UNIVERSITI PUTRA MALAYSIA

PRODUCTION OF XYLOOLIGOSACCHARIDES AND FERMENTABLE SUGARS FROM OIL PALM MESOCARP FIBER BY SUBCRITICAL H2O-CO2 PRETREATMENT

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By

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Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia,in Fulfilment of the Requirement for the Degree of Master of Science

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Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Master of Science

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Oil palm mesocarp fiber (OPMF) generated from oil palm processing is available abundantly and improper handling of OPMF has lead to serious pollution. High content of carbohydrate polymer in OPMF serves as feedstock for fermentable sugars production with appropriate pretreatments. The present works aimed to investigate xylooligosaccharide (XOs) and fermentable sugar production from OPMF in subcritical H_2O-CO_2 pretreatment at a temperature range of 150- 200°C and 20-60 min with pressure vary from 3-5 MPa. The pretreated solids and liquids from this process were separated by filtration and characterized. XOs, monomeric sugars, acids, furans and phenols in pretreated liquids were analyzed by using HPLC. XOs with a degree of polymerization DP X2-X4 which is xylobiose, xylotriose, xylotetraose were analysed by using HPAEC-PAD. Enzymatic hydrolysis was performed on cellulose-rich pretreated solids to study the xylose and glucose production. Morphological changes of pretreated solids were analyzed by SEM, WAXD and BET surface area. An optimal condition for XOs production was obtained at 180°C, 60 min, 3 MPa with a concentration of 81.60 mg/g which corresponded to 36.59% of XOs yield from xylan. On the other hand, the highest xylose and glucose yields obtained from conversion of hemicellulose and cellulose were 29.96% and 84.65%, respectively at cellulase loadings of 10 FPU/g-substrate. The results obtained clearly indicated that OPMF was a potential material for XOs and fermentable sugar production using subcritical H_2O-CO_2 method.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk Ijazah Master Sains

PENGHASILAN XYLOOLIGOSAKARIDA DAN GULA YANG DIHASILKAN SECARA BIOLOGIKAL DARIPADA MESOKAP KELAPA SAWIT MELALUI RAWATAN SUBKRITIKAL AIR-KARBON DIOKSIDA

Oleh

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Sisa OPMF terhasil daripada industri kelapa sawit dan ia boleh didapati sepanjang tahun. Kandungan karbohidrat polimer yang banyak yang terdapat di dalam sisa OPMF digunakan sebagai bekalan untuk menghasilkan gula dengan rawatan yang tertentu. Kajian yang sedang dijalankan ini bertujuan untuk mengkaji pengeluaran oligosakarida (XOs) dan gula daripada OPMF melalui rawatan subkritikal air-karbon dioksida pada suhu dalam lingkungan 150-200°C selama 20-60 minit dengan tekanan berbeza daripada 3-5 MPa. Pepejal dan cecair yang telah melalui rawatan awal daripada proses ini telah diasingkan dengan kaedah penapisan dan seterusnya diperincikan. XOs, gula monomer, asid, furan dan fenol yang terkandung di dalam cecair yang telah melalui rawatan awal kemudiannya dianalisa dengan menggunakan HPLC. XOs dengan darjah pempolimeran DP X2-X4 iaitu xilobiosa, xilotriosa, xilotatrosa dianalisa dengan menggunakan HPAEC-PAD. Hidrolisis enzimatik telah dilakukan ke atas pepejal yang telah melalui rawatan awal yang kaya dengan selulosa, untuk mengkaji penghasilan xilosa dan glukosa. Perubahan morfologi pepejal yang telah melalui rawatan awal dianalisa dengan menggunakan SEM, WAXD dan kawasan permukaan BET. Keadaan optima untuk penghasilan XOs telah diperolehi pada takat suhu 180°C, 60 minit, 3 MPa dengan kepekatan sebanyak 81.60 mg/g, yang bersamaan dengan 36.59% hasil XOs daripada xilan. Sebaliknya, hasil xilosa dan glukosa tertinggi yang diperoleh daripada proses penukaran hemiselulosa dan selulosa adalah masing-masing sebanyak 29.96% dan 84.65% pada pemuatan celulase bersamaan 10 FPU/g-substrat. Hasil eksperimen jelas menunjukkan XOs dan gula berpotensi dihasilkan daripada OPMF menggunakan rawatan subkritikal air-karbon dioksida.

