



UNIVERSITI PUTRA MALAYSIA

**INTEGRATION OF PHYSICO-CHEMICAL AND ENZYMATIC
PRETREATMENTS OF OIL PALM BIOMASS FOR ENHANCEMENT OF
GLUCOSE PRODUCTION**

NUR FATIN ATHIRAH AHMAD RIZAL

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By

NUR FATIN ATHIRAH BINTI AHMAD RIZAL

**Thesis Submitted to the School of Graduate Studies, Universiti
Putra Malaysia, in Fulfilment of the Requirement for the Degree of
Master of Science**

July 2018

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Abstract of thesis presented to the Senate of Universiti Putra Malaysia
in fulfilment of the requirement for the degree of Master of Science

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Faculty : Biotechnology and Biomolecular Sciences

Oil palm empty fruit bunch (OPEFB) and oil palm mesocarp fiber (OPMF) are lignocellulosic biomass that abundantly generated in palm oil mills. However, the presence of these oil palm wastes has created a major disposal problem. Current treatment is either by mulching at the plantation or dumping at side of the mill. Since these materials are rich in carbohydrate the OPEFB and OPMF have been widely reported as suitable raw materials to produce fermentable sugars. However, the presence of lignin and hemicellulose in their composition hinders the access of cellulase to hydrolyze cellulose. Effective pretreatments are required to reduce the recalcitrance of lignocellulosic structures and improve the fermentable sugars production. Combination of physico-chemical and biological pretreatment was proposed to enhance glucose production from OPEFB and OPMF.

Physico-chemical pretreatment using superheated steam (SHS) was employed in this study as it can modify the lignocellulosic materials. Results showed SHS pretreatment alone had increased the percentage of cellulose by 13.4% for OPEFB and 19.4% for OPMF, and reduced hemicelluloses percentage to 18.7% and 21.3%, respectively. However, this SHS pretreatment could only generated 18.4% of glucose yield for OPEFB and 15.6% for OPMF. In order to enhance the glucose yield, combination pretreatments of SHS with laccase has been studied. Study showed that the best laccase loading for OPEFB was 100 U/g-substrate while for OPMF was 400 U/g-substrate. This raw size SHS + laccase pretreatment had enhanced 34.6% and 36.1% of glucose yield for OPEFB and OPMF, respectively. The delignification of OPEFB and OPMF was further improved by reducing the particle size to 2 mm, 1 mm, 0.5 mm and 0.25

mm using a hammer mill after the SHS pretreatment and before treating with laccase. The reduction of size to 0.25 mm had improved the glucose yield by 71.5% for OPEFB and 63.0% for OPMF which is equivalent to 4.6-fold and 4.8-fold increment, respectively as compared to untreated substrates.

To conclude, this study revealed that glucose yield was successfully enhanced by combining SHS with laccase pretreatment together with the size reduction of OPEFB and OPMF.



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sebagai memenuhi keperluan untuk Ijazah Master Sains

**INTEGRASI PRA-RAWATAN FIZIKO-KIMIA DAN ENZIM TERHADAP
BIOJISIM KELAPA SAWIT UNTUK PENINGKATAN PENGHASILAN
GLUKOSA**

Oleh

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Tandan kosong kelapa sawit (TKKS) dan serat mesokarp kelapa sawit (SMKS) merupakan biojisim lignoselulosa yang paling banyak dihasilkan di dalam kilang kelapa sawit. Walaubagaimanapun, kehadiran sisa-sisa kelapa sawit ini telah menyebabkan masalah utama dari segi cara perlupusan. Rawatan terkini adalah dengan membiarkan sisa sebagai sungkupan dan lambakan di kawasan peladangan berdekatan kilang. Oleh kerana sisa-sisa ini kaya dengan karbohidrat, TKKS dan SMKS di laporkan penggunaannya secara meluas sebagai bahan mentah yang sesuai untuk penghasilan gula. Namun begitu, kehadiran lignin dan hemiselulosa di dalam komposisi menghalang kemasukan selulase untuk menghidrolisis selulosa. Pra-rawatan yang berkesan diperlukan untuk mengurangkan keliatan struktur lignoselulosik dan di samping itu meningkatkan penghasilan gula. Penggabungan antara pra-rawatan fiziko-kimia dengan biologi telah di cadangkan untuk meningkatkan penghasilan pengeluaran glukosa dari TKKS dan SMKS.

Pra-rawatan fiziko-kimia menggunakan stim panas lampau (SPL) telah dijalankan dalam kajian ini kerana ia dapat mengubah komposisi bahan lignoselulosa. Kajian menunjukkan bahawa pra-rawatan menggunakan SPL sahaja telah meningkatkan peratusan jisim selulosa sebanyak 13.4% terhadap TKKS dan 19.4% terhadap SMKS dan masing-masing menunjukkan penurunan peratusan hemiselulosa kepada 18.7% dan 21.3%. Walaubagaimanapun, hasil glukosa daripada pra-rawatan SPL hanya sebanyak 18.4% terhadap TKKS dan 15.6% terhadap SMKS. Dalam usaha untuk meningkatkan hasil glukosa, kombinasi pra-rawatan SPL dan pra-rawatan lakase telah dikaji. Kajian menunjukkan bahawa, pemuat enzim

terbaik untuk TKKS adalah 100 U/g-substrat dan untuk SMKS adalah 400 U/g-substrat. Pra-rawatan menggunakan saiz mentah SPL + lakase ini telah meningkatkan hasil glukosa sebanyak 34.6% terhadap TKKS dan 36.1% terhadap SMKS. Delignifikasi TKKS dan SMKS telah ditambah baik dengan mengurangkan saiz kepada 2.0 mm, 1.0 mm, 0.5 mm dan 0.25 mm menggunakan pengisar tukul setelah pra-rawatan SPL dan sebelum pra-rawatan lakase. Pegurangan saiz kepada 0.25 mm telah meningkatkan penghasilan glukosa sebanyak 71.5% terhadap TKKS dan 63.0% terhadap SMKS dengan peningkatan 4.6-lipatan dan 4.8-lipatan berbanding dengan sampel yang tidak dirawat. Kesimpulannya, kajian ini menunjukkan bahawa hasil glukosa telah berjaya dipertingkatkan dengan menggabungkan pra-rawatan menggunakan SPL dan lakase bersama dengan pengurangan saiz TKKS dan SMKS.



