J Wood Sci (2009) 55:381–386
DOI 10.1007/s10086-009-1038-0

NOTE

**Rumi Kaida · Tomomi Kaku · Kei'ichi Baba
Masafumi Oyadomari · Takashi Watanabe · Sri Hartati
Enny Sudarmonowati · Takahisa Hayashi**

Enzymatic saccharification and ethanol production of *Acacia mangium* and *Paraserianthes falcataria* wood, and *Elaeis guineensis* trunk

Received: September 17, 2008 / Accepted: April 3, 2009 / Published online: July 8, 2009

Abstract We examined the saccharification and fermentation of meals from *Acacia mangium* wood, *Paraserianthes falcataria* wood, and *Elaeis guineensis* trunk. The levels of enzymatic hydrolysis of cellulose and ethanol production were highest for *P. falcataria* wood and lowest for *A. mangium* wood. Ultrasonication pretreatment of meal further increased the rates of hydrolysis and ethanol production in meal from *P. falcataria* wood. Through this pretreatment, hemicelluloses (xylan and xyloglucan) and cellulose were released in the meal from *P. falcataria* wood. Loosening of hemicellulose associations can be expected to make *P. falcataria* wood more useful for bioethanol production.

Key words *Paraserianthes falcataria* · Saccharification · Bioethanol · Hemicelluloses · Ultrasonication

Introduction

Acacia mangium is a fast-growing dicotyledonous tree, native to northern Australia and Indonesia. The tree has shown a high level of silvicultural performance and a strong ability to grow in marginal lands with degraded soils due to its symbiotic relationship with nitrogen-fixing *Rhizobium*.[1] Large-scale plantations have been successfully established in tropical humid zones, including eastern Malaysia (Sabah) and Indonesia (Sumatra). *Acacia mangium* and certain related species (*Acacia auriculiformis*, *Acacia crassicarpa*, *Acacia difficilis*) are also grown on plantations in Indonesia, Vietnam, China, Brazil, and South Africa.[2]

R. Kaida (✉) · T. Kaku · K. Baba · M. Oyadomari · T. Watanabe · T. Hayashi
Kyoto University, RISH, Gokasho, Uji, Kyoto 611-0011, Japan
Tel. +81-774-38-3618; Fax +81-774-38-3618
e-mail: kaida@rish.kyoto-u.ac.jp

S. Hartati · E. Sudarmonowati
Research Centre for Biotechnology, LIPI, Cibinong 16911, Indonesia

Part of this report was presented at the 58th Annual Meeting of the Japan Wood Research Society, Tsukuba, March 2008

Paraserianthes falcataria, another dicotyledonous tree, belongs to the subfamily Mimosoideae of Leguminosae, and is native to Haiti, Indonesia, and Papua New Guinea. Like *A. mangium*, *P. falcataria* thrives in marginal lands, where it grows symbiotically with nitrogen-fixing *Rhizobium* and phosphorus-promoting mycorrhizal fungi.[3] It should be noted that *P. falcataria* is one of the fastest growing tree species in the world. A tree typically gains 7 m in height per year, reaching a mean height of 25.5 m and a bole diameter of 17 cm after 6 years, and a height of 39 m and a diameter of 63.5 cm after 15 years. It is therefore a recommended species for industrial timber estates in Southeast Asian countries.[4–6] The tree is useful not only as timber material but also in the production of pulp and paper. Its leaves are used as food for chickens, goats, and cattle.[7] Thus, the species fulfills a wider range of wood end-uses than *Acacia* species.[8,9] It is expected to be one of the most useful tropical tree species in terms of biomass in industrial forests.

