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Scalability of Cost Effective 2D Rocking Motion Bioreactors with Internal Filter Membrane for Perfusion Processes. High Cell Densities of up to 170 mln Cells per mL and Spectrometric PAT Sensors Allow for an Intensified Seed Train Handling and Automated Inoculation.

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Introduction

Standard bio-manufacturing processes commonly rely on seed ratios around 1:5 [1] that proofed to be sufficient and reliable for years. Modern technology platforms, like cost effective 2D rocking motion (RM) bioreactors in perfusion mode, can easily be implemented for process intensification thus increasing viable cell densities to 100E6 cells·mL^{-1} and more. These high cell density (HCD) processes provide powerful tools to further drive the seed ratio to 1:130 and beyond.
Furthermore, these highly versatile cultivation platform technologies allow to a) support the generation of HCD cell banks that can be directly thawed into seed train bioreactors, thus avoiding the initial shake flasks cell culture expansion (and the associated open handling), and b) reduce the number of intermediate scale batch stirred tank bioreactors, thereby reducing initial investment as well as reducing operational COGs.
Apart from reducing the duration of the seed train, high cell densities in the seed train also allow to inoculate production processes at elevated cell numbers and thus shorten the overall N-stage process [2]. Due to the potential of this approach this strategy is acknowledged and promoted by the biopharma industry and corresponding consortia (e.g. BPOG, DECHEMA).

Material & Methods

Perfusion seed culture in Biostat® RM
A perfusion protocol was developed based on a standard CHO-DG44- Fed-Batch platform (Sartorius Stedim Cellca) [3]. This method used a minimum cell specific perfusion rate (CSPR) of 50 pL·(c·d)$^{-1}$. The cultures were maintained at 36.8 °C, pH 6.9 and DO 60%. An oxygenation cascade was implemented to maintain DO by increasing the rocks per minutes at 10° angle. The goal of these N-1 perfusion cultures was to reach 100E6 cells·mL^{-1} in less than 7 days.

Automated inoculation of N-stage reactor
A viable cell density – capacitance model was generated during N-1 stage based on the signal from the BioPAT® Viamass probe. Due to this model the VCD can be measured online at any given time point during N-1 stage. A trigger point for inoculation was defined when reaching 100E6 c·mL^{-1} thus allowing to automatically transfer inoculum from Biostat® RM to Biostat STR®-200 systems.

Biostat STR®-200 cultivations
Fed-batch cultivations were carried out at 200 L scale to test the effect of intensified seed trains on N-stage performance. The process was run according to earlier publications [4], i.e. with temperature control at 36.8 °C, pH control of 7.1 and DO 60%. The feeding started after day 3 administering 4% of Feed A and 0.4% of Feed B with optional glucose feeding. The seed culture for the N-stage trial came a) from an intensified N-1 perfusion culture at 100E6 c·mL^{-1} and b) from a standard seed train with conventional cell densities of less than 3E6 c·mL^{-1}.

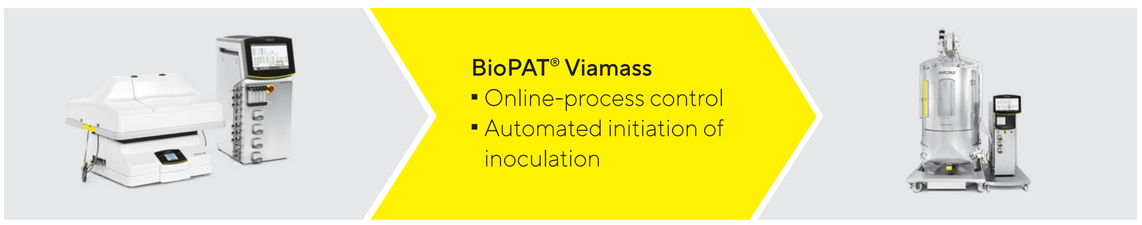

Figure 1: Automated inoculation from seed culture.

