Bioethanol production from bakery waste

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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 (Pietrzak & Kawa-Rygielska, 2014). It is one of the most heavily wasted food products in the developed world and is a particularly serious problem in most European nations. 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 (Narisetty, et al., 2021).

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.

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 in bread was measured using Megazymes method (www.megazyme.com, 2020) and it was 62 – 64 % of dry matter, while free glucose was 0.8 % of dry matter.

In the SHF process, the hydrolysis lasted 1 hour, in in different hydrolysis temperatures, enzyme dosages of Spirizyme and solid loadings according to the factorial design presented in Table 1. In the hydrolyzed bread waste, 2% w/w yeast, S. Cerevisiae was added and left for ethanolic fermentation for 48 hours at 35 °C. The optimum results were obtained at 65 °C, 60 $\mu L\, g^{-1}$ of initial starch and 20 % solid loading resulting at 91% saccharification yield. After the fermentation step, complete assimilation of glucose took place, providing final ethanol concentration of 76 $g\, L^{-1}$, which corresponds to ethanol yield of 0.34 $g\, g^{-1}$ of initial dry solid.

Table 1. Factorial Design of SHF trials.

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

In spite of the fact that different hydrolysis conditions led to lower saccharification yields after 1 hour of hydrolysis, it seems that ethanol yields in all cases were very high, with the highest ethanol concentration reaching 92 $g\, L^{-1}$. This fact demonstrates that saccharification is also achieved at 35 °C and at lower enzyme dosages (Table 2).

Table 1. Results of SHF process at **35** °C hydrolysis temperature, enzyme dosage $\mathbf{20\ \mu L\, g^{-1}}$ of initial starch, **20** % solid loading, **35**°C fermentation temperature, 1 h of hydrolysis, 48 h of fermentation.

Variant	Definition	Results
Ethanol Concentration	Ethanol/Liquid $(g\, L^{-1})$	92
Ethanol Yield	Ethanol/Initial dry bread $(g\, g^{-1})$	0.37
Starch Degradation	Degraded starch/Initial starch $(g\, g^{-1})$	0.99

Therefore, experiments were carried out under SSF conditions too. These results showed that similarly high ethanol yields could be obtained in the SSF experiments, indicating the insignificant effect of solid loading and enzyme dosage on ethanol yield. However, ethanol concentration reached up to 92 $g\, L^{-1}$, namely 0.37 $g\, g^{-1}$ of initial dry solid, (Table **2**).

Table 2. Results of SSF process with enzyme quantity $\mathbf{60\ \mu L\ g^{-1}}$ of initial starch $\mathbf{20}$ % solid loading, $\mathbf{35}$℃ fermentation temperature, 48 hours of fermentation.

Variant	Definition	Results
Ethanol Concentration	$\mathrm{Ethanol/Liquid\ (g\,L^{-1})}$	92
Ethanol Yield	$\mathrm{Ethanol/Initial\ dry\ bread\ (g\,g^{-1})}$	0.37
Starch Degradation	$\mathrm{Degraded\ starch/Initial\ starch\ (g\,g^{-1})}$	0.99

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 which 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 quantity $20\ \mu\mathrm{L\,g^{-1}}$ of initial starch at 35 ℃ for 48 hours. The highest ethanol concentration observed was $100\ \mathrm{g\,L^{-1}}$ after 31 hours (Figure $\mathbf{1}$).

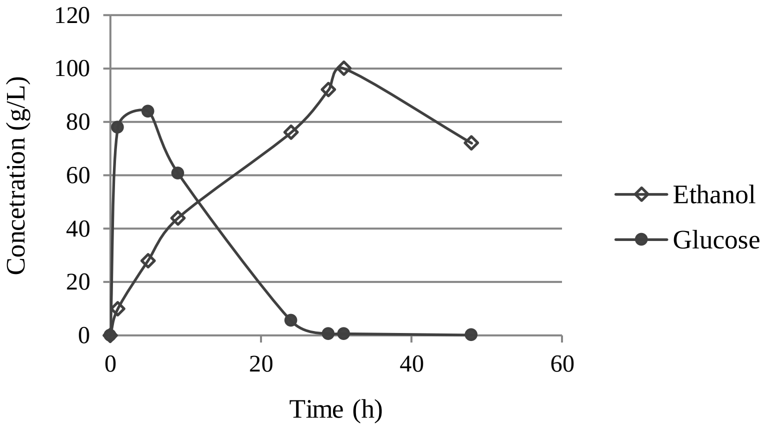

Figure 1 – The profile of glucose consumption and ethanol production of bakery waste (14 kg dry matter) on pilot scale in SSF conditions with $\mathbf{20}$ % solid loading, enzyme quantity $\mathbf{20\ \mu L\ g^{-1}}$ of initial starch at $\mathbf{35}$ ℃ for 48 hours.

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 (Gmoser, Sintca, Taherzadeh, & Lennartsson, 2019). 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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References

www.megazyme.com. (2020). Co. Wicklow, Leinster, Ireland. Retrieved November 2021, from https://www.megazyme.com/

Gmoser, R., Sintca, C., Taherzadeh, M. J., & Lennartsson, P. R. (2019, August 2). Combining submerged and solid state fermentation to convert waste bread into protein and pigment using the edible filamentous fungus. *Waste management*, pp. 63-70.

Narisetty, V., Cox, R., Willoughby, N., Aktas, E., Tiwari, B., Matharu, A., et al. (2021). Recycling bread waste into chemical building blocks using a circular biorefining approach. *Sustainable Energy & Fuels*, 4842–4849.

Pietrzak, W., & Kawa-Rygielska, J. (2014, June 12). Ethanol fermentation of waste bread using granular starch hydrolyzing enzyme: Effect of raw material pretreatment. *Fuel*, pp. 250-256.