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Hydrodynamics in a disposable rectangular parallelepiped stirred bioreactor with elliptic pendulum motion paddle

Marie-Laure Collignon [a,b,*], Laurent Droissart [a], Angélique Delafosse [a], Sebastien Calvo [a], Steven Vanhamel [c], Roman Rodriguez [c], Tom Claes [c], Fabien Moncaubeig [d], Ludovic Peeters [e], Michel Crine [a,b], Dominique Toye [a]

[a] *Laboratory of Chemical Engineering, University of Liège, Sart-Tilman, B6, B4000 Liège, Belgium*
[b] *F.R.S.-FNRS, Rue d'Egmont 5, B1000 Bruxelles, Belgium*
[c] *ATMI LifeSciences, Reugelstraat 2, B3320 Hoegaarden, Belgium*
[d] *Artelis, rue de Ransbeek 310, B1120 Bruxelles, Belgium*
[e] *GlaxoSmithKline Biologicals, rue de l'Institut 89, B1330 Rixensart, Belgium*

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ABSTRACT

Stainless steel bioreactors increasingly fall behind to their disposable counterparts in pharma research as they do not require cleaning or sterilization. This led company ATMI LifeSciences to develop the "Nucleo™". Original in design, this disposable bioreactor comprises a rectangular parallelepiped plastic bag stirred by a paddle revolving in elliptic pendulum motion. Studies covering this bioreactor showed good homogeneity of culture medium as well as good productivity for animal cell cultures. To further explain these good performances, the flow inside the "Nucleo™" had to be resolved. This paper focuses on the mean flow description, computed from stereo-PIV measurements performed in 20 verticals covering the whole volume of a 50 dm^3 Nucleo™ bioreactor. As the flow is already turbulent in the chosen agitation conditions, its dimensionless mean velocity field does not vary with the paddle rotational speed. Mean flow pattern exhibits an axial symmetry – same flow is observed in opposite quarters of the tank – and can be described as a three-dimensional helix coiled on itself to form a distorted horizontal torus which covers the whole tank volume. Mean velocity value is on average doubled in the cone swept by the paddle, and its two horizontal components are twice higher than its vertical ones. However, mean velocity remains significant everywhere and, in particular, no stagnant area is observed in tank corners. Our results thus confirm previous studies observations.

1. Introduction

In recent years, a significant shift towards disposable bioreactors occurred in pharma research. Dedicated to a single animal cell culture, they usually comprise a closed and sterile plastic bag attached to a steel structure and are equipped with connections for introducing culture medium and various probes. As their use brings many benefits, they have been gradually replacing their stainless steel counterparts [1]. The most obvious advantage is two costly steps, i.e. washing and sterilization between production campaigns, which in turn reduces global environmental impact in spite of higher solid waste [2]. Other major strategic advantages of single-use bioreactor are a significant reduction in time required to build and validate a new production facility together with a higher flexibility in the production capacity [2].

Recognizing high potential in this market, many companies have developed disposable bioreactors, such as the SUB (Hyclone), the Xcellerex (XDR) or the BioStat STR (Sartorius Stedim), which hold to the conventional stirred geometry of stainless-steel devices. Others have original design. It is the case of the disposable bioreactor studied in this paper, the Nucleo™ bioreactor commercialized by ATMI LifeSciences. As illustrated in Fig. 1, this device comprises a rectangular parallelepiped plastic bag stirred by a paddle integrated in the bag and covered by the same multilayer polymer. When oxygen supply is required, a sparger is fitted at the lower end of the blade. The bag rests in a stainless steel frame. The blade is connected to the motor through a metal rod which fits into the hollow axis of the blade. The blade is inclined at 13.5° with

* Corresponding author at: Laboratory of Chemical Engineering, University of Liège, Sart-Tilman, B6, B4000 Liège, Belgium. Tel.: +32 4 366 47 22; fax: +32 4 366 28 18.
E-mail address: mlcollignon@ulg.ac.be (M.-L. Collignon).

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Notations

A	major axis size for ellipse drawn by paddle external tip
k	turbulent kinetic energy ($m^2\,s^{-2}$)
N	paddle rotational speed (rpm)
Re	Reynolds number (–)
V_{tip}	linear velocity at paddle external tip ($m\,s^{1}$)
V_x, V_y, V_z	velocity components along x, y and z axes, respectively
x,y,z	Cartesian axes aligned along vessel walls
ε	dissipation rate of kinetic energy ($m^2\,s^{-3}$)
μ	dynamic viscosity (Pa s)
ρ	fluid density ($kg\,m^{-3}$)
rpm	rotation per minute
PIV	Particle image velocimetry

respect to the vertical and therefore draws an elliptical pendulum trajectory in the vessel, as illustrated in Fig. 2. The motion of the paddle through the bag can be visualized in the video available on the electronic version of this paper. The bag is equipped with several disposable sensors (pH, dissolved O_2, etc.) and with multiple sterile connections to enable gas injection, gas exhaust, to add the culture medium, and for sampling.

