IN THE FIELD

Pilot-scale processing with alkaline pulping and enzymatic saccharification for bioethanol production from rice straw

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Abstract
We examined pilot-scale bioethanol production from rice straw using sodium carbonate pulping as the alkaline pulping method and enzymatic saccharification. The yield of prewashed rice straw after the crushing and prewashing stage decreased with an increase in the input in rice straw. The pulp yield after alkaline cooking was 66–68% at kappa number ranging from 32 to 36, which was comparatively higher than the laboratory-scale study. The yield of enzymatic saccharized glucose was decreased with the increase in washed pulp and its saccharification rate was approximately 20%. We successfully produced approximately 100 liters of 95% ethanol from 1000 Bone-Dry kg (BDkg) rice straw. The results of our pilot-scale study indicated that the relationship between resource input and product yield for each operation exhibited exponential or logarithmic curves, rather than linear decreases or increases, which could suggest a high-cost structure for bioethanol production when the resource input is larger. However, we established an optimum quantity of resource input, approximately 2000–3000 BDkg in our pilot-scale study, for higher efficiencies.

Introduction
In recent decades, harvested material residues, including rice straw, have been the focus of bioethanol production. However, the use of rice straw faces several challenges before ethanol generation can be accomplished cost effectively [1]. One of the largest problems is the requirement for large quantities of energy for ethanol conversion. Therefore, highly efficient conversion techniques, which reduce energy requirements, need development [2]. Typically, enzymatic saccharification, which isolates glucose and xylose from polysaccharides such as cellulose and hemicellulose, is necessary to produce ethanol from botanical biomass resources. In general, the cellulose and/or hemicellulose contained in rice straw exist as lignocellulose, which is difficult to degrade by enzymes [3]. Therefore, a suitable method to enhance the chemical reactivity between enzyme molecules and lignocellulose has to be applied during a processing phase for bioethanol production from rice straw.

A pulping method, where lignin in the form lignocellulose is partially removed and/or denatured, is used as pretreatment processing in paper manufacturing. Alkaline pulping, which can be subdivided into kraft pulping, soda pulping, and sodium carbonate pulping, is one pulping method for paper manufacturing from wooden chips. Alkaline pulping was also used as a paper manufacturing method from rice straw almost 100 years ago in Japan [4], though it is not used nowadays, it has still remained in some countries for manufacturing specialized papers from rice straw. Kraft pulping, in which
NaOH, Na₂S, Na₂CO₃ are mainly employed as pulping chemicals, is the most advantageous alkaline pulping method, because a kraft pulp made by the kraft pulping method has better characteristics for paper manufacturing. However, kraft pulping generates a bad smell caused by sulfur compounds during the pulping operations. This problem is particular to kraft pulping and can be prevented using large preventive devices, which have high equipment costs and could be disadvantageous for large-scale bioethanol production. Sodium carbonate pulping, which is hard to employ as a wood pulping method because of its weak alkalinity, can be used for rice straw pulping for bioethanol production [5]. The alkaline chemicals used in sodium carbonate pulping can be recycled as black liquor, which is produced during the alkaline cooking operation and used as a resource for heat generation by burning it [6], thus, it can be regarded as a low-emission method and particularly good for bioethanol production. Pretreatment processing followed by sodium carbonate pulping is, therefore, a comparatively useful and efficient method for bioethanol production processing from rice straw.

In general, research about bioethanol production from harvested material residues has examined enzymatic saccharification and fermentation processes in the laboratory. However, few studies have reported on bioethanol production at larger processing scales with pretreatment processes, which is vital to elucidate cost effectiveness, because manufacturing costs of plant production are proportional to the 0.6th power at the production scale by the six tenths rule [7]. Commercial chemical plants are developed with a scale up approach, that is, from laboratory and pilot to commercial scales. Laboratory-scale studies are used to estimate basic examinations, and develop process design. Pilot-scale studies are applied to confirm the reproducibility of laboratory-scale examinations and yield versus input for larger resource amounts, and economic efficiency. Few reports, however, provide estimates of the scaled up pretreatment processing efficiency of alkaline pulping rice straw for bioethanol production.

In this study, an actual pilot-scale bioethanol production process with sodium carbonate pulping as an alkaline pulping method and enzymatic saccharification operation was performed and examined to investigate the yield efficiency of processed materials versus resource input for each operation.

