



Maestría en Ingeniería en Diseño de Bioprocesos

Title

**Microbial production of polyhydroxyalkanoates
using dairy industry waste**

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1. Introduction

Due to the current pollution trouble, some alternatives to avoid the use of non environmentally friendly products has been proposed. This is the case of polyhydroxyalkanoates (PHA) which are polymers synthesized intracellularly by some bacterial strains to be use like backup energy. It has an important application in the production of biodegradable plastics besides to find application in medical industry because its biocompatible properties.

On the other hand, whey is a byproduct of dairy industry which was regarded as pollutant due to its high organic load and elevated production volume. However, whey has been considered for obtaining industrial food and pharmaceutical products. Whey contain a considerable lactose amount (4.9 % approximately) whereby it can be use as substrate in bioprocess.

2. Objectives

2.1. General objective

Producing PHA by fermentation processes in batch culture and fed batch culture using whey as substrate.

2.2. Particular objectives

- To characterize the whey by proximate analysis.
- To determine pretreatment whey conditions.
- To select a microorganism to produce PHA, considering five bacterial strains, based on biomass and PHA production at flask level.
- To perform fermentation kinetics in batch culture and fed batch culture at bioreactor.

3. Method

Whey characterization was carry out using a Lactoscan LA 50 device to determine fat and protein. Reducing sugars were determined by DNS method. Water and ash were determined gravimetrically.

Figure 1 shows the whey treatment methodology and Figure 2 shows a comparison between hydrolyzate whey obtained with this methodology (left) and another one (right).

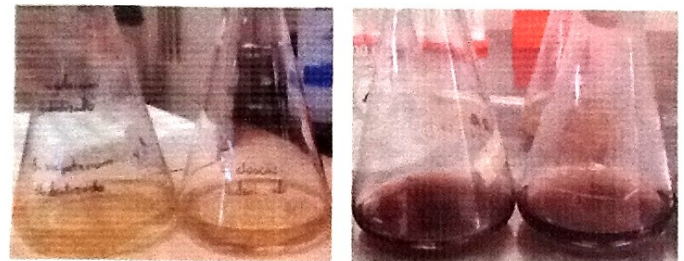
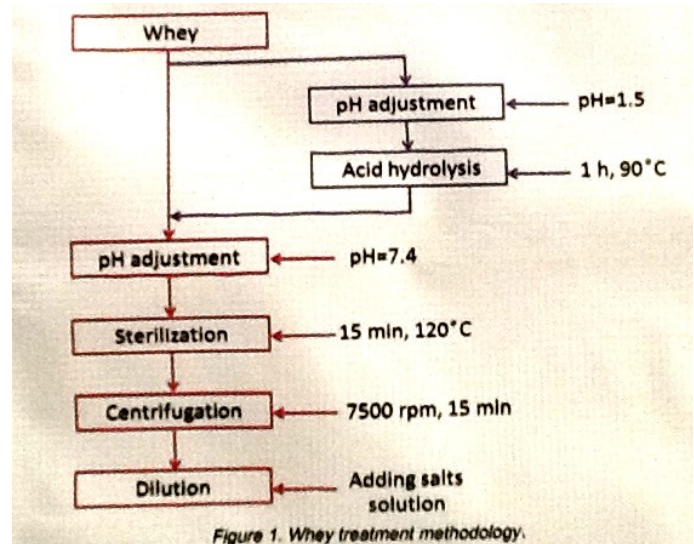
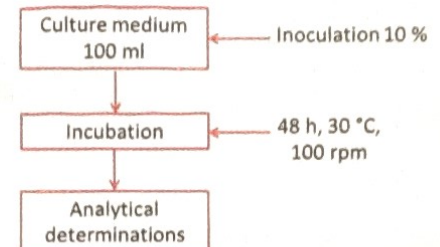


Figure 2. Comparison of whey treated by different procedures.

Microorganisms used in this work were *Pseudomonas putida*, *Pseudomonas aeruginosa*, *Azotobacter vinelandii*, *Bacillus megaterium* and *Enterobacter cloacae*. Figure 3 shows the methodology followed for fermentations



Fermentations were performed in shake flasks (Figure 4) by using conditioned whey or hydrolyzate whey added with salts, as culture medium. The fermentations at bioreactor level are in underway (Figure 5).



