

Analysis on Chemical Components of Woods to Predict Ethanol Production Values

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Abstract

This paper deals with analysis on chemical components of woods to predict ethanol production values. The aim is expected to give a reliable value of ethanol production, eliminating the effort needed to directly measure this ethanol production from each wood species. Since the data of wood chemical components is widely available, this result will be valuable in determining a potential use of a wood species as bio-ethanol feedstock. Saccharification and fermentation processes by enzymatic hydrolysis were applied for xylems derived from 49 branch trees of Cibodas, 32 branch trees of Purwodadi, and 19 branch trees of Bali Botanical Gardens in Indonesia. Three major wood components were analysed, i.e. cellulose, hemicellulose, and lignin. The results show varied relationships between ethanol production and chemical components of wood. The content of cellulose in wood was not exactly related to its ethanol production. This trend was also occurred for the relationship between hemicellulose and ethanol production. However, lignin content in woods gave an expected trend where the less lignin content, the higher the ethanol production. Furthermore, the ratios of cellulose-hemicelluloses and cellulose-lignin have been quantified. The result showed that the cellulose-lignin ratio can potentially be used to predict the value of ethanol production which is expressed by linear regression $y = 0.0616x + 0.8341$; where $R^2 = 0.4127$, x = ethanol production and y = cellulose-lignin ratio. *Gymnostoma sumatranum* with cellulose content of 43.8% and lignin content of 24.1% (cellulose-lignin ratio of 1.8) has actual ethanol production of 12.1 mg/100mg wood meal, compared to 15.7 mg/100 mg wood meal resulted from above equation. Therefore, by using its cellulose-lignin ratio, the woods having high ethanol production can be screened from literatures.

Keywords: enzymatic hydrolysis, chemical components, ethanol production, cellulose-lignin ratio.

Introduction

Tropical countries are the most productive places in producing tree biomass and an enormous amount of plants. These places experience abundant rain and no winter climate at any time during the year, generating various kinds of trees as natural resources. Many valuable tree plants have been found in Indonesian forests. In addition to native trees, many tropical trees were also collected from all over the world and planted in Indonesian Botanical Gardens, i.e. Cibodas, Purwodadi and Bali.

Recently, raw materials for bioethanol production are still dominated by food plants (generation I), such as corn, sugar-cane, cassava, etc. This condition will cause conflict of interest as it competes with food supply, causing increasing price of foods. This situation becomes worse as environmental issue such as global warming has been predicted to threaten world food supply. Furthermore, the availability of these raw materials is not guaranteed. Generally, even the total amount of starch and sucrose production of the world (1.5×10^9 tonnes/year) that can be converted into bioethanol can only fulfil at the most of 8×10^{11} L/year, while the demand for world fuel is 1.2×10^{12} L/year (Hayashi 2009). Therefore, alternative sources of raw materials other than food crops are needed, which is the non-food lignocellulosic materials (generation II).

Wood-derived lignocellulosic biomass is a good solution as an alternative raw material for bioethanol that should be developed. Wood has several advantages such

as having high cellulosic content ($\geq 80\%$ holocellulose). In spite of that, wood can be planted in marginal land, unlike agriculture crops (Kaida *et al.* 2009a). Mixing of 85% wood-based bioethanol with fossil fuel can reduce 65% carbon emission, while mixing of starch-based bioethanol with fossil fuel can only reduce it for 17~23% (Watanabe 2008). Bioethanol raw material from wood does not need spacious storage unlike other lignocellulosic materials. Wood can also be cultivated as Industrial Timber Plantation to more ensure material supply. Cellulose produced from this industrial forest can reach up to 5.0×10^9 tonnes/year that can be converted into 2.6×10^{12} L of bioethanol (Hayashi 2009). Wood plantation on Industrial Timber Plantation area suits Kyoto Protocol since these planted trees can increase carbon stock on earth which eventually will reduce greenhouse gasses in the atmosphere.

Plant-derived fuel will be one potential solution as an alternative energy source because abundant production of biomass in Indonesia. Therefore, continuity of plant-derived fuel is more guaranteed than that of fossil fuel. Indonesia has various wood species that strengthen its position as world producer for bioethanol in the future by exploring potential wood species.

The main problem on bioethanol production from lignocellulosic biomass, especially wood is the characteristic of plant cell wall which make it difficult for enzymatic hydrolysis. This drawback limits its utilization and cause uneconomical ethanol production from cellulose. Hayashi (2009) mentioned that wood is highly resistant to enzymatic

degradation which inhibits its degradation into fermented sugar. Therefore, easy accessibility for both saccharification and fermentation is also included as the most important factors in the conversion of wood into bioethanol.

