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A study of mixing by PIV and PLIF in bioreactor of animal cell culture

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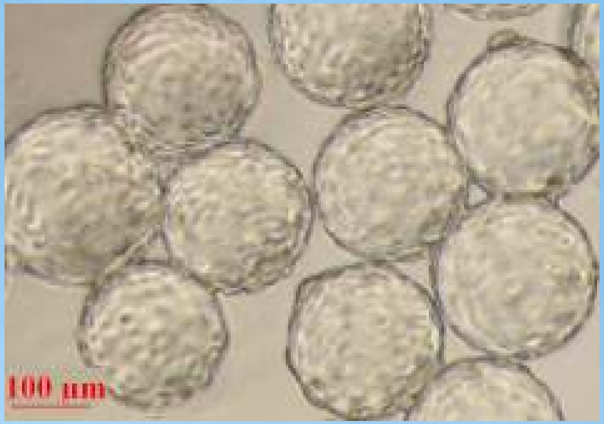
Background of the research

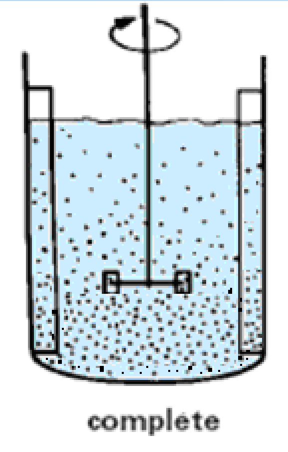
Collaboration between:

- the Laboratory of Chemical Engineering of Liege University
- the Company GlaxoSmithKline Biologicals

Process development of a **animal cell culture on microcarrier** in a **stirred tank** used for **vaccine production**



Introduction



Positive effect of mixing :

Keeping in complete suspension the microcarriers (N>Njs)

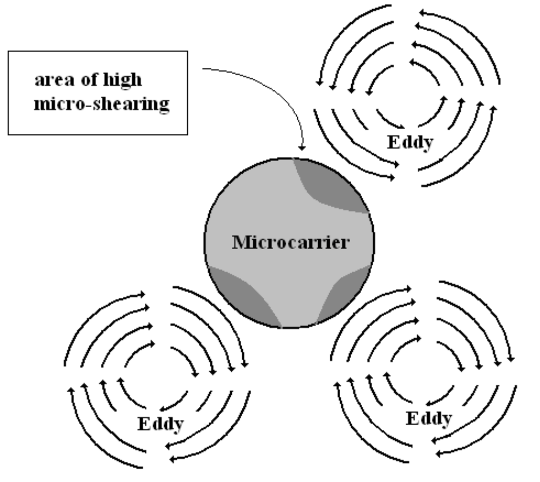
Homogenization of the culture medium

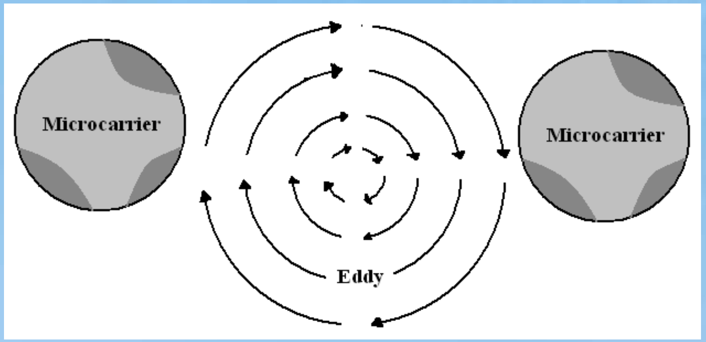
Negative effect of mixing:

Creation of mechanical constraints

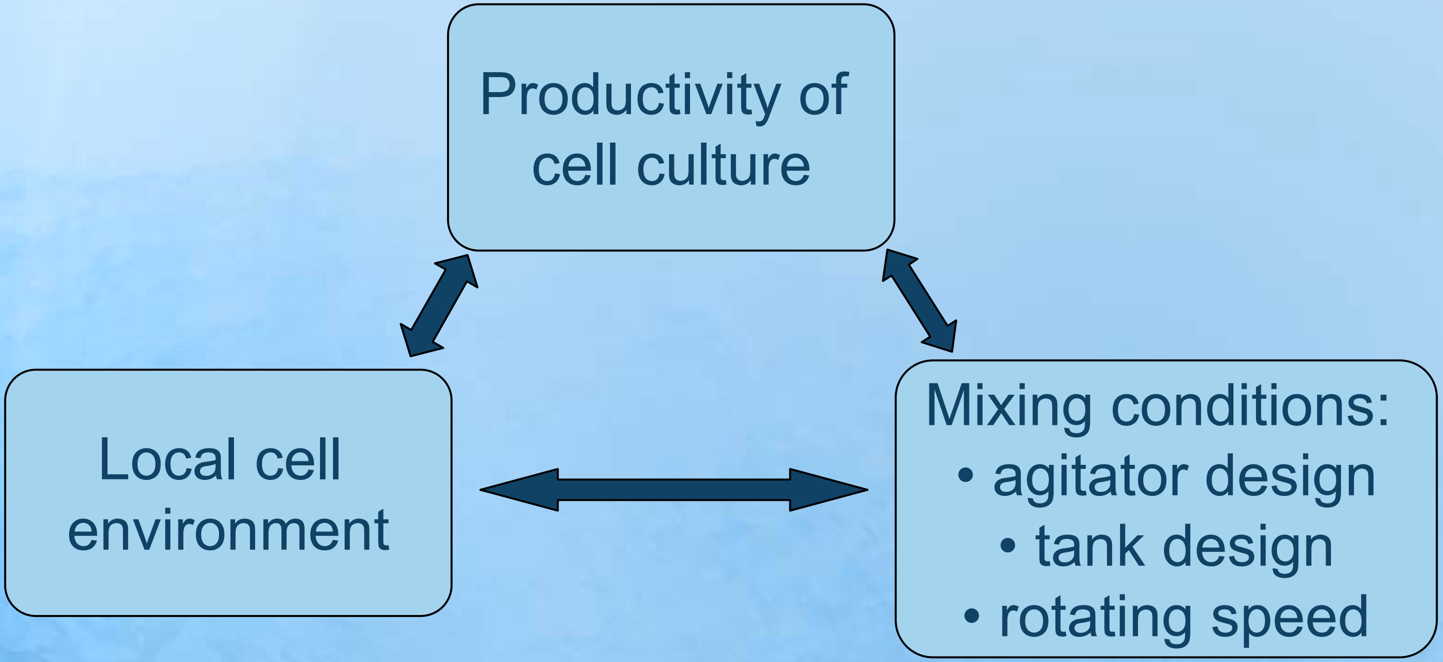
micro-shearing

collision





Goals of the research



Productivity of cell culture

Local cell environment

Mixing conditions:
- agitator design
- tank design
- rotating speed

➡ **Experimental characterization of the local cell environment depending on the mixing conditions**

