# Statistical Approach for Optimization of Ethanol Production from Fast-growing Trees: *Acacia mangium* and *Acacia* hybrid

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This is the first report of the potential of *Acacia* fast growing trees in Thailand, *A. mangium* and the *Acacia* hybrid (*A. mangium x A. auriculiformis*), as raw material for ethanol production through a simultaneous saccharification and fermentation process by *Saccharomyces cerevisiae* TISTR 5339. Alkaline pulping was applied as the pretreatment process. Optimization of ethanol production was studied using response surface methodology based on central composite design. The optimized conditions of 100 g/L solid loading and an $A_{600}$ of *S. cerevisiae* TISTR 5339 of 2 gave observed values of ethanol production of 35.7 and 27.3 g/L, which corresponded with the predicted values of 32.32 and 26.37g/L from *A. mangium* and *A.* hybrid, respectively. This condition was then used for up-scaling in a 10-L stirred bioreactor. The improved maximum ethanol concentrations of 37.84 and 36.52 g/L were obtained from *A. mangium* and *Acacia* hybrid, respectively, within 96 h of cultivation at 30 °C and no aeration rate.

*Keywords: Acacia mangium; Acacia hybrid; Ethanol; Optimization; Response surface methodology*

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## INTRODUCTION

With the depletion of the world's petroleum, alternative non-petroleum-based sources of energy are being looked at with greater interest. Many countries have begun focusing on renewable resources for production of ethanol-based fuels. Lignocellulosic biomass from woody plants has the potential to become a major source of fermentable sugars for the production of ethanol because trees are the most abundant source of biomass. Thus, lignocellulosic biomass has been considered a new resource for ethanol production.

Because of its chemical composition, lignocellulosic biomass is very different from types of biomass that have large content of sugars or starch, as is customarily used in the biofuel industry. The structure of these former materials, mainly composed by cellulose, hemicellulose, and lignin, requires the process for biofuels production to be adjusted for each type of biomass, according to their component characteristics. Thus, pretreatment has been recognized as a necessary upstream process to improve the formation of sugars for downstream microbial and enzymatic processing.

Different kind of pretreatment methods, under a large variety of conditions, have been studied to improve the fermentability and digestibility. Mechanical size reduction is a physical pretreatment to increase enzyme-accessible surface areas (Zhu *et al*. 2009). In addition to these other pretreatment options, steam explosion is a technique based on

subjecting the biomass to pressurized steam for a short duration of time and then suddenly depressurizing the system. Due to the explosive decomposition, the fibers are separated and changes to the microstructure are brought about by the suddenly reduced pressure (Brodeur *et al.* 2011). The treatments result in increasing cellulose digestibility of pretreated biomass and solubilizing a significant portion of the hemicellulosic component (Nibedita *et al*. 2012). To help with digestion of the lignin matrix and to make cellulose and hemicellulose more accessible for enzyme degradation, alkaline peroxide delignification and alkaline pulping have been further explored as viable options. The findings from said explorations reported enhancing enzymatic hydrolysis by an alkaline peroxide delignification treatment after a steam explosion. Up to 80% of the lignin in the wood was removed, leaving a cellulose-rich residue that led to a best case scenario of 51.3 g/L of a concentrated glucose solution (Cara *et al*. 2006). A pulping method also can be used as pretreatment processing in paper manufacturing. So far, few studies have been reported on ethanol production with pulping pretreatment processes. However, alkaline pulping from rice straw in pilot-scale was studied, which succeeded in higher the pilot-scale yield than that in the typical laboratory-scale (Sekine *et al*. 2014).

Because cost reductions are the primary motivation for research in bioethanol technologies, it follows that the feedstock price should be carefully considered. Woody biomass is of interest because it is a sustainable feedstock with low cost and large quantities and can be found in most regions of the world. *Acacia* is a type of woody biomass that originated in Australia and has spread to tropical to warm-temperate regions, including Europe, Africa, Southern Asia, and America. Some *Acacia* species grow rapidly and thus become extensively invasive in many countries (Lorenzo *et al*. 2010). *Acacia mangium* and the *Acacia* hybrid (*A. mangium x A. auriculiformis*), are fast-growing trees found in Thailand and are potentially useful tropical trees for biomass production. *Acacia* wood is currently only being acquisitioned by the paper industry and as biomass fuel, which leaves ethanol fuel production as a possible third use for the wood. The present research is the first in Thailand to report the improvement of ethanol production from *Acacia* wood by varying the pretreatments as well as optimizing the production condition, when compared with those previously reported in Japan (Kaida *et al*. 2009) and India (Santhi *et al.* 2014).

