UNIVERSITI PUTRA MALAYSIA

CHARACTERIZATION AND ANTIFUNGAL PROPERTIES OF OIL PALM MESOCARP FIBER SUPERHEATED STEAM CONDENSATE

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By

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

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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

CHARACTERIZATION AND ANTIFUNGAL PROPERTIES OF OIL PALM MESOCARP FIBER SUPERHEATED STEAM CONDENSATE

By

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June 2016

Chair: Hidayah Ariffin, PhD
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Degradation of hemicellulose, low molecular weight lignin and some amount of cellulose during pretreatment of lignocellulose by steam treatment produces acids, furans and phenolic compounds. These compounds are known to have antimicrobial properties. It has been recently reported that superheated steam (SHS) can be used as pretreatment method to alter the structure of lignocellulose prior to bioconversion or biocomposite production. Since SHS is a dry steam, the mechanism of lignocellulose degradation is unclear and hence its degradation products are unknown. Hence this study was conducted, with the aim to characterize the condensate from SHS treatment of oil palm mesocarp fiber (OPMF) and subsequently evaluating the effect of SHS treatment temperature on antifungal properties of the condensate. Results showed that OPMF condensate obtained (OS sample) was light yellowish-brown in colour with average pH of 3.04 ± 0.02. Detailed chemical compositional study by GCMS showed the presence of two different groups of compounds, with phenolic groups showed the most intense composition compared to the others. HPLC analysis showed that four types of carboxylic acids were generated during SHS treatment and formic acid was found as the most concentrated acid in the condensate (657 mg/L). Meanwhile, the concentrated OS sample (labeled as RF fraction) contained higher number of compounds (62 compounds), which were classified into ten different groups. Furthermore, the chemical composition of the condensates were exponentially increased by increases of SHS temperature; from 200ºC to 240ºC, while no significant increment was seen from 190ºC to 200ºC. This is in consistent with lignocellulose thermal degradation temperature. RF fraction of samples obtained from SHS treatment at 240°C showed 5.5 ± 0.17, 5.2 ± 0.1 and 8.0 ± 0.4 inhibition ratios on *Bacillus cereus, Escherichia coli* and *Staphylococcus aureus*, respectively. For fungal species, growth inhibitory test by agar dilution method showed that the growth of *Aspergillus fumigatus* UPM2 and *Trichoderma asperellum* UPM1 were suppressed by 51.8%, and 45.5%, correspondingly. This is supported by spore germination inhibition whereby no germination of spore was seen for both fungi tested. It is interesting to note that the OPMF condensate managed to fully inhibit the growth of *Ganoderma boninense* UPM13, which is a type of fungus causing basal stem rot (BSR) disease at the oil palm plantation. Results obtained herewith are very

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interesting as at present there is lack of information on the chemical composition of steam condensate obtained from SHS treatment of lignocellulose. The inhibitory effect of the OPMF SHS condensate on the growth of *G. boninense* UPM13 is indeed a new insight and should be used as a basis for future study on the development of antifungal agent for combating BSR disease.

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PENCIRIAN DAN SIFAT ANTIKULAT KONDENSAT GENTIAN SABUT KELAPA SAWIT STIM PANAS LAMPAU

