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Enzymatic hydrolysis of food waste and ethanol fermentation

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SUMMARY

Although food waste (FW) can serve as a valuable substrate containing large amounts of organic materials such as soluble sugar, starch, and cellulose, it is recognized as an environmental pollutant, and the hydrolysis of solids in FW still serves as a rate-limiting step in its biological processes. To evaluate a new potential application of FW as an alternative substrate for ethanol production through laboratory experiments, we investigated FW hydrolysis by using individual commercial enzymes and their mixtures; batch ethanol fermentation by *Saccharomyces cerevisiae*; and the effect of salt, which is inherently included in FW, on ethanol fermentation. A comparison of the glucose yields of the FW broth pretreated with amyloglucosidase, carbohydrase, and a mixture of both enzymes revealed that a higher glucose yield was obtained when the enzyme mixture was used (0.46 g g^{-1} of dry FW) than when amyloglucosidase (0.41) or carbohydrases (0.35) were used at 3 h from the initiation of the reaction. A high ethanol yield (0.23 g g^{-1} of dry FW) was obtained after 15 h of fermentation by *S. cerevisiae* by using the FW broth hydrolyzed by the enzyme mixture and was estimated to be nearly equivalent to the ethanol yields of lignocellulose biomasses. With regard to the effect of salt on ethanol fermentation, no alteration in the fermentation parameters was observed up to a salt content of 3% w/v. At a salt content of over 4%, however, substrate uptake and cell growth dramatically decreased, and a slight reduction in ethanol yield was observed. FW utilization for ethanol production by enzymatic hydrolysis and ethanol fermentation by *S. cerevisiae* suggests a promising practical approach to prevent environmental pollution and obtain a product of high value, ethanol. Copyright © 2008 John Wiley & Sons, Ltd.

KEY WORDS: food waste; enzyme; hydrolysis; ethanol fermentation; salt effect

1. INTRODUCTION

The global demand for ethanol has been increasing in recent years because of its wide use in chemical and motor-fuel industries, and its important role in reduction of greenhouse gas emissions. However, most of ethanol production is fermented using hexose sugars present in cane syrup and

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corn, which results in driving up the crop price [1]. Therefore, it is essential to research alternative and inexpensive substrate for ethanol production at a reduced cost.

Food waste (FW) discharged from households, restaurants, and refuse from the food industry accounts for approximately 23% of municipal solid wastes (MSWs) in Korea; in 2004, approximately 11 400 tonnes of FW per day were generated [2]. FW readily decomposes, generates odor and sometimes causes illness under natural conditions because of its high contents of biodegradable organic compounds and moisture (75–85%). MSWs, including FW, are usually incinerated or landfilled, but these processes generate many problems. Incineration facilities can be damaged by temperature fluctuations when FW with high water content is burned in a semi-continuous process. In addition, landfill space is limited, and uncontrolled fermentation of organic wastes in landfill causes emission of greenhouse gases, such as methane and carbon dioxide [3]. The major conventional recycling method for FW has been to employ it as animal feed and fertilizer. However, it is difficult to recycle FW efficiently because of the uncertainty with regard to the safety of its utilization as animal feed and a decrease in its demand as a fertilizer due to high salt content resulting from the traditional Korean food culture. Furthermore, direct landfill of raw FW was banned completely by the Korean government in 2005. Therefore, it is imperative to overcome the technological and systematic dilemma of the conventional recycling method for FW and simultaneously develop an environment-friendly recycling method that can convert FW to a high-value product such as bioethanol.

On the other hand, FW is characterized by a high organic content since it contains soluble sugars, starches, lipids, proteins, cellulose, and other compounds that make it a source of potential fermentative substrates. With an aim to develop a completely new recycling system for FW, Wang *et al*. [4] conducted a study on the preservation, deodorization, and suppression of the growth of putrefactive and food-poisoning bacteria by spontaneous fermentation. Moreover, there have been various biological processes employing FW as a substrate for the production of methane [5], hydrogen [6], lactic acid [7], and succinic acid [8] and biorefinery technologies to develop the purification of lactic and succinic acids [7,9] from FW fermentation broth. However, there are very few reports in the literature on the utilization of FW for ethanol production.

