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The Effect of Steam and Glycerol Pretreatment on Chemical Contents of Oil Palm Empty Fruit Bunch (EFB)

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Abstract

This research aimed to evaluate the effect of the type of solvent, pH, substrate loading, and reaction time on the chemical components of palm empty fruit bunches (EFB). Steam pretreatment was set up at a temperature of 121 °C and pressure of 1.18 bar, using an autoclave with substrate loading of 5, 10, 15 and 20 % w/v at reaction times of 15 and 60 min. Distilled water, waste glycerol, alkaline glycerol and acidic glycerol were compared as solvents during steam pretreatment. The results showed that with distilled water, better pretreatment was achieved at 5 % and 10 % loading for 60 min. During the pretreatment with waste glycerol at 5 % loading an increase on the reaction time from 15 to 60 min reaction resulted in a remarkable increase in reducing sugar in the liquid phase. Overall, the best steam pretreatment conditions were observed using alkaline glycerol at 5 % w/v and 15 min reaction time, resulting in holocellulose (cellulose plus hemicellulose) increase to 87.98 % and a lignin decrease to 9.17 %. However, pretreatment with glycerol for 15 min was better than those for 60 min using either glycerol or distilled water. The results suggest that waste glycerol during steam pretreatment of EFB can be utilized effectively at short reaction times and at an increased pH to achieve a high output of cellulose and hemicellulose for sugar conversion in the bioethanol fermentation process.

Keywords: Palm empty fruit bunch; Steam pretreatment; Waste glycerol; Alkaline and acidic pretreatment

Introduction

Exploring renewable energy sources for bio-ethanol production is imperative as alternatives to the use of fossil fuels. Lignocellulosic bio-mass from agricultural wastes offers one such source. According to Thailand's Alternative Energy Development Plan [1], 14 million L d^{-1} of biodiesel could be produced from oil palm fresh fruit brunch of about 30 million tons a^{-1}, which in turn, could generate about 6 million tons a^{-1} of empty fruit, bunches (EFB).

The chemical composition of EFB includes cellulose, hemicellulose and lignin. Cellulose and hemicellulose can be converted to sugars (C-6 and C-5) through enzymic digestion, and these sugars can then be fermented to ethanol. However, cellulose and hemicellulose are densely packed by layers of lignin, which render them highly recalcitrant to enzymic degradation [2]. Therefore, appropriate pretreatment of EFB is considered essential to accelerate the process.

Many pretreatment technologies have been applied including physical, chemical and bio-logical methods, or any combination thereof. For example, degradation of the complex che-mical structure of EFB can be accomplished by steam pretreatment [3]. Steam pretreatment exposes the lignocellulosic materials to high-pressure steam, which increases the potential of enzyme hydrolysis [4]. Additionally, chemicals such as hydrochloric acid, sulfuric acid, sodium hydroxide, ammonia and glycerol have been used for the pretreatment of EFB [5]. However, chemical pretreatment is expensive and has an impact on the environment. A promising chemical option could be crude glycerol, which is a by-product from biodiesel plants. For every 9 kg of biodiesel produced, 1 kg of crude glycerol is also formed [6]. Previous study has reported that glycerol has potential as a delignification agent in the presence of alkaline substances [7]. Zhang et al. has reported that glycerol thermal processing can disrupt the cell wall without noticeable change in chemical composition [8].

The objective of this research therefore is to study the effect of steam and waste glycerol pretreatment as a function of reaction time, pH and substrate loading on the organic chemical composition of oil palm empty fruit bunch, with emphasis on maximizing the holocellulose (cellulose plus hemicellulose) content and minimizing the lignin content in the treated product.

Materials and methods

1) Raw material

Palm EFB and waste glycerol were collected from Suksomboon oil palm mill factory located in the Chonburi Province, Thailand. Waste glycerol was analyzed for pH and soluble chemical oxygen demand (SCOD). To prepare the substrate for the experiment, EFB was washed to remove particles and dried at 100-105 °C for 5 h. Subsequently, it was chopped to about 1-3 mm size and stored in sealed plastic bags at room temperature until further use as carbon source (substrate) for the microorganisms involved.

