Dynamic Biomass-Based Feeding of an Industrial CHO Fed-Batch Process

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Background

The addition of highly concentrated feeds as well as its over- or underfeeding may stress cells by milieu changes that require unwanted frequent and labor-intensive human actions (Fig. 1).
An optimal nutrient supply of each cell can solely be guaranteed by the implementation of real-time cell-specific feeding strategies, concomitantly reducing human interference by automation of the process.

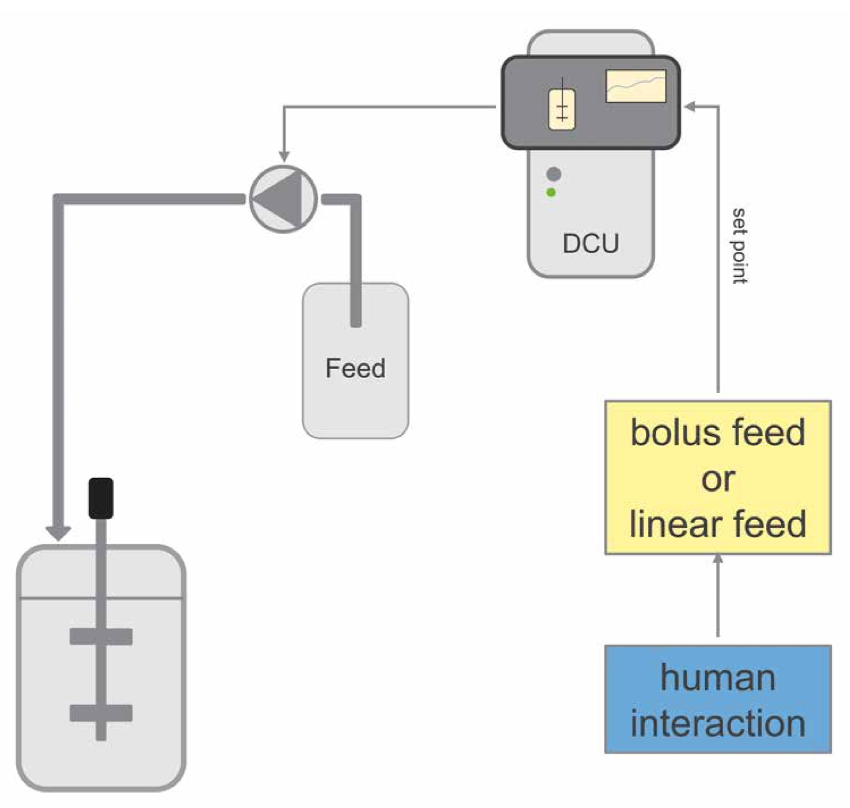

Figure 1: Batch-wise or linear addition of concentrated feed medium, which incrementally changes the volume and the composition of the cell broth.

Real-time biomass monitoring

The Incyte probe (Hamilton) generates an electrical field, in which cells with intact membrane act as capacitors. By alternating the field, the periodically polarization of the cell membranes is detected as permittivity signal.
The signal correlates well with the biovolume in the bioreactor (Fig. 2). Viable cell concentration [VCC] may be estimated by a cell line specific factor (equation I).

$$VCC_{t_x} = (p_{t_x} - offset) \cdot cf \qquad \text{(I)}$$

VCC_{t_x} = VCC at t_x [$10^6 \cdot mL^{-1}$]
p_{t_x} = permittivity at t_x [$pF \cdot cm^{-1}$]
$offset$ = background signal of medium [$pF \cdot cm^{-1}$]
cf = cell factor [$cm \cdot pF^{-1} \cdot mL^{-1}$]

