Chapter 15
Unlocking the Destructive Powers of Wood-Eating Termites: From Pest to Biopolymer Derivatives Extractor

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Abstract Termites are social insects that live in colonies and its incredible digestive system may provide a breakthrough in efficient biopolymer derivatives production. Termite has the ability to digest a kind of food that few other living organisms are able to; the woody material. It has mouthparts that chew up wood into pieces. But the secret is that it carries special microorganisms that can digest the lignocellulosic material. By being the host to these special microorganisms, termites are able to do something that most living organisms are unable to; the ability

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to digest lignocellulosic materials. Termites have been considered nothing more than nuisance pest which destroy woody materials. The termite is found chewing on frames, furniture, and flooring made out of wood. But new research shows how the termite digest lignocellulose could hold a key to the production of numerous biopolymer derivatives from lignocellulosic material. Annoying as they may be, termites are amazingly efficient at converting wood into sugars, and that ability is very useful for making numerous biochemicals and biofuels. Researchers are studying the termite's digestion process in order to synthetically copy the process so that lignocellulosic materials can be used as the source to derive numerous biochemicals. For decades, much effort was made to increase the utility of lignocellulosic materials. In consideration of the ever-growing demand for traditional usage such as fiber products, novel markets for lignocellulosic materials have been identified in recent years, in replacement of petrochemicals. The scientists believe that information learned from the termite could increase the efficiency of wood derivation, making these biopolymer derivatives even more cost-effective and utilizing lignocellulosic biomass as a sustainable source of chemicals and fuels by replacing fossil fuel. This chapter comprises information on recent conversion methods of biochemicals from lignocellulosic biomass for application enablement and commercialization, laying special emphasis on termite lignocellulolytic system.

Keywords Termites • Lignocellulosic material • Biopolymer derivatives • Biochemical • Lignocellulolytic digestion

15.1 Overview

The global demand of the chemicals and fuel satiated with derived forms of the fossil fuel (more than 80 %) has highlighted the importance of this finite resource. The continuous depletion of the fossil fuel reserves and the emission of greenhouse gases had resulted in an increased focus on production of biochemicals and biofuel by replacing fossil fuel. Wood derivatives had been seen as alternative chemical sources and its future use as fuel will be seen greatly increased due to the ever-rising cost of fossil fuel (current price USD 100 per barrel). Furthermore, wood derivatives are a primary target in many countries to help reduce greenhouse gas emissions, water pollution, and soil degradation. As reported by Puhan et al. (2005), there is a growing trend in developed countries, toward the application of modern technologies and efficient conversion involving a range of microorganism and insects, with their target to make the wood derivatives as competitive with fossil fuel derivatives as possible, in terms of costs.

Wood or biopolymer derivatives from lignocellulosic biomass had been studied intensively in recent years. There are many researches done previously to improve the production of biochemicals derived from lignocellulosic materials with different

new methods and enzymes to produce cheap and plentiful, ecologically sound biochemicals. Chin et al. (2010) found that bioethanol can be produced from lignocellulosic materials at conversion factor of 26 % over the dry weight of lignocellulosic biomass. Although the research result provided the novel knowledge about the amount of bioethanol that can be produced from lignocellulosic biomass, the problem with wood-derived chemicals is that it takes a lot of resources and energy to convert the lignocellulose into sugars using current technology. During ethanol production, the complex organic compounds of cellulosic materials need to be converted first into much simpler soluble compounds like sugars before reacting in further stages to produce bioethanol or other biochemicals. Nevertheless, the hard part is that the carbohydrate polymers of lignocellulosic materials form extremely dense, resilient bonds. Lignin molecules that deposited around the sugar molecules act as a barrier preventing the microorganisms from penetrating. Additional pretreatment steps such as heat, steam, or caustic acids and bases treatments are required to be used to break it down. Such steps cause the process to be more costly, and in often cases, generate hazardous waste. Ragauskas et al. (2006) stated that currently, the inability to efficiently derive simple, utilizable sugars from lignocellulosic material through depolymerization reactions is a significant limiting factor for this biochemical industry.

Therefore, continuous study is needed in finding a quick, efficient means of extracting wood derivatives from lignocellulosic materials to improve the production of biochemical with lower cost and energy consumption. Ohkuma (2003) reported that the ability of insects to convert recalcitrant lignocellulosic material into a usable or intermediate chemical source has attracted much interest recently due to the numerous potential applications in biofuel and biochemical production. The latest research on conversion of biochemicals using enzymes shifted their intention on using enzymes extracted from wood eating insects such as termites.

Termites are one of the most pervasive and destructive insects that efficiently digest lignocellulosic material and nurture on this apparently nutritionally poor diet. Wherever termite invasion happens, the termites cause tremendous damage to woody constructions and trees (Osbrink and Lax 2003). As mentioned earlier, the problematic part in producing wood-derived chemical is obtaining the metabolic intermediates from the lignocellulose but then has never been a problem to termites. Interpretation of digestive mechanisms underlying the termite's ability to hydrolyze the lignocellulose is a potential target for the development of effective bio-termiticides (Zhou et al. 2008) and also the hydrolyzing enzymes can be utilized for the conversion of wood cellulose into simple sugars for biochemical production (Warnecke et al. 2007; Scharf and Tartar 2008; Rubin 2008). Bacteria that help termites digest wood could be key to derive biochemical cheaply from nonfood crops such as wood and grass. This chapter comprises information on recent conversion methods of biochemicals from lignocellulosic biomass for application enablement and commercialization, laying special emphasis on termite lignocellulolytic system.