Elaeis guineensis (oil palm), native to South Africa, is a perennial monocotyledon widely planted for its oil; in fact, it produces a greater quantity of plant oil than any other single species in the world. In Malaysia and Indonesia, both of which are major producers of palm oil, trees are cut down for replantation at ~25-year intervals. Because no use has yet been devised for the felled *E. guineensis* trunks, a vast amount of material – about 4 million carbon tons per year in Malaysia alone – becomes waste through this process. Those wasted trunks are now required to be used either as biomaterial[10] or in the production of bioenergy.[11] Furthermore, in order to reduce this waste, it is necessary to look beyond the established material uses for the trunks and find new uses, such as the use of *E. guineensis* trunks as a source of glucose.

The trunk of *E. guineensis* has marked structural differences from the wood of angiosperms and gymnosperms. *Elaeis guineensis* is one of the monocotyledons, most of which exhibit neither growth rings nor secondary thickening. The "wood" of *E. guineensis* consists of primary vascular bundles embedded in parenchymatous tissue,[12,13] unlike the wood of *A. mangium* and that of *P. falcataria*, both of

which contain secondary xylem. Therefore, we do not use the term "wood" for *E. guineensis* trunk.

Cellulose microfibrils consist of many paracrystal 1,4-β-glucans that form nanofibers 3 to 4 nm in width and thickness. The surface glucans of these nanofibers may be irregularly intercalated with hemicelluloses to various degrees so that each nanofiber includes anywhere from one to several glucan layers.[14] The nanofibers associate to form bundles of compact lattices made up of hydrophobic and hydrogen bonds, which can result in I_β crystalline regions,[15] while multiple intercalation of some paracrystal glucans can result in noncrystalline regions. The degree of intercalation may be similar between primary and secondary walls, but an increased amount of cellulose occurs along with lignin deposition in the secondary wall.

Cellulase might not easily hydrolyze 1,4-β-glucan intercalated with hemicellulose, nor does hemicellulase efficiently attack hemicellulose intercalated tightly into microfibrils in wood, because the processes of plant cell elongation and expansion further tighten and fix the intercalation between 1,4-β-glucans and hemicellulose during growth. Lignin bound to xylan and glucomannan[16,17] is known to be a recalcitrant compound in cellulose hydrolysis.[18] The question we sought to answer, therefore, was whether the hydrolysis of cellulose in the wood of *A. mangium*, the wood of *P. falcataria*, and the trunk of *E. guineensis* could be accelerated by the loosening of hemicellulose association through treatment with ultrasonication. Our aim was to assess the recalcitrance of hemicellulose to the enzymatic saccharification of cellulose in *A. mangium*, *P. falcataria*, and *E. guineensis*.

Materials and methods

Preparation of meals from *Acacia mangium* wood, *Paraserianthes falcataria* wood, and *Elaeis guineensis* trunk

Parts of *Acacia mangium* and *Paraserianthes falcataria* stem wood were cut out from mature trees in Indonesia at a height of 2–3 m above the ground. Their barks were peeled off, and samples of wood were dried in an oven at 70°C for 16 h, and then milled to a powder using a ball mill (MM400, Retsch, Haan, Germany) at a speed of 15 rps for 10 min. Samples of *Elaeis guineensis* trunk (40 cm diameter) were also taken from 3 cm inward from the cortex and prepared in the same way. These meal samples were used for saccharification alone or in combination with fermentation.

Wall analysis

Polysaccharides of hemicelluloses were successively extracted from the meal samples four times with 24% KOH containing 0.1% $NaBH_4$. The insoluble wall residue (cellulose fraction) was washed twice with water and dissolved with ice-cold 72% sulfuric acid. The amount of cellulose was also determined by measuring the acid-insoluble residue; the samples were extracted with acetic/nitric reagent (80% acetic acid/concentrated nitric acid, 10:1) in a boiling waterbath for 30 min.[19] The resulting insoluble material was washed in water and dissolved with ice-cold 72% sulfuric acid. Total sugar in each fraction was determined by the phenol–sulfuric acid method.[20] The alkali-soluble fraction was neutralized, dialyzed, and freeze-dried for use in methylation analysis.[21] Partially methylated alditol acetates were analyzed by gas chromatography-mass spectrometry (GC-MS; Agilent, Santa Clara, CA, USA) with a glass capillary column (0.25 mm i.d. × 15 m, DB-225; Agilent). Each alditol acetate was identified by its retention time and mass spectrum. Lignin content was determined by the Klason method.[22]

