EFFECT OF CO$_2$-ADDED STEAM EXPLOSION ON OIL PALM EMPTY FRUIT BUNCH FOR BIOETHANOL PRODUCTION

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This study aimed to investigate the effect of adding CO$_2$ as an impregnation agent in steam explosion on oil palm empty fruit bunch (EFB) for bioethanol production. The influence of this treatment on the characteristics of EFB, enzymatic hydrolysis, and fermentation of EFB was evaluated in this investigation. CO$_2$-added steam explosion was conducted varying the CO$_2$ impregnation time (0, 30, 60 min). The results showed that the addition of CO$_2$ in steam explosion increased the surface area, pore area, and pore volume of EFB. Furthermore, this treatment enabled obtaining yields of glucose and ethanol of 84.14% and 56.01%, respectively, for 60 min CO$_2$ impregnation time. These results were higher than the glucose and ethanol yields of the sample treated by conventional steam explosion, which reached 58.12% and 41.37%, respectively. The findings illustrate the possibility of applying CO$_2$-added steam explosion (CO$_2$SE) for increasing the efficiency of biomass conversion.

Keywords: steam explosion, impregnating agent, carbon dioxide, oil palm empty fruit bunch, bioethanol

INTRODUCTION

Lignocellulose is considered an attractive raw material for second-generation bioethanol production.\textsuperscript{1} Biomass has the advantage of being abundantly available in nature and an example of accessible lignocellulosic sources can be oil palm solid wastes. Indonesia is the biggest oil palm producer in the world, but the production of crude palm oil (CPO) also results in huge amounts of wastes, such as oil palm empty fruit bunch (EFB). This waste has a cellulose content of about 37%, 14% hemicelluloses and 31% lignin, as well as 18% of ash and extractive compounds.\textsuperscript{2} Polysaccharides (cellulose and hemicelluloses) can be transformed into bioethanol, but lignin is an inhibitor in the process.\textsuperscript{3} Therefore, pretreatment should be conducted on lignocellulosic biomass in order to reduce the lignin content and increase biomass digestibility.\textsuperscript{4} Generally, there are four main steps for 2$^{nd}$ generation bioethanol production, \textit{i.e.} pretreatment, hydrolysis/saccharification, fermentation and purification.\textsuperscript{5-7}

The main targets of the pretreatment consist in swelling the lignocellulose, changing phases in cellulose crystallinity, and elimination of lignin.\textsuperscript{8} However, the pretreatment is still a bottleneck in the process of 2$^{nd}$ generation bioethanol production, even though various pretreatment ways have been investigated.\textsuperscript{9,10} Chemical pretreatment, such as using alkali, acids, organic solvents, and ionic liquids has been demonstrated to have a significant effect on the degradation of lignocellulose.\textsuperscript{10} However, it involves large amounts of consumed chemicals, which has a negative effect on the environment. Another pretreatment that has been most extensively studied and commonly applied to lignocellulosic biomass is steam explosion.

This method uses limited chemicals and does not result in unnecessary dilution of hydrolyzates,
but steam explosion has resulted in partial degradation of the carbohydrate-lignin matrix.\textsuperscript{10} To improve the effectiveness of the steam explosion method, the addition of H\textsubscript{2}SO\textsubscript{4}, SO\textsubscript{2}, or CO\textsubscript{2} as catalyst or impregnating agent to decrease the production of inhibitors and improve the enzymatic hydrolysis on the biomass has been reported.\textsuperscript{1,12}

The most interesting of the impregnating agents mentioned is CO\textsubscript{2}. This catalyst has several benefits, such as low cost, low toxicity and corrosion, as well as the fact that it allows the possibility of having high solids content in biomass.\textsuperscript{13} Moreover, the fermentation of lignocellulose for bioethanol production produces CO\textsubscript{2} as a by-product. This gas can be stored and sent to the pretreatment reactor, thus, CO\textsubscript{2} is already highly available in bioethanol plants. The use of CO\textsubscript{2} resulting as a by-product from the fermentation process is expected to have a double benefit: to decrease the operation cost and stop the release of CO\textsubscript{2} into the environment.