15.2 Lignocellulosic Material: Chemical Composition, Enzymatic Degradation and Depolymerization, and Biopolmer Derivatives

15.2.1 Chemical Composition

Chemical composition directly affects the efficacy of biomass energy conversion. Lignocellulosic biomass mainly contains a mixture of carbohydrate polymers (cellulose and hemicellulose), lignin, extractives, and ashes (Van-Dyne et al. 1999; Kamm and Kamm 2004). The composition of these constituents varies from one plant species to another. The ease of energy conversion of biomass reflects lignocellulosic structure and composition. Cost-effective conversion of potential feedstocks to fuels and products needs appropriate levels of cellulose, hemicellulose, extractive, and lignin. Understanding the effect of chemical composition on pretreatment chemistry and ultimately feedstock reactivity is the key in achieving the robust conversion processes in handling variety of feedstocks and biomass.

As cellulose is the main structural constituent in plant cell walls, it is often found in a fibrous structure of an organized manner (Sjostrom 1993). Cellulose molecules are completely linear without branching and intra- and intermolecular hydrogen bonds are easily formed. Cellulose molecules are thus aggregated to microfibrils in bundles which form either highly ordered (crystalline) regions or less ordered (amorphous) regions. The susceptibility of cellulose to enzymatic degradation is higher in its amorphous form. Fibrils are built up by microfibrils and, finally, the formation of cellulose fibers (Sjostrom 1993). Cellulose chains are converted into microfibrils which constitute the basic framework of the cell, delivering a great deal of resistance to the presence of tensile forces (Jarvis 1984). Cellulose is a homopolysaccharide composed of D-glucose subunits linked to each other by β-1,4-glycosidic linkages (Swati et al. 2013). While, β-1,4-glycosidic is the chemical repeating unit, the structural repeat unit of the cellulose chain is cellobiose constituent of wood, comprising approximately 40–45 % of dry wood (Fengel and Wegener 1983). Cellulose chains are packed into microfibrils that linked together by hydrogen bonds and Van der Waals forces (Hermans 1949).

Hemicellulose is covalently bound to lignin and bound to cellulose through hydrogen bonds (Sjostrom 1993). Hemicellulose is lower in molecular weight than cellulose and has branches with lateral and short chains that consist of various sugars. It is a heteropolymers of D-glucose, D-xyloses, D-mannose D-galactoses, and, L-arabinoses and various other sugars that is low in molecular weight. Hemicellulose is more susceptible to hydrolysis than to the rigid structure of cellulose because it has a low degree of crystallinity and microfibrils and with more amorphous regions. Its composition differs among wood. Hardwood hemicellulose mainly consists of xylose, but in softwood mannose and glucose are the dominating building blocks of carbohydrates (Sjostrom 1993). In comparison to cellulose, the polymers present in hemicelluloses can be hydrolyzed easily. Even when such polymers co-crystallize with cellulose chains, it do not aggregate.

Lignin is a complex polymer of aromatic alcohols known as monolignols. It is highly resistant to microbial attack due to its chemical complexity, insolubility, and lack of hydrolysable linkages (Taherzadeh 1999). In nature, cellulose and hemicellulose comprise the major energy source in lignocellulosic biomass and are encrusted with lignin and create an ability to protect against enzymatic attack in lignocellulosic materials. Lignin composes around 25 % of lignocelluloses and is an aromatic cross-linked polymer synthesized from phenylpropanoid precursors. Differing in the substituents of the phenylpropanoid skeleton, lignins are categorized into two classes, the guaiacyl lignins and guaiacyl-syringyl lignins, respectively. Guaiacyl lignins have a methoxy-group in the 3-carbon position, whereas syringyl-lignins have a methoxy-group in both the 3-carbon and 5-carbon positions (Galbe and Zacchi 2002). The lignin component of lignocellulosic biomass is responsible as a protective sheath around the cellulose microfibrils (Palmqvist and Hahn-Hagerdal 2000). The composition of lignin varies depending on the source of raw material. Softwood contains a higher amount of lignin (about 30 %) than hardwood (about 20 %).

