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

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# Introduction

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**.

Considering 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)

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 2L and 20L 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),

Third extraction and purification method using Acetic Acid was used according to the methodology of Aramvash et al (2017). Finally, the characterization, yield and purity of PHA were performed for each method.

# Materials and methods

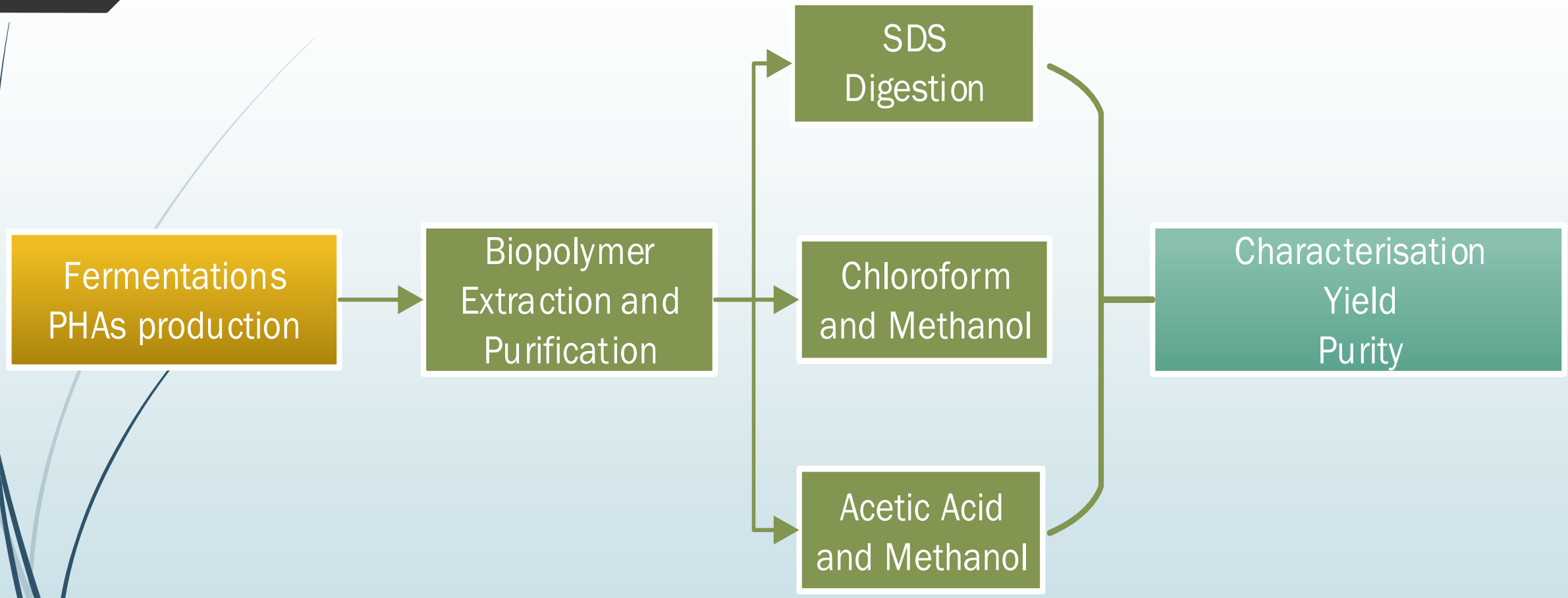
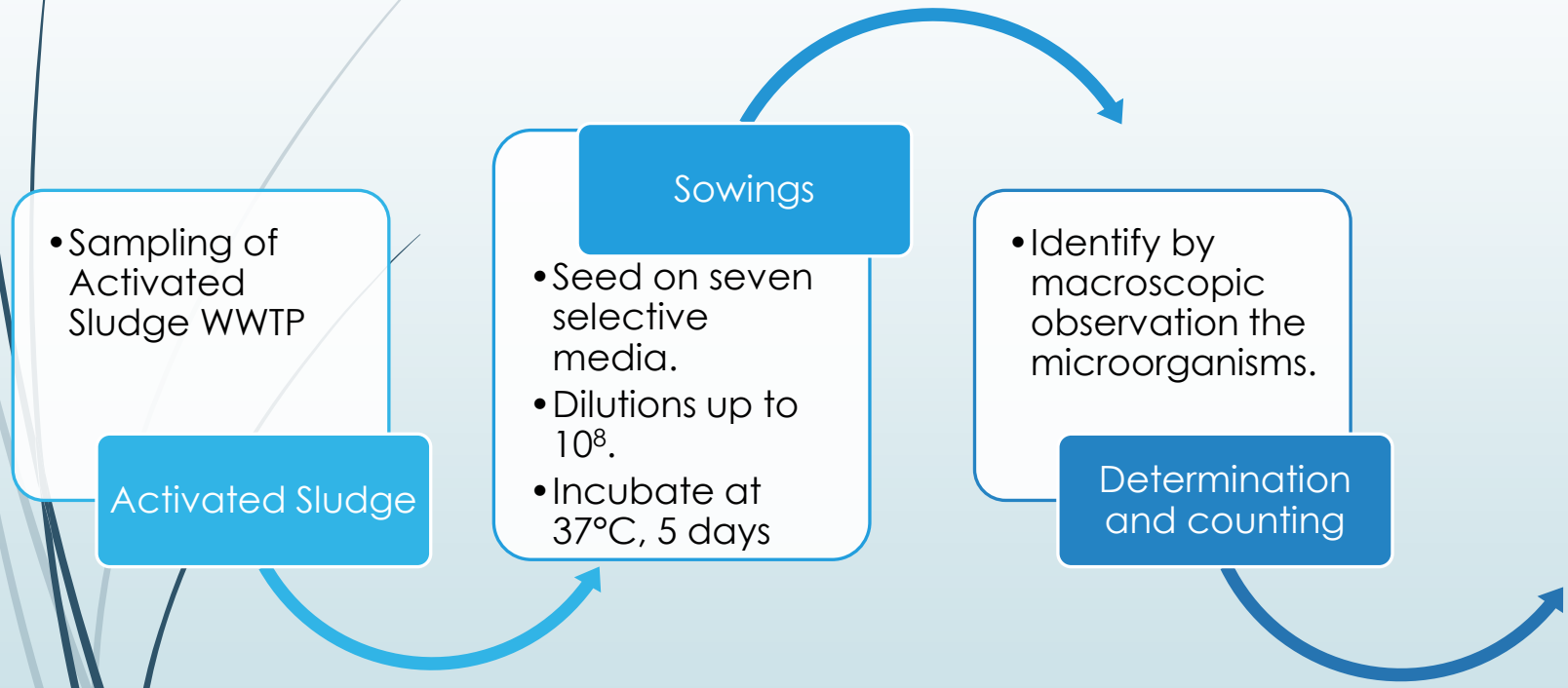



