



Kinetic modeling of enzymatic hydrolysis of pretreated kitchen wastes for enhancing bioethanol production

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ABSTRACT

It is well known that use of low cost and abundant waste materials in microbial fermentations can reduce product costs. Kitchen wastes disposed of in large amounts from cafeterias, restaurants, dining halls, food processing plants, and household kitchens contain high amounts of carbohydrate components such as glucose, starch, and cellulose. Efficient utilization of these sugars is another opportunity to reduce ethanol costs. In this study, the effect of pretreatment methods (hot water, acid solutions, and a control) on enzymatic hydrolysis of kitchen wastes was evaluated using a kinetic modeling approach. Fermentation experiments conducted with and without traditional fermentation nutrients were assessed at constant conditions of pH 4.5 and temperature of 30 °C for 48 h using commercial dry baker's yeast, *Saccharomyces cerevisiae*. The control, which involved no treatment, and hot water treated samples gave close glucose concentrations after 6 h. The highest and lowest rates of glucose production were found as 0.644 and 0.128 (h^{-1}) for the control (or no-pretreated (NPT)) and 1% acid solutions, respectively. The fermentation results indicated that final ethanol concentrations are not significantly improved by adding nutrients (17.2–23.3 g/L). Thus, it was concluded that product cost can be lowered to a large extent if (1) kitchen wastes are used as a substrate, (2) no fermentation nutrient is used, and (3) hydrolysis time is applied for about 6 h. Further optimization study is needed to increase the yield to higher levels.

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1. Introduction

Food wastes discharged from restaurants, food production plants and household kitchens constitute a considerable proportion of municipal solid waste (MSW) all over the world. OECD (OECD, 2002) statistics based on seven countries; Mexico, Greece, Japan, USA, Norway, France and Belgium revealed that the MSW includes 35–40% organic waste, 28% paper, and minor amounts of metal (5%), glass (7%), and plastic (10%). In Turkey, the annual generation of MSW was reported as 26 million tons. Approximately 34% of the collected solid waste consists of kitchen waste. This results in 8.84 million tons of kitchen waste per year (TUIK, 2006). Kitchen wastes contain 2–3% cellulose, 40–55% starch, and 55–67% total sugar (Wang et al., 2008), which can be converted to fermentable sugars. Thus, attention has been directed towards processing of kitchen wastes to produce value added products such as lactic acid (Wang et al., 2005; Ohkouchi and Inoue, 2006), ethanol (Wang et al., 2008; Yan et al., 2011; Tang et al., 2008), and biogas (Zhang et al., 2007). Recent studies also presented viable results for use of MSW in unsorted form to recover the fermentable sugars through enzymatic hydrolysis (Jensen et al., 2010, 2011).

Bioethanol is traditionally produced from sugar and starch containing crops such as potato, rice, and sugar cane in Brazil and corn in America and China (Thomsen et al., 2003; Varga et al., 2005). Starch is easily converted to glucose by commercial enzymes and subsequently fermented to ethanol by *Saccharomyces cerevisiae*. Since these materials are important food sources and abundant/low cost lignocellulosic wastes can reduce production costs, investigations have been performed to use unsorted MSW, wheat straw, crop residues, and kitchen wastes as alternative substrates (Jensen et al., 2011; Kim and Dale, 2003; Lissens, 2004; Nigam, 2000).

A pretreatment method is usually needed to have effective enzymatic hydrolysis when lignocellulosic materials are used (Sewalt et al., 1997; Kim and Holtzapple, 2005; Sun and Chen, 2007). The purpose of various pretreatment methods are to separate or remove lignin, hemicelluloses, and cellulose, reduce the crystalline structure of cellulose, and increase the surface area, which all improve penetration of hydrolytic enzymes (Dawson and Boopathy, 2007; Jørgensen et al., 2007). Alkaline and acid pretreatments have been successfully used (Dawson and Boopathy, 2007; Cara et al., 2008; Yu and Zhang, 2004). Dawson and Boopathy (2007) treated postharvest sugar cane residue with acid (H_2SO_4) and alkaline (H_2O_2) solvents. They reported that acid hydrolysis produced higher amounts of ethanol. Yu and Zhang (2004) produced high concentrations of ethanol from acid hydrolyzed cotton wastes. Alternative pretreatment methods are also

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available, such as hot water and steam pretreatment (Laser et al., 2002). In studies found on kitchen waste, which mostly focused on starchy fraction, no pretreatment method has been used prior to enzymatic hydrolysis (Kumar et al., 1998; Wang et al., 2008; Tang et al., 2008).