15.2.2 Enzymatic Degradation and Depolymerization

15.2.2.1 Lignin Biodegradation

The enzymatic degradation and depolymerization of lignocellulosic material into fermentable sugars is an intricate process that involves an extensive collection of enzymes. For first step in the process, it is of great importance to facilitate the degradation of cellulose and hemicellulose which involves depolymerization of hydrophobic lignin polymers (Scharf and Tartar 2008). Lignin is problematic substrate during biodegradation due to its insolubility behavior and high molecular weight. Theoretically, carbohydrolases such as hemicellulases and cellulases should have no significant effect in lignin degradation and depolymerization, as lignin is not a carbohydrate. The initial steps of lignin depolymerization can be catalyzed by extracellular, oxidative, and unspecific enzymes that are able to liberate highly unstable products which further undergo different oxidative reactions. Two major groups of enzymes that are involved in ligninolysis include laccases and peroxidases (Arora and Sharma 2010). Laccases are blue multicopper oxidases and with the presence of mediators it catalyze the oxidation of phenolic and non-phenolic compounds (Thurston 1994). The phenolic nucleus is by the removal of one electron, oxidized, creating a phenoxy-free radical which leads to polymer cleavage of the polymer. One-electron subtraction from phenolic hydroxyl group of lignin is catalyzed by laccase to provide phenoxy radicals which basically undergo polymerization through radical coupling along with partial depolymerization of propyl side chains via alkyl-aryl cleavage. It has been revealed that lignin biodegradation with laccase is mostly polymerized, but some parts are subject to depolymerization (Arora and Sharma 2010).

There are two groups of peroxidases function in delivering effective hydrolysis of the lignin polymer, lignin peroxidases, and manganese-dependent peroxidases. Lignin peroxidase is a glycoprotein which contains a heme as a group in its active center. Lignin peroxidase cannot enter the plant cell due to the size of this protein, thus lignin degradation occurs at the exposed regions of the lumen (Perez et al. 2002). Manganese-dependent peroxidases are molecularly very similar to lignin peroxidases; a glycosylated protein containing a heme group. Manganese-dependent peroxidase generates phenoxy radicals which further undergo various reactions, resulting in depolymerization (Gold et al. 2000). Manganese peroxidase oxidizes Mn^{2+} into Mn^{3+} by hydrolysis of hydrogen peroxide (Kirk and Cullen 1998). Mn^{3+} is a powerful oxidant that degrades lignin.

In addition, reductive enzymes including sugar oxidase and alcohol oxidase are a diverse group of enzymes that play major roles in ligninolysis (Eriksson et al. 1986; Nishida and Eriksson 1987; Kersten and Krik 1987; Kersten 1990). These enzymes are involved in the production of H_2O_2 which is vital in ligninase activity (Sarikaya and Ladisch 1997). In conventional processes, physicochemical treatments such as thermal treatment and chemical treatment are used to release fermentable sugars from raw material with high lignin content. However, extensive research to replace the physicochemical treatment with enzymatic treatments has been done. The main benefits of biological delignification include higher product yields, mild reaction conditions, less energy demand, and fewer side reactions.

15.2.2.2 Hemicellulose Biodegradation

Hemicellulose degradation is the second stage in the overall lignocellulose biodegradation process. This process is crucial to make the cellulose accessible for depolymerization. Enzymes required for efficient hemicellulose biodegradation include xylan esterases, p-coumaric and ferulic esterases, methyl glucuronosidases, and α-arabinofuranosidases (Subramaniyan and Prema 2002; Shallom and Shoham 2003). Compared to cellulose, more enzymes are required for total degradation and depolymerization due to the heterogeneity of hemicellulose. Hemicelluloses can be biodegraded to acetic acid and monomeric sugars. Xylan, the principle carbohydrate in hemicellulose, requires the combined activity of xylan 1,4-β-xylosidase and endo-1,4 β-xylanase during biodegradation process. The specific conversions achieved by each of these enzymes are as follows: (1) endo-xylanases hydrolyze the β-1,4-xylose linkages in the xylan backbone; (2) exo-xylanases hydrolyze reduced β-1,4 xylan linkages releasing xylobiose; (3) β-xylosidases act on xylobiose to liberate xylose and other short chain oligosaccharides; (4) α-arabinofuranosidases hydrolyze terminal non-reducing α-arabinofuranose from arabinoxylans; (5) α-uronidases release α-glucuronic, α-mannuronic, and α-galacturonic acids; and (6) esterases hydrolyze phenolic ester bonds (Collins et al. 2005). Xylanese is highly responsible to release the reducing sugars from xylan. For biodegradation of O-acetyl-4-O-methylglucuronxylan (one of the common hemicelluloses), four different enzymes are required for the degradation; endo-1,4-β-xylanase (endoxylanase),

acetyl esterase, a-glucuronidase, and β-xylosidase. The degradation of O-acetylgalactoglucomanann is as follows: (1) endomannases rupture the polymer; (2) acetylglucomannan esterases remove acetyl groups; (3) α-galactosidases eliminate galactose residues; and (4) β-mannosidase and β-glycosidase break down the endomannases (Saha 2003).

