Fluid flow and mixing dynamics in a shaken bioreactor with flat and conical bottom

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Aims and Objectives

The project aims at investigating the mixing and flow dynamics induced in cylindrical shaken bioreactors with different bottoms to obtain new insight on the type, nature and occurrence of flow transitions and instabilities and to evaluate the impact of operating conditions and mixing environment on GS-CHO expression system performance.

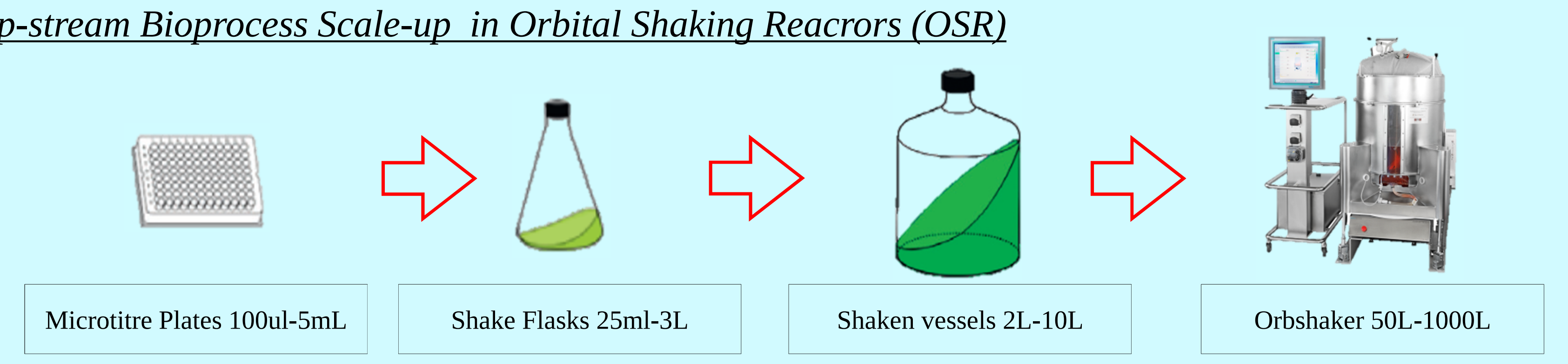
Project objectives

- Mixing time
  - Dual Indicator System for Mixing Time (DISMT, Melton, 2002)
  - Different operating parameters and geometrical conditions
- Particle Image Velocimetry (PIV) and Planar Laser Induced Fluorescence (pLIF)
  - Study of fluid mixing in model systems
  - Water-like and viscous liquids with different operating parameters
- Define and evaluate scale-up criteria for suspension and adherent large scale cell culture processes.

Figure 1. Kuhner SB200-X (OrbShake).
Website: www.kuhner.com accessed 10/04/15

Project Background

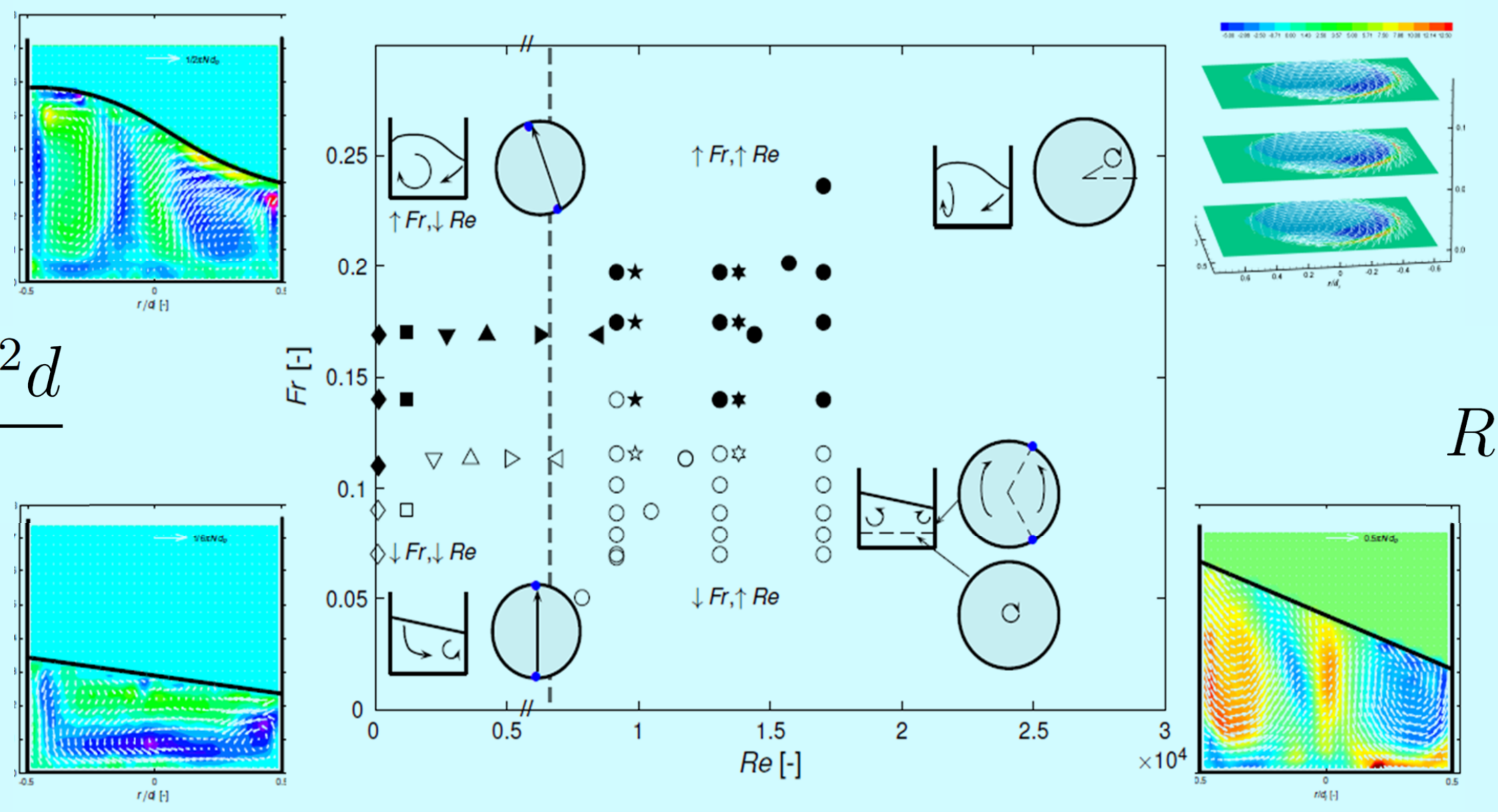
Up-stream Bioprocess Scale-up in Orbital Shaking Reacrors (OSR)