**Figure 1. General Research Methodology**

# Materials and methods



# Materials and methods

- PHA extraction with SDS from pilot fermentations.

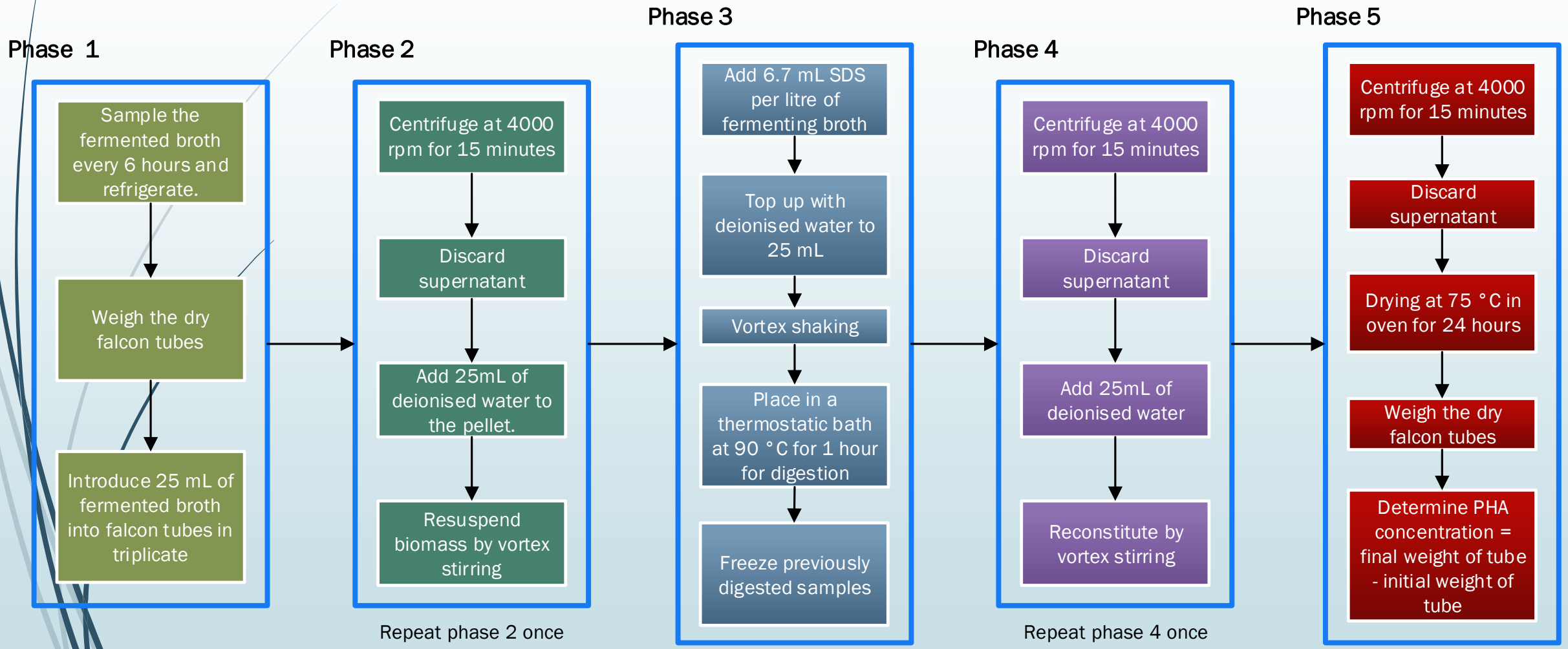



**Figure 3. SDS Extraction Process**

# Materials and methods

- **PHA Extraction and Purification with Chloroform and Methanol**

The pellet obtained in the digestion with SDS in dry state was pulverized with a mortar and suspended in analytical grade chloroform using the ratio of 8 mL of solvent per gram of biomass.

The mixtures were stirred in a shaker at room temperature for 2 hours.

Subsequently, the solution was filtered using a vacuum pump and paper filters to separate into two different phases, a liquid phase composed of polymer and chloroform, and a solid phase of the biomass retained on the filter.

