Extraction and purification of a polyhydroxyalkanoate-type biopolymer obtained from volatile fatty acids of mixed cultures and *B. Cepacia*

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The global consumption of plastics generates accelerated environmental pollution in landfills and marine ecosystems. Biopolymers and bio-based polymers are the materials with the greatest potential to replace synthetic polymers in the market due to their good biodegradation capacity, however, there are still several disadvantages, mainly related to their production cost and their physicochemical and mechanical characteristics. Taking into account the above, as an alternative solution, the generation of biodegradable and biocompatible bioplastics stands out, some made with renewable raw materials, among which are the PHAs Polyhydroxyalkanoates (Hao, et al., 2018). For the production of polyhydroxyalkanoates, it is usual to use methods of isolation of pure bacterial strains, this type of polyesters, which are thermoplastic and produced by numerous microorganisms under different culture conditions (Lai, et al., 2013). Although much research has been done on the bacteria that have the capacity for intracellular accumulation of PHAs, among others, it is also possible to produce PHAs using mixed microbiological cultures instead of a single microorganism by using naturally occurring microbial consortia that have the capacity to store high amounts of PHAs (Beun et al., 2002). A promising strategy for reducing the cost of producing PHAs is to use mixed microbial cultures such as activated sludge from wastewater treatment plants (Morgan et al., 2014); this process has additional beneficial effects on the environment by reducing the amount of excess sludge disposal (Lee, et al., 2017). In this contribution, three methods were evaluated for the extraction and purification of the PHAs produced by fermentation using volatile fatty acids as a carbon source at different concentrations, using the pure strain *Burkholderia cepacia* 2G-57 and the mixed cultures of the activated sludge from the WWTP El Salitre. The best method was selected from the point of view of environmental sustainability since this will contribute to the scalability of the process.

Materials and methods

The biopolymer was produced from the fermentation of volatile fatty acids produced from the acidogenic fermentation of primary and digested sludge from the El Salitre wastewater treatment plant in Bogotá. Using the Burkholderia cepacia 2G-57 strain and the Mixed Cultures of the Activated Sludge from the WWTP, in 1L and 5L reactors. The first extraction route consisted of digesting the biomass with SDS using the methodology of Fonseca (2016). The second extraction method was using chloroform and methanol following the process described by Rosengart et al (2015), and a third extraction and purification method using Acetic Acid was used according to the methodology of Aramvash et al (2017). Finally, the characterisation, yield and purity of PHA were performed for each method. See Figure 1.

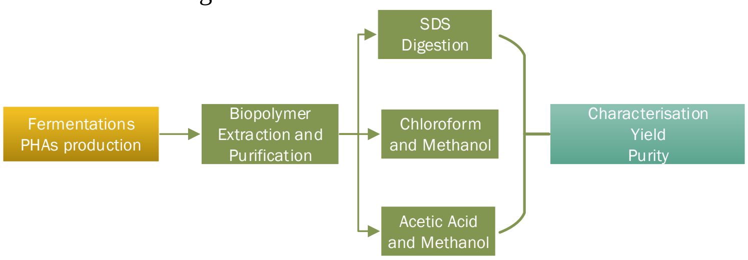

Figure 1. General Research Methodology

Results and discussion

In the case of PHA extraction with SDS, the best yield with respect to biomass was 92% and was given using 0.7g/L VFAs as carbon source, on the other hand, using both the chloroform and methanol method as well as the acetic acid and methanol extraction route the best yield was given by using 1.0 g/L, however these are lower yields. When using Mixed Cultures of the WWTP sludge, the concentrations of PHAs accumulation are lower with respect to the Pseudomonas Bulkolderia cepacia 2G-57 strain, however the best polymer yield with respect to the biomass was also using 0.7g/L of VFA's with 85.7%, and likewise the best yields are reported under these carbon source concentrations when extracting with chloroform, and only using the acetic acid route the best result is obtained when using 1.0g/L. The results obtained are consistent with those reported by Argiz et al., 2020; Zhang et al., 2019; Albuquerque et al., 2007; Dionisi et al., 2005 and Chua et al., 2003.

