# PRODUCTION OF FERULIC ACID VIA FERULOYL POLYSACCHARIDE HYDROLYSIS OF BANANA STEM WASTE USING SOIL MIXED CULTURE

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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 (Bio-process Engineering).

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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 of concurrently submitted for any other degree at Universiti Malaysia Pahang or any other institutions.

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Thesis submitted in fulfilment of the requirements

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Dedicated to

My parents, for constantly praying

My supervisor, for endless understanding

My husband, for never once doubting

My children, for evermore loving

My siblings, for always motivating

And to myself

For getting back up and start believing

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# ABSTRAK

Sumber lignoselulosa sangat mudah diperolehi daripada sisa-sisa pertanian terutamanya dari sisa tanaman dan sering dibiarkan mereput di kebun. Sisa tanaman ini bukan hanya boleh digunakan untuk menghasilkan kompaun organic, malah boleh juga digunakan sebagai sumber enzim. Kajian ini menggunakan kultur campuran tanah (SMC) bagi hidrolisis enzimatik *feruloyl-polysaccharide* daripada sisa batang pisang (BSW) untuk menghasilkan asid ferulik (FA). Kaedah analisis multivarian seperti model faktorial penuh (FFD) dan model komposit tengah (CCD) digunakan untuk mengkaji dan mengoptimumkan kesan dan interaksi antara lima faktor iaitu suhu fermentasi (A; 26 – 40 $^{o}$C), pergolakan (B; 0 – 150 rpm), nisbah air kepada BSW (C; 1 – 2 v/v), nisbah substrat kepada inokulum (D; 1 – 2 v/v), dan masa inkubasi (E; 24 – 120 jam). Kemudian, kajian kinetik dijalankan berdasarkan model *Michaelis-Menten* untuk memerhati tindak balas proses. Akhir sekali, lima bakteria dominan dari SMC dikenalpasti dan keupayaan mereka dalam penghasilan FA dibandingkan. Eksperimen fermentasi secara berkelompok dijalankan sebanyak tiga kali berdasarkan nilai parameter yang diperolehi daripada model. Model garis lurus lekap $2^5$ FFD didapati melengkapi nilai korelasi $R^2$ = 0.8019 dengan impak faktor pada kedudukan E > C > A > D > B. Faktor E menyumbang 27.37% impak menunjukkan kepentingan aktiviti pertumbuhan sel, manakala interaksi DE adalah yang tertinggi membuktikan kepentingan masa dalam penggunaan substrat bagi penghasilan FA. Faktor E dan C telah dipilih sebagai pembolehubah untuk $5^2$ CCD berdasarkan kaedah gerak-balas permukaan (RSM) dalam julat nilai diantara 12 sehingga 36 jam dan nisbah 0.5:1 sehingga 1.5:1. $R^2$ model kuadratik didapati melengkapi nilai 0.8068. Seperti yang dijangkakan, interaksi antara dua faktor ini membuktikan bahawa kesan masa lebih tinggi berbanding nisbah air kepada BSW. Hasil maksimum FA pada kondisi optimum 27 jam masa inkubasi dan 1.1:1 nisbah air kepada BSW adalah 1.1657 mg FA/g BSW. Dengan nilai ini, eksperimen baharu dijalankan dengan memanjangkan masa inkubasi sehingga 60 jam dimana hasil FA diperhatikan pada setiap 6 jam untuk membentuk model kinetik *Michaelis-Menten*. Pemalar kinetik $K_s$ (pemalar kadar tindak balas), $K_m$ (pemalar *Michaelis-Menten*) dan $V_{max}$ (kadar halaju maksimum) dari persamaan tindak balas kadar pertama dan model *Michaelis-Menten* telah dikenalpasti melalui jumlah ralat kuasa dua diantara data eksperimen dan teori menggunakan *Excel Solver*. Kepekatan awal biomas [$S_o$] dan hasil FA [$P_o$] adalah 7.4150 g biomas/L dan 1.3710 mg FA/g BSW. Nilai pemalar kinetik adalah $K_s$ = 0.0053 h$^{-1}$; $V_{max}$ = 4.1200x10$^{-5}$ µmol/min; $K_m$ = 0.0500 mmol/L. Nilai $R^2$ yang diperolehi adalah lebih dari 0.800 membuktikan kestabilan sistem. Kemudian, lima bakteria dominan telah dikenalpasti dan keupayaan masing-masing dalam penghasilan FA diperhatikan dimana: *Brevundimonas nasdae* s. W1-2B = 0.4535 mg FA/g BSW; *Pseudomonas monteilii* s. CIP 104883 (B) = 0.7919 mg FA/g BSW; *Pseudomonas monteilii* s. CIP 104883 (C) = 0.8302 mg FA/g BSW; *Lysinibacillus boronitolerans* s. 10a = 0.8249 mg FA/g BSW; dan *Bacillus anthracis* s. *Ames* = 0.8383 mg FA/g BSW. Walaubagaimanapun, hasil FA yang tertinggi dihasilkan dengan menggunakan SMC adalah pada 1.4597 mg FA/g BSW. Oleh itu, dapat disimpulkan bahawa BSW terbukti sangat berguna dan sesuai digunakan untuk menghasilkan produk seperti FA. Tambahan lagi, SMC dapat menjana enzim untuk hidrolisis BSW dalam penghasilan FA dengan lebih efisien.

