Effects of organosolv pretreatment using ethylene glycol on degraded empty fruit bunch for delignification and fractionation

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Abstract. Malaysia and Indonesia are the largest palm oil producers worldwide. In palm oil production, approximately one tonne of empty fruit bunch (EFB) will be generated as waste for every one tonne of palm oil produced. Currently, these fresh EFB are still underutilised and generally disposed under open environment. The fresh EFB are more susceptible to microbial attack under natural environment when exposed even for short period and become degraded empty fruit bunch (DEFB) which have lower quality. Consequently, disposal of DEFB becomes an enormous challenge as well as its following environmental problems including soil pollution and emission of greenhouse gases such as methane. However, DEFB remain as a promising lignocellulosic biomass feedstock with huge potential for production of high value added products entailing biofuels, bio-polymer and membrane with appropriate pretreatment. Therefore, DEFB was subjected to organosolv pretreatment in this research to recover its cellulose content. DEFB was discovered to possess higher cellulose content, lignin content and lower hemicellulose content as compared to fresh EFB. Organosolv pretreatment successfully fractionated DEFB to recover the cellulose portion by removing the lignin and hemicellulose content. Pretreatment with 50 v/v% ethylene glycol in the presence of 3 v/v% NaOH removed 75.1 wt.% lignin and 81.5 wt.% hemicellulose with 90.4 wt.% cellulose recovery. Furthermore, the cellulose purity of treated DEFB was improved drastically from 55.9% to 84.5%. For pretreatment liquor, the recoverable lignin was amounted to 74.6 % at pH 2.0. This study proved that organosolv pretreated DEFB exhibited the desirable properties for subsequent processes such as hydrolysis to synthesise the biomass waste into other high value added bio-products.

Keywords: Organosolv pretreatment, Ethylene glycol, Empty fruit bunch, Delignification, Fractionation

1. Introduction
The depletion of fossil fuel reserves and sustainable developments are the keys in developing renewable energy today. Production of renewable energy from lignocellulosic biomass is an active research topic with high potentials in future electricity generation. Numerous lignocellulosic biomass such as sugarcane bagasse, napier grass [1], corn stover [2] and cotton stalk [3] have been consistently applied in the studies of the production of biofuels.
Malaysia and Indonesia are the main palm oil producers which contributed 29% and 58% of worldwide palm oil production, respectively. During the palm oil production, approximately 1.05 tonnes of fresh empty fruit bunch (EFB) will be produced as waste with every tonne of palm oil produced [4]. These fresh EFB has been re-utilised as building materials for furniture such as bedding while minority of palm oil mills used the fresh EFB as solid fuel to generate electricity which partially offset their energy cost. However, problems associated with EFB such as high moisture content and higher capital investment limited its application as solid fuel. Therefore, enormous amount of fresh EFB were underutilised and majority were left to be composted in open environment. EFB is very susceptible to microbial attack and degradation in terms of physical and structural changes. This large quantity of degraded EFB (DEFB) was eventually disposed. As fresh EFB was difficult to store and susceptible to microbial attack, the state of art of this study was to show the feasibilities of re-utilisation of DEFB as the feedstock for biofuel and biochemical production instead of fresh EFB.

DEFB remains as lignocellulosic material and can be converted into biofuel which is able to enhance its commercial value and enhance the diversification of palm oil mills rather than single source of income from palm oil. Study had showed that DEFB possessed with high amount of cellulose content (55.9%) [5]. Owning to the recalcitrant properties of lignocellulosic biomass, pretreatment is necessary to remove the barrier in order to exploit the cellulose content. Organosolv pretreatment is a pretreatment method by using organic solvents in the presence or absence of catalyst. It has various advantages: (a) separation of high cellulose content (b) able to produce high quality lignin (c) organic solvents at low cost. Smit and Huijgen utilised 50 wt% acetone with sulfuric acid as catalyst at 140°C for 120 min to fractionate corn stover [2]. The delignification achieved 81.5% while the hemicellulose removal was recorded at 91.5% and recovered 89.3% cellulose. The objectives of this study were to investigate the applications of alkaline ethylene glycol pretreatment to pretreat DEFB for biochemical and biofuel production. In order to minimise the wastes produced and emerging applications of lignin, study on recoverable lignin from black liquor is included in this study.

