OPTIMIZATION OF BIOETHANOL PRODUCTION FROM OIL PALM TRUNK SAP

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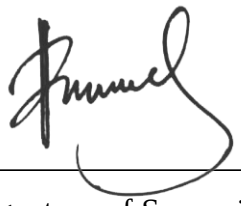

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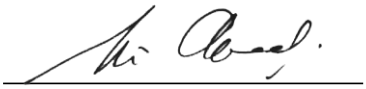

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TABLE OF CONTENTS

CHAPTER 3 MATERIALS AND METHODS

CHAPTER 4 RESULTS AND DISCUSSION

CHAPTER 5 CONCLUSION AND RECOMMENDATION

LIST OF TABLES

LIST OF FIGURES

LIST OF SYMBOLS

°C	degree celcius
$g.g^{-1}$	gram per gram
g/l	gram per liter
g/l.h	gram per liter per hour
ha	hectare
h	hour
l	liter
min	minute
nm	nanometer
rpm	revolution per minute
v/v	volume per volume
w/v	weight per volume

LIST OF ABBREVIATIONS

ANOVA	Analysis of variance
ATCC	American Type Culture Collection
BBD	Box-Behnken Design
CDW	Cell dry weight
DM	Dried matter
EFB	Empty fruit bunch
FFD	Full factorial design
GC-FID	Gas chromatography flame ionized detector
HPLC	High performance liquid chromatography
JCM	Japan Collection of Microorganism
KOS	*Saccharomyces cerevisiae* Kyokai no.7 and *Pichia stipitis*
MAS	*Saccharomyces cerevisiae* (local) and *Pichia stipitis*
OD	Optical density
OFAT	One-factor-at-a-time
OPT	Oil palm trunk
PBD	Plackett-Burman Design
RSM	Response surface methodology
TRS	Total reducing sugar

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ABSTRACT

Oil palm trunk (OPT) sap was generated from replantation of oil palm. As OPT was generally left at the oil palm plantation for nutrient recycling, research could be conducted to utilize OPT sap as the raw material for bioethanol production. OPT was chopped and pressed through a pressing machine to produce OPT sap, and then the sap was further characterized to determine its suitability as fermentation substrate.

The suitability of the OPT sap for bioethanol production was tested by investigating the relationship between temperature and shaking condition towards the fermentation of OPT sap using different microorganisms. At 96 h of fermentation in 30 °C, the highest ethanol yield was obtained by *S. cerevisiae* Kyokai no.7 in static condition which is 0.485 g/g and 94.92% of sugar conversion to bioethanol. This was followed by mixed culture of *S. cerevisiae* Kyokai no.7 and *Pichia stipitis* at 96 h (0.484 g/g and 94.69%).

Further investigation on the microorganism kinetic parameter was done. Monod equation and Hinshelwood model were used to relate the specific growth to the concentration of the limiting substrate and simulate bioethanol production rate. Among these three treatments, single *S. cerevisiae* Kyokai no. 7 microorganism produced the highest ethanol yield of 0.477 g/l.h within the shortest time (12 h).

Optimization of bioethanol production from OPT sap was performed using statistical methodology. Selection of the most important factor (10 factors) was done by using Plackett-Burman design (PBD) and it was found that initial pH, peptone and corn steep liquor (CSL) concentration have high influence towards bioethanol yield from OPT sap. Steepest-ascent experimental plan was performed to find suitable curvature of the experimental region. Selected factors were optimized using Box-Behnken design (BBD) at the identified region and it was predicted that the maximum ethanol yield of 0.5406 g/g can be obtained using OPT sap medium consisting of initial pH of 6.50, peptone 6.80 g/l and CSL 13.28 g/l. These conditions were validated experimentally. The predicted result after the optimization was in good agreement with the experimental data. The maximum bioethanol concentration of 0.480-0.500 g/g was obtained experimentally in the optimum condition which was 90.55-93% of the value predicted by *Design Expert* software.

Average bioethanol concentration of 30 g/l was obtained in repeated-batch. A kinetic analysis of bioethanol production showed that bioethanol yield and productivity were obtained from optimized OPT sap more stable compared to the corresponding values obtained by using non-optimized condition and by using pure glucose medium. The same condition was validated in 20 liter bioreactor and obtained 0.43-0.45 g/g of ethanol yield. It can be concluded that production of bioethanol from OPT sap as an alternative renewable energy in Malaysia is feasible.

