# Bioethanol production from bakery waste

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## Introduction

During the last few decades, demand for alternative sources of fuels has increased due to the excessive consumption of fossil fuels. Ethanol is considered as one of the most promising renewable fuels that can replace fossil-based transportation fuels. Bio-ethanol can be obtained from sucrose-containing feedstocks, starchy materials and lignocellulosic biomass. Bread is a starchy material and a rich source of easily extractable fermentable sugars. It is one of the most heavily wasted food products in the developed world and is a particularly serious problem in most European countries. The global **annual production of bread is 100 million tons** and it has been approximated that, globally and annually, **10% of bakery products are wasted**.

The aim of this study was to investigate the ethanol yield from bakery waste using two different experimental procedures: separate hydrolysis and fermentation (SHF) process and simultaneous saccharification and fermentation (SSF) process, in order to optimize the production of ethanol using factorial design. Experiments were carried out under a variety of operational conditions defined by three independent variables in the SHF process (hydrolysis temperature, enzyme quantity, solid loading) and two independent variables in the SSF process (enzyme quantity, solid loading), while hydrolysis time, fermentation time, fermentation temperature and quantity of yeast were kept constant. Thus, an analysis of the potential, challenges and technical advances in bioethanol production from bakery residues was provided.

## Results & Discussion

Bakery waste was obtained from local bakeries. After its delivery, it was dried, ground and stored at room temperature until use. The substrate was characterized to determine its composition. Starch content was 62 – 64 % of dry matter, while free glucose was 0.8 % of dry matter.

### Separate Hydrolysis and Fermentation (SHF)

In the SHF process, the enzymatic hydrolysis lasted 1 hour, in different hydrolysis temperatures, enzyme dosages of Spirizyme and solid loadings according to the factorial design (Table 1). In the hydrolysate, 2% w/w yeast S. Cerevisiae was added for 48 hours at 35℃.

**Table 1: Factorial Design of SHF trials.**

| Parameter | Low level (-) | High Level (+) | Center |
|---|---|---|---|
| Spirizyme excel (μL/ g starch) | 20 | 60 | 40 |
| Temperature (ºC) | 35 | 65 | 50 |
| Loading (%) | 10 | 20 | 15 |

Table 2 presents the saccharification and ethanol yields of all SHF trials. The optimum results were obtained at 65℃, 60 μL/g of initial starch and 20 % solid loading resulting at 91% saccharification yield. During the fermentation step, complete consumption of glucose took place, providing a final ethanol concentration of 76 g/L, which corresponds to ethanol yield of 0.34 g/g initial dry solid. In spite of the fact that different hydrolysis conditions led to lower saccharification yields after 1 hour of hydrolysis, it seems that ethanol yields remain very high, with the highest ethanol concentration reaching 92 g/ L. This fact demonstrates that saccharification is also achieved at 35 ℃ and at lower enzyme dosages.

**Table 2: Saccharification and Ethanol Yields of SHF trials.**

| A/A | Spirizyme (μL/g starch) | Temp. (ºC) | Loading (%) | Saccharification yield (%) | Ethanol yield (%) |
|---|---|---|---|---|---|
| 1 | 20 | 35 | 10 | 48.14 | 91.85 |
| 2 | 20 | 35 | 20 | 58.88 | 99.97 |
| 3 | 20 | 65 | 10 | 81.31 | 85.66 |
| 4 | 20 | 65 | 20 | 76.54 | 82.68 |
| 5 | 60 | 35 | 10 | 41.43 | 97.90 |
| 6 | 60 | 35 | 20 | 67.23 | 95.56 |
| 7 | 60 | 65 | 10 | 86.67 | 91.71 |
| 8 | 60 | 65 | 20 | 90.97 | 82.70 |
| Center | 40 | 50 | 15 | 87.29 | 73.15 |

### Simultaneous Saccharification and Fermentation (SSF)

SSF was executed for starch degradation and ethanolic fermentation for 24h at 35ºC with Spirizyme excel according to a $2^2$ factorial experiment (Table 3) and 2% w/w yeast S. Cerevisiae.

**Table 3: Factorial Design of SSF trials.**

| Parameter | Low level (-) | High Level (+) | Center |
|---|---|---|---|
| Spirizyme excel (μL/ g starch) | 20 | 60 | 40 |
| Loading (%) | 10 | 20 | 15 |

SSF trials results showed that similar ethanol yields were obtained in each experiment, indicating the insignificant effect of solid loading and enzyme dosage on ethanol yield. However, ethanol concentration reached up to 92 g/L, namely 0.37 g /g initial dry solid.

The residues from the experiments presented above were fully characterized to determine the degradation of starch. As expected, starch was converted to glucose, which, in turn, was fully consumed achieving **99 % starch degradation**.

The results obtained in the present study proved that the SSF process could lead to comparably high efficiencies. This process is advantageous considering that SSF is less energy demanding, less time consuming and more cost efficient than SHF.

Moreover, the overall conversion efficiencies indicate the potential of bakery waste as a biomass for **large scale bioethanol production**. Thus, an experiment was conducted on pilot scale (14 kg bakery waste) under SSF conditions with 20% solid loading, enzyme dosage 20 μL/g starch at 35 ℃ for 48 h. The highest ethanol concentration observed was 100 g/ L after 31 hours (Figure 1).

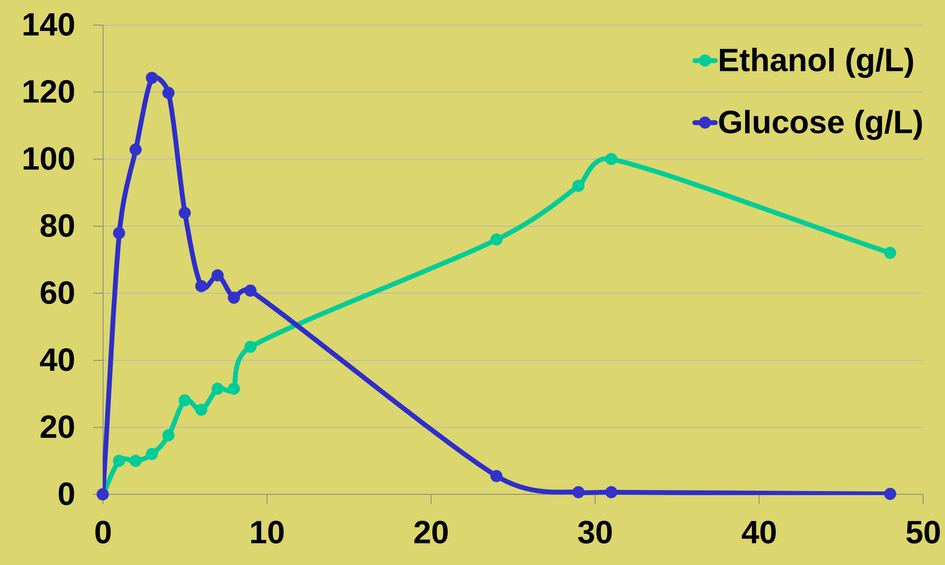


**Figure 1: Time profile of glucose consumption and ethanol production of SSF pilot trial with bakery waste 20% solid loading, enzyme dosage 20 μL/ g starch at 35 ℃ for 48 h.**

## Conclusions

In conclusion, the valorization of bakery waste via ethanolic fermentation provides an innovative solution for organic waste management and contributes to the sustainable production of bio-based products, such as bio-ethanol. Based on this study, bread waste possesses great potential from an economic viewpoint and demands further research for optimization of this process.

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