➡ **The choice of optimal agitation conditions to have:**

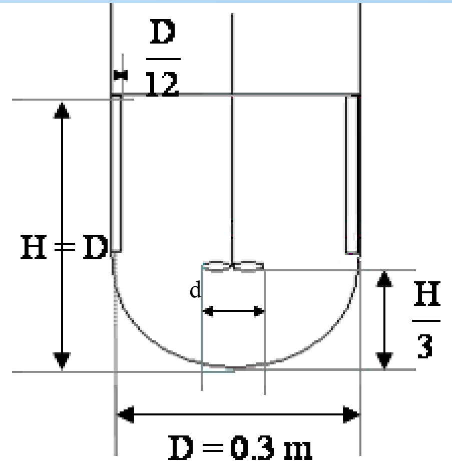
microcarrier in suspension,

small concentration gradient

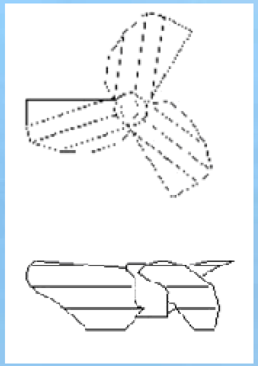
small mechanical constraints

Materials and methods

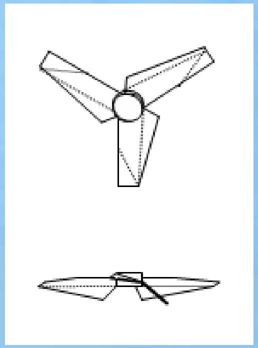
20 L standard tank :



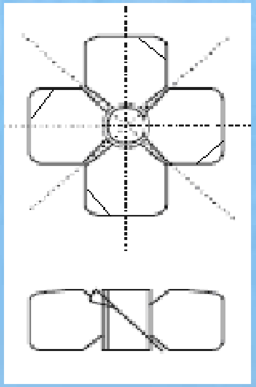
6 axial impellers :



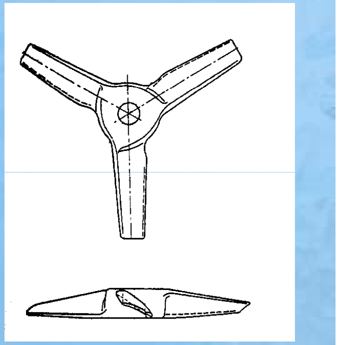
Propeller TTP
(Mixel)
d = 0.125 or 0.150 m
(TTP125) (TTP150)



Propeller 3 streamed blades (VMI)
d=0.160 m
(3SB 160)



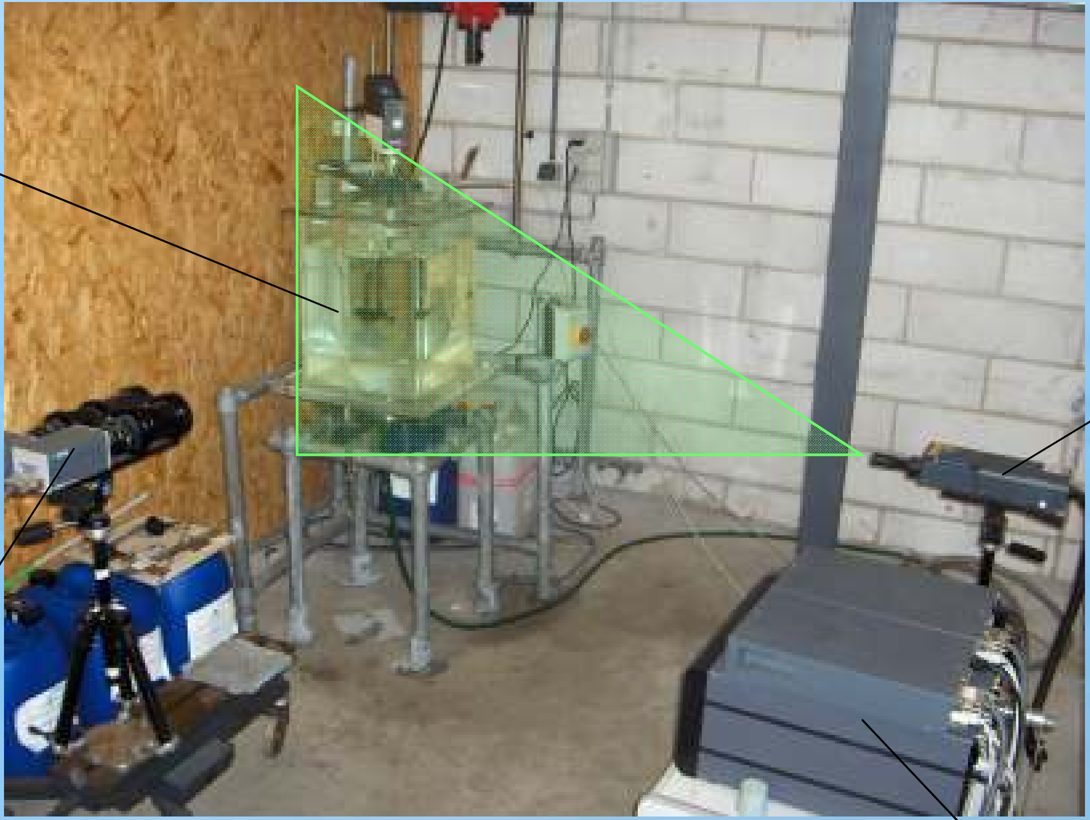
Propeller A315
(Lightnin)
d=0.125 or 0.150 m
(A315125) (A315150)



Propeller A310
(Lightnin)
d=0.156 m
(A310 156)

Materials and methods

Characterisation of local cell environment by P.I.V. and P.L.I.F. apparatus (Dantec Dynamics S.A., Denmark)



P.I.V. tracer:
polyamide particles, (20 µm, 1.03g/cm³)
P.L.I.F. tracer:
5ml of 8 mg/L fluorescent Rhodamine 6G
Injection position: Along the wall tank and same height than the impeller

Camera Hi/Sense
P.I.V/P.L.I.F: sensors CCD, 1280x1024 pixels
Lens AF Micro Nikkor 60 mm F2.8D (Nikon)

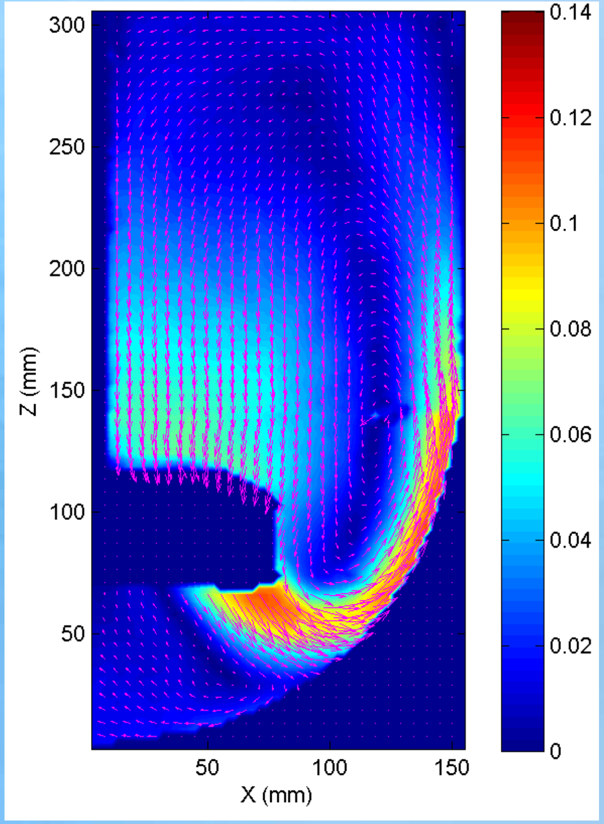
Laser Solo II-30
(New Wave Gemini): Nd-Yag, double cavity, 2X30 MJ, 532 nm

