Docosahexaenoic acid (DHA) production in the heterotrophic marine microalgae *Crypthecodinium cohnii*

Chair of Bioprocess Engineering

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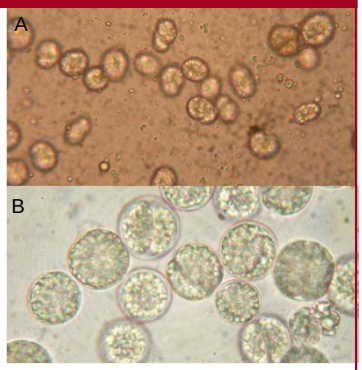
Motivation

Docosahexaenoic acid (DHA) is a polyunsaturated fatty acid (PUFA) with a beneficial effect on human health. The main source of DHA for the human diet is fatty sea fish. To maintain a high amount of DHA in fish grown in aquaculture, fishes are fed with fish oil. Fish oil is also used as an additive in the food industry. The utilization of this fish oil has several drawbacks, as it contributes strongly to the ocean's overfishing and contains environmental pollutions [1].
C. cohnii is a heterotrophic marine dinoflagellate which can be used for biotechnological DHA production [1]. The process is divided in two phases: in a growth phase and a shorter production phase, in which the amount of DHA per cell can be doubled.
In the project a screening system in deep well plates with the application of oxygen vectors was developed. With this screening system a DHA content of 25 % per cell dry weight was reached at the end of the growth phase. The possibility to obtain this results in larger scale has to be demonstrated.

Challenges in process development:
- High oxygen demand during growth and synthesis of fatty acids
- algal cells are sensitive to high shear forces → hamper the application of directly aerated systems [2].
- high chloride concentration of the media → corrosion of steel reactors.

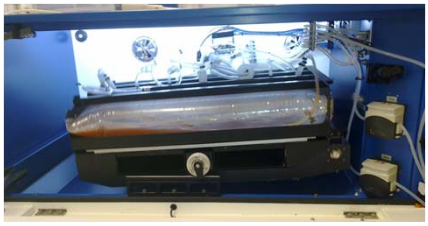
→ To overcome these limitations the application of polymer based bioreactors as alternative are evaluated.



C. cohnii cells A) growth phase B) production phase characterized by lipid particle enrichment

Results

CELL-tainer® – wave - mixed system (CELLution Biotech)



The two dimensional movement of the celltainer results in high oxygen mass transfer rates into the medium. The DO is regulated during the cultivation via shaker speed and the capability to inject pure oxygen instead of air when needed. The pH was adjusted automatically to pH 6.0

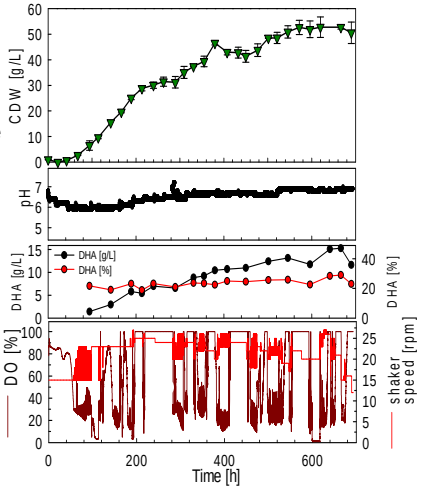
Cultivation:
- Start volume: 8 L
- Obtained cell dry weight (CDW): 53 g/L
- DHA content per CDW: 29 % (at the end of the growth phase)

→ The results are similiar to those obtained in a 5 L stirred tank bioreactor (data not shown)

→ The cell dry weight and the volumetric DHA content was higher than in the Kühner reactor due to sophisticated process controll and a higher oxygen transfer rate

→ Application of the celltainer for a fed-batch cultivation with *Crypthecodinium cohnii* was feasable

→ For the purpose of industrial application, a scale up of this system has to be performed



SB 200x orbital shaking system (Kühner)

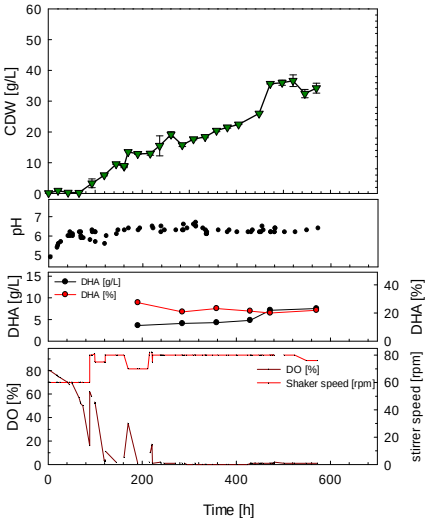
The SB 200 x is an orbital shaker, with aeration from the top. The cultivation was performed without DO controll. The shaker speed was adjusted to 80 rpm. After 560 h of cultivation the inlet gas was changed from pure air to air with 37 % O_2. The pH was controlled manually and therefore not stable over the cultivation time.

Cultivation:
- Start volume: 100 L
- CDW: 37 g/L
- DHA content per CDW: 28 % (at the end of the growth phase)

→ Cells could withstand oxygen limitation better than at other reactor systems

→ The cell dry weight and the volumetric DHA content was smaller than in the celltainer

→ In future application of improved process controll will allow for the achievement of higher amounts of biomass



Outlook

- Both system designs seem to be adequate for further development for the application in marine bioprocesses.
- A process control using the exhaust gas analysis will improve process robustness.
- Applying our developed plate screening system, the influence of different hydrophobic and hydrophilic additives on the DHA production is investigated to enhance the volumetric DHA content in the production phase.

Acknowledgements

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[1] Mendes A, Reis A, Vasconcelos R, Guerra P, da Silva TL. 2009. Crypthecodinium cohnii with emphasis on DHA production: a review. Journal of Applied Phycology 21:199-214.
[2] Hu WW, Gladue R, Hansen J, Wojnar C, Chalmers JJ. 2007. The sensitivity of the dinoflagellate Crypthecodinium cohnii to transient hydrodynamic forces and cell-bubble interactions. Biotechnology Progress 23:1355-1362.