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OPTIMIZATION AND CHARACTERIZATION OF OIL PALM EMPTY FRUIT BUNCH FERMENTATION FOR CELLULASE PRODUCTION BY *Botryosphaeria rhodina* UPM3

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By

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Thesis submitted to the School of Graduate Studies, Universiti Putra Malaysia, in fulfilment of the requirements for the degree of Doctor of Philosophy

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Dedicated to my beloved parents and brothers

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Doctor of Philosophy

OPTIMIZATION AND CHARACTERIZATION OF OIL PALM EMPTY FRUIT BUNCH FERMENTATION FOR CELLULASE PRODUCTION USING *Botryosphaeria rhodina* UPM3

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Chairman : Professor Suraini Abd Aziz, PhD

Faculty : Biotechnology and Biomolecular Sciences

Malaysia is well positioned as the major producers and exporters for palm oil industry worldwide. Hence, oil palm industry is currently producing the largest amount of biomass in Malaysia. In line with the Malaysian government approach to maximize the use of all by-products and waste streams, oil palm empty fruit bunch (OPEFB) is one of potential feedstock for industrial scale since it is abundant and available throughout the year. Integration of 'Waste to Wealth' concept is applicable to the palm oil industry in order reduce all production costs. Value added of oil palm solid waste into useful products such as organic acid, sugars, compost, biogas and enzymes may overcome the waste disposal problem in the mill.

Locally isolated fungus, namely *Botryosphaeria rhodina* UPM3 was found to be the best cellulase producing fungus among six fungi using rapid screening method. Solid state fermentation (SSF) is a strategic approach for bioconversion of lignocellulosic material by filamentous fungus. Results suggested that FPase (2.84 U/g) and CMCase (7.19 U/g) activities reached maximum production on day 3 of SSF. While β-glucosidase (0.09 U/g) indicated high activity on day 6 of SSF. Maximum FPase activity was obtained at the optimum levels of SSF parameters (fungal agar plug, 30°C incubation temperature, 20% initial moisture content, 5.0 g of substrate, initial pH of nutrient at 7.0 and without mixing). The OPEFB particle size of 0.42-0.60 mm contributed to the maximum activity of FPase and β-glucosidase whereas CMCase activity was maximized when 0.84-1.00 mm particle size was used in SSF. High cellulase production at low moisture content (20%) is a very rare condition for fungi cultured in SSF but *B. rhodina* UPM3 was capable to tolerate this condition and give a great advantage for large scale production.

Response surface method was applied in this study to improve the cellulase production from OPEFB by *B. rhodina* UPM3. An experimental design based on two-level factorial was employed to screen the significant environmental factors for cellulase production. From the analysis of variance (ANOVA), initial moisture content, amount of substrate and initial pH of nutrient supplied in the SSF system were significantly influenced the cellulase production. Then, the optimization of the variables was preceded in Central Composite Design (CCD). *B. rhodina* UPM3 exhibited its best performance with a high predicted value of FPase enzyme production (17.95 U/g) when the initial moisture content was 24.32%, initial pH of nutrient was 5.96 and 3.98 g of substrate. The statistical optimization from actual experiment resulted in a significant increment of FPase production from 3.26 to 16.83 U/g (5.16-fold). The model and design on the

optimization of the environmental factors in this study was dependable to predict the cellulase production by *B. rhodina* UPM3.

The enzyme productions under optimized condition of SSF were as follows: FPase (18.48 U/g), CMCase (20.54 U/g), xylanase (22.00 U/g) and β-glucosidase (1.13 U/g). In addition, fermented OPEFB by *B. rhodina* UPM3 was also analyzed and characterized to have a better understanding towards the macroscopic observation in SSF system. SEM micrographs showed a remarkable fungal growth cultivated on OPEFB for day 5 and 7. The craters of OPEFB provide a good anchorage for *B. rhodina* UPM3 mycelia to attach on the substrate. Cellulose (7.78%) and hemicellulose (22.6%) composition were gradually declined throughout the fermentation period. However, lignin content resided in the OPEFB fiber was remaining unchanged until the end of the fermentation. This finding suggested that *B. rhodina* UPM3 was unable to decompose lignin in a short period of time. Degradation of intra- and inter-linkage within lignocellulosic component in OPEFB indicated a vital finding of *B. rhodina* UPM3 capability to decompose these materials during SSF. Overall, OPEFB is one of promising lignocellulosic feedstock and can be employed as substrate in SSF by locally isolated fungus, *B. rhodina* UPM3 in order to produce cellulase enzymes.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

PENGOPTIMUMAN DAN PENCIRIAN FERMENETASI TANDAN KOSONG KELAPA SAWIT UNTUK PENGHASILAN CELLULASE MENGGUNAKAN *Botryosphaeria rhodina* UPM3

Oleh

EZYANA BINTI KAMAL BAHRIN

November 2012

Pengerusi : Profesor Suraini Abd Aziz, PhD

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Malaysia berkedudukan baik sebagai pengeluar dan pengekspot dalam industri kelapa sawit dunia. Maka, industri kelapa sawit adalah pengeluar biomas yang terbesar di Malaysia. Selaras dengan saranan kerajaan Malaysia untuk memaksimakan penggunaan kesemua hasil buangan dan aliran bahan buangan, tandan kosong kelapa sawit (TKKS) merupakan salah satu stok suapan yang berpotensi untuk skala industri kerana ia amat banyak dan boleh didapati sepanjang tahun. Penyepaduan konsep “Sisa ke Kekayaan” boleh diaplikasikan kepada industri kelapa sawit bagi mencapai pembuangan sisa sifar. Penambah baikkan bahan buangan pepejal kepada produk yang lebih berguna seperti asid organik, gula, kompos, biogas dan enzim dapat mengatasi masalah pembuangan sisa di kilang.

