รายงานวิจัยฉบับสมบูรณ์

โครงการ การพัฒนากระบวนการการแยกเศษวัสดุชีวมวลสำหรับการแปลงสัดส่วนเซลลูโลสให้เป็นผลิตภัณฑ์ที่มีมูลค่าเพิ่มผ่านทางแพลตฟอร์มของตัวเร่งปฏิกิริยาชีวภาพและเคมี

โดย ดร. มาริษา ไร่ทะ

มิถุนายน 2563

สัญญาเลขที่ TRG6180013

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สังกัด
บัณฑิตวิทยาลัยร่วมด้านพลังงานและสิ่งแวดล้อม
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สนับสนุนโดยสำนักงานคณะกรรมการการอุดมศึกษา และสำนักงานกองทุนสนับสนุนการวิจัย

รูปแบบ Abstract (บทคัดย่อ)

Project Code: TRG6180013
(รหัสโครงการ)

Project Title: Development of agricultural residue fractionation process for conversion of cellulose fraction to value added cellulose products via bio- and chemo-catalytic platforms

(ชื่อโครงการ) การพัฒนากระบวนการการแยกเศษวัสดุชีวมวลสำหรับการแปลงสัดส่วนเซลลูโลสให้เป็นผลิตภัณฑ์ที่มีมูลค่าเพิ่มผ่านทางแพลตฟอร์มของตัวเร่งปฏิกิริยาชีวภาพและเคมี

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Abstract:

The work focus on development of alternative processes both in one and two-stage pretreatment for separation of lignocellulosic biomass (palm residues, rice straw, and sugarcane trash) to cellulose, hemicellulose, and lignin, leading to industrial value-added chemicals and products. This research was mainly categorized in three routes: (i) Study on synthesis of cellulose particles from palm fiber using organosolv fractionation and acid hydrolysis process; (ii) Study on fractionation of sugarcane trash using glycerol-based organosolv pretreatment and acetone delignification; and (iii) Study on alkaline hydrogen peroxide pretreatment of rice straw in aqueous organic solvent systems with organic base. The abstracts of each work were described as followed;

Organosolv fractionation is an effective process for separating biomass components to cellulose, hemicellulose, and lignin for further conversion to industrial biofuels and chemicals. In this study, the work focus on recovery of cellulose fraction in palm fiber (PF) by removing the hemicellulose and lignin using a ternary solvent mixture comprising methyl isobutyl ketone (MIBK), ethanol, and water in the presence of H_2SO_4 as an acid promoter. The fractionation condition was performed at 160 °C for 40 min with MIBK/ethanol/water proportion of 0.25:0.42:0.33 and 0.025 M of H_2SO_4, which led to the highest cellulose recovery efficiency of 98.4 %(w/w) with hemicellulose and lignin removal of 79.8 %(w/w) and 71.4 %(w/w), respectively. The fractionated cellulose was subsequently bleached with sodium chlorite under acidic condition at 80 °C for 4 hour, which resulted in the cellulose enriched solid with 78.2% w/w cellulose content. Later, the production of cellulose particles obtained from bleached cellulose fiber was performed by acid hydrolysis with varying the reaction time (30, 60 and 120 min) for removing the amorphous region and increasing the cellulose crystallinity. X-ray diffraction (XRD) analysis showed that the highest crystallinity index of cellulose particle was 66.5% after hydrolysis in 40 %(v/v) H_2SO_4 at 40 °C for 60 min. The work demonstrated a promising approach for conversion of palm fiber to cellulose particles which can be production of microcrystalline and nanocrystalline cellulose for application in food, and pharmaceutical industries.

Sugarcane trash (SCT) is an under-utilized biomass with potential for conversion to fuels and chemicals. Here, we report an organosolv process involving pretreatment by aqueous glycerol followed by mild delignification with acetone for fractionation of SCT. The effects of glycerol and oxalic acid on efficiency and selectivity of the reaction were studied at different temperatures. The higher glycerol content was found to promote delignification efficiency and enhanced enzymatic digestibility of the solid. An optimal reaction using 80%v/v glycerol at 170℃, with 300 mM oxalic acid followed by acetone extraction at 30°C led to cellulose recovery of 71.7%, whereas 96.8% and 83.9% of the initial hemicelluloses and lignin was removed into the aqueous-glycerol and acetone fractions, respectively. The isolated lignin was recovered with 73.7% yield and 94.6% purity and showed similar chemical profiles with higher thermal stability compared to commercial organosolv lignin according to Fourier Transform Infrared Spectroscopy and Thermogravimetric analysis.

Alkaline hydrogen peroxide (AHP) pretreatment is a promising process for enhancing enzymatic digestibility of lignocellulosic biomass in biorefineries. In the present work, the effects of organic bases (NH_4OH and tri-ethylamine) and co-solvents (ethanol, isopropanol, *tert*-butyl alcohol, water) on AHP pretreatment efficiency of rice straw were studied and compared to the typical aqueous reaction with NaOH. It was found that the glucose recovery from enzymatic hydrolysis of the rice straw pretreated by AHP at 35°C for 24 h using NH_4OH in aqueous/ *tert*-butyl alcohol (73.6%) was higher than that achieved using ethanol and iso-propanol (31.6-48.6%) and water (71.2%) under the same experimental conditions. Increasing H_2O_2 concentration from 1-10 %v/v in the aqueous/ *tert*-butyl alcohol with NH_4OH led to enhancing sugar yield to from 349-623 mg/g pretreated rice straw, equivalent to the highest glucose recovery of 83.0%. Formation of highly porous structures in pretreated rice straw by removals of hemicelluloses and lignin was revealed by Fourier transform infrared spectroscopy and scanning electron microscopy while the increased crystallinity index was shown by X-ray diffraction. This modified low-temperature AHP pretreatment using organic solvent system is advantageous on recyclability potential of the reagents and potent for further implementation in lignocellulosic biorefineries.

Keywords: Biorefinery; Organosolv fractionation; Alkaline hydrogen peroxide; *tert*-Butyl alcohol; Ammonium hydroxide; Cellulose particle; Organosolv lignin; Palm fiber; Sugarcane trash; Rice straw

บทคัดย่อ:

งานวิจัยนี้มุ่งเน้นที่การพัฒนากระบวนการทางเลือกทั้งในขั้นตอนเดียวและสองขั้นตอนสำหรับการแยกสารชีวมวลลิกโนเซลลูโลสจากของเสียปาล์ม ฟางข้าวและใบอ้อย ไปเป็นเซลลูโลส, เฮมิเซลลูโลสและลิกนิน งานวิจัยนี้แบ่งออกเป็นสามส่วนใหญ่ ๆ คือ (i) ศึกษาการสังเคราะห์อนุภาคเซลลูโลสจากเส้นใยปาล์มโดยใช้การแยกส่วนของออกาโนซอลฟ์และกระบวนการไฮโดรไลซิสกรด (ii) ศึกษาการแยกส่วนของใบอ้อยโดยใช้การปรับสภาพตัวทำละลายอินทรีย์ด้วยกลีเซอรอล และการกำจัดลิกนินด้วยอะซิโตน (iii) ศึกษาการปรับสภาพอัลคาไลน์ไฮโดรเจนเปอร์ออกไซด์ของฟางข้าวในระบบตัวทำละลายอินทรีย์ที่มีน้ำโดยการปรับ pH ด้วยเบสอินทรีย์ บทคัดย่อของงานแต่ละชิ้นได้อธิบายไว้ดังนี้

การแยกด้วยตัวทำละลายอินทรีย์เป็นกระบวนการที่มีประสิทธิภาพสำหรับการแยกส่วนประกอบของชีวมวลเป็นเซลลูโลสเฮมิเซลลูโลสและลิกนินเพื่อเปลี่ยนเป็นเชื้อเพลิงชีวภาพและสารเคมีในอุตสาหกรรมต่อไป ในการศึกษานี้งานวิจัยนี้มุ่งเน้นการกู้คืนเซลลูโลสจากเส้นใยปาล์มโดยกำจัดเฮมิเซลลูโลสและลิกนินโดยใช้ตัวทำละลายแบบผสม 3 ส่วน ซึ่งประกอบด้วยเมธิลไอโซบิวทิลคีโตน (MIBK) เอธานอลและน้ำ ในสัดส่วน 0.25: 0.42: 0.33 ตามลำดับ ในสภาวะที่มีกรดซัลฟูริกเป็นตัวเร่งปฏิกิริยา 0.025 M ที่อุณหภูมิ 160 ° C เป็นเวลา 40 นาที จากเงื่อนไขดังกล่าวจะนำไปสู่ประสิทธิภาพการกู้คืนเซลลูโลสสูงสุด 98.4% (w / w) และกำจัดเฮมิเซลลูโลสและลิกนินเท่ากับ 79.8% (w/w) และ 71.4% (w/w) ตามลำดับ ต่อมาเซลลูโลสที่ผ่านการแยกจะถูกนำไปฟอกด้วยโซเดียมคลอไรต์ ภายใต้สภาวะที่เป็นกรด ณ อุณหภูมิที่ 80 ° C เป็นเวลา 4 ชั่วโมง ซึ่งส่งผลให้ปริมาณเซลลูโลสที่ได้เท่ากับ 78.2% w/w หลังจากนั้นเซลลูโลสที่ได้จะถูกดำเนินการย่อยด้วยกรดโดยใช้เวลาในการทำปฏิกิริยา (30, 60 และ 120 นาที) เพื่อผลิตอนุภาคเซลลูโลสจากการกำจัดพื้นที่อสัณฐานและเพิ่มผลึกเซลลูโลส และจากการวิเคราะห์ X-ray diffraction (XRD) พบว่าดัชนีผลึกสูงสุดของอนุภาคเซลลูโลสที่ได้เท่ากับ 66.5% หลังจากการไฮโดรไลซิสด้วย 40% (v/v) ด้วยกรดซัลฟูริก ที่อุณหภูมิ 40 ° C เป็นเวลา 60 นาที ซึ่งงานวิจัยนี้แสดงให้เห็นถึงการแปลงเส้นใยปาล์มเป็นอนุภาคเซลลูโลสซึ่งสามารถนำไปผลิตเซลลูโลสจำพวก microcrystalline และ nanocrystalline สำหรับการใช้งานในอุตสาหกรรมอาหารและยาต่อไป

ใบอ้อยเป็นชีวมวลที่ไม่ได้ใช้ประโยชน์ แต่มีศักยภาพในการเปลี่ยนเป็นเชื้อเพลิงและสารเคมี ซึ่งงานวิจัยนี้จะใช้กระบวนการตัวทำละลายอินทรีย์ที่เกี่ยวข้องกับการปรับสภาพด้วยกลีเซอรอลที่มีน้ำ ตามด้วยการกำจัดลิกนินด้วยอะซิโตนในสภาวะเงื่อนไขไม่รุนแรงของใบอ้อย จากการศึกษาผลของกลีเซอรีนและกรดออกซาลิกต่อประสิทธิภาพและการเลือกเกิดปฏิกิริยาที่อุณหภูมิต่างกัน พบว่าปริมาณกลีเซอรอลที่สูงขึ้นจะช่วยเพิ่มประสิทธิภาพในการแยกตัวและเพิ่มความสามารถในการย่อยของเอนไซม์ในของแข็งตัวอย่าง โดยปฏิกิริยาที่ดีที่สุดจะดำเนินการภายใต้ความเข้มข้นของกลีเซอรอล 80% v/v ที่อุณหภูมิ 170 ℃ ด้วยกรดออกซาลิก 300 mM ตามด้วยการสกัดอะซิโตนที่ 30 °C ซึ่งนำไปสู่การฟื้นตัวของปริมาณเซลลูโลส

71.7% ในขณะที่สามารถกำจัดเฮมิเซลลูโลสและลิกนิน 96.8% และ 83.9% ตามลำดับ สำหรับลิกนินที่แยกได้ถูกนำกลับมาและได้ผลผลิตเท่ากับ 73.7% ที่มีความบริสุทธิ์เท่ากับ 94.6% ร่วมกับการแสดงคุณสมบัติทางเคมีที่มีความเสถียรทางความร้อนสูงกว่าและมีลักษณะโครงสร้างหมู่ฟังก์ชันคล้ายกับลิกนินออร์กาโนโซลฟลิกนินในเชิงพาณิชย์ด้วยการวิเคราะห์ด้วยเทคนิคความเสถียรทางความร้อน และเทคนิคการกระตุ้นสารด้วยพลังงานช่วงแสงอินฟราเรด

การปรับสภาพอัลคาไลน์ไฮโดรเจนเปอร์ออกไซด์ (AHP) เป็นกระบวนการที่เพิ่มความสามารถในการย่อยได้ของเอนไซม์จากชีวมวลในอุตสาหกรรมไบโอรีไฟน์เนอรี่ ในงานวิจัยนี้ได้ศึกษาถึงผลของสารอินทรีย์ (NH_4OH และ tri-ethylamine) และตัวทำละลายร่วม (เอทานอล, ไอโซโพรพานอล, *tert*-butyl alcohol, น้ำ) ต่อประสิทธิภาพการปรับสภาพ AHP ของฟางข้าว เปรียบเทียบกับปฏิกิริยาที่ใช้โซเดียมไฮดรอกไซด์ในระบบน้ำ จากผลการทดลองพบว่าการกู้คืนกลูโคสจากกระบวนการไฮโดรไลซิสของเอนไซม์ของฟางข้าวที่ผ่านการเตรียมโดย AHP ที่อุณหภูมิ 35 °C เป็นเวลา 24 ชั่วโมง โดยใช้ NH_4OH ในน้ำ/*tert*-butyl alcohol จะมีค่าเท่ากับ 73.6% ซึ่งสูงกว่าที่ใช้เอธานอลและไอโซโพรพานในช่วง 31.6- 48.6% และน้ำ (71.2%) ภายใต้เงื่อนไขการทดลองเดียวกัน สำหรับการเพิ่มความเข้มข้นของไฮโดรเจนเปอร์ออกไซด์จาก 1-10% v/v ในน้ำ/*tert*-butyl alcohol ด้วย NH_4OH นำไปสู่การเพิ่มผลผลิตน้ำตาลจาก 349-623 mg/g ของฟางข้าวที่ผ่านการปรับสภาพ เทียบเท่าการกู้คืนกลูโคสที่สูงที่สุดเท่ากับ 83.0% และโครงสร้างที่มีรูพรุนสูงในฟางข้าวที่มีการกำจัดเฮมิเซลลูโลสและลิกนิน ถูกเปิดเผยเทคนิคการกระตุ้นสารด้วยพลังงานช่วงแสงอินฟราเรด (FT-IR) และกล้องจุลทรรศน์แบบส่องกราด (SEM) ในขณะที่ดัชนีผลึกเพิ่มขึ้นหลังจากวิเคราะห์ด้วยรังสีเอ็กซ์ (XRD) ซึ่งการปรับสภาพ AHP ที่อุณหภูมิต่ำด้วยการใช้ระบบตัวทำละลายอินทรีย์นี้จะได้ประโยชน์จากการรีไซเคิลของรีเอเจนต์ และมีศักยภาพสำหรับการนำไปใช้งานต่อไปในอุตสาหกรรมไบโอรีไฟน์เนอรี่ของชีวมวล

คำหลัก: ไบโอรีไฟน์เนอรี่; กระบวนการแยกองค์ประกอบชีวมวลด้วยตัวทำละลาย; การปรับสภาพด้วยเบสไฮโดรเจนเพอร์ออกไซด์; *บิลทิลแอลกอฮอล์*; แอมโมเนียมไฮดรอกไซด์; อนุภาคเซลลูโลส; ลิกนินจากการสกัดด้วยตัวทำละลายอินทรีย์; เส้นใยปาล์ม; ใบอ้อย; ฟางข้าว

Executive summary

In the study, the work focus on development of alternative processes both in one and two-stage pretreatment for separation of lignocellulosic biomass (palm residues, rice straw, and sugarcane trash) to cellulose, hemicellulose, and lignin, leading to industrial value-added chemicals and products. This progress work was mainly categorized in three routes: (i) Study on synthesis of cellulose particles from palm fiber using organosolv fractionation and acid hydrolysis process; (ii) Study on fractionation of sugarcane trash using glycerol-based organosolv pretreatment and acetone delignification; and (iii) Study on alkaline hydrogen peroxide pretreatment of rice straw in aqueous organic solvent systems with organic base. The first research part purposed to synthesize cellulose particle product in separating cellulose content of palm fiber (PF) from removing hemicellulose and lignin content based on organosolv fractionation and acid hydrolysis process, respectively. It was found that increase of fractionation temperature from 140 to 180 °C led to rising of lignin and hemicellulose removal and enhancing of cellulose content retained in solid fraction. The highest cellulose recovery efficiency was observed at 160 ^{O}C for 40 min with MIBK/ethanol/water proportion of 0.25:0.42:0.33 and 0.025 M of H_2SO_4 as acid catalyst. Efficiency of cellulose isolation by removing the hemicellulose and lignin content was demonstrated in bleaching step after organosolv fractionation. Later, the bleached cellulose was done by acid hydrolysis for increasing of crystallinity index, especially performed at 40 %(v/v) H_2SO_4 at 40 °C for 60 min. This work provides a platform for further cellulose isolation from palm fiber and conversion to value added cellulose products of industrial interest in biorefineries. Next, the second part aimed to develop a novel efficient GOP process for separation of lignocellulose-derived components from sugarcane trash. The result showed high yields and selectivities on separation of cellulose with high purity and enzymatic digestibility, along with the hemicellulose-derived fraction and high purity organosolv lignin. Including, the properties of isolated lignin were similar chemical profiles with higher thermal stability compared to commercial organosolv lignin according to Fourier Transform Infrared Spectroscopy and Thermogravimetric analysis. Finally, the research work aimed to study of alkaline hydrogen peroxide pretreatment (AHP pretreatment) using NH_4OH in the aqueous *tert*-butyl alcohol system. The effects of alkali types (NH_4OH and tri-ethylamine) and co-solvents (ethanol, isopropanol, *tert*-butyl alcohol) on AHP pretreatment efficiency of rice straw were studied and compared to the typical water-based reaction in the presence of NaOH. The results showed that the glucose recovery from enzymatic hydrolysis of rice straw using NH_4OH in aqueous/*tert*-butyl alcohol at 35^{O}C for 24 h was higher than that achieved using ethanol, iso-propanol and water under the same experimental conditions. Besides, the removals of hemicelluloses and lignin fraction after AHP pretreatment was analyzed by Fourier transform infrared spectroscopy and scanning electron microscopy while the increased crystallinity index was shown by X-ray diffraction. The AHP process could be performed at mild temperature which resulted in low energy consumption and low toxic compound with potential on recyclability of the reagents for effective pretreatment of lignocellulosic biomass in sugar platform biorefinery industry. Overall works demonstrated potential of the developed pretreatment processes for efficient conversion of different lignocellulosic biomasses into industrial-need chemicals for possibility to application in concept of biorefinery industry.

