Dynamic Feeding Enables Increased Total Protein Yield in Industrial Fed-Batch Bioprocesses

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Background

The addition of highly concentrated feed media as fixed bolus or in continuous rates may result in over- or underfeeding of the cells by incrementally changing the environment. In addition, frequent human interactions are labor intensive and error prone (Fig. 1).
A technical solution to avoid undesirable exceed of nutrients in the cell broth is the implementation of real-time cell-specific feeding strategies, supplying each cell with an optimal amount of nutrients at any time point of the cultivation.

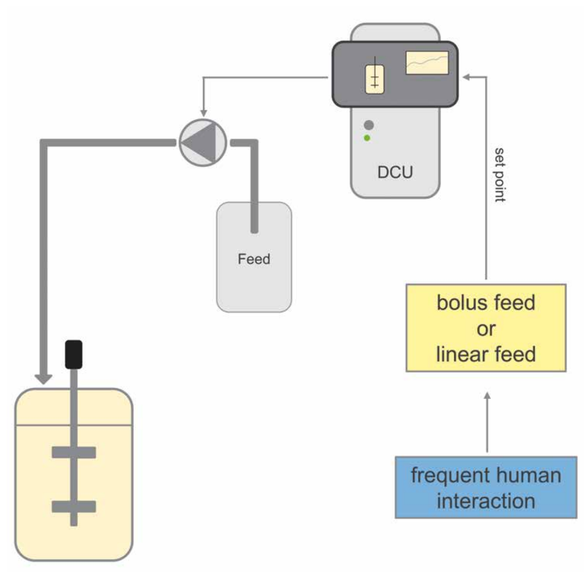

Figure 1: Bolus or linear addition of feed media is easy to implement, but shows the drawback of frequent human interactions.
Furthermore, volume and composition of the cell broth is incrementally changed by each addition.

Introduction

For optimized availability of nutrients in general, cell specific feeding strategies based on cell growth or consumption of key substrates have already shown their potential to improve the performance in bioprocesses.[1, 2]
Assuming, each cell has the same nutritional needs for survival and production, we programmed automated feed flows, based on a day-to-day calculation of either the viable cell concentration [VCC], the integral of the viable cell concentration [IVCC], or the glucose consumption rate, determined by inline measurement using a capacitance probe (Incyte, Hamilton) and offline measured VCC or nutrient concentration, respectively.

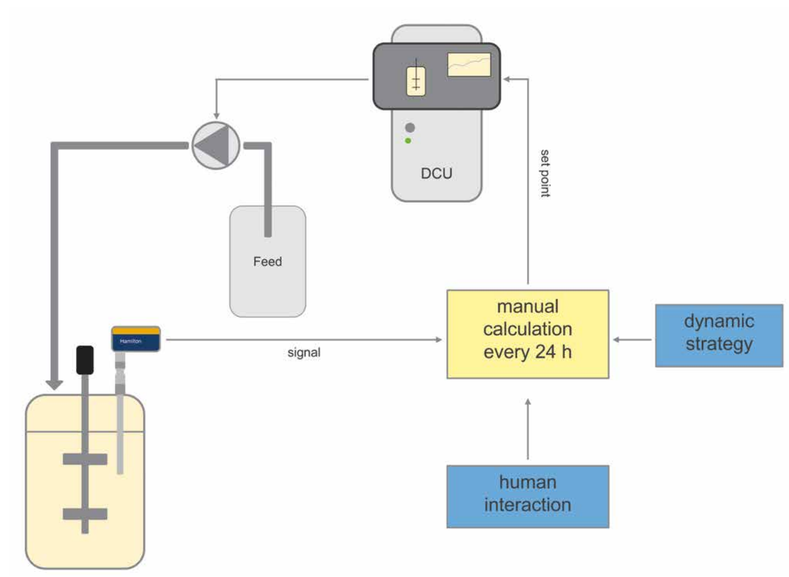

Figure 2: Dynamic adjustments of feeding rates based on retrospective calculations of growth or nutrient consumption on a day-to-day basis.

Results

Online biomass monitoring
The permittivity of the cell suspension correlates well with the bio volume in the bioreactor (Fig. 3) and may be correlated to the viable cell concentration [VCC] via a cell line specific factor (equation I).

