

# Life Cycle Assessment on Global Warming and Fine Particulate Matter Formation for Biological Extraction Method in Polyhydroxyalkanoates (PHA) Production: a Sustainable Alternative

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The production of polyhydroxyalkanoates (PHA), a promising class of biodegradable polymers, faces significant challenges due to the high costs associated with its downstream processing. Traditional methods for extracting PHA from microbial biomass are often expensive and environmentally taxing, involving the use of solvents and incalculable chemicals. There is a growing interest in developing non-conventional methods for PHA extraction to address these issues, such as utilizing insect model for the biological extraction of PHA. This method can potentially reduce costs, but its environmental feasibility is not inspected from a life cycle perspective. This study aims to evaluate the environmental impacts and identify the environmental hotspots of the biological extraction method in the PHA production with a focus on global warming (GW) and fine particulate matter formation (PMF) impacts using a life-cycle assessment (LCA) approach. The environmental impact of PHA production was assessed using the ReCiPe 2016 Midpoint (H) method, and results indicate that the biological extraction significantly contributes to GW (4.59 CO<sub>2</sub>-eq/kg of PHA) and PMF (4.59×10<sup>-3</sup> PM<sub>2.5</sub>-eq/kg of PHA), with steam used in drum drying process identified as major contributing activity. By addressing these environmental hotspots, the sustainability and environmental performance of biological extraction in PHA production can be significantly improved. These findings highlight the potential for biological extraction to contribute to more sustainable PHA production at an industrial scale, which could benefit stakeholders involved in PHA production.

## 1. Introduction

The environmental accumulation of petroleum-based polymers has raised significant concerns due to their negative impacts, prompting researchers to explore alternative materials that are biodegradable and environmentally friendly. PHA is a biopolymer that is biodegradable, non-toxic, thermoplastic, moldable, and flexible, suitable for various applications (Sudesh and Doi, 2000). It can completely degrade under specific environmental conditions and is produced from renewable resources such as sugars, plant oils, and vegetable oils, making it an attractive alternative to synthetic polymers (Loo and Sudesh, 2007). The expenses for feedstock, culture maintenance, and extraction constitute the most significant economic costs in PHA production (Koller, 2018). There is a scarcity of studies on downstream processes such as extraction and recovery (Koller, 2018). It is crucial to develop a more affordable and environmentally friendly PHA extraction method to advance towards a sustainable production process (Kunasundari and Sudesh, 2011). Currently, the primary challenge in the recovery process is achieving high recovery efficiency and purity in a cost-effective and environmentally friendly manner, while also preserving the molecular weight and thermal and mechanical properties of PHA (Kunasundari and Sudesh, 2011). To address these challenges, the extraction of PHA through biological processes represents a pioneering approach that harnesses the metabolic capabilities of living organisms to extract PHA. This method stands out for its environmental benefits, reducing the need for chemical solvents traditionally used in PHA extraction. The concept of using biological organisms for PHA extraction was first reported by Kunasundari et al. (2013). This groundbreaking work began when common house rats consumed

freeze-dried cells containing PHA and excreted white faecal pellets. Analytical tests revealed these pellets contained over 95 % pure PHA. Researchers conducted controlled studies with Sprague Dawley rats fed lyophilized cells of *Cupriavidus necator* H16 containing 39 wt% PHA. The results confirmed that the rats experienced no diet-related mortality and produced faecal pellets with 95 wt% PHA purity. Given the ethical and logistical challenges of using rats, researchers turned to mealworms (*Tenebrio molitor*) as an alternative for PHA extraction (Murugan et al., 2016). Mealworms can be bred in high densities with minimal water and space requirements, addressing some of the scalability issues. In experiments, mealworms were fed *Cupriavidus necator* Re2058/pCB113. The faecal pellets produced by the mealworms had a purity of 89 wt%, which increased significantly after treatment with a 1 % sodium dodecyl sulfate (SDS) solution at 50°C. Further studies by Ong et al. (2018) demonstrated that treating mealworm faecal pellets with water and 0.1 M sodium hydroxide achieved a purity of 94 wt%. These results indicated that mealworm gut contains enzymes such as proteases, cellulases, and lipases, which degrade bacterial cell walls and release PHA. Although the purity from mealworms was slightly lower than that of rats, this could be compensated with additional purification steps. Previous LCA studies on PHA extraction have primarily focused on laboratory-scale processes, highlighting significant environmental impacts associated with traditional chemical methods (Righi et al., 2017). A review by Pelliconi and Righi (2023), reveal notable environmental concerns but are limited by their small scale and hypothetical data, which may not reflect real-world conditions. Identified hotspots include high energy consumption and hazardous chemical use. In contrast, this study, based on a pilot-scale plant, offers a more realistic assessment of PHA production's environmental impacts, addressing past limitations and providing insights into the scalability and feasibility of biological extraction methods for industrial applications. Discovering the LCA of using insect models for PHA extraction is crucial, as these methods potentially offer a more sustainable alternative by reducing hazardous substances and energy use, making the findings more relevant to industrial-scale production. This first-of-its-kind study fills this gap by conducting a LCA of the biological extraction method for PHA production, analyzing its environmental hotspot, and identifying key areas for improvement.

