UNIVERSITI PUTRA MALAYSIA

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2013

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By

SALEHA BINTI SHAMSUDIN

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfillment of the Requirements for the Degree of Doctor of Philosophy

July 2013

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfillment of the requirement for the Degree of Doctor of Philosophy

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July 2013

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Faculty: Engineering

An abundant production of oil palm empty fruit bunch (EFB) from palm oil mill has created environmental issues to the surrounding localities primarily due to the inefficient disposal and incineration. Nevertheless, lignocellulosic EFB has a great potential as renewable feedstock. In addition, suitable pretreatment for EFB also have to be considered for commercial exploitation. Besides that, the conversion of EFB into biofuels and chemicals is one of the most feasible conversion route strategy in terms of sustainability compared to fossil fuel. Steam pretreatment has been chosen as a favourable pretreatment of EFB to be implemented in the palm oil mill due to the availability of steam being continuously generated as part of the mill's operation and the excess steam. In this study, we investigate the potential of using steam at 140°C from the palm oil mill back-pressure vessel as pretreatment for EFB. Major alterations occurred in the morphology of EFB as observed under the scanning electron microscope. The highest total conversion of 30% based on amount of pretreated EFB into sugar by Celluclast 1.5-L from *Trichoderma reesei* was achieved.

In order to increase the EFB biomass conversion to sugars, higher pressure of saturated steam pretreatment was then studied using 500 cm^3 reactor of steam pretreatment unit, whereby EFB is subjected to different saturated steam temperatures (130-230°C) for 2 to 8 minutes. The pretreated EFB concentration of 2% (w/v) with *Acremonium* cellulase was used in all saccharification experiments. As expected, the high pressure steam pretreatment can increase the digestibility by enzymatic hydrolysis of EFB. Using an excess of cellulase from *Acremonium cellulolyticus* demonstrated the greatly enhanced xylose (216.3 g kg^{-1} steam pretreated EFB) and glucose (621 g kg^{-1} steam pretreated EFB) digestibility by steam pretreatment at 140°C and 210°C, respectively. The results were compared by ANOVA and Turkey multiple range test using SPSS. The highest glucose achieved was 0.927 g glucose g^{-1} cellulose. The quantitative (BET) and the qualitative (FTIR/XRD/TG) analysis verified the effectiveness of steam pretreated EFB as a reactive substrate for biomass conversion via enzymatic hydrolysis system. Increased of EFB's surface area by fragmentation and development of cracks had enhanced the conversion of holocellulose, especially xylan into xylose, which is the second most important sugars of biomass. Higher pressure of saturated steam is necessary to modify the major parts of EFB, including the changes of inter/intra hydrogen bonding, biomass crystallinity and thermal stability behavior, which attributed to the removal of substantial hemicellulose part and enhanced the cellulose-lignin mixtures for high glucose production. From this study, a two-step steam pretreatment of EFB for the direct separation of xylose and glucose by enzymatic saccharification is proposed; 140°C for high yield of xylose and 210°C for high yield of glucose as low-cost substrates for biofuels and biomaterials production. Various chemical products could be generated from hemicellulose degradation at 210°C such as furfural (147.2

g kg^{-1} EFB), organic acids (39.1 g kg^{-1} EFB) and monosaccharides (66.9 g kg^{-1} EFB) from steam pretreatment of EFB which accounted for 91.4% of solubilized hemicellulose substances. The detail results of mass balance were well described and the energy consumption for saturated steam pretreatment at 210°C (32.8 MJ/kg) and 140°C (13.5 MJ/kg) from the 500 cm^3 steam pretreatment unit operation were estimated. Based on the energy excess found in the palm oil mill, the energy use to pretreat EFB for subsequent high xylose (140°C) and glucose production (210°C) were enough to be implemented in the palm oil mill. After steam pretreatment and saccharification, the calorific values of EFB residues were upgraded into solid product with favorable solid fuel characteristics (16.3 to 19.7 MJ/kg with 1–5 wt% moisture content).

