OPTIMIZATION OF SODIUM HYDROXIDE PRETREATMENT AND ENZYMATIC SACCHARIFICATION OF SPENT RUBBERWOOD SAWDUST FOR GLUCOSE PRODUCTION

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Optimization of sodium hydroxide pretreatment and enzymatic saccharification of spent rubberwood sawdust for glucose production

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SYMBOL

α	Alpha (axial distance from centre point which makes the design rotatable)
β_0	regression coefficients for the intercept coefficient
β_i	regression coefficients for the linear coefficient
β_{ii}	regression coefficients for the quadratic coefficient
β_{ij}	regression coefficients for the interaction coefficient
°C	Celsius
σ	standard deviation
χ_i , χ_j	coded independent variables
ε	residual associated to the experiments
g	gram
h	hour
K	number of variable
M	Molar
mM	mili Molar
µm	micro meter
min	minute
MPa	Mega pascal
N	number of measurements
n	sample size
rpm	rotation per minute
v	volume
v/v	volume to volume ratio

w/v	weight to volume ratio
w/w	weight to weight ratio
~	approximately

LIST OF ABBREVIATIONS

ADP	Adenosine di-phosphate
AFEX	Ammonia Fibre Explosion
a.i	After inoculation
ANOVA	Analysis of variance
A/O	Alkaline/ Oxidative
ASTM	American Standard Test Method
ATP	Adenosine tri-phosphate
Avg.	average
BBD	Box-Behnken Design
BG	ß-glucosidase
CBH	Cellobiohydrolases
CCD	Central Composite Design
CO_2	Carbon dioxide
conc.	concentration
CV	Coefficient variation
DoE	Design of Experiment
FP	Filter paper
EG	Endoglucanases
ES	Enzymatic saccharification
GM	Growth medium
H+	Hydrogen ion
HMF	Hydroxymethyl furfural

H_2O	Dihydrogen oxide (water)
H_2SO_4	Sulfuric acid
KBr	Potassium bromide
KOH	Kalium hydroxide
LiP	Lipase peroxide
MnP	Manganese peroxidise
MSM	Mushroom spent medium
n.a	not available
NaCl	Sodium chloride
NAD^+	Nicotinamide adenine dinucleotide
NADH	Nicotinamide adenine dinucleotide (in reduced from)
NaOH	Sodium hydroxide
OFAT	One Factor at a Time
OH^-	Hydroxide ion
OPFF	Oil palm fruit fibre
Pi	Phosphate group
PEG	Poly(ethylene)glycol
POME	Palm oil mill effluent
RSD	Rubberwood sawdust
RSM	Response Surface Methodology
RT	Room temperature
SSB	Sweat sorghum bagasse
SEM	Scanning Electron Microscope
vs	versus