Research on uncomplicated hydrolysis process and conversion of a wood species to become bioethanol is still uncommon so that big opportunity is widely opened to seek and explore these wood species. Recent studies revealed that sengon xylem consists of soft walls which are easily hydrolysable with commercial cellulase preparations. On the other hand, mangium xylem consists of hard walls which are less hydrolysable than those of sengon, although lignin content is lower for mangium than sengon (Kaida *et al.* 2009a). Genetically loosened walls increased the level of saccharification from 30% to 60% in the case of sengon and from 10% to 15% in the case of mangium (Kaida *et al.* 2009a). It is required to know the variety of saccharification level in the fast-growing tropical trees, in which sengon is the fastest growing tree species in Indonesia. Enzymatic saccharification was employed in this study because the hydrolysis is much more environmentally friendly than other processes such as acid hydrolysis.

Wood is mainly comprised of cellulose, hemicelluloses and lignin in which the crystalline bundles of cellulose are embedded in a covalently linked matrix of hemicellulose and lignin (Ingram and Doran 1995). The cellulose and hemicellulose fractions that comprise about two-thirds to three-quarters of lignocellulosic materials can be hydrolysed enzymatically to release the sugars that in turn can be converted into ethanol. Nevertheless, cellulose is considered as the most important ethanol source since it can be broken down into glucose that is further fermented into ethanol. Unlike cellulose, hemicellulose releases two forms of sugars, the pentoses and hexoses, where the pentoses are not readily fermented to ethanol, making the cellulose the only preferable component for hydrolysis. The condition of close association of the three major wood components resulted in many researches on effective pre-treatment process to disrupt this association with the main goal is to increase the enzyme accessibility in order to facilitate the digestibility of cellulose (Alvira *et al.* 2010; El-Zawawy *et al.* 2011). Therefore, the chemical components of certain materials are understandable to be the major consideration in choosing the most suitable feedstock for bioethanol.

The aim of this study was (1) to screen and to assess the xylems of Indonesian Botanical Garden trees for saccharification in order to be used in bioethanol production, (2) study on the relationship of wood chemical components and their ethanol production, (3) proposed that cellulose-lignin ratio can be used to predict the value of ethanol production.

Materials and Methods

Wood Sample Preparation

Branch of trees from Cibodas (49 branch trees), Purwodadi (32 branch trees), and Bali (19 branch trees) Botanical Gardens were cut out at the height of 2 to 3 m above the ground. Their barks were peeled and their xylems were dried in an oven at 70°C. The xylem was then milled to a powder using a ball mill at a speed of 15 rps for 30 min. The powder was used as a xylem preparation for saccharification alone or in combination with fermentation.

Cell Wall Analysis

Xylem preparation was ground in liquid nitrogen and the resulting fine powder was successively extracted 4 times with water and 24% KOH containing 0.1% NaBH₄. The insoluble wall residue (cellulose fraction) was washed twice with water. The amount of cellulose was 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 water bath for 30 min (Updegraff 1969) and the resulting insoluble material was washed in water and freeze-dried. Total sugar in each fraction was determined by phenol-sulfuric acid method (Dubois *et al.* 1956). Lignin content was determined by the Klason method (Chiang and Funaoka 1990).

Enzymatic Hydrolysis

Fifty mg of each xylem preparation was impregnated with water, autoclaved at 120°C for 3 min, and washed once with water by centrifugation. A commercial cellulase preparation (Accelerase, Palo Alto, USA) derived from *Trichoderma viride* was used to digest the xylem. The enzyme preparation contained endocellulases, exocellulases (CBHI and CBHII), xyloglucanase, xylanase, galactanase and polygalacturonase. Enzymatic hydrolysis of the xylem preparation was performed in 1 ml of 50 mM sodium acetate buffer, pH 4.8, containing 0.02% Tween 20 and 0.4 fpu (filter paper units) of a cellulase preparation (2.0 mg). The mixture was incubated at 45°C in a rotary shaker set at 75 rpm. About 100 µl of the supernatant was collected at 48 h of hydrolysis and used for sugar analysis. The sugar released was estimated as reducing sugar by the Nelson-Somogyi method (Somogyi 1952). Furthermore, free sugars released were directly analyzed according to their alditol acetates using gas chromatography (Hayashi 1989).

