PHYSIOCHEMICAL LIGNOCELLULOSE MODIFICATION BY THE FORMOSAN SUBTERRANEAN TERMITE *COPTOTERMES FORMOSANUS* SHIRAKI (ISOPTERA: RHINOTERMITIDAE) AND ITS POTENTIAL USES IN THE PRODUCTION OF BIOFUELS

Hongjie Li,[a] Jirong Lu,[a] and Jianchu Mo[a,]*

Formosan subterranean termites (*Coptotermes formosanus* Shiraki) and other wood-feeding insects have the ability to digest cellulose and structurally modify or degrade lignin. We examined the physical and chemical changes to lignocellulosic components of Chinese red pine (*Pinus massoniana*) after passing through the termite (*C. formosanus*) digestive system. The purpose of this research was to evaluate biochemical digestive processes in the *C. formosanus* gut as potential models for biofuels processing. Results suggest that demethylation, demethoxylation, and propyl side-chain modification are responsible for higher lignin removal and cellulose crystallinity reduction after structural alteration. SEM images also further indicated that unlike the fungus-growing termites *Odontotermes formosanus*, the lower termites *C. formosanus* disrupted the lignocellulose structure, and thus resulted in an increase of surface area to cellulase. Comparative enzymatic hydrolysis tests between raw wood and *C. formosanus* faeces revealed an enhanced level of enzymatic digestibility in digested material. Based on the results, *C. formosanus* can efficiently modify lignin at ambient temperatures and pressures in contrast to current methods used in biofuels production.

Keywords: Biofuel; Coptotermes formosanus; Cellulose crystallinity; Enzymatic digestibility; Lignin removal; Odontotermes formosanus; Pre-treatment

Contact information: Ministry of Agriculture Key Laboratory of Molecular Biology of Crop Pathogens and Insects, Institute of Insect Sciences, College of Agriculture and Biotechnology, Zhejiang University, Kaixuan Road 268, Hangzhou, Zhejiang 310029, P. R. China Tel/Fax: +86-571-86971695
**Corresponding author: mojianchu@zju.edu.cn*

INTRODUCTION

Pretreatment is a critical step in biomass-to-biofuel conversion and refers to processes in which plant lignin is broken down and cellulose crystalline structures are disrupted so that acids or enzymes can hydrolyse the cellulose (Chandra et al. 2007; Kumar et al. 2009). This is also the most expensive and energy-consuming step in biomass-to-biofuel conversion, requiring sufficient mechanical processing, as well as typically requiring high temperatures and pressure (Mosier et al. 2005). Biological pre-treatments offer an alternative and typically use microorganisms such as brown-, white-, and soft-rot fungi to enhance the suitability of lignocellulose for enzymatic hydrolysis (Galbe and Zacchi 2007; Tian et al. 2011). Pretreatment techniques that utilize natural processes have much lower energy requirements and are environmentally friendly

(Okano et al. 2005). The downside, however, is that most biological pre-treatment processes are slow because of low hydrolysis rates (Shary et al. 2007; Singh et al. 2008).

Insects that utilize wood as a food source include beetles, cockroaches, and termites. Termites are especially well known for their ability to overcome the lignin barrier and digest polymer carbohydrates (Brune 1998; Hyodo et al. 1999; Watanabe and Tokuda 2010). Several recent studies looking at lignocellulose digestion processes in these insects suggest that novel physiochemical processes may be involved, especially in *Coptotermes formosanus* Shiraki (Prins and Kreulen 1991; Breznak and Brune 1994; Geib et al. 2008; Ke et al. 2010).

A number of genes that encode candidate pretreatment enzymes have been identified in the termite guts by transcriptome analyses, including lignases and phenolic acid esterases (Tartar et al. 2009; Scharf and Boucias 2010). Furthermore, functional analyses of these enzymes have shown that they play roles in lignocellulosic pretreatment, such as lignin modification and hemicellulose solubilisation (Coy et al. 2010; Wheeler et al. 2010). The physiological and morphological information of digestive systems, especially those of microenvironment and masticating organs, which may result in the efficient use of biomass, were also measured (Sharma et al. 1984; Kim and Holtzapple 2006). These characteristics suggest that the termite digestive gut system may have potential as a model for more efficient pre-treatment of plant matter in biofuel production.

Little is known about the complex pretreatment mechanisms of lignocellulose in the termite gut system beyond current knowledge of single enzyme systems. Lignin degradation in the termite gut is well documented (Geib et al. 2008; Ke et al. 2010), but the high level of cellulose utilization despite apparently low lignin modification continues to be a mystery. Changes in the crystalline structure of cellulose and the morphology of cell walls during digestion in the termite gut have not been documented but may be of importance when trying to understand the exact nature of lignocellulose digestion in *C. formosanus*. In this paper, the extent of physiochemical changes to lignocellulose during digestion in the termite gut and the extent to which lignocellulose is modified after movement through the termite digestive system are presented and discussed.

