

A life cycle perspective on polyhydroxybutyrate (PHB) synthesis using mixed sludge cultures

Jeniffer Gracia^{a*}; Paola A Acevedo^b

^a Universidad Distrital Francisco José de Caldas, Bogotá D.C., Colombia; ^b Universidad EAN, Bogotá D.C., Colombia
jjgraciar@udistrital.edu.co; ppacevedo@universidadean.edu.co

Abstract.

This study addresses the need for sustainable, biodegradable plastic alternatives by evaluating polyhydroxybutyrate (PHB) production using mixed microbial cultures and wastewater treatment plant (WWTP) sludge as feedstocks, thereby promoting a circular-economy approach to organic residue valorisation. A material-energy balance and Life Cycle Analysis (LCA) using SimaPro were conducted for two PHB production processes, both involving initial extraction with sodium dodecyl sulphate (SDS) and purification with either chloroform-methanol or acetic acid-methanol. The results showed that the acetic acid process generally led to a 10% higher environmental impact across most categories. However, the chloroform process caused 21% greater pollution in the stratospheric ozone depletion category. Based on green chemistry principles, the SDS-acetic acid-methanol process is deemed the more sustainable option for PHB purification, as it poses a significantly lower risk to human health than chloroform.

Keywords: Life Cycle Analysis; Polyhydroxybutyrate; Environmental impacts; Mixed crops.

Materials and methods.



Microbial Selection and Identification.

The study began with the selection and identification of microorganisms from activated sludge at a Wastewater Treatment Plant (WWTP). Serial dilutions and inoculations were performed using selective media containing Volatile Fatty Acids (VFAs) as the sole carbon source. This process yielded 27 strains in total, of which 20 demonstrated tolerance and growth in the VFA medium. For taxonomic identification, bacterial DNA was extracted, purified, and the 16S gene was amplified via PCR and subsequently sequenced. Bioinformatic analysis, including Basic Local Alignment Search Tool (BLAST) and phylogenetic tree construction, was then used to determine the taxonomic identity of the strains and characterize microbial diversity in the activated sludge sample. (Gracia et al. 2023; Gracia et al. 2024)

Pilot-Scale Fermentation and Extraction Methodology.

Following selection, Polyhydroxyalkanoate (PHA) production was conducted at a pilot scale in three airlift reactors. The fermentation utilized the selected activated sludge strains and VFAs generated from the primary sludge of the El Salitre WWTP, under aerobic conditions for 72 hours. The culture medium consisted of essential inorganic salts and micronutrients. For polymer recovery, an extraction process involving Sodium Dodecyl Sulphate (SDS) was used, followed by a comparative analysis of two purification methods: chloroform/methanol and the more environmentally benign acetic acid/methanol system.

Yield Analysis and Biopolymer Characterization.

The analysis of Polyhydroxybutyrate (PHB) extraction showed an average yield of 59.79% with chloroform and 54.52% with acetic acid at the pilot scale. Despite the slightly higher yield with chloroform, the total PHA recovery using acetic acid and methanol was notably high (approx. 90%). The identity and properties of the extracted PHB were rigorously validated using analytical techniques. FT-IR confirmed the characteristic functional groups, while TGA and DSC provided thermal properties (e.g., crystallinity and melting point) that were comparable to those of commercial PHB. GC-MS confirmed hexadecenoic acid methyl ester as the predominant monomer.

Life Cycle Assessment (LCA) Framework.

Finally, the Life Cycle Assessment (LCA) methodology, as outlined in ISO 14040, was applied to evaluate the potential environmental impacts of the fermentation and PHB production processes under optimal mixed-culture conditions. The LCA was conducted using SimaPro, employing the ReCiPe 2016 Midpoint (H) method for impact assessment. This method was chosen for its extensive set of 18 impact categories and robust international characterization factors, which enable a comprehensive and precise environmental evaluation of the biopolymer production system. (Feng et al., 2023; Manjunatha et al., 2021).

Results and discussion.