Kulat yang telah dipencilkan dari kawasan tempatan dan diberi nama *Botryosphaeria rhodina* UPM3 adalah kulat pengeluar cellulase yang terbaik daripada enam kulat menggunakan kaedah penyaringan pantas. Fermentasi fasa pepejal (FFP) merupakan pendekatan strategik untuk biopenukaran bahan lignosellulose dengan menggunakan kulat berfilamen. Keputusan mencadangkan FPase (2.84 U/g) dan CMCase (7.19 U/g) aktiviti mencapai pengeluaran maksimum pada hari ke-tiga inkubasi. Sementara itu, β-glucosidase (0.09 U/g) menunjukkan aktiviti yang tinggi pada hari ke-enam. Maksimum cellulase aktiviti diperolehi pada tahap optima parameter FFP (kepingan agar kulat, 30°C suhu inkubasi, 20% kandungan kelembapan, 5.0 g substrat pH awal nutrisi pada 7 dan tanpa pengadukan). Saiz partikel TKKS 0.42-0.60 mm menyumbang kepada maksimum aktiviti FPase dan β-glucosidase manakala CMCase aktiviti adalah maksima apabila menggunakan 0.84-1.00 mm saiz partikel dalam FFP. Cellulase yang tinggi pada kelembapan rendah (20%) adalah keadaan yang jarang ditemui bagi kulat yang dikulturkan dalam FFP tetapi *B. rhodina* UPM3 berupaya untuk berdaptasi dengan keadaan tersebut dan ini memberikan satu manfaat yang baik untuk pngeluaran berskala besar.

Kaedah permukaan tindakbalas (KPT) telah digunakan dalam kajian ini adalah untuk reka bentuk eksperimen berdasarkan dua aras faktorial telah diguna pakai untuk menyaring faktor sekitaran yang penting untuk penghasilan cellulase. Daripada analisis variasi (ANOVA), kandungan kelembapan awal, jumlah substrat dan pH nutrisi awal yang dibekalkan pada SSF sistem sangat mempengaruhi penghasilan cellulase. Kemudian, pengoptimasasi pembolehubah diteruskan dengan KPT berdasarkan rekaan komposit pusat (RKP). *B. rhodina* UPM3 menghasilkan nilai ramalan FPase (17.95 U/g)

yang tinggi pada keadaan optimum dengan kandungan kelembapan awal pada 24.32%, pH awal nutrisi pada 5.96 dan 3.98 g substrat. Optimasasi berstatistikal dari eksperimen sebenar menunjukkan peningkatan yang ketara bagi penghasilan FPase daripada 3.26 kepada 16.83 U/g (5.16 kali ganda) Model dan reka bentuk pengoptimuman faktor sekitaran dalam kajian ini boleh dipercayai untuk menjangka penghasilan cellulase oleh *B. rhodina* UPM3.

Penghasilan enzim dalam keadaan optima adalah seperti berikut: FPase (18.48 U/g), CMCase (20.54 U/g), xylanase (22.00 U/g) dan β-glucosidase (1.13 U/g). Tambahan pula, TKKS yang telah difermentasi oleh *B. rhodina* UPM3 juga dianalisa untuk mendapatkan pemahaman yang lebih jelas tentang pemerhatian makroskopik dalam sistem SSF. Mikrograf Imbasan Mikroskop Elektron (IME) menunjukkan pertumbuhan kulat yang menonjol di atas TKKS pada hari ke-5 dan 7. Kawah yang terdapat pada TKKS memberikan tempat pautan yang baik bagi menempatkan *B. rhodina* UPM3 di atas substrat. Komposisi sellulose (7.78%) dan hemisellulose (22.6%) menurun beransur-ansur sepanjang fermentasi berlangsung. Walaubagaimanapun, kandungan lignin dalam TKKS tidak berubah sehingga penghujung fermentasi. Penemuan ini menunjukkan *B. rhodina* UPM3 tidak dapat meleraikan lignin dalam masa yang singkat. Penguraian intra- dan inter rangkaian dalam komponen lignosellulose TKKS menandakan penemuan penting yang menunjukkan *B. rhodina* UPM3 dapat menguraikan semasa FFP. Secara keseluruhan, TKKS adalah salah satu stok suapan lignosellulose yang berpotensi dan boleh dijadikan substrat dalam FFP oleh kulat yang dipencilkan iaitu *B. rhodina* UPM3 untuk menghasilkan enzim cellulase.

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I certify that a Thesis Examination Committee has met on 2 November 2012 to conduct the final examination of Ezyana binti Kamal Bahrin on her thesis entitled "Optimization and Characterization of Oil Palm Empty Fruit Bunch Fermentation for Cellulase Production using *Botryosphaeria rhodina* UPM3" in accordance with Universites andi Universiti College Act 1971 and the Constitution of the Universiti Putra [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the Doctor of Philosophy.

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Date: 26 February 2013

The thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement to the Doctor of Philosophy degree. The members of the Supervisory Committee were as follows:

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DECLARATION

I declare that the thesis is my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously and is not concurrently submitted for any other degree at Universiti Putra Malaysia or at any other institution.

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EZYANA BINTI KAMAL BAHRIN

Date: 2 November 2012

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