Kinetic models play an important role in describing performance and attributes of a process and can easily be used to control and predict these attributes. It is commonly agreed that more valuable information can be extracted from experimental data by simple inspection, e.g. assuming first order dynamics, performing statistical analysis, etc. The goal in kinetic modeling varies with the attributes of a chemical or biological process. As per pretreatment prior to enzymatic hydrolysis, short time and economy of the method are of great value while it is aimed to improve yields at the subsequent hydrolysis step (Zheng et al., 2009). Increasing yields of enzymatic hydrolysis would also improve the yields of ethanol.

Efficient utilization of sugars is also an opportunity to reduce costs (Taherzadeh and Karimi, 2008). Current literature is focused on use of (1) lignocellulosic and agro-industrial wastes and (2) various microbial strains in fermentation to improve ethanol production. In addition, pretreatment methods need to be settled down for commercial use. Therefore, the aim of this work was primarily two folds; (1) to evaluate the effect of two pretreatment methods (acid and hot water) and a control on glucose production during enzymatic hydrolysis, and (2) to study the kinetics of glucose production to select the best time and type of pretreatment for improvement of enzymatic hydrolysis prior to fermentation.

2. Materials and methods

2.1. Raw material

The kitchen wastes were collected from food courts of Middle East Technical University (METU), Ankara, Turkey. The plastic, metal, and glass pieces were separated if present in the waste, and remaining organic fractions were combined and ground in a chopper to form the composite substrate for experiments. The kitchen waste consisted of leftovers and peels of fruit and vegetables (potato, parsley, corn, mushroom, lettuce, zucchini, eggplant, etc.), bakery wastes (pasta, pizza, cookies), and others (coffee residues, beans, cereal foods). The composite waste was stored at 4 °C until use in a day or two.

2.2. Enzymes, inoculum, and fermentation medium

The enzymes used in liquefaction and saccharification steps were α -amylase (*Aspergillus oryzae*, A6211-1MU), amyloglucosidase (*Aspergillus niger*, AMG) (10115), cellulase (*Trichoderma viride*, C1794-10KU), and -glucosidase (almonds, 49290), which were all purchased from SIGMA-Aldrich. The activity of enzymes reported by the supplier was considered in our study.

A commercial dry baker's yeast *S. cerevisiae* was purchased from a local store and kept in a refrigerator until use. The dry yeast was dispersed in sterile water at room temperature at a concentration of 10 g/L (g dry bakers' yeast/liter of DI water) and 10 mL of this was used as an inoculum without any further cultivation and added to 90 mL of fermentation medium to obtain 10% (v/v) fraction (Chiang et al., 1981).

Fermentation medium contained pretreated and hydrolyzed waste, the yeast, *S. cerevisiae*, and fermentation nutrients where necessary. The fermentation nutrients used were: 6 g/L yeast extract, 1.5 g/L KH_2PO_4 , 1 g/L $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, and 4 g/L $(\text{NH}_4)_2\text{SO}_4$.

2.3. Pretreatment methods

The ground and mixed kitchen waste was subjected to pretreatment with two solutions (hot water and dilute acid) and a control

(no pretreatment). For dilute acid pretreatment, sulfuric acid at two concentrations of 1% and 4% (v/v) was added to the kitchen waste. Samples were kept at 60 °C for 3 h in all pretreatment methods (Li et al., 2007).

2.4. Enzymatic hydrolysis

For liquefaction of the starchy portion, α -amylase was added (120 U/g dry substrate) to the waste and kept at 95 °C for 1 h at 100 rpm and pH 5.5. Starch based oligosaccharides and the cellulosic fraction were then processed simultaneously adding the enzymes amyloglucosidase (120 U/g dry substrate) (Wang et al., 2008), cellulase (8 FPU/g dry substrate) and β -glucosidase (50 U/g dry substrate) (Krishna and Chowdary, 2000) after the liquefied mixture was cooled to 55 °C. Glucose production as representative of reducing sugars was monitored until it reached a constant value to indicate completion of hydrolysis under tested conditions (Kim et al., 2011; Yan et al., 2011). Agitation was applied at 100 rpm. To terminate the enzymatic activity, samples were boiled for 15 min at each time of sampling.