Figure 4. Cultures at 48 h of fermentation.



Figure 5. Bioreactor scale fermentation.

Biomass determination was performed by dry weight whereas reducing sugars were determined by DNS method using a standard curve of lactose from 0-2 g/L. PHA determination method is shown in Figure 6.

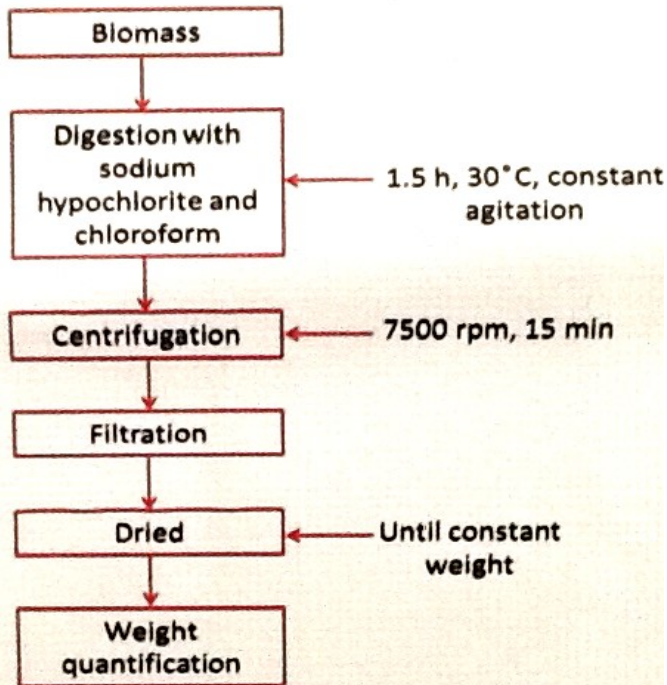


Figure 6. PHA extraction methodology.

In the figure 7 is shown PHA obtained from *Pseudomonas putida*.



Figure 7. PHA obtained from *Pseudomonas putida*.

4. Results

In Table 1 is shown the composition of whey used which contain 4.5 % of reducing sugars.

Component	Content %
Fat	0.05
Protein	3.47
Reducing sugars	4.35
Ash	0.54
Water	91.61

Table 1. Proximate analysis of whey.

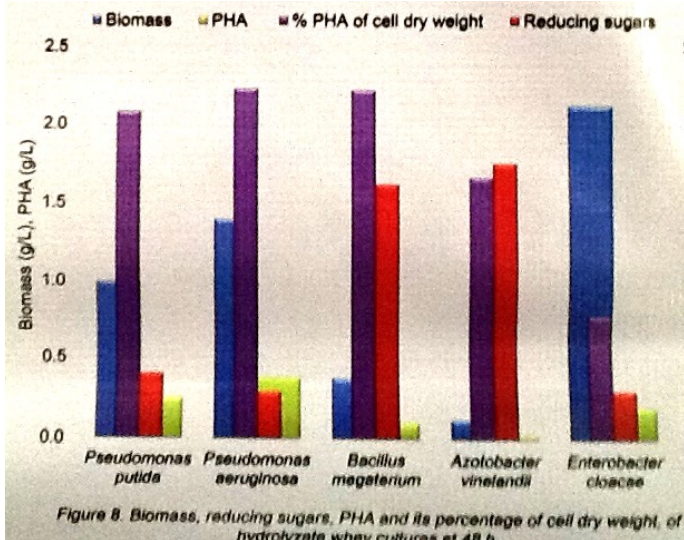


Figure 8. Biomass, reducing sugars, PHA and its percentage of cell dry weight, of hydrolyzate whey cultures at 48 h.

5. Conclusion

A maximum concentration of 0.38 g/L of PHA was achieved with *P. aeruginosa* using hydrolyzate whey, while the highest percentage of PHA of cell dry weight was obtained by *P. putida* with 38.89% using conditioned whey as substrate.

The highest biomass production was obtained by *E. cloacae* however PHA production was low, reaching a percentage of PHA of cell dry weight of 10.2% and 9.4% for hydrolyzate whey and conditioned whey respectively.

Acknowledgements

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