BIOLOGICAL PRE-TREATMENT OF RUBBER WOOD WITH WHITE ROT FUNGI FOR BIOETHANOL PRODUCTION

FOROUGH KALAEI NAZARPOUR

IB 2011 19

BIOLOGICAL PRE-TREATMENT OF RUBBER WOOD WITH WHITE ROT FUNGI FOR BIOETHANOL PRODUCTION

By

FOROUGH KALAEI NAZARPOUR

Thesis submitted to the School of Graduate Studies, Universiti Putra Malaysia in fulfillment of the Requirements for the Degree of Master of Science

April 2011

A Specially dedication

To Dad and Mom for all their love, care, support, and believe in me; they are the strongest inspiration in my life;

To my brother and sisters for encouragement and understanding;

To my dear Reza for standing by me in every thick and thin of life

To my uncle for instilling the importance of hard work and higher education;

To my nephews and nieces for their presence, that light up my life

Thank you all without you I would not be what I am today

Abstract of thesis presented to the Senate of University Putra Malaysia in fulfillment of the requirements for the degree of Master of Science

BIOLOGICAL PRE-TREATMENT OF RUBBER-WOOD WITH WHITE ROT FUNGI FOR BIOETHANOL PRODUCTION

By

FOROUGH KALAEI NAZARPOUR

April 2011

Chairman: Professor Dzulkefly Kuang Abdullah, PhD

Institute: Institute of Bioscience

In the present study, rubber-wood (*Hevea brasiliensis*) was used as a raw material for bioethanol production. The goal of this study was to investigate the efficiency of biological pretreatment using *Ceriporiopsis subvermispora* ATCC 90467, *Trametes versicolor* ATCC 20869, and a mixed culture of *C. subvermispora* and *T. versicolor* for the conversion of rubber-wood to bioethanol. There are numerous pre-treatment methods but they often lead to the losses of carbohydrate, generate toxic wastes that inhibit enzymatic hydrolysis and consume a lot of energy. In contrast, a biological pre-treatment method using fungi is advantageous because of low energy demand and mild treatment conditions but requires a long treatment time.

Change in chemical composition, structural modification and susceptibility to enzymatic saccharification and ethanol production in the degraded wood were analyzed. Results of this study showed that the selective lignin-degrading fungus *C. subvermispora* had greater selectivity for lignin degradation with the highest lignin and hemicellulose loss at 45.06 % and 42.08 %, respectively after 90 days among the tested samples. Meanwhile the cellulose loss was very low (9.50 %) compared to those of *T. versicolor* and mixed culture. X-ray analysis showed that pretreated samples had a higher crystallinity than untreated samples. The sample pretreated by *C. subvermispora* presented the highest crystallinity of all the samples which might be caused by the selective degradation of amorphous components. Fourier transform infrared (FTIR) spectroscopy demonstrated that the content of lignin and hemicellulose decreased in the biological pre-treatment process. The influence of particle size (0.25, 0.50, and 1.00 mm) on pretreatment effectiveness by *C. subvermispora* was also examined by X-ray and chemical analysis. The rubber-wood with particle size 1 mm was efficiently degraded to provide better aeration/respiration opportunities as compared to smaller particle size of samples. To evaluate the biological pre-treatment, cellulose in the pretreated woods was hydrolyzed using cellulase (Celluclast 1.5 L, produced by *Trichoderma reesei*) and β-glucosidase (Novozyme 188, produced by *Aspergillus niger*) at 50 °C for 168 hours and the released sugars were converted to bioethanol by simultaneous saccharification and fermentation process (SSF) using yeast *Saccharomyces cerevisiae* D5A at 37 °C for 120 hours. A study on hydrolysis of rubber-wood treated with *C. subvermispora*, *T. versicolor*, and mixed culture for 90 days resulted in an increase sugar yield about 27.67 %, 16.23 %, and 14.20 %, respectively as compared to untreated rubber-wood (2.88 %). The sample obtained using the best pretreatment (sample pretreated by *C. subvermispora*) was used for bioethanol production. After 120 hours, the maximum bioethanol concentration and yield were 17.9 g/L and 53 %, respectively. The results obtained demonstrate that white rot fungus *C. subevermispora* is a suitable fungus for improving the enzymatic hydrolysis and bioethanol production of rubber-wood. The results also demonstrated that rubber-wood is a potential raw material for bioethanol production.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagi memenuhi keperluan untuk jiazah Master Sains

PRARAWATAN BIO KAYU GETAH DENGAN CENDAWAN "WHITE ROT" UNTUC PENGHASILAN BIOETANOL

Oleh

FOROUGH KALAEI NAZARPOUR

April 2011

Pengerusi: Profesor Dzulkefly Kuang Abdullah, PhD

Institut: Institut Biosains

Pada masa kini kayu pokok getah (*hevea brasiliensis*) telah digunakan sebagai bahan asas untuk penghasiian bioetanol. Sasaran di dalam kajian ini adalah untuk mengenalpasti keberkesanan pra-rawatan biologi dengan menggunakan *Ceriporiopsis subvermispora* ATCC 90467, *Trametes versicolor* ATCC 20869, dan campuran kultur *C. subvermispora* dan *T. versicolor* untuk menukarkan kayu getah kepada bioetanol. Berbagai kaedah pra-rawatan telah dilaporkan tetapi kebanyakannya menyebabkan kehilangan karbohidrat, mengeluarkan sisa toksid yang boleh merencatkan hidrolisis enzim dan penggunaan tenaga tinggi. Sebaliknya prarawatan biologi menggunakan cendawan mempunyai kelebihan kerana penggunaan tenaga rendah, keadaan pra-rawatan yang lebih lembut kecuali masa rawatan panjang.