2.5. Fermentation

Fermentation experiments were conducted in 250 mL Erlenmeyer flasks with a working volume of 100 mL. The ratio of culture volume to flask size was kept constant according to other studies (Arapoglou et al., 2010; Man et al., 2010). The yeast was added at a ratio of 10% (v/v) to the fermentation mixture under aseptic conditions. Before inoculation, the flasks and medium were sterilized by autoclaving. Sulfuric acid (0.5 M) was used to adjust the initial pH to 4.5. The temperature and agitation speed were maintained constant throughout the experiment at 30 °C and 150 rpm, respectively. The fermentation period was kept at 48 h.

2.6. Analytical methods

The collected waste was analyzed for moisture, ash, protein, fat and total carbohydrate contents. Moisture and ash contents were determined according to analytical gravimetric methods (AOAC, 2001). Protein content was estimated as 6.25 times the Kjeldahl nitrogen. Glucose was determined by Dinitro Salicylic Acid (DNS) method (Miller, 1959). Ethanol concentration was measured by GC (SHIMADZU, Kyoto, GC-14A #124457), using 1%, 3%, and 5% (v/v) ethanol standards (Toro-Vazquez and Perez-Briceno, 1998).

3. Results and discussion

3.1. Composition of raw material

The composition of kitchen waste is shown in Table 1. The average moisture content of the kitchen waste was about 65% (w/w), which led to 35% (w/w) of total dry matter. Approximately 60% of the total dry matter was the carbohydrate fraction, which proved that the kitchen waste could be used as a valuable raw material for ethanol production.

Table 1
Characteristics of kitchen waste used in the experiments.

Constituent	Content (% w/w) ^a
Moisture	64.4 ± 5.9
Total solids	35.6 ± 5.9
Protein	4.5 ± 0.2
Fat	8.8 ± 0.1
Ash	1.8 ± 1.3
Total CHO's	20.5 ± 4.6

^aResults belong to two replicates.

3.2. Effect of pretreatment methods on glucose production

As stated previously, the kitchen waste used in this study contained a wide variety of leftovers in raw and cooked form as well as whole edible parts and peels of fruits and vegetables. Therefore, in order to improve the yield of enzymatic hydrolysis a pretreatment method was used (Table 2). Each method was followed by enzymatic hydrolysis conducted under the same conditions. Thus, the difference in final glucose concentrations was concluded to be due to the pretreatment method. This was also proved by the initial glucose concentrations after each pretreatment method (Table 2). According to the tabulated values, it was found that hot water pretreated and no pretreated samples (control) had higher glucose concentrations than the acid pretreated samples ($p < 0.05$). The two acid levels (1% and 4%) had similar glucose concentrations ($p > 0.05$).

The change in glucose concentration during enzymatic hydrolysis of pretreated samples over time is given in Fig. 1. The concentration of glucose increased gradually with time and reached a constant value within 6 h for all pretreatment methods. The highest glucose concentration was obtained as 64.8 g/L from the unpretreated samples after 6 h and followed by hot water pretreatment at 56.7 g/L. These values were found statistically similar ($p > 0.05$) right at the edge of Tukey's confidence interval, which even can be practically taken as different. The two acid concentrations (1% and 4%) also gave statistically similar results ($p > 0.05$) but still lower than no pretreatment case ($p < 0.05$), releasing glucose concentrations of 51.5 and 45.4 g/L, respectively. Furthermore, the glucose amount of 1% acid was similar to the amount of hot water ($p > 0.05$). Thus, the acid treatment was also concluded to be an effective method for mixed kitchen wastes as reported by Dawson and Boopathy (2007), who also applied acidic pretreatment on post-harvest sugarcane residue before fermentation. Alternatively, saccharification yield using the highest glucose level (64.8 g/L) was calculated as 0.70 g glucose/g dry waste (or 70%). This value was found to be close to the study of Aswathy et al. (2010), who observed 71% yield from hyacinth biomass and higher than the yield of Kim et al. (2011), who reported 63% at the end of enzymatic hydrolysis carried out at 35–45 °C for 12–48 h using higher enzyme loads and different enzyme combinations compared to this study. Glucose yield can be improved using higher enzyme loads, higher temperature in acidic pretreatment, and trying different solid loads and time in further studies (Aswathy et al., 2010; Zhang et al., 2010).

The following equation considering first order dynamics was used to calculate the rate of glucose production during enzymatic hydrolysis after each pretreatment method:

$$C = C_m(1 - e^{-kt}) \quad (1)$$

where C is the change in glucose concentration (g/L) with respect to initial glucose concentration (i.e. $C(t) - C_0$); C_m is the maximum glucose accumulated at an infinite hydrolysis time; k is the rate constant of glucose production (h^{-1}).