EXPERIMENTAL

Biomass Preparation

Samples of Chinese red pine *Pinus massoniana* (Lamb.) obtained from the market in Hangzhou, Zhejiang Province, P. R. China, were cut into blocks (5 cm × 5 cm × 20 cm) of the sapwood (Hyodo et al. 1999). The blocks were divided into two portions: one was used directly as food of termites and the other was milled and ground to pass a 1 mm screen, oven-dried at 60°C for 72 h, and stored in plastic bag at 4°C for all experiments.

Sample Collection and Preparation

A colony of *C. formosanus* was obtained from the National Center for Termite Control in Hangzhou, Zhejiang Province, P. R. China, in June 2009. The colony was fed sapwood blocks and maintained in a plastic box (40 cm × 40 cm × 50 cm) without soil in complete darkness at a constant temperature of 26°C and 69% RH. Fecal material was collected daily from surfaces of the sapwood food source and the plastic box. Collected material was stored in a sealed plastic bag at 4°C until processing for all experiments. In addition, fungus-growing termites *Odontotermes formosanus* (Shiraki), collected from Hangzhou Botanic Garden in Hangzhou, Zhejiang Province, P. R. China, were fed and maintained under the same conditions as described above.

For both species, *C. formosanus* and *O. formosanus*, 100 workers were selected at random and dissected to collect wood particles in various stages of digestion from the foregut, midgut, and hindgut of each insect (Fig.1). The contents of the three different gut segments of each termite were squeezed gently, diluted with 10 μL of deionised water, and collected by capillary tubes, using a dissecting microscope. The suspensions were stored in the capillary tubes until observation with scanning electron microscopy (SEM), described in following sections.

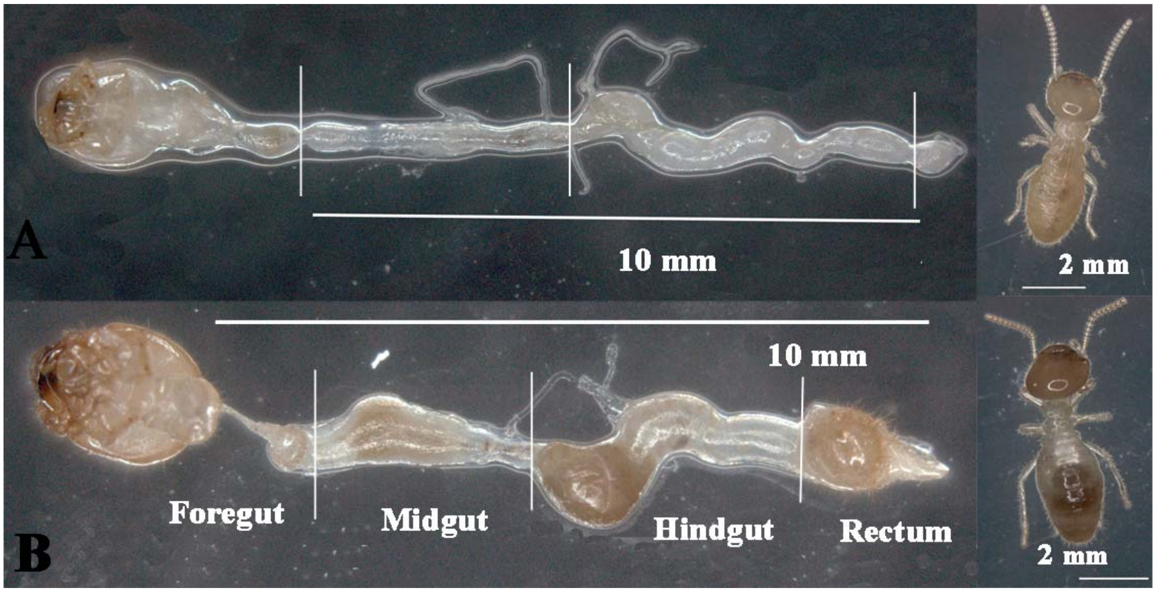

Fig. 1. Morphological characteristics of *C. formosanus* (a) and *O. formosanus* (b) worker gut system. The typical worker termite gut is divided into three major segments: foregut, midgut and hindgut

Chemical Analysis

Differences between carbohydrate and lignin structures in raw sapwood and digested faecal materials (*C. formosanus*) were measured using Laboratory Analytical Procedure (LAP) for biomass compositional analysis provided by the National Renewable Energy Laboratory (NREL). Hydrolysates were fractionated into acid-insoluble residues and filtrates containing carbohydrates and acid-soluble lignin by vacuum filtered through filtering crucibles. Filtrates were analysed for monosaccharides with a high performance liquid chromatography (HPLC) system (Agilent, USA) that was equipped with a refractive index detector and a Shodex sugar SP-0810 column. The column temperature was maintained at 80°C, and 0.2 μm filtered and degassed HPLC-grade water was used in the mobile phase and run at a flow rate of 0.6 mL/min for 35 min. Measurements of acid-soluble lignin were conducted with a UV-Vis spectrophotometer at 240 nm. The Klason lignin content was measured gravimetrically after washing and drying the acid insoluble residues collected as described above.