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

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LIST OF ABBREVIATIONS

5-HMF	5 hydromethylfurfural
BET	Brunauer-Emmett-Teller
CO_2	Carbon dioxide
$CSpco_2$	Combined severity partial carbon dioxide
DP	Degree of polymerization
FELCRA	Federal Land Consolidation and Rehabilitation Authority
FELDA	Federal Land Development Authority
FPU	Filter paper unit
H_2O	Water
HCW	Hot compressed water
HPAED-PAD	High-performance anion exchange chromatography detection system
HPLC	High performance liquid chromatography
kV	Kilo volt
mL	Mililiter
NaAC	Sodium acetate
NaOH	Sodium hydroxide
NDO	Non-digestible oligosaccharide
nm	Nanometer
OPEFB	Oil palm empty fruit bunch
OPFF	Oil palm frond fiber
OPT	Oil palm trunk
Pt-pd	Platinum-palladium
PV	Pore volume
RISDA	Rubber Industry Smallholders Development Authority
SEM	Scanning electron microscope
SSA	Specific surface area
t	Time
T	Temperature
UPM	Universiti Putra Malaysia
UV-Vis	Ultraviolet-visible spectroscopy
WAXD	Wide angle x-ray diffraction
wt	Weight
X2	Xylobiose
X3	Xylotriose
X4	Xylotetraose
X5	Xylopentose
XOs	Xylooligosaccharides

CHAPTER 1

INTRODUCTION

For the past few years, abundant of lignocellulosic biomass materials in the world were produced daily from many industries (Relvas et al., 2015). Lignocellulosic biomass can be found from many resources including oil palm industry. Currently, Malaysia has become the biggest oil palm producer. In the year 2011 alone, 4.92 million hectares of oil palm trees were planted in Malaysia. In the year 2016, 19.3 million tons of palm oil was produced in Malaysia (Abdullah & Sulaiman, 2013). The increasing demand for oil palm has resulted in many biomass generated such as oil palm empty fruit bunch (OPEFB), oil palm mesocarp fibre (OPMF), shell and oil palm frond fibre (OPFF) (Abdullah & Sulaiman, 2013). There are about 4.94 Mt/y of shell, 18.13 Mt/y OPEFB, 10.88 Mt/y OPFF and 10.9 Mt/y OPMF were produced. These biomass produced are looking for the way out to be managed in a proper way. The current practice of managing these biomass raising awareness among the society. For example, the burning of OPMF using incineration lead to air pollution and dumping of OPMF lead to water pollution. To this extend, each of the biomass are potential and can be further converted to many useful products. For example, OPMF are one of the potential and attractive biomass that can be used as a substrate to produce many bioproducts such as sugar, biogas, activated carbon and biocompost, which can be further used in various industries (Mustapa et al., 2011).

Generally, OPMF consists of cellulose (23-29%), hemicellulose (21-34%), lignin (21-32%), extractives and ash (Zakaria et al., 2015; Saidu et al., 2014; Mustapa et al., 2011; Iberahim et al., 2013). The high content of carbohydrate polymer in OPMF, which are cellulose and hemicellulose, make it a potential and preferable material to be exploited for the xylooligosacharide and fermentable sugar production. The advantages of using this local biomass as substrate are due to abundant availability throughout the year, low cost and solution to biomass disposal problems. OPMF needs to undergo pretreatment process to exploit its potential products such as XOs and fermentable sugars. From previous study, many experiments were performed on OPMF to study the preferable and more effective method for the pretreatment process of OPMF.

Some pretreatment process that have been done on OPMF are alkaline pretreatment and ball milling process to produce fermentable sugar (Zakaria et al., 2014b). There are also other method on OPMF pretreatment such as using sodium hydroxide and biological pretreatment (Iberahim et al., 2015). However, all these study are using chemical that are harmful to the environment.

