UNIVERSITI PUTRA MALAYSIA

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MOHAMAD FAIZAL BIN IBRAHIM

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By

MOHAMAD FAIZAL BIN IBRAHIM

Thesis submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirement for the Degree of Doctor of Philosophy

August 2013

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DEDICATED TO

My Mother and Father
My Brothers and Sisters
All of my family members

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

UTILISATION OF PRETREATED OIL PALM EMPTY FRUIT BUNCH FIBRES AS SUBSTRATE FOR ACETONE–BUTANOL–ETHANOL FERMENTATION BY *Clostridium* spp

By

MOHAMAD FAIZAL BIN IBRAHIM

August 2013

Chairman : Professor Suraini Abd. Aziz, PhD

Faculty : Biotechnology and Biomolecular Sciences

Oil palm empty fruit bunch (OPEFB) is the most abundant lignocellulosic biomass in Malaysia produced by the palm oil industry and can be used as an alternative carbon source for fermentation. This abundant renewable resource of lignocellulosic biomass is not yet well utilised for beneficial and useful product. Since the world now is facing with the limited resource of fossil fuel, a study on the utilisation of this lignocellulosic biomass into production of alternative renewable biofuel is necessary in order to reduce our dependency on fossil fuel. In this work, the conversion of OPEFB fibres into sugar monomers was performed using formulated crude cellulases cocktail prior to ABE fermentation. The crude cellulase cocktail was produced by *Trichoderma asperellum* UPM1 and *Aspergillus fumigatus* UPM2 using OPEFB fibres pretreated with 2% NaOH with autoclave, which were composed of 59.7% of cellulose, 21.6% of hemicellulose and 12.3% of lignin. Approximately 0.8 U/mL of FPase, 24.7 U/mL of CMCase and 5.0 U/mL of β–glucosidase were produced by *T.*

asperellum UPM1 at a temperature of 35°C and at initial pH 7.0. A 1.7 U/mL of FPase, 24.2 U/mL of CMCase, and 1.1 U/mL of β–glucosidase were produced by *A. fumigatus* UPM2 at a temperature of 45°C and at initial pH 6.0. The crude cellulase was best produced at 1.0% of substrate concentration for both *T. asperellum* UPM1 and *A. fumigatus* UPM2. The hydrolysis percentage of pretreated OPEFB fibres using 5% of crude cellulase loading from *T. asperellum* UPM1 and *A. fumigatus* UPM2 were 3.33% and 19.11%, respectively. Formulation of crude cellulases cocktail produced by *T. asperellum* UPM1 and *A. fumigatus* UPM2 improved the hydrolysis percentage to approximately 25.56% using the same enzyme concentration. The maximum hydrolysis percentage achieved was 73% with reducing sugars concentration equivalent to 30 g/L and it was comparable when using commercial cellulase (Celluclast) that produced 31 g/L of reducing sugars.

The two–step process of acetone–butanol–ethanol (ABE) fermentation by local isolate EB6 produced 2.61 g/L of total ABE while *Clostridium acetobutylicum* ATCC 824 produced 2.25 g/L of total ABE using 20 g/L of fermentable sugars derived from pretreated OPEFB fibres. ABE fermentation by *Clostridium butyricum* EB6 was not recommended for butanol production because it produced more ethanol (1.24 g/L) than acetone (0.69 g/L) and butanol (0.68 g/L). *C. acetobutylicum* ATCC 824 produced higher butanol (1.69 g/L) compared to acetone (0.37 g/L) and ethanol (0.19 g/L). A higher total ABE (2.93 g/L) was obtained in a fermentation using 20 g/L of glucose with buffer compared to without buffer that produced 1.34 g/L of total ABE by *C. acetobutylicum* ATCC 824. Approximately 8.77 and 9.15 g/L of total ABE were produced from fermentation using 40 g/L of glucose with and without buffer with butanol concentration of 5.33 and 5.37 g/L, respectively. The study found that by increasing the initial pH values, the formation of acids especially butyric acid

formation were increased. In addition, increased the buffer concentration to 0.2 M at initial pH 6.0 resulted in acids accumulation of 16.83 g/L but reduced the total ABE production to 1.36 g/L. Higher buffer concentrations were not favourable for butanol production but enhanced the formation of other solvents (acetone and ethanol).