Perubahan dari segi kompisisi kimia, perubahan struktur dan penerimaan kepada tindak balas enzim sakarifikasi dan penghasilan bioetanol di dalam penguraian kayu telah dianalisis. Keputusan di dalam kajian ini menunjukkan kulat pengurai lignin iaitu *C. subvermispora* mempunyai kebolehan untuk menguna lignin dan hemiselulosa yang paling tinggi dengan penurunan lignin dan hemiselulosa pada 45.06 % dan 42.08 %, selepas 90 hari berbanding dengan sampel yang diuji, manakala kehilangan selulosa adalah sangat rendah (9.50 %) berbanding dengan *T. versicolor* dan kultur campuran. Analisis X-ray menunjukkan pra-rawat sampel mempunyai pengkristalan yang tinggi berbanding dengan sampel yang tidak dirawat. Sampel yang dirawat dengan menggunakan *C. subvermispora* menunjukkan pengkristalan yang tinggi pada semua sampel yang mungkin disebabkan oleh penguraian terpilih komponen amorfus. Spektroskopi Jelmaan Fourier Inframerah (FTIR) menunjukkan kandungan lignin dan hemiselulosa menurun di dalam proses pra-rawatan. Saiz partikel (0.25, 0.50, and 1.00 mm) mempengaruhi keberkesanan pra-rawatan oleh *C. subvermispora* yang juga diteliti oleh X-ray dan analisis kimia. Kayu-getah dengan saiz partikel 1 mm adalah sangat berkesan dalam penguraian dengan menyediakan pengudaraan/respirasi yang lebih baik berbanding saiz partikel yang lebih kecil. Untuk mengukur pra-rawatan biologi, selulosa didalam kayu yang dirawat telah dihidrolisis dengan menggunakan selulasa (Celluclast 1.5 L, dihasilkan oleh *Trichoderma reesei*) dan β-glukosidase (Novozyme 188, dihasilkan oleh *Aspergillus niger*) pada suhu 50 °C selama 168 jam dan gula yang dihasilkan akan ditukar kepada bioetanol oleh proses sakarifikasi serentak dan proses penapaian menggunakan yis *Saccharomyces cerevisiae* D5A pada 37 °C selama 120 jam. Kajian hidrolisis oleh kayu-getah yang dirawat dengan *C. subvermispora*, *T. versicolor*, dan campuran kultur selama 90 hari menunjukkan kenaikan kandungan gula sebanyak 27.67 %, 16.23 %, dan 14.20 %, berbanding kayu-getah yang tidak dirawat (2.88 %). Sampel diperolehi dengan menggunakan pra-rawatan yang terbaik (sampel pra-rawat oleh *C. subvermispora*) telah digunakan untuk penghasilan bioetanol. Selepas 120 jam, kepekatan dan kandungan etanol yang tertinggi adalah 17.9 g/L dan 53 %. Keputusan diperolehi menunjukkan kulat *C. subevermispora* adalah sesuai untuk meningkatkan hidrolisis dan penghasilan etanol dari kayu-getah. Keputusan juga menunjukkan kayu-getah adalah berpontensi sebagai bahan asas untuk penghasilan bioetanol.

ACKNOWLEDGEMENTS

I would like to express by sincere gratitude to my supervisor, Prof. Dr. Dzulkefly Kuang Abdullah, for his guidance, encouragement and constructive criticisms, which brought to the completion of this thesis. I would also like to thank my co-supervisor, Assoc. Prof. Dr. Nurhafizah bt. Hj. Abdullah, for her valuable comments and critics during my study.

Acknowledgments are also extended to Prof. Dr. Ali Karimi, Dr. Reza Zamiri, Dr. Mehdi Jonoobi, and Mr. Mohammad Faseleh Jahromi for kindly providing information, facilities and materials that are required in this study.

My friends, who are like my sisters, thank you for being part of my life; Samira, Razieh and Nazila. Each of you means a lot to me and will always have a welcome place in my life.

Special appreciations are also extended to my labmates, Mr. Mirsasan, Mr. Farouk, Bahar and Mr. Alhasan for their supports and encouragements.

I would also like to thank Institute of Tropical Forestry and Forest Products, and current staffs working in the industrial biotechnology group in Institute of Bioscience and everyone, although not individually named here, who had contributed directly or indirectly to my project and thesis.

Last but not the least, extremely huge thanks to my family for their endless love and encouragement, without which nothing would have been possible for me to come along the way.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURS

LIST OF ABBREVIATIONS

α	alpha
β	beta
°C	degree centigrade
μL	microlitre (10^{-6} l)
μmole	micromole
AFEX	ammonium fiber explosion
CBU	cellobiase unit
d	day
DI	distilled water
FID	flame ionizing detector
FPU	filter paper unit
FTIR	fourier transform infrared
g	gram
g/l	gram per liter
ha	Hectare
l	liter
LAP	laboratory analytical procedure
mg	milligram (10^{-3} g)
mL	milliliter (10^{-3} L)
mm	millimeter (10^{-3} m)
NREL	national renewable energy laboratory

OD	optical density
PDA	potato dextrose agar media
rpm	rotation per minute
SAS	statistical analysis system
SEM	scanning electron microscopy
SHF	separate hydrolysis and fermentation
SSF	simultaneous saccharification and fermentation
TAPPI	technical association of pulp and paper
USA	United satates of America
UV	ultraviolet
v	volt
v/v	volume per volume
w/v	weight per volume
XRD	X-ray Diffraction
YPD	yeast extract, peptone and dextrose media

