***ENZYMATIC HYDROLYSIS OF LIGNOCELLULOSIC BIOMASS USING EXTRACT FROM THE TERMITE Coptotermes curvignathus HOLMGREN***

**WONG SIN-YEE**

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By

**WONG SIN-YEE**

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

**August 2015**

# DEDICATION

This thesis is dedicated to,

My father, **Wong Kar Ming**, who have given me the courage to endure; and

My mother, **Lee Kim Lin**, who introduced me to the joy of reading from birth.

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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**August 2015**

**Chairman : H'ng Paik San, PhD**
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Termites are by far the most successful wood-degraders on Earth, tunnelling and chewing on woody biomass for millions of years. To disintegrate the tough linear chains of cellulose, termites are loaded with different species of microorganisms in their relatively tiny guts. These gut microbes would collaborate to produce digestive enzymatic juice for degrading wood into consumable end products such as sugars, hydrogen, ethanol and acetate. By efficiently catalysing the conversion of lignocellulosic biomass, the key to generate customised cocktails lies within the termites' digestive enzymes. This study aimed to identify and evaluate the cellulolytic enzymes activities in the whole extracts of local wood-feeding termites (*Coptotermes curvignathus*) for enzymatic hydrolysis with biomass such as oil palm trunk (OPT) and cassava pomace. The enzymatic hydrolysis was controlled at three different sets of temperature (27˚C ± 2˚C, 32˚C ± 2˚C and 37˚C ± 2˚C) and evaluated based on reaction time (hours). Conversion of biomass was measured in reducing sugar yield and cellulase activities of the reaction. Ultimately the results showed that the fresh extracts of termite *Coptotermes curvignathus* contained reducing sugar and activities of endo-β-D-1,4-glucanase, exo-β-D-1,4-glucanase and β-D-1,4-xylanase that could potentially increase the digestion of lignocellulosic biomass. The highest reducing sugar recorded was 7.36 ± 0.65 g/L in the enzymatic hydrolysis with OPT occurring at the reaction temperature of 37˚C, while the highest enzyme activities recorded were endocellulase (31.58 ± 5.48 U/g) in incubated termite extract, exocellulase (14.94 ± 4.71 U/g) in hydrolysis with OPT, and xylanase (89.60 ± 20.87 U/g) in hydrolysis with cassava, all occurring at the incubation temperature of 32˚C.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk Ijazah Master Sains

**HIDROLISIS ENZIM BAGI BIOJISIM LIGNOSELULOSA DENGAN MENGGUNAKAN EKSTRAK DARI ANAI-ANAI *Coptotermes curvignathus* HOLMGREN**

Oleh

**WONG SIN-YEE**

**Ogos 2015**

**Pengerusi : H'ng Paik San, PhD**
**Fakulti : Perhutanan**


Anai-anai merupakan pemakan kayu yang paling berjaya di bumi setakat ini, disebabkan keupayaan mereka membuat terowong dalam kayu dan mengunyah biojisim berkayu sejak berjuta-juta tahun yang lalu. Untuk menghancurkan rantaian selulosa yang sangat tahan lasak, perut anai-anai yang kecil diisi dengan pelbagai jenis spesies mikroorganisma. Mikrob-mikrob perut ini akan berkerjasama untuk menghasilkan jus enzim pencernaan bagi mencernakan kayu untuk menghasil produk akhir yang berguna seperti gula, hidrogen, etanol dan asetat. Sebagai pemangkin bagi penukaran lignoselulosa yang cekap, kunci untuk menjanakan koktel pencernaan yang bersesuaian terletak pada enzim anai-anai tersebut. Penyelidikan ini bertujuan untuk mengenalpasti dan menilai aktiviti enzim selulosa yang terdapat di ekstrak keseluruhan anai-anai tempatan (*Coptotermes curvignathus*) bagi menjalankan hidrolisis enzim dengan biojisim lignoselulosa seperti batang kelapa sawit dan sisa ubi kayu. Proses hidrolisis enzim tersebut dikawal pada tiga set suhu yang berlainan (27˚C ± 2˚C, 32˚C ± 2˚C and 37˚C ± 2˚C) dan dinilai berdasarkan tempoh eksperimen (jam). Penukaran biojisim tersebut pula diukur melalui penghasilan gula dan kegiatan aktiviti selulase. Keputusan kajian tersebut menunjukkan bahawa ekstrak anai-anai *Coptotermes curvignathus* yang segar mengandungi gula dan aktiviti-aktiviti selulase seperti *endo-β-D-1,4-glucanase*, *exo-β-D-1,4-glucanase* dan *β-D-1,4- xylanase* yang berpotensi untuk meningkatkan pencernaan biojisim lignoselulosa. Kandungan gula yang paling tinggi dicatatkan adalah 7.36 ± 0.65 g/L yang terdapat di hidrolisis enzim dengan batang kelapa sawit pada suhu 37˚C, manakala aktiviti enzim yang paling tinggi dicatatkan merupakan *endocellulase* (31.58 ± 5.48 U/g) yang terdapat di ekstrak anai-anai, *exocellulase* (14.94 ± 4.71 U/g) yand terdapat di hidrolisis dengan batang kalapa sawit, dan *xylanase* (89.60 ± 20.87 U/g) yang terdapat di hidrolisis dengan ubi kayu, semuanya terjadi pada suhu 32˚C.