15.2.2.3 Cellulose Biodegradation

After hemicellulose degradation, cellulose is exposed for enzymatic degradation. Cellulose depolymerization requires the action of two primary cellulases; endoglucanases and cellobiohydrolases. The β-1,4-glycosidic linkages of cellulose are hydrolyzed by cellulases. Endoglucanases (endo β-1,4-glucanases) releasing new terminal ends by hydrolyzing internal bonds, preferably the amorphous regions in cellulose. Cellobiohydrolases (exo β-1,4-glucanases) act preferentially on the endoglucanase-generated or existing chain ends. Although both enzymes are able to degrade amorphous cellulose, the only enzymes that are able to degrade crystalline cellulose efficiently are cellobiohydrolases. Cellobiohydrolases and endoglucanases release cellobiose molecules. A requirement for effective hydrolysis of cellulose also includes β-glucosidases, which hydrolyze cellobiose and render two glucose molecules. Endoglucanases, cellobiohydrolases, and β-glycosidases must be stable in the exocellular environment and may form a ternary complex with the substrate to function properly (Perez et al. 2002). For microorganisms living in the environment where cellulose is being degraded, the products of cellulose hydrolysis are available as sources for carbon and energy. As a matter of fact, such release of sugars from cellulose is the main basis for microbial interactions occurring in such environments (Leschine 1995).

15.2.3 Biopolymer Derivatives from Lignocellulosic Material

Lignocellulosic material can be converted to a multitude of products. A vast number of chemicals and pharmaceuticals might be produced through enzymatic degradation and depolymerization of lignocellulosic biomass. Chemists and chemical engineers can eventually produce nearly any chemical from lignocellulosic material if they are given unlimited time and money. Though most of the produced chemicals will not be economically viable, various chemicals are potentially produced from lignocellulosic material with many uses. Seven chemicals derivable from lignocellulosic material that appear to have potential for production are: (1) furfural, (2) lactic acid, (3) acetic acid, (4) succinic acid, (5) ethanol, (6) methanol, and (7) hydrogen (Wang and Wang 1984; Zhan et al. 2003; Neureiter et al. 2004; Okino et al. 2008; Wang et al. 2011; Martin and Ignacio 2011; Agirrezabal-Telleria et al. 2013). These listed chemicals are often cited in the scientific research as potential products, and some have the potential to be platform chemicals. Acetic acid and

furfural are often used as chemical intermediate. These chemicals come from a range of technologies, many of which are still emerging technologies for biomass. The first four would more likely be created through low energy processes, while five, six, and seven through higher energy processes. Over the past two decades, gradual improvement has been made in the bioconversion of lignocellulosic material into chemicals and fuels, and the price of these products have dropped so much that nowadays biochemicals derived from lignocellulose can compete with petroleum derivatives. The conversion of lignocellulosic material into biochemical is accomplished in two main steps: (a) hydrolysis of the polymer, release the hemicellulose and cellulose from their composite with lignin through delignification, and depolymerization of carbohydrate polymer to produce fermentable sugars; and (b) fermentation using pentoses and hexoses obtained in the first step to produce biochemical. The main advantages of using biological process to derive chemical from lignocellulosic material include higher product yields, less energy demand, and fewer side reactions.

15.3 Nature-Inspired Technologies from Wood-Eating Termite

15.3.1 Lignocellulolytic Systems of Termite

Termites (order Isoptera), well-known pests to cause substantial economic damage to wood construction and landscape tree, are recently used as model for wood derivatives systems. The order Isoptera is separated into lower and higher level termites; characterized by the presence or the lack of symbiotic protists residing in hindgut (Ohkuma et al. 2007). Both higher and lower termites harbor a dense and diverse population of prokaryotic symbionts (Brune 2007). According to Scharf and Tartar (2008), the lignocellulolytic digestion model in termites is defined as group of host and symbiont genes in the termite gut that cooperate to achieve high efficiency of lignocellulose digestion. Besides as a host for numerous microorganism that assist the lignocellulose digestion, termites themselves contribute to the digestion by producing a series of cellulases, hemicellulases, and lignases (Scharf et al. 2010; Zhou et al. 2010).

Worker termites are the most numerous individuals in the colony and are majorly responsible on feeding and lignocellulose digestion. Three main body regions of a worker termite are the foregut, the midgut, and the hindgut (Wood and Johnson 1986). The foregut region which is responsible for mechanically grinding ingested wood fragments comprises of the esophagus, crop, and attached salivary glands. The termite produces and secretes numerous endogenous enzymes such as β-glucosidases, exoglucanases and endoglucanases by the salivary glands into the digestive tract. Apparently, the midgut is a location where some lignocellulose degradation and depolymerization happen. This slender tubular region continuously

secretes peritrophic membrane, a protective lining secreted by the midgut that surrounds food and other ingested materials (Tokuda et al. 2004). The midgut of higher termites is known to secrete endoglucanases as well. Attached at the junction of midgut and hindgut are the malpighian tubules which participate in nitrogen recycling and waste excretion. In comparison, less microbiota is to be found in the foregut and midgut, while microbiota is found more abundantly in the hindgut (Hongoh 2011; Köhler et al. 2012). The hindgut, which originates ectodermally, is the largest organ. The hindgut is fermentation compartment that is generally anaerobic, but does possess a microoxic region around its periphery (Leadbetter et al. 1999). The hindgut hosts the majority of gut symbionts and is where most cellulose degradation happens, as well as fermentation occurs. Additionally, Ke et al. (2010) report that lignin modification mainly occurs within termite foregut and midgut sections, then the ingested materials further depolymerize by protozoa in the hindgut.

