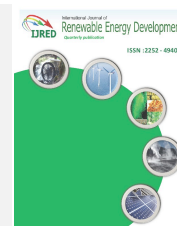




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Research Article

Optimizing the Synthesis of Lignin Derivatives from *Acacia mangium* to Improve the Enzymatic Hydrolysis of Kraft Pulp Sorghum Bagasse

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ABSTRACT. The present study is aimed at optimizing the synthesis of *Amphiphilic* lignin derivatives (A-LD) from the isolated lignin of *A. mangium* black liquor (BL), using the one and two step acid isolation method, and commercial lignin (LS) was used as comparison. The experimental design was conducted using Taguchi method, which consisted of four parameters and two level factors, with reference to the matrix orthogonal array, L₈, including temperature, reaction time, amount of polyethylene glycol diglycidylethers (PEGDE) and Kraft lignin (KL). Furthermore, the kraft pulp of sweet sorghum bagasse (SSB) was used as substrate in the enzymatic hydrolysis (NREL method), with addition of A-LD, whose functional group and surface tension were then characterised using ATR-FTIR and surface tension equipment. Conversely, an improvement in the reducing sugar yield (RSY) compared to the control was observed after adding various A-LDs to the substrate during enzymatic hydrolysis. This product was more prospective for L₂S than others products under milder circumstances, due to the fact that it possesses the lowest surface tension. Also, Taguchi analysis demonstrated the treatment at 60 °C for 1 h with 3.0 g and 0.6 g of PEDGE and lignin, respectively as the optimum condition, while the amount of lignin present was included as a factor with the propensity to significantly affect A-LD L₁S and LS. Therefore, it was established that the A-LDs from *A. mangium* kraft lignin require milder synthesis conditions, compared to other existing methods and despite the differences in optimum experimental condition for L₂S and LS, the functional groups in the IR spectra possessed very identical characteristics. ©2020. CBIORÉ-IJRED. All rights reserved

Keywords: Amphiphilic Lignin Derivatives, lignin, reducing sugar yield, Optimization, Taguchi analysis, A-LD synthesis

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1. Introduction

Lignin polymer is the second most abundant biopolymer after cellulose from the lignocellulosic biomass. The biorefinery concept efficiency is increased by converting lignin, a by-product obtained from the pre-treatment stage of second generation bioethanol production, into a value-added material. Conversely, technical lignin is usually isolated from black liquor (BL) as by-product in pulp and

paper industries. Pulp and paper industries has important contribution on Indonesian national economy because of this massive labour absorption and national export income (Ahmad et al. 2018). In 2015, Indonesia is listed on top 20 global pulp and paper producer i.e. the 6th and 10th for paper and pulp, respectively. With assumption annual growth of 3%, the pulp production capacity is forecasted about 11.6 million tons in 2020 (Asosiasi Pulp dan Kertas Indonesia, APKI 2016)..

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Silsia *et al.* (2011) reported a conventional pulping yield of 42.59%, and the isolate of kraft lignin obtained from *Acacia mangium* BL was 45.76% (Hermiati *et al.* 2017). Furthermore, about 50 % and 36% of the yield have been adopted as heat source in pulp mills, and for electricity generation, respectively (Mercer International Groups 2008). The potency of BL and lignin is predicted to be 6.66 and 3.05 million tons, respectively, where about 0.43 million tons of lignin (14%) is un-utilized as loss, hence the tendency for use as a valuable bioproduct. In addition, it is also possible to improve the efficiency of enzymatic hydrolysis during bioethanol production, using the resulting biosurfactant. This is attained through integration with the manufacturing process of pulp and paper mills. Conversely, the demand of surfactant in Indonesia has reached 95 thousand tons per year, while the domestic production capacity is limited to 55 thousand (www.lipi.go.id), hence the need to import approximately 40 thousand. Meanwhile, the wide use of surfactant, alongside the high domestic consumption facilitates the development of affiliated industries. These amphiphilic substances are known to contain hydrophilic molecules (water-soluble ionic groups) and hydrophobic (oil-soluble hydrocarbon) chains, with the ability to change a materials' surface property. Furthermore, they are soluble in water, as well as organic solvent (Uraki *et al.* 2012), due to the capability of decreasing surface and interfacial tension, improving the stability of dispersed particles, and also controlling the type of emulsion formed. For example, oil in water (O/W) or water in oil (W/O) emulsions (Swasono *et al.* 2012). Furthermore, Amphiphilic lignin derivatives (A-LD) are one of the lignin-based non-ionic surfactant, which is mainly utilized in elevating the efficiency of hydrolysis, as well as to improve the fermentation by yeast. Cheng *et al.* (2014) reported on the ability for A-LDs (DOPEG-SL and EPEG-SL) to improve the production of bioethanol during fed-batch simultaneous saccharification and fermentation (SSF). This 1.5 L unit with a substrate loading of 30% (w/v) generates high concentrations of 87.9 g/L at temperature of 38 °C, after adding 3.0 g/L A-LDs (Cheng *et al.* 2017).