The polymer solution was poured into Petri dishes and left at room temperature until the chloroform evaporated, resulting in a PHA film.

# Materials and methods

## PHA Extraction and Purification with Chloroform and Methanol

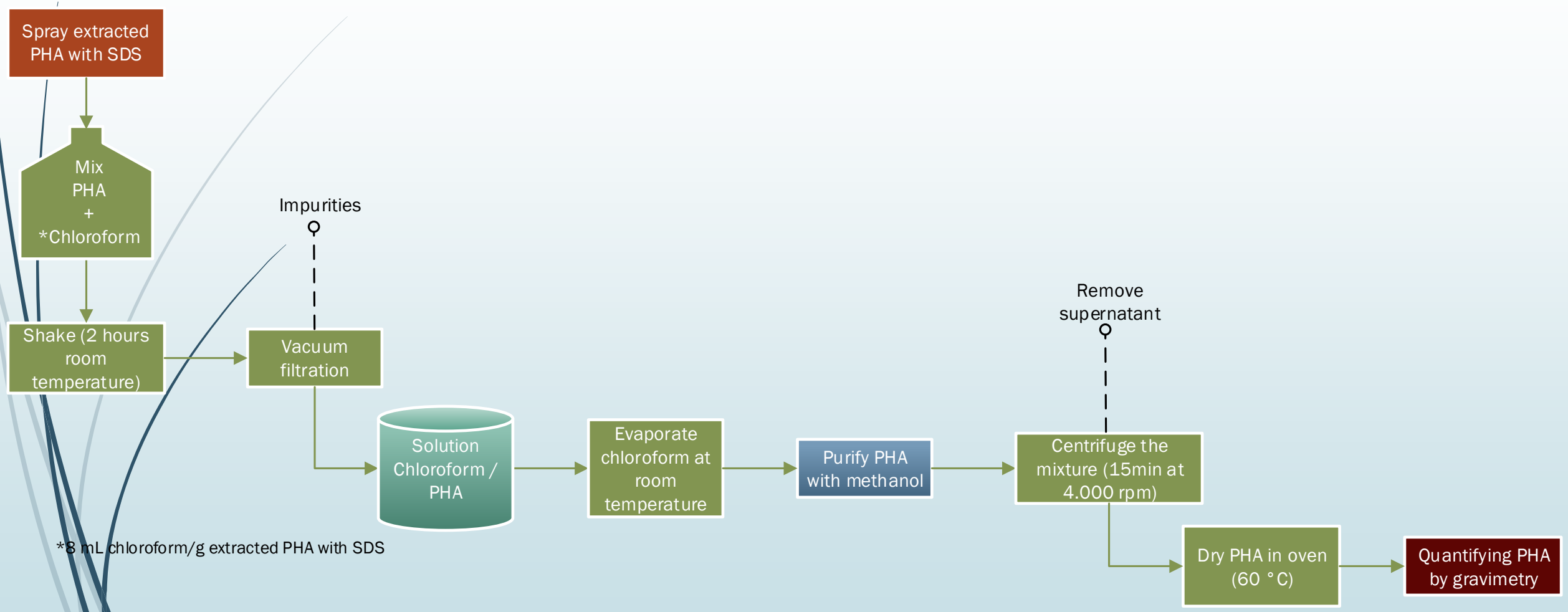



**Figure 4. PHA Extraction and Purification Process with Chloroform and Methanol**

# Materials and methods

- **PHA extraction with acetic acid and methanol.**


For the solubilization process the mixtures were brought to 90°C for one hour, following the methodology of Ramos (2019) and at an acid concentration of 1000g/L, then purified with methanol and centrifuged at 4000rpm for 15 minutes and dried in oven at 60°C for 24 hours to remove excess solvent.

