

The effect of amphipilic lignin derivatives addition on enzymatic hydrolysis performance of kraft pulp from sorghum bagasse

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Abstract. Previously, the chemical characteristics of isolated lignin from *Acacia mangium* black liquor of kraft pulping was characterized. This lignin was blended with natural rubber latex (NR-L) as adhesive in laminated wood. In addition, lignin has potency for biosurfactant materials by modification of the hydrophobic into hydrophilic properties. Therefore, this study was intended to develop lignin as material for amphipilic lignin derivatives (A-LD) biosurfactant by reacting lignin with epoxilated polyethylene glycol (PEG). A-LD addition in slurries was used to improve the enzymatic hydrolysis (EH) of kraft pulp sweet bagasse sorghum (SSB). The main observation in EH performance was to investigate the effect of lignin isolation method (one and two step) in A-LD and A-LD loading addition on reducing sugar yield (RSY) of SSB kraft pulp. The pulp was hydrolyzed at 50°C and 150 rpm for 72 h with 10 FPU cellulase loading in the shaking incubator. A-LD from one (L₁S) and two step (L₂S) lignin was added with A-LD loading of 0, 1, 2, 5, and 10% (b/v). The RSY of hydrolyzate has been observed after EH. A-LDs addition in EH of SSB kraft pulp enhanced RSY. L₁S worked better in reaction performance with PEDGE compared to L₂S and LS. A better performance was showed by PEDGE 500 than that of PEDGE 6000. Generally, the higher A-LDs loading resulted higher RSY. The highest RSY (81.33%) was resulted in addition of 10% A-LD L₁S using PEDGE 500. A 5% A-LD loading was more considered to be added in EH because the RSY was comparable with 10% A-LD loading.

1. Introduction

Previously, pulp kraft of Sweet sorghum bagasse (*Sorghum bicolor* L Moench) has been converted successfully to into fermentable sugar via enzymatic hydrolysis [1]. Pulping condition with 17% active alkali and 20% sulfidity resulted the highest reducing sugar yield (RSY) of 45.57%. Considering this result, this study was only used kraft pulp of sweet sorghum bagasse (SSB) as substrate in enzymatic hydrolysis (EH) by biosurfactant addition.

Acacia mangium is known as main raw materials in pulp and paper mills in Indonesia with kraft process as pulping method. Lignin is abundant materials that can be recovered from black liquor during pulping process. The main utilization of black liquor is to support energy generation in the mill with contribution of 66.2% from total energy needed at 2012 PT. Tanjung Enim Lestari pulp and paper (TELPP) mill. TELPP produces 1760 m³ per day black liquor with total solid content of 70% [2]. It is indicated that there is high potency of lignin to be converted into value added lignin-based materials. This isolated lignin has been also utilized as filler in Aqueous Polymer Isocyanate (API) adhesive with natural rubber latex (NRL) and polyvinyl alcohol (PVA) as based polymer (registered patent



P00201507466) [3]. Besides bioadhesives, isolated lignin can also utilize as biosurfactant materials to improve the enzymatic hydrolysis (EH) performance of biomass. Up to now, the various method has been employed to increase hydrolysis effectivity by removing the inhibitor during pretreatment stage. To improve the hydrolysis performance of pretreated biomass, surfactant can be added. Some of commercial surfactant such as Trixton X, tween, PEG (*poly ethylene glycol*) resulted from polymerization reaction [4, 5, 6] often become addition materials in EH. Surfactant can reduce surface tension thus enzyme activity will improve and increase reducing sugar yield (RSY) [7].