However, the hydrolysis of solids in FW still serves as a rate-limiting step in its application for biological processes. Nakamura and Sawada [10] investigated the effect of steam explosion on the physical and chemical changes in artificial household wastes. They reported that the explosion treatment beyond a specified stream pressure enhances the ethanol yield since the highly extractable components and low viscosity of the exploded products provide a high contact frequency of enzymes or cells with the substrate.

It was well known that NaCl affects ethanol fermentation by decreasing the cell growth and substrate uptake of *Saccharomyces cerevisiae*. Trainotti and Stambuk [11] reported that increasing the NaCl concentration significantly decreased the growth of *S. cerevisiae* in glucose and maltose media, and that particularly in case of maltose fermentation, the ethanol yield was significantly low at 0.7 M NaCl.

In the present study, the enzymatic hydrolysis of FW, batch ethanol fermentation by *S. cerevisiae*, and the effect of NaCl on each factor of ethanol fermentation were examined to estimate the feasibility of using FW as a substrate for ethanol production.

2. MATERIALS AND METHODS

2.1. Pretreatment of FW

The FW used in the present experiment was collected from a cafeteria in Gyeonggi Small and Medium Business Center (Suwon, Korea) during the summer. It was divided into four categories: vegetables (51% (w/w wet FW)), grains (22%), fish and meat (10%), and fruits (17%), approximately following the composition of Korean FW generated from various kinds of domestic and commercial kitchens. Wet FW was crushed in a lab blender (AM-11; ACE, Nissei, Japan) and

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Table I. Composition of food waste used in this study.

pH	4.2	Reducing sugars (w_{dm}%)	17.6
Moisture content (w%)	81.9	Crude protein (w_{dm}%)	21.1
Dry mass (w%)	18.1	Crude lipid (w_{dm}%)	8.3
Salt (w_{dm}%)*	1.7	Crude fiber (w_{dm}%)	14.9
Solid starch (w_{dm}%)	30.1	Ash (w_{dm}%)	5.0

*Weight percentage based on dry mass of food waste.

Table II. Characteristics of each enzyme used in this study.

Characteristics	Amyloglucosidase	Carbohydrase
Origin	*Aspergillus niger*	*Aspergillus aculeatus*
Product name	Spirizyme® Plus FG	Viscozyme® L
Activity	400 AGU g^{-1}*	100 FBGU g^{-1}*
Application and remarks	Hydrolysis of starch	Brewing industry
	Liquefaction of starch	Fruit water treatment
	Hydrolysis of grains	Cell-wall degrading enzyme

*The units of the enzyme activities of amyloglucosidase and carbohydrase that yield 1 μmol of glucose for 1 min from maltose and β-glucan, respectively.

sieved through a wire mesh (2×2 mm). Its characteristics are given in Table I in detail. The pH of the collected FW was very low, owing to a considerable amount of acidified or pickled food residues such as kimchi and the generation of volatile fatty acids during storage. The dry mass of FW contains various kinds of organic materials such as solid starch (30.1 w%), reducing sugar (17.6 w%), protein (21 w%), fiber (14.9 w%), and salt (1.7 w%).

2.2. Enzymes

Two types of commercial enzymes purchased from Novozyme (Novozymes Korea, Seoul, Korea) were employed for the enzymatic hydrolysis of FW. Their characteristics are shown in Table II. Amyloglucosidase (EC 3.2.1.3) extracted from genetically modified *Aspergillus niger* is commonly utilized for the hydrolysis of starch. Carbohydrase, a multi-enzyme complex containing arabinase, cellulase, β-glucanase, hemicellulase, and xylanase extracted from *A. aculeatus*, is used in the brewing industry and for fruit water treatment and cell-wall degradation.

2.3. Enzymatic hydrolysis of FW

One hundred grams of minced FW and 50 g of distilled water were mixed in a 250-mL Erlenmeyer flask. The pH was adjusted to 4.5 using 3 N NaOH. This mixture was used as a sample for the enzymatic hydrolysis of FW. First, the hydrolysis of FW using each enzyme separately was conducted at 50°C and 150 rpm for 3 h in a shaking incubator (DSK 512; Daeil Engineering, Korea) by adding different volumes of two commercial enzymes; for amyloglucosidase, we used control 0.0, 1.0, 2.0, 5.0, and 10.0 amyloglucosidase units (AGU) g^{-1} of dry FW, and for carbohydrase, control 0.0, 2.5, 5.0, 10.0, 20.0, and 40.0 fungal β-glucanase units (FBGU) g^{-1} of dry FW.

