Docosahexaenoic acid production with the marine organism *Crypthecodinium cohnii* in the wave-mixed single-use bioreactor CELL-tainer

Friederike Hillig, Nadine Porscha, Stefan Junne, Peter Neubauer
Technische Universität Berlin, Department of Biotechnology, Chair of Bioprocess engineering,
Ackerstraße 76, ACK 24, 13355 Berlin, Tel.: +49-30-314-72576, e-mail: f.hillig@tu-berlin.de, http://www.bioprocess.tu-berlin.de

gefördert durch Bundesanstalt für Landwirtschaft und Ernährung

Motivation

Docosahexanoic acid (DHA) belongs to the polyunsaturated fatty acids and exhibits a positive influence on human health because it protects against cardiovascular disease, cancer, diabetes, and depression, respectively [1]. Fish from aquaculture is fed with fish oil to ensure a sufficient supply with DHA, since fish is not able to synthesize DHA on its own [2]. The increasing demand of fish from aquaculture and the declining fish stocks lead to increasing fish oil prizes. The heterotrophic marine microalgae *Crypthecodinium cohnii* produces DHA in high concentrations [2] and therefore provide an economical and ecological alternative to fish oil.

The process development for the DHA production is part of the project: „Fischmehl- und –öl Ersatzstoffe für eine nachhaltige Aquakultur“. The aim of the project is to substitute fish oil and meal in the aquaculture with a mixture of biomass obtained from phototrophic microalgae (IGV), oleginous yeasts (TUB-Mibi) and heterotrophic microalgae (TUB-BVT).

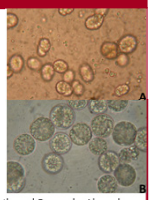


Fig. 1: Microscopic images of *C. Cohnii* suspension in the A: growth and B: production phase.

Challenges for the process development

The cultivation of *C. Cohnii* offers several challenges for the process development:

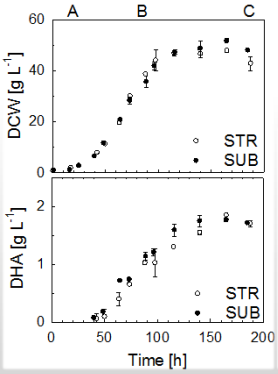
- High chloride ion concentration in the media hampers the application of stainless steel bioreactors
- High oxygen demand in the growth and production phase
- Cells are sensitive towards shear stress

Solution

Single-use bioreactors (SUBs) can circumvent the corrosion problem. Furthermore, the wave-mixed SUB CELL-tainer achieves high oxygen transfer rates and therefore provide an alternative to common stainless steal stirred tank reactors (STR). Recently, the CELL-tainer was successfully scaled up to 150 L [3].

Comparison between STR and SUB

Growth and production performance



- Comparison of fed-batch cultivations with 1 L working volume in the STR (Biostat® B plus Sartorius, Göttingen, Germany) and in the SUB CELL-tainer ® (CELLution Biotech BV, Assen, Netherlands)

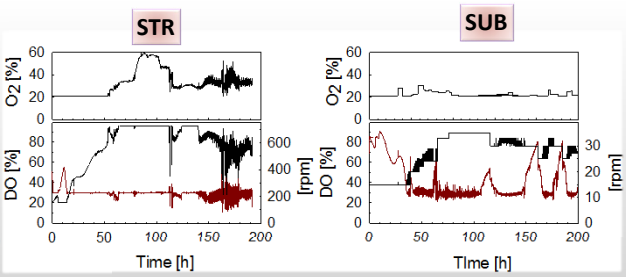


Fig. 2: Comparison of fed-batch cultivations in a STR Biostat B and SUB CELL-tainer. Cultivation carried out with 1 L working volume at similar nutrient conditions, DCW and DHA concentration, dissolved oxygen content (DO, red line), oxygen content of the inlet gas, stirrer and shaker speed, respectively.

- Growth and DHA production performance was comparable in both devices

→ Oxygen transfer rates were sufficient in the CELL-tainer for the cultivation of *C. cohnii*

Yields

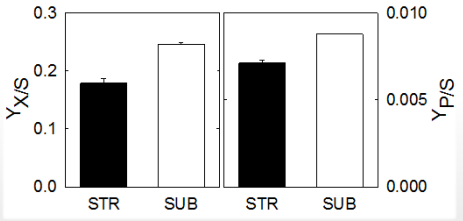


Fig. 3: Biomass and product yields of the fed batch cultivations in the STR Biostat B and the SUB CELL-tainer

- Biomass and DHA production yield higher in the SUB CELL-tainer than in the STR Biostat B

Influence of shear forces

Fig. 4: Foam formation during the fed-batch cultivation in the STR Biostat B. 1: gaseous phase, 2: culture broth.

