Phosphoric Acid Pretreatment of *Corchorus capsularis* L. Biomass for Enhancing Glucose Recovery

Suwanan Wongleang [1],Suchada Dana[1],
Duangporn Premjet[2-3] and Siripong Premjet[1,*]

[1]Department of Biology, Faculty of Science, Naresuan University, Muang, Phitsanulok, 65000, Thailand.
[2]Center of Excellence in Research for Agricultural Biotechnology, Faculty of Agriculture, Natural Resources and Environment, Naresuan University, Muang, Phitsanulok, 65000. Thailand.
[3]Department of Agricultural Science, Faculty of Agriculture, Natural Resources and Environment, Naresuan University, Muang, Phitsanulok, 65000, Thailand.

*Corresponding author. E-mail: siripongp@nu.ac.th

ABSTRACT

Lignocellulosic biomass (LB), a renewable resource, is an attractive feedstock for manufacturing biofuel and biochemical products because these products have emerged as cleaner alternatives to fossil fuels and minimize environmental implications. *Corchorus capsularis* L., often known as Jute, is a non-food feedstock fiber crop that produces high cellulose fiber. Therefore, Jute biomass (JB) is highly recognized as a sustainable lignocellulosic feedstock for sugar platform-based biorefineries synthesizing bioethanol and other chemicals with added value. In this study, JB containing bark and core fibers was pretreated with different concentrations of phosphoric acid (PA) under mild conditions. After pretreatment, it was observed that PA concentration has a substantial influence on the chemical composition of bark and core fibers. Additionally, when both feedstocks were pretreated with PA, hydrolysis efficiency (HE) and glucose recovery (GR) were greatly enhanced. The yield of HE was improved approximately 4.5 times for bark and 6.7 times for core fibers. However, GR yield was enhanced by approximately 4.2 folds for the bark and 6.2 folds for the core fibers. These findings indicate that PA pretreatment had a significant effect on the efficiency of cellulose hydrolysis by enzymes. In the material balance, the total theoretical ethanol yield from untreated and treated bark and core fibers was reported.

Keywords: *Corchorus capsularis, Jute biomass, Lignocellulosic feedstock, Pretreatment, Phosphoric Acid*

INTRODUCTION

Due to increased energy consumption, limited fossil fuel resources, and health concerns, the world must transition to alternative and renewable energy (Hatti-Kaul et al., 2007). Bioethanol is the most common biofuel for transportation and is recognized as renewable for its potential to replace fossil fuels. Additionally, due to its high oxygen content, it oxidizes better than benzene and emits less carbon dioxide (Mussatto et al., 2010). Bioethanol is produced by fermenting various plant materials, including sugar or starch and lignocellulosic biomass, such as agricultural residue,

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forest wastes, and grasses (Ajanovic, 2011; Mussatto et al., 2010). Its renewable resource, wide distribution, abundance, and high polysaccharides content (Sanchez et al., 2015; Wang et al., 2016). Lignocellulosic biomass (LB) is the most renewable resource, constituting 50% of total biomass (Kumar et al., 2009). The structure of LB mainly consists of cellulose (30-45%), hemicellulose (15-25%) and lignin (15-25%). Additionally, it contains approximately 5-10% ash and extractives. However, the composition of LB differs according to the source of biomass, age, and geographical distribution (S. K. Bhatia et al., 2020). *Corchorus capsularis* L., generally known as Jute, is a herbaceous plant belonging to *Tiliaceae* family. It is cultivated in tropical and subtropical regions and is a rain-fed crop that requires few fertilizers and pesticides (del Río et al., 2009; Ghosh & Jethi, 2013). Jute biomass is mainly composed of cellulose (58-63%), hemicellulose (20-22%), and lignin (12-14%) (Ragauskas et al., 2006). Jute biomass is widely utilized for industrial and commercial uses, including producing gunny bags, hessian for carpet backing, and decorative fabrics (Lavanya et al., 2020). However, the recalcitrance of the Jute biomass impact lowers the bioconversion's potential. Therefore, a pretreatment technique is required before utilizing the biomass to improve its efficiency (Zakaria et al., 2015).