It is assumed that these molecules confer stability on the enzyme responsible for denaturation during the process of enzymatic hydrolysis (Uraki *et al.* 2012; Kaar and Holtzapple 1998; Lee *et al.* 1996), and also the function as a cellulase-aid agent (Uraki *et al.* 2012). Previously, A-LDs have been developed from the acetic acid lignin (AL) and technical lignin via polyoxyethylation reaction. This requires the adsorption of epoxidized polyethylene glycol (PEGs) on the surface, subsequently serving as the hydrophilic moiety, including dodecyloxypoly(ethylene glycol) glycidyl ether (DOPEG), ethoxy (2-hydroxy) propoxy poly (ethylene glycol) glycidyl ether (EPEG), dodecyloxy-polyethylene glycol glycidyl ether (DAEO) and polyethylene glycol diglycidyl ethers (PEDGE) (Cheng *et al.* 2014; Homma *et al.* 2008; 2010; Winarni *et al.* 2013). In addition, the steric action of the bound lignin surface undergoes a hydrophobic interaction competition between the proteins and surfactants. This further reduces the possibility of un-reproductive enzyme bonding, facilitating the effectiveness of total enzyme applied in the hydrolysis process (Jeon *et al.* 1991; Börjesson *et al.* 2007).

A-LDs are also affiliated with the maintenance of high enzyme activity and significantly improved recovery

after the saccharification process (Uraki *et al.* 2001; Winarni *et al.* 2014; Winarni *et al.* 2013). Meanwhile, surfactants tend to prevent the incidence of reinteraction with the lignin, consequently increasing the possibility of enzyme re-use after reaction completion. Specifically, A-LD as a water soluble polymer prevents the incidence of non-reproductive absorption (Winarni *et al.* 2013). Based on the granted patent No US 8911976-B2 in 2014, there has been reports on the development of lignin surfactant derived from kraft, acetic, organosolv, steam explosion, sulphate, as well as alkali lignin from softwood (cedar, cypress and pine), hardwood (beech and oak), and Gramineae (rice straw, fir, bagasse). However, the type sourced from kraft lignin Indonesia has not been developed to serve biosurfactant, purposes as the A-LDs synthesized by Winarni *et al.* (2013) from the acetic lignin of cedar wood and sago alkali lignin.

Previously, Fatriasari *et al.* (2017) reported on the improvement of reducing sugar yield (RSY) after adding crude A-LD in the enzymatic hydrolysis of kraft pulp of sweet sorghum bagasse (SSB). Hence, the incorporation of a 5% crude loading was proposed for use in the reaction. This process involved the integration of isolated kraft lignin sourced from *A.mangium* with PEDGE, possessing a molecular weight (Mn) of 500, which demonstrated better synthesis performance, resulting in a higher RSY. In addition a better effect is attained through the optimization of synthesis condition and parameters. This has not been reported in any study to our best knowledge, as A-LD synthesis was conducted using the Taguchi method, which is well-known for simplicity and ease, consequently adopted for improved process quality and diminished production cost (Roy 1990). This approach is particularly adopted in the microwave based heating of cellular biomass, leading to reduced variation in parameter combination (Tseng *et al.* 2013), as well as the optimization of some other process, including the biodiesel production of soybean waste (Fajriutami *et al.* 2013). Therefore, Taguchi method adopted in the discovery of optimal parameters for A-LD synthesis, hence the product is subsequently added to the enzymatic hydrolysis of pulp sourced from kraft SSB. This was performed in an attempt to observe the effects on RSY. The aim of this study was to optimize the A-LD synthesis condition by using Taguchi method. Isolated lignin from *A.mangium* BL with one and two step method was used in A-LD synthesis.

2. Materials and Methods

2.1 Materials

Black liquor (BL) of *A. mangium* kraft pulping, characterized by 70% solid content, was obtained from PT TELPP, South Sumatera, Indonesia, and used as a lignin source in A-LD synthesis. For comparison, commercial lignin (LS), Sigma Aldrich, was also subjected to a similar design condition. Epoxylated PEG, i.e polyethylene glycol diglycidyl ethers (PEDGE) Mn 500 (analytical grade, Sigma-Aldrich) was adopted as hydrophilic molecules, while SSB pulped with 17% active alkali and 20% sulfidity at 170°C for 4 h was used as enzymatic hydrolysis substrate (Fatriasari *et al.* 2015). This condition was selected because prior study demonstrated the propensity to generate the highest RSY (Solihat *et al.* 2017).

Conversely, cellulase enzyme (*celluclast*) ATCC 26921 acquired from *Trichoderma reesei* with activity of 42.3 FPU/mL (NREL/TP-510-42628) was applied in the enzymatic hydrolysis of kraft pulp SSB.

2.2 Methods

2.2.1 Lignin Isolation

A.mangium BL was isolated using the one and two step acid isolation method according to Hermiati *et al.* (2017), with modification in the frequency of washing the solution. Furthermore, 1 mole/L of hydrochloric acid (HCl) was added to 100 g BL with pH 12 and above, in order to reduce the value to 2, while ethanol was incorporated at a pH of 7, in an attempt to remove the sugar embedded in solution. Meanwhile, lignin suspended in acid solution was reserved for 8 h, to ensure complete precipitation, which was followed by vacuum filtration, in order to achieve a separation from solution. The precipitates were then dried at 45 °C for 24 h, and the lignin isolates, L₁S (one step) and L₂S (two step), were grounded and sieved through mesh 40. Consequently, the individual moisture content was determined prior to use in the process of A-LD synthesis.

2.2.2 A-LD synthesis based on Taguchi statistical design

The product quality is possibly improved with the Taguchi method by reducing the impact of factor variations, including the application of orthogonal arrays to lessen the experiment frequency. Furthermore, signal to noise ratio (*S/N*) is used to determine and evaluate the quality characteristics of different parameter combinations, subsequently predict and verify the optimal values (Tseng *et al.* 2013).