ABSTRAK

Kuantiti yang banyak bongkah cendawan tiram terpakai (MSM) telah dilupuskan sebagai sisa pertanian ke alam sekitar dan dengan itu telah menimbulkan pencemaran alam sekitar. Penggunaan semula secara ekonomi bongkah cendawan terpakai untuk menghasilkan glukosa mungkin menjadi satu penyelesaian yang berjaya selain pelupusan. Oleh itu, kajian ini dijalankan untuk meningkatkan nilai tambah bongkah cendawan terpakai sebagai sumber glukosa alternatif kepada tanaman gula dan produk berkanji yang mahal sekarang. Pertama sekali, ciri-ciri kimia, morfologi permukaan, dan kesesuaian bongkah cendawan terpakai berbanding dengan habuk kayu getah (RSD), dan medium pertumbuhan (GM) telah pertama diperiksa untuk mengkaji potensinya sebagai sumber bahan mentah baru untuk glukosa. Analisis komposisi kimia membuktikan terdapat pengurangan kandungan lignoselulosik selepas penanaman Pleurotus sajor-caju. Jumlah kandungan lignin, hemiselulosa, dan selulosa di dalam MSM menunjukkan nilai yang lebih rendah daripada yang terkandung di dalam RSD dan GM iaitu masing-masing sebanyak 22.40, 29.30, dan 29.07%. Morfologi permukaan MSM pula kelihatan kasar dan terdapat banyak kesan rekahan pada permukaan gentian yang terhasil akibat daripada proses penanaman cendawan. RSD dan GM mempunyai permukaan licin dan rata serta menunjukkan struktur permukaan tegar dan sangat tersusun. Kajian awal mengenai keberkesanan dan kesenangan tiga teknik prarawatan (autoklaf pada suhu 121°C, pemanasan di dalam pemanas air, rendam pada suhu bilik) dengan kepekatan NaOH yang berbeza pada RSD, GM, dan MSM mendedahkan hasil yang terbaik dengan 30.13 g glukosa/100 g substrat kering dan 33.50% penurunan berat hidrolisis diperolehi daripada MSM yang telah dirawat di dalam pemanas air pada suhu 90°C selama 2 jam. Imej imbasan electron mikroskop (SEM) terhadap MSM hidrolisat berkenaan menunjukkan kerosakan teruk pada struktur biomas dan banyak retakan yang tidak teratur dan liang-liang pori. Kaedah satu faktor pada satu masa diaplikasi untuk menyaring nilai beberapa parameter dan kaedah respons permukaan (RSM) berdasarkan reka bentuk Box-Behnken telah diguna pakai untuk mengoptimumkan kondisi di dalam prarawatan NaOH (melalui kaedah pemanasan di dalam pemanas air) dan enzimatik sakarifikasi pada MSM. Keadaan optimum untuk prarawatan terhadap MSM pada konsentrasi substrat sebanyak 5.0% (w/v) adalah pada kepekatan NaOH sebanyak 2.63 M, suhu reaksi pada 92.26° C, dan masa rawatan sebanyak 112.92 minit dengan hasil glukosa maksimum sebanyak 34.55 g/100 g substrat kering selepas 48 jam enzimatik sakarifikasi pada kadar enzim yang tetap sebanyak 67 FPU/g substrat kering dan konsentrasi hidrolisat sebanyak 1.0% (w/v). Hidrolisat MSM yang diperoleh daripada prarawatan NaOH yang optimum telah digunakan untuk mengoptimumkan keadaan proses enzimatik sakarifikasi pada konsentrasi subtrat yang tetap sebanyak 1.0% (w/v). Di bawah keadaan optimum (kelajuan agitasi sebanyak 150.74 rpm, muatan enzim sebanyak 94.92 FPU/g substrat, dan masa hidrolisis sebanyak 56.89 jam), hasil glukosa maksimum sebanyak 71.21 g/100 g substrat kering telah dicapai. Analisis varian (ANOVA) menunjukkan bahawa model dan semua parameter dianggap penting secara statistik pada 95% untuk kedua-dua kajian pengoptimuman menggunakan persamaan polinomial peringkat kedua. Selain itu, pengesahan model menunjukkan perkaitan yang rapat antara keputusan eksperimen dan ramalan respon. Oleh itu, model-model ini boleh digunakan dengan jayanya untuk mengenal pasti kombinasi yang berkesan daripada tiga faktor yang berbeza di dalam kedua-dua kajian pengoptimuman untuk meramalkan hasil glukosa daripada MSM.

ABSTRACT

Large quantities of oyster mushroom spent medium (MSM) were disposed of as agricultural waste to the environment and thereby constituting environmental pollution. Economic reuse of the waste mushroom medium to produce glucose might be a viable solution instead of disposal. Therefore, the study was conducted to improve the added value of mushroom spent medium as an alternative glucose source to the current expensive sugar crops and starchy products. Firstly, the chemical characteristics, surface morphology, and suitability of mushroom spent medium in comparison to rubber sawdust (RSD) and growth medium (GM) were examined in order to investigate its potential as a new glucose feedstock. The composition analysis proved that decreases in lignocellulosic contents occurred after cultivation of Pleurotus sajor caju. The amount of lignin, hemicelluloses, and cellulose in MSM showed lower values than those in RSD and GM which were 22.40, 27.93, and 27.97% respectively. The surface morphology of MSM appeared to be rough and broken and traces of hyphen on the fibre surface were observed as a result from the mushroom cultivation process. RSD and FM had an even and smooth flat surface, indicating a rigid and highly ordered surface structure. Preliminary study on effectiveness and feasibility of the three pretreatment techniques (autoclaving at 121°C, heating in water bath, soaking at room temperature) with different NaOH concentration on RSD, GM, and MSM revealed the best result of 30.13 g glucose/100g dry substrate and 33.50% of hydrolysis weight decrease obtained from MSM, which had been treated in water bath at 90°C for 2 h. The scanning electron microscope (SEM) images of the corresponding MSM hydrolysate showed severe disruptions of biomass structure, irregular cracks, and pores. One factor at a time (OFAT) method was applied to screen the range of parameters in NaOH pretreatment via heating in water bath and enzymatic saccharification. With known parameters' range, the Response surface methodology (RSM) based on Box-Behnken Design (BBD) was adopted to optimize the conditions of NaOH pretreatment via heating in water bath method and enzymatic saccharification of MSM. The optimum conditions of MSM pretreatment at substrate loading of 5.0% (w/v) were found to be NaOH concentration of 2.63 M, reaction temperature of 92.26°C, and treatment time of 112.92 min with maximum glucose yield of 34.55 g/100g dry substrate after 48 h of enzymatic saccharification at constant enzyme loading of 67 FPU/g dry substrate and substrate loading of 1.0% (w/v). The MSM hydrolysate obtained under optimal NaOH pretreatment conditions were further used to optimize enzymatic saccharification conditions at constant substrate loading of 1.0% (w/v). Under optimized conditions (agitation rate of 150.74 rpm, enzyme loading of 94.92 FPU/g substrate, and hydrolysis time of 56.89 h), a maximum glucose yield of 71.21 g/100 g dry substrate was achieved. The Analysis of Variance (ANOVA) test revealed that the model and all independent parameters were considered statistically significant at 95% for both optimization studies using the second order polynomial equation. The model validation showed a good agreement between experimental results and the predicted responses. Therefore the models could be successfully used to identify the effective combinations of the three different factors in both optimization studies for predicting the glucose yield from MSM.

