Cell-tainer BIOTECH BV

Application Note

Application of the CT20 for the cultivation of the shear-sensitive heterotrophic algae *Cryptecodinium cohnii*

Introduction

The heterotrophic algae *Crypthecodinium cohnii* together with *Schizochytrium* sp. and other related genera are the major sources of the polyunsaturated fatty acid docosahexaenoic acid (DHA). The use of DHA from *C. cohnii*, especially for infant food, is preferred, since *Schizochytrium* sp. produces docosapentaenoic acid and other unfavorable fatty acids, such as pentadecylic acid, which has an unclarified influence on human health. Contrary to this, *C. cohnii* produces DHA as the sole PUFA (other PUFAs are less than 1 %), but it requires high concentrations of chloride ions and is very shear-sensitive to mechanical shear forces: a flagellum is required for growth. If it is disrupted, cells can't proliferate and higher lysis rates occur. At the same time, the oxygen demand of a culture is relatively high, exceeding requirements for cell culture by an order of magnitude.

In order to circumvent some of the difficulties that arise at *C. cohnii* cultures, single-use bioreactors were applied, which were based on shaking or rocking without any movable parts inside the reaction chamber. Thus, the mechanical shear forces were low. While using plastic bags for cultivation, no corrosion occurred due to the high chloride ion concentration like in stainless steel vessels.

Results

After several types of single-use bioreactors were applied, the CT20, which was operated up to a volume of 15 L in a non-carbon limited fed-batch mode, showed the best process performance with respect to growth and intracellular DHA accumulation. Thus, it was further investigated for *C. cohnii* cultivations of 10 to 14 days and up to dry biomass concentrations of 60 gL^{-1} [1].

Comparison between performance in a stirred tank reactor and the CELL-tainer CT20

A comparison was carried out between a conventional lab-scale stirred tank reactor (STR) made of glass and the CELL-tainer CT20. In order to obtain identical conditions in both devices, expansion channels were applied to use the CELL-tainer CT20 at a working volume of 1 L, the same was applied in the STR.

Both cultivations are shown in Fig. 1. The maximum dry biomass concentration was reached after 165 h. Maximum biomass concentrations were slightly higher in the CELL-tainer (Tab. 1).

Tab. 1: Comparison of 1 L C. cohnii fed-batch cutivations in a stirred tank reactor (STR) and the CELL-tainer CT20.

Parameter	*STR*	*CT20*
min. dry biomass concentration [gL^{-1}]	47.8	51.7
vol. DHA content [gL^{-1}]	1.8	1.8
max. production rate $mg(Lh)^{-1}$	22.5	22.1

The substrate-specific biomass yield $Y_{X/S}$ of the growth and the stationary phase were higher in the CELL-tainer cultivation. The amount of cells and a biofilm, which evolved on the vessel walls in the STR were not measurable, and thus not considered in biomass measurements. Additionally, the STR cultivation spilled over due to intensive foam formation, which was likely due to an elevated lysis rate. This also decreased the yield. The substrate-specific product yield $Y_{P/S}$ was higher in the growth phase in the CELL-tainer, too [1].

Influence of shear forces

Since the sensitivity against shear forces is crucial in *C. cohnii* cultivations, the impact of lower shear stress in the rocking-motion bioreactor CT20 on population homogeneity and cell lysis was compared with the STR

cultivation. Flow cytometric measurements revealed a comparable pattern of the populations (Fig. 2). Nevertheless, the amount of debris visible at the side scatter signal and the portion of cells with a collapsed cell membrane potential (if cells were stained with bis-oxonol (BOX)-Fig. 2)) was substantially higher in the STR than in the CT20 cultivations. The evolution of this subpopulation was detected after 80h. This indicates a higher amount of destroyed cells, if shear stress was higher. This portion of damaged cells might also have led to the vast amount of foam that was formed during the cultivation, especially in the late growth phase.

