Chapter 5

Development, Engineering and Biological Characterization of Stirred Tank Bioreactors

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Abstract

Stirred tank bioreactors are still the predominant cultivation systems in large scale biopharmaceutical production. Today, several manufacturers provide both reusable and single-use systems, whereas the broad variety of designs and properties lead to deviations in biological performance. Although the methods for bioreactor characterization are well established, varying experimental conditions and procedures can result in significantly different outcomes. In order to guarantee a reliable comparison and evaluation of different single-use and reusable bioreactor types, standardized methods for their characterization are needed. Equally important is the biological capability of bioreactors, which must be accessed by standardized cultivation procedures of industrially relevant organisms (bacteria, yeasts as well as mammalian and animal cell cultures). In addition, the implementation of well-defined uniform procedures for biological and engineering characterization during the development phase can support a fast assessment of the suitability of a bioreactor system. Based on stirred bioreactors, we describe the aspects of the engineering characterization in order to discuss further the biological characterization as a valuable complement. Finally, a case study is presented.

Keywords: stirred bioreactor, characterization, mixing time, power input, volumetric mass transfer coefficient, development, cultivation system

1. Introduction

Stirred bioreactor systems have been used on a large scale since the beginning of antibiotics and insulin production, and are indispensable in biopharmaceutical production today [1]. They are the most frequently used bioreactor systems as they are suitable for various

expression systems, currently using predominantly recombinant *Escherichia coli* (*E. coli*) strains or Chinese hamster ovary (CHO) cell lines [2–7].

Stirred bioreactors are available as reusable systems made of steel and glass or as single-use systems in different sizes. Many well-known manufacturers offer standard stainless steel systems with volumes from 2 to 1000 L, whereby larger systems with several cubic meters are also available according to customer specifications. The smaller scale glass bioreactors are used in research and process development [8]. The single-use systems, depending on their size, are either available as flexible bags or rigid vessels. They have become increasingly established in recent years and have found their way into biopharmaceutical productions with volumes of up to 2000 L. Eibl et al. [9] gives an overview of the currently available single-use systems.

In addition to the economic reasons for choosing one of the many reusable or single-use systems, they have to meet the requirements of the desired fermentation process. The design and equipment of stirred bioreactors differ in terms of their performance. The efficiency of the bioreactor is described with the help of process engineering parameters [10, 11]. Therefore, the mixing time θ_m of the system, the volumetric mass transfer coefficient $k_L a$ and the specific power input P/V are determined. This enables a comparison of different bioreactor types and the definition of suitable process parameters to achieve the desired product quality and quantity [12].

A new approach based on process engineering characterization is the biological characterization. This may be a standardized *E. coli* model process enabling a reveal in the performance limits of the bioreactor system.

It will be shown that process engineering characterization in combination with biological characterization is a simple standardized approach, which is not only necessary for the evaluation of existing bioreactor types, but also makes a valuable contribution during the development phase of new systems.

2. Theoretical background

The bioreactors used for the cultivation of microorganisms, mammalian and animal cells differ from reactors in the chemical industry in their aspect ratio (H/D). While H/D ratios of 1:1 occur in chemical production, these are usually 2:1 for bioreactors for cell cultures and 3:1 for microorganisms. The background to this is the longer residence time of oxygen or process air introduced into the system near the reactor bottom and the better temperature control due to the larger ratio of surface to volume [6, 10, 13]. However, with increasing reactor size, H/D ratios of up to 5:1 also occur [14]. For the cylindrical bioreactor vessel, the shape of the upper and lower end elements is also crucial. Curved heads with geometries from a hemisphere to a flat plate are used, whereby the dished head is the most common element. The reasons for this are the higher durability compared to planar end elements, and the geometry-related lower overall height compared to hemispherical elements. By avoiding dead zones and edges, cleaning of the system (hygienic design) and mixing is also favored. The upper end is usually a flat lid, which facilitates accessibility for the installation of probes or correction agents and feed [15, 16].

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2.1. Agitation

Besides the vessel geometry, the impeller is the central element of the bioreactor. The choice of the right agitator organ has a decisive influence on the success of cultivation, as it prevents local sources and sinks. It is now possible to choose from a variety of different impeller designs, while taking into account the type of microorganism, human or animal cell line to be cultivated. Shear-sensitive cell culture processes are characterized by low energy and low oxygen input ($P/V \approx 5–200\ W \cdot m^{-3}$/$OTR \approx 0.5–8\ mmol\ O_2 \cdot L^{-1} \cdot h^{-1}$) as well as small cooling capacities. Axial flow impellers are often used for this purpose. For most applications with microorganisms, however, especially in the high cell density range (≈ 100 $g \cdot L^{-1}$ dry cell weight), higher specific power inputs and oxygen transfer rates are required ($P/V > 5\ kW \cdot m^{-3}$ / $OTR \approx 300–500\ mmol\ O_2 \cdot L^{-1} \cdot h^{-1}$). For this purpose, radial flow impellers are used. Higher energy inputs lead to an improved gas dispersion and thus to higher oxygen transfer rates [6, 7, 10, 17–24]. Zlokarnik [19] and Mirro & Voll [17] provide an overview of the impeller types frequently used, and their field of application for the cultivation of various microbial and animal cell lines. Therefore, the process properties, in particular the mixing time, volumetric mass transfer coefficient and power input in combination with the resulting shear gradient are decisive for the impeller design to be selected [25]. Depending on the application and bioreactor size, multi-stage configurations with combinations of radial and axial flow impellers are also possible.