ATR-FTIR Spectrum

Attentuated total reflectance Fourier transform infrared (ATR-FTIR) analysis was conducted using a Nicolet Nexus 670 FTIR Spectrometer (Thermo Fisher, USA) in conjunction with an ATR single reflection crystal to detect structural changes in sapwood digested by the *C. formosanus* termites. Two milligram portions of raw wood and termite-digested samples were pressed with a spring-loaded anvil. Spectral analyses consisted of 128 scans at wave lengths ranging from 800 to 4000 cm^{-1} and a resolution of 2 cm^{-1}. All samples were done in triplicate. Baseline correction was completed using Omnic software.

Scanning Electron Microscopy (SEM) Analysis

SEM analyses were completed for raw sapwood, for particles contained in the termite gut sections, and for excreted faecal material. An ATM-1000 microscope (Hitachi, Japan) was used in accordance with the manufacturer’s instructions.

Enzymatic Digestibility

Samples of untreated sapwood and excreted faecal material (*C. formosanus*) were washed with deionized water. The water-insoluble fraction of each washed sample was collected for enzymatic hydrolysis testing. Each was placed in 5% substrate concentration using 50 mM sodium citrate buffer (pH 4.8) in 250 mL Erlenmeyer flasks. Forty FPU/g dry solid cellulase (Imperial Jade Biotechnology Co., Ltd., China) was then added to each flask as well as 0.08 g/L tetracycline to eliminate microbial contamination. Flasks were incubated at 50°C for 72 h and shaken at 150 rpm in a reciprocating shaker. The hydrolysates were centrifuged at 10,000 rpm for 5 min. Supernatants were collected, and glucose content in each sample was determined using HPLC based on the protocol described above. All assays were performed in triplicate.

RESULTS AND DISCUSSION

Compositional Analysis

Chemical characterization of Chinese red pine wood before and after termite digestion showed that large amounts of cellulose were removed by *C. formosanus* during the digestion process (Table 1). Glucan content was also reduced from 49.6% to 22.5%. Analysis of excreted faecal material showed a slight reduction in lignin content compared to the raw materials. Total lignin increased to 59.2% from 28.9%, with a final composition of 58.8% Klason lignin and 0.4% acid-soluble lignin. Lignin derivatives were concentrated in termite faeces compared to raw wood materials, apparently because of the metabolism of cellulose and hemicellulose.

The compositional analysis showed that termites selectively digest cellulose despite apparently low lignin modification, and this finding is consistent with previous results (Hyodo et al. 1999). There are several likely reasons that termites are able to efficiently digest cellulose without a large amount of lignin degradation: (1) mechanical processing includes mastication by mandible and grinding in the gizzard, leading to an increase of surface area and a lower crystallinity (Kumar et al. 2009), and (2) lignin modification includes propyl side-chain oxidation, demethylation of ring methoxyl groups, and lignin spatial rearrangement (Geib et al. 2008; Ke et al. 2010). These processes may increase the enzyme accessibility to carbohydrates.

Table 1. Compositional Analysis of Raw Wood Materials and Excreted Faecal Materials of *C. formosanus* Workers (Mean±1S.E, N=3)

Sample	Total solid (%)	Carbohydrate (%)					lignin (%)			Ratio of carbohydrate to lignin	Ash (%)
		Glucan	Xylan	Galcatan	Mannan	Total	Klason lignin	Acid soluble lignin	Total		
Raw materials	100±0.0 (300mg)	49.6±1.2 (148.7mg)	7.0±0.3 (21.0mg)	3.0±0.1 (8.9mg)	10.7±1.0 (32.1mg)	70.2±2.1 (210.7mg)	28.6±0.2 (85.8mg)	0.3±0.0 (0.9mg)	28.9±0.2 (86.7mg)	2.43	0.9±0.3 (2.6mg)
Termite Faeces	100±0.0 (300mg)	22.5±1.2 (67.6mg)	5.4±0.1 (16.3mg)	1.2±0.4 (3.6 mg)	1.8±0.2 (5.4 mg)	31.0±1.7 (93.0 mg)	58.8±0.1 (176.4mg)	0.4±0.0 (1.1mg)	59.2±0.1 (177.5mg)	0.52	6.9±0.1 (20.7mg)