Microtitre Plates 100ul-5mL | Shake Flasks 25ml-3L | Shaken vessels 2L-10L | Orbshaker 50L-1000L

Mean Flow Transition, (Weheliye et al 2013, Ducci et al. 2014)

$$Fr = \frac{2(\pi N)^2 d}{g}$$

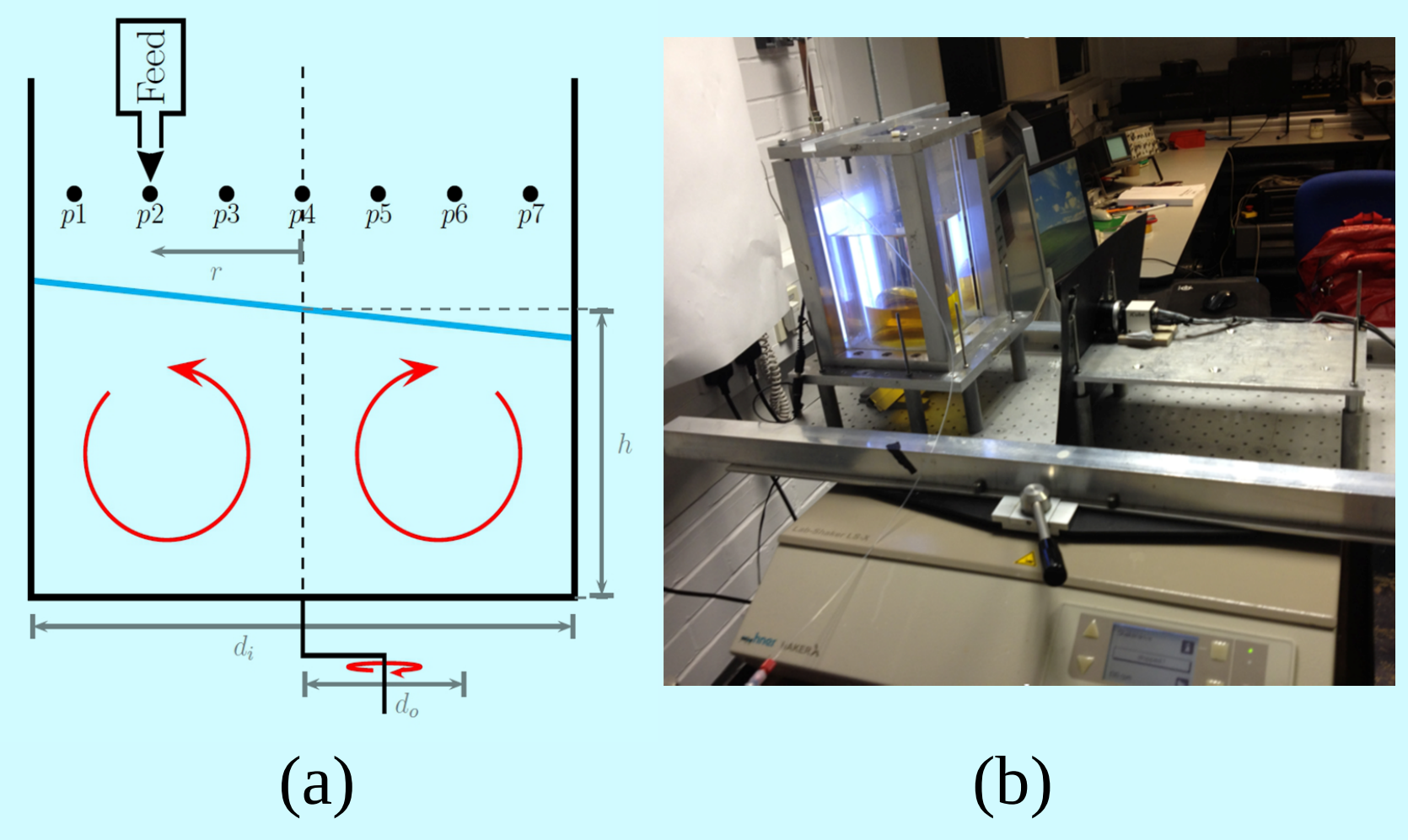


$$Re = \frac{\pi N d_o d_i}{\nu}$$

Scaling and feed insertion strategy for macro-mixing

Methodology

- Cylindrical flat-bottom vessel, d_i=10 cm, d_o=2.5 cm
- Colour camera mounted on the shaker table
- Phase resolved measurements (Encoder)
- Seven insertion points at different radial locations
- Dual Indicator System for Mixing Time (DISMT) based on fast acid-base reaction in presence of pH indicators



(a) (b)

Figure 2. a) Base feed radial locations;
b) Experiment set up.

Image processing software