# PHA Extraction and Purification with Acetic Acid and Methanol

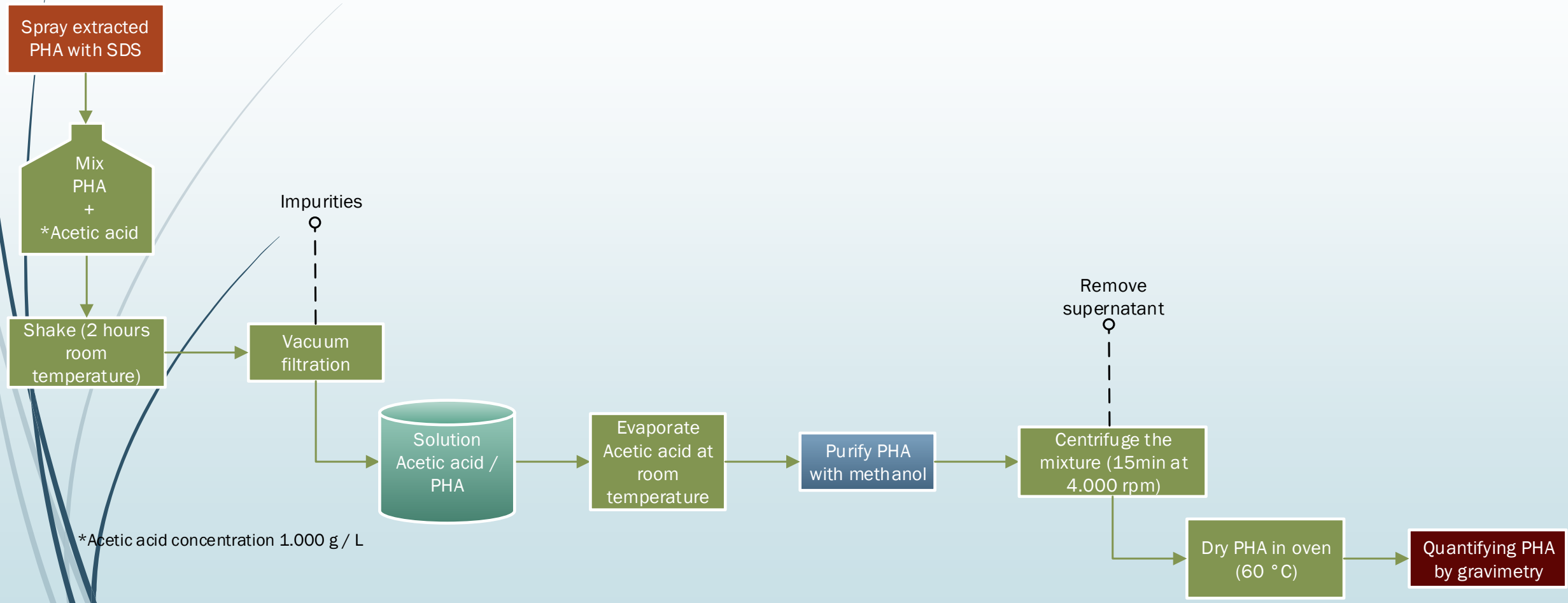



**Figure 5. Extraction and Purification Process of PHA with acetic acid and methanol.**

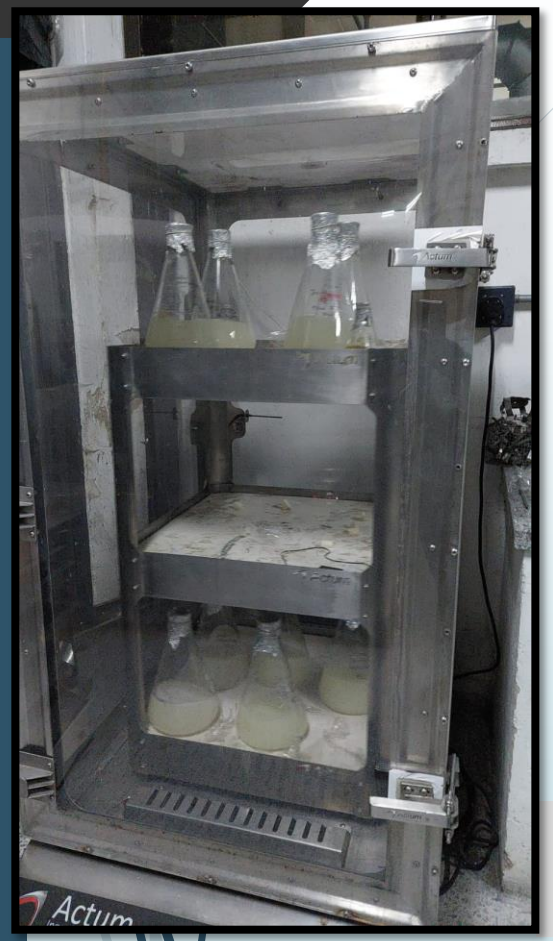
# Results

- **Fermentation to obtain PHA**

Photograph 1. Start of fermentation in Pilot Reactors R1-R2-R3. (0 hours)

Photograph 2. End of fermentation in Pilot Reactors R1-R2-R3. (72 hours)