## 2. Life-cycle Assessment Framework for Biological PHA Extraction

This research adheres to the life cycle assessment methodology outlined in ISO 14040/44, encompassing four key phases: (1) goal and scope definition, (2) inventory analysis, (3) impact assessment, and (4) interpretation (Chin et al., 2022).

### 2.1 Goal and Scope Definition

The primary goal of this study is to evaluate the environmental hotspot of the biological PHA extraction method from a life-cycle perspective. This assessment is crucial for understanding the potential environmental benefits and drawbacks associated with the use of biological method for PHA extraction. The scope of this study encompasses a "cradle-to-gate" system boundary, focusing exclusively on the biological extraction processes involved in PHA production (Figure 1). The functional unit (FU) for this analysis is defined as 1 kg of PHA generated by PHA biological extraction, ensuring that the results are standardized and comparable. It is important to note that this study does not include the end-of-life disposal and product use stages, limiting its boundaries to the production phase only.

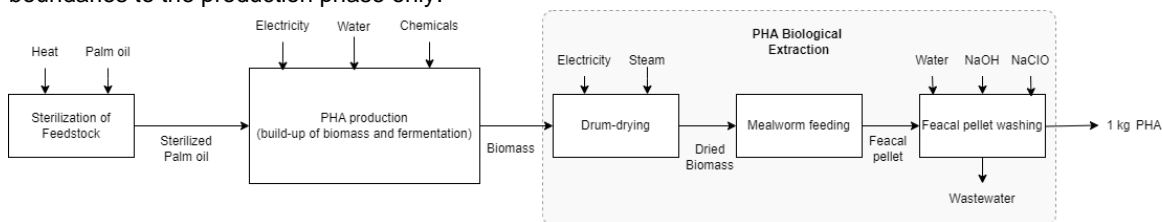


Figure 1: Flowchart of the biological extraction process.

### 2.2 Life-cycle Inventory

The life-cycle inventory for the production of 1 kg of PHA was compiled using data from laboratory experiments, pilot-scale operations, and literature. Inputs include raw materials, energy (electricity, heat), and water. Output include the water used for biological extraction. The data inventory per FU, is tabulated in Table 1. The process follows the method by Murugan et al. (2016) involving selecting and cultivating *Cupriavidus necator* Re2058/pCB113 bacterium in a minimal medium using crude palm kernel oil as the carbon source. Mealworms (*Tenebrio molitor*) were used to biologically extract PHA by feeding on dried bacterial cells containing PHA. The mealworms digest the cells and excrete PHA in their faecal pellets, which are collected and washed with water

to remove soluble impurities. Further purification involves multiple rounds of washing with mild solvents which are 0.25 M sodium hydroxide and 7.4 % sodium hypochlorite to ensure high purity of PHA, comparable to conventional solvent extraction methods. This method efficiently produces high-purity PHA while minimizing environmental impacts. To address data gaps, particularly regarding electricity consumption and steam generation at the facilities, life cycle inventory data from existing literature was utilized.