เนื้อหางานวิจัย:

The aim of project focuses on study of alternative processes both in one and two pretreatment steps for separation of potential lignocellulosic feedstocks (palm fiber, rice straw and sugarcane trash) to cellulose, hemicellulose, and lignin, resulting in further conversion of these biomass composition into interested value-added products. The present works were separated into three routes: (i) Study on synthesis of cellulose particles from palm fiber using organosolv fractionation and acid hydrolysis process; (ii) Study on fractionation of sugarcane trash using glycerol-based organosolv pretreatment and acetone delignification, and (ii) Study on alkaline hydrogen peroxide pretreatment of rice straw in aqueous organic solvent systems. This results from this project will provide promising alternative approaches for value-added utilization of the underused by-products, leading to increasing economic competitiveness of the local industry. Overall work on research of this project is presented as followed;

PART 1: Synthesis of cellulose particles from palm fiber residue using coupled organosolv fractionation and acid hydrolysis process

1. Objective

In this study, the aim of this research is to synthesize cellulose particle product in separating cellulose content of palm fiber (PF) from removing hemicellulose and lignin content based on organosolv fractionation and acid hydrolysis process, respectively. First, the removal of hemicellulose and lignin content for obtaining cellulose fiber was performed in different reaction temperatures of organosolv fractionation using ternary solvent mixture in presence of homogenous acid catalyst. Secondly, the purified cellulose was bleached with oxidizing agent and then depolymerized by acid hydrolysis with varying reaction time at optimized condition. Next, the corresponding product as cellulose particle was characterized by X-ray powder diffraction (XRD). This work provides a platform for further study on value-added utilization of cellulose in integrated biorefineries industry.

2. Materials and methods

2.1 Materials

Palm fiber was obtained from Suksomboon Palm Oil Industry, Chonburi, Thailand. It was physically milled using a cutting mill (Retsch ZM2000, Haan, Germany) and sieved to the size of <1 mm. This palm fiber was subsequently extracted by acetone at 50°C until the solvent was clear of any color for removal of extractives e.g. starch, wax, syrup, organic acids, soluble protein, and other soluble components based on TAPPI Standard T204 om-88 [1], followed by washing with hot water at 60 °C for 1 h according Ajuong and Bresse (1998) [2]. All processed raw materials were dried at 60^{o}C for overnight. Chemical composition of the raw materials was analyzed by NREL method [3]. Chemicals and reagents were analytical grade and obtained from major chemical companies.

2.2 Organosolv fractionation

The raw material (10 g) was fractionated using 100 ml ternary mixture solvent comprising methyl isobutyl ketone (MIBK), ethanol, and water (25% : 42% : 33% v/v) according to the method in solvent system from Klamrassamee et al. 2013 [4] in the presence of 0.025 M H_2SO_4 as acid promoter. The fractionation process was carried out in a 600 ml of stainless steel reactor heated by electric jacket with a thermocouple to measure temperature inside the reactor. The reaction was performed by different reaction temperatures (140, 160, and 180 °C) for 40 min based on initial pressure of nitrogen

(N_2) at 20 bars with stirring at 100 rpm for keeping the system homogeneous. After the organosolv treatment, the reaction was immediately stopped by quenching on ice for 10 min. The slurry was filtered through a 20-25 μm of the paper filter (Whatman No.4) using a Büchner funnel for separating liquid content (aqueous-organic fraction) from the solid. The solid fraction was subsequently soaked with 30 ml of MIBK and filtered before washing with distilled water to neutralize the samples to the final pH between pH 6.0-7.0. The Chemical composition of the solid fraction was determined using the NREL method [3]. The percentage of pulp yield was calculated according to the following equation 1.

$$\%\ \text{Pulp yield} = \frac{\text{Weight}_{(\text{f})}\ (\text{g})\times 100}{\text{Weight}_{(\text{I})}\ (\text{g})} \qquad (1)$$

Where pulp yield is percentage of remaining sample weight compared to initial weight (%); Weight $_{(f)}$ is weight of remaining solid sample after organosolv process (g); and Weight $_{(I)}$ is weight of initial raw material (g).

The percentage of biomass composition (cellulose, hemicellulose, and lignin) was determined based on the remaining contents in the solid residues compared to their respective contents in the native biomass. The method for calculation of cellulose recovery was described according to the following equation 2.

$$\%\text{cellulose recovery} = \frac{(\ \text{C}_\text{F}\times\%\ \text{pulp yield}/100)\times 100}{\text{C}_\text{I}} \qquad (2)$$

Where cellulose recovery is percentage of cellulose fraction after organosolv fractionation compare to initial cellulose content (%); C_I is percentage of initial cellulose content (%); C_F is percentage of remaining cellulose content in solid pulp after organosolv process (%).

2.3 Bleaching process

The bleaching process contained 5 g of pretreated samples from hydrothermal and organosolv process and 1% sodium chlorite ($NaClO_2$) into 250 mL breaker. The mixture was adjusted to pH = 5 using acetic acid before heated and stirred in water bath at 80 °C for 1 h. After that, liquid phase was removed and added fresh 1% $NaClO_2$ into breaker. This step was repeatedly done by four times. Next, the mixture was cooled to room temperature and separated by filter paper and washing with water and dried at 70 ºC in oven. The solid pulp after bleaching process was analyzed by National Renewable Energy Laboratory (NREL) method [3].

2.4 Acid hydrolysis

The bleached cellulose pulp was hydrolyzed by acid hydrolysis contained 40% H_2SO_4 at 40 ^{o}C for varying the reaction time (30, 60, and 120 min). After that, **t**he solid fraction was filtered through a 20-25 μm of the paper filter using a Büchner funnel for separating liquid content and then washed with distilled water to neutralize the samples to the final pH between pH 6.0-7.0. The obtained cellulose particle was dried at 70 ºC in oven before further study.

2.5 Analytical methods

2.5.1 Analysis of sugars by high-performance liquid chromatography (HPLC)

The amount of sugar products (glucose, xylose, and arabinose) after NREL method was determined on a Shimadzu HPLC system (SPD-M10A DAD, Shimadzu, Japan) equipped with a refractive index (RI) detector and a Bio-rad Aminex HPX-87H column (Bio-rad, Hercules, CA, USA). A 5 mM solution of H_2SO_4 was used as a mobile phase at a flow rate 0.5 mL/min with the column temperature of 65°C.

2.5.2 Analysis of the purified cellulose by X-ray diffraction (XRD)

The crystallinity of samples was determined by X-ray diffraction (XRD) using an X'Pert PRO diffractometer (PANalytical, Almelo, The Netherlands). The samples were scanned at a speed of 0.5°/min in a range of 2θ=5-40° with a step size of 0.02° at 40 kV, 30 mA and radiation at Cu Kα (λ=1.5418 Å). The crystallinity index (CrI) was calculated according to the following equation 3.

$$\text{CrI (\%)} = \left[\frac{I_{002} - I_{am}}{I_{002}}\right] \times 100 \qquad (3)$$

where CrI is the crystallinity index of sample (%); I_{002} is the highest intensity for the crystalline portion of biomass (i.e., cellulose) at 2θ = 22.03 and I_{am} is the peak for the amorphous portion (i.e., cellulose, hemicellulose, and lignin) at 2θ = 18.19.

3. Results and discussion

3.1 Compositional analysis of PF

According to the compositional analysis, the PF raw material contained 46.8% (w/w) carbohydrate (24.2% and 22.6% of cellulose and hemicellulose content, respectively) with the other major components including 30.8% (w/w) lignin content, 5.0% (w/w) of ash, and 17.5% (w/w) of extractives i.e. minerals, wax, organic acids, and soluble protein on a dry weight basis (**Table 1**). The composition of PF in this study was substantially different from that previous studies which contained in range of 59.6-72.3% (w/w) of holocellulose, 18.7-28.5% (w/w) of lignin, 3.4-5.6% (w/w) of ash, and 5.6-6.3% of extractive substances [5, 6]. This could be due to differences in crop variety and palm oil processing methods.

Table 1. PF composition based on dry weight basis.

Substrate	**Cellulose (%w/w)**	**Hemicellulose (%w/w)**	**Lignin (%w/w)**	**Ash (%w/w)**	**Extractive* (%w/w)**
Palm fiber	24.2±1.4%	22.6±1.2%	30.8±0.1%	5.0±0.3%	17.5±2.8%

*Extractive: wax, lipid, minerals, organic acids, and soluble protein

3.2 Organosolv fractionation of palm residues

The effect of organosolv fractionation process in presence and absence of acid catalyst from palm fiber was studied at a fixed temperature of 160 °C for 40 min using solvent mixture comprising methyl isobutyl ketone (MIBK), ethanol, and water (25%: 42% : 33% v/v) with and without 0.025 M H_2SO_4 as acid promoter. H_2SO_4 was generally used as the catalyst in most clean fractionation process reported due to its cost-effectiveness. Besides, the effect of reaction temperature at 140, 160, and 180 ^{O}C was also investigated under the same condition in presence of H_2SO_4 catalyst. In **Figure 1**, the result found that addition of acid promoter at led to increasing solubilization of hemicellulose and lignin into the aqueous/alcohol and MIBK phases, respectively, resulting in enrichment of cellulose in the solid fractions. It reflected to lower pulp yield in range of 31.8-58.5% at varying temperatures compared to the reaction with no acid catalyst (66.2%). Among the reaction temperatures in presence of H_2SO_4 catalyst, it was found that the condition at 180 ^{O}C gave the maximal cellulose content with 64.8% compared to that at 140 and 160 ^{O}C as 34.6 and 56.2%, respective. While, 5.8-17.8% and 17.0-27.7% of hemicellulose and lignin was still retained in the solid fractions. It indicated that increasing of reaction temperature from 140 to 180 ^{O}C affected to separate the hemicellulose content but a few significances with lignin removal under the experimental conditions in the presence of H_2SO_4. However, the removal of lignin content was further done in bleaching process.

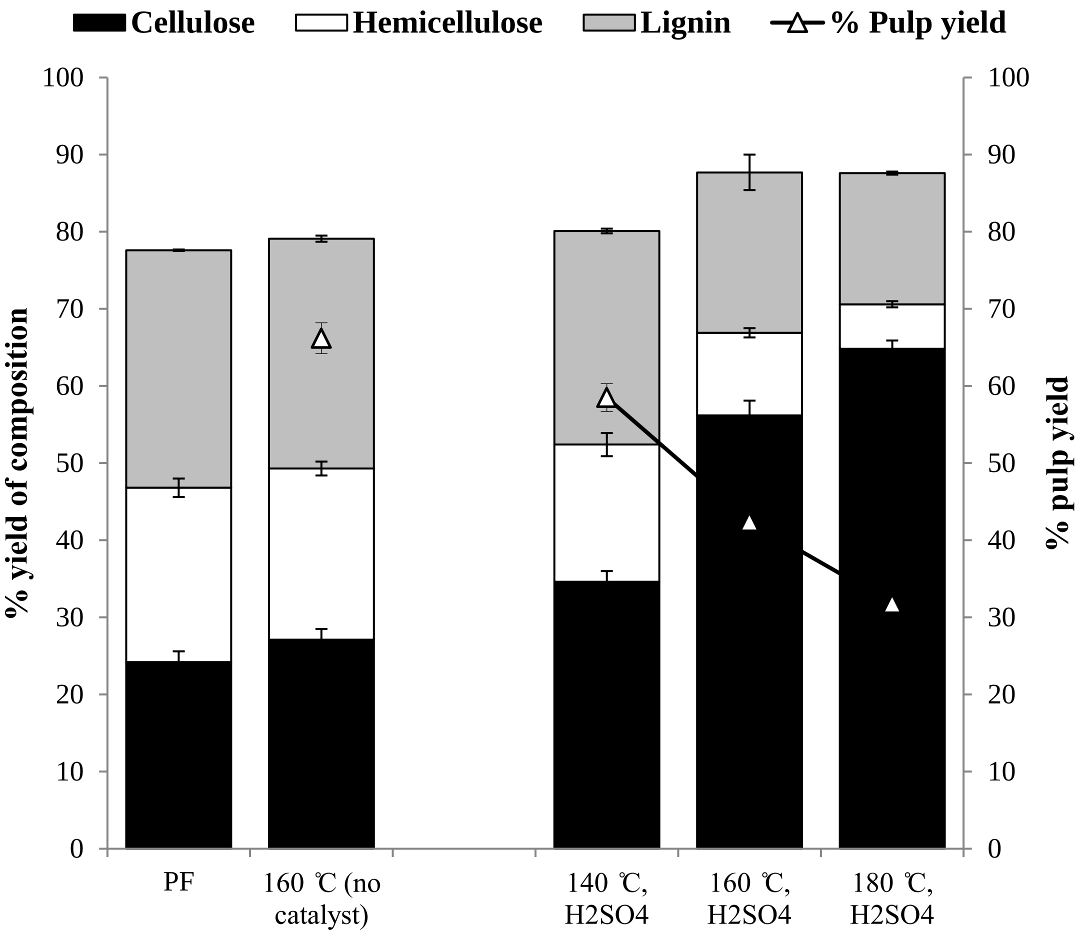


Figure 1. The effect of organosolv fractionation at different temperatures with/without addition of acid catalyst on biomass composition of the solid residues. The reaction contained 10% (w/v) palm fiber in ternary solvent mixture comprising MIBK, ethanol, and water (25%: 42%: 33% v/v) in the presence of 0.025 M H_2SO_4 as an acid catalyst and heated at 160-180°C for 40 min compared to the control reaction with no catalyst at 160 ^{O}C.

With regard to cellulose fraction based on varying the temperature between 140-180^{o}C, it was found that the highest cellulose recovery of palm fiber with 98.4% was observed at 160 ^{O}C in the presence of acid promoter **(Figure 2).** This included the removal of hemicellulose and lignin content with 79.8 %(w/w) and 71.4 %(w/w), respectively. It might suggest to utilize the high cellulose content for further converting to sugar and value-added products. The efficiency of organosolv fractionation of lignocellulosic biomasse in varying the reaction temperatures was reported several research works previously. Influence of temperature on organosolv fractionation of biomass has been recently investigated by several previous researches [7, 8]. Huijgen et al (2010) revealed that increase of temperature in the range of 160-220 °C in an acetone-based organosolv pretreatment of wheat straw resulted in enhancement of cellulose digestibility in enzymatic hydrolysis due to high efficiency of delignification and hemicellulose solubilization [8]. Moreover, the addition of acid catalyst such as H_2SO_4 on ethanosolv fractionation of wheat straw led to similar results in decrease of pulp yield, increase of delignification, xylan hydrolysis, and enzymatic digestibility of cellulose after rising the reaction temperature from 160 to 210^{o}C [7]. According to the results, the optimal condition of organosolv fractionation at 160 ^{O}C in presence of H_2SO_4 was a good candidate for further study.

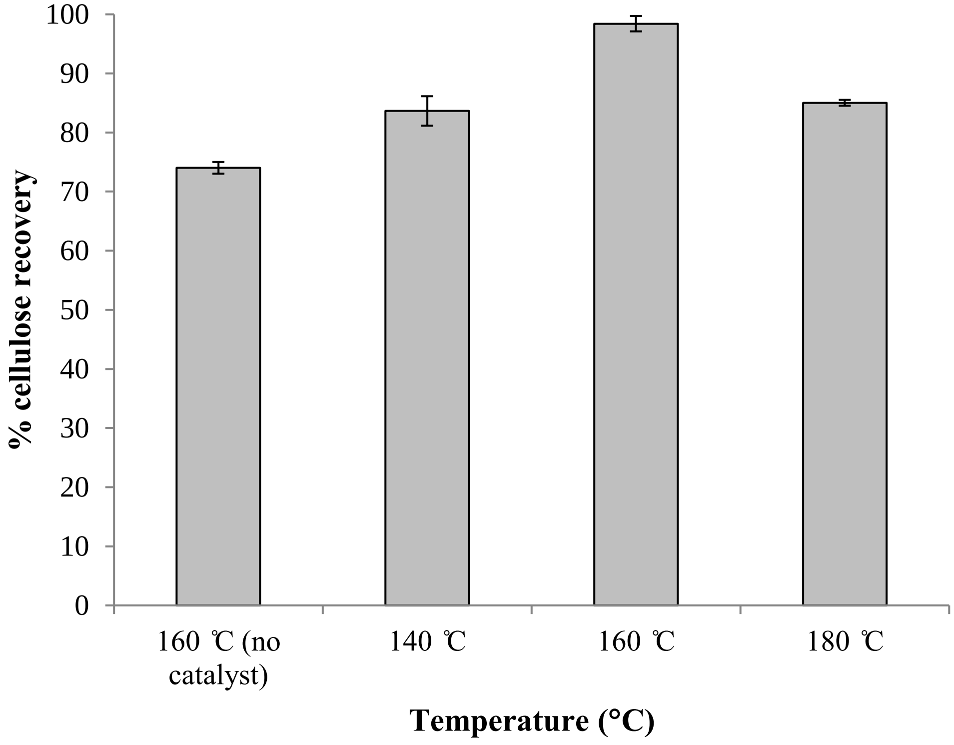


Figure 2. Cellulose recovery efficiency from organosolv fractionation of palm fiber at different temperatures. The reaction contained 10% (w/v) biomass in ternary solvent mixture comprising MIBK,

ethanol, and water (25% : 42% : 33% v/v) in the presence of 0.025 M H_2SO_4 as an acid catalyst and heated at 160-180 °C for 40 min compared to the control reaction with no catalyst at 160 °C.