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

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

To establish the online determination of the biomass in CHO cell cultivation we performed the correlation between offline VCC and online permittivity for several 10 L bioreactor runs (Fig. 3).

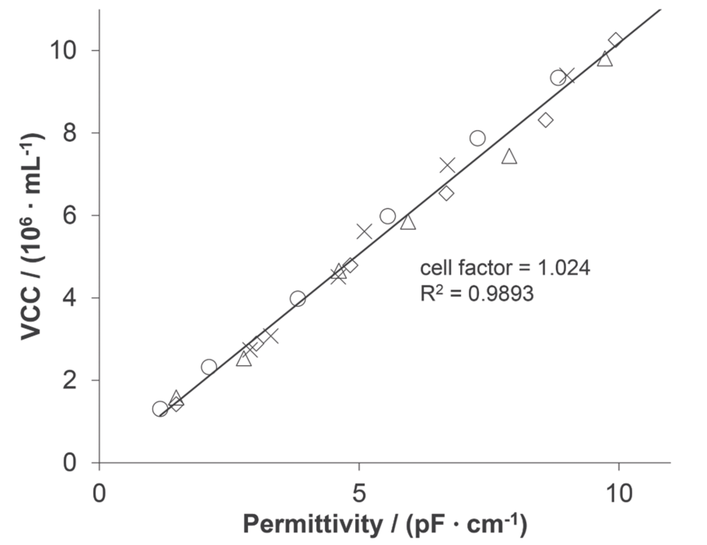

Figure 3: Correlation of *online* permittivity and *offline* VCC during exponential growth phase of the model process (X) and three bioprocesses with dynamic feed strategies (Δ, ○, ◊).

Dynamic feed strategies
To optimize the availability of nutrients in general, cell specific feeding strategies were implemented based on cell growth or consumption of key substrates calculated upon the basis of in process control [IPC] parameters (Fig. 2):

1. Feed flow [F_{Feed}] calculation based on the integral of viable cell concentration [IVCC] and a corresponding feed factor (equation II).

$$F_{Feed} = \frac{feed\ factor \cdot IVCC}{\Delta t} \qquad (II)$$

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

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

F_{Feed} = feed rate [mL 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]

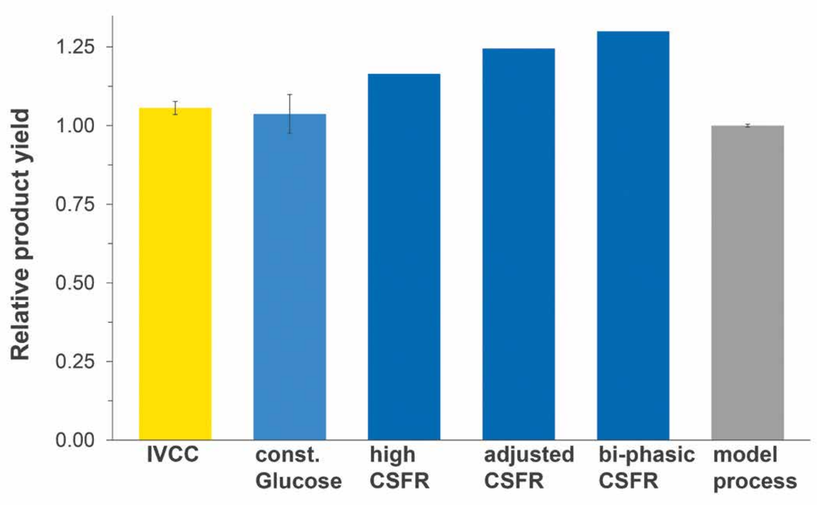

Figure 6: Relative product yield depending on different feed strategies in comparison to the model process. Mean values and standard deviations were calculated for biological duplicates (n = 2).