The aim of this study was thus to investigate the possibility of producing ethanol from two kinds of fast growing trees, *A. mangium* and the *Acacia* hybrid, planted in Thailand using different pretreatment techniques. The influence of the pretreatment step was studied to determine the highest amount of glucose after enzymatic saccharification. The ethanol production in SSF was investigated and optimized using a statistical method.

## EXPERIMENTAL

### Materials and Methods

*Raw materials*

*Acacia mangium* and *Acacia* hybrid tree wood were locally collected in Thailand and used as raw materials in this study. They were chopped to between 1 and 3 cm and air-dried at room temperature. They were then comminuted through milling, grinding, or chipping. This step is necessary in bioconversion to reduce cellulose crystallinity and improve the efficiency of downstream processing. Wood chips were milled to a particle

size of around 3 mm using a Wiley mill (Kinematica AG Co. Ltd., Tokyo, Japan) and stored until use.

*Content determination of wood component*

Each wood meal was milled to a particle size of 40-mesh using a Wiley mill (Kinematica AG Co. Ltd., Tokyo, Japan). The chemical compositions of *Acacia* sp. were determined according to standard TAPPI (Technical Association of Pulp and Paper Industry) methods: T204 om-88 (1997) for extractives; T211 om-02 (2002) for ash; T222 om-88 (1988) for Klason (acid-insoluble) lignin; T223 cm-01 (2001) for pentosan; and T203 om-88 (1992) for α-cellulose. To determine holocellulose content, the residual yield and its chemical composition during delignification by acidified sodium chlorite were studied (Browning 1967).

*Steam explosion*

The biomass was heated using high-pressure steam (20 to 50 bar, 160 to 290 °C) for a few minutes to expand within the lignocellulosic matrix (Neves *et al*. 2007; Balat *et al*. 2008). About 400 g of *A. mangium* and the *Acacia* hybrid wood chips were steam-exploded at 13 kgf/cm$^2$ (195 °C) and 15 kgf/cm$^2$ (200 °C), respectively, for 5 min in a 2.5-L stainless steel batch digester (Nitto Koatsu Co. Ltd., Tokyo, Japan). After that, the materials were separated into solid residue and liquid by filtration through a cheese cloth. The solid residue was washed with hot water until reaching neutral pH (T264 cm-07), dried in an oven at 60 °C for 24 h, and stored in a sealed plastic bag.

*Alkaline peroxide delignification*

Alkaline peroxide delignification was performed as described by Cristobal *et al*. (2005). The dried steam explosion-pretreated materials were slurried in 1% (w/v) $H_2O_2$ solution at 4% (w/v) solid concentration. The pH was adjusted to 11.5 using 4 M NaOH. The slurried sample was incubated at 80 °C for 45 min; then, the suspension was filtered and the solid residue was washed with hot water until reaching neutral pH. The delignified solid was dried in an oven at 60 °C for 24 h and stored in a sealed plastic bag for simultaneous saccharification and fermentation (SSF).

*Alkaline pulping*

Five hundred grams of each type of wood chip were reacted with a 6% (w/v) NaOH solution. The reaction was carried out in a 2-L stainless steel bucket using a solid-liquid ratio of 1:7 (w/v) at 170 °C for 3 h. The solid residue was separated from the liquid by filtration. The solid residue was then washed with hot water until it no longer produced a yellow-colored fluid resulting from the presence of lignin (neutral pH), dried in an oven at 60 °C for 24 h, and kept in a sealed plastic bag.

*Microorganism and culture*

*Saccharomyces cerevisiae* TISTR 5339 (Thailand Institute of Scientific and Technological Research, Thailand) was used in this study. Two loops of the colonies grown on 2% YPD for 2 d were transferred to 100 mL of 5% YPD containing (g/L) glucose, 50; yeast extract, 10; and peptone, 20, in a 250-mL flask and then cultured at 30 °C for 48 h with 200 rpm shaking using a rotary shaker. After centrifugation at 8000

rpm at 4 °C for 10 min, the cell pellets were washed twice with autoclaved distilled water and used as seed for further experiments.

*Enzymatic hydrolysis*

Each raw material (5% w/v) was autoclaved at 121 °C for 15 min and soaked in an acetate buffer (100 mM, pH 5.5). Enzyme loadings were 10 FPU cellulase (Celluclast 1.5 L, Novozyme A/S)/g raw material, 10 IU fungal β–glucosidase (Novozyme 188, Novo Ltd.) /g raw material, and 100% (v/w) solid concentration of 4% Optimash BG (Genencor, Finland). The experiments were performed at 30 °C on a rotary shaker at 150 rpm. Periodically, samples of hydrolysate were taken for glucose analysis by the Nelson-Somogyi method (Somogyi 1952).