Termite gut consisting of diverse microorganisms from all three domains of life: Bacteria, Archaea, and Eukarya (protists). Most of these symbiotic microorganisms inhabiting the termite hindgut, including the protists in lower termites and the bacteria responsible for lignocellulose degradation in higher termites, are culture-independent and difficult to isolate for growth and identification by traditional culturing methods. In the hindgut of lower termites, wood particles are endocytosed by symbiotic protists and decomposed within their food vacuoles (Yamaoka 1979). Using PCR technology, scientists identified several diverse genes encoding protist cellulases of the glycosyl hydrolase families 5, 7, and 45 (GHF 5, 7, and 45) from the hindguts of two lower termites *Reticulitermes speratus* and *Coptotermes formosanus* (Ohtoko et al. 2000; Nakashima et al. 2002; Inoue et al. 2005). These studies demonstrate that the symbiotic protists in lower termites may be a rich source of novel cellulase genes.

In consideration of the significant activity of xylanase that was observed in the hindgut of termites, diverse glycosyl hydrolases also are expected to exist in symbiotic protists (Inoue et al. 1997). Symbiotic bacteria in the hindguts of wood-feeding termite species decompose lignocellulosic substrates efficiently despite the lack of gut protists. The demand for the identification of novel lignocellulosic degrading enzymes from these symbiotic bacteria, the need for heterologous protein production at higher efficiencies, and the exigency of reducing the costs of commercial cellulases have generated an interest in obtaining genomic sequences of these symbiotic bacteria.

15.3.2 Integrating Nature-Inspired Technologies from Wood-Eating Termite

The world is on the inclining growth in the production and use of biochemicals and biofuel. Alternative and renewable source to derive fuels and chemicals such as lignocellulosic material offers the chance to potentially reduce the dependence on fossil fuels and prevent further global warming. In fact, there is still a

big gap in between the current state of lignocellulosic material conversion technology from being established for commercial application due to its efficiency and processing costs. Over the past 20 years, biochemical industrial technology has mostly laid its basis on the fermentation and biocatalysis technologies from fungal and bacterial lignocellulolytic enzymes, in tandem with discoveries in enzyme engineering, molecular genetics, and metabolic engineering. Hence, there is still improvement needed in developing the commercial techniques for converting lignocellulosic material to chemicals and fuels. As mentioned earlier, the hard part in deriving chemical and fuel from lignocellulosic material is obtaining the metabolic intermediates due to the complicated lignocellulosic biomass structure. To improve the wood-derived chemical and fuel technology, researchers explored and learned from the comprehensive digestive systems of wood-feeding termites in nature (Ohtoko et al. 2000; Nakashima et al. 2002; Inoue et al. 2005).

Termites possess a highly competent natural bioreactor, a specialized gut system that can efficiently process lignocellulosic material. The ability of termite to feed on lignocellulosic material has inspired extensive research into the termite digestion mechanisms of the structural and recalcitrant lignocellulose in their diet, which is potentially to advance current biochemical technologies and processing. The digestion of wood-feeding termites mainly comprises collaboration between two catalyst systems; catalysts from a variety of gut symbiotic microorganisms and termite endogenous catalyst systems, including lignocellulolytic bacteria and protozoa (Ohkuma 2003). The digestion efficiency of wood-eating termite is 65–87 % for hemicellulose and 74–99 % for cellulose (Prins and Kreulen 1991). Therefore, the critical goal development for efficient lignocellulose processing in the biochemical industry is dependent on the development of pretreatment methods that is suitable for vast diversity of lignocellulosic feedstock. Pretreatment procedures are important steps to improve cellulose dipolymerization where substrate that creates obstacles to cellulases is being removed. Researchers are now trying to see if we can use these same microorganisms in termite gut to ease the process for wood-derived chemicals. Current technologies to break down the cellulose in wood are nothing in comparison to termites. Termites are incredibly efficient at it and if we could somehow harness them to do the work for us, we may be able to make biochemicals in a cost-effective manner. One of the important goals behind the exploration of termite lignocellulolytic systems is to screen the genes or enzymes that are appropriate for biochemical industrial applications (Sun and Scharf 2010). Other than just shedding light upon the enigmas of the termite lignocellulolytic systems, researches have also been carried out on physiochemical microhabitats of termite gut systems which provide new information of the gut environments and the specific condition that may actually be essential for an efficient lignocellulolytic system. Hence, theoretically and practically speaking, such studies are important for understanding termite lignocellulolytic systems and evolving current biochemical and biofuel technology.

Zhang et al. (2010) defines two recombinant endogenous glycosyl hydrolases from a lower termite species that functionally convert cellulose to glucose.

This study provides better understanding to scientists in optimizing recombinant cellulolytic enzyme production and combinations for lignocellulose conversion. On the other hand, Ke et al. (2010) identified that all known termites possess a hindgut microbial community of high density and diversity, which is of aid in their digestion and is the source of lignocellulosic fermentation products like glucose, acetate, ethanol, and methane as they contain hydrolytic, acidogenic, acetogenic, and methanogenic bacteria. Methane and hydrogen emission from lower termites are reported to be by-products produced through termite gut lignocellulolytic and metabolic systems. These investigations suggest a distinctive mechanism for generating methane or hydrogen as a by-product during lignocellulose conversion (Cao et al. 2010).