FTIR Analysis of Functional Group Change Caused by Termite

The FTIR spectra of Chinese red pine wood and excreted termite faeces (*C. formosanus*) are shown in Fig. 2, and functional group assignments are provided in Table 2. Compared to untreated sapwood, the spectrum of termite faeces showed a decrease in peak 1, indicating deconstruction of C=O bonds. Peaks associated with aromatic rings had the greatest increase (supported by peaks 3, 4, 6, 9, 10, and 12), suggesting higher aromatic ring content in termite faeces. A decrease of peak 8 after passing through the termite gut indicates a structural modification of the syringyl derivatives. The change in peak 7 can be attributed to the metabolism of cellulose and hemicellulose. Peak 11 attributed to C-H bonds on benzene rings had changes in shape. No obvious changes were observed in peak 15 between raw sapwood and excreted fecal material, indicating that modification of C-H bonds on benzene rings occurred but without degradation of the aromatic ring. A reduction of amorphous cellulose bands at peak 16 and the elimination of a crystalline cellulose band at peak 13 were also observed.

Results of FTIR spectrum analysis indicate (1) deconstruction of the C=O bond occurs, causing it to become unconjugated from the aromatic ring, (2) cleavage of the C-H bond on the benzene ring, (3) cleavage of C-O bond from syringyl methoxyl groups, and (4) disruption of crystalline cellulose and removal amorphous cellulose. These findings suggest that lignin demethylation, demethoxylation, and side-chain modifications occur as material passes through termite gut. This was also observed by Geib et al. (2008), and they found propyl side-chain oxidation, ring demethylation, and ring hydroxylation of lignin in a lower termite in a tetramethylammonium hydroxide (TMAH) thermochemolysis analysis. Our results also concur with Ke et al. (2010), who reported that guaiacol and guaiacol derivatives were determined in termite faeces by a pyrolysis gas chromatography/mass spectrometry (Py-GC/MS).

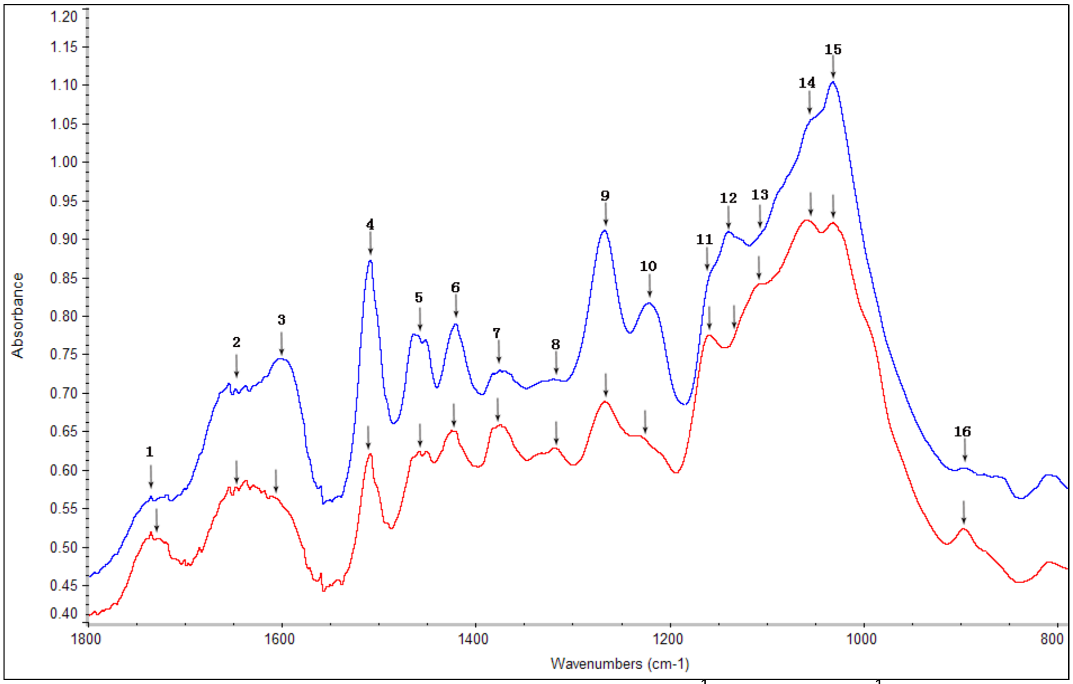

Fig. 2. Comparison of FTIR spectra ranging between 800 cm^{-1} and 1800 cm^{-1} for Chinese red pine wood (lower red curve) and faeces of *C. formosanus* (upper blue curve). Obvious changes in typical lignin and cellulose bands are denoted by black arrows. Assignments of band positions are listed in Table 2.

