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EXTRACTION OF STARCH, XYLOSE AND GLUCOSE FROM OIL PALM TRUNK FOR BIOETHANOL PRODUCTION

WONG LIH JIUN

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By

WONG LIH JIUN

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September 2019

Chair : H'ng Paik San, PhD
Faculty : Forestry and Environment

Use of oil palm trunk as sugars resource of biomass is currently under intensive study as an alternative growth substrate for bio-based chemical an energy production. It will benefit the environmental and reduce the agricultural waste. The complexity of chemical compositions in lignocelluloses as inhibits in degradation of lignin, hemicelluloses and cellulose which contain starch, five- and six-carbon sugars; prior to hydrolyzed and followed by co-fermentation for bioethanol production. Generally, this study aims to optimize the extraction of starch and mixed xylose-glucose from oil palm trunk and co-fermentation process of mixed sugars to bioethanol.

Chemical analysis was carried out based on TAPPI Test Methods (TAPPI, 2000) in determination of the chemical compositions of different levels and different portions of OPT. Low lignin and hemicelluloses and cellulose were found in this batch of study. It shows that OPT contains considerable extractives content which is valuable be source of chemical value added products like fermentable sugars.

For the study 2 (Starch extraction by Distilled Water and Chemical from OPT), starch in the oil palm trunk was extracted by 0.2 % sodium metabsisulphite (w/v) and 0.5 % lactic acid (v/v), and aqueous water respectively treated with levels of temperatures and times. Value $P \leq 0.01$ significance was observed for the main effects (temperature and time) on the starch yield with water solution and chemicals. A significant interaction was also found between two main effects (temperature and time). 19.7 % of starch yield was obtained by distilled water at 50 °C, 1 h, whereas 14.0 % of starch was obtained by chemicals at 26 ± 2 °C, 1 h). From the Response Surface Methodology (RSM) optimization analysis, optimum starch yield 12.8 % was extracted by room temperature 26 ± 2 °C and 1 h by chemical solution. Whereas, from the RSM optimization analysis, optimum

starch yield 16.3 % was extracted by distilled water under parameter 50 °C and 1 h. From the findings for study 2, the optimum starch yield can be obtained in lower temperature when OPT powder treated with chemicals solution than aqueous water.

Continuous dilute acid hydrolysis and simultaneous acid hydrolysis (115 °C, 120 °C and 130 °C reacted with 15 min, 30 min and 60 min) for producing xylose and glucose. 2 %, 4 % and 6 % of acid sulphuric was used for hydrolysis process. For continuous acid hydrolysis, optimum parameter of starch extraction method parameter (0.2 % sodium metabsisulphite (w/v) and 0.5 % lactic acid (v/v), 26 ± 2 ˚C and 1 h), the extracted starch was mixed with residue of oil palm trunk powder for dilute acid hydrolysis process. Simultaneous acid hydrolysis used untreated raw OPT powder for glucose and xylose production. The higher glucose yield occurred at dilute acid hydrolysis with the parameter of 6 % sulphuric acid concentration reacted for 60 min on 60 mesh of OPT powders at 100 °C with a total glucose yield of approximately 15.3 % by simultaneous hydrolysis. The optimum xylose yield occurred with the parameter of 2 % sulphuric acid concentration reacted for 30 min on 60 mesh of OPT powders at 115 °C with a total xylose yield of approximately 32.4 % by continuous acid hydrolysis. Value $P \leq 0.01$ significance was observed for the main effects (acid concentration, temperature and time). By RSM analysis, optimum glucose and xylose can be obtained by conducting continuous hydrolysis temperature 130 °C for 53 min with 2 % acid concentration. Conclusively, the total yield of glucose-xylose was improved and approximately range of 2.5 % - 28.8 % higher than total glucose-xylose yield from untreated OPT powder.

