

Determination of kinetic parameters of the anaerobic biotransformation process of corn cob (*Zea Mays* L.) with *Lactobacillus acidophilus*

Nohemí Sánchez-Valeriano ¹, Diana I. Romero-Mota ¹, Erik S. Rosas-Mendoza ¹, Eduardo Hernández-Aguilar ², Juan M. Méndez-Contreras ^{1,*}

¹ Laboratorio de Ambiental II. División de Estudios de Posgrado e Investigación. Tecnológico Nacional de México. Instituto Tecnológico de Orizaba. Veracruz. México.

² Laboratorio 112. Facultad de Ciencias Químicas, Universidad Veracruzana. Orizaba. Veracruz. México.

* Corresponding author: juan.mc@orizaba.tecnm.mx; Tel: +52 272 111 58 89

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Abstract: The cob is the residue generated from the separation of the grain from the cob, and due to its components, it is considered a lignocellulosic material, so its use is limited, which favors the burning of the cob and its spreading, thus generating a problem of environmental pollution. Probiotics are live microorganisms that, when supplied in adequate amounts, provide a beneficial effect to the host, reducing the carbohydrate content in lignocellulosic materials through fermentation, obtaining a product rich in nutrients. Therefore, this research aimed to determine the kinetic parameters of biotransformation of corn residues through the fermentative action of the probiotic bacteria *Lactobacillus acidophilus*. Anaerobic fermentation kinetics were performed using the bacterium *Lactobacillus acidophilus* at different concentrations (10 and 15% v/v) to obtain kinetic parameters of microbial growth and substrate consumption for the biotransformation of white corn (*Zea Mays* L.) residues. The kinetic parameters indicate that when using 15% inoculum, the bacteria adapt to a greater extent to the substrate since the K_s value was 0.5693 g/L and the adaptation time was 18.25 h, thus allowing the growth of *Lactobacillus acidophilus* and therefore the process of biotransformation of corn residues. While for the consumption of substrate, it was identified that the use of 10% inoculum was better since it presented a value of k of 0.2534 that is higher than the value obtained when 15% is used because the rate of substrate consumption is faster. Therefore, the dose of 15% inoculum seems to be the most suitable for the adaptation of the bacteria in the shortest time and therefore the biotransformation process is carried out.

Keywords: corn residue; fermentation; *Lactobacillus acidophilus*.

Introduction

In Mexico, a large part of maize production corresponds to the white variety, since it is mainly used for human consumption. Thus, 93% of the total production in the 2019/2020 seasons corresponds to this variety. According to the Report of the Corn Market in Mexico, the annual production of white corn in 2019 was 26.771 million tons (SADER 2020), while in 2021 the national production was 24,356,418 tons and it is expected that by the year 2022 the production increases to 24,291,726 tons according to the document "Expectativas Agroalimentarias 2022" issued by the Secretary of Agriculture and Rural Development (SADER 2021).

Agroindustry is the economic activity that combines agricultural and industrial processes for the generation of products destined to the market, resulting also in the generation of agro-industrial waste (AW) (Mejías-Brizuela *et al.*, 2016). Agro-industrial wastes are considered an environmental problem due to the pollution they produce when not properly treated, handled and disposed (Vargas-Corredor and Pérez-Pérez, 2018). Mexico is considered one of the main producers AW ranking thirteenth in the world in terms of the amount of food produced, generating approximately 76 million tons of organic waste per year, of which 79% are primary waste produced in harvesting (corn leaves and stalks, sorghum stalks and pods, sugarcane tips and leaves, wheat straw, barley straw, beans, and cotton hulls), while the remaining percentage corresponds to secondary waste generated from post-harvest processing (sugar cane bagasse, cobs, maguey or agave bagasse, and coffee pulp, among others) (Carrillo-Nieves *et al.*, 2018). Corn cob is considered a non-timber resource composed of large amounts of xylans; for this reason, it has been a source of interest to be used as an alternative to chemical compounds or biomass. The cob is the residue generated from the grain separation from the cob, and it is estimated that 170 kg of cob are generated for each corn ton. Due to its composition, which is rich in lignocellulosic materials, it is not considered a biodegradable material, so this residue is incinerated and subsequently scattered into the environment, causing an environmental pollution problem (Rodríguez- Martínez *et al.*, 2018). Sometimes this waste is dumped in landfills causing unpleasant odors, contamination of watercourses and the proliferation of vectors, among others (Vargas-Corredor and Pérez-Pérez, 2018).

