



Feasibility of producing ethanol from food waste

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ABSTRACT

Food waste generated in Korea is rich in carbohydrate as high as 65% of total solids. Using the food waste, the feasibility of ethanol production was investigated in a lab-scale fermentor. Pretreatment with hydrolyzing enzymes including carbohydrase, glucoamylase, cellulase and protease were tested for hydrolysis of food waste. The carbohydrase was able to hydrolyze and produce glucose with a glucose yield of 0.63 g glucose/g total solid.

Enzymatic hydrolysis and ethanol fermentation by using carbohydrase and *Saccharomyces cerevisiae* were conducted in the batch mode. For separated hydrolysis and fermentation (SHF), ethanol concentration reached at the level corresponding to an ethanol yield of 0.43 g ethanol/g total solids. For simultaneous saccharification and fermentation (SSF), the ethanol yield was 0.31 g ethanol/g total solids. During the continuous operation of SHF, the volumetric ethanol production rate was 1.18 g/l h with an ethanol yield of 0.3 g ethanol/g total solids. For SSF process, the volumetric ethanol production rate was 0.8 g/l h with an ethanol yield of 0.2 g ethanol/g total solids.

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1. Introduction

With the inevitable depletion of the world's petroleum supply, there has been an increasing worldwide interest in alternative, non-petroleum-based sources of energy. As petroleum supplies 97% of the energy consumed for transportation, industries and governments worldwide have been actively identifying, developing and commercializing technology for alternative transportation fuels over the past 20 years (Tomas-Pejo et al., 2008). The fermentation-derived ethanol is an alternative transportation fuel which is one of the most important renewable fuels contributing to the reduction of negative environmental impacts generated by petroleum-based source of energy.

The fermentation-derived ethanol can be produced by the transformation of biological resources such as energy-rich crops (sugarcane or corn) or lignocellulosic biomass. In Korea, however, those biological resources are not found in large quantity. Instead, the organic wastes such as food waste, livestock manure, vegetable waste and waste sludge are produced at approximately 82 million tons per year (Kim et al., 2008). Especially food waste contains carbohydrate as high as 65% of its total solid, is rich in carbon content and, therefore, is a good substrate for producing ethanol. But, for food waste, little information shows its utilization for ethanol production.

Food waste is a complex biomass containing various components such as starchy, fatty, and cellulosic materials. These materi-

als are difficult to be utilized by ethanol producing microorganisms such as *Saccharomyces cerevisiae*. A pretreatment is required to hydrolyze the food waste and produce fermentable sugars. This can have significant implications on the configuration and efficiency of the rest of process and, ultimately, also the economics. The pretreatment for biomass can be carried out in different ways such as acid hydrolysis, heat treatment, and enzymatic hydrolysis (Chen et al., 2008; Jeihanipour and Taherzadeh, 2008; Kadar et al., 2004; Sanchez and Cardona, 2008; Alfani et al., 2000; Jordan and Mullen, 2007). Utility cost of enzymatic hydrolysis is low compared to acid hydrolysis and heat treatment because it is usually conducted at mild condition and does not have any corrosion problem. Recently, the cost of enzyme has been reduced substantially by using an advanced biotechnology.

The hydrolyzing enzyme works on starchy and cellulosic materials to yield the monomeric unit, glucose, which can be subsequently fermented to produce ethanol. The hydrolyzing enzyme usually operates with highest efficiency at a temperature of at least 45 °C and pH of 4.8. In contrast, the optimum operating conditions for the fermentation of glucose to ethanol with commercial yeast, *S. cerevisiae*, are reported to be 35 °C and pH 4.5. Due to different reaction conditions between enzymatic hydrolysis and ethanol fermentation, the separated hydrolysis and fermentation (SHF) process can be considered as an alternative. However, the glucose produced during hydrolysis of food waste may inhibit the enzyme activity. In order to reduce its inhibition effect, the glucose produced during hydrolysis has to be rapidly converted to ethanol by the yeast in the simultaneous saccharification and fermentation (SSF). This continuous removal of inhibitor from the reaction

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medium may minimize depression of the enzyme activity. This leads to possibility of high production rate and cost reduction resulting from use of only one reactor.

In this study, feasibility of ethanol production using food waste was investigated in a lab scale fermentor. Food waste was hydrolyzed by using commercial enzymes. The commercial enzymes including carbohydrase, glucoamylase, cellulase and protease were used to be compared by the hydrolysis efficiencies for the food waste mixture. Glucose produced from enzymatic hydrolysis of food waste mixture was fermented by inoculating *S. cerevisiae* to produce ethanol. Separated hydrolysis and fermentation was compared with simultaneous saccharification and fermentation in term of volumetric ethanol production rate and ethanol yield.

2. Materials and methods

2.1. Food waste

Food waste was obtained from a cafeteria at the Seoul National University of Science and Technology. It was crushed by using a mechanical mixer. Representative characteristics of the collected food waste mixture used in this study are shown in the Table 1. This characteristic was compatible with others that have been reported (Kim et al., 2008). The food waste was stored in refrigerator at 4 °C. For experiment, food waste was transferred to a reactor.