Chemical pretreatment is the most studied technique for improving hydrolysis efficiency (HE). Especially, acid pretreatments are regarded as the most efficient (Sarkar et al., 2012). Acid pretreatment exposes cellulose to enzymatic hydrolysis by solubilizing hemicellulose and causing structural changes (de Carvalho et al., 2015). However, concentrated acids are highly corrosive and toxic. Phosphoric acid (PA) can be utilized as an alternative solvent for dissolving crystalline cellulose (Mosier et al., 2005). It showed non-corrosiveness, nontoxicity, and safety compared to other concentrated acids. In addition, it can dissolve crystalline cellulose at atmospheric pressure and moderate temperature (Shen et al., 2013; Zhang et al., 2010).

Therefore, the purpose of this study is to pretreat JB with various concentrations of PA for improvement of hydrolysis efficiency and glucose recovery.

MATERIAL METHODS

Raw material

The dry bark and core fibers of *Corchorus capsularis* L. were purchased from a farmer in Laem Sing District, Chanthaburi Province, Thailand. The method for preparing samples was utilized in previous reports (Premjet et al., 2018). In short, dry bark and core fibers were separated and cut into 3x5 cm pieces and milled with a wood grinder. The bark and core fibers were then passed through a 50–100-mesh laboratory test sieve. Both raw samples were placed in separate plastic containers with lids and kept at 25 °C for further experiment.

Chemical composition

In their previous report (Premjet et al., 2018), the chemical composition analysis was described: Extractive, ash, lignin, and carbohydrate contents were evaluated for both treated and untreated samples. For this investigation, we followed standard NREL protocols (Sluiter et al., 2012; Sluiter, et al., 2008; Sluiter, et al., 2008).

Analytical procedures

In this study, we followed the procedure by Premjet et al., 2018. In brief, Monomer sugars were investigated employing high-performance liquid chromatography (LC-20AD system, Shimadzu Co. Ltd., Kyoto, Japan) with a Bio-Rad Aminex HPX-87P column (Hercules, CA) and refractive index detector (RID-10A, Shimadzu). The column was run at 80 °C with a sample injection volume of 20 µL. 0.6 mL/min of filtered HPLC-grade water was utilized for the elution.

Phosphoric acid pretreatment

Pretreatment of JB was performed by following the protocol of Siripong et al., 2018. In brief, "bark and core fibers were treated with phosphoric acid in 50-mL polypropylene tubes by mixing 300 mg of dry biomass with 24 mL of 70%, 75%, and 80% (v/v) PA. The tube containing the mixture was then capped and heated in a water bath apparatus at 60 °C for 1 hour (Eyela Pro Cool Bath NCB-3300, Tokyo Rikakikai Co. Ltd., Tokyo, Japan)." "Approximately 30 mL of acetone was added to the slurry and thoroughly mixed in order to stop the process. After centrifuging at 2500 rpm for 10 minutes, the supernatant was removed. The solid component was resuspended in acetone and centrifuged once more." "The supernatant was discarded. This procedure was repeated three times, and then the solid portion was thoroughly washed with deionized water until the pH reached 6.5".

Enzymatic hydrolysis

Enzymatic hydrolysis was carried out using a methodology which was described in a previous report (Premjet et al., 2018). In brief, separating the treated and untreated JB for enzymatic hydrolysis in a 50-mL Erlenmeyer flask containing 0.1 g of biomass (dry basis), 0.05 M sodium citrate buffer (pH = 4.8), and 0.1 mL of 2% sodium azide (w/v) with a total reaction volume of 10 mL. β-glucosidase (Oriental Yeast Co. Ltd., Tokyo, Japan) and Cellulase (celluclast 1.5 L, Sigma-Aldrich, St. Louis, USA) and were loaded at and 60 U/g and 30 FPU/g of dry biomass, respectively. Two hundred microliters of hydrolysates were periodically collected (12 h, 24 h, 48 h, and 72 h) for HPLC analysis of monomer sugars.