Oleh

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Degradasi hemiselulosa, lignin yang mempunyai berat molekul rendah dan sebahagian selulosa semasa prarawatan lignoselulosa oleh rawatan wap menghasilkan sebatian asid, furan dan fenolik. Sebatian ini diketahui mempunyai ciri anti-mikrob. Baru-baru ini telah dilaporkan bahawa stim panas lampau (SHS) boleh digunakan sebagai kaedah prarawatan untuk mengubah struktur lignoselulosa sebelum penukaran bio atau pengeluaran biokomposit. Berikutan SHS adalah wap kering, mekanisme degradasi lignoselulosanya tidak jelas dan maka, produk degradasinya tidak diketahui. Maka kajian ini telah dijalankan, bertujuan untuk mencirikan kondensat daripada rawatan SHS gentian sabut kelapa sawit (OPMF) dan kemudiannya menilai kesan suhu rawatan SHS pada ciri antimikrob kondensat. Hasil kajian menunjukkan bahawa kondensat OPMF yang diperolehi (sampel OS) adalah berwarna kuning-keperangan dengan purata pH 3.04 ± 0.02. Kajian kimia terperinci oleh GCMS menunjukkan terdapat kehadiran dua kumpulan yang berbeza daripada sebatian tersebut, dengan kumpulan fenolik menunjukkan komposisi yang paling banyak berbanding yang lain. Analisis HPLC menunjukkan empat jenis asid karboksilik dijana semasa rawatan SHS dan asid formik merupakan asid yang paling pekat di dalam kondensat (657 mg/L). Sementara itu, sampel OS pekat (dilabelkan sebagai sampel RF) mengandungi bilangan sebatian yang lebih tinggi (62 sebatian), yang kemudiannya dikelaskan kepada sepuluh kumpulan yang berbeza. Tambahan lagi, komposisi kimia kondensat semakin meningkat dengan kenaikan suhu SHS; dari 200ºC ke 240ºC, manakala tiada kenaikan ketara dilihat dari 190ºC ke 200ºC. Ini adalah selari dengan suhu degradasi lignoselulosa. Sampel RF kondensat yang diperolehi daripada rawatan SHS 240°C masing-masing menunjukkan nisbah perencatan 5.5 ± 0.17, 5.2 ± 0.1 dan 8.0 ± 0.4 bagi *Bacillus cereus, Escherichia coli* dan *Staphylococcus aureus*. Bagi spesies kulat, ujian perencatan pertumbuhan dengan kaedah pencairan agar menunjukkan bahawa pertumbuhan *Aspergillus fumigatus* UPM2 dan *Trichoderma asperellum* UPM1 dibantutkan sebanyak 51.8%, dan 45.5%. Ini disokong oleh perencatan spora di mana tiada percambahan spora dilihat bagi semua spora kulat yang diuji. Menariknya, kondensat OPMF berjaya menghalang sepenuhnya pertumbuhan *Ganoderma boninense* UPM13, iaitu sejenis kulat yang menyebabkan penyakit reput pangkal batang (BSR) di ladang kelapa sawit. Keputusan yang diperoleh dari

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kajian ini adalah sangat menarik kerana pada masa kini terdapat kekurangan maklumat mengenai komposisi kimia wap kondensat daripada rawatan SHS lignoselulosa. Kesan rencatan daripada kondensat OPMF SHS kepada pertumbuhan *G. boninense* UPM13 merupakan satu penemuan baharu yang patut digunakan sebagai asas untuk kajian masa depan kepada pembangunan ejen antikulat bagi memerangi penyakit BSR.

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I certify that a Thesis Examination Committee has met on June 30, 2016 to conduct the final examination of Nur Sharmila Binti Sharip on her thesis entitled "Characterization and Antifungal Properties of Oil Palm Mesocarp Fiber Superheated Steam Condensate" in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the Master of Science.

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This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment 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 ABBREVIATION

Abs	Absorbance
DF 1	Distilled fraction 1
DF 2	Distilled fraction 2
GCMS	Gas performance mass spectroscopy
H^+	Hydrogen ion
H_3O^+	Hydronium ion
HPLC	High performance liquid chromatography
NA	Nutrient agar
OPEFB	Oil palm empty fruit bunch
OPF	Oil palm frond
OPMF	Oil palm mesocarp fiber
OPT	Oil palm trunk
OS	Original sample
PDA	Potato dextrose agar
PKS	Palm kernel shell
POME	Palm oil mill effluent
RF	Residue fraction
SHS	Superheated steam
UPM	Universiti Putra Malaysia
UV	Ultraviolet
UV-Vis	Ultraviolet-visible

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CHAPTER 1

INTRODUCTION

The concept of 'Waste to Wealth' focuses on utilization of unused resources to produce useful and high-end product. It has gained a lot of interest especially involving lignocellulose biomass. This biomass which came from agriculture and forestry industries, grasses and woody materials are largely available resources around the world. In Malaysia, largest biomass is contributed by palm oil industries which produce about 80 million dry tones of lignocellulose biomass annually (Agensi Inovasi Malaysia, 2011). Six types of biomass from oil palm industries are oil palm frond (OPF), oil palm trunk (OPT), oil palm empty fruit bunch (OPEFB), palm kernel shell (PKS), palm oil mill effluent (POME) and oil palm mesocarp fiber (OPMF) (Agensi Inovasi Malaysia, 2011).

To begin with, the utilization of these vast amounts of biomass as alternative sources to produce purposive products such as fermentable sugars, biofuel and biocomposite filler in various applications requires pretreatment in order to modify and alter its complex lignocellulose materials structure. This could be achieved by biological, chemical and physical pretreatments. For instance, it has been reported that acid and alkaline pretreatment of OPEFB managed to improve bioconversion of OPEFB as substrate for cellulase production due to the alteration of lignin content by the pretreatment (Umikalsom *et al.,* 1997). Hydrothermal pretreatment through hot water extraction has been reported to reduce hemicellulose and lignin content which in turn caused structural changes on the cellulose–hemicellulose–lignin matrix, resulting in the opening and expansion of specific surface area and pore volume and hence, provided better condition for hydrolysis of sugars (Zakaria *et al.,* 2015a; Zakaria *et al.,* 2015b).