In this study, a greener approach are being study that uses only steam and CO_2 as catalyst in hydrothermal pretreatment. Recently, the addition of CO_2 in hydrothermal pretreatment becomes attractive technology since it offers more benefits whereby less undesired by-product compounds produced and non-toxic gases applied (Benazzi et al., 2013). The reaction of high-pressure CO_2 and water has proven to help in the hemicellulose dissolution in acidic condition. The reaction of CO_2 and water is shown below in equation/ chemical formula (1). The addition of CO_2 will form carbonic acid (H_3CO^+) when reacted with water under subcritical condition (Agbor et al., 2011).

$$CO_2 + H_2O \rightarrow HCO_3^- + H_3CO^+;\ HCO_3^- + H_2O \rightarrow CO_3^{2-} + H_3O^+ \qquad (1)$$

The carbonic acid produced from the reaction facilitated the hydrolysis of hemicellulose and cellulose in biomass. The addition of CO_2 in hydrothermal pretreatment produced carbonic acid that offers the benefits of acid catalysis without any negative impact to the environment as the gas will be neutralized when the pressure released (van Walsum et al., 2007). High-pressure CO_2 is weak acid helps in penetration of small pores in biomass and help in disrupting the biomass structure. Thus, improve the hydrolysis rate of hemicellulose in the biomass (Silva et al., 2014). This pretreatment are known as physicochemical pretreatment where it combines the mechanism of physical and chemical effect during the process.

This process is also refer as subcritical CO_2-H_2O pretreatment. Subcritical condition are refers to liquid water at temperatures between the atmospheric boiling point and the critical temperature (374ºC) of water. The maximum pressure for subcritical condition is 22 MPa. In this study the temperature used are in the range 150-200ºC and pressure below 22 MPa. Thus pretreatment reaction in this study are best refer as subcritical CO_2-H_2O pretreatment.

To measure the intensity reaction of subcritical CO_2-H_2O pretreatment , combined severity factor (CS_{PCO2}) is used to evaluate the influence of temperature, time and high-pressure CO_2 on the hydrolysis of xylan (van Walsum, 2001). The relation between severity factor and subcritical CO_2-H_2O pretreatment process (such as xylan hydrolysis, cellulose digestibility) can be found and used to evaluate the pretreatment severity to predict the efficiency of the pretreatment process (Pedersen and Meyer, 2010).

Subcritical CO_2-H_2O pretreatment is one of the preferable pretreatment methods as it uses a greener approach such as compressed hot water with various temperature and time to hydrolyze the hemicellulose. XOs are products from hydrolysis of hemicellulose in lignocellulosic biomass. XOs are sugar oligomers of xylose from the hydrolysis process of xylan in hemicellulose of lignocellulosic biomass. It is made up of β-1,4 bonds that mainly consist of xylobiose, xylotriose, xylotetraose and xylopentose. As hot water contacts with the xylan-rich substrate, it will result in depolymerization of xylan into a shorter form such as xylose and XOs (Garrote et al., 2002). The liquid from this process contains soluble products from hemicellulose degradation such as xylooligosaccharides (XOs), monomeric sugars, acids, furans and aldehydes (Silva et al., 2014).This shows that hydrolysis of hemicellulose in OPMF using subcritical CO_2-H_2O pretreatment have produced xylose monomer and xylooligosaccharides (XOs) in the pretreated liquid (Zakaria et al., 2016). A liquid sample of wheat straw from autohydrolysis process with CO_2 assisted has proven to contain xylooligomer that can be used further.

The structure, degree of polymerization and yield of XOs depend on the types of biomass and the methods used in the production stage. The molecular weight of XOs is affected by the pretreatment severity during pretreatment process (Sabiha-Hanim et al., 2011). XOs can be obtained abundantly in hydrolyzates liquid, therefore it also contains other undesired by-products such as acetic acid, furfural, 5-HMF and tannic acid (Zakaria et al., 2016). Different types of XOs can be produced from xylans such as xylobiose (X2), xylotriose (X3), xylotetraose (X4) and xylopentaose (X5) depending on the pretreatment used (Bragatto et al., 2013).