2. Materials and methods

2.1. Raw material preparation

Fresh EFB and DEFB were collected from a plantation estate in Segamat, Malaysia. DEFB had been left in open environment for 14 days prior to collection. DEFB and fresh EFB were washed thoroughly with distilled water and cut into smaller pieces into less than 5 cm. The cut DEFB and fresh EFB were then dried in oven at 103°C overnight and underwent size reduction with powered grinder. Then, it was sieved with size 850 μm and stored in resalable container for further utilisation. The composition of DEFB and fresh EFB were determined as follow: (a) DEFB consisted of 23.9% lignin, 6.10% hemicellulose, 55.9% cellulose, 4.45% ash and 8.80% extractives (b) Fresh EFB composed of 22.1% lignin, 9.63% hemicellulose, 50.5% cellulose, 4.86% ash and 12.7% extractives.

2.2. Pretreatment with alkaline ethylene glycol

Different parameters were studied in the sequence of temperature and residence time for ethylene glycol pretreatment. For temperature study, powdered DEFB was pretreated (10 w/v%) with 50 v/v% ethylene glycol with 3 v/v% NaOH at temperature (60, 80, 100, 120 and 140°C) for 30 min. Treated DEFB was washed with distilled water for several times and dried in oven at 100°C [6]. The procedures were repeated similarly with different pretreatment residence times (15, 30, 45, 60 and 75 min).

2.3. Lignin recovery from ethylene glycol pretreatment liquor

Pretreatment liquor was collected immediately after the pretreatment and stored at 4°C. The initial pH of pretreatment liquors obtained was in the range of 12.3 to 12.5. The pretreatment liquor was then added with 5 mol/L sulfuric acid drop wise until desired pH (2.0, 3.25, 5.75 and 6.50) was reached. The acidified pretreatment liquor was filtered with vacuum filtration to recover precipitated lignin and dried at 103°C overnight and weighted gravimetrically.
2.4. Components analysis and equipment characterisation

The composition of holocellulose was determined according to TAPPI T203cm-09. The Klason lignin was determined with Klason method and the acid soluble lignin and lignin in pretreatment liquor were measured with UV-Vis spectrophotometer and the detection at 215 and 280 nm. The ash and extractives were determined with TAPPI T211om-02 and TAPPI T204cm-97, respectively. SEM was used to characterise the DEFAB morphologically by using Hitachi S-3400N with 500× magnifications.

3. Results and discussion

3.1. Effects of temperature of alkaline ethylene glycol pretreatment

Ethylene glycol pretreatment with concentration 50 v/v% with 3 v/v% sodium hydroxide for 30 min was conducted at temperature from 60°C to 140°C to study the effects of temperature as shown in Figure 1. Apparently, pretreatment temperature influenced the delignification achieved. The delignification increased from 56.1% to 71.5% as temperature increased from 60°C to 140°C. The cellulose recovery reduced from 92% to 78.5% while the hemicellulose removal increased from 52% to 78.0% as the temperature increased from 60°C to 140°C. Alkaline catalyst was able to cleave the benzyl ester and glycosidic bonds between hemicellulose [7]. The increment in temperature enhanced the cleavage of glycosidic bond of cellulose and hemicellulose. However, by referring to Table 1 and comparing to Figure 1., higher delignification from 100°C to 140°C did not result to the reduction in lignin composition of treated pulp. In addition, the cellulose composition in the treated pulp did not improve due to lower cellulose recovery. The composition of lignin, hemicellulose and lignin remained stagnant at temperature 100°C to 140°C which indicated that these components were removed concurrently during the pretreatment. For an ideal organosolv pretreatment, the organic solvent is able to selectively remove lignin and hemicellulose while retaining high cellulose content.

