# Stepwise verification and upscaling process for bioethanol production from sourceseparated food waste

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Keywords: bioethanol yield, enzymatic saccharification, factorial design, pilot plant, simultaneous saccharification

fermentation

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## Introduction

The management of biowaste frames a serious environmental, economic and social issue, given that one-third of food production is wasted annually worldwide. In fact, the main source contributing to food waste are households, in the case of developed economies. A promising way of management is to use household biowaste as a feedstock for ethanol production. Bioethanol is a renewable and sustainable liquid biofuel that is expected to have an auspicious future facing the current global energy crisis and the deteriorating quality of the environment. In this work, optimization and upscaling of ethanol production from real source separated biowaste using new enzymatic formulations developed by Novozymes was carried out.

### **Materials and Methods**

The raw material used within this paper was source separated biowaste that was compiled in the Municipality of Vari-Voula-Vouliagmeni in Greece. Then it was delivered to NTUA, School of Chemical Engineering, Unit of Environmental Science and Technology (UEST), where it was submitted to a simultaneous drying and milling process by a GAIA (GC-100) waste dryer. The initial moisture of the feedstock was around 76%. The characteristics of the dried raw material were as follows (%w/w dry basis): residual moisture 6.59  $\pm$  12.61, volatile solids 88.38  $\pm$  1.84, starch 7.63  $\pm$  3.94, cellulose 16.13  $\pm$  3.60, hemicellulose 9.07  $\pm$  7.23, fats and oils 13.14  $\pm$  2.14, acid soluble lignin 2.11  $\pm$  2.46, acid insoluble residue 11.90  $\pm$  4.38, water soluble solids 34.43  $\pm$  4.45.

All chemicals used were of analytical grade. Spirizyme XL which is an amylolytic formulation and noncommercial NS87014 which is a cellulolytic enzyme were provided by Novozymes (Denmark). The activity of Spirizyme XL was measured equal to 2337 U/mL (Xiao et al., 2006). Similarly, the activity of NS87014 was measured equal to 333 FPU/ mL (Ghose, 1987). S. cerevisiae was utilized as fermentation yeast.

The NREL laboratory analytical procedure was followed for the determination of lignin, cellulose and hemicellulose in biowaste (raw and pretreated) (Sluiter et al., 2012). Glucose, volatile fatty acids and ethanol were determined in the liquid fraction photometrically. All analyses took place in duplicate.

### Lab-scale

Within this study, simultaneous saccharification fermentation (SSF) was studied as fermentation mode. Initially, a  $2^3$  factorial experiment was designed at lab scale aiming to evaluate the impact of the process variables (amylase, cellulase and yeast dosages) to ethanol yield, Y<sub>EtOH</sub>. SSF at lab scale was conducted at 35 °C and 10% solid loading, based on preliminary experiments. In Table 1, the controlling parameters of the factorial experiment along with its levels are given.

Parameter	Low level (-)	High Level (+)	Center
SpirizymeXL (µL/g starch)	20	60	40
NS87014 (µL/g cellulose)	100	250	175
S. Cerevisiae (%)	1	3	2

Table 1. Controlling parameters and levels of the factorial experiment

The mean ethanol yield for all cases performed was 80.12% while the highest efficiency (84.73%) was obtained at the center of the experimental design ( $40\mu$ L/g starch for Spirizyme XL) and 175  $\mu$ L/g cellulose for NS87014 and 2% of TS yeast). Thus, these conditions were applied in a first scale-up step in 4L bioreactor where different solid loadings were applied from 10% to 30% with a step of 5%. From these experiments, the optimum results were at 25% solid loading with an ethanol yield of 84.58% and ethanol concentration equal to 37.5 g/L. Consequently, it was decided the pilot trials to be conducted under SSF fermentation mode at 35 °C for 24 h, 25% solids loading, Spirizyme 40 $\mu$ L/g starch, NS87014 175 $\mu$ L/g cellulose and 2% S. Cerevisiae.

### **Pilot plant**

The experiments of pilot scale were performed in a bioconversion pilot plant within the premises of UEST, which consists of two agitated horizontally rotating vessels (200L each) made of stainless steel. These reactors

may work independently under different operating conditions. Their temperature is controlled by water recirculation within their double walls. A distillation pilot unit is used to recover the produced ethanol at 70°C with the aid of low vacuum. The pilot plant operation is controlled via a Programmable Logic Controller.

During the saccharification and fermentation processes, with a view to better monitor the process, samples were collected at an hourly basis and were characterized in terms of ethanol and glucose. Ethanol yield  $Y_{EtOH}(g/g$  theoretical ethanol) was set as the optimization parameter for the bioprocess (Table 2).

Trials	Loading (%w/w)	Ethanol concentration (g/L)	Y <sub>EtOH</sub> (g/g)
1	25	34.66	0.87
2	25	20.40	0.58
3	25	39.26	0.89
4	25	39.00	0.74
5	25	42.00	0.86
6	25	30.00	0.91
7	25	40.00	0.99

Table 2. Bioethanol concentration and yield during the pilot trials

From Table 2 it is evident that the ethanol concentration produced by food waste presents satisfactory values in comparison with the results of (Kiran et al., 2015), (Konti et al., 2020), (Alamanou et al., 2015), (Matsakas et al., (2014) and (Kim et al., 2008) that observed bioethanol concentrations as high as 58 g/L, 53.9 g/L, 23.12 g/L, 42.78 g/L and 57.5 g/L respectively.

The mean degradation of starch ranged from 89 to 96% while the respective percentage of cellulose was  $73\pm6\%$ . Moreover, it was feasible to recover over 85% of the bioethanol produced via the distillation unit.

Finally, it is worth noting that, after the physicochemical characterizations, the results showed that the produced bioethanol is a high purity biofuel according to the EN 15376:2012, with purity of 99.55% v/v, and suitable for the production of biofuel E10 (reference gasoline + 10% bioethanol), according to the EN 228:2012 for unleaded petrol 95 RON.

#### Conclusions

The upscaling of bioethanol production at pilot scale by applying SSF was efficiently achieved. It was proved that the enzymatic mixtures utilized can treat efficiently at low temperatures ( $35^{\circ}$ C) the source-separated biowaste. Therefore, the yield of ethanol production and the degradation of structural polysaccharides achieved are very promising for the viability of the process. Finally, the purity of the ethanol recovered from the process reached 99.55% v/v ethanol.

#### Acknowledgements

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 818308.

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