SYNERGISTIC FERULIC ACID PRODUCTION FROM BANANA STEM WASTE BY CO-CULTURE

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DOCTOR OF PHILOSOPHY (CHEMICAL ENGINEERING)

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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 Doctor of Philosophy in Chemical Engineering.

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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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TABLE OF CONTENT

LIST OF TABLES

LIST OF FIGURES

LIST OF SYMBOLS

3-D	Three dimensional
β_0	Constant coefficient
β_i	Coefficient of the linear parameters
dS/dt	Rate of substrate utilization
dP/dt	Rate of product formation
$[S]$	Substrate concentration
V_{max}	Maximum rate of reaction
μ_{max}	Maximum cell growth rate
$[P]$	Product concentration
K_m	Michaelis constant
K_s	Substrate constant
R^2	Coefficient of determination
R_t	Retention time
g	Gram
h	Hour
L	Liter
min	Minute
mL	Milliliter
µm	Micrometer
M	Molar
mM	Milimolar
mol	Mole
µmol	Micromoles
pH	Potential Hydrogen
R^2	Coefficient of determination
%	Percentage
µL	Microliter
°C	Degree Celsius

LIST OF ABBREVIATIONS

ADF	Acid detergent fiber
ADL	Acid detergent lignin
ANOVA	Analysis of variance
BBD	Box-Behnken Design
BLAST	Basic Local Alignment Search Tool
BSG	Brewer's spent grain
BSW	Banana stem waste
CCD	Central composite design
CV	Coefficient of variation
DAD	Diode array detector
DCW	Dry cell weight
DNA	Deoxyribonucleic acid
DO	Dissolved oxygen
DX	Design Expert
FA	Ferulic acid
FAE	Ferulic acid esterase
FFD	Fractional factorial design
GDP	Gross domestic product
GN	Gram negative
GP	Gram positive
HPLC	High performance liquid chromatography
IF-A	Inoculating fluid A
LB	Lineweaver Burk
LCC	Lignin-carbohydrate complex
NA	Nutrient agar
NB	Nutrient broth
NCBI	National Center for Biotechnology Information
NDF	Neutral detergent fiber
NP	Natural product
OD	Optical density
ODE	Ordinary differential equation

OFAT	One factor at a time
PAL	Phenylalanine ammonia lyase
PBD	Plackett-Burman Design
PCR	Polymerase chain reaction
PDE	Partial differential equation
PRESS	Prediction residual error sum of squares
RK	Runge-Kutta
rpm	Revolutions per minute
rRNA	Ribosomal ribonucleic acid
RSM	Response surface methodology
RV	Revolution
SBP	Sugar beet pulp
TAL	Tyrosine ammonia lyase
TLC	Thin layer chromatography
WB	Wheat bran