Enhanced foam for-mation in the STR Biostat B was observed due to:

- Direct aeration
- Higher shear forces → higher amount of cell fragments

→ Antifoam (rapeseed oil) was not sufficient

→ Overspilling of the bioreactor

→ Decreasing Yields.

Fig. 5: SUB CELL-tainer

Proof with flow cytometry studies

Application of the flow cytometry to elucidate the impact of shear stress in both bioreactors

Unstained cells

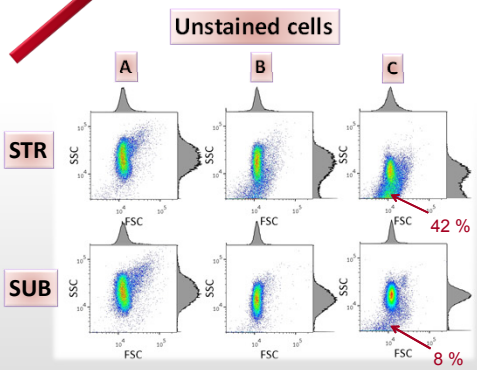
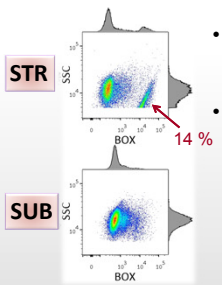


Fig 6: Flow cytometry dot plots of unstained cells obtained during the cultivation in the STR Biostat B and in the SUB CELL-tainer. Samples were taken A: in the beginning of the cultivation (18 h), B: at the end of the growth phase (89 h), and C: in the end of the cultivation 190 h), diluted with phosphate buffer to OD 0.5 and measured.

- Higher amount of lysed cells in the STR Biostat B than in the SUB CELL-tainer (Fig. 5: dots beneath the main cell population, 42 % (STR) in comparison to 8 % (SUB))
- Higher diversity in cell size and granularity in the STR

→ indication for higher stress conditions.

Cells stained with BOX



- BOX staining was applied to elucidate the energetic state of the cells
- The cell population with a collapsed membrane was visible in the STR cultivation (population in the same area as the positive control (Fig. 8)).

Fig. 7: Flow cytometry dot plots of BOX stained cells obtained during the cultivation in the STR Biostat B in the SUB CELL-tainer. Samples were taken in the end of the cultivation (190 h), diluted with phosphate buffer to OD 0.5, stained for 10 min with BOX (5µg mL^{-1}) and measured.

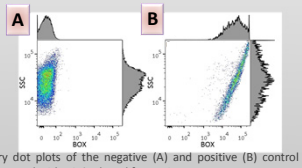


Fig. 8: Flow cytometry dot plots of the negative (A) and positive (B) control for BOX staining. Positive control: cells treated with 70% ethanol for 1 minute.

Discussion and Conclusion

- The SUB CELL-tainer provides sufficient gas transfer rates for the cultivation of *C. cohnii* and can therefore replace the common STR
  → avoidance of corrosion and reduction of investment costs in the process development stage
- Foam formation is avoided in the CELL-tainer, which makes the application of antifoam reagents dispensable
- Higher shear stress in the STR Biostat B resulted in higher amounts of damaged cells as proven with the flow cytometry
- Lower shear stress and reduced foam formation in the SUB CELL-tainer led to higher biomass and product yields.

Danksagung

We thank the Bundesministerium für Ernährung, Landwirtschaft und Verbraucherschutz, Bundesanstalt für Landwirtschaft und Ernährung (Project: **F**ischmehl und –öl **E**rsatzstoffe für eine **n**achhaltige **A**quakultur) for financial support.

[1] S. D. Doughman, S. Krupanidhi, C. B. Sanjeevi, *Current Diabetes Reviews* **2007**, *3*, 198.
[2] A. Mendes, A. Reis, R. Vasconcelos, P. Guerra, T. L. da Silva, *J. Appl. Phycol.* **2009**, *21*, 199
[3] S. Junne, T. Solymosi, N. Oosterhuis, P. Neubauer, *Chem. Ing. Tech.* **2013**, 85, 57