- **Normalisation** of green channel intensity, *G*, in each pixel (i, j)
- **Method A**: Mixing Time was measured as the time taken for the standard deviation of G^* to reach within 5% of the steady state value.
- **Method B**: Mixing Time measured as the time taken for 95% of pixels within the control volume to reach within 5σ of mixed *G* value.

$$G_{(i,j)}{}^{*} = \frac{G_{(i,j)_t} - G_{(i,j)_0}}{G_{(i,j)_\infty} - G_{(i,j)_0}}$$

	Method	*Mixing Condition*
A	$\sigma(t) = std\left(G^*_{ij}(t)\right)$	$\sigma(t_m) < \sigma(t_\infty) \times 1.05$
B	$N(t): \ \left\lvert G^*_{ij}(t) - 1 \right\rvert < 5\sigma_{ij}(t_\infty)$	$N(t_m)/N_{tot} = 0.95$

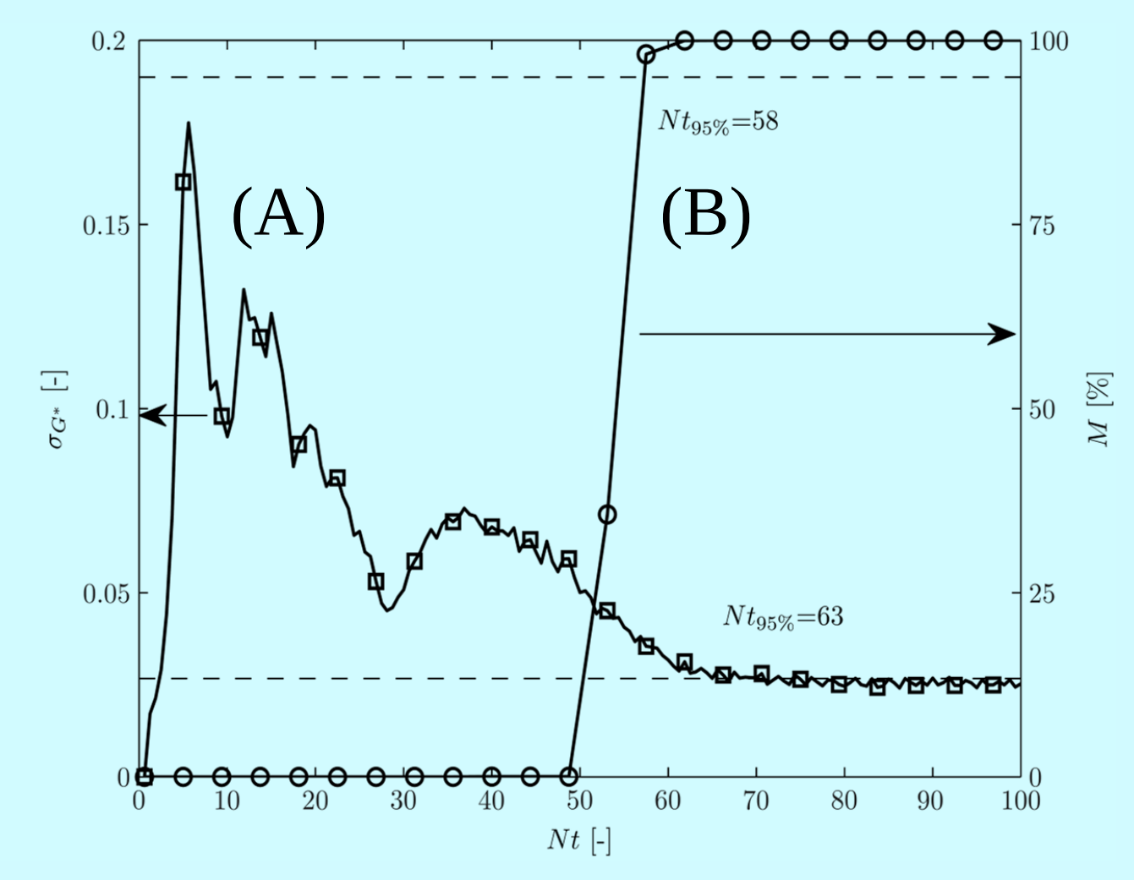


Figure 4. a) Methods A (☐) and B (○).