Biosurfactant which can be sulfonated directly from lignin such as sodium lignosulphonate (Na-LS). However, the major application of Na-LS in industry is for emulsifier, corrosion inhibitor, foaming prevention, or detergent. Lignin based biosurfactant concern in EH application is still limited. Therefore, the conversion lignin for Amphiphilic Lignin Derivatives (A-LD) surfactant to improve the EH performance is prospective to be developed. A-LDs are a water-soluble polymer that serves to prevent non-productive absorption of enzymes in EH process, and maintain high enzyme activity during the process. Recovery of enzyme after EH has occurred [8, 9, 10]. The improvement of EH by addition A-LDs made polyethylene glycol (PEG) and lignin in EH has reported previously [10]. This approach resulted increase hydrolysis efficiency as much as 80% and cellulase enzyme can be reused [8]. In addition, A-LDs can also accelerate the bioethanol production on large scale fed-batch SSF with substrate loading of 30% (w/v) producing high ethanol concentration of 87.9 g/L [11].

In Indonesia, A-LD synthesized from lignin as a byproduct of the kraft pulping mill has been conducted before. In the future, integration between pulp and paper mill with bioethanol pilot plant will support development of biorefinery concept. Patent No US 8911976-B2 [12] reported that lignin was reacted with hydrophilic compound i.e lauryl alcohol polyethylene oxide-glycidyl ether to result lignin derivative surfactant. This invention covered several lignin source including kraft lignin, acetic lignin, organosolve, steam explosion lignin, sulphate lignin and alkali lignin from softwood (cedar, cypress and pine), hardwood (beech and oak), and graminiae (ricestraw, fir, bagasse) to be used in this reaction. The addition of 0.5-20% A-LDs can improve enzymatic digestibility of biomass. Up to now, there is no report in utilization of lignin from *Acacia mangium* black liquor for A-LD surfactant to improve hydrolysis performance of kraft pulp SSB. Besides that this research emphasis was to evaluate the effect of lignin isolation method in A-LD performance during EH. The effect of molecular weight of PEDGE in A-LD was also has been reported before. Therefore, this research was required to observe the effect of A-LD addition from one and two step isolated lignin with different molecular weight of PEDGE in enzymatic hydrolysis of kraft pulp SSB.

2. Materials and Methods

The kraft pulp of SSB with active alkali (AA) of 17% and sulphidity of 20% was prepared as described in Fatriasari *et al.* [13]. The cellulose, hemicellulose, acid insoluble lignin (AIL), acid soluble lignin (ASL) of SSB kraft pulp *Acacia mangium* was 64.65%, 29.18%, 0.81%, 0.68%, respectively [1]. *Acacia mangium* black liquor with 70% solid content was obtained from PT TELPP, South Sumatera. Epoxylated polyethylene glycol (PEDGE) with Mn 500 and 6000 and celluclast enzyme was purchased from Sigma Aldrich. The chemicals for lignin isolation, polyethylene glycol (PEG) content analysis and reducing sugar determination were in analytical grade purchased from Sigma Adrich and Merck.

2.1 Lignin Isolation

One and two step acid isolation was used to isolate lignin from the *Acacia mangium* black liquor following Hermiati *et al.* [2] with modification in frequency of solution washing. Principally, the difference of one and two step isolation of lignin was in the precipitation of sugar embedded in solution by addition of ethanol when pH of solution reached 7. Lignin isolates, namely L₁S (one step)

and L₂S (two step) were then grounded and sieved through 40 mesh size sieve. The moisture content of them was also determined before used in A-LD synthesis.

2.2 A-LD Synthesis

Synthesis A-LD was performed based on Cheng *et al.* [14] with technical modification. Firstly, the lignin stock was prepared by dissolving 5 g of L₁S and L₂S in 50 mL of 1 M aqueous NaOH in room temperature. Before addition of 3 g PEDGE with Mn 500 and 6000 in 10 mL in lignin stock, the aquades placed in beaker glass was heated until 70°C. After that, the solution in reaction tubes was clamped and placed in heated water. The mixture was heated at 70°C for 2 h with mechanical stirring until homogenous. To stop the reaction, acetic acid was dropped wise about 2-4 mL in the solution to pH 4 and formed an un-settled emulsion. The A-LD mixtures were kept in refrigerator before using in EH. Besides that, A-LD from commercial lignin was also prepared following the mention synthesis method. It was used as control to compare with the EH performance of SSB kraft pulp by addition of A-LDs from L₁S and L₂S.

