In-line application of photon density wave spectroscopy as a PAT sensor: Monitoring and control of growth and PHA biopolymer formation in high-cell-density bioprocesses

B. Gutschmann[1], T. Schiewe[2], L. Aulich[1], P. Neubauer[1], M. Münzberg[2], S. L. Riedel[1]

[1] Technische Universität Berlin, Institute of Biotechnology, Chair of Bioprocess Engineering, Ackerstr. 76, 13355 Berlin, Germany
[2] University of Potsdam - innoFSPEC, Institute of Chemistry/Physical Chemistry, Am Mühlenberg 3, 14476 Potsdam, Germany

E-mail: bjoern.gutschmann@tu-berlin.de, riedel@tu-berlin.de, https://www.tu.berlin/bioprocess/

Motivation and Introduction

Biodegradable alternative: Polyhydroxyalkanoates (PHA)

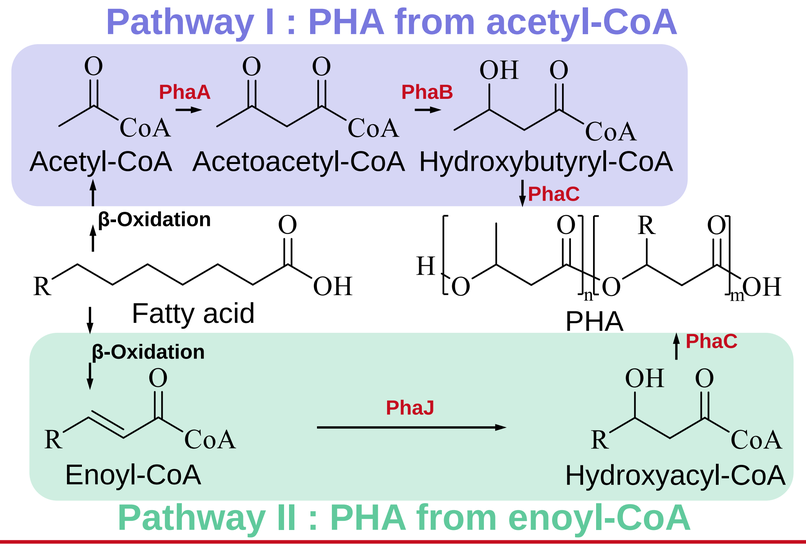
Issue: Convential plastics are not biodegradable

- Accumulation of plastic waste on land and in sea
- Plastic particles harm ecosystems and end up in human food chain

Proposed solution: Polyhydroxyalkanoates (PHA) as an alternative

- Fully biodegradable under aerobic and anaerobic conditions
- Can be produced from renewable resources and biogenic waste streams
- Oleaginous substrates are efficient feedstocks for PHA production with *Ralstonia eutropha*
- Use of hydrophobic substrates poses challenges for PAT applications and process development

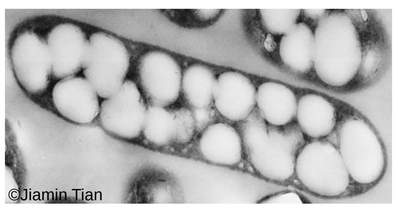
PHA synthesis from oleaginous feedstocks



Concept and Results

Ralstonia eutropha [1, 2]

- Model organism for PHA production
- Nutrient depletion triggers PHA formation
- Stores 90 wt% PHA per CDW
- Growth to high-cell-densities (>200 g L^{-1})



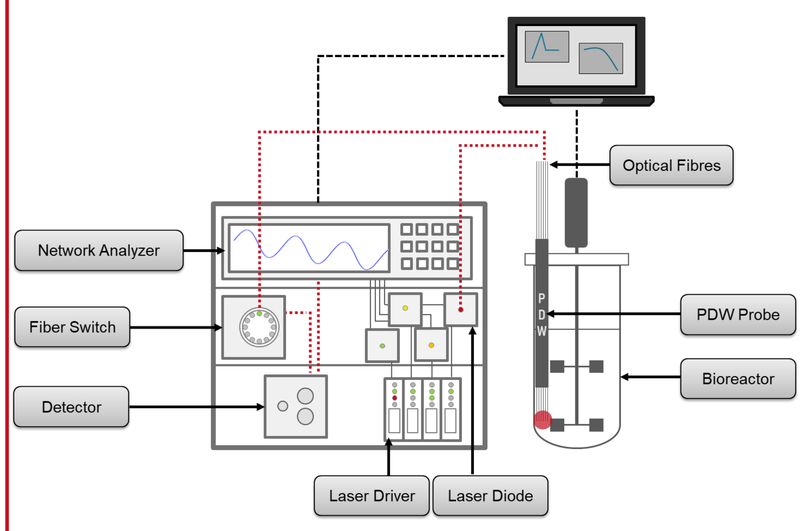
Ralstonia eutropha with high content of PHA granules

Experimental set-up

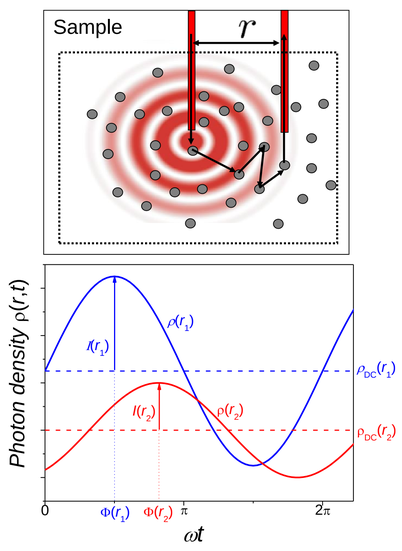
Oil in cultivation media

Integration of PDW probe

- Oleaginous feedstocks get emulsified by extracellular lipases from *R. eutropha* [2]
- Evaluation of photon density wave (PDW) spectroscopy during lab-scale cultivations