The effectiveness of hydrolysis was calculated using the following equation:

$$\text{Hydrolysis efficiency (HE; \%)} = (\text{Glucose released, g}) \times 0.9) / \text{Glucan in initial biomass}] \times 100$$

$$\text{Glucose recovery (GR; \%)} = (\text{solid recovery x glucan content x } 1.11 \text{ x HE}) \text{ x } 100$$

$$\text{Recovery yields (\%)} = \frac{[\text{solid recovery(\%)xTreated component of each content (\%)}]}{\text{Untreated component of each content (\%)}}$$

$$\text{Lignin removal (\%)} = 100 - \text{lignin recovery}$$

Analytical Statistics

The outcomes were statistically examined using variance and Turkey *t*-test. All data are shown as mean ± SD (n = 3, ± SD (P<0.05).

RESULTS AND DISCUSSION

Chemical compositions

In this experiment, bark and core fibers from JB were employed. The chemical compositions of bark and core fibers are presented in Table 1. The total carbohydrates (glucan and xylan) were approximately 60% and 62% for bark and core fibers, respectively. The amounts of xylan (18.0±0.8%), acid insoluble lignin (AIL) (18.3±0.2%) and total lignin content (21.2±0.1%) of core fiber were significantly greater than those of bark fiber (6.8±0.1%, 13.4±0.1%, and 16.3±0.1%, respectively). However, the acid soluble lignin (ASL) content of the bark (2.9±0.1%) and the core fibers (2.8±0.0%) was the same. In contrast, the bark fibers contained significantly higher glucan (53.9±0.8%), ash (6.8±0.1%), and ethanol extractive (8.2±0.9%) than the core fibers (43.9±0.8%, 3.5±.0.2%, and 5.8±.0.1%, respectively). The result indicated that glucan was a major component of both feedstocks. In the production of biofuels from biomass and sugar-based biorefineries, cellulose is the most essential component. The glucan content of bark fiber was greater than that of several feedstocks, including poplar (49.9%), hybrid poplar (48.6%), walnut (46.2%), white oak (43.6%) and red oak (43.4%) (Zhao et al., 2012a). In case of core fiber, the glucan content was higher than those observed in corn stover (40.9%), sugarcane bagasse (40.2%), wheat straw (38.2%), corn cob (36.4%) and switchgrass (31.0%) (Zhao et al., 2012a). The results indicate that JB has a great deal of potential for usage in sugar-based biorefineries to produce bioethanol and other chemicals with added value.

Table 1 Composition of bark and core fibers

Component	Bark % (DW)	Core %(DW)
Glucan	$53.9±0.8^a$	$43.9±0.8^b$
Xylan	$6.8±0.1^b$	$18.0±0.8^a$
Acid insoluble lignin (AIL)	$13.4±0.1^b$	$18.3±0.2^a$
Acid soluble lignin (ASL)	$2.9±0.1^a$	$2.8±0.0\ ^a$
Total lignin	$16.3±0.1^b$	$21.1±0.1^a$
Ash	$6.8±0.1^a$	$3.5±0.2^b$
Extractive	$8.2±0.9^a$	$5.8±0.1^b$

Data are presented as mean and standard deviation (n = 3). Statistically, the means in the same row with distinct superscript letters (a,b) differ when P > 0.05. The percentage DW indicates the percentage dry weight.

Effect of phosphoric acid concentration on pretreatment

In this study, the bark and core fibers of JB were prepared with different concentrations (70%, 75%, and 80%) of PA at 60 °C for 60 minutes. When both samples were pretreated with different concentrations of PA, it was found that their chemical composition changed substantially, as shown in Tables 2 and 3, respectively. The xylan content of each feedstock was entirely eliminated, however, after pretreatment with 70% PA. The range of total lignin reduction for bark fiber was 16.3±0.1% (raw material) to 10.1±0.2%, while for core fiber it was 21.1±0.1% (raw material) to 14.5±0.7% (Tables 2 and 3). This proportion of total lignin corresponds to a lignin removal efficiency of approximately 62.3% for bark fibers and 61.1% for core fibers (Figure 3). Moreover, while the concentration of PA increased, the solid, xylan, AIL, ASL, and total lignin recoveries of both feedstocks declined dramatically (Figures 1 and 2). This indicated that the concentration of PA had an effect on the removal of hemicellulose from biomass. Due to hemicellulose's greater solubilization susceptibility than cellulose and lignin (Liu et al., 2018). Nevertheless, the PA concentration was unable to remove all of the lignin in the bark and core fibers, so it only made a partial removal. This phenomenon was observed in several reports (Obeng et al., 2018; Sathitsuksanoh et al., 2013; Siripong et al., 2016; Takata et al., 2013). However, as the concentration of PA increased, the relative glucan content of each sample increased (Tables 2 and 3). During the PA pretreatment of lignocellulosic feedstock, it was observed that the linkages between cellulose, hemicellulose, and lignin were severed. Consequently, both cellulose and lignin are only partially soluble, while hemicellulose can be more dissolved (Liu et al., 2018). It was reported that, after pretreatment of feedstock with PA concentrations between 75% and 83%, hemicellulose was removed from lignocellulose most thoroughly. However, cellulose and lignin were only reduced considerably, resulting in an increase in relative glucan content (Kundu et al., 2021). In addition, it was shown that 80% PA concentration had a greater impact on glucan recovery, solid recovery (Figures 1 and 2), and lignin removal (Figure 3) of both bark and core fibers (Satari et al., 2019). The effect of PA concentration on chemical composition was observed in several biomasses such as napier grass (Takata et al., 2014), weedy biomass (Siripong et al., 2016), Thai kenaf (Premjet et al., 2018), and Durial peels (Obeng et al., 2018).

