**D-Lactic Acid Fermentation from Orange Peel Waste: Effect of Initial Hydrolysate Concentration.**

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**Highlights**

* D-Lactic acid production is carried out from cheaper carbon and nitrogen source
* 83.16 g/L of D-LA are obtained at highest OPW hydrolysate concentration
* 2.35 g/Lh of productivity is attained at the lowest OPW hydrolysate concentration
* A high OPW concentration improves D-LA final titer but worsens its productivity

**1. Introduction**

D-lactic acid (D-LA) is an organic acid produced mainly by lactic acid bacteria that has gained interest due to its application in the synthesis of poly-lactic acids (PLAs), a biodegradable and environmentally friendly polymer [1, 2]. The main obstacle to produce PLAs is the manufacturing costs caused largely by its monomer production (D-LA). This makes it necessary the use of cheaper carbon source like orange peel waste (OPW) [1]. The OPW is considered an optimum candidate for this applications due to: its high content in free sugars easily extracted (about 10 % in glucose, fructose and sucrose), its high content in polymers (37 % of cellulose and 11 % hemicellulose), which, after hydrolysis, release fermentable carbohydrates, and its low content in lignin (7.5 %), which inhibits enzyme action during hydrolysis and microbial activity in fermentation process. These residues are also notable for its high percentage of humidity [3].

**2. Methods**

OPW was milled down to 1–2-mm particle size, with a subsequent enzymatic hydrolysis carried out in batch run at 10.1% w/w of dry solid, 50 °C, 300 rpm, and initial pH of 5.2. Fed- batch saccharification was performed with 4 charges (initial + 3) of OPW partially dried by air convection, reducing the humidity from 80 to 60 % and 10.1% w/w of dry solid at initial time, 50 °C, 300 rpm and pH adjusted after added a charge to 5.2. The enzymes employed were Celluclast 1.5L, Novozyme 188 and Pectinex Ultra-SP. The hydrolysate was filtered through 0.2 µm size pore membranes of cellulose acetate for sterilization.

*Lactobacillus delbrueckii ssp delbrueckii* CECT 286 was employed as the biocatalyst to carry out D-Lactic acid production. Fermentation experiments were performed in 1L stirred tank bioreactor (STBR) BIOSTAT B-Plus with a volume broth of 0.5L and initial biomass concentration of 0.1g/L (5%v/v). The conditions were maintained at 40ºC, 200rpm, pH of 5.8 by adding NaOH 10M and anaerobic atmosphere by bubbling nitrogen before inoculation. The broth employed was OPW hydrolysate supplemented with 37 g/L corn steep liquor (CSL).

**3. Results and discussion**

D-Lactic acid production was carried out employing cheap carbon (OPW) and nitrogen (CSL) sources. If we pay attention to sugars uptake, glucose is consumed first in all experiments, while fructose and galactose, as observed in figures 1A and 1B, were metabolized later. All sugars were used to produce D-lactic acid, as suggested in those figures. In the figure 1C, results achieved when the hydrolysate concentration is highest are displayed, and it can be observed that the strain is not able to consume all sugars in this condition. If looking at final biomass concentrations, differences are not evident, although the growth rate is slower at higher hydrolysate concentration, as expected with increased inhibition due to either the substrates themselves or polyphenols and other components present in such hydrolysates. This LAB always grows from glucose, but it is not able to grow anymore in the presence of fructose and galactose. The final D-LA concentration improves when the OPW hydrolysate concentration increases, but the productivity decreases.

***Figure 1.****D-Lactic acid production form OPW hydrolysate.* ***A.*** *Saccharification carried out in batch,* ***B.*** *Saccharification carried out in Fed Batch, hydrolysate diluted 80% and* ***C.*** *Saccharification carried out in Fed Batch, hydrolysate without dilution. Legend: Glucose, squares; fructose and galactose, circles; D-lactic acid, triangles; and biomass, diamonds.*

**4. Conclusions**

D-Lactic acid production is possible from cheap carbon (OPW) and nitrogen (CLS) sources. An increment in hydrolysate concentration reduces the productivity and growth rate but increases the final D-LA concentration, suggesting that inhibition due to substrates or stress due to phenolics and other chemicals plays a role in D-lactic acid production. At the highest hydrolysate concentration, 83.16 g/L of D-lactic acid were obtained at a productivity of 1.53 g/L·h.

**References**

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