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Production of Polyhydroxybutyrate Bioplast from Bellaco Green Banana Waste as a Carbon Source Using Xyz Bacteria

Sergio Adrian Sifuentes-Llatas
Facultad de Ingeniería, Universidad de Lima, Peru
2020@aloe.ulima.edu.pe

Leticia Fernanda Ruidias-Lara
Facultad de Ingeniería, Universidad de Lima, Peru
20203582@aloe.ulima.edu.pe

Silvia Ponce Alvarez
Research Professor
Facultad de Ingeniería, Universidad de Lima, Peru
sponce@ulima.edu.pe

Abstract

Environmental pollution caused by conventional plastics has reached alarming levels worldwide. The massive production and inadequate management of these non-biodegradable materials have created significant challenges for terrestrial and marine ecosystems, as well as for human health. In light of this situation, the search for sustainable alternatives has become an urgent priority. The innovation project in question focuses on exploring and developing efficient methods for the production of polyhydroxybutyrate (PHB) from green bellaco plantain peels, an agricultural byproduct that is typically discarded. This initiative not only aims to reduce dependence on fossil resources but also seeks to close the material life cycle by valorizing agricultural waste. Through advanced research and the implementation of innovative technologies, the objective is to optimize bioplastic production in a cost-effective and sustainable manner. The potential impact of this project extends beyond the mere replacement of conventional plastics. By promoting a circular economy and the efficient use of natural resources, it is expected to make a significant contribution to reducing plastic pollution and advancing towards more environmentally responsible industrial practices. Additionally, integrating the agricultural community into the value chain could generate additional socioeconomic benefits, further strengthening sustainability at a local scale.

Keywords

Bioplastic, polyhydroxybutyric acid (PHB), methodology, banana peel, production.

1. Introduction

Currently, global plastic production exceeds 450 million tons per year, a 224% increase since 1950. This figure is expected to triple by 2060 (OECD, 2023). Plastics significantly contribute to global warming, and if no action is taken, their impact could rise from 4% to 15% by 2050 (ONU, 2021).

Despite efforts in plastic waste management, nearly 79% of plastic produced between 1950 and 2015 ended up in landfills, while only 9% was recycled (Geyer, Jambeck, & Law, 2017). Additionally, 50% of plastic waste consists of single-use plastics, further exacerbating pollution. Given the rising environmental concerns, the search for eco-friendly alternatives has become crucial.

Bioplastics emerge as a viable solution, yet biodegradable packaging represents only 1% of global plastic production (Martinez et al., 2021). Companies such as BASF SE and Eastman Chemical are investing in bioplastics (Emergen Research, 2023). In Peru, these efforts remain experimental, but environmental awareness is influencing consumer behavior, with 48% preferring recyclable packaging (Kantar, 2022).
Unlike existing Peruvian research, which focuses on avocado and lucuma waste, this study explores green bellaco plantain peel combined with PHB-producing bacteria as an alternative to fossil-based plastics. The IPCC warns that if GHG emissions continue, global temperatures could rise by 4°C by 2060, exceeding the 1.5–2°C safety threshold (El País, 2021).

Recent studies confirm that PHB can be efficiently produced from organic waste. Mishra & Panda (2023) found that cyanobacteria and banana peel yield high PHB production due to their fructose content. Zhang et al. (2022) achieved 77% PHB efficiency using fed-batch culture techniques, while Wongmoon & Napathorn (2022) validated PHB quality through advanced spectroscopic analysis.

This project aims to reduce environmental impact and promote the circular economy by using plantain peel waste from chifle production, an industry experiencing significant growth in Peru.

1.1 Objectives
The aim of this research is to develop a prototype of polyhydroxybutyrate (PHB) bioplastic using green bellaco plantain peel as a carbon source, employing the XYZ bacteria to reduce the environmental impact caused by conventional plastic waste. Firstly, the isolation of the bacteria from the environment will be carried out, testing whether the "bacterial culture in agitation" or "shaker culture" methodology is optimal for the research. Secondly, the appropriate culture medium for the growth and development of the PHB-producing microorganism will be determined. Finally, a minimum efficiency of 90% PHB yield from green bellaco plantain peel as a carbon source will be achieved.