*Table 1: Data inventory for PHA production using biological extraction.*

Hotspot	Unit per kg PHA	Data references
<b>Sterilization</b>		
Palm Oil	2 kg	Primary data
Steam	0.77 MJ	Harding et al. (2007)
<b>Fermentation</b>		
Potassium sulfate	0.065 kg	Primary data
Sodium hydroxide	0.57 kg	Primary data
Phosphoric acid	0.94 kg	Primary data
Urea	0.064 kg	Primary data
Calcium chloride	0.021 kg	Primary data
Water	0.15 m <sup>3</sup>	Primary data
Electricity	0.72 kWh	Meyer et al. (2017)
<b>Biological Extraction</b>		
Water	1.13 m <sup>3</sup>	Primary data
Sodium hydroxide	0.18 kg	Primary data
Sodium hypochlorite	0.13 kg	Primary data
Steam	13.5 kg	Koch et al. (2023)
Electricity	0.53 kWh	Righi et al. (2017)
<b>Output</b>		
Wastewater	1.13 m <sup>3</sup>	Primary data

### 2.3 Life-cycle impact assessment

The Life Cycle Impact Assessment (LCIA) is conducted using SimaPro 9.2 software, which is widely recognized for its robustness and comprehensive database, enabling accurate modeling and analysis of the environmental impacts of PHA production. This study focuses on two primary impact categories which are global warming (GW) and fine particulate matter formation (PMF). Global warming refers to the potential impact of greenhouse gas emissions on climate change, while PMF addresses the health impacts associated with the formation of fine particulate matter in the atmosphere. The ReCiPe 2016 Midpoint (H) method is used for characterization, translating emissions into potential impacts expressed in carbon dioxide equivalents (CO<sub>2</sub>-eq) for GW and particulate matter with a diameter of 2.5 micrometers or less equivalents (PM<sub>2.5</sub>-eq) for PMF. The chosen characterization factors facilitate a detailed assessment of the environmental impacts of PHA production. The selection of GW and PMF impacts is based on their critical environmental and health implications. Climate change is a major global concern, and the production of biodegradable polymers like PHA is often evaluated for its potential to reduce greenhouse gas emissions. Relevant literature, such as Brizga et al. (2020) highlights the importance of assessing the carbon footprint of bioplastics. The formation of fine particulate matter is closely linked to respiratory and cardiovascular health issues, as underscored by Fowler et al. (2020) which emphasize the need to evaluate the air quality impacts of industrial processes. By focusing on these two categories, this study aims to provide a comprehensive understanding of the key environmental impacts associated with the biological extraction of PHA.

## 3. Results and Discussion

### 3.1 Environmental Impact Assessment

The environmental impact of PHA production was assessed using the ReCiPe 2016 Midpoint (H) method. This study highlights the analysis focused on global warming (GW) and fine particulate matter formation (PMF). The results, illustrated in Figure 2, identify significant environmental impacts at different stages of PHA production. Figure 2(a) shows that sterilization is the highest contributor to GW, accounting for 46.40 % of the total impact. Biological extraction follows closely, contributing 36.94 %, while fermentation contributes 16.65 %. The detailed data in Table 2 further elucidate these findings. In biological extraction, the use of steam in drum drying process are major contributor, with steam contributing 4.59 CO<sub>2</sub>-eq/kg of PHA. Figure 2(b) indicates that biological extraction has the highest impact on PMF at 37.56 %, followed by fermentation at 36.12 %, and sterilization at 26.32 %. In biological extraction, the use of steam emerge as significant contributors, with steam contributing 4.59×10<sup>-3</sup> PM<sub>2.5</sub>-eq/kg of PHA.

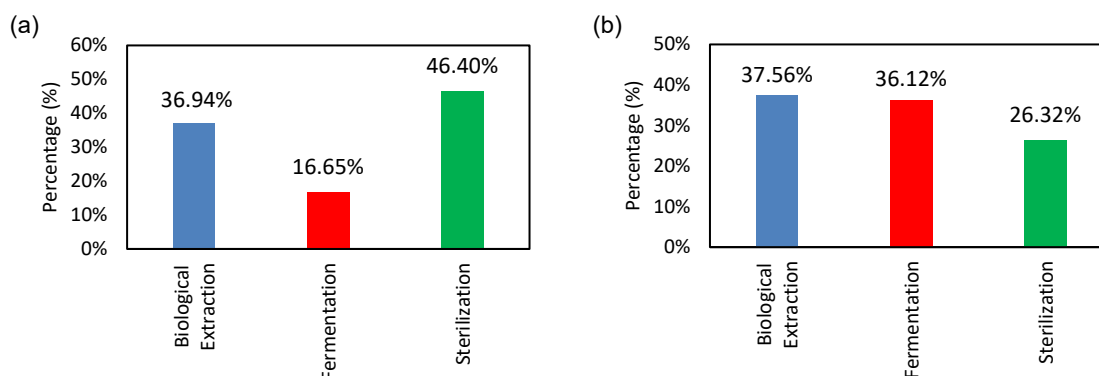


Figure 2: Human health impact on PHA production hotspots: (a) Global warming and (b) Fine Particulate Matter Formation.