Enzymatic hydrolysis

One hundred milligrams of each type of meal was autoclaved at 120°C for 3 min so that it became impregnated with water, and washed once with water by centrifugation. A commercial cellulase preparation (Meicelase, Meiji Seika, Tokyo, Japan) derived from *Trichoderma viride* was used to digest the meal samples. The enzyme preparation contained endocellulases, exocellulases (CBHI and CBHII), xyloglucanase, xylanase, galactanase, and polygalacturonase. Enzymatic hydrolysis of the meal samples was performed in 2 ml of 50 mM sodium acetate buffer, pH 4.8, containing 0.02% Tween 20 and 0.4 filter paper units of the cellulase preparation (2.0 mg). One filter paper unit is defined as 1 µg of glucose released per minute from filter paper. The mixture was incubated at 45°C in a rotary shaker set at 135 rpm. About 100 µl of the supernatant was collected at 6 h, 12 h, 24 h, and 48 h after the start of hydrolysis and used for sugar analysis. The quantity of sugar released was estimated as reducing sugar by the Nelson–Somogyi method.[23] Furthermore, free sugars released were directly analyzed according to their alditol acetates using gas chromatography as described above.[21]

Treatment of meal samples with ultrasonication

After each meal sample was autoclaved at 120°C for 3 min, it was washed once with water by centrifugation, suspended with 2 ml of water, and treated for 5 min with ultrasonication. Ultrasonication was achieved by immersing the radiating tip of a Branson Sonifier 250 (Danbury, CT, USA) directly into the solution. The intensity of the radiation was set at a microtip limit of 7 and a 50% duty cycle. The samples treated with ultrasonication were then used for saccharification followed by fermentation. The carbohydrates solubilized by the treatment were filtered on glass fiber, freeze-dried, and directly used for methylation analysis.

X-Ray diffraction

Each meal sample was subjected to X-ray diffraction measurement using a RINT 2000 diffractometer (Rigaku,

Tokyo, Japan) with Kβ-filtered CuKα radiation at 40 kV and 50 mA. The degree of crystallinity of native cellulose was calculated from the X-ray diffraction patterns according to the method described by Segal et al.[24] The line broadening and crystal size of cellulose microfibrils were calculated from the X-ray diffraction patterns according to Scherrer's equation.

Ethanol production

We produced three mixtures, each containing one type of meal in 2 ml of 50 mM sodium acetate buffer, pH 4.8, containing 0.02% Tween 20, 0.4 filter paper units of a cellulase preparation, and a seed culture of *Saccharomyces cervisiae* (SH1089) with yeast nutrients (4 mg $(NH_4)_2HPO_4$, 0.2 mg $MgSO_4 \cdot 7H_2O$, and 8 mg yeast extract). Each mixture was incubated at 45°C in a rotary shaker set at 135 rpm, and then subjected to simultaneous enzymatic saccharification and fermentation. About 100 µl of the supernatant was collected at 6 h, 12 h, 24 h, and 48 h after the start of hydrolysis and used for ethanol analysis. The ethanol formed was measured by gas chromatography on a Supelcowax-10 column (0.53 mm i.d. × 15 m, Supelco, Bellefonte, PA, USA) at 50°C using an Agilent gas chromatograph. Isopropanol was used as an internal standard.