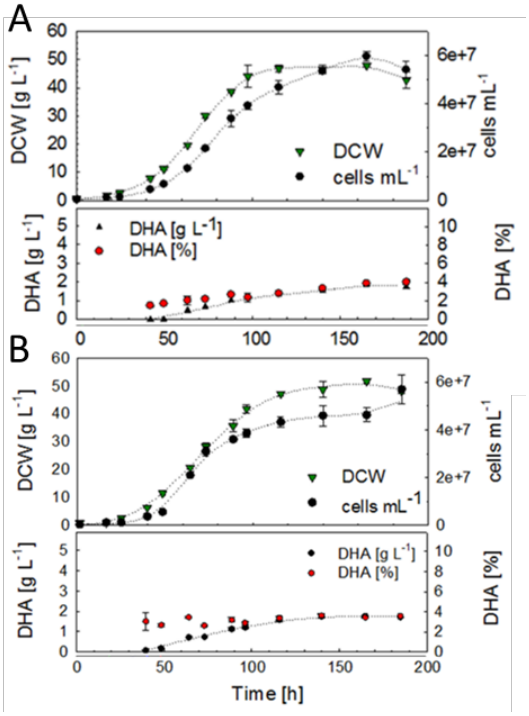

Fig.1: Process performance of 1L fed-batch cultivation of C. cohnii in a stirred tank reactor (A) and the CELL-tainer CT20 (B).

Scale up to the CT200

The C.cohnii cultivation of the 1 L scale was conducted in a 120 L scale in the CELL-tainer CT200. The process performance was maintained successfully also in the larger scale (Fig. 3). Cells exhibited the same viability and vitality than in the CT20 cultivations.

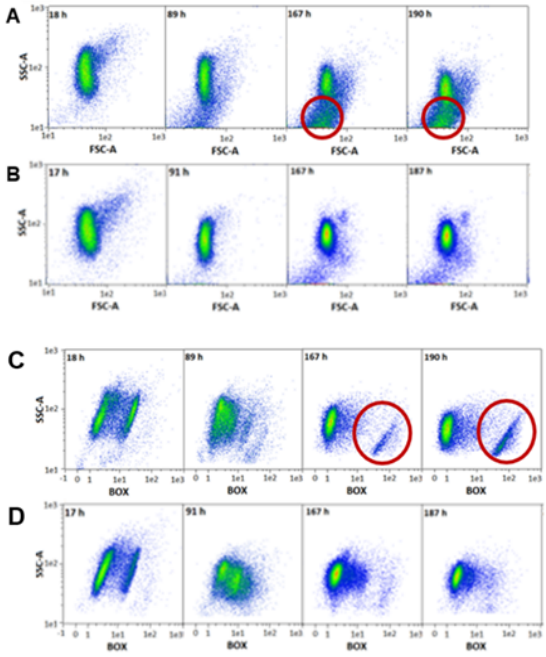

Fig.2: FSC/BOX signal at samples of C. cohnii from STR cultivation (A/C) and CT20 cultivation (B/D). Red circles indicate subpopulation of harmed cells of low polarity.

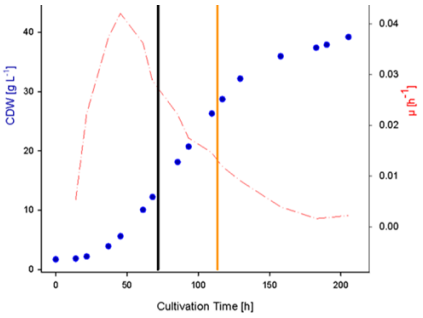

Fig.3: C. cohnii cultivation in the CT200.

Summary

The CELL-tainer is suitable for the cultivation of *C. cohnii*, while providing a sufficient oxygen gas mass transfer in both reactors, the CT20 and CT200, while exhibiting low shear forces, and a higher degree of culture homogeneity than in an STR.

Literature

Hillig, F., et al., *Growth and docosahexaenoic acid production performance of the heterotrophic marine microalgae Crypthecodinium cohnii in the wave-mixed single-use reactor CELL-tainer.* Engineering in Life Sciences, 2014. **14**(3): p. 254-263.