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Comparative Studies of *Bacillus thuringiensis* var. *israelensis* Metabolism in Different Concentrations of Cassava Flour Processing Waste Based Media

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Abstract

Techniques of production of enthomopatogenic bacteria are developed aiming to increase the productivity and to reduce the costs of the fermentative process. Like this, it has been using agro-industrial wastes or by-products as nutrient sources in culture medium, having been used, in this study, the manipueira, a by-product of the processing of the cassava flour. Fermentations were performed in flasks of Erlenmeyer of 500 mL containing 250 mL of culture media, conditioned in shaker at 180 r.p.m. and 28˚C, and the media were composed by manipueira, in concentrations that varied between 400 and 1000 mL/L. The time of the process varied between 48 and 120 hours. They appraised the following parameters: cellular growth, the production of spores, the reduction of organic matter (COD analysis) and the variation of reduction sugar. Although there was a proportional cellular growth to the manipueira concentration, the production of spores was similar in all the cases, at the end of the process, in spite of the smallest speed of production of the same ones in the highest concentrations. In relation to the variation of COD, it has, also, a percentile minor of reduction in the highest concentrations. In the analysis of variation of reduction sugars, the higher concentrations are the ones that they present larger slowness in the reduction of this.

Keywords

Bioinsecticides, Agroindustrial Wastes, *Bacillus thuringiensis* var. *israelensis*, Fermentation

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1. Introduction

The production process of cassava flour generates high pollutant load because of the large amount of organic material and potencially hydrolyzable cyanide glycosides. These substances have a negative impact on the environment. With these characteristics, these surpluses were handled with appropriate technologies, and could result in reuse of practically all discarded material as feedstock for other processes, which is currently not occurring in most of the factories in this sector. There are numerous opportunities that could be implemented to take advantage of such wastes so as to provide greater financial returns for the companies themselves, or to their possible future subsidiaries. One of these wastes, manipueira, also called "press water", is the juice or water building up the roots, removed by pressing of fresh, chopped or grated mass of the cassava flour industry. It contains a percentage of different constituents of cassava, such as starch, minerals, proteins and cyanogenic glycosides [1] [2].

Due to its composition, manipueira is a potentially viable cassava byproduct for the production of biopesticide based on *Bacillus thuringiensis* var. *israelensis* (Bti) bacteria used for biological pest control related to public health [3].

The use of Bti for mosquito control has inspired several adaptations. A key to the successful production of the bacterial insecticide and commercialization has been the development of the culture medium. Most culture media are employed using all-natural products such as carbon sources, nitrogen and salts thereof. Apparently, a medium containing waste from the agro-industry and agriculture reduces local generation costs. Thus, one can cite the molasses, water, corn steep liquor, and other potentially useful substances such as blood from the bird and pig [4] [5]. Thus, we studied the production of a biopesticide from manipueira, as a tool to help fight with the mosquito *Aedes aegypti* (*Ae. aegypti*), dengue vector, analyzing cell growth, the production of spores, the reduction of raw organic (COD) and the variation of reducing sugar.

2. Materials and Methods

2.1. Inoculum Preparation

Three heave growth of bacteria in the pipe stock, were inoculated into test containing 9 mL of sterile water pipe. After mixing by vortexing, 1 mL of this solution was added to 250 mL Erlenmeyer flasks containing 50 mL each of the culture media and autoclaved at 121˚C for 20 minutes. They were incubated in a "shaker" rotating at 180 rpm (revolutions per minute) and 28˚C for 15 hours. These cells in active growth were used as seed inoculum by transferring 5 mL to 500 mL Erlenmeyer flasks, containing 250 mL of media tested, sterilized as mentioned previously, giving a concentration of 1:50 (v/v).

2.2. Culture Media

The fermentation media were composed of manipueira, adjusted to pH 7.0 with 2 N NaOH at concentrations of 400, 500, 600, 700, 800, 900 and 1000 mL/L. The latter lasted for 120 hours. Fermentations were performed in Erlenmeyer flasks of 500 ml containing 250 mL of culture medium, placed in shaker-incubator at 180 rpm and 28˚C.

