



Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Elsevier hereby grants permission to make all its COVID-19-related research that is available on the COVID-19 resource centre - including this research content - immediately available in PubMed Central and other publicly funded repositories, such as the WHO COVID database with rights for unrestricted research re-use and analyses in any form or by any means with acknowledgement of the original source. These permissions are granted for free by Elsevier for as long as the COVID-19 resource centre remains active.



The RURAL (reciprocal upgrading for recycling of ash and lignocellulosics) process: A simple conversion of agricultural resources to strategic primary products for the rural bioeconomy

Kenji Yamagishi^a, Masakazu Ike^a, Akihiro Tanaka^b, Ken Tokuyasu^{a,*}

^a Bioresource Conversion Unit, Division of Food Biotechnology, Food Research Institute, National Agriculture and Food Research Organization, 2-1-12 Kannondai, Tsukuba 305-8642, Japan

^b Biomass Utilization Group, Division of Crop Production Systems, Central Region Agricultural Research Center, National Agriculture and Food Research Organization, 2-1-18 Kannondai, Tsukuba 305-8666, Japan

ARTICLE INFO

Keywords:

Rice straw
Poultry litter ash
Phosphorus
RURAL (reciprocal upgrading for recycling of ash and lignocellulosics) process
Strategic primary products

ABSTRACT

Rice straw (RS), an agricultural resource for lignocellulosic biorefineries, can deteriorate when sun-drying is ineffective. Poultry litter ash (PLA) has been considered as a renewable phosphorus source for crops but is highly alkaline. Here, a simple process was developed for their reciprocal upgrading. RS, PLA, and water were mixed for wet storage and alkali pretreatment of the RS at 25 °C for 14 d, and solid–solid separation was performed to obtain PLA-treated RS (PT-RS) and RS-treated PLA (RT-PLA). PT-RS was susceptible to enzymatic saccharification, and 65.5–68.6% of total sugar residues in PT-RS was converted to lactic acid by its nonsterile application for simultaneous saccharification and fermentation using *Bacillus coagulans*. RT-PLA exhibited 1.8-points lower pH and a more sensitive response of phosphorus solubilization to acid than those of PLA. This process could thus provide a breakthrough for the rural bioeconomy by manufacturing two strategic primary products for various commercial bioproducts.

1. Introduction

The impacts of climate change on ecosystems and living infrastructure are becoming severe, and a new virus COVID-19 is spreading around the world, destabilizing human lives and economic activities. The building and strengthening of a sustainable food supply system and local production, especially in areas where food is in short supply, are now of utmost importance as potential lifelines to consumers in the local microeconomy (Anser et al., 2020; Torero, 2020; Cappelli and Cini, 2020; Kanter and Boza, 2020).

The bioeconomy concept, incorporating approaches for sustainable production of valuable products such as building blocks for materials, chemicals, and energy, from renewable biological resources instead of fossil resources is expected to reduce greenhouse gas emissions and mitigate further climate change (McCormick and Kautto, 2013; Guo and Song, 2019). The upgrading agricultural waste and by-products from the food industry as biological resources could also serve to strengthen rural areas and the food supply system economically by the manufacture of new renewable products in synergy with existing agricultural- or food products (Ravindran and Jaiswal, 2016). Among these

waste products (or by-products), lignocellulosic feedstocks such as corn stover and sugarcane bagasse are regarded as the main sources of fermentable sugars for further conversion into valuable products without competing with food supply. In those feedstocks, fermentable sugars are mainly obtained by hydrolysis of two kinds of polysaccharides: cellulose, a polymer of β -1, 4-linked glucose residues, and xylan, a polymer of β -1, 4-linked xylose residues with various side chains and modifications. Because these polysaccharides are tightly packed with each other and surrounded with other components such as lignin and inorganic compounds to make the whole structure recalcitrant, an appropriate pretreatment step prior to enzymatic saccharification should be required. Various pretreatment methods have been developed including a steam explosion pretreatment, dilute acid pretreatments, and alkali pretreatments, all of which have advantages and disadvantages (Chandel et al., 2018). Many countries have carried out such upgrading processes termed lignocellulosic biorefineries and various projects for cellulosic ethanol production (Chandel et al., 2018; Hassan et al., 2019). Apart from a few specific cases, however, none of these projects has yet evolved into the worldwide deployment of commercial plants for cellulosic ethanol or for any other bioproducts. Instead, they have

* Corresponding author.

E-mail address: tokuyasu@affrc.go.jp (K. Tokuyasu).

<https://doi.org/10.1016/j.biteb.2020.100574>

Received 10 September 2020; Received in revised form 23 September 2020; Accepted 25 September 2020

Available online 08 October 2020

2589-014X/ © 2020 Elsevier Ltd. All rights reserved.

highlighted several bottlenecks for commercialization; unsolved problems exist in both feedstock supply and pretreatment at scale (Chandel et al., 2018; Dale, 2018).

A top-down manner should be used to fix the necessary amount of lignocellulosic feedstock for a large-scale plant according to the minimum profitable scale of production calculated with simulated feedstock- and non-feedstock costs (NREL, 2002). This top-down approach should also be adopted in selecting candidate areas for feedstock supply (for example, an area with a radius of 80 km around the plant, corresponding to 2000-t feedstock per day) at an affordable cost. The supply of agricultural waste as feedstock requires multi-year contracts with a huge number of farmers (in one case study, more than 20,000 farmers in Europe for the supply of corn stover at a scale of 300,000 t per year) (Valdivia et al., 2016). Such an approach, however, would not always satisfy feedstock suppliers because of the low benefits they would gain by selling cheap raw materials (NREL, 2002; Dale, 2018). In addition, rural areas not included in the areas selected for feedstock collection would have no opportunities of contributing to lignocellulosic biorefineries, although some of them may have good potential for feedstock supply at a small scale. The establishment, therefore, of the alternative bottom-up approach for the smaller lignocellulosic biorefineries depending on the qualities and quantities of available feedstocks in individual rural areas would facilitate individual feasible goals for value addition. This approach is in fact found, depending on available amounts of feedstock in individual areas, at various scales of biogas plants, and offers potential improvement in the conventional management of waste or unused materials in individual areas.

In small lignocellulosic biorefineries, a simple pretreatment step with low environmental impacts should be developed to reduce the cost of reagents, equipment, energy, and waste management. Calcium hydroxide ($\text{Ca}(\text{OH})_2$) is regarded as a candidate reagent for a simple alkali pretreatment because of its low price and environmental impacts, and an alkali pretreatment of corn stover with $\text{Ca}(\text{OH})_2$ at 55 °C with aeration for four weeks was found to be effective in facilitating the subsequent enzymatic saccharification, and a pile-style conversion system was proposed (Kim and Holtzappple, 2005). Additionally, wet storage of RS and sugarcane bagasse with $\text{Ca}(\text{OH})_2$ at room temperature for more than one week served as a pretreatment for fermentable sugar production (Shiroma et al., 2011, 2012). These simple pretreatments propose versatile bottom-up conversion systems enabling startups from a single pile, wrap silo or banker silo. They have, however, not yet attracted a sufficient number of feedstock suppliers to start commercial production of valuable products, probably because of the lack of profitable scenarios for valuable production using these upstream technologies. Additionally, the processes use $\text{Ca}(\text{OH})_2$ or calcium oxide (CaO), which is produced from non-renewable, mined limestone, and the production of 1 t of CaO from the limestone generates around 1.2 t of CO_2 , which could significantly impact the environment (Gutiérrez et al., 2012). The sustainability of the process would require the use of better alkali reagent for pretreatment.

Here, PLA was applied as an alternative alkali for pretreatment and wet storage of lignocellulosic feedstock. Poultry litter is discharged daily at the sites of chicken breeding, and left untreated may cause odors, spread water pollutants such as pathogens and antimicrobials, and generate greenhouse gases such as nitrous oxide (USEPA, 2013). Since the substance is produced in large amounts, its combustion to suppress its environmental impacts, reduce the amount of waste, and generate renewable heat and electricity has attracted intense interest (Lynch et al., 2013; Jeswani et al., 2019). In addition, PLA is rich in calcium, potassium, and phosphorus, and is thus regarded as an inorganic resource with a potential value as a fertilizer (Codling et al., 2002). Because of its highly alkaline characteristics and its powder-like form which may cause skin irritation or eye damage of workers and prevent its spreading on the fields in a uniform fashion, direct application of PLA as a fertilizer could be problematic (Chastain et al., 2012).

