UNIVERSITI PUTRA MALAYSIA

UTILIZATION OF *Trichoderma harzianum* SNRS3 FOR SUGAR RECOVERY FROM RICE STRAW FOR ACETONE-BUTANOL-ETHANOL PRODUCTION BY *Clostridium acetobutylicum* ATCC 824

NOOSHIN RAHNAMA

FBSB 2013 12

UTILIZATION OF *Trichoderma harzianum* SNRS3 FOR SUGAR RECOVERY FROM RICE STRAW FOR ACETONE-BUTANOL-ETHANOL PRODUCTION BY *Clostridium acetobutylicum* ATCC 824

By

NOOSHIN RAHNAMA

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfillment of the Requirements for the Degree of Doctor of Philosophy

December 2013

COPYRIGHT

Dedicated to My Beloved Parents

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

UTILIZATION OF *Trichoderma harzianum* SNRS3 FOR SUGAR RECOVERY FROM RICE STRAW FOR ACETONE-BUTANOL-ETHANOL PRODUCTION BY *Clostridium acetobutylicum* ATCC 824

By

NOOSHIN RAHNAMA

December 2013

Chair: Assoc. Prof. Umi Kalsom Md. Shah, PhD

Faculty: Biotechnology and Biomolecular Sciences

Acetone-Butanol-Ethanol (ABE) production from various agricultural residues has been a recent issue of interest for many scientists. However, research carried out on ABE production from rice straw is limited. Pretreatment and hydrolysis required prior to fermentation are costly. Hence, the aims of the study were to produce cellulolytic enzyme complex (FPase, CMCase, and β-glucosidase), and xylanolytic enzyme (xylanase) from untreated rice straw by local *Trichoderma harzianum* SNRS3 and to utilize the crude cellulolytic enzyme complex for sugar production through enzymatic hydrolysis of alkali-pretreated rice straw which was finally used as the substrate for ABE production.

The use of untreated rice straw as the substrate for enzyme production by *T. harzianum* SNRS3 under solid state fermentation resulted in the production of cellulase and xylanase at a higher activity (FPase 6.25 U/g substrate, CMCase 111.31 U/g substrate, β-glucosidase 173.71 U/g substrate, and xylanase 433.75 U/g substrate), as compared to when alkali-pretreated rice straw was used as fermentation substrate (FPase 1.72 U/g substrate, CMCase 23.01 U/g substrate, β-glucosidase 2.18 U/g substrate, and xylanase 45.46 U/g substrate). The results of XRD analysis indicated an increase in relative crystallinity of cellulose due to the hydrolyzation and peeling of amorphous regions during pretreatment. The SEM images showed that the pretreatment process disrupted the hemicelluloses and lignin, which might have caused the changes in the structure of cellulose. According to the results of FTIR analysis, alkali pretreatment caused lignin removal and the changes in the structure of cellulose. In fact, alkali pretreatment of the substrate caused the crystallinity of cellulose to decrease. Absolute crystallinity could most impact cellulase production. However, the overall complexity of the untreated substrate might have actually induced greater enzyme production.

The crude cellulase enzyme produced by *T. harzianum* SNRS3 via solid state fermentation was then characterized. At 60 ºC, β-glucosidase activity was still above 70% of its maximum activity and FPase and CMCase remained active almost up to 100% that could be an advantage for cellulases. FPase and CMCase retained their highest activity in the acidic region over an almost broad pH range that is

considered an advantage for industrial enzymes. At room temperature, FPase almost retained 60% of its original activity at the end of week 3 of storage. Whereas, CMCase retained 60% of its original activity at the end of the 2nd week of storage at room temperature. β-glucosidase activity only decreased to above 80% of its original activity at the end of the 3rd week of storage at room temperature.

Saccharification of alkali-pretreated rice straw was conducted to obtain reducing sugar for use as fermentation substrate. The use of rice straw pretreated with 2% (w/v) NaOH resulted in the production of 5.82 g/L reducing sugar after 72 h of enzymatic hydrolysis. At 5% (w/v) substrate, a reducing sugar yield of 0.6 g/g substrate was obtained that was equivalent to 60.75% saccharification.

