Interchangeability of stainless steel and single-use bioreactors in cell culture processes

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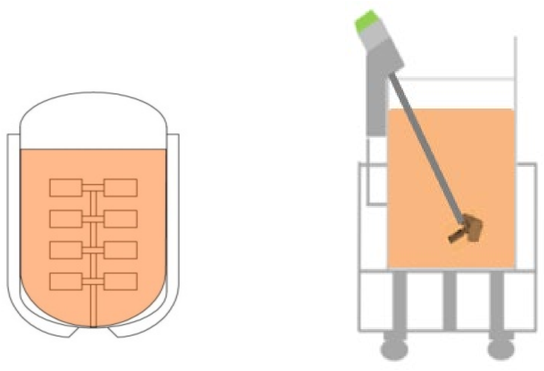
Himmelfahrtstagung on Bioprocess Engineering 2021 – New Bioprocesses, New Bioproducts, DECHEMA e.V., May 10-12 2021, Online Event

INTRODUCTION

Single-use bioreactors (SUBs) are becoming a state-of-the art technology in the pharmaceutical industry and are used in parallel with conventional stainless steel bioreactors. The advantages of single-use technologies are flexibility and speed, which need to be combined with the established systems and the gained expertise. Additionally, the environmental impact of single-use technologies is approximately ~ 40 % lower compared to traditional "fixed-in-place" process trains, which is primarily due to the reduced need for energy intensive water for injection, process water and clean steam[1].

The goal of this study was to evaluate how easily cell culture processes can be transferred from conventional stainless steel systems to single-use bioreactors. For this more than 20 bioreactor runs of different Chinese hamster ovary (CHO) clones producing standard monoclonal antibodies, bispecific antibodies and other products in stainless steel and single-use bioreactors were analyzed using multivariate data analysis. Especially critical process parameters (CPP) and critical quality attributes (CQA) of the products were compared in the different systems. Product concentrations, cell viability and CQAs were comparable between the reactor types.



Stainless steel bioreactor **Single-use bioreactor**

AIMS

- Establish flexible antibody production with easy process transfer between stainless steel and single-use processes
- Compare CPPs and CQAs in stainless steel and single-use bioreactors

METHODS

- More than 20 material supply, confirmation and consolidation runs of different antibody formats in stainless steel and single-use bioreactors are evaluated with multivariate data analysis (see Tab. 1)

Tab. 1: *Overview of the evaluated projects regarding product type and scale*

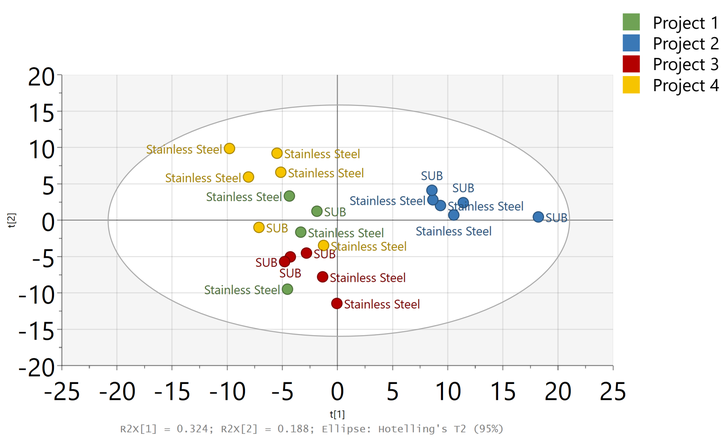
Project #	Product type	Stainless steel scale	Single-use scale
1	Bispecific mAb	80 – 200 L	100 – 500 L
2	Bispecific mAb	200 L	100 - 500 L
3	mAb	80 L	100 – 500 L
4	Bispecific mAb	200 L	500 L

- Partial least square models were combined with principal component analysis to assess the influence of the bioreactor type on CPPs and CQAs using SIMCA® 16

RESULTS

To get an overview of the data a Principal Component Analysis (PCA) was performed to assess whether clusters are visible for SUBs or stainless steel bioreactors. This is not the case as the clusters are divided based on the used production clone (project, see Fig. 1).

Figure 1: *Scores plot of Principal Component 1 and Principal Component 2 of the CPPs and CQAs colored by the evaluated project*



RESULTS

The multivariate data points cluster based on the project and not on the used bioreactor type as indicated in the PCA (see Fig. 1). To underly this observation, raw data of the most critical process parameters viable cell density, viability and product concentrations of the evaluated projects are plotted in Figure 2.

