# Disposable orbital shake bioreactor system from ml to 1000 Liter for cell culture - from concept to reality

**Florian M. Wurm[#*]**, Maria De Jesus[*], Markus Kühner[&]

[#] Laboratory of Biotechnology, Swiss Federal Institute of Technology Lausanne (EPFL), Switzerland

[*] ExcellGene SA, Monthey, Switzerland

[&] Kuhner AG, Birsfelden Switzerland

# Recombinant biopharmaceuticals made by mammalian cells in stirred (stainless steel) bioreactors

**Revenue of the Top 19 Protein-Biotech Drugs in 2008**

| Drug name (maker) | Indication | Revenue for 2008 |
|---|---|---|
| 1. **Enbrel** (Amgen) | Rheumatoid arthritis, psoriatic arthritis, ankylosing spondylitis, plaque psoriasis | $5,982M |
| 2. **Rituxan** (Genentech) | Non-Hodgkin's lymphoma, rheumatoid arthritis | $ 5,082M |
| 3. **Humira** (Abbott) | Rheumatoid arthritis, psoriatic arthritis | $4,521M |
| 4. **Avastin** (Genentech) | Colorectal cancer, non-small-cell lung cancer | $ 4,479M |
| 5. **Herceptin** (Genentech) | Breast cancer | $ 4,394M |
| 6. **Remicade** (J&J) | Crohn's disease, ankylosing spondylitis, arthritis, ulcerative colitis, rheumatoid arthritis, plaque psoriasis | $ 3,748M |
| 7. **Neulasta** (Amgen) | Infection associated with chemotherapy-induced-Neutropenia | $3,318M |
| 8. **Lantus** (Sanofis-Aventis) | Types I and II diatbetes | $ 3,159M |
| 9. **Aranesp** (Amgen) | Anemia | $ 3,137M |
| 10.**Prevnar** (Wyeth) | Prevention of diseases caused by S.pneumoniae | $2,716M |
| 11.**Procrit/Eprex** (Ortho-biotech) | Anemia | $ 2,460M |
| 12.**Epogen** (Amgen) | Anemia | $ 2,456M |
| 13.**Avonex** (Biogen Idec) | Multiple sclerosis | $ 2,203M |
| 14.**Truvada**(Gilead Sciences) | HIV | $ 2,110M |
| 15.**Lucentis** (Genentech) | Wet age-related macular degeneration | $ 1,761M |
| 16.**Humalog** (Eli Lilly) | Diabetes | $ 1,736M |
| 17.**Rebif (** Merck Serono) | Multiple sclerosis | $ 1,668M |
| 18.**Erbitux** (ImClone) | Colorectal cancer, head and neck cancer | $ 1,457M |
| 19.**Betaseron** (Bayer Schering) | Multiple sclerosis | $ 1,439M |
| *Source: BioWorld research from company press releases and SEC filings* | | |

**57 bio US $/year**

# Disposable bioreactors (>1 Liter)

- according to the story as told by Dr. V. Singh, the concept for the most famous disposable bioreactor, the Wave bioreactor, was developed in 1996.

## *The* Wave Bioreactor *Story*

by Vijay Singh Ph.D

The Wave Bioreactor® was conceived in August 1996 on a long Cathay Pacific flight from Sydney to New York. .....

(article can be downloaded from the internet via GE healthcare)

......but there were also a few other people thinking about simplifying and streamlining cell culture based manufacturing applying single use bioreactor systems

---

## ml scale to liter scale in single use* reactors

| | |
|---|---|
| 1999 | Girard, P., Wurm, F.M.: **Small Scale Bioreactor System for Process development and Optimisation**. *Genetic Engineering News*, 19, No 15, P 46.. |
| 2001 | P. Girard, M. Jordan, M. Tsao, Wurm, F.M. (2001) **Small scale bioreactor system for process development and optimization.** *Biochem. Eng. J.* 7 (2), pp.117-119. |
| 2004 | De Jesus, M., Girard, P., Bourgeois, Baumgartner, M., Jacko, G., Amstutz, H. and Wurm, F.M. (2004) **Tubespin satellites: a fast track approach for process development with animal cells using shaking technology.** *Biochemical Engineering Journal* 17 (3), pp. 217-223. |
| 2005* | Muller, N., Girard, P., Jordan, M. , Wurm, F.M. (2005) : **Orbital shaker technology for the cultivation of mammalian cells in suspension.** *Biotechnol. Bioeng.* 89 (4), pp. 400-406. |

* or simple, easy to clean bottles

# Literature on orbitally shaken bioreactors

> 1ml

5ml-30 ml

50 ml- 2 L

2 L- 10 L

20 L- 1000 L


1. P. Girard, M. Jordan, M. Tsao, Wurm, F.M. (2001) **Small scale bioreactor system for process development and optimization.** *Biochem. Eng. J.* 7 (2), pp.117-119.
2. **De Jesus,** M., Girard, P., Bourgeois, Baumgartner, M., Jacko, G., Amstutz, H. and Wurm, F.M. (2004) **Tubespin satellites: a fast track approach for process development with animal cells using shaking technology.** *Biochemical Engineering Journal* 17 (3), pp. 217-223.
3. Muller, N., Girard, P., Jordan, M. , Wurm, F.M. (2005) : **Orbital shaker technology for the cultivation of mammalian cells in suspension.** *Biotechnol. Bioeng.* 89 (4), pp. 400-406.
4. N. Muller, M. Derouazi, F. Van Tilborgh, S: Wulhfard, D.L. Hacker, M. Jordan and F.M. Wurm, (2007) : **Scalable transient gene expression in Chinese hamster ovary cells in instrumented and non-instrumented cultivation systems,** *Biotechnol Lett,* 29 (5), pp. 703-11
5. **Stettler, M.**, Zhang, X., Hacker, D.L., De Jesus, M., and Wurm, F.M. (2007). **Novel orbital shake bioreactors for transient production of CHO derived IgGs.**. *Biotechnol. Prog.* 23 (6), pp. 1340-1346.
6. **Zhang, X.**, Stettler, M., Reif, O., Kocourek, A., DeJesus, M., Hacker, D.L., and Wurm, F.M. (2008). **Shaken helical track bioreactors: providing oxygen to high-density cultures of mammalian cells at volumes up to 1000 liters by surface aeration with air**. *New Biotechnol.* 25 (1), pp. 68-75.
7. Zhang, X., Stettler, M, De Sanctis, D., Perrone, M., Parolini, N., Discacciati, M., De Jesus, M. D. Hacker, Quarteroni, A., F. Wurm (2008) **Use of Orbital Shaken Disposable Bioreactors for Mammalian Cell Cultures from the Milliliter-Scale to the 1000-Liter Scale** Adv. Biochemical Engineering/Biotechnology DOI:10.1007/10_2008_18.
8. Zhang, X., Bürki, C.-A., Stettler, M., De Sanctis, D., Perrone, M., Discacciati, M., Parolini, N., De Jesus, M., Hacker, D.L., Quarteroni, A., and Wurm, F.M. (2009). **Efficient oxygen transfer by surface aeration in shaken cylindrical containers for mammalian cell cultivation at volumetric scales up to 1000 L.** Biochemical Eng. J. doi: 10.1016/j.bej.2009.02.003

# Professor Jochen Büchs, RWTH, Aachen

Büchs J. 2001. Introduction to advantages and problems of shaken cultures. Biochem Eng J 7(2):91–98.

Büchs J, Zoels B. 2001. Evaluation of maximum to specific power consumption ratio in shaking bioreactors. J Chem Eng J 34(5):647–653.

Büchs J, Maier U, Milbradt C, Zoels B. 2000a. Power consumption in shaking flasks on rotary shaking machines: I. Power consumption measurement in unbaffled flasks at low liquid viscosity. Biotechnol Bioeng 68(6):589–593.

Büchs J, Maier U, Milbradt C, Zoels B. 2000b. Power consumption in shaking flasks on rotary shaking machines: II. Nondimensional description of specific power consumption and flow regimes in unbaffled flasks at elevated liquid viscosity. Biotechnol Bioeng 68(6):594–601.

# Dr. Chao-Min Liu

(Distinguished Research Leader, Roche, Nutley)

Cultivation of recombinant mammalian cells in shaking vessels of 20-36 L working volume (vessel volume 50 Liters)

(Biochemical Engieering Journal 7 (2001) 121-125)

# Orbitally Shaken Bioreactors for Mammalian Cell Culture

50 mL

De Jesus et al., 2004

1 L

Muller et al., 2005

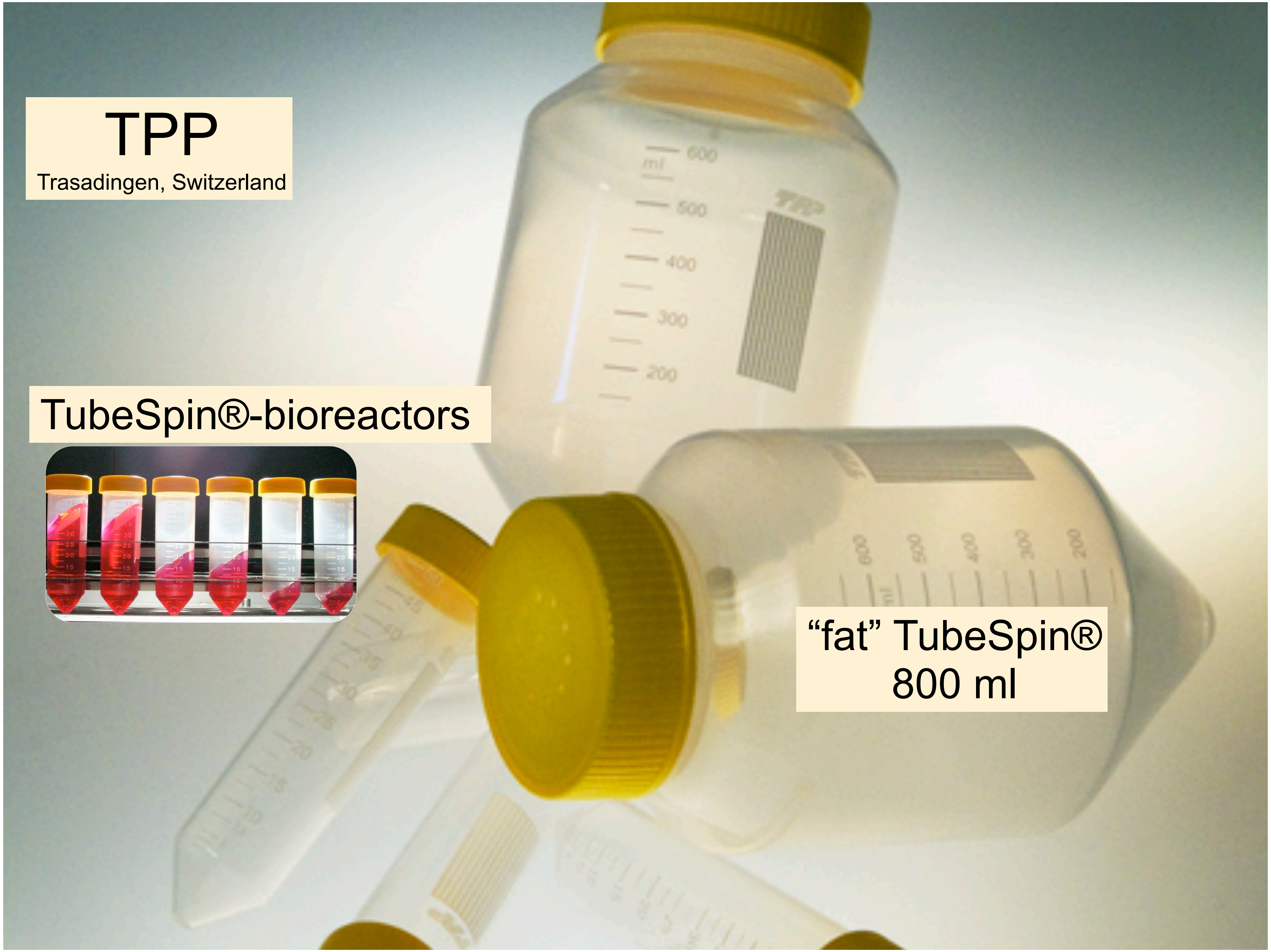
TubeSpin®-bioreactors
(invented by Dr. De Jesus, 2000)

200 L 2000 L


Stettler,2007

20 L 50 L

Stettler et al., 2007


TPP
Trasadingen, Switzerland
TubeSpin®-bioreactors
“fat” TubeSpin®
800 ml

# What are the benefits of disposable bioreactors?

- low upfront investment
- fast set-up
- low maintenance
- low cost? (short-term/long-term)
- low(er) human resource use ?
- low(er) risk in contaminations