2.3. Monitored Parameters

In this experiment, cell growth was investigated by optical density, measurement of the spores by plating on nutrient agar, variation in COD and reducing sugar.

2.4. Microorganism

The tests were performed with *Bacillus thuringiensis* var. *israelensis* (serotype H-14). The strain was routinely spiked with slant nutrient agar medium culture maintained at 28˚C for 72 hours and then kept at 4˚C in refrigerator.

2.5. Cell Growth

Determination of cell growth was performed at regular intervals. The withdrawn samples (3 mL) were centri-

fuged at 10,000 rpm three times for 10 minutes, performing a "cell washing" with distilled water twice in the past. Finally, the optical density of the suspension was determined in a spectrophotometer at 620 nm Ultropec unit 3000 UV/Visible Pharmacia Biotech.

2.6. Quantification of Spores

For quantification of spore a plating technique with heat shock was performed in which the dilutions after being prepared as usual, are inoculated into five or six points (volume of 5 μL/point) on the surface of nutrient agar culture taking care to leave the plates open in a sterile environment until drying the inoculated suspension [6]. Then the plates are incubated at 28˚C for about 10 hours, after which it performs the counting of colonies, preferably using a colony counter. Good accuracy is obtained when the count has, on average, between 20 and 40 colonies/point. For a better utilization of resources and material, the plates were divided in half; each half could be on different dilutions.

2.7. Determination of the Variation of COD (Chemical Oxygen Demand)

To measure the COD, the closed reflux colorimetric method using the apparatus Hach Co. was used [7].

2.8. Determination of the Variation in Reducing Sugar

The reducing sugar concentration in milligrams of glucose per milliliter, was determined using a standard curve prepared with glucose standard solutions [8] [9].

3. Results and Discussion

3.1. Cell Growth

Analyzed results indicated a direct relationship between growth and the concentration of manipueira used. As the cassava is a residue polluting and toxic at high power, data were promising, since they indicate the possibility of using a substrate with high concentration of manipueira, in addition to not cyanide interference (compound exists in cassava) in the aerobic process.

3.2. Determining the Amount of Spores

In this study, it was observed that the higher the concentration of manipueira, the slower sporulation, which raises indicative that it would not recommend the use of pure byproduct for the purpose of work.

For the plating technique, it was found that at all concentrations reached a similar number of spores at about 120 hours after fermentation of the order of 10^9, but at different fermentation times (**Figure 1**).

3.3. Determination of the Variation in Reducing Sugar

Manipueira presents reducing sugars and a high content of organic matter [3].

Analyzing **Figure 2**, where it is shown the variation of the content of reducing sugars, there was decrease after 24 hours of the procedure, which was most pronounced between 36 and 48 hours. This result may explain the stagnation of cell growth at 48 hours, which can be seen in **Figure 3**. At concentrations of 800, 900 and 1000 mL/L, the amount of sugar in 48 hours, is greater than at other concentrations, equating these in 60 hours.

3.4. Determination of the Variation in COD

It can be observed by examining **Figure 4**, a reduction in COD of from about 42% at 400 mL/L, 43% at 500 mL/L, 43% at 600 mL/L, 27.6% at 700 mL/L, 34% at 800 mL/L, 36% at 900 mL/L and 30.5% at 1000 mL/L. In higher concentrations of manipueira, the reduction of the organic load was lower. Although the reduction was less than 50%, it is important to reduce the organic load to obtain a high added value biocompound.

4. Conclusion

The observed results indicate that manipueira has proved to be an excellent culture medium for production of a