Second, based on the result of the performance of hydrolysis catalyzed by individual enzymes, 2.0 AGU of amyloglucosidase and 20.0 FBGU of carbohydrases per 1 g of dry FW were selected as the optimal amounts for the enzyme mixture. FW hydrolysis using this enzyme mixture was conducted under the same conditions as those used for hydrolysis using individual enzymes. All

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experiments were replicated three times, and the average values were evaluated. The performance of enzymatic hydrolysis was evaluated based on the glucose yield (Y_G) (g g^{-1} of dry FW); Y_G was calculated using Equation (1), where G indicates the glucose concentration:

$$Y_G = \frac{(G_{\text{final}} - G_{\text{initial}})\ (\text{g})}{\text{Substrate (g)}} \quad (1)$$

2.4. Yeast and ethanol fermentation

The *S. cerevisiae* strain KCTC 7107 purchased from KCTC (Korean Collection for Type Cultures, Daejeon, Korea) was used for the ethanol fermentations. Stock culture was maintained in yeast extract–peptone–dextrose (YPD) agar slants (Difco), which contained 1% of yeast extract, 2% of peptone, and 2% of dextrose and was stored at 4°C. Seed culture was prepared by inoculating a loop of stock culture to 30 mL of YPD broth in a 100-mL Erlenmeyer flask at 30°C in a static incubator for 24 h. The FW broth hydrolyzed by the enzyme mixture was sterilized at 121°C for 15 min and then used for batch ethanol fermentation. *S. cerevisiae* suspension (2% v/v; approximately 1E+07 cells mL^{-1}) was inoculated in 150 mL of FW broth in a 250-mL Erlenmeyer flask with a rubber stopper; it was incubated at 30°C at an initial pH of 4.5 and with mild agitation (100 rpm). The ethanol fermentation was estimated based on the ethanol yield ($Y_{E/S}$) (g g^{-1} of dry FW) obtained by Equation (2) [12]:

$$Y_{E/S} = \frac{\text{Ethanol produced (g)}}{\text{Substrate (g)}} \quad (2)$$

The effect of salt on ethanol fermentation was investigated using different FW samples that were prepared by adding varying amounts of NaCl to FW broth and control, which originally contained approximately 1.1% (w/v) of NaCl. The effect of salt was estimated by comparing fermentation parameters such as substrate uptake, cell growth, and ethanol yield of each FW sample under the same experimental conditions as those used in the above ethanol fermentation.

2.5. Analysis

Crude protein, lipid, and fiber contents were determined following the methods of AOAC [13]. Suspended solids in minced FW were washed twice with distilled water and filtered using a GFC filter. It was pretreated continuously according to the method used by Chandler *et al.* [14], and the solid starch was determined by the phenol sulfuric acid method [15]. Samples obtained from enzymatic hydrolysis and ethanol fermentation were centrifuged at 4000 rpm for 5 min and the supernatant was filtered through a chromato-disc filter (pore size = 0.45 μm); the reducing sugar was then analyzed by the 3,5-dinitrosalicylic acid method [16]. The glucose concentration was determined using a high-performance liquid chromatography system equipped with evaporative light-scattering detector (PL-ELS 2100; England). A 75% acetonitrile buffer solution was used as the mobile phase at an elution speed of 1.0 mL min^{-1} at 30°C. Ethanol concentration was measured using a gas chromatography-flame ionization detector (6890N system; Agilent Technologies, Hewlett-Packard, U.S.A.) equipped with an OPTIMA-624 column (50 m × 0.25 mm × 1.4 μm). The temperatures of the injector and detector were maintained at 220 and 200°C, respectively, and the column oven was isothermally operated at 50°C. Cell growth was measured using the dilution and plating method. After thorough dispersion, a 1-mL sample was serially diluted and plated (two plates per dilution) on YPD agar plates to obtain colony-forming units (CFUs). The plates were incubated at 30°C for 48 h, and the final colony count was calculated as the average of the CFUs of the two plates for the dilution containing 30–300 colonies per plate.

