Technical Report

Design space definition for a stirred single-use bioreactor family from 50 to 2000 L scale

Single-use bioreactors continue to gain large interest in the biopharmaceutical industry. They are frequently used for mammalian cell cultivations, e.g. production of monoclonal antibodies and vaccines. This is motivated by several advantages of these bioreactors such as reduced risk of cross contaminations or short lead times. Single-use bioreactors differ in terms of shape, agitation principle, and gassing strategy. Hence, a direct process transfer or scale-up can be a challenge. Conventional stirred tank bioreactor designs are therefore still considered as the gold standard due to their well-defined and characterized properties. Based on this knowledge, a stirred single-use bioreactor family from 50 to 2000 L scale was developed with geometrical ratios similar to conventional reusable systems. To follow a quality by design approach, the single-use bioreactor family evaluated here was characterized by using process engineering methods such as the power input per volume, the mixing time, and the $k_L a$ value. The process engineering characterization demonstrates that these systems are suitable for cultivations of mammalian cells, even for high cell density and high titer applications. Based on the data, a scale-up or process transfer is possible between this bioreactor family as well as reusable systems. Therefore, this bioreactor family represents a major progress for the single-use technology.

Keywords: Design space / Process engineering characterization / Scale-up / Quality by design / Stirred single-use bioreactor

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1 Introduction

For biopharmaceutical processes, the used bioreactor as well as its design is of major importance. The bioreactor is a key element for process optimization [1] as operations such as homogenization, dispersion, suspension of solid particles, and heat transfer [2] are performed. Due to the well-defined characteristics of stirred stainless steel and glass bioreactors, these cultivation platforms are the gold standard since many decades for biopharmaceutical requirements.

During the last years, single-use bioreactors have gained more and more acceptance in the field of biotechnology. This is motivated by several advantages these bioreactors offer, such as reduced operational and investment costs. Furthermore, the time to market is shortened by lower lead times and reduced qualification efforts [3, 4]. As a consequence, single-use technology is an answer to the increasing cost pressure in the industry for monoclonal antibody and vaccine production [5].

Many processes are carried out in stirred bioreactors where mixing and power input are achieved by – usually multiple – impellers. These systems are in general actively aerated. The design of most single-use bioreactors differs from conventional stainless steel or glass fermenters [6, 7] making a scale-up and process transfer to other systems challenging. To circumvent these difficulties, Sartorius Stedim Biotech GmbH developed the bioreactor family BIOSTAT® STR, which has the same well-known design criteria of reusable bioreactors [8].

The bioreactor needs to fulfill the specific process requirements of the respective cultivation. As a consequence, a detailed knowledge of the used cultivation system is necessary for a scale-up or process transfers [9], which can be obtained by process engineering characterization.

1.1 Process engineering characterization

Mammalian cells are sensitive against shear forces because they do not have a cell wall. The shear forces are proportional to the power input per volume $(P/V_L)$ [7], a generally used scale-up criterion. It describes the transferred energy of the agitation.
device into the medium and correlates with the ability to sus-
pense solids, disperse gas bubbles, and mixing behavior of a
bioreactor [2]. For cell culture processes $P/V_c$ between 10 and
250 W m$^{-3}$ have been reported from lab to production scale [7].
For the description of the power input, the Newton number ($Ne$)
is essential. With this value, conclusions about the efficiency of
an impeller configuration can be made [2]. By knowing $Ne$ of a
bioreactor, $P/V_c$ can be calculated.

One purpose of an agitation device is the homogenization,
necessary to compensate concentration and temperature gra-
dients. These gradients can have disadvantage effects on the cell
growth and protein expression [10]. For its quantification, the
mixing time can be used. They depend on the reactor geometry,
impeller design as well as $P/V_c$ [11]. The mixing time defines the
time needed to completely homogenize a solution after the oc-
currence of a concentration difference. Mixing times below 60 s
are described for the cultivation of mammalian cells to avoid
disadvantage effects on the cell growth [10]. Different methods
are established for the characterization of the mixing times [7].
In this report, the decolorization method was preferred, which
is based on the color change of a dye.