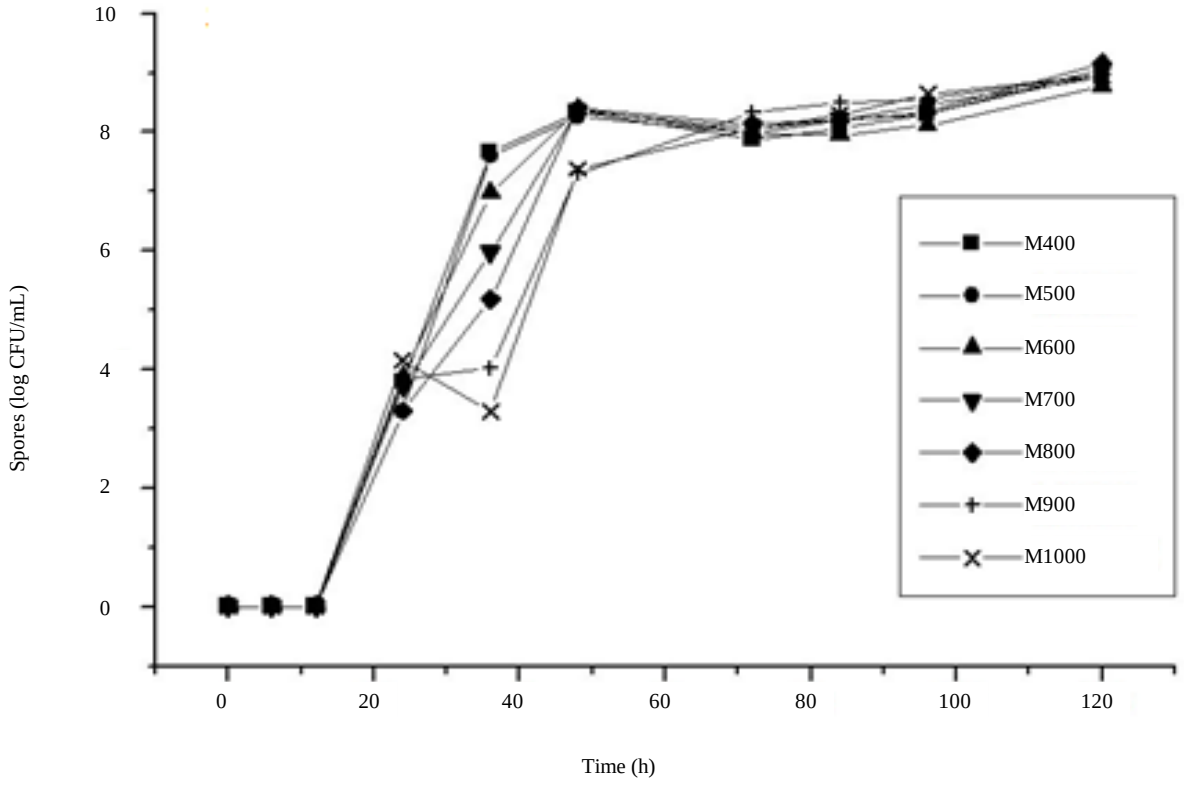

Figure 1. Bti spores formed during fermentation in media consisting of manupeira at concentrations of 400, 500, 600, 700, 800, 900 and 1000 mL/L.