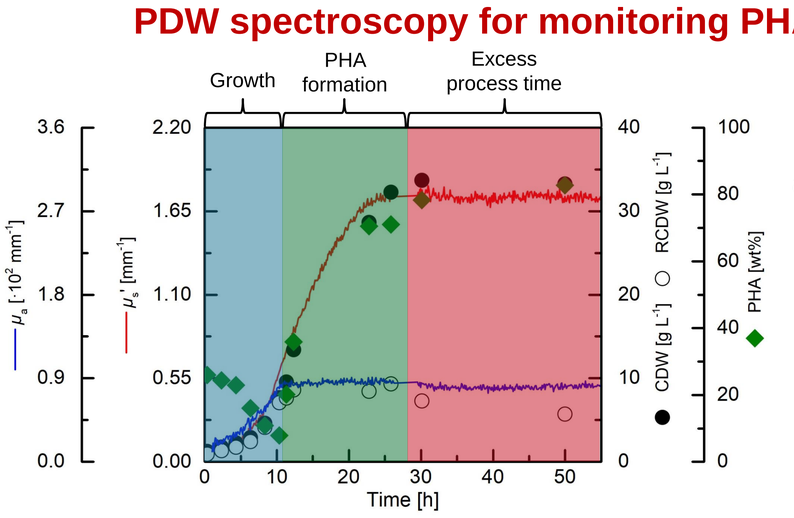
Measurement set-up for *in-line* PDW spectroscopy using an optical multifibre probe



Photon **D**ensity **W**ave**:** Variation of photon number by time

- Determination of optical properties using radiation transport theory, Mie theory and several approaches concerning dependent scattering
- Absorption (μ_a) and scattering properties (μ_s') can be determined independently [3,4]

PDW spectroscopy for monitoring PHA production [5]



Reduced scattering coefficient μ_s' allows estimation of total cell mass (CDW)

&

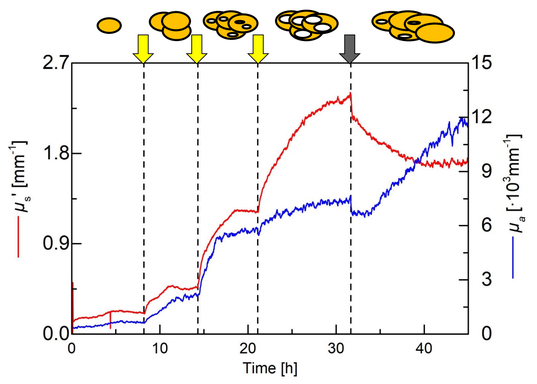
Absorption coefficient μ_a allows estimation of residual cell mass (RCDW)

Calculation of PHA content

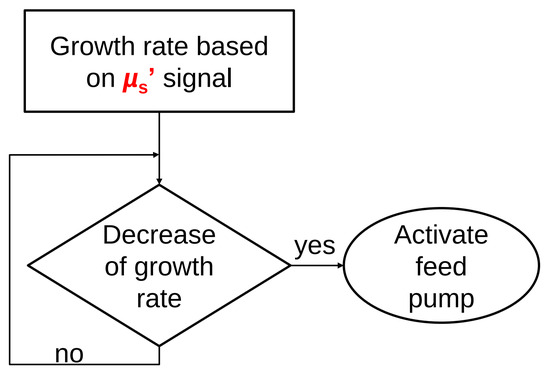
Development of a PDW based process control

Manual pulse experiment [5]

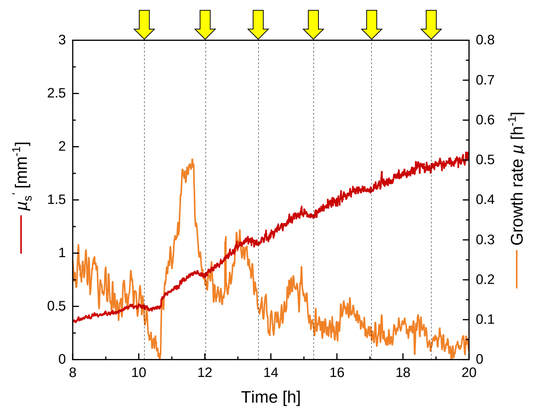
- Manual supply of pure plant oil (⇩) based on plateau of μ_s' signal allows control of growth and PHA formation
- Supply nitrogen (⇩) induces PHA consumption and enables further cell growth



Automated process control



Concept for automated process control



- *In-line* μ_s' signal can be used for an accurate calculation of the bacterial growth rate
- Automatic supply of waste frying oil (⇩) based on decrease of growth rate calculated from the *in-line* μ_s' signal

Conclusions and Outlook

- Successful integration of PDW spectroscopy at lab-scale as a novel optical PAT tool for *in-line* bioprocess monitoring of *R. eutropha* cultivations
- Independent quantification of absorption (μ_a) and scattering (μ_s') allows for monitoring of biomass accumulation, PHA formation and PHA consumption
- PDW signals can be used for manual feed control based on a signal plateau caused by nutrient depletion and subsequent interruption of cell growth
- Pulse feeding strategy was automated by detection of a decrease in growth rate calculated from scattering signal
- Control strategy is currently further developed into a continuous PID based feeding control to maintain a constant growth rate and thus increase process performance

References and Acknowledgements

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