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**BIORREFINERY, BIOECONOMY AND CIRCULARITY**

# **The integrated production of ethanol and 1,3-propanediol from enzymatic hydrolyses of food wastes in the context of biorefineries**

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## **ABSTRACT**

The sustainable management of Urban Solid Waste (USW) is one of the most relevant global environmental challenges. Food waste (FW) constitutes a significant fraction of USW. One way to valorize FW is to produce biofuels and high-value-added chemicals. Therefore, this study aimed to produce 1,3-propanediol (1,3-PDO) from vinasse obtained after the ethanolic fermentation of FW. Following the chemical characterization, it was produced the enzymatic hydrolysate to be fermented by *Meyerozyma caribbica*, which produced 26.88 g/L of ethanol and 6.36 g/L of glycerol. After ethanolic distillation, *Lentilactobacillus diolivorans* produced 1,3-propanediol with 74.28% efficiency. This shows that the integrated production of ethanol and 1,3-PDO is a promising alternative for the biorefinery of FW.

**Keywords:** Biomass. Waste. Bioprocess.

## **1 INTRODUCTION**

Solid Urban Solid Waste (USW) management is one of the main problems affecting populations worldwide, and food waste (FW) constitutes a significant fraction of USW. The Food and Agriculture Organization of the United Nations estimates that approximately 1.3 billion tons of food waste are discarded worldwide every year<sup>1</sup>. In Brazil, approximately 45.3% of municipal solid waste is composed of organic materials, a large part of which is food-derived<sup>2</sup>. The organic matter in food waste contains a variety of carbon sources such as carbohydrates, proteins, and lipids, whose enzymatic hydrolysis provides monomers such as monosaccharides, amino acids, fatty acids, and glycerol, which can easily be converted to value-added products using appropriate microorganism<sup>3</sup>. Thus, monomers, such as glucose and glycerol, resulting from enzymatic hydrolysis of FW can serve as substrates to produce compounds of industrial interest, such as ethanol and 1,3-propanediol<sup>4,5</sup>. Since food waste hydrolysates might contain both glucose and glycerol, simultaneous production of ethanol and 1,3-PDO can be achieved. The integrated process tends to establish what is conceptualized as a biorefinery. This industrial approach integrates processes to valorize biomass sources and generate different products. In this context, this study aimed to apply lab tests to evaluate the reliability of an integrated simultaneous process that converts glucose to ethanol and then glycerol to 1,3-PDO from food waste hydrolysates.

### **2 MATERIAL & METHODS**

Food waste was provided by the company Logica Ambiental. The biomass was dried (65ºC) and crushed (20 mesh) so that it could be enzymatically hydrolyzed using 15% w/v solid load, α-amylase (50 U/g) and amyloglucosidase (150 U/g) for 48 hours at 55 °C. Biomass has also been characterized<sup>6</sup>. To the enzymatic hydrolysate, 10% w/v of yeast *Meyerozyma caribbica* was added to convert carbohydrates into ethanol for 48 hours at 30 °C<sup>7</sup>. Samples were taken at 0, 2, 6, 12, 24, and 48 hours and the tests were performed in triplicate. After this stage, the fermentation was distilled and vinasse was used as a fermentation medium to produce 1,3-PDO by *Lentilactobacillus diolivorans* for 16 hours at 37 ºC (samples were collected every 4 hours), without stirring, and vitamin B12 was added to act as a co-factor. All the liquid fractions were analyzed by high-performance liquid chromatography, using an Aminex<sup>®</sup> column (HPX-87H, Bio-Rad, USA) and 5mM H<sub>2</sub>SO<sub>4</sub> as mobile phase with a flow rate of 0.6 mL/min at 35°C. The metabolites were detected using the refractive index (RID).

# **3 RESULTS & DISCUSSION**

Chemical characterization of the food waste biomass showed 85% moisture content and 15% dry mass. The dry mass was composed of 20.68% ( $\pm$  0.97) proteins, 13.64% ( $\pm$  1.63) lipids, and 11.52% ( $\pm$  0.89) extractive content. The biomass from this first extraction yielded 69.73%  $\pm$  3.88 glucan (starch and cellulose), 2.22%  $\pm$  0.89 lignin and 9.88%  $\pm$  0.97 ash. Taheri et al.<sup>8</sup> reported a hydrolysate containing approximately 27% carbohydrates, with 11.7% lipids and 13.5% proteins, while Modak et al.<sup>9</sup> reported contents of 45%, 25%, and 12% for the same components in different wastes. Due to the diversity of food waste sources, large differences are always expected for their constituents due to their origin, region, time of year, culture, economy, and climate of each country<sup>10</sup>.

After determining the carbohydrate content, hydrolysis was performed using enzymatic reactions. According to the conditions analyzed, the enzyme treatment produced a hydrolysate with 69 g/L glucose with 59.43% efficiency. Fermentation was performed, using *Meyerozyma caribbica*, producing approximately 27 g ethanol/L (Figure 1a), with an ethanol yield (YE) of 0.44 g/g, and 85% fermentation efficiency. Thus, fermentation resulted in an alcoholic solution of 3.4% (v/v). Ethanolic fermentation of food waste enzymatic hydrolysates have been reported in the literature with values varying from 75 g ethanol/L and 0.47 g/g<sup>11</sup> to 144 g/L and  $0.44$  g/g<sup>4</sup>.

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**Figure 1** Production of ethanol (1A) and 1,3-propanediol (1B) from the enzymatic hydrolysate of food waste.

At the end of the fermentation stage, 1,3-PDO is produced. The results showed that 1,3-PDO was produced to a titer of 2.59 g/L, representing a yield of 0.53 g per gram of glycerol consumed and 74.28% of production efficiency. During this stage, the bacterial fermentation biomass increased and reached 2.2 g/L. The fermentation kinetics can be divided into three phases (Figure 1B). In the first four hours, 1,3-PDO was rapidly produced at about 0.75 g/L.h driven by the large consumption of glucose was accelerated at 2.3 g/L.h to its complete consumption (Figure 1B). However, external glycerol was marginally utilized by the cells at this stage. This indicates that 1,3-PDO was produced from glycerol produced by the bacteria's central metabolism during glucose metabolization. Then, the cultures experienced a long second phase, with slight glycerol consumption and 1,3-PDO production. Finally, the cultures entered the third phase of accelerated glycerol uptake and slow1,3-PDO production in the absence of external glucose (Figure 1B). Alphy et al.<sup>5</sup> produced 25.90 g/L of 1.3-PDO from crude glycerol with sweet sorghum juice, using *Lactobacillus brevis* NIE9. Gupta et al.11, using *Clostridium butyricum* L4, produced 16.8 g/L of 1,3-PDO.

#### **4 CONCLUSION**

Efficiency of 85.4% for ethanol and 74.28% for 1,3-PDO were achieved. These values allow us to conclude that the subsequent integration of these processes can generate products at a lower cost and with more than 70% efficiency, even without adding nutrients that favor their production. Therefore, the integrated production of ethanol and 1,3-PDO is a promising alternative for biorefinery of food waste.

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