Co-fermentation of glucose and xylose to bioethanol was carried out using engineered strains of *Saccharomyces cerevisiae* 424A(LNH-ST) and *E.coli* strain B, respectively. There was a preferential order of sugar utilization: first using glucose followed by xylose. Generally, the optimum ethanol yield of 85.41 % using yeast as fermentation microbes was obtained at temperature 30 °C, pH 4. While, the optimum ethanol yield obtained from *E.coli* strain B was 86.01 % at temperature 34 °C, pH 6. Value $P \leq 0.0001$ significance was observed for the main effects (pH and temperature). From the optimization analysis, 87.9 % of ethanol conversion yield can be obtained by conducting the fermentation at temperature 34 °C and pH 6 by *E.coli* strain B; whereas 82.1 % of ethanol conversion yield can be obtained at 30 °C and pH 4 by *S.cerevisiae 424A(LNH-ST).*

Generally, Oil palm trunk contains considerable amount of extractives and lower lignin which suitable for starch and sugars extraction especially glucose and xylose and potentially be one of the biomass resource energy in bioethanol production.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

PENGEKSTRASI KANJI, GLUKOSA DAN XILOSA DARIPADA BATANG KELAPA SAWIT BAGI PENGELUARAN BIOETANOL

Oleh

WONG LIH JIUN

September 2019

Pengerusi : H'ng Paik San, PhD
Fakulti : Perhutanan dan Alam Sekitar

Kajian intensif atas penggunaan batang kelapa sawit sebagai alternatif sumber biomas untuk bahan kimia dan sumber pengeluaran tenaga. Oleh itu, idea pembangunan ini dapat menyelasai isu-isu alam sekitar dan mengurangkan sisa pertanian. Kerumitan komposisi kimia dalam lignocelluloses sebagai satu faktor penghalang degradasi lignin, hemicelluloses dan selulosa yang mengandungi kanji, lima dan enam karbon gula; sebelum terhidrolisis dan diikuti oleh rakan penapaian untuk pengeluaran bioetanol. Oleh itu, tujuan kajian ini untuk mendapat parameter pemprosesan optimum untuk kanji, campuran xylosa - glukosa yang diekstrak daripada batang kelapa sawit serta penapaian campuran gula untuk penghasilan bioetanol. Objektif umum kajian ini adalah untuk mendapat parameter optimum bagi penghasilan jumlah hasil kandungan kanji, glukosa dan xylosa; serta fermentasi proses.

Analisis komposisi kimia batang kelapa sawit dalam bentuk bentuk serbuk yang bersaiz 60 mesh siri analisis kimia dijalankan berdasarkan standard TAPPI (2000) untuk mendapatkan kandungan lignin, selulosa dan hemiselulosa dalam batang kelapa sawit. Nilai-nilai ini penting untuk pengiraan jumlah kandungan glukosa dan xylosa yang diekstrak dalam eksperimen ini. Keputusan menunjukkan kandungan lignin yang rendah , hemiselulosa dan selulosa didapati. Ini menunjukkan batang kelapa sawit mengandungi ekstrasi kimia yang sesuai untuk dijadikan bahan gula fermentasi.

Bagi kajian kedua iaitu pengesekstrasi kanji, serbuk batang kelapa sawit direndam dengan kepekatan 0.2 % metabisulfit natrium ($Na_2S_2O_5$), 0.5% laktat asid ($C_3H_6O_3$) dan air suling pada suhu-suhu berlainan dan masa yang berbeza. Nilai $P \leq 0.01$ dipadati *significant* bagi tiga parameter (suhu dan masa). 19.7 % kanji didapatkan melalui air suling pada 50 °C, 1 h, manakala 14.0 % kanji didapatkan melalui kimia pada 26 ± 2 °C, 1 h). Daripada analysis, 12.8 %

optimum kanji didapatkan melalui bahan kimia dengan parameter suhu bilik 26 ± 2 °C dan 1 jam. Di samping itu, 16.3 % optimum kanji didapatkan melalui air suling pada dengan parameter suhu bilik 50 ºC dan 1 jam. Keputusan ini menunjukkan bahawa optimum kanji boleh didapatkan pada suhu yang lebih rendah jika serbuk batang kelapa sawit direndamkan dengan bahan kimia.