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

PRA-RAWATAN STIM UNTUK PENAMBAHBAIKAN PENSAKARIDAAN ENZIM BAGI TANDAN KOSONG KELAPA SAWIT (*Elaeis guineensis*)

Oleh

SALEHA BINTI SHAMSUDIN

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Pengeluaran tandan kelapa sawit (TKS) yang banyak dari kilang minyak sawit telah mewujudkan masalah alam sekitar kepada penduduk tempatan dan kawasan sekitarnya terutama masalah yang berpunca daripada ketidakcekapan dalam proses pelupusan dan pembakaran. Sungguhpun demikian, lignoselulosa yang terdapat dalam TKS mempunyai potensi yang besar sebagai bahan mentah yang boleh diperbaharui. Di samping itu, prarawatan yang sesuai untuk TKS juga perlu dipertimbangkan untuk eksploitasi komersial. Selain itu, penukaran TKS kepada bahan bakar bio dan bahan kimia adalah kaedah strategi penukaran yang praktikal dari segi kemampanan dibandingkan dengan bahan api fosil. Prarawatan stim telah dipilih sebagai prarawatan yang baik bagi TKS untuk dilaksanakan di kilang minyak sawit kerana adanya stim yang sentiasa dijana sebagai sebahagian daripada operasi kilang dan stim yang berlebihan. Dalam kajian ini, kami mengkaji potensi menggunakan stim bersuhu 140°C yang berlebihan dari dandang kilang minyak sawit sebagai prarawatan bagi TKS. Perubahan yang ketara berlaku pada morfologi

TKS seperti yang diperhatikan di bawah mikroskop imbasan elektron. Peratus penukaran kepada gula tertinggi dicapai sebanyak 30% berdasarkan jumlah TKS terawat stim oleh Celluclast 1.5-L daripada *Trichoderma reesei*. Dalam usaha untuk meningkatkan peratusan penukaran biojisim TKS kepada gula, prarawatan dengan stim tepu yang bertekanan lebih tinggi dikaji menggunakan 500 cm^3 unit reaktor prarawatan stim, di mana TKS diprarawat pada suhu stim tepu yang berbeza (140-230°C) selama 2 hingga 8 minit. Peratus kepekatan prarawat tandan kosong kelapa sawit 2% (w/v) dengan selulase enzim *Acremonium* telah digunakan dalam semua eksperimen pensakaridaan. Seperti yang dijangka, prarawatan stim bertekanan tinggi boleh meningkatkan penghadaman dan hidrolisis enzim terhadap TKS. Penggunaan enzim selulase daripada *Acremonium cellulolyticus* yang berlebihan menunjukkan peningkatan yang tinggi terhadap xylose (216.3 g kg^{-1} TKS terawat) dan glukosa (621 g kg^{-1} TKS terawat) oleh prarawatan stim pada suhu 140°C dan 210°C, masing-masing. Keputusan telah dibandingkan dengan analisis varians menggunakan SPSS. Penghasilan glukosa tertinggi yang diperoleh adalah 0.927 g glukosa g^{-1} selulosa. Analisa kuantitatif (BET) dan kualitatif (FTIR/XRD/TG) mengesahkan keberkesanan stim-prarawat TKS sebagai substrat yang reaktif untuk penukaran biojisim melalui sistem hidrolisis enzim. Peningkatan luas permukaan TKS daripada kesan pemecahan dan keretakan telah meningkatkan penukaran holoselulosa, terutama xylan ke xylosa, gula kedua terpenting bagi biojisim. Stim tepu bertekanan tinggi adalah perlu untuk mengubah suai sebahagian besar TKS, ini termasuk perubahan pada inter/intra ikatan hidrogen, penghabluran biojisim dan tingkah laku kestabilan terma, yang dikaitkan dengan penyingkiran sebahagian besar hemiselulosa dan meningkatkan sebatian selulosa-lignin untuk penghasilan glukosa yang tinggi. Daripada kajian ini, dua langkah prarawatan stim dengan TKS untuk pemisahan langsung xylose dan glukosa oleh pensakaridaan enzim adalah dicadangkan; 140°C untuk hasil yang tinggi xylosa dan 210°C untuk hasil yang tinggi glukosa sebagai substrat kos rendah untuk penghasilan bahan bakar bio dan bio bahan. Pelbagai bahan kimia dapat dihasilkan daripada penurunan hemiselulosa pada 210°C seperti furfural (147.2 g kg^{-1} TKS), asid organik (39.1 g kg^{-1} TKS) dan monosakarida (66.9 g kg^{-1} TKS) dengan prapengolahan stim TKS yang menyumbang 91.4% daripada bahan-bahan hemiselulosa terlarut. Keputusan terperinci bagi keseimbangan jisim telah dijelaskan dan penggunaan tenaga untuk prarawatan stim tepu pada 210°C (32.8 MJ / kg) dan 140°C (13.5 MJ / kg) daripada 500 cm^3 unit operasi bagi prarawatan stim telah dianggarkan. Berdasarkan lebihan tenaga yang terdapat di kilang minyak sawit, penggunaan tenaga untuk prarawatan TKS untuk penghasilan xylosa (140°C) dan glukosa (210°C) yang tinggi adalah cukup untuk dilaksanakan di kilang minyak sawit. Selepas prarawatan stim dan pensakaridaan, nilai kalori sisa TKS telah dinaik taraf kepada suatu produk yang mempunyai ciri-ciri sebagai bahan bakar pepejal yang baik (16.3-19.7 MJ / kg dengan 1-5% berat kandungan kelembapan).