*Simultaneous saccharification and fermentation (SSF)*

For the SSF process, each sample was slurried in an acetate buffer (100 mM, pH 5.5). Then cellulase (10 FPU/g raw material), a fungal β-glucosidase (10 IU/g raw material), and 100% (w/v) of a solid concentration of 4% Optimash BG were added to initiate the hydrolysis. A seed culture of *Saccharomyces cerevisiae* TISTR 5339 and yeast nutrients (g/L: $(NH_4)_2HPO_4$, 0.5; $MgSO_4.7H_2O$, 0.025; yeast extract, 1; $NaH_2PO_4.H_2O$, 13.8) were also added to each sample. Each mixture was incubated at 30 °C on a rotary shaker at 150 rpm. Samples were taken every 24 h for ethanol concentration determination by gas chromatography (GC, Chromosorb-103, GC4000, GL Sciences, Japan). The GC was performed with an HP5 capillary (30 m x 0.32 mm x 0.25 µm, JW Scientific, USA) and an FID detector under the following conditions: splitless ratio 50:1; split flow 25.1 ml $min^{-1}$; air flow 400 mL $min^{-1}$; $H_2$ flow 40 mL $min^{-1}$; oven initial 40 °C $min^{-1}$ hold 5 min and 15 °C $min^{-1}$ to 250 °C 15 min; injection volume 1 µL). All experiments were carried out in triplicate.

*Experimental design*

Response surface methodology (RSM) was used to evaluate the variables and response data. The investigated factors were solid loading (3.98% to 11.03%) and inoculum size ($A_{600}$ of *S. cerevisiae* TISTR 5339 at 1.18 to 6.82) with five levels for each, based on the factorial design at two levels shown in Table 1. To identify the optimum levels of these two test variables, the RSM was applied. Central composite design (CCD) in the experiment consists of $2^2$ factorial points, so four axial points and three replicates of the center points could be designed. The experimental matrix was carried out with eleven runs. The observed and predicted values are shown in the table. The second-order model predicting the level of ethanol concentration is expressed as Eq. 1,

$$Y = a_0 + a_1X_1 + a_2X_2 + a_{11}X^2{}_1 + a_{22}X^2 + a_{12}X_1X_2 \quad \text{(1)}$$

where $Y$ is the predicted response (ethanol concentration, g/L); $a_0$ is a constant term; $a_1$ and $a_2$ are linear terms; $a_{11}$ and $a_{22}$ are quadratic terms; $a_{12}$ is an interaction term and $X_1$ and $X_2$ are test variables studied.

Statistical analysis of the data was performed by the assistance of software SPSS Statistics (version 17.0; IBM). Subsequently, additional confirmation experiments were conducted to verify the validity of statistical experimental strategies.

*Batch fermentation in stirred fermenter*

The batch fermentation was carried out in a 10-L stirred fermenter containing 3 L working volume of the optimized conditions according to the results of CCD. The fermentation temperature was varied at 30, 35, and 40 °C, and the aeration rate was varied at 0, 0.25, 0.5, 0.75, and 1.0 vvm. Agitation of 150 rpm and initial pH of 5.5 were fixed. The culture was sampled at intervals of 24 h for 96 h, after which the ethanol concentration was determined.

## RESULTS AND DISCUSSION

### Chemical Composition of *Acacia* sp.

The mass-based contents of ethanol/benzene extractives, holocellulose, α-cellulose, hemicellulose, lignin, and ash of *A. mangium* were 4.27, 74.13, 43.99, 30.14, 23.71, and 0.62% of dry wood, respectively. In the case of *Acacia* hybrid, the contents of ethanol/benzene extractives, holocellulose, α-cellulose, hemicellulose, lignin, and ash were 3.41, 73.82, 41.99, 31.83, 23.35, and 0.58% of dry wood, respectively. When compared to other reported lignocellulosic residues such as wheat straw (Chen *et al*. 2008), oil palm empty fruit bunches (Jeon *et al*. 2014), corn stover (Fenske *et al*. 1998), switchgrass (Zhang *et al*. 2013), and *A. dealbata* wood (Ferreira *et al*. 2011), *A. mangium*, and *Acacia* hybrid possessed a relatively higher cellulose content (42% to 44% *versus* 32, 35, 7.1, 34, and 43%, respectively). This result shows clearly that both raw materials contained a high enough cellulose content to be used for ethanol production.