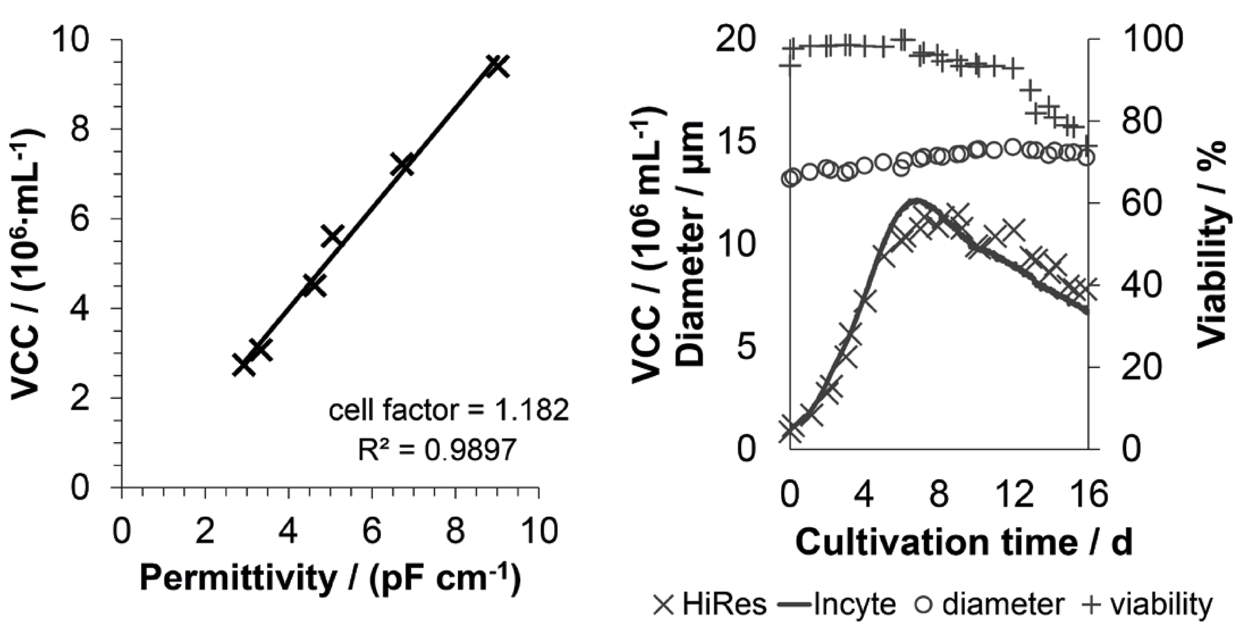

Figure 2: (left) correlation of online permittivity and offline VCC determination via trypan blue exclusion method; (right) VCC (online/offline), viability and cell diameter of model process (linear feed from day 2 to 10)

Biomass-based feeding strategies

Aim: To optimize nutrient availability in general, cell specific feeding strategies were implemented.
Assuming each cell has the same nutritional needs for survival and the same capacity to produce a certain product over a defined time period, dynamic feed flows with manual day-to-day tuning based on in process control (IPC) data were applied to the model process (Fig. 3):