A genomic study of the microbes that ultilizes the termite gut as their habitat has identified close to 1,000 possible enzymes that break down wood (Warnecke et al. 2007). This has proven that bacteria in termite guts encode a diversity of genes and enzymes that may play a role in wood degradation. The plethora of lignocellulolytic enzymes could explain the termite's well-known capacity of wood-eating and provides the suggestion of cheaper and more efficient ways for generating lignocellulosic-derived chemicals. Inspired by the termite gut system, simultaneous saccharification and fermentation is a compelling option to the two-step bioconversion, in which the presence of both hydrolytic enzymes and fermentative microorganism is in the same reactor. The great advantage of using simultaneous saccharification and fermentation is that hydrolysis and fermentation using the resulting sugar to desired biochemical happens at the same time, in the same reactor, thus, recovery of the products is easier. There are much efforts carried out by the laboratories throughout the world in order to obtain the production of biochemical or biofuel directly from lignocellulosic materials using a cocktail of microorganisms. The cocktail of symbiotic microbes and lignocellulolytic enzymes from the termite gut show promises for industrial use. The ability of termite to convert lignocellulose into fermentable sugars efficiently in a short time has made studying the insects a point of focus for number of scientists, to apply that capability to the large-scale generation of biochemical from lignocellulosic materials. From the evidence observed in laboratory bioassays, many of the enzymes isolated from termites could have a significant synergistic effect on wood degradation when mixed with other commercial enzyme preparations, e.g., >47 % more reducing sugar at 1:1 mixture than with pure commercial enzyme (Azuma et al. 1984). Therefore, the cellulases or other related hydrolases isolated from termite guts may act as unique catalysts and be an economically viable solution for the bioconversion of lignocelluloses. Besides, Nakashima et al. (2002) also stated that enzyme-producing genes from termite gut may be transferred to a creature that is more controllable, allowing cellulases to be mass produced to generate biofuels. Oftenly, this normally meant that the transfer of the genes would be done onto fungi or bacteria that are modified genetically, or into other bigger living organism, such as caterpillars, to industrially produce the enzymes. These diverse cellulase genes could potentially be cloned into *Escherichia coli* or other vector bacteria.

15.4 Conclusions and Future Perspective

Many researchers throughout the world are participating in the research on the various aspects of bioconversion of lignocellulosic material in hopes of a replacement for petrochemicals. Subsequently, practises that use microorganisms and enzymes are being industrialized to explore the possible applications to derive chemical and fuel from lignocellulosic material. This novel and evolving multidisciplinary area has emerged between entomology and bioengineering sciences; and thus, without a doubt, it will open the way for future discoveries and inventions in biotechnological application especially in the wood chemical industry. Through intensive studies, multiple genes discovered from the termite lignocellulolytic system and successful recombinant techniques with such enzyme genes have provided initial tools vital for further research. Notwithstanding the progress achieved so far, more effort is required for microorganisms and lignocellulolytic enzymes on the mechanism of crystalline microfibril depolymerization and lignin modifications/disruption on hemicellulose and cellulose digestion to obtain a significant industrial impact. Further understanding of the efficient lignocellulolytic system of termites by exploring the enzymes produced by termites and their symbiotic microorganisms will lead to the development of optimal enzyme cocktails with high efficiency of lignocellulose degradation, and in addition, provide novel approaches for biochemical and biofuel industrial applications.

References

Agirrezabal-Telleria I, Gandarias I, Arias PL (2013) Production of furfural from pentosan-rich biomass: analysis of process parameters during simultaneous furfural stripping. Bioresour Technol 143:258–264

Arora DS, Sharma RK (2010) Ligninolytic fungal laccases and their biotechnological applications. Appl Biochem Biotechnol 160:1760–1788

Azuma J, Nishimoto K, Koshijima T (1984) Studies on system of termites. II. Properties of carbohydrolases of termite Coptotermus formosanus Shiraki. Wood Res 70:1–16

Brune A (2007) Microbiology: woodworker's digest. Nature 450:487–488

Cao Y, Sun JZ, Rodriguez JM, Lee KC (2010) Hydrogen emission by three wood-feeding subterranean termite species (Isoptera: Rhinotermitidae): production and characteristics. Insect Sci 17:237–244

Chin KL, H'ng PS, Wong LJ, Tey BT, Paridah MT (2010) Optimization study of ethanolic fermentation from oil palm trunk, rubberwood and mixed hardwood hydrolysates using *Saccharomyces cerevisiae*. Bioresour Technol 101(9):3287–3291

Collins T, Gerday C, Feller G (2005) Xylanases, xylanase families and extremophilic xylanases. FEMS Microbiol Rev 29:3–23

Eriksson KE, Pettersson B, Volc J, Musilek V (1986) Formation and partial charectarization of glucose 2 oxidase, A H_2O_2 producing enzyme in Phanerochaete chrysosporium. Appl Microbiol Biotechnol 23:257–262

Fengel D, Wegener G (1983) Wood chemistry, ultrastructure and reactions. Walter de Gruyter, Berlin