2) Experimental approach

The efficiency of steam pretreatment was investigated employing laboratory Erlenmeyer flasks (125 mL volume) as batch reactors. The pretreatment was carried out by using steam in an autoclave (LS-2D, REXALL) at a tempera-ture of 121 °C and pressure of 1.18 bars. The four different solvents used in pretreatment were distilled water (DW), waste glycerol (WG) at pH = 8.7, alkaline waste glycerol at pH = 11.0, and acidic waste glycerol at pH = 3.8. The alkaline waste glycerol was prepared with the addition of 1M NaOH while the acidic waste glycerol was prepared using concentrated H_2SO_4. Each liquid was added to EFB to formulate substrate loadings of 5, 10, 15 and 20 %, respectively. Two reaction times of 15 and

60 min were tested for each substrate loading. After the completion of pretreatment, the samples were filtrated through a GF/C filter paper. A portion of the solid samples was washed with distilled water and dried at 100-105 °C for 2 hours. Subsequently, it was analyzed to determine the percentages of cellulose, hemicellulose and lignin following the forage fiber analytical method described in the Agriculture Handbook [9]. Briefly stated, this method involves three analytical procedures: The neutral-detergent fiber (NDF), the acid-detergent fiber (ADF), and the acid-detergent lignin (ADL) one. The percentages of the three organic components mentioned above can be calculated as follows:

- % Cellulose = % ADF – % ADL
- % Hemicellulose = % NDF – % ADF
- % Lignin = % ADL

The liquid phase from the pretreatment was analyzed for soluble chemical oxygen demand (SCOD) using the closed reflux, titrimetric method as outlined in the Standard Methods [10]. In addition, reducing sugar (RS) was determined by the 3, 5-dinitrosalicylic acid (DNS) method [11].

Results and discussion

1) Effect of pretreatment on the components of EFB

The EFB used as raw material contains cellulose, hemicellulose and lignin, typically expressed as percentages as shown in Table 1. The organic content of EFB, measured as volatile solids, is nearly 90 % (with cellulose comprising approximately half of the organic matter) while the remaining percentage is attributed to moisture and ash. The experimental results show that with distilled water, steam pretreatment for 15 min increased the % cellulose (% C) but decreased the % hemicellulose (% H), therefore resulting in no appreciable change in the % holocellulose (% HL) (the sum of cellulose and hemicellulose) and % lignin (% L), regardless of the substrate loading applied (Figure 1a). In contrast, employing distilled water for 60 min increased the %HL to a maximum value of 86.40 % at 5 % substrate loading (Figure 1b). A further increase in substrate loading however did not improve the process performance.

Table 1 Characteristics of raw EFB

Characteristics	Values (% weight)
Cellulose	48.19
Hemicellulose	29.44
Lignin	13.75
Volatile solids	89.57±0.27
Moisture	6.57±0.06
Ash	3.85±0.24

Steam pretreatment with waste glycerol at pH = 8.7 appeared to be more effective at 15 min (Figure 1) since the corresponding % HL values are higher than those obtained at 60 min (Figure 2). It was therefore decided that further experiments using alkaline glycerol (pH = 11.0) or acidic glycerol (pH = 3.8) be conducted at 15 min reaction time and at the three lower substrate loadings only (conditions E and F, Figure 1a). Alkaline glycerol proved to be more effective at the lowest substrate loading of 5 %, resulting in the highest % HL (87.98 %, condition 1E), obtained for all conditions investigated. Moreover, the use of acidic glycerol did not seem to improve the % HL content, while the % L was lower than the untreated (raw) sample for both alkaline and acidic conditions (Figure 2). It should also be noted that the mean value of the solid yield was 89.9±3.9 %. The small standard deviation denotes that there is only small variation among the conditions investigated.

It should be noted that the modification of pretreated EFB structure illustrates the effect of steam and waste glycerol on the biomass components. Baharuddin et al. [5] studied the effects of high pressure steam pretreatment on EFB structure using scanning electron microscope (SEM) observations which showed that the holes on the EFB surface were found to be swelling and the outer layers were disrupted along the structure. In addition, the previously-cited study reported that glycerolysis is a chemical process which can break the glycosidic bonds between carbohydrates and may also help increase delignification selectivity. This process can be accelerated by addition of small amounts of sulfuric acid or sodium hydroxide [7]. It should be also noted that at a pretreatment time of 60 min with waste glycerol, it was found that both the % C and % H decreased and were lower than the corresponding values obtained using distilled water. Such a decrease in cellulose and hemi-cellulose may indicate that these compounds were already degraded to sugars.