Macro-mixing map and effect of feed locations

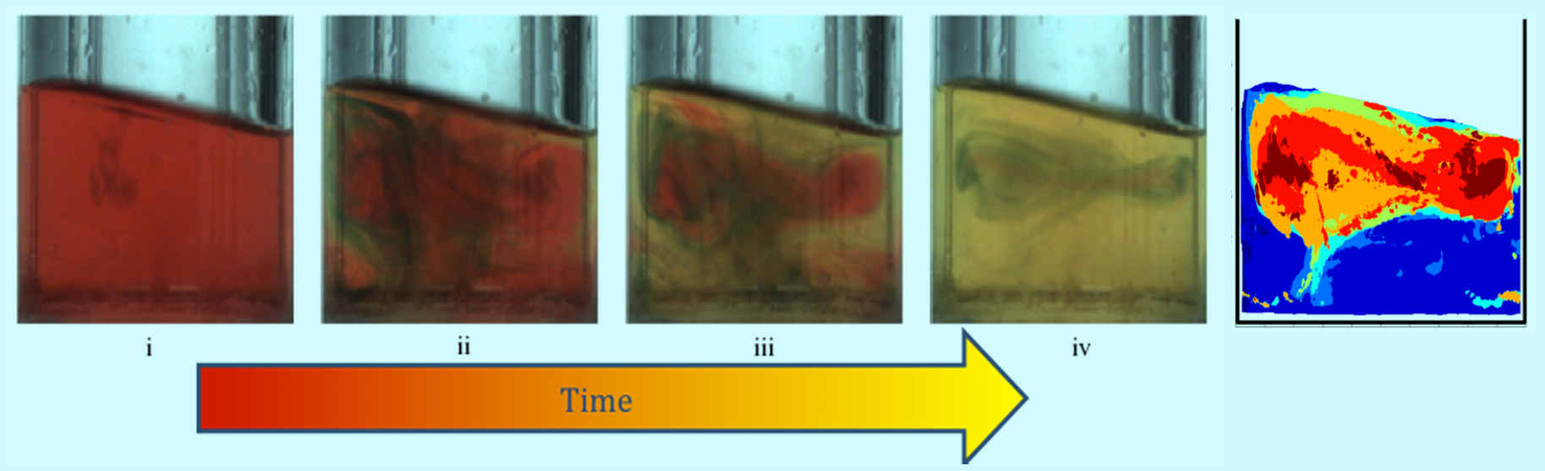


Figure 5. Before flow transition, P3, *N*=90 RPM (*Fr*=0.12).

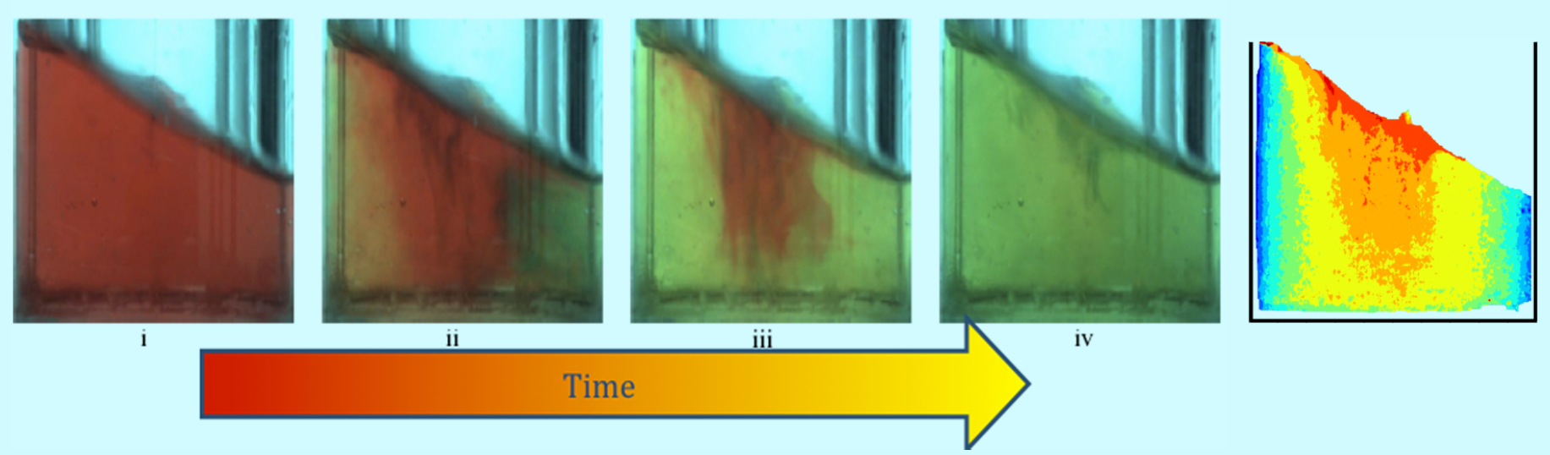


Figure 6. After flow transition, P4, *N*=130 RPM (*Fr*=0.24).