3. RESULTS AND DISCUSSION

3.1. FW hydrolysis by individual enzymes

FW normally consists of various polysaccharides, i.e. starch and cellulose, produced from grains and vegetables, respectively. A key factor in achieving high ethanol production is to convert

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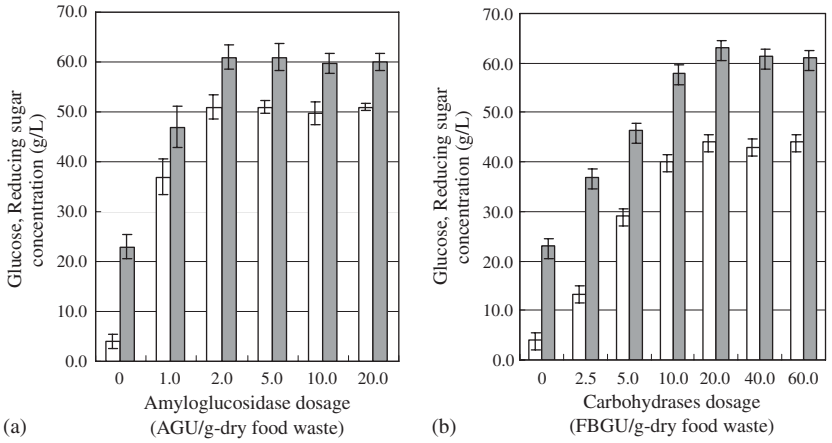

Figure 1. Profile of glucose and reducing sugar concentration in the products obtained by food-waste hydrolysis by amyloglucosidase (a) and carbohydrase (b) after 3 h of incubation. Symbols: ■, reducing sugar; □, glucose. The bar represents the standard deviation ($n = 3$).

polysaccharides into high monosaccharide content; monosaccharides are also fermentative sugars, e.g. glucose. We used amyloglucosidase and carbohydrase for the hydrolysis of starch and cellulose in FW, respectively.

Figure 1(a) and (b) shows the contents of glucose and reducing sugar extracted from the FW broth hydrolyzed by various units of amyloglucosidase and carbohydrase, respectively, after 3-h incubation. In the control samples, in which no enzyme was added, the amount of glucose increased to approximately 4.0 g L^{-1} after reaction. This appears to be a result of partial hydrolysis by the NaOH added for adjusting the pH to 4.5. A considerable amount of initial reducing sugar, i.e. approximately 23 g L^{-1}, was detected in the control. This indicates that FW itself contains a significant amount of water-soluble sugar, i.e. comparatively low-molecular-weight disaccharides, oligosaccharides, etc., which is extracted from the minced FW (17.6 w% in dry FW), as given in Table I. In case of amyloglucosidase, the concentration of glucose and reducing sugar noticeably increased with the increase in enzyme activity up to 2.0 AGU g^{-1} of dry FW and reached almost constant values of 52 and 61 g L^{-1}, respectively, at more than 2.0 AGU g^{-1} of dry FW (Figure 1(a)).

Figure 1(b) also indicates that both sugars were released at various doses of carbohydrases. The pattern of both sugars obtained using carbohydrases was similar to that obtained using amyloglucosidase. Approximately 44 and 63 g L^{-1} of glucose and reducing sugar, respectively, were produced at levels of more than 20.0 FBGU g^{-1} of dry FW. The difference in the amount of extractable glucose generated from FW hydrolysis by amyloglucosidase and carbohydrase may be attributed to the composition of FW, which contains a higher amount of starch (30.1 w% in dry FW) than of fiber (14.9 w%), as given in Table I.

3.2. FW hydrolysis by enzyme mixture and glucose yield

Based on the release of both sugars from the hydrolysis of FW catalyzed by individual enzymes, 2.0 AGU of amyloglucosidase and 20.0 FBGU of carbohydrase per 1 g of dry FW were selected and employed for the experiment of FW hydrolysis using the enzyme mixture. Figure 2 shows an outline of glucose and reducing sugar contents in accordance with the time course during FW hydrolysis catalyzed by the enzyme mixture. The concentration of both sugars dramatically increased till 2 h of incubation and reached almost constant values, of approximately 58 and 71 g L^{-1}, respectively, after 3 h of incubation. On the basis of these results, we selected the procedure involving 3 h of incubation using 2.0 AGU of