As mentioned above, the effect of substrate loading using alkaline and acidic waste glycerol on % C and % H release is also noticeable (Figure 1 and Figure 2). These results indicate an alkaline environment appears to enhance the effectiveness of steam pretreatment of EFB, resulting in higher output of holocellulose. Similarly, Choi et al. [12] reported that NaOH-catalyzed steam pretreatment of EFB can remove lignin efficiently and requires only a short reaction time [12]. Another study has also pointed out that glycerol used as delignification solvent was more efficient in the presence of acidic or alkaline environments [7]. Although the percent increase in holocellulose and the reduction in lignin during pretreatment, in general, appear to be modest compared to the raw sample (Figures 1 and Figure 2), the changes in the EFB structure can be significant as illustrated above, which can facilitate further degradation of the EFB.

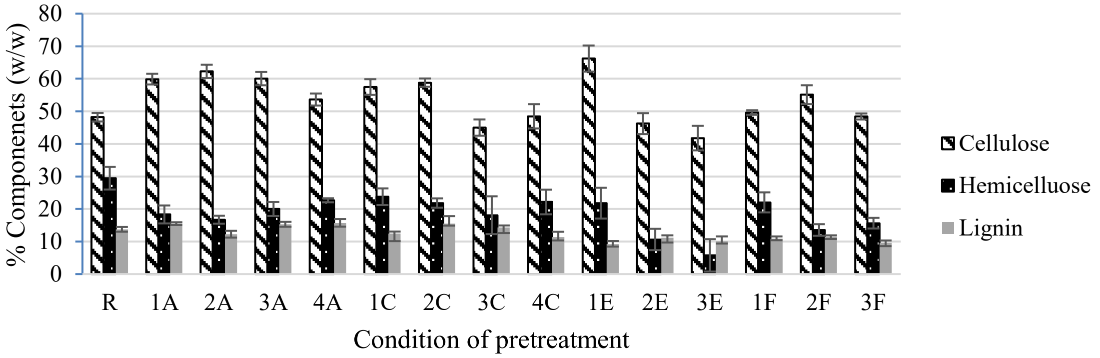

Figure 1 Effect of solvents on the components of pretreated EFB at 15 min (% w/w) : Raw material (R); DW 15 min (A); WG 15 min (C); Alkaline WG 15 min (E); Acidic WG 15 min (F) with substrate loading: 5 % (1), 10 % (2), 15 % (3) and 20 % (4).

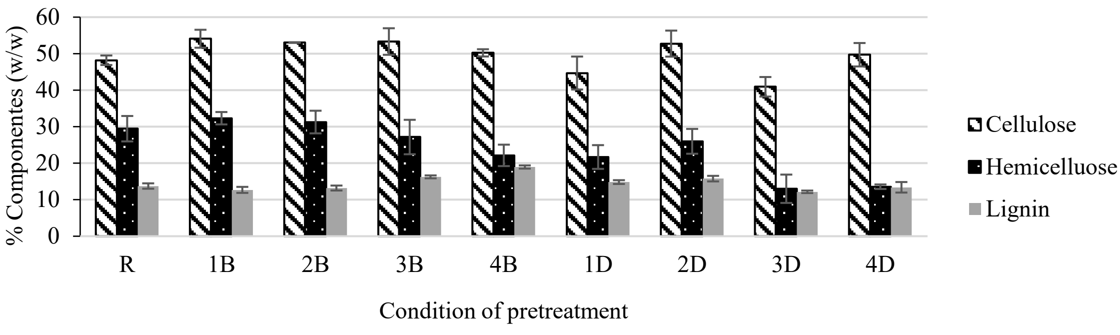

Figure 2 Effect of solvents on the components of pretreated EFB at 60 min (% w/w): Raw material (R); DW 60 min (B); WG 60 min (D); with substrate loading: 5 % (1), 10 % (2), 15 % (3) and 20 % (4).

