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# Production of ethanol from a mixture of waste paper and kitchen waste via a process of successive liquefaction, presaccharification, and simultaneous saccharification and fermentation

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## ABSTRACT

Efficient ethanol production from waste paper requires the addition of expensive nutrients. To reduce the production cost of ethanol from waste paper, a study on how to produce ethanol efficiently by adding kitchen waste (potentially as a carbon source, nutrient source, and acidity regulator) to waste paper was performed and a process of successive liquefaction, presaccharification, and simultaneous saccharification and fermentation (L+PSSF) was developed. The individual saccharification performances of waste paper and kitchen waste were not influenced by their mixture. Liquefaction of kitchen waste at 90 °C prior to presaccharification and simultaneous saccharification and fermentation (PSSF) was essential for efficient ethanol fermentation. Ethanol at concentrations of 46.6 or 43.6 g/l was obtained at the laboratory scale after fermentation for 96 h, even without pH adjustment and/or the addition of extra nutrients. Similarly, ethanol at a concentration of 45.5 g/l was obtained at the pilot scale after fermentation for 48 h. The ethanol concentration of L+PSSF of the mixture of waste paper and kitchen waste was comparable to that of PSSF of waste paper with added nutrients (yeast extract and peptone) and pH adjustment using H<sub>2</sub>SO<sub>4</sub>, indicating that kitchen waste is not only a carbon source but also an excellent nutrient source and acidity regulator for fermentation of the mixture of waste paper and kitchen waste.

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## 1. Introduction

Bioethanol is a sustainable alternative fuel that can reduce greenhouse gas emissions if substituted for gasoline. Bioethanol has been produced mainly from corn in America and China and from sugarcane in Brazil (Balat and Balat, 2009). However, because corn is a major food source, its use as a fuel raw material has been criticized, as it has led to an increase in the price of corn. Since 2006, the Chinese government has restricted the use of corn for ethanol production (Tao et al., 2011). Therefore, bioethanol pro-

duction from lignocellulosic biomass gained worldwide attention; however, the cost of lignocellulosic ethanol could not compete with gasoline, until now.

In Japan, kitchen waste, waste paper, and plastic, collected as combustible waste, are incinerated. However, incineration of this water-rich waste is energy-consuming and generates dioxins and dust when the incineration temperature is not high enough (Yan et al., 2011). In a recent study of ours, the combustible waste was successively subjected to a crusher and sorter, and the combustible waste was separated into heavy and light fractions. Approximately 80% of the kitchen waste was collected in the heavy fraction and approximately 90% of waste paper was collected in the light fraction (Tomiyama et al., 2015). Both waste paper and kitchen waste are rich in carbohydrates (cellulose and starch), and thus increasing attention has been given to the conversion of kitchen waste (Matsakas and Christakopoulos, 2015; Wang et al., 2017; Yan et al., 2013) and waste paper (Elliston et al., 2013; Nishimura et al., 2016; Rajasekaran et al., 2014) to ethanol.

**Abbreviations:** L+PSSF, successive liquefaction, presaccharification, and simultaneous saccharification and fermentation; PSSF, presaccharification and simultaneous saccharification and fermentation; HPLC, high-performance liquid chromatography; LHV, lower heating value.

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Waste paper contains clays (kaolin and calcite) and lacks fermentation nutrients. The clays in waste paper make a slurry (in water) of such alkali, which is higher than the suitable pH (4.8–5.0) for cellulase. Therefore, pH adjustment and the addition of fermentation nutrients are required to improve ethanol yield from waste paper (Nishimura et al., 2016). To obtain a suitable pH for saccharification of waste paper, waste paper slurry is commonly prepared using a buffer solution (Elliston et al., 2013), or in water with pH adjustment via a solution of H<sub>2</sub>SO<sub>4</sub> (Dubey et al., 2012; Nishimura et al., 2016; Wang et al., 2013). Elliston et al. (2013) used 0.1 M sodium acetate buffer (pH 5.0) to prepare waste paper slurry, and an ethanol yield of 65.8% was obtained without needing to add extra nutrients for fermentation. In our previous study, the pH of waste paper water slurry was adjusted using 20% w/w H<sub>2</sub>SO<sub>4</sub> solution, and an ethanol yield of 90.8% was obtained with the addition of yeast extract and peptone as fermentation nutrients (Nishimura et al., 2016). However, preparation of waste paper slurry using a buffer solution is impossible on a large scale (Elliston et al., 2013) due to high cost, and pH adjustment using a solution of H<sub>2</sub>SO<sub>4</sub> inhibits anaerobic treatment of the stillage eluted from the ethanol distillation. On the other hand, kitchen waste is rich in fermentation nutrients besides carbohydrates and has a relatively low pH of approximately 4.8 (Wang et al., 2017). Kitchen waste can serve as a nutrient source, acidity regulator, and substrate by mixing it with waste paper for ethanol production.

In this study, a process was developed to produce ethanol using a mixture of waste paper and kitchen waste without the need to add H<sub>2</sub>SO<sub>4</sub> and expensive nutrients. First, enzymatic saccharification of waste paper was optimized to maximize the sugar recovery efficiency. Then, the individual and combined saccharification performances of the mixture of waste paper and kitchen waste were investigated. Finally, ethanol was produced by combining waste paper and kitchen waste using a process of successive liquefaction, presaccharification, and simultaneous saccharification and fermentation (L+PSSF) at both the laboratory and pilot scales.

