OPTIMIZATION OF PRETREATMENT AND SACCHARIFICATION PROCESSES OF EMPTY FRUIT BUNCHES (EFB) FOR BIOETHANOL PRODUCTION

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LIST OF SYMBOLS

g/g	Gram per gram
g/l/h	Gram per liter per hour
IU/g	International Unit per gram
j/mol	Joule per mole
oz	Ounce
Pi	Isoelectric constant
V_{max}	Maximum rate of enzyme activity
^{o}C	Degree Celsius
h	Hour
IU	International Unit
mL	Milliliter
mM	Millimolar

LIST OF ABBREVIATIONS

ABTS	2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid)
AFEX	Ammonia fiber expansion
CCD	Central composite design
DNS	Dinitrosalicylic acid
DoE	Design of experiment
EC	Enzyme commission
EDTA	Ethylene diamine tetra acetic acid
EFB	Empty fruit bunches
FCCCD	Face centered central composite design
HMF	Hydroxyl methyl furfural
MW	Mega watt
OFAT	One factor at a time
PAP	Papanicolaou
POME	Palm oil mill effluent
ppm	Parts per million
ROS	Reactive oxygen species
rpm	Revolution per minute
RSM	Response surface methodology
RT	Room temperature
SHF	Separate hydrolysis and fermentation
SSF	Simultaneous saccharification and fermentation
V	Volts
Ro	Severity factor
%Cc	percentage cellulose content

%Hc	percentage hemicellulose content
%Lc	percentage lignin content

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ABSTRACT

Environmental degradation and episodes of global warming have facilitated studies into alternative sources of energy carriers for economic use. Green energy carriers such as bioethanol and biodiesel have waged the gap but not without a snag. Pretreatment of biomass for the production of biofuel has conventionally been done using energy and chemical agents which are not sustainable to the environment as well. Hence, in this study, a novel pretreatment method for the pretreatment of Empty fruit bunches (EFB) was studied and statistically optimized. The optimization of the process parameters for the pretreatment and saccharification of EFB using laccase and cellulase enzymes (enzyme and substrate concentrations, size of EFB, time, pH and temperature) were studied using one-factor-at-a-time (OFAT) and response surface methodology (RSM). The results of the study showed that the activity of laccase enzyme was more affected by the temperature of the reaction than any other factor. The optimized condition for the pretreatment of EFB with laccase enzyme was achieved as: temperature 35 ^{o}C, duration 4 h, enzyme concentration 20 IU/g of EFB, EFB concentration 5 % (w/v), and a reaction buffer of pH 5. The optimized saccharification condition of the enzyme pretreated EFB was studied and achieved as: temperature 50 ^{o}C, duration 24 h, enzyme concentration 30 IU/g of EFB, EFB concentration 5 % (w/v), and pH 5. Furthermore, the analysis of variance (ANOVA) of the statistically optimized parameters showed that temperature of pretreatment has higher significant effect ($P < .05$) compared to pH, while the pH during saccharification has higher significant effect ($P < .05$) compared to temperature. Ethanol production was evaluated at the optimized pretreatment and saccharification conditions and a yield of 29.13 % by total sugar content was achieved, as well as 31.12 % by biomass content. The pretreatment of EFB with laccase enzyme at the above modelled pretreatment conditions could contribute to the sustainability efforts aimed towards reduction of greenhouse gas emission from chemical agents and keep the environment safe from the harmful effect of global warming.

ÀBSTRAK

Pencemaran alam sekitar dan episod pemanasan global telah memudahkan kajian ke dalam sumber alternatif pembawa tenaga untuk kegunaan ekonomi. Pembawa tenaga hijau seperti bioetanol dan biodiesel telah melancarkan jurang tetapi tidak tanpa tergendala. Prarawatan biojisim untuk pengeluaran biofuel yang konvensional telah dilakukan dengan menggunakan ejen tenaga dan kimia yang tidak mampan kepada alam sekitar yang baik. Oleh itu, dalam kajian ini, satu kaedah rawatan awal baru untuk rawatan awal tandan buah kosong (EFB) telah dikaji dan statistik yang dioptimumkan. Pengoptimuman parameter proses untuk rawatan awal dan sakarifikasi EFB menggunakan enzim laccase dan enzim selulase (kepekatan enzim dan substrat, saiz EFB, masa, pH dan suhu) telah dikaji menggunakan satu faktor-pada-satu-masa (OFAT) dan kaedah gerak balas permukaan (RSM). Keputusan kajian menunjukkan bahawa aktiviti enzim daripada enzim laccase adalah lebih terjejas oleh suhu tindak balas berbanding mana-mana faktor lain. Keadaan optimum untuk prarawatan EFB dengan enzim laccase yang telah dicapai: suhu 35 ^{o}C, tempoh 4 j, kepekatan enzim 20 IU g EFB, kepekatan EFB 5 % (w/v), dan reaksi pemampan pH 5. Keadaan sakarifikasi optimum enzim EFB yang telah menjalani prarawatan telah dicapai: suhu 50 ^{o}C, tempoh 24 j, kepekatan enzim 30 IU/g EFB, kepekatan EFB 5 % (w/v), dan pH 5. Tambahan pula, analisis varians (ANOVA) daripada statistik parameter optimum menunjukkan bahawa suhu rawatan awal mempunyai kesan yang ketara lebih tinggi ($P < .05$) berbanding pH, manakala pH semasa sarafikasi mempunyai kesan yang ketara lebih tinggi ($P < .05$) berbanding dengan suhu. pengeluaran etanol telah dinilai pada keadaan rawatan awal dan sarafikasi yang telah dioptimumkan dan hasil sebanyak 29.13 % manakala jumlah kandungan gula adalah 31.12 % oleh kandungan biojisim. prarawatan EFB dengan enzime laccase dimodelkan pada keadaan rawatan awal boleh menyumbang kepada usaha kelestarian bertujuan ke arah pengurangan pelepasan gas rumah hijau daripada bahan kimia dan mengekalkan persekitaran yang selamat daripada kesan bahaya pemanasan global.