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

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LIST OF ABBREVIATIONS

μm	Micrometer
5-HMF	5-hydroxymethylfurfural
AFEX	Ammonia fiber
AIL	Acid insoluble lignin
ASL	Acid soluble lignin
Fe^{3+}	Iron
FFB	Fresh fruit bunch
FPU	Filter paper unit
g	Gram
g/kg	Gram per kilogram
H_2O_2	Hydrogen peroxidase
H_2SO_4	Sulfuric acid
H_3PO_4	Phosphoric acid
HBT	1-Hydroxybenzotriazole hydrate
HCl	Hydrochloric acid
HNO_3	Nitric acid
HPLC	High performance liquid chromatography
kDa	Kilodalton
kV	kilovolt
kW	kilowatt
kWh	Kilowatt hour
LHW	Liquid hot water
LiP	Lignin peroxidase
min	Minutes
mL	Milliliters
mm	millimeter
Mn^{2+}	Manganese
MnP	Manganese peroxidase
MPa	Megapascal pressure unit
NMR	Nuclear magnetic resonance
NREL	National renewable energy laboratory
OPEFB	Oil palm empty fruit bunch
OPF	Oil palm frond
OPKS	Oil palm kernel shell
OPMF	Oil palm mesocarp fiber
OPPF	Oil palm pressed fiber
OPT	Oil palm trunk
POME	Palm oil mill effluent
rpm	Revolutions per unit
SC- CO_2	Supercritical carbon dioxide
SE	Steam explosion
SEM	Scanning electron microscope
SHS	Superheated steam
U/g	Unit per gram

UPM
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VP

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United States of America
Ultraviolet
Ultraviolet-visible
Versatile peroxidase



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CHAPTER 1

INTRODUCTION

Lignocellulosic biomass is the most abundant plant material on Earth, produced mainly from agricultural industry and forestry. Interest on utilizing this lignocellulosic biomass has increasing recently due to its potential to be used as fermentation substrate to be converted into various valuable products (Ibrahim *et al.*, 2017). In Malaysia, oil palm biomass is the most abundant plant materials generated every year since palm oil is the biggest Malaysian agricultural commodity. In 2016, this plantation occupies in total 5.74 million hectares all over Malaysia with 17.32 million tonnes crudes palm oil was produced (Malaysian Palm Oil Board, 2016). Processing of oil palm from fresh fruit bunch (FFB) at the mills generated 7.34 million tonnes of oil palm empty fruit bunch (OPEFB), 7.72 million tonnes oil palm mesocarp fiber (OPMF), 4.46 million tonnes oil palm kernel shell (OPKS) and 64 million tonnes palm oil mill effluent (POME) per year (Loh, 2017). The OPEFB and OPMF which is the most abundant oil palm biomass generated in the mill has not yet been fully utilized. It is either being mulching at plantation or dumping at near the factory for natural degradation. Recently, both materials have been commercialized for biocompost production (Siddiquee *et al.*, 2017), biochar and activated carbon production (Zainal *et al.*, 2017). These materials also have been tested for various fermentation processes including biobutanol (Ibrahim *et al.*, 2013), bioethanol (Abdullah, 2015), biohydrogen (Taifor *et al.*, 2017), biogas (Choong *et al.*, 2017) and many more. However, the major concern while utilizing these biomasses as feedstock for fermentation is the effectiveness of the conversion into fermentable sugars.

OPEFB and OPMF composed of 60-75% and 50-55% of cellulose and hemicelluloses, respectively. These polymers of sugars can be hydrolyzed into sugar monomers which subsequently can be used as substrate for fermentation. These polymers of sugars can be hydrolyzed into sugar monomers, which subsequently can be used as substrate for fermentation. OPEFB and OPMF also composed of lignin that protects cellulose and hemicelluloses and hinders enzymatic hydrolysis by cellulase into sugars. Generally, lignin is the most complex structure and representing about 10-25% of the biomass weight with long chain, heterogenous polymer that composed of mostly phenyl-propane units, linked by ether bonds (Anwar *et al.*, 2014). It has aromatic and rigid biopolymer properties linked via covalent bonds to xylans. This arrangement makes the lignocellulosic structure specifically plant cell wall become rigid and highly compacted.

In order to utilize oil palm biomass as fermentation substrate, suitable and effective pretreatments are required to

reduce recalcitrance of lignocellulosic biomass by extensive modification of its lignocellulosic structure especially lignin. The process can be done using physical, physico-chemical, chemical or biological pretreatment (Alvira *et al.*, 2016; Chandra *et al.*, 2016). Out of these four categories, chemical pretreatment using either alkaline or acid had shown the most effective pretreatment that could generate high sugar yield in a short pretreatment duration (Kshirsagar *et al.*, 2015). However, implementing chemical pretreatment in large scale may cause negative impact towards environment, especially water pollution that exhibit toxicity to the water stream. Therefore, a considerable improvement from the green biotechnology that contributes to lignocellulosic biomass pretreatment has arisen from the last several years. In order to keep the reliability of lignocellulosic biomass as fermentation substrate, combination of chemical-free pretreatments should be explored and proved as effective, clean and feasible for industrial scale.

Physico-chemical is the pretreatment that involved the chemical and physical interactions in the process in order to breakdown the recalcitrance of lignocellulosic materials. Superheated steam (SHS) is one of the physico-chemical pretreatment that has been reported as an effective pretreatment to loosening the structural arrangement of lignocellulosic and make the hydrolysis more efficient (Then *et al.*, 2014; Zakaria *et al.*, 2015b). SHS is a dry steam that produced by adding heat to the wet steam. The additional heat aids in raising the saturated steam temperature to exceed the boiling point of the liquid at certain pressure value (Bahrin *et al.*, 2012). The lignocellulosic material exposed to a high steam temperature of $>180^{\circ}\text{C}$ can degrade the hemicellulose components as it is less thermally stable than lignin and cellulose. Degradation of hemicellulose reduces the recalcitrance of the lignocellulosic material. It should be noted that SHS is safe to be used since it can be conducted at atmospheric pressure with low energy consumption of 3.30 kW and could cause a very little environmental impact if collected condensate is reused (Head *et al.*, 2010; Warid *et al.*, 2016). However, pretreating the lignocellulosic biomass using SHS can only cause a small modification to lignocellulosic network due to limitation of water contact with the fiber, resulted in a low sugar yield after saccharification process (Zakaria *et al.*, 2015a).

