# *Continuous culture of the heterotrophic dinoflagellate Crypthecodinium cohnii*

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**Introduction**

*Crypthecodinium cohnii* is a heterotrophic dinoflagellate known to produce considerably amounts of docosahexaenoic acid (DHA), a polyunsaturated ω-3 fatty acid essential for the neurological development in infants. This dinoflagellate has also found use as feed in aquacultures. Both applications have made it to an object of intensive research regarding productivity of DHA and biomass.

**Aim**

The goal of our study is to develop continuous processes for the production of heterotrophic feed algae for aquaculture with stable and controllable biochemical composition. Since complex media were used in reported batch- and fed-batch cultures it is not definitely identified which factors affect growth and accumulation of storage compounds. Therefore, we are identifying relevant nutrients and designing defined media for continuous cultures of *Crypthecodinium cohnii*.

**Relevant growth factors**

*Crypthecodinium cohnii* respondes to nutrient limitation in a change from exponential to linear growth and accumulation of lipids. This change has previously been correlated to starvation in nitrogen. Our investigations, however, have shown that limitation in phosphorous leads to a similar response. Consumption of relevant nutrients in *Crypthecodinium cohnii* is depicted in Figure 1. While glucose and glutamic acid uptake is strongly correlated to growth, phosphate was almost totally consumed during the early exponential growth phase.

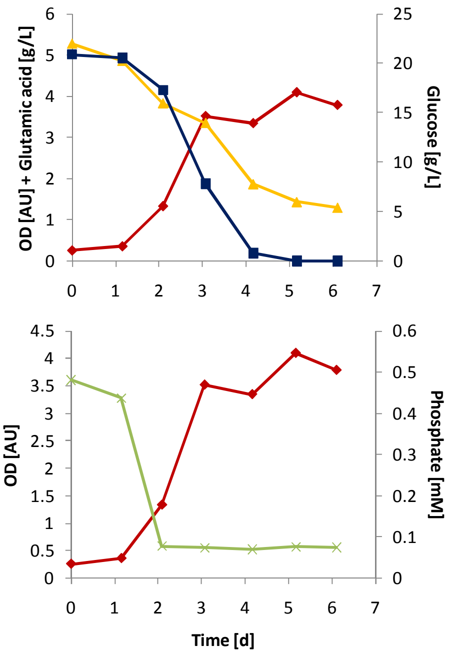


**Figure 1:** OD at 700 nm (red), glucose (blue), glutamic acid (yellow), and phosphate (green) in a batch culture of *Crypthecodinium cohnii* (CCMP 316).

**Figure 2:** Fluorescence micrograph of *Crypthecodinium cohnii* stained with Nile Red and excited at 500 nm, unpolar lipids appear as yellow-gold while polar lipids are visible as red-orange spots.

**Continuous culture**

Based on results found in batch cultures we designed a defined medium for continuous cultivation (Figure 3) containing 50 g $L^{-1}$ glucose as main carbon source, 8 g $L^{-1}$ glutamic acid as nitrogen source and 4 mM phosphate in artificial seawater (30 ppt).

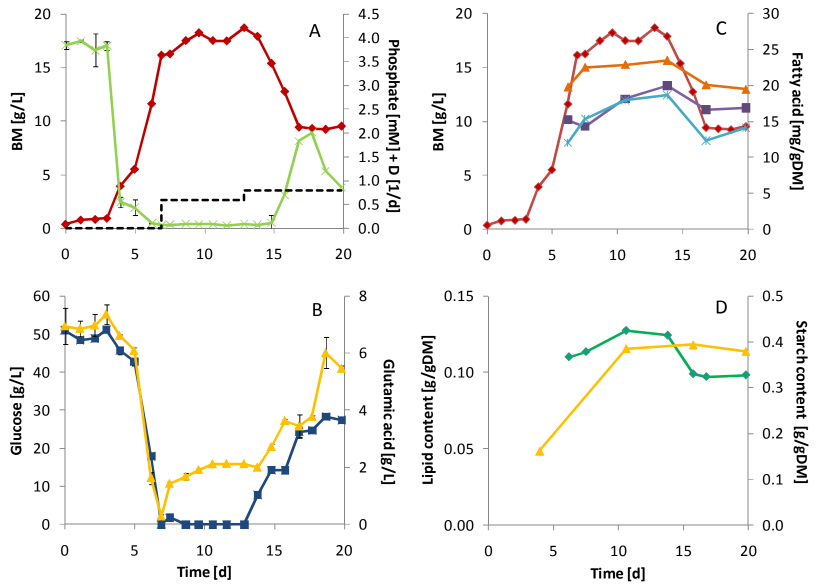


**Figure 3:** Continuous culture of *Crypthecodinium cohnii* (CCMP 316). **A:** Biomass concentration (red), phosphate (light green) and dilution rate (dished line). **B:** Glucose (blue) and glutamic acid (yellow). **C:** Lipid composition (myristic acid (orange), palmitic acid (violet) and DHA (light blue)). **D:** Total lipid- (green line) and starch contents (yellow line).

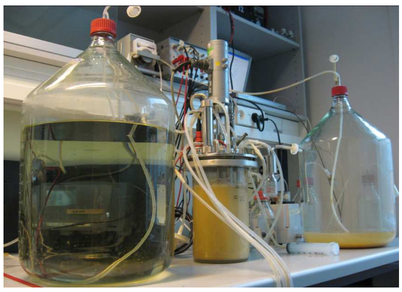

**Figure 4:** Continuous culture of *Crypthecodinium cohnii* was performed in a 3L controllable bioreactor.

In the initial batch phase, exponential growth occurred after a 3 days long lag-phase at a specific growth rate of 0.85 $d^{-1}$. The following steady state phase was C- and P-limited at a dilution rate of 0.6 $d^{-1}$, after increasing to a dilution rate of 0.8 $d^{-1}$ the cells were washed out until a new steady state was reached, where the maximum specific growth rate equals the dilution rate. All relevant nutrients were present in excess after increasing the flow and the DHA content decreased from 18 to 14 mg $g^{-1}$ DM. The starch content was stable at both dilution rates at 0.4 g $g^{-1}$ DM. Table 1 gives an overview about the productivity of biomass and DHA during nutrient limitation and excess.

**Table 1:** Productivity of biomass, DHA and DHA content per g dry matter (DM) during nutrient limitation and excess.

| | C- and P-limitation | Nutrients in excess |
|---|---|---|
| **Biomass** g $L^{-1}$ $d^{-1}$ | **12** | **8** |
| **DHA** mg $g^{-1}$ DM | **18** | **14** |
| **DHA** g $L^{-1}$ $d^{-1}$ | **0.2** | **0.1** |

**Conclusion**

- Relevant factors for developing continuous cultures have been identified
- Phosphorous has been found to play a crucial role for maintaining exponential growth of *Crypthecodinium cohnii* (CCMP 316)
- Starch seems to be the major storage compound
- Stable biochemical composition during steady-state has been observed