2.3 Enzymatic Hydrolysis

As substrate of EH, a 150 mg oven dry (OD) of SSB kraft pulp was placed into vial bottle with final volume of 15 mL. Each substrate was added 300 µl sodium azide (0.02 g/mL), 0.05 M citrate buffer (pH 4.8), 10 FPU/g substrate of cellulase from *Trichoderma reesei* ATCC 26921 with enzyme activity 42.3 FPU/mL (NREL/TP-510-42628) and A-LD dissolved in citrate buffer with concentration of 1-10% v/w. The samples were put in the shaking incubator (WiseCube WIS-30R) set at 50 °C with 150 rpm of shaking rate for 72 h. After hydrolysis finished, the samples were then centrifugated at 10.000 rpm for 5 min to separate hydrolyzate and solid fraction and then decanted the supernatant. Afterward the residue was added 10 mL aquades followed by centrifugation and decantation. The final residues were then oven dried at 60°C for 3 days. Weight loss of samples was calculated by subtracting the initial weight of samples with the final dried weight of residue. While, aliquots were taken from each hydrolyzates in microtubes and stored at 5-10 °C in freezer before sugar analysis (DNS method, [15]).

2.4 Reducing Sugar Analysis

Reducing sugar concentration of hydrolyzate was determined with DNS method [15]. Previously, standard stock glucose of 10 mg/mL was prepared to make glucose standard curve. And then glucose stock was diluted to obtain the series of glucose solution with a concentration of 2, 3.3, 5, and 6.7 mg/mL. The linear regression with its equation can be determined from the absorbance of glucose series. About 0.5 mL aliquots were placed in reaction tube and then added 1.0 mL distilled water and 3 mL DNS reagent. The mixture was vortexed to mix completely and then boiled for 5 min at 100°C. Subsequently, the mixture was transferred into ice-water bath for 20 min. A 0.2 mL sample was taken to be reacted in reaction tube then added 2.5 mL distilled water and then vortexed again. The absorbance was measured by UV VIS Hitachi U-2001 spectrophotometer at 540 nm [15] in absorbance range of 0.1-1 [15]. Each concentration of glucose standard solution was taken 0.5 mL and added with 1 mL aquades and 3 mL DNS reagent. The reducing sugar concentration of samples was calculated by using the regression equation which was built in series of standard curve. The RSY per biomass (g/100 g) was calculated with considering the weight loss of SSB during kraft pulping. The theoretical RSY was calculated by conversion factor of holocellulose into reducing sugar of 1.111 [16].

3. Results and Discussions

3.1 The effects of A-LD L₁S Addition in Enzymatic Hydrolysis

As seen in Fig. 1, the addition of A-LD L₁S caused the positive effect on RSY per biomass. The highest RSY was resulted after addition of 10% A-LD L₁S using PEDGE 500 which having similar RSY with addition of 5% A-LD L₁S loading. Theoretically, conversion of reducing sugar of SSB with

hydrolysis rate of 100% can produce 88.09g reducing sugar/100g initial SSB. It was considered the holocellulose content of SSB as much as 79.29% as reported by Solihat *et al.*[1] and conversion factor of holocellulose into reducing sugar [16]. After hydrolysis of SSB kraft pulp with addition of A-LDs, the highest RSY can obtain 92.32% of theoretical RSY of initial SSB. This result is in agreement with an improvement of EHefficiency of unbleached cedar pulp with addition of A-LDs. This A-LD was synthesized from acetic acid lignin (AL) and PEG-epoxide [8]. The improvement of enzymatic efficiency from 16% to 70.1% has been reported on application of water-soluble lignin based polyoxyethylene ether (EHL-PEG) of corn stover. In this present study, the sugar yield was relatively better than previous reports. Almost of potential cellulose can be converted into sugar monomer. The difference of lignin content in cedar pulp and SSB kraft pulp might affected it. The proposed mechanism was interaction EHL-PEG with cellulase and dispersion cellulase aggregates to form smaller aggregates that can reduce nonreproductive adsorption of cellulase on lignin and then EH of lignocellulose[17]. During EH, there was decrease in enzyme activities because of non-productive binding between enzyme and cellulose, and non-specific hydrophobic interaction between enzyme and lignin contained biomass [18]. Therefore, the presence A-LDs in the cocktails plays important roles to improve RSY by that proposed mechanism.