CHAPTER 1

INTRODUCTION

1.1 General Background

Researchers in the past couple of decades have studied on renewable sources of liquid fuels to replace fossil fuels. Burning fossil fuels such as coal and oil releases CO_2, which is a major cause of global warming (Kumar *et al.*, 2009). Unlike fossil fuel, bioethanol has the advantages of being renewable, cleaner burning and produces no greenhouse gases (Altintas *et al.*, 2002). At present, corn is the main raw material for bioethanol production in the United States (Kim and Dale, 2004). However, lignocellulosic biomass has the potential to provide a more economical feedstock as a result of its widespread availability, sustainable production and cheaply available (Shi *et al.*, 2008). Rubber-wood is one of the most abundant lignocellulosic materials in Malaysia. Rubber tree (*Hevea brasiliensis*) which is also known as *hevea* wood, is a major industrial crop grown in South East Asia with an estimated plantation area of 1.8 million ha (20 % of global plantation) in Malaysia alone (Srinivasakannan and Zailani Abu Bakar, 2004). Rubber wood can be used as a potential raw material for bioethanol production due to its high cellulose content (Alhasan *et al.*, 2010).

The general approaches for the conversion of lignocelluloses to ethanol include; 1) pre-treatment of lignocellulose materials to remove lignin and open the crystalline structure of cellulose, 2) hydrolysis of cellulose to glucose and 3) microbial fermentation of glucose to ethanol (Sun and Cheng, 2002). Even though challenges exist in the optimization of these steps, the pre-treatment step is considered to be one of the main barriers avoiding commercial success and makes up one third of the total ethanol production costs (McAloon *et al.*, 2000).

Existing pre-treatment methods have largely been developed on the basis of physicochemical technologies such as steam explosion, dilute acid and alkali pre-treatments, and oxidation or various combinations (Mosier *et al.*, 2005). However, typical physical and chemical pre-treatments need high-energy (steam or electricity) as well as corrosion resistant, high pressure reactors, which increase the cost of pre-treatment and requirement for specialty equipments. Additionally, chemical pre-treatments can be harmful to subsequent enzymatic hydrolysis and microbial fermentation apart from producing acidic or alkaline waste water which needs pre-disposal treatment to ensure environmental safety (Keller *et al.*, 2003). In contrast, microbial pre-treatment utilizes microorganisms especially fungi and their enzyme systems to degrade lignin and hemicellulose present in the lignocellulosic biomass. This environment friendly approach has recently received greater attention (Hadar *et al.*, 1993; Camarero *et al.*, 1994; Sawada *et al.*, 1995; Keller *et al.*, 2003; Amirta *et al.*, 2006). In bioethanol production white rot fungi can be employed for biological pre-treatment. Compared to other pretreatment alternatives the fungal treatment is advantageous because of low energy demand and mild treatment conditions but requires a long treatment time (Sun and Cheng, 2002).

White rot fungi are the only microorganisms that are able to efficiently degrade all the components of plant cell walls, both carbohydrates and lignin. There are two types of white rot fungi: simultaneous or nonselective and selective white rot (Blanchette *et al.*, 1985; Messner *et al.*, 2003). The present work employed two white rot fungi individually and mixed culture of both fungi to study the effect of biological pre-treatment on the rubber-wood for ethanol production. *Ceriporiopsis subvermispora* represents the selective degradation of wood that removes lignin preferentially than cellulose. It is the most promising fungi for biopulping (Eriksson *et al.*, 1990; Hatakka A., 2001). It is not only because it has the ability to degrade lignin selectively, but also because it grows on wood aggressively and is suitable for biotreatment of both soft and hardwood (Akhtar *et al.*, 1998; Ferraz *et al.*, 2003). Whereas, *Trametes versicolor* is a typical non selective rot type fungi which degrade cellulose, hemicelluloses and lignin simultaneously (Blanchette, 1991).

As mentioned before, the other general approaches for conversion of lignocellulosic biomass to ethanol are hydrolysis and fermentation. These two steps can be carried out simultaneously in a process known as simultaneous saccharification and fermentation (SSF) (Takagi *et al.*, 1977). SSF has several advantages over separate hydrolysis and fermentation like increase of hydrolysis rate by conversion of sugars that inhibit the cellulase activity. Additionally, SSF reduces contamination risk due to the presence of ethanol and eliminates equipment costs by performing the hydrolysis and fermentation in one reactor (Philippidis *et al.*, 1993; Nigam and Singh, 1995). SSF performs Saccharification by using enzymes instead of chemicals such as acids (Wright, 1988). The optimum temperature for cellulase activity is between 40 and 50 °C, whereas the ethanolgenic yeast *Saccharomyces cerevisiae* cannot exceed 38 °C (Bollók *et al.*, 2000). A number of thermotolerant species of *Saccharomyces*, *Kluyveromyces*, and *Fabospora genera* have been identified that have potential for use in the SSF process at high temperatures (Szczodrak and Targo ski, 1988).

1.2 Objectives

The objectives of this study are as follows;

1. To determine the effects on the physicochemical properties of rubber-wood after biological pre-treatment by white rot fungi (individually and mixed culture).

2. To evaluate the biological pre-treatment using white rot fungi for enzymatic hydrolysis of rubber-wood.
3. To investigate and develop a simultaneous saccharification and fermentation (SSF) method on the treated rubber-wood sample for production of bioethanol.

BIBLIOGRAPHY

Aden, A., Ruth, M., Ibsen, K., Jechura, J., Neeves, K., Sheehan, J., Wallace, B., Montague, L., Slayton, A., and Lukas, J., (2002). Lignocellulosic biomass to ethanol process design and economics utilizing co-current dilute acid prehydrolysis and enzymatic hydrolysis for corn stover.