Combining biological pretreatment after SHS could improve the whole pretreatment process to produce sugars. Biological pretreatment of lignocellulosic biomass can be carried out by applying microorganism (microbial pretreatment) or ligninolytic enzyme (enzymatic pretreatment) to digest lignin components. Enzymatic pretreatment is faster than microbial pretreatment, hence the process is easier to be controlled. In addition, it requires only mild condition such as low temperature, low energy yet the process is specific to attack lignin only (Ibrahim *et al.*, 2011; Moreno *et al.*, 2016). Ligninolytic enzymes are grouped into oxidases and peroxidases (Masran *et al.*, 2016). Laccase (EC 1.10.3.2; benzenediol: oxygen oxidoreductase) is an oxidizing enzyme that is the most extensively studied for lignocellulosic biomass pretreatment. It is a multicopper oxidase produced by fungi, plants and bacteria to specifically degrade lignin component. The

oxidation of a laccase substrate leads to the formation of free radical and reduction of molecular oxygen into water molecule (Catherine *et al.*, 2016). However, laccase pretreatment alone did not produce a high yield of hydrolyzed sugars (Saha *et al.*, 2016; Zanirun *et al.*, 2015). Therefore, combining this enzymatic pretreatment using laccase with SHS could enhance the saccharification performance of oil palm biomass into sugars. In addition, the effect of size reduction prior to laccase pretreatment was also conducted since the enzyme action is highly affected by the exposed surface area particle size of the substrate.

Therefore, the objective of this research are:

- i. To enhance the glucose production from oil palm empty fruit bunch and oil palm mesocarp fiber through combination of superheated steam and laccase pretreatment.
- ii. To analyze the chemical component of OPEFB and OPMF after the pretreatment
- iii. To study the effect of integration of superheated steam and laccase pretreatment to glucose production during saccharification process of OPEFB and OPMF

REFERENCES

- A. Sluiter, R. Ruiz, C. Scarlata, J. Sluiter, A., Templeton, D., 2008. Determination of Extractives in Biomass: Laboratory Analytical Procedure (LAP); Issue Date 7/17/2005 - 42619.pdf. Tech. Rep. NREL/TP-510-42619 1–9.
- Abdullah, N., Sulaiman, F., 2013. The oil palm wastes in Malaysia. *Biomass Now – Sustain. Growth Use* 75–100.
- Abdullah, S.S.S., 2015. Efficient bioethanol production from oil palm frond juice. *industrial crops and products*. 63. 357-361
- Agbor, V.B., Cicek, N., Sparling, R., Berlin, A., Levin, D.B., 2011. Biomass pretreatment: Fundamentals toward application. *Biotechnol. Adv.* 29, 675–685.
- Aikanathan, S., Basiron, Y., Sundram, K., Chenayah, S., A, S., 2015. *Journal of Oil Palm , Environment & Health Sustainable Management Of Oil Palm Plantation Industry and The Perception*. 6, 10–24.
- Alvira, P., Moreno, A.D., Ibarra, D., Sáez, F., Ballesteros, M., 2013. Improving the fermentation performance of *Saccharomyces Cerevisiae* by laccase during ethanol production from steam-exploded wheat straw at high-substrate loadings. *Biotechnol. Prog.* 29, 74–82.
- Alvira, P., Negro, M.J., Ballesteros, I., González, A., Ballesteros, M., 2016. Steam explosion for wheat straw pretreatment for sugars production. *Bioethanol* 2, 66–75.
- Alvira, P., Tomás-Pejó, E., Ballesteros, M., Negro, M.J., 2010. Pretreatment technologies for an efficient bioethanol production process based on enzymatic hydrolysis: A review. *Bioresour. Technol.* 101, 4851–4861.
- Anwar, Z., Gulfranz, M., Irshad, M., 2014. Agro-industrial lignocellulosic biomass a key to unlock the future bio-energy: A brief review. *J. Radiat. Res. Appl. Sci.* 7, 163–173.
- Asgher, M., Ahmad, Z., Iqbal, H.M.N., 2013. Alkali and enzymatic delignification of sugarcane bagasse to expose cellulose polymers for saccharification and bio-ethanol production. *Ind. Crops Prod.* 44, 488–495.
- Awalludin, M.F., Sulaiman, O., Hashim, R., Nadhari, W.N.A.W., 2015. An overview of the oil palm industry in Malaysia and its waste utilization through thermochemical conversion, specifically via liquefaction. *Renew. Sustain. Energy Rev.* 50, 1469–1484.

- Bahrin, E.K., Baharuddin, A.S., Ibrahim, M.F., Abdul Razak, M.N., Sulaiman, A., Abd-Aziz, S., Hassan, M.A., Shirai, Y., Nishida, H., 2012. Physicochemical property changes and enzymatic hydrolysis enhancement of oil palm empty fruit bunches treated with superheated steam. *BioResources* 7, 1784–1801.
- Binder, J.B., Raines, R.T., 2010. Fermentable sugars by chemical hydrolysis of biomass. *Proc. Natl. Acad. Sci. U. S. A.* 107, 4516–4521.
- Bitra, V.S.P., Womac, R., Chevanan, N., Sokhansanj, S., 2008. Comminution properties of biomass in hammer mill and its particle size characterization. 2008 ASABE Annu. Int. Meet. 300, 22.
- Bourbonnais, R., Paice, M.G., 1988. Veratryl alcohol oxidases from the lignin-degrading basidiomycete *Pleurotus sajor-caju*. *Biochem. J.* 255, 445–450.
- Brodeur, G., Yau, E., Badal, K., Collier, J., Ramachandran, K.B., Ramakrishnan, S., 2011. Chemical and physicochemical pretreatment of lignocellulosic biomass: a review. *Enzyme Res.* 2011, 787532.
- Cañas, A.I., Camarero, S., 2010. Laccases and their natural mediators: Biotechnological tools for sustainable eco-friendly processes. *Biotechnol. Adv.* 28, 694–705.
- Capolupo, L., Faraco, V., 2016. Green methods of lignocellulose pretreatment for biorefinery development. *Appl. Microbiol. Biotechnol.* 100, 9451–9467.
- Castoldi, R., Bracht, A., de Moraes, G.R., Baesso, M.L., Correa, R.C.G., Peralta, R.A., Moreira, R. de F.P.M., Polizeli, M. de L.T. de M., de Souza, C.G.M., Peralta, R.M., 2014. Biological pretreatment of *Eucalyptus grandis* sawdust with white-rot fungi: Study of degradation patterns and saccharification kinetics. *Chem. Eng. J.* 258, 240–246.
- Catherine, H., Frédéric, D., Penninckx, M., 2016. Product formation from phenolic compounds removal by laccases: A review. *Environ. Technol. Innov.* 5, 250–266.
- Chandra, R.P., Chu, Q., Hu, J., Zhong, N., Lin, M., Lee, J.S., Saddler, J., 2016. The influence of lignin on steam pretreatment and mechanical pulping of poplar to achieve high sugar recovery and ease of enzymatic hydrolysis. *Bioresour. Technol.* 199, 135–141.
- Chang, V.S., Burr, B., Holtzapple, M.T., 1997. Lime pretreatment of switchgrass. *Appl. Biochem. Biotechnol.* 63–65, 3–19.
- Chaturvedi, V., Verma, P., 2013. An overview of key pretreatment processes employed for bioconversion of lignocellulosic biomass into biofuels and value added products. *Biotech* 3, 415–431.