2.2. Drive

Traditionally, the agitator is driven via a centrally mounted shaft with the aid of a motor located above or below the bioreactor. The feedthrough of the shaft into the bioreactor has to be sealed. In the simplest case, a single-acting mechanical seal reduces the escape of organisms from the bioreactor, but bears the risk of contamination [16]. For reasons of product safety, as well as maintaining a tight containment, double mechanical seals are predominantly used. Two pairs of sliding rings are arranged one behind the other and form an intermediate space through which a barrier fluid flows. The pressurized barrier liquid, which is often sterile condensate, prevents leakage from the fermenter [26]. Magnetic couplings offer an alternative to complex double-acting mechanical seals. The magnetic field transfers the torque from the motor through the closed bioreactor to the impeller. The risk of contamination is further decreased by contactless power transmission [13]. In industrial applications, both free-floating and bearing-supported impellers can be found. Bearing-supported impellers are manufactured by MAVAG AG, Millipore Corporation and ZETA Holding GmbH, among others. The impeller with one part of the magnetic coupling sits on a bearing journal where the second part of the magnetic coupling is also located. The mounting is often done by means of ceramic plain bearings [27–29]. However, friction with insufficient lubrication may result in attrition of the material [30]. The levitation technology is used, for example, by Sartorius AG and Pall AG for mixing systems. Only the impeller with one part of the magnetic coupling is located in the vessel. The magnetic field applied causes the impeller to lift off the bottom of the container. This simple type of drive does not require a bearing, and is therefore ideally suited for use in single-use systems, whereas radially acting forces are difficult to absorb [31, 32]. As shown in our case study (see Section 3.1), the levitation technology is also suitable for new stirred bioreactors.

2.3. Characterization according to parametric and experimental approaches

Due to the large number of bioreactors available and their different process engineering properties, the choice of the right system for the requirements of a desired and successful process is decisive. The process engineering characterization allows the comparison of different systems and supports process optimization and scale-up strategies by using parametric as well as experimental approaches [11, 12]. Therefore in January 2016, DECHEMA issued a recommendation with standardized methods for obtaining reliable experimental data, which can be applied to both reusable and single-use bioreactor systems [33].

The dimensionless Reynolds number describes the ratio of inertial to viscous forces in a flow and describes it as laminar, transient or turbulent (Re_{crit} 1–10·10^4) [34, 35]. For stirred bioreactor systems, the Reynolds number can be determined parametrically as a function of the impeller speed N, the impeller diameter d, the density ρ and the viscosity η according to Eq. (1).

$$Re = \frac{\rho \cdot N \cdot d^2}{\eta} \tag{1}$$

Another parameter is the maximum fluid velocity (u_{max}), which usually corresponds to the tip speed u_{tip} (Eq. (2)).

$$u_{tip} = \pi \cdot d \cdot N \tag{2}$$

In order to avoid sources and sinks in the bioreactor, a homogeneous distribution of all components is required. A benchmark of homogeneity is the mixing quality, which is regarded as adequate at 95% [36]. The mixing time θ_m defines the time required after adding a disturbance variable to the system (e.g. change in temperature, concentration, conductivity or density) to achieve the required mixing quality [12, 35, 36]. Eq. (3) applies in completely turbulent flows, in which case the mixing number c_H is calculated according to Eq. (4).

$$\theta_m \propto (P/V)^{-1/3} \tag{3}$$

$$c_H = \theta_m \cdot N = \text{const.} \tag{4}$$

One of the most important parameters is the specific power input P/V, as this is responsible for maintaining sufficient mixing and mass transfer. There are several methods for determining the power input. The most common is the direct torque measurement [12, 37]. For the calculation according to Eq. (5), the effective impeller torque (difference between the torque when stirring in liquid M and the dead torque in air M_d) will be measured by means of a torque sensor. If a DC motor with a known motor torque constant K_t is used, it is also possible to determine the respective torque by measuring the required current I and using Eq. (6) [38].

$$P/V = \frac{(M - M_d) \cdot 2 \cdot \pi \cdot N}{V} \tag{5}$$

$$M = K_t \cdot I \tag{6}$$

Oxygen supply is essential for aerobic cultivation processes. This is ensured by the use of spargers, gassing via membranes or the fluid surface [21, 39]. The oxygen transition is defined by the oxygen transfer rate (*OTR*) and depends on the mixing efficiency, the power input, the gassing rate and the fluid properties [40, 41]. It results from the product of the mass transfer coefficient k_L and the volume-specific interface area a as well as the oxygen concentration difference $C^*_{O_2} - C_{O_2}$ as the driving force (Eq. (7)).

$$\frac{d\,C_{O_2}}{dt} = OTR = k_L a \cdot \left(C^*_{O_2} - C_{O_2}\right) \tag{7}$$

2.4. Characterization by biological approaches

Biological characterization focuses on the evaluation and comparison of bioreactor systems with respect to their biological performance. With the help of a model organism, it should be possible to make an exact prediction of the suitability of a bioreactor system for a desired purpose with a standardized cultivation procedure [42]. For example, two biological test procedures with respiratory yeast and mycelium-forming fungi were developed by Adler and Fiechter [43] and Wagner [44], since the physical characterization often only provides information about optimal bioreactor design conditions and information for improved scale transfer. For this reason, DECHEMA's *'Single-use technology in biopharmaceutical production'* working group is currently working on a new standardized procedure for the biological characterization of classical stirred bioreactors and single-use systems using batch and fed-batch cultivations in addition to the recommendation for process engineering characterization. *Escherichia coli* W3110 is used as a model organism. This is a subspecies of the *E. coli* K12 strain, which is one of the most frequently used and best characterized microorganisms. The suitability of *E. coli* as a model organism can be explained by its high availability, short generation time and extensively investigated growth behavior as well as its high relevance in the biopharmaceutical industry [20, 45, 46].

3. Case study

In this case study, the methodical procedures described above are used to develop a bearing-free magnetically driven 2 L benchtop bioreactor system, which is based on Levitronix's freely levitating impeller technology.

3.1. Bioreactor and setup

The use of a magnetic drive without bearings enables the establishment of a seal-free, contactless and magnetically mounted bottom impeller, which offers an almost unlimited speed range and a minimized risk of contamination (**Figure 1**).