Table 2 Chemical composition of bark fiber after PA pretreatment

Composition (% dw)	**Untreated**	**PA 70%**	**PA 75%**	**PA 80%**
Glucan	53.9±0.8^{d}	71.3±0.6^{c}	78.1±0.5^{b}	79.8±0.1^{a}
Xylan	6.8±0.1^{a}	n.d.	n.d.	n.d.
AIL	13.4±0.1^{a}	11.2±0.3^{b}	10.0±0.3^{c}	9.2± 0.2^{d}
ASL	2.9±0.1^{a}	1.9±0.0^{b}	1.1±0.0^{c}	0.9±0.0^{d}
Total lignin	16.3±0.1^{a}	13.0±0.4^{b}	11.1±0.3^{c}	10.1±0.2^{d}

Data are presented as mean and standard deviation (n = 3). Statistically, the means in the same row with distinct superscript letters (a,b) differ when $P > 0.05$. The percentage DW indicates the percentage dry weight.

Table 3 Chemical composition of core fiber after PA pretreatment

Composition (% dw)	**Untreated**	**PA 70%**	**PA 75%**	**PA 80%**
Glucan	43.9±0.8^{d}	57.5±0.0^{c}	64.9±0.1^{b}	70.0±0.1^{a}
Xylan	18.0±0.8^{a}	n.d.	n.d.	n.d.
AIL	18.3±0.2^{a}	17.8±0.0^{b}	15.5±0.0^{c}	13.7±0.7^{d}
ASL	2.8±0.0^{a}	0.9±0.0^{b}	0.8±0.0^{c}	0.8±0.0^{c}
Total lignin	21.1±0.1^{a}	18.7± 0.0^{b}	16.3±0.0^{c}	14.5±0.7^{d}

Data are presented as mean and standard deviation (n = 3). Statistically, the means in the same row with distinct superscript letters (a,b) differ when $P > 0.05$. The percentage DW indicates the percentage dry weight.