ACKNOWLEDGEMENTS

In the name of Allah, Most Gracious and Most Merciful. Praise be to Allah, the Cherisher and Sustainer of the Worlds, and Peace and Prayer be upon the Final Prophet and Messenger.

Thanks be to Allah. Most grateful am I to Allah, for through with His support and blessing was I able to successfully complete this research study. I would like to express my deepest gratitude to my supervisor, Professor Dr. Mohd Ali Hassan and to all my co-supervisors, Professor Dr. Yoshihito Shirai, Professor Dr. Suraini Abd Aziz and Associate Professor Siti Mazlina Mustapa Kamal. Many thank to my supervisory committee who contributed their time and supported me in making the completion of studies. My gratitude also goes out to Associate Professor Wakisaka Minato, Professor Dr. Nishida, Dr. Yoshihito Ando, for their help during my time at Kyushu Institute of Technology.

I would like to acknowledge Universiti Putra Malaysia (UPM), Kyushu Institute of Technology (KIT), Advance Institute of Science and Technology, Japan (AIST) and Federal Land Development Authority (FELDA) for funding and technical support for this research. Special thanks to the Ministry of Higher Education Malaysia (MOHE) and Universiti Malaysia Perlis (UNIMAP) for providing the PhD scholarship.

My deepest gratitude to my husband Muhammad Fairuz A. Adi, my children Muhammad Ali Fatih and Sofea Raihanah, my beloved parent and my parent in laws, family and relatives. I sincerely thank to Ezyana, all lecturers, friends in EB group and to all the individuals who are involved directly and indirectly in the completion of this research study. Thanks very much for giving me the spirit and motivation in facing the obstacles and challenges in my life.

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

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DECLARATION

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I hereby confirm that:

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Declaration by Members of Supervisory Committee

This is to confirm that:

- the research conducted and the writing of this thesis was under our supervision;
- supervision responsibilities as stated in Rule 41 in Rules 2003 (Revision 2012-2013) were adhered to.

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LIST OF ABBREVIATIONS

EFB	Empty fruit bunch
PKS	Palm kernel shell
PFF	Pressed fruit fiber
MF	Mesocarp fiber
OPT	Oil palm trunk
OPF	Oil palm fiber
POME	Palm oil mill effluent
psi	Pounds per square inch
OPA	Oil palm ash
MPa	Megapascal
AFEX	Ammonia fiber explosion
DA	Dilute acid
CAFI	Consortium for Applied Fundamentals and Innovation
ABE	Acetone-butanol-ethanol
DM	Dry matter
DSIA	Direct steam-injection apparatus
HPST	High-pressure steam pretreatment
WIS	Water insoluble solid
FPU	Filter paper unit
CBU	Cellobiase unit
FELDA	Federal land development authority
CPO	Crude palm oil
MPOB	Malaysian palm oil board

FPase	Filter paper activity
CMCase	Carboxymethyl cellulase
ADF	Acid detergent fiber
ADL	Acid detergent lignin
NDF	Neutral detergent fiber
SEM	Scanning electron microscope
FTIR	Fourier transform infrared
ATR	Attenuated Total Reflectance
TGA	Thermalgravimetric analysis
XRD	X-ray diffraction
CV	Calorific value
ANOVA	Oneway analysis variance
HMF	Hydroxymethylfurfural
CF	Correction factor
BET	Analysis
DTG	Differential thermogravimetric
GC-MS	Gas chromatography mass spectrometry
%	percentage
(w/v)	Weight per volume
(w/w)	Weight per weight
M	Molar
HPLC	High performance liquid chromatography
g/kg	Gram per kilogram