Generally, CO\textsubscript{2} explosion applies supercritical CO\textsubscript{2} or high-pressure CO\textsubscript{2} to enhance biomass digestibility.\textsuperscript{9,14,15} However, adding CO\textsubscript{2} under mild conditions as an impregnation agent in steam explosion has not been performed yet. Moreover, the pretreatment of EFB using CO\textsubscript{2}SE has not been reported yet either. Therefore, this research intends to study the possibility of bioethanol production from EFB using steam explosion with CO\textsubscript{2} as an impregnating agent. The effect of CO\textsubscript{2}SE on the characteristics of EFB was investigated in this study. The influence of this treatment on the glucose and ethanol yields obtained by separate enzymatic hydrolysis and fermentation (SHF) was also evaluated.

**EXPERIMENTAL**

**Materials**

In this study, EFB was collected from an oil palm plantation in Palembang, South Sumatra, Indonesia. CO\textsubscript{2} gas was obtained from PT WAP Andalan, Indonesia. The cellulolytic enzymes used were Celli\textsuperscript{c}® Ctec2 and Celli\textsuperscript{c}® Htec2, which were purchased from Novozymes Korea Ltd. Instant active dry yeast *Saccharomyces cerevisiae* was used in the fermentation process. All the chemicals used were of analytical grade.

**Methods**

**CO\textsubscript{2}-added steam explosion**

Chopping and milling were applied to EFB in order to provide a particle size of about 3 mm and, then, the ground EFB was dried until the moisture content of ±10%. For steam explosion, a mass ratio of EFB to water of 1:5 was used. After EFB and water were put into the pretreatment reactor, CO\textsubscript{2} gas was added until the reactor pressure reached 4 kg/cm\textsuperscript{2}. The reactor was manufactured by Changhae Ethanol Co. Ltd. and was designed for operation at a maximum 5 L volume and 230 °C temperature. Then, EFB, water and CO\textsubscript{2} gas were mixed using a blade-type agitator, at room temperature, while varying the time of impregnation (0, 30, 60 min). The temperature in the reactor was increased to 150 °C and the pressure – up to ±7 kg/cm\textsuperscript{2} after ±1 h of heating. After reaching the temperature of 150 °C, the process lasted for 30 min. Treated EFB was washed by water until neutral pH and then dried up. A blank pretreatment (steam explosion without adding CO\textsubscript{2}) was also run. Air was used to increase the reactor pressure up to 4 kg/cm\textsuperscript{2} in the blank pretreatment.

**Separate hydrolysis and fermentation process**

To carry out the hydrolysis, 10 g/L of treated EFB was added by 30 FPU/g biomass of Celli\textsuperscript{c}® Ctec2. Celli\textsuperscript{c}® Htec2 was also added as much as 20% of the volume of added Celli\textsuperscript{c}® Ctec2. The temperature, agitation speed and pH were controlled at, respectively, 50 °C, 150 rpm and 4.8 using sodium citrate buffer. The hydrolysis was conducted for 96 h and the sample was taken at the end of the process. Enzymatic hydrolysis was carried out in duplicate experiments.

After hydrolysis, 1% w/w of *Saccharomyces cerevisiae* yeast was added into the hydrolyzate for the fermentation process. The fermentation was operated at 32 °C, with an agitation speed of 150 rpm for 72 h. The SHF process was conducted in duplicate experiments.

**Analytical methods**

The chemical composition (cellulose, hemicelluloses and lignin) was analyzed using standard biomass analytical procedures from National Renewable Energy Laboratory (NREL).\textsuperscript{16} The crystallinity index and structural changes of EFB before and after the pretreatment were analyzed using a Philips PW 1710 X-ray diffractometer, with CuK\textsubscript{α} irradiation at 40 kV and 30 mA, and a secondary graphite monochromator. The structural changes of EFB were determined by a Shimadzu FTIR spectrometer. Brunauer-Emmett-Teller (BET) analysis was conducted to analyze the total surface area of the samples. Scanning electron microscopy (SEM, JEOL JSM-IT200), with SE 10 kV and 1000x magnification, was performed to observe the surface morphology of the treated and untreated biomass samples.