# Some disposable bioreactors in the industry (> 10 L)

XDR-2000, *Xcellerex (2000L)*


BIOSTAT® CultiBag RM, *Sartorius-Stedim (50 L)*


CellMaker, *Cellexus (50L)*



11

# Disposable bioreactors in the industry

*three different mixing systems*

Stirred

XDR-2000, *Xcellerex (2000L)*

Rocking


BIOSTAT® CultiBag RM, *Sartorius-Stedim (50L)*

Bubble column


CellMaker, *Cellexus (50L)*



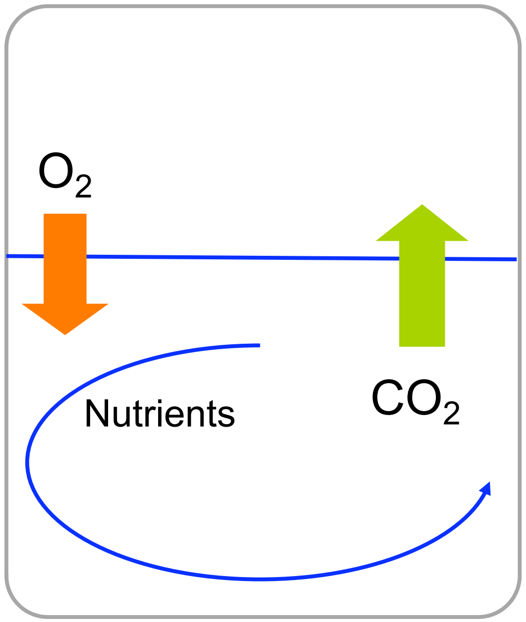
12

Goals: Making Bioreactors EASY to use, rapid to set up, run **and** *disposable*

*The two tasks in bioreactors are to provide for:*

# Homogeneity: Generating and maintaining it **(mix efficiently)**

# Gas transfer : gas in and out (**transfer efficiently**




Mixing

Oxygen transfer

# What is the purpose of any (disposable) bioreactor?

- To provide a **cell-friendly environment** (appropriate cell physiology) for growth and high level productivity



14

# Cell-friendly?

- homogeneous environment within the entire volume of the reactor that provides

1. temperature (37ºC)
2. $O_2$ supply and maintenance of $O_2$ level within desired conc. range
3. physiological pH range
4. low shear stress
5. rapid response to operator-desired changes of physico-chemical conditions

# Stirred-tanks

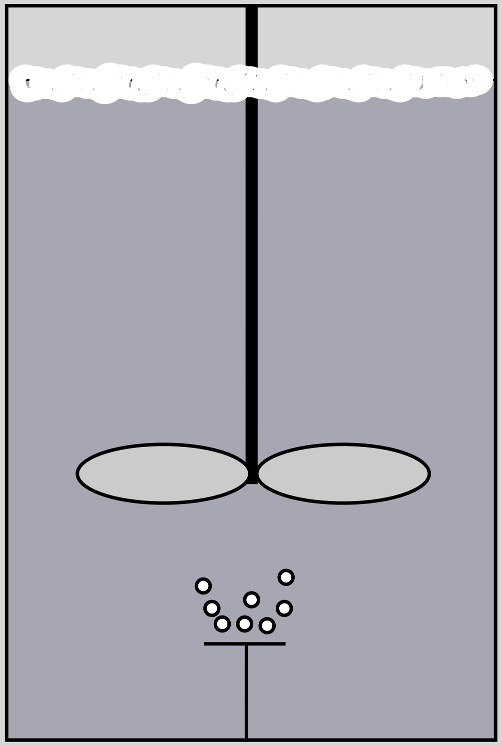
- Most commonly used in industry

---

- Mixing
  - Impeller - shear stress
- Oxygen transfer
  - Sparger - foam formation

<u>Strengths</u>
+++ Proven applicability from small scale to very large scale!
+++ Scale-up parameters are known

<u>Weakness</u>
- Mixing times can be long, depending on scale
- Oxygen supply by air not sufficient (under low rpms and gas flow rates used in mammalian cell culture)
- Foam formation can be a problem (implications for DSP - antifoam)





16

The strong confidence in stirred tank technology drove (after the success of the Wave technology - and the recognition of certain limitations) the rapid emergence of disposable **stirred** tanks...

*... so why would anybody invest time and money into something different, especially for the very large scale.... ?*

# Shaken, not stirred?



18

# Disposable orbitally shaken bioreactors

- Fully scalable technology from a few mL to 1000 L

TubeSpin bioreactor

2000 Liter Orbshake bioreactor

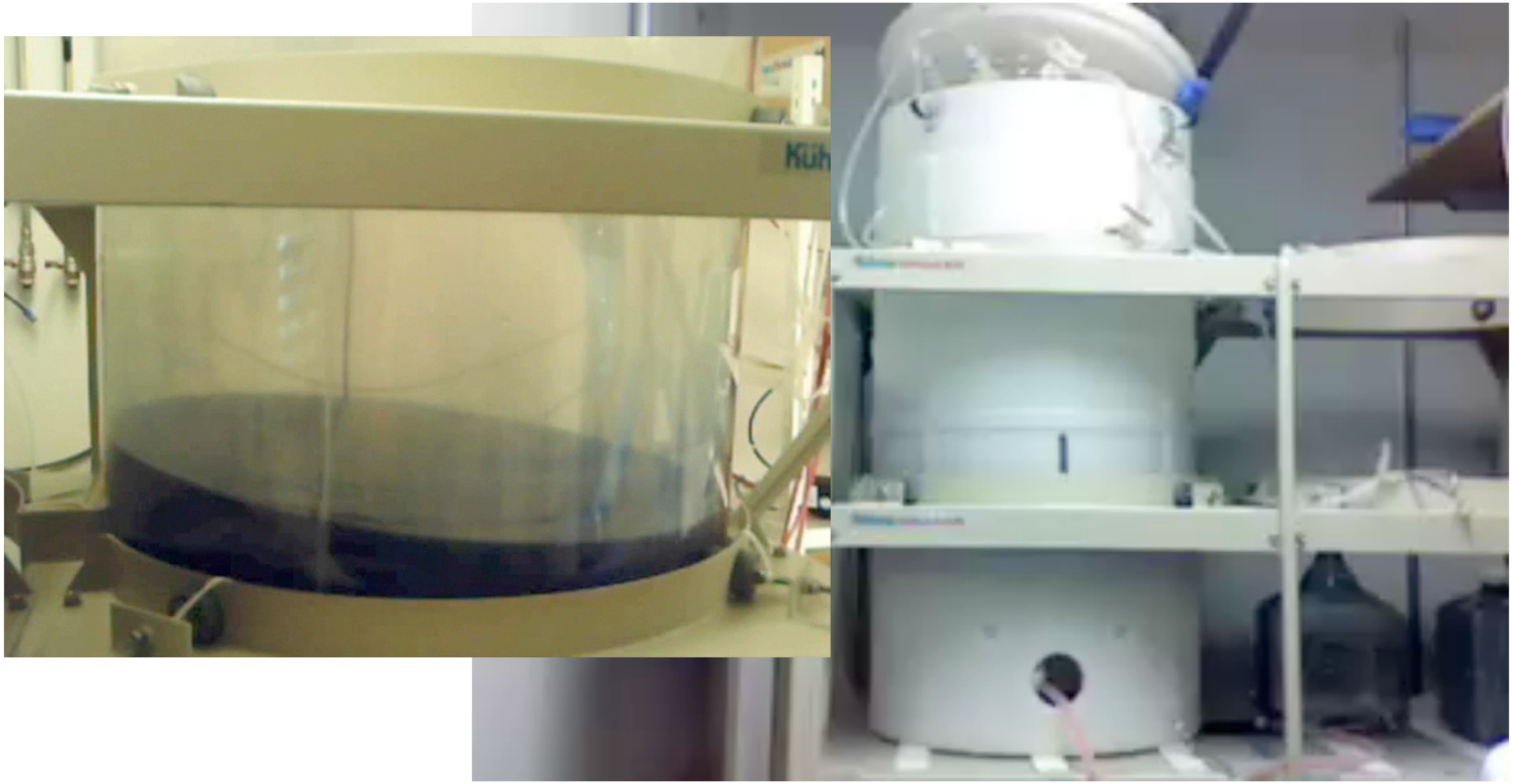
- Instrumented or non-instrumented bioprocesses
- No sparging (no excessive stripping of $CO_2$)
- Aeration through the “free” surface

Kuhner SHAKER

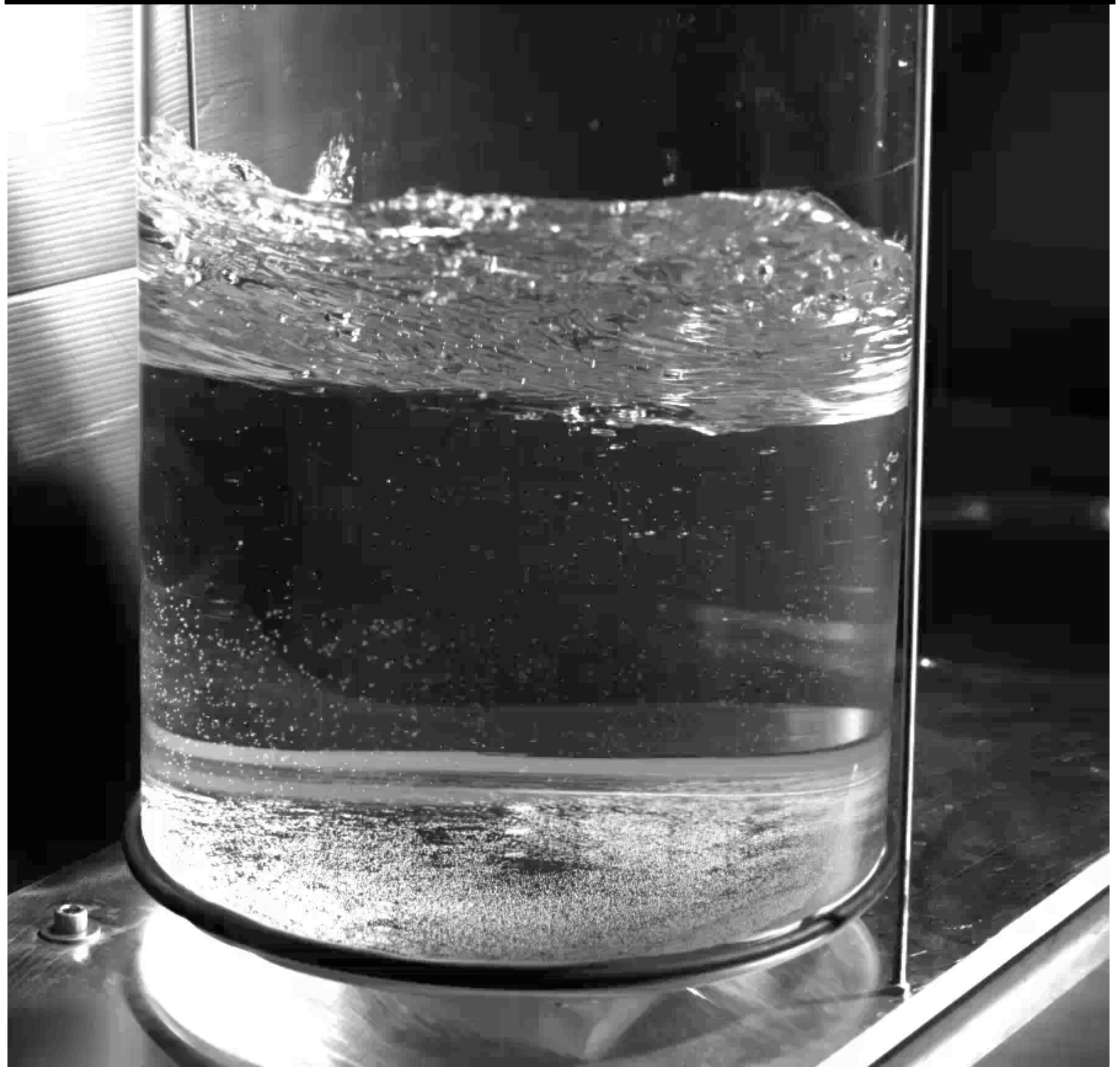
sartorius provided bag



19



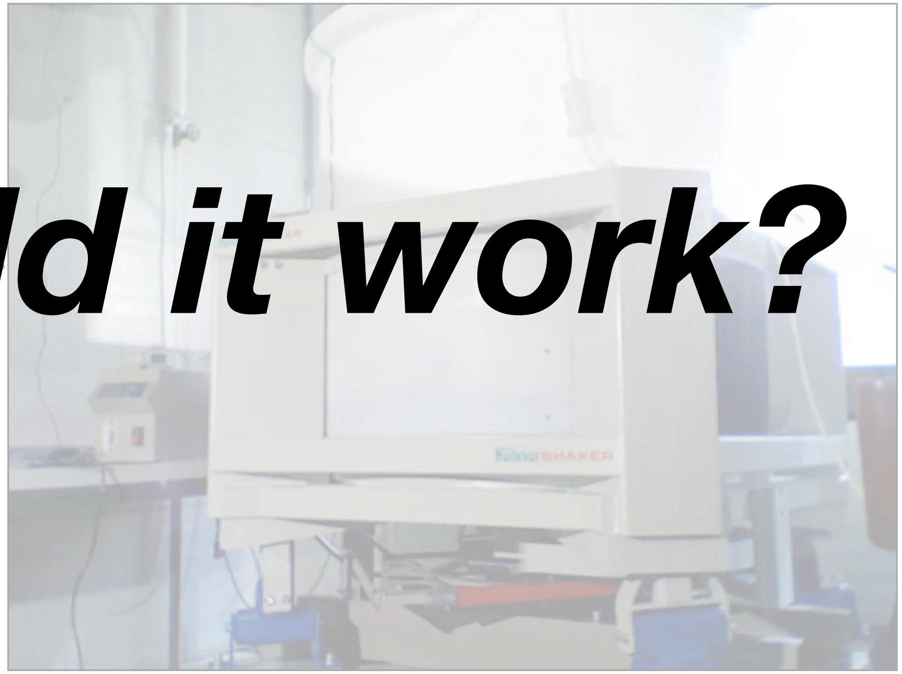
# 95 rpm time lapse



22

# Disposable orbitally shaken bioreactors

2000 Liter Orbshake bioreactor



- Fully scalable technology from a few mL to 1000 L

TubeSpin bioreactor

**but would it work?**

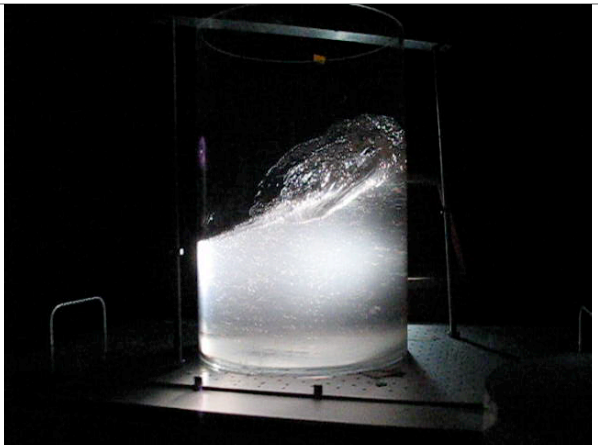
- Instrumented or non-instrumented bioprocesses
- No sparging (no excessive stripping of $CO_2$)
- Aeration through the “free” surface