Adney, B., and Baker, J., (1996). Measurement of cellulase activities. *Laboratory Analytical Procedure*, 6.

Akerholm, M., Hinterstoisser, B., and Salmen, L., (2004). Characterization of the crystalline structure of cellulose using static and dynamic FT-IR spectroscopy. *Carbohydrate research*, 339(3): 569-578.

Akhtar, M., Attridge, M. C., Myers, G. C., and Blanchette, R. A., (1993). Biomechanical pulping of loblolly pine chips with selected white-rot fungi. *Holzforschung International Journal of the Biology, Chemistry, Physics and Technology of Wood*, 47(1): 36-40.

Akhtar, M., Blanchette, R. A., Myers, G., and Kirk, T. K., (1998). An overview of biomechanical pulping research.

Akin, D. E., Rigsby, L. L., Sethuraman, A., Morrison 3rd, W. H., Gamble, G. R., and Eriksson, K. E., (1995). Alterations in structure, chemistry, and biodegradability of grass lignocellulose treated with the white rot fungi Ceriporiopsis subvermispora and Cyathus stercoreus. *Applied and environmental microbiology*, 61(4): 1591.

Akin, D. E., Sethuraman, A., Morrison Iii, W. H., Martin, S. A., and Eriksson, K. E. L., (1993). Microbial delignification with white rot fungi improves forage digestibility. *Applied and environmental microbiology*, 59(12): 4274.

Alemdar, A., and Sain, M., (2008). Biocomposites from wheat straw nanofibers: Morphology, thermal and mechanical properties. *Composites Science and Technology*, 68(2): 557-565.

Alharan, A. M., Kuang, D., Mohammad, A. B., and Sharma-Shivappa, R. R., (2010). Combined effect of nitric acid and sodium hydroxide pretreatments on enzymatic saccharification of rubber wood (Heavea brasiliensis). *Int. J. Chem. Technol*, 2: 12-20.

Altintas, M. M., ÜLgen, K. O., Kirdar, B., ÖNsan, Z. I., and Oliver, S. G., (2002). Improvement of ethanol production from starch by recombinant yeast through manipulation of environmental factors. *Enzyme and microbial technology*, 31(5): 640-647.

Alvira, P., Tomás-Pejó, E., Ballesteros, M., and Negro, M. J., (2009). Pretreatment technologies for an efficient bioethanol production process based on enzymatic hydrolysis: A review. *Bioresource technology*.

Amirta, R., Tanabe, T., Watanabe, T., Honda, Y., and Kuwahara, M., (2006). Methane fermentation of Japanese cedar wood pretreated with a white rot fungus, Ceriporiopsis subvermispora. *Journal of Biotechnology*, 123(1): 71-77.

Amoroso, A., Mancilla, R. A., González, B., and Vicuña, R., (2009). Hydroquinone and H2O2 differentially affect the ultrastructure and expression of ligninolytic genes in the basidiomycete Ceriporiopsis subvermispora. *FEMS Microbiology Letters*, 294(2): 232-238.

Azzam, A. M., (1989). Pretreatment of cane bagasse with alkaline hydrogen peroxide for enzymatic hydrolysis of cellulose and ethanol fermentation. *Journal of Environmental Science and Health, Part B*, 24(4): 421-433.

Bak, J. S., Kim, M. D., Choi, I. G., and Kim, K. H., (2010). Biological pretreatment of rice straw by fermenting with Dichomitus squalens. *New biotechnology*, 27(4): 424-434.

Balat, M., and Balat, H., (2009). Recent trends in global production and utilization of bio-ethanol fuel. *Applied Energy*, 86(11): 2273-2282.

Banerjee, S., Mudliar, S., Sen, R., Giri, B., Satpute, D., Chakrabarti, T., and Pandey, R. A., (2009). Commercializing lignocellulosic bioethanol: technology bottlenecks and possible remedies. *Biofuels, Bioproducts and Biorefining*, 4(1): 77-93.

Béguin, P., and Aubert, J. P., (1994). The biological degradation of cellulose. *FEMS Microbiology Reviews*, 13(1): 25-58.

Blanchette, R. A., (1991). Delignification by wood-decay fungi. *Annual Review of Phytopathology*, 29(1): 381-403.

Blanchette, R. A., (1995). Degradation of the lignocellulose complex in wood. *Canadian Journal of Botany*, 73(S1): 999-1010.

Blanchette, R. A., Otjen, L., Effland, M. J., and Eslyn, W. E., (1985). Changes in structural and chemical components of wood delignified by fungi. *Wood science and technology*, 19(1): 35-46.

Bodîrl u, R., Teac, C. A., and Spiridon, I., (2008). Chemical modification of beech wood: Effect on thermal stability. *BioResources*, 3(3): 789-800.

Bollók, M., Réczey, K., and Zacchi, G., (2000). Simultaneous saccharification and fermentation of steam-pretreated spruce to ethanol. *Applied biochemistry and biotechnology*, 84(1): 69-80.

Bowyer, J. L., Shmulsky, R., and Haygreen, J. G. (2007). Forest products and wood science: an introduction: Wiley-Blackwell.

Brett, C. T., Waldron, K. W., and Waldron, K. (1996). Physiology and biochemistry of plant cell walls: Springer.

Brown, R. C. (2003). Biorenewable resources: engineering new products from agriculture: Wiley-Blackwell.

Camarero, S., Galletti, G. C., and Martinez, A. T., (1994). Preferential degradation of phenolic lignin units by two white rot fungi. *Applied and environmental microbiology*, 60(12): 4509.

Chandra, R., Bura, R., Mabee, W., Berlin, A., Pan, X., and Saddler, J., (2007). Substrate pretreatment: The key to effective enzymatic hydrolysis of lignocellulosics? *Biofuels*: 67-93.