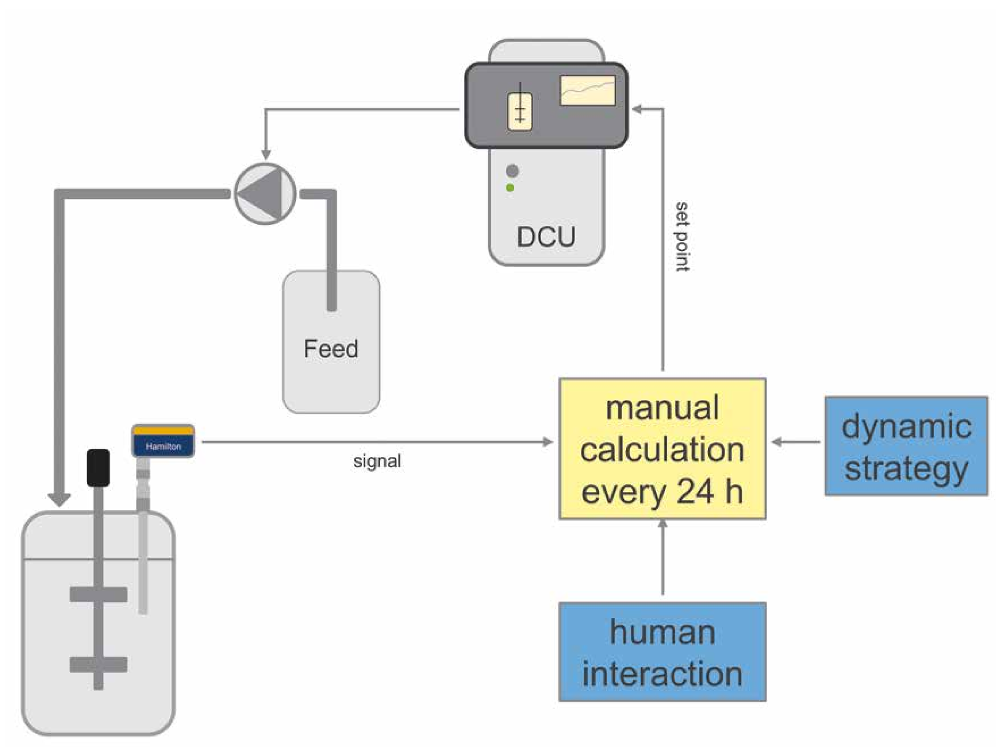

Figure 3: Dynamic feeding based on manual day-to-day adjustment of feed rates.

1. Feed addition based on the integral of viable cell concentration [IVCC] and a corresponding feed factor (equation II).

$$F_{Feed} = \frac{feed\ factor \cdot IVCC}{t_{x+1} - t_x} \qquad \text{(II)}$$

F_{Feed} = feed rate [$mL\ h^{-1}$]
$feedfactor$ = feed factor based on model process [$mL\ 10^6\ d^{-1}$]
$IVCC$ = IVCC [10^6 d]
t_{x+1} = time next IPC [h]
t_x = time current IPC [h]

2. Feed addition with a cell-specific feeding rate [CSFR] by retrospective calculation of expected VCC based on growth rate μ (equation III).

$$F_{Feed} = CSFR \cdot \frac{VCC_{t_{x+1}} \cdot V_{t_{x+1}} + VCC_{t_x} \cdot V_{t_x}}{2} \cdot 10^{-3} \qquad \text{(III)}$$

F_{Feed} = feed rate [$mL \cdot d^{-1}$]
$CSFR$ = CSFR based on model process [$pL\ d^{-1}$]
$VCC_{t_{x+1}}$ = $\frac{VCC_{t_x} \cdot V_{t_x}}{V_{t_{x+1}}} \cdot e^{\mu_{t_x} \cdot (t_{x+1} - t_x)}$
VCC_{t_x} = VCC at t_x [$10^6\ mL^{-1}$]
V_{t_x} = bioreactor filling volume at t_x [mL]
$V_{t_{x+1}}$ = bioreactor filling volume at t_{x+1} [mL]
μ = specific growth rate [d^{-1}]