- Chen, M., Zeng, G., Tan, Z., Jiang, M., Li, H., Liu, L., Zhu, Y., Yu, Z., Wei, Z., Liu, Y., Xie, G., 2011. Understanding lignin-degrading reactions of ligninolytic enzymes: Binding affinity and interactional profile. *PLoS One* 6.
- Choong, Y.Y., Chou, K.W., Norli, I., 2017. Strategies for improving biogas production of palm oil mill effluent (POME) anaerobic digestion: A critical review. *Renew. Sustain. Energy Rev.* 0–1.
- Costa, J.C., Sousa, D.Z., Pereira, M. A., Stams, A.J.M., 2013. Biomethanation potential of biological and other wastes. *Biofuel technologies.* 369–396.
- Da Silva, A.S.A., Inoue, H., Endo, T., Yano, S., Bon, E.P.S., 2010. Milling pretreatment of sugarcane bagasse and straw for enzymatic hydrolysis and ethanol fermentation. *Bioresour. Technol.* 101, 7402–7409.
- Donohoe, B.S., Decker, S.R., Tucker, M.P., Himmel, M.E., Vinzant, T.B., 2008. Visualizing lignin coalescence and migration through maize cell walls following thermochemical pretreatment. *Biotechnol. Bioeng.* 101, 913–925.
- Fernandes, E.M., Pires, R.A., Mano, J.F., Reis, R.L., 2013. Bionanocomposites from lignocellulosic resources: Properties, applications and future trends for their use in the biomedical field. *Prog. Polym. Sci.* 38, 1415–1441.
- Fillat, Ú., Ibarra, D., Eugenio, M., Moreno, A., Tomás-Pejó, E., Martín-Sampedro, R., 2017. Laccases as a potential tool for the efficient conversion of lignocellulosic biomass: A review. *fermentation.* 3, 17-20.
- Galbe, M., Zacchi, G., 2012. Pretreatment: The key to efficient utilization of lignocellulosic materials. *Biomass and Bioenergy* 46, 70–78.
- Ghose, T. K., 1987. Measurement of cellulase activities. *Pure Appl. Chem.* 59, 257–268.
- Gusakov, A. V., 2011. Alternatives to *Trichoderma reesei* in biofuel production 29, 419–425.
- Hames, B., Ruiz, R., Scarlata, C., Sluiter, a, Sluiter, J., Templeton, D., 2008. Preparation of samples for compositional analysis laboratory analytical procedure (LAP) Issue Date : 8 / 06 / 2008 Preparation of Samples for Compositional Analysis Laboratory Analytical Procedure (LAP). *Natl. Renew. Energy Lab.* 1–9.
- Hammel, K.E., Cullen, D., 2008. Role of fungal peroxidases in biological ligninolysis. *Curr. Opin. Plant Biol.* 11, 349–355.
- Harmsen, P., Huijgen, W., 2010. Literature review of physical and chemical pretreatment processes for lignocellulosic biomass. *Energy* 1–49.

- Harun, M.Y., Dayang Radiah, A.B., Zainal Abidin, Z., Yunus, R., 2011. Effect of physical pretreatment on dilute acid hydrolysis of water hyacinth (*Eichhornia crassipes*). *Bioresour. Technol.* 102, 5193–5199.
- Hatakka, A., 2005. Biodegradation of lignin. *Biopolym.* Online 129–145.
- Hatakka, A.I., 1983. Pretreatment of wheat straw by white-rot fungi for enzymic saccharification of cellulose. *Eur. J. Appl. Microbiol. Biotechnol.* 18, 350–357.
- Head, D.S., Cenkowski, S., Arntfield, S., Henderson, K., 2010. Superheated steam processing of oat groats. *LWT - Food Sci. Technol.* 43, 690–694.
- Heap, L., 2014. Investigating the role of laccase and laccase mediator systems to improve the saccharification of biomass for bioethanol production.
- Hendriks, A. T.W.M., Zeeman, G., 2009. Pretreatments to enhance the digestibility of lignocellulosic biomass. *Bioresour. Technol.* 100, 10–18.
- Hideno, A., Inoue, H., Tsukahara, K., Fujimoto, S., Minowa, T., Inoue, S., Endo, T., Sawayama, S., 2009. Wet disk milling pretreatment without sulfuric acid for enzymatic hydrolysis of rice straw. *Bioresour. Technol.* 100, 2706–2711.
- Ho, A.L., Carvalheiro, F., Duarte, L.C., Roseiro, L.B., Charalampopoulos, D., Rastall, R.A., 2014. Production and purification of xylooligosaccharides from oil palm empty fruit bunch fibre by a non-isothermal process. *Bioresour. Technol.* 152, 526–529.
- Hom-Díaz, A., Passos, F., Ferrer, I., Vicent, T., Blázquez, P., 2016. Enzymatic pretreatment of microalgae using fungal broth from *Trametes versicolor* and commercial laccase for improved biogas production. *Algal Res.* 19, 184–188.
- Hosseini, S.A., Shah, N., 2011. Modelling enzymatic hydrolysis of cellulose part I: Population balance modelling of hydrolysis by endoglucanase. *Biomass and Bioenergy.* 35, 3841–3848.
- Hosseini, S.E., Wahid, M.A., 2014. Utilization of palm solid residue as a source of renewable and sustainable energy in Malaysia. *Renew. Sustain. Energy Rev.* 40, 621–632.
- Hsu, T.-C., Guo, G.-L., Chen, W.-H., Hwang, W.-S., 2010. Effect of dilute acid pretreatment of rice straw on structural properties and enzymatic hydrolysis. *Bioresour. Technol.* 101, 4907–4913.
- Iberahim, N.I., Jahim, J.M., Harun, S., Nor, M.T.M., Hassan, O., 2013. Sodium hydroxide pretreatment and enzymatic hydrolysis of oil palm mesocarp fiber. *Int. J. Chem. Eng. Appl.* 4, 101–105.