Results and discussion

Analysis of *Acacia mangium* wood, *Paraserianthes falcataria* wood, and *Elaeis guineensis* trunk

Stemwood of *Acacia mangium*, stemwood of *Paraserianthes falcataria*, and trunk of *Elaeis guineensis* revealed different specific gravities; that of *A. mangium* was high, while those of *P. falcataria* and *E. guineensis* were low (Table 1). Crystallinity was high for *A. mangium* and *P. falcataria* and low for *E. guineensis*; this is probably because *E. guineensis* trunk contains higher quantities of parenchyma cells than the other species.[12,13] Chemical analysis of the samples confirmed the composition of cellulose, hemicellulose, and lignin, together with hemicellulose components, based on the methylation analysis of hemicellulose fraction (alkali-soluble polysaccharides) (Table 1).

The cellulose content was highest in *A. mangium* and lowest in *E. guineensis*; this is because *E. guineensis* trunk contains a relatively large concentration of primary wall. The lignin content was highest in *P. falcataria*, and the lignin levels in *A. mangium* and *P. falcataria* were within the range typically found in hardwoods, 20%–26%. The levels of hemicelluloses in *A. mangium* and *P. falcataria*, in which 4-linked xylose was the predominant sugar component, were similar (about 20%), indicating that xylan might be the largest hemicellulose. *Elaeis guineensis* contained more hemicelluloses than the other species. Its hemicellulose content included large quantities of xyloglucan, due to 4,6-linked glucose; (1→3),(1→4)-β-glucan, due to 3-linked and 4-linked glucoses; and xylan, due to 4-linked xylose.

Enzymatic saccharification

We measured the levels of enzymatic saccharification of the meal samples from *A. mangium*, *P. falcataria*, and *E. guineensis* (Fig. 1). At 48 h, *A. mangium* had released 8 mg of sugars, *P. falcataria* had released 29 mg, and *E. guineensis* 18 mg. The highest incidence of cellulose hydrolysis was observed in *P. falcataria*. Table 2 shows the compositions of the monosaccharides released by enzymatic hydrolysis at 48 h. Fermentable sugars (glucose, galactose, and mannose) made up about 70% of all sugars produced during saccharification. At 48 h, cellulose was hydrolyzed up to 8.2%

Table 1. Physical properties and components of wood from *Acacia mangium* and *Paraserianthes falcataria*, and trunk from *Elaeis guineensis*

	A. mangium	*P. falcataria*	*E. guineensis*
Property			
Specific gravity (g/cm^3)	0.51	0.28	0.25
Crystallinity (%)	46	45	40
Components (mg/100 mg xylem)			
Cellulose	59.7	52.5	45.9
Lignin	21.2	26.5	19.9
Hemicellulose	20.1	21.0	34.4
Fucosyl terminal	nd	nd	nd
Arabinosyl terminal	1.0	0.3	0.5
Xylosyl terminal	0.2	0.3	0.3
2- or 4-linked	7.7	11.9	14.7
Glucosyl 3-linked	nd	nd	1.6
4-linked	7.7	4.6	10.4
4,6-linked	0.6	0.6	1.2
Galactosyl terminal	0.1	0.5	0.2
4-linked	1.1	1.6	3.3
3,6-linked	1.1	0.3	0.8
Mannosyl 4-linked	0.2	0.7	0.6

Fuc, Fucosyl; Ara, arabinosyl; Xyl, xylosyl; Glc, glucosyl; Gal, galactosyl; Man, mannosyl; nd, not detected