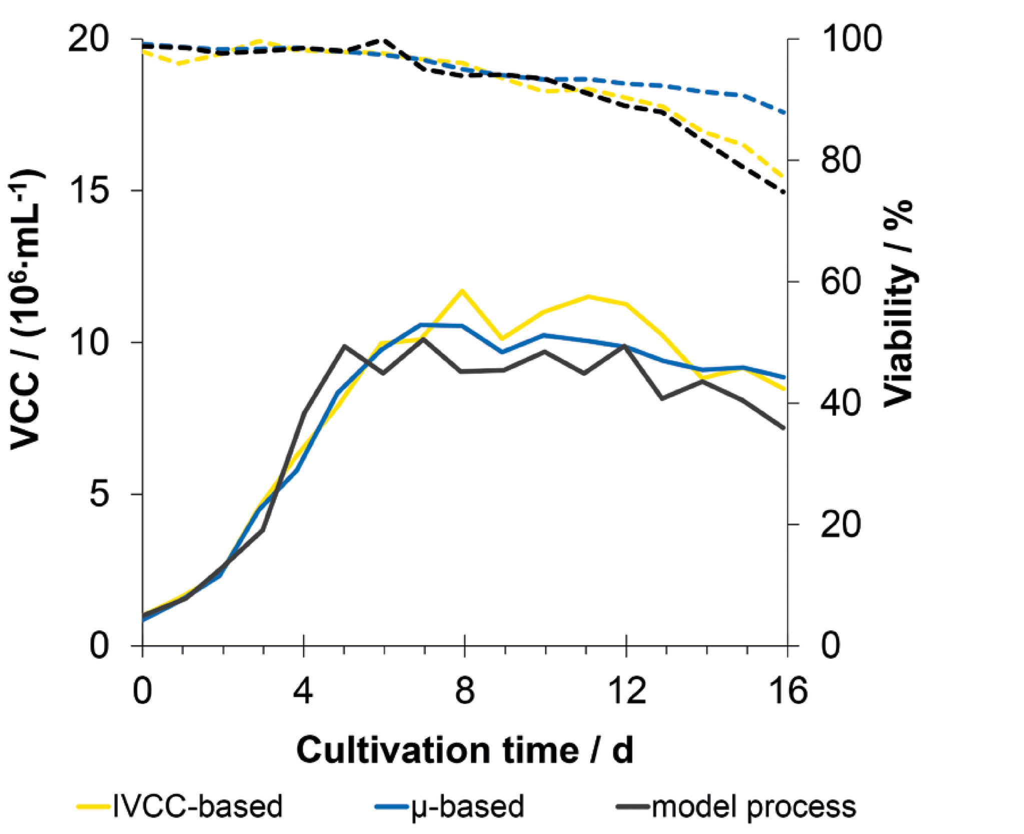

Figure 4: Exemplary course of growth (solid lines) and viability (dashed lines) applying different feed strategies in comparison to linear feed (model process).

