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Euler–Lagrange approach to model heterogeneities in stirred tank bioreactors – Comparison to experimental flow characterization and particle tracking

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HIGHLIGHTS

- A Euler–Lagrange approach, coupling a CFD/CM and a stochastic model, is proposed.
- The CFD/CM is based on CFD simulations of hydrodynamics of a 20 L bioreactor.
- The stochastic model is based on a Continuous-Time Markov Chain (CTMC).
- The CFD/CM predicts the mixing of an inert tracer measured by tracer experiments.
- The CTMC reproduces well the experimental residence/circulation time distributions.

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ABSTRACT

The aim of this work is the validation of an Euler–Lagrange modeling approach coupling a CFD-based compartment model (Eulerian approach) and a stochastic model based on a Continuous-Time Markov Chain (Lagrangian approach). The turbulent flow structure and the mixing process in a bioreactor stirred by an axial Mixel TT impeller is characterized by PIV and tracer experiments. Comparison between experimental and numerical data shows that the CFD-based compartment model is able to reproduce accurately the spatial heterogeneities inside the bioreactor. The trajectory of a small tracer particle which perfectly follows the fluid flow is measured by optical trajectography. It is then simulated by a stochastic model which is either based on an homogeneous or on an inhomogeneous Continuous Time Markov Chain (CTMC). Comparison of residence and circulation time distributions in three zones defined inside the bioreactor shows that the inhomogeneous CTMC model predicts with an excellent accuracy the particle trajectories inside the bioreactor. The modeling approach proposed here could be an useful tool to design scale-down bioreactors in order to reproduce at lab-scale the stress levels encountered in large-scale production bioreactors and to characterize and compare different bioreactor configurations.

1. Introduction

When dealing with bioprocesses, such as human stem cell, animal cell or microbial cell cultures in stirred tank bioreactors, it is of great importance to be able to predict the performance of a new process before its scale up to large-scale production conditions. For several obvious reasons, selection and characterization of new cell lines are mainly performed at lab-scale. However, hydrodynamics significantly differ between small lab-scale and much larger production-scale bioreactors. As a result, cells circulating in large-scale bioreactors are subjected to environmental conditions and thus to stresses which may significantly differ from those encountered in lab-scale bioreactors. A perfectly mastered scale-up strategy should thus make use of scale-down bioreactors reproducing at lab-scale the conditions encountered in production bioreactors.

In stirred tank bioreactors, environmental stresses which may have an impact on the biological response depend on the bioreactor hydrodynamics. Two kinds of stresses may be identified: those related to the mixing process (substrate and metabolite concentrations, pH) and those related to the hydromechanical shear stresses generated by the turbulent flow. The latter are only relevant in animal or stem cell cultures which are reputed to be

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more sensitive to mechanical stresses when compared to bacteria or yeast, due to their lack of cell wall (Nienow, 2006). Mechanical stresses can also be generated by the presence of bubbles when dealing with aerated cultures but this point will not be addressed in this work.

Because mixing efficiency decreases when the bioreactor volume increases, concentration gradients are likely to appear at larger scale. Cells circulating in large-scale bioreactors are thus experiencing composition fluctuations in their local environment that can lead to modifications of the biological responses, and ultimately to a decrease of the productivity compared to lab-scale cultures (Enfors et al., 2001). In the case of bacteria or yeast cultures, many authors have attempted to study the influence of substrate or pH gradients encountered at large-scale using scale-down bioreactors (George and Larsson et al., 1993a; Bylund et al., 1999; Hewitt et al., 2000; Amanullah et al., 2001; Delvigne et al., 2005a, 2006; Junne et al., 2011). Fewer studies are available for animal cell cultures and only concerning the influence of pH gradients (Osman et al., 2002; Nienow et al., 2013). The scale-down bioreactors proposed in those works are usually composed of:

- a small stirred-tank bioreactor ($V \approx 1$–5 L) which reproduces the low concentration levels far from the feeding position,
- a plug-flow reactor (or in some case a smaller stirred-tank reactor) where the substrate or acid/base (for pH regulation) is added to mimic the high concentration levels encountered near the feeding point in large-scale bioreactors.

Mechanical stresses induced by the turbulent flow are related to the turbulent energy dissipation rate ϵ. In stirred tank bioreactors, turbulent dissipation rates are far from homogeneous, with high values encountered in the vicinity of the impeller and significantly smaller values in the bulk flow. So, cells are also experiencing fluctuating mechanical stress levels. Several works have been devoted to the study of the mechanical stress required to damage animal cells using either microfluidic flow contraction devices (Gregoriades et al., 2000; Godoy-Silva et al., 2009a,b; Nienow et al., 2013) or lab-scale stirred tank reactors (Sieck et al., 2013, 2014; Nienow, 2006). These studies showed that free-suspended commercial cells such as CHO or insect cells are not sensitive to mechanical stress levels generated by agitation alone in bioreactors. However the 'shear sensitivity' seems to be dependent on the cell line and most specifically human cell lines can be significantly more sensitive to hydrodynamic stress (Hu et al., 2011). Moreover, adherent cells which are cultivated on microcarriers may be sensitive to mechanical stress levels typically encountered in stirred bioreactors (Gregoriades et al., 2000).