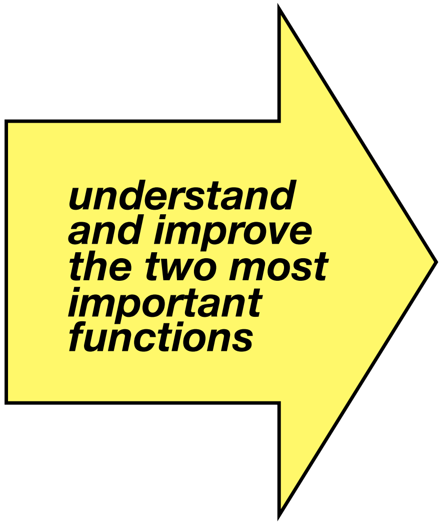
Kuhner SHAKER

***let’s find out!!***

# Engineering studies - ExcellGene/EPFL:

- **Physical Parameters:**
    1. Liquid height
    2. Agitation speed (rpm)
    3. Shaking diameter
    4. Inner diameter of the container






- **Homogeneity - mixing**

  Homogeneity in orbitally shaken bioreactor ?

  Mixing times

  Mixing patterns /simulation

- **Gas transfer ($O_2$ supply)**

  Sufficient for mammalian cell growth ?

  Free surface characterization / simulation

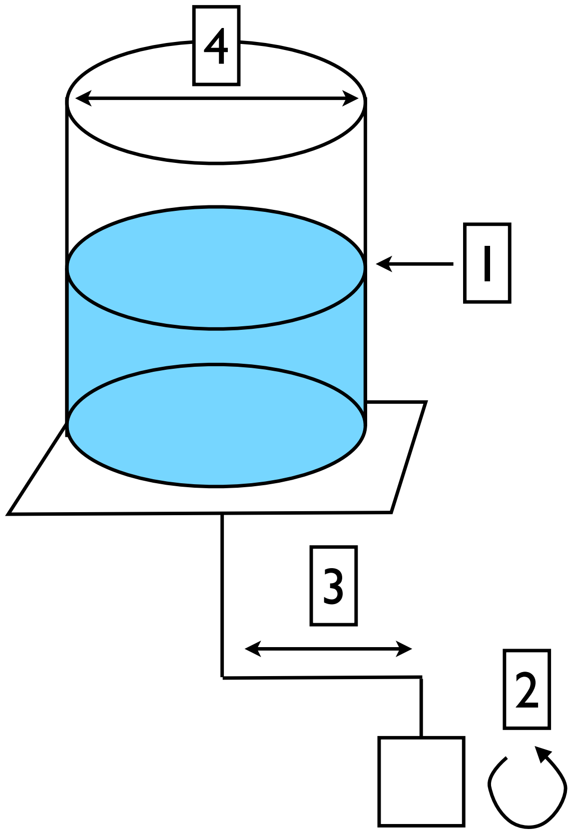
  Scalability

what about combining it with disposable bags?

# Key engineering principles

## Physical parameters:

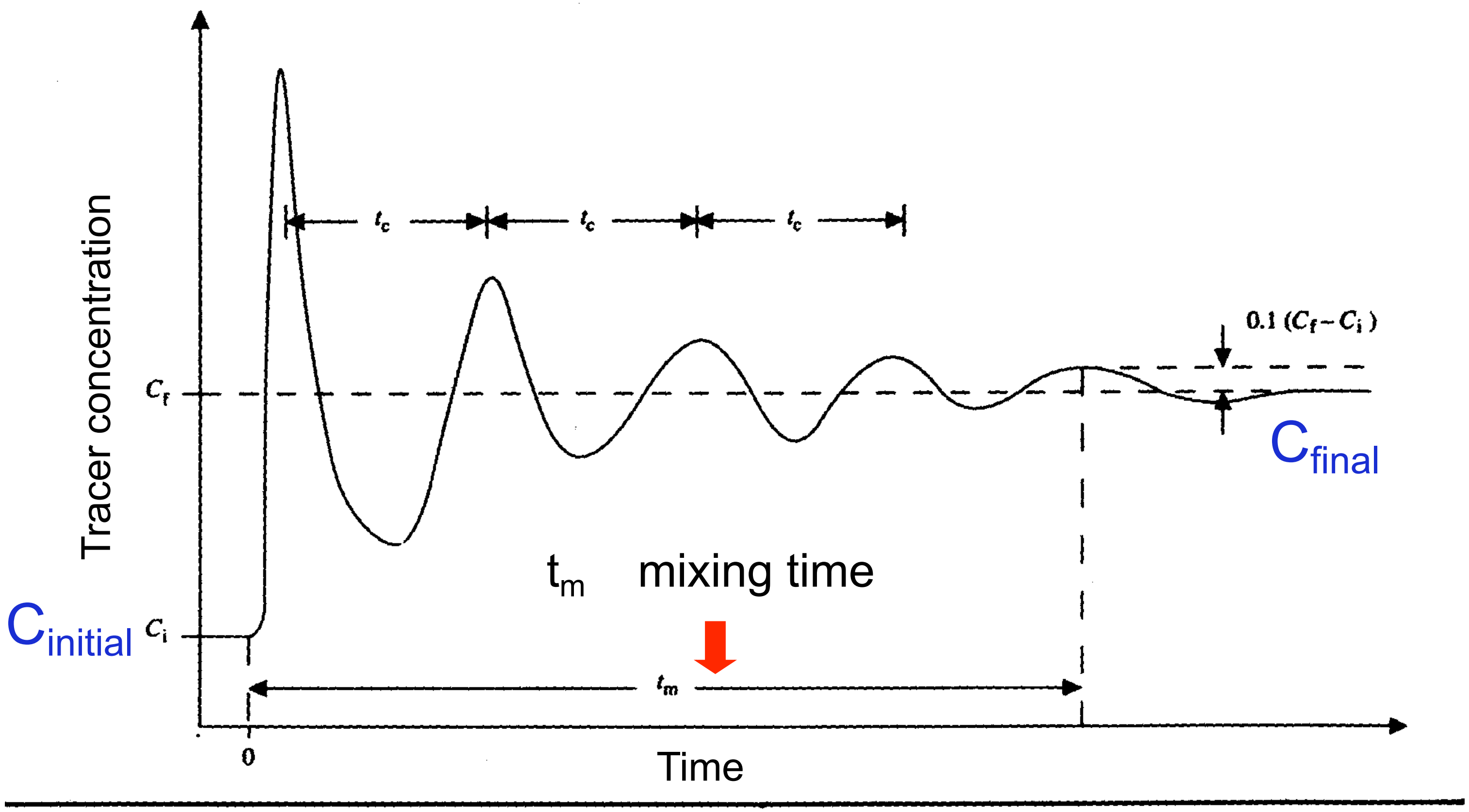
1. **Liquid height**
2. Agitation speed (rpm)
3. Shaking diameter
4. Inner diameter of the container






25

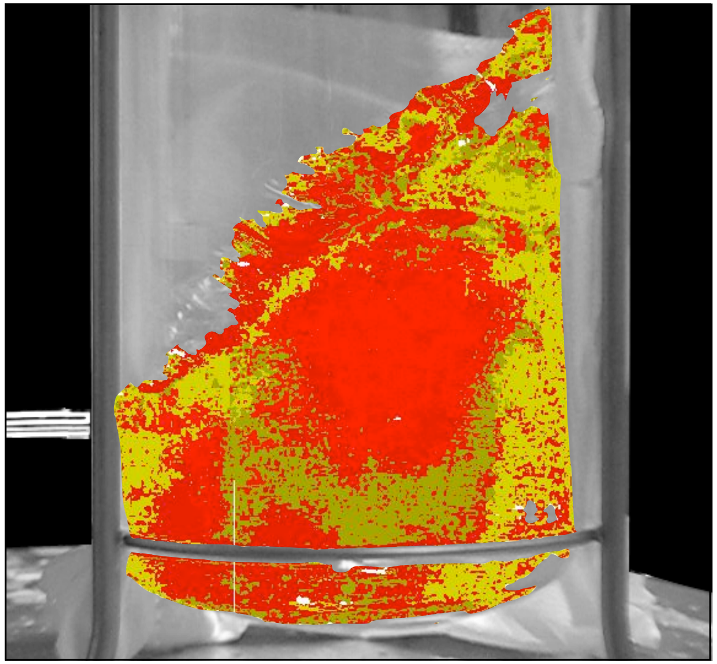
# How to measure mixing time: (to obtain **homogeneity**)