Oxygen is an essential nutrient for aerobic processes and is
consumed by the cells for growth, maintenance, and metabolic
production [12]. The oxygen demand is described by the oxygen
uptake rate ($OUR$ [mmol/(L·h)], see Eq. (1)). The $OUR$ corre-
lates with the cell density ($X$ [cells L$^{-1}$]), specific growth rate
($\mu$ [h$^{-1}$]), and the oxygen yield coefficient ($Y_{XO_{2}}$ [cells/
mmol(O$_2$)], which depends on the used organism.

$$OUR = \frac{\mu}{Y_{XO_{2}}} \cdot X \tag{1}$$

To ensure a sufficient supply of oxygen a permanent transfer
to the medium by e.g. sparger has to be facilitated. The transfer
of oxygen from gas bubbles into the medium is defined by the
oxygen transfer rate ($OTR$ [mmol L$^{-1}$·h$^{-1}$]) (see Eq. (2)). It
depends on the difference of the oxygen saturation concentra-
tion ($C^*$ [mmol L$^{-1}$]) and the dissolved oxygen concentration ($C$
[mmol L$^{-1}$]) defining the driving force [12]. Furthermore, it is
affected by the volumetric mass transfer coefficient ($k_{la}$ [h$^{-1}$])
being the reciprocal time needed for the oxygen transfer from
the gas bubble to the liquid phase. This value is influenced by the
bioreactor and impeller design as well as $P/V_c$ and the gassing
strategy [12]. For most mammalian cell cultivations, a $k_{la}$ of
$\sim 10$ h$^{-1}$ is sufficient [6].

$$OTR = k_{la} \cdot (C^* - C) \tag{2}$$

1.2 Scale-up

After a cultivation process is established on laboratory scale, the
aim is to transfer the process into larger bioreactors (scale-up).
Deviations can occur during a process scale-up because mass
transfer phenomena are highly dependent on the scale and the
presence of spatial gradients [10].

A basic prerequisite for a successful scale-up is the geomet-
rical similarity (e.g. height to diameter ratio, impeller to vessel
diameter ratio) [13]. Furthermore, the material properties of the
medium such as the pH, temperature, and composition have to
remain the same. It is impossible to keep all process engineering
parameters constant during a scale-up. Hence, a relevant param-
eter has to be defined, which remains constant. A commonly used
scale-up criterion is to maintain $P/V_c$ [9] or for shear sensitive
cells, the tip speed [14].

Based on a detailed knowledge from reusable bioreactors,
important design criteria can be given for the cultivation of
mammalian cells. An aspect often considered is the height to
vessel diameter ratio (H:D), which varies between 1:1 and 3:1
for stirred tank reactors [13]. The ratio has an influence on the gas
transfer because it correlates with residence time of gas bubbles
for bioreactors with direct sparging [15]. For the cultivation of
mammalian cells, a ratio of 2:1 is recommended [16].

A further parameter is the impeller to vessel diameter ratio
having an effect on the mixing efficiency and the generated shear
rates. For cell culture applications, the ratio should be between
0.33 and 0.5 [17]. For Newtonian culture broths like mammalian
cell suspensions often segment impellers are used to achieve mixing
generating an axial flow pattern. Segment impellers trans-
form the transferred energy efficient into hydrodynamic power
and generate large circulation loops. This correlates with a suit-
able mixing behavior, and therefore they are preferred for ho-
mogenization. Furthermore the transferred dissipative energy
is low, and consequently they are often used for shear sensitive
cells. However, in the past decade, optimization of different cell
strains resulted in less shear sensitive cells. This also allows the
usage of a disk impeller on the bottom and a segment impeller
on the top of the stirrer shaft. In this way, the efficient mixing
performance of the segment impeller gets combined with the
enhanced bubble distribution of the disk impeller, making this
configuration interesting for high density applications [18, 19].

It is common to install multiple impellers in bioreactors with a
H:D ratio above 1:1.4 to ensure an efficient mixing through the
entire cultivation chamber. A distance between the impellers
of 1.2–1.5 times the impeller diameter ($d_I$) should be selected
because the impellers act independent of each other.