The concentration of glucose and ethanol was determined using high-performance liquid chromatography (HPLC), with a HPX-87P (Bio-RAD, CA, USA) column, and analyzed with an RID detector.
The eluent used as mobile phase was 5 mM H$_2$SO$_4$ solution, at a flow rate of 0.6 mL/min.

**Calculations**

**Crystallinity index**

The index of crystallinity was determined by Segal’s method, and was calculated from the height ratio between the intensity of the crystalline peak ($I_{200}$) and total intensity ($I_{200}$)\textsuperscript{17,18}

\[
I_c = \left( \frac{I_{200} - I_m}{I_{200}} \right) \times 100\%
\]  

where $I_c =$ crystallinity, $I_{200} =$ peak at 20 = 22°, and $I_m =$ peak at 20 = amorphous peak.

**Glucose yield on cellulose content**

\[
\%W_g = \frac{W_g}{W_c} \times 100\% \tag{2}
\]

where $W_g =$ glucose content (g/L); $W_c =$ theoretical glucose content (g/L); $W_c =$ cellulose content on substrate (g/L); anhydro correction = 1.1 (cellulose conversion to equivalent glucose)\textsuperscript{19}

**Glucose yield on substrate**

\[
\%W_g = \frac{W_g}{W_{substrate}} \times 100\% \tag{3}
\]

where $W_g =$ glucose content (g/L); $W_{substrate} =$ substrate content in hydrolysis (g/L).

**Ethanol yield calculation**

\[
\%\text{Ethanol yield} = \frac{[\text{EtOH}]_{f} - [\text{EtOH}]_{i}}{0.51 \times (f \times [\text{biomass}] \times 1.1)} \times 100\% \tag{4}
\]

where $[\text{EtOH}]_{f} =$ final ethanol concentration in fermentation process (g/L), $[\text{EtOH}]_{i} =$ ethanol concentration at initial time of fermentation (g/L), 0.51 = conversion factor from glucose to ethanol based on stoichiometric biochemistry of yeast, 1.1 = cellulose conversion to equivalent glucose, $[\text{biomass}] =$ biomass concentration in SHF process (g/L), f = cellulose fraction of dry biomass (g/g).

**RESULTS AND DISCUSSION**

**Influence of CO$_2$-added steam explosion on recovery of biomass and chemical composition of EFB**

EFB was steam-exploded using CO$_2$ as an impregnating agent for 0, 30 and 60 min of impregnation time. Table 1 shows the recovered weight of the samples after the CO$_2$SE treatment and conventional steam explosion (the blank sample). As a result, the weight of the samples after this pretreatment decreased as compared to the weight of the samples before the pretreatment. The recovered weight accounted for 66.3-67.77% (w/w) of the initial biomass on CO$_2$SE. On the other hand, the conventional steam explosion allowed a recovered biomass weight of 65.84%. These results indicate that the weight loss of the samples subjected to CO$_2$SE was similar to that of the blank that underwent conventional steam explosion. The weight loss of the samples might be caused by several reasons: it is possible that a part of the materials was blown from the vortex into the exhaust hole, inhibitory products may have evaporated\textsuperscript{20} some components of biomass may have dissolved, or some losses may occur during the filtration process at the end.

Table 2 shows the chemical composition of EFB before and after the pretreatment. CO$_2$SE provided a slightly higher cellulose percentage, as compared to that of the untreated biomass. The results revealed a tendency towards a higher cellulose content with longer CO$_2$-impregnation time. After the pretreatment, the cellulose content in the biomass reached 34.84%-35.95%, while in untreated EFB, it was 30.12%.