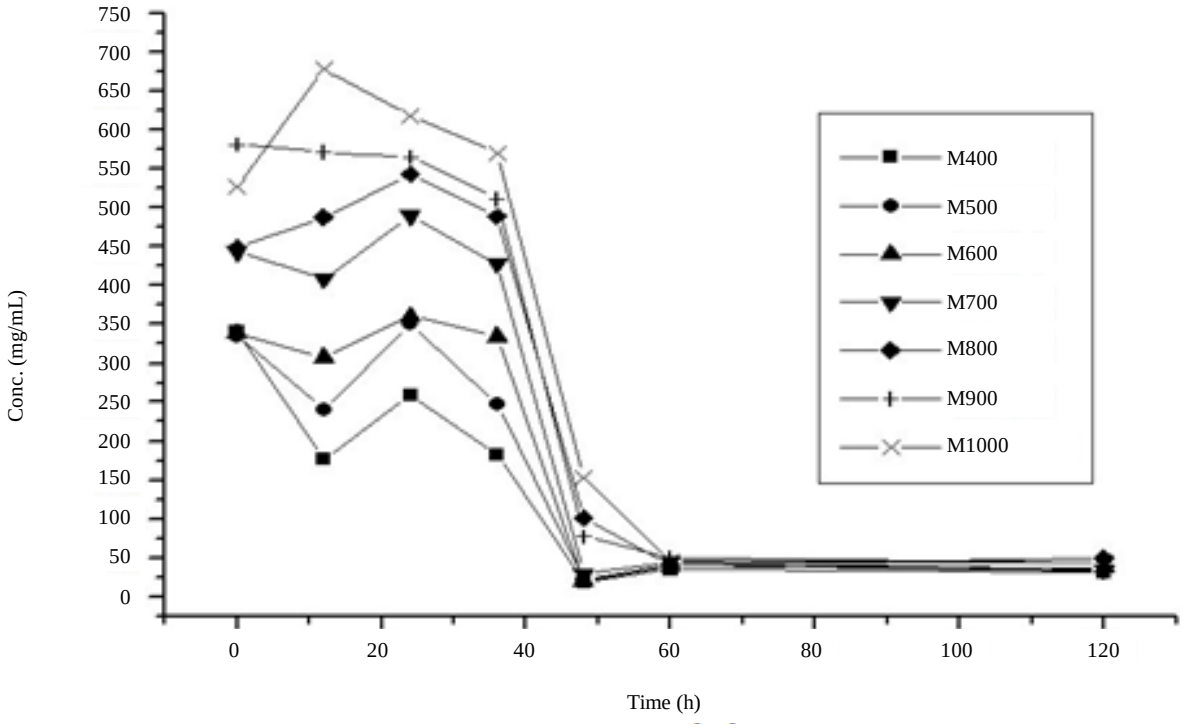

Figure 2. Variation of the content of reducing sugars during Bti growth on media consisting of cassava at concentrations of 400, 500, 600, 700, 800, 900 and 1000 mL/L.