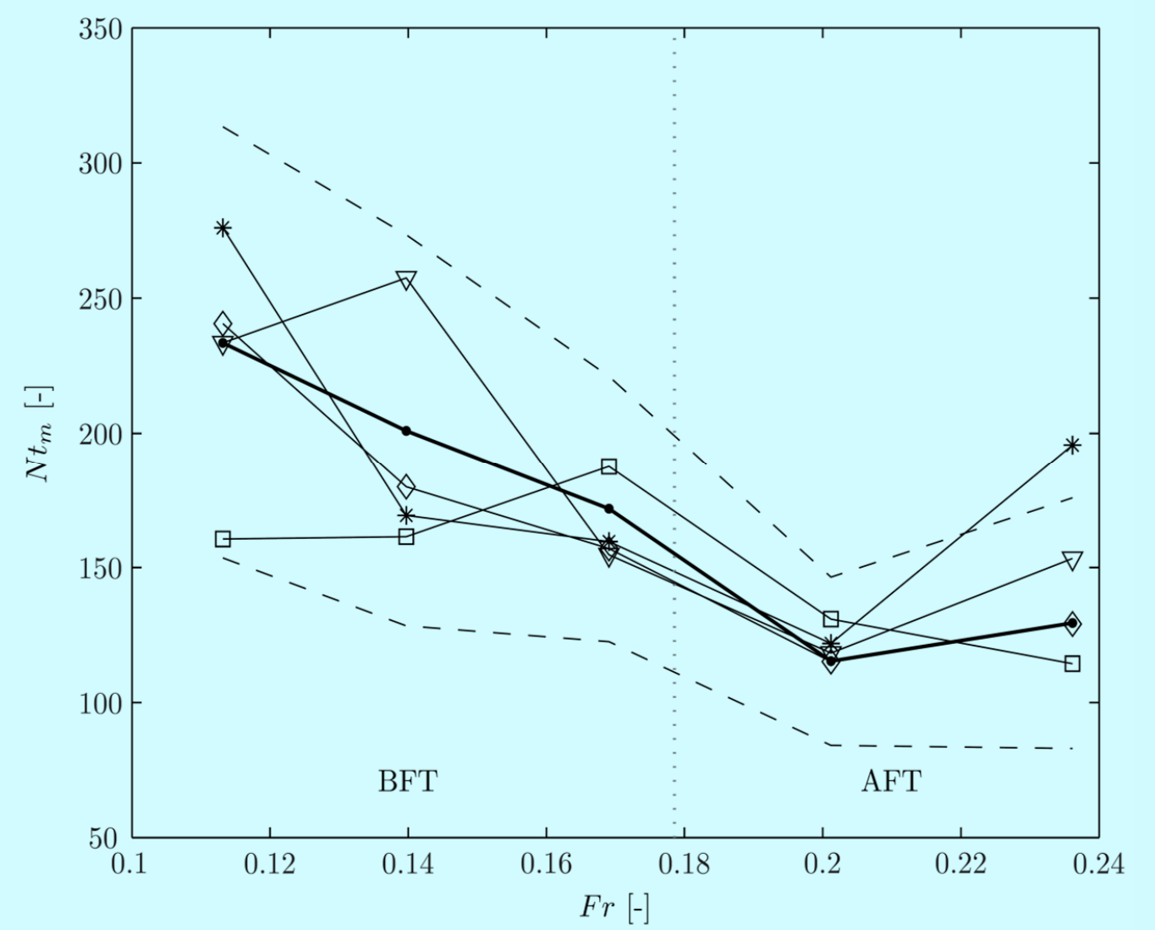
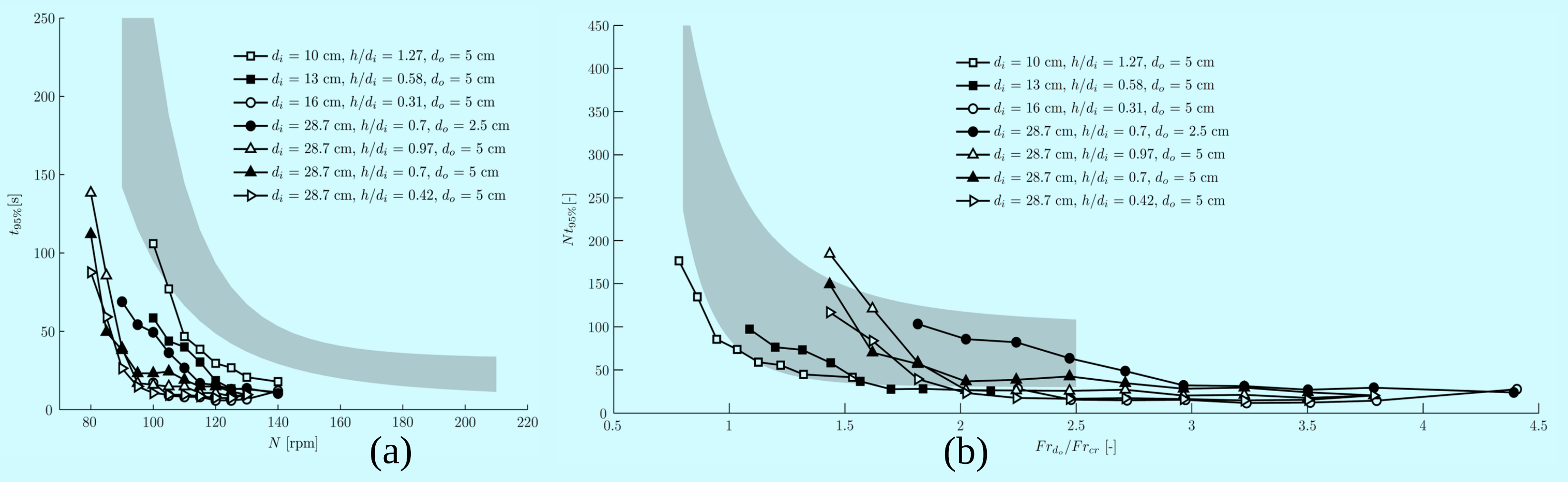


