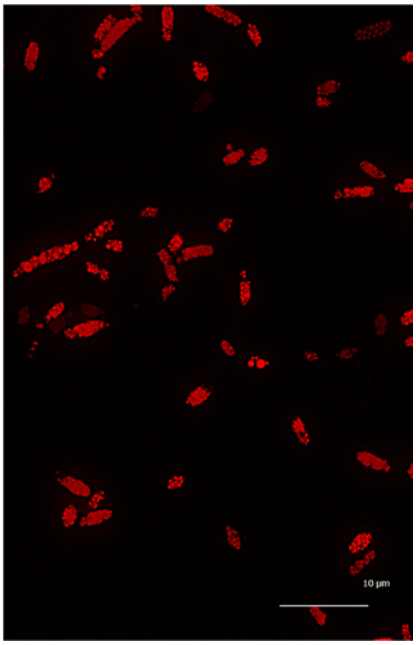

Bachelor's Thesis
| 2016 |

Degree programme
Life Technology

Field of application
Biotechnology

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Gearing up engineered platform for high-density lactate-based polymer production

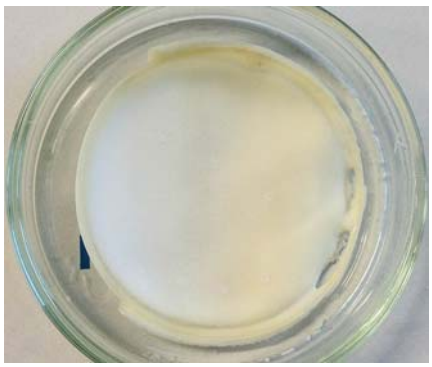
Graduate Ribordy Greg

Objectives

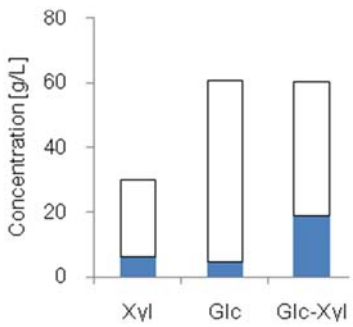
Define a bioprocess leading to high cell densities and high product yields for the poly(lactate-co-3-hyroxybutyrate), a novel type of PHA polymers.

Methods | Experiences | Results

In order to gearing up a bioprocess for high-density cultivation of engineered *Escherichia coli* producing the copolymer poly(lactate-co-3-hydroxybutyrate) [P(LA-co-3HB)], the genes coding for the LA-polymerizing enzyme and monomer supplying enzymes were placed under lac operon regulation. Thereupon, the copolymer accumulation could be controlled. Three Fed-batch cultures were carried out with different carbon sources (glucose and/or xylose), in a chemically defined medium, under aerobic condition and without induction. It has been shown that culture with xylose accumulated polymer in the early phase of growth, leading to a lower cell density; oppositely, cultivation with glucose resulted in enhanced cell concentration and reduced polymer accumulation. Based on this data, a new feeding strategy with both sugars was developed. By using this feeding, the dry cell weight concentration reached 60.4 $g \cdot L^{-1}$ with a polymer content of 31.6 wt% and a productivity of 0.23 $g \cdot h^{-1} \cdot L^{-1}$, constituting the highest yield obtained. Concerning the copolymer, its accumulation was observed in 50% of the cell population, the LA fraction was measured at 4mol% and the weight average-molecular weight (M_w) at 138 000.



Solvent cast film of copolymer poly(lactate-co-3-hydroxybutyrate) with a lactate fraction of 4 mol%.



Concentration of the true cell weight (white) and copolymer (blue) for different sugar feedings.