3.3 Bleaching process of pretreated palm fiber

The cellulose pulp in solid fraction after fractionation process at 160 °C was bleached in 1% $NaClO_2$ solutions contained in water bath at 80° C for 4 hours under acidic condition at pH 5 and repeated 4 times. The overall step of cellulose bleaching from optimal condition of organosolv fractionation was showed in **Figure 3.** It was found that the physical property of bleached cellulose showed the white fiber after containing in water bath at 80°C for 4 hours.

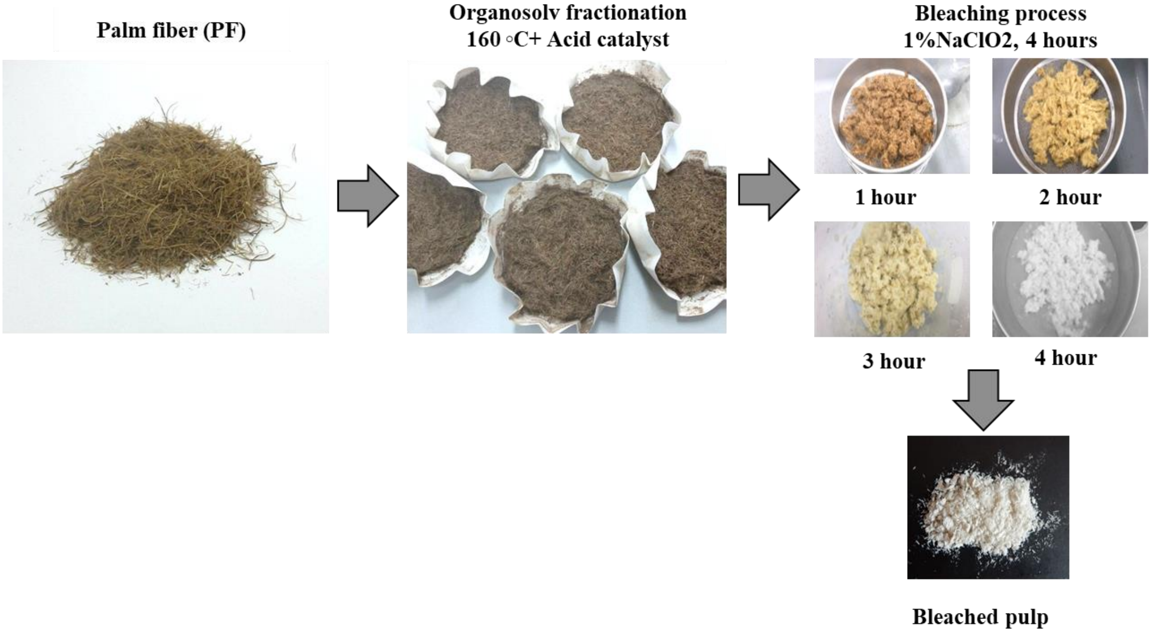


Figure 3. Overall process of cellulose bleaching from optimal condition of organosolv fractionation at 160 °C in presence of H_2SO_4 catalyst.

Later, the composition of bleached cellulose after organosolv fractionation process at 160 °C was compared to that of bleached palm fiber as raw material without fractionation step, as listed in **Table 2**. The result demonstrated that the cellulose pulp through organosolv fractionation and bleaching process showed increase of cellulose content to 78.2% and contained a much lower hemicellulose and lignin content with 4.0% and 3.2%, respectively compared to those of bleached sample with no fractionation process (20.7 and 23.8% of hemicellulose and lignin content). It reflected to the efficiency of organosolv fractionation in opening and attacking the biomass structure, leading to enhancement of delignification and hemicellulose solubilization in bleached cellulose.

Table 2. Bleached cellulose after organosolv fractionation at optimal condition.

Processes	Conditions	% Composition		
		Cellulose (%)	Hemicellulose (%)	Lignin (%)
Raw material	PF	24.2	22.6	30.8
Organosolv fractionation	PF at 160 °C	56.2	10.7	20.8
Bleaching	PF with no fractionation	27.1	20.7	23.8
	PF at 160 °C	78.2	4.0	3.2

3.4 Production of cellulose particle via acid hydrolysis

After that, the bleached cellulose pulp was hydrolyzed by acid hydrolysis contained 40% H_2SO_4 at 40 ^oC for varying the reaction time (30, 60, and 120 min), leading to depolymerization of cellulose fiber in degradation and removal of amorphous structure separated from crystalline region. The crystallinity index of cellulose samples obtained from several steps and native biomass was analyzed by an X-ray diffractometer (XRD), as shown in **Table 3**. It found that each process in production of cellulose particle directly influenced on increasing the crystallinity index. Importantly, the highest crystallinity index (66.5%) was obtained from acid hydrolysis in presence of 40% H_2SO_4 at 40 ^oC for 60 min but it was still lower than that of commercial MCC (Avicel). Therefore, development in fractionation process, including optimization of reaction parameters will be considered for further study.

Table 3. The crystallinity index of cellulose samples.

Samples	Reaction time (min)	Crystallinity index (%)
Native PF	-	31.8
Pretreated cellulose in organosolv fractionation	-	49.0
Bleaching cellulose	-	56.5
Cellulose particles	30	57.9
	60	66.5
	120	61.9
Commercial MCC*	Avicel	79.1

4. Conclusion

Isolation of cellulose from palm fiber using the clean fractionation based organosolv process for converting to value added cellulose by using acid hydrolysis was demonstrated in this study. Increase of fractionation temperature from 140 to 180 °C led to rising of lignin and hemicellulose removal and enhancing of cellulose content retained in solid fraction; however, the loss of pulp yield was more increased in harsher temperature condition. The highest cellulose recovery efficiency was observed at 160 ^{O}C for 40 min with MIBK/ethanol/water proportion of 0.25:0.42:0.33 and 0.025 M of H_2SO_4. Efficiency of cellulose isolation by removing the hemicellulose and lignin content was demonstrated in bleaching step after organosolv fractionation. Depolymerization of bleached cellulose by acid hydrolysis showed increasing of crystallinity index, especially performed at 40 %(v/v) H_2SO_4 at 40 °C for 60 min. The work provides a platform for further cellulose isolation from palm fiber and conversion to value added cellulose products of industrial interest in biorefineries.

PART 2: Fractionation of sugarcane trash by oxalic-acid catalyzed glycerol-based organosolv followed by mild solvent delignification

1. Objective

In this study, we aimed to develop a novel efficient glycerol organosolv pretreatment (GOP) for separation of lignocellulose-derived components from sugarcane trash. The process involved GOP process of the raw material using oxalic acid as the catalyst followed by acetone delignification of the solid pulp. Efficiency of the process was evaluated based on the recoveries and selectivities of the reaction on separating cellulose, hemicellulose-derived products, and lignin into different fractions. Moreover, the physico-chemical properties of the lignin recovered from the GOP hydrolysate and delignification solvent were determined in order to provide insights for its further application.

2. Materials and methods

2.1 Materials

Sugarcane trash (SCT) was obtained from Eastern Sugar and Industries Ltd (Srakaew, Thailand). The biomass was sun dried for 48 h, milled and sieved to give 0.5-1.0 mm particles. The particles were treated with acetone to dissolve the extractives according to TAPPI T 204 cm-97 standard procedure and stored dry at room temperature. The composition of extractive-free SCT was analyzed according to the standard NREL Laboratory Analytical Procedures [9]. The extractive-free SCT contained 38.1±1.5 wt% cellulose, 25.4±2.6 wt% hemicelluloses, 21.1±1.5 wt% lignin, 8.2±0.1 wt% ash and minerals, and 7.2±0.4 wt% moisture. Commercial organosolv lignin (CP8068-03-9-BULK) was obtained from Chemical Point, city, Germany. Analytical grade organic solvents and chemicals were purchased from major chemical suppliers i.e., Sigma-Aldrich, Merck, and Fluka.

2.2. Glycerol-organosolv fractionation of SCT

In this work, the major lignocellulosic components of SCT (cellulose, hemicelluloses, and lignin) were fractionated by GOP followed by an acetone washing (Ac) step (GOP/Ac process). The process scheme is shown in **Figure 4**. For the GOP step, effects of different reaction parameters were investigated including (i) operating temperature (150-170℃), (ii) glycerol concentration (20-80%v/v), and (iii) concentration of acid catalyst. Reactions (100 ml total volume) contained 10% solid loading of SCT in aqueous glycerol with varying glycerol concentrations supplemented with oxalic acid (75-300 mM) in a 1-L high pressure reactor (Parr Instruments, Moline, Illinois, USA). The initial pressure was set to 10 bars with nitrogen gas and heated to the desired temperature with stirring at 100 rpm for 40 min. Subsequently, the fractionated SCT was separated from the liquid fraction by vacuum filtration on Whatman filter paper no. 4 and washed with distilled water until the pH was neutral. The washed SCT was extracted with acetone (100 ml acetone/ 10 g of pretreated SCT) at 30°C for 5 min. The solid and liquid were separated by vacuum filtration to recover the adsorbed lignin. The solid pulp was oven dried at 70℃ for 12 hours and left in a desiccator until a constant weight was reached for solid pulp yield determination (Equation 1).

$$\%\ \text{Solid pulp yield} = \frac{\text{Final solid pulp weight (g)}}{\text{Initial SCT weight (g)}} \times 100 \qquad (1)$$

The hydrolysates generated from the GOP step were analyzed for cellulose and hemicellulose derived compounds including mono-, oligo-saccharides, organic acids, hydroxymethyl furfural (HMF), and furfural (FF). The compounds were quantified both directly and after acid post-hydrolysis treatment (121℃, 4%w/w H_2SO_4, and 30 min) on a high performance liquid chromatograph (SPD-M10A DAD, Shimadzu, Japan) equipped with an Aminex HPX-87H (Biorad, Hercules, CA) and a refractive index detector. The amount of cellulose solubilized during the GOP step was calculated based on the amounts of glucose and HMF found in the generated hydrolysates (Equation 2) while the amount of solubilized hemicelluloses was calculated from the xylose, arabinose, and FF contents (Equation 3).

$$\%\ \text{Solubilized cellulose} = \frac{\big(0.90 \times \text{Glucose (g)}\big) + (\text{HMF (g)}/0.78)}{\text{Initial amount of cellulose (g)}} \times 100 \qquad (2)$$

Where cellulose solubilized is the percentage of cellulose fraction degraded and dissolved to the liquid phase during the GOP process compared with the initial cellulose amount; 0.90 and 0.78 are the mass conversion factors related to the stoichiometry for conversion of glucose to cellulose and cellulose to HMF, respectively. Levulinic acid was not included due to its low concentration that could not be measured accurately by HPLC.

$$\%\ \text{Solubilized hemicelluloses} = \frac{0.88 \times (\text{Xylose (g)} + \text{Arabinose (g)}) + (\text{FF (g)}/0.73)}{\text{Initial amount of hemicelluloses (g)}} \times 100 \qquad (3)$$

Where solubilized hemicelluloses is the percentage of hemicellulose fraction degraded and dissolved to the liquid phase during the GOP step compared with the initial hemicellulose amount; 0.88 and 0.73 are the mass conversion factors related to the stoichiometry for conversion of xylose and arabinose to hemicelluloses and hemicelluloses to FF respectively.

Finally, the dissolved lignin in both the GOP hydrolysate and extracted from the Ac step were precipitated as residual GOP lignin (GOL) and acetone washed organosolv lignin (AOL), respectively by adding 3 ml of distilled water/ ml of liquid fractions, centrifuged at 8,000x g for 10 min, dried at 50℃ and weighed. The purity of recovered lignin was determined based on the Klason lignin according to the method provided by the National Renewable Energy Laboratory (NREL) [10].

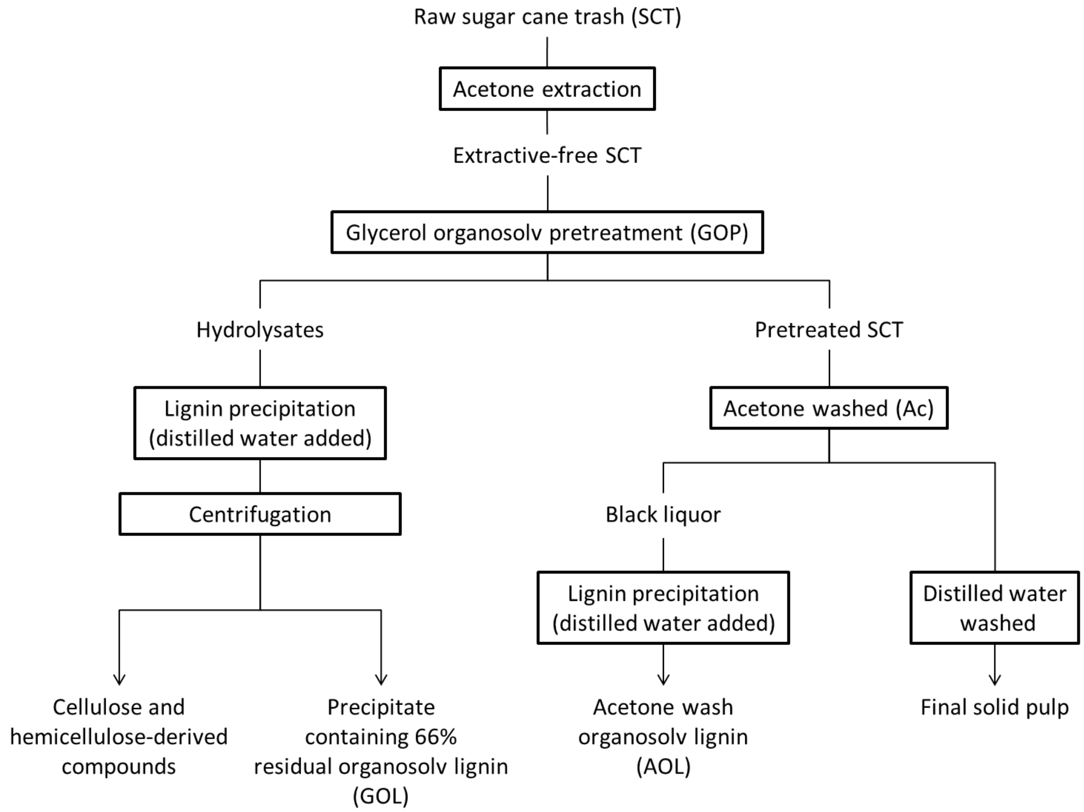


Figure 4 Scheme of the GOP/Ac fractionation process.

2.3. Characterization of the pretreated SCT solid pulps

2.3.1. Chemical composition

Chemical composition of the pretreated SCT solid pulp was determined according to the standard NREL analysis method [9]. The %cellulose recovery, %hemicellulose removal, and %lignin removal were calculated according to Equation 4-6.

$$\%\ \text{Cellulose recovery} = \left(\frac{\text{C}_\text{F} \times \%\ \text{Solid pulp yield}/100}{\text{C}_\text{I}}\right) \times 100 \qquad (4)$$

Where cellulose recovery is the percentage of cellulose remaining after the GOP/Ac process compared with the initial cellulose content; C_I is the percentage of initial cellulose content; C_F is the percentage of remaining cellulose content in the final solid pulp.

$$\%\ \text{Hemicellulose removal} = \left(\frac{\text{H}_\text{I} - (\text{H}_\text{F} \times \%\ \text{Solid pulp yield}/100)}{\text{H}_\text{I}}\right) \times 100 \qquad (5)$$

Where hemicellulose removal is the percentage of hemicelluloses removed after GOP/Ac process compared with the initial hemicellulose content; H_I is the percentage of initial hemicellulose content; H_F is the percentage of remaining hemicellulose content in the final solid pulp.

$$\%\ \text{Lignin removal} = \left(\frac{\text{L}_\text{I} - (\text{L}_\text{F} \times \%\ \text{Solid pulp yield}/100)}{\text{L}_\text{I}}\right) \times 100 \qquad (6)$$

Where lignin removal is the percentage of lignin removed after GOP/Ac process compared with the initial lignin content; L_I is the percentage of initial lignin content; L_F is the percentage of remaining lignin content in the final solid pulp.

2.3.2. Scanning electron microscopy

The microstructure and morphology of pretreated SCT were analyzed by scanning electron microscope (SEM) using the S-3400 N Type II SEM (Hitachi, Tokyo, Japan) with an electron beam at 10.0 kV energy voltage. The samples were dried and coated with gold prior to analysis.

2.3.3. Enzymatic hydrolysis of the solid pulps

The enzymatic digestibility of the final solid pulp was analyzed as follows. Reactions (1 ml) contained 5% w/v final solid pulp in 50 mM sodium acetate buffer (pH 5.5) with 5 mg/g substrate Cellic® CTec2 (Novozymes A/S, Bagsvaerd, Denmark) and 0.1%v/v sodium azide. The hydrolysis reactions were incubated at 50℃ with shaking at 220 rpm for 72 h. The released sugar profiles were analyzed by high performance liquid chromatography as described above.

2.4. Organosolv lignin characterization

2.4.1. Thermogravimetric analysis

Thermal behavior of the isolated organosolv lignin (GOL and AOL) and standard lignin (CP8068-03-9-BULK) samples were determined by thermogravimetric analysis (TGA) in Mettler Toledo TGA 2 equipment (Mettler Toledo Co., Schwerzenbach, Switzerland). The measurements were operated in the range of 30-800℃ with a linear increasing temperature of 10℃/min under N_2 atmosphere.

2.4.2. Fourier-transform infrared (FT-IR) analysis

Functional groups present in the isolated organosolv lignin (GOL and AOL) and standard lignin (CP8068-03-9-BULK) samples were analyzed by Fourier transformed infrared spectroscopy (FT-IR) (Perkin-Elmer System 2000, Waltham, USA). The FT-IR spectra were obtained in the frequency range of 4000-400 cm^{-1}.