Hidrolisis berterusan and hidrolisis serentak (115 ºC, 120 ºC dan 130 ºC dengan 15 min, 30 min dan 60 min) digunakan untuk hidrolisis xilosa dan glukosa. 2 %, 4 % dan 6 % asid sulfurik digunakan bagi proses hidrolisis. Daripada analisis RSM, optimum parameter bagi penghasilan xylosa-glukosa ialah pada suhu 130 °C, 53 min, 2 % asid sulfurik. Daripada analisis, optimum parameter ialah pada suhu bilik 26 ± 2 ºC dan 1 jam daripada bahan kimia. Parameter ini dipilihkan sebagai parameter pra-rawatan hidrolisis berterusan. Kanji yang diekstrasi dicampurkan dengan serbuk kelapa sawit untuk hidrolisis gula-gula yang seterusnya. Bagi hidrolisis serentak, serbuk kepala sawit yang belum direndamkan dengan bahan kimia digunakan untuk menghasilkan xylosa dan glukosa. 15.3 % glukosa yang optimum boleh didapatkan melalui proses hidrolisis serentak pada suhu 100 °C, 6 % asid sulfurik dan 60 min. 32.4 % xylosa yang optimum boleh didapatkan melalui proses hidrolisis berterusan pada suhu 115 °C, 2 % asid sulfurik dan 300 min. Nilai P≤0.01 dipadati *significant* bagi tiga parameter (kepekatan asid, masa dan suhu).Daripada analisis RSM, optimum glukosa dan xylosa boleh dihasikan daripada hidrolisis berterusan pada suhu 130 ºC, 53 min dan 2 % asid sulfurik. Kesimpulannya, terdapat penambahan jumlah hasil glukosa dan xylosa sebanyak 2.5 % - 28.8 % daripada jumlah hasil glukosa dan xylose yang dihasilkan daripada batang kelapa sawit yang belum direndamkan bahan kimia.

Proses fermentasi glukosa dan xylosa untuk bioetanol dilaksanakan dengan menggunakan rekombinan strain *Saccharomyces cerevisae 424A(LNH-ST) dan E.coli* strain B masing-masing. Optimum 85.41 % ethanol dihasilkan pada suhu 30 °C, pH 4 dengan menggunakan yeast manakala optimum 86.01 % ethanol dihasilkan pada suhu 34 °C, pH 6 dengan menggunakan *E.coli.* Nilai P≤0.0001 dipadati *significant* bagi dua parameter (pH dan suhu). Daripada analisis, 87.9 % hasil etanol boleh diperolehi dengan suhu penapaian 34 °C dan pH 6 oleh *E.coli*; manakala 82.1 % hasil etanol boleh diperolehi dengan suhu penapaian 30 °C dan pH 4 daripada fermentasi oleh *S.cerevisae 424A(LNH-ST).*

Kesimpulanya, batang kelapa sawit mengandungi bahan ekstrasi dan lignin yang rendah jadi ia sesuai untuk proses ekstrasi kanji dan gula terutamanya glukosa dan xylosa serta berbakat sebagai salah satu sumber biomas untuk pengeluaran tenaga

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I certify that a Thesis Examination Committee has met on (date of viva voce) to conduct the final examination of Wong Lih Jiun on her thesis entitled "Extraction of Starch, Xylose And Glucose From Oil Palm Trunk for Bioethanol Production" in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the (insert the name of relevant degree).

Members of the Thesis Examination Committee were as follows:

Edi Suhaimi Bakar, PhD
Associate Professor
Faculty of Forestry and Environment
Universiti Putra Malaysia
(Chairman)

Rosfarizan binti Mohamad, PhD
Professor
Faculty of Biotechnology and Bimolecular Science
Universiti Putra Malaysia
(Internal Examiner)

Rasmina binti Halis, PhD
Associate Professor
Faculty of Forestry and Environment
Universiti Putra Malaysia
(Internal Examiner)

Wasrin Syafii, PhD
Professor Ir.
Faculty of Forestry
Bogor Agricultural University
Indonesia
(External Examiner)

ZURIATI AHMAD ZUKARNAIN, PhD
Professor Ts. and Deputy Dean
School of Graduate Studies
Universiti Putra Malaysia

Date: 1 November 2021

This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Doctor of Philosophy. The members of the Supervisory Committee were as follows:

H'ng Paik San, PhD
Associate Professor
Faculty of Forestry and Environment
Universiti Putra Malaysia
(Chairman)

Luqman Chuah Abdullah, PhD
Professor
Faculty of Engineering
Universiti Putra Malaysia
(Member)

Paridah binti Md Tahir, PhD
Professor
Institute of Tropical Forestry and Forest Product
Universiti Putra Malaysia
(Member)