### Table 1

<table>
<thead>
<tr>
<th>Sample code</th>
<th>Pretreatment time (min)</th>
<th>Pretreatment conditions</th>
<th>Recovered weight after pretreatment (w/w %)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blank</td>
<td>-</td>
<td>0</td>
<td>65.84±0.02</td>
</tr>
<tr>
<td>Sample A</td>
<td>+</td>
<td>0</td>
<td>66.38±0.72</td>
</tr>
<tr>
<td>Sample B</td>
<td>+</td>
<td>30</td>
<td>67.43±2.85</td>
</tr>
<tr>
<td>Sample C</td>
<td>+</td>
<td>60</td>
<td>67.77±2.69</td>
</tr>
</tbody>
</table>

Hemicelluloses and lignin underwent a certain degree of degradation after the pretreatment. The highest delignification was of 11.65%, which was obtained after steam explosion with CO$_2$ for 60 min of impregnation time. Carbon dioxide, added in steam explosion, will dissolve in the water and form carbonic acid, which will slightly help the delignification process\textsuperscript{14}. The delignification in
EKA TRIWAHYUNI et al.

CO₂SE was categorized as low, compared to that achieved in alkali-steam explosion. The delignification of EFB from alkali-steam explosion using 10% NaOH solution has been reported to reach 69.43% due to the high solubility of lignin in alkali. However, the utilization of CO₂ is believed to be more environmentally friendly than the use of an alkali solution, as it is nontoxic and leaves no residue.

Influence of CO₂-added steam explosion on characteristics of EFB

Crystallinity index of EFB

Table 2

<table>
<thead>
<tr>
<th>Sample code</th>
<th>Cellulose</th>
<th>Hemicelluloses</th>
<th>Lignin</th>
<th>Ash</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(w/w %)</td>
<td>(w/w %)</td>
<td>(w/w %)</td>
<td>(w/w %)</td>
</tr>
<tr>
<td>Untreated sample</td>
<td>30.12±0.10</td>
<td>22.84±0.29</td>
<td>37.16±0.25</td>
<td>2.51±0.02</td>
</tr>
<tr>
<td>Blank</td>
<td>34.84±0.12</td>
<td>22.70±0.10</td>
<td>33.10±0.21</td>
<td>2.59±0.11</td>
</tr>
<tr>
<td>Sample A</td>
<td>35.08±0.80</td>
<td>22.42±0.26</td>
<td>32.81±0.40</td>
<td>2.51±0.16</td>
</tr>
<tr>
<td>Sample B</td>
<td>35.67±0.10</td>
<td>22.31±0.07</td>
<td>32.65±0.14</td>
<td>2.31±0.05</td>
</tr>
<tr>
<td>Sample C</td>
<td>35.95±0.01</td>
<td>21.12±0.10</td>
<td>32.83±0.07</td>
<td>2.47±0.05</td>
</tr>
</tbody>
</table>

Table 3

<table>
<thead>
<tr>
<th>Sample code</th>
<th>Crystallinity index (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated sample</td>
<td>68.84</td>
</tr>
<tr>
<td>Sample A</td>
<td>65.82</td>
</tr>
<tr>
<td>Sample B</td>
<td>66.45</td>
</tr>
<tr>
<td>Sample C</td>
<td>64.71</td>
</tr>
</tbody>
</table>

Figure 1: XRD patterns of untreated EFB sample, sample A, sample B and sample C

Influence of CO₂-added steam explosion on characteristics of EFB

Crystallinity index of EFB

Figure 1 shows the X-ray diffraction patterns of EFB before and after the pretreatment during different impregnation times. The patterns illustrate an almost similar trend of the peaks. According to Narayanaswamy et al., the presence of lignin and hemicelluloses in lignocellulosic biomass makes it more resistant to changes in the crystalline structure of cellulose that could occur upon the CO₂SE pretreatment. Moreover, it is observed that the crystallinity index of the cellulose after the pretreatment tends to slightly decrease, compared to the control (untreated), as can be seen in the data in Table 3. EFB-CO₂SE treated during 60 min of impregnation time exhibited the lowest crystallinity index of 64.71%. According to Zheng et al., there are intimate interactions between the hydrophobic molecules of carbon dioxide and cellulose during the pretreatment using CO₂, thus, CO₂ molecules can access the cellulose crystal lattices.