3. Results and discussion

3.1. Effects of temperature on SCT fractionation

The effects of temperature in the GOP step on yields and selectivities of SCT component separation were studied initially using the reactions with 20%v/v glycerol supplemented with 300 mM oxalic acid as the catalyst at different temperatures (150°C-190˚C) followed by acetone extraction. Increasing temperature led to marked decreases in solid recovery from 54.0-45.0% (**Figure 5**). This was mainly attributed to the effective solubilization of hemicelluloses along with partial loss of cellulose and lignin under the experimental conditions. More than 95% of the initial hemicelluloses was solubilized resulting in the final hemicellulose content of less than 2.05% remaining in the solid. The GOP/Ac process resulted in partial loss of lignin from the solid pulp with an increasing trend from 18.8-33.9% at 150-190˚C as determined in the final solids. Along with the increasing removal of hemicelluloses, this led to the enrichment of lignin content from 18.8-31.0% in the final solid. Remarkable loss of cellulose was found under the experimental conditions with a decreasing trend from 72.1% at 150^{o}C with a marked decrease to 57.2% cellulose recovery at 190^{o}C with the increasing temperature. This led to a decreasing trend of cellulose content in the final solid pulp, which also reflected the decreasing glucose yield of 464-428 mg/g pretreated biomass (**Figure 5**). Due to the significant cellulose lost and lower glucose yield occur at 190˚C, the temperatures of 150˚C and 170˚C were selected for the subsequent experiments.

At higher temperature, non-specific removal of all major components was observed. In aqueous media without the presence of lignin dissolving solvents, hydrolysis of hemicelluloses to soluble products initiated at a lower temperature range with a sharp increase at 160^{o}C, followed by the hydrolysis of lignin and cellulose at the higher temperature. However, the dissociated derivatives of lignin could condense with themselves and with other derived compounds (from cellulose and hemicellulose fractions) forming insoluble (oligo-) polymeric compounds [11]. Although the fractionation process we developed provides a lignin post-extraction process using acetone after the GOP process, the condensate of lignin derived compounds may not dissolve as easily in acetone as native lignin. This could result in the relatively higher lignin content found in the final solid pulp along with the excessive degradation of cellulose at high temperatures.

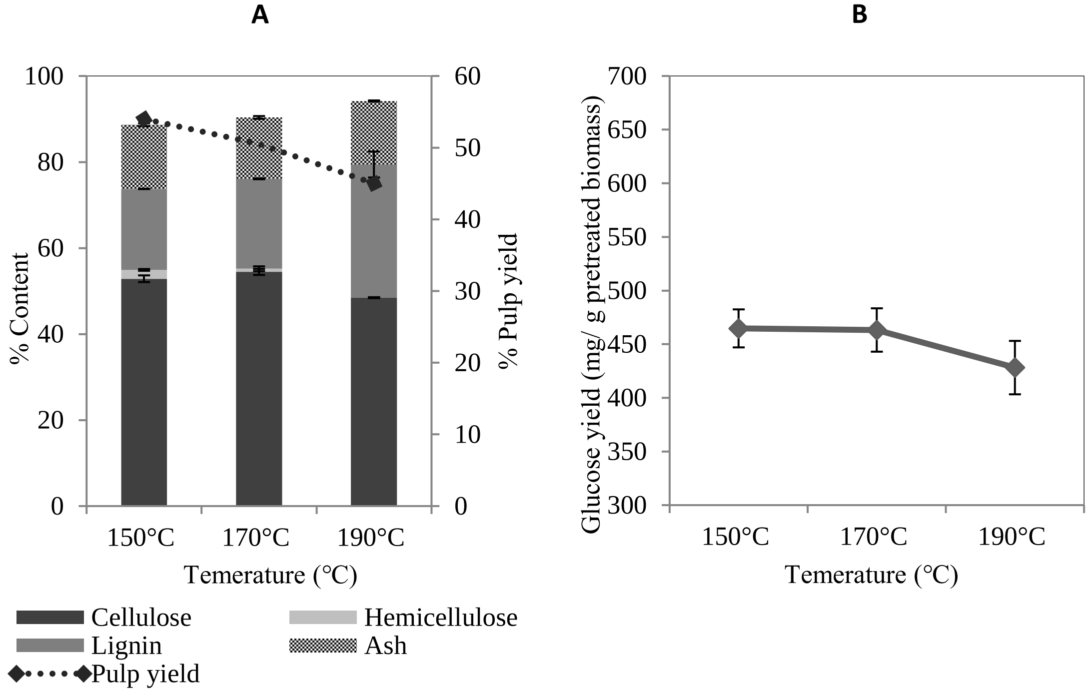


Figure 5 Effects of reaction temperature on composition (A) and glucose yield from enzymatic hydrolysis (B) of SCT treated by the GOP/Ac process. Reactions contained 10%w/v SCT in 20%v/v glycerol with 300 mM oxalic acid and were treated at different temperatures for 40 min followed by acetone extraction.

3.2. Effects of glycerol concentration on SCT fractionation

The influence of glycerol concentration on the fractionation process of SCT was investigated by comparing the composition of final solid pulp obtained using 20-80%v/v glycerol supplemented with 300 mM oxalic acid catalyst at 150℃ and 170℃ in the GOP step before subjecting the pulp to acetone delignification (**Figure 6**). Dissociation of hemicelluloses from SCT was suppressed with increasing glycerol concentration at both temperatures. At 170℃, a slight increase in residual hemicellulose content of 0.7 to 2.0 %w/w retained in the final solid pulp was observed when the glycerol concentration was increased from 20-80%. This resulted in the highest hemicellulose removal of 96.8% using the glycerol content of 20%. In contrast, increasing glycerol concentration at both temperatures led to increasing delignification efficiency, reflecting the lower residual lignin content of 18.1-8.5% in the final solid with the increasing glycerol concentration. Similar trends on hemicellulose and lignin removals were observed at 150^{o}C, but with lower efficiencies compared with reactions performed at 170^{o}C.

The effects of glycerol on enhancing delignification while suppressing xylan solubilization agreed well with a previous study on glycerol-based pretreatment of sugarcane bagasse [12]. Likewise, the pulping liquor with higher water content was also found to enhance better polyoses removal while the lignin removal efficiency was increased along with the higher glycerol content of the reaction mixture [13]. The highly polar polyalcohol structure of glycerol could effectively penetrate the fiber tissue. This thus enhanced the accessibility of pretreating solvents to the lignocellulose structure and facilitated lignin dissociation (Sun et al., 2015). Moreover, similar to the effects of water and ethanol on fragmented lignin previously proposed (Zhang et al. 2016), the three hydroxyl groups in the glycerol structure could potentially form linkages with carbonium ion intermediates generated during lignin fragmentation. This thus competed with the pathway on condensation of lignin derived compounds and facilitated subsequent delignification process. The reaction conditions at 170^{o}C with 80% glycerol were considered optimum for the GOP step. This resulted in the final solid pulp containing 68.2%w/w cellulose, 2.0%w/w hemicelluloses, and 8.5% lignin. According to the respective contents in the raw material, up to 71.7% of cellulose was recovered while 96.8 and 83.9% of hemicelluloses and lignin were removed, respectively. This led to the highest glucose yield of 592 mg/g pretreated SCT, equivalent to 78.1% enzymatic conversion based on the pretreated solid.

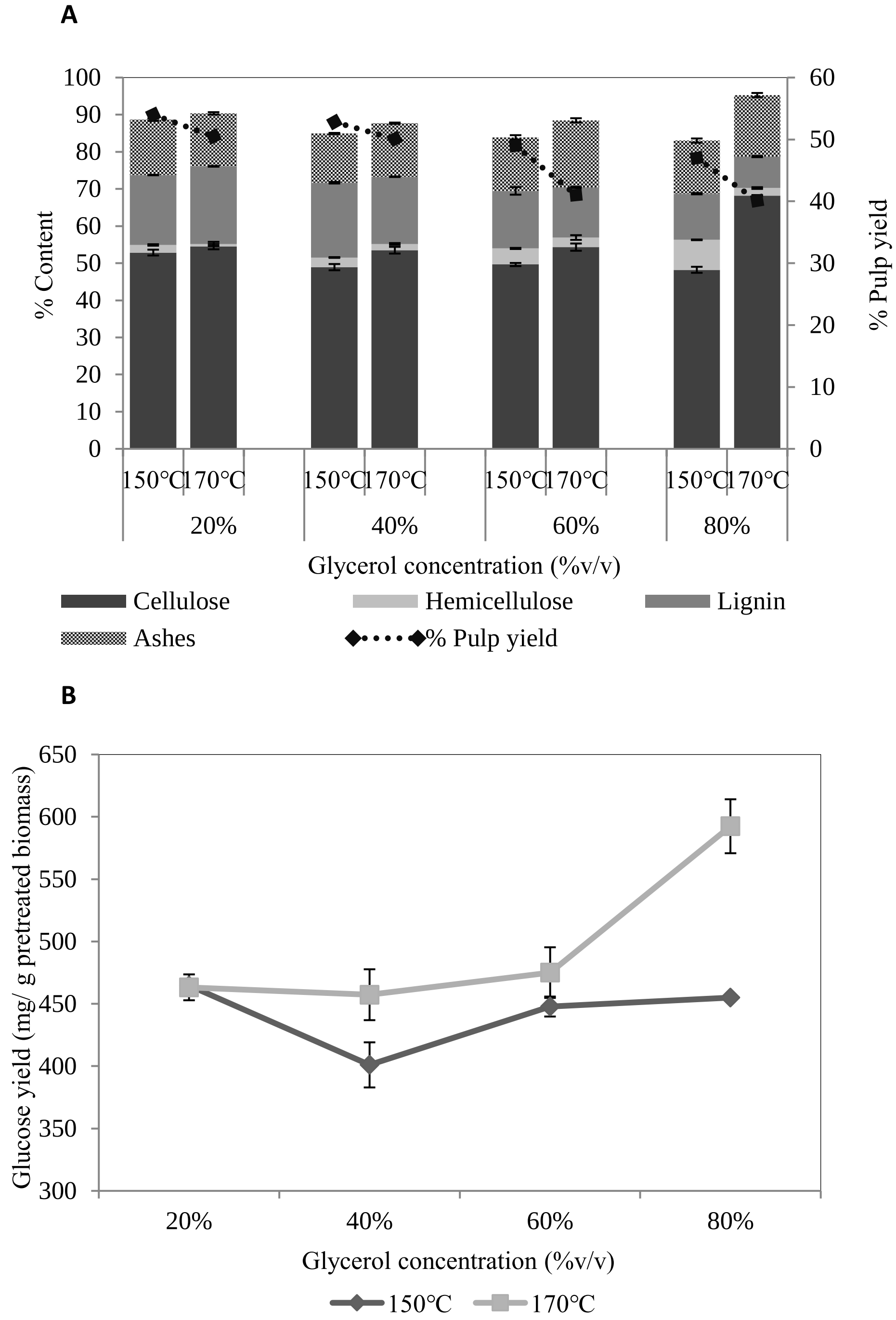


Figure 6 Influences of glycerol concentration on composition (A) and glucose yield achieved from enzymatic hydrolysis (B) of SCT final solid pulps from the GOP/Ac process. Reactions contained

10% SCT in aqueous glycerol containing varying glycerol concentrations with 300 mM oxalic acid at 150℃ and 170℃. The solid pulp was further treated with acetone for lignin extraction.

3.3. Effect of acid catalyst on SCT fractionation

The influence of oxalic acid as the catalyst on efficiency of the fractionation process was studied under the optimal temperature and glycerol concentration (170°C and 80% glycerol). The reaction was first studied by comparing the yields and selectivities of the fractionation process in the absence of the acid catalyst using water and aqueous glycerol (80%) as the reaction media before subjecting to acetone extraction. Compared with the liquid hot water process (LHW) using water solely as the reaction medium under the same conditions, the presence of glycerol led to higher lignin removal of 70.7% while only 51.9% was achieved by the liquid hot water process (**Figure 7**). In contrast, lower hemicellulose removal of 78.5% was found in the final solid pulp pretreated by the GOP/Ac process while the one pretreated by the LHW/Ac process showed up to 96.5% hemicellulose removal. The effects of glycerol on suppressing hemicellulose removal agreed well with the finding previously reported (See section 3.2.) when higher glycerol content was applied to the GOP step. The suppressed hemicellulose dissociation could be due to the lower amount of H_2O molecules available for autohydrolysis in the GOP step [14]. No significant differences in the cellulose content in the final solid were found between the GOP/Ac (54.3 %w/w) and LHW/Ac (58.2 %w/w) processes. Interestingly, glucose yield from the enzymatic hydrolysis experiment of the final solid pulp pretreated by the GOP process (440 mg/g) was significantly higher (p-value < 0.05 according to two-tail Student's t-test) compared with that obtained using the LHW process (415 mg/g), which also contained a higher lignin content in the final solid. This could be attributed to the effect of lignin on inhibition of cellulase by physical blockage and non-specific adsorption or binding to the enzyme [15].

Addition of oxalic acid to the GOP step resulted in higher hemicellulose removal from SCT. The hemicellulose content of SCT final solid pulps pretreated by GOP/Ac process decreased to 8.4-2.0%w/w with increasing oxalic concentration of 75-300 mM (**Figure 7**). This was equivalent to the hemicellulose removal efficiency of 85.3-96.8%. For the lignin fraction, the delignification efficiency was also slightly improved by a higher concentration of supplemented oxalic acid. Lignin contents of the SCT final solid pulps pretreated by GOP with oxalic acid supplement followed by acetone extraction decreased to 11.4-8.5%w/w. The highest lignin removal of 83.9% was achieved by the pretreatment supplemented with the highest oxalic acid concentration tested of 300 mM. For the cellulose fraction, the final solid pulp pretreated with higher oxalic acid supplement showed an increased cellulose content of 59.5-68.2%w/w compared with the final solid pulp pretreated without acid (54.3%w/w). The improved cellulose purity of the solid pulps pretreated with higher oxalic acid supplement was associated with higher glucose yields from enzymatic hydrolysis. Glucose yields of 509-592 mg/g pretreated biomass, equivalent to the enzymatic conversion of 77.0-78.1% based on the pretreated solids, was achieved from an enzymatic hydrolysis of the final solid pulp pretreated with 75-300 mM oxalic acid supplement whereas 440 mg/g was obtained without acid supplementation.

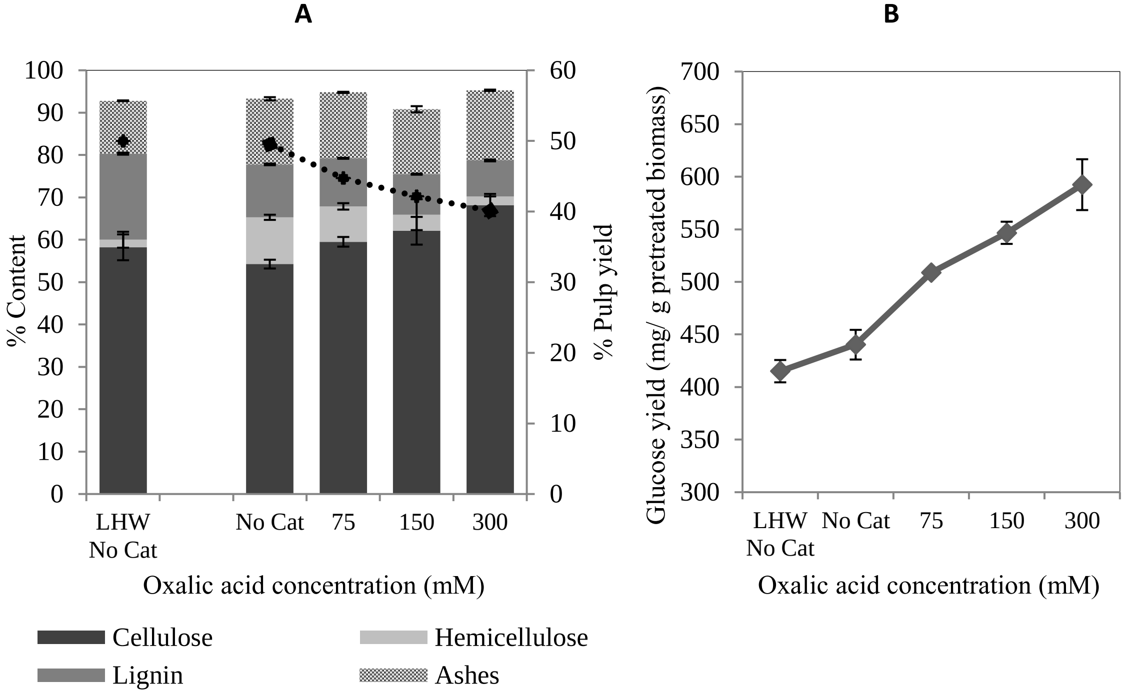


Figure 7 The influence of oxalic acid supplementation on composition (**A**) and glucose yield achieved from enzymatic hydrolysis (**B**) of SCT final solid pulps from the GOP/Ac process. Reactions contained 10% SCT in 80% glycerol with 0-300 mM oxalic acid at 170°C for 40 min. The solid pulps were further treated with acetone for lignin extraction.

The efficiency and selectivity of the GOP reaction on SCT in this study was higher compared with most previous works using the GOP process on different biomasses (**Table 4**), in which hemicellulose and lignin removal efficiencies of 68-96% and 54-81%, respectively were reported [12, 13, 16-18]. Moreover, [19] (2015a) also demonstrated that the implementation of post extraction processes (water extraction, dioxane extraction, and alkaline extraction) after the glycerol thermal processing of sweet gum wood could further improve delignification efficiency and provide up to 80%

lignin removal resulting a cellulose-rich solid pulp with 84% homogeneity. Raghavi et al. (2016) also reported the effects of sugarcane trash pretreatment using crude glycerol assisted transition metal and sodium hydroxide on enzymatic digestibility of the pretreated solid. However, the process was performed at lower temperature (121°C) with low glycerol concentration (3-6%) and did not show the efficiency and selectivity of the process on fractionation of the biomass components (Raghavi et al., 2016) [20].

