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On the measurement and scaling of mixing time in orbitally shaken bioreactors

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ABSTRACT

Accurate determination of the mixing time in orbitally shaken bioreactors (OSRs) is essential for the optimization of mixing processes and minimization of concentration gradients that can be deleterious to cell cultures. The Dual Indicator System for Mixing Time (DISMT) was employed to measure mixing times in cylindrical and Erlenmeyer flask bioreactors. Various aspects of importance for the acquisition of accurate data from the measurement methodology are discussed, utilizing also comparisons of DISMT and pH probe results obtained in two stirred reactors. The OSR results are juxtaposed with data previously reported in the literature for both cylindrical reactors and Erlenmeyer flasks. The employment of a critical Froude number shows promise for the establishment of a scaling law for mixing time across the various types and sizes of shaken bioreactors.

1. Introduction

Mammalian cell cultures have been widely used for the production of therapeutic proteins and vaccines. Monoclonal antibodies, in particular, represent a class of therapeutics that has shifted the treatment paradigm in the fields of oncology and immunology by improving the quality of life for the patient during cancer treatment [1,2]. Commercial production of these antibodies relies on the development of cost-effective large-scale cultivation methods of genetically engineered mammalian cells. At laboratory scale, cells are usually grown in low shear devices, with orbitally shaken bioreactors (OSRs) being largely employed in the early stages of bioprocess development because they offer an effective solution to screen several conditions in parallel. This low energy demand and the well-defined gas–liquid interface, make orbitally shaken bioreactors a favourable solution that provides a promising environment for mammalian cell cultivation in terms of oxygen transfer and nutrient requirements. Once the process is optimized at small scale, it is then adapted to stirred tank reactors (STRs), which is the type of bioreactor most commonly used at production level. STRs have been thoroughly characterized in the literature (for example, [3–6]).

The differences between the mixing mechanisms occurring at the two scales and geometries represent a challenge for scale-up to commercial manufacture. Such differences may result in inadequate mass transfer characteristics, cell damage and reduced antibody productivity. Studies of animal cell cultures have focused on optimizing the chemical environment and culture media in order to enhance antibody productivity [7,8], but studies of the engineering characteristics relevant to the optimization of bioreactor geometry and operating conditions are still lacking. While growth rates in mammalian cell cultures are slower than those in microbial systems, the need for a well-mixed environment has been acknowledged in terms of the quality of cell suspension and gas supply and removal [9–12]. Recent studies in STRs have shown that shear stresses do not significantly affect cell growth of animal cells [13,14], however there is a growing interest in understanding the effect of shear stress on stem cells and cells for therapy, and the low shear levels present in OSRs could potentially make this device a suitable option for scaling up cell expansion using suspension cultures [15]. From this point of view operating conditions should be carefully selected by comparing the Kolgomorov length scale to

Abbreviations: OSR, orbital shaken reactor; STR, stirred tank reactor; 2D, two-dimensional; 3D, three-dimensional; CFD, computational fluid dynamics; PIV, particle image velocimetry; DISMT, Dual Indicator System for Mixing Time; RGB, red green blue colour model; HSV, hue saturation value colour model.

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a_o	constant
d_i	inner diameter of the cylinder (m)
d_o	orbital or shaking diameter (m)
d_b	Erlenmeyer bottom diameter (m)
Fr	Froude number
Fr_{d_i}	Froude number based on the inner diameter of the cylinder
Fr_{d_o}	Froude number based on the orbital diameter
$G^*_{i,j}$	normalized intensity of the green channel in the ith jth pixel
g	gravitational acceleration ($m\,s^{-2}$)
H	cylinder height (m)
h	fluid height (m)
Δh	free surface height (m)
I_a	free surface interfacial area (m^2)
M	global degree of mixing
N	shaker table rotational speed (s^{-1})
N_{cr}	critical shaker table rotational speed (s^{-1})
Re	Reynolds number
T_θ	circulation time around the toroidal vortex axis (s)
T_z	circulation time around the cylinder axis (s)
$t_{95\%}$, t_m	mixing time (s)
V	nominal volume (m^3)
V_L	liquid/filling volume (m^3)
X	local degree of mixedness

Greek symbols

θ	mixing number
φ	phase angle of the table (°)
σ_G	standard deviation of the normalized green channel across the field of view at time t

the size of the microcarriers or cell aggregates [16]. The intrinsic differences between stirred vessels and orbitally shaken bioreactors have motivated the development of production scale OSRs [17,18] and industrial efforts to provide a single piece of equipment for different cell culture processes at multiple scales. Despite recent progress of shaken bioreactor platforms, with the development of optical sensors and control loops to improve automation in millilitre scale bioreactors, the number of publications which address scaling up/down aspects of shaken bioreactors is very limited [19].