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Without these kind souls, none would have happened. Thank you.

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 ABBREVIATIONS

| | |
|---|---|
| AIM | Agensi Inovasi Malaysia |
| BGL | Beta-glucosidase |
| CBD | Cellulase Binding Domain |
| CBH | Cellobiohydrolase |
| CMC | Carboxymethyl Cellulose |
| CMCase | Carboxymethyl Cellulase |
| DNA | Deoxyribonucleic Acid |
| DNS | Dinitrosalicyclic Acid |
| EFB | Empty Fruit Bunches |
| EG | Endoglucanase |
| FAO | Food and Agricultural Organisation of the United Nations |
| GHF | Glycosyl Hydrolase Family |
| IUPAC | International Union of Pure and Applied Chemistry |
| MC | Moisture Content |
| MFG | Manufacturing |
| MPOB | Malaysian Palm Oil Board |
| NaOH | Sodium Hydroxide |
| NBS | National Biomass Strategy |
| OD | Optical Density |
| OPF | Oil Palm Frond |
| OPT | Oil Palm Trunk |
| PKC | Palm Kernel Cake |
| POME | Palm Oil Mill Effluent |
| POPF | Pruning Oil Palm Frond |
| RE | Renewable Energy |
| SSF | Simultaneous Saccharification and Fermentation |
| TAPPI | Technical Association of the Pulp and Paper Industry |
| TCA | Tricarboxylic Acid |
| XOS | Xylooligosaccharide |

# CHAPTER 1

# INTRODUCTION

## 1.1 General

### 1.1.1 Ensuring Sustainability

In the alarming wake of global warming, rising food and fuel costs, much interest has been shifted to use alternative resources for green energy conversion, such as the use of wasteful lignocellulosic biomass, because the existing practice of deriving energy from edible crops such as corn, beets or sugar cane will threaten the food supply (Held, 2012). Examples of green energy include the colourless and biodegradable ethanol or ethyl alcohol ($C_2H_5OH$). It is one of the most commonly used biofuels worldwide, especially in Brazil and the United States (U.S.), and can be harvested by fermenting the sugary components of cellulosic plants (Bon and Ferrara, 2007; Chin and H'ng, 2013). Burning of bioethanol results in reduced carbon emission compared to fossil fuels and therefore will not burden the natural resources.

However, major setback of the process is due to the recalcitrant nature of lignocellulosic structures. Cellulose being the basic components of plant cell wall, is a tough linear chain of glucose joined by β-1,4-glycosidic and hydrogen bonds wrapped in the matrix of insoluble lignin and hemicelluloses (Jeffries, 1994; Tokuda *et. al.*, 1997; Rubin, 2008). Common industrial degradation methods of lignocelluloses depend heavily on acid treatments (Sun and Cheng, 2002). Employment of enzymes in deconstructing lignocelluloses is relatively stable.

### 1.1.2 The Nature will find its Own Way

Among the wild battle of survival, termites have evolved to find food in the woods. These insects survive strictly on a cellulose-rich diet, which is attributed to the significant decomposition activities of symbiotic microbes in their gut. The cellulose in their food is turned into consumable acetate in just a day (Martin, 1983). To disintegrate these tough polysaccharides, termites are loaded with different species of microorganisms in their relatively tiny guts (Brune and Friedrich, 2000; Nadin, 2007; Wong *et. al.*, 2014). Different species of microbes in termite gut have different needs and release different end-products, but they share a common goal – that is to degrade lignocelluloses. Termites provide the needed settlement for the microbes and feed on wood, while the microbes digest the food for their hosts in return. Such exchange reflects a mutual symbiotic relationship that benefits both the host and the symbiont.