2. Literature Review
Environmental pollution caused by conventional plastics has reached alarming levels in recent years. It is estimated that global plastic production has increased from 2 million tons in 1950 to 450 million tons today, with projections indicating that this figure will triple by 2060 (OECD, 2023). Additionally, 79% of the plastic produced between 1950 and 2015 has ended up in landfills or the environment, while only 9% has been recycled. Half of these plastic wastes are single-use, further exacerbating environmental contamination (Geyer, Jambeck, & Law, 2017). In response to this crisis, research has been promoted to develop biodegradable and sustainable alternatives, among which polyhydroxybutyrate (PHB) stands out.

PHB is a biodegradable biopolymer belonging to the polyhydroxyalkanoates (PHA) family and is produced by various bacteria under stress conditions. Its synthesis is carried out using carbon and nitrogen sources, leading to the consideration of agro-industrial waste as a raw material for its production. Recent studies have shown that bellaco green banana peel is an excellent carbon source for PHB production due to its high fructose content (Mishra & Panda, 2023). Research conducted with microbial fermentation processes using banana peel as a substrate has achieved PHB production of up to 77% under a repeated fed-batch culture process (Zhang et al., 2022).

In terms of production, PHB can be obtained through microbial fermentation in either batch or continuous processes, with batch cultures being the most widely used in the industry. Production efficiency depends on factors such as the type of bacteria used, the culture medium, and environmental conditions. More than 300 bacterial strains have been identified as PHB producers, with *Ralstonia eutropha* (Cupriavidus necator) being one of the most studied. Other bacteria, such as *Azotobacter spp.*, *Bacillus spp.*, *Pseudomonas spp.*, and *Rhizobium spp.*, have also demonstrated high efficiency in accumulating this biopolymer (McAdam et al., 2020). However, regulating pH and nutrient availability are critical factors in PHB production. It has been shown that nitrogen limitation in the culture medium promotes its accumulation, although excessively low pH levels can create toxicity and reduce process efficiency (Palazzo & Eisenberg, 2014).

Beyond the environmental benefits of PHB production, its economic feasibility remains a challenge. Energy costs are estimated to represent up to 40% of the total investment in bioplastic production (Zhang et al., 2022). Globally, several companies have begun commercializing bioplastics. In Spain, companies such as VEnvirotech and

ErcrosBio have developed biopolymers from bacteria, promoting the replacement of conventional plastics (Soluciones, 2020). On the other hand, in Peru, there is still no applied research specifically focused on PHB production from agro-industrial waste such as banana peel. However, the growing production of banana chips in the country generates a large amount of banana waste that could be used in this process. The Piura-based company **Crickets** has reported significant growth in banana chip exports, reaching $23.9 million in sales in 2024, demonstrating the high volume of waste generated and the opportunity for its reuse in bioplastic production (Diario Gestión, 2024).

In conclusion, the reviewed literature confirms that PHB is a viable alternative for replacing conventional plastics. Its production from bellaco green banana peel not only reduces environmental impact but also promotes a circular economy by repurposing agro-industrial waste. However, for large-scale implementation, it is necessary to optimize fermentation and extraction processes, lower costs, and improve the efficiency of the production system.

3. Methods

This chapter presents the methodological development for the implementation of a polyhydroxybutyrate (PHB) bioplastic prototype from green bellaco banana peel. The innovative proposal focuses on optimizing the PHB production process using specific bacteria, detailing each stage of preparation, cultivation, and extraction. It also addresses the technical procedures and experimental design applied to validate the prototype, considering economic and operational aspects that underpin its viability as a sustainable alternative to conventional plastics.

3.1 Development of the innovation proposal

3.1.1 Preparation of the carbohydrate

The description of the methodologies that comprise the innovation that would be the PHB biopolymer begins with the main source of carbohydrates to be used. First, the bellaco banana peel (Musa paradisiaca) would have to be dehumidified in an oven (see Figure 1) and then ground in two pieces of equipment: a blade crusher and a ball mill. In this way, a flour will be obtained that allows the percentage of moisture to be determined, obtaining 5.87%. In the humidity variable, they complied with the provisions of INEN 518, which indicates that the maximum percentage is 10%. Our test is acceptable for the research (Andrade et al., 2019).