Ethanol Production

The mixtures from previous enzymatic hydrolysis were inoculated with a seed culture of *Saccharomyces cerevisiae* (SH1089) and yeast nutrients (4 mg (NH₄)₂HPO₄, 0.2 mg MgSO₄·7H₂O and 8 mg yeast extract). The mixtures were incubated at 37°C in a rotary shaker set at 100 rpm. About 100 µl of the supernatant was collected after 48 h

fermentation 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. Butanol was used as an internal standard.

Results and Discussion

Composition of Xylem and Enzymatic Saccharification

Cibodas Botanical Garden Woods. Appendix 1 shows the amounts of celluloses varied between 24.3% (*Pterospermum javanicum*) and 60.0% (*Araucaria glauca*). Hemicelluloses varied between 3.3% (*Agathis borneensis*) and 18.5% (*Firmiana malayana*). Lignin contents varied between 22.2% (*Casuarina junghuhniana*) and 39.6% (*Michelia montana*).

Previous results showed the levels of enzymatic saccharification varied among xylems from the 49 wood species. At 48 h, the xylem of *G. sumatranum* released 42.5 mg of sugars/100 mg wood meal (Kaida *et al.* 2011) while poplar xylem released only 30.3 mg/100 mg wood meal (Kaida *et al.* 2009b). The higher incidence of cellulose hydrolysis was also observed in *A. microsperma* (37.1 mg/100mg wood meal) which was higher than in poplar (Kaida *et al.* 2011). *Araucaria glauca* has the highest cellulose content (60%) while its sugar released was only 17.1 mg/100mg wood meal. Therefore, the levels of enzymatic saccharification were regardless of the cellulose content (Dwianto *et al.* 2011).

Purwodadi Botanical Garden Woods. Appendix 2 shows the amounts of celluloses varied between 34.7% (*Diospyros celebica*) and 54.8% (*Acacia catechu*). Hemicelluloses varied between 8.0% (*Alstonia scholaris*) and 26.9% (*Pterocymbium javanicum*). Lignin contents varied between 23.3% (*A. catechu*) and 36.9% (*Syzygium polyanthum*).

Previous results showed the levels of enzymatic saccharification varied among xylems from the 32 trees. At 48 h, the highest level of saccharification was obtained from the xylem of *F. malayana*, which had released 36.9 mg of sugars/100 mg wood meal (Sakata *et al.* 2012), while sengon only released 29 mg of sugars/100 mg wood meal (Kaida *et al.* 2009a). The higher incidence of cellulose hydrolysis was also observed in *P. indicus* (34.0 mg/100mg wood meal) which was higher than in sengon (Sakata *et al.* 2012).

A. catechu has the highest cellulose content while its sugars released was only 28.4 mg/100 mg wood meal (Sakata *et al.* 2012). Therefore, the levels of enzymatic saccharification was regardless of the cellulose content (Dwianto *et al.* 2011). The amount of hemicellulose in xylems varied between 8.0 to 26.9%, in which xyloglucan content varied between 0 to 0.1425%. *Alstonia scholaris* has the lowest hemicellulose content while its sugar released was only 12.6 mg/100 mg wood meal (Sakata *et al.* 2012). Therefore, the levels of enzymatic saccharification was

regardless of the hemicellulose content (Dwianto *et al.* 2011). Although xyloglucanase activity improved the total hydrolysis of lignocelluloses (Benko *et al.* 2008), there was no correlation between ethanol production and xyloglucan content. Lignin is known to be a recalcitrant compound in cellulose hydrolysis (Chen and Dixon 2007). The data shows the correlation between the high level of enzymatic saccharification and the low lignin content, but it does not happen all the time. *A. catechu* also has the lowest lignin content but its sugar released was only 28.4 mg/100mg wood meal. Therefore, the levels of enzymatic saccharification were regardless of the lignin content (Dwianto *et al.* 2011).

Bali Botanical Garden Trees. Appendix 3 shows that the amounts of cellulose varied between 35.9% (*Mimusops elengi*) and 51.2% (*Tabernaemontana macrocarpa*). Hemicelluloses varied between 5.7% (*Podocarpus neriifolius*) and 22.4% (*Flacourtia rukam*). Lignin contents varied between 23.2% (*Toona sureni*) and 39.1% (*Calophyllum soulattri*).

Previous results showed the levels of enzymatic saccharification varied among xylems from the 19 wood species. At 48 h, the highest level of saccharification was obtained from the xylem of *A. scholaris* which released 38.0 mg sugars/100 mg wood meal (Kaida *et al.* 2012), while sengon only released 29.0 mg sugars/100 mg wood meal (Kaida *et al.* 2009a). Higher levels of cellulose hydrolysis when compared to that of sengon were also observed in *F. rukam* (36.9 mg/100mg wood meal), *S. rarak* (34.9 mg/100mg wood meal) and *F. padana* (30.3 mg/100mg wood meal).