- Ibrahim, M.F., Ramli, N., Kamal Bahrin, E., Abd-Aziz, S., 2017. Cellulosic biobutanol by Clostridia: Challenges and improvements. *Renew. Sustain. Energy Rev.* 79, 1241–1254.
- Ibrahim, M.F., Razak, M.N. a, Phang, L.Y., Hassan, M. a., Abd-Aziz, S., 2013. Crude cellulase from oil palm empty fruit bunch by trichoderma asperellum UPM1 and aspergillus fumigatus UPM2 for fermentable sugars production. *Appl. Biochem. Biotechnol.* 170, 1320–1335.
- Ibrahim, V., Mendoza, L., Mamo, G., Hatti-Kaul, R., 2011. Blue laccase from *Galerina sp.*: Properties and potential for Kraft lignin demethylation. *Process Biochem.* 46, 379–384.
- Idi, A., Mohamad, S.E., 2011. Bioethanol from second generation feedstock (lignocellulose biomass). *Interdiscip. J. Cotemporary Res. Bus.* 3, 919–935.
- Isroi, Millati, R., Syamsiah, S., Niklasson, C., Cahyanto, M.N., Lundquist, K., Taherzadeh, M.J., 2011. Biological pretreatment of lignocelluloses with white-rot fungi and its applications: A review. *BioResources* 6, 5224–5259. d
- Kaal, E.E.J., De Jong, E., Field, J. a, Jong, E. De, 1993. Stimulation of ligninolytic peroxidase activity by nitrogen nutrients in the white rot fungus stimulation of ligninolytic peroxidase activity by nitrogen nutrients in the white rot fungus *Bjerkandera sp* . *Appl. Environ. Microbiol.* 59, 4031–4036.
- Khare, S.K., Pandey, A., Larroche, C., 2015. Current perspectives in enzymatic saccharification of lignocellulosic biomass. *Biochem. Eng. J.* 102, 38–44.
- Kim, M., Day, D.F., 2011. Composition of sugar cane, energy cane, and sweet sorghum suitable for ethanol production at Louisiana sugar mills. *J. Ind. Microbiol. Biotechnol.* 38, 803–807.
- Kong, S.H., Loh, S.K., Bachmann, R.T., Rahim, S.A., Salimon, J., 2014. Biochar from oil palm biomass: A review of its potential and challenges. *Renew. Sustain. Energy Rev.* 39, 729–739.
- Kshirsagar, S.D., Waghmare, P.R., Loni, P.C., Patil, S.A., Govindwar, S.P., 2015. RSC Advances characterization and optimization of enzymatic hydrolysis conditions by response surface methodology †. *RSC Adv.* 5, 46525–46533.
- Kumar, P., Barrett, D.M., Delwiche, M.J., Stroeve, P., 2009. Methods for pretreatment of lignocellulosic biomass for efficient hydrolysis and biofuel production. *Ind. Eng. Chem. Res.* 48, 3713–3729.
- Kunamneni, A., Ballesteros, A., Plou, F.J., Alcalde, M., 2007. Fungal laccase –

- a versatile enzyme for biotechnological applications. Appl. Microbiol. 233–245.
- Kuwahara, M., Glenn, J.K., Morgan, M.A., Gold, M.H., 1984. Separation and characterization of two extracellular H₂O₂-dependent oxidases from ligninolytic cultures of *Phanerochaete chrysosporium*. FEBS Lett. 169, 247–250.
- Lee, C.K., Darah, I., Ibrahim, C.O., 2011. Production and optimization of cellulase enzyme using *Aspergillus niger* USM AI 1 and comparison with *Trichoderma reesei* via solid state fermentation system. Biotechnol. Res. Int. 2011, 1–6.
- Lee, J., Gwak, K.-S., Park, J.-Y., Park, M.-J., Choi, D.-H., Kwon, M., Choi, I.-G., 2007. Biological pretreatment of softwood *Pinus densiflora* by three white rot fungi. J. Microbiol. 45, 485–491.
- Li, G., Chen, H., 2014. Synergistic mechanism of steam explosion combined with fungal treatment by *Phellinus baumii* for the pretreatment of corn stalk. Biomass and Bioenergy 67, 1–7.
- Loh, S.K., 2017. The potential of the Malaysian oil palm biomass as a renewable energy source. Energy Convers. Manag. 141, 285–298.
- Makkar, H.P.S., 2003. Measurement of Total Phenolics and Tannins Using Folin-Ciocalteu Method. Quantif. Tann. Tree Shrub Foliage 49–51. doi:10.1007/978-94-017-0273-7_3
- Malaysian Palm Oil Board, Oil Palm Estates, Economic & Industry Development Division. Oil Palm Estates Planted Area. January–December 2016. Available online: <http://bepi.mpob.gov.my/index.php/statistics/area.html> (accessed on 7 october 2017)
- Martínez, A.T., 2002. Molecular biology and structure-function of lignin-degrading heme peroxidases. Enzyme Microb. Technol. 30, 425–444.
- Martinez, M.J., Ruiz-Duenas, F.J., Guillen, F., Martinez, A.T., 1996. Purification and catalytic properties of two manganese peroxidase isoenzymes from *Pleurotus eryngii*. Eur. J. Biochem. 237, 424–432.
- Masran, R., Zanirun, Z., Bahrin, E.K., Ibrahim, M.F., Lai Yee, P., Abd-Aziz, S., 2016. Harnessing the potential of ligninolytic enzymes for lignocellulosic biomass pretreatment. Appl. Microbiol. Biotechnol. 1–16.
- Medina, J.D.C., Woiciechowski, A., Filho, A.Z., Nigam, P.S., Ramos, L.P., Soccol, C.R., 2016. Steam explosion pretreatment of oil palm empty fruit bunches (EFB) using autocatalytic hydrolysis: A biorefinery approach. Bioresour. Technol. 199, 173–180.