Processor Correlator 2500 (Dantec Dynamics)
Software FlowManager 4.71 (Dantec Dynamics)

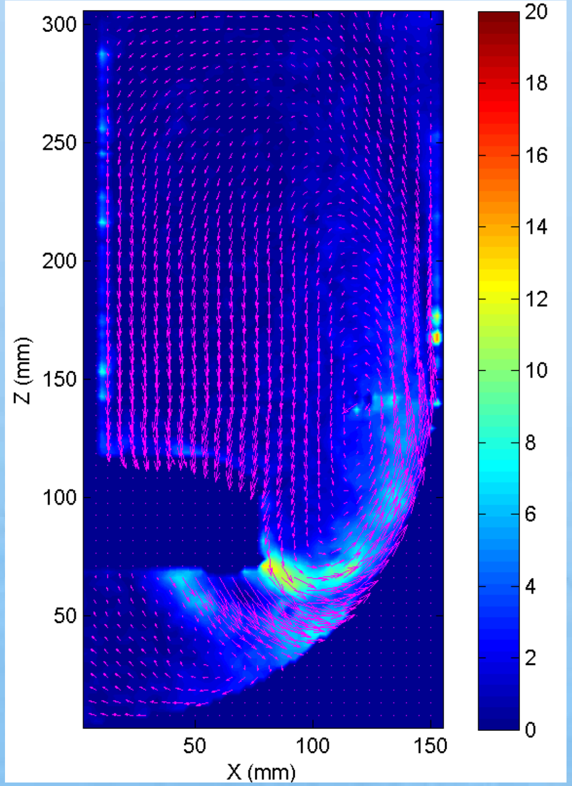
Materials and methods

Exploitation of P.I.V. measurements:

impeller A315 150 at 38 rpm



Time average velocity field (m.s^1) for a half tank

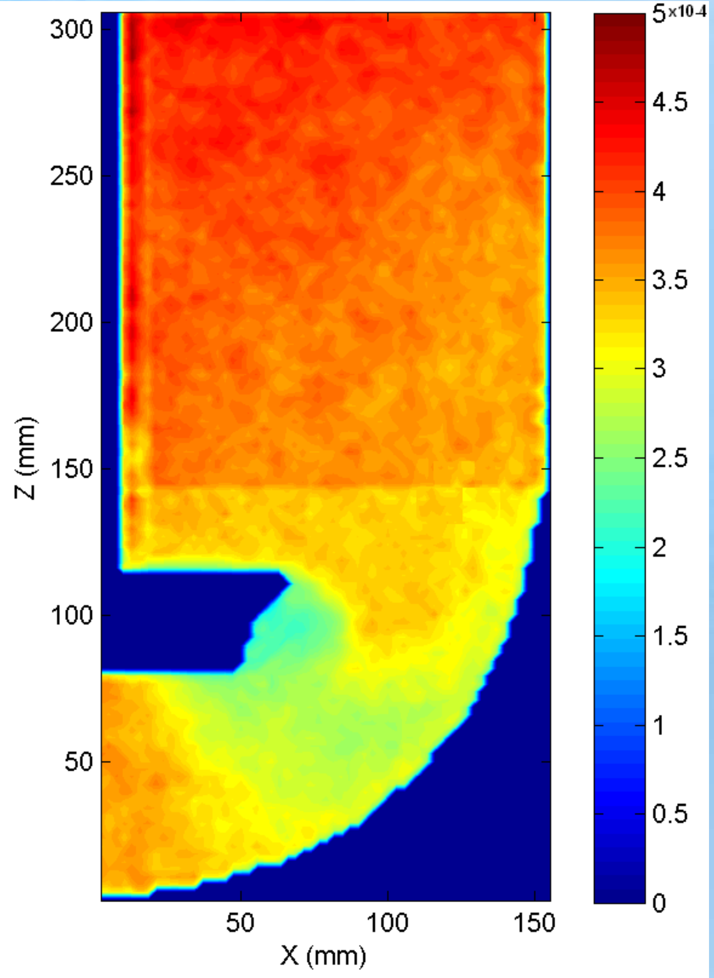


Time average macro-shearing field (s^{-1}) computed by

$$\left|\frac{\partial U_x}{\partial z}\right| + \left|\frac{\partial U_z}{\partial x}\right|$$

Materials and methods

Exploitation of P.I.V. measurements: Kolmogorov scale field



Fluctuation velocity field :

$$u' = u - U$$

Local rate of energy dissipation :

$$\varepsilon = \upsilon \cdot \left\{ \begin{array}{l} 2 \cdot \overline{\left(\frac{\partial u'_r}{\partial r} \right)^2} + 2 \cdot \overline{\left(\frac{\partial u'_z}{\partial z} \right)^2} + 3 \cdot \overline{\left(\frac{\partial u'_r}{\partial z} \right)^2} \\ + 3 \cdot \overline{\left(\frac{\partial u'_z}{\partial r} \right)^2} + 2 \cdot \overline{\frac{\partial u'_r}{\partial z} \cdot \frac{\partial u'_z}{dr}} \end{array} \right\}$$

Kolmogorov scale field :

