The role of enzyme loading on cellulose hydrolysis of food waste

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Introduction

Food waste (FW) discharged from households, restaurants, and refuse from the food industry accounts for approximately 43% of municipal solid wastes in Greece. 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. Therefore, it is imperative to develop an environment-friendly method that can convert FW to a high value product such as bioethanol. There are several 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. In the present study, the enzymatic hydrolysis of FW was examined to estimate the feasibility of using FW as a substrate for ethanol production. The aim of this paper was to gain better understanding of the dependence of cellulose hydrolysis upon enzyme loading.

Materials and Methods

The household food waste utilized in the present study came from houses of the Papagos-Cholargos and Aspropyrgos Municipalities, Attica, Greece. The biowaste was source separated and was transferred to the Unit of Environmental Science and Technology (UEST), School of Chemical Engineering, NTUA, where it was dried by a GAIA food waste dryer (model GC-100). The dried material was milled to an average particle size of 3 mm in a laboratory mill. The raw material had the following composition (%w/w dry base): cellulose 8.6 ± 0.3, hemicellulose 9.4± 0.2, starch 8.6 ± 0.3, fats and oils 11.7 ± 0.4, proteins 13.5 ± 0.4, total soluble solids 35.0 ± 1.9, ash 13.2 ± 0.3. moisture 9.1 ± 0.1.

All chemicals used were of analytical grade. Non-commercial enzymatic formulations NS22109 (amylase) and NS22177 (cellulase) were kindly donated by Novozymes (Denmark). Total cellulase activity (FPU) was measured against filter paper by the standard IUPAC method, as described by Ghose (1987), and found to be 227 FPU/mL.

Moisture, extractives, ash, total starch, cellulose, fat in raw and pretreated materials were analyzed following NREL laboratory analytical procedures (Sluiter et al., 2012). All the samples were centrifuged at 3600 rpm for 10 min and filtered before being analyzed. In the liquid phase, glucose concentration was determined using a commercially available kit (Biosis S.A., Athens, Greece) that employed the Glucose Oxidase–Peroxidase (GOX–PER) method. All analyses were performed in duplicate.

Enzymatic hydrolysis

Enzymatic hydrolysis of untreated and pretreated raw material solids (20%w/w) was performed in 250 mL Erlenmeyer flask. The initial pH of the mixture was around 4,8 and was not adjusted since it was within the range of optimum pH for the operation of the enzymes. Firstly, the hydrolysis of starch in biowaste using NS22109 (amylase) (36μL/g starch) was conducted at 65ºC for 1h in a water bath for all samples based on non-published research of the NTUA group. The hydrolysis of cellulose was the next step. Cellulase (NS22177) was used. Enzyme loadings of 110, 170, 220, 280, 560, 830 and 1120 μL/g cellulose were used. The reaction mixtures were incubated at 50ºC and 150 rpm for 5 h in a water bath. At the end of each experiment, the samples were analyzed. The performance of enzymatic hydrolysis was also evaluated based on the glucose yield (Y_G) (gg^{-1} of dry FW); Y_G was calculated using Equation (1), where G indicates the glucose concentration:

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

Second, based on the result of the performance of hydrolysis catalyzed by NS22177, a dosage of cellulase was selected as the optimal amount for the conduction of kinetic experiments. FW kinetic experiments were conducted under the same conditions as those used for enzymatic hydrolysis mentioned before. All experiments were replicated three times, and the average values were evaluated.

Results and discussion

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 polysaccharides into high monosaccharide content; monosaccharides are also fermentative sugars, e.g. glucose. In this study, NS22109 and NS22177 were used for the hydrolysis of starch and cellulose in FW, respectively.

Figure 1 presents the glucose yields as well as the % cellulose degradation evaluated from the FW hydrolysis of all enzyme loadings. Cellulose degradation ranged from 26 to 50%. At low loadings (110, 170, 220 μL/g cellulose), the % cellulose degradation was almost steady (~27%), whereas further increasing of enzyme loading resulted in increase of cellulose hydrolysis. The total glucose yield is fractionated according to its origin. Thus, it derives from the sum of glucose produced from hydrolysis of starch and cellulose. It is evident that the amount of glucose yields

attributed to the first step of enzymatic hydrolysis of starch is almost identical for all cases examined, since in all cases the % degradation of starch reached up to 92%.

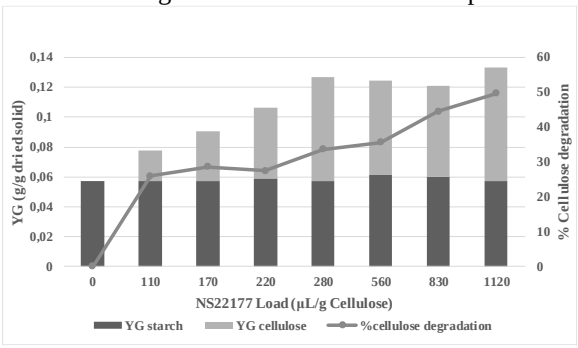

Figure 1. Glucose yields obtained from hydrolysis by NS22109 and NS22177 after 6 h of the reaction. The bar represents standard deviation (n=3).

The glucose yield (0.146 g g^{-1} of dry FW) obtained using the enzyme dosage of 280 μL/g cellulose is the first enzyme dosage that almost reaches the maximum glucose yield. Further increase of enzyme dosage just slightly increases the glucose yield. More specifically, by increasing the enzyme loading 300%, the resulting glucose efficiency increased by just 4%.

On the basis of these results, the enzyme dosage of 280μL/g cellulose was selected as the most suitable one for the enzymatic hydrolysis of FW and used it hereafter at the kinetic experiments.

Figure 2 shows an outline of glucose content in accordance with the time course during FW hydrolysis catalyzed by 280μL NS22177 /g cellulose. The concentration of free glucose dramatically increased till 2 h of incubation and reached almost constant values, of approximately 30 gL^{-1} after 5 h of incubation. Cellulose degradation ranged from 17 to 52% following an exponential pattern. On the basis of these results, 5 hours incubation time with cellulase NS22177 were selected as the optimal time for the operation of the non-commercial enzyme provided.

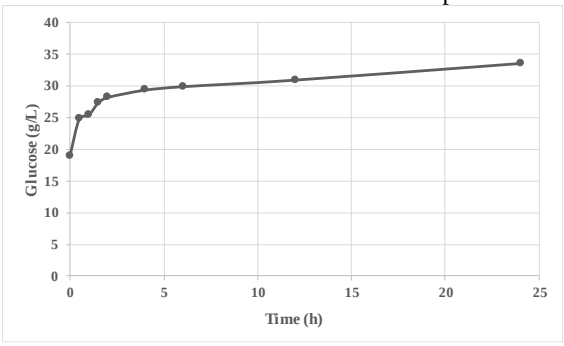

Figure 2. Glucose concentration in the products obtained from food-waste hydrolysis by cellulase NS22177 according to time course. The bar represents standard deviation (n=3).

Conclusions

Enzymatic hydrolysis of food waste (FW) was experimentally investigated for estimating the feasibility of using it as an alternative substrate for ethanol production. The following findings were obtained. The experiments on FW hydrolysis by cellulase NS22177 revealed that it was more effective for producing glucose (0.146 g glucose g^{-1} of dry FW), if a dosage of 280 μL/g cellulose were used. The hydrolysis performed by using 280 μL NS22177 /g cellulose for 5 h of incubation was found to be the most appropriate for FW hydrolysis in view of producing large amounts of fermentable glucose (30 gL^{-1}).

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