Materials and Methods

Materials

Rice straw (Oryza sativa L., cv. Koshihikari) cultivated in Kashiwa, Chiba, Japan in 2009 was used for this study. Harvested plants were threshed, and the straw was placed on the field and subsequently field dried in the sun for 3 months, facilitated by the climate conditions around our test site and to reduce operating costs. The dried straw was baled and tied with sisal-twine (30 × 40 × 80 cm size bale). The average weight of a single bale was approximately 12.5 kg, and the average bale moisture content before examination was approximately 16.2%. Cellulase (Accellerase DUET, Nagase ChemteX Co., Osaka, Japan) was used as the hydrolysis enzyme for enzymatic saccharification, and bakery yeast (Oriental yeast, Oriental Yeast Co., Tokyo, Japan) was employed for fermentation procedures.

Chemical analysis method

Total sugar was measured using phenol–sulfuric acid method [8]. Monosaccharide was measured using TAPPI Standards T12 os-75 and T249 cm-09 [9]. Acid-insoluble ash was measured using LAP-003 [10]. Ash, acid-soluble ash, and acid-insoluble ash were measured using high-temperature firing at 575°C [11].

Bioethanol production processing

A flowchart of the bioethanol production process applied to this study is depicted in Figure 1. Baled off rice straw was pretreated for the saccharification and fermentation operations. The straw was shredded, then crushed, and prewashed. The prewashed straw was cooked with alkaline chemicals, and the cooked pulp was subsequently washed. After pretreatment, the slurry state washed pulp was treated to enzymatic saccharification, which was subsequently treated to an alcoholic fermentation, distilled, and dehydrated to obtain a 95% ethanol solution. The bioethanol production process employed in this study was developed and constructed in Kashiwanoha, Chiba, Japan. Specifications of each device are provided in Table 1, and photographs of the main devices and operations are shown in Figure 2.

Crushing and prewashing

Baled off rice straw was shredded and crushed using a masher (E-Gimmick, Taizén Co., Shizuoka, Japan), fitted with a rotary blade knife. The crushed straw was prewashed with running 80°C hot water. The rice straw to masher input was 500 kg/h. The prewashed straw (Fig. 2A) was drained by self-weight consolidation, and packed into a flexible 1 m³ volume container bag. The bag filled with prewashed straw was weighed using a crane scale, and the straw moisture content was measured to calculate the dry weight of input prewashed rice straw.
for weight calculation of alkaline chemicals to alkaline cooking.

**Alkaline cooking**

Less than 200 BDkg of prewashed straw was added to the glove rotary digester (inner volume 1.8 m³), with a maximum input of 200 BDkg per batch digester. Na₂CO₃ and a NaOH were also added to the digester, and the concentrations, respectively, diluted to 3.45% (0.5 mol/L) and 0.07% (0.026 mol/L) with water. The ratio of alkaline liquor to dry weight of prewashed rice straw was 5:1. The digester was subsequently closed, and heated to 165°C by steam injection with 1.0 rpm of rotation for approximately 1 h. After heating, the digester was air-cooled for 1 h while maintaining its rotation. The rice straw pulp, so-called cooked pulp, and the cooking spent liquor, so-called black liquor, were the products of this procedure.

The cooked pulp was separated from the black liquor using a drainer with self-compression (Fig. 2B). This batch process was carried out repeatedly for larger amounts of prewashed straw than 200 BDkg, and the total mass for each cooking operation was divided by the mass of prewashed straw input to calculate cooked pulp yield. A pulp kappa number was calculated, which had a correlation with residual lignin amount in pulp; TAPPI Standard T236m-60 (same to JIS P 8211 and ISO 302:2004) was applied for this calculation.

**Pulp washing**

Cooked pulp separated from the black liquor was moved to a 5 m³ dilution tank, and diluted with tap water to form pulp slurry. The quantity of added water was approximately 10 times the cooked pulp weight. The pulp slurry was moved into a drum-type washer (Taizen Co.,

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**Table 1. Specifications of integrated devices into bioethanol production processing.**