A-LD synthesis was performed based on Taguchi design with matrix L_n(I⁹), and conducted to detect the effect of temperature, reaction time, amount of lignin, and PEDGE. Conversely, the RSY per biomass was adopted as response parameter, with independent variables of temperature at 60 °C and 70 °C, reaction time (1 and 2h), amount of PEDGE (2 and 3 g) and lignin (0.6 and 1g) during A-LD synthesis. The orthogonal matrix design selected for variable was L₈(2³), being the smallest factorial, where the level chosen was (l), with 2 and 4 factors (f), respectively. In addition, the software Minitab series 17 was used to determine the experimental design. A-LD synthesis was conducted in duplicate, based on modified Cheng *et al* (2014).

Table 1.
Experiment design L₈(2³)

| Synthesis condition | Level | | | |
|---------------------|-----------|-------------------|-----------|------------|
| | Temp (°C) | Reaction time (h) | PEDGE (g) | Lignin (g) |
| 1 | 60 | 1.0 | 2.0 | 0.6 |
| 2 | 60 | 1.0 | 3.0 | 1.0 |
| 3 | 60 | 2.0 | 2.0 | 1.0 |
| 4 | 60 | 2.0 | 3.0 | 0.6 |
| 5 | 70 | 1.0 | 2.0 | 1.0 |
| 6 | 70 | 1.0 | 3.0 | 0.6 |
| 7 | 70 | 2.0 | 2.0 | 0.6 |
| 8 | 70 | 2.0 | 3.0 | 1.0 |

Furthermore, lignin stock was prepared by dissolving lignin (L₁S, L₂S, LS) in 1 M aqueous NaOH, followed by the addition of PEDGE to the 10 mL reaction tube. This was then placed in a beaker glass containing heated distilled water set at the temperature and reaction time stated in Table 1. In addition, the reaction was continuously stirred to attain homogeneity. Subsequently, the process was terminated through the dropwise incorporation of glacial acetic acid (2-4 mL) at pH 4, leading to the formation of an emulsion. This was placed in a tightly covered vial bottle, and reserved in a refrigerator prior to use in enzymatic hydrolysis.

2.2.3 Enzymatic hydrolysis and reducing sugar analysis

The effectiveness of crude A-LD added to the substrate in the enzymatic hydrolysis of kraft pulp SSB was evaluated using the National Renewable Energy Laboratory (NREL) method. This involved placing about 0.2 g of oven dried (OD) pulp in a conical flask, followed by the addition of 200 µl 2% b/v sodium azide, 10 mL sodium citrate buffer (0.05 M, pH 4.8), 30 FPU/g substrate of cellulase enzyme, and 5% A-LD/OD substrate. Prior to use, the A-LD stock at a concentration of 15 mg/mL was dissolved in citrate buffer, which was used to adjust the samples by 20 g, followed by incubation in an incubator shaker (WiseCube WIS-30R) at a rate of 150 rpm, and 50 °C for 72 h. Conversely, the control of substrate, enzyme, and buffer were also considered alongside the samples, and the hydrolysis process was performed in duplicate. Furthermore, the hydrolyzed substrates were then centrifugated at 500 rpm for 5 min to separate the supernatant from the residue, which was specifically placed in a freezer at temperatures of 5-10 °C. Therefore, the reducing sugar analysis was conducted on the supernatant following DNS method (Miller 1959) using UV-Vis Hitachi U-2001 at wavenumber of 540 nm. Subsequently, quality characteristics were evaluated with Taguchi statistical analysis, using the equation of Larger, which is better calculated with Minitab 17 statistical software. Based on this assessment, the equation obtained from ANOVA is possibly used to analyse data, followed by the determination of significant factors of the response, and model accuracy.

2.2.4. Functional group analysis and surface tension

The Attenuated Total Reflectance-Fourier Transform Infra-Red (ATR)-FTIR (a spectrum two Perkin Elmer, Shelton, USA) was used to characterize the functional groups of A-LD L₁S, L₂S and LS, in the optimum synthesis condition. Subsequently, approximately 0.1 mg of each was placed in a plate and hand-pressed, then the IR spectra were recorded using the scan number of 10 per sample. This was performed in the absorption mode and at a spectral resolution of 4.0 cm⁻¹, within the range of 4000 to 600 cm⁻¹, using the software (Perkin Elmer, USA). The surface tension of A-LDs in the optimum condition was measured using Spinning Drop Tensiometer using the method of SOP TX 500D Full Range interfacial Tensiometer.

3. Results and Discussions

3.1 The effect of lignin sources in A-LD on RSY

Lignin is known to dissolve in alkali, including NaOH. Furthermore, Cheng *et al.* (2014) reported on the absence of sediment formation in completed A-LD reactions, after the addition of glacial acetic to attain pH 4. Fig. 1 shows the proposed reaction between *A.mangium* KL and PEDGE in A-LD surfactant, and no significant differences were identified with the physical characteristics of L₁S, L₂S, and LS biosurfactant. Previous reports have demonstrate the presence of higher acid insoluble lignin (AIL) content in L₂S than L₁S, while the acid soluble lignin (ASL) composition was vice versa. In addition, the smaller particle size of L₂S was affiliated with the creation of a larger surface area, which is characterized by a relatively brighter colouration compared to L₁S (Hermiati *et al.* 2017). Meanwhile, both A-LDs tend to possess an unpleasant odor, due to the inherent chemical compounds, encompassing methyl mercaptan, dimethyl sulfide ((CH₃)₂S) and dimethyl disulfide (CH₃-S-S-CH₃) (Gilligan and Pyle 1974). The formation of these gases is initiated by the break down reaction of methyl aryl ether in one of the lignin composing units.