# PHA extraction with SDS




Photograph 3, Laboratory scale fermentations


Photograph 4. Vortex Stirrer

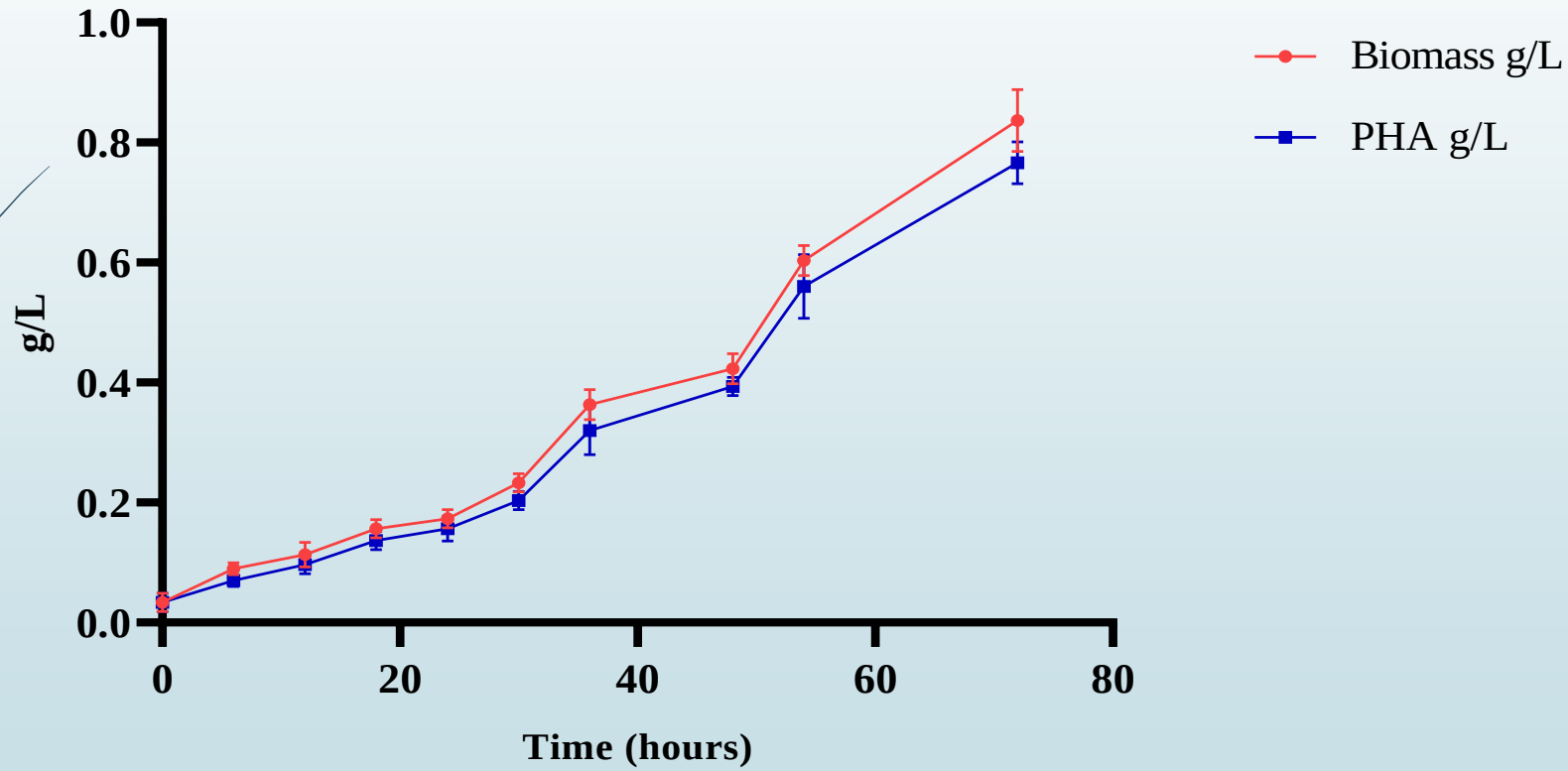

Photograph 5. Digestion with SDS

Photograph 6. Oven drying

# PHA extraction with SDS

- **PHAS production using mixed crops and VFAs as carbon source in pilot reactors**

The result shows the amount of PHA and biomass, accumulating up to 0.77 g/L of PHA and an accumulation of 83% biomass.




**Graph 1. Biomass and PHA production from mixed crops at pilot scale.**

# Extraction and Purification of PHA *B.cepacia* 2G-57 and Mixed Cultures laboratory scale.

Table 1. Comparison of methods for the extraction and purification of PHA.

| Sample | Weight Dry Biomass (g) | Extraction weight with chloroform (g) | Weight purification with methanol (g) |
|---|---|---|---|
| **2G57 – Sucrose** | 1.0661 | 0.498 | 0.315 |
| **2G57 – VFAs 1 g/L** | 0.478 | 0.135 | 0.101 |
| **2G57 – VFAs 0.7 g/L** | 0.498 | 0.113 | 0.089 |
| **2G57 – VFAs 0.5 g/L** | 0.2302 | 0.038 | 0.024 |