The impeller levitating in the bioreactor at the bottom creates a constant gap, which is made possible by the passive stabilization of the stirring element by a constantly changing magnetic field [47, 48]. For design reasons, a flat end element was chosen for the bottom, into which the BPS-i30 and BPS-i100 drives from Levitronix GmbH were introduced for the investigations. A glass cylinder with a diameter of 124.5 mm and a planar lid with nozzles for probes and the

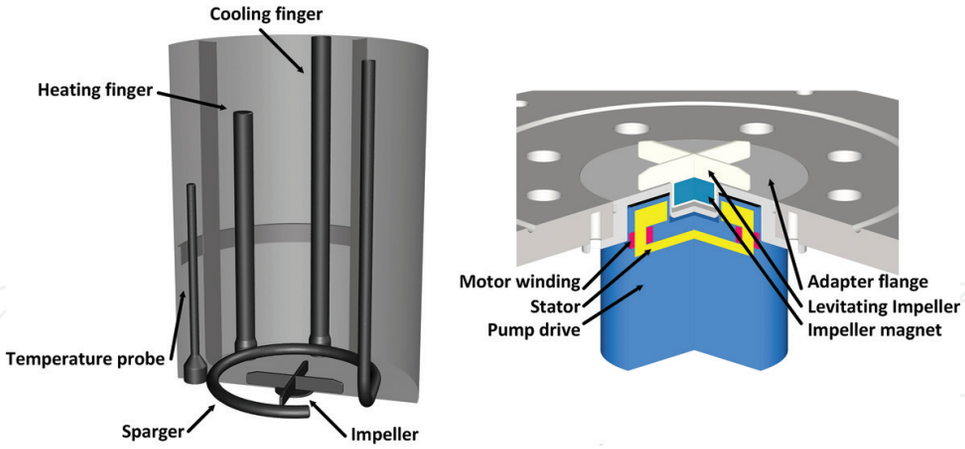

Figure 1. Setup and design of 2 L vessel (left) and construction of the designed impeller unit in the bioreactor bottom plate (right).

possibility of adding correction agents and feed solutions was mounted on top of it. The impellers used for the BPS-i30 drive are the geometries shown in **Figure 2** with diameters of 20, 30, 40 and 50 mm and, based on this, 40, 50, 60 and 74 mm for the more powerful BPS-i100 drive. The oxygen input was made possible by means of a ring sparger with holes facing upwards. The temperature was controlled by using an electric heating and a water-flow cooling finger.

3.2. Process engineering characterization

All process engineering parameters to be investigated were determined by means of design of experiments, and the experimental data were evaluated using MODDE 10.1 (Umetrics, Sweden).

3.2.1. Power input

The specific power input (non-gassed conditions) was determined with water at a constant temperature of 25°C, and a maximum working volume according to Ref. [33]. Because of the constructive conditions of the vessel and motor geometry, the sensor method for determining the torque was not applicable. Due to the known motor constants K_t with 1.13 and 2.0 Ncm·A^{-1} (BPS-i30/BPS-i100), the torque can be recalculated with the desired current for agitation using Eqs. (5) and (6). Likewise, the examined torque of the empty vessel (dead weight torque) was subtracted from the measured torque of the filled vessel.

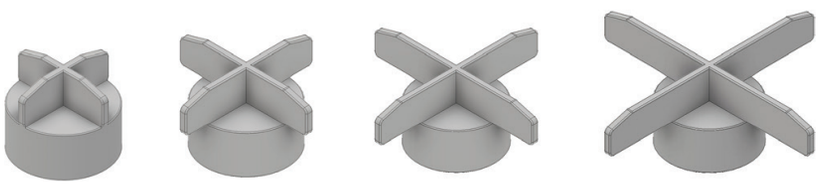

Figure 2. Magnetic impeller with increasing blade diameter.

Additionally, the torque was determined by numerical simulations (computational fluid dynamics (CFD)). Based on the predicted fluid flow, the power inputs of the impellers were obtained from the torque acting on the impeller and the shaft. Therefore, the fluid flow inside the bioreactor equipped with the different impellers was modeled using the finite volume solver ANSYS Fluent (ANSYS Inc., Version 16.2, USA) by using the realizable k-ϵ turbulence model for water at 25°C [49]. The vessel walls and the impeller were treated as non-slip boundaries with standard wall functions. The axial velocity at the fluid surface was set to zero. All equations were discretized using the first-order upwind scheme and the COUPLED algorithm was chosen for pressure-velocity coupling. The fluid domain was discretized by an unstructured mesh consisting of about 8×10^6 to 11×10^6 tetrahedrons.

3.2.2. Mixing time

The mixing times were examined by the decolorization method (iodometry) at maximum working volume according to [33]. Therefore, the bioreactor was filled with water and 2 $mL\cdot L^{-1}$ iodine potassium iodide solution (potassium iodide 40 $g\cdot L^{-1}$, iodine 20 $g\cdot L^{-1}$) and 5 $mL\cdot L^{-1}$ starch solution (1% w/v) were added under agitation at a constant temperature of 25°C. After ensuring a completely homogeneous chemical solution and a quasi-stationary fluid flow pattern, 4 $mL\cdot L^{-1}$ sodium thiosulfate solution were added and the time was measured until the color change from dark blue to colorless was achieved.

3.2.3. Volumetric mass transfer coefficient

The k_La values were determined by the gassing-out method with phosphate-buffered saline (PBS) at 37°C for gassing rates between 0.5 and 2 vvm with a maximum working volume according to Ref. [33]. An OIM-PSt3 probe in combination with a prototype sensor (OEC-PSt3-UF) without protection membrane (PreSens GmbH, Germany, sensor response time 4 s) was used for measurement of dissolved oxygen *DO*. For a measurement, a quasi-stationary fluid flow pattern was ensured and the dissolved oxygen in the PBS in the bioreactor was eliminated by introducing nitrogen. Afterwards, the data acquisition was started, the nitrogen supply stopped, the process air supply set to the desired aeration rate and the aeration started. The measurement was completed when a saturated oxygen concentration had been reached, indicated by a stable *DO* value of 100%. The evaluation and calculation of the k_La value according to Meusel et al. [33] was done for a *DO* saturation rate between 10 and 90%.

