Simplifying Progress

Superior Scalability of a Single-Use Bioreactor Family from 0.25 to 2000 L

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Introduction

The use of single-use bioreactors continues to grow in the biopharmaceutical industry and especially for mammalian cell cultivations [1]. The reasons for this are the advantages of single-use systems compared to reusable bioreactors. These include time saving factors like reduced lead times and safety factors e.g. a reduced risk of cross contaminations [2].

Compared to reusable bioreactors, which generally use stirred agitation, single-use bioreactors strongly differ significantly in terms of agitation principle and shape [3]. For example, classical stirring, rocking motion and orbital shaking are all used for single-use

bioreactors. These differences can complicate the scale-up and scale-down. Due to this, a stirred 250 mL system for process development and a bioreactor family for scaling up to production scales was developed with geometrical dimensions similar to common reusable systems.

The key process parameters for mammalian cell cultivations, kLa-value, mixing time and power input per volume, were evaluated to allow a Quality by Design approach as well as an easy up/down scaling.

2. Single-Use Bioreactors: Ambr® 250 High Throughput and Biostat STR® family

The process development system Ambr® 250 High Throughput and the Biostat STR® family were designed to have dimensional ratios, which are comparable to widely accepted reusable systems (Tab. 1). They have a cylindrical cultivation chamber, two impellers and a sparger for aeration.

The Ambr® 250 High Throughput is equipped with a central impeller configuration 2x3-blade segment impellers and sparging is performed by an open pipe (1.7 mm).

In comparison the Biostat STR® family can be used with central 2x3-blade segment impellers or 6-blade disk impeller + 3-blade segment impeller. The sparging below the bottom impeller is implemented by a ring (hole diameter 0.8 mm), micro (hole diameter 0.15 mm) or combi sparger, which contains an independent ring and micro part.

For the process engineering trials 2x3-blade segment impellers and open pipe / ring sparger is used to allow an efficient comparison between Ambr® 250 High Throughput and Biostat STR® family.

Table 1:
Ratios of Ambr® 250 High Throughput and Biostat STR® family

Ratio	Ambr® 250 High Throughput	STR® 50	STR® 200	STR® 500	STR® 1000	STR® 2000
Vessel Height / Vessel Diameter	2	1.8	1.8	1.8	1.8	1.8
Liquid Height / Vessel Diameter	1.44	1.3	1.34	1.23	1.36	1.29
Impeller Diameter / Vessel Diameter	0.42	0.38	0.38	0.38	0.38	0.38

3. Quality by Design Approach

The Biostat STR® family and Ambr® 250 High Throughput mammalian bioreactors were developed for cultivation of mammalian cells. To verify the performance of the single-use bioreactors for mammalian cells, a modern CHO process was considered with a peak cell density of 27 - 28 x 10^6 cells/mL. This process defines the key process parameters relevant for the design space definition [3] [4] [5]:

- Moderate shear rates (tip speeds < 2.0 m/s)
- Sufficient oxygen transfer rate (kLa > 7 1/h, supply pure oxygen assumed)
- Suitable homogeneity (mixing times < 60 s)
- A sufficient minimal power input of 10 W/m^3
- The theory of kolmogorov shows that cell damages by shear stress can occur only for a specific power input above 2000 W/m^3
- Turbulent flow behavior (Re > 10.000)

4. Oxygen Transfer Capabilities

To describe the oxygen transfer efficiency of a bioreactor the volumetric mass transfer coefficient (kLa-value) can be used. This was determined by the gassing-out method at 37 °C and for a maximal filling volume of 1 x PBS [6].

The kLa-value is shown in Fig. 1 as a function of the tip speed for a constant gas flow rate of 0.1 vvm. The required kLa-value of 7 1/h can be achieved in all bioreactors.

5. Mixing Time

Sufficient mixing is required to achieve homogeneity and therefore, to avoid e.g. concentration or temperature gradients. The mixing time in the single-use bioreactor family was determined by the decolourization method for maximal filling volume [7].