### Pretreatment

Because carbohydrates in wood are not completely accessible for enzymatic hydrolysis, the use of pretreatment is necessary for the ultimate production of biofuel. The objective of a pretreatment is to reduce the recalcitrance of lignocelluloses and improve the accessibility of the cellulose, which is the most suitable compound for enzymatic attack. Improved accessibility can be achieved by removing hemicelluloses and/or lignin, milling the biomass to small particles to increase surface area, or destroying cellulose crystalline structure. A variety of pretreatments have been tested over the past years, including mechanical size reduction, pyrolysis, steam explosion, acid pretreatment, and alkaline pretreatment (Nibedita *et al*. 2012). In this work, we compared the influence of selected pretreatments on the efficiency of the carbohydrate conversion process using enzymes.

The influence of selected pretreatments on enzymatic hydrolysis is shown in Fig 1. Alkaline pulping was found to be the most effective pretreatment for enzymatic hydrolysis. At 72 h, the highest reducing sugar yields of 492.88 and 452.92 mg/g substrate from *A. mangium* and *Acacia* hybrid, respectively, were obtained. Other pretreatments of *A. mangium* wood yielded reducing sugars at 46.45, 62.24, 71.45, and 91.14 mg/g substrate from steam-exploded material, milled-steam-exploded material, alkaline peroxide-steam-exploded material, and alkaline peroxide-milled-steam-exploded material, respectively. Likewise, reducing sugar yields of 44.75, 37.73, 84.61, and 60.83 mg/g substrate from steam-exploded material, milled-steam-exploded material, alkaline peroxide-steam-exploded material, and alkaline peroxide-milled-steam-exploded material, respectively, were received from *Acacia* hybrid wood. The alkaline pulping process generated a readily digestible cellulose substrate, which resulted in an easy

enzymatic hydrolysis, because the removal of hemicellulose and lignin can also reduce hydrophobic interaction, depolymerization of cellulose, and size reduction (Zhu and Pan 2010). Therefore, the alkaline pulping pretreatment was selected for further study in SSF by statistical experimental design.

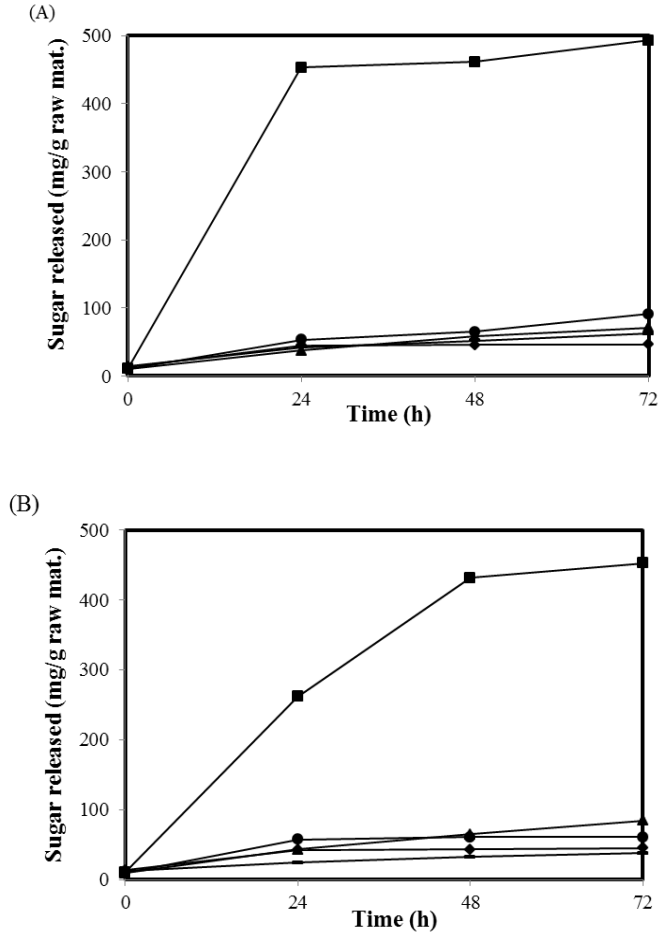


**Fig. 1.** Time course of enzymatic hydrolysis of (A) *Acacia mangium* and (B) *Acacia* hybrid woods with different pretreatments. (-♦-) steam explosion materials; ( ) mill+steam explosion materials; (-▲- ) alkaline peroxide+steam explosion materials; (-●-) mill+alkaline peroxide+steam explosion materials, and (-■-) alkaline pulping materials.

## Simultaneous Saccharification and Fermentation of *A. mangium* wood

To optimize the ethanol production from alkaline pulping pretreated *A. mangium* wood, the effect of solid loading and inoculum size were investigated. The averages of the triplicate measurements of ethanol concentration are summarized in Table 1. The results obtained by CCD were analyzed by standard analysis of variance (ANOVA). The second-order regression equation modeled the ethanol production, which can be predicted by the following equation,

$$Y=20.453+7.772X_1+1.647X_2+2.68X_1^2+2.884X_2^2-0.175X_1X_2\ldots\ldots \quad (2)$$

where $Y$ is the predicted response (ethanol concentration, g/L); $X_1$ and $X_2$ are coded values of solid loading (g/L) and inoculum size ($A_{600}$), respectively.