Rice straw hydrolysate containing 10 g/L glucose was then utilized as the substrate for ABE production by *Clostridium acetobutylicum* ATCC 824. Rice straw was shown to be a potential substrate for ABE production and a maximum total ABE of 2.73 g/L (0.82 g/L acetone, 1.62 g/L butanol, and 0.29 g/L ethanol) at 72 h was obtained. Fermentation of rice straw hydrolysate resulted in an ABE yield of 0.27 g/ g glucose.

Therefore, untreated rice straw serves as a better substrate than alkali-pretreated rice straw for cellulase production and produced a higher cellulase enzyme activity for the subsequent use in saccharification for production of glucose which finally can be utilized as the substrate for ABE production. The results of this study could contribute to future research on production of cellulolytic enzymes from lignocellulosic agricultural waste including rice straw for their various industrial applications such as their use in biomass-biofuel conversion.

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

PENGGUNAAN *Trichoderma harzianum* SNRS3 BAGI PENGHASILAN GULA DARIPADA JERAMI PADI UNTUK PENGELUARAN ASETON-BUTANOL-ETANOL OLEH *Clostridium acetobutylicum* ATCC 824

Oleh

NOOSHIN RAHNAMA

Disember 2013

Pengerusi : Prof. Madya Umi Kalsom Md Shah, PhD

Fakulti : Bioteknologi dan Sains Biomolekul

Penghasilan Aseton-Butanol-Etanol (ABE) daripada pelbagai sisa pertanian telah menjadi isu yang mendapat perhatian daripada ramai saintis. Walau bagaimanapun, penyelidikan yang dijalankan ke atas penghasilan ABE daripada jerami padi adalah terhad. Kos prarawatan dan hidrolisis yang diperlukan sebelum fermentasi adalah mahal. Oleh itu, tujuan kajian ini dijalankan adalah untuk menghasilkan kompleks enzim selulolitik (FPase, CMCase dan β-glucosidase), dan enzim xylanolitik (xylanase) daripada jerami padi yang tidak dirawat oleh *Trichoderma harzianum* SNRS3 dan menggunakan kompleks enzim selulolitik mentah untuk pengeluaran gula melalui hidrolisis enzim ke atas jerami padi yang telah dirawat dengan alkali yang akhirnya digunakan sebagai substrat untuk penghasilan ABE.

Penggunaan jerami padi yang tidak dirawat sebagai substrat untuk pengeluaran enzim oleh *T. harzianum* SNRS3 melalui fermentasi dalam keadaan pepejal menghasilkan aktiviti selulase dan xylanase yang lebih tinggi (FPase 6.25 U/g substrat, CMCase 111.31 U/g substrat, β-glukosidase 173.71 U/g substrat, dan xylanase 433.75 U/g substrat), berbanding menggunakan jerami padi yang telah dirawat dengan alkali sebagai substrat fermentasi (FPase 1.72 U/g substrat, CMCase 23.01 U/g substrat, β-glukosidase 2.18 U g substrat /, dan xylanase 45.46 U/g substrat). Keputusan analisis XRD menunjukkan peningkatan dalam penghabluran relatif selulosa disebabkan oleh hidrolisis dan pengupasan kawasan amorfus semasa prarawatan. Imej SEM menunjukkan bahawa proses prarawatan telah mengganggu hemiselulosa dan lignin, yang menyebabkan perubahan dalam struktur selulosa. Menurut keputusan analisis FTIR, prarawatan alkali menyebabkan penyingkiran lignin dan perubahan dalam struktur selulosa. Malah, prarawatan substrat dengan alkali menyebabkan penghabluran selulosa berkurangan. Penghabluran mutlak paling memberi kesan kepada pengeluaran selulase. Walau bagaimanapun, kerumitan substrat yang tidak dirawat mungkin mendorong pengeluaran enzim yang lebih tinggi.