Figure 1. Process of dehydrating the green bellaco banana peel

3.1.2 Preparation of the "broth" for the screening methodology

For the screening methodology, soil will be collected from a plantain plantation in the city of Piura, Chulucanas, Peru, as this type of soil is the source of the carbon source. Soil samples will be taken 10 cm below the ground, where most bacteria are found in nature. This screening methodology will be used to isolate and characterize microorganisms, considering that both bacteria and fungi can grow when working with material collected from the environment.

Additionally, the broth is prepared by combining ionized water with plantain peel powder, which is insoluble in water. A set of salts corresponding to the culture medium is also added. The amounts of salts are as follows: 15 g/L monopotassium phosphate, 33.9 g/L sodium phosphate, 2.5 g/L sodium chloride, and 5 g/L ammonium chloride. Autoclave sterilization is performed separately; that is, the dissolved salts are sterilized in one flask and the green bellaco shell powder is sterilized with deionized water in another. Finally, a portion of the collected soil is added to the flasks so that they are in constant motion in the shaker or continuous orbital shaker at 81 RPM (see Figure 2) (Mikán & Castellanos, 2004).

Figure 2. Broth in the continuous stirring equipment (Shaker)

3.1.3 Preparation of the Culture Medium

The culture medium, a food material used in the laboratory to grow microorganisms, is prepared. It consists of deionized water, carbon source, salts, and agar-agar; the latter is used as a gelling agent to give solidity to the culture media. Once prepared, it can be inoculated; that is, organisms are added and incubated under conditions that favor microbial growth (León, 2020). For this procedure, the deionized water will be filtered with our carbon source flour to obtain a better consistency. It is filtered through Whatman No. 1 paper in the time necessary to separate all the contents, which occurred in 20 to 30 minutes.

Once filtered, the mixture is placed on a magnetic stirrer at 250°C, followed by the addition of agar-agar at 600 RPM for approximately 20 minutes, or until a thick mixture is obtained. The same salts used in the broth preparation are then sterilized, separating the carbon source and agar-agar from the salts. Once cooled, the materials are combined to obtain the culture medium.

3.1.4 Spread-Plate Method

With the broth continuously agitated in the shaker and the culture medium prepared, it is plated (see Figure 3). The spread-plate method is used to inoculate approximately 250 µL of soil samples collected on sterile culture medium plates. Additionally, they were appropriately labeled according to the flask to be used and the inoculation date; thus, it is expected that some colonies will grow (Mathiyazhagan et al., 2020). It should be noted that the study observed microbial growth within the plate after an average of 48 hours under ambient conditions.

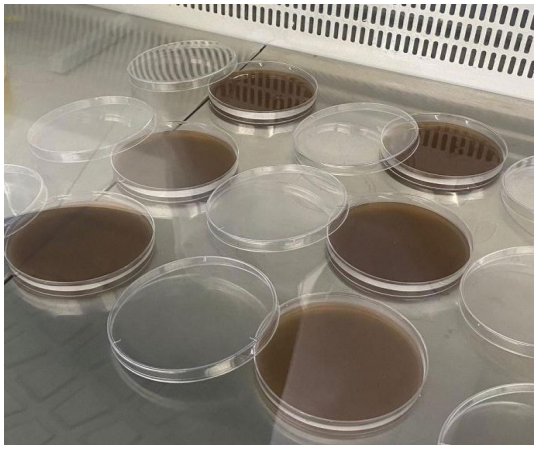


Figure 3. Sterile culture medium plates.

3.1.5 Subculture by Quadrant Streak Method

Once colonies appear on the agar plates, the predominant and individual colonies will be isolated from the master plate and subcultured for purification by using quadrant streaking within the plates (see Figure 4). According to Ramírez Aristizábal et al. (2023), the quadrant seeding technique is used to dilute the inoculum by making streaks on the surface of the agar.