| **Mixed crops– Sucrose** | 1.524 | 0.6603 | 0.054 |
| **Mixed crops – VFAs 1 g/L** | 1.179 | 0.4793 | 0.123 |

Comparison of Chloroform extraction method for *B. cepacia* 2G-57 and Mixed cultures in laboratory scale.
Yields are low in these extractions, so the technique is improved.

# PHA Extraction and Purification using Mixed Cultures in Pilot Reactors

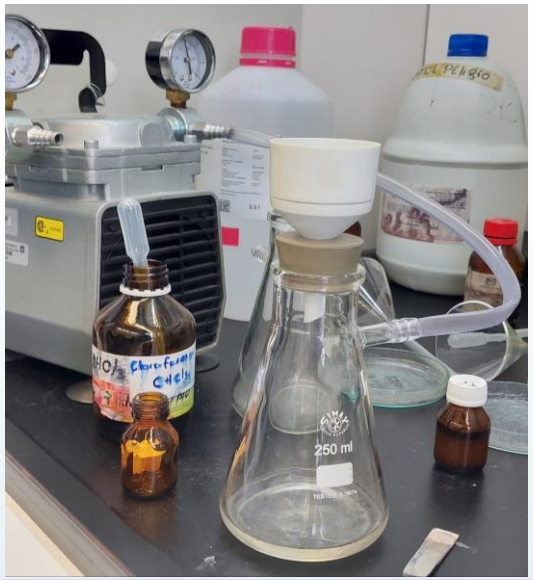
**Table 2. Comparison of methods for the extraction and purification of PHA.**

| Reactor | Chloroform | | | Acetic Acid | | |
|---|---|---|---|---|---|---|
| | R1 | R2 | R3 | R1 | R2 | R3 |
| Dry Biomass Initial [g] | 5.7154 | 5.0147 | 4.9362 | 5.6842 | 5.0061 | 4.8745 |
| Polymer Obtained [g] | 3.4178 | 2.9436 | 3.0058 | 3.1376 | 2.6825 | 2,6712 |
| Yield [%] | 59.79 | 58.69 | 60.89 | 55.19 | 53.58 | 54.79 |
| Average [%] | 59.79 | | | 54.52 | | |
| Recovery [%] | 100 | | | 91.18 | | |

Comparison of extraction and purification methods for *R1-R2-R3: Pilot scale reactors.