Simultaneous saccharification and ABE fermentation (simultaneous process) using pretreated OPEFB fibres as substrate was further conducted in order to reduce the number of steps involved in bioconversion of cellulosic biomass into ABE. In this study, the saccharification process was tested at the condition similar to the ABE fermentation. The fermentable sugars (31.58 g/L) was comparable as in normal saccharification process. The simultaneous process by *C. acetobutylicum* ATCC 824 produced 4.45 g/L of ABE with butanol concentration of 2.75 g/L, while the total sugars consumption was equivalent to 25 g/L. This fermentation generated a butanol yield of 0.11 g/g and ABE yield of 0.18 g/g which were higher than ABE fermentation using 25 g/L of fermentable sugars from pretreated OPEFB fibres (two–step process). The simultaneous process by *C. butyricum* EB6 produced a similar ABE concentration when compared to the two–step process. Furthermore, both *C. acetobutylicum* ATCC 824 and *C. butyricum* EB6 produced more cumulative hydrogen in the simultaneous process compared to the two–step process.

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

PENGGUNAAN FIBER TANDAN KOSONG KELAPA SAWIT TERAWAT SEBAGAI SUBSTRAT UNTUK FERMENTASI ASETON–BUTANOL–ETANOL OLEH *Clostridium* spp

Oleh

MOHAMAD FAIZAL BIN IBRAHIM

Ogos 2013

Pengerusi : Professor Suraini Abd. Aziz, PhD

Fakulti : Bioteknologi dan Sains Biomolekul

Tandan kosong kelapa sawit (TKKS) merupakan biomas lignoselulosa yang paling banyak dihasilkan oleh industri minyak sawit di Malaysia, di mana ianya boleh digunakan sebagai sumber karbon alternatif untuk proses fermentasi. Biomas lignoselulosa ini belum lagi digunakan secara sepenuhnya untuk penghasilan produk tambah yang bernilai. Oleh kerana dunia pada ketika sekarang menghadapi kekurangan bahan api fosil, kajian terhadap penggunaan biomass lignosellulosa untuk penghasilan biotenaga alternatif adalah penting dalam mengurangkan kebergantungan kita terhadap bahan api fosil. Dalam kajian ini, proses penukaran TKKS kepada monomer gula telah dilakukan dengan menggunakan koktail selulase mentah. Selulase mentah telah dihasilkan oleh kulat UPM1 dan *Aspergillus fumigatus* UPM2 menggunakan TKKS yang mengandungi 59.7% selulosa, 21.6% hemiselulosa dan 12.3% lignin setelah dirawat menggunakan 2% NaOH dengan autoklaf. Sebanyak 0.8 U/mL FPase, 24.7 U/mL CMCase dan 5.0 U/mL β–

glucosidase telah dihasilkan oleh kulat *T. asperellum* UPM1 pada suhu 35°C dan pH awal 7.0. Kulat *A. fumigatus* UPM2 menghasilkan sebanyak 1.7 U/mL FPase, 24.2 U/mL CMCase dan 1.1 U/mL β–glucosidase pada suhu 45°C dan pH awal 6.0. Pada kepekatan 1% substrat, kedua–dua kulat *T. asperellum* UPM1 dan *A. fumigatus* UPM2 menghasilkan selulase mentah yang tertinggi. Proses hidrolisis menggunakan 5% selulase mentah menghasilkan peratus hidrolisis sebanyak 3.33% bagi *T. asperellum* UPM1 dan 19.11% bagi *A. fumigatus* UPM2. Proses formulasi selulase koktail mentah daripada *T. asperellum* UPM1 dan *A. fumigatus* UPM2 telah meningkatkan peratus hidrolisis kepada 25.56% menggunakan kepekatan enzim yang sama. Peratus hidrolisis maksimum yang dapat dicapai ialah sebanyak 73% dengan kepekatan gula penurun yang dicatat sebanyak 30 g/L dan ianya adalah setanding apabila dibandingkan dengan selulase komersil (Celluclast) yang menghasilkan 31 g/L gula.

Proses dua-langkah fermentasi aseton–butanol–etanol (ABE) oleh bakteria pencilan tempatan *Clostridium butyricum* EB6 telah menghasilkan 2.61 g/L ABE manakala *Clostridium acetobutylicum* ATCC 824 telah menghasilkan 2.25 g/L ABE dengan menggunakan 20 g/L gula fermentasi dari TKKS terawat. Proses fermentasi ABE oleh *C. butyricum* EB6 tidak disarankan untuk penghasilan butanol kerana ia menghasilkan lebih banyak etanol (1.24 g/L) daripada aseton (0.69 g/L) dan butanol (0.68 g/L). *C. acetobutylicum* ATCC 824 menghasilkan lebih banyak butanol (1.69 g/L) daripada aseton (0.37 g/L) dan etanol (0.19 g/L). Sebanyak 2.93 g/L ABE telah dihasilkan oleh *C. acetobutylicum* ATCC 824 melalui fermentasi menggunakan 20 g/L glukosa dengan penimbal berbanding tanpa penimbal yang menghasilkan hanya 1.34 g/L ABE. Sebanyak 8.77 dan 9.15 g/L ABE telah dihasilkan dari fermentasi menggunakan 40 g/L glukosa, masing–masing dengan penimbal dan tanpa penimbal.