By efficiently catalysing the conversion of cellulose into acetate, glucose and ethanol, the key to generate customised cocktails lies within the termites' digestive enzymes. In recent years, significant progress has been made to isolate cellulolytic strains from termites and optimise the digestion efficiency of cellulose. Thus, the local wood feeding termite *Coptotermes curvignathus* is known to have the ability to kill immature palm (Lim and Silek, 2001; Yeoh and Lee, 2007, Chan *et. al.*, 2011), posing as a good candidate to study its incredible digestion capability.

## 1 .2 Problem Statement and Justification

Microbial strategies for degrading lignocelluloses are diverse, yet the fundamental understanding of the enzymes involved is limited. More findings should highlight the isolation of cellulolytic strains from termites, detail the evaluation of the enzymatic activities and conduct genetic modification or immobilisation of the microbes which produce the desired enzymes. In evaluating the effectiveness of the enzyme application, comparison between two biomass substrates was attempted. Hence, oil palm trunk (OPT) and cassava pomace were used in this experiment as the raw materials and respective biomass substrates for enzymatic hydrolysis with the whole enzymatic extracts of local wood-feeding termites, *Coptotermes curvignathus*.

Resources such as OPT and cassava pomace are readily available in Malaysia as a wasteful residue from the industry. According to the Malaysian Oil Palm Statistics, the oil palm plantations now covered 5.23 million hectares in 2013 (MPOB, 2014). National Biomass Strategy Blueprint also revealed that the Malaysia's palm oil industry is expected to generate about 100 million dry tonnes of solid biomass by the year 2020 (AIM, 2013). Consequently large quantities of oil palm plantation residues are produced annually the during replanting activities, averagely every 25 years, whereby the unproductive oil palm trees are replaced with young tree. Current waste practise is to leave the huge mass to rot and return to the field as fertiliser.

In addition, cassava processing, especially in concentrated areas, is regarded as a major polluting factor (FAO, 2001). Thailand, Indonesia, India, China, Vietnam, Malaysia and the Philippines all produce cassava starch, yet the conventional forms of processing are water intensive, generate huge amounts of organic effluents and visible dust wastes. If left untreated, this will be displayed in the form of stagnant ponds and emit strong odours. These agricultural residues are generally found in the vicinity of factories or plantations. In a growing nation such as Malaysia, there are clearly more than enough biomass residues that can be utilised for more lucrative purposes. In other words, both materials contain relatively high starch and total sugar contents that can be easily converted into sugar and fermented to bioethanol.

As mentioned, wood-feeding termites exhibit an incredible degradation capability via their specialised intestinal symbiotic association. By combining the termites' enzymes and lignocellulosic biomass, researchers may yield breakthrough solutions for renewable energy production. Therefore, the cellulolytic activities in the enzyme extracts of lower termite (*Coptotermes curvignathus*) were analysed. The reducing sugar yield and specific enzymatic activities such as endocellulase, exocellulase and xylanase, based on reaction hours, were presented in this study. Several bio-reaction parameters, such as temperatures and reaction hours, were studied in detail and their significances were analysed. Upon treating OPT or cassava pomace with the termites' enzymes, the reducing sugar yield, cellulase activities and degradation rate were also evaluated in this study.

### 1.3 Research Objectives

Based on the mentioned scenario, the main objective of this study is to evaluate the cellulolytic enzyme activities in the whole extracts of local wood-feeding termites (*Coptotermes curvignathus*) for enzymatic hydrolysis with OPT and cassava pomace. Therefore, this study aims to achieve the following objectives:

1. To determine the chemical compositions of OPT and cassava pomace.

2. To determine the reducing sugar yield and cellulase activities (endo-β-D-1,4-glucanase, exo-β-D-1,4-glucanase and β-D-1,4-xylanase) in the whole extracts of local wood-feeding termites (*Coptotermes curvignathus*) controlled at three different sets of temperature (27˚C ± 2˚C, 32˚C ± 2˚C and 37˚C ± 2˚C) and based on reaction time (hours).

3. To determine the reducing sugar yield, cellulase activities and degradation rate of OPT or cassava pomace upon enzymatic hydrolysis with the termite's extract.

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