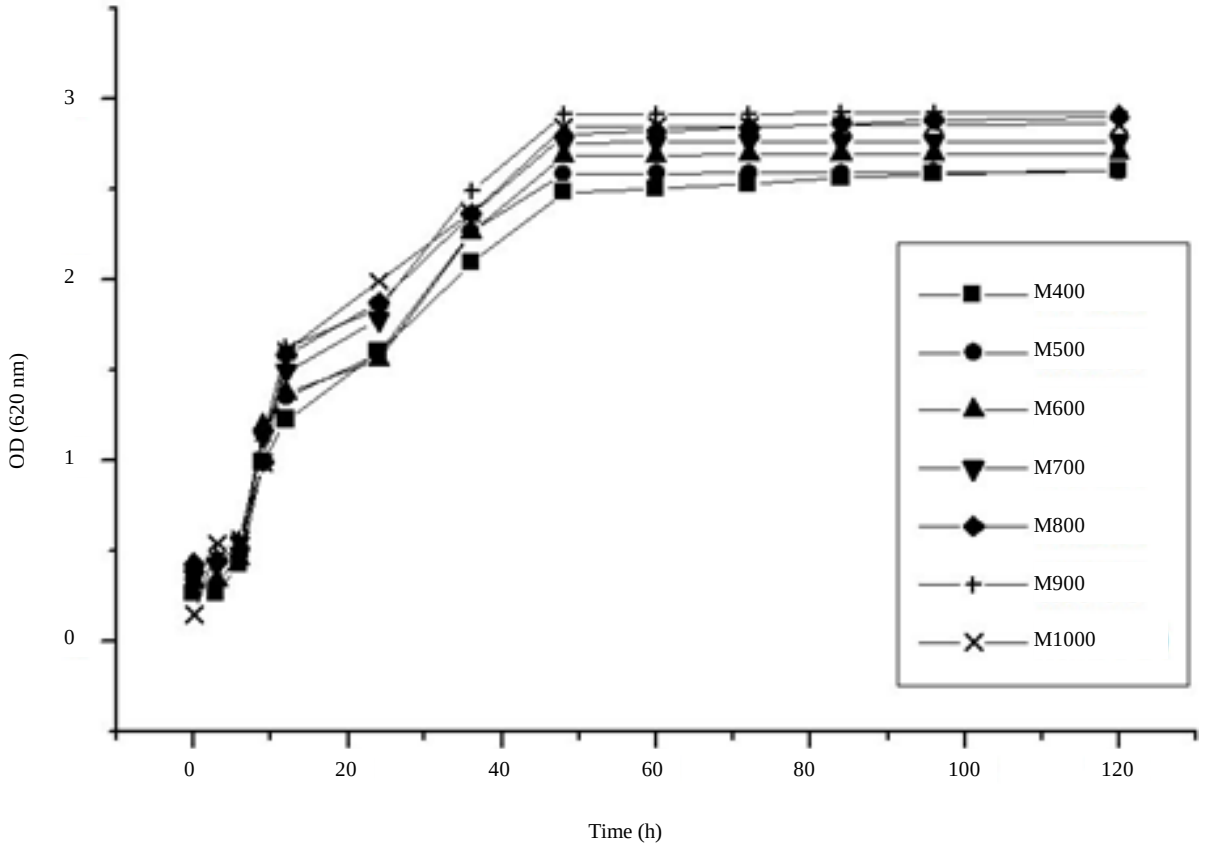

Figure 3. Bti growth media consisting of manipueira at concentrations of 400, 500, 600, 700, 800, 900 and 1000 mL/L.

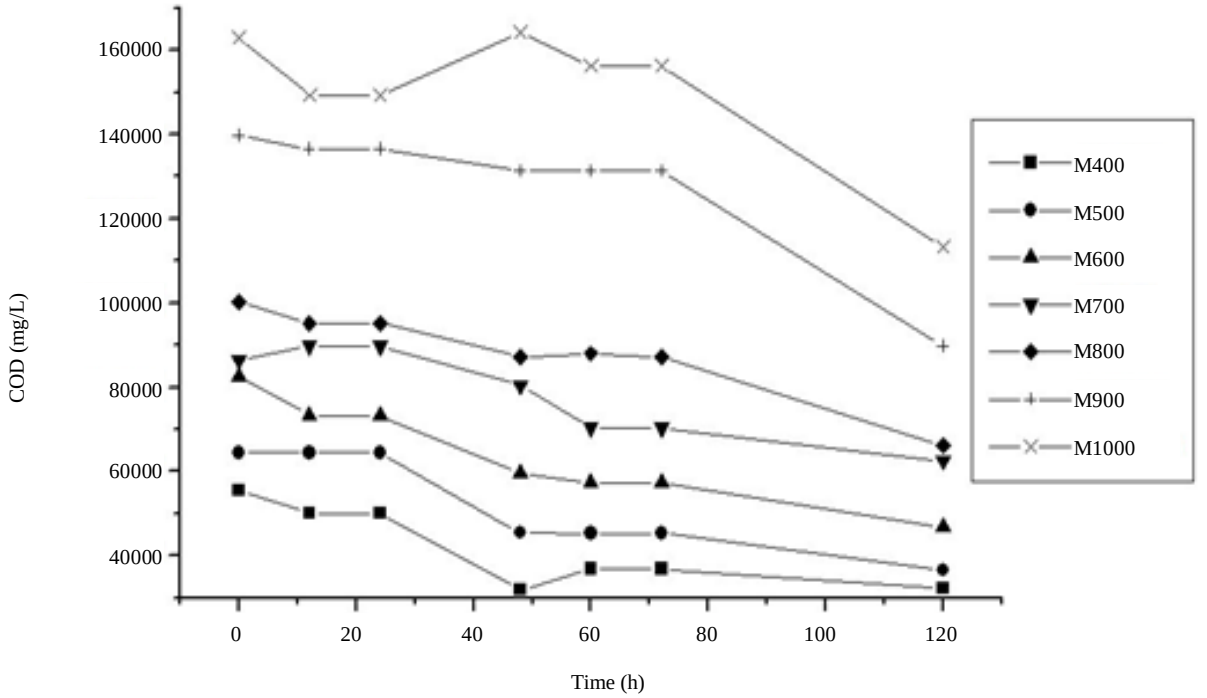

Figure 4. Variation of COD during Bti growth on media consisting of manipueira at concentrations of 400, 500, 600, 700, 800, 900 and 1000 mL/L.

Bti based biopesticide. Although there is a cell growth proportional to the concentration of manipueira, the production of spores was similar in all cases, at the end of the process, despite the lower output speed of the same at higher concentrations. Regarding the variation of COD, there is also a lower percentage of reduction at higher concentrations. In the analysis of variation of reducing sugars, the highest concentrations present larger delays in this reduction satisfying than the proposed concerning the production of a low-cost biopesticide objective, involving the recovery of waste/by-product of pollutant character.

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