Different acid catalysts in GOP have been shown previously to have enhancing effects on various biomass. For instance, Martin et al. (2011) reported that the cellulose content of solid pulp pretreated by H_2SO_4-assisted GOP was higher than the solid pulp pretreated in the presence of NaOH and in the absence of the catalyst due to the improved xylan hydrolysis intensity. [21] (2010) reported that the sugar yield of the solid pulps pretreated by a GOP process was correlated with the pKa of the supplemented acid catalysts [21]. The advantages of oxalic acid as the catalyst were further examined by comparing the efficiency of the GOP process supplemented with oxalic acid (300 mM) and H_2SO_4 (at equal dissociated hydronium ion concentration). Under the optimized GOP conditions (170˚C and 80% glycerol), only 2.1% of the initial cellulose was recovered in the final solid pulp of H_2SO_4 supplemented GOP. Moreover, 17.7 g of levulinic acid was found in the generated hydrolysate, which corresponded to 64.8% of the initial cellulose. These results indicated an excessive degradation and successive dehydration reactions of cellulose fraction when H_2SO_4 was applied as the catalyst in the GOP process. In contrast, the cellulose fraction was highly conserved (71.7% recovered in the final solid pulp) and fewer dehydration products were formed when oxalic acid was applied as the catalyst in the GOP process optimized in this study. Overall, the higher selectivity of oxalic acid to hemicellulose removal with minimized formation of sugar dehydration products makes oxalic acid a promising catalyst for GOP under harsh conditions (low water content, high temperature, and high acidity). In the hydrothermal pretreatment process, Scordia et al. (2011) reported that dilute oxalic acid pretreatment was an efficient catalyst for selective hemicellulose removal from giant reed biomass. According to its two pKa values and relatively lower ionization potential than H_2SO_4, oxalic acid was suggested to be an effective catalyst for β-(1-4) linkage hydrolysis while suppressing successive dehydration reactions.

Table 4 Hemicellulose and lignin removal efficiencies of glycerol-based organosolv pretreatment on different lignocellulosic biomass.

Substrate	**T (℃)**	**t (min)**	**Catalyst**	**Glycerol concentration (%)**	**Removal (%)**		**References**
					Hemicelluloses	**Lignin**	
Sugarcane bagasse	198	150	-	80	69.33	81.4	[13]
	190	60	0.94% H_2SO_4	80	96.32	54.4	[12]
	130	30	1.2% H_2SO_4	90	77.2	56.8	[22]
	220	120	-	70	67	71	[17]
Wheat straw	220	180	-	70	68	64	[16]
Sweet gum wood	240	12	-	100	N/A	~80	[19]
Sugarcane trash	170	40	300 mM oxalic acid	80	96.78	83.88	This study

3.4. Mass balance of the products from the optimized GOP/Ac process

Mass balance of the major fractions in the final solid pulps generated from the GOP/Ac process (**Figure 8**) showed that the optimized GOP condition (80%v/v glycerol, 170℃, 40 min, 300 mM oxalic acid) resulted in the highest efficiency and selectivity on separating hemicelluloses and lignin from SCT while effectively conserving the cellulose fraction. The soluble products derived from cellulose, hemicellulose, and lignin fractions from the GOP/Ac process under the optimized conditions are shown in **Figure 9**. For the cellulose fraction, 71.5% of the initial cellulose was recovered in the final solid pulp while only a slight fraction of cellulose was solubilized and obtained as glucose (3.5%) and hydroxymethyl furfural (HMF) (0.6%) in the GOP hydrolysate. This marginal amount of HMF suggested much less dehydration occurred in the oxalic acid supplemented GOP/Ac process compared with the process using H_2SO_4 as described in section 3.3.

For the hemicellulose fraction, only 3.2% of the initial amount was retained in the final solid pulp whereas 60.8% of the initial hemicelluloses was found in the GOP hydrolysates as its derivative compounds. The major sugar in the liquid phase was xylose, equivalent to 38.3% of the initial hemicelluloses whereas only a minor fraction was found as xylo-oligosaccharides (1.3%).

Furthermore, furfural was found as the major dehydration product of the pentose sugars which corresponded to 17.3% of the initial hemicelluloses. According to the severe and low water content condition applied to the pretreatment, the total cellulose and hemicellulose yields (including furfural, hydroxyl methyl furfural, and sugars) were below the theoretical maximum value, which could be due to the condensation of derived compounds to unidentified heterogenous residues (humins) [23-25]. Moreover, previous studies on biomass pretreatment using acidified glycol solvents under harsh conditions reported the formation of glycoside and xyloside complexes, which may also contribute to the reduced amount of cellulose and hemicellulose derived compounds in the GOP hydrolysate [18, 26, 27].

In contrast to the cellulose and hemicellulose yields, the mass of lignin fraction in this study almost reached the theoretical maximum. Residual fraction (16.1%) of the initial lignin was retained in the final solid pulp. Some of the lignin (10.7%) was recovered from the GOP hydrolysate as GOL which had relatively low purity (66.7%). The precipitation of this GOL and its low purity were evidence that a condensation reaction had occurred among lignin derivatives and with other lignocellulosic derivatives during the GOP process described in section 3.1. The majority of the initial lignin (73.2%) was recovered as AOL after the Ac step with high purity (94.6%).

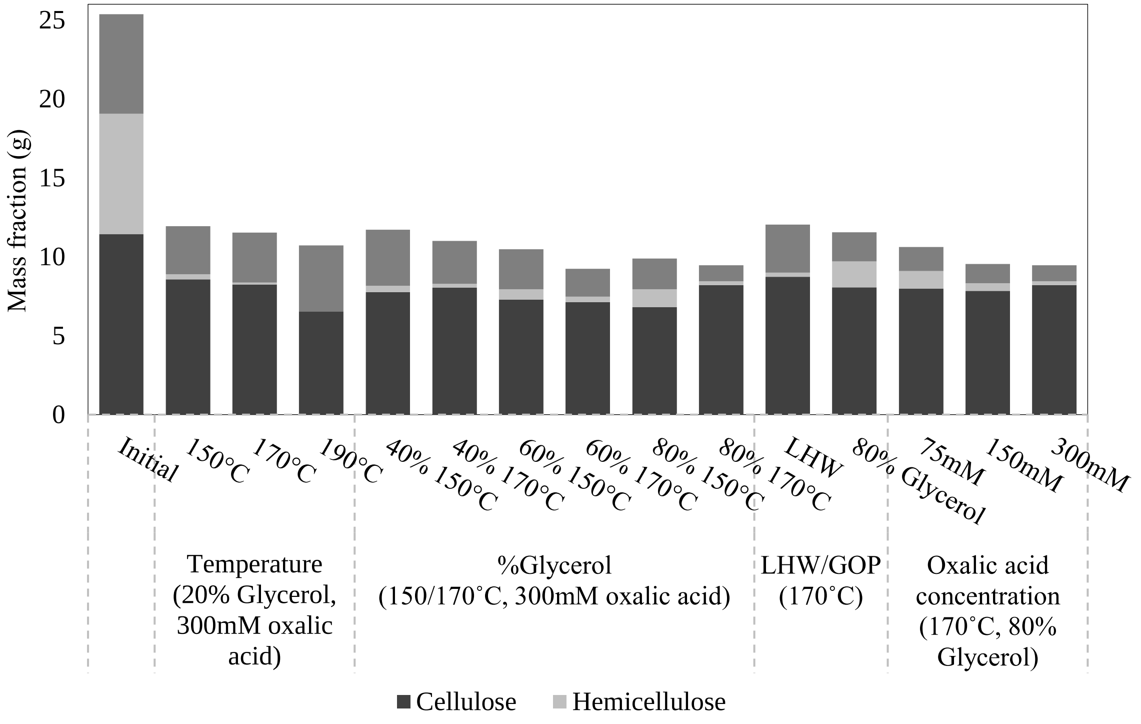


Figure 8 Mass balance of major components in SCT solid fraction from different processes (GOP/Ac and LHW/Ac) under different conditions (150-190˚C, 0-80%v/v glycerol, 0-300 mM oxalic acid).

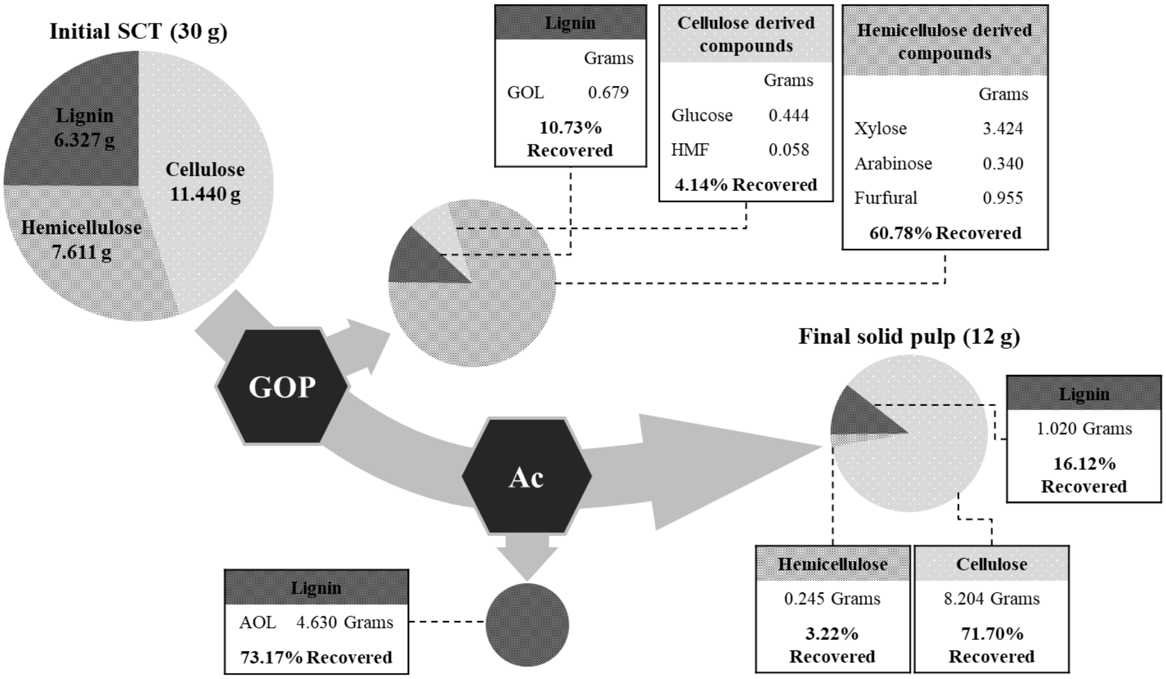


Figure 9 Compositions of products derived from SCT in the GOP/Ac process under optimized conditions.

3.5. Structural characterization of the solid pulp

Effects of the GOP/Ac process on the alteration of SCT structure were studied by electron scanning microscopy (**Figure 10**). The intact structure of SCT pretreated by the GOP/Ac process was effectively altered. Higher fiber accessibility was observed when compared with the raw material and the biomass pretreated by the conventional LHW/Ac process under the same pretreatment condition. Glycerol is an efficient solvent for opening the structure of lignocellulose owing to its highly polar polyol structure [13]. [28] (2008a) also reported that the lignocellulose structure of wheat straw fibers is degraded more efficiently by the GOP process compared with fibers pretreated by steam explosion [28].

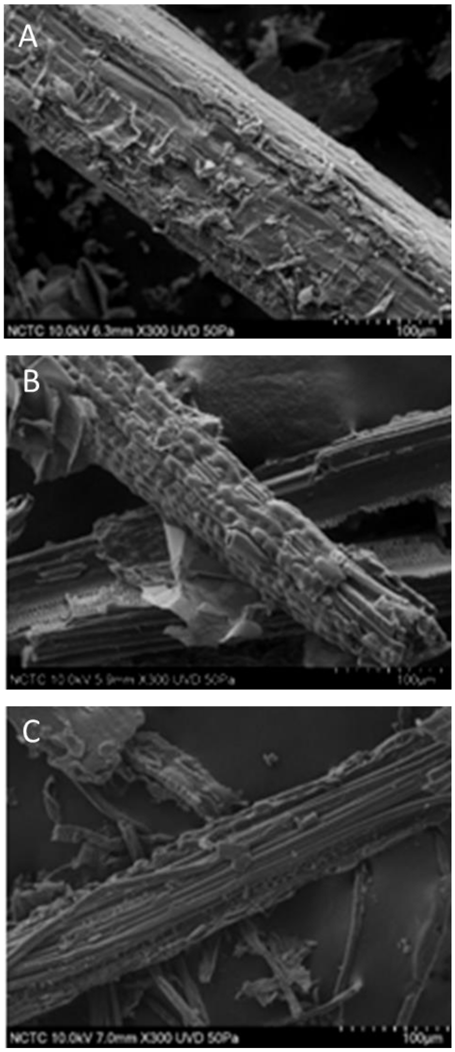


Figure 10 Electron scanning micrographs of SCT raw material (Left), solid fraction pretreated by LHW/Ac process (Middle), and GOP/Ac process (Right).

3.6. Organosolv lignin characterization

The recovered organosolv lignin samples (GOL and AOL) were characterized by TGA and FT-IR. The results were compared with those obtained from commercial organosolv lignin. The TGA profile at 25-800°C of the lignin samples showed a typical thermal degradation pattern (**Figure 11A**). However, a sharp thermal degradation of the commercial organosolv lignin was observed at approximately 180℃, whereas degradation of the GOL and AOL samples was observed in the range of 100 to 230℃ with 1.8-2.0% initial weight lost per degree Celsius. Between 250 and 410℃, the GOL and AOL samples were degraded with an initial weight loss <35%. Overall, the lignin fractions generated in this study were more thermally stable than the commercial organosolv lignin. AOL was found to have higher thermal stability than GOL. The results obtained from the organosolv lignin samples in this study were similar to those of lignin prepared by the organosolv process reported previously [29, 30].

Functional group profiles of the commercial organosolv lignin and GOL- and AOL-derived lignin samples were determined by FT-IR and compared with FT-IR spectra data reported in published literature [30] (**Figure 11B**). Transmittance percentages patterns indicated similar functional group profiles in all lignin samples, including O-H stretching at 3200-3600 cm^{-1} for the hydroxyl groups of phenolic compounds, symmetric and asymmetric C-H stretching at 2841-2849 cm^{-1}, 2916-2937 cm^{-1} for methyl and methylene groups within aliphatic chains, and methoxyl groups within aromatic ring of lignin. Stretching vibrations of carbonyl and carboxyl groups at 1700 cm^{-1} were identified. Aromatic ring stretching and vibration at 1500 and 1462 cm^{-1} combined with C=C and C-C stretching at 1603-1605 cm^{-1} confirmed the presence of aromatic ring. Although the investigated lignin samples showed similar FT-IR spectra pattern, a higher intensity of absorbed bands between 1135 and 952 cm^{-1} related to the C-O and C-O-H bonds of saccharides were identified in the GOL sample compared with other lignin samples. The result thus indicated higher cross-contamination of residual cellulose and hemicellulose derived components in the GOL lignin compared with the AOL lignin, which showed remarkably higher purity.

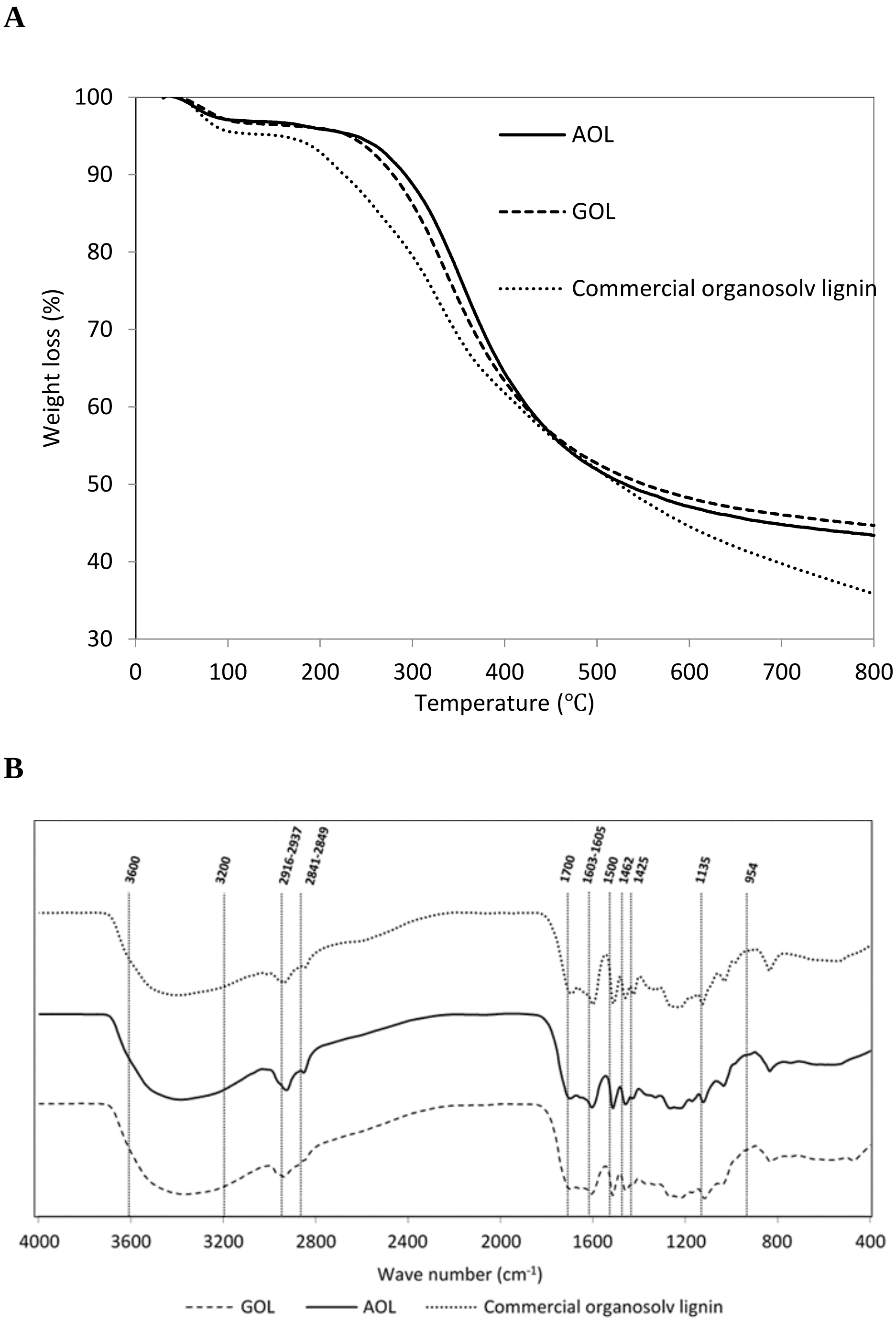


Figure 11 Physico-chemical analysis of the lignin samples. (A) Thermogravimetric patterns obtained from different organosolv lignin samples including (i) commercial organosolv lignin, (ii) organosolv lignin precipitated from acetone phase (AOL), and (iii) organosolv lignin precipitated from glycerol phase (GOL); (B) FT-IR spectra of commercial and recovered (AOL and GOL) organosolv lignin samples.