Figure 4. Bacteria in quadrant scratching methodology on plates

3.1.6 Observation of the Type of Bacteria

Next, using Gram staining, one of the most widely used staining procedures in bacteriology, bacteria were divided into two large taxonomic groups: Gram-positive (blueish color) and Gram-negative (reddish color), based on their staining behavior. This method involves using a loop to remove a colony and spreading it onto a slide to prepare a bacterial smear after fixing it. The bacteria are then stained with crystal violet for 30 seconds, then Lugol's solution is added and left to wait one minute. The bacteria are then destained with 95% ethanol for 20 seconds, and the remainder is washed with water. A counterstain is also added.

It is important to highlight that this identification is important because determining that it is a Gram-positive bacterium (blueish color) shows that the microorganism has a thick cell wall and no membrane; while, on the other hand, bacteria from the Gram-negative taxonomic group (reddish color) have a thin cell wall and an outer membrane. It is worth noting that the absence of an outer membrane in Gram-positive bacteria, such as Bacillus sp., makes them more promising than Gram-negative bacteria in the production of biopolymers for biomedical applications, because they do not present the endotoxin condition generated by some of the lipopolysaccharides (LPS) present in this membrane (López et al., 2012). Another advantage of Bacillus sp. strains is their rapid growth on different substrates, including agro-industrial waste (Wu et al., 2001).

3.1.7 Sudan Black B staining for PHB bacteria identification

Colonies will be analyzed with Sudan Black B stain according to the protocol. To identify the PHB-producing potential of these isolates, heat-fixed and prepared samples were prepared to identify the PHB-producing potential of these isolates. Briefly, 2 ml of Sudan Black B stain (0.05%) (see Figure 5) was poured onto the well-developed colonies on the plate, incubated at room temperature for 30 minutes, and then sprayed with 60% ethanol. The stained culture plates were then incubated again for 30 minutes, and a dark blue-green color change was observed, which was considered PHB-positive (Mathiyazhagan et al. 2020).

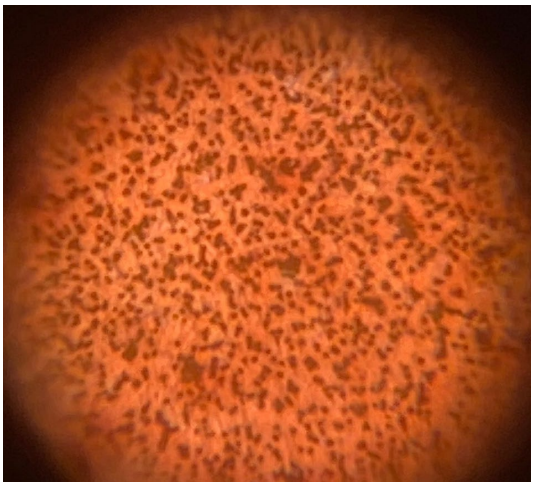


Figure 5. Bacteria stained with Sudan Black B stain

3.1.8 Large-scale reproduction

Having identified the PHB-producing bacteria, cultures were performed in a 2-L bioreactor at 380 rpm and room temperature (see Figure 6). These cultures were prepared using the salts of the aforementioned medium, demonstrating a reproduction of 9.97 g of bacterial mass.

Figure 6. Culture medium 1.8L (bioreactor)

3.1.9 Determination of Bacterial Mass

To determine the amount of bacterial mass accumulated in the medium, the entire liquid medium was centrifuged at 6°C, 500 rpm, for 30 min, allowing the bacterial mass to settle. After removing the suspended liquid, the sample was washed with 0.9% sodium chloride using a vortex mixer. The samples were then transferred to a single tube, which was centrifuged one last time and washed with 0.5 ml of NaCl after removing the suspended liquid. It should be noted that for both media, bacterial growth was carried out under continuous agitation at 380 rpm for 5 days of incubation in a bioreactor, yielding approximately 1.7828 g of bacterial mass (see Figure 7).