Table 1. Improved yields of PHAs using three extraction and purification methods for *B. cepacia 2G-57* and Mixed Cultures from the WWTP

Microorganism (s)	**Condition of VFAs**	**Percentage % of PHAs in biomass**		
		SDS	**Chloroform**	**Acetic Acid**
B. cepacia	1.0 g/L	81.7	21.1	54.6
	0.7 g/L	92.0	18.9	52.3
	0.5g/L	65.0	10.4	41.8
Mixed cultures	1.0 g/L	38,3	10.4	39.6
	0.7 g/L	85.7	16.7	34.2
	0.5 g/L	81.0	12.3	28.7

Conclusions

This research made it possible to compare three different extraction routes for the PHAs that were produced by fermentations using Mixed Cultures from the El Salitre Wastewater Treatment Plant and the Pseudomona Bulkolderia cepacia strain and volatile fatty acids in different concentrations as a carbon source, which allows identify the best extraction and purification route with a view to scaling the process to an industrial level. Extraction with acetic acid as an alternative solvent to chloroform is encouraging in terms of the results obtained and the reduction of environmental impacts. However, it is necessary to perform more tests to validate the best method to use.

References

Albuquerque T., Reis., 2010, Polyhydroxyalkanoate (PHA) production by a mixed microbial culture using sugar molasses: Effect of the influent substrate concentration on culture selection, Water Research, 44(11), 3419–3433.

Argiz L., Fra-Vasquez A., Val del Río A., Mosquera-Corral, A., 2020, Optimization of an enriched mixed culture to increase PHA accumulation using industrial saline complex wastewater as a substrate, Chemosphere. 247, 125873.

Aramvash A., Zavareh F., Banadkuki N., 2018, Comparison of different solvents for extraction of polyhydroxybutyrate from Cupriavidus necator, Engineering in Life Sciences, 18, 20 – 28.

Beun J., K. Dircks K., Van Loosdrecht M., Heijnen J, 2002, Poly-beta-hydroxybutyrate metabolism in dynamically fed mixed microbial cultures.

Fonseca J., 2016, Extracción de PHAs con SDS, Instituto de Biotecnología de la Universidad Nacional de Colombia, Bogotá.

Hao J., Wang H., Wang, X., 2018, Selecting optimal feast-to-famine ratio for a new polyhydroxyalkanoate (PHA) production system fed by valerate-dominant sludge hydrolysate. Applied Microbiology and Biotechnology, 102(7), 3133–3143.

Lai S. Y., Kuo P. C., Wu W., Jang M. F., Chou Y. S., 2013, Biopolymer production in a fed-batch reactor using optimal feeding strategies. Journal of Chemical Technology and Biotechnology, 88(11), 2054–2061.

Lee Kim., Chung Kim., Rhee., 2017, Analysis of Medium-Chain-Length Polyhydroxyalkanoate-Producing Bacteria in Activated Sludge Samples Enriched by Aerobic Periodic Feeding. Microbial Ecology, 75(3), 720–728.

Morgan., Valentino F., Hjort M., Cirne D., Karabegovic L., Gerardin F., Werker A., 2014, Polyhydroxyalkanoate (PHA) production from sludge and municipal wastewater treatment. Water Science and Technology, 69(1), 177–184.

Rosengart A., Cesário M., Almeida M., Raposo R., Espert S., Apodaca A., Fonseca M, 2015, Efficient P(3HB) extraction from Burkholderia sacchari cells using non-chlorinated solvents, Biochemical Engineering Journal, 103, 39 – 46.

Chua A., Takabatake H., Satoh H., Mino T., 2003. Production of polyhydroxyalkanoates (PHA) by activated sludge treating municipal wastewater: effect of pH, sludge retention time (SRT), and acetate concentration in influent, Water Res, 37 (15), 3602-3611.

Dionisi D., Carucci G., Petrangeli P., Riccardi C., Majone M., Carrasco F., 2005, Olive oil mill effluents as a feedstock for production of biodegradable polymers, Water Res, 39 (10), 2076-2084.

Zhang D., Jiang H., Chang J., Sun J., Tu W., Wang H., 2019. Effect of thermal hydrolysis pretreatment on volatile fatty acids production in sludge acidification and subsequent polyhydroxyalkanoates production, Bioresour Technol, 279, 92-100.