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ABSTRAK

Penghasilan asid ferulik (FA) menggunakan mikrob kini telah diperluaskan kerana kandungan antioksidan yang terdapat di dalamnya. Kulat dan bakteria telah digunakan dalam penghasilan asik ferulik daripada sisa pertanian. Namun, tidak banyak penyelidikan yang telah dijalankan terhadap bakteria berbanding kulat. Oleh itu, teknik fermentasi yang sesuai oleh ko-kultur bakteria menggunakan sisa batang pisang (BSW) sebagai substrat telah dilaksanakan untuk meningkatkan penghasilan FA. Mikrob daripada tanah telah dipencilkan dan kajian awal dilaksanakan untuk memilih strain bakteria yang terbaik. Setelah itu, 26 jenis ko-kultur bakteria telah dihasilkan dan dinilai keupayaannya berdasarkan kandungan FA yang dihasikan. Pengoptimuman penghasilan FA telah dijalankan menggunakan kaedah gerak balas permukaan menggunakan ko-kultur yang terpilih sebagai inokulum. Kajian kinetik telah dijalankan untuk menentukan pemalar kinetik menggunakan persamaan Michaelis-Menten. Manakala, pengasaian enzim telah dijalankan untuk mengkaji mekanisma penghasilan FA melalui enzim hidrolisis. Eksperimen telah dilaksanakan melalui fermentasi tenggelam dan sampel dianalisis oleh HPLC untuk mengukur kandungan FA. Hasil kajian mendapati bahawa 5 daripada 46 strain bakteria yang dipencil menunjukkan kecekapan dalam membebaskan FA daripada BSW. Namun, penghasilan FA yang paling tinggi telah diperhatikan dalam ko-kultur A (*Bacillus cereus* CCM 2010, *Bacillus pumilus* SAFR-032 dan *Bacillus thuringiensis* Bt407), diikuti oleh ko-kultur B (*Bacillus cereus* CCM 2010, dan *Bacillus thuringiensis* Bt407). Dalam usaha menambah baik proses fermentasi, penyaringan oleh rekabentuk pecahan faktorial telah dijalankan dan hasil yang diperoleh menunjukkan hanya empat faktor termasuk pH, kadar putaran, jenis ko-kultur, dan jumlah inokulum mempunyai kesan yang nyata terhadap penghasilan FA. Selanjutnya, kadar putaran pada 150 rpm dan jumlah inokulum 5 % adalah optimum dalam mempengaruhi peningkatan hasil FA sehingga 510.24 mg/kg dalam tempoh 24 jam menggunakan ko-kultur B sebagai inokulum. Sementara itu, pemalar kinetik V_{max}, K_m dan K_s telah dilaporkan masing-masing sebagai 0.0003 g L^{-1} h^{-1}, 1.636 g L^{-1} dan 0.0095 h^{-1} dengan menggunakan kaedah Runge-Kutta Keempat. Manakala, aktiviti esterase asid ferulik (0.046 mU/mL) adalah maksimum semasa keadaan fasa pegun pertumbuhan ko-kultur. Hasil yang diperoleh membuktikan bahawa hidrolisis enzim telah berlaku dengan menguraikan dinding sel BSW dalam proses membebaskan FA. Penemuan dalam kajian ini mencadangkan bahawa ko-kultur bakteria berupaya merangsang kesan sinergistik dalam meningkatkan penghasilan FA daripada sisa batang pisang semasa proses fermentasi.

ABSTRACT

Ferulic acid (FA) production using microbes has currently become extensive owing to its antioxidant properties. Both fungi and bacteria have been used in producing ferulic acid from agricultural waste. However, less work has been done on bacteria compared to fungi. Therefore, an appropriate bacterial co-culture fermentation technique using banana stem waste (BSW) as substrate was implemented to improve FA production. Soil microbes were isolated and preliminary study was performed to select the best bacterial strain. Subsequently, 26 type of bacterial co-cultures were formed and evaluated their ability dependent on FA production. Optimization of the FA production was conducted by employing response surface methodology using the selected co-culture as inoculum. A kinetic study was carried out to determine kinetic constants using Michaelis-Menten equation. Meanwhile, the enzyme assay was performed to investigate the mechanism release of FA by enzymatic hydrolysis. Ferulic acid production was accomplished through submerged fermentation and the sample was analyzed by HPLC to quantify the FA content. The result found that 5 out of 46 isolated bacterial strains were efficient in releasing FA from BSW. However, the highest production of FA was observed in co-culture A (*Bacillus cereus* CCM 2010, *Bacillus pumilus* SAFR-032 and *Bacillus thuringiensis* Bt407), followed by co-culture B (*Bacillus cereus* CCM 2010, and *Bacillus thuringiensis* Bt407). In improving the fermentation process, screening by fractional factorial design was performed and the result obtained presented four factors including pH, rotation rate, type of co-culture, and volume of inoculum have significant effects on FA production. Furthermore, rotation rate at 150 rpm and volume of inoculum of 5 % were found to be optimum in increasing FA yield up to 510.24 mg/kg within 24 h using bacterial co-culture B as an inoculum. Meanwhile, the kinetic constant of V_{max}, K_m and K_s were reported as 0.0003 g L^{-1} h^{-1}, 1.636 g L^{-1} and 0.0095 h^{-1}, respectively using Runge-Kutta Forth method. Besides, the maximum activity of ferulic acid esterase (0.046 mU/mL) was detected in the stationary phase of co-culture growth. The result proved that the enzymatic hydrolysis was taking place to break down the cell wall of BSW in releasing FA. The finding of this study suggests that co-culture could induce the synergistic work in improving the FA production from banana stem waste during the fermentation process.

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