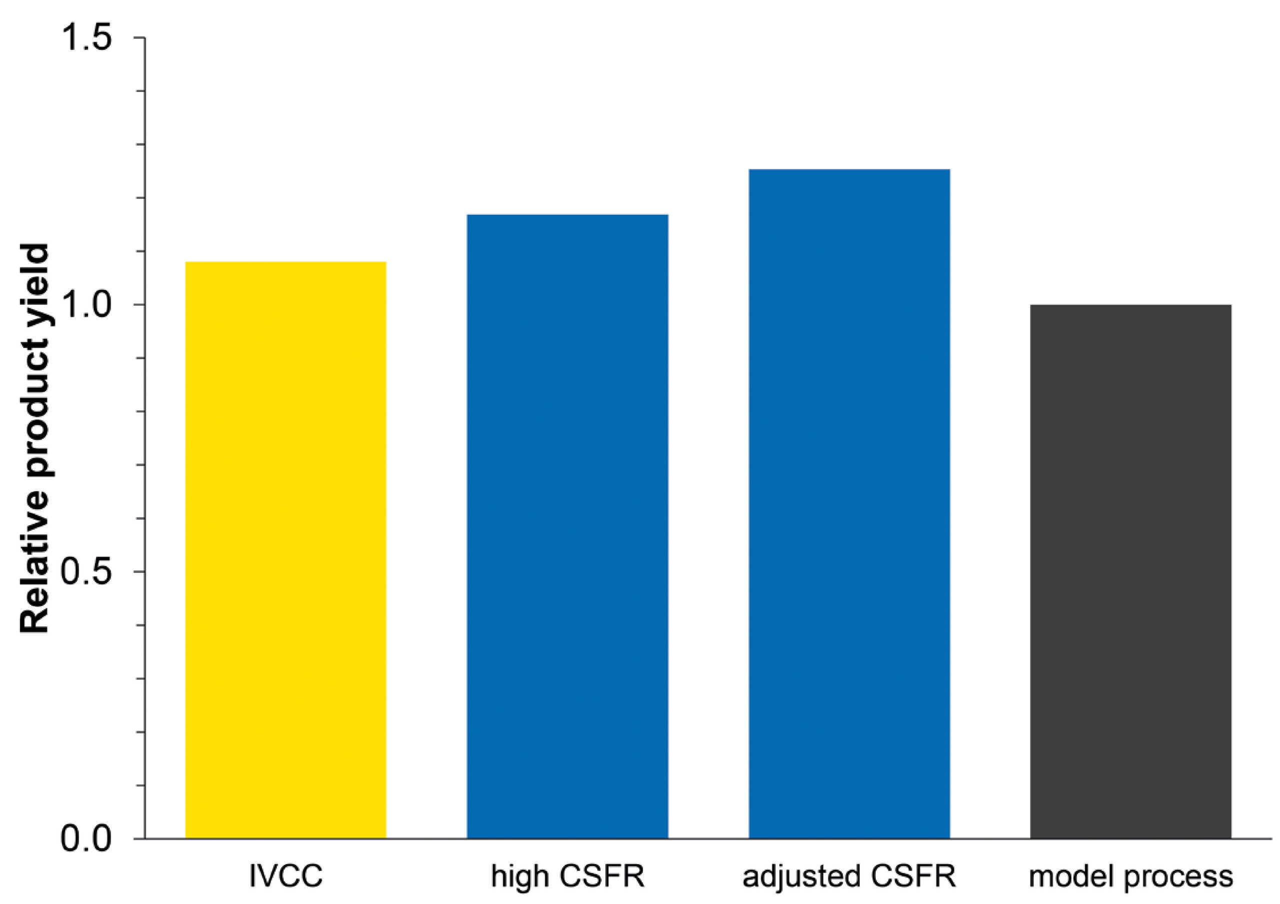

Figure 6: Impact of different feeding strategies (yellow IVCC-based, blue μ-based) on the relative product yield in comparison to the model process.

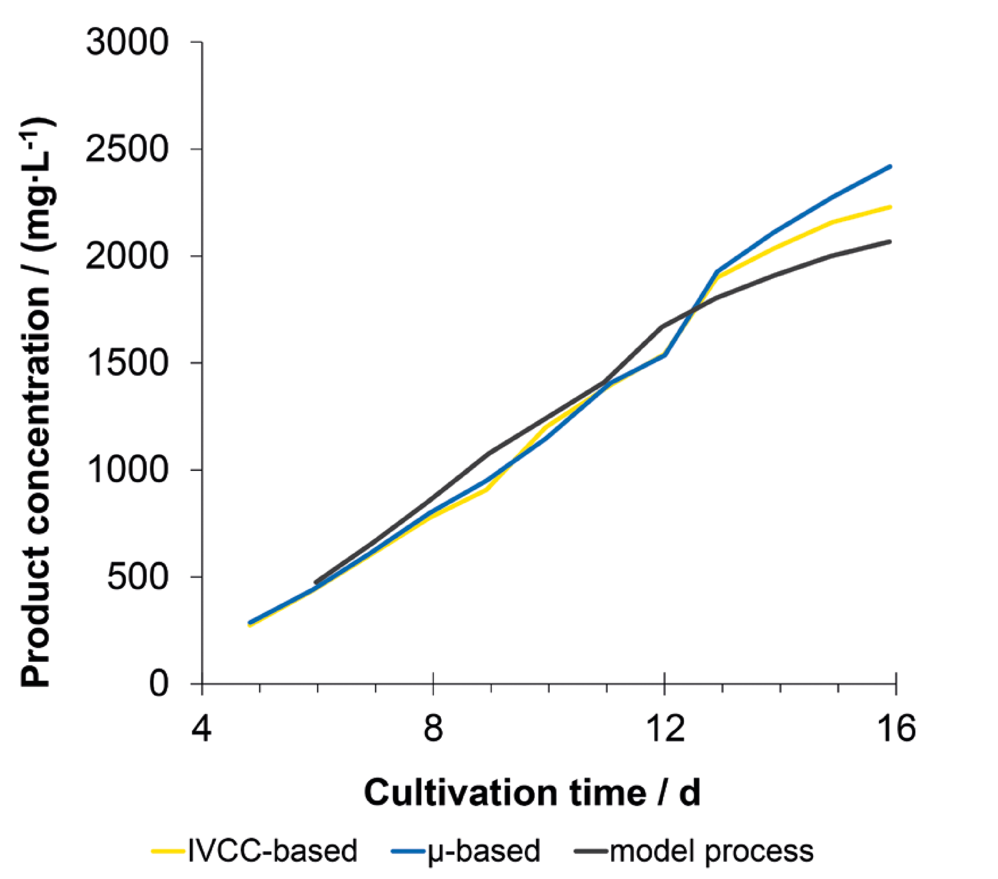

Figure 5: Exemplary IgG production curves, applying different feeding strategies in comparison to the linear feed (model process).

Conclusion

Both strategies resulted in slight elevation of the peak cell concentrations (Fig. 4). The final product titer gradually increased in dependence on the feeding strategy (Fig. 5).
In total, feeding based on IVCC, as well as by calculating the expected VCC, respectively, promotes an increase of relative product yields (Fig. 6). The μ-based method is dependent on a well-adjusted CSFR, preventing over- or underfeeding of single cells. The application can be simplified, combining this dynamic feeding strategy with the Incyte probe, not only improving but also automating the bioprocess.