The LCA comparing polyhydroxybutyrate (PHB) production streams—purified with either chloroform-methanol or acetic acid-methanol—revealed that the fermentation and initial extraction processes are the dominant environmental burdens across most impact categories. Specifically, in the chloroform-methanol stream, fermentation and extraction generated an average of 87% of total impacts (see Figure 1). In comparison, the purification step accounted for 13%, with the notable exception of the stratospheric ozone depletion category, where chloroform-based purification contributed 35% due to the emission of an ozone-depleting precursor. In the acetic acid-methanol stream, which used SDS and acetic acid for purification, the fermentation and extraction phase similarly accounted for the majority of the impacts, at 79% of the total environmental effects, leaving the purification phase responsible for 21%. This comparative analysis underscores the need to optimize upstream fermentation and initial extraction processes to reduce the overall environmental footprint of PHB production significantly.

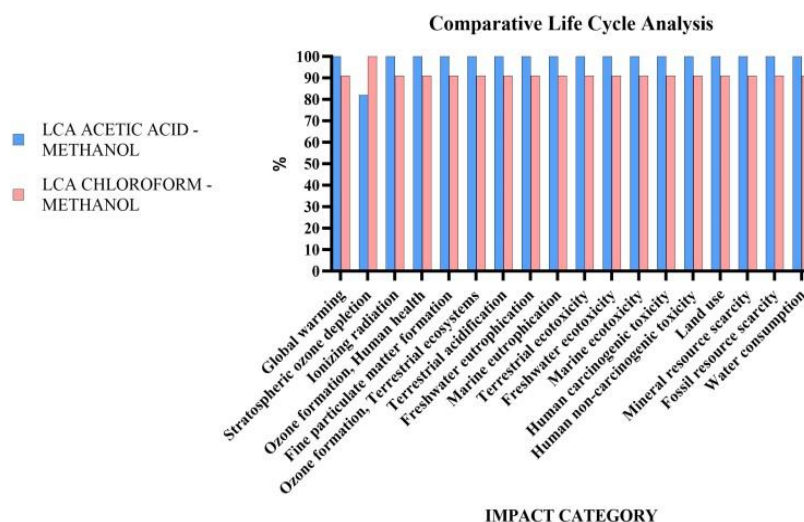


Figure 1. Comparative LCA of PHB purification processes

Conclusion.

This research successfully analysed a PHB production process from WWTP sludge, demonstrating a viable circular-economy approach to bioplastic synthesis as an alternative to conventional, high-impact plastics. Comparative LCA showed that both extraction methodologies achieved similar efficiencies and comparable environmental impact profiles across multiple categories. However, the acetic acid-methanol system is deemed more viable for large-scale implementation due to its significantly lower risk to human health compared to chloroform. The LCA identified that the high electrical consumption for PHB production is the primary contributor to the assessed environmental impacts, highlighting that future efforts should focus on integrating renewable energy.

References.

Feng, H., Zhao, J., Hollberg, A., & Habert, G. (2023). Where to focus? Developing an LCA impact category selection tool for manufacturers of building materials. *Journal of Cleaner Production*, 405. <https://doi.org/10.1016/j.jclepro.2023.136936>

- Gracia, J., Montenegro, C., Moreno, N., & Cabeza, I. (2023). Production of Polyhydroxyalkanoates using Volatile Fatty Acids from Municipal Wastewater Treatment Plant Sludge. *Chemical Engineering Transactions*, 100. <https://doi.org/10.3303/CET23100094>
- Gracia, J., Espinosa, A., Moreno, N., & Cabeza, I. (2024). Evaluation of the production and extraction of polyhydroxybutyrate from volatile fatty acids by means of mixed cultures and *B. cepacia*. *Environmental Research*, 250. <https://doi.org/10.1016/j.envres.2024.118448>
- Manjunatha, M., Preethi, S., Malingaraya, H., Mounika, H., Niveditha, K., & Ravi. (2021). Life cycle assessment LCA of concrete prepared with sustainable cement-based materials. *Materials Today: Proceedings*, 47, 3637–3644. <https://doi.org/10.1016/j.matpr.2021.01.248>