3.3. Biological characterization

Based on the results of the process engineering characterization, the process parameters for the *E. coli* cultivations were set to values resulting in the highest k_La values by maintaining a constant gassing and tip speed of 2 vvm (process air) and 7000 and 2900 rpm (20 mm – BPS-i30/40 mm – BPS-i100). Therefore, a cryopreserved culture of *E. coli* W3110 thyA36 supO λ- (ATCC: 27325) was incubated for 24 h at 37°C on lysogenic broth (LB) agar. Pre-culture 1 (1-L baffled shake flask, Corning, USA) was inoculated in 200 mL LB medium with one colony from the petri dish and incubated for 8 h at 37°C in a shaking incubator.

The second pre-culture was also incubated in a 1-L shake flask with 150 mL medium at an initial optical density at 600 nm (*OD600*) of 0.1 for 16 h at 30°C in a shaker. The medium of the second pre- and main culture correspond to the composition described by Biener et al. [50] with concentrations ($g \cdot L^{-1}$): glucose (pre-culture 2: 10, batch: 80 and fed-batch: 20), $MgSO_4 \cdot 7H_2O$ (0.54), $(NH_4)_2$H-citrat (1.01), Na_2SO_4 (2.02), $(NH_4)_2SO_4$ (4.03), NH_4Cl (0.51), K_2HPO_4 (15.17), $NaH_2PO_4 \cdot H_2O$ (3.55), $CaCl_2 \cdot 2H_2O$ ($2.25\ 10^{-3}$), $ZnSO_4 \cdot 7H_2O$ ($0.81 \cdot 10^{-3}$), $MnSO_4 \cdot H_2O$ ($0.45 \cdot 10^{-3}$), Na_2-EDTA$\cdot 2H_2O$ ($45 \cdot 10^{-3}$), $FeCl_3 \cdot 6H_2O$ ($37.6 \cdot 10^{-3}$), $CuSO_4 \cdot 5H_2O$ ($0.72 \cdot 10^{-3}$) and $CoCl_2 \cdot 6H_2O$ ($0.81 \cdot 10^{-3}$).

For the fed-batch process, a concentrated feed with a high glucose concentration was added into the bioreactor after the initial glucose had depleted. To maintain a constant growth rate, an exponential profile was used [51, 52]. The feed medium was formulated with the following concentrations ($g \cdot L^{-1}$): glucose (655.3), $MgSO_4 \cdot 7H_2O$ (16.02), $CaCl_2 \cdot 2H_2O$ ($43 \cdot 10^{-3}$), $ZnSO_4 \cdot 7H_2O$ ($15 \cdot 10^{-3}$), $MnSO_4 \cdot H_2O$ ($85 \cdot 10^{-3}$), Na_2-EDTA$\cdot 2H_2O$ ($85 \cdot 10^{-3}$), $FeCl_3 \cdot 6H_2O$ ($71 \cdot 10^{-3}$), $CuSO_4 \cdot 5H_2O$ ($14 \cdot 10^{-3}$) and $CoCl_2 \cdot 6H_2O$ ($15 \cdot 10^{-3}$). In contrast to the batch process, the *DO* was regulated by the substitution of process air with pure oxygen.

The batch fermentations had a starting volume of 2 L, whereas the fed-batch started with 1.3 L to ensure an appropriate covering of all sensors and heating and cooling devices. After reaching an *OD600* of 150, a second feed with $(NH_4)_2HPO_4$ was immediately added to a concentration of 4 $g \cdot L^{-1}$ to the bioreactor. The pH was regulated automatically by adding 20% (w/w) ammonia solution and foaming was controlled by the addition of 1:5 diluted Antifoam 204 (Sigma-Aldrich). Cultivations were terminated at $DO \approx 0\%$.

3.4. Results

In the run-up to the experimental investigations, the new bioreactor system with the magnetic drive was numerically examined with regard to the process engineering parameters regarding its suitability for the cultivation of microorganisms. As expected, the specific power input shows an exponential increase with rising rotational speed (**Figure 3**). However, it also becomes apparent that with the weaker BPS-i30 drive, only the impellers with a diameter of 20 and 30 mm are in the range of microbial requirements with $P/V > 5\ kW \cdot m^{-3}$ and $u_{tip} > 1.5\ m \cdot s^{-1}$ [10]. With the more powerful BPS-i100 drive, this is the case for all impellers.

The experimentally determined specific power inputs show only minor deviations compared to the numerically determined values, whereby larger differences result in increasing rotational speed. The largest deviation for the 20 mm impeller at 7000 rpm ($u_{tip} = 7.3\ m \cdot s^{-1}$) is around $3.5\ kW \cdot m^{-3}$. This circumstance can result from the radial forces not taken into account in the simulations, which increase with rising rotational speed due to possible impeller imbalances. Therefore, in the present case, the power inputs are estimated as slightly too low with the help of the CFD.

With regard to the mixing time, **Figure 4** shows that all impellers with a specific power input of $1\ kW \cdot m^{-3}$ and above meet a required mixing time of $\theta_m < 10$ s [53]. A turbulent flow regime with $Re > Re_{crit}$ is also present from this value on (see **Table 1**). The slope of the regressions of the mixing times is between −0.19 and − 0.4, which is close to the theoretical value of −0.33 (see Eq. (3)). Thus, mixing numbers in the range of 65–183 result according to Eq. (4).