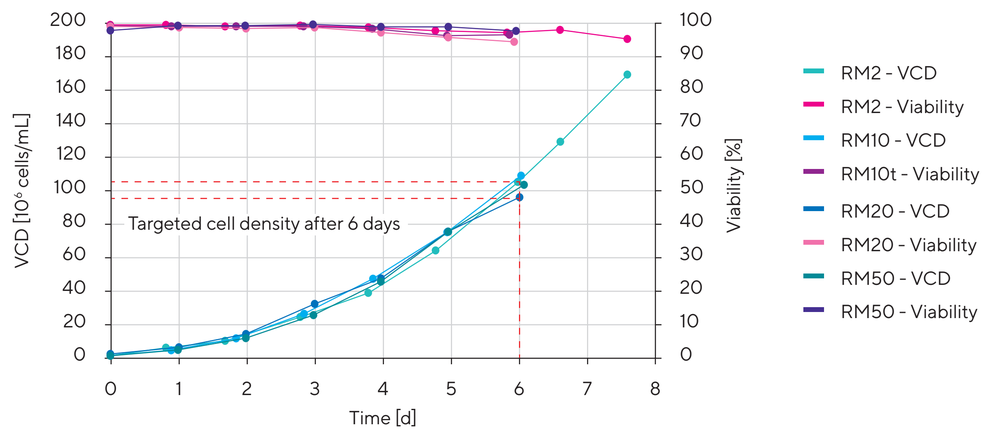

Figure 2: Scalability of viable cell densities and viabilities of high cell density cultivations in Biostat® RM systems, ranging from 2 to 50 L bag size. The goal was to reach 100E6 cells·mL^{-1} in less than 7 days. For the Biostat® RM 2 L system the maximal cell density was tested.

References
[1] Wittmann, C., Lia, J. C., Lee, S. Y., Nielsen, J., Stephanopoulos, G., Industrial Biotechnology: Products and Processes, Wiley-VCH Verlag GmbH & Co. KgaA, 2017
[2] Grauf, M., Erb, N., Vogt, F., Stipek, G., Zijlstra, G., Müller, D., Zehe, C., Poster presentation at Continuous Biomanufacturing: Achievements and Challenges for Commercial Implementation, Oxford (2018): Development of a commercial cell line generation program for intensified biomanufacturing.
[3] Matuszczyk, J-C., Schulze, M., Janoschek, S., Zijlstra, G., Greller, G., Poster presentation at Bioprocessing Summit Europe (2018): High cell density cell cultures (>100E6 c·mL^{-1}) in 2D bags with integrated filter for seed train process intensification.
[4] Ruhl, S., Stacey, A., Matuszczyk, J.-C., Greller, G., Poster presentation at ESACT (2017): Bioreactor scaling thought new – from 5 to 2000 L with utility functions

Results & Discussion

The N-1 perfusion seed cultures in Biostat® RM 2 to 50 L scale successfully reached 100E6 cells·mL^{-1} in less than 7 days with more than 90% viability (Fig. 2). Maximum viable cell densities were tested for the Biostat® RM 2 L and resulted in 170E6 cells·mL^{-1}. For all scales the cellular oxygen demand at 100E6 cells·mL^{-1} can easily be satisfied and cells show excellent viabilities. Viable cell densities of 100E6 cells·mL^{-1} allow impressive reductions of seed trains as indicated in Tab. 1.

RM scale	Possible inoc. volume [L] at 3E5 cells·mL^{-1}	Possible inoc. volume [L] at 2E6 cells·mL^{-1}	Possible inoc. volume [L] at 10E6 cells·mL^{-1}
2	333	50	10
10	1.667	250	50
20	3.333	500	100
50	8.333	1.250	250
100	16.667	2.500	500
200	33.333	5.000	1.000

Tab. 1: Possible inoculation scenarios based on an intensified seed train in Biostat® RM

As presented in Fig. 3 fed-batch processes inoculated from standard and intensified seed trains showed comparable growth characteristics over the entire process time. The automated PAT sensor-controlled inoculation procedure resulted in starting cell densities of 3.7E5 cells·mL^{-1} while targeting for 3.3 ± 0.4E5 cells·mL^{-1}, i.e. reaching manual precision at first trial. In total 0.4 L of the intensified seed train was used for inoculation and 14 L for standard seed trains.

