Abstract: Bio-ethanol is such a promising renewable fuel. However, today it is produced from sugar or starch—raw materials that are relatively expensive. To lower the production cost of bio-ethanol the cost of the raw material must be reduced and the production process made more efficient. The production of bioethanol from banana peel was studied by using separate hydrolysis and fermentation (SHF) and simultaneous saccharification and fermentation (SSF). Banana peel is an agricultural by-product and thus has a low economic value. Different methods of pretreatment were investigated for improving the saccharification in ethanol fermentation. Biomass pretreatment procedures include: distilled water diluted sulphuric acid and diluted sodium hydroxide at 120°C and 135°C. The fermentations were performed on the diluted acid-pretreated banana peel at 1.5% water-insoluble solids (WIS). The pretreated substrates were hydrolysed with a blend of amylase, amyloglucosidase, xylanase, cellulose and pectinase. The fermentable sugars hydrolysate were bioconverted to ethanol with SHF and SSF, using co-culture of S. cerevisiae and C. tropicalis. The sugars formed varied from 11.42 to 14.85 g/l and the saccharification yield from 81.93 to 99.0 %, depending on time and temperature of the pretreatment methods. The highest ethanol yield (99.24% of theoretical value) was obtained with coculture of S. cerevisiae and C. tropicalis in SHF. The SHF process time was longer (≈48 h) than that for the SSF (16–20 h) and leading to significantly increased ethanol concentration for the former process (8.48 g/l for SSF; 10.26 g/l for SHF).

Keywords: Ethanol, SHF, SSF

I. INTRODUCTION

Energy consumption has increased steadily over the last century as the world population has grown and more countries have become industrialized. Bioethanol, a renewable fuel is becoming increasingly important as a consequence of major concern for depleting oil reserves, rising crude oil prices and greenhouse effect [1]. Lignocellulosic feedstock is considered as an attractive raw material not only for the liquid transportation fuel but also for the production of chemicals and materials, i.e. the development of carbohydrate-based biorefineries [2] because of its availability in large quantities at low cost [3]. Besides terrestrial plants, aquatic plants are also promising renewable resource.

Over the past few years, ever since the energy crunch began, there has been a tremendous interest in energy saving both on new and existing structures. Using certain materials and techniques can result in big savings. Today, the idea of utilizing biomass from agricultural and livestock wastes as a
raw material for production of ethanol has attracted the interest of researchers especially in agricultural practicing countries. Thailand has an abundance of agriculture by-products available which are usually directly discharged as solid waste; causing environmental issues.

When banana becomes mature, the chemical compound is changed such as moisture content, pulp and peel. After banana are harvested, the moisture content inside the peel continuously increased and moisture content in the pulp continuously increased by the time.

Productions of ethanol from different materials can be reported but there is not information on production and utilization of banana shell as ethanol precursor. Thus, this study is aimed to investigate bioethanol production from banana peel cellulose and enzyme hydrolysate using mono-culture and co-culture of yeasts fermentation in SHF and SSF.

II. MATERIALS AND METHODS

Substrate preparation
Banana peels were collected were washed manually using tap water to remove adhering dirt, dried at 45 °C in a hot-air oven for 4 days, milled, screened to select the fraction of particles with a size of 45-697 μm, homogenized in a single lot and stored until needed.

Hydrolysate preparation
Hydrolysate was prepared by autoclaving under 15 lb/inch² the 1.5 g dried powder of banana peels with 100 ml of 0.1 M sulfuric acid, in conical flasks. Then, 250-ml filter-sterilized cellulase (Sumitime C; Shin Nihon Chemical Co. Ltd., Japan) solution (cellulase activity: 20 Filter paper units (FPU) (g substrate)¹, α-amylase 100 unit (g substrate)², amyloglucosidase 100 unit (g substrate)¹, xylanase activity: 500 unit (g substrate)¹) and pectinase activity: 250 unit (g substrate)¹) in 0.1 M sodium phosphate (pH 5.0) was added to the flask and reacted at 50°C and 120 rpm for 48 h for hydrolysis. After the enzymatic reaction, the hydrolysate was centrifuged at 21,000 x g for 10 min. The supernatant was supplemented with additional nutrients to give a base medium composition of: 1 g/l yeast extract; 2 g/l (NH₄)₂SO₄; 1 g/l MgSO₄·7H₂O.

Banana peel cellulose hydrolysate medium
Fermentation medium composed of (g/l): yeast extract 1; (NH₄)₂SO₄ 2; MgSO₄·7H₂O.

Batch fermentation
Batch fermentation was conducted in a 250 ml conical flask with a working volume of 100 ml. The fermentation medium was inoculated with 5% v/v inoculum (20 h culture, 1 x 10⁷ cells/ml). The fermentation temperature was kept constant at 30 ± 0.2°C in an Incubation shaker. The broth was kept under agitation at 50 rpm. Samples were taken at regular time intervals during fermentations to determine the concentrations of cell mass, ethanol and residual sugars in the broth. All experiments were carried out in duplicate.