Besides the mixing efficiency, the gas transfer is crucial for the
design of a bioreactor. For the aeration, generally ring spargers
with drilled holes are in general installed below the impellers
[20]. The gas transfer is influenced by the size and amount of
generated gas bubbles. Smaller bubbles have a higher specific
transfer area ($a$), which significantly improves the overall gas
transfer [12]. On the other hand, a disadvantage is that cells can
be damaged when bubbles burst. Bubble sizes are generally in
the region of 4–6 mm. Larger bubbles have the advantage that
CO$_2$-stripping is enhanced, and consequently an accumulation
in the cultivation medium can be reduced.

2 Materials and methods

2.1 Design criteria for the CultiBag STR

To overcome the existing challenges during a process scale-up, a
stirred single-use bioreactor family (BIOSTAT® STR, Sartorius
Stedim Biotech GmbH, Germany) was developed based on the
proven and trusted principles of stainless steel fermenters [21].
The main application of this bioreactor family is the cultivation
of mammalian cells and is available from 50 to 1000 L scale,
while a 2000 L scale is under development. These systems have a
Table 1. Summary of key geometrical dimensions\(^a\) for the CultiBag STR family [8].

| CultiBag STR | 50   | 200  | 500  | 1000 | 2000 |
|--------------|------|------|------|------|------|
| Total volume [L] | 68   | 280  | 700  | 1300 | 2800 |
| Max. working volume [L] | 50   | 200  | 500  | 1000 | 2000 |
| Min. working volume [L] | 12.5 | 50   | 125  | 250  | 500  |
| Bag diameter \(D\) [mm] | 370  | 585  | 815  | 997  | 1295 |
| Bag height \(H\) [mm] | 666  | 1055 | 1467 | 1800 | 2330 |
| Ratio \(H:D\) [-] | 1.8  | 1.8  | 1.8  | 1.8  | 1.8  |
| Liquid height \(h_1\) [mm] | 480  | 783  | 1005 | 1360 | 1670 |
| Ratio \(h_1/D\) [-] | 1.3  | 1.34 | 1.23 | 1.36 | 1.29 |
| Impeller diameter \(d_2\) [mm] | 143  | 225  | 310  | 379  | 492  |
| Ratio \(d_2/D\) [-] | 0.38 | 0.38 | 0.38 | 0.38 | 0.38 |
| Distance between impellers \(\Delta z\) [mm] | 186  | 300  | 403  | 493  | 640  |
| Size drilled holes ring sparger part [mm] | 0.8  | 0.8  | 0.8  | 0.8  | 0.8  |
| Number drilled holes ring sparger part [-] | 5    | 25   | 100  | 100  | 200  |
| Size drilled holes micro sparger part [mm] | 0.15 | 0.15 | 0.15 | 0.15 | 0.15 |
| Number drilled holes micro sparger part [-] | 25   | 100  | 500  | 500  | 1000 |

\(^a\)Key geometrical dimensions are represented in Fig. 1.

— single-use cultivation chamber (bag) made of synthetic materials. The bags have a cylindrical shape with a H:D ratio of 2:1, an impeller to bag diameter ratio of 0.38 and \(\Delta z = 1.3d_2\). Based on this, the design criteria are inside the specifications for mammalian cell cultivations. Table 1 shows a detailed list of the geometrical dimensions for the CultiBag STR family (Fig. 1).

For the 50 and 200 L scale, the impellers are mounted on a rigid shaft. For scales greater or equal to 500 L, a telescopic shaft is used. Since the impeller shaft is connected to the motor via a magnetic coupling, the cultivation chamber remains close and sterile. For the CultiBag STR 50–1000 an axial and for the CultiBag STR 2000 L a radial magnetic coupling is used. Therefore, torque measurements were carried out for the 50 and 200 L scale and \(\text{Ne} \) was determined by Eq. (3).

\[
\text{Ne} = \frac{2 \cdot \pi \cdot M_i}{\rho \cdot N^2 \cdot d_2^4} \tag{3}
\]

where \(\text{Ne}\) is the Newton number [-] and \(M_i\) is the torque [Nm]. For the measurements, a torque transducer with a measuring range up to 10 Nm (ETH-Messtechnik) was used. The single-use bioreactors were filled with RO water to their maximal filling volume. For the determination, only values under turbulent conditions were considered because a constant \(\text{Ne}\) is achieved. For the trials, \(P/V_i\) in an ungassed system was determined because the gassing rates for cell culture processes are generally low. Hence, the influence on the measurement can be neglected.