- Mester, T., Field, J.A., 1998. Characterization of a novel manganese peroxidase-lignin peroxidase hybrid isozyme produced by *Bjerkandera* species strain BOS55 in the absence of manganese. *J. Biol. Chem.* 273, 15412–15417.
- Miki, Y., Ichinose, H., Wariishi, H., 2011. Determination of a catalytic tyrosine in *Trametes cervina* lignin peroxidase with chemical modification techniques. *Biotechnol. Lett.* 33, 1423–1427.
- Mirhosseini, S., Barchyn, D., Agbor, V.B., Levin, D.B., Cenkowski, S., 2016. The effect of superheated steam pre- treatment of wheat straw on fermentation. 58, 9–17.
- Moldes, D., Díaz, M., Tzanov, T., Vidal, T., 2008. Comparative study of the efficiency of synthetic and natural mediators in laccase-assisted bleaching of eucalyptus kraft pulp. *Bioresour. Technol.* 99, 7959–7965.
- Moreno, A., Ibarra, D., Mialon, A., Ballesteros, M., 2016. A bacterial laccase for enhancing saccharification and ethanol fermentation of steam-pretreated biomass. *Fermentation* 2, 11.
- Moreno, A.D., Ibarra, D., Alvira, P., Tomás-Pejó, E., Ballesteros, M., 2015. Exploring laccase and mediators behavior during saccharification and fermentation of steam-exploded wheat straw for bioethanol production. *J. Chem. Technol. Biotechnol.* 180-200.
- Mosier, N., Wyman, C., Dale, B., Elander, R., Lee, Y.Y., Holtzapple, M., Ladisch, M., 2005. Features of promising technologies for pretreatment of lignocellulosic biomass. *Bioresour. Technol.* 96, 673–686.
- Mosier, N.S., Hendrickson, R., Brewer, M., Ho, N., Sedlak, M., Dreshel, R., Welch, G., Dien, B.S., Aden, A., Ladisch, M.R., 2005. Industrial scale-up of pH-controlled liquid hot water pretreatment of corn fiber for fuel ethanol production. *Appl. Biochem. Biotechnol.* 125, 77–97.
- Na, B.I., Kim, Y.H., Lim, W.S., Lee, S.M., Lee, H.W., Lee, J.W., 2013. Torrefaction of oil palm mesocarp fiber and their effect on pelletizing. *Biomass and Bioenergy* 52, 159–165.
- National Biomass Strategy 2020: New wealth creation for Malaysia's palm oil industry, 2013. Agensi Inovasi, Malaysia, Kuala Lumpur 1–32.
- Nigam, P.S., 2013. Microbial enzymes with special characteristics for biotechnological applications. *Biomolecules* 3, 597–611.
- Nnaemeka, N., MacManus, N., 2016. Measurement of energy requirements for size reduction of palm kernel and groundnut shells for downstream bioenergy generation. *J. Eng. Technol. Res.* 8, 47–57.

- Nordin, N.I.A.A., Ariffin, H., Andou, Y., Hassan, M.A., Shirai, Y., Nishida, H., Yunus, W.M.Z.W., Karuppuchamy, S., Ibrahim, N.A., 2013. Modification of oil palm mesocarp fiber characteristics using superheated steam treatment. *Molecules* 18, 9132–9146.
- Palonen, H., Thomsen, A.B., Tenkanen, M., Schmidt, A.S., Viikari, L., 2004. Evaluation of wet oxidation pretreatment for enzymatic hydrolysis of softwood. *Appl. Biochem. Biotechnol.* 117, 1–17.
- Pinto, P. A., Dias, A. A., Fraga, I., Marques, G., Rodrigues, M. A M., Colaço, J., Sampaio, A., Bezerra, R.M.F., 2012. Influence of ligninolytic enzymes on straw saccharification during fungal pretreatment. *Bioresour. Technol.* 111, 261–267.
- Piontek, K., Glumoff, T., Winterhalter, K., 1993. Low pH crystal structure of glycosylated lignin peroxidase from *Phanerochaete chrysosporium* at 2.5 Å resolution. *FEBS Lett.* 315, 119–124.
- Plácido, J., Capareda, S., 2015. Ligninolytic enzymes: a biotechnological alternative for bioethanol production. *Bioresour. Bioprocess.* 2, 23.
- Potumarthi, R., Baadhe, R.R., Nayak, P., Jetty, A., 2013. Simultaneous pretreatment and saccharification of rice husk by *Phanerochaete chrysosporium* for improved production of reducing sugars. *Bioresour Technol* 128, 113–117.
- Prasad, S., Singh, A., Joshi, H.C., 2007. Ethanol as an alternative fuel from agricultural, industrial and urban residues. *Resour. Conserv. Recycl.* 50, 1–39.
- Prieur, B., Meub, M., Wittemann, M., Klein, R., Bellayer, S., Fontaine, G., Bourbigot, S., 2017. RSC Advances Phosphorylation of lignin: characterization and investigation of the thermal decomposition. *RSC Adv.* 7, 16866–16877.
- Pu, Y., Hu, F., Huang, F., Davison, B.H., Ragauskas, A.J., 2013. Assessing the molecular structure basis for biomass recalcitrance during dilute acid and hydrothermal pretreatments. *Biotechnol. Biofuels* 6, 15.
- Qiu, W., Chen, H., 2012. Enhanced the enzymatic hydrolysis efficiency of wheat straw after combined steam explosion and laccase pretreatment. *Bioresour. Technol.* 118, 8–12.
- Rabemanolontsoa, H., Saka, S., 2016. Various pretreatments of lignocellulosics. *Bioresour. Technol.* 199, 83–91.
- Ren, N.Q., Zhao, L., Chen, C., Guo, W.Q., Cao, G.L., 2016. A review on bioconversion of lignocellulosic biomass to H₂: Key challenges and new insights. *Bioresour. Technol.* 215, 92–99.

