# Production of lactic acid from different lignocellulose biomass for polylactic acid as a biodegradable plastic

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## Introduction

Lignocellulosic biomass considered as cheap, abundant, and renewable source for the production of different by-products such as biofuels, biomolecules, biomaterials, bio-energy, and represents a more sustainable alternative resource. Lignocellulosic residues composed of cellulose, hemicellulose, and lignin, following pretreatment and enzymatic hydrolysis processes, where the released polysaccharides from its cell wall can be converted into fermentable sugars to be utilized for the production of economical products including bioethanol, bacteriocins, lipoteichoic acid, probiotics, lactic acid (LA) and biogas (Azaizeh *et al.*, 2020). The bioconversion of lignocellulose to LA is an important alternative for its valorization to produce LA to be utilized in food industry, in cosmetics and pharmaceuticals industries (Mora-Villalobos *et al.*, 2020). The global interest in LA production is due to its utilization in various products including poly lactic acid (PLA), a biodegradable plastic to replace un-biodegradable synthetic plastic (Lo´pez-Go´mez *et al.*, 2018).

Carob is a perennial leguminous, where it covers hills and large mountain areas for reforestation purposes of the arid and the semi-arid regions with high amounts of bagasse after syrup extraction (Ben Othmen et al., 2019). Bananas are important crop cultivated worldwide where the peduncles represent ca 13% of the biomass and discarded as organic waste (Pazmino-Hernandez *et al.*, 2017). Another interested biomass is sugarcane which is known rich with lignocellulosic biomass is remaining after sugar extraction as bagasse waste.

The main aim of the current study is to investigate the production of LA from different biomass residues including carob, banana peduncles and sugarcane bagasse.

## Materials and methods

Bananas, sugarcane, and carob were collected from the Galilee region, Israel. The banana peduncles, sugarcane, and carob biomass remaining after juice extraction were dried in air for a few days before use. The main components of the lignocellulosic bagasse were determined using the laboratory analytical protocol.

Enzymatic hydrolysis of the lignocellulosic biomass were carried using a mixture of 4% (v/w) Accellerase BG and 3% (v/w) Cellic CTec2 for 24 h using a 2 L BIOSTAT bioreactor.

The lab scale fermentations were carried out at 52 °C, with stirring at 400 rpm, using banana peduncle biomass (15% (w/v) dry matter, DM), sugarcane bagasse (15% (w/v) DM), or carob biomass (30% (w/v) DM). For the batch fermentations we used the strain *Bacillus coagulans* isolate A107 for carob, *B. coagulans* isolate A166 for banana and sugarcane bagasse using 2 L fermenter (one liter working volume) at 52 °C. Samples were withdrawn aseptically for the analysis of the different sugars and LA concentrations.

Carob biomass was used for the scale-up using fermenters of 50 L where each containing 35 L of carob biomass at 30% DM using the strain *B. coagulans* isolate A107. In addition, a 2 L (one liter working volume) of bioreactor was used for comparison purposes. Sampling and analysis were carried out as previously described.

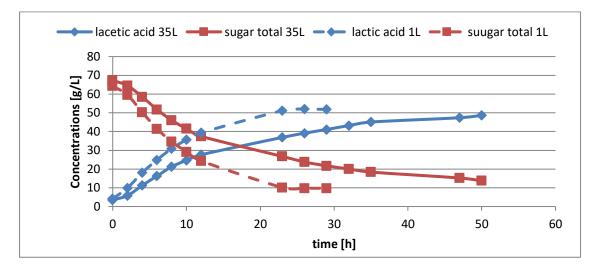
#### **Results and Discussion**

The composition of carob, banana peduncle and sugarcane biomass are summarized in Table 1. Table 1: Composition of carob, banana peduncle and sugarcane biomass (% of dry matter, DM)

	<b>DM</b> [%]	Proteins [%DM]	Sugars [%DM]	Cellulose [%DM]	Hemicellulose [%DM]	Lignin [%DM]
Banana	93.2	8.2	0.1	35.8	20.7	6.16
Sugarcane	91.1	12.5	11.3	27.9	25.6	2.79
Carob	85.8	9.3	27.7	19.0	0.35	28.4

The calculated LA yield of banana peduncles was 0.90 g LA.g<sup>-1</sup> sugars, and the calculated productivity at the log phase was 3.61 g.L<sup>-1</sup>.h<sup>-1</sup>. The calculated LA yield of sugarcane biomass was 0.88 g LA.g<sup>-1</sup> sugars, and the productivity at the log phase was 6.67 g.L<sup>-1</sup>.h<sup>-1</sup>).

Lactic acid production using carob in one liter scale fermenter showed LA yield of  $0.89 \text{ g LA.g}^{-1}$  sugars, and the productivity at the log phase was  $3.28 \text{ g.L}^{-1}$ . h<sup>-1</sup>(Fig. 1). Lactic acid production using the 35 L pilot scale fermenter resulted in the production of  $48.7 \text{ g.L}^{-1}$  LA (Fig. 1). The calculated LA yield was  $0.84 \text{ g LA.g}^{-1}$  sugars, and the productivity at the log phase was  $2.30 \text{ g.L}^{-1}$ .h<sup>-1</sup>.



**Figure 1:** Lactic acid production and remaining sugars trend during fermentation after sugar hydrolysis followed by protein hydrolysis of carob biomass (30% DM) conducted in one-liter fermenter compared to 35 L pilot scale without yeast extract using isolate A107.

Recent studies suggested that continuous fermentation process is advantageous for high LA productivity due to dilution of the produced LA in the broth resulted from feeding new medium and high operational stability by achieving steady state of cell growth, LA production, and sugar consumption, compared with batch or fedbatch fermentation processes where usually difficult to sustain homogeneities in large scales (Abdel-Rahman *et al* 2016; 2020). The pilot scale using carob biomass which is just a lignocellulose bagasse when fermented even without yeast extract supplementation resulted in high yield of 0.84 gLA.g<sup>-1</sup> sugars, and productivity of 2.30 g.L<sup>-1</sup>.h<sup>-1</sup> which is very promising process for industrial production of LA.

### Conclusions

The LA production from bagasse of banana peduncles, sugarcane or carob showed very promising results where carob and sugarcane were the best biomass, where we can use high % of DM during the fermentation process. The pilot scale of 35 L using carob biomass without yeast extract resulted in high yield of 0.84 gLA.g<sup>-1</sup> sugars, and productivity of 2.30 g.L<sup>-1</sup>.h<sup>-1</sup>.

#### References

Abdel-Rahmana, et al 2020. Non-Carbon Loss Long-Term Continuous Lactic Acid Production from Mixed Sugars using Thermophilic *Enterococcus faecium* QU 50. Biotechnol. Bioeng.

Azaizeh *et al* 2020. Production of lactic acid from carob, banana and sugarcane lignocellulose biomass. Molecules Journal 25(13), 2956.

Ben Othmen et al 2019. Evolution of phytochemical and antioxidant activity of Tunisian carob (*Ceratonia siliqua* L.) pods during maturation. EuroBiotech. J. 3, .135-142.

Mora-Villalobos et al 2020. Multi-product lactic acid bacteria fermentations: A Review. Fermentation 6, 23.

Pazmiño-Hernandez et al 2017. Feasibility assessment of waste banana peduncle as feedstock for biofuel production. Biofuels.

Lo'pez-Go'mez et al 2018. A review on the current developments in continuous lactic acid fermentations and case studies utilising inexpensive raw materials Process Biochem. 79, 1-10.