![Figure 1. Pretreatment performances of alkaline ethylene glycol with variations in pretreatment temperatures from 60°C to 140°C](image)

As a result, 80°C was selected for following studies due to its lowest lignin composition (11.9%), 90.8% cellulose recovery and 70.4% hemicellulose. The treated pulp at 80°C retained high cellulose content.
purity at 81.2%. Alkaline ethylene glycol pretreatment was able to achieve similar delignification at milder temperature compared to acidified ethylene glycol pretreatment. Lee and co-workers pretreated newspaper by acidified ethylene glycol at 150°C for 15 min and achieved 75% delignification [8]. Figure 2, shows the morphology of treated DEFB after alkaline ethylene glycol pretreatment at 60°C and 80°C. Higher temperature resulted to higher formation of opened and deepened surface structures which indicated that the removal of lignin and hemicellulose.

| Temperature (℃) | Pulp Yield (g) | Composition (%) |
|-----------------|----------------|-----------------|
|                 |                | Lignin | Hemicellulose | Cellulose | Ash | Extractive |
| Untreated       | -              | 23.9   | 6.10         | 55.9      | 4.45 | 8.80       |
| 60              | 6.87           | 15.3   | 3.93         | 75.4      | 2.33 | 3.06       |
| 80              | 6.29           | 11.9   | 2.70         | 81.2      | 1.91 | 2.23       |
| 100             | 5.89           | 12.4   | 2.38         | 81.2      | 2.72 | 1.36       |
| 120             | 5.55           | 12.1   | 2.34         | 79.8      | 4.32 | 1.44       |
| 140             | 5.42           | 12.0   | 2.40         | 81.5      | 3.14 | 0.92       |

**Figure 2.** Surface morphology of treated DEFB after alkaline ethylene glycol pretreatment at (a) 60°C and (b) 80°C under 500× magnifications

**3.2 Effects of residence time of alkaline ethylene glycol pretreatment**

Figure 3 and Table 2 show the effects of residence time on the pretreatment performances of alkaline ethylene glycol pretreatment. The delignification improved from 63.1% to 75.1% as time increased from 15 to 45 min and reduced 72.8% as time was increased to 75 min. On the other hand, increased time enhanced the hemicellulose removal significantly but cellulose recovery was minimally affected which decreased from 91.8% to 88.0%. As compared to temperature, prolong residence time had lesser influencing effects on cellulose recovery. This could be related to mild pretreatment temperature (80°C) was used. Sun and co-workers also reported the similar trend that the cellulose recovery decreased from 93.1% to 85.6% when pretreatment time was increased from 30 min to 90 min at 80°C while cellulose loss was amplified which cellulose recovery decreased from 46.1% to 35.7% when pretreatment time was increased from 30 min to 90 min at 140°C [9]. Therefore, pretreatment temperature was likely a dominant factor in cellulose recovery. The hemicellulose removal improved from 53.2% to 85.2% as the residence time increased from 15 min to 75 min. Similarly, the composition of hemicellulose reduced from 6.10% to 1.39% over the time tested. Similar to increased temperature, prolong residence time did not effectively reduce the lignin content and increase the cellulose content. Residence time 45 min was selected due to its highest delignification (75.1%), 81.5% hemicellulose removal and 90.4% cellulose recovery. The treated pulp had high cellulose content at 84.5%.