4. Conclusion

An efficient fractionation process comprising glycerol-based organosolv pretreatment with subsequent acetone extraction step was developed for SCT. The reaction showed high yields and selectivities on separation of cellulose with high purity and enzymatic digestibility, along with the hemicellulose-derived fraction and high purity organosolv lignin. The separated lignocellulose streams could be further used for conversion to a spectrum of potential products. Further studies on process design and solvent recycling are warranted for improving the potential of the process for industrial implementation.

PART 3: Investigation of alkaline hydrogen peroxide in aqueous organic solvent to enhance enzymatic hydrolysis of rice straw

1. Objective

In this work, we aimed to study the effects of co-solvents (ethanol, iso-propanol, and *tert*-butyl alcohol) and types of organic bases (NH_4OH and tri-ethylamine) on AHP pretreatment of rice straw, one of the world's most abundant agricultural wastes with the average annual production of 900 million tons/year [31, 32], compared to the use of conventional AHP process. Given the known benefits of co-solvents, organic bases, and the delignification effect of the oxidative H_2O_2 agent, these combined effects could result in improvement in AHP pretreatment strategy in terms of efficiency, environmental friendliness and reagent reusability. Physicochemical characteristics and microstructures of the cellulose-enriched solid fraction after alkaline peroxide pretreatment were investigated providing supports for improved processability of the biomass.

2. Materials and methods

2.1 Materials

Rice straw (RS) was obtained from a paddy field in Pathumthani province, Thailand. Initially, it was physically grinded by a cutting mill (Retsch ZM2000, Haan, Germany) and sieved through a 0.5 mm size screen. The milled rice straw was dried at 70^{o}C for overnight and stored in plastic bag at room temperature before use. The composition of raw biomass contained 35.5±1.2 wt% of cellulose, 22.5±0.7 wt% of hemicelluloses, 25.0±0.5 wt% of lignin, and 14.2±0.2 wt% of ash determined by the standard NREL analysis [33]. All analytical grade chemicals and organic solvents were provided from major chemical suppliers such as Sigma-Aldrich and Merck.

2.2 Alkaline peroxide pretreatment

2.2.1 Conventional AHP process

Based on the conditions from our preliminary study, the dried rice straw (2.5, 5, 7.5, 10 and 12.5%, w/v) was pretreated with 10 mL of hydrogen peroxide solution at different concentrations (1, 2.5, 5, 7.5 and 10% v/v) pre-adjusted to pH 11.5 (approx. 0.4-4.5% w/v of NaOH final concentration depending on concentrations of hydrogen peroxide). The reaction was performed in aqueous media by incubating the samples in different reaction temperatures (25, 30, 35, 40 and 45 ^{O}C) for various reaction times (6, 18, 24, 36 and 48 h). After that, the solid residue was collected by vacuum filtration on a paper filter (No. 4) with a pore size of 20-25 μm, washed by distilled water until pH reached to neutral. The residue was dried at 70^{o}C for overnight and stored at room temperature for further use in enzymatic hydrolysis process.

2.2.2 AHP process using organic solvents and organic bases

AHP pretreatment in aqueous organic solvent system was performed in a solvent mixture comprising different organic solvents (ethanol, isopropanol, or *tert*-butyl alcohol) and water in the ratio of 70:30. The reaction was performed using an organic bases (NH_4OH or tri-ethylamine) for pre-adjusting the reactions to pH 11.5 (approx. 5% v/v final concentration) under the starting condition obtained from the conventional AHP process. The effect of H_2O_2 concentrations (1, 2.5, 5, 7.5, 10%v/v) at 35°C for 24 h was then further studied. The collected solid residues were processed for studying the lignocellulosic composition, and tested for enzymatic digestibility.

2.3 Enzymatic hydrolysis

Enzymatic hydrolysis reaction consisted of 5% (w/v) pretreated rice straw with cellulase dosage at 20 FPU/g pretreated rice straw using Accellerase® 1500 (Dupont, Rochester, NY) containing 45 FPU/ml of initial activity [34] in 50 mM sodium citrate buffer at pH 4.8 and 0.25% (w/v) of NaN_3. The reaction was incubated at 50^{o}C for 72 h. The sugar product profile was examined using a high-performance liquid chromatograph (SPD-M10A DAD, Shimadzu, Japan) equipped with a refractive index detector and an Aminex HPX-87H column (Bio-rad, Hercules, CA). Sulfuric acid solution (0.005 M) was employed as the mobile phase using a flow rate of 0.5 mL/min with column temperature at 65°C.

Sugar yield represented the total amount of fermentable sugars (glucose, xylose, and arabinose) obtained from enzymatic hydrolysis of the pretreated biomass calculated based on the weight basis of the substrate used in the hydrolysis reaction (mg/g pretreated RS) according to equation 1. Sugar recovery was defined as the amount of total sugars obtained from the hydrolysis reaction taken weight recovery of the solid fraction from the pretreatment step into an account (mg/g raw RS) according to equation 2. %Glucose recovery was calculated based on the glucose recovered from the hydrolysis reaction compared to the cellulose content (×1.11) in the raw material on a dried weight basis according to equation 3.

$$\text{Sugar yield (mg/g pretreated RS)} = \frac{\text{Total sugar(glucose + xylose + arabinose) (mg)}}{\text{Initial weight of pretreated RS (g)}} \qquad (1)$$

$$\text{Sugar recovery (mg/g raw RS)} = \frac{\text{Total sugars of glucose, xylose, arabinose (mg)}}{\text{Initial weight of pretreated RS (g)}} \times \frac{\text{Final weight after pretreated RS (g)}}{\text{Initial weight of raw RS (g)}} \qquad (2)$$

$$\%\ \text{Glucose recovery} = \frac{\text{Recovered glucose (mg/g raw RS)}}{\text{Theoretical glucose in raw RS (mg/g raw RS)}} \times 100 \qquad (3)$$

2.4 Analytical methods

2.4.1 Chemical composition

Chemical composition of the pretreated RS solid recovery was determined according to the standard NREL analysis method [33]. The %cellulose recovery, %hemicellulose removal, and %lignin removal were calculated according to Equation 4-6.

$$\%\ \text{Cellulose recovery} = \left(\frac{C_F \times \%\ \text{Solid recovery}/100}{C_I}\right) \times 100 \qquad (4)$$

Where cellulose recovery is the percentage of cellulose remaining after the AHP pretreatment compared with the initial cellulose content; C_I is the percentage of initial cellulose content; C_F is the percentage of remaining cellulose content in the final solid recovery.

$$\%\ \text{Hemicellulose removal} = \left(\frac{H_I - (H_F \times \%\ \text{Solid recovery}/100)}{H_I}\right) \times 100 \qquad (5)$$

Where hemicellulose removal is the percentage of hemicelluloses removed after AHP process compared with the initial hemicellulose content; H_I is the percentage of initial hemicellulose content; H_F is the percentage of remaining hemicellulose content in the final solid recovery.

$$\%\ \text{Lignin removal} = \left(\frac{L_I - (L_F \times \%\ \text{Solid recovery}/100)}{L_I}\right) \times 100 \qquad (6)$$

Where lignin removal is the percentage of lignin removed after AHP process compared with the initial lignin content; L_I is the percentage of initial lignin content; L_F is the percentage of remaining lignin content in the final solid recovery.

2.4.2 Scanning electron microscopy

The microstructure and morphology of the raw and pretreated biomass obtained from AHP pretreatment in aqueous and organic solvent systems was determined by scanning electron microscope (SEM) using a S-3400N Type II SEM (Hitachi, Tokyo, Japan). The samples were dried and coated with gold according to the standard protocol [35]. An electron beam energy of 5 kV was applied for analysis.

2.4.3 X-ray diffraction analysis

The crystallinity of raw and pretreated biomass was analyzed by X-ray diffraction (XRD) using an X'Pert PRO diffractometer (PANalytical, Almelo, The Netherlands). The samples were scanned at a speed of 0.5°/min in a range of 2θ=10–30° with a step size of 0.02° at 40 kV, 30 mA and radiation at Cu Kα (λ=1.54 Å). The average crystallite size was calculated by the Scherrer equation. The crystallinity index (CrI) were calculated according to the equations 7 [36].

$$\text{CrI (\%)} = \left[\frac{I_{002} - I_{am}}{I_{002}}\right] \times 100 \qquad (7)$$

where CrI is the crystallinity index (%); I_{002} is the highest intensity for the crystalline portion at 2θ = 22.40 and I_{am} is the peak for the amorphous portion at 2θ = 18.00.

2.4.4 Fourier transform infrared spectroscopy (FTIR)

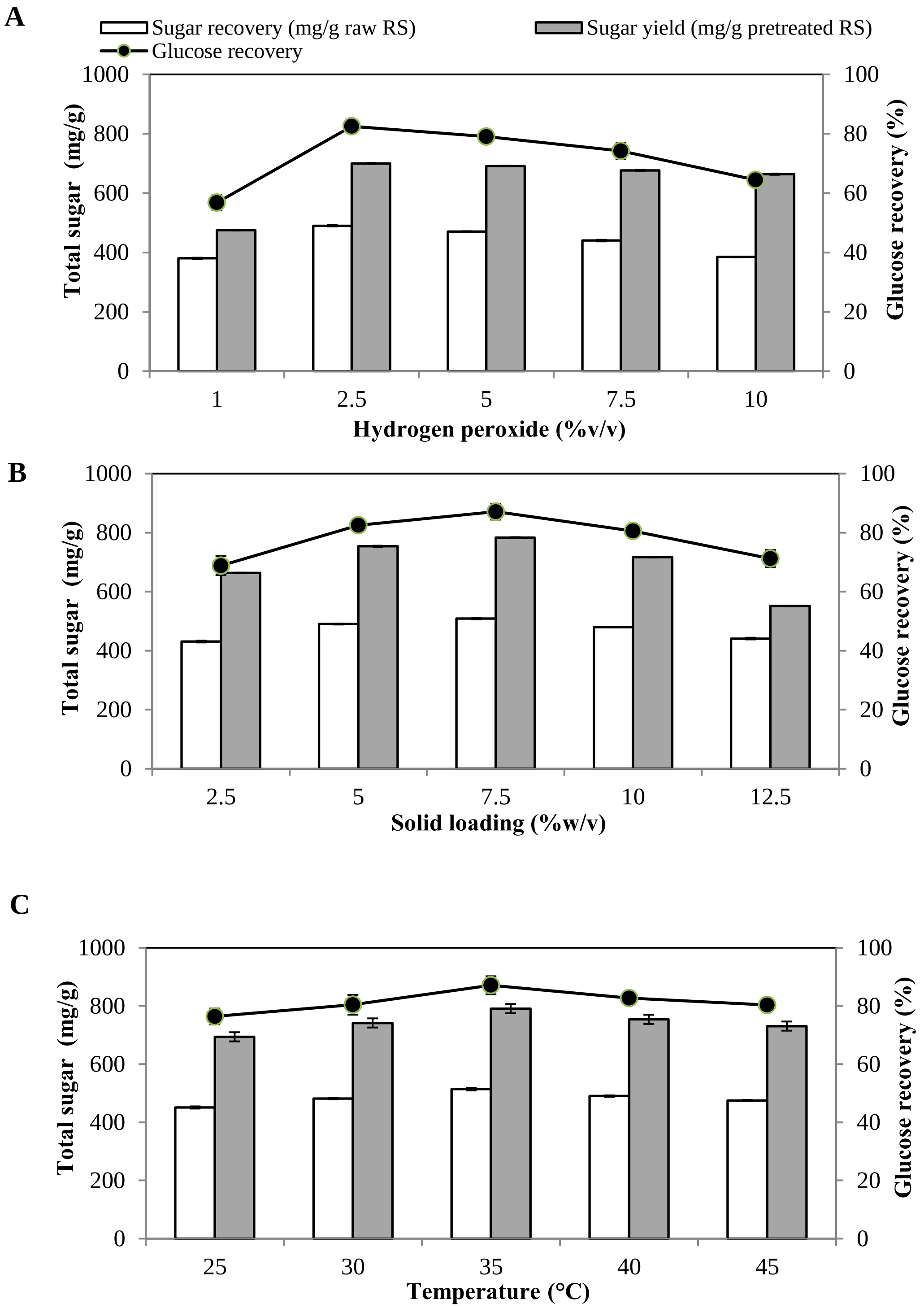
The functional groups on the lignin samples were analyzed by Fourier-Transformed Infrared Spectroscopy (FT-IR) (Perkin-Elmer System 2000, Waltham, United States) with infrared spectra collected in the wave number range of 600–4000 cm^{-1}.

3. Results and Discussion

3.1 AHP pretreatment in aqueous system

Firstly, the conventional AHP pretreatment of rice straw using water as the medium and pH adjusted with NaOH was studied under different reaction conditions with varying concentrations of H_2O_2, solid loading, temperature, and reaction time to study the optimized conditions for maximizing the glucose recovery (**Fig. 12**). The effect of H_2O_2 dosage was initially studied in the range of 1–10 %v/v in the reactions containing 5% initial solid loading at 35°C for 18 h. The result showed that increasing H_2O_2 dosage from 2.5% to 10% led to decreasing sugar yield from 700 to 664 mg/g pretreated RS with a decrease on solid recovery from 70.0 to 58.0%. This led to the decreasing sugar recovery from 490 to 385 mg/g raw RS, corresponding to the glucose recovery in the range of 82.5 to 64.5 % (**Fig. 12A**). The lower sugar yield obtained from the reactions with higher H_2O_2 dosages could be due to the excessive dosage of H_2O_2 which led to lower cellulose recovery together with formation of inhibitory compounds from the delignification reaction [37, 38]. Next, the effect of solid loading was studied in the range of 2.5–12.5 %w/v with a fixed reaction volume using the optimum H_2O_2 concentration (2.5%v/v) at the fixed temperature and time. It was found that the optimal solid loading at 7.5%w/v gave the highest sugar yield and recovery as shown in **Fig. 12B.** The excessive biomass loading at 10 %w/v resulted in reduction of free water which caused reduced efficiency in mass transfer in the reaction and could lead to higher energy consumption for mixing in an up-scaled process [37]. The effect of temperature was subsequently studied at 25-45°C in the reactions containing 2.5%v/v of H_2O_2 and 7.5%w/v of solid loading for 18 h. The highest sugar product and glucose recovery were achieved at 35°C (**Fig. 12C).** Higher temperature (45°C) could lead to an excessive generation of radicals in AHP pretreatment which reflected in the decrease in the solid recovery and thus the yield of sugar products. Optimization of reaction time was then studied in the range of 6-48 h based on optimal condition identified. The result demonstrated that increasing reaction time from 6 to 36 h led to an increase in the sugar yield of 720-834 mg/g pretreated RS (**Fig. 12D**). The reaction time at 24 h under the optimal AHP conditions (7.5% biomass loading, 2.5% H_2O_2 at 35 ^{o}C) was a good candidate for achieving the sugar recovery of 540 mg/g raw RS, equivalent to 93.3% glucose recovery which was 3.1 times compared to that obtained from hydrolysis of untreated RS (105 mg/g raw RS).

The glucose recovery obtained using the conventional AHP process in this study was higher than that reported from most previous works on several lignocellulosic feedstocks such as rice husk, sugarcane bagasse, and corn stover where the glucose recovery yields of 54.3-75.0% were obtained under varying AHP pretreatment conditions [39-41]. Banerjee et al (2012) studied scaling-up of AHP pretreatment of corn stover for converting the released sugars to ethanol. The optimal conditions of AHP process were at 0.125 g H_2O_2 /g biomass at 22°C for 48 h under atmospheric pressure, resulting in a glucose recovery of 75.0% after saccharification [41]. The AHP pretreatment of rice husk using 7.5% H_2O_2 v/v at 35 °C for 24 h showed a lower glucose yield of 240 mg/g rice husk, equivalent to 60.7% glucose recovery after enzymatic hydrolysis at 45°C for 120 h [40]. It is noted that a lower glucose yield of 69.4% after enzymatic hydrolysis was also obtained from AHP pretreated sugarcane bagasse using contained 5% H_2O_2 v/v at 20 °C for 24 h [39]. The results thus suggested high efficiency and selectivity of the conventional AHP process for pretreatment of rice straw under the optimized conditions in this study.