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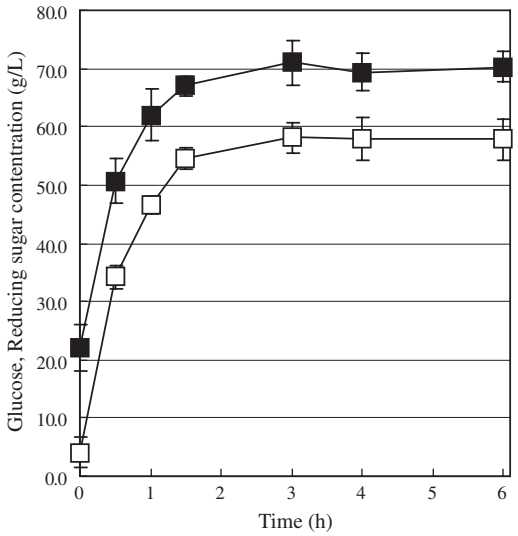

Figure 2. Glucose and reducing sugar concentration in the products obtained from food-waste hydrolysis by the enzyme mixture (amyloglucosidase, 2.0 AGU and carbohydrase, 20.0 FBGU per gram of dry food waste) according to time course. Symbols: ■, reducing sugar; □, glucose. The bar represents standard deviation ($n = 3$).

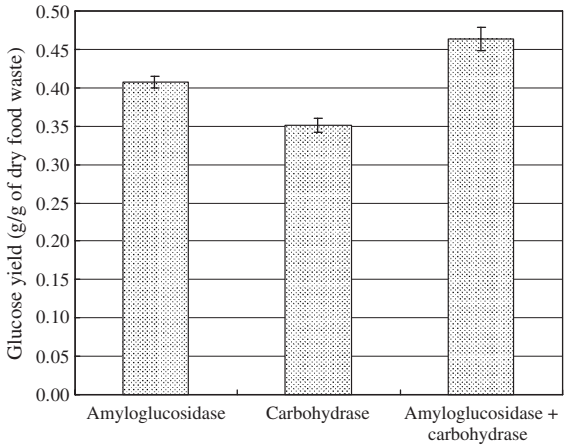

Figure 3. Glucose yields obtained from hydrolysis by amyloglucosidase, carbohydrase, and the enzyme mixture (2.0 AGU, 20.0 FBGU, and 2.0 AGU+20.0 FBGU per gram of dry food waste, respectively) after 3 h of the reaction. The bar represents standard deviation ($n = 3$).

amyloglucosidase and 20.0 FBGU of carbohydrase per 1 g of dry FW as the most suitable one for the enzymatic hydrolysis of FW and used it hereafter as a pretreatment prior to ethanol fermentation.

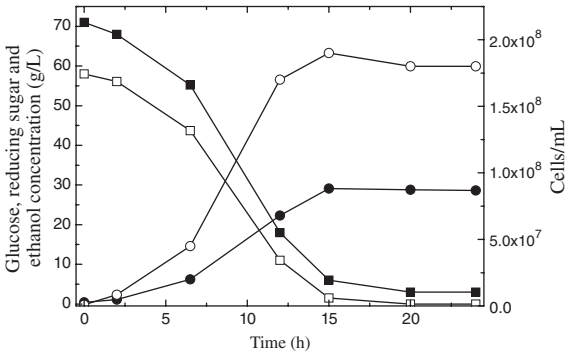

Figure 4. Ethanol fermentation of food waste hydrolyzed by the enzyme mixture. Symbols: ■, reducing sugar; □, glucose; ○, CFU; ●, ethanol.

Figure 3 presents the glucose yields evaluated from the FW hydrolysis by 2.0 AGU of amyloglucosidase, 20.0 FBGU of carbohydrase, and the enzyme mixture containing 2.0 AGU and 20 FBGU after a 3-h reaction under the same experimental conditions. The glucose yield ($0.46\,g\,g^{-1}$ of dry FW) obtained using the enzyme mixture was significantly higher than those obtained using amyloglucosidase and carbohydrase (0.41 and $0.35\,g\,g^{-1}$ of dry weight of FW, respectively).