Although a washing of PLA with water reduces its alkalinity, low solubility of $\text{Ca}(\text{OH})_2$ in water could make the process inefficient in terms of management of dilute alkaline wastewater.

In this study, a novel process (the reciprocal upgrading for recycling of ash and lignocellulosics (RURAL) process) was developed for simultaneous modifications of RS and PLA in two steps: a wet storage/alkali pretreatment of lignocellulosics and solid–solid separation for recovery of the upgraded products. PLA as a recycled alkali reagent could be substituted for $\text{Ca}(\text{OH})_2$ in the conventional lime pretreatment of biomass for the subsequent enzymatic saccharification. Additionally, the alkalinity of PLA could be simultaneously reduced during the pretreatment of RS, because calcium ions could transfer from PLA to lignocellulosics and water under basic conditions. Successful solid–solid separation of PT-RS and RT-PLA, the other critical step in the RURAL process, could lead to the production of multiple products such as fermentable sugars, lactic acid, and RT-PLA as a recycled phosphorus source for a fertilizer, which would attract interest of feedstock suppliers by providing them with various profitable scenarios for the rural bioeconomy.

2. Materials and methods

2.1. Materials

Sun-dried RS (Cultivar Koshihikari) was collected from fields of the Central Region Agricultural Research Center, National Agriculture and Food Research Organization, Japan. The RS was chopped into approximately 2–3 cm pieces using a forage cutter (SU-16, COWA CUTTER corporation, Shizuoka, Japan). Yokohama Farm (Shimotsuma, Japan) kindly donated poultry litter of hens for egg, and its moisture was reduced to 9.3% by ventilation with 3-time mixings during 7 d. Cellulase (Cellic CTec2) and β -glucosidase (Novozyme 188) were purchased from Novozymes Japan Ltd. (Chiba, Japan). Calcium oxide (CaO), calcium hydroxide ($\text{Ca}(\text{OH})_2$), calcium carbonate (CaCO_3), and all other chemicals were of analytical grade.

2.2. Preparation of PLA

The dried poultry litter and wood pellets were combusted in a rotary kiln burner (J-R100, MIS Ltd., Fukuoka, Japan). The wood chips were ignited with a gas ignition device and supplied to the burner continuously to warm up the system until the flame temperature increased to approximately 800 °C. Then, the dried poultry litter (9.8 kg/h) and wood pellets (3.1 kg/h) were supplied to the burner. The airflow rates for combustion were set at 129 m³/h for the primary air and 15.3 m³/h for the secondary air. The average combustion gas temperature was set at 904 °C during the measurement period of 3.72 h. The PLA (6.6 kg) was recovered after cooling to ambient temperature. The cooled PLA was immediately put in a plastic vessel, the lid was closed tightly, and the vessel with the lid was put in an airtight stainless-steel vessel filled with nitrogen gas for storage at ambient temperature. The PLA was ground with a continuous mill with a cutter type head (MF-10 basic and MF-10.1, IKA Japan K.K. Osaka, Japan) to recover as 4-mm mesh pass flakes prior to use in further experiments.

2.3. Evaluation of PLA as a reagent for RS pretreatment

The chopped RS was milled with the mill (MF-10 basic and MF-10.1) to 2-mm mesh pass flakes. The RS flakes (1.5 g) were mixed with various amounts of alkali reagent (either $\text{Ca}(\text{OH})_2$ or the PLA flakes) in airtight glass vials, and deionized water (15 mL) was added to the mixture. Each vial was heated at 95 °C for 1 h and cooled to room temperature. The appropriate amount of 5 N HCl was added to the cooled glass vials to lower the pH to below 1. The neutralized samples were centrifuged at 3000g for 5 min at room temperature to remove the supernatants. An enzyme mixture solution was added to each

precipitate, and the total volume was raised to 15 mL with H₂O to finally include 50 mM sodium acetate buffer (pH 4.5); Cellic CTec2 (12 filter paper units (FPU)/g-biomass (BM)); Novozyme 188 (12 cellobiase units (CbU)/g-BM); NaN₃ (0.5 g/L); chloramphenicol (0.1 g/L); and tetracycline (0.1 g/L). The suspensions were shaken at 130 rpm and 50 °C for 48 h, and centrifuged at 3000g for 5 min. The supernatants were used for analysis of the liberated sugars (monomers and oligomers) of glucose and xylose in the hydrolysate as previously reported (Yamagishi et al., 2019). The recovery of glucose residues (monomer + oligomers) was calculated as follows: [total solubilized glucose residues (as glucose equivalent) (mg)] – (glucose residues in enzyme preparations (as glucose equivalent) (mg)) × 0.9 / (glucan content in the RS flakes (mg)) × 100 (%). The recovery of xylose residues (monomer + oligomers) was calculated as follows: [total solubilized xylose residues (as xylose equivalent) (mg)] × 0.88 / (xylan content in the RS flakes (mg)) × 100 (%).

2.4. Treatment of RS and PLA for reciprocal upgrading

The chopped RS (corresponding to 800 g dry matter (DM)) was mixed with the PLA flakes (200 g) and tap water to adjust its water content by weight to 60%. Aliquots of the mixture (200 g) were packed in airtight aluminum bags, and the bags were pressed to remove air with a hydraulic press machine (AH-2003, AS ONE Corporation, Osaka, Japan). The pressed bags were sealed to prevent contact with CO₂ in air, and they were allowed to settle at 25 °C for 14 d. Then, a part of the mixture (125 g) was taken from the bag and mixed with tap water (2 L). The suspension was agitated at 150 rpm for 30 min with a stirring blade. After stirring, the fibrous fraction (PT-RS) was recovered by both scooping up floating parts and passing the suspension through a stainless-steel filter (3 mm mesh) to separate the precipitated part from the ash fraction. The PT-RS was squeezed with a hydraulic press machine (AH-2003) at 10 MPa, and the leached water was returned to the suspension. Most of the ash fraction (RT-PLA) precipitated after agitation, and the remainder dispersed in the suspension. Therefore, the suspension after the recovery of the PT-RS fraction was centrifuged at 8000g for 5 min to recover the RT-PLA fraction as a precipitate. The supernatant was used for the next washing step of the pretreatment mixture for a sequential recovery of PT-RS and RT-PLA. The preliminary washing sequence was performed 4 times (sequences #P1–#P4) to equilibrate the wash water. The wash water (crude water #P4) was used as the first wash water for the main experiments (experiments #1 and #2). The wet fractions recovered as PT-RS and RT-PLA were dried in an oven at 105 °C to constant weights, and then cooled to room temperature. Some of the dried samples were powdered with the continuous mill and the subsequent ball mill (MM-310, Verder Scientific Co., Ltd. Tokyo, Japan) for 4 min for further experiments and component analyses.

2.5. Release of phosphorus from PLA and RT-PLA

The PLA flakes (201 mg) or RT-PLA #1 powders (192 mg) were suspended in 40 mL deionized water. The quantities of individual samples were determined according to the quantity of acid-soluble phosphorus (15 mg). After a vigorous stirring for 3 min using a magnetic stirrer, the pH was measured, and 0.2 mL of the solution was sampled for phosphorus ion measurement. Then, a small quantity of 5 N HCl solution was added to arrive at the acid concentration of 6.25 mM, and 0.2 mL of the suspension was taken after stirring for 3 min. In the same manner, the concentration of HCl was raised to 12.5 mM, 18.8 mM, 25 mM, 37.5 mM, 50 mM, 67 mM, 83 mM, 100 mM, and 150 mM, and individual samples were taken from the suspension. The phosphorus elution ratio was calculated as follows: (eluted phosphorus (mg)) / (total acid-soluble phosphorus in the powder (mg)) × 100 (%).