CHAPTER 1

INTRODUCTION

1.1 Overview

Glucose from sugar crops (sugarcane, sugar beets) and starchy food (potato, cassava, corn) can be converted into ethanol via fermentation process with the assist of microbes or enzymes. However, concerns about its production and use related to the increased food prices due to the large amount of arable land required for crops, as well as the energy and pollution balance of the whole cycle of ethanol production has caused a new source of glucose being introduced such as lignocellulosic biomass that may allay these concerns. Concomitantly, over the last decades, research efforts have been devoted to converting the promising feedstock of lignocellulosic biomass into biofuel, especially residues from agricultural and forestry operations due to its great availability, sustainability, and low cost compared to other energy feedstock (Pan et al., 2005).

The rubber tree or scientific name "Hevea brasiliensis" is one of the major agricultural crops grown in Malaysia besides oil palm, cocoa, rice, and coconut. The trees are logged off after 25 to 30 years and utilized mainly for making furnitures. As a result of logging and lumber processes, large amount of residual biomass were generated, which have no significant value except its usage in the making of products like briquetted fuel and compressed powder boards (Srinivasakannan & Bakar, 2004). However, due to its high cellulose content, rubber wood waste represents a potential raw material for bioethanol production (Alhassan, 2010).

Apart from its potential as bioethanol feedstock, rubberwood sawdust maybe used to cultivate oyster mushroom and then, further used the remaining medium wastes to produce glucose for bioethanol production. Extra income could be generated and necessary pretreatment steps could be reduced prior to the enzymatic hydrolysis process with high reducing sugars yield via the aforementioned processes. According to previous study, lignocellulosic biomass, which has been biologically treated by fungi have been proven to increase the sugar produced during enzymatic saccharafication and lower lignin contents was detected as a result from the fully/partially digested lignocellulosic materials. Wan and Li (2010) has reported that, with the result of selective delignification, enzymatic digestibility as high as 60-80% has been obtained from fungal-pretreated corn stover, which was comparable to that obtained from chemical pretreatment. Unlike biologically pretreated biomass, the raw lignocellulosic biomass could not be saccharified by enzymes for high glucose yield without first undergoing several pretreatment including, physical, mechanical, and chemical pretreatment. The main cause for the recalcitrance of lignocellulosic biomass is the presence of lignin and hemicelluloses on the surface of cellulose. They formed a barrier and prevented cellulase from accessing the cellulose in the substrate (Koshy & Nambisan, 2012). The pretreatment of lignocellulosics was primarily employed to increase the accessible surface area of cellulose to enhance the conversion of cellulose to glucose in enzymatic saccharification.

Pretreatment of lignocellulosic materials was considered as the rate-limiting step in an economically feasible process for enzymatic hydrolysis of cellulose. Combination of biological and chemical pretreatment was expected to increase the cellulose amount, which could be feasibly accessed by cellulase in subsequent enzymatic saccharification,