

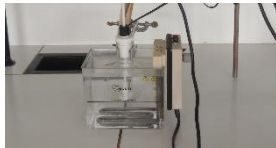
Forsøgsprotokol: Syntese af PHB (polyhydroxybutyrate)

1. Filling of fermenter

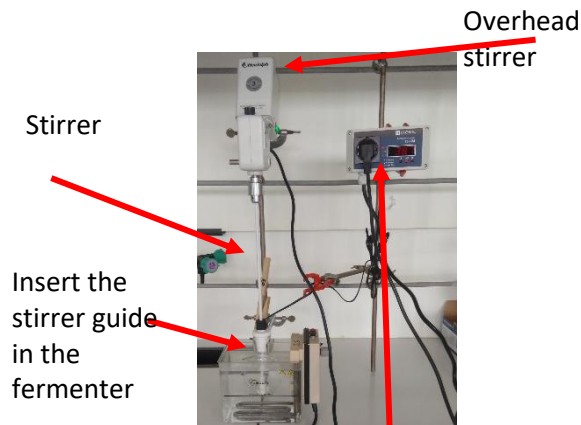
- 1.1 Take 200 mL of solution A, with a volumetric cylinder.
- 1.2 Take 20 μ L of solution B
- 1.3. Pour them in the fermenter (250mL), and add 2 mL of culture (with a pipette bulb)

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 acetone.

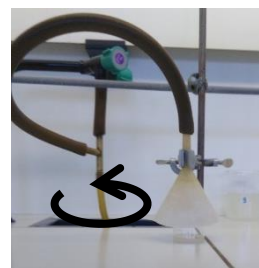
Keep pellets in the tube (discard supernatant).
Washed with 5 mL of acetone (measured with a volumetric cylinder).
Centrifugate the mixture for 5 minutes.

3.4.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.

3.5.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

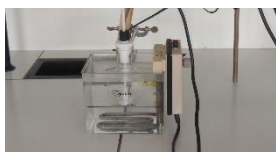
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1. Fyldning af fermentoren

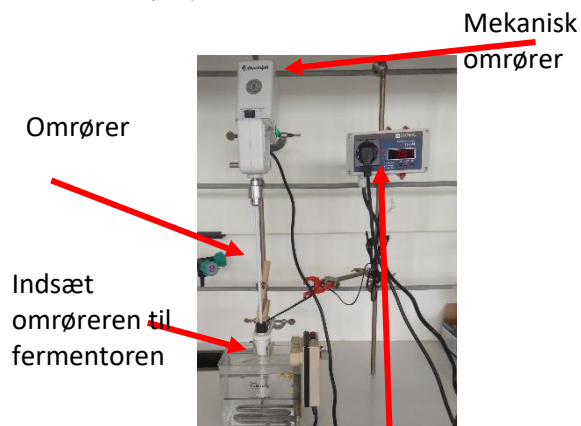
- 1.1 Fyld 200 mL af opløsning A, I et måleglas.
- 1.2 Tag 20 μ L af opløsning B med en mikropipette
- 1.3. Hæld opløsning A og B op I fermentoren (250 mL), og tilføj 2 mL bakteriekultur med en pipette

2. Klargøring af vandbad.

- 2.1 Klargør vandbadet med varmesystemet og temperatur kontrol systemet.



- 2.2 Sæt fermentoren i vandbadet og fastgør den med en klemme.
Monter over fermentoren en mekanisk omrører. Rotationshastigheden skal være på minimum.



Temperaturkontrolløren skal indstilles:
Lav: 36°C
Høj: 38°C

- 2.3. Fermentation
Lad fermentoren blive omrørt og opvarmet I 45 timer..

3. Udvinning af PHB.

- 3.1. Centrifugering
Tag fermentoren op af vandbadet.
Lad det bundfælde i 15 minutter.

Udtag forsigtigt 150 mL af supernatanten (den klare væske, uden bundfald) med en pipette (opbevar i et bægerglas).
Resten (dvs. Bundfaldet) centrifugeres (15 minutter ved 10000 rpm).

3.2. Hypoklorit behandling.

Efter centrifugering:

Behold pellets/bundfald i røret (kasser supernatanten)

Tilføj 10 mL natrium hypoklorit til pellets/bundfald (målt i måleglas).

Inkuber blandingen ved 60° i 45 minutter.
Centrifuger i 5 minutter (5000 rpm)

After centrifugation:

- Keep pellets in the tubes (discard the supernatant)
- Washed with 5 mL of distilled water.
- Centrifugate the mixture for 5 minutes

3.3. Vask med acetone.

Behold pellets/bundfald I røret (kasser supernatanten)

Vask med 5 mL acetone (målt I et måleglas).
Centrifuger blandingen I 5 minutter

3.4. Vask med methanol

Behold pellets/bundfald I røret (kasser supernatanten)

Vask med 5 mL methanol (målt I et måleglas).
Centrifuger blandingen I 5 minutter

3.5. Chloroform behandling

Behold pellets/bundfald I røret (kasser supernatanten)

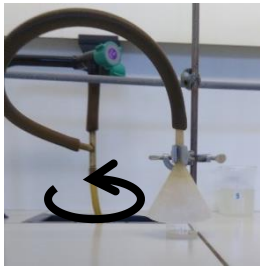
Overfør pellets/bundfald I en petri skål.

Mål 5 mL chloroform under et røgfang/udsugning

Hæld det I petri skålen (under røgfandet/udsugning)

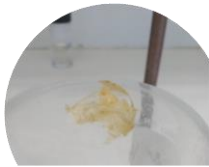


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Læg petriskålen under en omvendt tragt, tilkoblet til en vakkumpumpe.
Roter i blandingen med roterende bevægelse.

4. Indhentning af PHB.



Når chlorofomen er helt fordampet, kan du fjerne plastic film.