2.6. Saccharification of PT-RS

PT-RS #1 was used for the substrates for the saccharification experiments. The wet sample (equivalent to 5 g DM) was mixed with distilled water (68 mL), and the mixture was neutralized with 1 M acetic acid until the pH reached 5.1. The fibrillation of PT-RS #1 was performed as follows: the neutralized PT-RS #1 suspension was dehydrated with a plastic mesh sheet (Mesh Sheet (28 mesh), Advantec Co., Ltd., Tokyo, Japan) set on a Buchner funnel, and the trapped fibers were manually divided to five parts (each equivalent to 1 g dry weight). One of the parts was mixed with the filtrate, and the suspension was set on a blender (Foodmill TML17, Tescom Denki Co., Ltd., Tokyo, Japan) for fibrillation for 30 s. The fibrillated suspension was passed again through the plastic mesh sheet on the Buchner funnel to trap fibrillated fibers and prepare a new filtrate. The next part of the divided fibers was added to the new filtrate for fibrillation. After repeating this procedure four more times, all the divided fibers were obtained in fibrillated form, and the four fractions of the fibrillated fibers were mixed with the fifth suspension after fibrillation. Then, samples of the neutralized PT-RS#1 suspension with and without the fibrillation procedure were mixed with the enzyme mixture solution as described in Section 2.3 for the enzymatic saccharification reaction at a substrate concentration of 5% (w/v). Aliquots (0.5 mL) were taken from the suspension for analysis of solubilized sugars as described in Section 2.3.

2.7. Nonsterile simultaneous saccharification and fermentation for lactic acid production

The nonsterile simultaneous saccharification and fermentation (SSF) for lactic acid production was performed twice (runs #1 and #2) using fibrillated PT-RS as a substrate. In the detailed procedure for run #1, the chopped RS (equivalent to 480 g DM) was first mixed with the PLA flakes (120 g) and tap water to adjust its water content by weight to 60%. The mixture was settled at room temperature for 14 d, the solid fraction was washed with 24 L of water and the wet PT-RS was prepared as described in Section 2.4. A part of the PT-RS (equivalent to 180 g DM) was divided into 20 parts (9 g of DM each), and the first part was suspended in 600 mL of distilled water and fibrillated with a blender (VA-G15, HITACHI, Ltd., Tokyo, Japan) for 30 s. The filtrate was obtained by a solid–liquid separation of the blended suspension using the plastic mesh sheet as described in Section 2.6, and the filtrate was reused for blending the next part of the PT-RS. This procedure was repeated to fibrillate all 20 parts of the PT-RS, and two fractions were obtained: one batch of large fibrillated PT-RS, collected as fibers on the mesh sheet and a suspension of small PT-RS fibers which passed the mesh sheet. A part of the former fraction (equivalent to 137 g DM) was divided to 16 parts (each 1/16 of the large PT-RS) for fed batch applications during the SSF, and a part of the latter fraction (the small PT-RS suspension, 610 mL, equivalent to 17.0 g DM) was used as a part of the initial culture broth for the SSF. The initial culture broth comprised the small PT-RS suspension, the first 1/16 large PT-RS, lactic acid (2.3 g, for pH adjustment to 5.6), Cellic CTec2 (306 FPU), Novozyme 188 (306 CbU), and yeast extract (4.4 g). *Bacillus coagulans* CP1-33 was isolated at a composting facility in Ibaraki, Japan. The strain was inoculated in Luria broth (100 mL × 4 flasks) and agitated at 180 rpm for 10 h at 50 °C. The culture fluid was centrifuged at 8000g for 5 min, and the precipitate was suspended in 40 mL of water for use as an inoculum for the SSF. The SSF was performed by using a jar fermenter (Type MDL N-2L, Marubishi Bioengineering Co. Ltd., Tokyo, Japan) at 190 rpm and 50 °C for 36 h under anaerobic conditions by continuously bubbling nitrogen gas through the suspension. Another 1/16 large PT-RS sample was added to the fermenter when the pH decreased below 5.2, and the remaining 1/16 large PT-RS samples were added one by one in the same manner. Cellic CTec2 (103 FPU) and Novozyme 188 (103 CbU) were also added simultaneously with the addition of the 1/16 large PT-RS samples. After adding all 1/16 large PT-RS samples, the pH was

further controlled at 5.2 by the automated addition of 5 N NaOH solution. Although run #2 was performed in the same way as run #1, the amount of substrate differed slightly from that of run #1 because of several manual separation steps. The final components in the culture broth of runs #1 and #2 after a 36-h incubation were as follows: the large PT-RS (137 g DM; 144 g DM), the small PT-RS (17.0 g DM; 11.9 g DM), Cellic CTec2 (1850 FPU; 1870 FPU), Novozyme 188 (1850 CbU; 1870 CbU), NaOH (10.2 g; 8.5 g), final volume (1160 mL; 1130 mL). The lactic acid yield was calculated as $(L_{\text{output}} - L_{\text{input}}) / [(TG \times 1.11) + (TX \times 1.14) + TG_E \times 1.11] \times 100$ (%), where L_{output} – lactic acid content accumulated after 36 h incubation time (g); L_{input} – lactic acid content initially added for pH adjustment (g); TG – total glucan content in the PT-RS (g); TX – total xylan content in the PT-RS (g); and TG_E – total glucan content (as glucose equivalent) in the enzyme preparations (g).

2.8. Component analysis

The total amounts of glucan and xylan were analyzed as described previously (Shiroma et al., 2011). Phosphorus was defined in this study as phosphorus atoms in solubilized phosphate and measured using a Malachite Green Phosphate Assay Kit (Sigma-Aldrich, St. Louis, MO, USA) with phosphoric acid as a standard. The calcium ions in the solutions liberated in acidic conditions with HCl were measured using a Metalloassay Calcium LS (Metallogenics Co., Ltd., Chiba, Japan) with CaCl_2 as a standard. Potassium ions in the solutions were measured using a pH/ion meter (F-53, HORIBA, Ltd. Kyoto, Japan) equipped with a potassium electrode (#6582, HORIBA, Ltd.) with KCl as a standard. *p*-Coumarate, ferulate, acetic acid and lactic acid were measured with the corresponding standards using an isocratic HPLC system (LC Prominence, Shimadzu Corporation, Kyoto, Japan). An ODS column (CAPCELL PACK C18 MGII, Osaka Soda Co., Ltd., Osaka, Japan) and a UV detector (SPD-20A, Shimadzu Corporation, Kyoto, Japan) were used for the measurements of *p*-coumarate and ferulate (mobile phase: 20% acetonitrile in 0.1% H_3PO_4 , $\lambda = 310$ nm). Acetic acid and lactic acid were measured by using an Aminex HPX-87H column (Bio-Rad Laboratories Co., Ltd., Tokyo, Japan, mobile phase: 5 mM H_2SO_4) with a refractive index detector (RID-16A, Shimadzu Corporation, Kyoto, Japan).

2.9. Calculation

The following experiments were designed and performed to prove the theory shown in the Introduction section. The properties of PLA as an alkali reagent were investigated in terms of the contents of acid-soluble elements and alkalinity of the suspension in water. The efficiency of PLA for alkali pretreatment of RS was also evaluated in comparison with that of $\text{Ca}(\text{OH})_2$. A wet-storage/pretreatment experiment of RS in the presence of PLA was performed for the subsequent solid–solid separation of PT-RS and RT-PLA, and the wash water used for the separation was reused for monitoring the stability of the separation step in the RURAL process. The properties of RT-PLA as a primary product were also investigated to compare with those of PLA, including its phosphorus solubilization property under acidic conditions. An enzymatic saccharification of PT-RS was performed to evaluate the pretreatment efficiency in the wet storage/pretreatment experiment. Lactic acid production by a nonsterile SSF was also performed to demonstrate the versatility of PT-RS as feedstock.

3. Results and discussion

3.1. Characterization of PLA

In the upper part of Table 1 the contents of the main acid-soluble inorganic elements (calcium, potassium, and phosphorus) in the poultry litter before combustion and in the PLA flakes were shown. The fraction

of phosphorus extracted with 1 N HCl reflects the total available phosphorus in the analysis of the fertilizer properties (Codling, 2006). Similarly, acid-soluble calcium and potassium fractions soluble in 1 N HCl were defined as corresponding acid-soluble elements in this study. Water-soluble fractions of individual elements were also determined; for the PLA flakes, the ratio of water-soluble calcium to acid-soluble calcium was 38.6%, whereas that for the poultry litter before combustion was 12%, suggesting that conversion of CaCO_3 to CaO during combustion at 900 °C could result in a substantial increase in its solubility in water. This suggests that CaCO_3 in poultry litter could be converted to CaO during combustion, thereby significantly raising the pH (Codling, 2006). The pH of the liquid part of the 5% (w/v) suspension of the PLA flakes in deionized water was 13.0, while that of the poultry litter before combustion was 7.7 (Table 1).