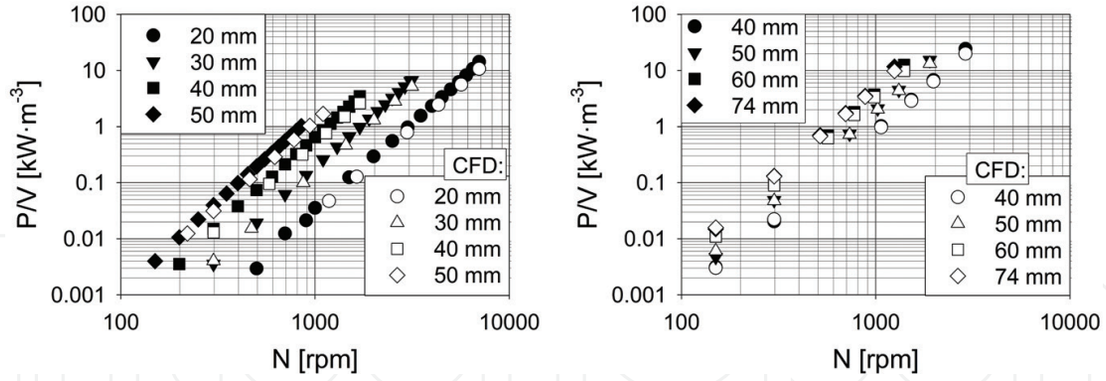

Figure 3. Double logarithmic representation of the numerically and experimentally determined P/V values for all used impellers as a function of N (left: BPS-i30/right: BPS-i100). P/V, specific power input; N, impeller speed.

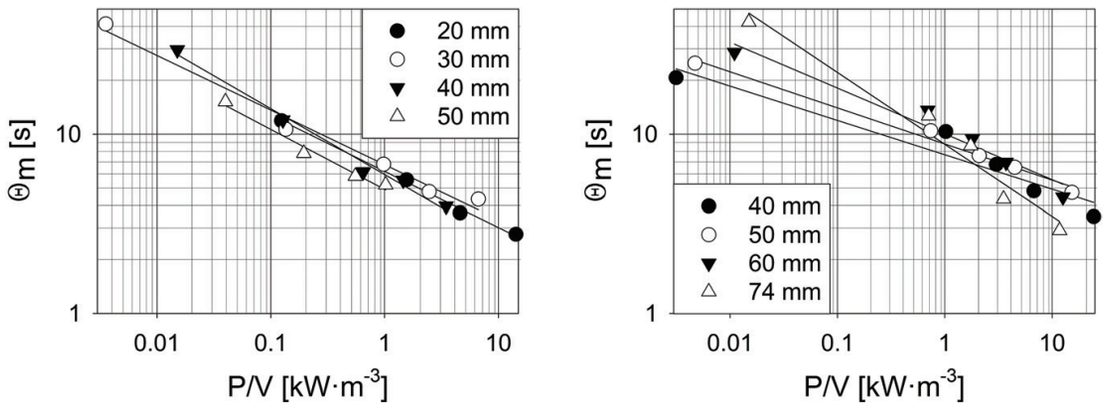

Figure 4. Double logarithmic representation of the experimentally determined θ_m values for all impellers used as a function of P/V (left: BPS-i30/right: BPS-i100). θ_m, mixing time; P/V, specific power input.

The experimentally determined $k_L a$ values for the two impellers 20 and 40 mm (BPS-i30/BPS-i100) are shown in **Figure 5**. The values explain the highest volumetric mass transfer coefficients found at the highest possible rotational speeds of 7000 and 2900 rpm (14.3 kW·m^{-3}/24.4 kW·m^{-3}) (see **Table 1**). The strong influence of the impeller speed becomes clear, as at low rotational speeds most of the gas reaches the fluid surface with very low dispersion due to the insurmountable buoyancy force of the introduced gas. This effect is often also referred to as "flooding" [35, 54], whereby in the present bioreactor design, the impeller running on the bottom does not pull down the bubbles emerged by the higher lying sparger, and is therefore not able to disperse them sufficiently due to radially acting forces. Compared with experiments on the 30 and 100 L scale, the value determined with the 20 mm impeller is three times smaller [42].

Based on the process engineering investigations, the cultivation for biological characterization was carried out. The impellers 20 and 40 mm (BPS-i30/BPS-i100) used demonstrated identical behavior during the process up to hour 6 with respect to biomass, glucose and acetate concentrations as well as in the *DO* profile (see **Figure 6**). This can also be seen in the growth rates, which after approximately 3 h reach a value of $\mu \approx 0.4$ h^{-1}. Due to the high glucose concentration, which inhibits growth with values of more than 50 g·L^{-1} [24], the maximum growth rate

Impeller diameter [mm]	BPS-i30		BPS-i100		*Re* at *P/V* of 1 W·m^{-3}
	N_{max} [rpm]	k_La [h^{-1}]	N_{max} [rpm]	k_La [h^{-1}]	
20	7000	206	—	—	≈ 19,900
30	3150	142	—	—	≈ 25,400
40	1700	172	2900	694	≈ 30,000
50	1100	170	1900	657	≈ 34,000
60	—	—	1400	560	≈ 37,000
74	—	—	1250	591	≈ 52,200

k_La, volumetric mass transfer coefficient; N_{max}, maximum impeller speed; Re, Reynolds number; P/V, specific power input.

Table 1. Representation of the highest experimentally determined k_La values (n = 5) for both drive systems used with the corresponding maximum impeller speeds and Reynolds numbers of the different impellers at a P/V of 1 W·m^{-3}.