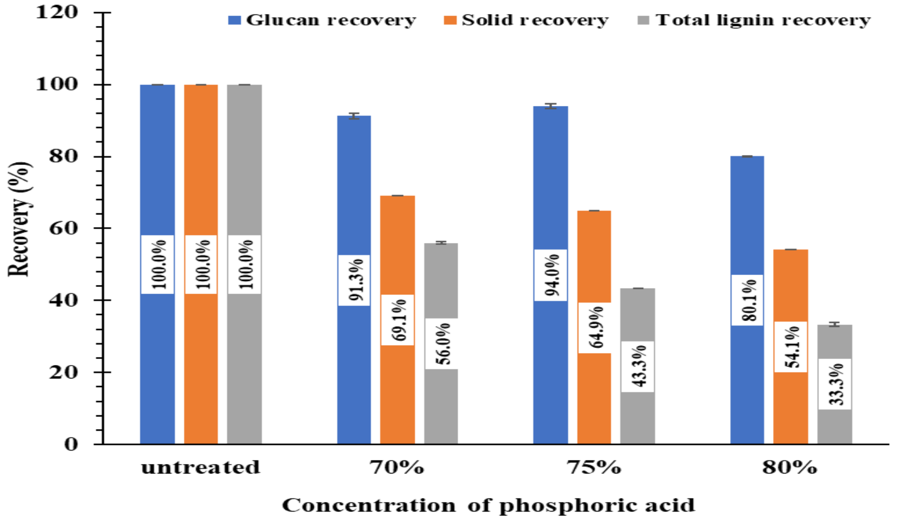

Figure 1 Recovery yield of each component from pretreated bark fiber

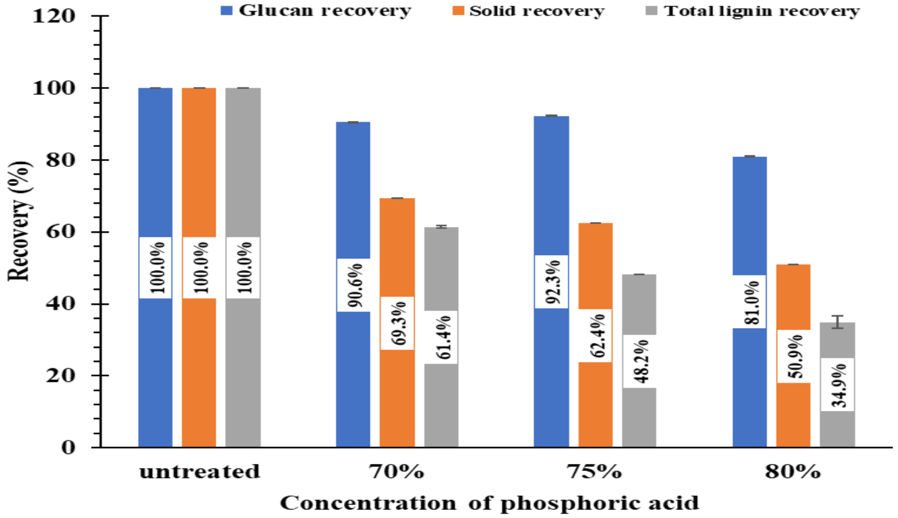

Figure 2 Recovery yield of each component from pretreated core fiber

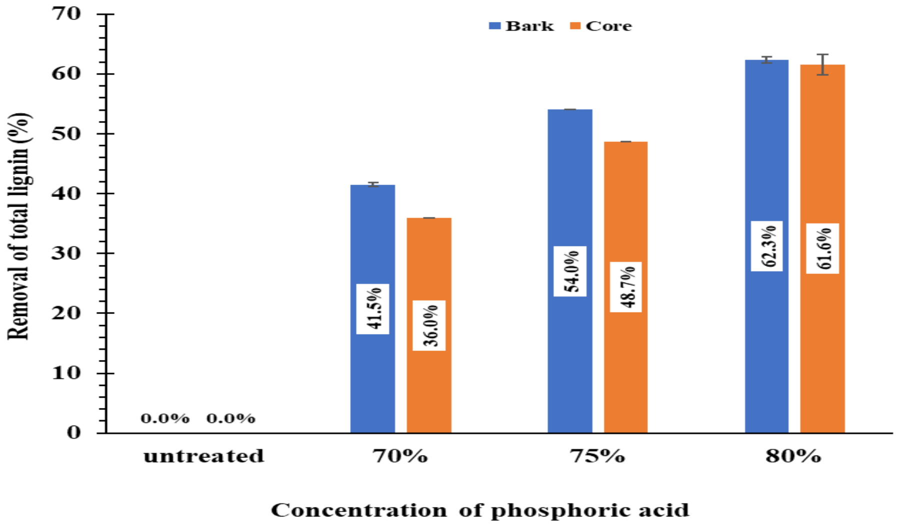

Figure 3 Lignin removal of both treated bark and core fibers

Influence of phosphoric acid on enzyme hydrolysis

Before JB has utilized in sugar platform-based biorefineries, the rigid and compact structure of this feedstock was subdivided by pretreatment, which is a necessary step for maximizing enzymatic hydrolysis in biochemical processes (Satari et al., 2019; Zhao et al., 2012a). The influence of PA concentrations on the yields of hydrolysis efficiency (HE) and glucose recovery (GR) of both samples was evaluated. The results for bark and core fibers are shown in Figures 4 and 5, respectively. The HE and GR yields of untreated bark fiber were 18.3±0.0% and 12.2±0.0%, respectively. However, untreated core fiber’s HE and GR yields were 12.2±0.0% and 6.6±0.0%, respectively. After these feedstocks were treated with 70%, 75%, and 80% PA, the yields of HE and GR were greatly enhanced compared to untreated samples. During in the enzymatic hydrolysis process, the yields of HE and GR increased considerably after 12 hours and then gradually improved after 24, 48, and 72 hours. Consequently, it was found that 72 hours of enzymatic hydrolysis generated the highest HE and GR yields from both treated and untreated materials (Figures 4 and 5). However, pretreating these feedstocks with 75% PA resulted in the highest yields of HE and GR. The highest HE and GR of treated bark fiber were 82.3±0.2% and 51.5±0.1%, respectively. In contrast, treated core fiber’s greatest HE and GR were 81.8±0.2% and 40.9±0.1%, respectively (Figures 4 and 5). The HE values of these feedstocks are insignificant ($P > 0.05$) for both treated samples. However, the GR of treated