Interest in AW has recently increased in different areas due to low cost, high availability, and the need to reduce the environmental impact they caused. For this reason, new technologies have been studied which, based on the composition of the waste, employ the appropriate method and technology for waste utilization (Peñaranda-González *et al.*, 2017). The conversion of lignocellulosic biomass, from agricultural residues, into value-added compounds has become a sustainable strategy for their use. The degradation of lignocellulosic materials by the action of microorganisms allows the production of various compounds such as proteins, enzymes, organic acids, secondary metabolites, and oligosaccharides (Matos-Trujillo *et al.*, 2020).

On the other hand, fermentation is the process by which a substrate, solid or liquid, is transformed through prokaryotic microorganisms (bacteria) and eukaryotes (microalgae, yeasts, and fungi) into a product with value and utility for the man (Hernández-Melchor *et al.*, 2019). Lactic acid bacteria (LAB) are a group of probiotic bacteria that have the form of cocci or bacilli, whose main characteristic is the production of lactic acid (LA) in the fermentation process (Heredia-Castro *et al.*, 2017).

Lactobacillus acidophilus can be found naturally in a wide variety of foods. Most strains of this bacteria are anaerobic, and their optimal growth is achieved within the temperature range of 30-45 °C with a pH of 4-5 (Fina-Martín, 2019). The growth media for *Lactobacillus acidophilus* is selected for the ability to provide the necessary nutrients for its development. However, it is an expensive medium due to its nitrogen sources, such as meat extract and protease peptone. It is possible to replace nitrogen sources with sources from agro-industrial residues, to reduce costs (Domínguez-Gutiérrez *et al.*, 2019).

Materials and Methods

Collection and conditioning of white corn residues

The collection of corn residues was carried out at the Emiliano Zapata market located in the municipality of Orizaba, Veracruz, Mexico. The samples obtained were subjected to a drying process for 48 hours in an oven at 115 °C. After drying, a particle size reduction was carried out to approximately 2000 µm. Once the powdered sample was obtained, a 1:15 dilution was made with purified water to obtain a total solids value (TS) of 4-5%. The diluted corn residue was refrigerated at 4 °C until use.

Characterization of corn residues

The physicochemical and microbiological characterization of the waste was carried out to know the properties, composition, and possible contamination levels. In the characterization, the content of total solids (TS), total volatile solids (TVS) (2540 B Standard Methods), pH (ORION Research, Inc. Model 250 A), total nitrogen (N) (4500-NTK C SM), proteins (Micro Kjeldahl), carbohydrates (Anthrone-Sulfuric), fecal coliforms and salmonella spp (NOM-004-SEMARNAT-2002) was determined.

Thermal treatment

The treatment consisted of heating the substrate obtained from corn residues at a temperature of 90 °C for 30 minutes. After the thermal treatment, a second characterization was carried out with the physical, chemical, and microbiological parameters indicated in the previous section. The thermal treatment was carried out to promote the hydrolysis making possible the splitting and denaturation of complex organic compounds present in the corn residue and the inactivation of the microbiological populations that may represent a contamination problem due to pathogens or even generate competition for the substrate during the bioconversion process.