Table 2. Functional Group Assignments in FTIR Spectra of Chinese Red Pine Wood and *C. formosanus* Faeces

Number	Band position	Assignment
1	1714-1725	Carbonyl bonds (C=O) unconjugated to aromatic rings (oxidized side-chains)
2	1655	Carbonyl bonds (C=O) conjugated to aromatic rings
3	1595	Aromatic ring vibrations
4	1510	Aromatic skeletal from lignin
5	1462-1464	Asymmetric C-H bending (in CH_3 and $–CH_2$-)
6	1421-1424	Aromatic ring vibrations
7	1375	C-H deformation in cellulose and hemicellulose
8	1320-1330	C-H in cellulose and C_1-O vibration in syringyl derivatives
9	1270	Vibrations of guaiacyl rings and stretching vibrations of C-O bonds in lignin, C-O in guaiacyl $–OCH_3$ group
10	1245	Syringyl ring and C-O stretch in lignin and xylan
11	1160	Deformation vibrations of C-H bonds on benzene rings
12	1122	Aromatic skeletal and C-O stretch
13	1098	C-O stretching (crystalline cellulose)
14	1056	C-O stretch in cellulose and hemicellulose
15	1030-1033	Deformation vibrations of C-H bonds in aromatic rings
16	900	C-H deformation in cellulose (amorphous cellulose)

Scanning Electron Microscopy

Representative SEM images of raw wood materials and wood particles from each of the three termite gut segments (*C. formosanus*) are shown in Fig. 3. Compared to morphologically intact raw wood (Fig. 3a), it was obvious that some cracks and kinks

