Long Duration Plant Cell Culture in SB200-X

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The sensitivity of plant cells to shear forces sets high demands on cultivation vessels (Zhong, 1994). At the same time, the use of pharmaceutical-grade sterility is a major cost factor in food production with plant cell culture (PCC). Food Brewer addresses this issue by extending the duration of individual cultivation runs to the maximum.

Experimental setup

The cell line used to produce cell-cultured cocoa biomass is derived from non-genetically modified cells obtained from the callus tissue of *Theobroma cacao* pods sourced from Peru.

The orbitally shaken Bioreactor SB200-X was inoculated from a 50 L stirred bioreactor. Multiple feeds with fresh media were performed to bring the bioreactor to its final volume of 200 L. Then the repeated feed batch process started and multiple partial harvests and feeds were made. No active pH control was used.

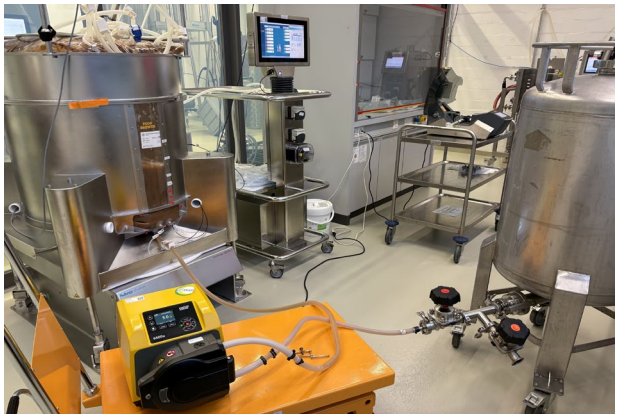

Figure 1

For partial harvests cell suspension is transferred from the SB200-X to a pre-sterilized steel vessel. By using weldable tubing, the sterility in the reactor can be maintained.

The main goal of this experiment was to prove that sterility can be maintained during long cultivation time and that the shear sensitive plant cells are able to grow in the orbitally shaken bioreactor SB200-X. In addition, different feeding strategies were tested to improve the productivity of biomass production in larger scale.

Figure 2

Freshly harvested cacao biomass.

Outcome

The SB200-X bioreactor was running with one single-use bag continuously during more than 280 days. During this time more than 100 manipulations like sampling, harvest and feed were performed on the SB200-X without contamination.

Given the extreme duration of this experiment, far exceeding standard sensor specifications, it is expected that sensor performance may decline over time. The pH sensor showed reduced reliability over the course of the 280 day cultivation, most likely due to the maximum flash frequency (life span) of the built-in disposable sensor pads being exceeded. In contrast, the dO2 sensor's fluorescence based mechanism proved more robust to these long term effects, providing reliable readings throughout the full 280 day cultivation

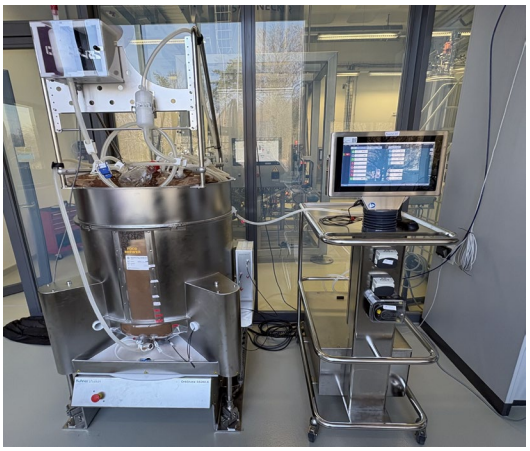

Figure 3

The SB200-X has been operating for more than 280 days. The single-use bag remained intact throughout the entire cultivation period. No tubing damage or filter clogging was observed.

After more than 20 million shaking cycles, the single-use bag was still intact and in shape. Long term effects of aging on media, cell culture and equipment could be monitored, while at the same time more than 400 kg of fresh biomass, later dried and used for chocolate production, was generated without contamination. This demonstrates that the SB200-X can reliably support long term, large scale biomass production for the food industry, combining operational robustness with significant cost savings through extended single-use bag lifetime.

References:

Zhong, J.-J., Fujiyama, K., Seki, T. and Yoshida, T. (1994). A quantitative analysis of shear effects on cell suspension and cell culture of perilla frutescens in bioreactors. Biotechnol. Bioeng., 44: 649-654. https://doi.org/10.1002/bit.260440512