Galbe M, Zacchi G (2002) A review of the production of ethanol from softwood. Appl Microbiol Biotechnol 59:618–628

Gold MH, Youngs HL, Sollewijn Gelpke MD (2000) Manganese peroxidase. In: Sigel H, Sigel A (eds) Metalions biological systems. Marcel Dekker, New York, pp 559–587
Hermans EH (1949) Physics and chemistry of cellulose fibres. Elsevier Publishing Company, Inc, New York, p 543
Hongoh Y (2011) Toward the functional analysis of uncultivable, symbiotic microorganisms in the termite gut. Cell Mol Life Sci 68:1311–1325
Inoue T, Murashima K, Azuma JI, Sugimoto A, Slaytor M (1997) Cellulose and xylan utilisation in the lower termite *Reticulitermes speratus*. J Insect Physiol 43(3):235–242
Inoue T, Moriya S, Ohkuma M, Kudo T (2005) Molecular cloning and characterization of a cellulose gene from a symbiotic protist of the lower termite, *Coptotermes formosanus*. Gene 349:67–75
Jarvis MV (1984) Structure and properties of pectin gels in plant cell walls. Plant Cell Environ 7:164
Kamm B, Kamm M (2004) Biorefinery systems. Chem Biochem Eng 18:1–6
Ke J, Sun JZ, Nguyen HD, Singh D, Lee KC, Beyenal H, Chen SL (2010) In-situ oxygen profiling and lignin modification in guts of wood-feeding termites. Insect Sci 17:277–290
Kersten PJ (1990) Glyoxal oxidase of Phanerochaete Chrysosporium: its characterization and activation by ligning peroxidase. Proc Natl Acad Sci U S A 87:2936–2940
Kersten PJ, Krik TK (1987) Involvement of a new enzyme. Glyoxal oxidase, in extracellular H2O2 production by Phanerochaete chrysosporium. J Bactriol 169:2195–2201
Kirk K, Cullen D (1998) Enzymology and molecular genetics of wood degradation by white rot fungi. In: Young RA, Akhtar M (eds) Environmental friendly technologies for pulp and paper industry. Wiley, New York, pp 273–307
Köhler T, Dietrich C, Scheffrahn RH, Brune A (2012) High-resolution analysis of gut environment and bacterial microbiota reveals functional compartmentation of the gut in wood-feeding higher termites (*Nasutitermes* spp.). Appl Environ Microbiol 78:4691–4701
Leadbetter JR, Schmidt TM, Graber JR, Breznak JA (1999) Acetogenesis from H2 plus CO2 by spirochetes from termite guts. Science 283:686–689
Leschine SB (1995) Cellulose degradation in anaerobic environments. Annu Rev Microbiol 49:399–426
Martin M, Ignacio E (2011) Grossmann energy optimization of hydrogen production from lignocellulosic biomass. Energ Syst Eng 35(9):1798–1806
Nakashima K, Watanabe H, Azuma J (2002) Cellulase genes from the parabasalian symbiont *Pseudotrichonympha grassii* in the hindgut of the wood-feeding termite *Coptotermes formosanus*. Cell Mol Life Sci 59:1554–1560
Neureiter M, Danner H, Madzingaidzo L, Miyafuji H, Thomasser C, Bvochora J, Bamusi S, Braun R (2004) Lignocellulose feedstocks for the production of lactic acid. Chem Biochem Eng Q 18(1):55–63
Nishida A, Eriksson KE (1987) Formation, purification and partial charectarization of methanol oxidase, a H2O2 producing enzyme in Phanerochaete chrysosporium. Biotechnol Appl Biochem 9:325–338
Ohkuma M (2003) Termite symbiotic systems: efficient bio-recycling of lignocellulose. Appl Microbiol Biotechnol 61:1–9
Ohkuma M, Sato T, Noda S, Ui S, Kudo T, Hongoh Y (2007) The candidate phylum "Termite Group 1" of bacteria: phylogenetic diversity, distribution, and endosymbiont members of various gut flagellated protists. FEMS Mbiol Ecol 63(3):467–476
Ohtoko K, Ohkuma M, Moriya S, Inoue T, Usami R, Kudo T (2000) Diverse genes of cellulase homologues of glycosyl hydrolase family 45 from the symbiotic protists in the hindgut of the termite *Reticulitermes speratus*. Extremophiles 4:343–349
Okino S, Noburyu R, Suda M, Jojima T, Inui M, Yukawa H (2008) An efficient succinic acid production process in a metabolically engineered Corynebacterium glutamicum strain. Appl Microbiol Biotechnol 81(3):459–464
Osbrink WLA, Lax A (2003) Effect of imidacloprid tree treatments on the occurrence of formosan subterranean termites, *Coptotermes formosanus* Shiraki (Isoptera: Rhinotermitidae). J Econ Entomol 96:117–125