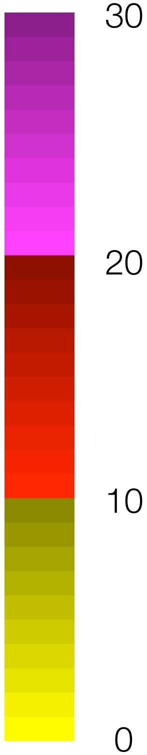


# A side-by-side comparison between orbital shaking and stirring: mixing efficiency

## Orbital shaking

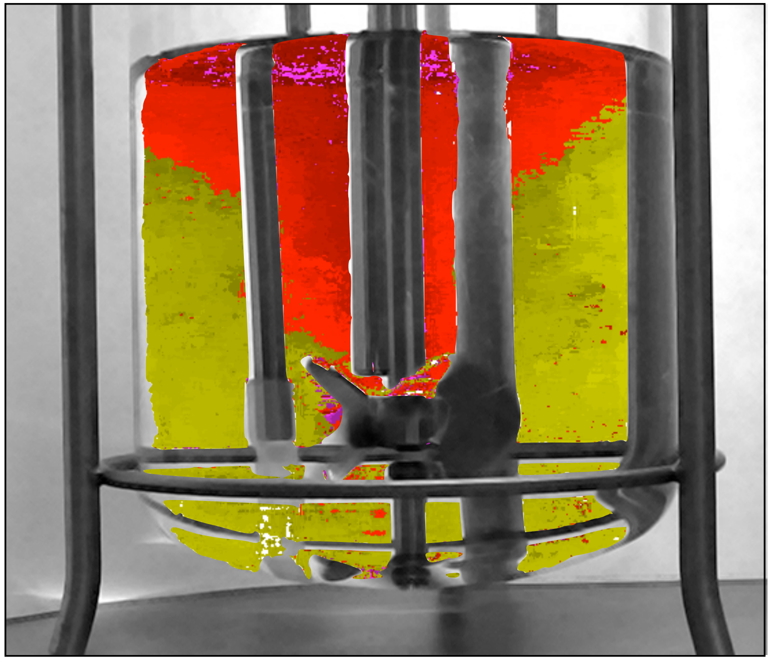
140 rpm






## Stirring

150 rpm

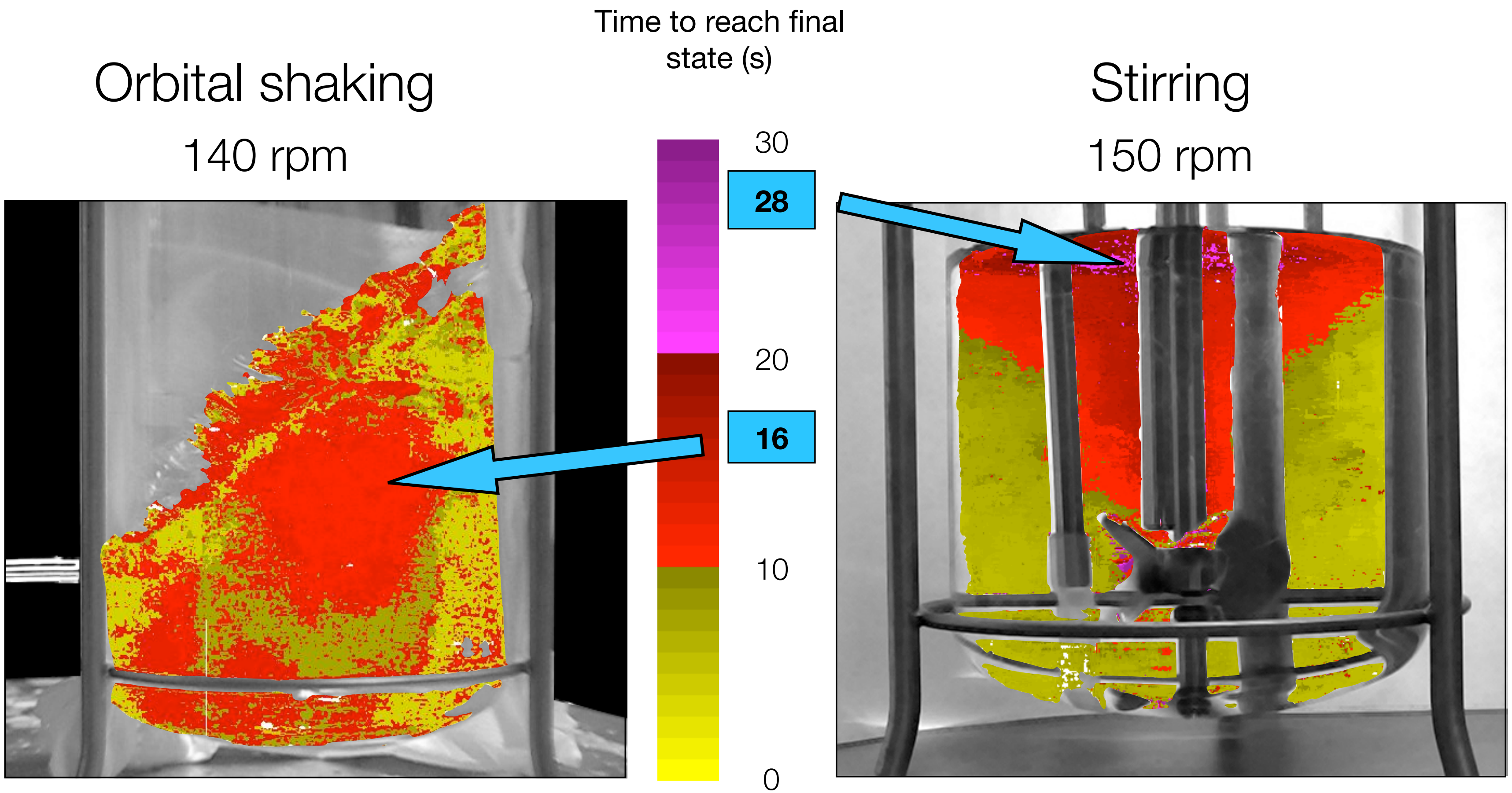


***Container diameter: 13 cm, working volume: 1.5 L***



27

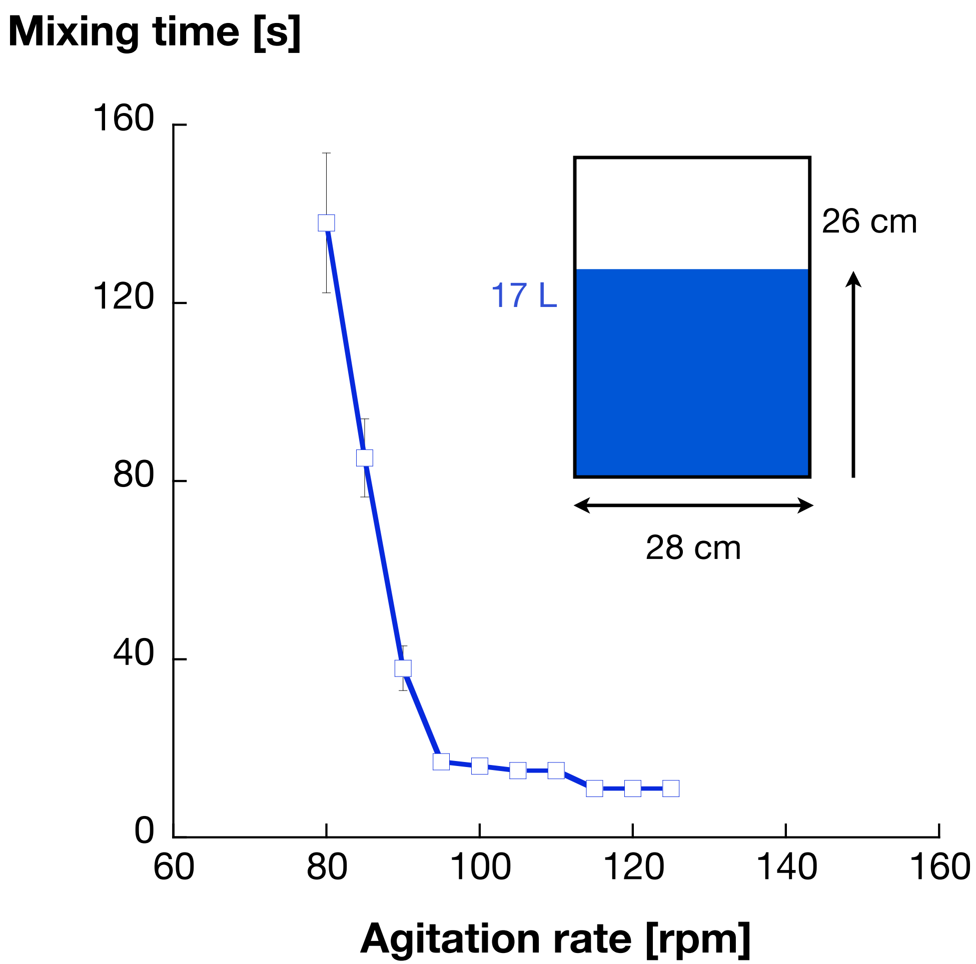
# A side-by-side comparison between orbital shaking and stirring: mixing efficiency