bark fiber was significantly ($P > 0.05$) greater than treated core fibers. The yield of HE was improved approximately 4.5 times for bark and 6.7 times for core fibers compared to untreated feedstocks. However, GR yield was enhanced by approximately 4.2 folds for the bark and 6.2 folds for the core fibers. These findings indicate that PA pretreatment significantly affected the efficiency of cellulose hydrolysis by enzymes.

In this study, the lowest HE and GR yields were obtained from both raw materials because the recalcitrance structure of the lignocellulosic material performed as a physical barrier for both enzymatic and chemical degradation (Shashi Kant Bhatia et al., 2020; Satari et al., 2019; Zhao et al., 2012a, 2012b; Zoghlami & Paës, 2019). Hemicelluloses in lignocellulosic material serve as a physical barrier that restricts enzyme access, but they were eliminated with acid pretreatment (Satari et al., 2019; Zoghlami & Paës, 2019). In this investigation, the entire hemicellulose was removed from both feedstocks by treating them with 70% PA. However, the maximum yields of HE and GR were derived from both feedstocks when they were pretreated with 75% PA. When cellulose was treated with PA concentrations lower than 80%, it showed splitting, roughening, fibrillation, and peeling or delamination. This caused the cellulose to swell and become amorphous rather than crystalline. Consequently, enzyme digestibility improved (Satari et al., 2019; Yoon et al., 2015). When they were pretreated with 80% PA, the amount of HE yields increased slightly, however, the amount of GR yields declined due to the solubilized glucan during pretreatment. Several studies have demonstrated that cellulose can be made more accessible to enzymes by removing hemicelluloses with an acid pretreatment, which is essential for enhancing enzyme digestibility (Auxenfans et al., 2017; Herbaut et al., 2018; Santos et al., 2018).