2.3 Mixing time determination

The mixing times were determined by the decolorization method, which is based on the color change of a dye [23]. 2 mL L\(^{-1}\) potassium iodide (stock solution containing: 40 g L\(^{-1}\) potassium iodide and 20 g L\(^{-1}\) iodine) and 5 mL L\(^{-1}\) starch solution (containing: 10 g L\(^{-1}\) starch) were added into the bioreactors filled with RO water to their maximal filling volume leading to a dark blue colorization. Afterwards, 4 mL L\(^{-1}\) sodium thiosulfate...
Figure 2. Results of the process engineering characterization of the CultiBag STR family from 50–2000 L scale with the configuration $2 \times 3$-blade-segment impeller. (A) Power input per volume, where a single determination was performed. Based on previous measurements, a standard deviation of $\pm 10\%$ was assumed. (B) Mixing times in dependence of the tip speed. Based on previous studies, a standard deviation of $\pm 2\ s$ was assumed. (C) Shows the $k_{L}a$ values for the aeration with the ring and micro sparger part. Based on previous studies, a standard deviation of $\pm 10\%$ was assumed. The error bars show the standard deviation, which was calculated from triplicates of prior experiments.

$\text{(Na}_{2}\text{S}_{2}\text{O}_{2}\text{)}$ solution with 0.1 M was added, which decolorizes the solution. The time between $\text{Na}_{2}\text{S}_{2}\text{O}_{2}$ addition to complete decolorization (goodness of mixture $= 1$) is defined as the mixing time. The mixing time is determined by visual judgment, hence it is a subjective procedure and can lead to deviations. An advantage is that possible zones of poor mixing are detectable.

2.4 Volumetric mass transfer coefficient ($k_{L}a$) measurements

For the quantification of the oxygen transfer rate of the bioreactor family, the $k_{L}a$ value was determined by the gassing out method [24]. The bags were filled with 1X PBS buffer, and the liquid was deoxygenated by the supplementation of nitrogen to achieve a $pO_{2}$ below 10%. After $N_{2}$ addition air was sparged and the $pO_{2}$ increase was recorded. All measurements were carried out at room temperature. The $k_{L}a$ was calculated from the slope of the mass balance (see Eq. (4)).

$$In\left(\frac{C_{1} - C_{2}}{C^{*} - C_{2}}\right) = k_{L}a \cdot (t_{2} - t_{1}) \quad (4)$$

where $C_{1}$ and $C_{2}$ [mmol L$^{-1}$] are the dissolved oxygen concentrations at time $t_{1}$ and $t_{2}$ [h]. For the measurements, a preinstalled optochemical probe (PreSens) was used. All measurements were performed at a gas flow rate of 0.1 vvm.

3 Results

To characterize $P/V_{L}$, the Newton number was determined (see Section 2.2). For the $2 \times 3$-blade-segment impeller, a $Ne$ of $\sim 1.3$ was calculated for the scales 50 and 200 L. Due to geometrical similarities, it is suggest that the other scales have a same $Ne$. Because for all scales $Re > 10,000$, turbulent flow conditions are present [25] and it can be concluded that $Ne$ is constant for the tip speeds between 0.6–1.8 m/s. The power input per volume ($P/V_{L}$) is an important process engineering parameter
and was calculated based on the Newton number (Eq. (5)) (see Fig. 2A).

$$\frac{P}{V_L} = \frac{Ne \cdot \rho \cdot N^3 \cdot \delta^2}{V_L}$$

(5)

where $P/V_L$ is the power input per volume [W m$^{-3}$] and $V_L$ is the filling volume [m$^3$]. $P/V_L$ increases with the tip speed and decrease with the scale. For example $P/V_L$ increases for the CultiBag STR 50 L from 4 W m$^{-3}$ at a tip speed of 0.6 m s$^{-1}$ to 97 W m$^{-3}$ at $u_{tip} = 1.8$ m s$^{-1}$. $P/V_L$ is for the CultiBag STR 2000 L three times lower compared to the CultiBag STR 50 L at the same tip speeds. As a consequence, to achieve the same $P/V_L$ in larger scales, the tip speed has to be increased.