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LIST OF ABBREVIATIONS

Am:Ap	Amylose:Amylopectin ratio
ANOVA	Analysis of variance
CZ	Central zone
EFB	Empty fruit bunches
GOS	Galactoologosaccharides
ha	hectares
HMF	5-hydroxymethly-furfural
HPLC	High Performance Liquid Chromatography
h	Hour
IZ	Inner zone
LSD	Least Significant Difference
Min	Minute
Mpa	Pressure unit in SI system
OPF	Oil palm fronds
OPT	Oil palm trunk
Pm	Maximum ethanol concentration rate
POME	Palm shells and palm oil mill effluent
PPF	Palm pressed fibres
PZ	Peripheral zone
RSM	Response surface methodology
SEM	Scanning Electron Microscope
SY	Starch yield
w/w	Weight/weight
w/v	Weigh/volume

CHAPTER 1

INTRODUCTION

1.1 General

With the inevitable depletion of the world's energy supply, there has been an increasing worldwide interest in alternative sources of energy (Chin et al., 2010; Zaldivar et al., 2001; Aristidou & Penttila, 2000; Jeffries & Jin, 2000; Wheals et al., 1999; Kerr, 1998). The United States is looking to wean itself off of fossil fuels, especially petroleum used in gasoline. This may due to the factors such as global climate change, dependence on other countries, and increased of world political instability are only the reasons why. One possible way to decrease dependence on petroleum as a transportation fuel is by using more ethanol. Currently, many countries regulated the policy to have gasoline contains approximately 10% ethanol as an additive. Special flex-fuel vehicles in Brazil can use E85 gasoline, which is 85% ethanol (Chris, 2019).

In Brazil, ethanol is made by using sugarcane as the feedstock. The main product of sugarcane is sucrose, which is a disaccharide of glucose and fructose. Most species of yeast have the enzyme sucrase and are able to cleave the glucose – fructose bond. However, these raw materials are also sources of food for humans. Utilizing plants, wood, wood waste, paper waste, and food waste as raw materials for bioethanol production is extremely effective and environmentally friendly. These kinds of biomass are the most abundant raw materials on earth and are a potential carbon-neutral source for alternative natural resources in place of petroleum.

Oil Palm is cultivated in 43 countries throughout the World and the plantation currently cover more than 27 million hectares of the Earth's surface (The Oil Palm, 2017). Oil palm trunk (OPT) is considered as one of the lignocellulosic biomass waste due to the replanting season; it is an abundantly renewable resource on the Earth and typically nonedible plant materials for the production of biofuel, chemicals and polymers (Abdullah & Sulaiman, 2013). Oil palm trunk generated from replanting activity is left over at the plantation site or burned after replanting activity. There are some usages of the trunk for veneer and plywood production. Nonetheless, the utilization of OPT in plywood production is not so economic due to great variations in physical and mechanical properties which caused many difficulties during working and application (Dungani et al., 2013). Therefore, planters still preferred the trunks to be left over in the field to decompose and provide nutrient for the next replanting tree. However, this technique sometime takes several years. Furthermore, it has been reported that trunks are easily attacked by pests and can cause diseases. While burning of OPT can lead to environmental issues such as global warming. The management of these huge amounts of oil palm solid waste has become a problem for Malaysia, and other countries that also produce palm oil such as Indonesia.

With the expected large volume of oil palm trunk available annually due to replanting, the task of finding ways to utilize this enormous amount of lignocellulosic biomass is great. Many researchers have reported on the bioconversion of oil palm trunk into sugar production especially fermentable sugars and starch (Tay et al., 2013; Chin et al., 2010). Whereby, this method will benefit the environment by reducing the agricultural wastes, meanwhile increases the economic return for the country (Chin et al., 2010).

These kinds of biomass are mainly composed of cellulose, hemicellulose, and lignin, with cellulose being used as the main raw material for bioethanol production. Cellulose is a linear homopolymer of glucose which is rigid and requires special treatment for breakdown. Hemicellulose is a polymer of five different sugars. It consists of two, five-carbon sugars (d-xylose and l-arabinose) and three, six carbon sugars (d-galactose, d-glucose, and d-mannase) (Naraian, & Gautam, 2018) which they are the second most abundant type of polysaccharide in nature (Saha, 2003). Xylose, can be converted in to bioethanol as well (Rodrussamee et al., 2018; Toivari et al., 2001). Oil palm trunk have advantages compare to other plant based materials, oil palm trunk contain starch. Sulaiman et al. (2012) found 29.36% of starch in the trunk which the highest content of all parts of tree. These fermentable sugars can be converted into bioethanol (Wangpor et al., 2017; Okamoto et al., 2014; Prawitwong et al., 2012).