T. macrocarpa has the highest cellulose content while its sugar-released was only 24.8 mg/100 mg wood meal (Kaida *et al.* 2012). From this condition, it can be said that the level of sugar released was not directly related to cellulose content (Dwianto *et al.* 2011). The amount of hemicellulose in xylems varied between 5.7 to 22.4%, in which xyloglucan contents varied between 0 to 0.0877%. *P. neriifolius* has the lowest hemicellulose content yet its sugar-released was only 26.6 mg/100 mg wood meal (Kaida *et al.* 2012). This result showed that lower level of hemicellulose can not ensure that higher sugar released could be obtained (Dwianto *et al.* 2011). Although xyloglucanase activity improved the total hydrolysis of lignocelluloses (Benko *et al.* 2008), there was no correlation between ethanol production and xyloglucan content. Lignin is known to be a recalcitrant compound in cellulose hydrolysis (Chen and Dixon 2007). The data shown the correlation between the high yield of enzymatic saccharification and the low lignin content, but this condition was not a must. *T. sureni* has the lowest lignin content (23.2%) but its sugar-released was only 23.0 mg/100mg wood meal. Therefore, the yield of enzymatic saccharification was also not directly related to lignin content (Dwianto *et al.* 2011).

Relationships between Chemical Components of Wood and their Ethanol Production

The results from Appendix 1 to 3 were then plotted on the graphs of chemical components of wood against ethanol production as shown in Figure 1. Analysis of relationship between chemical components and ethanol production of all wood samples tested revealed various patterns of cellulose, hemicellulose and lignin content. Cellulose and hemicellulose contents demonstrated weak correlation with ethanol production. Correlation between lignin content and ethanol production was more pronounced, were the lower the lignin content of wood samples, the higher the ethanol production. The complexity of wood structure and high lignin content were estimated to be the major cause that inhibits contact between cellulose and the enzyme in saccharification process. We assumed that lignin was the

primary obstacle to releasing sugar in several wood samples tested. The wood with lower lignin content will give higher cellulosic ethanol production which ultimately will reduce the processing cost. This statement goes along with some previous researches (Jørgensen *et al.* 2007; Taherzadeh and Karimi 2008; Yamashita *et al.* 2010) saying that removal of lignin can effectively increase cellulose hydrolysis, in other words result in higher sugar release. The structure of lignin -which is made up of cross-linked network polymers gives the plant structural support and impermeability resistance against microbial attack and oxidative stress which limits the accessibility of enzymes to cellulose and the rate of hydrolysis by acting as a shield, preventing the digestible parts of the substrate from being hydrolyzed (Yamashita *et al.* 2010).

