PRODUCTION OF FERULIC ACID FROM OIL PALM FROND BAGASSE HYDROLYSIS USING MIXED CULTURE FERMENTATION

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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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LIST OF ABBREVIATIONS

ANOVA	Analysis of Variance
BLAST	Basic Local Alignment Search Tool
CCD	Central Composite Design
DNA	Deoxyribunocleic acid
FA	Ferulic acid
FAE	Feruloyl-esterase
FFD	Full Factorial Design
GDP	Gross domestic product
HPLC	High-Performance Liquid Chromatography
LPCB	Lactophenol cotton blue
MPOB	Malaysian Palm Oil Board
MPOC	Malaysian Palm Oil Council
NA	Nutrient agar
NB	Nutrient broth
NCBI	National Centre for Biotechnology Information
NREL	National Renewable Energy Laboratory
OD	Optical density
ODE	Ordinary differential equation
OFAT	One Factor at a Time
OPF	Oil palm frond
OPFB	Oil palm frond bagasse
PCR	Polymerase chain reaction
RK	Runge-Kutta
rRNA	Ribonucleic acid
RSM	Response Surface Methodology

LIST OF SYMBOLS

[*P*]	Product concentration
[*S*]	Substrate concentration
°C	Degree Celsius
µL	Microliter
µm	Micrometer
μ_{max}	Maximum cell growth rate
µmol	Micromoles
3-D	Three dimensional
dP/dt	Rate of product formation
dS/dt	Rate of substrate utilization
g	Gram
h	hour
k	Kilo
K_m	Michaelis constant
K_S	Substrate constant
L	Litre
M	Molar
min	Minute
mL	Millilitre
mM	Millimolar
mol	Mole
pH	Potential Hydrogen
R^2	Coefficient of determination
R_t	Retention time
rpm	Revolutions per minute
V_{max}	Maximum rate of reaction
β_0	Constant coefficient
β_i	Coefficient of the linear parameters

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ABSTRAK

Penghasilan asid ferulik (FA) semakin menjadi fokus penyelidikan pada masa kini kerana kandungan antioksida yang terdapat di dalamnya. Enzim hidrolisis merupakan antara kaedah yang efisien dalam penghasilan FA. Kulat dan bakteria merupakan faktor penting dalam penghasilan FA melalui kaedah enzim hidrolisis kerana kebolehan kedua-dua mikrob ini untuk menghasilkan enzim asid ferulik (FAE). Namun, tidak banyak penyelidikan pada masa kini memberi fokus pada penggunaan kultur campuran sebagai inokulum. Dalam penyelidikan ini, teknik penapaian kultur campuran menggunakan hampas pelepah kelapa sawit (OPFB) telah dilaksanakan bagi meningkatkan penghasilan FA. Kultur campuran diperoleh dari tanah ladang kelapa sawit. Kultur campuran telah dibiarkan menyesuai dengan substrat OPFB sebelum digunakan dalam penyelidikan ini. Isi kandungan kultur campuran telah dikenalpasti bagi menentukan mikroorganisma yang telah memberi kesan pada penghasilan FA. Dua bakteria dikenalpasti sebagai penyumbang kepada penghasilan FA iaitu *Bacillus cereus* CCM2010 dan *B. thuringiensis* ATCC 10792. Kedua-dua bakteria ini pernah dilaporkan mempunyai keupayaan untuk menghasilkan FAE. Penghasilan FA telah dicapai dengan menggunakan teknik penapaian tenggelam. Sampel yang diperolehi kemudiannya dianalisis menggunakan kromatografi cecair berprestasi tinggi (HPLC) bagi menentukan kandungan FA. Pengoptimuman penghasilan FA menggunakan kultur campuran telah dilaksanakan dengan menggunakan kaedah gerak balas permukaan. Keadaan optimum bagi penghasilan FA daripada OPFB menggunakan kultur campuran telah ditentukan pada suhu 26°C, pH 9, kadar putaran pada 150 rpm, 1 hari tempoh penapaian dan 2% isi padu inokulum. Nilai optimum bagi penghasilan FA ialah sebanyak 205.724 mg FA/kg OPFB dengan ralat sebanyak 8.413%. Kajian kinetik juga telah dilaksanakan sebagai proses seterusnya dengan menggunakan model kinetik Michaelis-Menten sebagai persamaan kinetik rujukan. Tiga pemalar kinetik, iaitu V_{max}, K_m dan K_s telah masing-masing dilaporkan sebagai 3.725×10^{-3} $gL^{-1}j^{-1}$, 28.231 gL^{-1} and 0.013 j^{-1} dengan menggunakan kaedah Runge-Kutta Turutan Keempat. Mekanisme FAE merupakan proses yang dikaji seterusnya. Aktiviti maksimum FAE telah diperoleh pada jam ke-28 semasa proses penapaian dengan jumlah aktiviti sebanyak 3.7 x 10-3 mU/mL. Analisis dari kajian mekanisme ini membuktikan bahawa hidrolisis enzim telah berlaku semasa proses penapaian. Hasil kajian ini adalah selari dengan hasil penyelidikan lain yang melaporkan bahawa hidrolisis enzim bertanggungjawab ke atas pemecahan dinding sel OPFB semasa proses penghasilan FA. Hasil daripada penyelidikan ini menunjukkan bahawa penggunaan kultur campuran mempunyai potensi tersendiri dalam merangsang hidrolisis enzim bagi meningkatkan penghasilan FA daripada OPFB semasa proses penapaian.