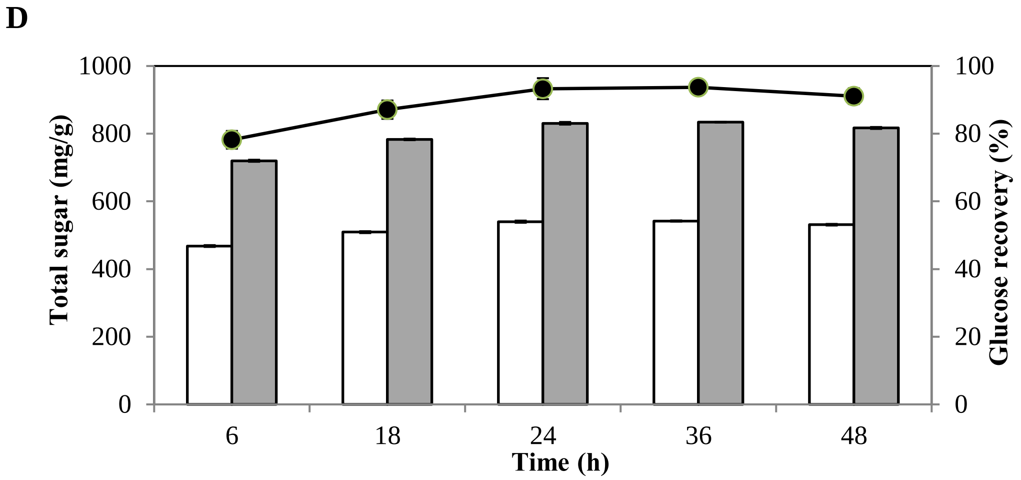


Figure. 12 Effects of reaction parameters on AHP pretreatment with NaOH in water as the medium. The standard reaction contained 7.5%w/v solid loading with 2.5%v/v H_2O_2 and incubated 35°C for 24 h. The total reaction volume was fixed at 10 ml. The reaction parameters were varied for (A) H_2O_2 concentration; (B) solid loading; (C) temperature; and (D) reaction time.

3.2 Effects of base types and organic solvents in AHP pretreatment

The effects of base (NH_4OH and TEA) in the presence of water and various co-solvents (ethanol, isopropanol, and *tert*-butyl alcohol) was initially studied based on the optimal AHP condition obtained from the conventional process (7.5% w/v solid loading in presence of 2.5% v/v H_2O_2). The pH was adjusted using different bases to pH 11.5 and incubated at 35°C for 24 h **(Fig. 13)**. The use of NH_4OH for pH adjustment in AHP pretreatment showed a high sugar yield in the range of 178-427 mg/g pretreated RS and sugar recovery of 164-320 mg/g raw RS, equivalent to the glucose recovery of 35.3-73.6% in the aqueous-organic solvent system. A lower sugar yield of 176-208 mg/g pretreated RS and sugar recovery of 165-185 mg/g raw RS, equivalent to 31.6-36.8% glucose recovery was obtained using TEA. For both bases, higher sugar yield and recovery were obtained using the AHP process in *tert*-butyl alcohol system compared with those from the water system. The highest glucose recovery of 73.6% was achieved using NH_4OH in *tert*-butyl alcohol as the co-solvent, which was higher than that obtained using the water system under the same experimental conditions (71.2%), suggesting advantages on using this co-solvent in AHP process. The result agreed well with the higher sugar yield obtained from AHP in ethanol system with KOH compared to that from the water system previously demonstrated [42].

Aqueous ammonia or NH_4OH has several advantages including high selectivity on delignification of biomass by cleaving the C-O-C linkages in lignin molecule together with breaking the ester and ether bonds in the lignin-carbohydrate complexes with less degradation of cellulose [43]. The use of NH_4OH could also allow recycling of the base reagent by evaporation due to the high volatility of ammonia at relatively low temperatures [44]. *Tert*-butyl alcohol was shown as a superior solvent related to selectivity to cellulose fraction in the pretreatment step and enzymatic hydrolysis compared to the other alcohols (ethanol and isopropanol) and water under the same experimental conditions. The use of *tert*-butyl alcohol (partition coefficient: log P *tert*-butyl alcohol = 0.35) has been reported to result in higher solubilization of lignin compared to the use of other alcohols (ethanol, 1-propanol, and 2-propanol; log P = −0.31, 0.25, and 0.05, respectively), as demonstrated in organosolv pretreatment of sorghum bagasse [45].

Compared to water, the aqueous organic solvent can lead to efficient swelling of the biomass during the pretreatment step due to its larger molecular size. A strong linear correlation between the activation energy on biomass swelling and the molecular weight of solvent was reported [42]. This swelling effect can result in higher accessibility of H_2O_2 radicals and subsequently by enzymes to the polysaccharide fractions. According to its partition coefficient [45], the use of organic solvent can also allow more efficient solubilization of lignin and lignin-degraded products generated from oxidative cleavages on the ester and ether bonds by the actions of superoxide ($O_2^{\bullet}$) and hydroxyl radical ($OH^{\bullet}$) species during the pretreatment step [37, 43]. Overall, this can result in increase in pretreatment efficiency and sugar yield from enzymatic hydrolysis of the biomass. The results thus suggested the possibility of using the organic base and co-solvent for the modified low-temperature AHP pretreatment with high efficiency and selectivity.

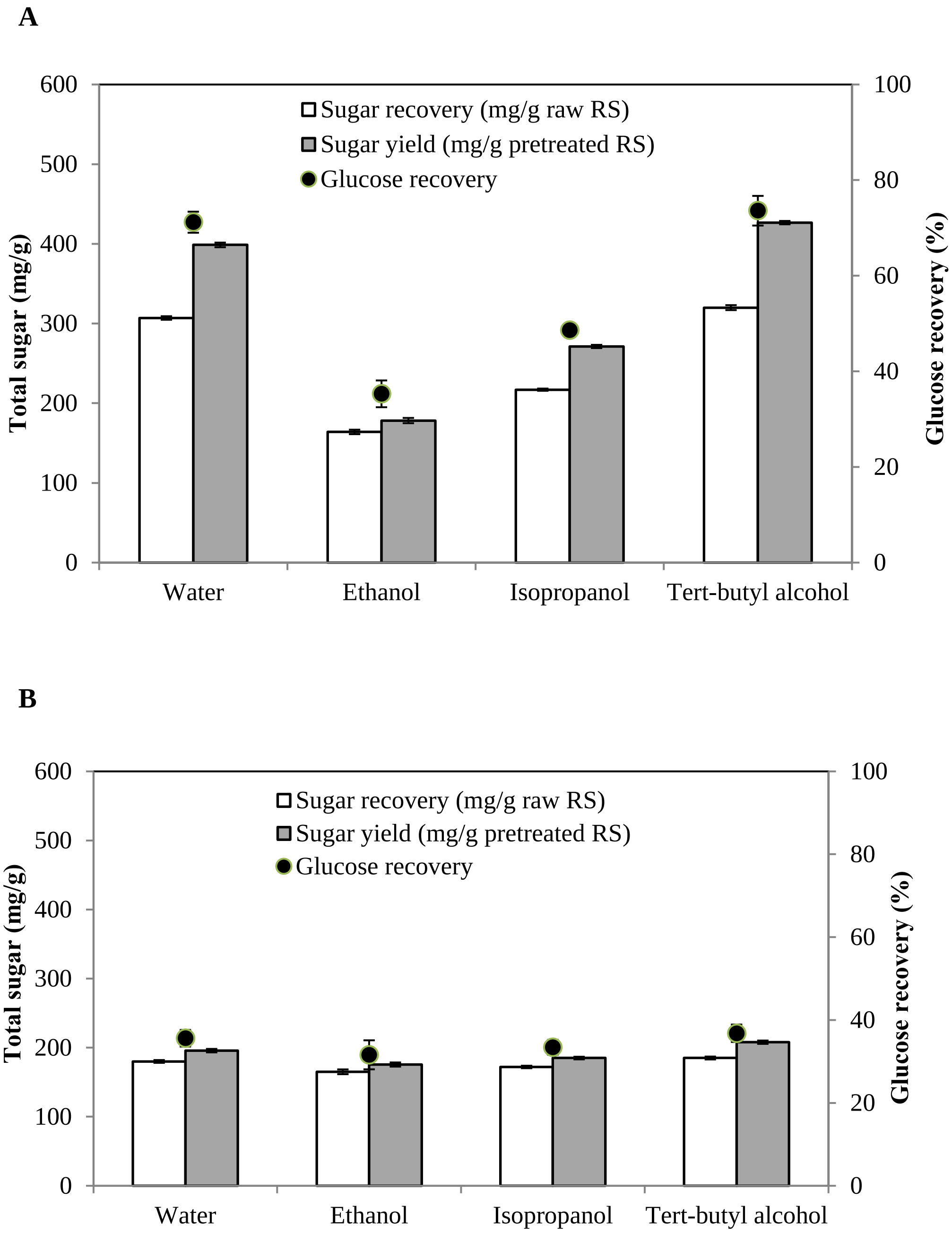


Fig. 13 Effects of co-solvents and water in AHP pretreatment of rice straw using different organic bases on total sugar product and glucose recovery based on enzymatic digestibility; (A) NH_4OH; (B) TEA. Reactions contained 7.5 %w/v solid loading of rice straw in different aqueous organic systems (70% v/v) in the presence of 2.5 %(v/v) H_2O_2 with 5 % (w/v) organic base and incubated at 35 $^{\circ}$C for 24 h.

3.3 Effects of peroxide loading in the aqueous *tert*-butyl alcohol system using NH_4OH

The effects of H_2O_2 concentration in the *tert*-butyl alcohol system using NH_4OH on total sugar product and glucose recovery are shown in **Fig. 14**. In contrast to the trend observed for the conventional AHP process, it was found that increasing H_2O_2 concentration from 1 to 10 %v/v led to higher sugar yield (349 – 623 mg/g pretreated RS) and sugar recovery (290-411 mg/g raw RS), equivalent to the maximum glucose recovery of 83.0% at 7.5%v/v of H_2O_2 concentration. The glucose recovery achieved in this study was higher than those reported in many of the previous works using conventional AHP process on rice straw (44.4-80.0%) [46, 47]. The optimal concentration of H_2O_2 shown in this study agreed well with the previous reports using conventional AHP processes on different rice wastes. Saha and Cotta (2007) reported that increasing H_2O_2 concentration from 0 to 7.5 %v/v improved release of sugars from rice husk with the highest glucose recovery of 52.2% [40]. The increase in H_2O_2 concentration (1-4 %w/v) also improved enzymatic digestibility of rice husk as reported by Cabrera et al. (2014) [48]. However, excess H_2O_2 concentration was reported to result in a progressive decrease in sugar product due to non-specific degradation of cellulose along with degradation of released sugars to inhibitory compounds [49].

The chemical compositions of the solids obtained under the optimal pretreatment conditions and enzymatic hydrolysis using NH_4OH in aqueous *tert*-butyl alcohol and NaOH in water were studied compared with the raw RS (**Fig. 15)**. The solid recovery after pretreatment was in the range of 65-66%. According to **Fig 15A**, the hemicellulose removal and lignin removal after AHP pretreatment were 10.7% and 58.0% for the NaOH/water process and 9.4% and 52.5% for the NH_4OH/*tert*-butyl alcohol process, respectively with more than 90% cellulose recovery. Enzymatic hydrolysis led to conversion of the cellulose fraction to glucose with further removal of the hemicellulose and lignin fractions **(Fig 15B)**. This led to a further increase in accumulated hemicellulose to 73.8% and 39.1% for the NaOH/water and NH_4OH/*tert*-butyl alcohol processes, respectively while the lignin fraction was mainly not degraded and enriched in the solid residues. Interaction efficiency between hemicellulose and lignin removals by both AHP processes under the optimal conditions and glucose recovery after enzymatic hydrolysis was significant with high correlation based on a statistical analysis of the regression model (R^2 = 0.92 and 0.96 and *P*- value <0.05). This agree with strong correlation of xylan and lignin removals to glucose yield from AHP pretreatment of sugarcane bagasse [14]. Mass balance of the pretreatment processes with the focus on the cellulose fraction is shown in **Figure 16**, showing balance of starting cellulose, the released glucose, and the residual non-digested cellulose in the solid residues. The results thus showed effective delignification of the biomass with slight hemicellulose removal while the majority of the cellulose fraction was obtained with high enzymatic digestibility. The results on degrees of hemicellulose and lignin removals were correlated to several previous works using water system with NaOH [50-53]. Xing et al (2013) reported that AHP pretreatment of bamboo using 30%w/w H_2O_2 concentration at 80 °C for 1 h led to reduction of the lignin fraction from 26% to 12.7%, with no significant decrease of glucan and xylan [52]. The AHP process of corn stover containing 1% (w/w) of H_2O_2 with 4% of solid loading at 37 °C for 3 h resulted in hemicellulose and lignin removals of 6.25% and 41.1%, respectively together with 87.3% cellulose recovery [51].

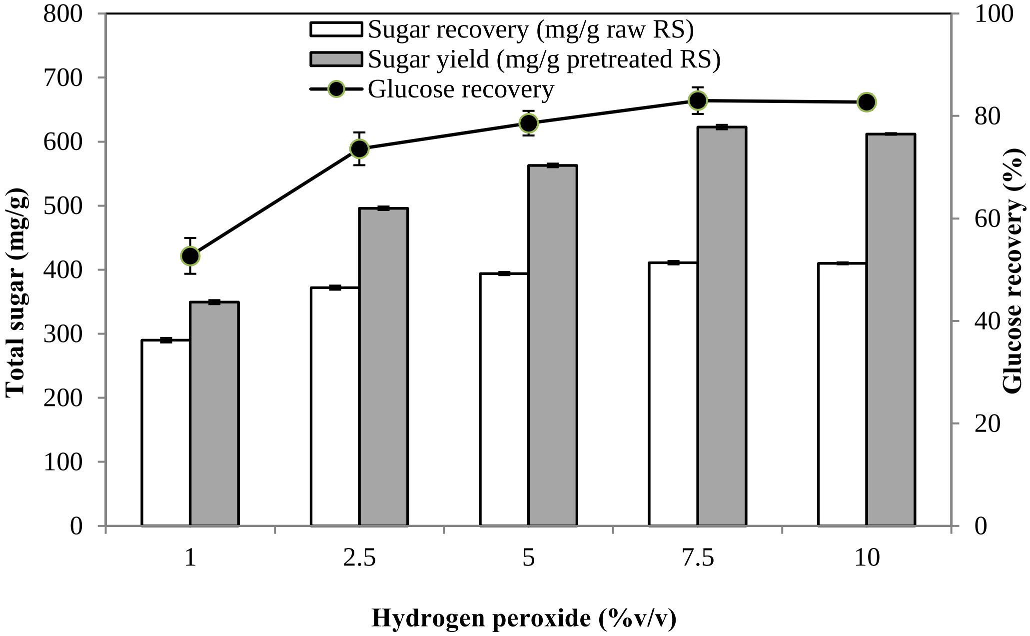


Fig. 14 Effects of H_2O_2 concentration in AHP pretreatment using NH_4OH in 70% v/v aqueous *tert*-butyl alcohol system. Reactions contained rice straw at 7.5 %w/v solid loading with varying level of H_2O_2 in aqueous *tert*-butyl alcohol systems with 5% (w/v) NH_4OH and incubated at 35 °C for 24 h.

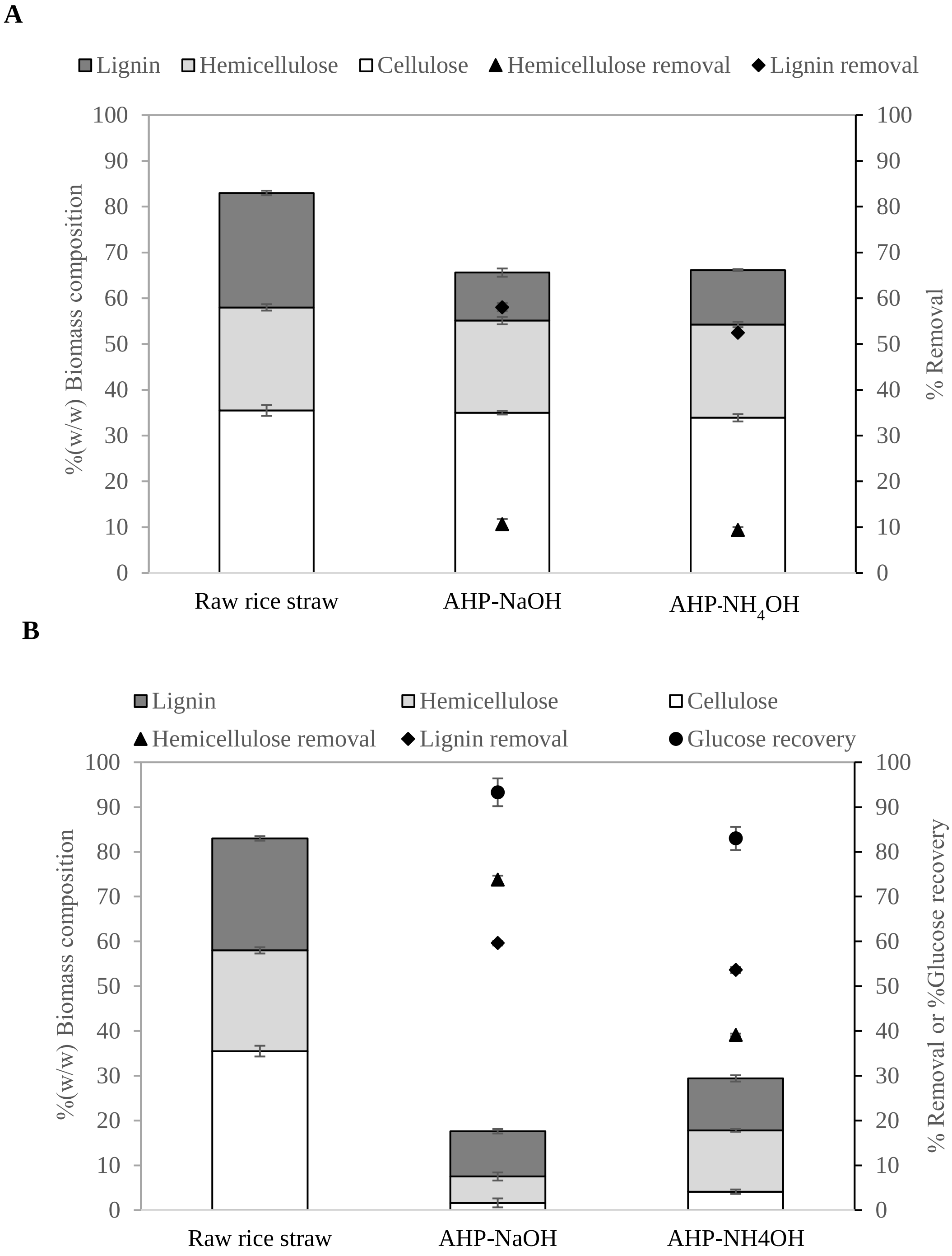
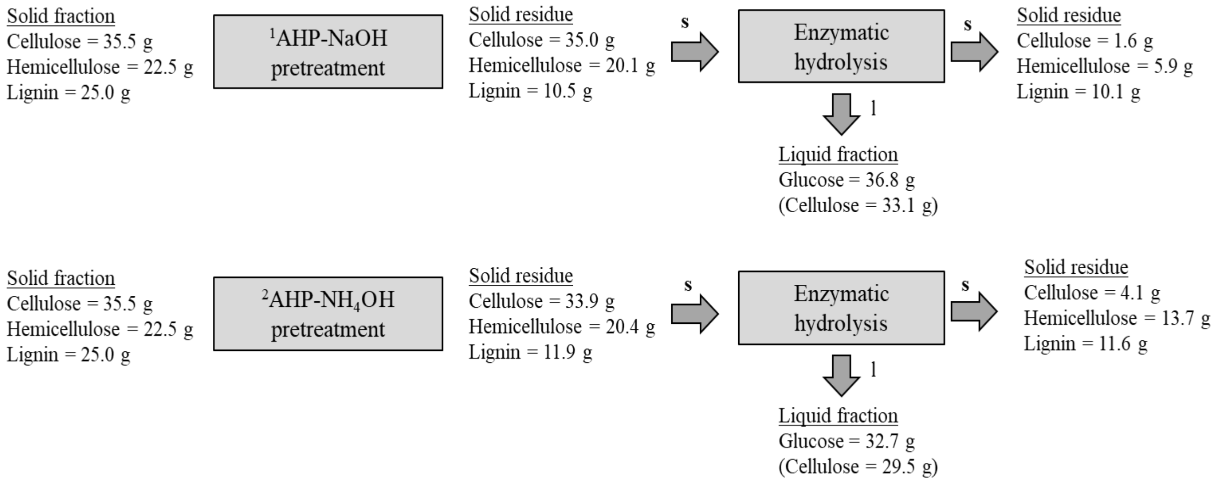