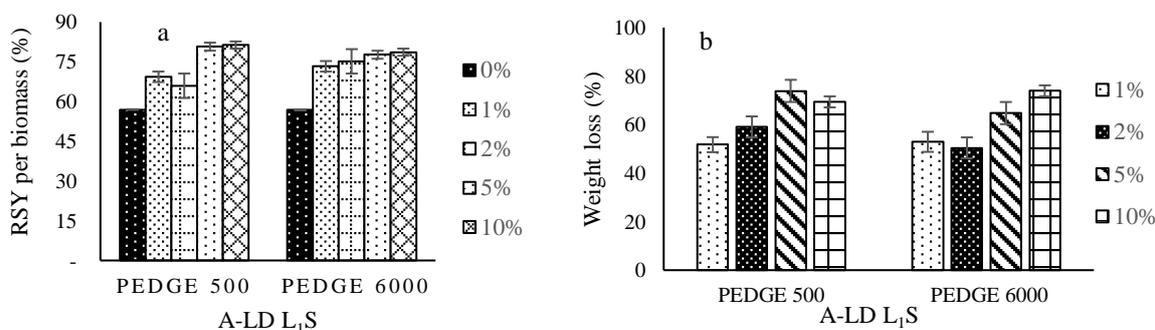


Figure 1. The effects of A-LD L₁S loading and molecular weight of PEDGE on reducing sugar yield (RSY) after enzymatic hydrolysis (EH) (a) and weight loss (b)

The higher A-LD L₁S loading, the higher of RSY obtained. It can be understood caused by more presence of A-LD to improve digestibility in enzymatic hydrolysis of SSB kraft pulp. A-LD with PEDGE 500 showed better performance of RSY per biomass compared to PEDGE 6000. A-LD was resulted from reaction of PEDGE and lignin solution with heating process. In same reaction condition, L₁S particle with bigger size (visual observation) might more difficult to react completely. PEDGE 500 itself has lower molecular weight than that of PEDGE 6000 (unpublished data), therefore it might easier to react with bigger particle size of L₁S. The subsequent isolation of lignin resulted light colour of lignin as shown in Fig. 2.



Figure 2. Physical performance of L₁S (a) and L₂S (b) isolated from black liquor of *Acacia mangium* wood

Six A-LD kinds (PEDGE 500-L₁S, PEDGE 6000-L₁S, PEDGE 500-L₂S, PEDGE 6000-L₂S, PEDGE 500-LS, PEDGE 6000-LS) were added in enzymatic hydrolysis of kraft pulp SSB. Two glycidyl groups of PEDGE interacted with lignin polymer. Winarni *et al.* [8] also reported the hydrophobic

properties of PEDGE-acetic lignin (AL) has become as inhibitor of cellulase covering the active sites of enzymes and then prevented the substrate to insert into these sites. Amongst lignin derivatives including EPEG-, DAEO-, PEDGE-AL_s, the most recovery of cellulose activity was resulted by utilization of PEDGE-AL [8]. DOPEG-SL and EPEG-SL derived from Japanese cedar has been reported capable to reduce enzyme adsorption and end-product inhibition, and improve bioethanol production [11].

The weight loss of SSB kraft pulp after enzymatic hydrolysis is presented in Fig. 1b. This loss indicated that there was breaking down process of cellulose polymer in SSB kraft pulp into its glucose monomer. The presence of glucose in the hydrolyzate was determined in reducing sugar concentration. Generally, the higher A-LD loading results the higher weight loss both PEDGE 500 and 6000 (Fig. 1b). These results are inline with higher RSY obtained by increasing of A-LD loading. At same A-LD loading except 10%, A-LD with PEDGE 500 demonstrates higher weight loss than that of A-LD PEDGE 6000. It means that PEDGE 500 worked more effective with L₁S compared to PEDGE 6000.