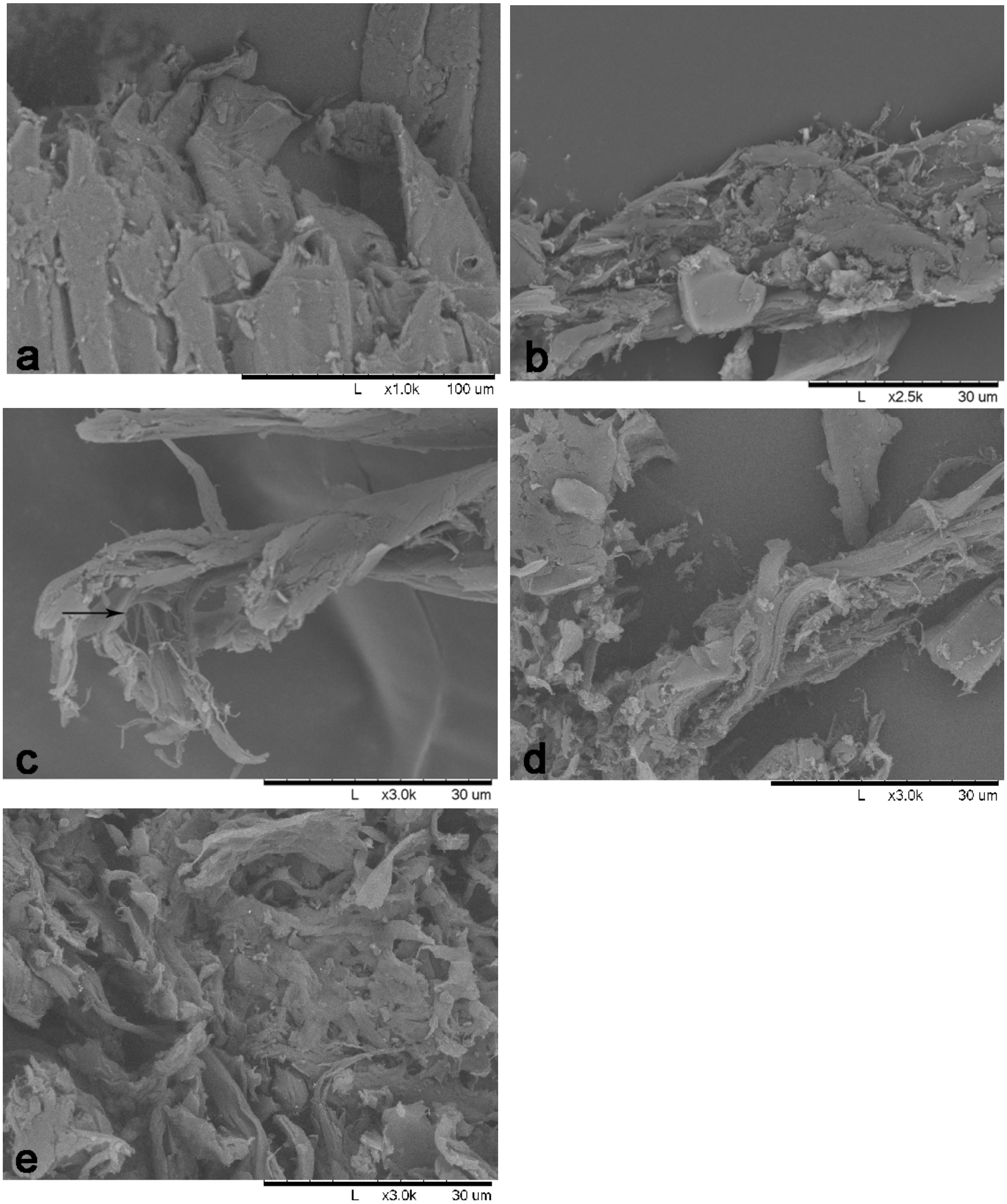

Fig. 3. SEM images for wood particles by obtaining from raw wood materials (a) and *C. formosanus* gut segments of foregut (b), midgut (c), hindgut (d), and rectum (e)

existed on the surface of wood particles from the termite foregut, and that particle sizes of wood ranged from 20 to 110 μm (Fig. 3b). These results are supported by previously reported work showing that mastication of wood particles by the mandibles and the gizzard in a termite produces a range of particle sizes (Yoshimura et al. 1995).

Wood particles collected from the midgut revealed a disruption of the biomass structure, causing an increase in surface area (Fig. 3c). This may indicate that hemicellulose and lignin were removed while in the midgut segment of the termite (Kumar et al. 2009). These results are consistent with the previous reports by Ke et al. (2010) that lignin content changed with progression through the termite digestive system using Py-GC/MS and phenol along with other lignin-derived monomers, which indicate lignin ring demethylation, decarboxylation, and side-chain oxidation taking place in the midgut first. Notably, side-chain oxidation supposedly leads to lignin depolymerization (Shary et al. 2007). Taken together, these findings suggest that the symbiont-free midgut plays an important role in loosening packed lignin or even removing it altogether.

