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Power input correlation to characterize the hydrodynamics of cylindrical orbitally shaken bioreactors

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ABSTRACT

Disposable cylindrical shaken bioreactors using plastic bags or vessels represent a promising alternative to stainless steel bioreactors, because they are flexible, cost-effective and can be pre-sterilized. Unlike conventional well-established steel bioreactors, however, such disposable bioreactor systems have not yet been precisely characterized. Thus, the aim of this current work is to introduce a new power input correlation as a potential means to characterize the hydrodynamics of these new systems. A set of relevant power input variables was defined and transformed into dimensionless numbers by using the Buckingham's π-Theorem. These numbers were then experimentally varied to quantify the relationship among the numbers. A simple correlation was generated for the power input with seven variables. The application of this new correlation was validated using 200 L and 2000 L orbitally shaken bioreactors. In conclusion, the proposed correlation is a useful tool to predict the power input and hydrodynamics during cell cultivation in cylindrical shaken bioreactors of all scales.

1. Introduction

Disposable bioreactors are increasingly being used to enhance the time to market, flexibility and cost-efficiency of bioprocesses. One of the first successful disposable bioreactor systems was the Wave Bioreactor for mammalian cell cultivation. The first characterization of this bioreactor type with a wave-induced liquid motion for cell cultivation was reported in 1985 [1]. During the past decade, demands for different biological cultures led to other disposable reactor systems [2]. Orbitally shaken bioreactors have grown in importance in biotechnology; in particular, for animal and plant cell cultivation and recombinant protein production [3–6]. These bioreactors have been extensively used on a small scale for clone and media screening, process development and more recently have been shown to be scalable up to 2000 L for mammalian cell cultivation [7].

To optimize culture conditions and to ensure efficient scale-up, the gas transfer, mixing and hydromechanical stress all need to be characterized. Among orbitally shaken bioreactors much effort has been made to investigate these process parameters in conventional conical Erlenmeyer flasks. A model for the prediction of k_La values in Erlenmeyer flasks was proposed by Maier et al. [8]. Correlations for mass transport and ventilation in conical Erlenmeyer flasks were reported in several publications [9,10]. Compared to Erlenmeyer flasks only few publications have focused on the gas transport phenomena in cylindrical orbitally shaken reactors. One study on the gas transfer in these bioreactors has reported k_La values in the range of 1–30 h^{-1} in cylindrical vessels from 50 mL to 2000 L [11]. Another study on mixing in orbitally shaken cylindrical bioreactors reported mixing times of 10–30 s for vessels with volumes of 2–1500 L, and showed that these bioreactors ensure homogeneity for mammalian cell cultures for these scales of operation [12]. However, the operating conditions for sufficient mixing and gas transfer should not damage the cells through excessive hydromechanical stress [13,14]. This latter parameter can be estimated by determining the volumetric power input (P/V_L), defined as the amount of energy required to maintain fluid motion within a vessel in a given period of time [15]. Furthermore P/V_L is a commonly used criterion for bioreactor scale-up, and it directly influences mass transfer and fluid flow in the bioreactor [15]. Studies on the hydromechanical stress in Erlenmeyer flasks have shown that for the same P/V_L, the maximum energy dissipation rate in orbitally shaken flasks is about 10 times lower than in stirred tank reactors [14,16].

2. Objectives

The aim of this work is to introduce a scale- and volume-independent correlation for calculating the volumetric power input for characterizing the hydrodynamics of cylindrical orbitally shaken bioreactor systems. Such bioreactors with volumes ranging

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from 10 L to 2000 L are used for determining and validating the correlation. The final correlation for the volumetric power input is compared to previously published correlations.

3. Theory

3.1. Volumetric power input

Power input can be described as the required power to maintain the motion of a liquid in a bioreactor. The power is transferred to the liquid by a rotating stirrer in a conventional stirred tank reactor or by the reactor wall in an orbitally shaken reactor system. The transferred physical power is first converted into kinetic energy in the form of liquid motion and finally transferred into heat energy due to friction losses in the liquid [17].

A dimensionless correlation for P/V_L in conical Erlenmeyer flasks was already reported by Büchs et al. [18]. A modified Newton number (Ne') was defined to consider the influence of the filling volume on P/V_L in Erlenmeyer flasks [19]:

$$Ne' = \frac{P}{\rho \cdot n^3 \cdot d^4 \cdot V_L^{1/3}} \quad (1)$$

Whether the modified Newton number can be applied to cylindrical vessels is discussed in Section 5. The already published correlation for Erlenmeyer flasks allows a precise calculation of P/V_L for different flask size and operation conditions. However, the application of this dimensionless correlation implies geometrical similarity [20], which is not fulfilled between conical flasks and cylindrical vessels. Thus, the published equation for P/V_L in conical flasks is not directly transferable to cylindrical vessels. However, Raval et al. adapted the power input equation for Erlenmeyer flasks to cylindrical vessels [21]. The following resulting equation was determined with measuring values from 20 L and 50 L vessels:

$$\frac{P}{V_L} = \frac{\rho \cdot n^3 \cdot d^4}{(V_L)^{2/3}} \cdot (15 \cdot Re^{-0.6} + 3.5 \cdot Re^{-0.2}) \quad (2)$$

