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RESEARCH ARTICLE OPEN ACCESS

Polyhydroxyalkanoates production and extraction

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ABSTRACT
Polyhydroxyalkanoates (PHA) are considered candidates to replace petrochemical-based plastics. Halophilic bacteria have the ability to use carbohydrates as a carbon source and accumulate polyhydroxyalkanoates. The research aims to evaluate the production of PHA by halophilic bacteria isolated from the Paramonga area, coded as BH 6, BH 7 and BH 8 in different broths and PHA was extracted. It was determined that BH 7 is the bacterium with the highest yield in the 3 culture media used: nutrient broth, molasses broth plus salts and molasses broth without salts. On the other hand, BH 6 presented a better PHA production, and the 1% molasses broth was the medium that represented the highest efficiency in PHA production. Likewise, PHA production can be carried out with 1% molasses medium + water, since it represents a low-cost alternative.
Keywords - *Polyhydroxyalkanoates, halophilic, broth, absorbance.*

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I. INTRODUCTION

Petroleum-based plastics (synthetic, non-biodegradable) have been used since the 1940s. In the USA alone, in 2009, 31 million tons were generated between plastics, durable objects such as household appliances and non-durable objects such as plates and cups. Poly-B-hydroxyalkanoates (PHA) are presented as ideal candidates to replace petrochemical plastics [1,2]. The production costs depend on the culture medium, fermentative process and PHA extraction [3].

The economic problem can be decreased by improving the fermentation process, separating the product and searching for more efficient microorganisms. Quillahuaman et al. (2008) demonstrated that halophilic bacteria are heterotrophs capable of using various carbohydrates as a carbon source and accumulating PHA [4].

The objective of this study is to evaluate the production of PHA produced by halophilic bacteria isolated from Paramonga, Lima in 3 culture media: nutrient broth, molasses broth plus salts and molasses broth without salts; and the extraction of PHA produced by halophilic bacteria.

II. MATERIALS AND METHOD:

The present work was carried out at the company Agro Industrial Paramonga S.A.A. from 01/04/2018 to 03/11/2019.

The extraction process involved several operations to ensure the extraction of the biopolymer from the cells, which are detailed below [5,6]:

Preparation of culture media
Two hundred milliliters of minimal salt medium (MMS) supplemented with 1% molasses, 1% molasses broth, and nutrient broth were prepared in a 500 ml flask.

Selection and activation of halophilic bacteria

Three isolated halophilic bacteria (coded as BH6, BH7 and BH8) were seeded in the 1% molasses broth and incubated for 4 days under agitation at 28 C. Then, the bacteria that had the highest amount of biomass in the molasses broth were selected and seeded in 20 ml of nutrient broth to evaluate the growth kinetics.

Kinetics of growth and PHA production

After 3 days of inoculum incubation, the 20 ml of inoculum was transferred into a 500 ml flask with 180 ml of sterilized nutrient broth. 2 ml of sample were taken from time zero. 2 ml of sample was collected every 4 hours (8am, 12 pm, 4pm, 8pm) for 4 days and read in the spectrophotometer at 575 nm. For PHA production, we waited for the bacteria to reach the stationary phase to start PHA extraction.

Spectrophotometer reading

To perform the reading in the spectrophotometer, the medium in which the kinetics for the reference sample was initiated was needed.

Then, the sample was placed in the cell and the measurement was performed at 575 nm.

Sudan staining

A hoe was taken from the growth kinetics flask and a smear was made on a slide. Then, Sudan dye was added, left for 5 minutes and the excess was removed for complete drying. Then, xylol was added over the smear and left for approximately 20 seconds, the excess was removed and allowed to dry. Finally, safranin was added as a contrast dye and allowed to dry, and then taken to the microscope to observe the granules present in the bacteria at 100x.

PHA extraction

For PHA extraction, 10 ml of the culture medium was taken and placed in test tubes and centrifuged at 4700 RPM for 20 minutes. Then, the supernatant was discarded and 3 ml of concentrated sodium hypochlorite was added to the sediment and incubated at 37°C for 2 hours. After the incubation time, the pellet was centrifuged at 4700 RPM for 20 minutes and the pellet obtained was re-suspended in 99% chloroform. Finally, to dry the sediment, it was placed in a flask at 60°C for 15 hours and then weighed.

III. RESULTS

Figure 1 shows the absorbance obtained from BH 6, BH 7 and BH 8 in relation to the time in hours in nutrient broth. It is observed that BH 7 presented higher absorbance with respect to BH 6 and BH 8.

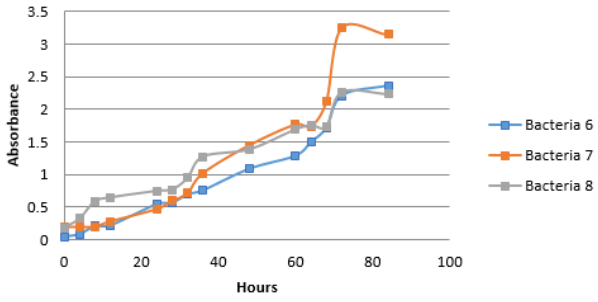

Figure N°1: Growth kinetics in nutrient broth of halophilic bacteria (BH 6, BH 7 and BH 8).