Structural changes of EFB

The structural changes that occurred in EFB during steam explosion were determined based on
the FTIR spectra obtained for the pretreated EFB, with various CO$_2$ impregnation times, in comparison with the untreated sample. The results are shown in Figure 2. Band assignments and band shifts were made according to the literature and are listed, along with the characteristic wavenumbers, in Table 4. This study found that there was a shift of the peak at 648 cm$^{-1}$ observed for the untreated EFB to a smaller vibration for the EFB samples treated with CO$_2$. The longer the treatment time, the more significant the shift. The shifting at this wavenumber refers to the C-O out-of-plane bending mode of cellulose, which could be related to the crystallinity change of the cellulose. Moreover, this finding is supported by a similar pattern for the absorption band located at 3338 cm$^{-1}$ in the spectrum of the untreated cellulose and shifting to a smaller vibration after the treatment with CO$_2$. It has been previously mentioned that A3308/A1330 is known as hydrogen-bond intensity (HBI). Intramolecular and intermolecular hydrogen bonds indicated by the broad absorption at 3340.19 cm$^{-1}$ show the stretching frequency of the –OH group. It is assumed that hydrogen-bond intensity will be lower for a lower crystallinity index of cellulose.

**Surface area of EFB**

The surface area of EFB was analyzed using BET calculations. The samples were heated under vacuum at a temperature of 353.15 K for 10 hours before analysis. The adsorption/desorption experiments were conducted at a liquid nitrogen temperature of 77.15 K and were analyzed on a Micromeritics ASAP 2020 automatic surface area and pore radius distribution analyzer. The results showed that the longer impregnation time with CO$_2$ increased the BET surface area, pore volume and pore size of EFB, as can be seen in the data in Table 5. It means that the accessible surface area of EFB increased after the treatment by CO$_2$-added steam explosion. The accessible surface area is one of the most important factors for the digestibility of lignocellulose during the hydrolysis process. The contact between the enzyme molecules and the cellulose surface is essential for the hydrolysis to proceed.

**Surface morphology of EFB**

The changes in the surface morphology of EFB were observed using SEM. Photomicrographs of untreated EFB and treated EFB are presented in Figure 3. Before the pretreatment, the untreated EFB fiber had a rigid surface, with a layer of matrix material, such as waxes and silica covering the entire surface of the fiber (Fig. 3a).

After CO$_2$-added steam explosion, there appeared uniform pores in the surface of EFB. It indicated that some silica was removed from EFB. According to Figure 3, the longer time of CO$_2$ impregnation promotes deeper penetration of CO$_2$ molecules into the micropores of EFB. The biomass structure exhibited greater disruption after 60 min of pretreatment time.

![Figure 2: FTIR spectra of untreated EFB sample, sample A, sample B, and sample C](image-url)
Table 4
Assignments of IR band maxima to various components of EFB according to literature