Pencirian ke atas selulase mentah yang dihasilkan oleh *T. harzianum* SNRS3 melalui fermentasi dalam keadaan pepejal telah dijalankan. Pada suhu 60˚C, aktiviti β-glucosidase masih melebihi 70% daripada aktiviti maksimum manakala FPase

dan CMCase kekal aktif hampir 100% yang merupakan satu kelebihan untuk enzim sellulase. FPase dan CMCase mengekalkan aktiviti yang tinggi dalam kawasan berasid dengan julat pH yang lebih luas yang dianggap sebagai satu kelebihan untuk industri enzim. Pada suhu bilik, aktiviti Fpase masih di atas separuh hayat dan FPase dapat mengekalkan 60% daripada aktiviti asal pada minggu ke-3. CMCase dapat mengekalkan 60% daripada aktiviti asal pada minggu ke-2 penyimpanan pada suhu bilik. Aktiviti β-glukosidase hanya menurun sebanyak 80% daripada aktiviti asal pada minggu ke-3 penyimpanan pada suhu bilik.

Sakarifikasi jerami padi yang tidak dirawat dan yang telah dirawat telah dijalankan bagi menghasilkan gula penurun untuk digunakan sebagai substrat fermentasi. Penggunaan jerami padi yang telah dirawat dengan 2% (w/v) NaOH dapat menghasilkan 5.82 g/L gula penurun selepas 72 j hidrolisis enzim. Pada kepekatan substrat 5% (w/v), hasil gula penurun sebanyak 0.6 g/g substrat telah diperolehi bersamaan 60.75% pengsakaridaan.

Hidrolisat jerami padi yang mengandungi 10 g/L glukosa kemudian digunakan sebagai substrat untuk pengeluaran ABE oleh *Clostridium acetobutylicum* ATCC 824. Jerami padi terbukti menjadi substrat yang berpotensi untuk pengeluaran ABE dan penghasilan ABE maksimum sebanyak 2.73 g/L (1.62 g/L butanol, 0.82 g/L aseton dan 0.29 g/L ethanol) telah diperolehi oleh *C. acetobutylicum* ATCC 824 selepas 72 j. Fermentasi hidrolisat jerami padi dapat menghasilkan ABE sebanyak 0.27 g/g glukosa.

Oleh itu, jerami padi yang tidak dirawat berfungsi sebagai substrat yang lebih baik berbanding jerami padi yang telah dirawat dengan alkali untuk pengeluaran selulase yang menghasilkan aktiviti enzim selulase lebih tinggi bagi penggunaan berikutnya dalam sakarifikasi bagi pengeluaran glukosa yang akhirnya boleh digunakan sebagai substrat untuk pengeluaran ABE. Hasil kajian ini boleh menyumbang kepada penyelidikan masa depan dalam pengeluaran enzim selulolitik daripada sisa pertanian lignoselulosa termasuk jerami padi untuk pelbagai aplikasi industri seperti penukaran biojisim kepada bahan api secara biologi.

ACKNOWLEDGEMENTS

First and above all, I praise God, the almighty for providing me this opportunity and granting me the capability to proceed successfully. This thesis appears in its current form due to the assistance and guidance of several people. I would therefore like to offer my sincere thanks to all of them.

Foremost, I would like to express my deepest gratitude to my supervisor, Assoc. Prof. Dr. Umi Kalsom Md Shah for the continuous support of my PhD study and research, for her patience, moral support, motivation, enthusiasm, and thoughtful guidance.

I would like to acknowledge my supervisory committee members, Assoc. Prof. Dr. Foo Hooi Ling, Dr. Nor Aini Abdul Rahman, and Prof. Dr. Arbakariya Ariff for their guidance and insightful comments.

I would like to thank all my fellow labmates and friends in MTDC Laboratory who were all kind and friendly to me. My special thanks go to Zuraida and Suhaila who were very kind and always willing to share their knowledge with me. My sincere thanks also go to Firdaus, Atika, Marini, Muhaimin, and Huey Kheng for all their help and kindness to me. I would like to appreciate Mr. Rosli, all the staff and laboratory assistants in Biotechnology Laboratory for all their assistance and guidance.

Last but not the least; I would like to thank my lovely parents for their endless love, prayers, sacrifices, and support throughout my life. I am really grateful to my dearest, lovely sisters for their encouragement, understanding, and support. I am also greatly indebted to my brother and sisters for having taken care of my parents while I was away for my PhD study.

TABLE OF CONTENTS