CHAPTER 1

INTRODUCTION

1.1 Research background

Currently about 50 000 km^2 or 5 million hectares of oil palm are cultivated in Malaysia, the world's largest exporter of palm oil (Malaysian Palm Oil Board, 2012). As one of the biggest exporters of palm oil and palm oil products, palm oil industries in Malaysia generate huge quantities of biomass in the form of oil palm empty fruit bunch (EFB), palm kernel shells (PKS) and pressed fruit fiber (PFF). The potential of these are yet to be exploited. Out of these biomass, the EFB generated during processing of palm oil, can be considered as primary feedstock due to their abundant production, high cellulosic, non-food biomass and inexpensive (waste or by products from palm oil industries) availability for the production of sugars that can be further used as carbon source for biological conversion through fermentation.

Several biomass pretreatments have been investigated over the years involving physical, chemical and biological approaches. In order to disrupt the lignocellulosic's recalcitrant nature which is highly crystalline cellulose structure in the presence of lignin and possesses limited surface area for enzyme attack, the conversion into more hydrolysable forms under a combination of physical, chemical and thermal methods is often needed (Taherzadeh and Karimi, 2007). Efficient pretreatment methods which change the physical and chemical structure of the lignocellulosic biomass by removing the compositional barriers, opens up the structure or altering the structure can greatly enhance the hydrolysis of biomass for efficient downstream processing.

According to Sun and Cheng (2002), successful pretreatment must lead to improve production of sugars or the ability to subsequently form sugars by hydrolysis, with minimal loss of carbohydrate into degradation products and should be cost effective.

In this study, the non-chemical pretreatment strategy which combines physical, chemical and thermal action could be the best pretreatment option for EFB to be implemented in the palm oil mill. Since no chemicals is used in pretreating the EFB, then the whole process being environmentally friendly technology for biomass utilization. The pretreatment step is intended to open up the hemicellulose and cellulose biomass (EFB) by enzymatic attack into sugars. Lignin and other components not converted can be burned to provide heat and electricity needed to run the overall process involved (Wyman, 2007) or be used in palm oil mill production. On the other hand, lignin could also be potentially used as a substrate for the production of lignophenol a highly value added product.

The physicochemical method of steam pretreatment is one option for pretreating the biomass. Steam has the potential to degrade (and thereby pretreat) the complex structure of the lignocellulosic biomass. In steam pretreatment, the biomass is simultaneously treated at high pressures with high steam temperatures. Exposure of lignocellulosic biomass to temperatures of 120-210°C for few to several minutes can remove most of the hemicellulose and produce cellulose-rich solids from which high glucose yields are possible with cellulase enzymes (Yang and Wyman, 2010). Besides the hydrolyzing of hemicelluloses, steam pretreatment has been reported to modify the lignin, increase access to surface area, decrease the crystallinity of cellulose and its degree of polymerization (Ramos, 2003).

Steam pretreatment of EFB for sugar production is the best pretreatment option owing to the availability of excess steam that is continuously being generated during palm oil processing. The boilers produce superheated steam which is used to generate electricity through turbine generator. Steam is generated in the boiler at a pressure of 20 Bar (300°C) and expended in steam turbines at 18.5 Bar at 260°C (Simarani, 2010). There is some reported data which stated that the typical final steam produced from the boiler is saturated steam with pressure and temperature of <21 Bar and 210°C, respectively. The high pressure steam from the boiler enters the back pressure turbine at 250-300 psi or 17-20 Bar and expands through the turbines blades for the mechanical work. The ranges of inlet and outlet pressures of the steam turbines are reported to be 18-20 Bar and 2.9-3.2 Bar, respectively (Nasrin *et al.*, 2011).

Steam is then led into the process where the latent heat contained in exhaust steam in the back pressure receiver (BPR) is about 3.16 Bar. When the steam pressure at BPR exceeds more than 3.5 Bar, the steam is released to the atmosphere. The 3 Bar steam that leaves the steam turbine still have a great deal of energy. According to Simarani (2010), there is about 5.55×10^7 kg of excess saturated steam (140°C) is produced in the palm oil mill which is equivalents to 12.6% of energy loss as steam via the sterilizer exhauster. Therefore, in this study we propose to use the excess steam at the mill for pretreating the EFB.