Kajian ini mendapati, peningkatan nilai pH awal akan menyebabkan lebih banyak asid dihasilkan terutamanya asid butirik. Selain itu, peningkatan kepekatan penimbal sehingga 0.2 M pada pH awal 6.0 akan menghasilkan pengumpulan asid sebanyak 16.83 g/L tetapi mengurangkan penghasilan ABE kepada 1.36 g/L. Penimbal dengan kepekatan yang tinggi adalah tidak sesuai untuk penghasilan butanol tetapi meningkatkan penghasilan aseton dan etanol.

Proses sakarifikasi dan fermentasi ABE secara serentak menggunakan TKKS terawat telah dijalankan bagi mengurangkan langkah–langkah dalam proses penukaran selulosa biomas kepada ABE. Kajian ini dijalankan bagi menentukan keberkesanan proses hidrolisis TKKS terawat oleh selulase pada keadaan yang diperlukan untuk menjalankan proses fermentasi ABE, yang mana telah menghasilkan sebanyak 31.58 g/L gula fermentasi. Proses sakarifikasi dan fermentasi ABE secara serentak oleh *C. acetobutylicum* ATCC 824 menghasilkan 4.45 g/L ABE dengan kepekatan butanol sebanyak 2.75 g/L dan mencatatkan penggunaan gula fermentasi adalah sebanyak 25 g/L. Fermentasi ini menghasilkan hasil butanol sebanyak 0.11 g/g dan hasil ABE sebanyak 0.18 g/g, di mana ianya lebih tinggi jika dibandingkan dengan proses dua–langkah fermentasi ABE yang menggunakan 25 g/L gula fermentasi dari TKKS terawat. Proses fermentasi serentak untuk *C. butyricum* EB6 menghasilkan kepekatan ABE yang sama apabila dibandingkan dengan proses fermentasi dua–langkah. Kajian ini juga mendapati, kedua-dua *C. acetobutylicum* ATCC 824 dan *C. butyricum* EB6 menghasilkan lebih banyak gas hidrogen terkumpul dalam proses fermentasi serentak berbanding proses fermentasi dua–langkah.

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I certify that a Thesis Examination Committee has met on 2nd August 2013 to conduct the final examination of Mohamad Faizal bin Ibrahim on his thesis entitled "Utilisation of Pretreated Oil Palm Empty Fruit Bunch Fibres as Substrate for Acetone–Butanol–Ethanol Fermentation by *Clostridium* spp" in accordance with the Universities and University Colleges Act 1971 and the constitution of the Universiti Putra Malaysia [P.U.(A) 106] March 15, 1998. The committee recommends that the candidate be awarded the Doctor of Philosophy.

Members of the Thesis Examination Committee were as follows:

Foo Hooi Ling, PhD
Associate Professor
Faculty of Biotechnology and Biomolecular Sciences
Universiti Putra Malaysia
(Chairman)

Arbakariya Ariff, PhD
Professor
Faculty of Biotechnology and Biomolecular Sciences
Universiti Putra Malaysia
(Internal Examiner)

Mohd. Yunus Abd. Shukor, PhD
Associate Professor
Faculty of Biotechnology and Biomolecular Sciences
Universiti Putra Malaysia
(Internal Examiner)

Wen–Chien Lee, PhD
Professor
National Chung Cheng Universiti
(External Examiner)

NORITAH OMAR, PhD
Associate Professor and Deputy Dean
School of Graduate Studies
Universiti Putra Malaysia

Date: 19 September 2013

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

Suraini Abd. Aziz, PhD
Professor
Faculty of Biotechnology and Biomolecular Sciences
Universiti Putra Malaysia
(Chairman)

Mohd Ali Hassan, PhD
Professor
Faculty of Biotechnology and Biomolecular Sciences
Universiti Putra Malaysia
(Member)

Phang Lai Yee, PhD
Senior Lecturer
Faculty of Biotechnology and Biomolecular Sciences
Universiti Putra Malaysia
(Member)

BUJANG BIN KIM HUAT, PhD
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DECLARATION

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This is to confirm that:

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Signature : _____
Name of Chairman of
Supervisory Committee : **SURAINI ABD. AZIZ, PhD**

Signature : _____
Name of Member of
Supervisory Committee : **MOHD ALI HASSAN, PhD**

Signature : _____
Name of Member of
Supervisory Committee : **PHANG LAI YEE, PhD**

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