De Vries, R. P., and Visser, J., (2001). Aspergillus enzymes involved in degradation of plant cell wall polysaccharides. *Microbiology and Molecular Biology Reviews*, 65(4): 497.

Demirbas, A., (2008). Biofuels sources, biofuel policy, biofuel economy and global biofuel projections. *Energy Conversion and Management*, 49(8): 2106-2116.

Dowe, N., and McMillan, J., (2001). SSF experimental protocols: lignocellulosic biomass hydrolysis and fermentation. *LAP-008. Golden, CO.: NREL.*

Duff, S. J. B., and Murray, W. D., (1996). Bioconversion of forest products industry waste cellulosics to fuel ethanol: a review. *Bioresource technology*, 55(1): 1-33.

Ehrman, T., (1994). Standard method for determination of total solids in biomass. *Laboratory Analytical Procedure*, 1.

Eriksson, K. E. L., Blanchette, R. A., and Ander, P. (1990). Microbial and enzymatic degradation of wood and wood components: Berlin.

Evans, C. S., and Palmer, J. M., (1983). Ligninolytic activity of Coriolus versicolor. *Microbiology,* 129(7): 2103.

Faix, O., Bremer, J., Schmidt, O., and Tatjana Stevanovic, J., (1991). Monitoring of chemical changes in white-rot degraded beech wood by pyrolysis--gas chromatography and Fourier-transform infrared spectroscopy. *Journal of Analytical and Applied Pyrolysis,* 21(1-2): 147-162.

Fan, L., Lee, Y. H., and Gharpuray, M., (1987). cellulose hydrolysis. *Berlin: Springer-Verlag*: 198 p.

Fan, L. T., Gharpuray, M. M., and Lee, Y. H., (1987). Cellulose hydrolysis. Biotechnology monographs. Volume 3.

Ferraz, A., Córdova, A. M., and Machuca, A., (2003). Wood biodegradation and enzyme production by Ceriporiopsis subvermispora during solid-state fermentation of Eucalyptus grandis. *Enzyme and microbial technology,* 32(1): 59-65.

Ferraz, A., Parra, C., Freer, J., Baeza, J., and Rodriguez, J., (2000). Characterization of white zones produced on Pinus radiata wood chips by Ganoderma australe and Ceriporiopsis subvermispora. *World Journal of Microbiology and Biotechnology,* 16(7): 641-645.

Galbe, M., and Zacchi, G., (2007). Pretreatment of lignocellulosic materials for efficient bioethanol production. *Biofuels*: 41-65.

Genestar, C., and Palou, J., (2006). SEM–FTIR spectroscopic evaluation of deterioration in an historic coffered ceiling. *Analytical and Bioanalytical Chemistry,* 384(4): 987-993.

Gharpuray, M. M., Lee, Y. H., and Fan, L. T., (1983). Structural modification of lignocellulosics by pretreatments to enhance enzymatic hydrolysis. *Biotechnology and bioengineering,* 25(1): 157-172.

Ghose, T. K., (1987). Measurement of cellulase activities. *Pure Appl Chem,* 59(2): 257-268.

Gray, K. A., Zhao, L., and Emptage, M., (2006). Bioethanol. *Current Opinion in Chemical Biology,* 10(2): 141-146.

Hadar, Y., Kerem, Z., and Gorodecki, B., (1993). Biodegradation of lignocellulosic agricultural wastes by Pleurotus ostreatus. *Journal of Biotechnology,* 30(1): 133-139.

Hakala, T. K. (2007). Characterization of the lignin-modifying enzymes of the selective white-rot fungus Physisporinus rivulosus. University of Helsinki.

Hamelinck, C. N., Hooijdonk, G., and Faaij, A. P. C., (2005). Ethanol from lignocellulosic biomass: techno-economic performance in short-, middle-and long-term. *Biomass and Bioenergy,* 28(4): 384-410.

Hatakka, A., (2001). Biodegradation of lignin. *Biopolymers,* 1: 129-180.

Hatakka, A. I., (1983). Pretreatment of wheat straw by white-rot fungi for enzymic saccharification of cellulose. *Applied microbiology and biotechnology,* 18(6): 350-357.

Hendriks, A., and Zeeman, G., (2009). Pretreatments to enhance the digestibility of lignocellulosic biomass. *Bioresource technology,* 100(1): 10-18.

Highley, T. L., Kirk, T. K., and Ibach, R. (1989). *Properties of cellulose degraded by the brown-rot fungus Postia placenta. Document no*: IRG/WP/1350. International Research Group on Wood Preservation, Stockholmo. Document Number).

Howell, C., Paredes, J., Shaler, S., and Jellison, J., (2008). Decay resistance properties of hemicellulose-extracted oriented strand board. *International Research Group on Wood Protection IRG/WP*: 08-10644.

Hu, G., Heitmann, J. A., and Rojas, O. J., (2008). Feedstock pretreatment strategies for producing ethanol from wood, bark, and forest residues. *BioResources,* 3(1): 270-294.

Hult, E. L., Iversen, T., and Sugiyama, J., (2003). Characterization of the supermolecular structure of cellulose in wood pulp fibres. *Cellulose,* 10(2): 103-110.

Itoh, H., Wada, M., Honda, Y., Kuwahara, M., and Watanabe, T., (2003). Bioorganosolve pretreatments for simultaneous saccharification and fermentation of beech wood by ethanolysis and white rot fungi. *Journal of Biotechnology,* 103(3): 273-280.