| Operation                                      | Appliance                                   | Performance (×unit) | Manufacturer                              |
|------------------------------------------------|---------------------------------------------|---------------------|-------------------------------------------|
| Crushing and prewashing                        | Musher                                      | 500 kg/h × 1        | Taizen Co., Ltd.                          |
| Alkaline cooking                               | Digester (pressure vessel)                  | 1.8 m³ × 1          | Kato Seikan Co.                           |
| Pulp washing                                   | Drum-type washer                            | 460 kg/h × 1        | Taizen Co., Ltd.                          |
| (Pulp-density control)                         | Dilution tank                               | 5.0 m³ × 2          | Masuko Sangyo Co., Ltd.                   |
| Enzymatic saccharification (Concentration)     | Saccharification tank (with agitator)       | 3000 L × 4          | KK. Takasugi Seisakusho                   |
| Fermentation                                   | Evaporator                                  | 200 kg/h × 1        | Okawara MFG Co., Ltd.                     |
| Distillation and dehydration (for confirmation test) | Thermo vapor distillation               | 20 L × 1            | KK. Takasugi Seisakusho                   |
| Heating (utility)                              | Once-through boiler                         | 2000 kg/h × 1       | Miura Co., Ltd.                           |
Fig. 2C) using a tube pump for viscous fluid, and the drum was rotated. The washer input rate was 460 kg/h. The washed pulp was separated from pulp slurry during drum rotation (Fig. 2D), collected into a drainer, and drained by self-weight consolidation. The black liquor and the washed pulp drainage were tested by the TAPPI Test Method T625ts-64 for estimation of residual alkali [12].

Enzymatic saccharification

Washed and dehydrated pulp was transferred into a saccharification jar (Fig. 2E), diluted with water up to 20 times of the dry weight of dehydrated pulp, and adjusted to pH 6.0 using a diluted H$_2$SO$_4$ solution. The jar was heated to 50°C with indirect heating of continuous steam, then 101 g of hydrolysis enzyme per 1 kg of dried pulp was added to the jar, and stirred for 24 h to accelerate the enzymatic reaction. A Brix meter (PAL-BX/RI, Atago, Tokyo, Japan) was used to measure a glucose concentration in the saccharified mixture.

Fermentation and postfermentation

The saccharified mixture was released from the jar bottom stop valve, and separated into saccharified liquid containing glucose (Fig. 2F) and residues using a drainer. The saccharified liquid was moved to a concentrating device (centrifugal-flow thin-film vacuum evaporator, CEP-5S, Okawara MFG. Co., Shizuoka, Japan), and condensed to an approximately 20% glucose solution. A volume of 1000 liters of condensed liquid were moved into fermentation jars, and bakery yeast was added to ferment the liquid at 28°C. The amount of added yeast was 20 g per 1 L of 20% glucose solution. After 24 h of fermentation, the fermentation liquor was sampled, and the ethanol concentration was evaluated by J. TAPPI No.42-84 using gas chromatography [13]. The liquor was distilled until 90% ethanol concentration was achieved. The concentrated ethanol solution was dehydrated for 10 h by PSA-column with a 3A molecular sieve up to 95% of ethanol concentration, and subsequently evaluated for adequacy as a biofuel by JASO standard (JIS K 2190:2011).

Results and Discussion

The relationships between rice straw input and prewashed rice straw yield after crushing and prewashing operations are shown in Figure 3. The relationship with the moisture content of prewashed rice straw is also shown in this figure. The yield exponentially decreased with increases in input. In contrast, moisture content showed a logarithmic increase. The approximate exponential and logarithmic curves exhibited high correlation values ($R^2 = 0.98$, prewashed rice straw yield to rice straw input; $R^2 = 0.86$, moisture content of prewashed rice straw to rice straw input, respectively); therefore, yield and moisture content should become constant at larger inputs. In this case, yield tended to show constant values (96–97%) over 3000 BDkg input. The moisture content should also show

![Figure 3. Relationships between rice straw input and prewashed straw yield and its moisture content at the crushing and prewashing operation.](image-url)
constant values over 3000 BDkg input, which was approximately 65%. Shredded (A) and prewashed (B) rice straw sample images are shown in Figure 4. The prewashed sample was darker in color, due to washing in hot water, and high moisture content. The prewashed sample also exhibited a wadding-like state compared with the shredded sample, which had low handling properties for processing. Overall, the straw samples were fragmented and pulverized by the cutter mill or atomizer in the laboratory-scale experiments, which is analogous to the coarse-crushing operation, thus results in the wadding-like state (Fig. 4B) cannot be produced and observed. Furthermore, the yield from crushing and prewashing operations cannot be estimated from laboratory-scale experiments [14]. The pilot-scale processing study provided a value for rice straw resource losses, and results indicated that prewashed rice straw exhibits the possibility of low handling properties. In addition, the prewashed straw wadding-like state is associated with its moisture content.