Analytical methods
Total solids (TSs) moisture and crude protein in banana peel were determined according to standards [4]. Cellulose, hemicellulose and lignin contents were determined by the detergent extraction method [5].

Biomass estimation
Culture dry weight was measured by centrifugation and drying at 105 °C, until no weight change between consecutive measurements was observed.

Sugar estimation
Total reducing sugar was estimated by using dinitrosalicylic acid (DNS) reagent [6].

Ethanol determination
The fermentation was carried out at 30°C for 48 h. The fermentation broths were filtered through a 0.45 μm Millipore filter. Ethanol in the samples was determined by gas chromatograph using a 60:80 Carbopack B: 5% Carbowax 20 M glass column. The detector was operated at 200°C. The flame
ionization detector (FID) was kept at 200°C. Nitrogen gas was used as carrier gas at a flow rate of 30 ml/min. The temperature was programmed at 120°C for 1.4 min, from 120°C to 240°C at 30°C/min, then held 5 min at 240°C.

III. RESULTS AND DISCUSSION

Composition of banana peel

The average composition of banana peel is summarized in Table 1.

<table>
<thead>
<tr>
<th>Constituents</th>
<th>% of wet weight</th>
<th>TSs 14.53–17.12</th>
<th>Moisture 82.47–86.21</th>
</tr>
</thead>
<tbody>
<tr>
<td>Organic components (% TSs)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hemicellulose</td>
<td>12.57 ± 1.41</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cellulose</td>
<td>15.48 ± 0.24</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lignin</td>
<td>11.48 ± 1.67</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Crude protein</td>
<td>6.41 ± 0.43</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Starch</td>
<td>32.75 ± 0.21</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Starch, cellulose and hemicelluloses contents of banana peel were relatively high. The result indicated that banana peels could be a good source of carbon source for bioconversion.

Banana peel acid and enzyme hydrolysate preparation

Dilute sulfuric acid hydrolysis (0.1 M) under autoclaving under pressure of 15 lb/inch² at 135°C for 10 min and enzyme hydrolysis as described in materials and methods was very effective in releasing a good amount of sugar from banana peels (Table 2). Higher temperature, higher yield of glucose and reducing sugars were released. Approximately 18% and 9% of glucose and reducing sugars were released at 120°C less than that of at 135°C, respectively. So, temperature at 135°C was suitable to hydrolyse the banana peels for sugar production. Approximately 79% of the reducing sugars were released in the first 10 min of autoclaving and enzyme hydrolysis, at 30 min of autoclaving and enzyme hydrolysis, 95% of the reducing sugars were released after which only slight increase was observed. After 30 min of autoclaving, sugar yield was 100% of dry biomass of D-glucose, 5.66 g/L (Table 3). These sugars were derived primarily from starch component. The glucose yield (5.66 g/L) was rather high, showing that starch almost practically hydrolyzed.

Ethanol production in SHF

The highest values of ethanol yield per unit biomass (C_E), the maximum ethanol production (P_max), ethanol production rate (Q_E) and product (ethanol) yield coefficient (Y_p/s) were found to be 0.26 g (g-biomass)⁻¹, 3.91, 0.19 g/l/hour and 0.45 (g-total sugar)⁻¹, respectively, by the fermentation of co-culture of S. cerevisiae 5229 and C. tropicalis 5045. The lowest values of ethanol yield per unit biomass (C_E), the maximum ethanol production (P_max), ethanol production rate (Q_E) and product (ethanol) yield coefficient (Y_p/s) were found to be 0.19 g (g-biomass)⁻¹, 2.84, 0.15 g/l/hour and 0.32 (g-total sugar)⁻¹, respectively, by the fermentation of C. tropicalis 5045. It was found that mono-culture of S. cerevisiae 5229 could produce relatively higher ethanol yield than C. tropicalis 5045 (Table 4).

Ethanol production in SSF

The highest values of ethanol yield per unit biomass (C_E), the maximum ethanol production (P_max), ethanol production rate
Fig. 1 The time course of growth (×), reducing sugars (♦), glucose (■) and ethanol (▲) concentration in SHF by S. cerevisiae 5229 (a), C. tropicalis 5045 (b) co-culture of S. cerevisiae 5229 and C. tropicalis 5045 (c); in SSF by S. cerevisiae 5229 (d), C. tropicalis 5045 (e) and co-culture of S. cerevisiae 5229 and C. tropicalis 5045 (f) at 30 ± 0.2 °C and pH 5.0 ± 0.2 using simulated synthetic hydrolysate medium.