2) Parameters of liquid phase

Following pretreatment, the treated samples were filtrated and the filtrate was analyzed for SCOD and RS concentration. In general, as the EFB loading increased the reducing sugar concentration also increased (Figure 3). The highest concentration was observed during pretreatment with waste glycerol at 60 min (condition D), regardless of the loading variation, suggesting that glycerol can release more reducing sugar at a longer reaction time. Whereas at 15 min, both alkaline and acidic conditions encourage a higher reducing sugar generation from glycerol than that observed with waste glycerol without pH adjustment. The results of alkaline waste glycerol pretreatment relate to the composition of EFB, that is, a low initial percentage of cellulose and hemicellulose may increase the reducing sugar content. Overall, it is apparent that steam pretreatment can convert the components of EFB to reducing sugar.

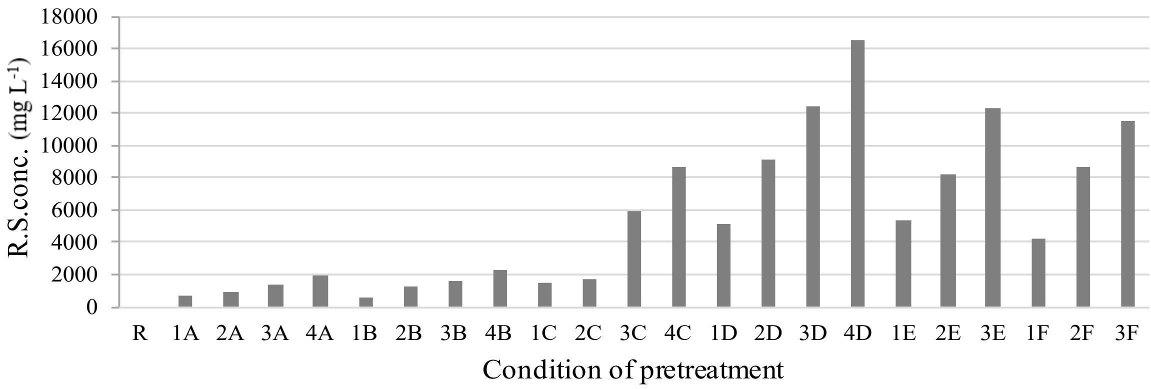

Figure 3 Concentration of reducing sugar in liquid phase (mg L^{-1}): Reducing sugar (R.S.); Raw material (R); DW 15 min (A); DW 60 min (B); WG 15 min (C); WG 60 min (D); Alkaline WG 15 min (E); Acidic WG 15 min (F) with substrate loading: 5 % (1), 10 % (2), 15 % (3) and 20 % (4). (S.D. < 25 mg L^{-1})

Regarding the SCOD data, pretreatment with distilled water produced the lowest concentrations at both reaction times of 15 and 60 min, designated as conditions (A) and (B) (Figure 4). The values corresponding to distill water pretreatment are significantly lower than those obtained with glycerol as a solvent, because glycerol as an organic compound itself contributes to SCOD of the sample. An EFB loading of 5 % achieved the highest SCOD in each pretreatment method, however, a reduction in SCOD was observed with an increase in the loading. This indicates that a low organic loading may favor the production of soluble organic compounds. Since EFB has been shown to have absorptive properties regarding SCOD-contributing compounds [13], higher organic loadings may result in overall lower SCOD concentrations in the liquid phase. Moreover, pretreatment with alkaline and acidic glycerol for a shorter period (15 min) generated a moderately lower SCOD concentration than waste glycerol without pH adjustment, indicating that the pH of a sample may affect the release of organic matter.

Figure 5 illustrates the percent of reducing sugar in the form of SCOD. Overall, reducing sugar appears to be a minor component of the SCOD in the liquid phase. It is interesting to note that the use of distilled water as a solvent (conditions A and B) resulted in higher RS content in the liquid phase compared to the other solvents applied. When EFB was pretreated with crude glycerol, not only reducing sugar was produced but also other soluble organic compounds were present. The ratio of

RS/SCOD signifies that most of the SCOD occurring in the liquid phase of the glycerol solvent did not originate from reducing sugar. Therefore, concerns about the treatment and disposal of the high organic-content wastewater generated should be also addressed during the pretreatment with glycerol. Moreover, it is apparent that regardless of the type of solvent used, an increase in the substrate loading caused a gradual increase in the RS content, which can be attributed to the higher initial organic content of the samples.

