Photoautotrophic 3-hydroxybutyrate production and optimization in continuous cultivation PBR system

Frans Uusipaavalniemi, M.Sc. Lauri Kakko, B.Sc. Samuli Pyytövaara, Ph.D. Pauli Kallio
Department of Life Technologies, University of Turku
SUSTAINABLE BIOTECHNOLOGICAL PROCESSES M.Sc. (TECH)

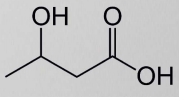
Introduction

Synechocystis sp. PCC 6803, a unicellular organism, works as a model for studying both photosynthesis and genetic modification tools. It produces organic compounds by using sunlight and carbon dioxide. We are able to alter its natural metabolic pathways and produce commercially essential chemicals, traditionally derived from crude oil. In this study, we employed a continuous photobioreactor (PBR) setup alongside genetically modified *Synechocystis* strains. Instead of natural polyhydroxybutyrate (PHB) biosynthesis, the engineered strains produce 3-hydroxybutyrate (3HB) and secrete it into culture medium.

Aims of the study

- Establish a continuous cultivation setup
- Assess changes in cultivation conditions to optimize the bioprocess
- Obtain quantitative data from 3HB production in a continuous cultivation photobioreactor
- Study the utilization of *Synechocystis sp.* PCC 6803 in a PBR

3-HYDROXYBUTYRATE



- Our target molecule
- Water-soluble, organic acid
- Suitable for bioplastic manufacturing

1. BG11 medium
2. Pumps in / fresh medium
3. Humidifier for aeration
4. Engineered *Synechocystis* producing 3HB
5. Eight separate cultivation tubes with LED lights
6. Integrated control unit
7. Tubing for medium and aeration system
8. Pump out / cultivation
9. Water bath with pump and coil heat exchanger
10. Sample bottles for 3HB quantification

Photo by Suvi Harvisalo, UTU tiedotus

Materials and methods

- Three genetically engineered strains of *Synechocystis sp.* PCC 6803 were evaluated and compared for their capability to produce 3HB.
- Two strains were cultured in eight parallel channels, (threshold 60 ml, OD_{720}=1) within a customized, continuous turbidostat PBR (MC1000, PSI) under three sets of cultivation conditions, involving variations in light intensity (100, 150, 200 µmol $m^{-2}s^{-1}$) and CO_2 aeration (1-5 %).
- Quantification of 3HB was performed using gas chromatography-mass spectrometer (GC-MS).
- Biomass cultivation rate was assessed by measuring the dry cell weight accumulation from 24 hours in every cultivation setup.
- Analysis of changes in photosynthetic pigment was conducted by measuring light absorption spectra.

Results

- Selection of two target strains based on their evaluated 3HB production.
- Successful establishment of the continuous PBR system.
- Integration of a CO_2 supply and recycling unit into the PBR.
- Demonstrated system capability for maintaining steady-state cultivation.
- Sample collection and analysis conducted across three cultivation conditions.

Discussion

This study marks the first continuous photoautotrophic production of 3HB, using engineered *Synechocystis sp.* PCC 6803. Findings highlight the potential of interdisciplinary research. Future efforts will focus on genetic enhancements and photobioreactor optimization. This research is necessary for advancing towards industrial-scale applications.

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References:

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2. Wang, B., Xiong, W., Yu, J., Maness, P-C., Meldrum, DR: **Unlocking the photobiological conversion of CO_2 to (R)-3-hydroxybutyrate in cyanobacteria.** *Green Chemistry* 2018, **20**(16):3772-3782.

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