%)]	A [Cellulose (U/kg)]				Average
	A1	A2	A3	A4	
B1	5.75^{b}	3.47^{g}	3.78^{fg}	7.22^{a}	5.06^{a}
B2	4.70^{cde}	4.07^{efg}	4.37^{cdef}	4.84^{cd}	4.50^{b}
B3	5.10^{bc}	4.18^{defg}	4.03^{efg}	3.65^{fg}	4.24^{b}
Average	5.18^{a}	3.91^{b}	4.06^{b}	5.24^{a}	–

the highest reducing sugar compared to other treatments.Not increased levels of reducing sugars during enzyme concentration was increased due to the concentration of substrate may affect the rate of production and catalytic activity of the enzyme. According to Yetti[16], the addition of enzyme concentration increased the rate of reaction when the substrate is available in excess. However, increasing the reaction rate decreased for each additional enzyme concentration, so that the speed of enzyme kinetics has been saturated with substrate and the total sugar produced was decreased.

Effect of degree of polymerization: The average degree of polymerization of data generated after oil palm trunks reacted with the thermostable enzyme cellulose according to the treatment can be seen in Table-3.

The results of variance analysis showed that there is a significant effect ($P < 0.05$) to the level of cellulase enzymes used (factor A), the level of palm trunks (factor B) and the interaction between the enzyme levels with palm trunks. After further testing using DMRT between factor A and factor B (interaction) showed that the levels of cellulase enzyme 1000 U/kg and 60 % palm trunk level (A4B3 treatment) resulted in the lowest degree of polymerization compared to the other treatments.

The lowest value indicates the degree of polymerization of the polymer chains of polysaccharides has been decomposed into monomers. Value of the degree of polymerization in this study ranged from 3.47 to 7.22. Anggraini[17], adding the value of the degree of polymerization of dwindling showed that a growing number of polysaccharides which terdepolimerisasi into compounds with shorter chains, where the degree of polymerization is a bound variable, depending on the value of total sugar and reducing sugar produced.

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