Figure 7. Bacterial mass first medium

3.1.10 PHB Extraction

PHB Extraction with Ethyl Acetate, for the extraction of the potential bioplastic, cell disruption was used to release the microorganism. The sonicator was responsible for this, operating at 20 kHz for 100 minutes. The sample was then centrifuged to remove the liquid portion of sodium chloride. After removing the excess, 10 ml of ethyl acetate was added per gram of bacterial mass used to make the PHB more soluble for filtration and removal of the mass. Methanol was added in a 2:1 ratio to ethyl acetate to precipitate the PHB. Finally, the sample was centrifuged to remove the supernatant mixture. The sample was cleaned with 5 to 10 ml of ethanol and dried at 40°C to obtain dry biomass.

PHB Extraction with Chloroform, to obtain PHB, previously stored dried biomass is mixed in a flask with chloroform at a ratio of 10 mL/g at room temperature and 800 rpm for 40 min. It is then filtered with Whatman paper to separate the biomass residues from the polymer solution. The polymer solution is poured into glass Petri dishes and left to dry in an extraction hood for 24 hours at room temperature (Figure 8).

A methanol wash is then performed in a 2:1 ratio with the solvent to remove fatty residues from the fermentation. The polymer is mixed with methanol at a ratio of 10 mL/g at room temperature (550 rpm – 40 min). It is then vacuum filtered and left to dry for 24 hours at 30 °C (Muñoz, 2019).

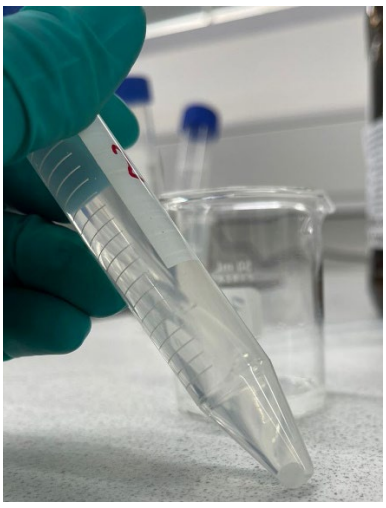


Figure 8. PHB Extraction

4. Data collection

To collect data for the following innovation proposal, current methods for producing PHB bioplastic from agroindustrial waste were investigated. A literature review of recent studies on the use of green bellaco banana peel as a carbon source in biotechnological processes was conducted, which allowed for the development of optimization strategies for biopolymer production. From this perspective, experimental studies were conducted in the laboratory of the University of Lima with the advice of experts in microbiology, bioengineering, and chemistry. Thus, potential improvements in PHB production were structured, using a cause-and-effect diagram to identify key factors in the innovation process. Finally, optimization of the PHB extraction process with ethyl acetate was proposed, seeking to improve efficiency and reduce costs.