# Photographs Extraction and Purification Process

Shaker agitation



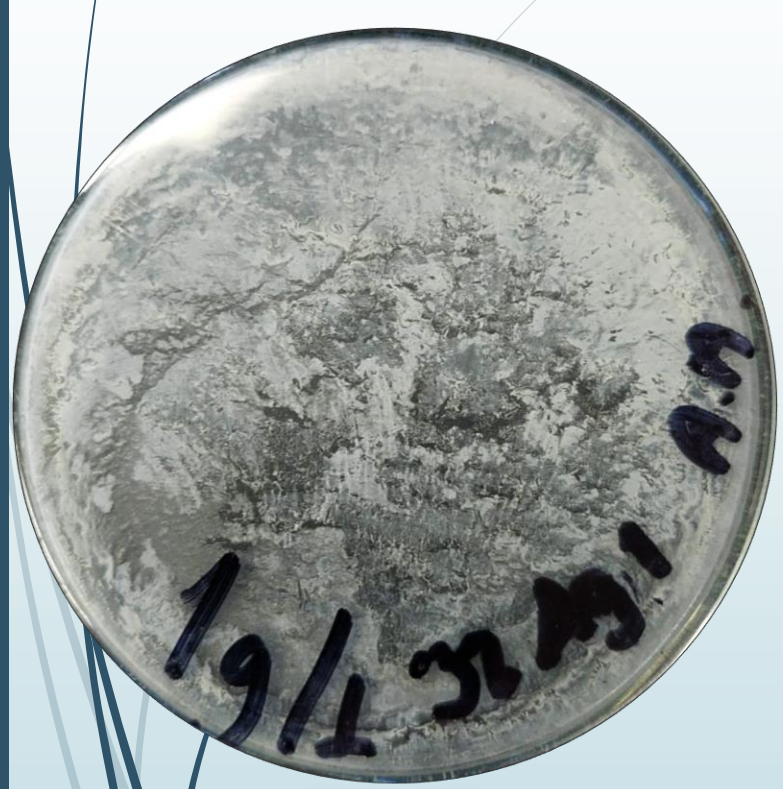

Filtration and separation

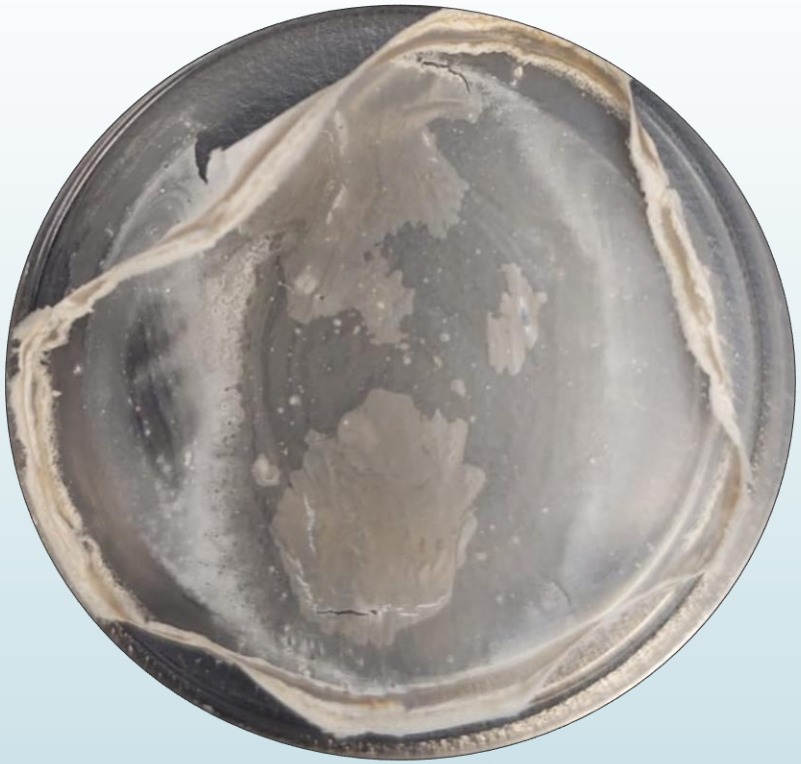
PHA + Chloroform Liquid Phase

# Extraction and Purification Results with Chloroform-Methanol Mixed Cultures

PHA obtained under different conditions

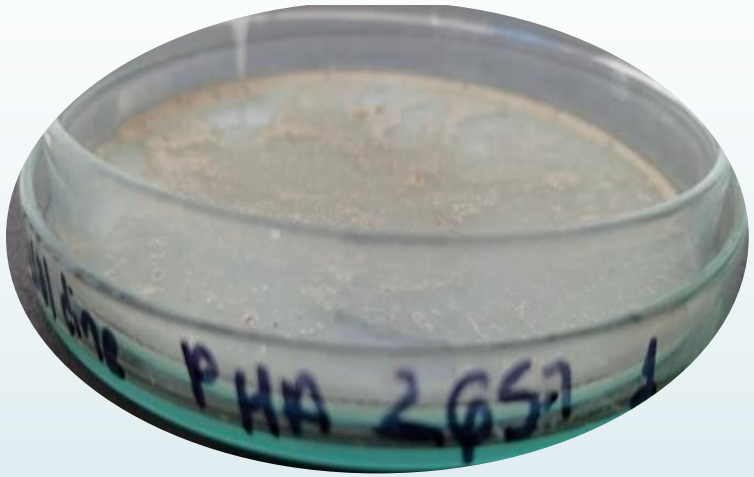
# Results of Extraction and Purification with Acetic Acid-Methanol Mixed Cultures