3.3. Ethanol fermentation and potential ethanol yield of FW

Batch ethanol fermentation using *S. cerevisiae* was carried out for 24 h using the FW broth treated by the enzyme mixture. Changes in cell growth, consumption of glucose and reducing sugar, and ethanol yield in accordance with the time course were completely determined as illustrated in Figure 4. After a few hours of lag phase, the cell growth of *S. cerevisiae* continued until the exponential phase and reached the stationary phase (2×10^8 CFU mL^{-1}). After the stationary phase (assumed to be at 15 h), a slight reduction in cell density was observed, indicating nutrient depletion in the fermentation broth. The end of fermentation was indicated by the prompt assimilation and complete consumption of glucose accompanied by a concomitant increase in cell growth after 15 h and the nearly constant content of reducing sugar thereafter. The highest yield of ethanol (total

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Table III. Comparison of potential ethanol yields, processing temperature, and enzyme hydrolysis for food waste and raw biomasses [17].

Raw material	Temperature (°C) used for pretreatment/ enzymatic hydrolysis	Enzymes (type)	Ethanol potential ($g\,g^{-1}$)
Sucrose and starch			
Molasses	None	None	0.32
Sugar cane	None	None	0.28
Corn	130–160/52	Amylases	0.32
Wheat	130–160/52	Amylases	0.31
Rice	130–160/52	Amylases	0.34
Rye	130–160/52	Amylases	0.30
Barley	130–160/52	Amylases	0.31
Potato	130–160/52	Amylases	0.24
Lignocelluloses			
Bagasse	190–210/50	Cellulases	0.26
Corn stover	190–210/50	Cellulases	0.25
Wheat straw	190–210/50	Cellulases	0.23
Aspen	190–210/50	Cellulases	0.26
Willow	190–210/50	Cellulases	0.19
Spruce	190–210/50	Cellulases	0.25
Food waste*	None/50	Enzyme mixture†	0.23

*Our results.
†The mixture of amyloglucosidase and carbohydrase.

ethanol content, $29.1\,g\,L^{-1}$) was achieved at this time. Ethanol yield is defined as the ratio of the amount of ethanol produced to the amount of the mass of dry FW (according to Equation (2)); the ethanol yield in this experiment was approximately $0.23\,g\,g^{-1}$ of dry FW, which was slightly higher than that obtained by Nakamura and Sawada [10]. They reported that an ethanol yield of $0.20\,g\,g^{-1}$ of dry artificial household waste was obtained through simultaneous saccharification and fermentation of artificial domestic household waste pretreated by steam explosion.

The potential ethanol yield ($0.23\,g\,g^{-1}$ of dry FW) of FW is lower than that (approximately $0.30\,g\,g^{-1}$ of dry starchy substrates) of starchy biomass, i.e. molasses, sugar cane, corn, wheat, rice, rye, barley, and potato, but comparable to those (approximately $0.23\,g\,g^{-1}$ of dry lignocellulose substrates) of lignocellulose biomass, i.e. bagasse, corn stover, wheat straw, aspen, willow, and spruce (Table III) [17]. Depending on the technology employed, starchy and lignocellulose biomasses require more rigorous physical or chemical pretreatments prior to enzymatic reaction, i.e. high temperature, acidic treatment, and/or high pressure, in order to increase the saccharification efficiency and achieve a high level of ethanol fermentation [1]. As shown in Table III, high-temperature cooking of various feedstocks for bioethanol production involves high energy consumption and results in higher costs of ethanol production. In this regard, using FW is more advantageous than using starchy and lignocellulose biomasses since it is primarily derived from the remains of meals normally cooked in kitchens; therefore, no additional treatment of FW except for enzymatic hydrolysis will be required.

Nearly all fuel ethanol is produced by fermentation of corn glucose or sucrose since the conversion process of lignocellulose biomasses to ethanol still requires high cost, which results in driving up the crop price. In this regard, utilization of FW, organic waste, as feedstock for alternative energy resources could be a promising strategy for significantly reducing ethanol production cost.

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Table IV. Effect of salt on ethanol fermentation of food waste by *S. cerevisiae*.

Salt (% w/v)	Residual glucose ($g\,L^{-1}$)	Ethanol yield (%)*	Cells mL^{-1}
Control†	0.0	0.47	2E+08
2	0.0	0.47	2E+08
3	8.8	0.47	1E+08
4	16.7	0.45	6E+07
5	30.7	0.41	4E+07
6	40.4	0.39	2E+07

*Based on glucose consumption. All results were obtained for ethanol fermentation for 24 h by using a food-waste hydrolysate containing $58\,g\,L^{-1}$ of initial glucose concentration.
†Control medium had a salt content of 1.1% that was originally present in food waste.