3.2 The effects of A-LD L₂S Addition in Enzymatic Hydrolysis

Fig. 3a shows the effects of the addition of A-LD L₂S in the EH of SSB kraft pulp on RSY per biomass. The RSY improvement after addition of A-LD L₂S was occurred that has similar tendency with effect of A-LD L₁S addition. Prevention of non-productive enzymes absorption during EH facilitated high enzyme activity during the process [8, 9, 10] thus the RSY improvement occurred.

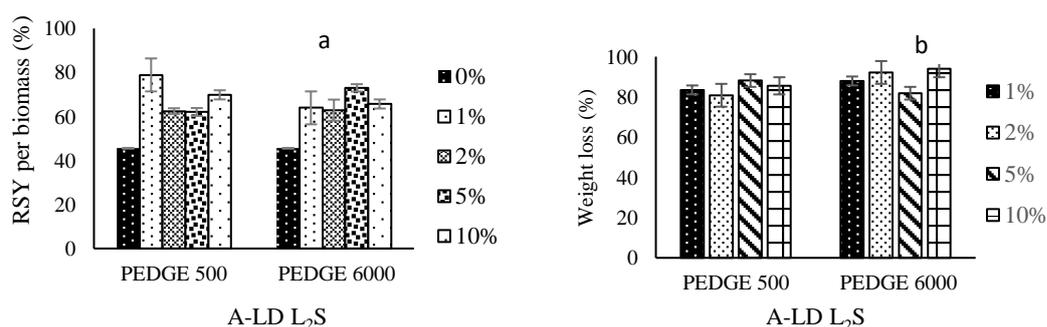


Figure 3. The effects of A-LD L₂S loading and molecular weight of PEDGE on reducing sugar yield (RSY) after enzymatic hydrolysis (EH) (a) and weight loss (b)

The highest RSY was obtained in 1% A-LD loading using PEDGE 500 as much as 78.89%. This yield is also higher than that of 5% A-LD loading using PEDGE 6000. It was proposed that PEDGE 500 resulted better efficiency compared to PEDGE 6000. And these results are higher than that of Winarni *et al.* [8] and Lin *et al.* [17] by using AL-PEG epoxide and EHL-PEG. In the same reaction condition, the RSY of A-LD L₂S produces lower RSY slightly compared to A-LD L₁S. It indicates that the further step in the lignin isolation is not required. Furthermore, PEDGE 500 is better to be used in the reaction with L₁S and L₂S as well. With hydrolysis rate of 100%, the highest RSY can produce 89.56% of theoretical RSY of initial SSB. Compared to RSY of A-LD L₁S, this yield has lower value. Previously, Cheng *et al.* [11] reported that the utilization of GC 220 enzyme in EH demonstrated higher sugar yield (73.7%) than that of meicellase enzyme in EH (63.9%) of softwood unbleached kraft pulp (NUKP) by addition DOPEG-SL (soda lignin). It was indicated that GC 220 enzyme relatively prefer to be proposed in improving performance reaction with DOPEG-SL, one of A-LD types. In this present research, we used celluclast with PEDGE-KL (kraft lignin) in which the RSY was higher than that of DOPEG-SL with GC 220 enzyme and meicellase.

Weight loss has also occurred during EH of SSB kraft pulp with addition of A-LD L₂S (Fig. 3b). As mention before, cellulose in the pulp has been hydrolyzed into sugar monomer. A-LD L₂S using

PEDGE 6000 tended to yield higher weight loss than that of A-LD L₂S using PEDGE 5000. The highest weight loss (92.14%) was found in A-LD loading of 2% with PEDGE 6000. Eventhough, weight loss after EH with A-LD L₂S is higher than that of A-LD L₁S, the RSY isnot higher anymore. Thus, high weight loss isnot always related with high RSY after EH. The other factors might affected it which need to be further studied.