Figure 7. Mixing number for different feed location P1=◊, P2=▽, P3=☐, P4=*.

Mixing time scaling, (Rodriguez et al. 2014)



(a) (b)

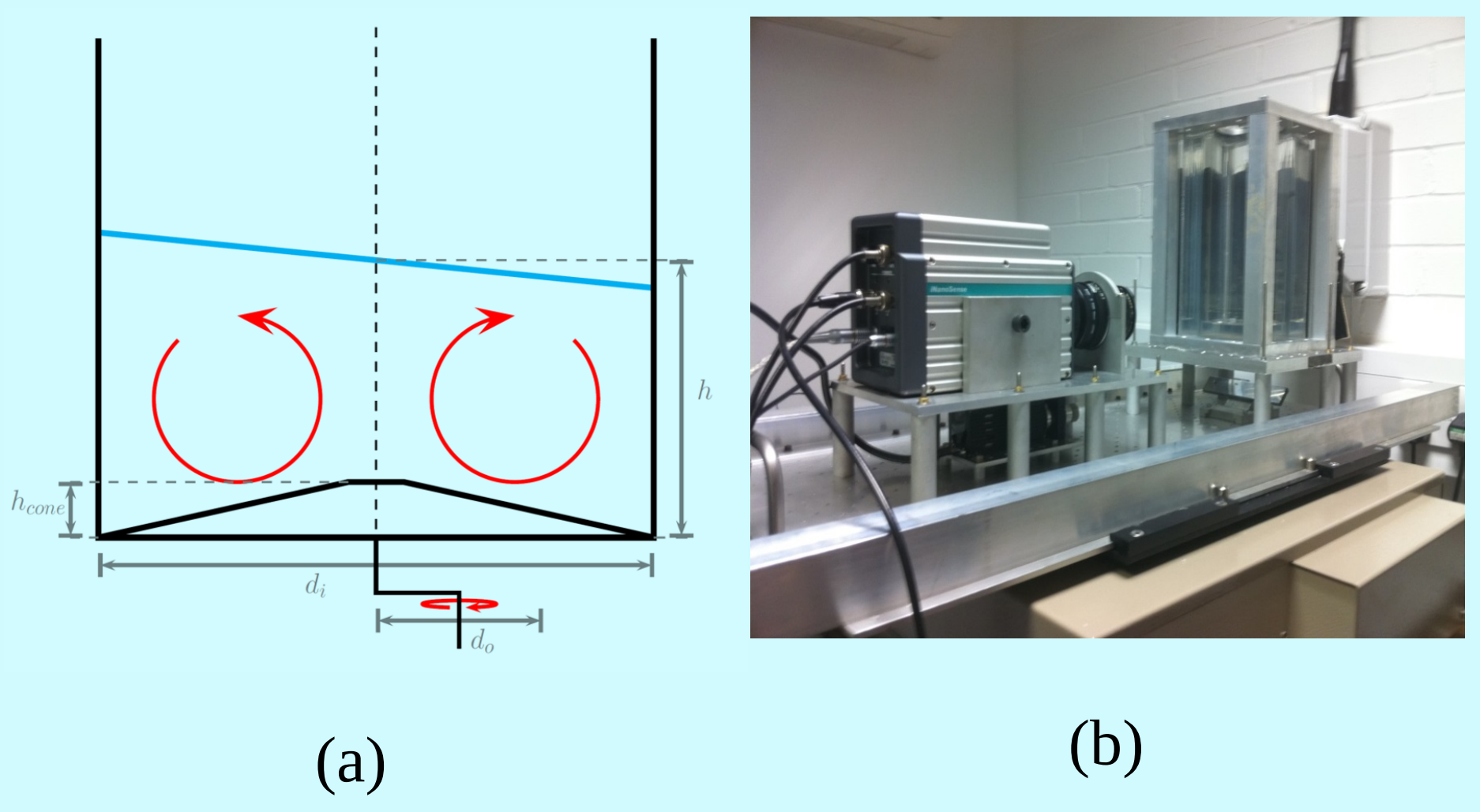
Figure 6. Fig. 9. Data comparison between *Rodriguez 2014* (shaded gray) and Tissot (2010). a) dimensional, b) non dimensional with scaling factor Fr_{do}/Fr_{cr}