Lactobacillus acidophilus propagation

Lactobacillus acidophilus was obtained from a commercial drugstore. Man, Rogosa y Sharpe (MRS) agar (Difco BD) was used as a culture medium, which is appropriate for the enrichment of *Lactobacillus spp* and other lactic acid bacteria (LAB) (Vera-Mejía *et al.*, 2021). The agar was previously sterilized in an autoclave at 121 °C for 15 minutes, and then poured into Petri dishes and kept in a culture oven at 37°C for 24 hours. Subsequently, platinum loop streaking was

performed in triplicate, and the boxes were left to incubate at 37 °C for 24 hours. After incubation, the strains were transferred to previously sterilized tubes with MRS broth and kept refrigerated at 4°C until use.

Anaerobic fermentation

After the bacteria propagation, the kinetic process of anaerobic fermentation was carried out, which consisted of placing in 250 mL Erlenmeyer flasks, whose volume was 150 mL, the substrate, and the inoculum of probiotic bacteria in two different concentrations (10 and 15% v/v). The flasks were placed in an incubator (ZHWY-100B) at a temperature of 36 °C and shaking at 110 rpm. This process was monitored for three days, and samples were taken every 8 hours.

Kinetic parameters

The Gompertz model (Equation 1) was used to estimate the kinetic parameters that predict behavior of carbohydrate consumption from the determination of latency time and the maximum rate of carbohydrate consumption. Equation 2 was used to calculate the value of μ_{max} , while Equation 3 was used to obtain λ . The values of the kinetic parameters of substrate consumption rate (k^* and m) were obtained using the modified Hom model, presented in Equation 4. During the corn residues fermentation and biotransformation tests, carbohydrate consumption was used as a response variable. It is worth mentioning that the GraphPad Prism 8.0.1 software was used as a tool to obtain the kinetic parameters.

$$LN(N(t)) = B + (A - B)e^{-e^{(-K_s(t-T_i))}} \quad (1)$$

$$\mu_{max} = \frac{K_s}{e} \quad (2)$$

$$\lambda = T_i - \frac{1}{K_s} \quad (3)$$

$$\text{Log} \frac{C}{C_0} = -k^* \cdot t^m \quad (4)$$

Results and Discussion

It was observed that the average pH value of the sample was 6 and 6.27 after being heat treated. This value is within the optimal pH range used for the growth of the bacteria *Lactobacillus acidophilus*, which is 4.5 – 6 (Zhang *et al.*, 2021). The percentage of TS was 5.95%, whose value increased slightly to 6.13% after heat treatment, while the TVS decreased from 96.29% to 92.90%.

On the other hand, the protein percentage was 1.08% before heat treatment, which decreased to 0.94% after heat treatment; these values are close to 1.25% of crude protein in corn husks reported by Ponce-Ross and Romero- de Armas (2015). Similarly, the nitrogen percentage decreased from 0.17% to 0.15%; this value is close to that reported by Romero *et al.*, (2020), who obtained a nitrogen percentage of 0.15% when characterizing agro-cane residues, which are also considered lignocellulosic material. The initial concentration of carbohydrates was 10.19 g/L increased to 13.42 g/L after heat treatment because a slight loss of water caused an increase in the concentration of this parameter and the TS.

Before and after heat treatment, the corn residue showed a concentration of fecal coliforms and Salmonella spp of <2000 and <1000 NMP/g ST, respectively. The low concentration of pathogenic agents is attributed to the drying that was initially carried out on the corn residue and to the thermal treatment to which it was subjected since fecal coliforms, and Salmonella spp are not capable of resisting temperatures above 60 °C for more than 20 minutes (Cota-Espéricueta and Ponce-Corral, 2008).