Considerable interest in cellulosic ethanol exists due to its important economical potential. The polymers present in a collective matrix which is lignocelluloses; mainly comprised of cellulose, hemicelluloses, and lignin (Harmsen et al., 2010). In addition, fermentable sugars refer to glucose and xyloses are mainly used for production of bioethanol for energy consumption (Liguori et al., 2016). The fermentable sugars can be converted from OPT through acid hydrolysis process which is a potential source of fermentable sugar. After the sugars extractions process, the resulting sugars (glucose and xylose) can be converted to bioethanol through co-fermentation by engineered strain of *Saccharomyces cerevisiae 424A(LNH-ST)* (Casey et al., 2010) and *E.coli* strain B (Munjal et al., 2012) which play a role as biocatalysts.

1.2 Statement of Problem

As mentioned early, the oil palm exhibits substantial amount of starch and fermentable sugars, however, there is lack of effective technology in extracting total available starch and fermentable sugars from OPT and convert into bioethanol; especially the starch content in trunk. The uneven distribution of vascular bundle and parenchyma tissues along the trunk and the starch content is highly depends on the rate of photosynthesis process of a plant (Omar et al., 2011).

Oil palm belongs to monocotyledon and its trunks consist of vascular bundles and parenchymas. Oil palm trunks have such special characteristics as high moisture content (1.5 to 2.5 times the weight of the dry matter), low cellulose and lignin content and high content of water solubles and NaOH solubles (Tomimura, 1992). In order to maximize the production of fermentable sugar, pre-treatment of OPT is needed under controlled condition. Pre-treatment is needed to improve the digestibility of lignocellulose materials. It is hard to define which pre-treatment method is the best as it depends on several factors such as type of the lignocellulosic, the reaction factors on lignocelluloses, and the desired of products. Each of the pre-treatment method has its own effects on hemicelluloses, cellulose and lignin fractions. As for industrial practice, most commonly use pre-treatment method is acid-based and this pre-treatment normally used for the production of ethanol from biomass.

Moreover, it is very little known on how the starch extraction parameters affect the OPT hydrolysates and co-fermentation process of glucose-xylose sugars to form bioethanol at the final stage. Pre-treatment and hydrolysis are the crucial steps to determine total extractable fermentable sugars yield from OPT. In addition, starch is unable to be converted into ethanol directly. It needs to be hydrolyzed into fermentable monomer sugar first before the process of co-fermentation. The additional of starch extracted through the pre-treatment process is aims to increase the fermentable sugars yield from oil palm trunk through acid hydrolysis so that optimized the bioethanol yield from OPT.

During the fermentation process, several phases of cell growth can be observed including lag phase, acceleration phase, exponential phase, stationary and death phase (Norhazimah & Faizal, 2014b). A series of experimental rate, including specific cell mass growth rate, specific sugar consumption rate, specific ethanol production rate, to monitor the sugar consumption and ethanol production. As different strains ferment sugars at different rates depending on the process conditions especially temperature and pH, there is a need to use different temperatures and pH when making a selection (Norhazimah & Faizal, 2014a).

1.3 Justification

As mentioned early, the oil palm trunk contains substantial amount of starch due to its natural characteristics. Extraction of starch, glucose, xylose for the conversion into bioethanol involved several steps. There are many factors that will affect the total starch, xylose and glucose yield, and bioethanol from OPT and the extraction methods.

The pre-treatment employed in this study was functioned as starch extraction as well as preparation of oil palm trunk prior to the hydrolysis process. Chemical steeping method is a method for the production of starch by soaking the materials in dilute sulphur dioxide or bisulfate solution at certain time until saturated with soluble ingredient. The sulphur dioxide will disrupt the protein matrix that surrounds starch granules by breaking inter and intra molecular disulfide bonds, thus making the physical separation of starch easier (Pérez et al., 2001). Nonetheless, the yield of starch depends on the condition during chemical steeping process and this includes incubation time. Whereas, substantial time was required for the chemical reaction to take place so that starch can be released from the matrix during the chemical steeping. Industrial steeping times may range from 36-60 hrs (Watson et al., 1995). In most laboratory milling procedures, a steeping time of 48-50 hrs is frequently used (Eckhoff & Tso, 1991). Therefore, the effect of pre-treatment using chemical steeping process on the starch yield from OPT was evaluated.