Effects of conical bottom on flow dynamics

Methodology

- PIV system, phase resolved measurements at φ=0.
- Experimental conditions investigated:

Bottom geometry	d_i, [cm]	h_{cone} [cm]	d_o, [cm]	h, [cm]	N [RPM]
Flat	10	0	25	5	80-130
Conical A	-	0.5	-	5.2	-
Conical B	-	1.5	-	5.6	-



(a) (b)

Figure 9 a) Cross-sectional view of the cylindrical bioreactor and conical bottom; b) Experimental rig with vessel, laser and camera secured to the shaker

Flow field and tangential vorticity

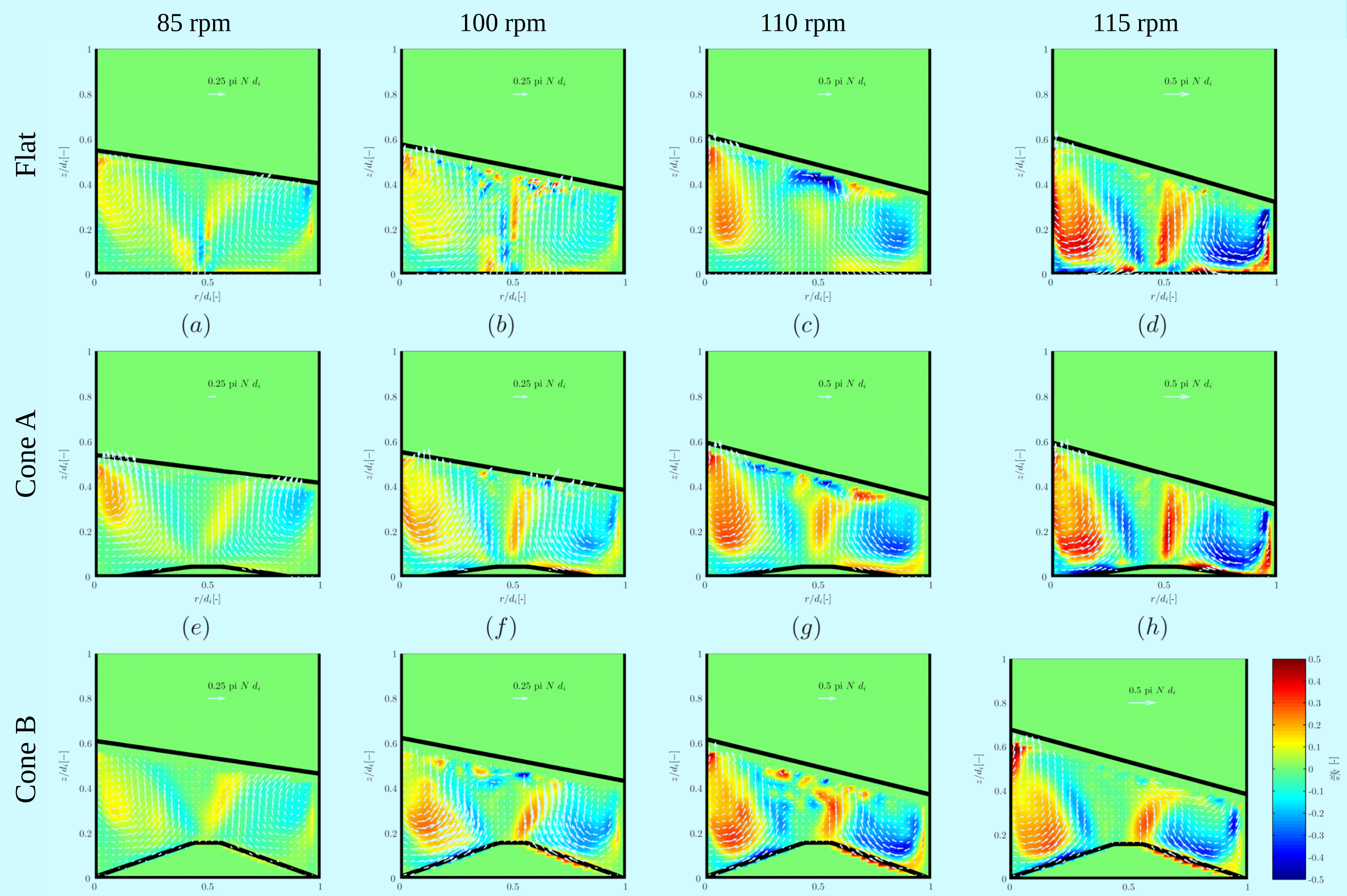
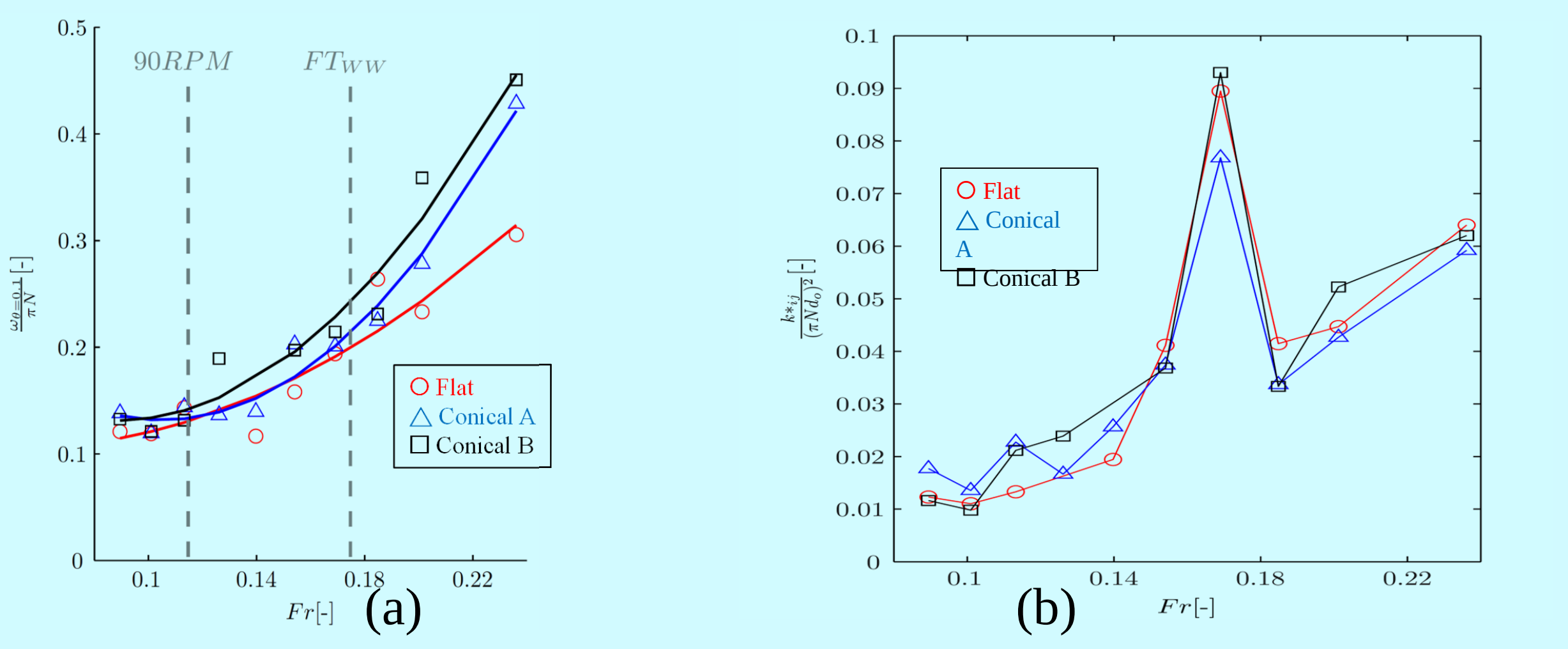


Figure 10. Comparison of the flow for different geometries and *N*.

- Counter-rotating toroidal vortices expanding towards the bottom
- Vortices reach bottom at lower speed for higher conical bottoms
- Vortices are more stable for higher conical bottoms (i.e. flow transition is not affected)

Spaced averaged vorticity and kinetic energy



(a) (b)

Figure 11 a) Spaced-averaged non dimensional vorticity increases at greater rate for conical B. b) Spaced-averaged non dimensional kinetic energy, k_{ij}*, shows that Fr_{CR} remains unchanged for all bottoms

Conclusions

- Improved mixing efficiency feed insertion close to vessel walls
- Scaling law based on critical Froude number
- Toroidal vortices more stable as the inclination of conical bottom is increased
- *Fr* associated with flow transition does not change between bottoms
- Conical bottom enhances the space-averaged vorticity
- Suspension of cell and microcarrier culture can benefit from greater circulation at lower *N*

ACKNOWLEDGEMENTS

The authors would like to thank the Engineering and Physical Sciences Research Council and *KühnerAG* for providing funding for the current work in the form of a studentship..

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