While all acid-soluble potassium was water-soluble in the poultry litter before combustion, only 45% of acid-soluble potassium was water soluble in the PLA flakes. The property of phosphorus was also changed by combustion: no water-soluble phosphorus was detected in the PLA flakes, while before combustion, 30% of acid-soluble phosphorus in poultry litter was soluble in water. The combustion step is reported to significantly change the properties of inorganic ions and reduces the amount of water-soluble fraction of phosphorus which could cause water pollution when overloaded on agricultural fields (Codling, 2006). The change in the water-soluble property of phosphorus after combustion is also crucial in the design of the process to include the washing step of PLA in this study, as outlined below. The calcium/phosphorus ratios before and after combustion of the poultry litter were 8.6 and 5.3, respectively. The discrepancy would be attributed to both the heterologous nature of the poultry litter before combustion and changes in the acid-solubility of parts of elements after combustion.

Then, the activity of PLA flakes as an alkali reagent for pretreatment of the RS flakes at 95 °C for 1 h was evaluated in comparison with that of $\text{Ca}(\text{OH})_2$ (Fig. 1). The amounts of solubilized glucose- and xylose residues (corresponding monomers and oligomers) after a 48-h enzymatic saccharification gradually increased with increases in the amounts of alkalis for pretreatment. The effect of $\text{Ca}(\text{OH})_2$ on the pretreatment was greater than that of the PLA flakes of the same weight ratio (reagent/RS), but with the application of a large amount, the PLA flakes could be substituted for $\text{Ca}(\text{OH})_2$ as observed at closer points to the plateaus of the sugar recoveries (the weight ratios of alkali reagent / RS ≥ 0.2 , Fig. 1). Thus, although the alkalinity was lower than that of $\text{Ca}(\text{OH})_2$, PLA flakes can be used as an alkali reagent for pretreatment of RS.

The severity of $\text{Ca}(\text{OH})_2$ pretreatment can be controlled by various factors such as the amount of alkali, reaction temperature, and reaction time (Chang et al., 1998; Kim and Holtzapple, 2005). The addition of PLA for pretreatment of RS on the recoveries of fermentable sugars after enzymatic saccharification became less effective at the weight ratio of PLA/RS ≥ 0.2 (Fig. 1). This would be attributed to low solubility of $\text{Ca}(\text{OH})_2$, the main alkali component in PLA, in water. Therefore, the severity of $\text{Ca}(\text{OH})_2$ pretreatment has been raised by oxidation with O_2 or a reaction at a high temperature for a long time (Chang et al., 1998; Kim and Holtzapple, 2005). In the RURAL process, the reaction was performed at ambient temperature for simplicity, whereas the severity could be raised for efficient sugar recoveries in shorter times. The minimum reaction time for $\text{Ca}(\text{OH})_2$ pretreatment of herbaceous biomass at 25 °C was reported as one week for rice straw (Shiroma et al., 2011) or some weeks for corn stover (Kim and Holtzapple, 2005).

In Fig. 1 the pH of the pretreatment slurry supernatant was also indicated on each bar; the conditions at pH 12.5 and 12.6 scored the highest recoveries of both residues after saccharification. The alkali pretreatment modified the structure of xylan to make it more susceptible to enzymatic attacks, which in turn affected the recovery of glucose residues by exposing new cellulose surfaces by the removal of xylan (Santos et al., 2018). In this study, a fixed enzyme preparation was used for all saccharification experiments, whereas it is expected

Table 1
Contents of acid-soluble inorganic elements in poultry litter and its derivatives.

Samples	Contents (% ^b ; [] : water-soluble part)			pH ^c	Calcium/phosphorus ratio
	Calcium	Potassium	Phosphorus		
Poultry litter ^a	8.6 ± 0.0 [1.0 ± 0.2]	2.1 ± 0.0 [2.2 ± 0.1]	1.0 ± 0.0 [0.3 ± 0.0]	7.7 ± 0.0	8.6
Poultry litter ash (PLA)	42.0 ± 1.4 [16.2 ± 0.0]	5.6 ± 0.0 [3.1 ± 0.0]	7.9 ± 0.1 [0.0 ± 0.0]	13.0 ± 0.0	5.3
Rice-straw treated PLA (RT-PLA) #1	21.8 ± 0.2	3.5 ± 0.0	7.8 ± 0.2	11.2 ± 0.0	2.8
Rice-straw treated PLA (RT-PLA) #2	25.2 ± 0.4	3.8 ± 0.0	9.5 ± 0.1	11.2 ± 0.1	2.7

Inorganic elements extracted in 1 N HCl solution are defined as acid-soluble inorganic elements; a sample (100 mg) was added to 20 mL 1 N HCl solution at room temperature.

The average values and the standard deviations for major inorganic elements in two samples are shown.

The schemes for production of RT-PLAs are shown in Fig. 2.

^a The sample is a part of the feedstock for combustion to PLA, whereas it may not properly represent the characteristics of the whole feedstock.

^b Weights of acid-soluble or water-soluble elements per those of the corresponding samples (%).

^c The value of 5% (w/v) suspension in water.

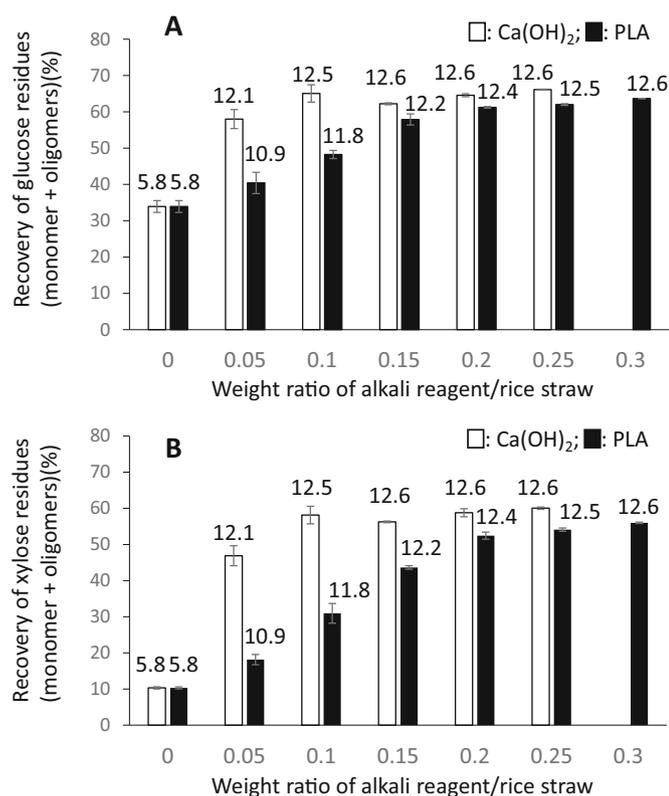


Fig. 1. Evaluation of PLA as a reagent for RS pretreatment.

Open bars, the RS flakes treated with Ca(OH)₂; closed bars, the RS flakes treated with the PLA flakes. Panels A and B show the glucan and xylan recovery ratios, respectively. The number above each bar indicates the pH value of the cooled sample after the pretreatment step at each condition. The saccharification tests were performed in duplicate. The average values ± standard deviations are shown.

that optimization of xylan-degrading enzymes to feedstock could raise the total sugar recovery.

In addition to its environmental value as a recycled alkali reagent for the pretreatment of biomass as described in the Introduction section, PLA has advantages over Ca(OH)₂ in that it would be produced daily at the site of combustion of poultry litter, and possibly even at the vicinity of a small lignocellulosic-biorefinery plant for collection of lignocellulosic biomass for conversion. If PLA is also upgraded as a fertilizer especially as a phosphorus source for crops in the rural areas, the pretreatment system of lignocellulosic biomass would contribute greatly to the achievement of a circular economy (Jurgilevich et al., 2016). Ash from manure samples from cattle, pigs, and other animals

could be expected to have the same potential as an alkali reagent for the pretreatment of biomass. Manure samples rich in potassium are generally supplemented with CaCO₃ for the stable running of combustion, which could result in an increased alkalinity of the ash.