Since the chemical steeping using lactic acid and sodium metabisulfite as medium for extraction of starch, there is possible of mild hydrolysis occur on the hemicellulose and cellulose of the OPT. This may remove some of lignin and make the hemicelluloses to be more easily hydrolyzed into xylose and glucose. During the past few years, pre-treatment uses various techniques, including physical treatment, chemical treatment, physicochemical treatment and biological treatment, to alter the structure of lignocellulosic more accessible to enhance the yield of xylose and glucose (Kumar & Sharma, 2017). Therefore, the interaction of present pretreatment on hydrolysis efficiency was evaluated.

In order to produce glucose and xylose, dilute acid hydrolysis should be carried out in fractionation of lignocellulose. On other hand, the acid hydrolysis depends mainly on operational conditions, which is also known as hydrolysis parameters and the properties of wood. The properties are proportion of easily hydrolysable hemicelluloses and cellulose, amount and rate of hydrolysis of the difficult-to-hydrolyze materials, the length of the macromolecules, degree of polymerization of cellulose, configuration of cellulose chain and association of cellulose with other protective polymeric structures within the plant cell wall such as lignin, pectin, hemicelluloses, proteins, minerals elements, etc. (Taherzadeh & Karimi, 2008). According to previous study, acid concentration is an important parameter for release of sugars (Neureiter et al., 2002). In acid hydrolysis of lignocelluloses material, it is believed that the mechanisms are leading to a scission of glycosidic bonds, which initially catalyzed by the action of proton (H^+) existing in the aqueous medium (Fengel & Wegener, 1984). Therefore, the higher the concentration of H+, the more glycosidic bonds will be broken and the more xylose will be produced from amorphous structure of hemicelluloses and glucose from cellulose. However, the extremely high concentration of acid may lead to the decomposition of xylose to its by-products such as furfural, acetic acid and so on. Hence, the suitable range of acid concentration was determined in this study in order to reduce the formation of decomposed products.

In recent, introduction of fermentation process on OPT has been started involved in research to optimise their utilisation. Derived fermentable sugars from OPT mainly glucose and xylose are the main sugars to converted into bioethanol. Fermentation is a critical steps in producing bioethanol as it dependent mainly on the processing condition; the fermentation parameters. The optimal fermentation parameters; temperature and pH contribute to high fermentation efficiency (Lin et al., 2012). Temperature plays a major role in the fermentation process. It is one of the important parameters that can affect the ethanol yield because it has an impact on the *Saccharomyces sp.* and *E.coli* growth rate and activity used in fermentation (Azhar et al., 2017). Yeasts and bacteria growth rate and metabolism increase as the temperature increases until it reaches the optimum value. Increase in ethanol concentration during fermentation can cause inhibition to microorganism growth and viability (Alexandre & Charpentier, 1998; Attfield, 1997). Continuous hydrolysis combined with co-fermentation of glucose-xylose at the same time to convert bioethanol using yeast and bacterium respectively under controlled reaction parameters. In this study, co-fermentation of glucose-xylose mixtures from OPT was studied using *Saccharomyces cerevisiae* 424A(LNH-ST) and *E.coli*. B strain.

In this study, the starch in OPT was extracted using steeping method; whereas, xylose and glucose from OPT were extracted using dilute acid hydrolysis. In between, extracted starch is added into hydrolysate together with OPT powders for sugar production by dilute acid hydrolysis. The starch extraction process is treated as pre-treatment of lignocelluloses in this study. Next, the optimized glucose-xylose mixture sugar hydrolysate was continued for bioethanol co-fermentation process by *Saccharomyces cerevisiae* 424A(LNH-ST) and *E.coli*. *B strain.*

1.4 Objectives of Study

The general objective of this study is to produce bioethanol from the fermentable sugars extracted from oil palm trunk.