***Container diameter: 13 cm, working volume: 1.5 L***


28

# Liquid height (filling volume of the reactor) does not affect mixing times (significantly)



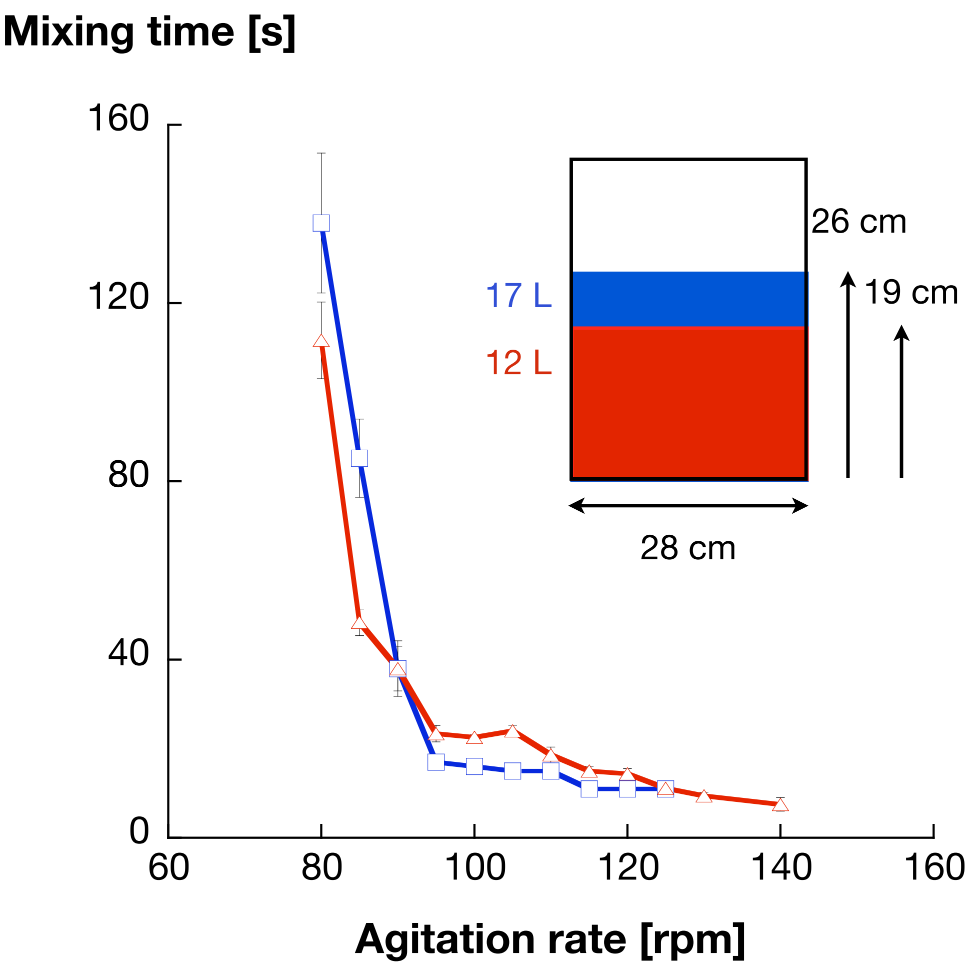

**Mixing time:**

Time needed to reach 95% homogeneity in the bioreactor



29

# Liquid height (filling volume of the reactor) does not affect mixing times (significantly)



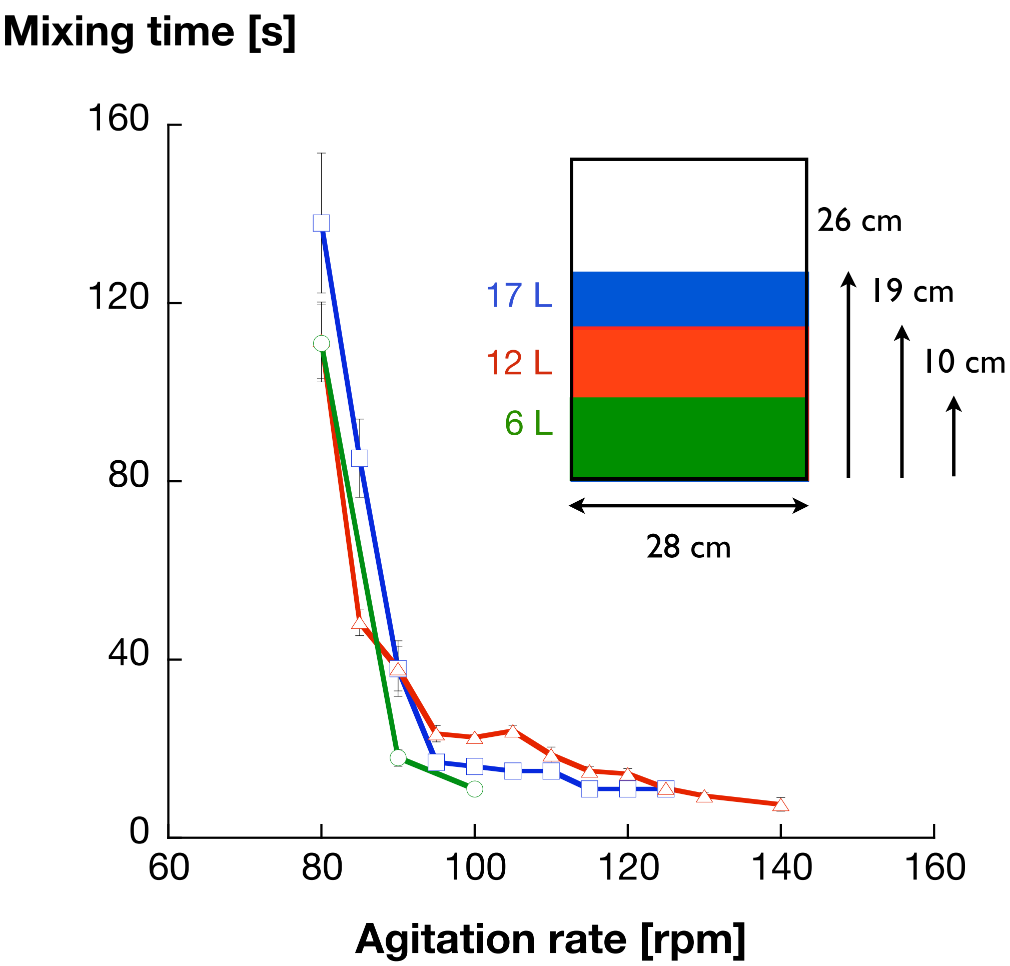

**Mixing time:**

Time needed to reach 95% homogeneity in the bioreactor



30

# Liquid height (filling volume of the reactor) does not affect mixing times (significantly)




**Mixing time:**

Time needed to reach 95% homogeneity in the bioreactor



31

# Mixing times in a 200 Liter OrbShake® bioreactor

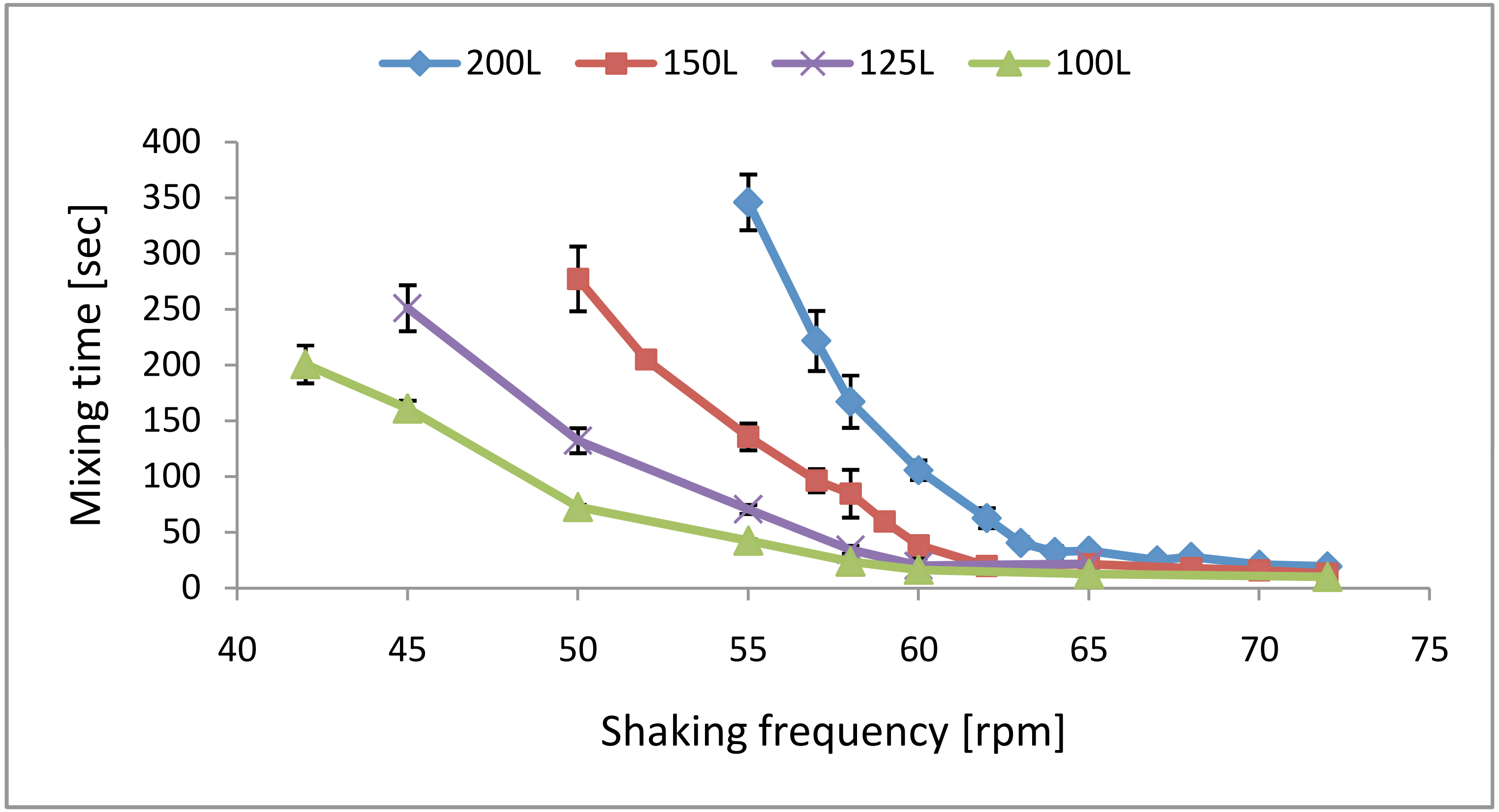
Kuhner SHAKER (bioreactor)

sartorius disposable bioreactor bag

*inspired by*

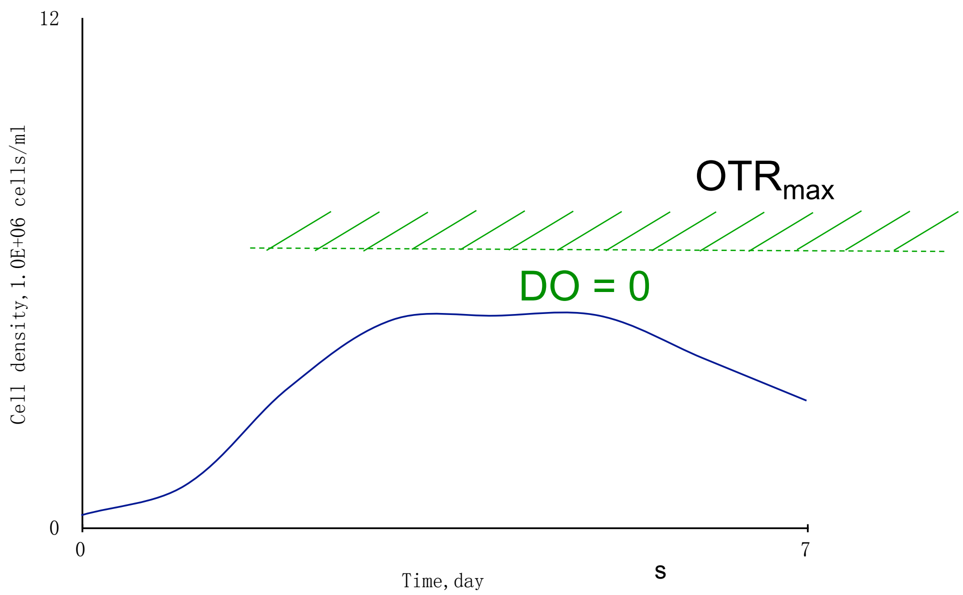
ExcellGene

# Mixing times in a 200 Liter OrbShake® bioreactor



# **Gas (oxygen) Transfer** (in and out of the liquid)

Insufficient oxygen supply due to low gas transfer rate limits cell growth




Theoretical batch culture with $O_2$ limitation

# Oxygen transfer rate (OTR)

is dependent on the kLa value of the reactor (under conditions for cell culture)

Henry's law

$$c_L^{sat} = \frac{p_{O_2}}{k_H}$$

Mass transfer coefficient

Specific surface of the interface

$$\underbrace{k_L a}_{\text{Volumetric mass transfer coefficient}} \cdot \underbrace{\left(c_L^{sat} - c_L\right)}_{\text{Driving force}} = \frac{dc_L}{dt}$$



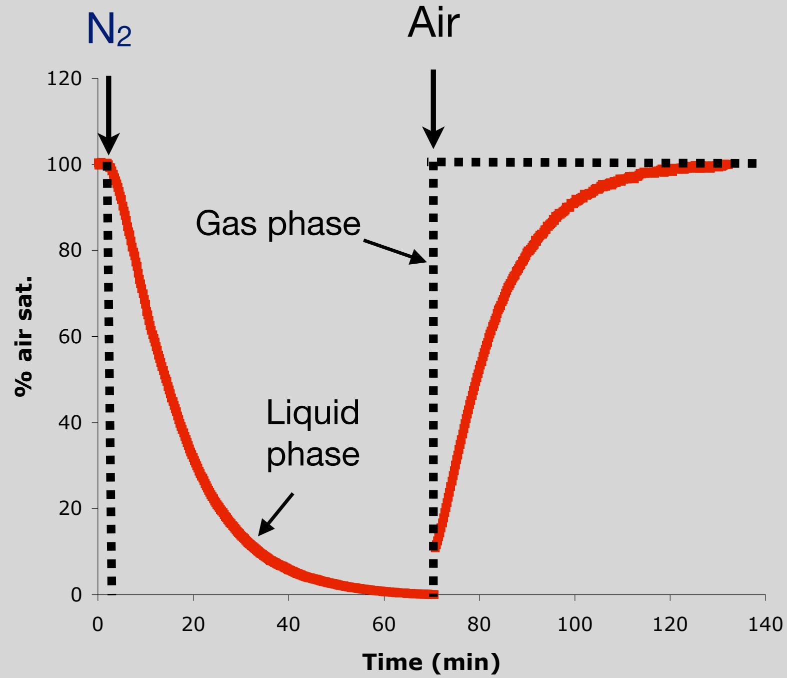
35

# $O_2$ transfer rate

- Measurement of $k_La$ using gassing-in method
- Nitrogen is flushed into the system until a dissolved oxygen concentration (DO) of 0% air saturation is reached
- Air is flushed in the headspace to replace nitrogen and the DO is recorded

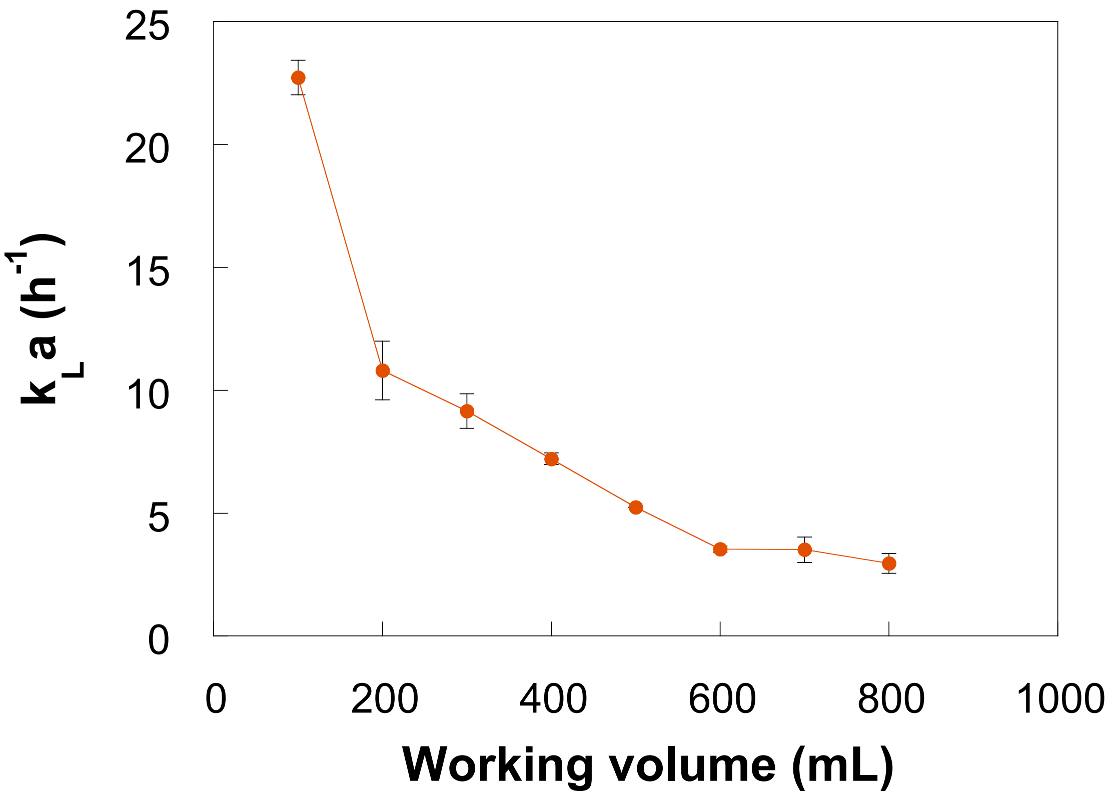
$$\frac{dc_L}{dt} = k_La \cdot (c_L^* - c_L)$$