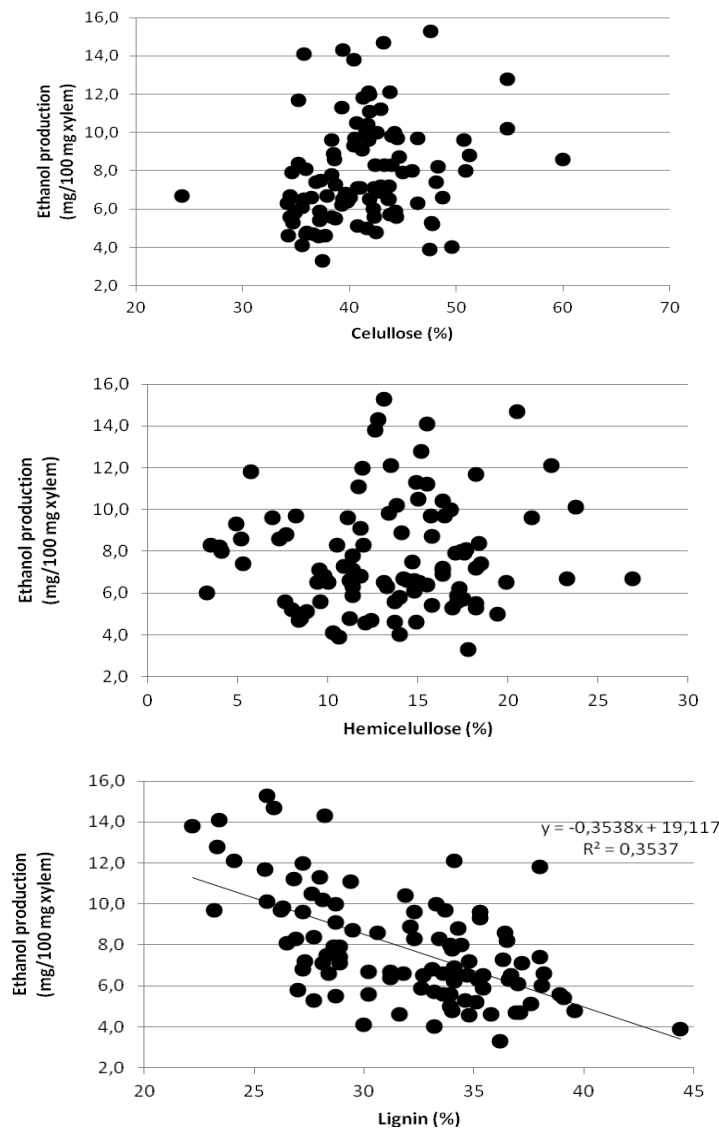


Figure 1. Relationships between major chemical components of wood and their ethanol production (Dwianto *et al.* 2012).

Since we expect woods with higher cellulose content should produce higher ethanol production, this research confirmed that any wood should be investigated individually to see its suitability for bioethanol production. It might occur that even though the wood has high level of cellulose, the composition of its other chemical components such as hemicellulose and lignin may act as inhibitors in the conversion of its cellulose into reducing sugar that will be further converted into ethanol. However, even though this study shows lignin is the main factor affecting the ethanol production and the same result is also obtained by many other studies stated above, other study shows that improving the surface area accessible to cellulase is a more important factor for achieving a high sugar yield (Rollin *et al.* 2011). Since the cellulose, hemicellulose and lignin are present in various amounts in different parts of the plant and form the structural framework of the plant cell wall which depends on plant species, age and growth conditions (Jørgensen *et al.* 2007), their contribution on the amount of ethanol production is not just in the matter of their quantity on plants, especially woods, but also on how they are distributed and growth condition involved. It is also mentioned by Chandra *et al.* (2007) that the chemical, physical and morphological characteristics of the heterogeneous lignocellulosic substrates influenced the effectiveness of enzymatic hydrolysis.

Analysis on Chemical Components of Woods to Predict Ethanol Production Values

A previous study on the relationship between chemical components of woods and their ethanol production showed varied relationships between ethanol production and chemical components of woods (Dwianto *et al.* 2012). The content of cellulose in wood did not exactly relate to its ethanol production. This trend also occurred for the relationship between hemicelluloses and ethanol production. However, lignin content in woods gave an expected trend where the less lignin content, the higher the ethanol can be obtained. The study concluded that high cellulose content of wood is not the only indicator of its high ability to result in high ethanol production. The complexity of wood structure makes it necessary to always examine the other two components, hemicelluloses and lignin where lignin seems to be the most prominent component affecting the ethanol production.

On the other hand, ratio of wood component seems to be acceptable to use in determining the potential use of wood as bioethanol feedstock. It is due to the assumption that wood with high cellulose content and low lignin and hemicellulose content will generally produce more ethanol than the opposite. However, an exact value of the way these components affect ethanol production is still not known. Therefore, this study was undertaken to get a closer approach to quantify this issue. The scattered graphs of around 100 wood species showing their cellulose-hemicellulose ratio against ethanol production is shown in Figure 2 and the graph of cellulose-lignin ratio against ethanol production is shown in Figure 3.

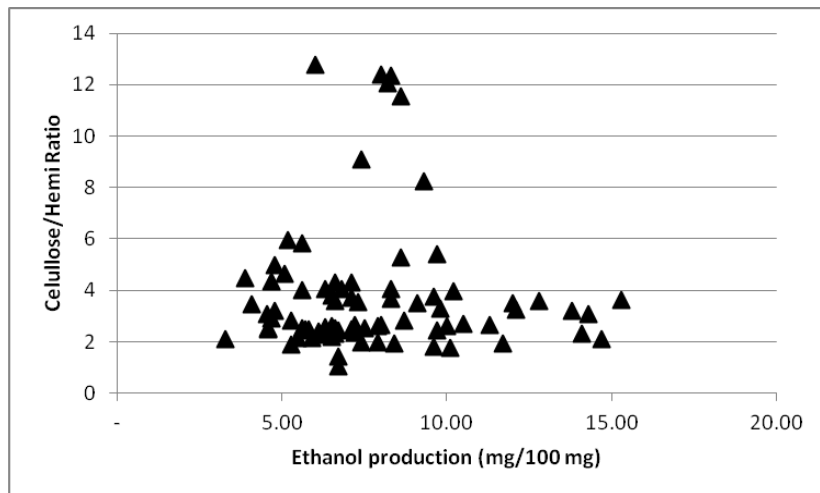


Figure 2. Relationship between cellulose-hemicellulose ratio and ethanol production.

