Effect of pretreatment techniques on enzymatic hydrolysis of biowaste

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Introduction

Food waste (FW) is an organic waste discharged from various sources, e.g. households, cafeterias, restaurants. Given its organic-rich nature, FW should be considered as a useful resource for producing bioethanol. However, microorganisms generally cannot directly assimilate the nutrients in FW without proper pretreatment. A pretreatment is required to hydrolyze the food waste and produce fermentable sugars. Obviously, high-concentration of glucose favors the subsequent bioethanol production. A pretreatment stage could have significant implications on the configuration and efficiency of the rest of the process and, ultimately, also the economics. The pretreatment for biomass can be carried out in different ways such as acid hydrolysis and heat treatment. In order to make the enzymatic hydrolysis of FW more efficient and maximize sugar release, different pretreatment methods should be examined. The present study aims to compare the effects of six pretreatment methods vis-a-vis improving enzymatic hydrolysis of FW.

Materials and Methods

The household FW 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. Enzymatic formulations NS22109 (amylase) and NS22177 (cellulase) were kindly donated by Novozymes (Denmark).

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, TOC, TN were also measured according to standard methods (APHA, 2005) while glucose concentration in the liquid phase 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.

Pretreatments of biowaste

Six different thermal, sonochemical, electrochemical and chemical pretreatments were applied in this study.

(A) Hydrothermolysis: Samples (10% w/w solid) were placed into an autoclave (ISOLAB Laborgeräte GmbH). Temperature was set at 105°C for 1h.

(B) Sonolysis - Sonochemical oxidation: Samples (10% w/w solid) were exposed to ultra-sonication for 1h. A Branson Ultrasonics Sonifier S-450 Digital Ultrasonic Cell Disruptor/Homogenizer operating at a fixed frequency of 20 kHz and power output 225 W was employed.

Electrochemical oxidation: Samples (10% w/w solid) were electrochemically pretreated for 1h. Experiments were performed at constant power supply adjustment at continuous 39V and 1A electrical current. Two different sets of electrode classes were used. For pretreatment C, catalytic electrochemical oxidation - electrocatalysis, this single-compartment, squared cell comprised of a boron-doped diamond (BDD) anode (Adamant Technologies SA, Switzerland; B/C 1000 ppm) and a stainless-steel cathode. For pretreatment D, electrochemical oxidation - Electrolysis, a graphite anode and a stainless-steel cathode were used. The active surface area of the cathode and anode in the liquid phase was [25 mm x 15mm] for all of the electrodes.

(E) Sono-electro-chemical oxidation/Sono-electrolysis: Samples (10% w/w solid) were exposed for 15 minutes to continuous 225Watt and 20kHz ultrasound radiation, and then they were exposed to continuous 39V and 1A electric current for 15 minutes, through a first class electrode set [Graphite (Anode) & Stainless Steel (Cathode)].

(F) Solid-Liquid fat extraction: The oil of the dried food waste was extracted by means of Soxhlet extraction.

Enzymatic hydrolysis of pretreated biowaste

Enzymatic hydrolysis of untreated and pretreated raw material solids was performed in a 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. The hydrolysis of cellulose was the next step.

Cellulase (NS22177) (304μL/g cellulose) was used at 50ºC for 5h in a water bath. At the end of each experiment, the samples were analyzed. All experiments were carried out in triplicate and average data are shown.

Results and discussion

In terms of solid fraction composition, different variations were observed in starch compared to the raw material depending on the pretreatment. These results are shown in Table 1.

Table 1. Main components of solid and liquid fractions after different pretreatments of dried biowaste. Hydrothermolysis (A), Sonolysis (B), Electrocatalysis (C), Electrolysis (D), Sono-electrolysis (E) and Solid-Liquid fat extraction (F).

	Solid Fraction (%)		**Aqueous Liquid Fraction (g/100g raw material)**		
Pretreatment	Starch	Cellulose	TOC	TN	Free Glucose
No pretreatment	7,39	7,16			
A	9,86	16,54	13,42	1,31	2,14
B	9,53	18,10	11,66	0,55	1,80
C	10,85	17,38	10,57	0,52	1,38
D	13,06	17,74	10,77	0,55	1,60
E	11,24	17,73	11,00	0,54	1,64
F	9,34	15,06	-	-	-

- No aqueous fraction derives from this pretreatment.

In all cases, starch was solubilized from 26,8 to 42,8% releasing from 1,38 to 2,14 g glucose/100g raw material, whereas cellulose solubilization and degradation proved much lower than was experienced by starch, resulting in a relative increase in cellulose percentage in the solid compared to raw material. The higher the solubilization and reduction of solids, the greater the cellulose composition increase of the pretreated solid. The percentage of cellulose in samples from all pretreatments underwent a sharp increase of 50-60%, a rise which was due to the drastic decrease of 40-50% in solids composition caused by the high solubilization with the pretreatment steps.

Figure 1 shows the effect of the different pretreatments on the percentages of starch and cellulose degradation and glucose release after 6 h of enzymatic hydrolysis. Glucose concentrations in the hydrolysates were higher than those of the control (No pretreatment) for all the experiments, except in hydrolysates from pretreatment A.

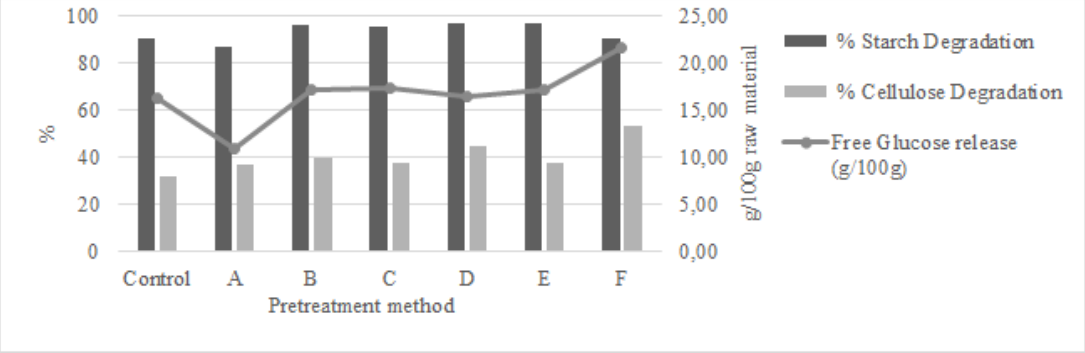

Figure 1. Starch and cellulose degradation and glucose release after 6 h of enzymatic hydrolysis of dried and pretreated food waste. Hydrothermolysis (A), Sonolysis (B), Electrocatalysis (C), Electrolysis (D), Sono-electrolysis (E) and Solid-Liquid fat extraction (F).

The highest glucose concentrations were found in hydrolysates from food waste pretreated with method F, with 21,66 g glucose/100 g raw material. Glucose concentration in the control hydrolysate was slightly lower than in hydrolysates B-E, yet still proved quite significant, revealing that food waste drying could stand as a satisfactory pretreatment method by itself.

Conclusions

In this study, fat extraction (method F) was considered the most suitable pretreatment for food waste, as high sugar yield and starch and cellulose degradations were obtained in enzymatic hydrolysis. Thermal autoclaving proved unsatisfactory as a pretreatment method since glucose yield was lower even for the control experiment, implying possible inhibitory compounds production. It was also revealed that food waste drying could stand as a satisfactory pretreatment method by itself.

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