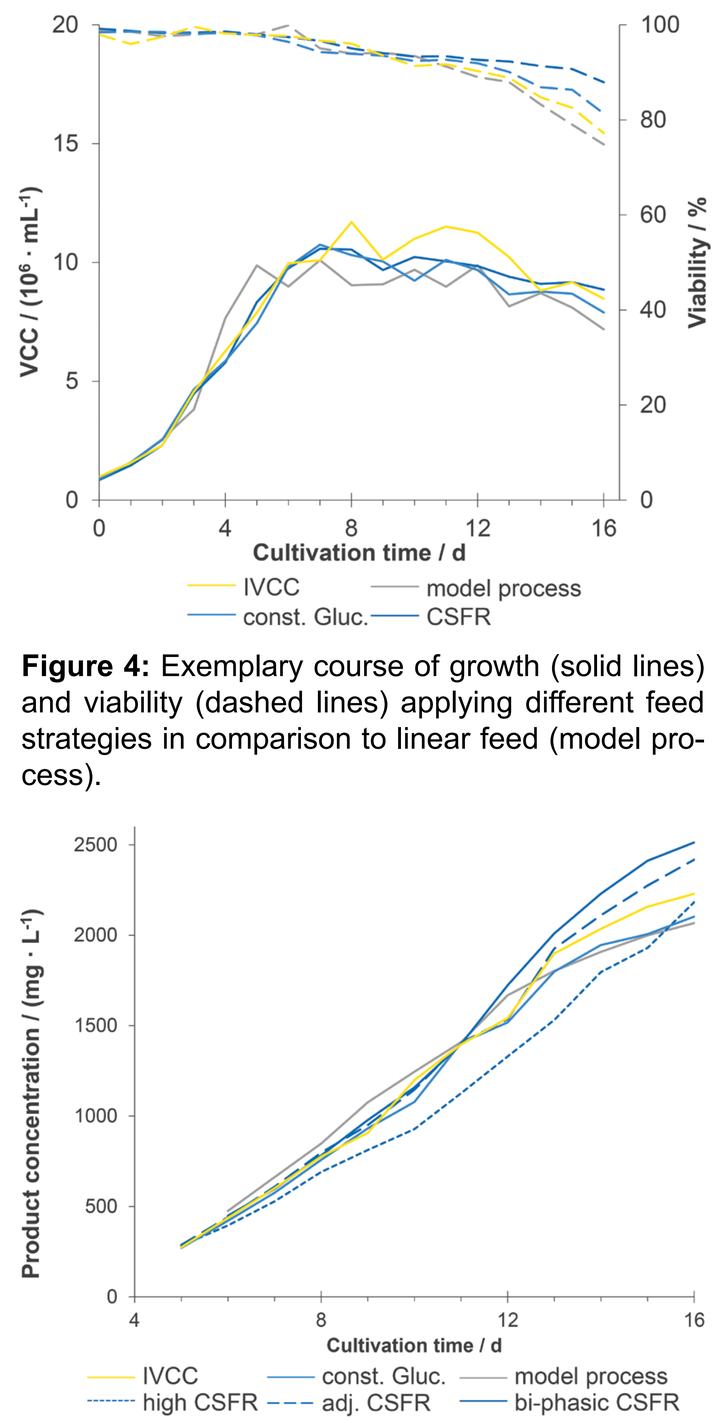

Figure 4: Exemplary course of growth (solid lines) and viability (dashed lines) applying different feed strategies in comparison to linear feed (model process).

Figure 5: Exemplary IgG production over cultivation time, applying different feed strategies in comparison to linear feed (model process).

Conclusion

In conclusion, the use of a real-time capacity measurement shows the potential to facilitate the determination of biomass and in partial of VCC in bioprocesses. By applying dynamic instead of linear feeding strategies, we were able to increase titer and total mAb yield. Especially the bi-phasic feeding, adjusting CSFR when cells reach stationary phase, resulted in 21% higher titer and 30% increase in relative mAb yield (Fig. 5 and 6). Our findings endorse the potential of dynamic feeding strategies to increase the space-time-yield of upstream bioprocesses in the absence of complicated cell retention systems.
This in combination with real-time measured biomass can besides increasing yield and titer, simplify and automate monitoring and control of bioprocesses.

[1]Lu et al. 2013. Biotechnol Bioeng 110: 191–205
[2]Konakovsky et al. 2017. American Institute of Chemical Engineers Biotechnol. Prog 33: 317-336

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