3.2. Process for reciprocal upgrading of RS and PLA

In the scheme of Fig. 2, the proposed RURAL process was shown. In the boxes on the left parts, it is indicated that the chopped RS, PLA flakes, and water were mixed and settled at ambient temperature (25 °C) for two weeks for both wet storage and pretreatment of the chopped RS. This step is also regarded as a crucial calcium-transfer step from PLA to RS or water. After wet storage/pretreatment, water was added for a solid–solid separation to obtain two fractions: the PT-RS and the RT-PLA. The water for the solid–solid separation was recovered and reused as crude water for the next solid–solid separation step. In the four preliminary sequences (sequences #P1–#P4) for reuse of the water, the amount of DM in the wash water increased as the number of reuses increased. The recoveries of PT-RS in the four preliminary sequences were between 772 g and 790 g, whereas those in the experiments #1 and #2 were 746 g and 809 g, respectively, due to an instability in the manual procedure of the solid–solid separation; small particles of RS might have been transferred to the RT-PLA fraction in the experiment #1, as suggested by the weight loss after combustion of organic compounds in the RT-PLA samples (data not shown).

Solid–solid separation technologies have been incorporated in lignocellulosic biorefineries at various steps, one of which is the removal of solid inorganic matter contaminated in agricultural residues during collection (Hassan et al., 2019). For feedstock preparation, the use of a cyclone-type separator for separation of the dense part (culms) of the chopped RS from the remaining low-density part was also proposed (Park et al., 2011a). A two-step centrifugation technique and a floating bead reactor system were proposed for reuse of yeast cells after SSF of pretreated lignocellulosic biomass (Matano et al., 2013; Guan et al., 2019). In the RURAL process, a separation was performed based on a difference in both size and specific gravity between the chopped RS and the PLA flakes; the size difference may cause a trade-off between the efficiency of the separation and that of the subsequent enzymatic saccharification (Chang et al., 1997). The use of finely chopped RS would result in a reduced recovery of PT-RS due to the release of small fibers, an increased contamination of the fibers in the RT-PLA, and a larger cost for milling, whereas the use of coarsely chopped RS at a large size would suppress the formation of small fibers, while also reducing the efficiency of the alkali pretreatment.

Fig. 3 depicts a schematic representation of material flow of the main substances—glucan, xylan, calcium, potassium, phosphorus, and so on—of experiments #1 and #2 in Fig. 2. The recoveries of glucan and xylan in the PT-RS #1 were 89.4% and 90.5%, respectively, while the

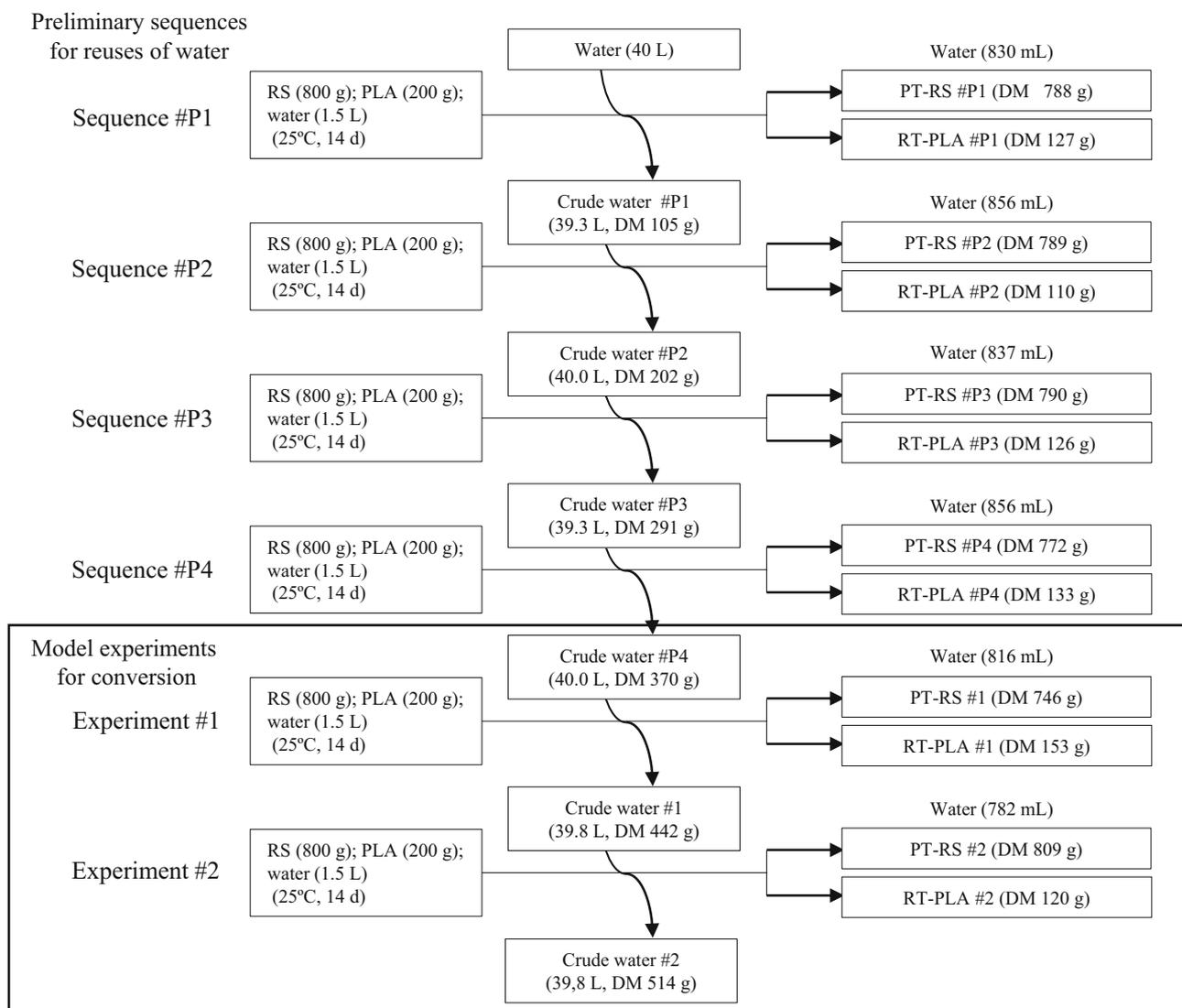


Fig. 2. Overall illustration of treatment of RS and PLA for reciprocal upgrading.

All the values in this figure were converted to 1 kg dry matter (dry RS and dry PLA) as start material for convenience of calculation and clear communication of the trend. The values were calculated based on the measured value; material balances may not always be stoichiometric because of some operational measurement errors. DM: dry matter.

corresponding values in the PT-RS #2 were 102% and 96.3%, respectively, reflecting the corresponding data variation in Fig. 2. The crude water #P4 contained calcium and potassium which had accumulated during the preliminary sequences (Fig. 2), and further increased during the two-time reuses in the main experiments (crude waters #1 and #2). Some of the calcium and potassium in the PLA existed in a water-soluble form (Table 1), which is in accordance with their gradual accumulations in the wash water fractions. Alkali pretreatment of RS cleaved ester bonds in the cell wall to liberate acetate, *p*-coumarate, and ferulate in solution (Zhao et al., 2013), which also gradually accumulated in the crude water fractions.

3.3. Characterization of RT-PLA

All acid-soluble phosphorus in the PLA exists in a water-insoluble form (Table 1), which is reflected in the high phosphorus recoveries of 75.3% and 71.5% in oven-dried RT-PLA #1 and #2, respectively. In addition, 12% and 13% of the total phosphorus in PLA were transferred to PT-RS #1 and #2, respectively, suggesting that some flakes of RT-PLA would be physically entrapped in the chopped RS. The RURAL process exploits the high affinity of calcium with lignocellulosics

(Chang et al., 1998) for efficient removal of calcium from the PLA; PT-RS adsorbed 41.5%–44.6% of the total calcium in the PLA (Fig. 3). The calcium/phosphorus ratios of RT-PLA #1 and #2 were therefore reduced to 2.8 and 2.7, respectively, values which are about a half of that of PLA (Table 1). The pH in the water of the RT-PLA powders was 11.2, which is 1.8 points lower than that of the PLA flakes. The lower pH of the RT-PLA would significantly reduce the risk for workers handling it as a fertilizer. In addition, the wet form of the RT-PLA slurry just after solid–solid separation would facilitate the improvement of structure through pellet production by the addition of an appropriate binder for better handling properties and/or mixing with other components to further raise its value as a fertilizer.