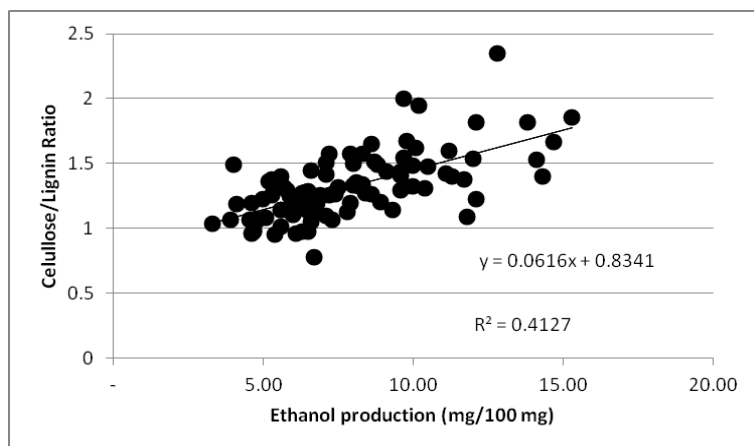


Figure 3. Relationship between cellulose-lignin ratio and ethanol production.

Analysis of relationship between cellulose-hemicellulose ratio and ethanol production of all wood samples tested revealed broad patterns. Cellulose-hemicellulose ratio demonstrated weak correlation with ethanol production. However, correlation between cellulose-lignin ratio and ethanol production was more pronounced where the higher cellulose-lignin ratio of wood samples, the higher amount of ethanol can be produced. Cellulose-lignin ratio is expressed by linear regression $y = 0.0616x + 0.8341$; where $R^2 = 0.4127$, x = ethanol production and y = cellulose-lignin ratio. This result justifies that wood with lignin content that is much lower than its cellulose content will strongly produce higher ethanol production. Kaida *et al.* (2011) reported that *Gymnostoma sumatranum* has the higher ethanol production among other Indonesian botanical gardens' wood species. *G. sumatranum* with cellulose content of 43.8% and lignin content of 24.1% (cellulose-lignin ratio of 1.8) has actual ethanol production of 12.1 mg/100 mg wood meal, compared to 15.7 mg/100 mg wood meal resulted from above equation. The coefficient determination actually is not high ($R^2 = 0.4127$). Other factors outside the chemical composition to affect the equation were probably density and anatomical features of the woods. However, the two factors were not observed on these results.

By using its cellulose-lignin ratio, we could select the woods potentially having high ethanol production from literatures. Some major and minor timbers from literatures (Soerianegara *et al.* 1994; Lemmens *et al.* 1995) having cellulose-lignin ratio more than 3.0 are *Parishia paucijuga*, *Sterculia macrophylla*, *Pterocymbium sp.*, *Artocarpus lanceifolius*, *Lithocarpus sp.*, *Swintonia sp.*, and *Sterculia ceramica*.

However, since the cellulose, hemicelluloses and lignin are present in various amounts in the different parts of the plant and form the structural framework of plant cell wall depending on plant species, age and growth conditions (Jørgensen *et al.* 2007), their contribution on the amount of ethanol production is not just in the matter of their quantity

on plants, especially woods, but also on how they are distributed and growth condition involved. It is also mentioned by Chandra *et al.* (2007) that the chemical, physical and morphological characteristics of the heterogeneous lignocellulosic substrates influenced the effectiveness of enzymatic hydrolysis. Even though each wood species has to be investigated thoroughly to see its potential for bioethanol feedstock, still this equation using cellulose-lignin ratio can be used as an alternative option to save time and effort in screening the remarkable number of wood species.

Conclusion

High cellulose content of wood is not the only indicator of its high ability to produce high ethanol production. The complexity of wood structure makes it necessary to always examine the other two components, hemicellulose and lignin where lignin seems to be the most prominent component that affects the ethanol production. Cellulose-lignin ratio can be used to predict the value of ethanol production which is expressed by linear regression $y = 0.0616x + 0.8341$; where $R^2 = 0.4127$. This regression also confirms that wood with lignin that is much lower than its cellulose content will strongly produce higher ethanol production. Therefore, by using its cellulose-lignin ratio, woods having high ethanol production can be selected from literatures. Still, further analysis is needed to confirm this result.