Palmqvist E, Hahn-Hagerdal B (2000) Fermentation of lignocellulosic hydrolysates. II: inhibitors and mechanisms of inhibition. Bioresour Technol 74:25–33
Perez J, Munoz-Dorado J, de la Rubia T, Martınez J (2002) Biodegradation and biological treatments of cellulose, hemicellulose and lignin: an overview. Int Microbiol 5:53–63
Prins RA, Kreulen DA (1991) Comparative aspects of plant cell wall digestion in mammals. In: Hoshino S, Onodera R, Minoto H, Itabashi H (eds) The rumen ecosystem. Japan Scientific Society Press, Tokyo, pp 109–120
Puhan S, Vedaraman N, Rambrahaman BV, Nagarajan G (2005) Mahua (Madhuca indica) seed oil: a source of renewable energy in India. J Sci Ind Res 64:890–896
Ragauskas AJ, Williams CK, Davison BH, Britovsek G, Cairney J, Eckert CA, Frederick WJ Jr, Hallett JP, Leak DJ, Liotta CL (2006) The path forward for biofuels and biomaterials. Science 311(5760):484–489
Rubin EM (2008) Genomics of cellulosic biofuels. Nature 454:841–845
Saha BC (2003) Hemicellulose bioconversion. J Ind Microbiol Biotechnol 30:279–291
Sarikaya A, Ladisch M (1997) Mechanism and potential application of bioligninolytic systems in a CELSS. Appl Biochem Biotechnol 62(2):131–149
Scharf ME, Tartar A (2008) Review: termite digestomes as sources for novel lignocellulases. Biofpr J 2:540–552
Scharf ME, Kovaleva ES, Jadhao S, Campbell JH, Buchman GW (2010) Functional and translational analyses of a beta-glucosidase gene (glycosyl hydrolase family 1) isolated from the gut of the lower termite *Reticulitermes flavipes*. Insect Biochem Mol Biol 40:611–620
Shallom D, Shoham Y (2003) Microbial hemicellulases. Curr Opin Microbiol 6:219–228
Sjostrom E (1993) Wood chemistry: fundamentals and applications. Academic, Espoo, Finland, pp 51–108
Subramaniyan S, Prema P (2002) Biotechnology of microbial xylanases: enzymology, molecular biology and application. Crit Rev Biotechnol 22:33–46
Sun JZ, Scharf ME (2010) Exploring and integrating cellulolytic systems of insects to advance biofuel technology. Insect Sci 17(3):163–165
Swati G, Haldar S, Ganguly A, Chatterjee PK (2013) Review on Parthenium hysterphorus as a potential energy source. Renew Sustain Energy Rev 20:420–429
Taherzadeh MJ (1999) Ethanol from lignocellulose: physiological effects of inhibitors and fermentation strategies, chemical reaction engineering. Chalmers University of Technology, Sweden
Thurston CF (1994) The structure and function of fungal laccases. Microbiology 140:19–26
Tokuda G, Lo N, Watanabe H, Arakawa G, Matsumoto T, Noda H (2004) Major alteration of the expression site of endogenous cellulases in members of an apical termite lineage. Mol Ecol 13:3219–3228
Van-Dyne DL, Blase MG, Clements LD (1999) A Strategy for returning agriculture and rural America to long-term full employment using biomass refineries. In: Janeck J, Alexandria VA (eds) Perspectives on New crops and new uses. ASHS Press, United States, pp 114–123
Wang G, Wang DI (1984) Elucidation of growth inhibition and acetic acid production by Clostridium thermoaceticum. Appl Environ Microbiol 47:294–298
Wang D, Li QA, Yang MH, Zhang YJ, Su ZG, Xing JM (2011) Efficient production of succinic acid from corn stalk hydrolysates by a recombinant Escherichia coli with ptsG mutation. Process Biochem 46:365–371
Warnecke F, Luginbuhl P, Ivanova N, Ghassemian M, Richardson TH, Stege JT (2007) Metagenomic and functional analysis of hindgut microbiota of a wood feeding higher termite. Nature 450:560–565
Wood TG, Johnson RA (1986) The biology, physiology, and ecology of termites. In: Vinson SB, Johnson RA (eds) Economic impact and control of social insects. Praeger, New York, pp 1–68
Yamaoka I (1979) Selective ingestion of food by the termite protozoa, Trichonympha agilis. Dobutsugaku Zasshi 88(2):174–179
Zhan X, Wang D, Tuinstra MR, Bean S, Seib PA, Sun XS (2003) Ethanol and lactic acid production as affected by sorghum genotype and location. Ind Crop Prod 18:245–255

Zhang D, Lax AR, Bland JM, Yu J, Fedorova N, Nierman WC (2010) Hydrolysis of filter-paper cellulose to glucose by two recombinant endogenous glycosyl hydrolases of *Coptotermes formosanus*. Insect Sci 17:245–252

Zhou X, Wheeler MM, Oi FM, Scharf ME (2008) RNA interference in the termite *Reticulitermes flavipes* through ingestion of double-stranded RNA. Insect Biochem Molecularmol 38: 805–815

Zhou X, Kovaleva ES, Wu-Scharf D, Campbell JH, Buchman GW (2010) Production and characterization of a recombinant beta-1,4-endoglucanase (glycohydrolase family 9) from the termite *Reticulitermes flavipes*. Arch Insect Biochem Physiol 74:147–162