The relationships between prewashed rice straw input and alkaline cooked pulp yield following the alkaline cooking operation are shown in Figure 5. The relationship with cooked pulp Kappa number is also provided. The yield shown in this pilot-scale study was 66–68% at kappa number ranging from 32 to 36. This was comparatively higher than the typical laboratory-scale study [5], which reported the yield was 60–62% at same kappa number range. A continuous digester has usually been employed to cook large amounts of resources during the alkaline cooking operation. The digester employed in this pilot-scale study was a comparatively smaller batch type for total cost reduction. However, results for yield and kappa number in each batch seemed to be stabilized.

Relationships between input of alkaline cooked pulp and washed pulp yield resulting from the pulp washing operation are indicated in Figure 6. The washed pulp yielded between 90% and 95% throughout the input range (0–4000 BDkg). The fiber length range of pretreated rice straw pulp in this study was approximately 0.3–0.6 mm, which was less than the fiber of hardwood bleached kraft pulp (LBKP) (0.6–0.7 mm) for ordinary paper resources [15]. A mesh size of drainage screen incorporated into the pulp washing machine was designed for LBKP; therefore, the washed pulp would not yield 100% and device modification should be optimized for the rice straw attributes. Straw sample compositions for each operation and yields for the rice straw resource at each operation in the pretreatment processing are provided in Table 2. Lignin content in alkaline cooked pulp was less than in prewashed straw, and washed pulp lignin content was less than in cooked pulp. These results indicated lignin content decreased during the
alkaline cooking and washing operations; in contrast, cellulose and hemicellulose contents increased relative to the other contents following cooking operations. Ash content, showed no change throughout the pretreatment processing, and remained at 23% to 25%. These results indicated that the ash, similar to silica contained in rice straw, would dissolve at the same ratio as the rice straw dissolved during pretreatment processing. The remaining ash content might affect processing efficiency.

The relationships between washed pulp input and the saccharification rate during enzymatic saccharification are shown in Figure 7. The saccharification rate exhibited an exponential decrease with increased input ($R^2 = 0.9761$). The saccharified mixture exhibited high stirring resistance by the unreacted pulp fibers, therefore the actual stirring rate for the mixture in the saccharification jar was 20–30 rpm using a direct drive-type stirrer. This result also suggested that larger-scale processing would need a stronger agitator for the enzymatic saccharification operation. Relationships between rice straw input and converted 95% ethanol are shown in Figure 8, which were produced by multiple distillations and dehydrations after the fermentation process. The resulting 95% ethanol showed a logarithmic increase consistent with an increased straw quantity ($R^2 = 0.9313$). The pilot-scale processing employed in this study produced approximately 100 L of 95% ethanol per 1000 BDkg of rice straw when the rice straw quantity exceeded 2000 BDkg. This result showed a comparatively lower bioethanol production efficiency than laboratory-scale bioethanol processing [16].

**Conclusions**

In general, construction costs, operating costs, and energy consumption of bioethanol production processing increases when the system scale and devices become larger. However, larger systems might result in increased efficiency by implementing larger biomass resource inputs, and subsequently reduced costs. In this pilot-scale study, the relationship between input and yield for each operation indicated exponential or logarithmic curves rather than linear decreases or increases, suggesting a high-cost structure for bioethanol production when the resource inputs are larger. However, there is an optimum quantity of resource input, approximately 2000–3000 BDkg in our pilot-scale study, for higher efficiencies.

**Table 2.** Straw sample composition and yield for each operation in the pretreatment processing.

| Sample                  | Composition (%) | Yield at each operation (%) (vs. rice straw, weight base) |
|-------------------------|----------------|----------------------------------------------------------|
|                         | Cellulose  | Hemicellulose | Lignin | Ash | Others |                          |
| Rice straw (bale)       | 29.1 ± 1.0 | 21.3 ± 1.0  | 13.4 ± 3.0 | 24.8 ± 3.0 | 11.4 ± 8.0 | 100.0 |
| Prewashed rice straw    | 27.0 ± 1.0 | 16.5 ± 1.5  | 15.3 ± 4.0 | 23.4 ± 2.0 | 17.8 ± 8.5 | 95.0  |
| Alkaline cooked pulp    | 51.1 ± 0.5 | 17.1 ± 1.0  | 5.05 ± 0.5 | 23.9 ± 0.5 | 2.9 ± 2.5  | 63.8  |
| Washed pulp             | 51.5 ± 0.5 | 17.1 ± 0.5  | 1.7 ± 2.0  | 25.5 ± 1.0 | 4.2 ± 4.0  | 60.1  |

Mean ± standard deviation ($n = 3$).
at each operation. This should be confirmed by larger quantities of resource input with optimum modifications for each device in future studies.

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Conflict of Interest

None declared.

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