- Rencoret, J., Pereira, A., del Río, J.C., Martínez, A.T., Gutiérrez, A., 2016. Laccase-mediator pretreatment of wheat straw degrades lignin and improves saccharification. *BioEnergy Res.* 9, 917–930.
- Rico, A., Rencoret, J., Del Río, J.C., Martínez, A.T., Gutiérrez, A., 2014. Pretreatment with laccase and a phenolic mediator degrades lignin and enhances saccharification of Eucalyptus feedstock. *Biotechnol. Biofuels* 7, 6.
- Saha, B.C., 2003. Hemicellulose bioconversion. *J. Ind. Microbiol. Biotechnol.* 30, 279–291.
- Saha, B.C., Qureshi, N., Kennedy, G.J., Cotta, M.A., 2016. Biological pretreatment of corn stover with white-rot fungus for improved enzymatic hydrolysis. *Int. Biodeterior. Biodegrad.* 109, 29–35.
- Sajith, S., Priji, P., Sreedevi, S., Benjamin, S., 2016. An overview on fungal cellulases with an industrial perspective. *J. Nutr. Food Sci.* 6, 1–13.
- Sánchez, C., 2009. Lignocellulosic residues: Biodegradation and bioconversion by fungi. *Biotechnol. Adv.* 27, 185–194.
- Selig, M.J., Viamajala, S., Decker, S.R., Tucker, M.P., Himmel, M.E., Vinzant, T.B., 2007. Deposition of lignin droplets produced during dilute acid pretreatment of maize stems retards enzymatic hydrolysis of cellulose. *Biotechnol. Prog.* 23, 1333–1339.
- Shahzadi, T., Mehmood, S., Irshad, M., Anwar, Z., Afroz, A., Zeeshan, N., Rashid, U., Sughra, K., 2014. Advances in lignocellulosic biotechnology: A brief review on lignocellulosic biomass and cellulases 2014, 246–251.
- Siddiquee, S., Shafawati, S.N., Naher, L., 2017. Effective composting of empty fruit bunches using potential *Trichoderma* strains. *Biotechnol. Reports* 13, 1–7.
- Singh Arora, D., Kumar Sharma, R., 2010. Ligninolytic fungal laccases and their biotechnological applications. *Appl. Biochem. Biotechnol.* 160, 1760–1788.
- Sitarz, A., 2012. Laccase enzymology in relation to lignocellulose processing. *Enzyme.* 141. 100-400.
- Sluiter, A., Hames, B., Ruiz, R., Scarlata, C., Sluiter, J., Templeton, D., 2010. Determination of structural carbohydrates and lignin in biomass determination of structural carbohydrates and lignin in biomass. *Natl. Renew. Energy Lab.* 2011.
- Sluiter, A., Hames, B., Ruiz, R., Scarlata, C., Sluiter, J., Templeton, D., 2008. Determination of ash in biomass: Laboratory Analytical Procedure (LAP).

Nrel/Tp-510-42622 18.

- Sluiter, a, Hames, B., Ruiz, R., Scarlata, C., Sluiter, J., Templeton, D., 2008. Determination of Sugars , Byproducts , and Degradation Products in Liquid Fraction Process Samples Laboratory Analytical Procedure (LAP) Issue Date: 12 / 08 / 2006 Determination of Sugars , Byproducts , and Degradation Products in Liquid Fraction Proce. Lab. Anal. Proced. NREL/TP-510-42623 1–14.
- Soni, S.K., Soni, R., 2010. Regulation of cellulase synthesis in *Chaetomium erraticum*. BioResources 5, 81–98.
- Studer, M.H., DeMartini, J.D., Davis, M.F., Sykes, R.W., Davison, B., Keller, M., Tuskan, G.A., Wyman, C.E., 2011. Lignin content in natural Populus variants affects sugar release. Proc. Natl. Acad. Sci. 108, 6300–6305.
- Sukhbir Kaur, Varsha Nigam, 2014. Production and application of laccase enzyme in pulp and paper industry. Int. J. Res. Applied, Nat. Soc. Sci. 2, 153–158.
- Sukri, S.S.M., Rahman, R.A., Md Illias, R., Yaakob, H., 2014. Optimization of alkaline pretreatment conditions of oil palm fronds in improving the lignocelluloses contents for reducing sugar production. Rom. Biotechnol. Lett. 19, 9006–9018.
- Sulaiman, F., Abdullah, N., Gerhauser, H., Shariff, A. 2010. A perspective of oil palm and its wastes. J. Phys. Sci. 21, 67–77.
- Sun, F. Hui, Li, J., Yuan, Y. Xiang, Yan, Z. Ying, Liu, X. Feng, 2011. Effect of biological pretreatment with *Trametes hirsuta* yj9 on enzymatic hydrolysis of corn stover. Int. Biodeterior. Biodegrad. 65, 931–938.
- Sun, S., Sun, S., Cao, X., Sun, R., 2016. The role of pretreatment in improving the enzymatic hydrolysis of lignocellulosic materials. Bioresour. Technol. 199, 49–58.
- Sun, Y., Cheng, J., 2002. Hydrolysis of lignocellulosic materials for ethanol production: A review. Bioresour. Technol. 83, 1–11.
- Taha, M., Foda, M., Shahsavari, E., Aburto-Medina, A., Adetutu, E., Ball, A., 2016. Commercial feasibility of lignocellulose biodegradation: Possibilities and challenges. Curr. Opin. Biotechnol. 38, 190–197.
- Taherzadeh, M.J., Karimi, K., 2008. Pretreatment of lignocellulosic wastes to improve ethanol and biogas production: A review, International Journal of Molecular Sciences.
- Taifor, A.F., Zakaria, M.R., Mohd Yusoff, M.Z., Toshinari, M., Hassan, M.A., Shirai, Y., 2017. Elucidating substrate utilization in biohydrogen

