



POLYHYDROXYBUTYRATE ACCUMULATION IN *RALSTONIA EUTROPHA* IN LIPID CONTAINING MEDIA

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Introducere

The most common type of PHA produced by microorganisms is poly-β-hydroxybutyrate, a short-chain homopolymer also called poly-D(-)-3-hydroxybutyric acid, P(3HB) or PHB. Some heterotrophic microorganisms produce this polymer in batch or fed-batch crops. The use of vegetable oils is closely related to the specifics and climate of each country, their field of interest and the need for efficient removal of their residues, resulting from various technological processes. For this, olive oil, sesame oil, palm oil, soybean oil, rapeseed oil, corn oil, cold-pressed or heat-processed oils are used. The use of sunflower oils it is estimated that represent only 8% of all vegetable oils used in the world. Among the microorganisms used, *Ralstonia eutropha* grows well in mineral environments, at temperatures of 30°C, on many carbon sources, structurally correlated or not with our product of interest. Therefore, this microorganism was chosen for testing various vegetable oils for the production of PHAs and were tested the following: cultivation duration; the maximum amount of PHB for different oils; the maximum amount of dry biomass; the maximum amount of PHA in relation to dry biomass.

Materials and methods

- ✓ **Bacterial strains:** *Ralstonia eutropha* DSM 545.
- ✓ **Polymer biosynthesis:** fermentation in three different ICCF media with the following composition: sunflower oil in different quantities (0.92g%, 1g%, 1.38g%) and mineral salts, 48 hours of cultivation, temperature: 30°C-34°C, initial pH = 7.5, aeration by stirring on a rotary stirrer at 220 rpm.
- ✓ **Recovery of intracellular PHB including:**
 - medium is centrifuged,
 - the native wet biomass is subjected to a series of washes with distilled water and methanol,
 - the biomass is suspended in a mixture of hypochlorite and chloroform 1:3,
 - kept for 2 hours at 37-40°C, under moderate stirring.
- ✓ **Measurements:** optical density, pH, dry biomass, concentration of PHA.



Figure 1. Biopolymer pellicle (PHB)

Results

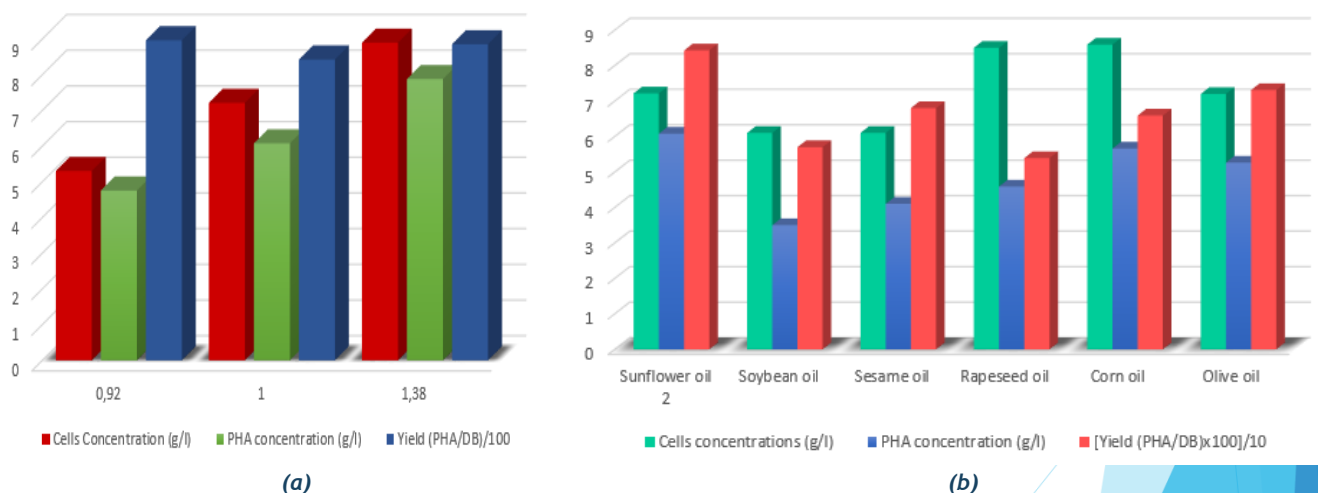


Figure 2. Comparative results obtained from the three culture media with sunflower oils (a) and other oils (b)

Conclusion

Following the study, PHAs can be obtained in quantities and yields of biotechnological interest with the help of the microorganism *Ralstonia eutropha* DSM 545, based on an environment containing a lipid substrate, namely sunflower oil in different concentrations. We were obtaining PHA in different concentrations of 4.747 g/l, 6.080 g/l and 7.880 g/l. Therefore, PHAs are relative to dry biomass of 89.67%, 84.27% and 88.54% are obtained depending on the concentration of the lipid substrate (the concentration of sunflower oil).