Table 2: Life-cycle impact assessment data for PHA production using biological extraction

Hotspot	Unit kg <sup>-1</sup> PHA produced	Global warming, human health	Unit kg <sup>-1</sup> PHA produced	Fine particulate matter formation
<b>Sterilization</b>				
Palm Oil	CO <sub>2</sub> -eq	9.19	PM <sub>2.5</sub> -eq	7.68×10 <sup>-3</sup>
Steam	CO <sub>2</sub> -eq	9.52×10 <sup>-2</sup>	PM <sub>2.5</sub> -eq	9.52×10 <sup>-5</sup>
<b>Fermentation</b>				
Potassium sulfate	CO <sub>2</sub> -eq	9.88×10 <sup>-2</sup>	PM <sub>2.5</sub> -eq	3.10×10 <sup>-4</sup>
Sodium hydroxide	CO <sub>2</sub> -eq	7.86×10 <sup>-1</sup>	PM <sub>2.5</sub> -eq	1.82×10 <sup>-3</sup>
Phosphoric acid	CO <sub>2</sub> -eq	1.38	PM <sub>2.5</sub> -eq	6.23×10 <sup>-3</sup>
Urea	CO <sub>2</sub> -eq	2.17×10 <sup>-1</sup>	PM <sub>2.5</sub> -eq	3.76×10 <sup>-4</sup>
Calcium chloride	CO <sub>2</sub> -eq	1.63×10 <sup>-2</sup>	PM <sub>2.5</sub> -eq	4.21×10 <sup>-5</sup>
Water	CO <sub>2</sub> -eq	2.26×10 <sup>-1</sup>	PM <sub>2.5</sub> -eq	5.31×10 <sup>-4</sup>
Electricity	CO <sub>2</sub> -eq	6.04×10 <sup>-1</sup>	PM <sub>2.5</sub> -eq	1.36×10 <sup>-3</sup>
<b>Biological Extraction</b>				
Water	CO <sub>2</sub> -eq	1.75	PM <sub>2.5</sub> -eq	4.11×10 <sup>-3</sup>
Sodium hydroxide	CO <sub>2</sub> -eq	2.48×10 <sup>-1</sup>	PM <sub>2.5</sub> -eq	5.73×10 <sup>-4</sup>
Sodium hypochlorite	CO <sub>2</sub> -eq	3.54×10 <sup>-1</sup>	PM <sub>2.5</sub> -eq	8.19×10 <sup>-4</sup>
Steam	CO <sub>2</sub> -eq	4.59	PM <sub>2.5</sub> -eq	4.59×10 <sup>-3</sup>
Electricity	CO <sub>2</sub> -eq	4.44×10 <sup>-1</sup>	PM <sub>2.5</sub> -eq	1.00×10 <sup>-3</sup>

The biological extraction poses significant environmental challenges. The reliance on high steam inputs underscore the need for more sustainable practices. Despite these impacts, biological extraction presents substantial sustainability benefits over conventional plastic production, such as reducing dependence on fossil fuels and providing a biodegradable alternative to conventional plastics by mitigating plastic pollution (Walker and Rothman, 2020). Addressing the environmental impacts associated with biological extraction can significantly enhance the sustainability of PHA production. Key solutions for these hotspots include implementing advanced engineering solutions to retrofit existing production facilities, such as incorporating L-shaped baffles and utilizing a closed-loop drying system. L-shaped baffles improve the homogeneity of particle distribution across the drum, resulting in faster drying times by positioning more wood chips in regions with hot, unsaturated air (Scherer et al., 2016). Closed-loop drying systems offer a wide range of benefits, including reduced emissions, lower power consumption, and higher energy recovery potential. These systems operate by recycling the drying medium, thereby maintaining a consistent drying environment and enhancing overall efficiency (Hosseinabadi et al., 2014). Installing effective air pollution control systems like filters to reduce pollutant release, establishing chemical recycle systems to minimize waste by reusing chemicals within the production process, and enhancing wastewater treatment processes while developing water recirculation systems to reduce water consumption and prevent contamination are crucial steps to further improve environmental performance and sustainability. To promote the adoption of these sustainable methods, supportive policy measures and regulatory frameworks are essential. Implementing policies that incentivize research and development in biological extraction methods, providing subsidies or tax incentives for industries adopting these techniques, and establishing guidelines and standards for environmentally sustainable PHA

production are crucial steps. Enforcing regulations to limit emissions and waste associated with PHA manufacturing processes will be important.