As shown in Fig. 2 the mixing time improves with increasing tip speed. The mixing times of the STR 2000 and 1000 are very similar. For Ambr® 250 High Throughput up to STR 500 the mixing time decreases with the scale.

Mixing times below 30 s can be achieved for all scales.

6. Power Input per Volume

To achieve homogenization and gas dispersion in a bioreactor, power has to be contributed by agitation. For the Biostat STR® family the power input per volume (P/VL) was calculated with the dimensionless power number (Ne=1.3), which was determined by torque measurement [6].

Due to the small scale, the power in the Ambr® 250 High Throughput was determined by the motor power. The P/VL for the single-use bioreactor family was determined for the turbulent flow zone and is shown in Fig. 3. The P/VL increases with the tip speed and decreases with the bioreactor scale. A specific power input of 30 W/m3 can be achieved for all bioreactors.

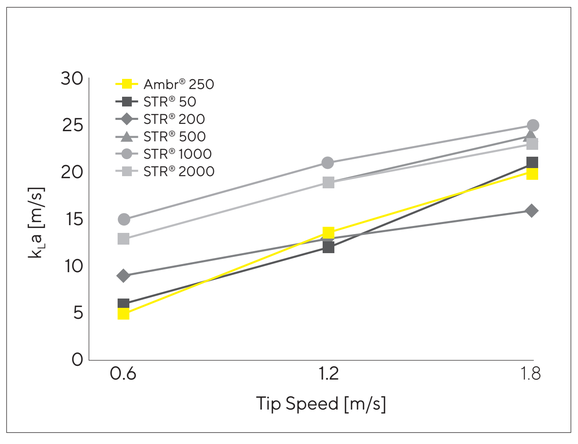

Figure 1: Volumetric mass transfer coefficient

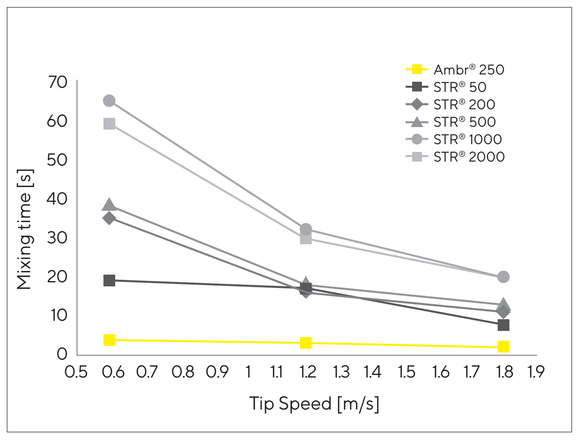

Figure 2: Mixing time

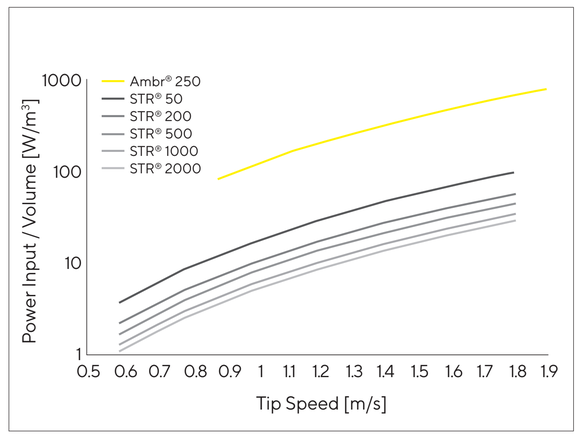

Figure 3: Power input per volume

Conclusion

- Due to geometrical similarity of the single-use bioreactor family the prerequisite for a successful scale-up and process transfer is given
- All bioreactors fulfill the requirements of high cell density CHO processes
- The single-use bioreactor family gives the possibility for small scale process development and easy scale up to production bioreactors

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