Shear forces correlate directly with $P/V_L$ because the generation of micro eddies is influenced. If these eddies are of the same magnitude like the diameter of the cultivated organisms, the cell walls can be disrupted [14]. The size of the eddies can be calculated with the kinematic viscosity and $P/V_L$ (see Eq. (6)).

$$I = \left( \frac{\nu^3}{P/V_L} \right)$$

(6)

where $I$ is the diameter of the eddies [m] and $\nu$ is the kinematic viscosity [m$^2$ s$^{-1}$] ($\nu$ of water was assumed). For the $P/V_L$ described above, an eddy size between 56 and 178 $\mu$m is achieved. Mammalian cells like CHO have a diameter of 10–20 $\mu$m [26] and consequently cell damage can be excluded.

For the verification of the mixing efficiency, the mixing times were determined as a function of the tip speed (see Fig. 2B). It is obvious that the mixing time decreased by increasing tip speed resulting from the higher $P/V_L$ and higher mixing times were determined for increasing scales. The CultiBag STR 50 L had at $u_{tip} = 0.6$ m s$^{-1}$, a mixing time of 19 s and at $u_{tip} = 1.8$ m s$^{-1}$ of 8 s. The lowest mixing time which could be achieved for the CultiBag STR 2000 L was 20 s.

Figure 2C shows the characteristics of the $k_a$ value for different tip speeds and scales. For the trials the influence of the ring and micro sparger part was investigated. The $k_a$ values increase with tip speed independent of the scale and configuration. This can be explained by the increased $P/V_L$. It was observed that the $k_a$ value is higher for larger scales. The reason is presumably the increased liquid height and the associated longer residence time of the gas bubbles resulting in a more efficient oxygen transfer [12]. This is also indicated by a linear correlation between the filling height and the $k_a$ value. For $u_{tip} = 0.6$ ms$^{-1}$ and e.g. ring sparger aeration a dependency of $k_a = 0.077h_i$ is given ($R^2 = 0.99$).

Volumetric mass transfer coefficients of 23 h$^{-1}$ can be achieved for the CultiBag STR 2000 L using ring sparger aeration. Due to the smaller gas bubbles, the $k_a$ achieved by the aeration with micro sparger part is higher and the $k_a$ values are approximately doubled. The results demonstrate that oxygen limitations can be excluded during a process scale-up because the oxygen transfer rate is higher for larger scales.

### Table 2. Alterations of the process engineering parameters for the scale-up from the CultiBag STR 50 to the CultiBag STR 2000. The initial CHO process in the CultiBag STR 50 was performed at a stirrer speed of 150 rpm [27].

| Process engineering parameters | N [rpm] | $u_{tip}$ [m/s] | $Re$ [-] | $P/V_L$ [W/m$^3$] |
|-------------------------------|--------|----------------|--------|-----------------|
| CultiBag STR 50               | 150    | 1.1            | 49,420 | 22.3            |
| Equal N for CultiBag STR 2000 | 150    | 3.9            | 605,406| 293.1           |
| Equal $u_{tip}$ for CultiBag STR 2000 | 42 | 1.1 | 171,936 | 6.7 |
| Equal $Re$ for CultiBag STR 2000 | 12 | 0.31 | 49,420 | 0.15 |
| Equal $P/V_L$ for CultiBag STR 2000 | 63 | 1.6 | 254,270 | 22.3 |

#### 4 Concluding remarks

The main application of the presented single-use bioreactor family is the cultivation of mammalian cells. To verify if this bioreactor family guarantees optimal growth conditions for mammalian cells, different process engineering parameters were determined. With a $P/V_L$ below 100 W m$^{-3}$ the bioreactor can be regarded as suitable for mammalian cell lines. The generated micro eddies have a significantly larger diameter than animal cells, and therefore damaging can be excluded. For all scales, mixing times below 30 s can be achieved. This is comparable to stainless steel bioreactors used for cultivation of mammalian cells [10].