$$\lambda = \left(\frac{\upsilon^3}{\varepsilon} \right)^{\frac{1}{4}}$$

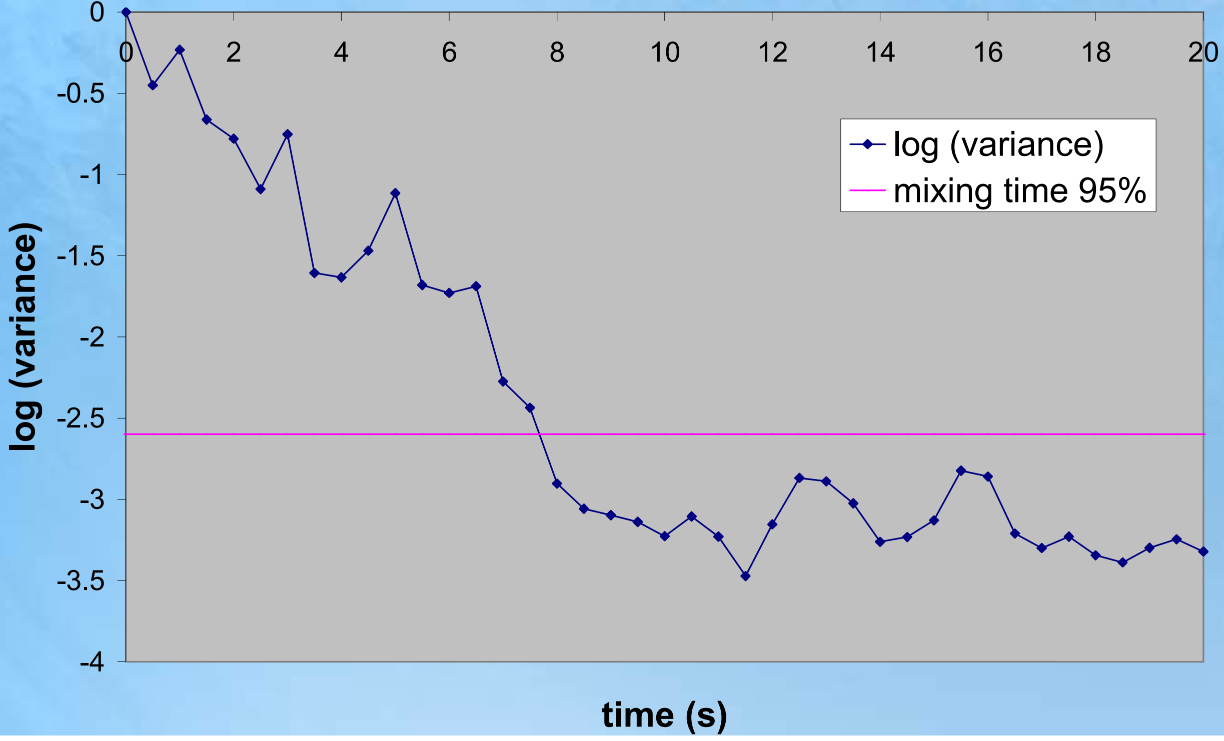
Materials and methods

Exploitation of P.L.I.F. measurements:

a global mixing time of 95% homogeneity by log variance method

(Brown & al, 2004, Handbook of Industrial mixing, Science and Practice, 145-256, John Wiley&Sons Inc.)

$$\log \sigma^2 = \log\left[\frac{1}{\mathrm{R}}\sum\left(\left(\frac{G_i - G_0}{G_\infty - G_0}\right) - 1\right)^2\right]$$



Results

Results division in two parts:

1. **Impellers comparison at the just-suspended rotating speed Njs**

2. Impellers comparison as function of the evolution of their hydrodynamic quantities while the rotating speed increases

Impeller comparison at N_{js}

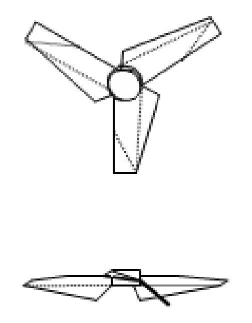
Interest ?

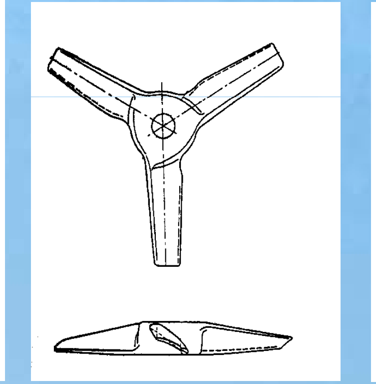
1st agitation goal : keeping microcarriers in complete suspension

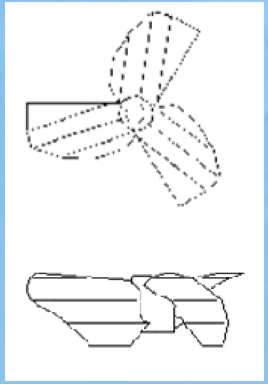
➡ Impellers comparison at the same conditions regarding to microcarrier suspension

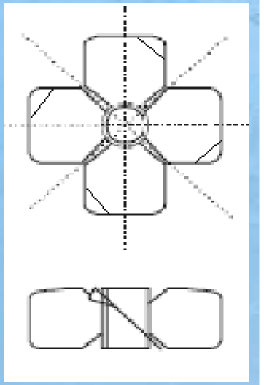
Results:

	N_{js} (rpm)
A315 150	38
TTP 150	40
A310 156	49
TTP 125	50
3SB 160	53
A315 125	54


3SD

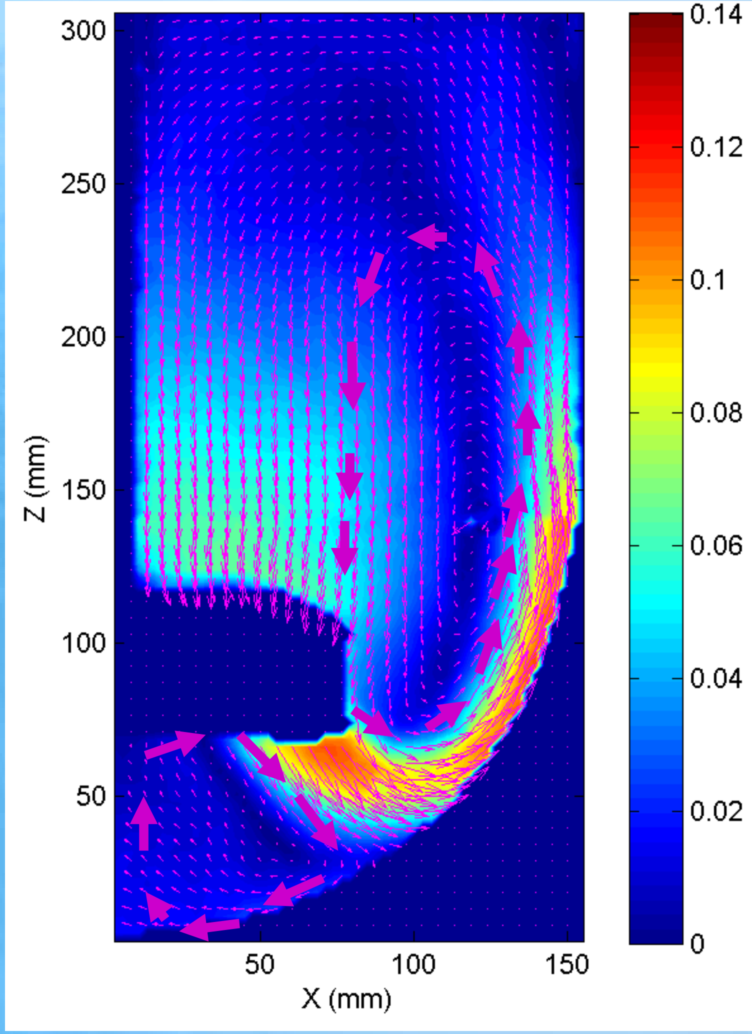

A310


TTP


A315

Impeller comparison at N_{js}

Time average velocity field :



A315 150 at 38 rpm

	N_{js} (rpm)	$V_{average}$ ($m.s^{-1}$)	$V_{90\%}$ ($m.s^{-1}$)
A315 150	38	0.029	0.055
TTP 150	40	0.030	0.055
A310 156	49	0.031	0.06
TTP 125	50	0.027	0.050
3SB 160	53	0.032	0.065
A315 125	54	0.030	0.06