Next, phosphorus solubilization properties of the PLA flakes and RT-PLA #1 powders were evaluated (Fig. 4). The RT-PLA #1 sample solubilized phosphorus under conditions of far smaller amounts of HCl than those required for the PLA sample, reflecting the alkalinity of both samples in that the trends of relationships between the ratio of its solubilization and the pH appears almost the same in both samples. The data suggest that the release of phosphorus from RT-PLA as a fertilizer would be more sensitive to acids produced at the roots of crops than that from PLA.

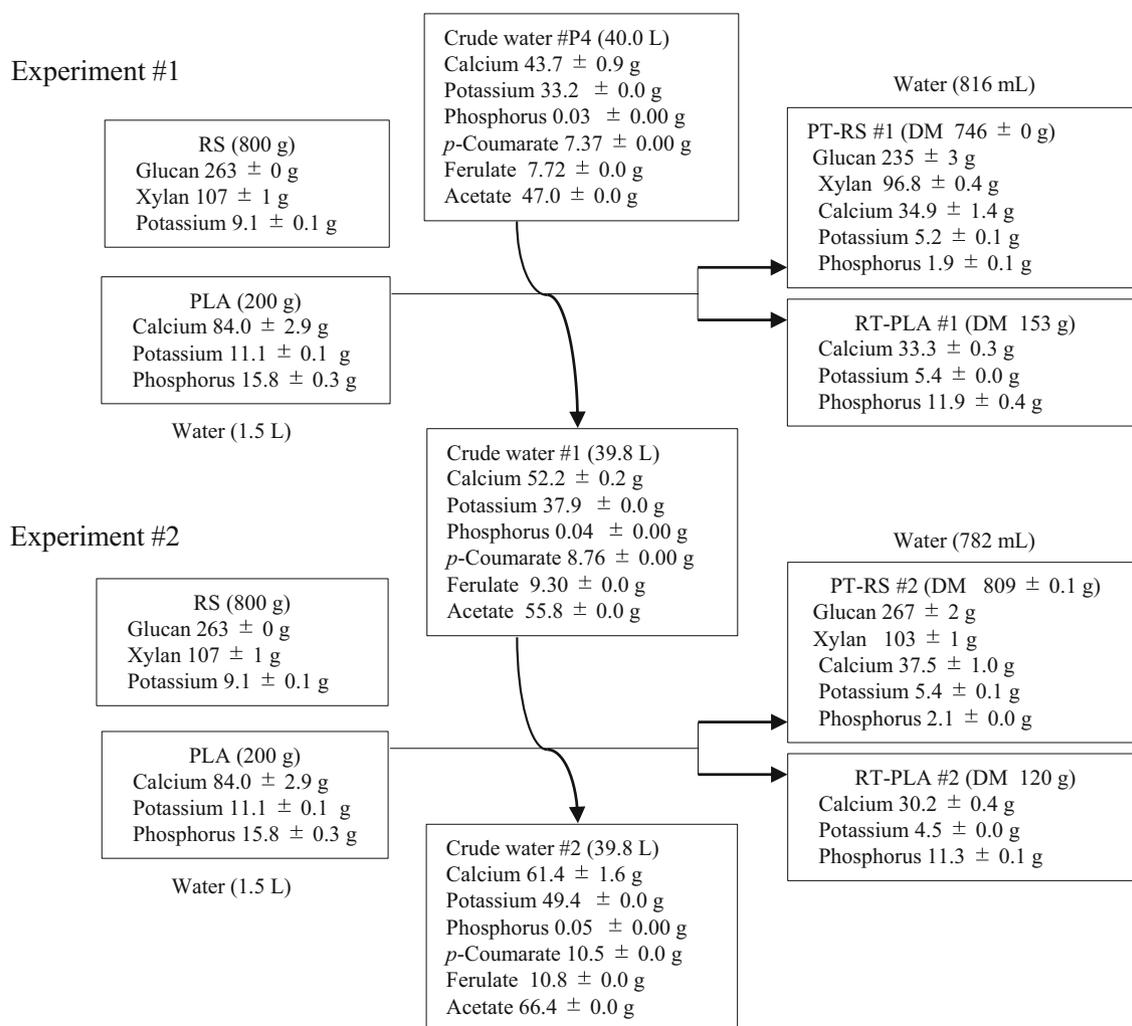


Fig. 3. Distribution of major components in the reciprocal upgrading process.

Samples in the model experiments for conversion (experiments #1 and #2, Fig. 2) were used for component analysis. All the values in this figure were converted to 1 kg dry matter (dry RS and dry PLA) as start material as shown in Fig. 2. The measurements were taken in duplicate, and the average values ± standard deviations are shown.

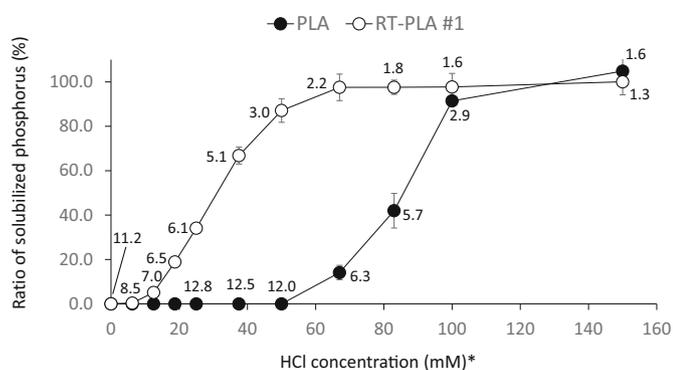


Fig. 4. Release of phosphorus from PLA and RT-PLA #1 under acidic conditions.

Closed circles and open circles indicate PLA and RT-PLA #1, respectively. The elution tests were performed in duplicate, and the average values ± standard deviations are shown. The numbers above individual circles indicate the average pH values.

*The values were calculated under the assumption that the added HCl stock was used for preparation of the final HCl solution, whereas the value does not reflect the actual pH because of the presence of alkaline ash.

3.4. Saccharification of PT-RS

The pH of a slurry of PT-RS #1 was lowered by the addition of 1 M acetic acid to a weak acidic range for efficient enzymatic saccharification. Calcium and phosphorus in PT-RS #1 were gradually solubilized according to the pH reduction; when the pH was adjusted to 5.1, 80% of total calcium and 60% of total phosphorus in PT-RS #1 were solubilized (data not shown). Acetic acid for pH adjustment was used to omit a washing of the pretreated lignocellulosics which would otherwise have needed additional wash water and caused a loss of the sample during the solid–liquid separation, although the presence of calcium acetate was reported to reduce the efficiency of enzymatic saccharification (Chang et al., 1997). As an alternative to neutralization with acid reagents, tightly bound calcium in an RS sample after pretreatment with $\text{Ca}(\text{OH})_2$ was reportedly recovered in a recyclable form (that is, CaCO_3) by stepwise washings with water and carbonated water (Yamagishi et al., 2019). This enabled the minimum use of acidic reagents, although the entire washing step would have been complex. A unique method of skipping a washing step of pretreated biomass prior to saccharification was also reported for feedstock containing significant amounts of water-soluble sugars, adopting bubbling or pressurization of the reaction vessel by carbonation for both neutralization and further reduction of pH for saccharification in the presence of CaCO_3 (Park

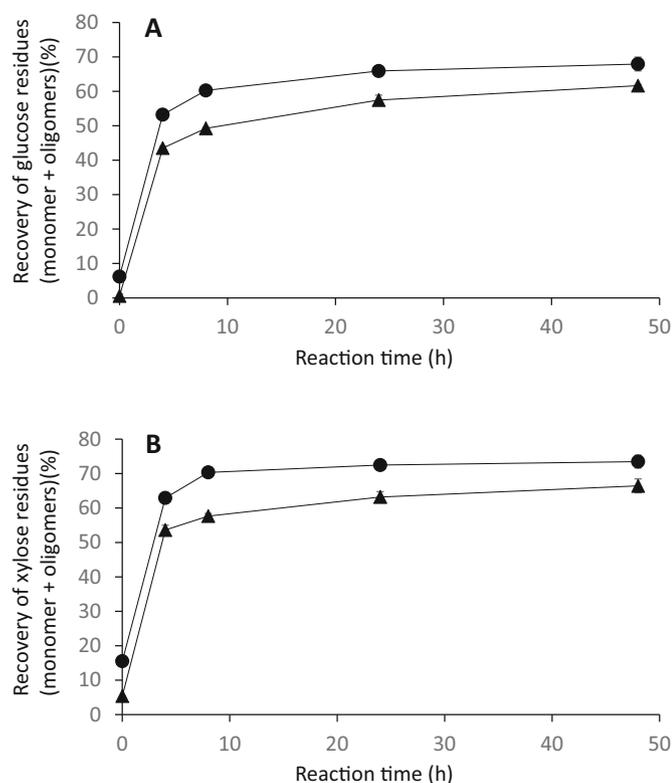


Fig. 5. Enzymatic saccharification of PT-RS #1.