Polyethylene and its derivatives were applied as hydrophilic molecules because of the characteristic ability to degrade easily in nature, with low toxicity level. However, epoxidized polyethylene was synthesized as the result of a reaction between polyethylene glycol (PEG) and epichlorohydrin in the BF₃-Dibutylamine-Complex of PEG. This process was terminated by the incorporation of dehydrochlorination in base condition (Lin *et al.* 2014).

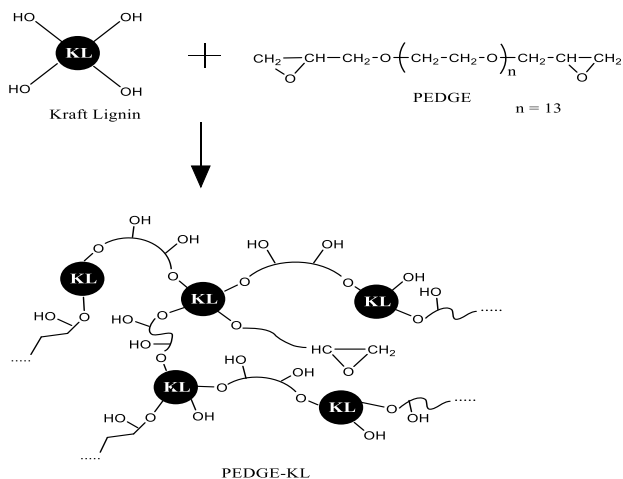


Fig.1. Proposed reaction of PEDGE and *A.mangium* kraft lignin (KL) on A-LD synthesis

Furthermore, PEDGE demonstrated hydrophilic attributes, hence the tendency to facilitate the water solubility properties of A-LD. These characteristics are due to the presence of oxide ethylene unit on the structure, which is enhanced in the presence of more replications (Homma *et al.* 2008). Moreover, the addition of hydrophilic PEGs for derivatization of hydrophobic lignin has been proven to improve surface activity. Furthermore, the addition of A-LD significantly lowered the surface tension of water (Uraki *et al.* 2012). Therefore, the results of adding A-LD to kraft pulp SSB show higher RSY per

biomass (75.99 %) in L₁S, compared with the control (Fig.2a), and obtained in experiment condition 2. This was characterized by a synthesis temperature of 60 °C, reaction time of 1 h, with the amount of PEGDE and lignin, at 3.0 g and 1.0 g, respectively. However, the outcome was slight lower than the report of Fatriasari *et al.* (2017), which used similar A-LD loading, although the current study used milder synthesis condition, encompassing lower temperature and shorter reaction time.

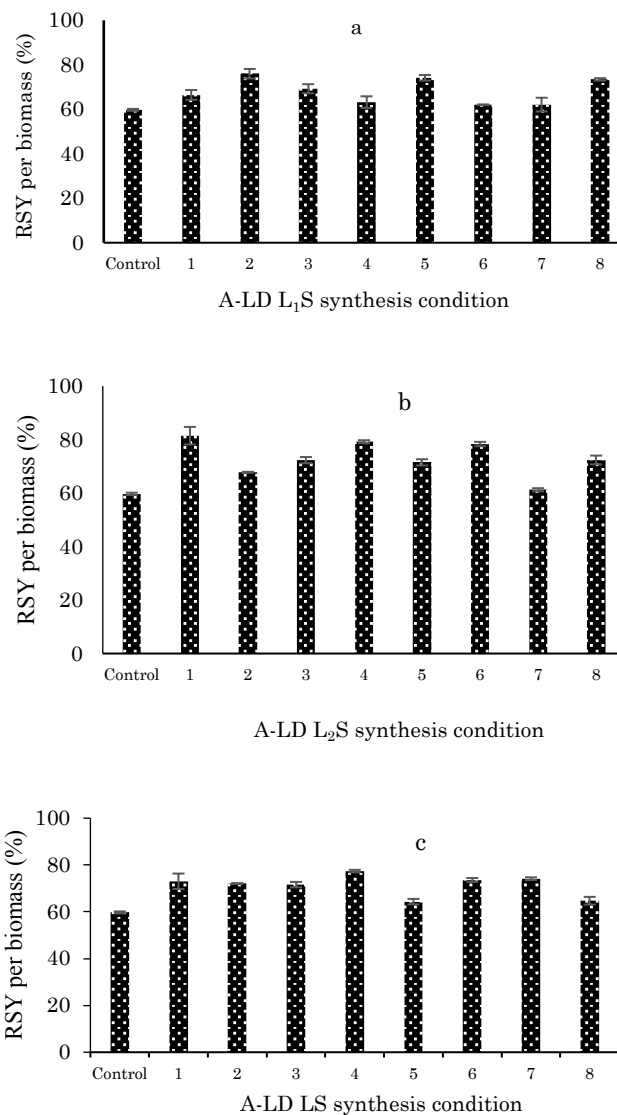


Fig. 2. Reducing sugar yield after addition of A-LD L₁S (a), A-LD L₂S (b), A-LD LS (c) in enzymatic hydrolysis of kraft pulp SSB