This original stirred bioreactor is a joint development by three companies, ATMI LifeSciences, Pierre Guerrin and Artelis, which aims at a better answer to specific requirements of animal cell culture. Indeed, as other microorganisms, animal cells require a constant physico-chemical environment, which means good homogenization and aeration of the culture medium. However, unlike bacteria or yeast, animal cells do not possess a rigid cell wall but a fragile plasma membrane, which leads to consider them as particularly shear-sensitive. Mechanical constraints generated inside the culture medium due to mixing and aeration must thus be as small as possible [3,4]. Fulfilling these two opposite requirements becomes even more of a challenge in anchorage-dependent cell culture, i.e. when cells are fixed on the surface of microcarriers. To maximize surface available for cell development, the latter must remain in complete suspension in the culture medium but will also collide with each other, thus creating additional mechanical constraints.

Studies show that the Nucleo™, thanks to its original design, reconciles (i) liquid and solid homogenization and (ii) minimizing mechanical constraints on cells. As a matter of fact, even at low paddle motion (i.e., 30–40 rpm), good homogeneity of the culture medium, total dispersion of the gas phase and effective suspension of microcarriers are observed [5–8]. Efficient animal cell culture processes were also demonstrated in this bioreactor for free suspended cells [9] as well as anchorage-dependent cells [10]. Furthermore, as research performed by Goedde et al. [9] highlighted, cell concentration and secreted protein production are at least 30% higher with the Nucleo™ disposable bioreactor as opposed to conventional stainless-steel stirred bioreactors under equivalent operating conditions.

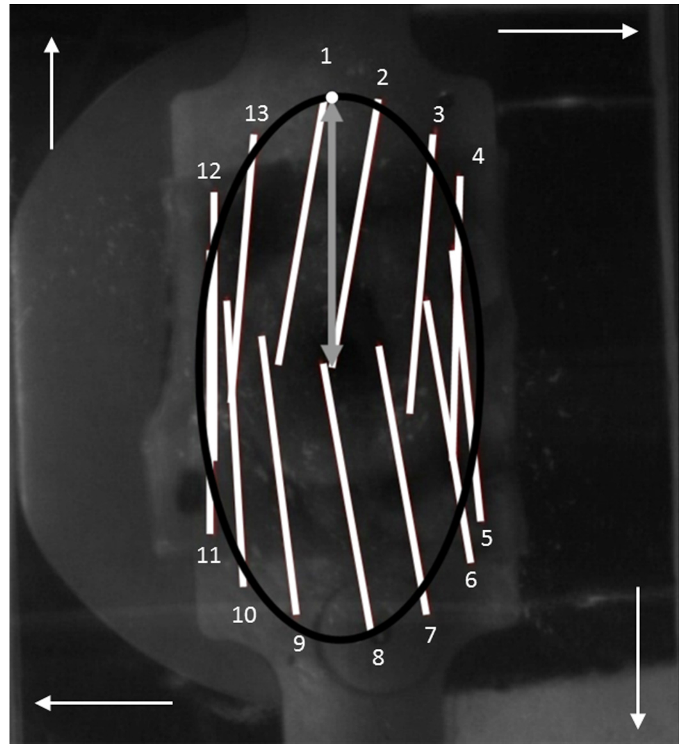

Fig. 2. Paddle tip position sequence (white lines) during its rotation, observed through tank bottom. White arrows show direction of paddle displacement in each tank corner. Grey arrow materializes major semi-axis of ellipse swept by the paddle.

Although above performances were experimentally observed, their theoretical basis has yet to be clarified further. The US Food and Drug Administration also promotes an approach called "Quality by design" [11] in characterizing new biotechnological processes. Per said approach, new processes should no longer be developed empirically but on the basis of robust models which represent as closely as possible the physics, the chemistry and the biology involved in the process.

A key step in the development of such a model for the Nucleo™ disposable bioreactor is the detailed description of the flow produced by the elliptic pendulum motion of the paddle in the

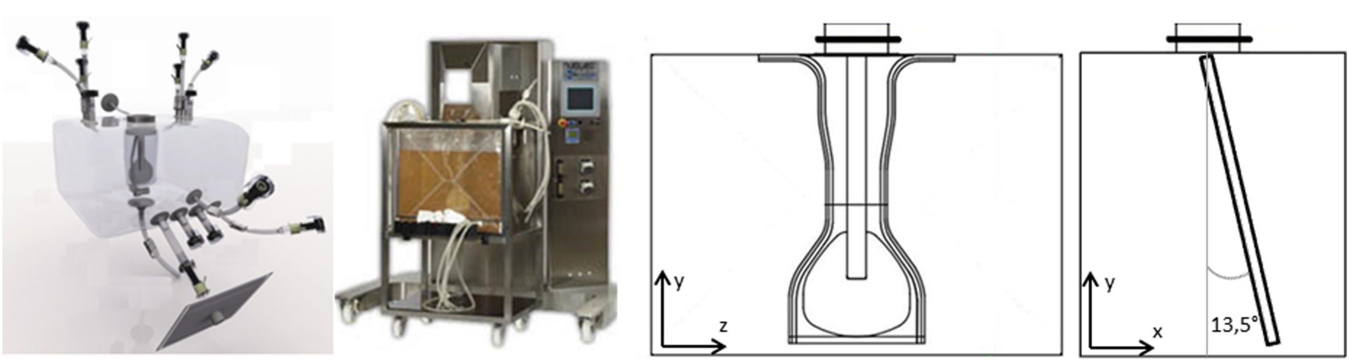

Fig. 1. Design of the Nucleo™ disposable bioreactor.