[a] Component of hemicellulose

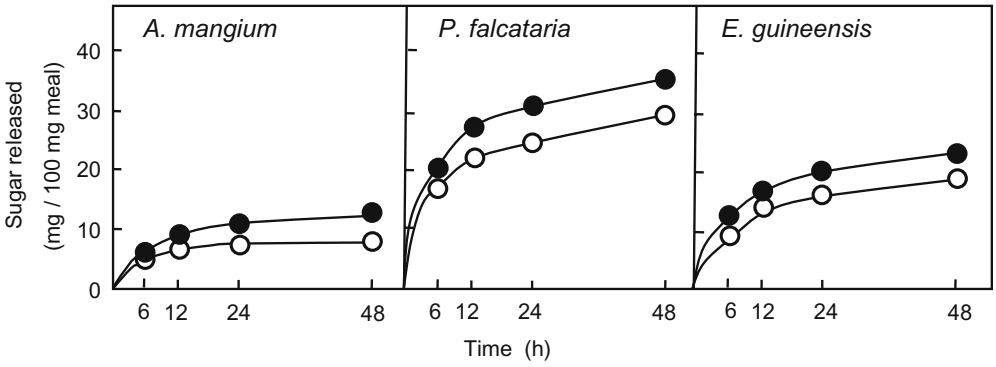

Fig. 1. Sugar released by enzymatic hydrolysis of meals of *Acacia mangium* wood, *Paraserianthes falcataria* wood, and *Elaeis guineensis* trunk. The amounts of sugar were calculated from the reducing power. *Open symbols*, control samples; *closed symbols*, samples pretreated with ultrasonication

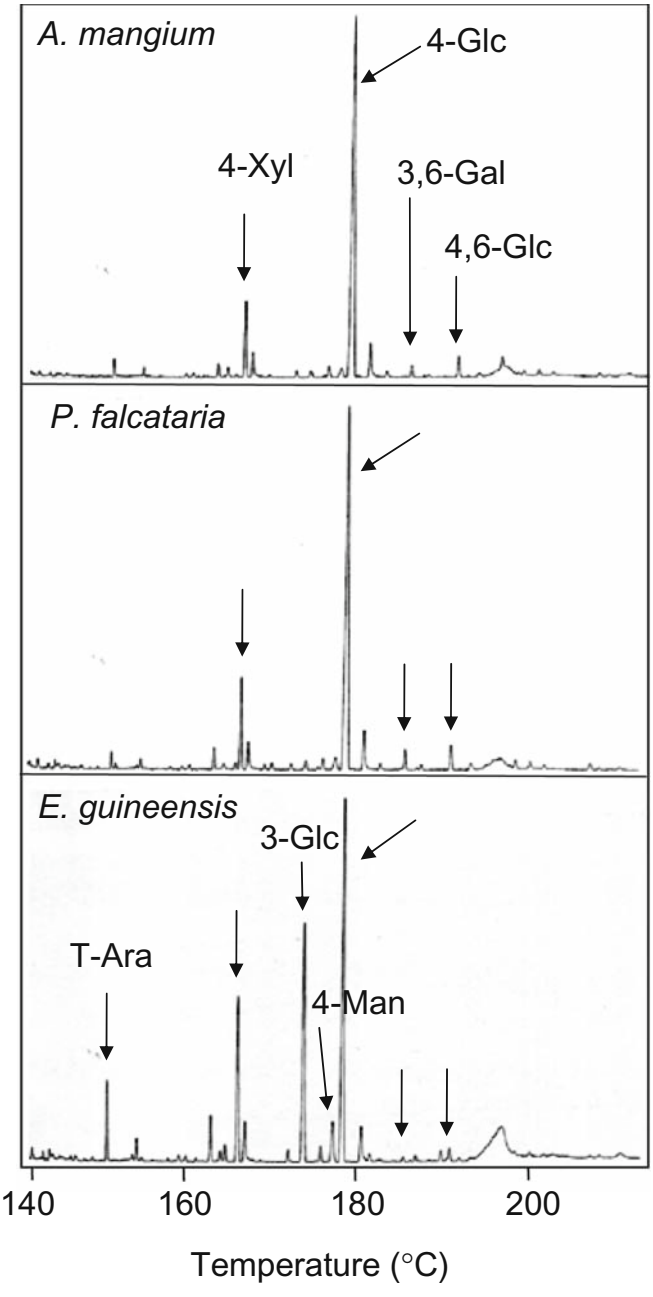

Fig. 2. Results of methylation analysis of carbohydrate solubilized from meal after treatment with ultrasonication for *Acacia mangium* wood (*top*), *Paraserianthes falcataria* wood (*middle*), and *Elaeis guineensis* trunk (*bottom*). *Xyl*, xylosyl; *Glc*, glucosyl; *Gal*, galactosyl; *Ara*, arabinosyl; *Man*, mannosyl. *Unlabeled arrows* coincide with *labeled arrows* in higher figures

(4.9 mg of 59.7 mg) in *A. mangium*, 38.1% (20.0 mg of 52.5 mg) in *P. falcataria*, and 24.6% (11.3 mg of 45.9 mg) in *E. guineensis*.