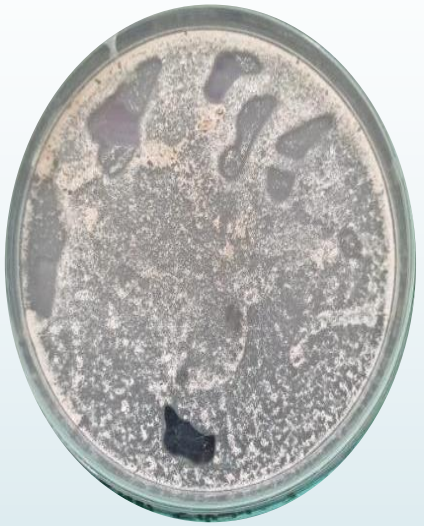


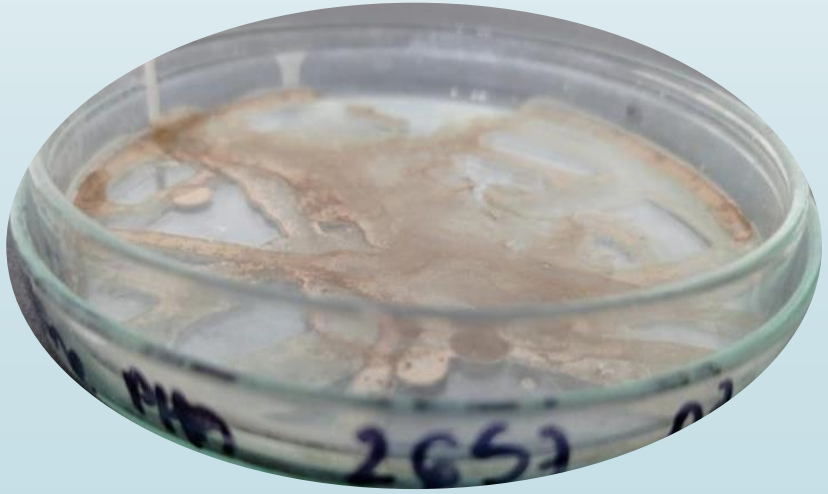


PHA obtained under different conditions

# Extraction and Purification Results with Chloroform-Methanol *B.2G57*







PHA obtained under different conditions

# Extraction and Purification of PHA at Laboratory Scale

**Table 3. Comparison of Chloroform and Acetic Acid-Methanol extraction under different conditions (mixed cultures)**

| Condition | Chloroform | | | Acetic Acid | | |
|---|---|---|---|---|---|---|
| | C1 | C2 | C3 | C1 | C1 | C3 |
| Dry Biomass Initial [g] | 1.5659 | 1.4972 | 1.3712 | 1.5974 | 1.5201 | 1.4257 |
| Polymer Obtained [g] | 0,9583 | 0,8963 | 0,8355 | 0,8416 | 0,8226 | 0,7651 |
| Yield [%] | 61.19 | 59.86 | 60,93 | 52,68 | 54,11 | 53,66 |
| Average [%] | 60.66 | | | 53,48 | | |
| Recovery [%] | 100 | | | 88,16 | | |

In order to evaluate the fermentation conditions, the following conditions were chosen: Condition 1 (C1): 100rpm agitation, 1g/L VFA, temperature 37°C, Condition 2 (C2): 100rpm agitation, 1g/L VFA, temperature 32°C and Condition 3 (C3): 100rpm agitation, 0.7g/L VFA, temperature 32°C.

# Conclusions

The difference obtained between extractions with chloroform and acetic acid in pilot reactors is 5.27 percentage points and in the evaluation of laboratory scale conditions was 7.18 percentage points below with respect to PHA accumulation using chloroform. According to Aramvash et al., 2018 when using acetic acid like solvent for PHA extraction the yield was 36.7% for PHA obtained by fermentation of sugars with *Cupriavidus necator* which is sensibly lower than the results of this research, however, in the results reported by Ramos, 2019 the yield of PHA extraction was 89.3%, for fat fermentations using *B. cepacia*, coinciding with the results obtained.

The percentage recovery of PHA obtained by extraction with acetic acid and methanol in the samples analyzed was 89.7%.

The use of less toxic solvents in the extraction of PHA should be further developed in order to reduce environmental impacts, costs and adverse health effects.

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