36




# $k_La$ value decreases as the working volume increases

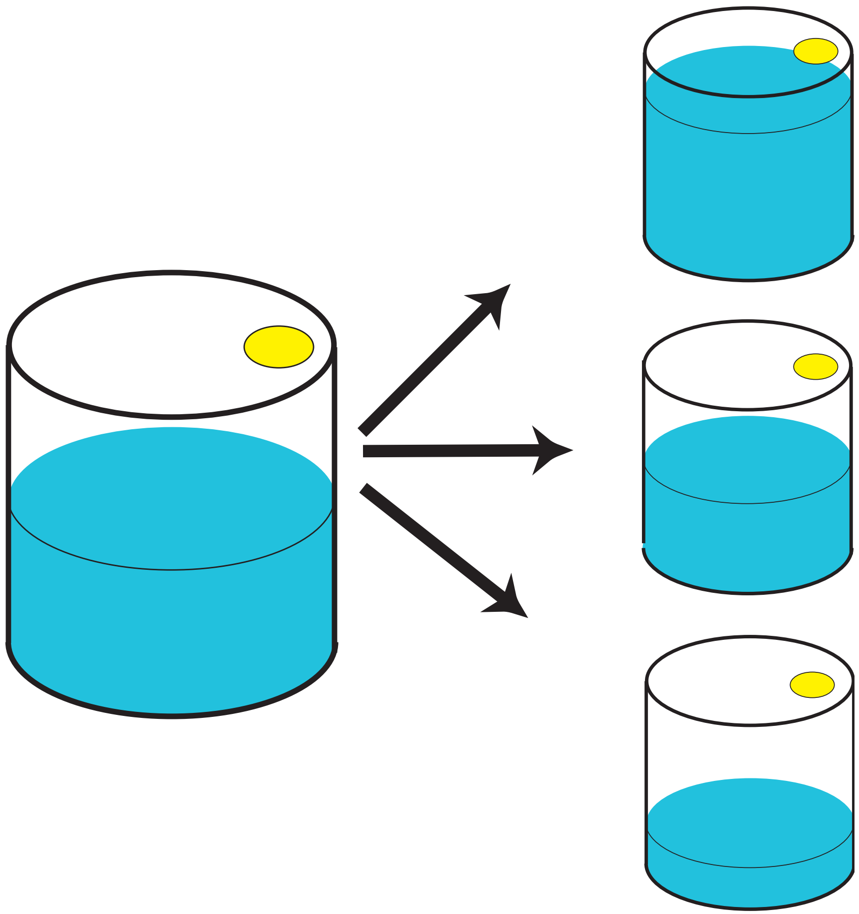
- 1L round bottle
- 110 rpm



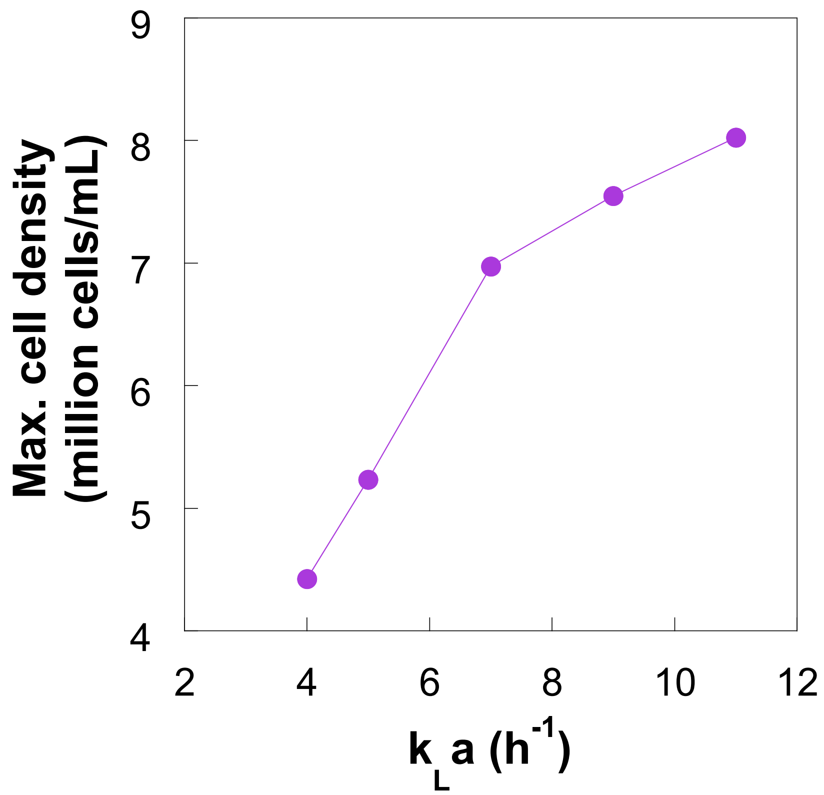


37

# kLa and maximal cell density (CHO)



# The maximal cell density is correlated to $k_La$



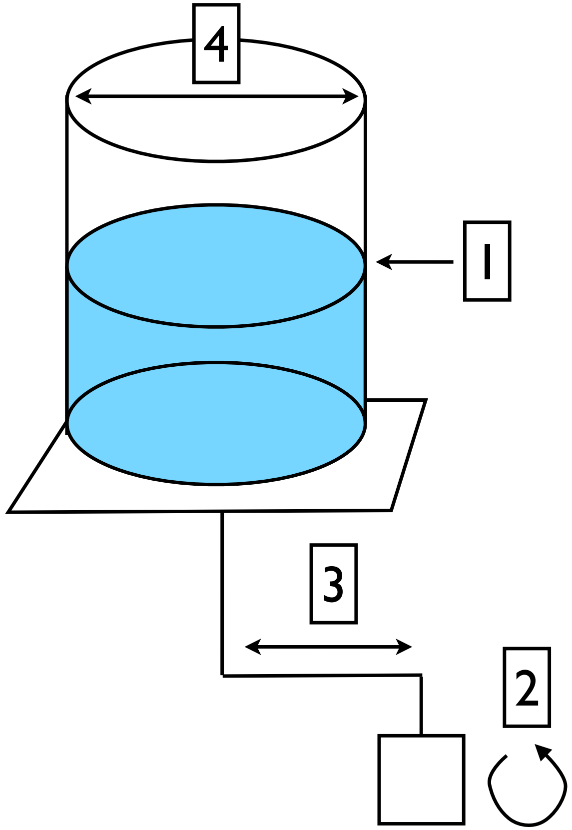



39

# Key engineering principles

## Physical parameters:

1. Liquid height
2. **Agitation speed (rpm)**
3. Shaking diameter
4. Inner diameter of the container



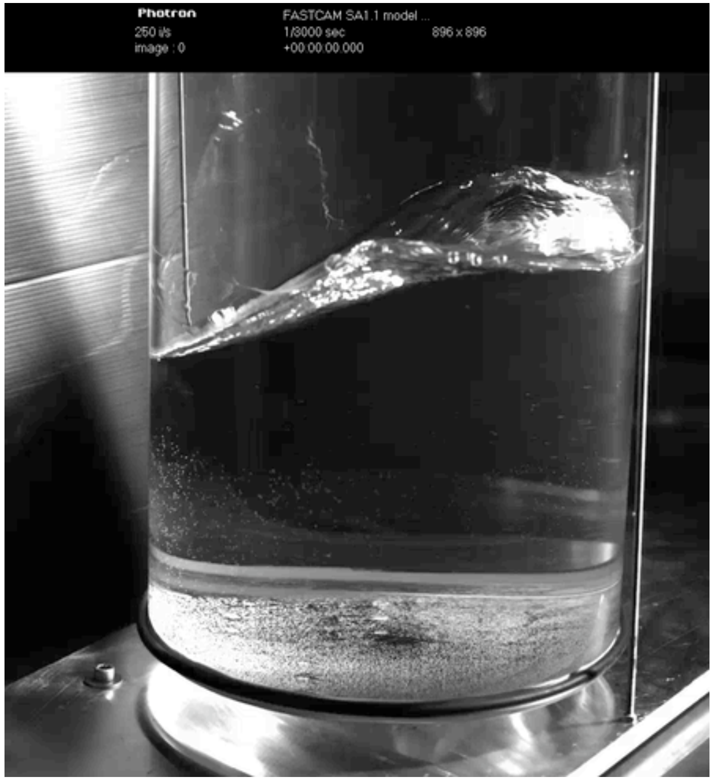



40

# Shaking speed and gas transfer rates in orbitally shaken bioreactors are closely correlated

# Free surface shape

80 rpm

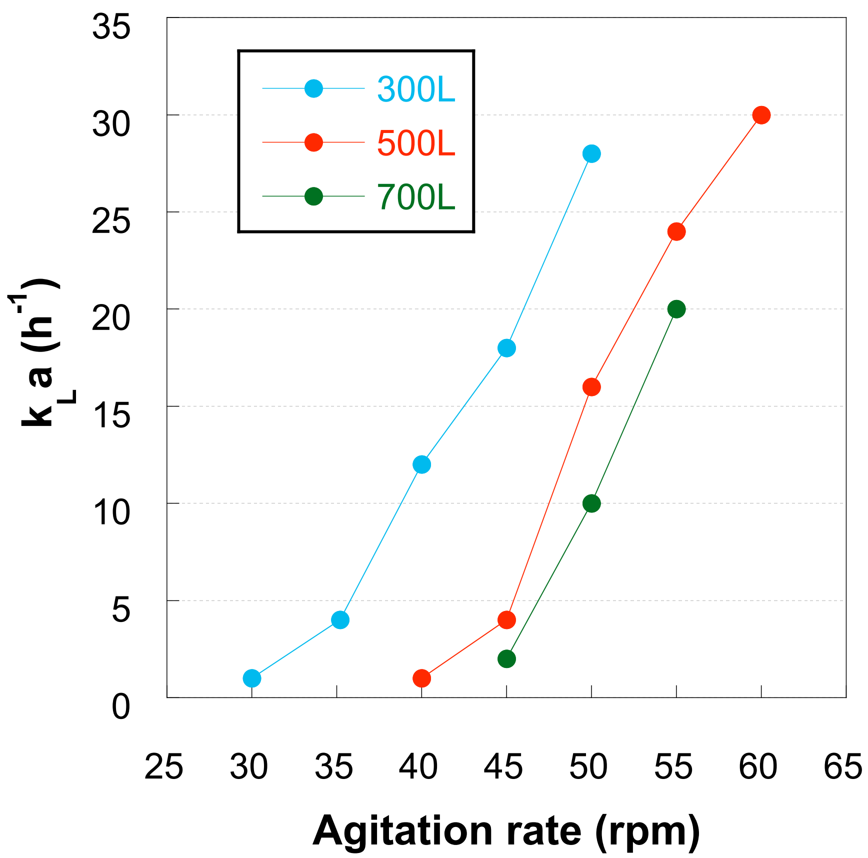



110 rpm


***Fast camera: 250 images/sec.***
***Thanks to Dr. Mohamed Farhat, LMH, EPFL***

42

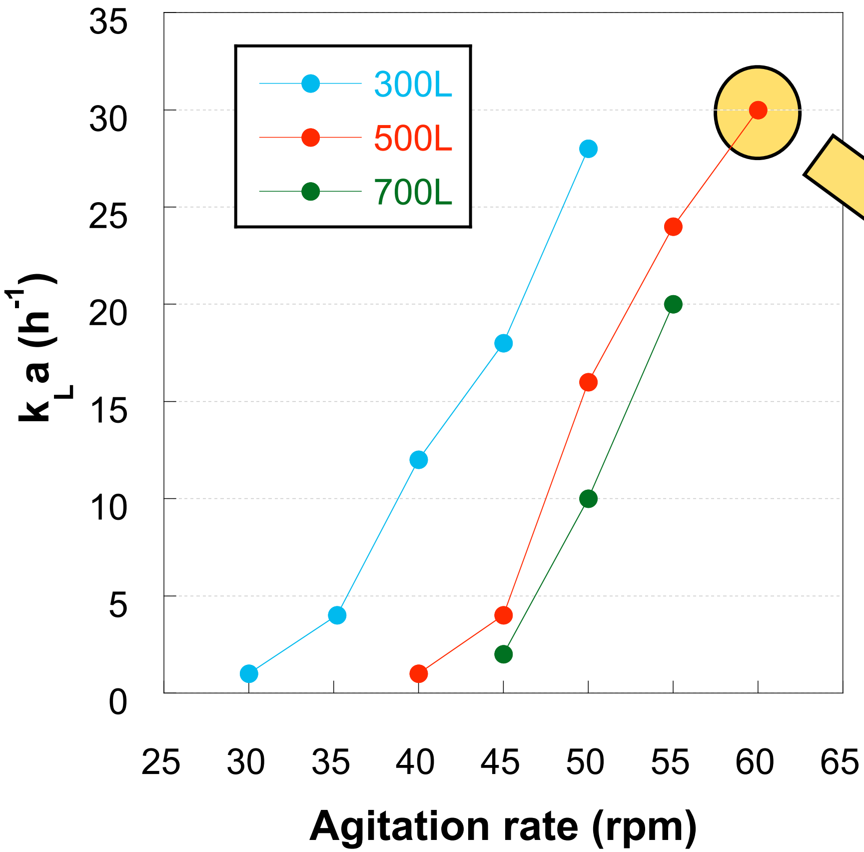
# $k_La$ in our prototype 2000L bioreactor






43

# $k_La$ in the 2000L bioreactor

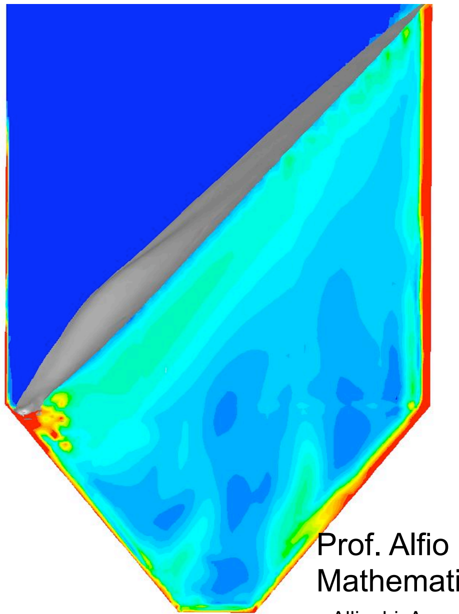





44

# Shear stress?

# Shear Stress in the TubeSpin® - bioreactors
## - now a widely used system in the industry

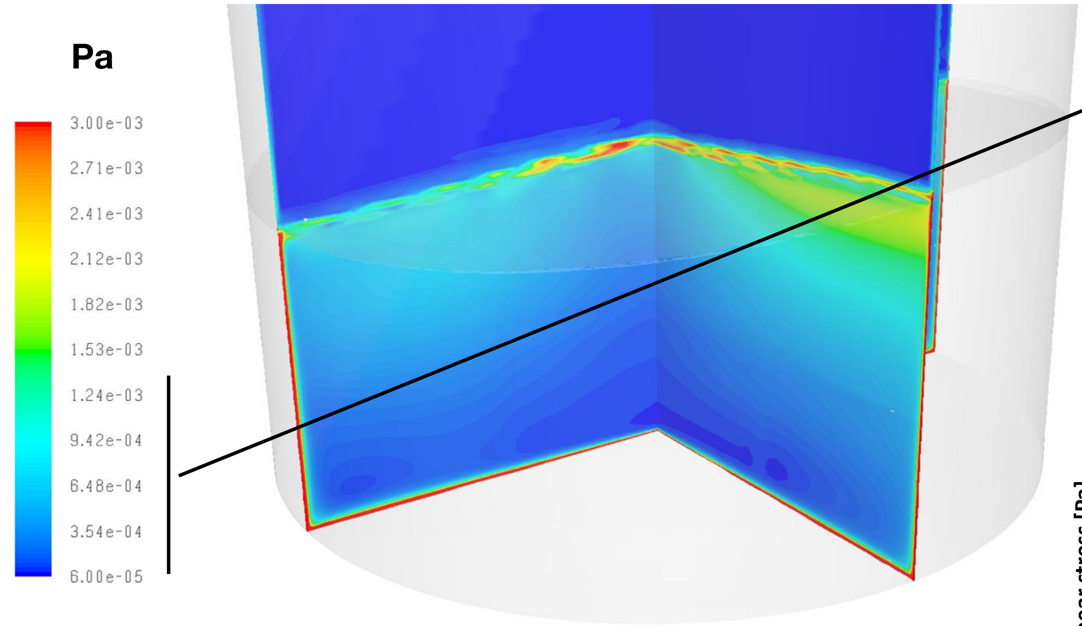


Prof. Alfio Quateroni, Dept. Mathematics, EPFL
Allinghi, America's Cup

180 rpm - 300 rpm:
*up to 30 mio cells/ml cultivated in high seed cultures - no indications of shear stress as determined by viability assessment*

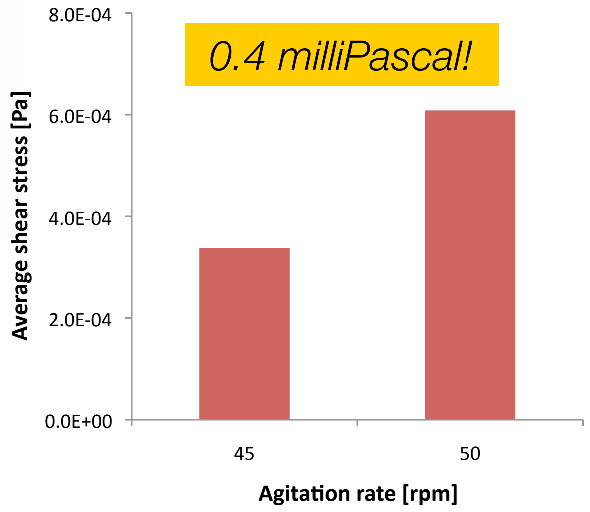
# What about shear stress?

below 1 milliPascal




***Inner diameter: 1.3 m, agitation rate: 44 rpm, shaking diameter: 5 cm, working volume: 690 L***

**Shear stress calculation from power input measurement**




Calculated shear stress is 2 logs below cell killing value!