The addition of surfactant in enzymatic hydrolysis is assumed to inhibit the bonding between enzymes and lignin residue at the initiation of reaction. This further increases the possibility of subsequent enzyme reuse after completing the process (Eriksson *et al.* 2002; Li *et al.* 2016). In addition, A-LD is implicated in the reduced surface tension of solution, attained through the mechanism of enzyme and lignin absorption, consequently enhancing the reaction effectiveness (Fig. 3). The addition of A-LD is possibly improved by reducing non-productive

binding of enzyme with cellulose, as well as the non-specific hydrophobic interaction with the lignin in biomass as substrate (Uraki *et al.* 2012). Conversely, there was also a competition of hydrophobic interaction between protein and surfactant, which leads to a decline in the possibility of unfavourable enzyme bonding. The mechanism of resolution involves the ability for surfactant to prevent the occurrence of a reconnection between the enzyme and lignin, subsequently increasing the chance of reuse by immobilization. Moreover, A-LD is a water soluble polymer, characterized by the capacity to prevent the absorption of non-reproductive enzyme in the reaction process (Winarni *et al.* 2013), leading to enhanced working effectiveness (Jeon and Andrade 1991; Börjesson *et al.* 2007). Using similar procedure (72 h), the RSY of the control, using celluclast with an enzyme loading of 30 FPU/g was higher than meicellase at 293 FPU/g (45.57% (Solihatet *et al.* 2017). This outcome was congruent with the report of Cheng *et al.* (2017), where DOPEG-SL was added in the saccharification of softwood unbleached kraft pulp (NUKP) with Genecor (GC) 220 enzyme. This subsequently resulted in higher sugar yield, and the recovery of enzyme activity, compared to the use of meicellase, hence the variation affects RSY. Therefore, the higher effectiveness of celluclast (42.3 FPU/mL) compared to meicellase (14.65 FPU/mL) is implicated in this study result.

The addition of RSY to A-LD L₂S is shown in Fig.2b, where the highest amount resulted from synthesis condition 1, characterized by temperature of 60 °C, reaction time of 1 h, and amount of PEGDE 2.0 g and lignin of 0.6 g, *i.e.* 81.49%. Compared with the previous study of Fatriasari *et al.* (2017), where a lower A-LD loading of 5% with milder reaction condition was used, this current investigation provided a higher RSY. Therefore, the optimization endorsed effectively facilitates the loading and synthesis factors applied. During several experiment conditions, the RSY of A-LD L₂S was observed to be higher than L₁S, which contains lower carbon content, despite the relatively same hydrogen, oxygen, nitrogen, and sulphur contents.

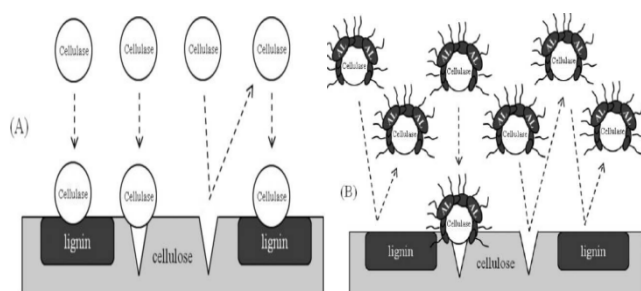


Fig. 3. Mechanism of A-LD-AL as cellulase-aid agent (a) without, and (B) with the addition of PEGDE-AL (Uraki *et al.* 2012)

Conversely, L₂S possesses more significant total lignin with comparably lower attachment of sugar and other impurities, although both consist of similar functional groups (Hermiati *et al.* 2017). Therefore, further isolation of L₂S causes a decline in the lignin yield of L₁S, which affects the interaction with PEDGE in the A-LD synthesis,

consequently affecting the individual sample performance in the process of enzymatic hydrolysis.

The addition of A-LD L₁Sto EPEG and DOPEG as hydrophilic polymers in the hydrolysis of unbleached cedar pulp reported by Cheng *et al.* (2014) resulted in RSY yield of 70.1% and 74.3%, respectively. Furthermore, the prerequisites for synthesis adopted were similar to experimental condition 4 in the current study, using L₂S and PEDGE. This yield was higher than the RSY with addition of L₁S. Meanwhile, the longer reaction time and higher amount of PEDGE observed in the synthesis condition 3 with L₂S, was characterized by an enhanced possibility to produce more polyethylene compounds. This facilitates the consequent reaction with lignin, therefore resulting in better A-LD characteristics. Fig. 2c shows the RSY obtained from the addition of A-LD LS in the enzymatic hydrolysis process. This involved the use of synthesis condition 4, characterized by temperature of 60 °C, reaction time of 2 h, amount of PEGDE and lignin of 2.0 g and 0.6 g, respectively, which provided the highest value at 77.39%. Furthermore, these prerequisites were selected as the optimum synthesis condition, although the RSY produced was comparably lower than the output obtained from adding L₂S, which was the highest, and closer to L₁S. Therefore, it is proposed that KL isolated from *A.mangium* BL is prospective sources of lignin for its derivatives. The RSY obtained for each treatment shows the effect of lignin isolation technique on the A-LD characteristics.

3.2 Optimum Condition Based on Taguchi Method

Signal to Noise (S/N) ratio, where Larger is the Better approach is used for RSY in the presence of A-LD L₁S and L₂S. This is determined based on the Taguchi method, which is chosen considering the positive value of RSY and the quality characteristic observed, as S/N ratio demonstrates the effect of noise factor on variations in the effect of temperature, reaction time, amount of PEDGE and lignin. Fig. 4 shows the quality characteristics in presence of A-LD L₁S, with the most influential factors exhibited with the following sequence: amount of lignin > reaction time > temperature > amount of PEDGE.