Panels A and B show the glucon and xylan recovery ratios, respectively. Circles and triangles indicate the data of PT-RS #1 with and without fibrillation, respectively. The saccharification tests were done in duplicate, and the average values \pm standard deviations are shown.

et al., 2011b; Ike and Tokuyasu, 2020).

In this study, PT-RS #1 was produced from chopped RS, and its large size could have affected the efficiency of the enzymatic saccharification in terms of a limited surface area on the substrate for enzymatic attacks. A wet fibrillation step of the PT-RS was therefore performed with a blender after the pH adjustment. In Fig. 5 the ratios of solubilized glucose- and xylose residues to their total amounts in the substrate during the enzymatic saccharification of both PT-RS #1 and fibrillated PT-RS #1 were shown. The fibrillation of the substrate clearly resulted in higher recoveries of glucose- and xylose residues. After a 48-h enzymatic saccharification of the fibrillated PT-RS #1, the recoveries of glucose- and xylose-residues were 67.9% and 73.5%, respectively. The corresponding data for the non-fibrillated PT-RS #1 were 61.6% and 66.4%, respectively. In both samples, the solubilization of xylose residues proceeded slightly faster than that of glucose residues; for example, in the fibrillated sample, the ratio of yield of glucose residues at 8 h after reaction to that at 48 h after reaction was 0.888, whereas the corresponding data of xylose residues was 0.956. In addition to the speculation in Section 3.1 that the solubilization ratio of xylose residues would affect the saccharification ratio of glucan, the rapid solubilization of xylose residues would affect the total reaction time to achieve the plateau phases for recoveries of both residues. Low-cost preparation and optimization of xylan-degrading enzymes for individual lignocellulosic substrate would be among the breakthroughs in the small lignocellulosic biorefineries.

3.5. Strategic primary products

The aim of this study is to develop a breakthrough for small-scale lignocellulosic biorefineries using a bottom-up approach. The feedstock is a natural waste product or unused resource located at the most

upstream side of the conversion processes, and the characteristics and availability in any given situation should primarily determine the possibilities and limitations in a bottom-up approach. It contrasts well with the top-down approach in which both the minimum profitable scale of production and the feedstock price would determine them, as described in the Introduction section. However, neither the current characteristics of the feedstock, which are not powerful, nor its availability, which is inefficient, justify the initiation of new process developments for valuable production beyond its conventional applications. The breakthrough RURAL process should be a way to improve the characteristics and availability of feedstock for the activation of new process developments.

In this study, the importance of manufacturing the strategic primary products (SPPs) from the feedstock is emphasized (Ubando et al., 2020). The SPP defined here is a more versatile primary product made from feedstock, providing merits such as suppressed quality fluctuations, improved storability, and improved processability. The versatility of SPPs is expected to lead to new ideas and strategies for production of various new SPPs and final products. In this study, the poultry litter, which is bulky and carries a high environmental load, was converted by combustion into heat and the PLA as an SPP. PLA was further converted to RT-PLA as another SPP with enhanced handling properties and characteristics of phosphorus solubilization. The chopped RS was converted to PT-RS, which is regarded as an SPP in terms of its superior storability and saccharification property to those of RS. The enhanced saccharification property of PT-RS gave fermentable sugars by enzymatic saccharification (Fig. 5), thereby providing various SPPs by adoption of fermentation technologies developed for large-scale lignocellulosic biorefineries.

In Fig. 6, as an example of such valuable production by fermentation, the time course of lactic acid production by SSF using fibrillated PT-RS #1 as a substrate was shown. Here, a fed-batch method was adopted to use the alkali in PT-RS for suppressing the pH decrease by lactic acid production, where alkalis in PLA initially used for a pre-treatment of RS were reused for pH control of the broth (Maas et al., 2008). After fermentation for 36 h, lactic acid was successfully produced in yields of 68.6% and 65.5% from the total fermentable sugars in PT-RS #1 at runs #1 and #2, respectively. In industry, calcium lactate in the fermentation broth is acidified with sulfuric acid to generate free lactic acid for further purification and gypsum. This simple

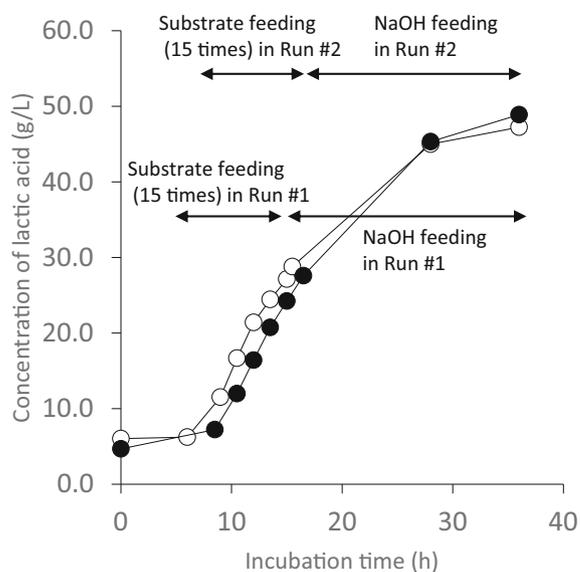


Fig. 6. Time course of a nonsterile SSF experiment for lactic acid production. Open and closed circles indicate the data of Runs #1 and #2, respectively. Spans of substrate feeding and NaOH feeding in individual runs are also shown as arrows in the figure. Limit of lactic-acid quantification: 1.0 g/L.

conversion process omits heating processes above 50 °C for pretreatment of the RS and sterilization, which would facilitate small-scale production by saving on capital expenditure. Lactic acid from RS is expected to be a new SPP or a final product in various industries after appropriate purification. The water-insoluble residue after fermentation comprised mainly microbial cells, lignocellulosics, and inorganic salts, and could therefore also be regarded as a potential SPP in that it could be further composted to return to the field for maintenance of soil quality or further purified to produce specific SPPs such as lignin and silica.

4. Conclusions

The RURAL process was proposed for SPP productions which could provide a breakthrough in realizing small-scale lignocellulosic biorefineries and upgrading PLA for recycling as a fertilizer. The simplicity of the process would enable implementation from a silo scale in combination with a local combustion system of poultry litter for recycling of energy and inorganic compounds. This process is expected not only to facilitate small-scale production and the use of fermentable sugars from lignocellulosics but also to spark the generation of ideas and strategies inspired by the new properties of SPPs for achieving the rural bioeconomy.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

CRediT authorship contribution statement

K.Y.: Data curation, Investigation, Methodology, Writing - original draft; M.I.: Data curation, Investigation, Methodology; A.T.: Data curation, Investigation, Methodology; K.T.: Conceptualization, Supervision, Project administration, Writing - review & editing.

Declaration of competing interest

The authors submitted a patent on the process.

Acknowledgments

The authors thank Ms. Kana Hiramoto and Ms. Hiromi Nakayama for excellent technical assistants. The authors would like to thank Enago (www.enago.jp) for the English language review.

