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

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

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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 dijalankan 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-ethylbenzthiazoline-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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