The boilers have the capacity in generating the saturated and superheated steam at 210°C to 260°C which can potentially be used to pretreat the EFB for bioconversion. The higher operating steam pretreatment temperatures in the examined range might increase the accessibility of enzymatic hydrolysis of EFB. Figure 1.1 shows the proposed pretreatment system in the palm oil mill for EFB conversion into fermentable sugar. Every year, palm oil mills produce 50 million tons of PKS and PFF globally; but only 60% are used as solid fuel for steam boilers (Mohamed, 2006). This amount might be sufficient to support the application of steam for the pretreatment of EFB in the palm oil mill. Overall, this pretreatment is attractive to be practiced in the palm oil mill as it includes renewable resources (water, PKS and PFF) that, could be considered as inexpensive resources readily available in the mill.

1.2 Problem statement

The palm oil industry generates an abundance of oil palm biomass such as EFB which is now considered as by-product or 'waste'. As an important Malaysia's agricultural industry, the palm oil industry which plays a vital role in the socio-economy and well being of the country had an annual production increment of this resource due to the rising of fresh fruit bunch (FFB) output from plantations each year. At present, current practice of EFB's disposal method by incineration has created problem by producing a considerable amount of smoke that released to pollute the environment. Only 10% of the EFB is used as mulching material while the rest are dumped in areas adjacent to the mill besides burning. In short, the inefficient of EFB's utilization in the palm oil plantation had generated air and odor pollution in the nearby localities.