The specific objectives of this study are:

1. To analyze the chemical compositions of OPT.

2. To analyze the effect of chemical steeping on the starch content extracted from OPT.

3. To evaluate the effect acid hydrolysis on the yield of glucose and xylose extracted from OPT.

4. To analyze the effect of co-fermentation parameters on ethanol yield from mixed fermentable sugars substrate using engineered yeast strain *Saccharomyces sp.*, and *Escherichia coli B strain.*

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BIODATA OF STUDENT

Wong Lih Jiun is born on 27th August, 1986 in Teluk Intan, Perak. She completed her primary (UPSR) at S.R.J.K.C San Min (1), secondary school (PMR, SPM) at S.M.J.K.C San Min and STPM at S.M.J.K. Horley Methodist from 1993-2005. She entered University Putra Malaysia in 2006 and graduated with the degree of Bachelor of Forestry Science in 2009. She entered the School of Graduate Studies at University Putra Malaysia in July 2009 to pursue her Master's degree in Faculty of Forestry and graduated with the Master of Science in 2012. Her research areas are mainly in wood science and technology. She then furthers her study at the same university as a full time Doctor of Philosophy (Ph.D.) student in faculty of forestry in year 2013. In year 2014, she has successfully passed her comprehensive examination as a PhD student. Her research areas are mainly in hydrolysis process of lignocellulosic biomass and process of co-fermentation of polysaccharides.

LIST OF PUBLICATIONS

Wong, L. J., H'ng P. S., Abdullah, L. C., Paridah, M. T., Chin, K. L. (2022). Effect of chemical steeping on yields of glucose and xylose from dilute acid hydrolysis of extract from oil palm trunk. *BioResources*, *17*(1), 207-222.

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This study focused to optimize the extraction of starch, xylose and glucose from OPT for bioethanol production. Chemical analaysis showed that OPT contained low lignin and hemicelluloses and cellulose were found in this batch of study hence OPT contains considerable extractives content which is valuable be source of chemical value added products like fermentable sugars. Starch in the OPT powder was extracted by soaking with 0.2 % sodium metabsisulphite (w/v) and 0.5 % lactic acid (v/v), and distilled water respectively under levels of temperatures and times. Value P≤0.01 significance was observed for the main effects on the starch yield with water solution and chemicals. 19.7 % of starch yield was obtained by distilled water at 50 °C, 1 h, whereas 14.0 % of starch was obtained by chemicals at 26 ± 2 °C, 1 h). RSM optimization analysis showed the optimum starch yield can be obtained in lower temperature when OPT powder treated with chemicals solution than water. Glucose and xylose were hydrolyzed by continuous and simultaneous dilute acid hydrolysis process. Optimum parameter of starch extraction method parameter (0.2 % sodium metabsisulphite (w/v) and 0.5 % lactic acid (v/v), 26 ± 2 ˚C and 1 h) was chosen as a pretreatment for continuous acid hydrolysis. Value P≤0.01 significance was observed for the effects (acid concentration, temperature and time). The higher glucose yield occurred at dilute acid hydrolysis with the parameter of 6 % sulphuric acid concentration reacted for 60 min on 60 mesh of OPT powders at 100 °C with a total glucose yield of approximately 15.3 % by simultaneous hydrolysis. The optimum xylose yield occurred with the parameter of 2 % sulphuric acid concentration reacted for 30 min on 60 mesh of OPT powders at 115 °C with a total xylose yield of approximately 32.4 % by continuous acid hydrolysis. RSM analysis showed that the optimum glucose and xylose can be obtained by conducting continuous hydrolysis temperature 130 °C for 53 min with 2 % acid concentration. The total yield of glucose-xylose was improved and approximately range of 2.5 % - 28.8 % higher than total glucose-xylose yield from untreated OPT powder. Co-fermentation of glucose and xylose to bioethanol was carried out using engineered strains of *Saccharomyces cerevisiae* 424A(LNH-ST) and *E.coli* strain B, respectively. The optimum ethanol yield of 85.41 % using yeast as fermentation microbes was obtained at temperature 30 °C, pH 4. While, the optimum ethanol yield obtained from *E.coli* strain B was 86.01 % at temperature 34 °C, pH 6. Value P≤0.0001 significance was observed for the main effects (pH and temperature). From the optimization analysis, 87.9 % of ethanol conversion yield can be obtained by conducting the fermentation at temperature 34 °C and pH 6 by *E.coli* strain B; whereas 82.1 % of ethanol conversion yield can be obtained at 30 °C and pH 4 by *S.cerevisiae 424A(LNH-ST).* In conclusion, OPT contains considerable amount of extractives and lower lignin which suitable for starch and sugars extraction especially glucose and xylose and potentially be one of the biomass resource energy in bioethanol production.