The results of the second-order response surface model fitting in the form of ANOVA are given in Table 2. To test the fit of the model equation, the regression-based determination coefficient $R^2$ was evaluated. The model presented a determination coefficient ($R^2$ = 0.93), explaining that 93% of the variability in the response could be accepted, and the other 7% was affected by other variables.

## Simultaneous Saccharification and Fermentation of *Acacia* hybrid wood

The results of *Acacia* hybrid materials corresponded with the results of *A. mangium* wood. The effects of solid loading and inoculum size were investigated, and the averages of triplicate measurements of the ethanol concentration are summarized in Table 1. The ethanol production can be predicted by the following equation:

$$Y=21.570+6.499X_1+0.551X_2-0.526X_1^2-0.211X_2^2+0.407X_1X_2\ldots\ldots \quad (3)$$

The results obtained by CCD were analyzed by standard analysis of variance (ANOVA), and the mean predicted and observed responses are presented in Table 2. The model presented a determination coefficient ($R^2$ = 0.98), explaining that 98% of the variability in the response could be accepted and the other 2% was affected by other variables.

Model coefficients estimated by regression analysis for each variable of *A. mangium* and *Acacia* hybrid wood are shown in Tables 3 and 4, respectively. The result revealed that only solid loading ($X_1$) had a significant effect on ethanol production. No interactions between the two variables were found to contribute to the response at a significant level. Response plot between the effect of solid loading ($X_1$) and inoculum size ($X_2$) on ethanol production from *A. mangium* and *Acacia* hybrid materials is shown in Figs. 2A and 2B, respectively. These figures revealed that the ethanol concentration continuously increased when the concentration of solid loading was increased, whereas yeast inoculum size had no effect on ethanol production. Addition of solid loading could increase ethanol production, but an excess of added solid loading affected agitation; therefore, the optimized value was selected from the highest ethanol production in Table 1. Solid loading (100 g/L) and inoculum size ($A_{600}$ of *S. cerevisiae* TISTR 5339 of 2) as shown in treatment no. 2 were selected as the optimized conditions for further experimentation. As a result, this treatment yielded near to the highest ethanol production, consumed less yeast inoculum, and did not affect agitation.

The validity of the experimental model and regression equation was tested by carrying out ethanol production in the optimized value. The predicted and actual responses for ethanol production of *A. mangium* wood were 32.32 and 35.7 g/L, respectively, whereas those for ethanol production of *Acacia* hybrid wood were 26.37 and 27.32 g/L, respectively. Singh *et al*. (2013) optimized ethanol production from pretreated wheat straw hydrolysate using sequential statistical process. First, the factors affecting ethanol production were screened by Placket-Burman design. The significant factors pH, temperature, initial total reducing sugar concentration and inoculum size were further studied by the Box-Behnken design. The highest ethanol production obtained at pH 5.5, temperature 30 °C, inoculum size 3.3%, and initial total reducing sugar concentration 6.5% was 16.4 g/L. These results indicated that ethanol production can be enhanced by optimization of fermentation conditions. Moreover, Luo *et al*. (2014) studied ethanol

production from sweet sorghum juice using RSM. The variable factors investigated included juice solid concentration, inoculum size, and temperature. It was found that inoculum size had no significant effect on the final ethanol concentration. However, the juice solid concentration had a significant effect on ethanol concentration. When the results from this study were compared with other agricultural residues for ethanol concentration, maximum ethanol concentration obtained from *A. mangium* and *Acacia* hybrid woods were 0.35 and 0.27 g/g raw material, respectively, whereas ethanol concentrations of 0.14, 0.37, and 0.16 g/g raw material were produced from elephant grass (Eliana *et al*. 2014), oil palm empty fruit bunch (Jeon *et al*. 2014), and corn stover (Bondesson *et al.* 2013), respectively. Even though ethanol concentration from *A. mangium* and *Acacia* hybrid was equivalent to that from oil palm empty fruit bunch, both *Acacia* are relatively abundant and grow easily and quickly without the need for fertile cultivable land for cropping. Therefore, it could be a promising option for ethanol production.