Figure 2 shows the absorbance obtained from BH 6, BH 7 and BH 8 in relation to the time in hours in broth Half water + 1% molasses. It is observed that bacterium 7 (BH 7) presented higher absorbance, while bacterium 8 (BH 8) presented constant absorbance values through time, without experiencing significant increase or decrease.

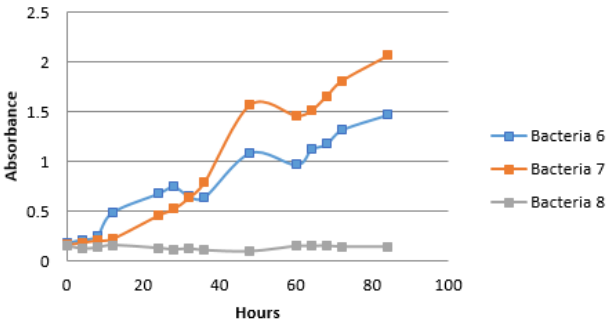

Figure N°2: Growth kinetics of halophilic bacteria (BH 6, BH 7 and BH 8) in water + 1% molasses medium.

Figure 3 shows the absorbances obtained from BH 6, BH 7 and BH 8 in relation to the time in hours in medium salt broth + 1% molasses. It is observed that BH 7 presented higher absorbance than BH 6 and BH 8.

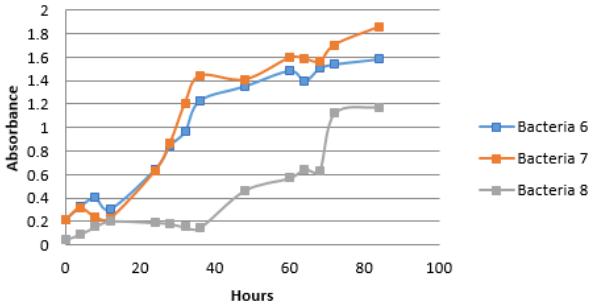

Figure N°3: Growth kinetics of halophilic bacteria (BH 6, BH 7 and BH 8) in salt medium + 1% molasses.

Table 1 relates the data of the different media used with the bacteria under experimentation.

Table 1. PHA extraction data.

Half	Bacteria	Test tube weight	Final tube weight	Weight of PHA in grams/10ml	grams PHA/L
Broth Nutricio	6	3.5046	3.5378	0.0332	3.32
	7	3.5033	3.5202	0.0169	1.69
	8	3.4795	3.4972	0.0177	1.77
Medium salt free	6	3.4654	3.4774	0.012	1.2
	7	3.4767	3.4885	0.0118	1.18
	8	3.505	3.5168	0.0118	1.18
Medium with salts	6	3.8072	3.8371	0.0299	2.99
	7	3.4217	3.4408	0.0191	1.91
	8	3.4843	3.4981	0.0138	1.38

The following graph (Figure 4) shows the amount of grams of PHA/Liter obtained in the different experimental media. It is evident that BH 6 obtained a higher concentration of grams of PHA/Liter with respect to BH 8 and BH 7.

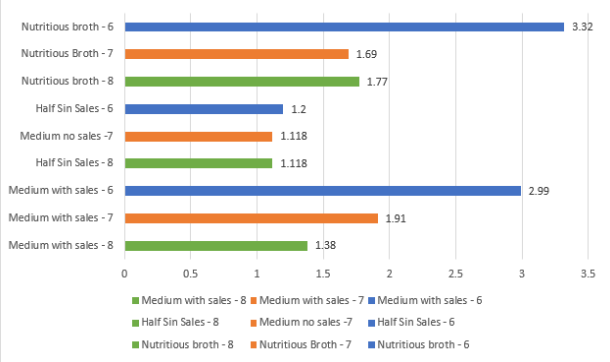

Figure N° 4: *PHA/Liter gram chart*

IV. DISCUSSION

Figure 1, 2 and 3 show the growth kinetics of the 3 bacteria in nutrient broth, it is observed that they have a good biomass yield.

The growth kinetics was performed in molasses medium, with water and with salts, separately. The results were satisfactory since biomass was obtained from strains BH 6 and BH 7 in water medium with 1% molasses, discarding BH 8 because it did not grow in this medium.

According to the data obtained (Table 1), the highest values were obtained with BH 6 in nutrient broth and in medium with salts, 3.32 g/L and 2.99 g/L, respectively. In the medium without salts the lowest PHA values were obtained in the 3 bacteria evaluated BH 6, BH 7 and BH 8 giving values of 1.2 g/L, 1.18 g/L and 1.18 g/L, respectively. It is observed that bacterium 8 (BH 8) in the media without salts and with salts presented the lowest values in their respective groups.

V. CONCLUSION

- Bacterium 7 (BH7) had a high yield in the three culture media.
-PHA production can be carried out in 1% molasses medium and water.
-Bacterium 6 (BH 6) has a better PHA production.
-The 1% molasses broth supplemented with salts is a good alternative for PHA production.

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