In order to fit the data in Fig. 1 to Eq. (1), all values were first transformed by subtracting the initial concentration from glucose

Table 2
Glucose concentrations after each pretreatment.

Pretreatment method	Glucose concentration (g/L)
1% Acid	13.1 ± 2.9
4% Acid	12.9 ± 2.1
Hot water	22.7 ± 1.3
NPT ^a	24.7 ± 0.7

^aNPT: No pretreatment method (control).

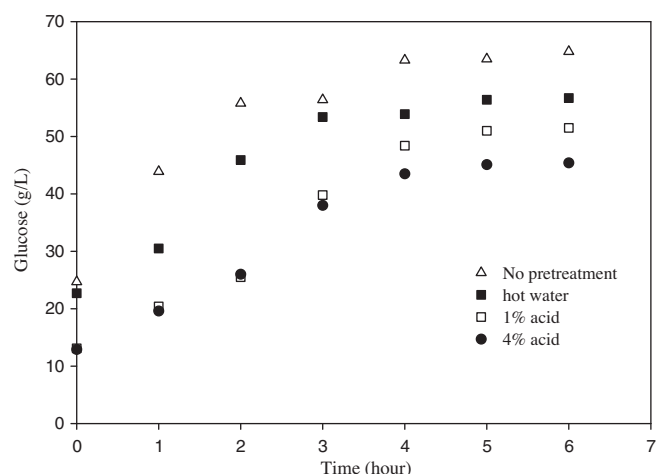


Fig. 1. Glucose production from hydrolyzed wastes subjected to different pretreatments.

produced at an instantaneous time, t , and the resulting plots with model fits are presented in Fig. 2. Notice that all curves passed through the origin in the transformed form. The results of kinetic analysis for each pretreatment method are given in Table 3. Expectedly, the kinetic results revealed that the rate of glucose production was higher for 4% acid than the rate of 1% acid, although the final glucose concentrations for the two acid pretreatments were similar (Fig. 1). The rate constants in a descending order can be written as $k_{\text{NPT}} > k_{\text{HW}} > k_{4\%} > k_{1\%}$ for no pretreatment (NPT), hot water, 4% acid, and 1% acid, respectively. When the time constant (τ), which is defined as the time required for the glucose level to reach 63.2% of the final steady level during hydrolysis process, is considered, the NPT method had the smallest time constant value (1.55 h) while the 1% acid method had the highest value (7.81 h), as per the rate constants.

3.3. Effect of fermentation nutrients on ethanol production

Upon completion of the enzymatic hydrolysis step, the pretreated and hydrolyzed samples were subjected to batch ethanol fermentation at pH 4.5 and 30 °C for 48 h to check for the fermentability of the hydrolysates and to evaluate if chemical nutrients are necessary or not in the fermentation medium. The experimental plan and the results of fermentation experiments are shown in

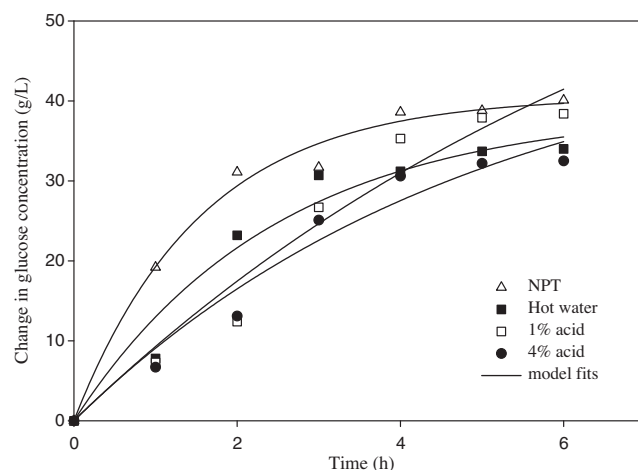


Fig. 2. Transformed results for glucose production after each pretreatment method.

Table 3

First order kinetics model parameters for the enzymatic hydrolysis after each pretreatment method.

Pretreatment	C_m (g/L)	R^2	k (h ⁻¹)	τ (h)
NPT	40.54	0.989	0.644	1.55
Hot water	38.90	0.962	0.406	2.46
1% Acid	77.52	0.958	0.128	7.81
4% Acid	49.81	0.963	0.201	4.98

Table 4

Fermentation results of kitchen wastes subjected to different pretreatment methods.