Projects 1 and 3 show an overall comparable trajectory with low variation for the evaluated parameters. In projects 2 and 4 higher variabilities between the evaluated bioreactor types are visible for the viable cell concentration and the viability. A deeper analysis on which parameters are influencing the models most is discussed in the right column.

Figure 2: *CPPs of the evaluated runs performed in single-use bioreactors (circles) and stainless steel bioreactors (squares)*

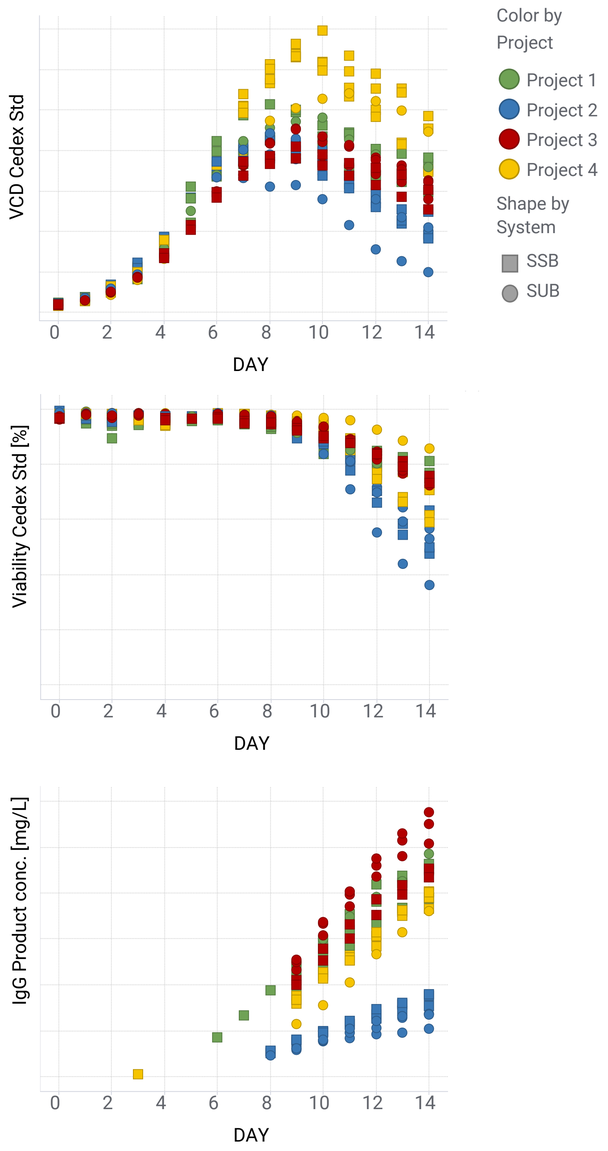
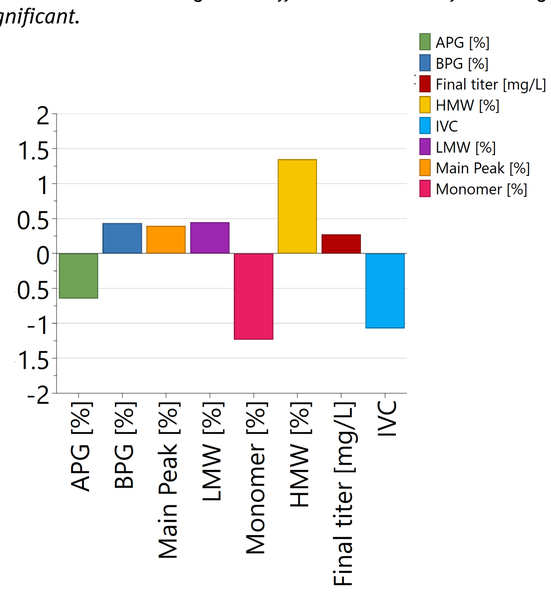


Figure 3 shows the differences of the CQAs of the different products depending on the bioreactor type. Positive values mean a higher value in SUBs compared to stainless steel bioreactors. The largest differences between SUBs and stainless steel bioreactors are observed for the product aggregation (HMW), which is approximately 1.3 scaled and centered units, followed by the monomer content and integral viable cell density with values of approximately -1.2 and 1, respectively.

Charge variants and product concentrations show a difference of lower than ±0.6 scaled units between stainless steel bioreactors and SUBs.

To conclude, there is no significant difference between stainless steel bioreactors and SUBs for the evaluated CQAs. In a next step, the model validity and the influence of different evaluated scales is evaluated.