SHS pretreatment has been recently reported as one of the pretreatment methods for oil palm biomass meant for sugar (Mahmud *et al.*, 2013; Bahrin *et al.,* 2012) and biocomposite (Then *et al.*, 2014a; Then *et al.*, 2014b; Nordin *et al.*, 2013) production. SHS can be defined as a type of unsaturated steam with temperature higher than its boiling point at given pressure (Nordin *et al.*, 2013; Bahrin *et al.,* 2012). It was found that hemicellulose was removed during SHS treatment of OPMF, which eventually provided better surface for polymer-fiber interaction in biocomposite making (Nordin *et al.*, 2013). In the same report, it was mentioned that lignin, hemicellulose, and cellulose degraded within temperature ranges of 160°C to 900°C, 220°C to 315°C and 315°C to 400°C, respectively. This indicates the possibility of having lignocellulose components degradation products as by-products during SHS pretreatment. Degradation products of lignocellulose varied from sugar degradation product of cellulose and hemicellulose such as sugar oligomers, furfural, acetic acid and formic acid, as well as phenol and extractive from lignin degradation (Garrote *et al.*, 2004). These products are toxic to microbial growth, thus it could be potentially used as antifungal agent (Cruz *et al.,* 2001; Garrote *et al.*, 2004). In relation to microorganism's inhibitory potential of lignocellulose degradation products, the

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study on its application as antifungal agent could be very beneficial. The control of fungal growth is important in various areas of industrial, medical, pharmaceutical, food processing and environment.

Studies on pretreatment of oil palm biomass by SHS have been carried out by several researchers (Then *et al.*, 2014a; Then *et al.*, 2014b; Nordin *et al.*, 2013; Mahmud *et al.,* 2013; Bahrin *et al.*, 2012). It has been postulated that application of SHS for pretreatment had partially degraded the lignocellulose structure of the biomass into smaller compounds which can be easily volatile at high temperature. In regard with high temperature used during SHS, it is expected that the lignocellulose degradation products were vaporized and released out to the environment along with the steam. As it is known that lignocellulose degradation products have microbial growth inhibitory properties, therefore this research will be conducted, mainly to characterize and identify the degradation products obtained from SHS pretreatment of oil palm biomass, and eventually to determine its potential as an antifungal agent.

CHAPTER 1 of this thesis briefly introduces about the palm oil biomass, its utilization as alternative sources and the pretreatment methods; the SHS pretreatment; the degradation products from lignocellulose materials and its inhibition potential. Problem statements and objectives of this research were also included in this chapter. Details explanation and other's findings on topics related to this study were reviewed in CHAPTER 2. For the next chapter (CHAPTER 3), overview of the experimental procedure, materials and methods were explained. It covers the growth and maintenance of the bacteria (*Bacillus cereus*, *Escherichia coli*, *Staphylococcus aureus)* and fungi (*Trichoderma asperellum* UPM1, *Aspergillus fumigatus* UPM2, *Ganoderma boninese* UPM13) strain; the SHS treatment and condensation process of OPMF; fractionation of the OPMF condensate; analytical methods; antimicrobial screening and antifungal tests using agar dilution method and spore germination method.

CHAPTER 4 discusses on the findings of this research. Physical properties of OPMF condensate from 240ºC SHS were characterized by means of its appearance and acidity while chemical properties were determined using HPLC and GCMS analysis. The effect of SHS treatment temperature on OPMF condensate composition and characteristics were also discussed. The selection of fraction with strongest inhibitory potential using antibacterial screening test was explained in this chapter. The antifungal properties of chosen OPMF condensate fraction on *Trichoderma asperellum* UPM1, *Aspergillus fumigatus* UPM2, and *Ganoderma boninense* UPM13 were also discussed in this chapter. Last chapter covers summary of the research and few recommendations for future works. It followed by references, appendices, biodata and list of publications.

The study aims to characterize condensate from SHS pretreatment of OPMF and subsequently identify the potential use of the steam condensate as antifungal agent. The specific objectives of this research are:

1. To characterize the condensates obtained from superheated steam (SHS) pretreatment of oil palm mesocarp fiber (OPMF) at various pretreatment temperatures.

2. To evaluate antifungal properties of OPMF SHS condensate in relation to the chemical composition of the condensate.

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