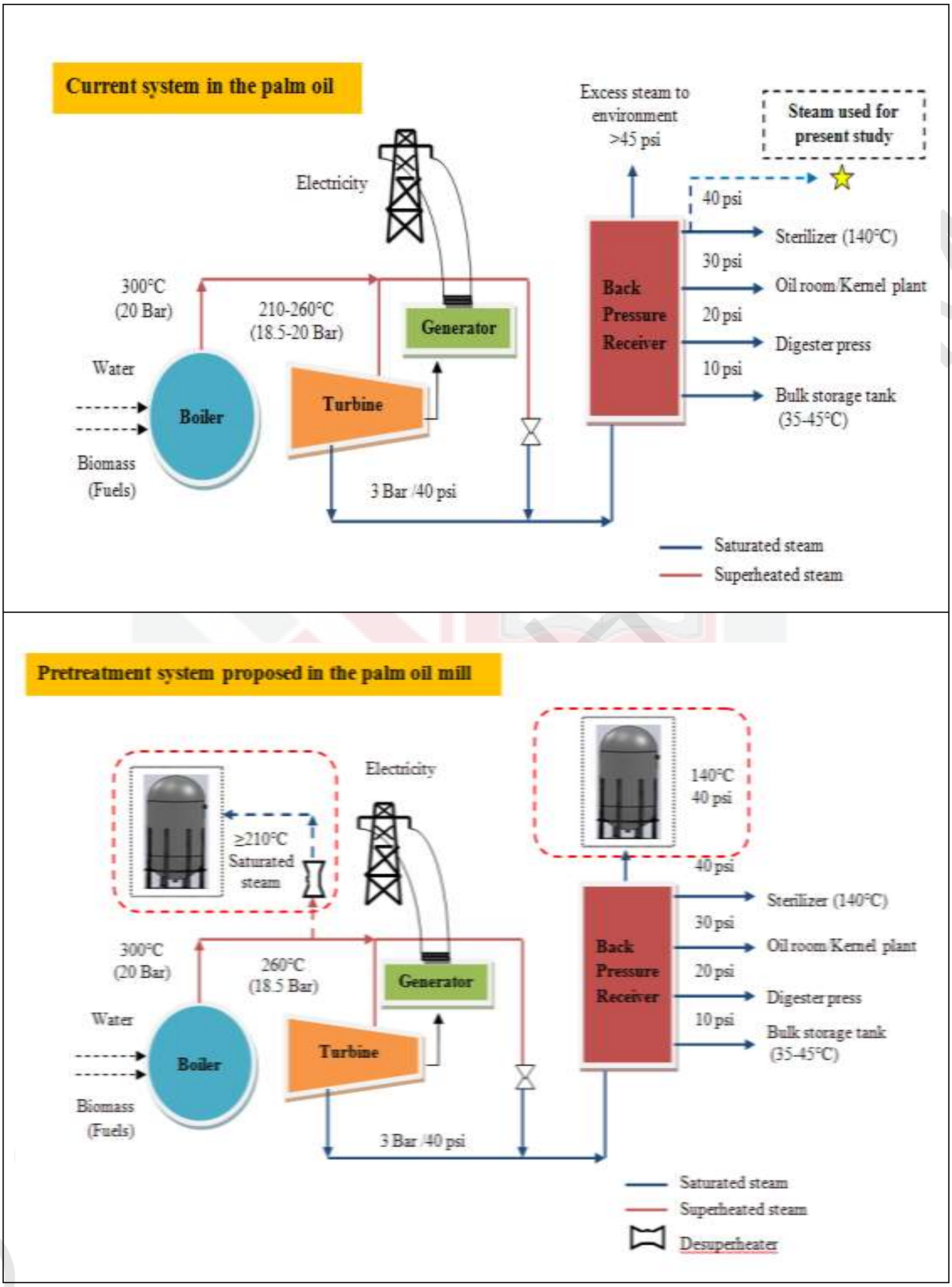

Figure 1.1 The proposed pretreatment system of EFB in the palm oil mill

Much research work has been carried out and still on going to utilize EFB for value-added product. However, its commercial utilization is hindered due to lack of appropriate energy management towards environmental friendly industrial activity. EFB is a plentiful source of renewable cellulosic material which is easily decomposed by a combination action of suitable pretreatment. The physicochemical method of steam pretreatment is one option for pretreating the biomass. A trial of steam pretreatment on EFB have been reported, however it was not in depth study on the effects of steam pretreatment for EFB on sugars production. A concise enumeration of the steam pretreatment effect on EFB and their changes characteristics after steam pretreatment are important aspects to be understood by render the factors such as steam temperature, residence time and form of EFB to increase sugars conversion from EFB for fully exploits its benefit through research.

1.3 Research hypothesis

The hypothesis evaluated in this research was that steam pretreatment can enhance the production of sugars during the subsequent enzymatic saccharification of EFB.

1.4 Research objectives

The primary goal of this study was to investigate the potential of saturated steam to pretreat EFB for sugar production. The specific objectives of this study were:

1. To investigate the feasibility of using excess steam from the palm oil mill back-pressure vessel for EFB pretreatment to enhance the digestibility of EFB using enzyme for sugar production
2. To select the most favourable conditions of saturated steam pretreatment that results in maximum enhanced digestibility of EFB for xylose and glucose production
3. To estimate the energy consumption in EFB steam pretreatment for fermentable sugar production based on enzyme-catalyzed process and determine the potential energy of EFB residues as boiler fuels

1.5 Scope of study

This thesis is focused on pretreatment of EFB by saturated steam for enhancement of sugars production by enzymatic hydrolysis. It was divided into five chapters (Figure 1.2). Chapter 1 contains a research background, problem statement, research hypothesis, research objectives and scope of study. Chapter 2 reviews the literature concerning the characteristics of EFB, biomass pretreatment technologies, historical background, fundamental and degradation products of biomass steam pretreatment. Chapter 3 outlines the materials used and the experimental conditions, equipment set up used in this study. Results obtained in this study are presented in Chapter 4 which divided into five major topics: preliminary study on the feasibility of using steam from the palm oil mill back-pressure vessel as a pretreatment to enhance the digestibility of EFB for sugars production, selection of the most favorable condition of saturated steam pretreatment that increase EFB digestibility into fermentable sugars, physical and chemical characteristics of steam pretreated EFB and steam condensate and the estimation of energy consumption for the direct separation of glucose and xylose from steam pretreatment of EFB. The conclusion of this study and the recommendations for further research presents in the last chapter (Chapter 5) for this thesis.

The study was conducted at two locations namely FELDA Serting Hilir Palm Oil Mill, Negeri Sembilan (Malaysia) and Kyushu Institute of Technology (Japan). Because of their respective availability, two different cultivars were used in this study. The trials conducted in FELDA Serting Hilir Palm Oil Mill is the preliminary study consisted of pretreating different forms of EFB using the saturated steam temperature (140°C) from the mill back-pressure vessel. This study was performed by increase the pretreatment time from 15 to 60 minutes of saturated steam from raw or controls EFB. EFB were then analyzed on enzymatic saccharification performance, lignocellulosic content analysis, structural changes by scanning electron microscope and mass changes durin steam pretreatment.