Regarding the kinetic parameters obtained from the Gompertz model, the data are adjusted appropriately since the value of R^2 is greater than 90, these values being 0.9950 and 0.9668 for the dose of 10 and 15% of inoculum, respectively; in addition to that, the graphed data present a sigmoidal behavior characteristic of the model used (Figure 1). The experiment in which 10% inoculum was used presents an adaptation time of $\lambda = 21.87$ h, while when using 15%

inoculum, the time in which the bacteria adapted to the substrate was $\lambda = 18.2534$ h. In addition, the K_s value is higher when using the 15% inoculum, being this 0.5693 g/L, whose value is similar to that reported by Estrada (2021), who performed anaerobic fermentation kinetics with agro-cane residues through the action of *Lactobacillus acidophilus* using a stirring speed of 100 rpm, he obtained a K_s value of 0.5884 g/L. Figure 2 shows the bacterial growth in CFU/mL, as in Figure 1 a sigmoidal behavior is observed. After 24 hours, an increase in cell concentration is observed, which is established at 42 hours and a rise in the population occurs again at 50 hours, with a final concentration of 7.56×10^{10} and 7.57×10^{10} CFU/mL for the inoculum of 10 and 15 % respectively. Table 1 shows the parameters obtained from the Gompertz model in the two doses applied. These parameters allow us to analyze the behavior of the growth curve of the bacteria and the adaptation time so that the development of *Lactobacillus acidophilus* can be predicted under the same experimental conditions and make the design bases and operation for the scaling of this fermentative process.

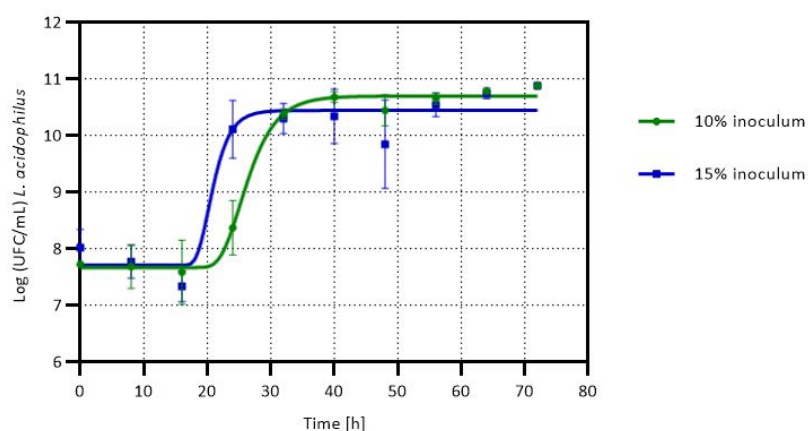


Figure 1. Fit of the experimental data to the Gompertz model, with standard deviation.

Table 1. Kinetic parameters of cell growth of *Lactobacillus acidophilus*.

Inoculum	K_s (g/L)	μ_{max} (h ⁻¹)	λ (h)	R^2
10%	0.3096	0.1138	21.8700	0.9950
15%	0.5693	0.2094	18.2534	0.9668

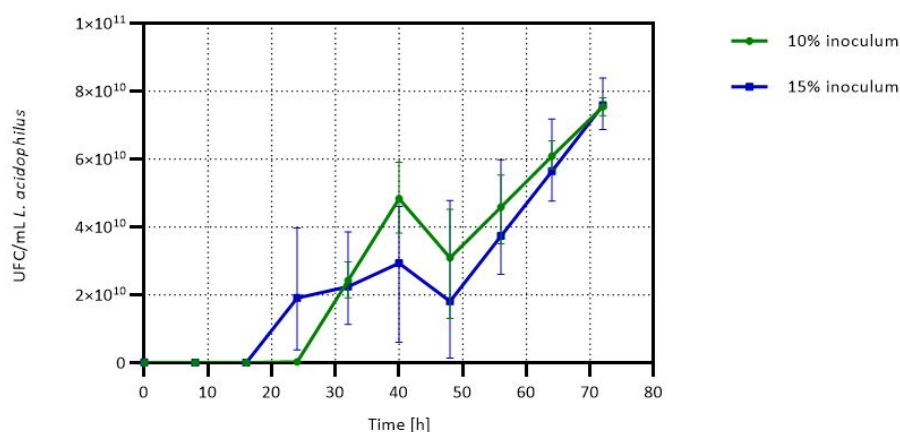


Figure 2. Population growth in CFU/ mL.