Same hydrodynamic pattern

average and maximum velocity values very close to each other

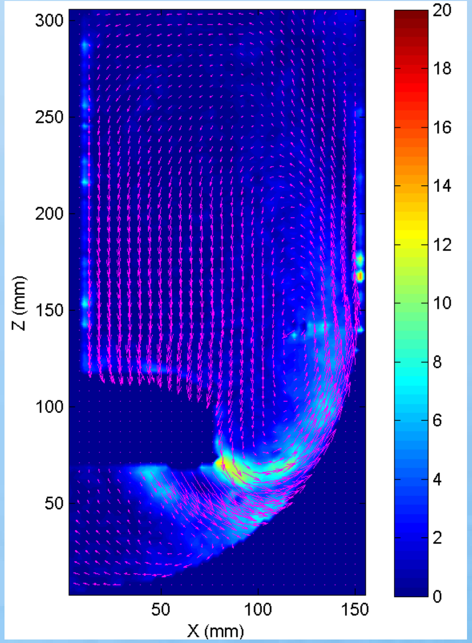
Impeller Comparison at N_{js}

Macro-shearing distributions

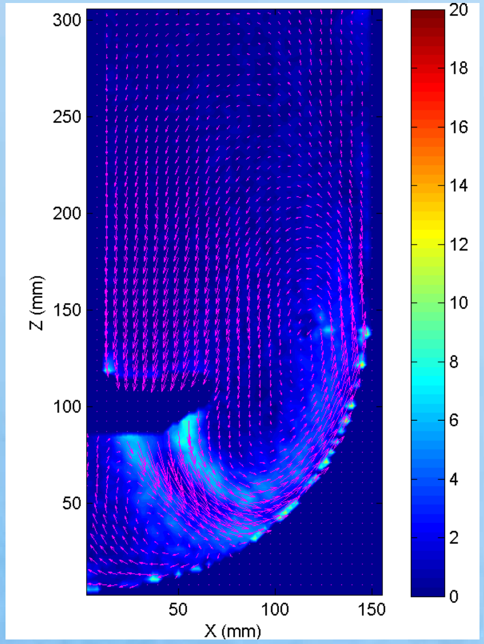
Calculated by: $$\left|\frac{\partial U_x}{\partial z}\right| + \left|\frac{\partial U_z}{\partial x}\right|$$

Comparison criterion

Similar approaches available in the literature (Croughan & al, 1987; Hu,1983; Sinskey & al, 1981)



A315 150 at 38 rpm



TTP 125 at 50 rpm

	TTP 125		A310 156		TTP 150		A315 150		3SB 160		A315 125
$cis_{average}$ (s^{-1})	1.299	<	1.387	<	1.437	<	1.485	<	1.541	<	1.609
$cis_{90\%}$ (s^{-1})	3		3.4		3.4		3.6		3.6		4.2

TTP 125 creates the smallest macro-shearing

Impeller comparison at N_{js}

Rate of energy dissipation and Kolmogorov scale :

Gradients situated outside the measurement plane estimated by supposing an isotropic turbulence

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

But under-estimation of local rate of energy dissipation if

PIV resolution > kolmorogov scale

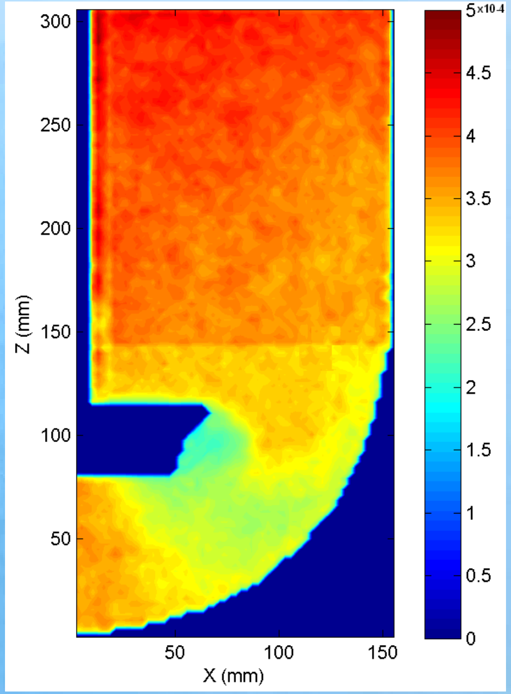
(Baldi & al, 2002, On the measurement of turbulence energy dissipation in stirred vessels with PIV techniques, Proceedings of the 11th International Symposium on Applied Laser Techniques in Fluid Mechanic,Lisbon, Portugal, July 8-11).

⇨ Over-estimation of kolmogorov scale

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

Impeller comparison at N_{js}

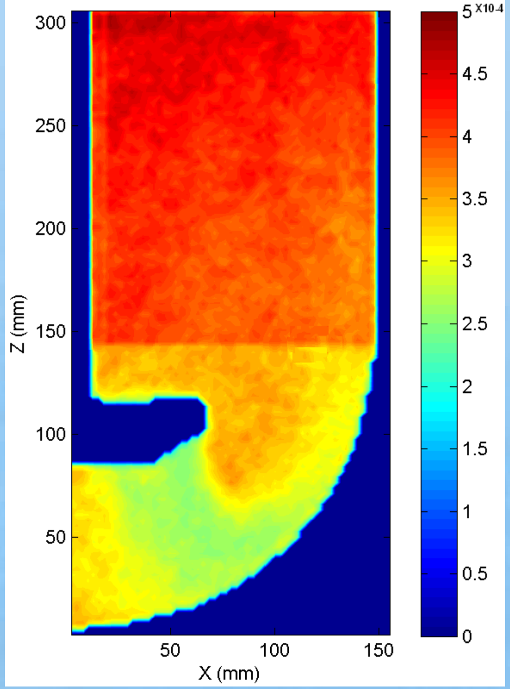
Kolmogorov scale distribution :



Microcarrier

Size (250 µm)

A315 150 à 38 rpm



Microcarrier

Size (250 µm)

TTP 125 à 50 rpm

	TTP 125		A310 156		TTP 150		3SB 160		A315 150		A315 125
Relative size of the area (%) where $\lambda \leq d_{microcarrier}$	0.90	<	1.45	<	1.97	<	3.48	<	3.72	<	5.16

➡ TTP 125 creates the smallest area where the micro-shearing could be high

Impeller comparison at N_{js}

Characterization of the constraints created by collisions:

Based on Cherry et Papoutsakis model (1989):

Turbulent Collision Severity

$$TCS\,[W] = \frac{\text{kinetic energy of the interaction }[\text{J}] \times \dfrac{\text{interaction frequency}}{\text{volume}}\left[{}^{\text{s}^{-1}}\!/_{\text{m}^{3}}\right]}{\text{microcarrier concentration}\left[\text{m}^{-3}\right]}$$

$$TCS_s = \left(\frac{P/V}{\mu}\right)^{3/2} \cdot \left(\frac{\pi^2 \cdot \rho_S \cdot d_p^5 \cdot \varepsilon_S}{72}\right)$$