ABSTRACT

Ferulic acid (FA) production has become a frequent focus on today's research due to its antioxidant properties. Enzymatic hydrolysis stands as one of the most efficient method in producing FA. Bacteria and fungi proved to be crucial in enzymatic hydrolysis of FA as they possess the ability to release ferulic acid esterase (FAE). However, there has been little to none studies reported on the usage of mixed culture as inoculum. In this study, an appropriate mixed culture fermentation approach using oil palm frond bagasse (OPFB) as substrate was applied for FA production improvement. Mixed culture was obtained from soil of a palm oil plantation. The mixed culture was acclimatized with OPFB substrate before being used in the research. The content of this mixed culture was identified to determine the microorganisms that contributed to the FA production. Two bacteria were discovered to be affecting FA production which were *Bacillus cereus* CCM 2010 and *B. thuringiensis* ATCC 10792. Both bacteria are reported to possess the ability to release FAE. The FA production was achieved using submerged fermentation. The enzymatic hydrolysis of FA took place during this process. Samples were analysed using High-Performance Liquid Chromatography (HPLC) to determine the FA content. Optimization of FA production using mixed culture was carried out with response surface methodology. The optimum condition for FA production from OPFB using mixed culture was determined to be at 26°C of temperature, pH value at 9, 150 rpm of agitation speed, 1-day of response time and 2% of inoculum volume. The optimum value of FA produced was 205.724 mg FA/kg OPFB with an error of 8.413%. Kinetic study was conducted as the next step with Michaelis-Menten kinetic model used as a reference kinetic equation. Three kinetic constants, V_{max}, K_m and K_s were determined as 3.725×10^{-3} $gL^{-1}h^{-1}$, 28.231 gL^{-1} and 0.013 h^{-1} respectively using Runge-Kutta Fourth Order approach. The FAE mechanism was also studied in the next step of the process. Maximum FAE activity was achieved at the 28-hour of fermentation process at 3.7×10^{-3} mU/mL. This result proved that enzymatic hydrolysis occurred during fermentation process. This finding is aligned with other studies that suggested enzymatic hydrolysis was responsible for the OPFB cell wall breakdown during FA production. The outcome of this study suggest that the usage of mixed culture has the potential to induce enzymatic hydrolysis hence improving FA production from OPFB during fermentation process.

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