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Appendix 1. Chemical components and ethanol production of Cibodas Botanical Garden's woods.

Wood species	Cellulose (%)	Hemicellulose (%)	Lignin (%)	Ethanol production (mg/100 mg wood meal)
<i>Gymnostoma sumatranum</i>	43.8	13.5	24.1	12.1
<i>Adenanthera microsperma</i>	35.7	15.5	23.4	14.1
<i>Casuarina junghuhniana</i>	40.4	12.6	22.2	13.8
<i>Tabernaemontana macrocarpa</i>	44.5	8.2	33.7	9.7
<i>Flacourtia rukam</i>	37.3	14.7	28.3	7.5
<i>Taxus sumatrana</i>	48.3	4.0	36.5	8.2
<i>Pinus merkusii</i>	40.4	4.9	35.3	9.3
<i>Garcinia parvifolia</i>	40.7	15.0	27.6	10.5
<i>Mangifera odorata</i>	35.2	18.2	25.5	11.7
<i>G. beccarii</i>	35.2	18.4	27.7	8.4
<i>Araucaria cunninghamii</i>	50.9	4.1	33.9	8.0
<i>Agathis beccarii</i>	43.3	3.5	32.3	8.3
<i>Lithocarpus indutus</i>	44.0	13.4	26.3	9.8
<i>Artocarpus heterophyllus</i>	41.9	11.9	27.2	12.0
<i>Ficus padana</i>	34.6	17.6	28.9	7.9
<i>Acer laurinum</i>	39.3	14.9	28.0	11.3
<i>Platea latifolia</i>	39.6	9.8	33.1	6.8
<i>Bischofia javanica</i>	41.8	11.1	32.3	9.6
<i>Weinmannia blumei</i>	42.3	11.4	28.1	7.1
<i>Actinodaphne glomerata</i>	41.2	11.8	28.7	9.1
<i>Symplocos acuminata</i>	36.4	14.4	31.8	6.6
<i>Persea rimosa</i>	34.4	13.7	33.9	5.6
<i>Lithocarpus pseudomoluccus</i>	42.4	10.5	26.9	8.3
<i>Ostodes paniculate</i>	34.4	14.2	30.2	6.7
<i>Rauvolfia javanica</i>	37.1	12.1	34.8	4.5
<i>Toona sureni</i>	38.7	18.2	28.7	5.4
<i>Firmiana malayana</i>	36.9	18.5	28.9	7.4
<i>Araucaria var. Glauca</i>	60.0	5.2	36.4	8.6
<i>Sloanea sigun</i>	34.9	14.0	27.0	5.8
<i>Lithocarpus rotundatus</i>	38.7	10.9	36.3	7.3
<i>Litsea firma</i>	39.4	15.1	34.7	6.5
<i>Decaspermum fruticosum</i>	34.5	14.8	28.4	6.6
<i>Podocarpus neriifolius</i>	48.1	5.3	38.0	7.4
<i>Neonauclea obtusa</i>	35.7	9.4	36.7	6.5
<i>Lithocarpus pallidus</i>	34.3	13.7	35.8	4.6
<i>Michelia champaca</i>	40.1	11.2	38.2	6.6
<i>Manglietia glauca</i>	40.8	8.8	37.6	5.1
<i>Neonauclea lanceolata</i>	36.6	8.4	37.1	4.7
<i>Manglietia calophylla</i>	44.4	7.6	38.9	5.6
<i>Mastixia trichotoma</i>	35.6	14.8	37.0	6.1
<i>Alstonia scholaris</i>	38.6	7.3	30.6	8.6
<i>Macaranga rhizinoides</i>	40.8	9.5	37.2	7.1
<i>Acmena acuminatissima</i>	36.0	11.2	34.0	4.8
<i>Michelia montana</i>	42.5	8.5	39.6	4.8
<i>Syzygium polyanthum</i>	34.2	13.3	35.2	6.3
<i>Agathis borneensis</i>	42.2	3.3	38.1	6.0
<i>Persea excelsa</i>	37.7	14.9	31.6	4.6
<i>Pterospermum javanicum</i>	24.3	23.3	31.2	6.7
<i>Radermachera gigantea</i>	35.6	10.3	30.0	4.1

Appendix 2. Chemical components and ethanol production of Purwodadi Botanical Garden's woods.

