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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.

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 $MgSO_4 \cdot 7H_2O$, and 4 g/L $(NH_4)_2SO_4$.

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 Salisylic 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

[a]Results belong to two replicates.