If it is possible to predict the maximum levels of stress in a stirred-tank bioreactor, it is far more difficult to also predict the exposure time and frequency of the cells to these stresses, and thus the correct way to recirculate the cells in a scale-down bioreactor. In order to design scale-down bioreactors representative of large-scale ones, ones need to characterize the production-scale bioreactors in an Euler–Lagrange framework. This characterization could possibly be attained using Computational Fluid Dynamics with an Euler–Lagrange model to simulate both the spatial heterogeneities and the displacements of the cells. But, the computation time required is prohibitive, especially if complex biological reaction schemes are taken into account to reproduce the temporal evolution of concentration fields.

Therefore, the aim of this paper is to propose and to validate an Euler–Lagrange model that enables to predict, with a reasonable computation time, both the stress spatial distribution and the cell trajectories inside a 20 L bioreactor stirred by an axial impeller (Mixel TTP). The modeling approach proposed here is inspired by the work of (Delvigne et al., 2005a,b, 2006) and consists in coupling two models:

(1) a CFD-based compartment model (CFD/CM) describing the continuous phase (culture medium),
(2) a stochastic model based on a Continuous Time Markov Chain (CTMC) describing the discrete phase (cultivated cells).

Originally proposed by Bezzo et al. (2003), the CFD/CM consists in dividing the bioreactor into a limited number of volumes interconnected by mean and turbulent flow rates that are computed from a CFD simulation of the bioreactor hydrodynamics. In previous papers (Delafosse et al., 2011b, 2014), the CFD/CM has been developed for a flat-bottom 20 L bioreactor stirred by two 4-blade Rushton turbines. Comparison to PIV and tracer experiments showed that the CFD/CM is able to reproduce with an excellent accuracy the mixing of an inert tracer if compared to 'classical' compartment model or even to CFD. Moreover, the computational cost is far smaller than for CFD and it is possible to add biological kinetics without adding a prohibitive computational cost. In the present paper, a similar CFD/CM is developed for a hemispheric bottom bioreactor stirred by an axial impeller and then validated by comparison to PIV and tracer experiments.

A stochastic model is then added to the CFD/CM to simulate the displacement of virtual particles between the compartments defined in the CFD/CM. The stochastic model is validated by comparing the residence and circulation time distributions simulated in different zones of the bioreactor to corresponding experimental distributions obtained from the displacement of one particle measured by optical trajectography (Collignon, 2012; Collignon et al., 2013).

2. Experimental set-up

All experiments are performed in a hemispheric bottom vessel stirred by an axial Mixel TTP impeller and equipped with two baffles positioned at 180° from each other. The vessel diameter is $T = 0.305$ m and the liquid height is $H = T = 0.305$ m, corresponding to a working volume of 20 L. The impeller, composed of three curved blades (Figs. 1a and b), has a diameter $D \approx 0.41T = 0.125$ m and is located at a distance $C = T/3 \approx 0.1$ m from the tank bottom. The two baffles have a width of $b \approx T/10 = 0.03$ m. The configuration is represented in Fig. 2 and the dimensions are reported in Table 1.

The vessel is made in transparent glass. It is installed in a square aquarium filled with water to minimize optical distortion due to the curvature of the cylindrical wall.

The bioreactor used in this work has already been experimentally characterized in previous studies by PIV (Collignon et al., 2010a; Delafosse et al., 2011a), PLIF (Collignon et al., 2010b; Collignon, 2012) and optical trajectography (Collignon, 2012).

All these experimental results are used in the present paper to validate the modeling approach. The corresponding measurement techniques are briefly described below.

2.1. Tracer experiments

Tracer experiments are used to validate the model in terms of mixing: the concentration evolution of an inert tracer is measured using the conductivity technique. For each measurement, a pulse injection of 4 mL of a NaCl solution ($C_0 = 300\ \mathrm{g \cdot L^{-1}}$) is made at the liquid surface ($2r/D = 1.2$, $\theta = -\pi/4$). The temporal evolution of NaCl concentration is measured by two conductivity probes located near the tank wall, just under the liquid surface and at two angular positions as pictured in Fig. 2b (P1: $\theta = \pi/4$; P2: $\theta = 3\pi/4$). For each injection position, several injections are performed. The averaged mixing time at 95% of homogeneity is then computed for each probe position.