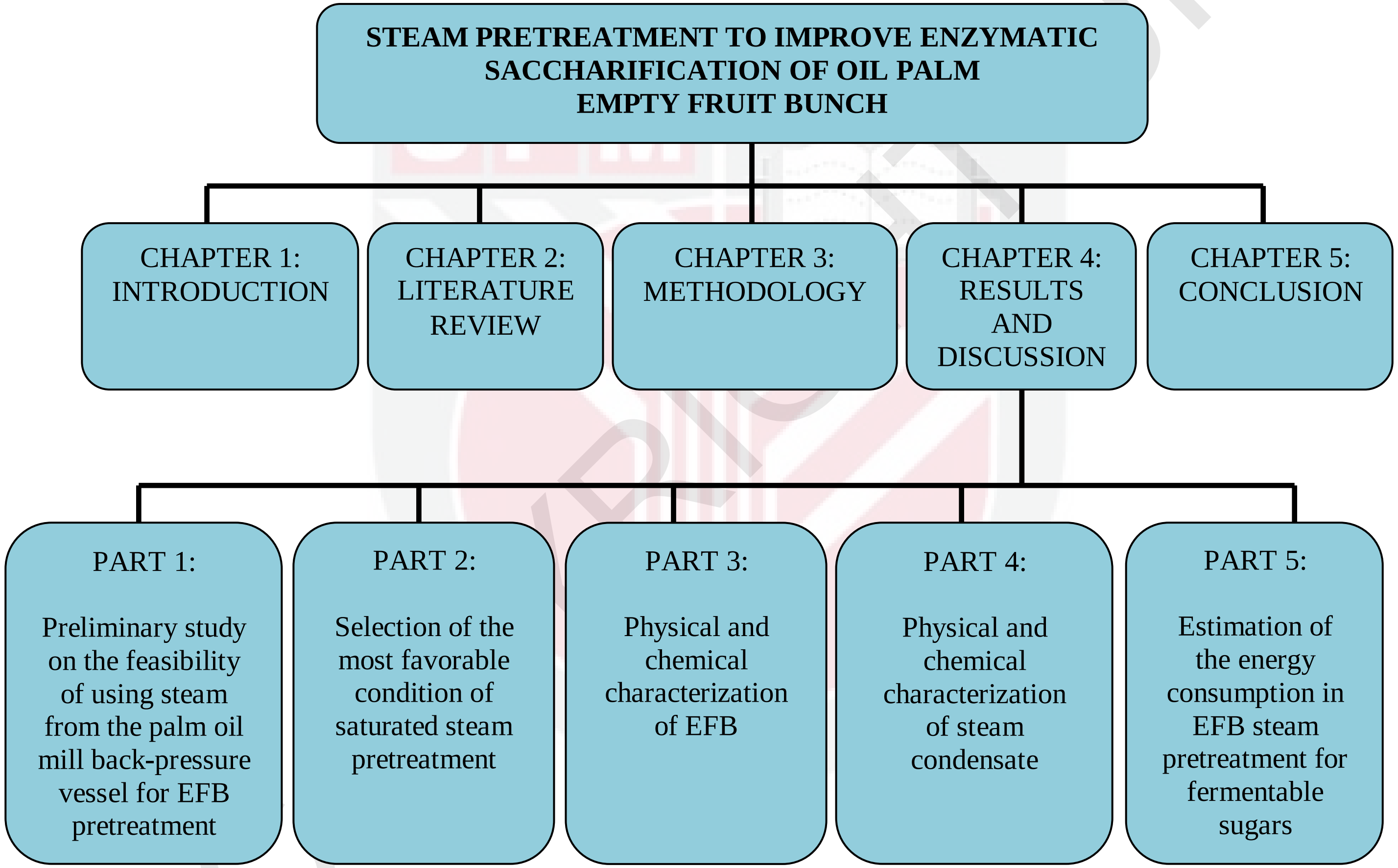

Figure 1.2 Flow diagram of the thesis "Steam pretreatment to improve enzymatic saccharification of oil palm empty fruit bunch"

The trials performed at Kyushu Institute of Technology were done using saturated steam temperature (130-230°C) generated by 500 cm^3 reactor of steam pretreatment unit and the starting substrate material was the pressed-shredded EFB obtained from Sri Ulu Langat Palm Oil Mill for the entire experiments. An in-depth study on the effect of steam pretreatment on EFB was then assessed by digestibility, physical and chemical analyses to the steam pretreated EFB fibers and condensate. In addition to that, a study on estimating the energy consumption in EFB steam pretreatment was done in order to evaluate the efficiency of available excess steam as a pretreatment to EFB for implementation in the palm oil mill. Besides that, after pretreatment and saccharification the determination of potential energy of EFB residue as boiler fuels had also been done.

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