ABSTRAK

Batang pokok kelapa sawit terhasil dari penanaman semula pokok kelapa sawit. Oleh sebab kebiasaannya ia dtinggalkan di kawasan penanaman untuk proses kitar semula nutrien, kajian menggunakan air sap sebagai bahan mentah bagi penghasilan bioetanol boleh dijalankan. Batang pokok kelapa sawit dipotong dan diperah menggunakan mesin pemerah untuk mendapatkan air sap batang pokok kelapa sawit, yang mana ia kemudiannya dikaji kesesuaiannya sebagai bahan fermentasi.

Kesesuaian air sap batang pokok kelapa sawit diuji dengan menyiasat hubungan di antara suhu dan keadaan goncangan terhadap fermentasi bioetanol menggunakan mikroorganisma yang berbeza. Pada tempoh 96 jam fermentasi dengan suhu 30 °C, hasil etanol yang tertinggi dicatatkan oleh *S. cerevisiae* Kyokai no.7 dalam keadaan statik iaitu 0.485 g/g dan 94.92% gula ditukar kepada bioethanol. Ini diikuti oleh campuran *S. cerevisiae* Kyokai no.7 dan *Pichia stipitis* pada 96 jam (0.484 g/g dan 94.69%).

Kajian mendalam terhadap kinetik parameter mikroorganisma juga telah dilakukan. Persamaan Monod dan model Hinshelwood telah digunakan untuk mengaitkan pertumbuhan spesifik terhadap had kepekatan substrat, dan juga untuk mensimulasikan kadar penghasilan bioetanol. Antara ketiga-tiga rawatan, mikroorganisma tunggal *S. cerevisiae* Kyokai no. 7 memperolehi keberhasilan etanol sebanyak 0.477 g/l.h dalam masa terpendek (12 h).

Pengoptimuman bioetanol daripada air sap dijalankan menggunakan kaedah statistik. Pemilihan faktor yang penting (10 faktor) menggunakan *Plackett-Burman design* (PBD) mendapati pH permulaan, kepekatan *peptone* dan *corn steep liquor* (CSL) mempunyai pengaruh yang paling besar terhadap penghasilan bioetanol dari sap. Pelan menuruni kecerunan telah digunakan untuk mengenalpasti lekuk yang sesuai sebagai kawasan eksperimen. Faktor-faktor terpilih telah dioptimumkan menggunakan *Box-Benhken design* (BBD) pada kawasan yang dikenalpasti dan diramalkan bahawa jumlah maksimum hasil bioetanol sebanyak 0.5406 g/g mampu diperolehi menggunakan sap yang mengandungi pH permulaan 6.50, kepekatan *peptone* 6.80 g/l dan CSL 13.28 g/l. Kondisi ini disahkan dengan eksperimen. Keputusan jangkaan adalah sejajar dengan keputusan eksperimen yang diperolehi melalui makmal. Kepekatan maksimum bioetanol sebanyak 0.480-0.500 g/g telah diperolehi melalui eksperimen pada kondisi optimum. Kepekatan bioetanol yang diperolehi ni adalah 90.55-93.00 % dari nilai yang telah dijangkakan oleh perisian *Design Expert*.

Purata kepekatan bioetanol sebanyak 30 g/l telah diperolehi dalam setiap kitaran. Analisis kinetik terhadap penghasilan bioetanol menunjukkan keberhasilan etanol dan produktiviti yang diperolehi daripada air sap yang telah dioptimumkan kondisinya adalah lebih stabil berbanding dengan daripada air sap yang tidak dioptimumkan kondisi, dan juga glukosa tulen. Berdasarkan kondisi yang sama yang dijalankan dalam bioreaktor 20 liter telah menghasilkan 0.43-0.45 g/g keberhasilan etanol. Ini dapat disimpulkan bahawa penghasilan bioetanol dari air sap batang kelapa sawit sebagai sumber alternatif tenaga yang boleh diperbaharui di Malaysia adalah realistic.