Similarly, when using the Hom model, both doses are adjusted appropriately since the correlation factor was 0.9648 and 0.9492 for 10 and 15% of inoculum, respectively. Figure 3 shows the behavior of substrate consumption using the Hom model. According to the graph, using 10% inoculum, the curve is closer to the slope, so there is a better fit for the model; this is verified with R^2 shown in Table 2, whose value is higher as well as the value k (0.02534), a compared 15% and the value of m (0.7609) the smallest whose case is that at lower values of m , the affinity of the bacteria for the

substrate is greater, so that when using the 10% inoculum, the bacteria adapts to the substrate and therefore carry out the process of biotransformation of the waste, using the anaerobic fermentation strategy. It is worth mentioning that when using the 10% dose, the percentage of carbohydrate removal was 52.15%, while in the experiment in which 15% of inoculum was added, 40.79% of the carbohydrates were removed. In Figure 4 it can see the corn cob residue before (Figure 4A) and after (Figure 4B) performing the anaerobic fermentation.

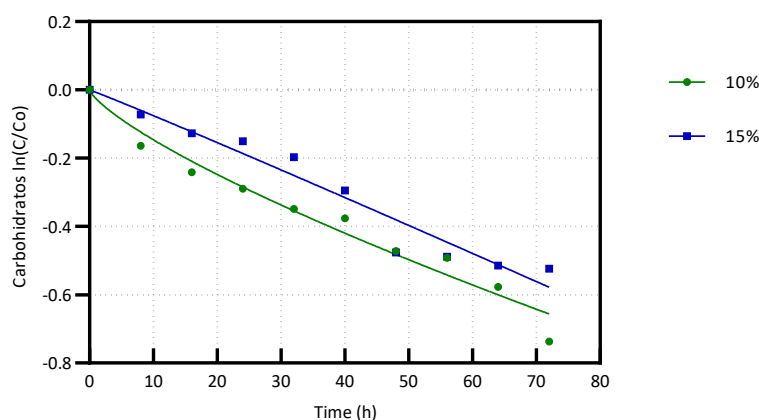


Figure 3. Substrate consumption through the Hom model with *Lactobacillus acidophilus*.



Figure 4. Corn residues. (A) Before the fermentative process. (B) Biomass generated from anaerobic fermentation.

Table 2 shows the kinetic parameters obtained from the anaerobic fermentation using *Lactobacillus acidophilus* bacteria with the two inoculum concentrations that were used for the experiments, highlighting that when using 10% of inoculum, the bacteria adapts to the substrate more quickly, allowing the biotransformation of waste into products such as biomass and lactic acid.

Table 2. Kinetic parameters of carbohydrate consumption with *Lactobacillus acidophilus*.

Inoculum	k^*	m	R^2
10%	0.02534	0.7609	0.9648
15%	0.007023	1.031	0.9492

Conclusions

The nutrient content in corn cob residue was sufficient for the growth of *Lactobacillus acidophilus*. The kinetic parameters of microbial growth show an affinity between the bacteria and the substrate and that a shorter adaptation time (18.25 h) is required when using the 15% v/v inoculum, which allows the growth and development of *Lactobacillus*

acidophilus. Regarding substrate consumption, the kinetic parameters indicate that when using 10% v/v inoculum, carbohydrate consumption is slightly faster than when using the 15% v/v inoculum. However, in both cases, there is an effective removal of the carbohydrates present in the residue.

Anaerobic fermentation from white corn residues using *L. acidophilus* bacteria has proven to be a sustainable strategy for the use of lignocellulosic residues, by adding value to them, so that it could be used as a possible feed for livestock. For future research, it is recommended to use other species such as *Bacillus subtilis*, which are also considered a probiotic.

This study lays the foundations for designing and operating new organic waste treatment processes, which generate biomass of high commercial value due to the production of probiotics; they increase the profitability of agricultural activity and minimize environmental pollution problems.

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