47

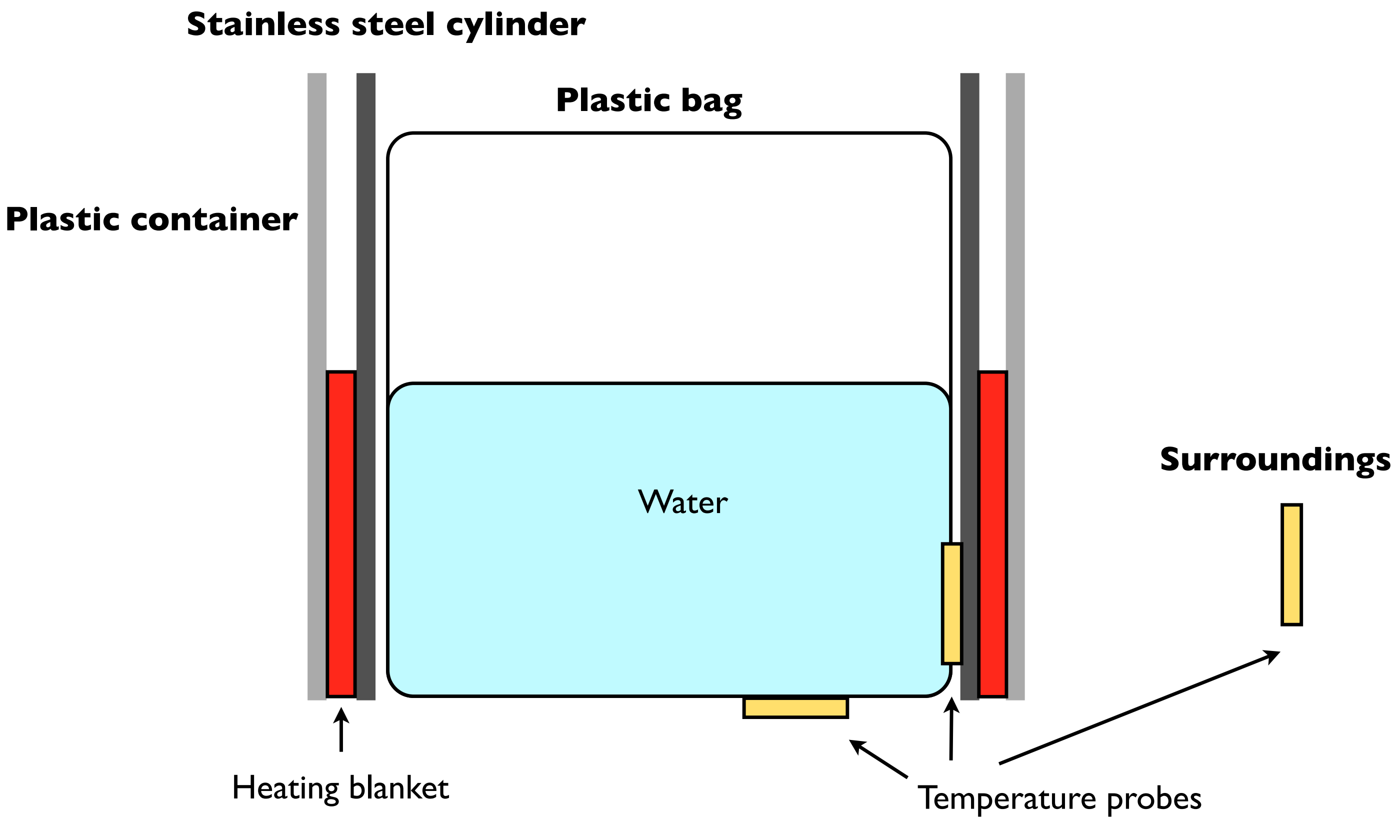
# Shear stress: .... but this is only a calculated value

## What about Power input?

isn't this a good number to know in order to assess
the energy distributed (and possibly damaging cells?)

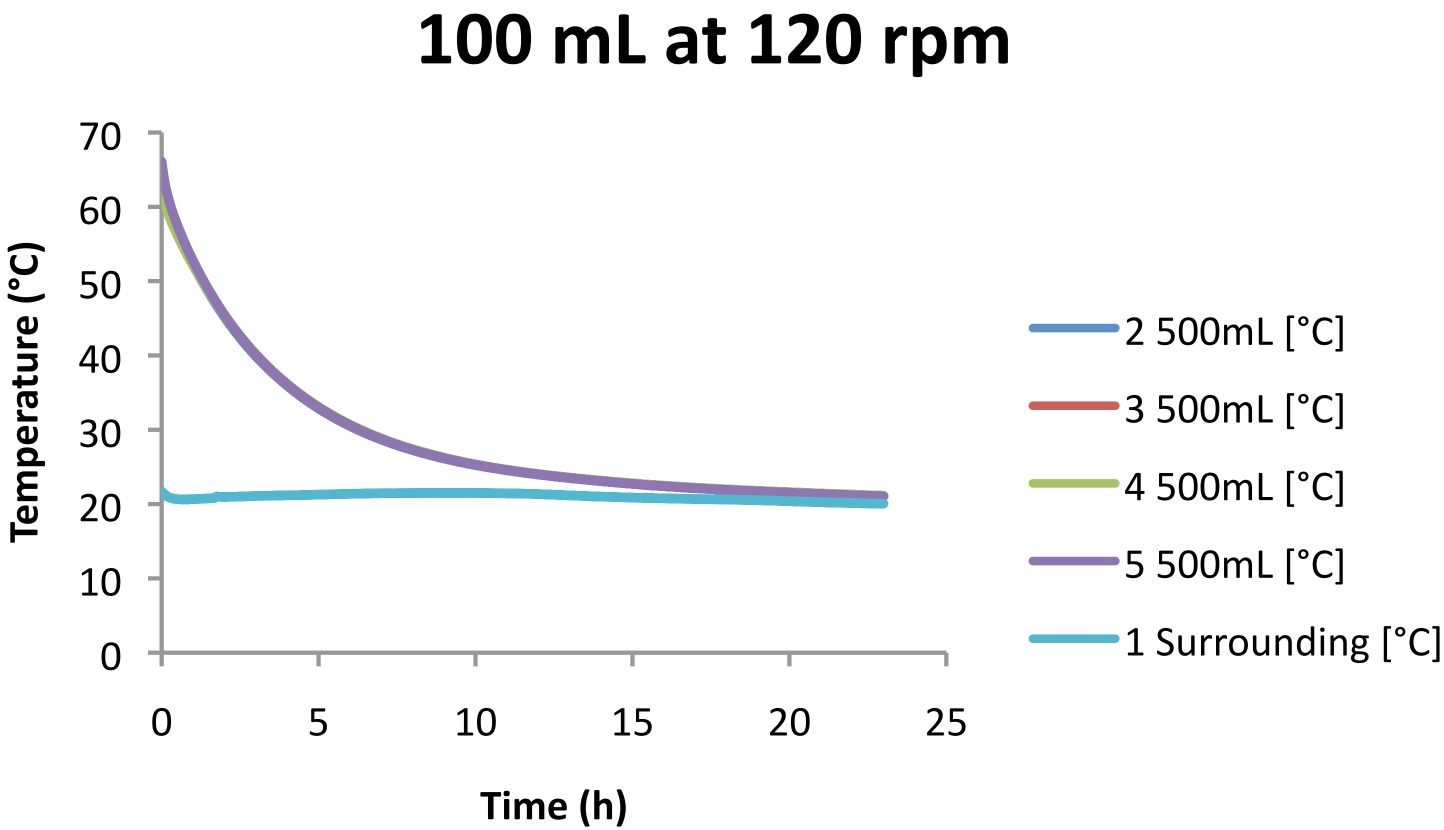
# Volumetric power input by the "thermodynamic method"