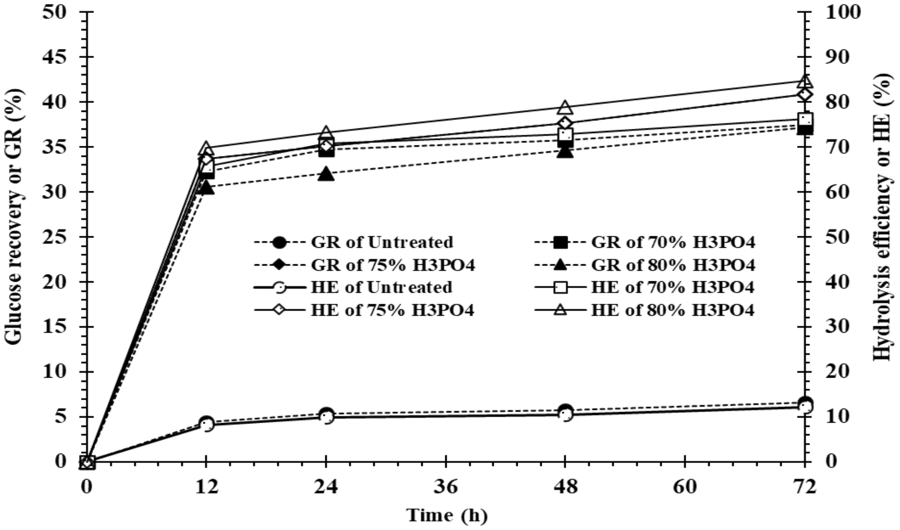

Figure 4 Glucose recovery (GR) and hydrolysis efficiency (HE) of bark fiber

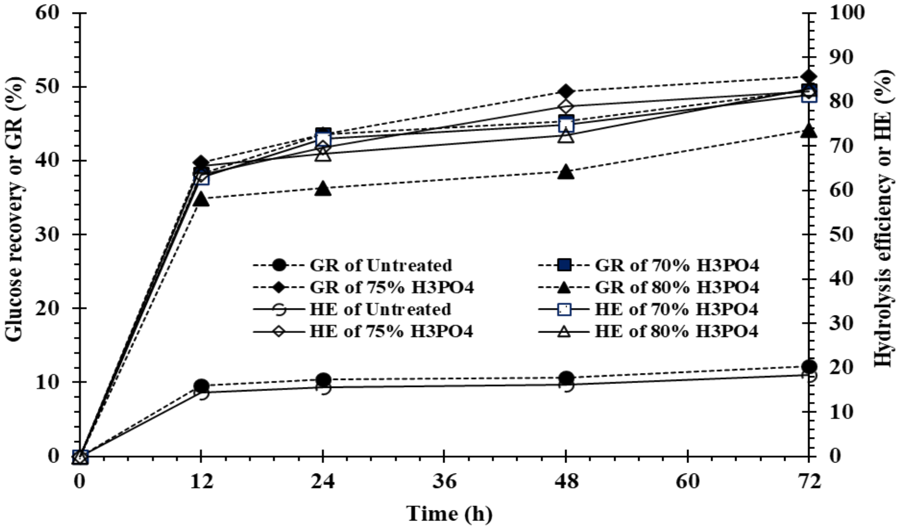

Figure 5 Glucose recovery (GR) and hydrolysis efficiency (HE) of core fiber

Lignin serves as a physical barrier that prevents enzymes from accessing cellulose (Zoghlami & Paës, 2019). In this experiment, the removal of total lignin was determined to be 41.5±0.4%, 54±0.3%, and 62.3±0.2% for bark fiber, and 36.0±0.0%, 48.7±0.0%, and 61.6±0.7% for the core at a PA concentration of 70%, 75%, and 80%, respectively. At 80% PA, the highest delignification was observed. Nonetheless, the highest yields of HE and GR were produced from feedstocks treated with 75% PA. At this condition, total lignin removal was 54±0.3% for bark fiber and 48.7±0.0% for core fiber. This suggested that about 50% of the lignin was still retained in both treated feedstocks. Since lignin is one of the primary factors that prevents cellulose's accessibility to cellulase by irreversibly binding hydrolytic enzymes. Therefore, eliminating lignin is essential for enhancing biomass enzymatic hydrolysis. However, in some cases, lignin removal is unnecessary to improve the accessibility of cellulose. However, there are various factors that affect the enzymatic hydrolysis of lignocellulose, including accessible surface area and crystalline cellulose. (Zhao et al., 2012a; Zoghlami & Paës, 2019).

To further determine the best conditions for the generation of fermentable sugar from both bark and core fibers, an overall mass balance based on the conversion of bark and core fibers from JB into glucose was constructed (Figure 6). The maximum HE and GR of both untreated samples after 72 hours of hydrolysis were about 18.3% and 12.2% for bark fiber and 12.2% and 6.6% for core fibers, respectively, per 1000 g of biomass processed. The optimum pretreatment conditions of 75% PA, 60°C, and 60 minutes increased the HE and GR of both treated biomasses by up to 82.3% and 51.5% for bark fiber and 81.8% and 40.9% for core fiber, respectively. When 1000 g of each feedstock is loaded into the process. The mass balance data suggest that glucose production from bark and core fibers was increased by roughly 4.2 and 6.2 times, respectively. However, the yield of HE was improved approximately 4.5 times for bark and 6.7 times for core fibers. Based on the glucose recovered under optimal pretreatment conditions, an estimated total theoretical ethanol production was calculated for both feedstocks, assuming 100% conversion. The total theoretical ethanol yield for untreated and treated bark fiber was 62.3 g and 263.2 g, an approximately 4.2-fold increase. For core fiber, an estimated total theoretical ethanol yield of 33.7 g and 209.0 g for untreated and treated biomass, respectively, corresponding to an approximately 6.2-fold increase, was determined (Figure 6).