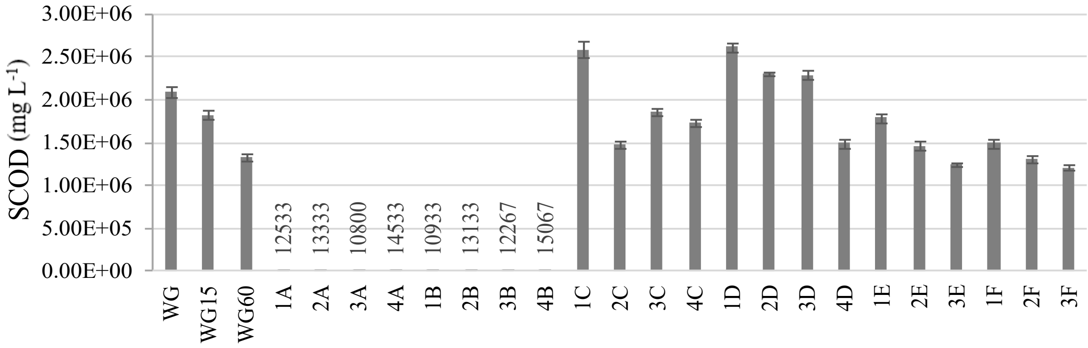

Figure 4 Soluble Chemical Oxygen Demand (SCOD) in liquid phase (mg L^{-1}): Glycerol 15 min (1G); Glycerol 60 min (2G); DW 15 min (A); DW 60 min (B); WG 15 min (C); WG 60 min (D); Alkaline WG 15 min (E); Acidic WG 15 min (F) with substrate loading: 5 % (1), 10 % (2), 15 % (3) and 20 % (4).

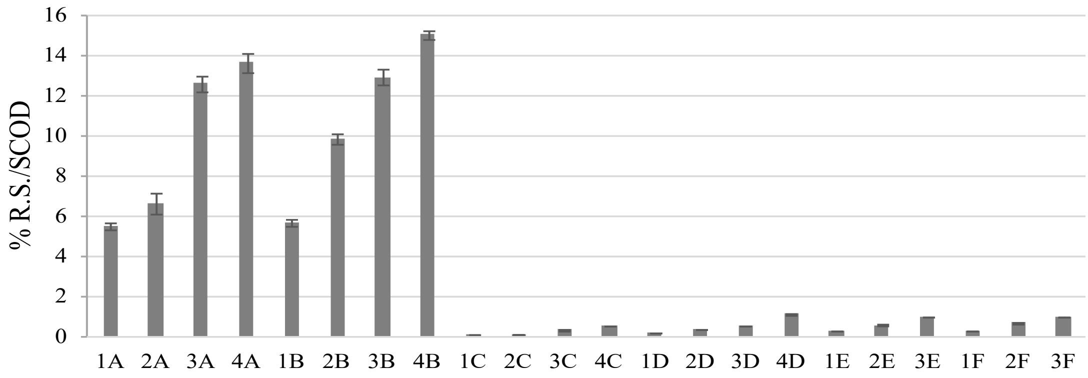

Figure 5 Ratio of RS to SCOD in the liquid phase (%): DW 15 min (A); DW 60 min (B); WG 15 min (C); WG 60 min (D); Alkaline WG 15 min (E); Acidic WG 15 min (F) with substrate loading: 5 % (1), 10 % (2), 15 % (3) and 20 % (4).

Conclusions

The chemical composition of treated EFB during steam pretreatment was found to vary according to the type of solvent use, the pH of the liquid phase, and reaction time. The steam pretreatment with distilled water for 60 min produced a higher content of holocellulose than that for 15 min. However, steam pretreatment with distilled water for 60 min achieved similar holocellulose concentrations when compared to pretreatment with waste glycerol for 15 min. It is interesting to note that a shorter reaction time was needed when waste glycerol was used as a solvent in steam pretreatment process. Furthermore, the highest content of holocellulose and the lowest content of lignin were found during pretreatment with alkaline glycerol at a low substrate loading. The reducing sugar results also demonstrate that a limited degradation of the EFB components was achieved under such conditions. Overall, it can be concluded that alkaline pretreatment of EFB with waste glycerol can be achieved at a shorter reaction time, which could result, among other benefits, in lower energy requirements for the process.

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