3.4. Effect of salt on ethanol fermentation of FW

The influence of NaCl on ethanol fermentation was investigated by adding varying predetermined amounts of NaCl to the FW broth that initially contained approximately 1.1% w/v of NaCl and $58\,g\,L^{-1}$ of glucose. The effect of salt on ethanol fermentation was evaluated by comparing the amount of residual glucose, degree of cell growth, and ethanol yield after 24 h of incubation. No alterations in the level of glucose uptake, cell growth of *S. cerevisiae*, and ethanol yield were observed up to salt content of 3% w/v. At salt content of more than 4% w/v, however, the growth of *S. cerevisiae* was severely inhibited and gradually decreased; the glucose consumption dramatically decreased in accordance with the increasing salt content. Therefore, the residual glucose content at 4, 5, and 6% of NaCl was 16.7, 30.7, and $40.4\,g\,L^{-1}$, respectively. Ethanol yield slightly reduced, as shown in Table IV. Trainotti and Stambuk [11] also reported that while salt stress dramatically affected specific growth rate and cell mass of *S. cerevisiae,* ethanol yield was not significantly reduced in the yeast extract peptone media containing 2% w/v glucose at 0.7 or 1.4 M NaCl. Hirasawa *et al.* [18] observed that when *S. cerevisiae* was exposed to different concentrations of NaCl during its exponential growth, the specific growth rate radically decreased with the increasing salt content. These facts imply that although the presence of NaCl does not drastically affect ethanol yield, it inhibits the cell growth and substrate uptake so that eventually it extends the period of ethanol fermentation. Therefore, our results suggest that when FW broth contains more than 4% w/v of NaCl, dilution is required for achieving higher efficiency of ethanol fermentation.

4. CONCLUSION

Enzymatic hydrolysis of food waste (FW), batch ethanol fermentation, and the effects of salt on ethanol fermentation were experimentally investigated for estimating the feasibility of using FW as an alternative substrate for ethanol production. The following findings were obtained.

The experiments on FW hydrolysis by individual enzymes revealed that amyloglucosidase was more effective than carbohydrase for producing glucose (52 and $44\,g\,L^{-1}$, respectively). The hydrolysis performed by using 2.0 AGU of amyloglucosidase and 20.0 FBGU of carbohydrase per gram of dry FW for 3 h of incubation was found to be the most appropriate for FW hydrolysis in view of producing large amounts of fermentable sugars, glucose ($58\,g\,L^{-1}$) and reducing sugars ($71\,g\,L^{-1}$), and high glucose yield ($0.46\,g\,g^{-1}$ of dry FW).

High ethanol content ($29.1\,g\,L^{-1}$) was obtained from batch ethanol fermentation by *S. cerevisiae* using the FW broth pretreated with the enzyme mixture; the ethanol yield estimated based on the mass of dry FW corresponded to $0.23\,g\,g^{-1}$ of dry FW. This value is lower than that of starchy biomass (approximately $0.30\,g\,g^{-1}$ of dry mass) but is equivalent to that of lignocellulose substrates (approximately $0.23\,g\,g^{-1}$ of dry mass). However, considering that starchy and

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lignocellulose feedstocks require further processing at high temperatures prior to enzymatic reaction for ethanol production, using FW would be more economical than using other biomasses since no additional treatment except for the enzymatic hydrolysis is required for FW.

The cell growth and substrate uptake of *S. cerevisiae* were drastically hindered, and the ethanol yield slightly decreased at an NaCl content of over 4% w/v. This result indicates that when FW broth has a salt content of more than 4% w/v, it should be appropriately diluted for efficient ethanol fermentation.

In conclusion, FW was revealed to be a promising substrate for ethanol production via enzymatic hydrolysis and batch ethanol fermentation. Moreover, the environmental benefits of employing FW as an alternative energy resource for ethanol production could be significant driving force encouraging expanded use of biomass for energy production. The fundamental environmental reasons for employing FW, as ethanol production will be global greenhouse gas control and local or regional solid waste and water quality control. In addition, recycling FW for ethanol production could be a beneficial alternative to conventional incineration and landfill disposals of FW, which generated second environmental problems such as toxic or greenhouse gas emission, and underground-water contamination by leachate, respectively.

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