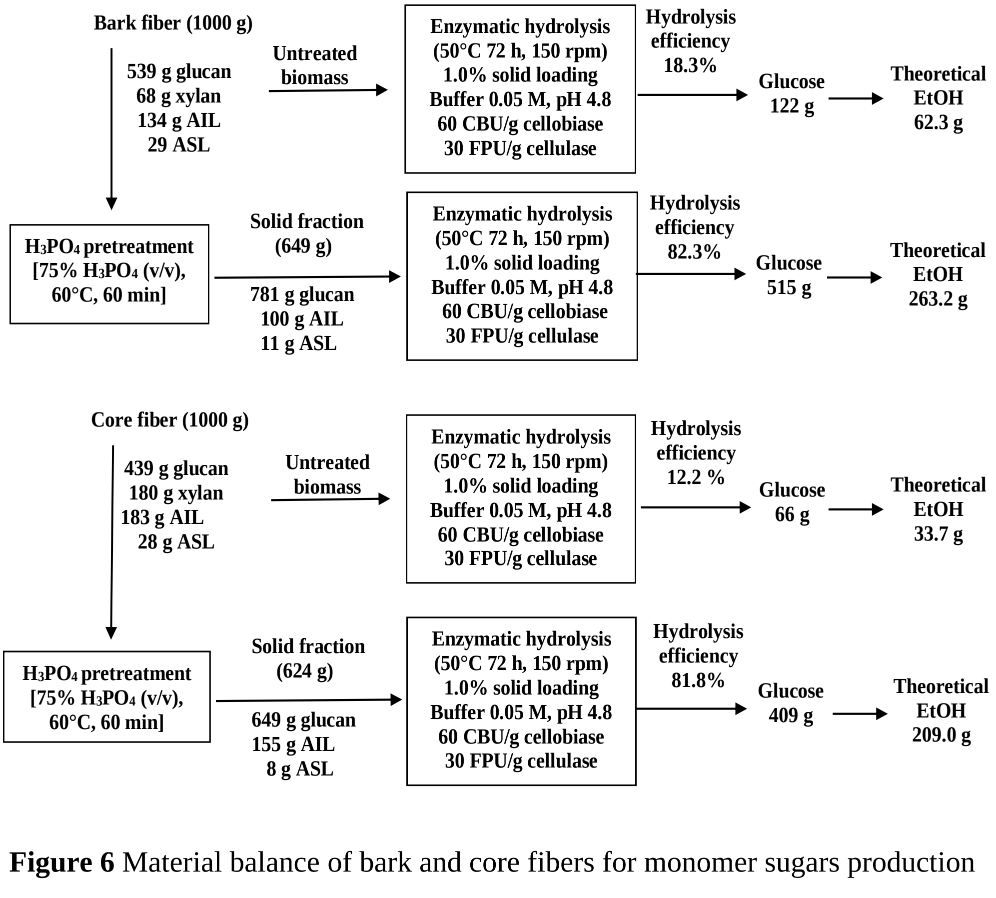

Figure 6 Material balance of bark and core fibers for monomer sugars production

CONCLUSION

According to the results, JB has great potential for use as a feedstock in sugar platform-based biorefineries to produce bioethanol and other chemicals with added value. In order to obtain the maximum hydrolysis efficiency and glucose recovery yield, 75% PA has a substantial impact on the pretreatment of both bark and core fibers. In comparison to untreated feedstocks, the yield of HE was improved approximately 4.5 times for bark and 6.7 times for core fibers. However, GR yield was enhanced by approximately 4.2 folds for the bark and 6.2 folds for the core fibers. These findings indicate that PA pretreatment had a significant effect on the efficiency of cellulose hydrolysis by enzymes. Furthermore, the elimination of hemicellulose and partial removal of lignin have been associated with an increase in hydrolysis efficiency and glucose recovery. PA could be employed to pretreat not only JB, but also a variety of other lignocellulosic feedstocks.

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