Sixth International Symposium on Mixing in Industrial Process Industries- ISMIP VI
Niagara on the Lake, Niagara Falls, Ontario, Canada
August17-21, 2008

A study of the mixing by PIV and PLIF in bioreactor of cells animals culture

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ABSTRACT

Selecting the optimal agitation conditions (rotating speed, type of impeller) is crucial in stirred tank bioreactors used for the culture of animal cells. In the present study, hydrodynamics and mixing quantities are experimentally determined by PIV and PLIF in a 20 litre bioreactor. The results shows that optimal agitation conditions in terms of homogenisation, suspension and mechanical constraints on cells are obtained if the impeller A310 (Lightnin) rotating at 49 rpm is used.

NOMENCLATURE

$u_{r,z}$	fluctuation of the velocity (m/s)
$U_{x,z}$	component of velocity vectors (m/s)
ε	rate of energy dissipation(W)
ν	cinematic viscosity (m^2/s)

INTRODUCTION

Bioreactors are very specific and complex gas-liquid-solid reactors, because the solid particles are living organisms. The use of bioreactors for animal cells culture is relatively recent and finds applications especially in pharmaceutical industry. The company GlaxoSmithKline Biologicals is interested in this type of bioreactors to implement an industrial scale culture of animal cells in a stirred tank, in which the cells are adsorbed on microcarriers. Microcarriers are non-porous beads made of reticulated dextran. Their mean size is 250 µm and they are suspended in the culture medium.

In this type of process, the optimization of mixing conditions is very complex. The agitator design and the rotating speed have to be chosen to meet the two following goals:

- The microcarrier beads must be perfectly suspended in order to make their external surface available for cellular development.
- The concentrations must be homogeneous in the culture medium and if an additive (acid or base for pH regulation, nutrients …) is injected, it must be very rapidly distributed.

But the agitation conditions must not be too severe in order to limit the mechanical constraints (macro and micro shearing) imposed to animals cells that can be harmful for their development and their metabolism.

Thus, the aim of the present work is two-fold. The first one is to characterize experimentally local quantities such as velocity fields and their corresponding shear fields, concentration fields and mixing time for different commercials impellers operating at different rotating speeds. The second one is to optimise the agitation conditions (type of impeller and its rotating speed).

APPARTUS AND METHODS

In order to characterise local quantities, two optical techniques are used: PIV and PLIF. PIV (Particle Image Velocimetry) is a technique which is used to measure 2D velocity fields in the vertical plane that contains the agitation shaft. PLIF (Planer Laser Induced Fluorescence) is used to measure the concentration field of a tracer in the same plane. A limitation of these optical techniques is that the tank as its contents must be transparent. The presence of microcarriers makes opaque the culture medium. Measurements must thus be performed on a model fluid which has same rheological properties as the culture medium. Rheological measures relative to the culture medium (with no cell) show that water can be used as model fluid.

The glass tank used has a volume of 20 litres, with a height to diameter ratio equals to 1 and a hemispheric bottom. It is equipped with two baffles (figure1). The tank is disposed in a cubic container filled with water to limit errors due to optical distortions. Impeller is placed at one third of the tank height. Measurements are realised for 5 rotation speeds ranging between 50 rpm and 120 rpm. Four axial impellers have been tested: Propellers TTP (Mixel), Propellers A315 and A310 (Lightnin) and Propeller VMI with 3 streamlined blades (VMI-Rayneri). The direction of rotation is chosen to obtain a down-pumping through the impeller.

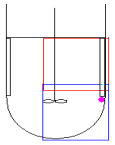

Figure 1: Tank geometry and measurement zones.

For P.I.V measure, water is seeded by tracer polyamid particles which emit broadcast visible radiation. The tracer particle mean size is 20 µm and their density is 1.03, which is similar to water density. For each operating condition, 200 image pairs are recorded. The time interval between the two images of a pair is set between 2250 µs and 5000 µs depending on the rotation speed of the impeller. In order to increase spatial resolution (6 pixels per millimeter), images are not measured on a whole plane of the tank but only on the two rectangular areas represented on figure1. Images are treated by cross-correlation in order to extract instantaneous velocity fields and the corresponding time averaged velocity field in the measurement plane. The flow quantities relative to the whole tank volume are then computed assuming a rotational symmetry around the impeller shaft.

During PLIF measurement, 5 ml of 0.008 g/l fluorescent Rhodamine 6G is injected at the point in the tank

represented by a pink dot in the figure 1. The injection spread inside the tank is followed during time by an image record each 250 ms. PLIF images are measured on a whole plane of the tank and a macroscopic mixing time can be determined from P.L.I.F measures.
For each impeller, the minimum speed of rotation that leads to a complete suspension of microcarriers is measured. To this end, 20 litres of culture medium containing microcarriers is put in the tank and the (possible) decantation of microcarriers is followed during 45 minutes by a video camera for different rotation speeds. For each impeller, PIV and PLIF measurements are then performed at the minimum rotation speed in order to determine the corresponding velocity fields and macroscopic mixing time.