Impeller Collision Severity

$$ICS = \frac{\text{kinetic energy}}{\left[\dfrac{\text{reactor volume}}{\text{(window area)(velocity past blade)}}\right]}$$

$$ICS = \frac{9 \cdot \pi^4 \cdot \rho_S \cdot n_B \cdot N^3 \cdot d^4 \cdot d_P^4}{512 \cdot V}$$

Classification :

TCS TTP 125 < TTP 150 < 3SB 160 < A310 156 < A315 125 < A315 150

ICS TTP 125 < TTP 150 < A315 150 < A315 125 < A310 156 < 3SB 160

➡ TTP 125 creates the smallest mechanical constraints due to collisions

Impellers Comparison at N_{js}

Mixing time:

Obtained from P.L.I.F measurements :

Mixing time (s)	3SB 160		A315 150		A310 156		A315 125		TTP 150		TTP 125
	18	<	20	<	22	<	23	<	23	<	33

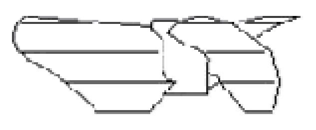
➡ TTP 125 creates the highest mixing time

➡ But small in comparison to the response time of cell metabolism to a perturbation of their environment ~ 1 hour

Impellers Comparison at N_{js}

Conclusion on the impeller comparison at Njs

Choice of the impeller TTP 125 in view of its characteristics :



- the smallest macro-shearing;
- the smallest area where micro-shearing could be high;
- the smallest mechanical constraints due to collisions;
- the highest mixing time but small in comparison to the response time of cell metabolism.

Results

Results division in two parts:

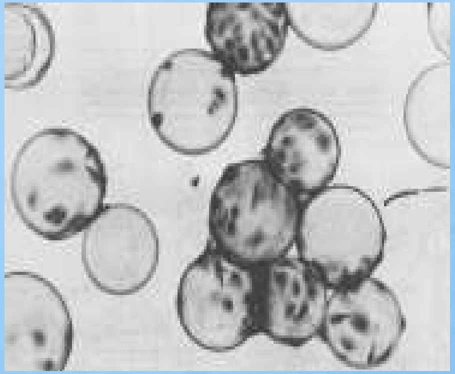
1. Impellers comparison at the just-suspended rotating speed Njs

2. **Impellers comparison as function of the evolution of their hydrodynamic quantities while the rotating speed increases**

Variation of quantities as function of N

Interest ?

❖ Formation of 3 beads agglomerate on average during the cell culture



(Cherry & Papoutsakis, 1988)

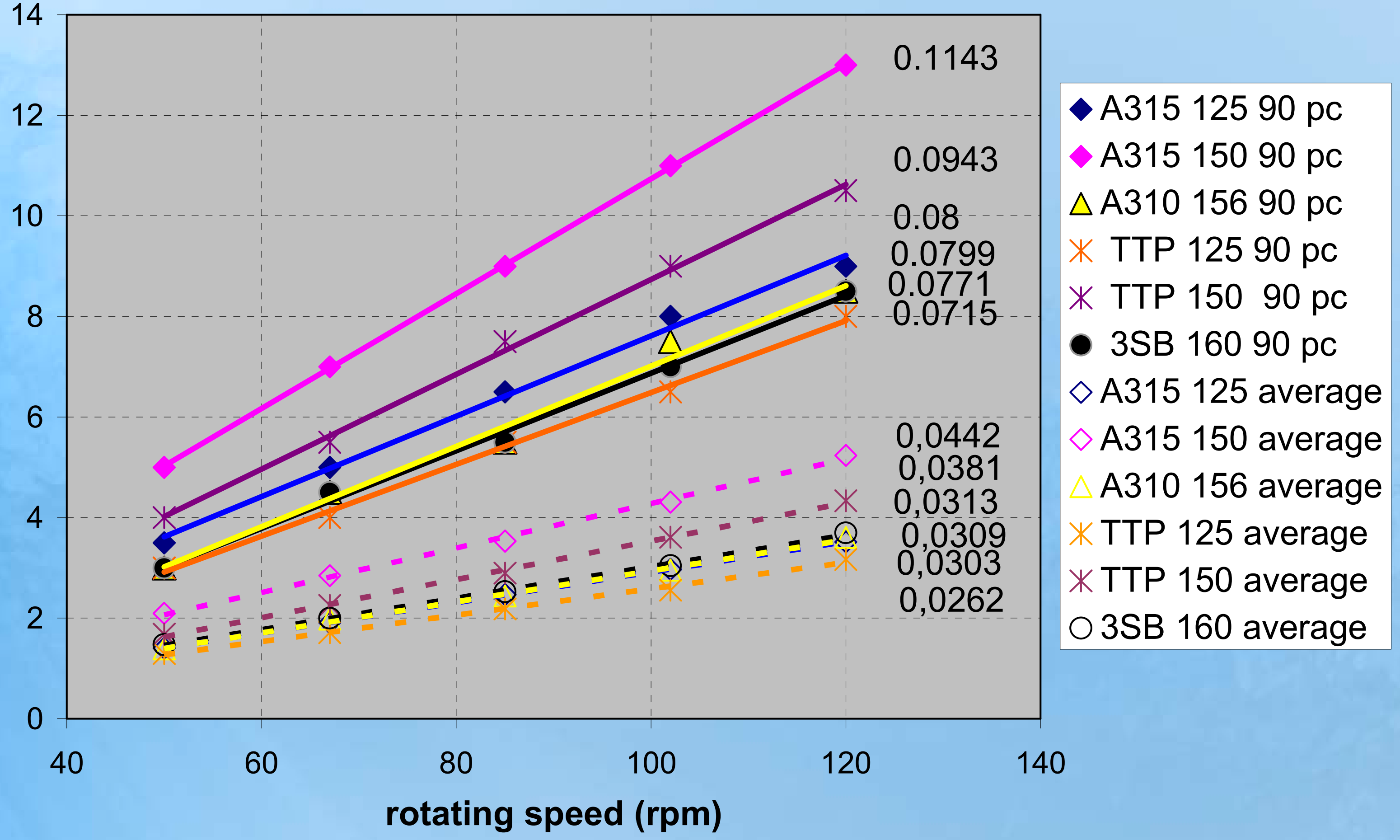
❖ Aeration not taken into account

➡ N_{js} insufficient

➡ Interest of knowing the quantity variations with the rotating speed

Variation of quantities as function of N

Macro-shearing distribution (s^{-1}) :