Pretreatment of the meal samples with ultrasonication led to a small but definitive increase in the incidence of enzymatic hydrolysis of cellulose (Fig. 1). The pretreatment increased the incidence of hydrolysis for *A. mangium* by a factor of 1.5, for *P. falcataria* by a factor of 1.2, and for *E. guineensis* by a factor of 1.2. The quantities of fermentable sugars produced were also increased in both *A. mangium* (1.6-fold) and *P. falcataria* (1.2-fold) by the pretreatment; in *E. guineensis*, however, fermentable sugars were not increased by the pretreatment (Table 2).

The pretreatment solubilized polysaccharides at the following levels: 1.12 mg per 100 mg of meal for *A. mangium*, 1.44 mg per 100 mg of meal for *P. falcataria*, and 1.47 mg per 100 mg of meal for *E. guineensis*. Methylation analysis revealed that 4-linked xylose and 4-linked glucose were the main derivatives in the solubilized carbohydrate, indicating that xylan and cellulose were solubilized from all the meal samples under the ultrasonication treatment (Fig. 2). In the samples of *A. mangium* and *P. falcataria*, 3,6-linked galactose and 4,6-linked glucose were also identified, showing that arabinogalactan and xyloglucan were released. In *E. guineensis*, 3-linked glucose was a second major derivative, showing that (1→3),(1→4)-β-glucan was solubilized from the meal.

Ethanol production

When saccharification was accompanied by fermentation with yeast, ethanol production was higher in *P. falcataria* than in the other species (Fig. 3). In *P. falcataria* and *E. guineensis*, the levels of ethanol production were theoreti-

Table 2. Monosaccharides released by enzymatic hydrolysis at 48 h

Monosaccharide	*A. mangium*		*P. falcataria*		*E. guineensis*	
	Control	US	Control	US	Control	US
Glucose	4.9	7.7	20.0	23.8	11.3	11.6
Galactose	0.4	0.6	0.7	0.9	0.9	1.0
Mannose	0.2	0.3	0.4	0.5	0.3	0.4
Xylose	2.0	2.8	7.3	8.4	4.7	7.6
Arabinose	0.2	0.2	0.3	0.5	0.5	0.7
Fucose	nd	nd	nd	nd	nd	nd
Rhamnose	0.4	0.5	0.6	1.0	0.3	0.7
Total	8.1	12.1	29.3	35.1	18.0	22.0