Pretreatment	Glucose before fermentation (g/L) ^a	Ethanol (g/L) ^a	Yield (g ethanol/g glucose) ^a
Hot water + EH + WM	56.7 ± 3.4	14.6 ± 2.3	0.26 ± 0.02
Hot water + EH + NM	56.7 ± 3.4	17.2 ± 2.2	0.31 ± 0.02
NPT + EH + WM ^b	64.8 ± 1.8	17.4 ± 1.3	0.27 ± 0.01
NPT + EH + NM ^b	64.8 ± 1.8	23.3 ± 0.6	0.36 ± 0.00

^aResults are averages of two replicates.

^bEH = Enzymatic hydrolysis, WM = With fermentation medium, NM = No fermentation medium, and NPT = No pretreatment method (control).

Table 4. The initial glucose concentration, final ethanol concentration and yield values are also given in Table 4. Statistical analysis of the results indicated that the ethanol concentrations and the yields were similar for the samples with nutrients added and samples without nutrients regardless of the pretreatment method applied ($p > 0.05$). It was found that the highest ethanol concentration and yield values were obtained as 23.3 g/L and 0.36 g/g from samples without nutrients added and unpretreated samples, which was significantly higher than the samples pretreated with hot water and supplemented with nutrients. This result proved that there was no need for nutrient addition and also indicated the advantage of starting fermentation with higher glucose concentration. This result also meant that a productivity of about 0.49 g/L h and 69% of the theoretical yield were achieved. Similar crude fermentation trials with low ethanol concentration (4.4 g/L) were performed to check for the fermentability of hydrolysates (Aswathy et al., 2010) without any attempt at optimization. The ethanol yield of these hydrolysates was improved in another study (Uncu and Cekmecelioglu, 2011). These results supported the idea that fermentation of kitchen wastes was feasible without adding the traditional fermentation nutrients. Thus, it can be concluded that nutrients already present in the kitchen waste were sufficient for functioning of *S. cerevisiae* to produce ethanol. Our results are consistent with results of others. Wang et al. (2008) reported fermentation of *S. cerevisiae* with kitchen garbage at pH values of 4–6.63 and temperatures of 26.8–40 °C. They obtained ethanol concentration of 22.13 g/L at 26.8 °C within 48 h at pH 5, which can be taken as similar to conditions of our study. Their productivity for these conditions was 0.46 g/L h, close to our value. It should also be noted that Wang et al. (2008) used a pure yeast culture and working volume of 150 mL for fermentation experiments. In batch experiments using *S. cerevisiae* KF-7 Tang et al. (2008), reported 29.9 g/L of ethanol in 24 h at pH 4.5 and 30 °C from 64 g/L of glucose using 300 mL of working volume. This difference was explained by the yeast strain, working volume and thus the higher initial glucose amounts they used in their study. In another study of Kumar et al. (1998), mixed bakery wastes, potato chips, and grain flour was fermented by distiller's yeast at pH 5 and 30 °C. They obtained ethanol concentration of 245.4 g/kg from 609.95 g/kg of glucose after 15 h, which gives ethanol yield of 0.40 g/g. Therefore, it can be concluded that kitchen waste was successfully utilized in ethanol fermentation without adding fermentation nutrients using dry baker's yeast.

Alternatively, if the same food wastes were to be used for methane generation, the concentration of methane to be reached and the relevant yield would be found as 12 g/L and 0.19 g/g, respectively, multiplying the stoichiometric yield of methane (0.27 g/g) by the fraction of theoretical yield achieved for ethanol in this study (~0.70) and using the initial glucose concentration of 64.8 g/L. Although these values are lower than those of ethanol, the calorific value of 0.19 g/g of methane will be approximately the same as 0.36 g/g of ethanol. Thus, the market value can indicate which is more feasible to produce, currently ethanol's being higher. However, the need for both is continuously growing due to different places of use.

4. Conclusions

It is evident that a pretreatment method prior to enzymatic hydrolysis is not strictly needed for production of high glucose levels from the kitchen wastes tested in this study. The hydrolysis time is as short as 6 h. Addition of fermentation nutrient is found to be not necessary for yeast to produce ethanol. The nutrients present in the original kitchen waste provide enough nutritive medium for *S. cerevisiae* to produce high yields of ethanol. Thus, it is concluded that ethanol production costs could be lowered using kitchen wastes as substrate and by excluding the fermentation nutrients traditionally used in fermentation practice.

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