OPTIMIZATION OF BIOETHANOL FROM OIL PALM FROND JUICE BY USING *Saccharomyces cerevisiae*

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Master of Engineering (Bioprocess)

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SUPERVISOR'S DECLARATION

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 Engineering (Bioprocess).

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STUDENT'S DECLARATION

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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Thesis submitted in fulfillment of the requirements
for the award of the degree of
Master of Engineering (Bioprocess)

Faculty of Chemical & Natural Resources Engineering

UNIVERSITI MALAYSIA PAHANG

OCTOBER 2018

ACKNOWLEDGEMENTS

In the name of Allah, the Most Gracious and the Most Merciful Alhamdulillah, all praises to Allah for the strength, His blessing and opportunity for me to step in the excellent world of science and completing this paper. I am grateful and would like to express my special profound gratitude to my supervisor, Dr. Mior Ahmad Khushairi Mohd Zahari for his great support, patience, germinal ideas, invaluable guidance and continuous support in making this research possible and supervision throughout the thesis. He has always impressed me with positive enthusiasm and constant support in able for me to finish the research. I appreciate his consistent support from the beginning to the end of these concluding moments.

Sincere thanks also goes to my co-supervisor, Dr. Nina Suhaity binti Azmi for her experience and motivation are valuable as guidance to successful research.

My gratitude goes to the community of Felda Lepar Hilir 3, Chini, Pahang for providing me raw material which is oil palm frond and to all my friends for their togetherness, kindness and moral support. Thanks for being with me through all the hardship during the project. Thank You. Appreciation also goes to all staff of laboratory Faculty of Chemical and Natural Resources Engineering (FKKSA) for helping me along to complete this study. I want to thank Universiti Malaysia Pahang (UMP) for providing me the financial support.

My deepest gratitude goes to my beloved parents, Mat Zani bin Mohd Yusof and Norlijah binti Ahsan for their endless love, prayers and continuous support that giving me strength and encouragement to complete my study. To all my family members, all kindness means a lot to me.

Last but not least, I would like to give my special thanks to my husband, Mohd Bukhari bin Salleh, my daughter, Hana Azzahra binti Mohd Bukhari and my son, Umar Yusuf bin Mohd Bukhari whose patient love enabled me to complete this work and inspired to increase great success in my life.

I am indebted to all of you from the bottom of my heart. I am truly grateful for having such good people around me who gave endless support throughout the study even though it takes very challenging time.

ABSTRAK

Dianggap sebagai bahan api cecair yang mesra alam, bioetanol dipercayai boleh menjadi sumber tenaga yang boleh diperbaharui untuk menggantikan bahan api fosil. Penapaian bioetanol telah menjadi minat berterusan dalam kalangan penyelidik untuk mencari kaedah bagi menghasilkan tenaga alternatif melalui penapaian mikrob di mana ianya boleh dihasilkan melalui proses penapaian langsung komponen gula daripada bahan-bahan tumbuhan. Kajian ini memfokuskan kepada penggunaan jus pelepah kelapa sawit (OPF) sebagai sumber mampan bagi menghasilkan produk melalui proses penapaian kerana ia mempunyai kandungan gula yang tinggi dan mudah diperolehi setiap hari di Malaysia. Jus OPF digunakan sebagai sumber karbon untuk pengeluaran bioetanol secara efektif dengan menggunakan yis *Saccharomyces cerevisiae* Kyokai No. 7 (ATCC 26622). Hasil daripada analisis dengan menggunakan Kromatografi Cecair Berprestasi Tinggi (HPLC) menunjukkan bahawa jumlah kepekatan gula di dalam jus OPF yang digunakan di sepanjang kajian ini adalah sebanyak 56.87 g/L. Seterusnya, untuk menentukan keadaan terbaik bagi parameter yang boleh mempengaruhi penghasilan bioetanol, kesan suhu, pH awal media dan kadar putaran telah dikaji dengan menggunakan kaedah Satu Faktor Pada Satu Masa (OFAT) diikuti dengan kajian pengoptimuman proses penapaian bagi penghasilan bioetanol dengan menggunakan kaedah gerak balas permukaan (RSM) untuk meneroka corak tindak balas dan nilai-nilai yang tepat mengenai parameter penapaian. Lima tahap-tiga faktor reka bentuk komposit berpusat (CCD) telahpun dikaji dan titik pusat setiap pemboleh ubah proses telah dipilih berdasarkan keadaan terbaik yang diperolehi daripada kaedah OFAT. Julat parameter yang telah ditetapkan adalah seperti berikut; pH awal media (5-9), suhu (27.5-37.5°C), kadar putaran kelalang goncang (80-120 rpm). Bioetanol dan sisa kepekatan gula telah dianalisa dengan menggunakan peralatan HPLC. Keadaan optimum untuk pengeluaran bioetanol adalah dicadangkan pada pH awal media (6.60), suhu (33°C) dan kadar putaran (96 rpm). Berdasarkan eksperimen validasi, hasil bioetanol pada keadaan optimum adalah 0.50 ± 0.02 g/g gula dan nilai ini adalah hampir sama dengan ramalan model di mana perbezaannya hanyalah 4.17%. Dalam keadaan yang optimum, bioetanol yang diperolehi adalah 47.06% lebih tinggi berbanding dengan keadaan yang tidak optimum. Penghasilan bioetanol dengan menggunakan kaedah berkelompok secara berulang pada skala besar telah dijalankan di dalam bioreaktor 2-L untuk mengkaji prestasi S. cerevisiae bagi jangka masa panjang untuk meniru proses penghasilan bioetanol secara jangka masa panjang pada skala industri. Bagi eksperimen berkelompok secara berulang, keputusan menunjukkan hasil bioetanol yang terhasil adalah paling tinggi pada kadar kelajuan pengaduk 50 rpm, isipadu pengeluaran (larutan penapaian) dan penambahan (jus OPF segar) pada kadar 75% dan sepuluh kitaran kumpulan berulang. Kepekatan bioetanol bagi sepuluh kitaran eksperimen berkelompok berulang pada kadar 75% isipadu pengeluaran (larutan penapaian) dan penambahan (jus OPF segar) adalah 0.41 g/g. Kesimpulannya, kajian ini menunjukkan bahawa jus OPF berpotensi untuk digunakan sebagai sumber bahan mentah penapaian yang boleh diperbaharui dan lengkap untuk penghasilan bioetanol untuk menyokong industri bioteknologi tersebut.