## 2. Materials and methods

### 2.1. Biomass, enzymes, and yeast strain

The waste paper used in this study was from the light fraction of combustible waste in Japan, which was prepared by the Hitachi

Zosen Corporation. The waste paper was hard block because it was dried before it was sent to our laboratory. Kitchen waste was procured from a canteen at Sichuan University and chopped (MKBC-32; Masuko Sangyo Co. Ltd., Saitama, Japan) into small pieces after the bones and shells were separated. The chopped material was stored at –20 °C and thawed before use. A 50 g sample was freeze-dried to a moisture content of less than 10%. This dried sample was then used for components analysis.

Alpha-amylase and glucoamylase were purchased from Sichuan Shanye Co., Ltd. (Sichuan, China). Cellulase Cellic CTec2 was provided by Novozymes (Bagsværd, Denmark).

The flocculating yeast *Saccharomyces cerevisiae* strain KF-7, which cannot convert xylose to ethanol, was used for ethanol fermentation.

### 2.2. Effect of liquefaction on enzymatic saccharification of kitchen waste

Table 1 shows the outlines of enzymatic liquefaction, enzymatic saccharification, and simultaneous saccharification and fermentation.

As optimized in our previous study (Wang et al., 2017), starch in kitchen waste was converted to glucose using a process of successive liquefaction at 90 °C and saccharification at 50 °C. Briefly, kitchen waste (300 g wet weight) was added into a 1 L jar fermenter (MDL-1L; B.E. Marubishi, Chiba, Japan) and preheated to 90 °C with agitation at 400 rpm. Liquefaction was initiated by adding  $\alpha$ -amylase at 0.3  $\mu$ l/g glucan. After liquefaction for 30 min, the kitchen waste was cooled to 50 °C and glucoamylase at 4  $\mu$ l/g glucan was added to initiate saccharification. In contrast, a process of simultaneous liquefaction and saccharification at 50 °C was tested to evaluate the effect of liquefaction at 90 °C on enzymatic saccharification of kitchen waste. Briefly, kitchen waste (300 g wet weight) was preheated to 50 °C with agitation at 400 rpm. Then,  $\alpha$ -amylase at 0.3  $\mu$ l/g glucan and glucoamylase at 4  $\mu$ l/g glucan were added together to initiate saccharification. Saccharification was carried out for 20 h, and samples were taken at various time points to measure the released sugar. Prior to sugar analysis, sample of 1 g was weighted and diluted with addition of deionized water, and the supernatant was filtrated by a 0.45  $\mu$ m membrane filter. Glucose yield (Y) after saccharification was calculated using Eq. (1):

**Table 1**  
Summary on experimental methods.

Tests (in MM)	Purpose	Scale	Conditions					Remarks
			Materials	Enzyme	Temp.	Revol.	Time	
2.2	Liq. of KW	Lab	KW 300 g	AA (0.3 $\mu$ l/g glucan from KW)	90 °C	400 rpm	1 h	Liq. → Sacchari. (GA, 50 °C)
2.3	Sacchari. of WP	Lab <sup>a</sup>	WP (15.5% w/v)	Cellulase (10–20 FPU/g WP)	50 °C	150 rpm	72 h	Liq. of KW → Liquefied KW + WP → Sacchari. Non-liquefied KW + WP → Sacchari.
2.4.1	Sacchari. of KW	Lab <sup>a</sup>	WP:KW (15.5:6.5, dry base)	GA (4 $\mu$ l/g glucan from KW)	50 °C	150 rpm	72 h	
2.4.2	Sacchari. of WP	Lab <sup>a</sup>	WP:KW (15.5:6.5, dry base)	Cellulase (15 FPU/g WP)	50 °C	150 rpm	72 h	
2.4.3	Sacchari. of KW and WP	Lab <sup>a</sup>	WP:KW (15.5:6.5, dry base)	GA + Cellulase	50 °C	150 rpm	72 h	Liq. of KW → Liquefied KW + WP → Sacchari.
2.5.1	Optimization of SSF	Lab <sup>b</sup>	Enzymatic hydrolysate	Precultivated yeast	35 °C 38 °C	150 rpm	120 h	Liq. → Presacchari. → SSF
2.5.2	L+PSSF	Pilot <sup>c</sup>	KW 5.5 kg wet Liquefied KW + WP (3.1 kg dry)	AA	90 °C	20 rpm	1 h	Liq. →
				GA + Cellulase	50 °C	20 rpm	16 h	Presacchari. →
				Precultivated yeast	35 °C	20 rpm	96 h	SSF

Abbreviations: MM, Materials and Methods; Liq., liquefaction; Sacchari., saccharification; Presacchari., presaccharification; Revol., revolution; SSF, simultaneous saccharification and fermentation; L+PSSF, successive liquefaction, presaccharification, and SSF; KW, kitchen waste; WP, waste paper; AA,  $\alpha$ -amylase; GA, glucoamylase.

<sup>a</sup> Total volume was 0.1 L.

<sup>b</sup> Total volume was 0.11 L.

<sup>c</sup> Total volume was 22 L.

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