A further critical parameter, especially for high cell density cultivations, is the oxygen transfer rate. A modern CHO process with a peak cell density of 27–28 × 10$^6$ cells mL$^{-1}$ had an OUR of 7 mmol (Lh)$^{-1}$ [27]. Therefore, a $k_a > 7$ h$^{-1}$ is needed when pure oxygen aeration is assumed, which is the case for all scales.

Besides the feasibility for mammalian cell line cultivation, care was taken regarding the scalability of the single-use bioreactor family. Regarding the possibility for scale-up and scale-down, the fact that the design directly relates to those of stainless steel bioreactors represents a large advantage. Care was taken that the geometrical properties of the cultivation chamber and impellers are similar for all scales. This similarity is a prerequisite for a successful scale-up/scale-down or process transfer.

To illustrate a possible process transfer a scale-up matrix is given in Table 2. A CHO process, which was performed at a stirrer speed of 150 rpm in the CultiBag STR 50 L, was assumed [27], and the alterations of the process engineering parameters in the CultiBag STR 2000 L are listed. If the stirrer speed is selected as scale-up criterion, the tip speed is increased to 3.86 m s$^{-1}$, which is outside the specifications for mammalian cell cultivations. This can result in damaging of the cells due to shear forces, which is
also evident by $P/V_L$ which is above the proposed values for mammalian cells. When $u_{tip}$ remains constant, the stirrer speed and $P/V_L$ decreases. This results in a decrease of the mixing efficiency and $k_{La}$ value, and consequently limitation can occur.

The same phenomena in an enhanced way can be concluded if $Re$ is kept constant.

For the use of $P/V_L$ as scale-up criterion, the tip speed is slightly increased, and the stirrer speed decreases making cell damage unlikely. An insufficient mixing can be excluded if the process is performed at $u_{tip} = 0.6–1.8$ m/s because the mixing times are below the specifications [10]. Furthermore, oxygen limitations are not possible when the same cultivation process is used because the $k_{La}$ is higher for larger scales. Based on the results, $P/V_L$ is recommended when a scale-up is performed for the CultiBag STR family because the changes of the process engineering parameters are in good agreement with the key process parameters for mammalian cell cultivations.

### Practical application

This study deals with the process engineering characterization of the stirred single-use bioreactor family BIOSTAT® STR from 50–2000 L scale. For the characterization, parameters commonly used as scale-up criteria like the power input per volume, mixing time, and volumetric mass transfer coefficient ($k_{La}$) were measured. Furthermore, the dimensionless Newton number was determined to describe the system properties. These parameters were compared to the demands of modern mammalian cell cultivations, which define the design space. Hence, a conclusion about the suitability for cell culture applications can be made. Such a complete characterization study of a single-use bioreactor family across the scales will therefore significantly facilitate the scale-up and process transfers of processes based on these technologies.

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### Nomenclature

| Symbol | Units | Description |
|--------|-------|-------------|
| $L$ | [m] | Eddy diameter |
| $M_t$ | [Nm] | Torque |
| $N$ | [s$^{-1}$] | Stirrer speed |
| $Ne$ | [–] | Newton number |
| OUR | [mol(Lh)$^{-1}$] | Oxygen uptake rate |
| OTR | [mol(Lh)$^{-1}$] | Oxygen transfer rate |
| $pO_2$ | [%] | Oxygen partial pressure |
| $P/V_L$ | [W m$^{-3}$] | Power input per volume |
| $Re$ | [–] | Reynolds number |
| $u_{tip}$ | [m s$^{-1}$] | Tip speed |
| $V_L$ | [L] | Filling volume |
| $X$ | [cells mL$^{-1}$] | Cell density |
| $Y_{O2}$ | [cells:mmolO$2$] | Oxygen yield coefficient |
| $Δz$ | [m] | Distance between impellers |
| $μ$ | [h$^{-1}$] | Specific growth rate |
| $N$ | [m$^3$s$^{-1}$] | Kinematic viscosity |
| $P$ | [kg m$^{-3}$] | Density |

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