Kato et al. published a P/V_L correlation that was specified for liquid height equal to the vessel diameter ($h = d$) [22]:

$$\frac{P}{V_L} = 934 \cdot g^{-1.5} \cdot \rho^{0.75} \cdot d^5 \cdot n^{5.75} \cdot \eta^{0.25} \cdot V_L^{-1} \cdot d_0 \quad (3)$$

Even though several correlations have been developed to predict P/V_L in cylindrical shaken reactors for a restricted range of experimental conditions, a scale-independent and volume-independent correlation for P/V_L in cylindrical bioreactors with volumes from 10 L to 2000 L has not yet been published. Thus, a set of relevant power input variables using dimensional analysis was defined in the following section to determine a new power input correlation.

3.2. Dimensional analysis

The theory of dimensional analysis was used to reduce the number of variables during the development of a P/V_L correlation. Eight variables were assumed to influence the P/V_L (see Table 1):

These variables were categorized into the following 5 dimensionless groups by using Buckingham's π-theorem [23]:

Conventional Newton number: $\frac{P}{\rho \cdot n^3 \cdot d^5}$

Reynolds number: $\frac{\rho \cdot n \cdot d^2}{\eta}$

Acceleration ratio: $\frac{n^2 \cdot d}{g}$

Volume number: $\frac{V_L}{d^3}$

Geometric number: $\frac{d_0}{d}$

Table 1
Relevant variables used for a P/V_L correlation.

Variable	Abbreviation	Units
Power	P	$kg\,m^2\,s^{-3}$
Density of the liquid	ρ	$kg\,m^{-3}$
Vessel diameter	d	m
Shaking frequency	n	s^{-1}
Dynamic viscosity	η	$kg\,m^{-1}\,s^{-1}$
Liquid volume	V_L	m^3
Shaking diameter	d_0	m
Gravitational acceleration	g	$m\,s^{-2}$

A power law was used as mathematical correlation among the dimensionless numbers (Eq. (4)):

$$\overbrace{\left(\frac{P}{\rho \cdot n^3 \cdot d^5}\right)}^{Convent.\ Newton\ no.} = C_1 \cdot \overbrace{\left(\frac{\rho \cdot n \cdot d^2}{\eta}\right)^{\alpha}}^{Reynolds\ no.} \cdot \underbrace{\left(\frac{n^2 \cdot d}{g}\right)^{\beta}}_{Acceleration\ ratio} \cdot \underbrace{\left(\frac{V_L}{d^3}\right)^{\gamma}}_{Volume\ no.} \cdot \underbrace{\left(\frac{d_0}{d}\right)^{\delta}}_{Geometric\ no.} \quad (4)$$

The exponents α, β, γ and δ were determined experimentally. One dimensionless number was modified in each experiment, while the others were kept constant. The general approach according to Eq. (4) differs from the specific approach that was used to determine a P/V_L correlation for Erlenmeyer flasks [18,19]. The reason for the application of a more general approach is explained in 5.

4. Materials and methods

4.1. Equipment

Studies about the factors influencing power input in cylindrical orbitally shaken bioreactors were carried out in various vessels (Table 2).

4.2. Torque method for power input determination

For reactor systems with a nominal volume of 10–50 L the transferred power is determined by measuring the required mechanical power for shaking. Mechnical power is generally defined as $P = 2 \cdot \pi \cdot n \cdot M$. The shaking frequency and the measured torque signal are specified with n and M, respectively. A torque sensor (Type ViscoPakt -rheo 110, HiTec Zang, Herzogenrath, Germany) with a range from 0 to 1.1 Nm is integrated in the shaker drive as shown in Fig. 1. Büchs et al. [19] have described a similar setup for power input measurements in Erlenmeyer flasks. Friction losses due to the aerodynamic resistance of the vessel and the friction in the ball bearing of the shaker were considered by reference measurements [19]. During these reference measurements, the liquid was replaced by a solid mass with the same weight as the liquid. The volumetric power input was calculated according to

$$\frac{P}{V_L} = 2 \cdot \pi \cdot n \cdot \frac{M}{V_L} = 2 \cdot \pi \cdot n \cdot \frac{M_{liquid} - M_{solid}}{V_L} \quad (5)$$

whereby M_{liquid} specifies the measured torque signal in the reactor system filled with liquid and M_{solid} in the empty reactor system weighted with a solid mass.

4.3. Temperature method for power input determination

The volumetric power input of the 200 L and 2000 L orbitally shaken bioreactors (Kühner AG, Birsfelden, Switzerland) was