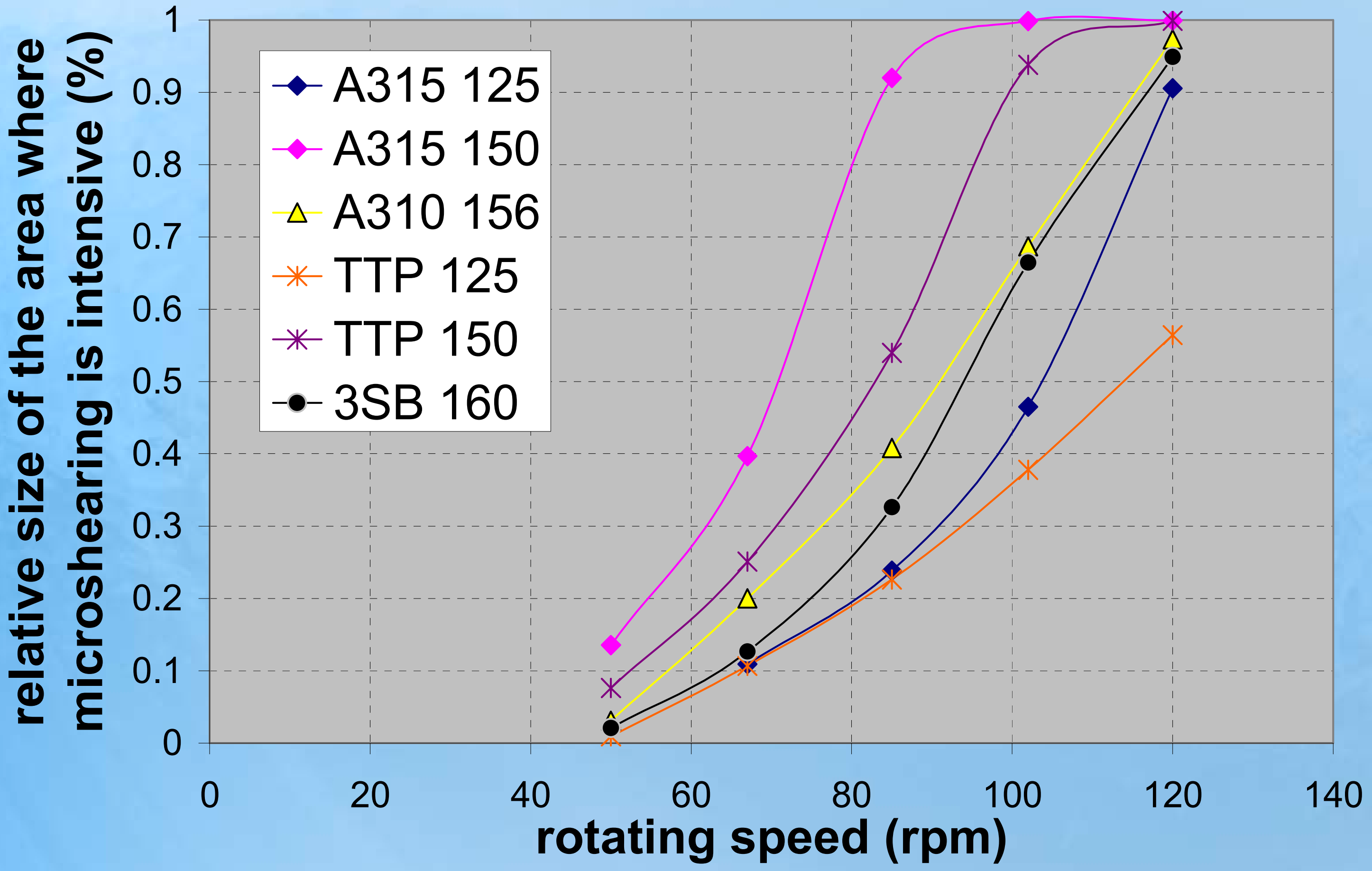


Linear evolution with the impeller rotating speed

Smallest increase (slope of the straight line) associated to the impeller TTP 125

Variation of quantities as function of N

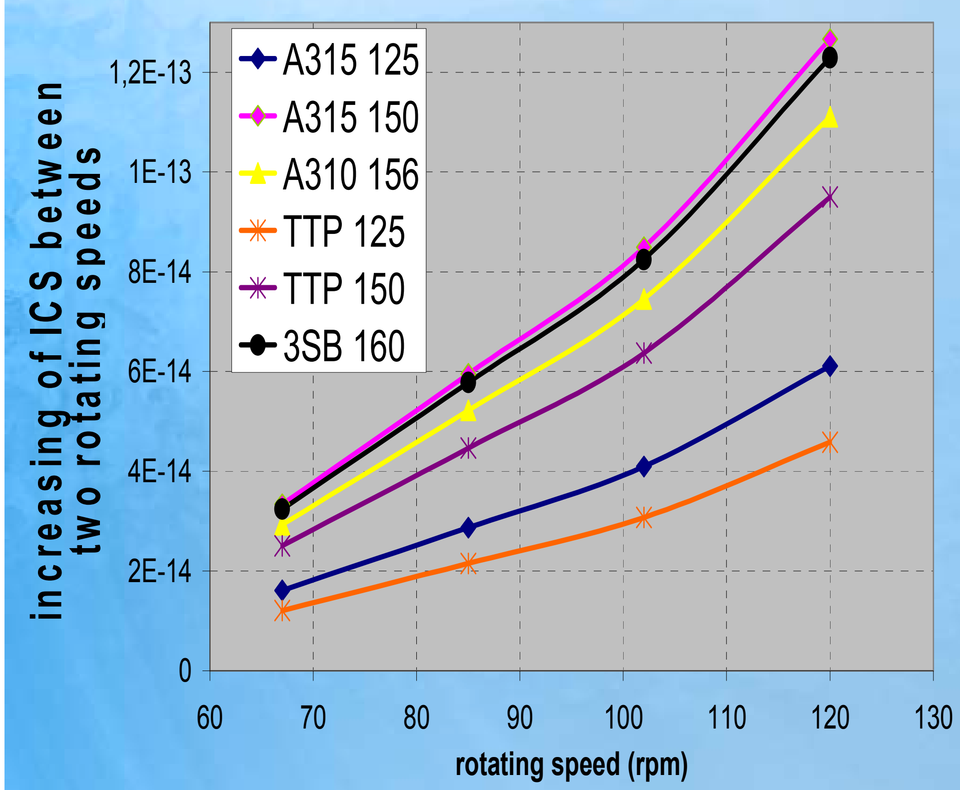
Micro-shearing :