<table>
<thead>
<tr>
<th>Untreated EFB</th>
<th>Sample A</th>
<th>Sample B</th>
<th>Sample C</th>
<th>Assignments (^{2,26})</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>625.93</td>
<td>549.71</td>
<td>545.85</td>
<td>543.93</td>
<td>C-O out-of-plane bending mode rocking vibration</td>
<td>Cellulose</td>
</tr>
<tr>
<td>1036.78</td>
<td>1035.77</td>
<td>1033.85</td>
<td>1029.99</td>
<td>Deformation in primary alcohols; plus C=O stretch</td>
<td>Lignin</td>
</tr>
<tr>
<td>1241.25</td>
<td>1238.30</td>
<td>1259.52</td>
<td>1249.87</td>
<td>C=O stretch, OH i.p. bending G-ring plus C=O</td>
<td>Lignin</td>
</tr>
<tr>
<td>1319.37</td>
<td>1325.1</td>
<td></td>
<td></td>
<td>O-H blending of alcohol groups</td>
<td>Carbohydrates</td>
</tr>
<tr>
<td>1412.92</td>
<td>1402.25</td>
<td></td>
<td>1436.97</td>
<td>Aromatic skeletal vibrations with C-H in plane deformation CH(_2)</td>
<td>Lignin</td>
</tr>
<tr>
<td>1519.91</td>
<td></td>
<td></td>
<td>1597.06</td>
<td>G &gt; S Lignin</td>
<td>Lignin</td>
</tr>
<tr>
<td>1555.66</td>
<td>1593.2</td>
<td></td>
<td></td>
<td>S &gt; G; G condensed &gt; G etherified</td>
<td>Lignin</td>
</tr>
<tr>
<td>1634.74</td>
<td>1629.85</td>
<td></td>
<td></td>
<td>Lignin C O stretch in conjugated p-substituted aryl ketones</td>
<td>Lignin</td>
</tr>
<tr>
<td>2920.35</td>
<td>2918.3</td>
<td>2916.37</td>
<td>2910.58</td>
<td>Symmetric CH(_2) valence vibration</td>
<td>Lignin</td>
</tr>
<tr>
<td>3352.43</td>
<td>3311.78</td>
<td>3329.14</td>
<td>3311.78</td>
<td>Intermolecular O(3)H...O(3) in cellulose</td>
<td>Cellulose</td>
</tr>
</tbody>
</table>

Table 5
BET surface area, pore volume and pore size of EFB

<table>
<thead>
<tr>
<th>Sample code</th>
<th>BET surface area (m(^2)/g)</th>
<th>Pore volume (cm(^3)/g)</th>
<th>Pore size (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample A</td>
<td>0.585 ± 0.021</td>
<td>0.0026</td>
<td>17.6</td>
</tr>
<tr>
<td>Sample B</td>
<td>0.781 ± 0.030</td>
<td>0.0040</td>
<td>20.5</td>
</tr>
<tr>
<td>Sample C</td>
<td>0.867 ± 0.024</td>
<td>0.0045</td>
<td>20.6</td>
</tr>
</tbody>
</table>

Influence of steam explosion with CO\(_2\) impregnation agent on enzymatic hydrolysis of EFB

Table 6 shows the production and yield of glucose after hydrolysis. The results indicate that EFB could be hydrolyzed using a cellulolytic enzyme. The EFB treated by CO\(_2\)-added steam explosion recorded higher glucose concentration, as compared to untreated EFB and EFB treated by conventional steam explosion. The hydrolysis of untreated EFB and that treated by conventional steam explosion provided glucose yields of 51.12% and 58.12% (based on cellulose), respectively. On the other hand, the hydrolysis of EFB after the pretreatment by steam explosion with 60 min of CO\(_2\) impregnation time resulted in the highest yield of glucose, namely 84.14% (based on cellulose) and 33.27% (based on the substrate). Several studies regarding the lignocellulosic pretreatment using CO\(_2\) have been reported, for example, the pretreatment of EFB using supercritical CO\(_2\) produced 24% glucose yield, as compared to untreated EFB, which yielded only 17% glucose.\(^{28}\) The hydrolysis of corn stover treated using supercritical CO\(_2\) provided 30% of glucose yield, while the untreated biomass produced 12% of glucose yield.\(^{23}\) Another study demonstrated that 86.6% of
The results indicated that adding CO$_2$ as an impregnating agent to the steam explosion treatment can increase the accessibility of biomass to enzymatic hydrolysis. The pretreatment method using water and CO$_2$ in steam explosion causes swelling of the lignocellulose, which enlarges the micropores of the biomass for CO$_2$ molecules to penetrate deeper; also, carbonic acid is formed when CO$_2$ is dissolved in water, which can increase the hydrolysis rate. According to Zheng et al., the pretreatment with supercritical carbon dioxide increases the efficiency of cellulose hydrolysis for glucose production by approximately 50%, compared to the untreated biomass. Thus, biomass treated with CO$_2$ is more vulnerable to hydrolytic enzymes.