ABSTRACT

Considered to be the environmental-friendly liquid fuel, bioethanol can be a reliable renewable source of energy to substitute fossil fuels. Bioethanol fermentation became an ongoing interest among researcher to find out ways to produce an alternative energy by microbial fermentation in which can be produced by direct fermentation of sugar components of plant materials. This study focused on the utilization of oil palm frond (OPF) juice as sustainable source of fermentation products as it's have high sugar contents and easily obtained daily in Malaysia. OPF juice was used as the carbon source for the effective bioethanol production by the yeast *Saccharomyces cerevisiae* Kyokai No. 7 (ATCC 26622). Based on the sugar analysis using High Performance Liquid Chromatography (HPLC), it showed that the total sugars concentration in OPF juice used throughout the study was 56.87 g/L. In order to further evaluate the best condition of parameters which affecting the production of bioethanol, investigation on the effect of temperature, medium initial pH and agitation speed was carried out by using One-Factor-At-Time (OFAT) method. This was followed by optimizing the fermentation process of bioethanol production using response surface methodology (RSM) to explore the response pattern and accurate values of the fermentation parameters. A five-level-three-factor central composite design (CCD) was attempted in this study and the central point of each process variable was chosen based on the best condition obtained from the OFAT method. The parameters range were set as follows; medium initial pH (5-9), temperature (27.5-37.5°C), and agitation speed (80-120 rpm). Bioethanol and residual sugars concentration were determined by using HPLC analysis. The optimum conditions for bioethanol production were proposed to be medium initial pH (6.60), temperature (33°C) and agitation speed (96 rpm). Based on the validation experiment, the optimum bioethanol yield was 0.50 ±0.02 g/ g sugars and this value was in close agreement with the model prediction where the difference was only 4.17%. Under the optimal conditions, the bioethanol yield obtained was 47.06% higher compared with non-optimized condition. Repeated batch of bioethanol production was carried out at larger scale by using 2-L bioreactor to study the performance of *S. cerevisiae* for long term experiment to mimic a long term industrial bioethanol production process. For the repeated batch experiment, results showed that highest bioethanol yield at agitation speed of 50 rpm, drain (fermentation broth) and fill (fresh OPF juice) volume at 75% and ten cycles of repeated batch. The average bioethanol yield of ten successive batches at 75% drain and fill volume were 0.41 g/ g sugars. As a conclusion, the present research has shown that OPF juice is promising to be used as a renewable and complete fermentation feedstock for bioethanol production to support the biotechnology industry.

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LIST OF SYMBOLS

α	Alpha
DF	Degree of Freedom
P	Bioethanol concentration
Q_p	Bioethanol production
$Y_{p/s}$	Bioethanol yield of the ten-successive cycle
N	Number of factors
2^k	Axial runs
C_o	Central points's run
%	Percentage
g/L	Grams per liter
v/v	Volume per volume
^oC	Degree Celsius
µm	Micro meter
mg	Milligram

LIST OF ABBREVIATIONS

OPF	Oil Palm Frond
OPFj	Oil Palm Frond Juice
P (3HB)	Poly (3-Hydroxybutyrate)
OFAT	One Factor At Time
CCD	Central Composite Design
CO_2	Carbon Dioxide
OPT	Oil Palm Trunk
RSM	Response Surface Methodology
YPD	Yeast Peptone Dextrose
HPLC	High Performance Liquid Chromatography
ANOVA	Analysis of Variance
(BMM1M)Cl	1-butyl-3-methylimidazolium chloride
EHCW	Ethanolic hot compressed water
CP	Crude Protein
EE	Ether Extract
FFB	Fresh Fruit Bunch
PKS	Palm Kernel Shells
MF	Mesocarp Fibre
POME	Palm Oil Mill Effluent
DOE	Design of Experiment
OD	Optical Density
MPOC	Malaysian Palm Oil Council
DE	Entner-Doudoroff
GP	Glyceraldehyde-3-Phosphate-to-Pyruvate
PE	Pyruvate-to-Ethanol
ATP	Adenosine Triphosphate
CO_2	Carbon Dioxide
mRNA	Specific Messenger RNA
CDW	Cell dry weight
H	Hour

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