RESULTS

1. Hydrodynamic and mixing parameters

The velocity field in the bioreactor is measured by PIV as a function of the rotation speed for different types of impeller. The **hydrodynamic pattern** is the same for all the impellers. It is composed by two ejection areas and by four recirculation loops. The time averaged velocity field represented on figure 2 is relative to half of the tank. The correspondence between the background colour and the norm of the velocity vector is given by the colorbar situated on the right of the image. Pink arrows indicate the direction of the velocity vectors. Depending on the type of impeller and on its rotating speed, average and maximum velocity values range between 0.024 m/s - 0.105 m/s and between 0.1 m/s - 0.38 m/s, respectively.

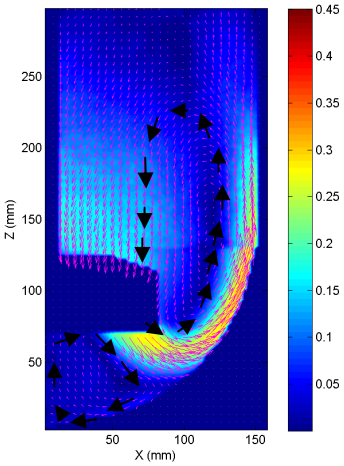

Figure 2: Time average velocity field created by the impeller A315 at 102 rpm

The **macro-shearing** is evaluated at each point by: $\left|\frac{\partial U_x}{\partial z}\right| + \left|\frac{\partial U_z}{\partial x}\right|$ where U_x and U_z are components of the time averaged velocity which are included in the vertical measurement plane. For each impeller, macro-shearing values increase with the rotating speed. They are high at the periphery of the ejection areas and near the tank walls (10 s^{-1} to 40 s^{-1} depending on the impeller and its rotating speed). They are smaller in other regions of the tank (1 to 5 s^{-1} respectively). Given that the way macro-shearing is evaluated, that result appears logical when the figure 2 is observed.
The **local energy dissipation rate** is obtained by applying the following formula, proposed by Baldi&al, at each point of the vertical plane:

$$\varepsilon = \upsilon \cdot \left\{ 2 \cdot \overline{\left(\frac{\partial u_r}{\partial r}\right)^2} + 2 \cdot \overline{\left(\frac{\partial u_z}{\partial z}\right)^2} + 3 \cdot \overline{\left(\frac{\partial u_r}{\partial z}\right)^2} + 3 \cdot \overline{\left(\frac{\partial u_z}{\partial r}\right)^2} + 2 \cdot \overline{\frac{\partial u_r}{\partial z} \cdot \frac{\partial u_z}{dr}} \right\}$$

where u_r and u_z are fluctuations of the velocity in the vertical plan. The rate of energy dissipation also increases with the rotating speed. It is high in the ejection areas (6.10^{-4} W to 6.10^{-3} W depending on the impeller and its rotating speed) and very small in the remaining part of the flow (0 to $1{,}2.10^{-3}$ W respectively).The knowledge of the rate of energy dissipation at each point of the vertical measurement plane is useful to obtain the spatial distribution of the size of the smallest eddies (**Kolmogorov scale**) and thus **to characterise micro-shearing**. Indeed, if the size of the smallest eddy is equal or smaller than the microcarrier size, micro-shearing applied to the cells adsorbed on the microcarrier surface can be high. For all the impellers, the micro-shearing could be problematic because the area where the Kolmogorov scale is smaller than the microcarrier diameter includes at least the ejection area at 50 rpm and spreads for higher rotating speed.
The macroscopic mixing time is evaluated from P.L.I.F measurements for different agitation conditions. The computed mixing time ranges between 7 s and 32 s depending on the type of impeller and on its rotating speed. That macroscopic mixing time is very small compared to the response time of cell metabolism to a perturbation of their environment (approximately 1 hour).

2. Impeller comparison at minimal rotating speed

The minimal rotating speed that leads to a complete suspension of microcarriers Nmin was experimentally determined for each impeller. All the above mentioned quantities were then evaluated at Nmin. The macroscopic mixing time at Nmin is always negligible compared to the response time of cell metabolism. The analysis of micro and macro-shearing distribution at Nmin shows that impeller A310 products the smallest mechanical constraints compared to those which are produced by other impellers.

CONCLUSION

The characterisation of local quantities for each impeller demonstrated that the mixing time is never the limiting parameter in the choice of agitation conditions. It was shown that the micro and macro-shearing increase with the rotating speed and the rotating speed must thus be chosen as small as possible, equal to the minimal rotating speed which leads to a complete suspension of micro carriers. When impellers are compared at their respective minimal rotating speed, the impeller A310 products the smaller mechanical constraints. In view of these facts, we can conclude that the optimal agitation is obtained with the impeller A310 rotating at 49 rpm.

ACKNOLEDGEMENTS

M-L Collignon is grateful to the FNRS (National Fund for Scientific Research, Belgium) for a grant of research fellow.

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