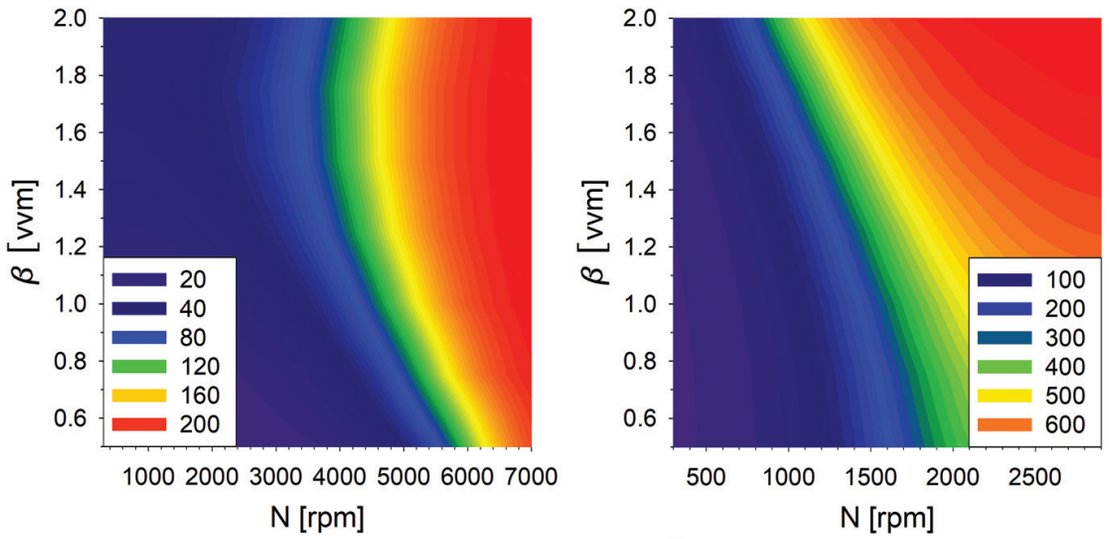

Figure 5. Representation of the experimentally determined k_La values (n = 5) as a function of N and β (left: BPS-i30, 20 mm, $R^2 = 0.76$/right: BPS-i100, 40 mm, $R^2 = 0.96$). k_La, volumetric mass transfer coefficient; N, impeller speed; β, gassing rate.

of 0.61 h^{-1} for this *E. coli* strain is not reached [45]. From hour 6, the use of the smaller impeller shows a successive decrease in the growth rate and a faster increase in acetate. This effect is ascribed to the lower oxygen content in the medium and a so-called salt effect by the increased addition of base, which could be observed during fermentation [24, 55–57]. This finally leads to a decrease in growth and glucose consumption, as acetate concentrations from 2 g·L^{-1} can have an inhibiting effect [58]. Thus, when using the smaller impeller, an *OD600* of 35.4 ± 0.1 or a dry cell weight DCW of 13.0 ± 1.8 g·L^{-1} is achieved. When using the larger impeller in combination with a more powerful drive and a resulting higher k_La value, an optical density of 65.3 ± 3.4 (21.6 ± 1.9 g·L^{-1} *DCW*) is reached.

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In reusable pilot bioreactors for microbial applications with 30 and 100 L previously tested, only optical densities of 39 ± 5 at higher oxygen transport rates of 735 and 745 h^{-1} were obtained [42]. This fact can only be attributed to the considerably shorter mixing times of 2.77 and 3.47 s (20 mm/40 mm) in the 2 L scale shown here. These were determined in the mentioned larger systems with 8–10 s. The additional oxygen uptake rate *OUR* determined during cultivation with the 40 mm impeller shows a maximum of 256 mmol·L^{-1}·h^{-1} (see **Figure 6**).

The results of the fed-batch cultivations with the BPS-i100 system presented in **Figure 7** show an expected higher biomass concentration with an *OD600* of 262.4 ± 0.3, which corresponds to a *DCW* of 86.6 ± 1.9 g·L^{-1}. Due to the lower glucose concentration in the starting medium, a growth rate of >0.6 h^{-1} could be achieved after 3 h. With a further steady decrease in glucose concentration, the growth rate drops to values between 0.3 and 0.4 h^{-1}, which were controlled by exponential feed addition. The feed was started between hours 6 and 7 since the glucose in the medium was depleted at this time, which is also expressed by the corresponding *DO* peak. To keep the oxygen content constant at 40% during the further cultivation, the process air was gradually substituted with oxygen from hour 7 on. From hour 12.5 on, 2 vvm pure oxygen was required. Interestingly, after a cultivation time of 11.5 h, there were signs of insufficient cooling of the system, as the bioreactor temperature rose steadily to a maximum of 40.7°C by the end of the cultivation.