# ABSTRACT

The source of lignocellulose is abundantly available through agricultural wastes and often discarded in the landfill. These wastes are not just potentially produce organic compound, but also could be the source to generate crude enzyme. This study proposed the used of soil mixed culture (SMC) for feruloyl-polysaccharide hydrolysis from banana stem waste (BSW) to produce ferulic acid (FA). Method used was comprised of multivariate analysis through full factorial design (FFD) and central composite design (CCD) to investigate and optimize the effect and interaction of five factors; fermentation temperature (A; 26 – 40 $^oC$), agitation (B; 0 – 150 rpm), water-to-BSW ratio (C; 1 – 2 v/v), substrate-to-inoculums ratio (D; 1 – 2 v/v), and incubation time (E; 24 – 120 h). Then, a study of kinetic modelling was performed based on Michaelis-Menten model to observe the process behaviour. Lastly, five most dominant bacteria from SMC were identified and later be compared on their ability to produce FA. Series of batch fermentation were conducted at triplicate according to the parameter values of experimental runs generated by the design. A $2^5$ linear model of FFD was well fitted at $R^2$=0.8019 with factors contribution in the order of E > C > A > D > B. Factor E had 27.37% contribution with an optimum range at 12 – 36 h, indicating the significance of cell growth activities, while interaction of DE was highest revealing the importance of sufficient time for substrate utilization to get high FA yield. Parameter E and C were selected as the variables for a $5^2$ CCD based on response surface methodology (RSM) at values range of 12 to 36 h and 0.5:1 to 1.5:1 v/v, respectively. The $R^2$ value for the quadratic model was fitted at 0.8068. Interaction between these factors revealed that effect of time was greater than ratio of water-to-BSW, as expected. The maximum FA produced was 1.1657 mg FA/g BSW at the optimum condition of 27 h incubation time and 1.1:1 water to BSW ratio. With this value, experiments were conducted at a prolonged of 60 h incubation time, where FA yield was observed at every 6 h time interval to develop the study of Michaelis-Menten kinetic model. The kinetic constant, $K_s$ (reaction rate coefficient), $K_m$ (Michaelis-Menten constant) and $V_{max}$ (maximum forward velocity) from first-order reaction equation and Michaelis –Menten model were determined based on the sum squared error between experimental data and theoretical data by using Excel Solver. The initial concentration of biomass [$S_o$] and FA yield [$P_o$] were 7.4150 g biomass/L and 1.3710 mg FA/g BSW, respectively. The values of calculated kinetic parameters were: $K_s = 0.0053\ h^{-1}$; $V_{max} = 4.1200x10^{-5}$ μmol/min; $K_m = 0.0500$ mmol/L. The $R^2$ values obtained from squared error calculation were satisfactorily more than 0.800 which verified the stability of the system. Then, five most stand out pure strains were identified and their performance on FA yield were observed as follows: *Brevundimonas nasdae* s. W1-2B = 0.4535 mg FA/g BSW; *Pseudomonas monteilii* s. CIP 104883 (B) = 0.7919 mg FA/g BSW; *Pseudomonas monteilii* s. CIP 104883 (C) = 0.8302 mg FA/g BSW; *Lysinibacillus boronitolerans* s. 10a = 0.8249 mg FA/g BSW; and *Bacillus anthracis* s. *Ames* = 0.8383 mg FA/g BSW. Meanwhile, using SMC, highest FA yield was 1.4597 mg FA/g BSW. It can be concluded that, BSW was proven to be useful and highly feasible for producing FA, which is useful in pharmaceutical industry. Furthermore, using SMC as the source of inoculum to generate enzymes for hydrolysis of BSW was potentially abled to produce FA as efficiently.

## TABLE OF CONTENT

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