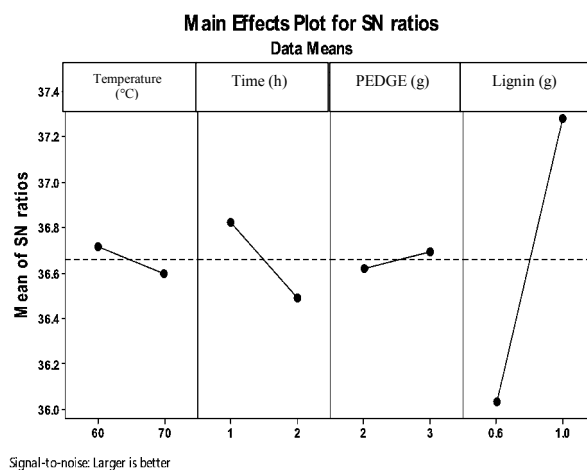


Fig. 4. Response of S/N ratio Larger is Better in A-LD L₁S synthesis

Table 2.
Analysis of Variance (ANOVA) of A-LD L₁S

| Factor | DF | Seq SS | Adj SS | Adj MS | F | P | F _{Table} |
|----------------|----|--------|--------|--------|-------|--------|--------------------|
| Temperature | 1 | 1.32 | 1.32 | 1.32 | 0.19 | 0.695 | 10.13 |
| Time | 1 | 14.06 | 14.06 | 14.06 | 1.99 | 0.253 | 10.13 |
| PEGDE | 1 | 0.91 | 0.910 | 0.91 | 0.13 | 0.744 | 10.13 |
| Lignin | 1 | 190.35 | 190.35 | 190.35 | 26.93 | 0.014* | 10.13 |
| Residual Error | 3 | 21.20 | 21.203 | 7.07 | | | |
| Total | 7 | 227.85 | | | | | |

$\alpha = 0.05$, confidence level of 95%, *) significant factor

Analysis of variance (ANOVA) is used to determine the optimum condition for A-LD synthesis, which was performed by observing the factors and levels responsible for the significant effects on L₁S (Table 2). Furthermore, it was established that only the amount of lignin conferred a significant effect on RSY ($F_{\text{count}} > F_{\text{table}}$). Based on S/N ratio, the optimum condition (indicated by the highest RSY) was identified in experiment 2, at a temperature of 60°C, reaction time of 1 h, amount of PEDGE and lignin of 3 g and 1 g, respectively.

S/N ratio with Larger is Better approach was also used to determine the optimum condition of A-LD L₂S (Fig. 5), which was identified as experiment 1, with temperature of 60°C, reaction time of 1 h, and amount of PEDGE, and lignin of 2.0 g and 0.6 g, respectively. Meanwhile, the ANOVA result is presented in Table 3, which shows the absence of any significant effect from all involved factors on A-LD characteristics ($F_{\text{count}} < F_{\text{table}}$) at a confidence level of 95%. Therefore, H₀ is accepted, as there are no dominant influencing factors in the various

levels, as their close nature to one another is assumed to have an effect on the result. Despite this study outcome, it was also established that each factor confers a different effect, as observed in Fig. 5, with sequence of most influential following the order; reaction time > amount of PEDGE > amount of lignin > temperature.

The optimization of A-LD L₂S synthesis as a control was also conducted with the Taguchi method, where Fig. 5 demonstrates the S/N ratio with Larger is Better approach, while Table 4 presents the ANOVA results. Furthermore, the optimum synthesis condition was observed in experiment 4, characterized by a temperature of 60°C, reaction time of 2 h, PEDGE and lignin amount of 3 g and 0.6 g, respectively. These prerequisites were compared with the optimum synthesis condition of A-LD L₂S, and the need to add more PEDGE, and the exposure to longer reaction time was established. Consequently, the RSY obtained with LS was also lower, and the significant factor of synthesis, organised in the order of most influential, include amount of lignin > temperature > reaction time > amount of PEDGE.

Table 3.
Analysis of Variance of A-LD L₂S

| Factor | DF | Seq SS | Adj SS | Adj MS | F | P | F _{Table} |
|----------------|----|--------|--------|--------|------|-------|--------------------|
| Temperature | 1 | 20.29 | 20.29 | 20.29 | 0.48 | 0.540 | 10.13 |
| Time | 1 | 144.21 | 144.21 | 144.21 | 3.38 | 0.163 | 10.13 |
| PEGDE | 1 | 51.39 | 51.39 | 51.39 | 1.21 | 0.352 | 10.13 |
| Lignin | 1 | 24.16 | 24.16 | 24.16 | 0.57 | 0.506 | 10.13 |
| Residual Error | 3 | 127.8 | 127.8 | 42.63 | | | |
| Total | 7 | 367.9 | | | | | |

$\alpha = 0.05$, Confidence level of 95%, *) significant factor

Table 4.
Analysis of Variance of A-LD LS

| Factor | DF | Seq SS | Adj SS | Adj MS | F | P | F _{Table} |
|----------------|----|---------|--------|--------|-------|--------|--------------------|
| Temperature | 1 | 37.329 | 37.329 | 37.329 | 5.15 | 0.108 | 10.13 |
| Time | 1 | 2.865 | 2.865 | 2.865 | 0.40 | 0.574 | 10.13 |
| PEGDE | 1 | 2.684 | 2.684 | 2.684 | 0.37 | 0.586 | 10.13 |
| Lignin | 1 | 80.020 | 80.020 | 80.020 | 11.15 | 0.044* | 10.13 |
| Residual Error | 3 | 21.741 | 7.247 | | | | |
| Total | 7 | 145.439 | | | | | |