$$-C_p \frac{dT}{dt} = UA(T_{out} - T) - Q_{agitation}$$



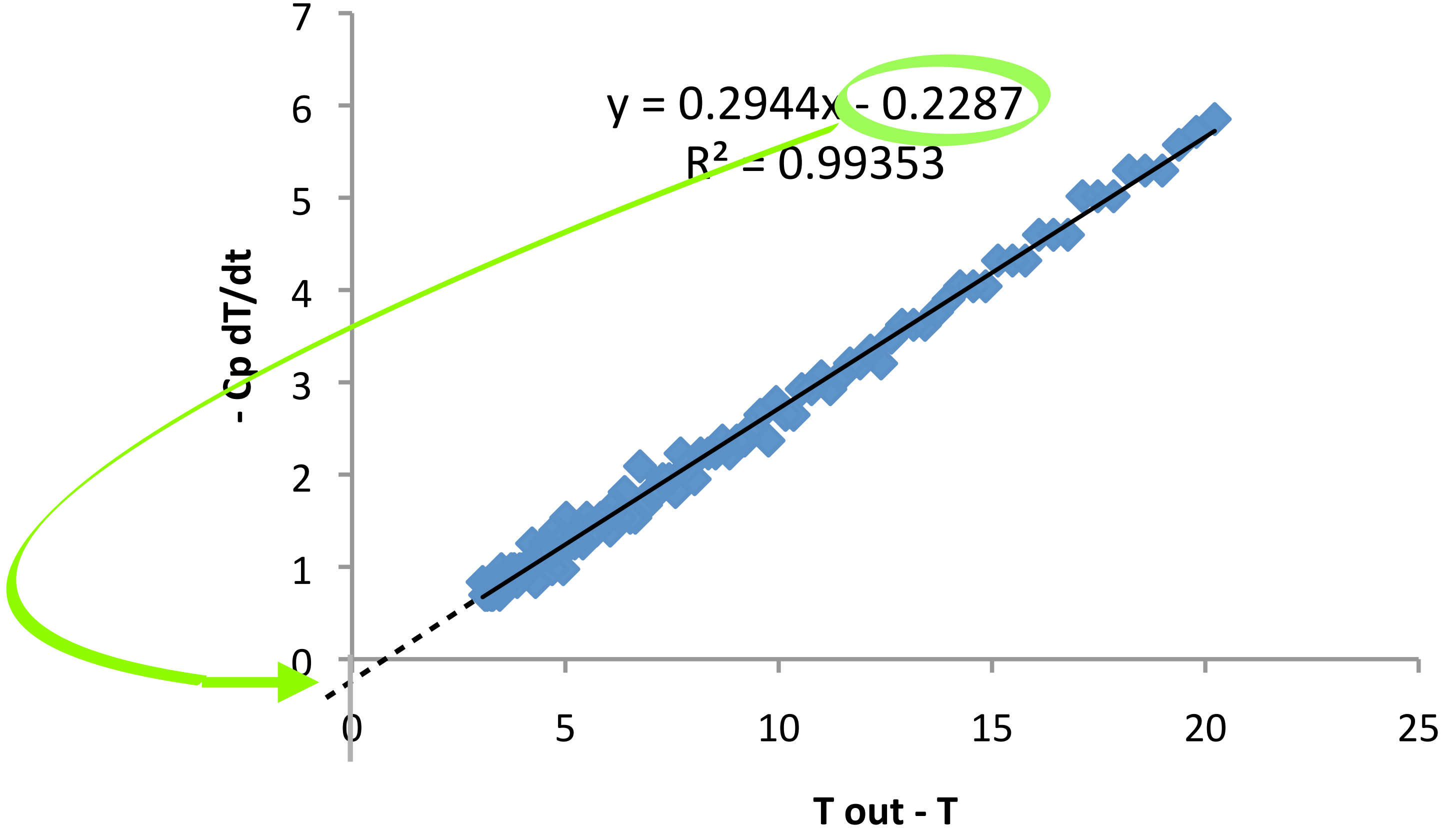
# Volumetric power input

$$-C_p \frac{dT}{dt} = UA(T_{out} - T) - Q_{agitation}$$

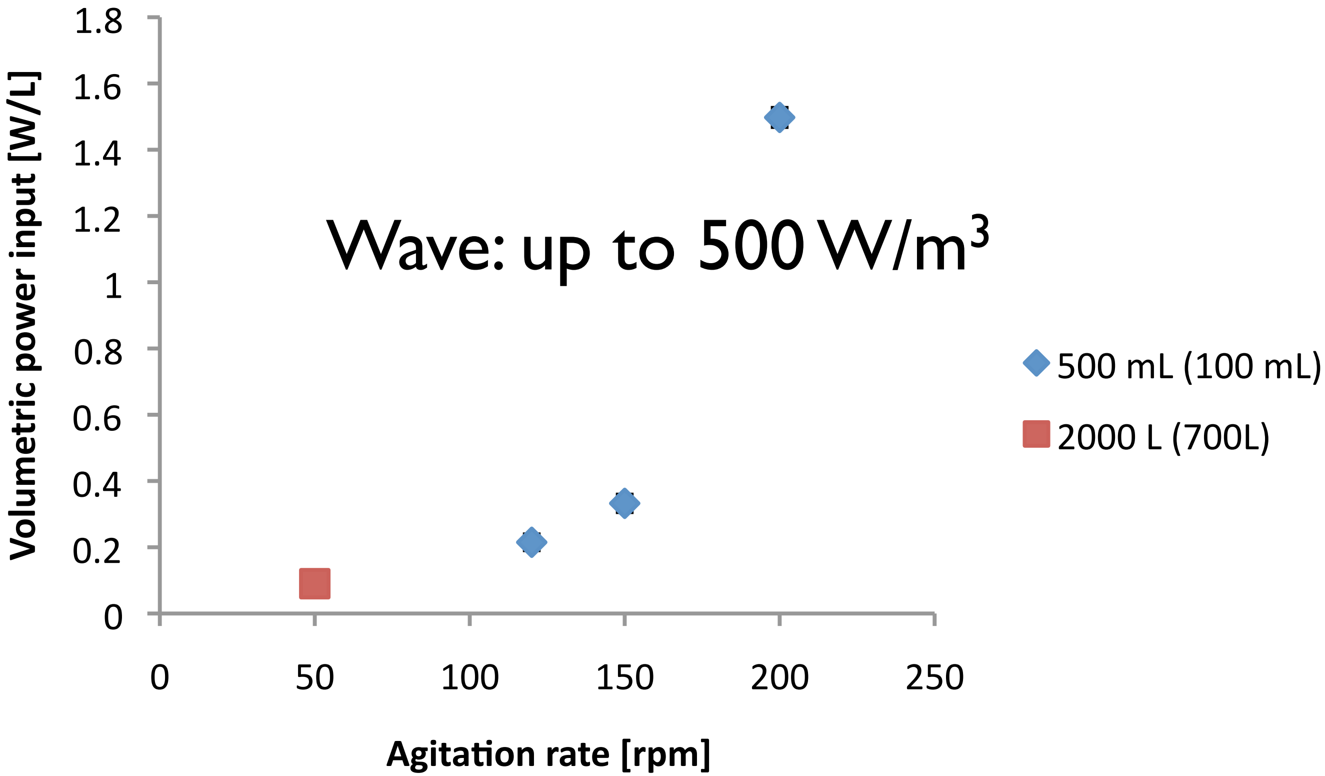


# Volumetric power input

$$-C_p\frac{dT}{dt} = UA(T_{out} - T) - Q_{agitation}$$



# Volumetric power input at 100 ml and 700 Liter scale



# Volumetric power input

... the larger the scale, the smaller the volumetric power input?

Why is that ?

# Volumetric power input

... lets look where the energy may be absorbed/used for example at the wall, where the highest **friction is occurring**

500 mL bottle - **100 mL**: friction surface: **0.01 $m^2$**

**S/V ratio:** about **100 $m^2/m^3$**

2000L bioreactor - **700 L**: friction surface: **3.5 $m^2$**

**S/V ratio** about **5 $m^2/m^3$**

# Orbital shaking of a 2000 L reactor with a working volume of 1000 Liter (one metric ton of liquid!)

Electric power consumption:

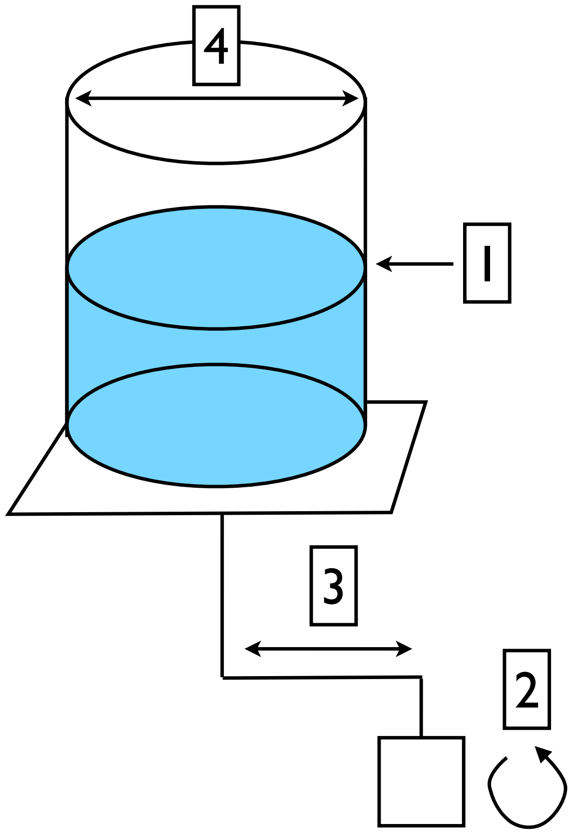
**< 200 Watts**

(when liquid in synchrony with shaking)

# Key engineering principles

## Physical parameters:

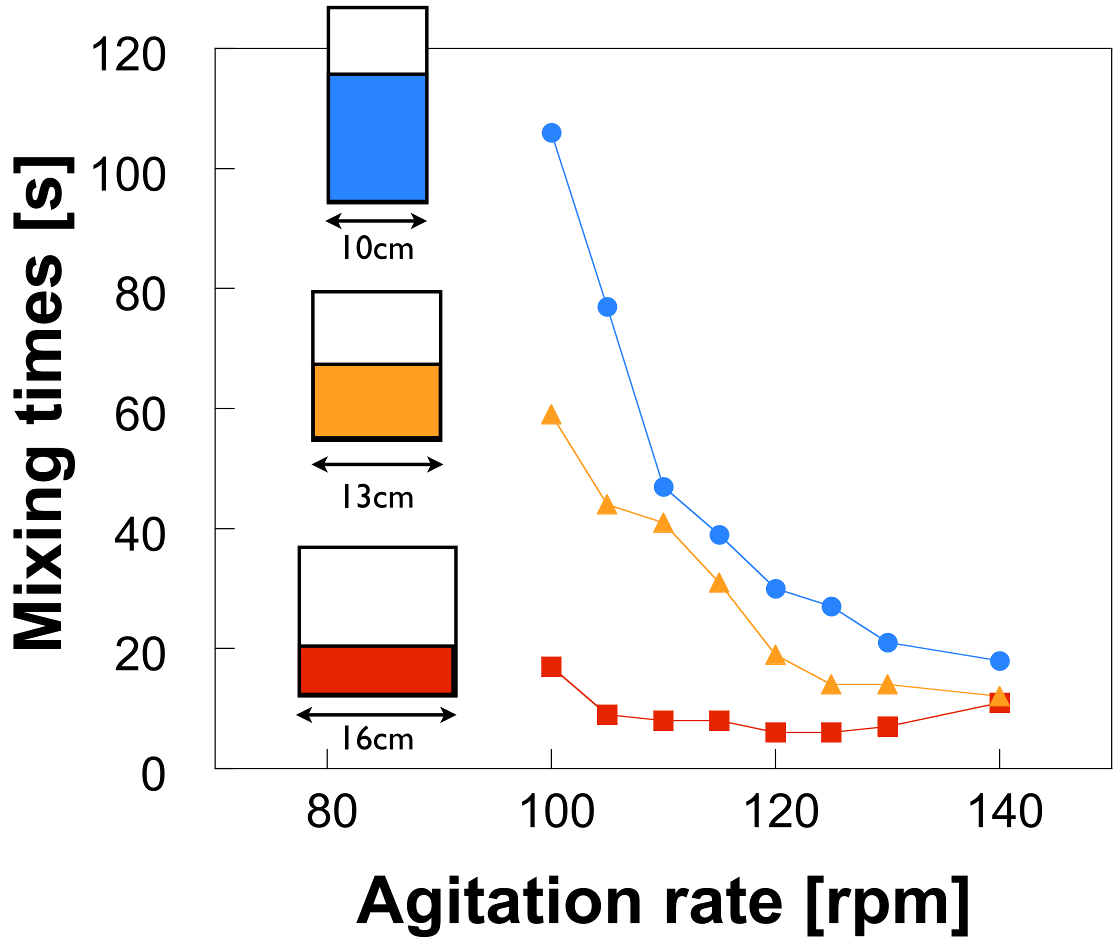
1. Liquid height
2. Agitation speed (rpm)
3. Shaking diameter
4. **Inner diameter of the container**






56

# Effect of vessel diameter on mixing time

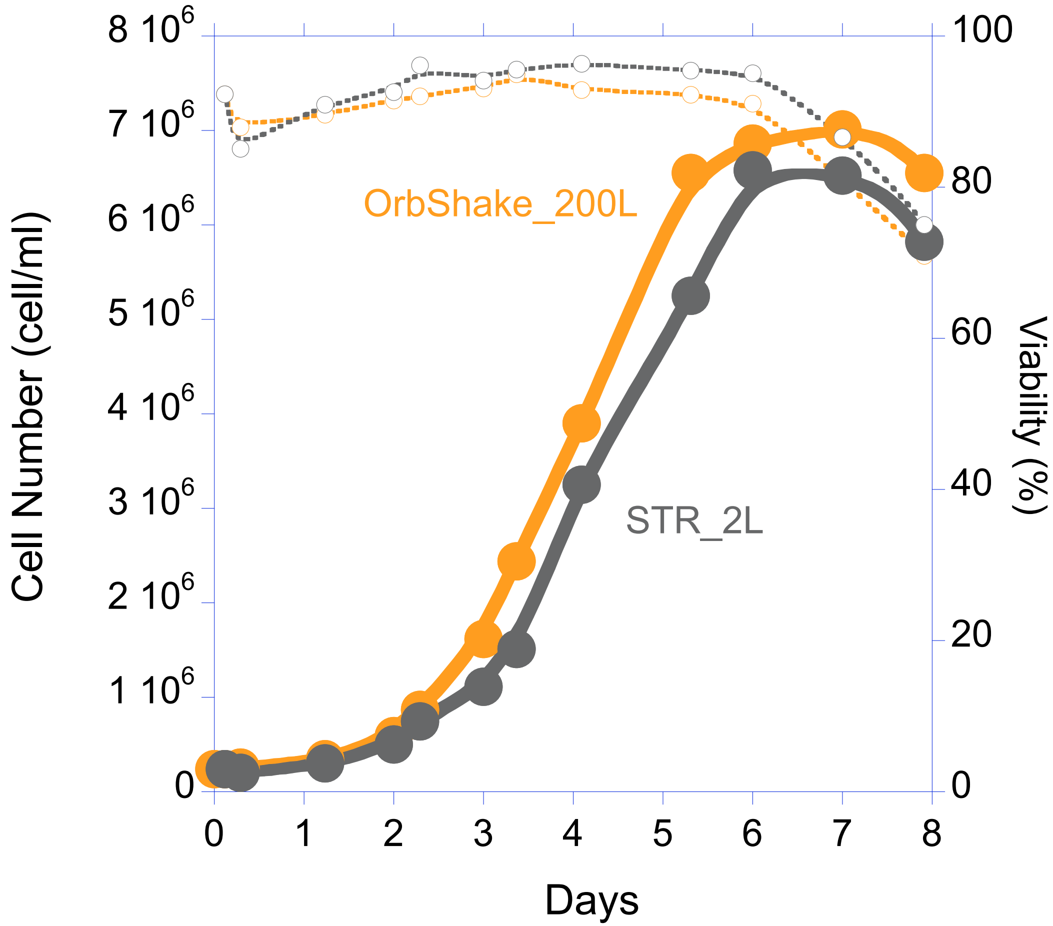



***Working volume: 1 L***



57

# Comparison of a 2 L stirred tank with a 200 Liter OrbShake bioreactor (both controlled)



# Orbitally shaken cylindrical bioreactors

- more efficient for mixing and $O_2$ transfer than stirred-tanks under conditions for mammalian cell culture
- low shear stress

***Orbitally shaken bioreactors appear to be a suitable alternative to stirred tanks for mammalian cell culture, even at the large scale.***

# Thank you:

- large team of scientists and engineers in my laboratory at EPFL and at ExcellGene SA, working over the last 10 years on orbital shaking

- Dr. Xiaowei Zhang
- Dr. Matthieu Stettler
- Stephanie Tissot
- Dr. Maria De Jesus (ExcellGene)
- Dr. Martin Jordan (Merck-Serono)
- Cedric Bürki (ExcellGene)

Kuhner SHAKER

sartorius

**TPP Techno Plastic Products AG**

- Special thanks to the KTI of Switzerland for funding far 3 doctoral students
- Dr. M. Farahd (Fluid Dynamics Laboratory of EPFL)
- Prof. Quateroni (Mathematics) and his team for modeling