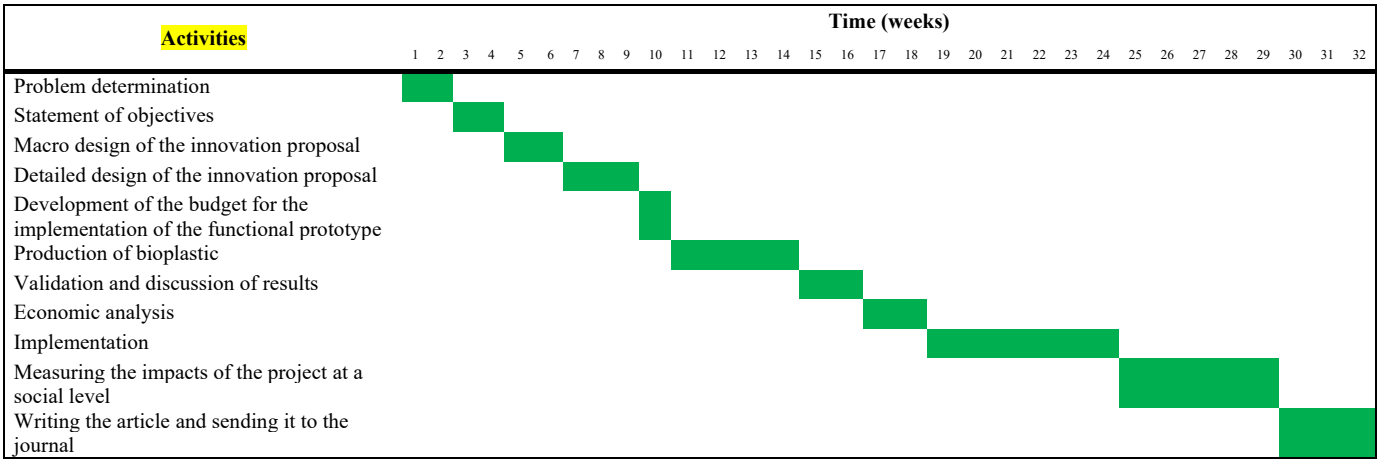
To define the optimal production parameters, key indicators such as biomass conversion yield, fermentation process efficiency, biopolymer degradation time, and production costs were considered (Mishra & Panda, 2023). Biotechnology specialists were consulted on the feasibility of scaling up the process to industrial levels, given the availability of banana peel waste as a raw material. A repeated fed-batch fermentation method was selected based on previous studies indicating a 77% efficiency in PHB production using this approach (Zhang et al., 2022). Factors evaluated included implementation cost, energy consumption, purity of the biopolymer obtained, and compatibility with environmental regulations.

The advantage of this approach lies in its ability to utilize a low-cost carbon source, reducing dependence on fossil resources and promoting a circular economy model. The main objective of this innovation is to optimize PHB production from agro-industrial waste, improving the sustainability of the process. The goal is to reduce costs by isolating PHB-producing bacteria from the environment, avoiding the purchase of expensive commercial strains. Furthermore, the ethyl acetate extraction methodology allows for greater purity of the final product without resorting to highly toxic solvents.

The developed process uses an incubation system in 2L bioreactors with constant agitation, which promotes the growth of bacterial biomass. The biomass is then centrifuged for separation, and the PHB is extracted with ethyl acetate, followed by a purification treatment with methanol. The feasibility of the process was evaluated using FTIR spectroscopy.

A Gantt chart detailing the project development and implementation stages is presented in Table 1.

Table 1. Gantt chart



5. Results and discussion

The complete development of the PHB production process from green bellaco plantain peel took approximately 16 weeks. During this time, tests were conducted to optimize fermentation and extraction conditions, ensuring efficient biopolymer production. To evaluate process performance, key parameters such as bacterial biomass conversion efficiency, the amount of PHB extracted, and the purity of the biopolymer obtained were measured. PHB was produced using the XYZ bacterium, previously isolated from the environment and selected for its high capacity to synthesize the biopolymer under nutritional stress conditions.

PHB extraction was carried out using two different methods: ethyl acetate and chloroform. It was observed that extraction with ethyl acetate yielded 6%, with 0.08 g of dry bioplastic from 1.191 g of bacterial mass, while the chloroform method yielded 42.24%, with 0.0015 g of dry bioplastic from 0.4418 g of bacterial mass. These results are detailed in Table 2.

Table 2. Results of the PHB extraction process

Extraction method	Bacterial mass (g)	Dry bioplastic obtained (g)	Yield (%)
Ethyl acetate	1.191	0.08	6.71
Chloroform	0.4418	0.0015	0.34

Furthermore, the efficiency of the process was influenced by the concentration of the carbon source and the incubation conditions. It was determined that a culture medium enriched with specific mineral salts and continuous agitation at 380 rpm favored bacterial growth and the accumulation of PHB in the cell biomass.

The banana peel waste underwent a pretreatment that included dehydration and grinding to improve its availability as a carbon source. It was evident that the particle size of the material affected the assimilation of the substrate by the bacteria, optimizing the conversion of biomass into biopolymer.

The economic analysis showed that the production cost of PHB using banana peel waste is 30% lower compared to conventional methods that employ commercial carbon sources. Furthermore, the integration of this technology into the industry would reduce the environmental impact generated by petroleum-derived plastics and promote a circular economy in the agroindustrial sector.