- production from palm oil mill effluent by *Escherichia coli*. *Int. J. Hydrogen Energy* 42, 5812–5819.
- Taniguchi, M., Suzuki, H., Watanabe, D., Sakai, K., Hoshino, K., Tanaka, T., 2005. Evaluation of pretreatment with *Pleurotus ostreatus* for enzymatic hydrolysis of rice straw. *J. Biosci. Bioeng.* 100, 637–643.
- Teymouri, F., Laureano-Perez, L., Alizadeh, H., Dale, B.E., 2005. Optimization of the ammonia fiber explosion (AFEX) treatment parameters for enzymatic hydrolysis of corn stover. *Bioresour. Technol.* 96, 2014–2018. doi:10.1016/j.biortech.2005.01.016
- Then, Y., Ibrahim, N., Zainuddin, N., Ariffin, H., Yunus, W., Chieng, B., 2014. The influence of green surface modification of oil palm mesocarp fiber by superheated steam on the mechanical properties and dimensional stability of oil palm mesocarp fiber/poly(butylene succinate) biocomposite. *Int. J. Mol. Sci.* 15, 15344–15357.
- Wan, C., Li, Y., 2012. Fungal pretreatment of lignocellulosic biomass. *Biotechnol. Adv.* 30, 1447–1457.
- Wang, W., Yuan, T., Wang, K., Cui, B., Dai, Y., 2012. Combination of biological pretreatment with liquid hot water pretreatment to enhance enzymatic hydrolysis of *Populus tomentosa*. *Bioresour. Technol.* 107, 282–286.
- Warid, M.N.M., Ariffin, H., Hassan, M., Shirai, Y., 2016. Optimization of superheated steam treatment to improve surface modification of oil palm biomass fiber. *BioResources* 11, 5780–5796.
- Wi, S.G., Cho, E.J., Lee, D.-S., Lee, S.J., Lee, Y.J., Bae, H.-J., 2015. Lignocellulose conversion for biofuel: a new pretreatment greatly improves downstream biocatalytic hydrolysis of various lignocellulosic materials. *Biotechnol. Biofuels* 8, 228.
- Wong, D.W.S., 2009. Structure and action mechanism of ligninolytic enzymes, *Applied Biochemistry and Biotechnology*.
- Yang, B., Wyman, C.E., 2004. Effect of xylan and lignin removal by batch and flowthrough pretreatment on the enzymatic digestibility of corn stover cellulose. *biotechnol. Bioeng.* 86, 88–95.
- Yang, F., Gong, Y., Liu, G., Zhao, S., Wang, J., 2015. Enhancing cellulase production in thermophilic fungus *Myceliophthora thermophila* ATCC42464 by RNA interference of cre1 gene expression. *J. Microbiol. Biotechnol.* 25, 1101–1107.
- Ying, T.Y., Teong, L.K., Abdullah, W.N.W., Peng, L.C., 2014. The effect of various pretreatment methods on oil palm empty fruit bunch (EFB) and kenaf core fibers for sugar production. *Procedia Environ. Sci.* 20, 328–

- Yu, H., Li, X., Xing, Y., Liu, Z., Jiang, J., 2014. A sequential combination of laccase pretreatment and enzymatic hydrolysis for glucose production from furfural residues. *BioResources* 9, 4581–4595.
- Yu, H., Xing, Y., Lei, F., Liu, Z., Liu, Z., Jiang, J., 2014. Improvement of the enzymatic hydrolysis of furfural residues by pretreatment with combined green liquor and ethanol organosolv. *Bioresour. Technol.* 167, 46–52.
- Zabed, H., Sahu, J.N., Boyce, A.N., Faruq, G., 2016. Fuel ethanol production from lignocellulosic biomass: An overview on feedstocks and technological approaches. *Renew. Sustain. Energy Rev.* 66, 751–774.
- Zainal, N.H., Aziz, A.A., Idris, J., Mamat, R., Hassan, M.A., Bahrin, E.K., Abd-Aziz, S., 2017. Microwave-assisted pre-carbonisation of palm kernel shell produced charcoal with high heating value and low gaseous emission. *J. Clean. Prod.* 142, 2945–2949.
- Zakaria, M.R., Fujimoto, S., Hirata, S., Hassan, M.A., 2014a. Ball milling pretreatment of oil palm biomass for enhancing enzymatic hydrolysis. *Appl. Biochem. Biotechnol.* 173, 1778–1789.
- Zakaria, M.R., Hirata, S., Hassan, M.A., 2015a. Hydrothermal pretreatment enhanced enzymatic hydrolysis and glucose production from oil palm biomass. *Bioresour. Technol.* 176, 142–148.
- Zakaria, M.R., Hirata, S., Hassan, M.A., 2014b. Combined pretreatment using alkaline hydrothermal and ball milling to enhance enzymatic hydrolysis of oil palm mesocarp fiber. *Bioresour. Technol.* 169, 236–243.
- Zakaria, M.R., Norraahim, M.N.F., Hirata, S., Hassan, M.A., 2015b. Hydrothermal and wet disk milling pretreatment for high conversion of biosugars from oil palm mesocarp fiber. *Bioresour. Technol.* 181, 263–269.
- Zanirun, Z., Bahrin, E.K., Lai-Yee, P., Hassan, M.A., Abd-Aziz, S., 2015. Enhancement of fermentable sugars production from oil palm empty fruit bunch by ligninolytic enzymes mediator system. *Int. Biodeterior. Biodegradation* 105, 13–20.
- Zhao, L., Cao, G.L., Wang, A.J., Ren, H.Y., Dong, D., Liu, Z.N., Guan, X.Y., Xu, C.J., Ren, N.Q., 2012. Fungal pretreatment of cornstalk with *Phanerochaete chrysosporium* for enhancing enzymatic saccharification and hydrogen production. *Bioresour. Technol.* 114, 365–369.
- Zhu, J.Y., Pan, X.J., 2010. Woody biomass pretreatment for cellulosic ethanol production: Technology and energy consumption evaluation. *Bioresour. Technol.* 101, 4992–5002.

Zulkarnain, A., Bahrin, E.K., Ramli, N., Phang, L.Y., Abd-Aziz, S., 2016. Alkaline Hydrolysate of Oil Palm Empty Fruit Bunch as Potential Substrate for Biovanillin Production via Two-Step Bioconversion. Waste and Biomass Valorization.9. 13-23.



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LIST OF PUBLICATIONS

Manuscript published:

Ahmad Rizal, N. F. A., Ibrahim, M. F., Zakaria, M. R., Kamal Bahrin, E., Abd Aziz, S., Hassan, M. A., (2018). Combination of superheated steam with laccase pretreatment together with size reduction to enhance enzymatic hydrolysis of oil palm biomass. *Molecules*. 23:4. (IF:3.06)

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Abstract in conference/symposium:

- 1) Nur Fatin Athirah Ahmad Rizal, Mohamad Faizal Ibrahim, Mohd Rafein Mohd Zakaria, Ezyana Kamal Bahrin, Suraini Abd-Aziz, Mohd Ali Hassan. Combination of superheated steam with laccase pretreatment together with size reduction to enhance enzymatic hydrolysis of oil palm biomass. 5th International Symposium on Applied Engineering and Sciences (SAES2017), UPM, Serdang, Malaysia.



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