Data given as mg per 100 mg of meal
US, Ultrasonication; nd, not detected

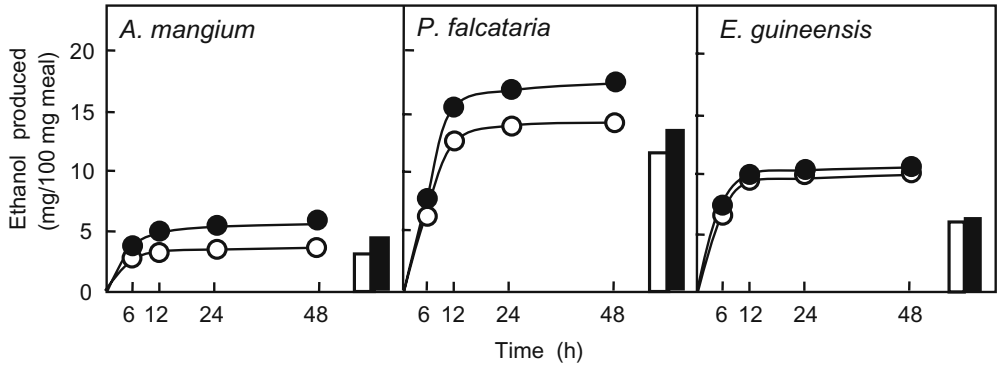

Fig. 3. Ethanol production from meal. Time course of ethanol production due to the simultaneous enzymatic saccharification and fermentation is shown using *symbols*. *Open symbols*, control samples; *closed symbols*, samples pretreated with ultrasonication. *Bars* show levels of ethanol production achieved through saccharification (48 h) followed by fermentation. *Open bars*, control samples; *closed bars*, samples pretreated with ultrasonication

cally 1.2-fold and 1.7-fold higher, respectively, in simultaneous saccharification and fermentation than in saccharification followed by fermentation. The bars in Fig. 3 show the levels of ethanol produced through saccharification (48 h) followed by fermentation. These results show that ethanol production was higher in the case of simultaneous saccharification and fermentation than in the case of saccharification followed by fermentation. A possible explanation for this is that there might have been some product inhibition during saccharification, but there was none or little during simultaneous enzymic saccharification and fermentation. It should be noted that although ethanol fermentation was reported to be inhibited by 5-hydroxymethyl furfural, furfural, and vanillin formed in acid hydrolysis or thermal pretreatment,[25] other inhibitory compounds could be formed rather than those in the enzymatic hydrolysate.

The pretreatment increased ethanol production in *A. mangium* and *P. falcataria*, but not in *E. guineensis*; this is because the pretreatment increased the amounts of fermentable sugars in *A. mangium* and *P. falcataria*, but not in *E. guineensis* (Table 2). In the cases of *A. mangium* and *P. falcataria*, pretreatment resulted in the release of some xylan, arabinogalactan, and xyloglucan from the meal samples, in which the loosening of hemicellulose association could accelerate the saccharification of cellulose microfibrils (Figs. 1 and 2). Both saccharification and fermentation occurred at high rates in *P. falcataria*, which exhibits saccharification and ethanol production rates similar to those of poplar wood. We have concluded that *P. falcataria* is a suitable tree species for bioethanol production. Genetic improvements can be expected to transform *P. falcataria* so that it is more easily hydrolysable using enzyme preparations alone.

Acknowledgments Funding for this work was provided by JSPS Kakenhi (grant nos. 19208016 and 19405030). This work is also part of the outcome of the JSPS Global COE Program (E-04): In Search of Sustainable Humanosphere in Asia and Africa.

References

1. Galiana A, Gnahoua GM, Chaumont J, Lesueur D, Prin Y, Mallet B (1998) Improvement of nitrogen fixation in *Acacia mangium* through inoculation with rhizobium. Agroforest Syst 40: 297–307
2. Orchard AE, Maslin BR (2005) The case for conserving Acacia with a new type. Taxon 54:509–512
3. Binkley D, Senock R, Bird S, Cole TG (2003) Twenty years of stand development in pure and mixed stands of *Eucalyptus saligna* and nitrogen fixing *Falcataria moluccana*. Forest Ecol Manag 182:93–102
4. Shivery GE, Zelek CA, Midmore DJ, Nissen TM (2004) Carbon sequestration in a tropical landscape: an economic model to measure its incremental cost. Agroforest Syst 60:189–197