Wood species	Cellulose (%)	Hemicellulose (%)	Lignin (%)	Ethanol production (mg/100 mg wood meal)
<i>Firmiana malayana</i>	43.2	20.5	25.9	14.7
<i>Pterocarpusa indicus</i>	47.6	13.1	25.6	15.3
<i>Sapindus rarak</i>	39.4	12.8	28.2	14.3
<i>Hibiscus macrophyllus</i>	40.5	16.5	26.2	9.7
<i>Acacia catechu</i>	54.8	15.2	23.3	12.8
<i>Canarium asperum</i>	44.2	16.8	33.3	10.0
<i>Schizolobium amazonicum</i>	54.8	13.8	28.1	10.2
<i>Parkia timoriana</i>	44.7	15.8	29.5	8.7
<i>Melia azedarach</i>	44.0	12.0	33.4	8.3
<i>Albizia procera</i>	41.5	23.8	25.6	10.1
<i>Leucaena leucocephala</i>	42.9	18.2	27.3	7.2
<i>Enterolobium cyclocarpum</i>	45.9	17.4	34.4	8.0
<i>Dalbergia latifolia</i>	45.0	17.1	28.6	7.9
<i>Mimusops elengii</i>	38.3	21.3	27.2	9.6
<i>Lagerstromia loudonii</i>	48.7	11.3	33.6	6.6
<i>Pterocymbium javanicum</i>	37.9	26.9	34.0	6.7
<i>Swietenia mahagoni</i>	43.7	16.4	34.8	7.2
<i>Artocarpus heterophyllus</i>	42.3	17.2	30.2	5.6
<i>Homalium tomentosum</i>	39.3	17.3	34.1	6.2
<i>Flacourtia rukam</i>	41.0	16.4	28.9	7.1
<i>Guioa diplopetala</i>	-	11.8	27.2	6.8
<i>Anthocephalus cinensis</i>	46.4	11.4	36.6	6.3
<i>Albizia lebbekoides</i>	43.7	19.9	34.0	6.5
<i>Cananga odorata</i>	43.8	17.5	33.2	5.7
<i>Alstonia scholaris</i>	47.8	8.0	35.1	5.2
<i>Lagerstroemia speciosa</i>	47.7	16.9	34.6	5.3
<i>Diospyros celebica</i>	34.7	18.2	27.7	5.3
<i>Wrightia tomentosa</i>	38.3	9.6	33.6	5.6
<i>Michelia alba</i>	37.2	17.2	32.6	5.9
<i>Tectona grandis</i>	47.5	10.6	44.4	3.9
<i>Syzygium polyanthum</i>	35.9	12.4	36.9	4.7
<i>Bischofia javanica</i>	37.5	17.8	36.2	3.3

Appendix 3. Chemical components and ethanol production of Bali Botanical Garden's woods.

Wood species	Cellulose (%)	Hemicellulose (%)	Lignin (%)	Ethanol production (mg/100 mg wood meal)
<i>Alstonia scholaris</i>	42.9	15.5	26.8	11.2
<i>Flacourtia rukam</i>	41.8	22.4	34.1	12.1
<i>Sapindus rarak</i>	41.9	11.7	29.4	11.1
<i>Ficus padana</i>	42.6	16.8	28.7	10.0
<i>Podocarpus neriifolius</i>	41.3	5.7	38.0	11.8
<i>Pometia pinnata</i>	38.5	14.1	32.1	8.9
<i>Tabernaemontana macrocarpa</i>	51.2	7.7	34.3	8.8
<i>Toona sureni</i>	46.4	15.7	23.2	9.7
<i>Araucaria cunninghamii</i>	50.7	6.9	35.3	9.6
<i>Artocarpus heterophyllus</i>	41.7	16.4	31.9	10.4
<i>Mimusops elengii</i>	35.9	17.7	26.5	8.1
<i>Guioa diplopetala</i>	42.8	16.4	34.1	6.9
<i>Firmiana malayana</i>	41.6	19.4	33.9	5.0
<i>Syzygium polyanthum</i>	41.9	10.0	32.7	6.5
<i>Michelia champaca</i>	43.6	13.1	35.4	6.5
<i>Bischofia javanica</i>	38.3	11.4	34.0	7.8
<i>Hibiscus tiliaceus</i>	39.8	15.5	31.2	6.4
<i>Decaspermum fruticosum</i>	44.3	11.4	35.4	5.9
<i>Calophyllum soulattri</i>	37.2	15.8	39.1	5.4
<i>Pterospermum javanicum</i>	49.6	14.0	33.2	4.0