### 3.2 Sensitivity Analysis

The sensitivity analysis aims to evaluate the impact of varying steam demand in biological extraction on environmental indicators GW and PMF. The baseline for steam demand is set at 100 %, with variations examined at 50 %, 60 %, 70 %, 80 %, 90 %, and 150 %. The data used for the sensitivity analysis are presented in Figures 3(a) and 3(b), illustrating the impact of changing steam input solely for the biological extraction stage, with the fermentation and sterilization stages remaining constant.

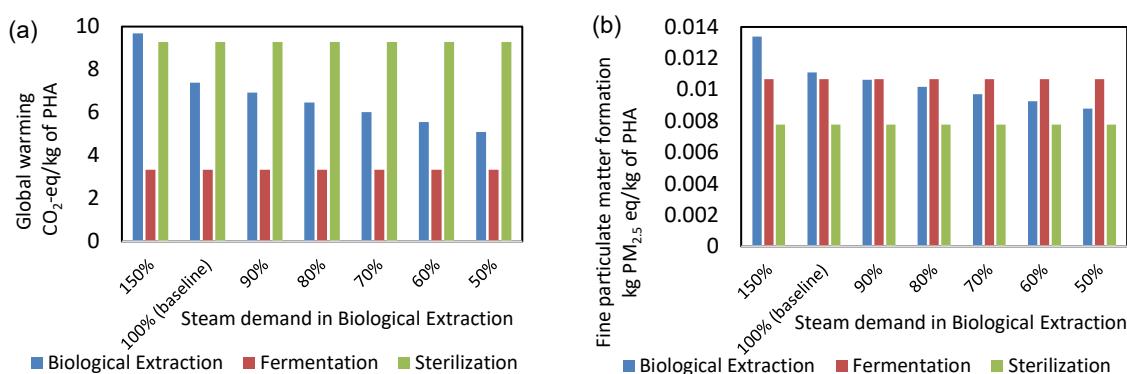


Figure 3: Sensitivity analysis of the steam used in biological extraction: (a) Global warming and (b) Fine Particulate Matter Formation.

The global warming varies significantly with changes in steam demand, reaching its peak at 9.68 CO<sub>2</sub>-eq/kg of PHA for biological extraction at 150 % steam demand and the lowest value of 5.09 CO<sub>2</sub>-eq/kg of PHA at 50 % steam demand. Similarly, the formation of fine particulate matter is highest at  $1.34 \times 10^{-2}$  kg PM<sub>2.5</sub>-eq/kg of PHA at 150 % steam demand and lowest at  $8.80 \times 10^{-3}$  kg PM<sub>2.5</sub>-eq/kg of PHA at 50 % steam demand. The fermentation and sterilization stages remain constant in their contributions. The sensitivity analysis indicates that reducing steam demand from 100 % to 50 % results in a 31.05 % decrease in global warming and a 20.72 % decrease in fine particulate matter formation. Conversely, increasing steam demand from 100 % to 150 % results in a 31.06 % increase in global warming and a 20.72 % increase in fine particulate matter formation. This analysis underscores the critical role of steam demand management in the biological extraction stage, suggesting that optimizing steam usage can significantly reduce the environmental footprint of PHA production, contributing to more sustainable and eco-friendly bioprocesses.

## 4. Conclusions

The life-cycle assessment of the biological extraction method for PHA production, conducted using the ReCiPe 2016 Midpoint (H) method, revealed critical insights into the sustainability and environmental footprint of the process. The analysis focused on global warming (GW) and fine particulate matter formation (PMF), identifying biological extraction, specifically steam, as a significant contributor with 4.59 CO<sub>2</sub>-eq/kg of PHA for GW and  $4.59 \times 10^{-3}$  kg PM<sub>2.5</sub>-eq/kg of PHA for PMF. Future research should focus on optimizing the biological extraction process to reduce its environmental impact and enhance efficiency. This involves developing innovative methods to lower energy consumption and emissions, exploring alternative microorganisms or enzymes for more efficient and less impactful processes, and conducting comprehensive life-cycle assessments of different PHA applications to understand their overall sustainability. Future research can significantly contribute to making PHA production more sustainable and environmentally friendly by addressing these areas.

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