5. Kurinobu S, Prehatin D, Mohanmad N, Matsune K, Chigira O (2007) A provisional growth model with a size–density relationship for a plantation of *Paraserianthes falcataria* derived from measurements taken over 2 years in Pare, Indonesia. J For Res 12:230–236
6. Siregar UJ, Rachmi A, Massijaya MY, Ishibashi N, Ando K (2007) Economic analysis of sengon (*Paraserianthes falcataria*) community forest plantation, a fast growing species in East Java, Indonesia. Forest Policy Econ 9:822–829
7. Merkel RC, Pond KB, Burns JC, Fisher DS (2000) Rate and extent of dry matter digestibility in sacco of both oven- and freeze-dried *Paraserianthes falcataria*, *Calliandra calothyrsus*, and *Gliricidia sepium*. Trop Agr 77:1–5
8. Otsamo R (1998) Effect of nurse tree species on early growth of *Anisoptera marginata* Korth. (Dipterocarpaceae) on an *Imperata cylindrica* (L.) Beauv. grassland site in south Kalimantan, Indonesia. Forest Ecol Manag 105:303–311
9. Alamsyah EM, Nan LC, Yamada M, Taki K, Yoshida H (2007) Bondability of tropical fast-growing tree species I: Indonesian wood species. J Wood Sci 53:40–46
10. Hayashi T, Park YW, Isogai A, Nomura T (2008) Cross-linking of plant cell walls with dehydrated fructose by smoke-heat treatment. J Wood Sci 54:90–93
11. Singhal RS, Kennedy JF, Gopalakrishnan SM, Kaczmarek A, Knill CJ, Akmar PF (2008) Industrial production, processing, and utilization of sago palm-derived products. Carbohydr Polym 72:1–20
12. Lim SC, Khoo KC (1986) Characteristics of oil palm trunk and its potential utilization. Malaysian Forest 49:3–22
13. Husin M (2000) Utilization of oil palm biomass for various wood-based and other products. In: Basiron Y, Jalani BS, Chan KW (eds) Advances in oil palm research. Malaysian Palm Oil Board, Kuala Lumpur, pp 1346–1412
14. O'Sullivan AC (1997) Cellulose: the structure slowly unravels. Cellulose 4:173–207
15. Hackney JM, Atalla RH, VandeHart DL (1994) Modification of crystallinity and crystalline structure of *Acetobacter xylinum* cellulose in the presence of water-soluble β-1,4-linked polysaccharides: ^{13}C-NMR evidence. Int J Biol Macromol 16:215–218
16. Imamura T, Watanabe T, Kuwahara M, Koshijima T (1994) Ester linkages between lignin and glucuronic acid in lignin–carbohydrate complexes from *Fagus crenata*. Phytochemistry 37: 1165–1173
17. Lawoko M, Henriksson G, Gellerstedt G (2006) Characterization of lignin–carbohydrate complexes from spruce sulfite pulp. Holzforschung 60:162–165
18. Chen F, Dixon RA (2007) Lignin modification improves fermentable sugar yields for biofuel production. Nat Biotechnol 25: 759–761
19. Updegraff DM (1969) Semimicro determination of cellulose in biological materials. Anal Chem 32:420–424
20. Dubois M, Gilles KA, Hamilton JK, Roberts PA, Smith F (1956) Colorimetric method for determination of sugars and related substances. Anal Chem 28:350–356
21. Hayashi T (1989) Measuring β-glucan deposition in plant cell walls. In: Linskens HF, Jackson JF (eds) Modern methods of plant analysis: plant fibers. Springer, Berlin Heidelberg New York, pp 138–160
22. Chiang VL, Funaoka M (1990) The difference between guaiacyl and guaiacyl–syringyl lignins in their responses to kraft delignification. Holzforschung 44:309–313
23. Somogyi M (1952) Notes on sugar determination. J Biol Chem 195:19–23
24. Segal L, Creely JJ, Martin AE Jr, Conrad CM (1959) An empirical method for estimating the degree of crystallinity of native cellulose using the X-ray diffractometer. Text Res J 29:786–794
25. Miyafuji H, Nakata T, Ehara K, Saka S (2005) Fermentability of water-soluble portion to ethanol obtained by supercritical water treatment of lignocellulosics. Appl Biochem Biotechnol 121–124: 963–971