XOs have novel applications in many industries such as food, pharmaceutical, and health industries. It was reported that the production of XOs was obtained from bamboo shoot, almond shells, corn cob (Garrote & Parajó, 2002), cotton stalk, tobacco stalk, sunflower stalk, wheat straw (Akpinar et al., 2009a), mushroom cultivation waste (Sato et al., 2010), wheat bran, sugarcane bagasse (Bian et al., 2013), OPFF (Sabiha-Hanim et al.,2011) and OPEFB (Ho et al., 2014). XOs with a shorter degree of polymerization range DP X1-X6 obtained are beneficial and advantageous to function as prebiotics in food related products (Reddy & Krishnan, 2016). XOs are a potential compound that can behave as prebiotics when ingested as it can stimulate good bacteria inside the colon (Moure et al., 2006). Xylobiose has been found to be an important oligosaccharide in the food industry. Xylobiose was found to have 30% sweetness of sucrose, while other XOs exhibited less sweetness. This caused xylobiose to be the main target in the food related product (Vázquez et al., 2000).

On top of that, fermentable sugar can also be produced from subcritical CO_2-H_2O pretreatment process. Fermentable sugar is sugar monomer with empirical formula ($C_6H_{12}O_6$) from the hydrolysis process of glycan in cellulose. It consist of C6 sugars (glucose, mannose, galactose) and C5 sugars (xylose and arabinose). The removal of hemicellulose makes the cellulose more accessible to enzymatic attack for fermentable sugar production. The saccharification or enzymatic hydrolysis of cellulose constituents from OPMF after pretreatment can produce fermentable sugar or known as glucose. Many different types of pretreatments are performed to disrupt the hemicellulose structure and give maximum access of enzyme on cellulose. Higher treatment severity with higher carbon dioxide pressure will resulted to higher removal of hemicellulose and provide more accessibility for enzyme to attack on the cellulose to produce sugar (glucose) yield. This show that higher pressure will help CO_2 penetration better into the cellulose in the biomass (Zheng et al., 1998). The penetration cause distruption of cellulose and help better enzymatic attack by *cellulase* as the surface area of the solid biomass increased.

Apart from XOs that have high potential value in industry fermentable sugar are widely used in sugar platform globally. Fermentable sugar can be potentially exploited as renewable carbon sources to produce biofuel which included bioethanol and biobutanol. Fermentable sugar also used to produce chemical such as succinic acid, lactic acid, sorbitol, furfural and xylitol. These chemical and polymer are widely used to produce many products in industry. The main application of fermentable that are currently used in industry is as a fermentation feedstock for production many biobased products such as bioplastic (Zahari et al., 2014).

1.1 Problem Statement

OPMF is one of the massive biomass generated from oil palm industry. The massive production of OPMF has led to many environmental problem as it is not managed properly. For example, the burning of OPMF using incineration lead to air pollution and dumping of OPMF cause water pollution. Thus, in this study, utilization of OPMF as feedstock for XOs and fermentable sugar production will help to reduce the OPMF generated and solve the environmental issues.

Secondly, the conventional methods approach to produce XOs and fermentable sugars are using chemical pretreatments such as acid and alkali hydrolysis. These method approach which are not recommended as it will lead to environmental issue post discharged. The chemical disposed in the river will cause water pollution. Another approach is using biological pretreatment that uses fungi as addition in pretreatment are costly and not economic friendly. This kind of pretreatment will later pollute the water and environment if we fail to manage them carefully and wisely. To that extent, this study was carried out by using greener approach subcritical H_2O-CO_2 pretreatment or known as physicochemical pretreatment that combined the effectiveness of physical and chemical reaction to overcome this problem. This method is preferable as it only uses steam and CO_2 as catalyst. However, the XOs and fermentable sugars yield produced using hydrothermal without CO_2 is not a promising method. Thus, in this study, carbon dioxide (CO_2) was added to improve the method and as a result, XOs and high sugar yield were obtained at optimal condition.

1.2 Objectives

The objectives of the study are:

1) To obtain optimal XOs production from OPMF by subcritical H_2O-CO_2 pretreatment.
2) To produce glucose and xylose yield from subcritical H_2O-CO_2 pretreated OPMF solids.

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