3.3 The effects of A-LD LS Addition in Enzymatic Hydrolysis

In the same method, commercial lignin (LS) was also reacted with PEDGE used as comparison to observe the effect of isolation method in the performance of A-LDs. A-LD LS can alsoincrease RSY compared to without A-LD addition. The higher A-LD LS loading in EH, the higher RSY obtained (Fig 4a). The highest RSY (76.02%) is still lower than that of the highest RSY of A-LD L₁S and L₂S. These results has been supported by previous report of Winarni *et al.*[8]; Cheng *et al.* [11] and Lin *et al.* [17]. This highest RSY can produce about 86.29% of the theoretical RSY of initial SSB with hydrolysis rate of 100%. It is indicated that lignin which was isolated from black liquor of *Acacia mangium*kraft pulping for A-LDs can become new prospective utilization compared to commercial lignin. Eventhough commercial lignin and lignin two step physically have finer particles, however prior to the synthesis process, they were dillutedin alkali solution. Thus, they can react with PEDGE easily to yield A-LDs.

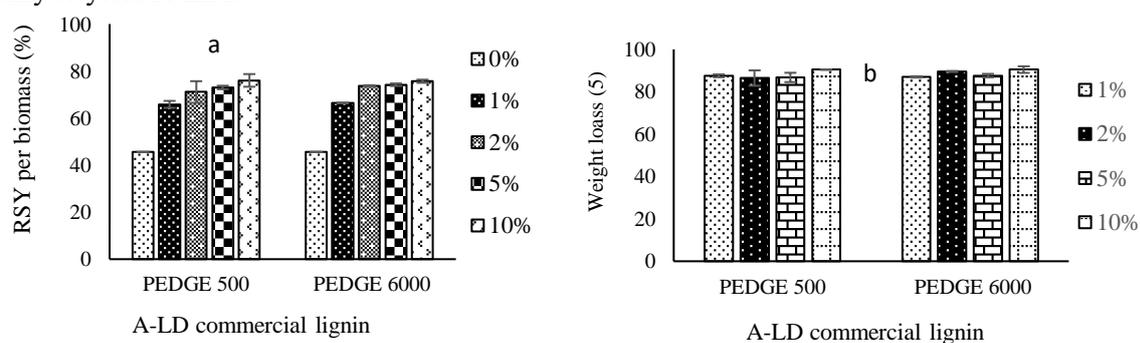


Figure 4.The effects of A-LD LS loading and molecular weight of PEDGE on reducing sugar yield (RSY) after enzymatic hydrolysis (EH) (a) and weight loss (b)

As mention before, A-LDs was effective to allow enzyme in working effectively by preventing non-productive enzymeabsorption [8, 9, 10]. PEDGE 500 worked better reaction with LS than that of PEDGE 6000. Therefore, based on the results, PEDGE 500 is prefer to be used than that of PEDGE 6000. EH of SSB kraft pulp with addition of A-LD LS caused weight loss (Fig. 3b). The formation of sugar monomer from cellulose contained in the pulp contributed on the loss. There is no significant difference in weight loss between A-LD LS using PEDGE 500 and 6000. The higher A-LD LS loading, the higher weight loss. This weight losstendency is in line with RSY. During EH, the weight lossoccurred due to conversion cellulose in the SSB pulp in the sugar monomer.

4. Conclusions

In this present study, A-LD L₁S, L₂S and LS was successfully made by reacting PEDGE and lignin. The addition of A-LDs in EH of SSB kraft pulp improved the RSY compared to control. Further step lignin isolation was not required to yield higher RSY. Isolated lignin performed more effective with PEDGE than that of LS.PEDGE 500 showed better reaction performance with lignin compared to PEDGE 6000. The higher A-LD loading tends to result the higher RSY. Utilization of 5% A-LD loading was more considered to achieve high RSY yield.

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