Experimental analysis by P.I.V. and P.L.I.F of the local hydrodynamic environment of animal cells cultivated in a stirred tank bioreactor

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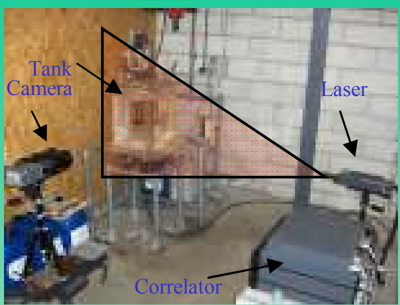
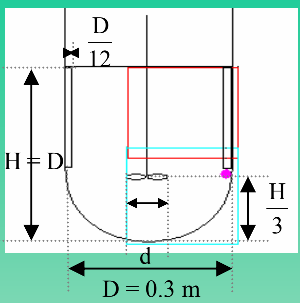
THE BIOTECHNOLOY ASPECT OF THE RESEARCH

- The research is a collaboration between the company GlaxoSmithKline Biologicals and Laboratory of Chemical Engineering of Liege University.
- GSK BIO develops an industrial scale culture of animals cells in stirred tank, in which cells are adsorbed on microcarriers (non-porous beads made of reticulated dextran, mean size = 250 µm, density=1.03)
- The choice of agitation conditions (impeller type, rotating speed…) is complex in order to optimize the culture productivity which depends on local cell environment
- The agitation conditions have to be chosen to :
    - maintain microcarrier beads in suspension in order to maximize the external surface available for cellular development
    - get rid of concentrations gradient

  But they must not be too severe in order to limit mechanical constraints imposed to animal cells
- The aim of this study is to collect experimental data in a 20 L tank by P.I.V. and P.L.I.F measurements on the influence of the agitation conditions on the cell local environment.

APPARATUS





P.I.V.-P.L.I.F apparatus: Dantec 2500 (Dantec Dynamics,DK):

- Laser Solo II-30 (New Wave Gemini): Nd-Yag, double cavity,2X30 MJ, 532 nm
- Camera Hi/Sense P.I.V/P.L.I.F: sensors CCD, resolution 1280x1024 pixels
- Lens AF Micro Nikkor 60 mm F2.8D (Nikon)
- Processor Correlator 2500 (Dantec Dynamics)
- Software FlowManager 4.71 (Dantec Dynamics)

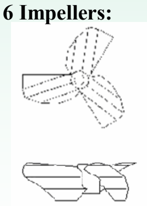
P.I.V measurements conditions:

- Tracers: polyamide particles, average diameter = 0.20 µm, density = 1.03g/cm³
- Field of view of the camera fixed on each rectangular areas (blue and red)
- 200 image pairs recorded for each impeller and each rotating speed
- time interval between the two image of a pair set between 2250 µs and 5000µs
- Cross-correlation treatment

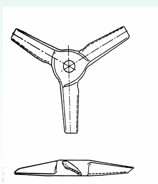
P.L.I.F measurements conditions:

- Injection of 5ml of 0.008 g/L fluorescent Rhodamine 6G at the pink dot position

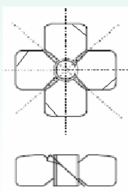
6 Impellers:



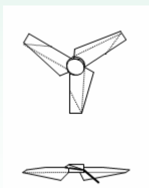
Propeller TTP (Mixel)
d = 0.125 or 0.150 m
(TTP125) (TTP150)



Propeller A310 (Lightnin)
d=0.160 m
(A310)



Propeller A315 (Lightnin)
d=0.125 or 0.150 m
(A315125) (A315150)

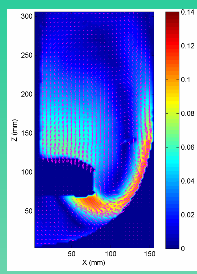


Propeller 3 streamed blades (VMI)
d=0.156 m
(3SB)

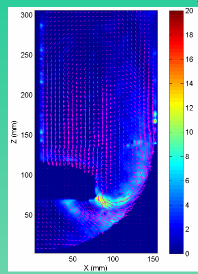
RESULTS

1. Experimental characterization of the local cell environment

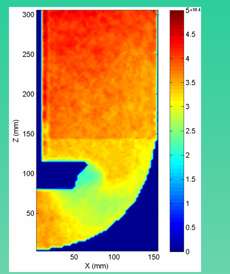
Results obtained for impellers A315 150 at 38 rpm



Time average velocity field ($m.s^{-1}$) for a half tank



Macro-shearing field computed by $\left|\frac{\partial U_x}{\partial z}\right| + \left|\frac{\partial U_z}{\partial x}\right|$



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

$$\lambda_{k-\max} = \left(\frac{\upsilon^3}{\varepsilon_{\min}}\right)^{\frac{1}{4}}$$

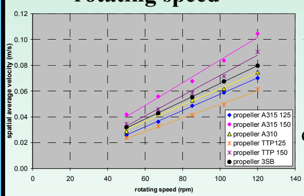
2. Impellers comparison at just-suspended speed N_{js}

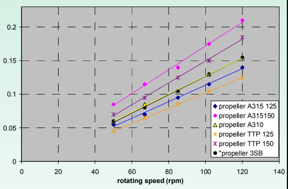
Propellers	N_{js} (rpm)	$V_{average}$ ($m.s^{-1}$)	$V_{90\%}$ ($m.s^{-1}$)	$Shear_{average}$ (s^{-1})	$Shear_{90\%}$ (s^{-1})	Relative size of area where microshearing is intensive (%)	t^M (s)
3SD	53	0.032	0.065	1.541	3.6	3.48	18
A310	49	0.031	0.06	1.387	3.4	1.45	22
A315125	54	0.030	0.06	1.609	4.2	5.16	23
A315150	38	0.029	0.055	1.485	3.6	3.72	20
TTP 150	40	0.030	0.055	1.457	3.4	1.97	23
TTP 125	50	0.024	0.045	1.299	3.0	0.90	33

Similar   Smallest mechanicals constraints

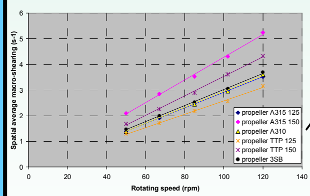
Highest macroscopic mixing time << response time of cell metabolism

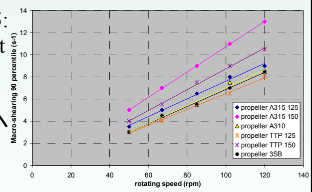
3. Variation of characteristic quantities as a function of rotating speed



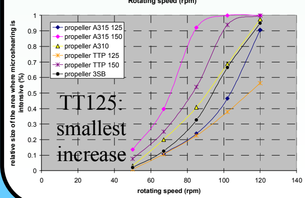


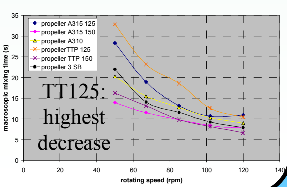
Linear dependency to impeller tip speed





TTP125: smallest slope





Power law evolution

CONCLUSIONS

At the just-suspended speed, the propeller TT125 creates the most suitable local cell environment. The evolution of its hydrodynamic characteristic parameters is such that it remains the best impeller at higher rotating speed.

ACKNOWLEDGEMENT

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