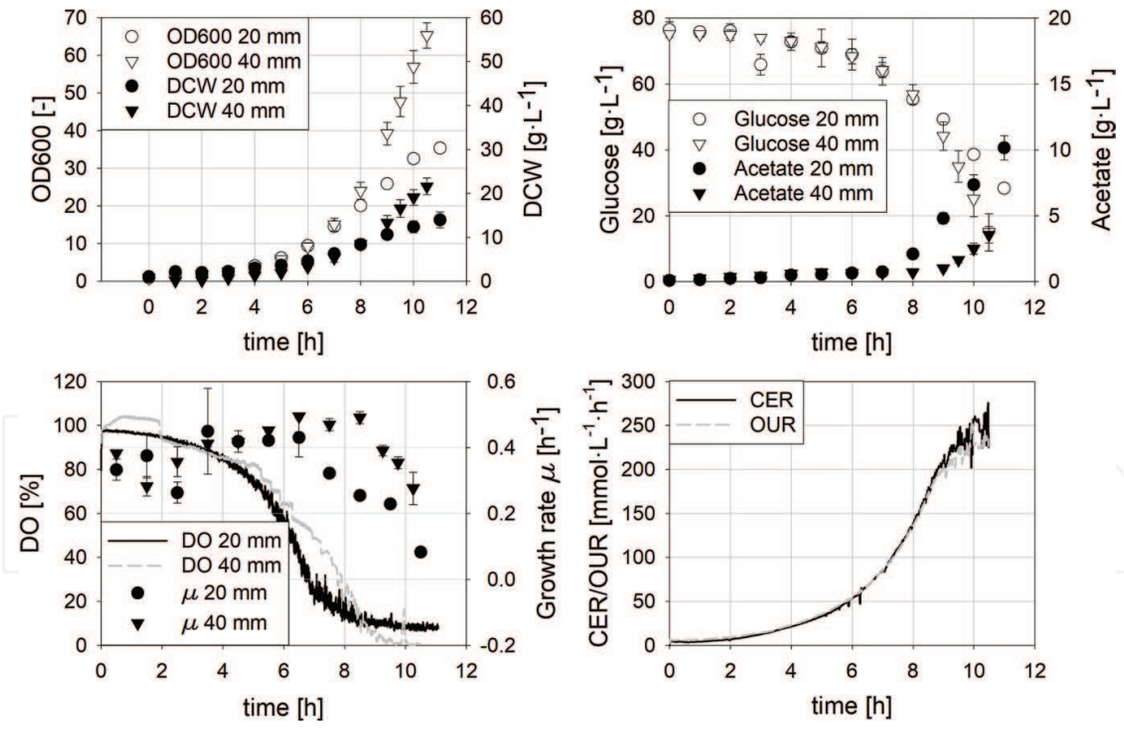

Figure 6. Determined biomass profiles for OD600 and DCW (top left), glucose and acetate concentrations (top right) and DO profiles as well as the growth rates (bottom left) over the process time from the batch cultures (n = 2) when using impellers with a diameter of 20 and 40 mm (BPS-i30/BPS-i100). The lower right diagram shows the OUR and CER determined in the exhaust air over the cultivation time for the latter bioreactor configuration (40 mm). OD600, optical density; DCW, dry cell weight; DO, dissolved oxygen; μ, growth rate; OUR, oxygen uptake rate; CER, carbon dioxide formation rate.

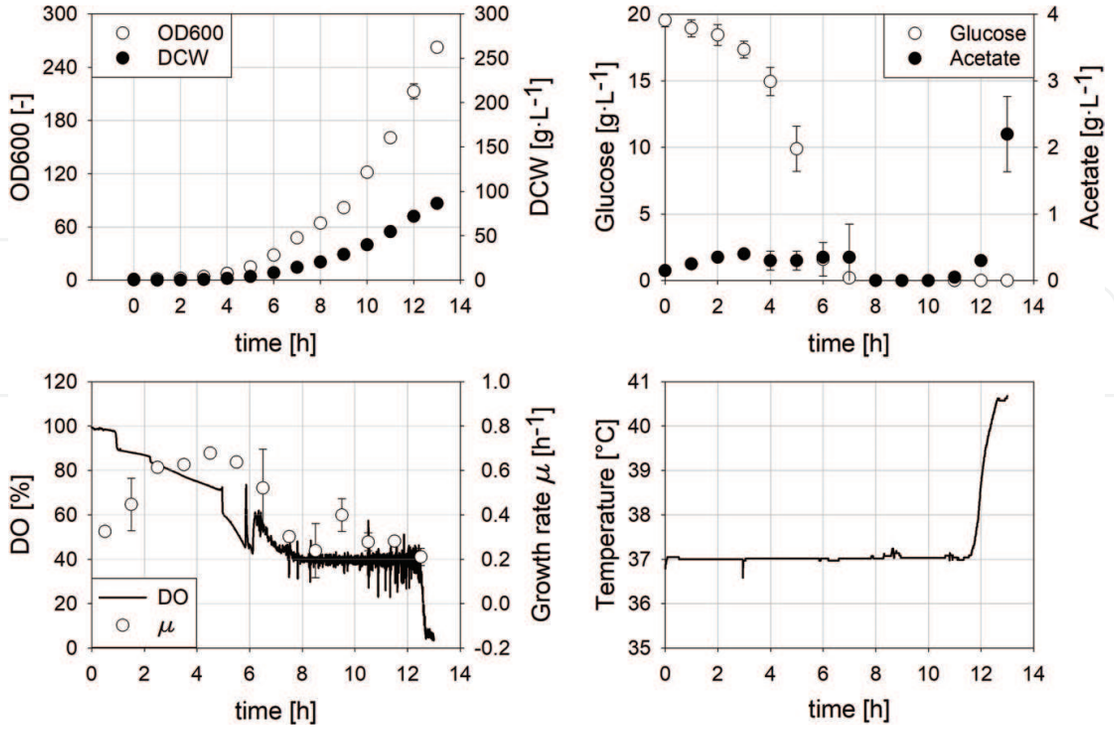

Figure 7. Certain biomass profiles for OD600 and DCW (top left), glucose and acetate concentrations (top right) and DO profile as well as the growth rates (bottom left) over the process time from the fed-batch cultures (n = 2) using the impeller with a diameter of and 40 mm (BPS-i100). The lower right diagram shows the temperature curve in the bioreactor over the cultivation time. OD600, optical density; DCW, dry cell weight; DO, dissolved oxygen; μ, growth rate.

4. Discussion

The determined process engineering parameters demonstrate that the newly developed bioreactor system can be used for the cultivation of shear-sensitive animal cells as well as microbial cells up to the high cell density range. In this way, the process engineering parameters for all impellers with specific power inputs of up to 500 $W \cdot m^{-3}$ show suitability for animal cell culture processes. The $k_L a$ values are within a range of 20 and 100 h^{-1}, and the resulting mixing times are ≤10 s. These are also typical values described in the literature for animal cell culture bioreactors, such as the Finesse SmartGlass bioreactor [59], the Mobius CellReady 3 L bioreactor [60] or the reusable BIOSTAT UniVessel and BIOSTAT UniVessel SU [11]. A first proof of concept batch cultivation with CHO XM 111-10 suspension cells (SEAP secreting cell line, the secreted alkaline phosphatase of the placenta, CCOS No. 837) in a chemically defined minimal medium using the BPS-i30 drive resulted in middle cell densities of up to $4 \cdot 10^6$ cells·mL^{-1}(data not shown), which are in the same order of magnitude as in the previously mentioned bioreactors, and in the BioBlu 0.3c as well as BIOSTAT® A [61–66]. Taking into account the shear stress acting on the cells, theoretically higher cell densities may also be achievable, since the performance limits of the system have not been reached, and can be complemented by suitable feeding strategies.