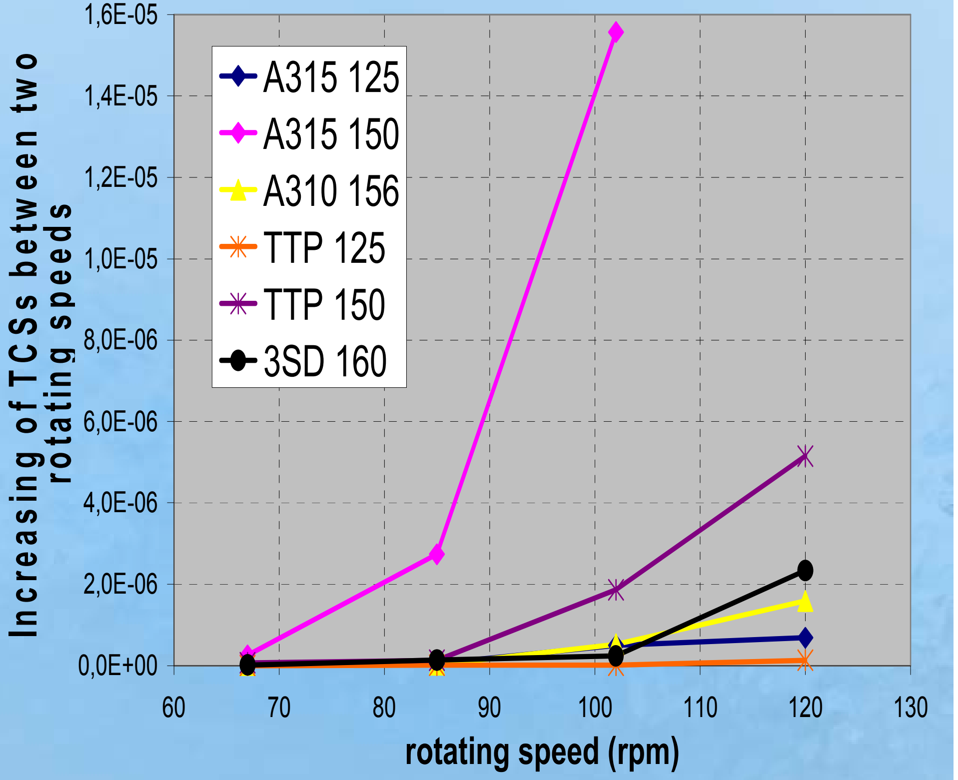


Smallest increase of the area where the micro-shearing could be high associated to the impeller TTP 125

Variation of quantities as function of N

Mechanical constraints due to collisions :

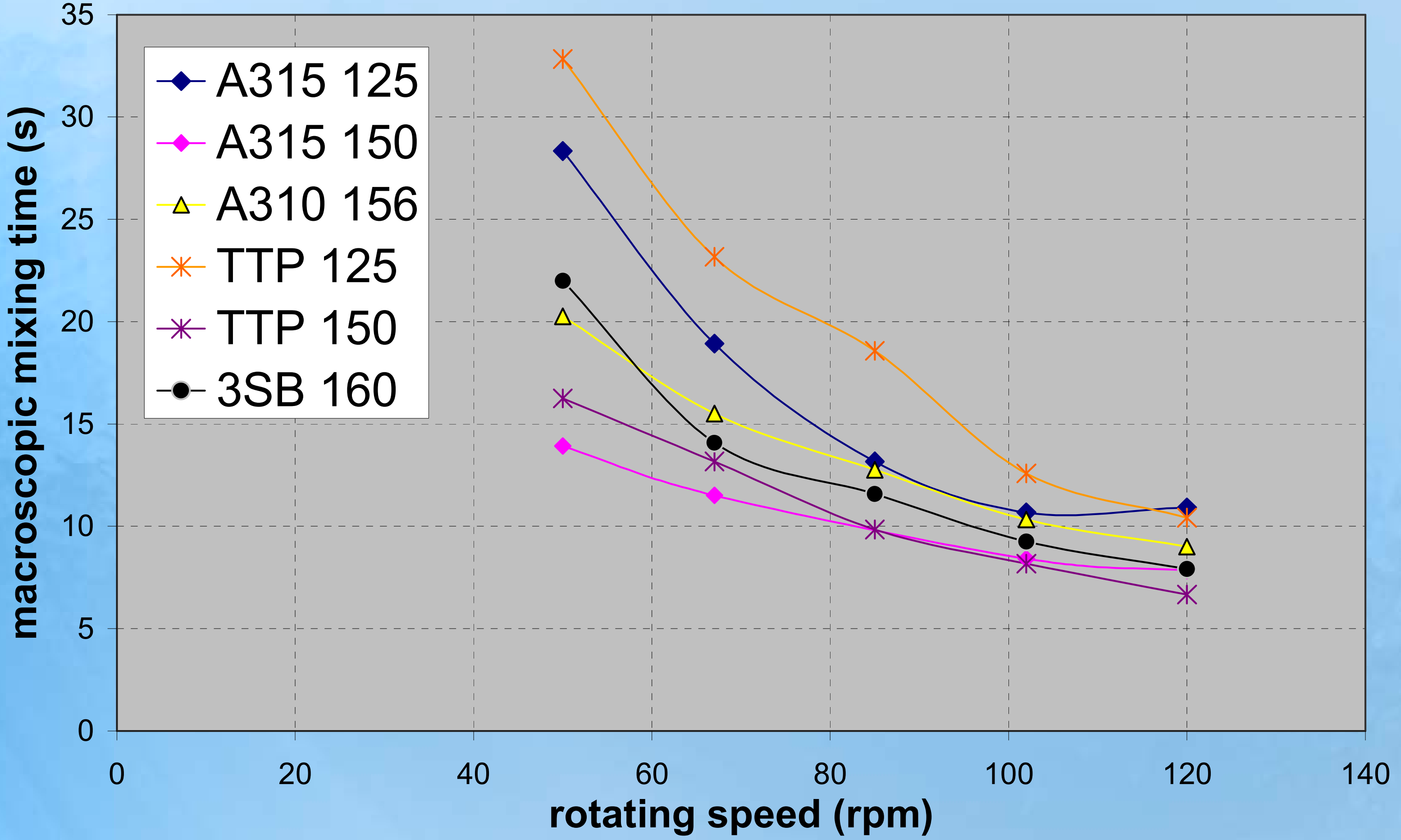




Smallest increase of mechanical constraints associated to the impeller TTP 125

Variation of quantities as function of N

Mixing time:

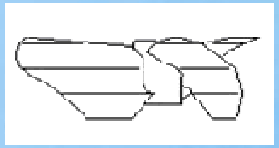


Highest decrease associated at the impeller TTP 125

Variation of quantities as function of N

Conclusions on that part of the results

Favourable evolution of hydrodynamic quantities of the impeller TTP 125 :



- Smallest increase of macro-shearing
- Smallest increase of micro-shearing
- Smallest increase of mechanical constraints due to collisions
- Highest decrease of mixing time

Conclusions

In summary:

Goals : 1) Study the influence of agitation conditions on cell local environment

2) Determine the optimal agitation conditions

Tools Use of P.I.V. and P.L.I.F. techniques to compare 6 impellers

Results 1) Impellers comparison at Njs

Choice of the impeller TTP 125: smallest mechanical constraints

mixing time < metabolism response time

2) Impellers comparison when the rotating speed increases

Choice of the impeller TTP 125: smallest increase of mechanical constraints

highest decrease of mixing time

Conclusions

→ **Goals achieved regarding to impeller selection**

not achieved regarding to rotating speed choice

Futures:

Improvement of the knowledge on animal cell behaviour

1. Determination of the animal cell resistance to hydrodynamic constraints
2. Experiment these agitation conditions on animal cell cultures

Improvement of measurement techniques

1. Use of 3-D PIV to obtain the 3rd velocity component
2. Refining the Kolmogorov scale measurements

Acknowledgements

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I thank the society GlaxoSmithKline Biologicals for the fruitful collaboration

Thank you for your attention