![Figure 3: SEM images of EFB before and after pretreatment](image)

**Table 6**  
Glucose production and yield of EFB after hydrolysis

<table>
<thead>
<tr>
<th>Sample code</th>
<th>Hydrolysis time (h)</th>
<th>Glucose production (g/L)</th>
<th>Glucose yield (cellulose basis) (%)</th>
<th>Glucose yield (substrate basis) (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated sample</td>
<td>96</td>
<td>20.1±0.5</td>
<td>51.1</td>
<td>20.1</td>
</tr>
<tr>
<td>Blank</td>
<td>96</td>
<td>22.3±0.6</td>
<td>58.1</td>
<td>22.3</td>
</tr>
<tr>
<td>Sample A</td>
<td>96</td>
<td>31.5±0.4</td>
<td>80.2</td>
<td>31.5</td>
</tr>
<tr>
<td>Sample B</td>
<td>96</td>
<td>32.2±0.5</td>
<td>82.2</td>
<td>32.2</td>
</tr>
<tr>
<td>Sample C</td>
<td>96</td>
<td>33.3±0.6</td>
<td>84.1</td>
<td>33.3</td>
</tr>
</tbody>
</table>

**Table 7**  
Ethanol concentration and yield after fermentation

<table>
<thead>
<tr>
<th>Sample code</th>
<th>Fermentation time (h)</th>
<th>Ethanol (g/L)</th>
<th>Ethanol yield (cellulose basis) (%)</th>
<th>Ethanol yield (substrate basis) (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated sample</td>
<td>72</td>
<td>3.7±0.1</td>
<td>18.3</td>
<td>3.7</td>
</tr>
<tr>
<td>Blank</td>
<td>72</td>
<td>8.1±0.2</td>
<td>41.4</td>
<td>8.1</td>
</tr>
<tr>
<td>Sample A</td>
<td>72</td>
<td>10.4±0.2</td>
<td>52.6</td>
<td>10.4</td>
</tr>
<tr>
<td>Sample B</td>
<td>72</td>
<td>10.7±0.5</td>
<td>53.7</td>
<td>10.7</td>
</tr>
<tr>
<td>Sample C</td>
<td>72</td>
<td>11.8±0.6</td>
<td>58.8</td>
<td>11.8</td>
</tr>
</tbody>
</table>

**Influence of steam explosion with CO$_2$ impregnation agent (CO$_2$SE) on fermentation**

Table 7 shows the ethanol production after fermentation of untreated EFB and EFB treated by CO$_2$SE and conventional steam explosion (blank). As may be noted in Table 7, the highest concentration and yield of ethanol were obtained from the fermentation of EFB treated by CO$_2$SE with 60 min impregnation time, namely, of 10.2 g/L and 51.13%, respectively. This confirms that CO$_2$ opens the surface pores of the lignocellulose, which makes it easier for enzymes to enter.
enabling greater cellulose conversion into glucose, which is subsequently fermented into ethanol by the yeast.  

CONCLUSION

The pretreatment of EFB using steam explosion with the addition of CO\(_2\) as an impregnating agent was demonstrated to provide higher glucose and ethanol concentration, as compared to the pretreatment using the conventional steam explosion. The CO\(_2\) pretreatment resulted in a slightly lower crystallinity index, more disrupted biomass and increased enzymatic hydrolysis of EFB. However, further studies are needed to utilize CO\(_2\) as a by-product from bioethanol fermentation as an impregnating agent in steam explosion pretreatment for applying the biorefinery concept in bioethanol production.

REFERENCES
