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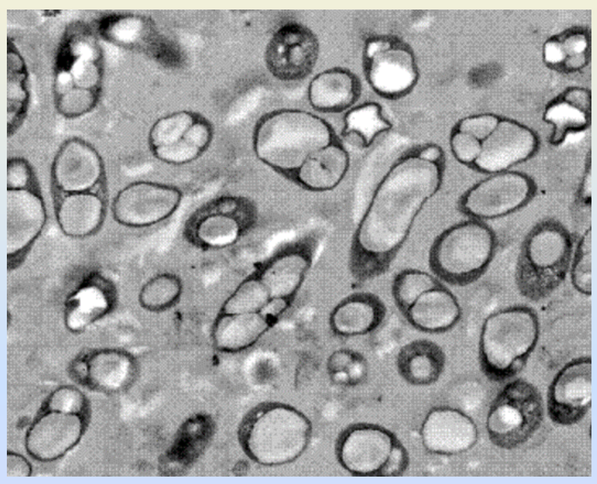
Development of a synthetic mixed culture to produce polyhydroxyalkanoates using molasse as substrate

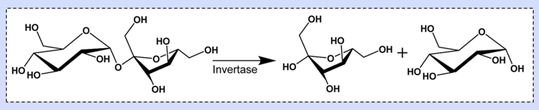
Introduction

Polyhydroxyalkanoates (PHA) are bioplastics accumulated in certain organisms as an energy/carbon source and represent an attractive alternative to traditional plastics from petroleum. However, some challenges need to be overcome to make PHA genuinely competitive. As part of this work, the goal was to develop a viable alternative to reduce the costs of substrates for PHA production by using molasses, a low-valued by-product of the Swiss sugar industry, in a defined mixed culture composed of yeast and bacteria.

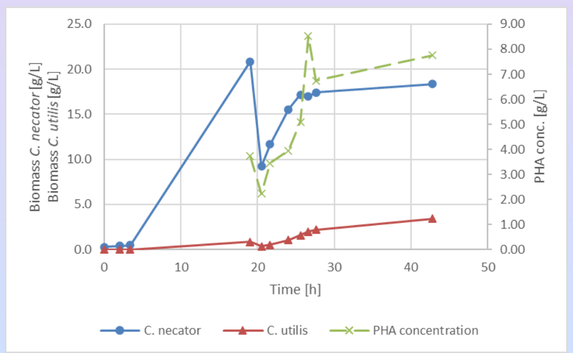


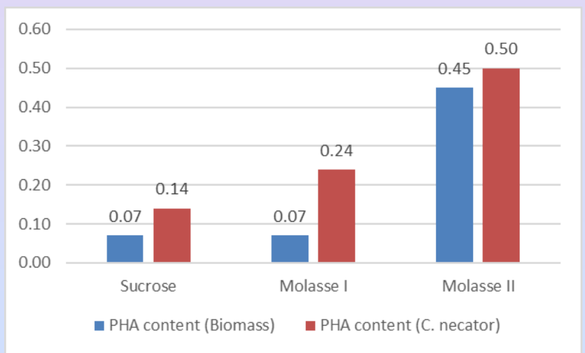
Polyhydroxyalkanoates accumulation in *C. necator* DSM545

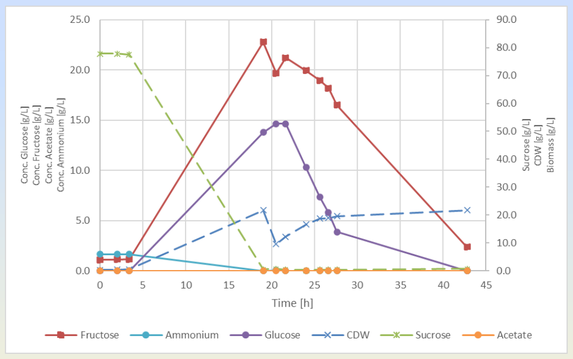
Molasse, by-product from the sugar industry

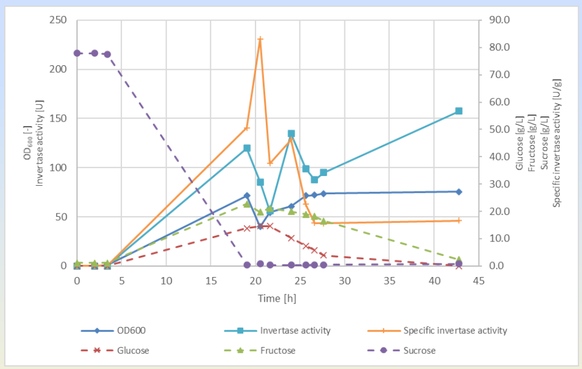


Hydrolysis of the sucrose by the invertase









Results

Screening of microorganisms in shake flasks cultivation determined that *Candida utilis* and *Cupriavidus necator* DSM545 were the strains that would be combined in the mixed culture. This study also made it possible to find a defined medium suitable for both strains and to develop two methods: one for measuring the enzymatic activity of invertase and the other for qualitatively and quantitatively monitoring the concentration of each strain during fermentation using flow cytometry (FCM).
Finally, three mixed-culture fermentation of *C. necator* DSM and *C. utilis* made in batch mode with pulses allowed to produce PHA from molasses without any pretreatment, showing a proof-of-concept of the collaboration between the yeast and the bacteria. The results led to a maximal PHA concentration of 8.53 g/L with 50% [$g_{PHA}/g_{Bacteria}$] and 45% [$g_{PHA}/g_{Totalbiomass}$].

Conclusion

This works showed the possibility of producing by a one-step process a biodegradable bioplastic, PHA, from a low valued by-product of the Swiss sugar industry, the molasse, in a mixed culture of yeast *Candida utilis*, and bacteria, *Cupriavidus necator* DSM545. The content of PHA could reach 45% of the bacterial biomass content and 50% of the total biomass, with a concentration of 8.53 g/L. In the future, those values can be even more improved by adjusting the parameters, such as pH, temperature, oxygen concentration, medium composition, to favor even more the growth of the bacteria and the accumulation of PHA. The work showed another essential development using the FCM to measure the evolution of both strains in the medium, qualitatively and quantitatively. This technique can be adapted to work for a defined-mixed culture of bacteria (for example, *C. necator* with *A. lata*) and to follow the PHA concentration at-line, thus permitting an easier way to optimize the PHA content in the mixed culture.