References

- Anser, K.M., Hina, T., Hameed, S., Nasir, H.M., Ahmad, I., Naseer, A.R.M., 2020. Modeling adaptation strategies against climate change impacts in integrated rice-wheat agricultural production system of Pakistan. *Int. J. Environ. Res. Public Health* 17, 2522. <https://doi.org/10.3390/ijerph17072522>.
- Cappelli, A., Cini, E., 2020. Will the COVID-19 pandemic make us reconsider the relevance of short food supply chains and local productions? *Trends Food Sci. Technol.* 99, 566–567.
- Chandel, K.A., Garlapati, K.V., Singh, K.A., Antunes, F.A.F., de Silva, S.S., 2018. The path forward for lignocellulose biorefineries: bottlenecks, solutions, and perspective on commercialization. *Bioresour. Technol.* 264, 370–381.
- Chang, S.V., Burr, B., Holtzapfel, T.M., 1997. Lime pretreatment of switchgrass. *Appl. Biochem. Biotechnol.* 63–65, 3–19.
- Chang, S.V., Nagwani, M., Holtzapfel, T.M., 1998. Lime pretreatment of crop residues bagasse and wheat straw. *Appl. Biochem. Biotechnol.* 74, 135–159.
- Chastain, P.J., Coloma-del Valle, A., Moore, P.K., 2012. Using broiler litter as an energy source: energy content and ash composition. *Appl. Eng. Agric.* 28, 513–522.
- Codling, E.E., 2006. Laboratory characterization of extractable phosphorus in poultry litter and poultry litter ash. *Soil Sci.* 171, 858–864.
- Codling, E.E., Chaney, L.R., Sherwell, J., 2002. Poultry litter ash as a potential source for agricultural crops. *J. Environ. Qual.* 31, 954–961.
- Dale, B., 2018. Time to rethink cellulosic biofuels? *Biofuels Bioprod. Bioref.* 12, 5–7.
- Guan, D., Zhao, R., Li, Y., Sakakibara, Y., Ike, M., Tokuyasu, K., 2019. Reusable floating beads with immobilized xylose-fermenting yeast cells for simultaneous saccharification and fermentation of lime-pretreated rice straw. *J. Appl. Glycosci.* 66, 21–28.
- Guo, M., Song, W., 2019. The growing U.S. bioeconomy: drivers, development and constraints. *New Biotechnol.* 49, 48–57.
- Gutiérrez, S.A., Caneghem, V.J., Martínez, B.C.J., Vandecasteele, C., 2012. Evaluation of environmental performance of lime production in Cuba. *J. Clean. Prod.* 31, 126–136.
- Hassan, S.S., Williams, A.G., Jaiswal, K.A., 2019. Lignocellulosic biorefineries in Europe: current status and prospects. *Trend Biotechnol.* 37, 231–234.
- Ike, M., Tokuyasu, K., 2020. Control of pH by CO₂ pressurization for enzymatic saccharification of Ca(OH)₂-pretreated rice straw in the presence of CaCO₃. *J. Appl. Glycosci.* 67, 59–62.
- Jeswani, K.H., Whiting, A., Martin, A., Azapagic, A., 2019. Environmental and economic sustainability of poultry litter gasification for electricity and heat generation. *Waste Manag.* 95, 182–191.
- Jurgilevich, A., Birge, T., Kentala-Lehtonen, J., Korhonen-Kurki, K., Pietikäinen, J., Saikku, L., Schösler, H., 2016. Transition towards circular economy in the food system. *Sustainability* 8, 69. <https://doi.org/10.3390/su8010069>.
- Kanter, R., Boza, S., 2020. Strengthening local food systems in times of concomitant global crises: reflections from Chile. *Am. J. Public Health* e1–e3. <https://doi.org/10.2105/AJPH.2020.305711>.
- Kim, S., Holtzapfel, T.M., 2005. Lime pretreatment and enzymatic hydrolysis of corn stover. *Bioresour. Technol.* 96, 1994–2006.
- Lynch, D., Henihan, M.A., Bowen, B., Lynch, D., McDonnell, K., Kwapinski, W., Leahy, J.J., 2013. Utilisation of poultry litter as an energy feedstock. *Biomass Bioenergy* 49, 197–204.
- Maas, H.W.R., Bakker, R.R., Jansen, L.A.M., Visser, D., de Jong, E., Eggink, G., Weusthuis, A.R., 2008. Lactic acid production from lime-treated wheat straw by *Bacillus coagulans*: neutralization of acid by fed-batch addition of alkaline substrate. *Appl. Microbiol. Biotechnol.* 78, 751–758.
- Matano, Y., Hasunuma, T., Kondo, A., 2013. Cell recycle batch fermentation of high-solid lignocellulose using a recombinant cellulose-displaying yeast strain for high yield ethanol production in consolidated bioprocessing. *Bioresour. Technol.* 135, 403–409.
- McCormick, K., Kautto, N., 2013. The bioeconomy in Europe: an overview. *Sustainability* 5, 2589–2608.
- NREL: National Renewable Energy Laboratory, 2002. Lignocellulosic biomass to ethanol process design and economics utilizing co-current dilute acid prehydrolysis and enzymatic hydrolysis for corn stover. Available at: <https://www.nrel.gov/docs/fy02osti/32438.pdf>, Accessed date: 28 July 2020.
- Park, J.-Y., Ike, M., Arakane, M., Shiroma, R., Li, Y., Arai-Sanoh, Y., Kondo, M., Tokuyasu, K., 2011a. DiSC (direct saccharification of culms) process for bioethanol production from rice straw. *Bioresour. Technol.* 102, 6502–6507.
- Park, J.-Y., Al-Haq, M.I., Zhang, Y., Ike, M., Arai-Sanoh, Y., Ida, A., Kondo, M., Tokuyasu, K., 2011b. A novel lime pretreatment for subsequent bioethanol production from rice straw - calcium capturing by carbonation (CaCCO) process. *Bioresour. Technol.* 101, 6805–6811.
- Ravindran, R., Jaiswal, K.A., 2016. Exploitation of food industry waste for high-value products. *Trend Biotechnol.* 34, 58–69.
- Santos, O.T.V., Siqueira, G., Milagres, F.M.A., Ferraz, A., 2018. Role of hemicellulose removal during dilute acid pretreatment on the cellulose accessibility and enzymatic hydrolysis of compositionally diverse sugarcane hybrids. *Ind. Crop. Prod.* 111, 722–730.
- Shiroma, R., Park, J.-Y., Al-Haq, I.M., Arakane, M., Ike, M., Tokuyasu, K., 2011. RT-CaCCO process: an improved process for rice straw by its incorporation with a step of lime pretreatment at room temperature. *Bioresour. Technol.* 102, 2943–2949.
- Shiroma, R., Park, J.-Y., Ishikawa, S., Terajima, Y., Ike, M., Tokuyasu, K., 2012. Fraction of wet sugarcane bagasse as an advanced feedstock for monosaccharide production via the RT-CaCCO process. *Bioresour. Technol.* 116, 529–532.
- Torero, M., 2020. Without food, there can be no exit from the pandemic. *Nature.* 580, 588–589.
- Ubando, T.A., Felix, B.C., Chen, W.-H., 2020. Biorefineries in circular economy: a comprehensive review. *Bioresour. Technol.* 299, 122585.
- USEPA: United States Environmental Protection Agency, 2013. Literature review of contaminants in livestock and poultry manure and implications for water quality. Available at: https://www.adeq.state.ar.us/regs/drafts/reg06/14-004-r/comments/reg_6_cafa_noi_comments_of_beaver_water_district-attachment_i_7-28-14.pdf, Accessed date: 10 June 2020.
- Valdivia, M., Gelan, L.J., Laffarga, J., Ramos, J.-L., 2016. Biofuels 2020: biorefineries based on lignocellulosic materials. *Microb. Biotechnol.* 9, 585–594.
- Yamagishi, K., Ike, M., Guan, D., Tokuyasu, K., 2019. Washing lime-pretreated rice straw with carbonated water facilitates calcium removal and sugar recovery in subsequent enzymatic saccharification. *J. Appl. Glycosci.* 66, 11–19.
- Zhao, R., Yun, M.-S., Shiroma, R., Ike, M., Guan, D., Tokuyasu, K., 2013. Integration of a phenolic-acid recovery step in the CaCCO process for efficient fermentable-sugar recovery from rice straw. *Bioresour. Technol.* 148, 422–427.