2.2. Enzymes and microorganisms

Carbohydrase (*Aspergillus aculeatus*, Viscozyme L.), glucoamylase (*A. niger*, Spirizyme Plus FG.), protease (*Bacillus licheniformis*, Alcalase 2.4 L FG, Subtilisin) and cellulase (*Humicola insolens*, Novozym.) were purchased from Novozymes, Denmark. The activity of carbohydrase was 80 Fungal Beta Glucanase Units (FBG)/ml. 1 FBG is the amount of enzyme required under the standard condition (30 °C, pH 5.0, reaction time 30 min) to degrade barley β -glucan to reducing carbohydrates with a reduction power corresponding to 1 μ mol glucose/min. The specific activity of glucoamylase was 400 AGU/g (one unit is defined as the amount of enzyme which hydrolyze 1 μ mol of maltose per minute under specified conditions). The specific activity of protease and cellulase were 2.5 Anson-U/g and 90 endoglucanase unit (EGU)/g, respectively. *S. cerevisiae* (KTCM No. 11293) was purchased from Korea type culture collection (KTC) because it is able to be cultivated at 35 °C and pH 4.5 (pH of food waste). Seed culture of *S. cerevisiae* was carried out in a 250 ml Erlenmeyer flask containing 100 ml YM medium (3 g/l of yeast extract, 3 g/l of malt extract, 5 g/l of peptone, and 10 g/l dextrose) at 35 °C and pH 4.5 for 20 h. Shaking speed was adjusted to 150 rpm. Cells were inoculated into 5 l fermentor (KF-5L, Kobiotech Co., Incheon, Korea).

2.3. Batch test for enzymatic hydrolysis

Enzymatic hydrolysis experiments were carried out in 200 ml serum bottles with working volume of 100 ml shown in Fig. 1. About 100 ml of food waste crushed by mechanical mixer was transferred to a serum bottle and adjusted to pH 4.5 (if necessary). After pH adjustment, food waste was sterilized at 121 °C for

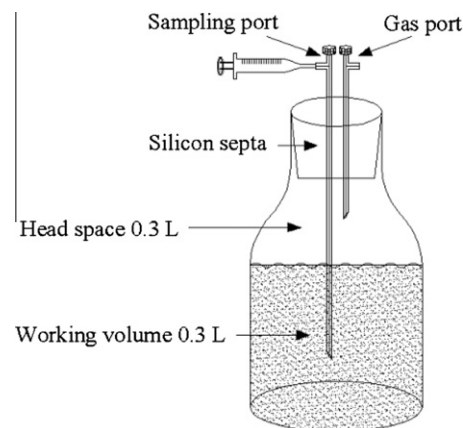


Fig. 1. Experimental set-up for batch test of enzymatic hydrolysis of food waste.

15 min. One hundred milliliter of food waste was mixed with the hydrolyzing enzymes from 0.1% to 10% on basis of volume. The hydrolysis was performed in a laboratory incubator at 150 rpm and 35 °C for 12 h. Liquid phase of food waste hydrolyzate obtained from enzymatic hydrolysis was analyzed to determine glucose concentration.

2.4. Enzymatic hydrolysis and ethanol fermentation in batch and continuous mode

Enzymatic hydrolysis and ethanol fermentation were carried out in 5 l fermentor with working volume of 3 l. Experimental system for enzymatic hydrolysis and ethanol fermentation is shown in Fig. 2. The amount of enzyme addition was determined from preliminary experiments. Batch and continuous experiments were started out with 1.5 kg of food waste mixed with 1.17 l of phosphate buffer solution by adding 30 ml of enzyme solution. The food waste mixture was sterilized at 121 °C for 15 min and controlled at pH 4.5 and 35 °C. In order to separate enzymatic hydrolysis from ethanol fermentation for a batch test, *S. cerevisiae* was inoculated into the batch reactor when glucose concentration reached to a maximum. For continuous operation of separate hydrolysis and fermentation (SHF), two fermentors were used for enzymatic hydrolysis and ethanol fermentation. Both fermentors were operated at pH 4.5 and 35 °C. For simultaneous saccharification and ethanol fermentation (SSF), both of enzyme and *S. cerevisiae* were added to the fermentor at the beginning of reaction. The fermentor was operated at pH 4.5 and 35 °C. Liquid phase of the food waste mixture was collected to determine glucose and ethanol concentration. To ensure anaerobic condition, all inoculations and manipulations were performed in the anaerobic chamber.

2.5. Analytical methods

During hydrolysis and ethanol fermentation, aqueous phase of food waste mixture was collected to determine glucose and ethanol concentration. Glucose concentration was measured by using liquid chromatography. Before analysis, aqueous samples were centrifuged at 1000 g for 10 min and filtered by using 0.45 μ m syringe filter. The filtered samples were analyzed by high performance liquid chromatography (HPLC Acme 9000, YoungLin, Korea) equipped with ELS detector. A separation of hydrolyzed products was done by using a guard column, Zorbax NH2(4.6 $\times$ 12.5 mm, 5 μ m, Agilent, US) and an analytical column, Zorbax carbohydrate(4.6 $\times$ 250 mm, 5 μ m, Agilent, US). The temperature of the column oven was maintained at 35 °C. The ELS detector was optimized for the analytes (2 l/min for a nitrogen gas flow). The

Table 1
Characteristics of food waste used in this study.

pH	Alkalinity (mg/l)	Volatile solid (g/l)	Total solid (g/l)	Soluble chemical oxygen (g/l)	Total chemical oxygen demand (g/l)
4.5–4.8	0.1–0.3	130–138	163–190	62–98	150–180

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