

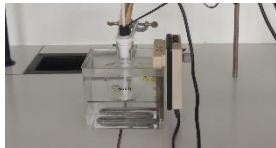
Plan of procedures: Synthesis of PHB (polyhydroxybutyrate)

1. Filling of fermenter

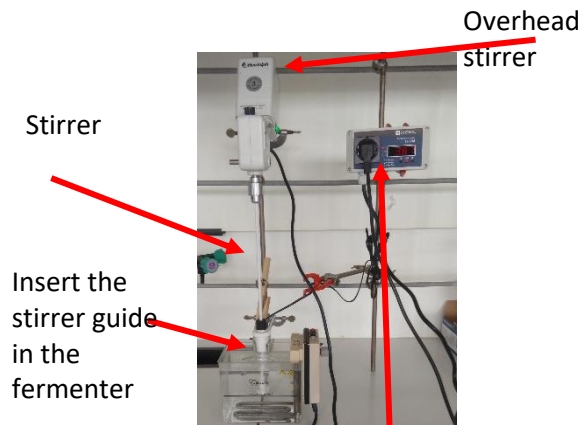
- 1.1 Take 200 mL of solution A, with a volumetric cylinder.
- 1.2 Take 20 μ L of solution B
- 1.3. Pour them in the fermenter (250mL), and add 2 mL of culture (with a pipette bulb)

2. Preparation of water bath.

- 2.1 Prepare the water bath with the heating system, and the temperature control system.



- 2.2 Put the fermenter in the water bath and fix it with a clamp.
Adapt above the fermenter a mechanical stirrer. The rotating speed must be at the minimum.



Temperature controller must be adjusted:

Low: 36°C

High: 38°C

- 2.3. Fermentation
Let the fermenter stirred and heated for 45h.

3. Extraction of PHB.

3.1.Centrifugation.

Take the fermenter off the water bath.
Let it decant 15 minutes.
Extract gently 150 mL of the supernatant with a pipette (store it in a beaker).

The rest (which is at the bottom of the fermenter) is centrifugated. (15 minutes at 10000 rpm).

3.2.Hypochlorite treatment.

After centrifugation:

Keep pellets in the tube (discard supernatant)

Add to pellets 10mL (measured with a volumetric cylinder) of sodium hypochlorite.

Incubate the mixture at 60° for 45 minutes.

3.3.Washing with distilled water.

Keep pellets in the tube (discard supernatant).

Washed with 5 mL of acetone (measured with a volumetric cylinder).

Centrifugate the mixture for 5 minutes.

3.4.Washing with acetone.

Keep pellets in the tube (discard supernatant).

Washed with 5 mL of acetone (measured with a volumetric cylinder).

Centrifugate the mixture for 5 minutes.

3.5.Washing with methanol

Keep pellets in the tube (discard supernatant).

Washed with 5 mL of methanol (measured with a volumetric cylinder).

Centrifugate the mixture for 5 minutes.

3.6.Chloroform treatment

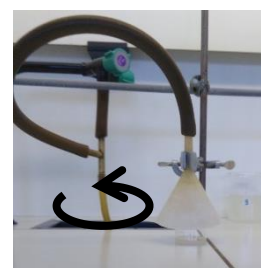
Keep pellets in the tube (discard supernatant).

Transferred pellets in a glass petri dish.

Under a filter fumed hood measure 5 mL of chloroform.



Poor it in the glass petri dish (under a filter fumed hood).



Put the glass petri dish below an upside-down funnel, connected to a venturi vacuum.
Stir the mixture with a rotating movement.

4. Obtaining PHB.



When the chloroform is entirely evaporated.

You can put off the plastic film