$\alpha = 0.05$, confidence level of 95%, *) significant factor

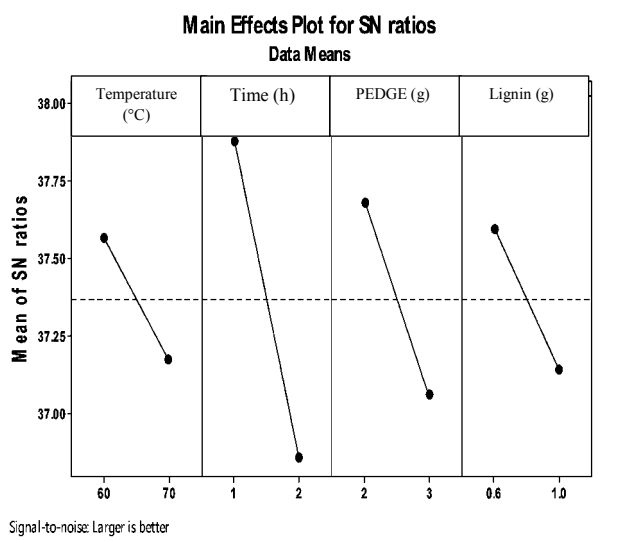


Fig.5. Response S/N ratio Larger is Better in A-LD L₂S synthesis

Based on the RSY obtained for each A-LDs, the synthesis condition is possibly listed on the basis of milder optimum, encompassing A-LD L₂S (81.49 %), LS (77.39 %), and L₁S (75.99 %). These variations were attributed to the lignin isolation method, based on the results of S/N ratio analysis and ANOVA (Table 4). Furthermore, the presence of impurities in lignin has also been implicated as a possible influencer of A-LD characteristics. Cheng *et al.* (2014) reported on the propensity for epoxylated PEGs used to confer an effect on surface activity, which is independent of the lignin origin or isolation method. Previously, Winarni *et al.* (2013) reported on the 69% yield from the addition of A-LD obtained from PEDGE and AL in enzymatic hydrolysis of cedar kraft pulp, with a cellulase loading of 20 FPU/g. Furthermore, the synthesis condition used involved 10 g lignin, 30 g PEDGE, and exposure to a temperature of 70 ° C for 2 h. Hence, a comparison with previous study showed a better RSY in optimum condition using A-LD sourced from *A.mangium* KL (1 and 2 step isolation), than PEDGE-AL, which was exposed to more severe conditions, compared to L₁S and L₂S.

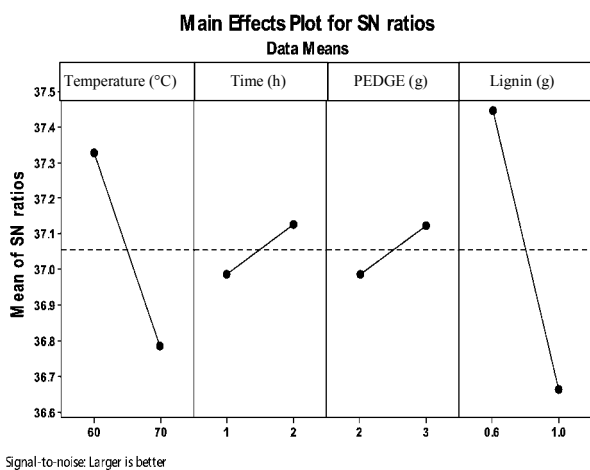


Fig. 6. Response of S/N ratio larger is better of A-LD LS

3.3 Analysis of functional groups of the optimum A-LD synthesis

The A-LDs obtained using the optimum synthesis condition was then analysed for functional group characteristics, using FTIR, as seen in Fig. 7 and Table 5. There were identical features between L₂S and LS, compared to L₁S, as some were only present in L₂S and LS, but not L₁S, including the C-H stretch at 3000-2842 cm⁻¹. Meanwhile, the effect of isolation method on the lignin characteristics is shown by the presence of impurities embedded in L₁S, which subsequently leads to an imperfect reaction with PEDGE. Physically, the internal observation shows a darker colouration with bigger particle size compared to L₂S and LS, hence the synthesis process completion is affected by the lignin type used as a main raw material. This study outcome is supported by previous results indicating the amount of lignin included as a significant factor, leading to the formation of varying characteristics in each A-LD type. Meanwhile, all samples in optimum condition tend to demonstrate low absorption of the epoxy moiety (C=O), with weak intensity and some typical lignin absorption spectra.

Basically, the characteristic of lignin is assigned by the following groups; -OH (3460-3412 cm⁻¹), C-H (3000–2842 cm⁻¹), C=O (1738–1593 cm⁻¹), syringil (1330 cm⁻¹), and C-H (925–832 cm⁻¹) (Chen *et al.*2015). Furthermore, the Infrared (IR) of PEDGE shows absorption in the range of O-H groups at 3424 cm⁻¹, C-H alkane at 2885 cm⁻¹, -CH₂ at 1464 cm⁻¹, C-O at 1183 cm⁻¹, linier-CH₂-OCH₂- at 1096 and 1150 cm⁻¹, -CH- cyclic epoxy at 1647 cm⁻¹ and epoxy groups at 837 and 1238 cm⁻¹ (Motawie *et al.* 2010). Hermiati *et al.*(2017) also reported on the functional groups of KL (1 and 2 steps) isolated from *A.mangium*BL, where L₁S and L₂S possess typical lignin absorption characteristics, although syringyl at wave number of 1330-1325 cm⁻¹ was not identified. Uraki *et al.* (2001) reported on the IR spectra of A-LD AL-PEDGE, which showed aromatic nucleus absorption at 1590-1510 cm⁻¹ and intense absorption at 2900 cm⁻¹, corresponding to the presence of a C-H bond in methylene.