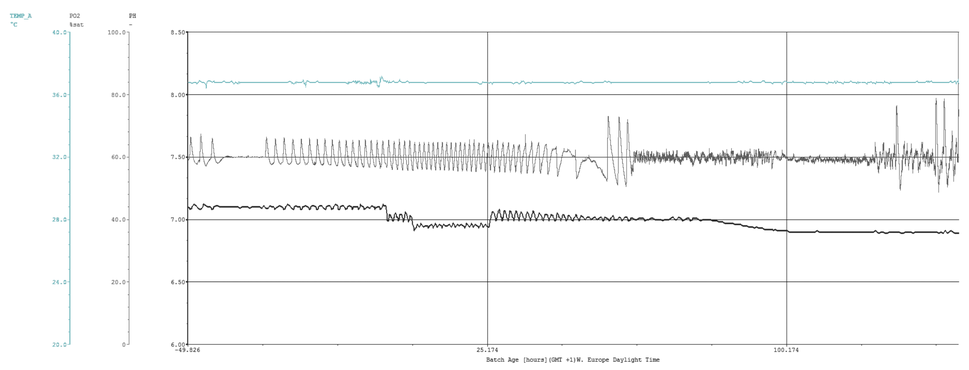

Figure 3: Process controller output for a representative Biostat® RM-system. T and DO were maintained at the indicated level, pH was allowed to drop to 6.9 and then controlled at this value.

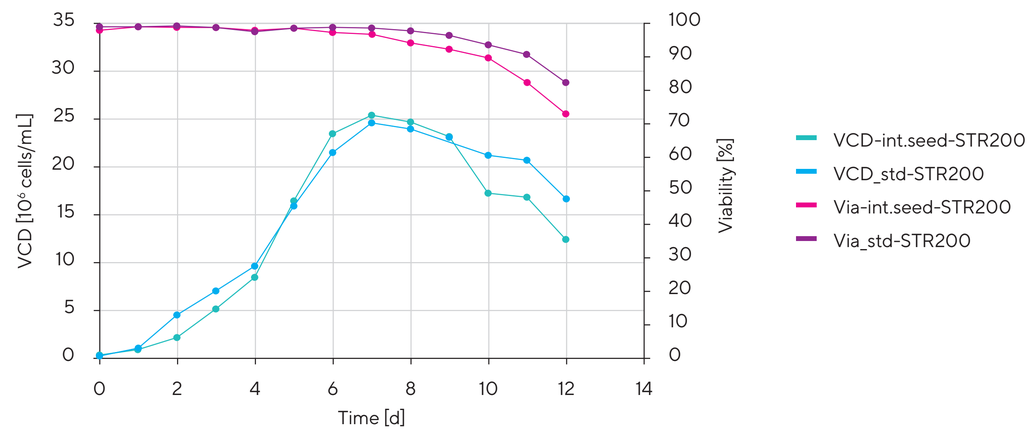

Figure 4: Comparison of N-stage fed-batch processes at 200 L scale inoculated either from standard or intensified seed trains.

Conclusions

- High cell densities can easily be achieved using the Biostat® RM platform. This allows to significantly reduce established seed trains as well as implementing sophisticated intensified strategies like HCD cell banking or subsequent seed train steps.
- Online PAT sensors, like BioPAT® Viamass, are highly beneficial for a) monitoring the state of the culture and b) implementing novel process control strategies. Due to constant surveillance of process parameters, like viable cell density, specific trigger points for automated inoculation of subsequent cultures can be set and executed. This allows for more process automation ultimately resulting in better process control.

Outlook

As presented in the given data set, intensified seed trains using Biostat® RM with Flexsafe® RM bags for HCD cultures are highly suitable for subsequent fed-batch processes. So far growth characteristics were evaluated, the next steps will focus on critical quality attributes like titer, glycan patterns and charge variants. Furthermore additional PAT sensors like BioPAT® Trace will be tested.

Acknowledgements:
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