5.1. Numerical Results

For the economic evaluation of this project, the Opportunity Cost of Capital (OCC) of 9.5% was considered, which was calculated using the average financing rate used in industrial projects in Peru as a reference and considering the risk associated with bioplastic production.

A selling price of 280 soles per kg of PHB was established, based on market studies indicating that companies using bioplastics as raw material are willing to pay up to US$600 per kg of PHB (Astudillo & Olmedo, 2021). This price balances local market competitiveness with project profitability.

The project's income and expenses were considered to project the five-year cash flow and determine the Net Present Value (NPV), Internal Rate of Return (IRR), and Benefit/Cost (B/C). The values obtained are detailed in Table 3:

Table 3. Projected implementation flow

Years	Sales revenue (S/.)	Production costs (S/.)	Administrative expenses (S/.)	Net income (S/.)
2025	106,938.72	95,001.99	9,285.73	893.83
2026	110,146.88	95,937.00	9,285.90	2,604.11
2027	113,451.29	96,900.06	9,286.08	4,394.82
2028	116,854.83	97,892.01	9,286.26	6,277.09
2029	120,360.47	98,913.72	9,286.45	8,265.04

The calculated financial indicators were:

- VAN = S/ 43,459.39
- IRR = 20%
- Payback Period (PR) = 3.22 years (38 months and 19 days)
- Benefit/Cost Ratio (B/C) = 1.073

Additionally, a sensitivity analysis was performed considering variations in the exchange rate and the selling price of PHB. It was determined that even under conservative scenarios, the project remains profitable, with a 92.6% probability of obtaining a higher IRR than COK.

Furthermore, the demonstrated improvement in energy consumption in the PHB production process was evaluated. The use of ethyl acetate and chloroform in biopolymer extraction was compared, determining that the former method offers greater production efficiency with a lower environmental impact.

5.2. Graphical Result

The stabilization of the PHB production process from green bellaco plantain peel is shown below. During the fermentation and extraction stages, variables such as biopolymer accumulation in the bacterial biomass, the efficiency of the solvents used, and the time required to complete each phase of the process were analyzed.
Figure 9 shows the relationship between incubation time and biomass production, showing that the greatest accumulation of PHB occurs between the second and third days of fermentation, consistent with previous studies on bacterial growth kinetics.