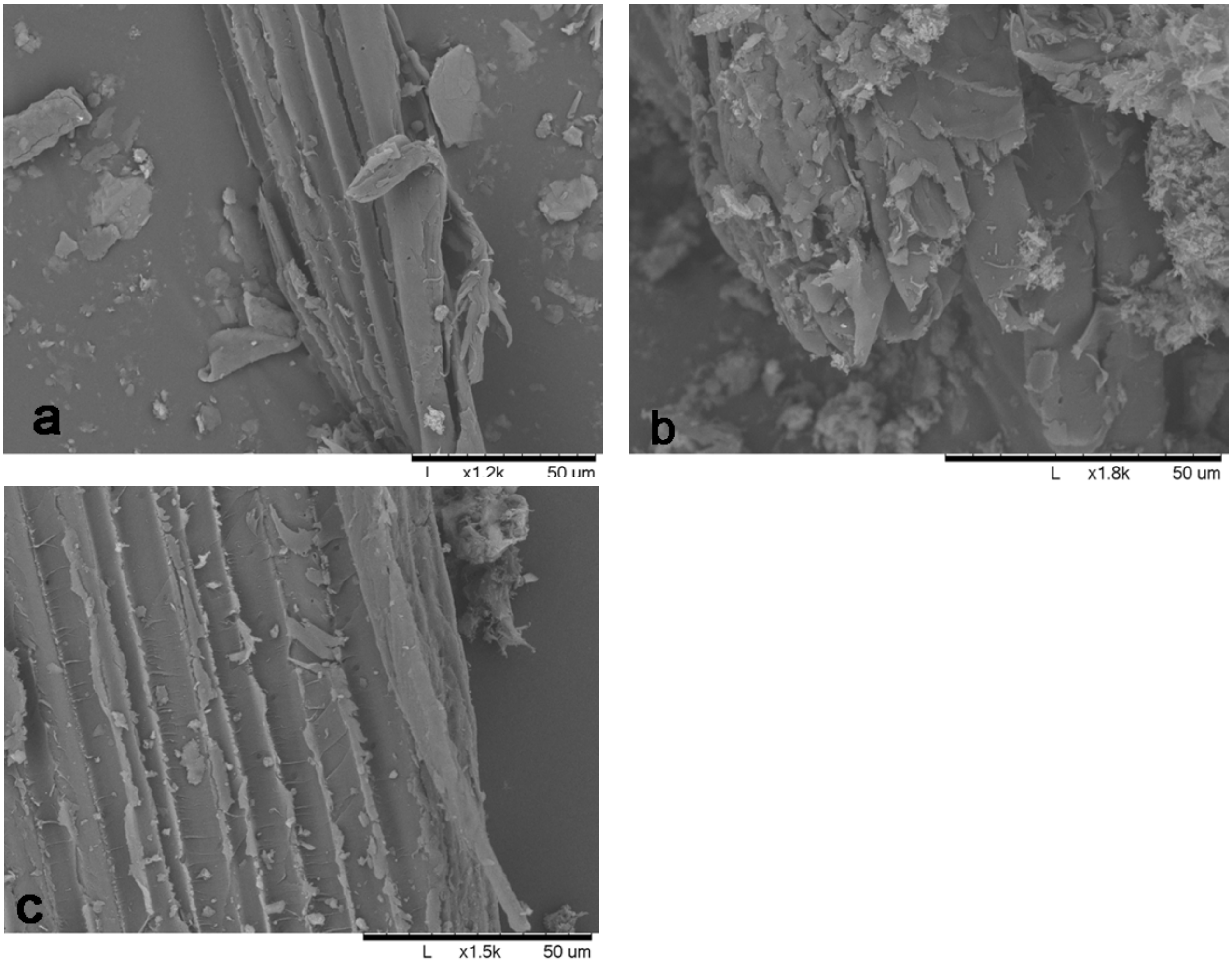

Fig. 4. SEM images for wood particles by obtaining from *O. formosanus* gut segments of midgut (a), hindgut (b), and rectum (c)

The SEM images for wood particles from the hindgut show a reduction in length of the fibrils (Fig. 3d). More lignin or lignin-derived content, along with fewer micro-fibrils, were observed in termite faecal material, confirming that the termite hindgut is the most important digestive segment in cellulose digestion. In addition, a large amount of lignin compounds are present in digested sapwood samples, as shown in Fig. 3e.

By contrast, the fungus-growing termite, *Odontotermes formosanus*, degrades lignin with the help of the mutualistic fungi and enhances the digestibility of cellulose (Hyodo et al. 2000). The wood particles passage though the gut of *O. formosanus* showed no obviously changes both on the morphological surface and length of the fibrils (Fig. 4a-c). *Odontotermes formosanus* termites might have little effect on delignification without the aid of mutualistic fungi.

It was hypothesized that mechanical grinding of wood in the mandible and gizzard regions could not be an important factor for further cellulose digestion (Ford 1983). In addition, the ligninolytic enzymes responsible for lignin modification existing in the lower termite, *C. formosanus*, might be the crucial factor breaking down the plant cell walls, therefore, improving cellulase access to the cellulose surface.

Effect of Termite Treatment on the Improvement of Enzymatic Digestibility of Wood Particles

Some wood particles were observed in termite faeces (*C. formosanus*) using SEM, and in order to know the effect of termite pre-treatments such as potential lignase attack and special physicochemical action in gut, enzymatic hydrolysis tests were carried out using raw sapwood. Termite faecal materials were evaluated for comparison. Findings indicate that low amorphous cellulose and high lignin content in termite faeces did not result in lower enzymatic digestibility. Faecal material showed improvement in enzymatic digestibility, reaching 28.5% within 72 h, whereas digestibility of raw materials only reached 14.7% in the same period of time (Table 3). These data suggest glucan chain of faecal materials have better access to cellulase, which is in agreement with the results of FTIR data and SEM observation.

Table 3. Comparison of the Enzymatic Digestibility by Raw and Termite-Digested Materials (*C. formosanus*) at 72 h (Mean±1S.E, N=3)

Sample	Total potential glucose in sample (mg)	Glucose yield in 72 h enzymatic hydrolysis (mg)	Digestibility (%)
Raw materials	991.2±1.2	145.8±8.3	14.7±0.8
Termite faecal materials	451.0±1.2	128.5±4.8	28.5±1.1

CONCLUSIONS

1. During the softwood digestion process, the lower termite *C. formosanus* showed great lignin removal ability, resulting in high surface area of cellulose micro-fibrils.

2. In the termite digestion system, the cellulose crystallinity was reduced. Surplus wood in faeces was more easily degraded by cellulase, suggesting that such pre-treatment strategies of the termite promote the enzymatic hydrolysis of the softwood.
3. Wood-feeding termite digestion systems could provide new insight for pre-treatment processes, which could be worthwhile for the conversion process of lignocellulosic biomass to biofuels.

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REFERENCES CITED

Breznak, J.A., and Brune, A. (1994). “Role of microorganisms in the digestion of lignocellulose by termites,” *Annu. Rev. Entomol.* 39, 453-487.

Brune, A. (1998). “Termite guts: The world's smallest bioreactors,” *Trends in Biotechnology* 16, 16-21.