Jeoh, T., Ishizawa, C. I., Davis, M. F., Himmel, M. E., Adney, W. S., and Johnson, D. K., (2007). Cellulase digestibility of pretreated biomass is limited by cellulose accessibility. *Biotechnology and bioengineering,* 98(1): 112-122.

Kang, K. Y., Jo, B. M., Oh, J. S., and Mansfield, S. D., (2003). The effects of biopulping on chemical and energy consumption during kraft pulping of hybrid poplar. *Wood and Fiber Science,* 35(4): 594-600.

Kasahara, K., Sasaki, H., Donkai, N., and Takagiski, T., (2004). Modification of Tencel with treatment of ferric sodium tartrate complex solution. *Sen'i Gakkaishi,* 60(2): 65-69.

Kaygusuz, K., (2002). Sustainable development of hydropower and biomass energy in Turkey. *Energy Conversion and Management,* 43(8): 1099-1120.

Keller, F. A., Hamilton, J. E., and Nguyen, Q. A., (2003). Microbial pretreatment of biomass. *Applied biochemistry and biotechnology,* 105(1): 27-41.

Keshwani, D. R. (2009). Microwave pretreatment of switchgrass for bioethanol production.

Khalil, H., Ismail, H., Rozman, H. D., and Ahmad, M. N., (2001). The effect of acetylation on interfacial shear strength between plant fibres and various matrices. *European Polymer Journal,* 37(5): 1037-1045.

Kim, S., and Dale, B. E., (2004). Global potential bioethanol production from wasted crops and crop residues. *Biomass and Bioenergy,* 26(4): 361-375.

Kim, S., and Holtzapple, M. T., (2006). Effect of structural features on enzyme digestibility of corn stover. *Bioresource technology,* 97(4): 583-591.

Kleman-Leyer, K., Agosin, E., Conner, A. H., and Kirk, T. K., (1992). Changes in molecular size distribution of cellulose during attack by white rot and brown rot fungi. *Applied and environmental microbiology,* 58(4): 1266.

Kumar, P., Barrett, D. M., Delwiche, M. J., and Stroeve, P., (2009). Methods for pretreatment of lignocellulosic biomass for efficient hydrolysis and biofuel production. *Ind. Eng. Chem. Res,* 48(8): 3713-3729.

Kumar, R., Singh, S., and Singh, O. V., (2008). Bioconversion of lignocellulosic biomass: biochemical and molecular perspectives. *Journal of Industrial Microbiology and Biotechnology,* 35(5): 377-391.

Le Troedec, M., Sedan, D., Peyratout, C., Bonnet, J. P., Smith, A., Guinebretiere, R., Gloaguen, V., and Krausz, P., (2008). Influence of various chemical treatments on the composition and structure of hemp fibres. *Composites Part A: Applied Science and Manufacturing,* 39(3): 514-522.

Lee, J., Gwak, K. S., Park, J. Y., Park, M. J., Choi, D. H., Kwon, M., and Choi, I. G., (2007). Biological pretreatment of softwood Pinus densiflora by three white rot fungi. *Journal of Microbiology,* 45(6): 485-491.

Martínez, Á., Speranza, M., Ruiz-Dueñas, F. J., Ferreira, P., Camarero, S., Guillén, F., Martínez, M. J., Gutiérrez, A., and Río, J. C., (2005). Biodegradation of lignocellulosics: microbial, chemical, and enzymatic aspects of the fungal attack of lignin. *International Microbiology,* 8: 195-204.

McAloon, A., Taylor, F., Yee, W., Ibsen, K., and Wooley, R., (2000). Determining the cost of producing ethanol from corn starch and lignocellulosic feedstocks. *National Renewable Energy Laboratory Report.*

McMillan, J. D. (1994). *Pretreatment of lignocellulosic biomass.*

Messner, K., Fackler, K., Lamaipis, P., Gindl, W., Srebotnik, E., Watanabe, T., Goodell, B., Nicholas, D. D., and Schultz, T. P. (2003). *Overview of white-rot research: where we are today.*

Mooney, C. A., Mansfield, S. D., Touhy, M. G., and Saddler, J. N., (1998). The effect of initial pore volume and lignin content on the enzymatic hydrolysis of softwoods. *Bioresource technology,* 64(2): 113-119.

Mosier, N., Wyman, C., Dale, B., Elander, R., Lee, Y. Y., Holtzapple, M., and Ladisch, M., (2005). Features of promising technologies for pretreatment of lignocellulosic biomass. *Bioresource technology,* 96(6): 673-686.

Mtui, G. Y. S., (2009). Recent advances in pretreatment of lignocellulosic wastes and production of value added products. *African Journal of Biotechnology,* 8(8): 1398-1415.

Nacos, M. K., Katapodis, P., Pappas, C., Daferera, D., Tarantilis, P. A., Christakopoulos, P., and Polissiou, M., (2006). Kenaf xylan-A source of biologically active acidic oligosaccharides. *Carbohydrate Polymers,* 66(1): 126-134.

Nass, L. L., Pereira, P. A. A., and Ellis, D., (2007). Biofuels in Brazil: An Overview.

Neville, A. C. (1993). Biology of fibrous composites: development beyond the cell membrane: Cambridge Univ Pr.

Nigam, P., and Singh, D., (1995). Enzyme and microbial systems involved in starch processing. *Enzyme and microbial technology,* 17(9): 770-778.

Novotn, C., Svobodova, K., Erbanová, P., Cajthaml, T., Kasinath, A., Lang, E., and Sasek, V., (2004). Ligninolytic fungi in bioremediation: extracellular enzyme production and degradation rate. *Soil Biology and Biochemistry,* 36(10): 1545-1551.