Fig. 15 Biomass composition on AHP pretreatment (A), and enzymatic hydrolysis (B) using modified and conventional AHP process based on analysis of hemicellulose removal, lignin removal, and glucose recovery. Rice straw was pretreated using NH_4OH in 70% v/v aqueous *tert*-butyl alcohol (7.5% solid loading, 7.5% v/v H_2O_2, 35^{o}C, 24 h), and NaOH in water (7.5% solid loading, 2.5% v/v H_2O_2, 35^{o}C, 24 h) under the optimal condition compared to raw rice straw.



[1] Reactions of pretreated rice straw in water with NaOH contained 7.5% solid loading, 2.5% v/v H_2O_2, 35 °C, 24 h.

[2] Reactions of pretreated rice straw in 70% v/v *tert*-butyl alcohol with NH_4OH contained 7.5% solid loading, 7.5% v/v H_2O_2, 35 °C, 24 h.

Fig. 16 The mass balance diagram of AHP process using NaOH/water and NH_4OH/*tert*-butyl alcohol systems.

3.4 Physicochemical characterization of pretreated rice straw in organic solvent system

The structural alteration of RS samples pretreated by the AHP process in the presence of NaOH in the aqueous medium and NH_4OH in the aqueous organic of *tert*-butyl alcohol system were analyzed by SEM compared with the native RS (**Fig. 17**). It can be seen that the native RS showed intact physical structure with no cracks and cavities and covered with an intact wax-coated surface. Overall, AHP pretreatment in the presence of different bases both in water and solvent systems resulted in structural changes in the biomass surface due to efficient removals of the cuticle wax and silica layers. The use of NaOH/aqueous and NH_4OH/*tert*-butyl alcohol systems led to formation of higher porous structure and increased surface area of the biomass. Their modified structure allowed increasing accessibility of the enzymes to the inner cellulose microfibers in the substrate. This result was corresponded to the previous work reported by Qi et al (2009) [54], which showed that the AHP pretreatment of wheat straw in the aqueous medium using NaOH led to rugged, rough, and broken surface as well as appearance of porous structure in the pretreated solid fraction compared to the native biomass which showed an even and smooth flat surface.

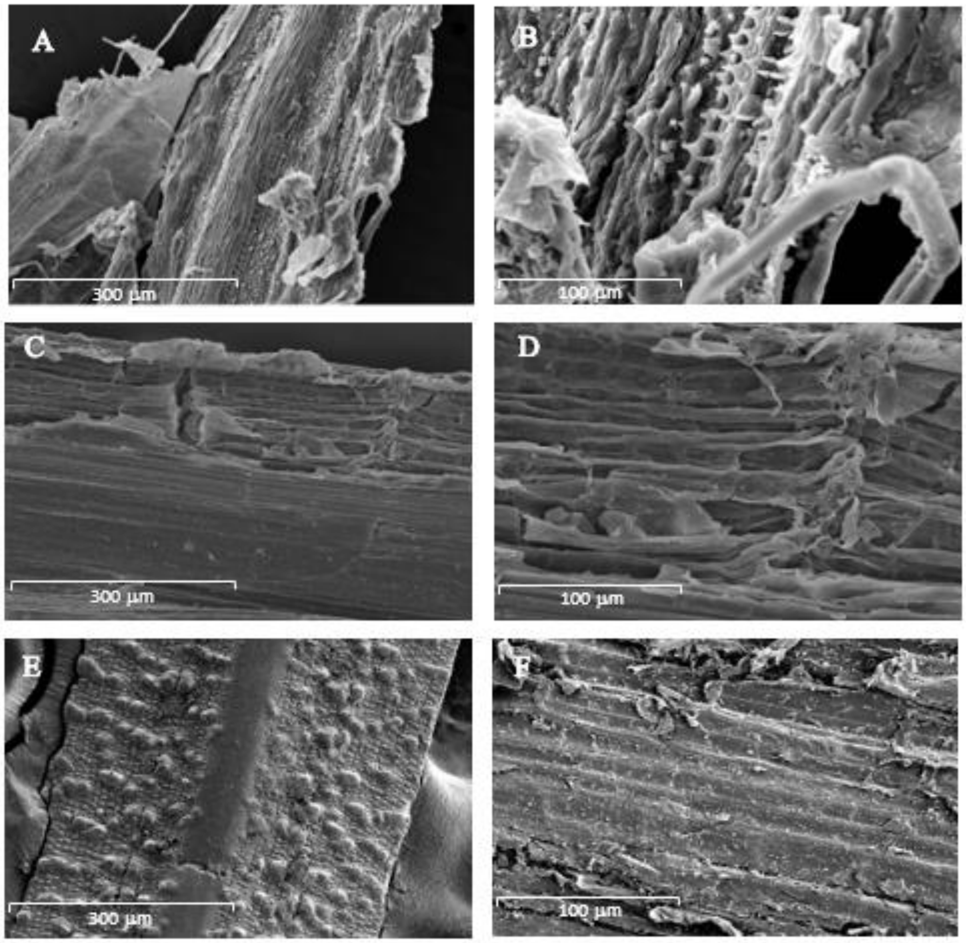


Fig. 17 SEM analysis of AHP pretreated rice straw using modified and conventional AHP process under the optimal condition compared to raw rice straw; (A-B) pretreated rice straw in water with NaOH (7.5% solid loading, 2.5% v/v H_2O_2, 35°C, 24 h) at 200x, 500x; (C-D) pretreated rice straw in 70% v/v *tert*-butyl alcohol with NH_4OH (7.5% solid loading, 7.5% v/v H_2O_2, 35°C, 24 h) at 200x, 500x; and (E-F) native rice straw at 200x, 500x.

The crystallinity index of AHP pretreated samples pretreated under the optimal condition was analyzed by XRD. It was found that the use of NH_4OH in aqueous *tert*-butyl alcohol systems resulted in an increase in crystallinity index (56.1%) compared with the native RS (45.2%) but lower than that obtained using NaOH in aqueous media (59.1%). This could be due to the effect of AHP process which led to more solubilization of amorphous lignin and hemicellulose fractions. The effect of AHP pretreatment on crystallinity index of different lignocellulosic biomass has been reported by several researches. Reis et al (2016) reported the increase of crystallinity index to 47.6 and 47.5% after AHP pretreatment of sugarcane bagasse and sweet sorghum bagasse, respectively compared to 34.0% and 33.8% of these biomasses in their native forms, respectively [55]. Moreover, the effect of aqueous ammonia addition could also result in removal of the amorphous lignin and hemicellulose, leading to increasing crystallinity index after biomass pretreatment and enhancing the enzymatic digestibility as reported in the ammonia soaking pretreatment process of corn stover [56].

FT-IR spectra of the rice straw pretreated under the optimal conditions for NaOH /water and NH4OH/*tert*-butanol system compared to the native biomass are shown in **Figure 18**. The functional groups of AHP pretreated samples were corresponded to the assignment of FT-IR spectra of pretreated oak wood, sugarcane and sweet sorghum bagasse reported earlier [57, 58]. The raw rice straw showed the peak of C=C stretching of aromatic ring of lignin at 1604 cm^{-1}, C=C aromatic skeletal vibration stretching of the benzene ring in lignin at 1513 cm^{-1}, and C–O–C stretching of primary alcohol in cellulose and hemicelluloses at 1053 cm^{-1}. All functional groups in the raw rice straw were identified in the pretreated rice straw samples according to the FT-IR spectra. However, the lower intensity of these peaks was observed after pretreatment by the AHP process, suggesting solubilization of the lignin and partial hemicellulose fractions. In addition, the higher intensity of C–H deformation of glucose ring in cellulose and hemicellulose at 889 cm^{-1} of the AHP pretreated samples compared to that of the raw material also suggested the change in cellulose structure after the pretreatment step.

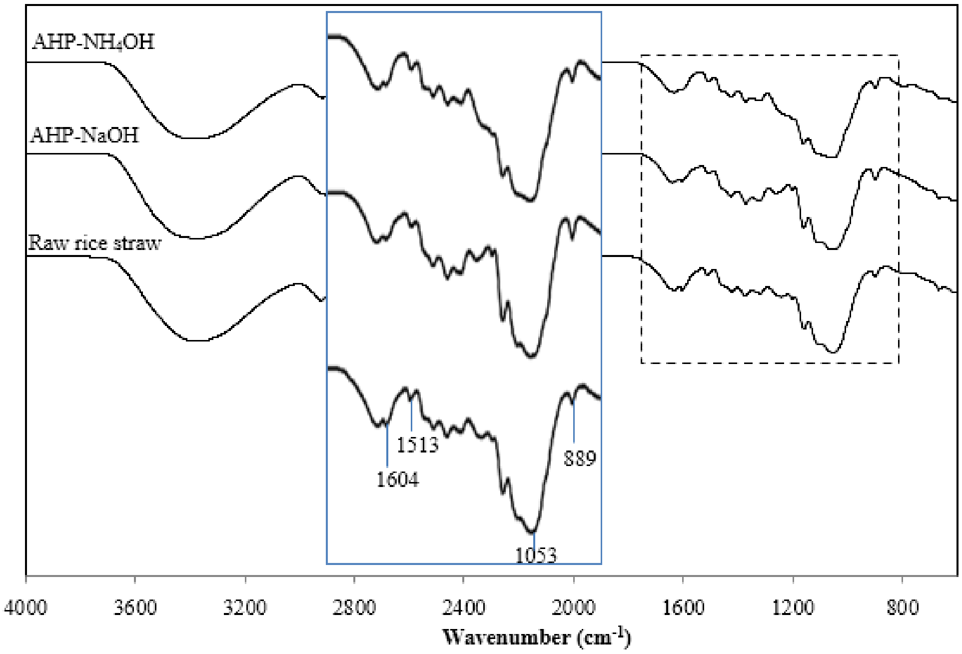


Fig. 18 FT-IR analysis of AHP pretreated rice straw using modified and conventional AHP process under the optimal condition compared to raw rice straw.

Comparison of total sugar product and glucose recovery achieved in our study and previous works on pretreatment of rice straw by different methods is summarized in **Table 5**. The glucose recovery achieved in this study using the modified *tert*-butyl alcohol/ NH_4OH-based AHP process or the optimized conventional AHP process were higher or at least comparable compared to that previously reported using different chemical (e.g. acid and ionic liquid) and hydrothermal (e.g. liquid hot water and steam explosion) methods or their combinations which led to a varying glucose recovery in the range of 57.0-83.0% [59-64]. This can reflect the synergized effect of high lignin solubility in *tert*-butyl alcohol and efficient biomass delignification caused by H_2O_2. Compared to previous studies on pretreatment of rice straw using different chemical and hydrothermal methods, the developed AHP processes in this study were performed under markedly milder conditions and hence could result in less energy requirement and lower equipment cost. The developed AHP pretreatment by NH_4OH/*tert*-butyl alcohol system is also advantageous compared to the conventional AHP pretreatment using NaOH/aqueous system in term of reusability of the organic solvent and organic base which can lead to generation of less waste water and chemical waste. Recycling of *tert*-butyl alcohol using distillation method was demonstrated [65] while NH_4OH can be recovered by simple evaporation technique [44]. However further study on the solvent and base recycling step is needed. The results thus demonstrated the potential of the modified AHP pretreatment developed in this work for biomass utilization in biorefineries.

Table 5 Comparison of sugar product from rice straw pretreated by different methods

Process	**Optimal conditions**	**Sugar products**	**Ref.**
Alkaline hydrogen peroxide	7.5% (w/w) solid loading, 7.5% (v/v) H_2O_2 at 35°C for 24 h in NH_4OH/*tert*-butyl alcohol (70:30 %v/v) system	411 mg/g raw RS and 623 mg/g pretreated RS 83.0% glucose recovery	this study
Alkaline hydrogen peroxide	7.5% (w/w) solid loading, 2.5% (v/v) H_2O_2 at 35°C for 24 h in NaOH/water system	540 mg/g raw RS and 831 mg/g pretreated RS 93.3% glucose recovery	this study
Alkaline hydrogen peroxide	5% (w/w) solid loading, 1% (w/v) H_2O_2 at 30°C for 24 hours and adjusted to pH 11.5 using NaOH in water	91.6% reducing sugar yield	[48]
Nitric acid pretreatment	10% (w/w) solid loading, 0.65% HNO_3, 158.8 ^{o}C for 5.86 min	83.0% glucose recovery	[59]
Dilute acid pretreatment	5% (w/w) solid loading, 1% (w/w) H_2SO_4 at 160°C for 5 min	210 mg/g pretreated RS 57.0% glucose recovery	[60]
Liquid hot water pretreatment with alkaline catalyst	10% (w/w) solid loading, 0.25% (w/v) NaOH at 140°C for 10 min	74.6% glucose recovery	[63]
Acidified glycerol pretreatment	5% (w/w) solid loading, 1% HCl at 190°C for 10 hours	58.2% glucose recovery	[64]

Combined pretreatment of steam explosion and alkaline pretreatment	Steam explosion at 170 °C for 10 min, sequentially treated with 2%(w/v) KOH in autoclave at 121 °C for 30 min	664 mg/g pretreated RS 82.6% total sugar yield	[61]
Alkaline hydrogen peroxide	5% (w/w) solid loading, 16% (v/v) H_2O_2, 8% (w/v) NaOH at 70°C for 12 h 5% (w/w) solid loading, 16% (v/v) H_2O_2, 8% (w/v) NaOH for 10 min at room temperature	80.0% glucose recovery	[46]
Alkaline hydrogen peroxide with wet air oxidation pretreatment	4% H_2O_2 with 6 bars of air at 190 °C for 24 h soaking time	44.4% glucose recovery	[47]
Combined pretreatment of ionic liquid and inorganic salt and water	Mixing of 50% ([C_2mim]Cl) and 49.5% water and 0.5% K_2CO_3 at 95 °C for 3 h	82.4% glucose recovery	[62]

4. Conclusions

AHP pretreatment using NH_4OH in the aqueous *tert*-butyl alcohol system was reported in this study. The developed process led to marked improvement on enzymatic digestibility of rice straw, resulting in high glucose recovery with high selectivity on separation of the cellulose. This was associated efficient delignification with partial hemicellulose removal, and hence increase in crystallinity of the cellulose-enriched fraction. The developed AHP pretreatment using the organic base and co-solvent allowed high pretreatment efficiency under mild reaction conditions leading to advantages on low energy consumption and lower formation of toxic compound during the pretreatment step with potential on solvent and base recycling. This work presented a promising modified AHP pretreatment for rice straw and other agricultural wastes for further application in biomass conversion industry.

Output จากโครงการวิจัยที่ได้รับทุนจาก สกว. :

ผลงานตีพิมพ์ในวารสารวิชาการนานาชาติ

Chotirotsukon, C., **Raita, M***., Champreda., V., Laosiripojana, N. (2019). Fractionation of sugarcane trash by oxalic-acid catalyzed glycerol-based organosolv and mild solvent delignification, Industrial crops and products, 141, 111753

Damaurai, J., Preechakun, T., **Raita, M***., Champreda., V., Laosiripojana, N. (2020). Investigation of alkaline hydrogen peroxide in aqueous organic solvent to enhance enzymatic hydrolysis of rice straw (DOI: 10.1007/s12155-020-10152-5)

การเสนอผลงานในที่ประชุม

Marisa Raita, Verawat Champreda, Navadol Laosiripojana (2018). Synthesis of Cellulose Particles from Palm Fiber Residue using Coupled Organosolv Fractionation and Acid Hydrolysis Process, International Conference on Engineering, Technology, and Applied Science (ICETA), Hokkaido, August 17-19.

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