**TABLE 4**

**ETHANOL PRODUCTION BY SHF WITH MONO-CULTURE AND CO-CULTURE**

<table>
<thead>
<tr>
<th>Strain</th>
<th>C_E</th>
<th>P_max</th>
<th>Q_E</th>
<th>Y_p/s</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. cerevisiae 5229</td>
<td>0.22</td>
<td>3.23</td>
<td>0.22</td>
<td>0.37</td>
</tr>
<tr>
<td>C. tropicalis 5045</td>
<td>0.19</td>
<td>2.84</td>
<td>0.15</td>
<td>0.32</td>
</tr>
<tr>
<td>Co-culture*</td>
<td>0.26</td>
<td>3.91</td>
<td>0.19</td>
<td>0.45</td>
</tr>
</tbody>
</table>

*Co-culture of S. cerevisiae 5229 and C. tropicalis 5045

C_E: Ethanol yield per unit biomass (g(g-biomass)^{-1})

Q_E: Ethanol production rate (g/l/hour)

P_max: Maximum ethanol production (g/l)

Y_p/s: Product (ethanol) yield coefficient (g(g-total sugar)^{-1})

**TABLE 5**

**ETHANOL PRODUCTION BY SSF WITH MONO-CULTURE AND CO-CULTURE**

<table>
<thead>
<tr>
<th>Strain</th>
<th>C_E</th>
<th>P_max</th>
<th>Q_E</th>
<th>Y_p/s</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. cerevisiae 5229</td>
<td>0.25</td>
<td>3.72</td>
<td>0.24</td>
<td>0.42</td>
</tr>
<tr>
<td>C. tropicalis 5045</td>
<td>0.21</td>
<td>3.16</td>
<td>0.12</td>
<td>0.36</td>
</tr>
<tr>
<td>Co-culture*</td>
<td>0.29</td>
<td>4.34</td>
<td>0.35</td>
<td>0.49</td>
</tr>
</tbody>
</table>

*Co-culture of S. cerevisiae 5229 and C. tropicalis 5045

C_E: Ethanol yield per unit biomass (g(g-biomass)^{-1})

Q_E: Ethanol production rate (g/l/hour)

P_max: Maximum ethanol production (g/l)

Y_p/s: Product (ethanol) yield coefficient (g(g-total sugar)^{-1})
(Q_E) and product (ethanol) yield coefficient (Y_p/s) were found to be 0.29 g (g-biomass)^{-1}, 4.34, 0.35 g/l/hour and 0.49 g (g-total sugar)^{-1}, respectively, by the fermentation of co-culture of *S. cerevisiae* 5229 and *C. tropicalis* 5045. The lowest values of ethanol yield per unit biomass (C_E), the maximum ethanol production (P_max), ethanol production rate (Q_E) and product (ethanol) yield coefficient (Y_p/s) were found to be 0.21 g (g-biomass)^{-1}, 3.16, 0.12 g/l/hour and 0.36 g (g-total sugar)^{-1}, respectively, by the fermentation of mono-culture of *C. tropicalis* 5045. It was found that mono-culture of *S. cerevisiae* 5221 could produce relatively high ethanol yield almost equal to the coculture (Table 5). Fig. 1 shows the time-course for growth, sugar utilization and ethanol concentration in the banana peel hydrolysate medium at initial pH 5.0 ± 0.2 of *S. cerevisiae* and *C. tropicalis* and co-culture.

The fermentation parameters are summarized in Table 4 and 5. The yield (C_E) and productivities (P_max, Q_E and Y_p/s) increased 1.32-, 1.34-, 1.59 and 1.32-fold, respectively, in SHF and 1.16-, 1.17-, 1.46 and 1.17-fold, respectively, in SSF; when mono-culture of *S. cerevisiae* 5221 was grown in a medium banana acid hydrolysate as compared with the co-culture of *S. cerevisiae* and *C. tropicalis*. This showed that co-culture of *S. cerevisiae* and *C. tropicalis* fermentation employed for the treatment of banana acid hydrolysate has partially used reducing sugars as substrate and improved the fermentability. The fermentation period of co-culture in SHF and SSF comparing to mono-culture of *S. cerevisiae* 5229 was also reduced to about 55%. However, the fermentation period of co-culture for the banana acid hydrolysate was rather similar to that obtained with *S. cerevisiae* 5229 (Table 4 and 5). This shows that there might be some leftover reducing sugars in the treated cellulose acid hydrolysate that are not used in the fermentation performance of *S. cerevisiae* 5229.

**IV. CONCLUSIONS**

The maximum values of ethanol yield (C_E), productivity (P_max, Q_E and Y_p/s) and percent sugar utilization were 0.29 g (g-biomass)^{-1}, 4.34, 1.39 g/l/hour and 0.35 g (g-total sugar)^{-1}, and 96.1%, respectively, at temperature 30 ± 0.2°C and pH 5.0 ± 0.2, when co-culture of *S. cerevisiae* 5229 and *C. tropicalis* 5045 was grown in treated cellulose hydrolysate medium in SSF. However, fermentation by co-culture of *S. cerevisiae* 5229 and *C. tropicalis* 5045 in SHF and SSF improves the ethanol yield and productivity compared to the mono-culture of *S. cerevisiae* 5229 or *C. tropicalis* 5045. Therefore, the fermentation of banana peel for ethanol production was carried out in a high yield by optimum treatment and co-culture of yeast strains.

**ACKNOWLEDGMENT**

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