OPTIMIZATION OF ENZYMATIC PRETREATMENT AND SACCHARIFICATION PROCESSES OF SAWDUST FOR BIOETHANOL PRODUCTION

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We hereby declare that we have checked this thesis and in our opinion, this thesis is adequate in terms of scope and quality for the award of the degree of Master of Science.

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I hereby declare that the work in this thesis is based on my original work except for quotations and citations, which have been duly acknowledged. I also declare that it has not been previously or concurrently submitted for any other degree at Universiti Malaysia Pahang or any other institutions.

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ABSTRAK

Penghasilan etanol dari bahan yang boleh diperbaharui dan mampan adalah penting bagi menangani krisis bahan api berasaskan fosil pada masa akan datang. Biojisim lignoselulosa seperti, habuk papan adalah bahan mentah biojisim yang menarik, banyak, murah dan dari sumber bukan makanan untuk pengeluaran etanol yang lebih murah. Walau bagaimanapun, kerana struktur bahan ini yang keras, prarawatan perlu dijalanka untuk pemecahan lignin. Selain itu, kebanyakan teknik prarawatan menggunakan bahan kimia dan tenaga yang mendedahkan ancaman terhadap alam sekitar tehadap prosesnya serdi. Tesis ini mengkaji pengoptimuman secara statistik prarawatan mesra alam dan proses *saccharification* habuk papan untuk pengeluaran bioetanol. Proses pengoptimuman dilakukan terlebih dahulu dengan analisis *one-factor-at-a-time* (OFAT), untuk menunjukkan julat optimum dan kepentingan setiap parameter dalam proses tersebut. Pengoptimuman kedua menggunakan *Face-Centred Central Composite Design* (FCCCD) bagi Kaedah *Response Surface Methodology* (RSM) untuk mengkaji kesan gabungan parameter tertentu. Dalam kajian ini, enzim laccase digunakan untuk prarawatan habuk papan dan hasilnya dinilai dari segi peratusan penurunan berat (%) dan jumlah gula yang dihasilkan (mg / mL). Keadaan prarawatan yang optimum adalah pada suhu 35°C; pH 5; masa, 10 jam; kepekatan enzim, 20 IU/g habuk papan; kepekatan substrat, 5% (w/v); saiz sampel, 1 mm dan agitasi, 150 rpm. Manakala keadaan *saccharification* yang optimum untuk laccase prarawatan habuk papan telah dicapai pada suhu 48 °C, masa 36 jam, kepekatan enzim 30 IU / g habuk papan dan pH 5. Selepas proses pengoptimuman, prarawatan enzim dan proses *saccharification* telah dinilai untuk pengeluaran bioetanol menggunakan *Saccharomyces cerevisae* sebagai organisma fermenter. Hasil bioetanol telah didapati melalui jumlah gula dan kandungan biojisim sampel masing-masing sebanyak 29.4% dan 9.78%. Proses enzimatik yang optimum ini boleh dianggap sebagai salah satu usaha kemapanan yang boleh diperbaharui yang direka untuk mengurangkan pelepasan gas rumah hijau dan melindungi alam sekitar dari pemanasan global.

ABSTRACT

A widespread interest has been devoted to the production of ethanol from sustainable renewable materials to compensate for the impending fossil fuel crisis. Lignocellulosic biomasses, such as sawdust and others, are an attractive, abundant, cheap and non-food competitive biomass feedstock for cheaper ethanol production. However, the recalcitrant structure of these materials necessitates a pretreatment process for a complete or partial breakdown of the lignin in the cell wall of these biomasses. Most of the pretreatment techniques require the use of chemicals and energy which poses a threat to the environment and the whole process as well. This study embarked on a statistical optimization of the pretreatment and saccharification processes of sawdust for bioethanol production in an environmentally-friendly manner. The study process involved the screening of the process parameters using One-Factor-At-a-Time (OFAT) to determine the optimum range and significance of each parameter in the process, followed by a statistical optimization process using Face-Centered Central Composite Design (FCCCD) of Response Surface Methodology (RSM) to study the combined effect of certain parameters. In this study, laccase enzyme was used as the ligninolytic agent for sawdust pretreatment. The performance of the pretreatment process at each stage of the study was evaluated based on the weight loss (%) and total sugar produced (mg/mL). The optimized condition for the pretreatment of sawdust using laccase enzyme was achieved as temperature 35 °C, pH 5, time 10 h, laccase enzyme concentration 20 IU/g of sawdust, substrate concentration 5% (w/v), sample size 1 mm, and agitation rate 150 rpm. The optimized condition for the saccharification of the laccase pretreated sawdust was achieved as temperature 48 °C, time 36 h, enzyme concentration 30 IU/g of sawdust, and pH 5. After the optimization of the pretreatment and saccharification processes, the enzyme-pretreated and saccharified sawdust was evaluated for bioethanol production using *Saccharomyces cerevisiae* as the fermentation organism. The bioethanol yield based on the total sugar content and biomass content was achieved as 29.4% (w/v) and 9.78% (w/v), respectively. These enzymatic pretreatment and saccharification processes can be considered as one of the renewable efforts towards reducing greenhouse gas emissions and environmental protection from global warming.

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LIST OF SYMBOLS

g/g	Gram per gram
IU/g	International Unit per gram
vMax	Maximum velocity (rate of enzyme activity)
°C	Degree Celsius
Hr	Hour
IU	International Unit
mM	mill Molar
mL	Millilitre
Ppm	Part per million
MPa	Mega Pascal
Rpm	Revolution per minute
MW	Mega watt
kDa	Kilo Dalton
Pi	Iso-electric point
Ha	Hectare

LIST OF ABBREVIATIONS

%Cc	Cellulose content percentage
%Hc	Hemicellulose content percentage
%Lc	Lignin content percentage
ABTS	2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid)
AFEX	Ammonia fiber expansion
CCD	Central composite design
DNS	Dinitrosalicylic acid
DoE	Design of experiment
EDTA	Ethylene diamine tetra acetic acid
EtOH	Ethanol
FCCCD	Face centered central composite design
FTIR	Fourier Transform Infrared
GC-FID	Gas Chromatography with Flame Ionization Detector
HMF	Hydroxyl methyl furfural
OFAT	One factor at a time
ROS	Reactive oxygen species
RSM	Response surface methodology
RT	Room temperature
SEM	Scanning Electron Microscope
SGB	Second Generation Bio-ethanol
SHF	Separate hydrolysis and fermentation
SSF	Simultaneous saccharification and fermentation

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