Öhgren, K., Bura, R., Saddler, J., and Zacchi, G., (2007). Effect of hemicellulose and lignin removal on enzymatic hydrolysis of steam pretreated corn stover. *Bioresource technology,* 98(13): 2503-2510.

Okano, K., Yuko, I., Samsuri, M., Prasetya, B., Usagawa, T., and Watanabe, T., (2006). Comparison of in vitro digestibility and chemical composition among sugarcane bagasses treated by four white-rot fungi. *Animal Science Journal,* 77(3): 308-313.

Pandey, A., (2003). Solid-state fermentation. *Biochemical Engineering Journal,* 13(2-3): 81-84.

Pandey, K. K., and Nagveni, H. C., (2007). Rapid characterisation of brown and white rot degraded chir pine and rubberwood by FTIR spectroscopy. *European Journal of Wood and Wood Products,* 65(6): 477-481.

Pandey, K. K., and Pitman, A. J., (2003). FTIR studies of the changes in wood chemistry following decay by brown-rot and white-rot fungi. *International Biodeterioration & Biodegradation,* 52(3): 151-160.

Pathan, A. K., Bond, J., and Gaskin, R. E., (2008). Sample preparation for scanning electron microscopy of plant surfaces--Horses for courses. *Micron,* 39(8): 1049-1061.

Percival Zhang, Y. H., Himmel, M. E., and Mielenz, J. R., (2006). Outlook for cellulase improvement: screening and selection strategies. *Biotechnology advances,* 24(5): 452-481.

Pérez, V., Troya, M. T., Martínez, A. T., González-Vila, F. J., Arias, E., and González, A. E., (1993). In vitro decay of Aextoxicon punctatum and Fagus sylvatica woods by white and brown-rot fungi. *Wood science and technology,* 27(4): 295-307.

Philippidis, G. P., Smith, T. K., and Wyman, C. E., (1993). Study of the enzymatic hydrolysis of cellulose for production of fuel ethanol by the simultaneous saccharification and fermentation process. *Biotechnology and bioengineering*, 41(9): 846-853.

Playne, M. J., (1984). Increased digestibility of bagasses by pretreatment with alkalis and steam explosion. *Biotechnology and bioengineering*, 26(5): 426-433.

Redding, A. P. (2009). An Assessment of the Dilute Acid Pretreatment of Coastal Bermudagrass for Bioethanol Production. North Carolina State University.

Reid, I. D., (1989). Optimization of solid-state fermentation for selective delignification of aspen wood with Phlebia tremellosa* 1. *Enzyme and microbial technology*, 11(12): 804-809.

Rowell, R. M., Pettersen, R., Han, J. S., Rowell, J. S., and Tshabalala, M. A., (2005). 3 Cell Wall Chemistry.

Rüttimann, C., Schwember, E., Salas, L., Cullen, D., and Vicuna, R., (1992). Ligninolytic enzymes of the white rot basidiomycetes Phlebia brevispora and Ceriporiopsis subvermispora. *Biotechnology and applied biochemistry*, 16(1): 64-76.

Saha, B. C., (2003). Hemicellulose bioconversion. *Journal of Industrial Microbiology and Biotechnology*, 30(5): 279-291.

Sakakibara, A., (1980). A structural model of softwood lignin. *Wood science and technology*, 14(2): 89-100.

Sánchez, C., (2009). Lignocellulosic residues: Biodegradation and bioconversion by fungi. *Biotechnology advances*, 27(2): 185-194.

Sanchez, O. J., and Cardona, C. A., (2008). Trends in biotechnological production of fuel ethanol from different feedstocks. *Bioresource technology*, 99(13): 5270-5295.

Sanders, J., Scott, E., Weusthuis, R., and Mooibroek, H., (2007). Bio-refinery as the bio-inspired process to bulk chemicals. *Macromolecular bioscience*, 7(2): 105-117.

Sawada, T., Nakamura, Y., Kobayashi, F., Kuwahara, M., and Watanabe, T., (1995). Effects of fungal pretreatment and steam explosion pretreatment on enzymatic saccharification of plant biomass. *Biotechnology and bioengineering,* 48(6): 719-724.

Segal, L., Creely, J. J., Martin Jr, A. E., and Conrad, C. M., (1959). An empirical method for estimating the degree of crystallinity of native cellulose using the X-ray diffractometer. *Textile Research Journal,* 29(10): 786.

Shi, J., Chinn, M. S., and Sharma-Shivappa, R. R., (2008). Microbial pretreatment of cotton stalks by solid state cultivation of Phanerochaete chrysosporium. *Bioresource technology,* 99(14): 6556-6564.

Shrestha, P., Rasmussen, M., Khanal, S. K., Pometto Iii, A. L., and Van Leeuwen, J., (2008). Solid-substrate fermentation of corn fiber by Phanerochaete chrysosporium and subsequent fermentation of hydrolysate into ethanol. *Journal of Agricultural and Food Chemistry,* 56(11): 3918-3924.

Smith, J. E., Heath, L. S., and Jenkins, J. C., (2003). Forest volume-to-biomass models and estimates of mass for live and standing dead trees of US forests. *Notes.*

Srinivasakannan, C., and Zailani Abu Bakar, M., (2004). Production of activated carbon from rubber wood sawdust. *Biomass and Bioenergy,* 27(1): 89-96.

Sternberg, D. (1976). *Production of cellulase by Trichoderma.*

Sun, X. F., Sun, R. C., Tomkinson, J., and Baird, M. S., (2004). Degradation of wheat straw lignin and hemicellulosic polymers by a totally chlorine-free method. *Polymer Degradation and Stability,* 83(1): 47-57.

Sun, Y., and Cheng, J., (2002). Hydrolysis of lignocellulosic materials for ethanol production: a review* 1. *Bioresource technology,* 83(1): 1-11.

