Study of turbulent flow inside a stirred tank used in animal cell culture

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Abstract

Nowadays, the culture of animal cells has become an inescapable step to produce proteinic compounds. These cultures are commonly performed inside stirred tank bioreactors. Some animal cell species must be fixed on a support to grow and divide. They are usually fixed on non-porous beads, so-called microcarriers, with a diameter ranging around 250 µm. To maximise the surface available for cell development, these microcarriers are maintained in suspension inside the stirred bioreactor by the rotation of an axial propeller. The choice of agitation conditions in this type of process is not an easy task as it has to fulfil three potentially conflicting goals: (1) maintaining microcarriers in complete suspension, (2) homogenizing the culture medium, and (3) limiting mechanical constraints. The first two goals are easily reached inside a highly turbulent liquid flow containing a large range of eddies which exchange energy from larger ones (with a size similar to the impeller diameter) to smaller ones (with a size equal to a few microns). However, eddies the size of which are similar or smaller than the microcarrier diameter, interact with animal cells and generate potentially harmful shear at their surface. Moreover, animal cells fixed on microcarriers surface, can not rotate to reduce the shear experienced. Works conducted these last 20 years by several researchers ([1], [2]) have clearly linked the viability of cells fixed on microcarriers to the turbulent nature of the liquid phase flow. They also have correlated the cells viability with the air sparging. However, damages on cells due to air sparging may be strongly reduced by adding a surfactant such as Pluronic F-68 [2].

In order to select the better impeller for the specific case of animal cell culture on microcarriers, a previous study [3] compared seven axial impellers rotating at their respective just-suspended speed N_{js}. They were classified on the basis of the shearing they produced inside the liquid phase estimated from overall quantities as the power input P. This study concluded that the impeller TTP with a diameter to tank diameter ratio, d/T, equal to 0.4 produces the lowest shear at the just-suspended speed Njs. The aim of the present work is to study, at the local scale, the turbulent flow generated by the propeller TTP at its just-suspended speed in order to evaluate the turbulent shear, no longer on the basis of overall quantities but, on the basis of its spatial distribution.

To this aim, 300 instantaneous velocity fields are acquired by 3D P.I.V. in a 20 L cylindrical tank filled with water. P.I.V. measurements are performed inside a vertical plan crossing the tank symmetry axis, with a spatial resolution of 1.25 mm.

The turbulent flow of liquid is characterised, using Reynolds decomposition: the time

average velocity field $\vec{V}(x,y)$ is computed and subtracted from each instantaneous velocity fields $\vec{v}(x,y)$ to obtain fluctuating velocity fields $\vec{v}'(x,y) = [u'(x,y), v'(x,y), w'(x,y)] = \vec{v}(x,y) - \vec{V}(x,y)$. The root mean square of each component of the fluctuating velocity as well as the terms of the Reynolds stress tensor are then computed from the 300 fluctuating fields.

To compute the spatial distribution of the turbulent shear stress, the Reynolds tensor is multiplied by the water density. The diagonal elements (i.e. normal shear stress) are ten times higher than the off-diagonal elements (i.e. shear stress). The former ranges between 0 and 2 Pa. Both are maximal in the impeller discharge stream. The results are in good agreement with the study of Kumaresan et al. [4].

Another classical way to characterize the shear stress undergone by cells is to compute the local specific energy dissipation rate ε. In view of the spatial P.I.V. resolution, it is not feasible to compute that quantity directly from the spatial derivative of the fluctuating velocity fields. Indeed, according to Saarenrinne et al.[5], the spatial P.I.V. resolution should be as small as the size of the Kolmogorov scale where turbulent kinetic energy is dissipated into heat. This scale corresponds to the smallest eddies inside the flow and is of the order of magnitude of one hundred microns. That is the reason why, that in the present study, the spatial distribution of the local specific energy dissipation rate is estimated via the dimensional analysis: $\varepsilon = k^{3/2}/\Lambda$ where k is the turbulent kinetic energy and Λ is the integral length scale. The turbulent kinetic energy is computed from the root mean square of fluctuating velocity components. The spatial distribution of the integral length scale is computed by an autocorrelation method as described in Escudié et al. [6]. The computed values of the turbulent kinetic energy and of the integral length scale are in good agreement with literature. Moreover, the numerical and spatial distributions of the local specific energy dissipation rate, computed in this study from the dimensional analysis show very good accordance with: (1) results obtained by Delafosse and al. [7] who computed ε from the spatial derivative of the fluctuating velocity fields acquired by 2D PIV with a spatial resolution of 0.36 mm (ten times smaller than the spatial resolution of this present study) (2) mean values computed from global power input provided by the impeller $P/\rho V$ and $P/\rho d^3$

Keywords: Animal cell; Turbulent Flow; Reynolds stress, Local specific energy dissipation rate

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