**Table 1.** Experimental Design Used in Response Surface Methodology of 2 Independent Variables, Solid Loading ($X_1$), and Inoculum Size ($X_2$)

| Treatment number | Level | | Actual level | | Ethanol concentration (g/L) | | | |
|---|---|---|---|---|---|---|---|---|
| | | | | | *Acacia mangium* materials | | *Acacia* hybrid materials | |
| | $X_1$ | $X_2$ | $X_1$ | $X_2$ | Observed | Predicted | Observed | Predicted |
| 1 | 1 | 1 | 100 | 6 | 37.49 | 35.26 | 29.15 | 28.29 |
| 2 | 1 | -1 | 100 | 2 | 35.70 | 32.32 | 27.30 | 26.37 |
| 3 | -1 | 1 | 50 | 6 | 20.95 | 20.07 | 13.54 | 14.48 |
| 4 | -1 | -1 | 5 | 2 | 18.46 | 16.42 | 13.32 | 14.19 |
| 5 | 1.41 | 0 | 110.3 | 4 | 33.64 | 36.74 | 28.42 | 29.69 |
| 6 | 0 | 1.41 | 75 | 6.82 | 27.18 | 28.51 | 21.98 | 21.93 |
| 7 | -1.41 | 0 | 39.8 | 4 | 13.63 | 14.82 | 12.64 | 11.36 |
| 8 | 0 | -1.41 | 75 | 1.18 | 20.90 | 23.86 | 20.33 | 20.37 |
| 9 | 0 | 0 | 75 | 4 | 20.58 | 20.45 | 21.52 | 21.57 |
| 10 | 0 | 0 | 75 | 4 | 20.01 | 20.45 | 22.07 | 21.57 |
| 11 | 0 | 0 | 75 | 4 | 20.82 | 20.45 | 21.12 | 21.57 |

## Ethanol Production in a Stirred Fermenter

Optimum conditions as determined by the previous experiments were further implemented in a 10-L stirred fermenter. The results indicated that an aeration rate of 0 vvm (no aeration) and temperature of 30 °C gave the highest ethanol concentrations from both *A. mangium* and *Acacia* hybrid wood (Figs. 3 , 4, respectively). The maximum ethanol productions of 0.38 and 0.37 g/g raw material from *A. mangium* and *Acacia* hybrid wood, respectively, were achieved within 96 h of cultivation at a temperature of 30 °C, no aeration rate, agitation of 150 rpm, and initial pH of 5.5. It was far higher than those reported from the previous studies; 0.04 g/g raw material from *A. mangium* wood (Kaida *et. al.* 2009), 0.08 g/g raw material from *A. mangium* leaves, and 0.06 g/g raw material from *A. mangium* pods (Santhi *et al.*, 2014). It was also observed that the ethanol yield from the fermenter was slightly higher than that from a volumetric flask; however, fermentation time was extended from 72 to 96 h. Generally, when a fermentation system with lignocellulose as a substrate is scaled up from flask to stirred fermenter scale, the increased amount of substrate always interferes with the agitation system, resulting in a non-homogeneous raw material.

**Table 2.** Analysis of Variance (ANOVA) for the Model Regression Representing Ethanol Concentration of *A. mangium* and *Acacia* Hybrid

| Source | Sum of Squares | | DF | | Mean Square | | F | | *p*-value | |
|---|---|---|---|---|---|---|---|---|---|---|
| | *Am*[a] | *Ah*[a] | *Am* | *Ah* | *Am* | *Ah* | *Am* | *Ah* | *Am* | *Ah* |
| Regression | 570.897 | 341.580 | 5 | 5 | 114.179 | 68.316 | 13.193 | 49.236 | 0.007[b] | 0.000[b] |
| Residual | 43.273 | 6.938 | 5 | 5 | 8.655 | 1.388 | | | | |
| Total | 614.170 | 348.518 | 10 | 10 | | | | | | |

[a]*Am*= *A. mangium*; *Ah*= *Acacia* hybrid
[b]significance level = 95%

**Table 3.** Regression Coefficients and their Significance for Ethanol Production of *A. mangium* Wood

| Variables | Coefficient | *t*-value | *p*-value |
|---|---|---|---|
| Intercept | 20.453 | 12.042 | 0.000 |
| $X_1$ | 7.772 | 7.462 | 0.001[a] |
| $X_2$ | 1.647 | 1.581 | 0.175 |
| $X_1^2$ | 2.680 | 2.156 | 0.084 |
| $X_2^2$ | 2.884 | 2.320 | 0.068 |
| $X_1X_2$ | -.175 | -.119 | 0.910 |