Figure 4.(a) and (b) show the surface morphology of treated DEFB after 15 min and 45 min of alkaline ethylene glycol pretreatment, respectively. Uneven and opened surface structures were increased when the pretreatment time was increased from 15 min to 45 min which indicated that the
lignin and hemicellulose were depolymerised by the alkaline ethylene glycol. Enhanced pretreatment severity led to higher lignin and hemicellulose removal which was leaving the treated DEFB with enlargement of pores and deepened structures. The significant improvements of cellulose purity in treated DEFB from 55.9% to 84.4%, the high cellulose purity in treated DEFB rendered it to be a better feedstock for biochemical and biofuel production. The high amount cellulose in treated DEFB can be hydrolysed by enzymatic hydrolysis in to glucose which can be further converted into bioethanol (biofuel), biobutanol (biofuel), 5-hydroxymethylfurfural (important platform chemical for packaging, textile, health and polymer industries), levulinic acid (platform chemical and additives in adhesives and lubricants) and succinic acid (intermediate chemical for plasticizers and coating).

Figure 3. Pretreatment performances of alkaline ethylene glycol with variations in residence time from 15 min to 75 min

Table 2. The compositional analysis of treated pulp with variation in residence time

| Time (min) | Pulp Yield (g) | Composition (%) |
|------------|----------------|-----------------|
|            |                | Lignin | Hemicellulose | Cellulose | Ash | Extractive |
| Untreated  | 10.0           | 23.9   | 6.10          | 55.9      | 4.45 | 8.80       |
| 15         | 6.71           | 13.6   | 3.56          | 77.0      | 3.27 | 2.10       |
| 30         | 6.29           | 11.9   | 2.70          | 81.2      | 1.91 | 2.23       |
| 45         | 5.98           | 10.0   | 1.89          | 84.4      | 1.73 | 1.98       |
| 60         | 5.85           | 11.3   | 1.50          | 83.2      | 1.76 | 2.19       |
| 75         | 5.84           | 11.6   | 1.39          | 84.3      | 1.86 | 0.92       |
3.3 Lignin recovery from spent pretreatment liquor

The acid precipitation was the most common method that applied in lignin recovery from pretreatment liquor. Figure 4(a) shows the percentile of precipitated lignin and its purity against the reduction of pH of pretreatment liquor with addition of sulfuric acid. The precipitated lignin increased substantially from 47.7% at pH 6.50 to 74.6% at pH 2.0. The purity of precipitated lignin was obtained in the range of 94.5% to 97.2%. The remaining composition was mainly composed of polysaccharides from hemicellulose sugars which attached at the side chain of lignin [4]. It was resulted mainly due to incomplete cleavage of linkages between lignin and hemicellulose. Furthermore, incomplete lignin precipitation was observed although the pH of pretreatment liquor was dropped to very acidic condition at pH 2.0. It was similarly observed in the studies conducted by Mussatto et al. to recover precipitated lignin from sodium hydroxide delignified brewer’s spent grain [10]. The yield of precipitated lignin was reported as 81.4% at pH 2.15 which was higher than this study which recorded 74.6% at pH 2.0. This could be justified with the presence of different phenolic composition due to different biomass was being used which lead to different precipitation rates.

Figure 5. Precipitated lignin and its purity from acidification of spent liquor
The recovered lignin from organosolv pretreatment had been regarded as high quality lignin due to lack of sulfur content. The current emerging trends of incorporating recoverable lignin from black liquor are bioplastics, composites, nanoparticles, adsorbents, dispersants and carbon fibres [11]. Therefore, the method applied in this study can be utilised to recover the lignin for promising future applications.

Conclusions
DEFB was proven as potential lignocellulosic biomass feedstock for biofuel production. Ethylene glycol pretreatment with 3 v/v% sodium hydroxide removed 75.1% lignin and 81.5% hemicellulose and recovered 90.4% cellulose. Its cellulose purity enhanced from raw 55.9% to 84.5% which resulted to DEFB was suitable to be utilised as substrate for hydrolysis process. Addition of acid was able to precipitate the lignin from pretreatment liquor. The optimum of lignin recovery stood at 74.6% at pH 2.0.

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