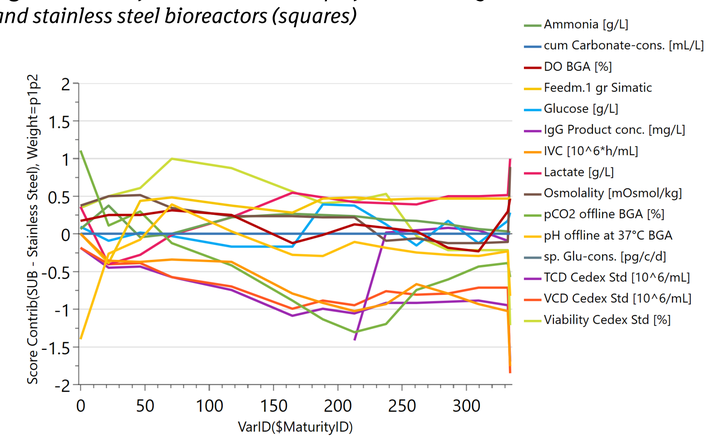
Figure 3: *In this contribution plot the scaled and centered differences between single-use and stainless steel bioreactor CQAs are shown. Largest variations occur for the monomer content and IVC. All values are within the ± 3 standard deviation range and differences can therefore be regarded as not significant.*



RESULTS

Main variations between SUB and stainless steel bioreactors were identified using the empirical model in SIMCA® 16 and were: pH and pCO_2 variations, cell viability in the first process phase, lactate concentration in the later process phase (see Figure 4).

Figure 4: *CPPs of the evaluated runs performed in single-use bioreactors (circles) and stainless steel bioreactors (squares)*



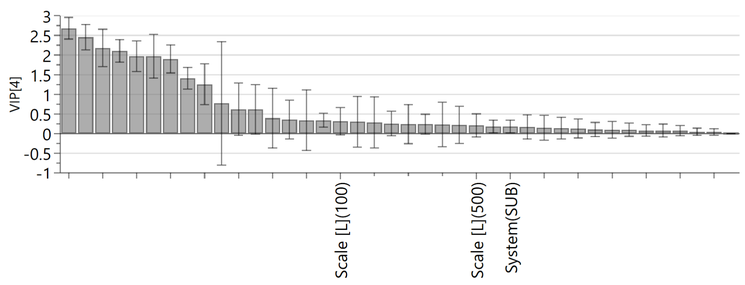
In order to evaluate the model, different parameters were considered. One of them being the model predicted variables vs. the measured (observed) variables of CPPs and CQAs.

The models for final titer, charge variants and integral viable cell density showed R^2 values of 0.96, 0.78 or higher, respectively, indicating a very good model fit. Models for product related impurities were also of a good quality (R^2 values were 0.66 – 0.75).

Production runs conducted in different scales were evaluated. Variable importance on protection (VIP) vectors were evaluated to exclude an impact caused by scale differences rather than single-use vs. stainless steel bioreactors. The VIP values reflect the importance of terms in the model both with respect to Y, i.e. its correlation to all the responses, and with respect to the projection. Its attraction lies in its intrinsic parsimony; for a given model and problem there will always be only one VIP vector summarizing all components and Y-variables. One can compare the VIP of one term to the others. Terms with a VIP larger than 1 are the most relevant for explaining Y.

The most important variables for the models (VIP > 1) were clone dependent parameters such as cell growth and metabolic production rates. Scale or bioreactor type variables showed values lower than 0.4 and standard deviations including 0 indicating a low contribution to the model (see Figure 5). This confirms the experimental values that the bioreactor type has no influence on CPPs and CQAs.

Figure 5: *Variable importance plot of the PLS model. Cell related factors are made unrecoanizable*



Preliminary results on the environmental assessment reveal that stainless steel needs 50 to 75 % less kg CO_2 equivalents compared to single-use technologies depending on the considered scale, which is contrary to studies conducted in literature, because Pietrzykowski et al. assumed material recycling of the single-use material[1].

For stainless steel systems cleaning in place (CIP) & sterilization in place (SIP) and for single-use systems the fully equipped single-use containers are the main environmental impact drivers, respectively.

CONCLUSION

- There is no significant difference in CPP and CQA between the production in stainless steel and single-use bioreactor systems, therefore antibody production can be transferred flexibly between stainless steel and single-use bioreactors
- For a holistic analysis on how to move forward in using single-use and/or stainless steel bioreactor systems, also sustainability and environmental aspects must be considered in the future in the light of production at Boehringer Ingelheim

LITERATURE

1. Pietrzykowski, Matthew, et al. "An environmental life cycle assessment comparison of single-use and conventional process technology for the production of monoclonal antibodies." *Journal of cleaner production* 41 (2013): 150-162.

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