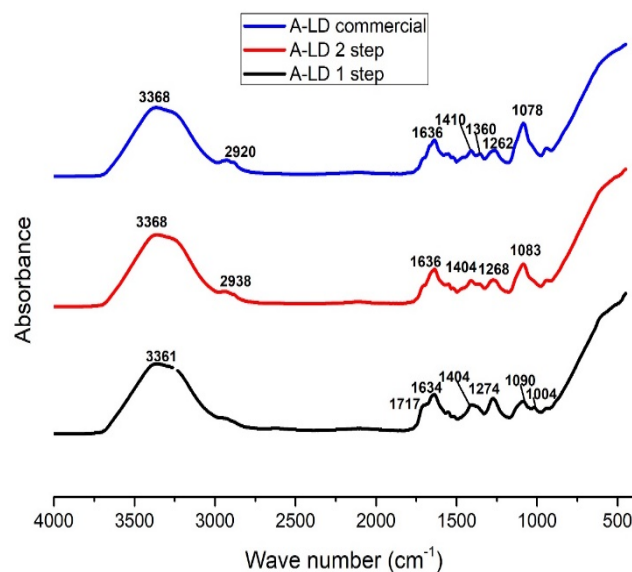


Fig. 7. FTIR spectra of A-LDs in the optimum synthesis condition

Table 5.

Functional groups analysis of A-LD L₁S, A-LD L₂S and A-LD LS in the optimum condition

| A-LD L ₁ S | A-LD L ₂ S | A-LD LS | Wave number (cm ⁻¹) (Chen <i>et al.</i> 2015) | Functional groups |
|-----------------------|-----------------------|---------|--------------------------------------------------------------|----------------------------------------------------------|
| 3356.95 | 3356.61 | 3356.85 | 3460-3412 | O-H stretch |
| - | 2938.83 | 2926.73 | 3000-2842 | C-H stretch/bond in methylene groups |
| 1638.82 | 1637.45 | 1638.08 | 1675-1655 | C=O stretch, unconjugated ketone |
| 1551.06 | 1549.54 | 1551.16 | 1605-1505 | Aromatic ring |
| - | 1525.27 | - | 1605-1505 | Aromatic ring |
| 1405.29 | 1409.26 | 1409.60 | 1470-1365 | Asymmetric C-H in -CH ₃ and CH ₂ - |
| - | 1362.68 | 1354.46 | 1270-1365 | C-H stretch aliphatic in -CH ₃ and phenol |
| 1273.46 | 1272.94 | 1261.58 | 1270-1266 | Guaiacyl ring |
| 1086.84 | 1084.89 | 1084.21 | 1086 | Deformation C-O on secondary alcohol |
| 1017.79 | - | - | 1030 | C-H in plane aromatic |
| - | 940.19 | 942.82 | 990-966 | -HC=CH- out of plane |

This is present in L₂S and LS, but absent in L₁S, due to the impurities embedded, which possibly prevent the complete bond formation with PEDGE, consequently leading to a lower output of RSY. In addition, the intensity of PEDGE and lignin is decreased at wave number 1200 cm⁻¹ and 837 cm⁻¹, due to the assignment of epoxy and finger prints for PEDGE, and the absorption intensity of C=O occurs at 1600 cm⁻¹ for lignin. This is possibly caused by a reaction between both materials, involving the opening of the epoxy group, thus exposing the linking O atom.

3.4 Surface Activity of A-LDs

The feasibility of A-LD L₁S, L₂S and LS as surfactants were determined by evaluating the ability to reduce water surface tension. Consequently, the resulting preparation dissolves both in water and organic solvents, encompassing methanol, chloroform, pyridine and benzene, hence all samples depressed water surface tension (Cheng *et al.* 2014; Homma *et al.* 2008). However, the least effect was observed with PEGDE-AL, which possesses the highest ethylene oxide (EO) content (Homma *et al.* 2008). In this current investigation, the lowest surface tension was identified in A-LD L₂S (38.87 dyne/cm), subsequently contributing to the higher RSY produced, followed by L₁S (41.34 dyne/cm) and LS (42.73 dyne/cm), which were not congruent with the RSY produced. This manifestation was, therefore, attributed other factors, including the absence of functional groups (C-H bond in methylene groups in L₁S, and aromatic ring in LS and L₁S). A previous study by Cheng *et al.* (2014) affiliated the dependence of A-LD surface activity with the nature of corresponding epoxylated PEGs used during synthesis, resulting from the crosslink of KL by PEGDE.

6. Conclusion

The addition of A-LD L₂S in the enzymatic hydrolysis of kraft pulp SSB leads to improved RSY productivity, compared to L₁S and LS. Subsequent evaluation

demonstrated the optimum synthesis condition, based on the ability to produce the highest amount of RSY. For L₁S, 75.99% yield was recorded in experiment 2, which was characterized by a temperature of 60°C, reaction time of 1 h, amount of PEGDE and lignin of 3.0 g and 1.0 g, respectively. Moreover, a 81.49% yield was obtained in L₂S, through experiment 1 (temperature of 60 °C, reaction time of 1 h, amount of PEGDE and lignin of 2.0 g and 0.6 g, respectively), while, the experiment condition 4 (temperature of 60°C, reaction time of 2 h, amount of PEDGE and lignin of 3.0 g and 0.6 g, respectively) was the best synthesis condition for LS, with RSY of 77.39%. Furthermore, it was established that the amount of lignin significantly affects L₁S and LS, hence the ideal synthesis prerequisites for *A.mangium* KL requires milder condition compared to existing approaches. The method for lignin isolation used results in varied characteristics, which consequently affects the binding reaction with PEDGE. Based on identified IR spectra evaluation, there were similarities in the functional group characteristics of A-LD LS and L₂S obtained with optimum conditions, characterized by the presence of C-H stretch at 3000-2842 cm⁻¹, which is absent in L₁S.

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