1.1. Flow characterization and transition in orbitally shaken bioreactors

Gardner and Tatterson [20] were the first to report the flow and mixing dynamics in a shaken cylindrical vessel. Dye visualization techniques with mixtures of different viscosity were employed to assess the variation of the homogenization time with increasing Reynolds number, *Re*. Büchs et al. [21] estimated the power consumption in Erlenmeyer flasks by measuring the torque on the drive acting upon the shaker table and taking into account friction and other system losses. The effects of size of the container, filling volume, shaking or orbital diameter, shaking frequency and liquid viscosity were investigated. At certain agitation regimes an 'out-of-phase' condition occurred, when the liquid in the shaken flask did not move in synchronization with the shaker table. Klöckner et al. [22] extended the work of [21] to estimate the power consumption of cylindrical shaken bioreactors. Contrary to the results obtained for Erlenmeyer flasks, the orbital to internal diameter ratio d_o/d_i was found to affect the power number. The correlation of Eq. (1) was proposed to estimate the critical shaking speed, N_{cr}, which for cylindrical OSRs may be used to identify 'suitable' shaking conditions associated with a measurable power number and in-phase flow.

$$N_{cr} = \frac{1}{d_i^2}\sqrt{0.28Vg} \tag{1}$$

The study of Weheliye et al. [23] was the first to provide a thorough insight into the dynamics of the flow occurring inside a cylindrical shaken bioreactor, and to determine a flow scaling law based on physical considerations, without resorting to the use of power law correlations. The work covered different internal diameters, filling volumes, orbital diameters, and agitation speeds and made use of phase-resolved particle image velocimetry (PIV) and free surface measurements. The scaling law was formulated based on the consideration that the free surface orientation, $\Delta h/d_i$, is orthogonal to the resultant force obtained from the vectorial sum of the centrifugal force due to the orbital motion and the gravitational one (see Eq. (2)).

$$\frac{\Delta h}{d_i} = a_o\left(\frac{2(\pi N)^2 d_o}{g}\right) \tag{2}$$

where Δh is the maximum height difference between diametrically opposite points on the free surface, and is hereafter denoted as the wave amplitude induced by the orbital motion. The constant of proportionality, a_o, depended on the fluid considered. The relationship of Eq. (2) was tested for several combinations of non-dimensional fluid height, $h/d_i = 0.3$–0.7, and orbital to cylinder diameter ratio $d_o/d_i = 0.14$–0.5. The measured data and the correlation of Eq. (2) showed very good agreement at low speed or Froude number *Fr*. At greater *Fr* the free surface exhibited a complex three-dimensional shape, which is due to local non-uniformities of the inertial to the gravitational force ratio, and its mean inclination started deviating from the linear relationship of Eq. (2). Weheliye et al. [23] showed that the variation of free surface shape corresponded to the onset of a flow transition from a horizontal toroidal vortex to a vertical one processing around the cylinder axis, before and after the transition, respectively. This flow transition is determined by the growth in size of the toroidal vortex with increasing rotational speed, N (i.e. *Fr*), and it occurs when the toroidal vortex extends over the entire fluid height. They concluded that the flow transition in a shaken cylindrical bioreactor can be predicted using either Eq. (3) or (4) depending whether $h/d_i < (d_o/d_i)^{0.5}$ or $h/d_i > (d_o/d_i)^{0.5}$, respectively.

$$\frac{h}{d_i} = a_o\left(\frac{d_i}{d_o}\right)^{0.5} Fr_{d_o} \tag{3}$$

$$a_o Fr_{d_i} = 1 \tag{4}$$

where the Froude numbers Fr_{d_o} and Fr_{d_i} are estimated from Eq. (5) using the orbital and cylinder diameters, respectively.

$$Fr = \frac{2\pi^2 N^2 d}{g} \tag{5}$$

Given the broad range of definitions of Froude number available in literature, the definition of Eq. (5) was selected for consistency with the works of [21,23–25].

1.2. Mixing time in bioreactors

Macro-mixing time studies based on a colorimetric method have been carried by Tissot et al. [26] and Tan et al. [27] in shaken cylinders and flasks, respectively. This approach has been widely used in stirred tanks, employing either a single or a combination of two pH indicators, and it has been thoroughly analyzed by Cabaret et al. [28] to optimize both the experimental and post-processing aspects of the colorimetric methodology. Tissot et al. [26] used a