Chandra, R. P., Bura, R., Mabee, W. E., Berlin, A., Pan, X., and Saddler, J. N. (2007). “Substrate pretreatment: The key to effective enzymatic hydrolysis of lignocellulosics,” *Biofuels* 108, 67-93.

Coy, M. R., Salem, T. Z., Denton, J. S., Kovaleva, E. S., Liu, Z., Barber, D. S., Campbell, J. H., Davis, D. C., Buchman, G. W., Boucias, D. G., and Scharf, M. E. (2010). “Phenol-oxidizing laccases from the termite gut,” *Insect Biochem. Mol. Biol.* 40, 723-732.

Ford, C. W. (1983). “Effect of particle-size and delignification on the rate of digestion of hemicellulose and cellulose by cellulase in mature pangola grass stems,” *Aust. J. Agric. Res.* 34, 241-248.

Galbe, M., and Zacchi, G. (2007). “Pretreatment of lignocellulosic materials for efficient bioethanol production,” *Biofuels* 108, 41-65.

Geib, S. M., Filley, T. R., Hatcher, P. G., Hoover, K., Carlson, J. E., Jimenez-Gasco, M. D., Nakagawa-Izumi, A., Sleighter, R. L., and Tien, M. (2008). “Lignin degradation in wood-feeding insects,” *Proc. Natl. Acad. Sci. USA* 105, 12932-12937.

Hyodo, F., Azuma, J., and Abe, T. (1999). “Estimation of effect of passage through the gut of a lower termite, *Coptotermes formosanus* Shiraki, on lignin by solid-state CP MAS C-13 NMR,” *Holzforschung* 53, 244-246.

Hyodo, F., Inoue, T., Azuma, J.-I., Tayasu, I., and Abe, T. (2000). “Role of the mutualistic fungus in lignin degradation in the fungus-growing termite *Macrotermes gilvus* (Isoptera; Macrotermitinae),” *Soil Biol. Biochem.* 32, 653-658.

Ke, J., Sun, J. Z., Nguyen, H. D., Singh, D., Lee, K. C., Beyenal, H., and Chen, S. L. (2010). “In-situ oxygen profiling and lignin modification in guts of wood-feeding termites,” *Insect Sci* 17, 277-290.

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

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

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

Okano, K., Kitagawa, M., Sasaki, Y., and Watanabe, T. (2005). “Conversion of Japanese red cedar (*Cryptomeria japonica*) into a feed for ruminants by white-rot basidiomycetes,” *Anim. Feed Sci. Technol.* 120, 235-243.

Prins, R. A., and Kreulen, D. A. (1991). “Comparative aspects of plant-cell wall digestion in insects,” *Anim. Feed Sci. Technol.* 32, 101-118.

Scharf, M. E., and Boucias, D. G. (2010). “Potential of termite-based biomass pre-treatment strategies for use in bioethanol production,” *Insect Sci.* 17, 166-174.

Sharma, B. R., Martin, M. M., and Shafer, J. A. (1984). “Alkaline proteases from the gut fluids of detritus-feeding larvae of the crane fly, *Tipula-abdominalis* (Say) (Diptera, Tipulidae),” *Insect Biochem.* 14, 37-44.

Shary, S., Ralph, S. A., and Hammel, K. E. (2007). “New insights into the ligninolytic capability of wood-decay ascomycete,” *Appl. Environ. Microbiol.* 73(20), 6691-6694.

Singh, P., Suman, A., Tiwari, P., Arya, N., Gaur, A., and Shrivastava, A. K. (2008). “Biological pretreatment of sugarcane trash for its conversion to fermentable sugars,” *World J. Microbiol. Biotechnol.* 24, 667-673.

Tartar, A., Wheeler, M. M., Zhou, X. G., Coy, M. R., Boucias, D.G., and Scharf, M. E. (2009). “Parallel metatranscriptome analyses of host and symbiont gene expression in the gut of the termite *Reticulitermes flavipes*,” *Biotechnol. Biofuels* 2, 25.

Watanabe, H., and Tokuda, G. (2010). “Cellulolytic Systems in Insects,” *Annu. Rev. Entomol.* 55, 609-632.

Wheeler, M. M., Tarver, M. R., Coy, M. R., and Scharf, M. E. (2010). “Characterization of four esterase genes and esterase activity from the gut of the termite *Reticulitermes flavipes*,” *Arch. Insect Biochem. Physiol.* 73, 30-48.

Yoshimura, T., Imamura, Y., Tsunoda, K., and Takahashi, M. (1995). “Biodegradation of wood fragments in the digestive tube of *Coptotermes formosanus* Shiraki (Isoptera: Rhinotermitidae),” *Holzforschung* 49, 385-388.

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