In addition, due to the simple design of the system, with the elimination of seals and bearings, by using a magnetic drive with a freely levitating impeller the bioreactor is almost maintenance-free and the risk of contamination is reduced. It also facilitates cleaning and the

easy and fast change of impeller types for different applications. Furthermore, the bioreactor system offers a high turn down ratio allowing an easy-to-scale process.

While conventional microbial processes can also be implemented with the less powerful BPS-i30 drive, the BPS-i100 in combination with the 40 mm impeller is recommended for high cell density microbial processes. This is demonstrated by nearly a doubling of the optical densities in the *E. coli* batch cultivations, and the results of the fed-batch procedure.

So it comes as no surprise that the specific power inputs obtained in the case study with the new bioreactor provide comparable results to other microbial bioreactor systems described in the literature. For bioreactors with sizes of 1–100 L, these are between 2.5 and 20 $kW \cdot m^{-3}$ [42, 67–69], whereby a minimum requirement of >5 $kW \cdot m^{-3}$ is generally assumed [10]. The achieved mixing numbers are partly $c_H > 100$ due to the impeller position at the vessel bottom, which are above the usual values for bioreactors equipped with different impeller types [35]. Mixing times are also below the 10 s recommended for microbial requirements [53], as is also the case in other conventional bioreactors up to pilot scale [42, 70, 71]. However, compared to systems with larger volumes, a possible increase in mixing time of up to 2 min must also be taken into account [10]. According to manufacturers and previously published data, for several bioreactors k_La values between 300 and 745 h^{-1} are reached and are sufficient for microbial processes with a resulting *OTR* from 250 to 500 $mmol \cdot L^{-1} \cdot h^{-1}$ [20, 42, 67, 69, 70, 72–74]. Against this background, only the impellers with the BPS-i100 drive shown in the case study appear to be relevant for microbial industrial processes. The impellers driven on the bioreactor bottom with the weaker BPS-i30 drive are not able to disperse the gas bubbles sufficiently. In conventional bioreactors, this problem is circumvented by the fact that the impellers are located above the aeration organ. So far, the fed-batch cultures have shown, one of the main problems of microbial high cell density cultures is the removal of the heat generated in the system, which means that this either has to be countered with larger heat exchange surfaces or lower coolant temperatures [10]. Nevertheless, with the performed fed-batch model process, a higher cell density (>260) could be achieved as in other microbial systems. These given results of *OD600* values between 100 and 201 in a very similar high-demanding *E. coli* process [67, 70, 72, 74, 75].

5. Conclusion

The combination of DECHEMA's recommendation for process engineering characterization and the *E. coli* standard model process described above provides an easy-to-implement approach for the standardized qualification of existing microbial bioreactor systems, and for those currently under development, as shown for the novel benchtop-scale bioreactor equipped with Levitronix's magnetic drives.

The investigated process engineering parameters allow the estimation of its optimal working areas and limits. In addition, it allows a selection of a suitable impeller design to increase the productivity of biopharmaceutical processes. The impeller with a diameter of 40 mm in combination with the more powerful BPS-i100 drive shows the highest k_La value and a mixing time of <4 s at the highest specific power input of 24.4 $kW \cdot m^{-3}$. In line with expectations, the largest biomass with an optical density of 65.3 in batch mode and 262.4 in fed-batch mode is achieved.

Surprisingly, the use of the smallest impeller with the smaller BPS-i30 drive shows a comparable biomass concentration to bioreactors on a pilot scale despite a very low k_La value for microbial processes. This circumstance is not foreseeable by the sole process engineering characterization, so that the additional use of a biological characterization approach becomes evident. The suitability of the presented developed bioreactor concept for microbial applications could be clearly demonstrated, even if it seems rather unusual in comparison to commercial systems due to its bottom drive without bearings. The complete characterization provides the possibility for an easier transfer to the industrial biopharmaceutical scale. Finally, the currently available bioengineering data of the new developed bioreactor indicate that the bioreactor operated with the BPS-i30 drive can also be used to grow animal cells. More detailed investigations are planned in the future.

Abbreviations

CCOS	culture collection of Switzerland
CFD	computational fluid dynamics
CHO	Chinese hamster ovary cell line
CHO XM 111–10	SEAP secreting cell line
E. coli	*Escherichia coli*
LB	lysogenic broth
PBS	phosphate-buffered saline
SEAP	secreted alkaline phosphatase of the placenta

Nomenclature

C_{O_2}	present oxygen concentration [mmol·L^{-1}]
$C^*_{O_2}$	maximum oxygen concentration [mmol·L^{-1}]
μ	specific growth rate [h^{-1}]
a	phase boundary interface [m^{-1}]
c_H	mixing number [–]
d	impeller diameter [m]
D	vessel diameter [m]
DCW	dry cell weight [g·L^{-1}]
DO	dissolved oxygen [%]
H	vessel height [m]

H/D	ratio of vessel height to diameter [−]
I	current [A]
k_L	mass transfer coefficient [m·h^{-1}]
k_La	volumetric mass transfer coefficient [h^{-1}]
K_t	motor torque constant [N·m·A^{-1}]
M	torque [N·m]
M_d	dead weight torque [N·m]
N	number of impeller revolutions [rps]
$OD600$	optical density at 600 nm [−]
OTR	oxygen transfer rate [mmol·L^{-1}·h^{-1}]
OUR	oxygen uptake rate [mmol·L^{-1}·h^{-1}]
P/V	specific power input [W·m^{-3}]
R^2	regression coefficient [−]
Re	Reynolds number [−]
u_{tip}	tip speed [m·s^{-1}]
V	volume [L]
β	gassing rate [vvm]
η	viscosity [Pa·s]
θ_m	mixing time [s]
π	3.14159 [−]
ρ	density [kg·m^{-3}]

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