[a]Statistically significant at 95% confidence level

**Table 4.** Regression Coefficients and their Significance for Ethanol Production of *Acacia* hybrid Wood

| Variables | Coefficient | *t*-value | *p*-value |
|---|---|---|---|
| Intercept | 21.570 | 31.717 | 0.000 |
| $X_1$ | 6.499 | 15.583 | 0.000[a] |
| $X_2$ | 0.551 | 1.322 | 0.244 |
| $X_1^2$ | -0.526 | -1.057 | 0.339 |
| $X_2^2$ | -0.211 | -0.425 | 0.689 |
| $X_1X_2$ | 0.407 | 0.692 | 0.520 |

[a]Statistically significant at 95% confidence level

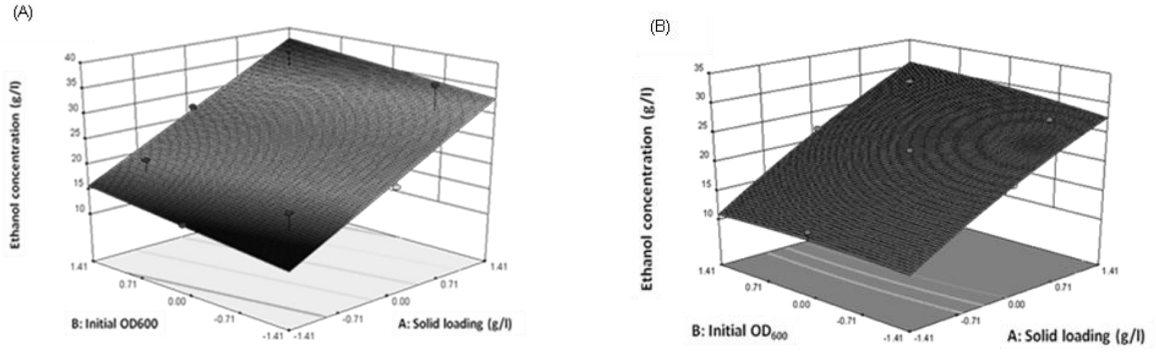


**Fig. 2.** Response plot of the combined effects between solid loading and inoculum size on the ethanol production by *S. cerevisiae* TISTR 5339 of *A. mangium* (A) and *Acacia* hybrid woods (B)

As a result, enzyme and yeast could not effectively function, leading to lowered amounts of ethanol and otherwise increasing fermentation time. In this study, homogeneous raw material was observed after 24 h of fermentation, after which the enzyme and yeast were able to function efficiently. This study was the first to report a scale up of SSF using fast-growing *Acacia* wood as substrates. The wood consisted of approximately 42% to 44% cellulose, which is rather high and is very promising as a substrate for ethanol production.