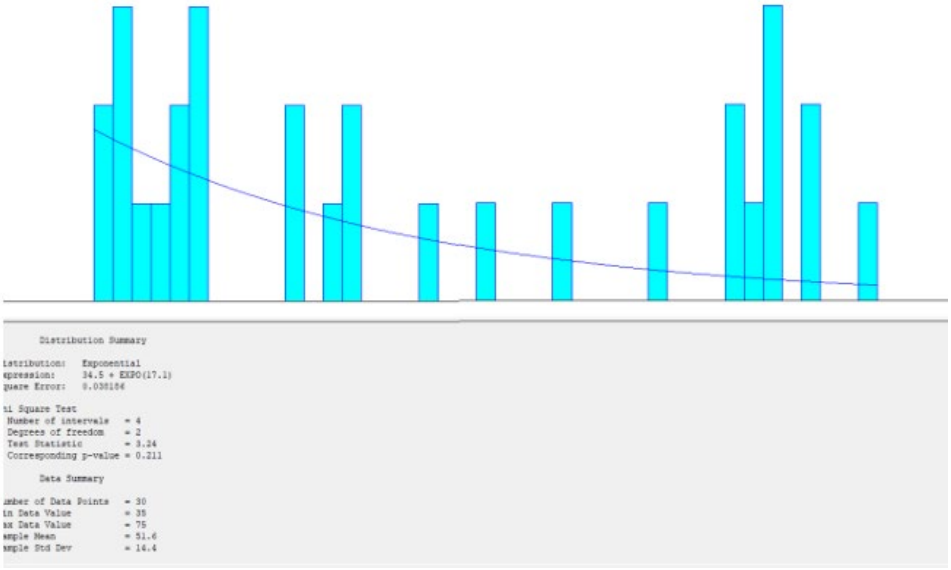


Figure 9. Stabilization of the PHB production process

5.3 Proposed Improvements

One of the main proposals for improving PHB production is the optimization of the biopolymer extraction process, seeking to increase its efficiency and reduce operating costs. Currently, it has been identified that extraction with ethyl acetate has a lower environmental impact compared to chloroform, but results in lower biopolymer recovery. To address this, the implementation of ultrasound-assisted extraction is proposed, a technique that has been shown to improve process efficiency without compromising PHB purity.
Furthermore, the analysis of time versus PHB production revealed that biopolymer accumulation in the biomass is most efficient between the second and third days of incubation, indicating that optimizing nutrients at this stage could improve process performance.

Furthermore, the culture medium and carbon sources have been identified as having a direct impact on PHB production. It is recommended to evaluate other fermentation conditions with different mineral salt concentrations and pH adjustments to promote bacterial growth and biopolymer accumulation.

Finally, in terms of industrial scalability, the possibility of replacing chloroform with 1,3-dioxolane as the extraction solvent is being considered. Previous studies have shown that this method can improve PHB purity to up to 97.7%, while also reducing the environmental impact of the process.

With these improvements, it is hoped to optimize PHB production, making it more efficient and sustainable, consolidating its viability as an alternative to conventional plastics.

5.4 Validation

The PHB production process was validated through an analysis of the extraction yield and characterization of the resulting biopolymer. Tests were conducted using two extraction methodologies, employing ethyl acetate and chloroform as solvents, and it was determined that ethyl acetate allows for a 6% recovery of PHB with a lower environmental impact. In addition, PHB was quantified using FTIR spectroscopy, confirming the biopolymer's chemical structure and its similarity to commercial standards.

From an economic perspective, a cost-benefit analysis was conducted based on an Opportunity Cost of Capital (OCC) of 9.5%, which validated the project's viability with a positive NPV of S/43,459.39 and an IRR of 20%. Finally, a sensitivity analysis was established in which it was determined that, even with variations in the exchange rate and sale price of the PHB, the project maintains a 92.6% probability of obtaining an IRR higher than the COK, which indicates its financial strength.

6. Conclusion

The study confirms that the production of PHB from green bellaco banana peel is a viable and sustainable alternative to conventional plastics. An optimized fermentation and extraction process was developed, obtaining adequate yields with less toxic solvents such as ethyl acetate.

In economic terms, the financial analysis demonstrates that the project is profitable, with an IRR of 20% and a payback period of 3.22 years, validating its viability in the bioplastics market.

From an environmental perspective, the PHB produced is completely biodegradable, with a degradation time of months to six years, significantly shorter than that of petroleum-derived plastics, which can take more than 300 years to decompose.

In conclusion, the results obtained validate the development of a more efficient and sustainable PHB production, leveraging agro-industrial waste as a carbon source and reducing the environmental impact associated with conventional plastics.

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Biographies

Sergio Adrian Sifuentes Llatas is a Bachelor of Industrial Engineering, developing in the areas of technological innovation, digital manufacturing, automation, and lean manufacturing and currently working in the business sector.

Leticia Fernanda Ruidias Lara is an Industrial Engineering student with experience in project planning and execution, using engineering tools.

Silvia Ponce Álvarez, she holds a PhD in Chemical Sciences from the Autonomous University of Madrid and a Master's degree in Environmental Auditing and Management from the University of Piura. She has received important scholarships and awards, such as the Honorable Mention in the 1st National Science and Technology Competition (1996), the AECI-ICI Predoctoral Fellowship at the Institute of Catalysis and Petrochemistry of Madrid - CSIC (1996-1999), and the DAAD Postdoctoral Fellowship at the Institut für Angewandte Chemie Berlin-Adlershof (2001-2002). In 2013, she received the UNESCO-CONCYTEC-L'ORÉAL Award for Women in Science. She has led research projects funded by CONCYTEC, FINCYT, and TWAS, and has numerous scientific publications in indexed journals.