Szczodrak, J., and Targo ski, Z., (1988). Selection of thermotolerant yeast strains for simultaneous saccharification and fermentation of cellulose. *Biotechnology and bioengineering,* 31(4): 300-303.

Taherzadeh, M. J., and Karimi, K., (2008). Pretreatment of lignocellulosic wastes to improve ethanol and biogas production: a review. *International Journal of Molecular Sciences,* 9(9): 1621.

Takagi, M., Abe, S., Suzuki, S., Emert, G. H., and Yata, N., (1977). A method for production of alcohol directly from cellulose using cellulase and yeast. *Proceedings of Bioconversion of Cellulosic Substances into Energy, Chemicals and Microbial Protein. New Delhi, India*: 551-571.

Tan, K. T., Lee, K. T., and Mohamed, A. R., (2008). Role of energy policy in renewable energy accomplishment: The case of second-generation bioethanol. *Energy Policy,* 36(9): 3360-3365.

Tanaka, H., Itakura, S., and Enoki, A., (1999). Hydroxyl radical generation by an extracellular low-molecular-weight substance and phenol oxidase activity during wood degradation by the white-rot basidiomycete Trametes versicolor. *Journal of Biotechnology,* 75(1): 57-70.

Taniguchi, M., Suzuki, H., Watanabe, D., Sakai, K., Hoshino, K., and Tanaka, T., (2005). Evaluation of pretreatment with Pleurotus ostreatus for enzymatic hydrolysis of rice straw. *Journal of bioscience and bioengineering,* 100(6): 637-643.

Tarkow, H., and Feist, W. C. (1969). In: a mechanism for improving the digestibility of lignocellulosic materials with dilute alkali and liquid NH3 advance chemistry series 95: American Chemical Society, Washington, DC.

Teramoto, Y., Tanaka, N., Lee, S. H., and Endo, T., (2008). Pretreatment of eucalyptus wood chips for enzymatic saccharification using combined sulfuric acid-free ethanol cooking and ball milling. *Biotechnology and bioengineering,* 99(1): 75-85.

Turner, J. A., (1999). A realizable renewable energy future. *Science,* 285(5428): 687.

Urzua, U., Kersten, P. J., and Vicuna, R., (1998). Manganese peroxidase-dependent oxidation of glyoxylic and oxalic acids synthesized by Ceriporiopsis subvermispora produces extracellular hydrogen peroxide. *Applied and environmental microbiology,* 64(1): 68.

Vincent, J. F., (1999). From cellulose to cell. *Journal of Experimental Biology,* 202(23): 3263-3268.

Vinzant, T. B., Ehrman, C. I., Adney, W. S., Thomas, S. R., and Himmel, M. E., (1997). Simultaneous saccharification and fermentation of pretreated hardwoods. *Applied biochemistry and biotechnology,* 62(1): 99-104.

Wan, C., and Li, Y., (2010). Microbial pretreatment of corn stover with Ceriporiopsis subvermispora for enzymatic hydrolysis and ethanol production. *Bioresource technology.*

Wooley, R., Ruth, M., Glassner, D., and Sheehan, J., (1999). Process design and costing of bioethanol technology: a tool for determining the status and direction of research and development. *Biotechnology Progress,* 15(5): 794-803.

Wright, J. D., (1988). Ethanol from biomass by enzymatic hydrolysis. *Chem. Eng. Prog.;(United States),* 84(8).

Wyman, C. E., (2001). Twenty years of trials, tribulations, and research progress in bioethanol technology. *Applied biochemistry and biotechnology,* 91(1): 5-21.

Wyman, C. E., Dale, B. E., Elander, R. T., Holtzapple, M., Ladisch, M. R., and Lee, Y. Y., (2005). Coordinated development of leading biomass pretreatment technologies. *Bioresource technology,* 96(18): 1959-1966.

Xu, C., Ma, F., Zhang, X., and Chen, S., (2010). Biological Pretreatment of Corn Stover by Irpex lacteus for Enzymatic Hydrolysis. *Journal of Agricultural and Food Chemistry*: 1-11.

Yang, B., and Wyman, C. E., (2008). Pretreatment: the key to unlocking low-cost cellulosic ethanol. *Biofuels, Bioproducts and Biorefining,* 2(1): 26-40.

Yu, H., Guo, G., Zhang, X., Yan, K., and Xu, C., (2009). The effect of biological pretreatment with the selective white-rot fungus Echinodontium taxodii on enzymatic hydrolysis of softwoods and hardwoods. *Bioresource technology,* 100(21): 5170-5175.

Zaldivar, J., Nielsen, J., and Olsson, L., (2001). Fuel ethanol production from lignocellulose: a challenge for metabolic engineering and process integration. *Applied microbiology and biotechnology,* 56(1): 17-34.

Zhang, X., Xu, C., and Wang, H., (2007). Pretreatment of bamboo residues with Coriolus versicolor for enzymatic hydrolysis. *Journal of bioscience and bioengineering,* 104(2): 149-151.

Zhao, X. B., Wang, L., and Liu, D. H., (2008). Technical Note Peracetic acid pretreatment of sugarcane bagasse for enzymatic hydrolysis: a continued work. *Journal of Chemical Technology and Biotechnology,* 83: 950-956.

Zhu, L., O'Dwyer, J. P., Chang, V. S., Granda, C. B., and Holtzapple, M. T., (2008). Structural features affecting biomass enzymatic digestibility. *Bioresource technology,* 99(9): 3817-3828.

Ziegler, J., (2007). Report of the Special Rapporteur on the right to food. *United Nations General Assembly, Sixty-Second Session*.