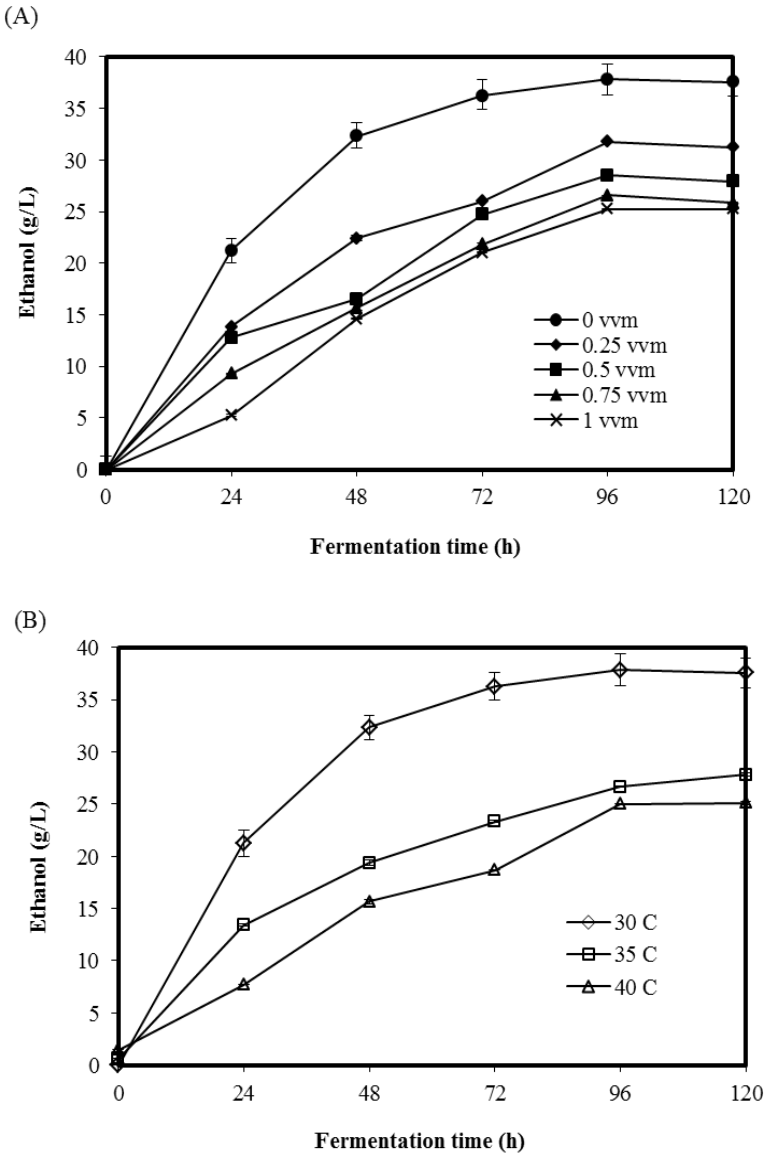


**Fig. 3.** Time course of batch fermentation for ethanol production from *A. mangium* wood under optimized conditions at various (A) aeration rates and (B) temperatures

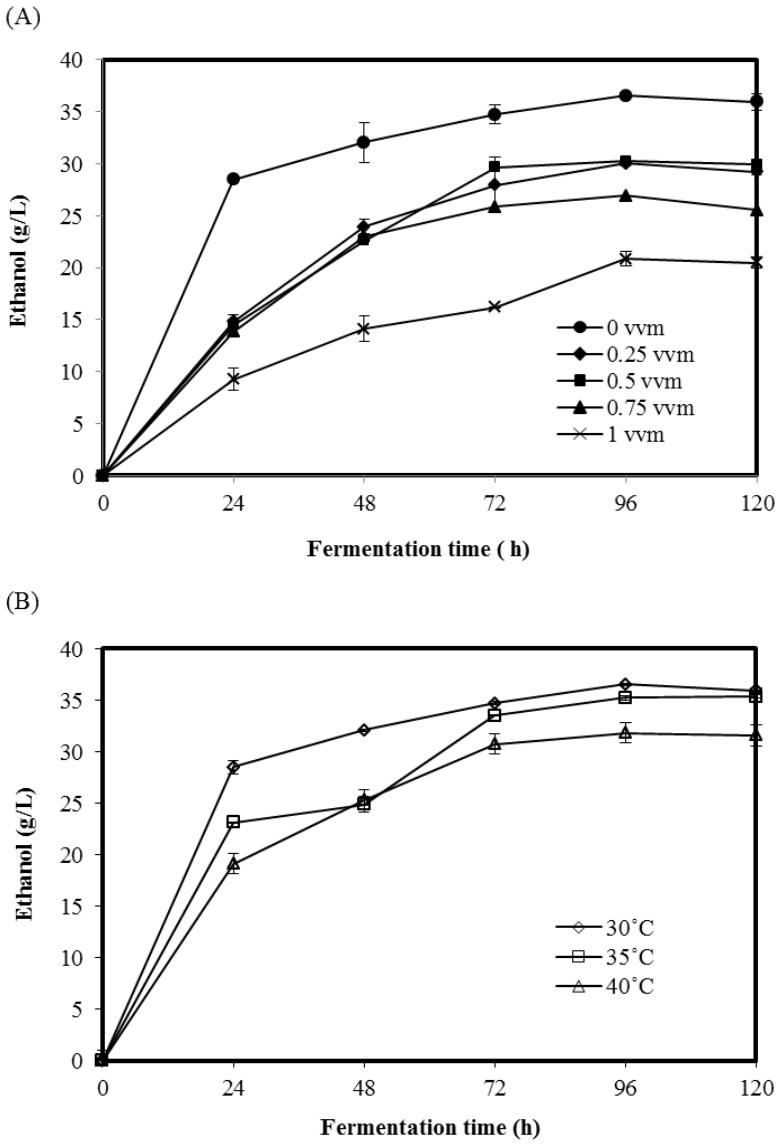


**Fig. 4.** Time course of batch fermentation for ethanol production from *Acacia* hybrid wood under optimized conditions at various (A) aeration rates and (B) temperatures

## CONCLUSIONS

1. This study was the first to show the possibility of using fast-growing *Acacia* trees as a substrate for ethanol production in Thailand.
2. The ethanol fermentation in a 10-L stirred bioreactor, with 10% (w/v) solid loading and an $A_{600}$ of *S. cerevisiae* TISTR 5339 of 2, reached maximum ethanol production after 96 h at 37.84 g/L (87.34% of the theoretical value) and 36.52 g/L (86.72% of the theoretical value) and productivity of 0.4 and 0.38 g/L/h from *A. mangium* and *Acacia* hybrid woods, respectively.

3. Data obtained from this study provide promising information of ethanol production from the alternative source of lignocelluloses and could be further applied on an industrial scale.

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