# Characterisation of a reciprocally shaken 20 L single-use bioreactor

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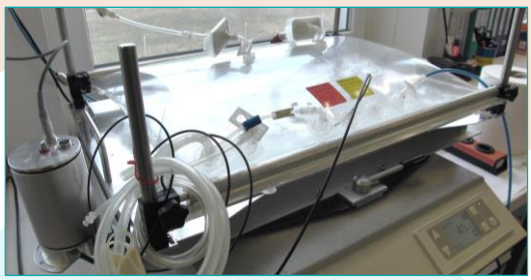
## Introduction

On the pharmaceutical market there is an increasing demand of single-use bioreactors for culturing shear sensitive mammalian cells [1]. All available 2D-bag-systems with pillow-shaped bags are using several tilt-techniques but no one is shaking in a reciprocal way. Researching the values of a 2D-bag on a solid RSB (Reciprocally Shaken Bioreactor) on the robust LS-X seemed interesting. A RSB would be a space-saving and mechanically robust alternative to wave induced mixing bioreactors.
In this project a reciprocally shaken bioreactor with a 20 L single-use 2D-bag was characterised and a fitting tray was developed. Important scale-up parameters such as power input, mixing time and oxygen transfer rate were determined.

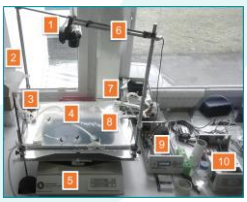


2D-bag tray prototype

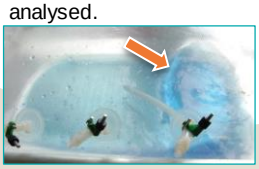
## Materials and methods

**Volumetric electric power input $P/V_L$ [kW m$^{-3}$]**
The power input was measured using a measuring device for electric power (Christ CLM 1000 Professional +). The device plots every second the actual power uptake. For the calculations, the average power over 4 minutes was used. The power needed for shaking the liquid ($P_{Vo}$) subtracted with the power of the corresponding amount of solid weight ($P_{Vc}$), divided by the infilled volume, results in the volumetric electric power input $P/V_L$.

**Mixing time $\theta_{100}$ [sec]**
The mixing times were determined using iodometry: thiosulfate redox reaction with iodine and a starch indicator. Discolouration is a result of the reduction of iodine and the oxidation of thiosulfate [2]. Discolouration was filmed and the videos analysed.




camera (1), funnel (2), gas outlet with valve (3), 20 L 2D-bag (4), reciprocal shaker 50 mm (5), LED-element (6), sodium thiosulfate injection (7), air supply (8), LED-controller (9), gas mixing station (10)



Example of the wave while shaking (4 L, 25 rpm): This kind of wave results in a good mixing time.

Example of the discolouration and a good parameter for the mixing time (4L, 25 rpm)

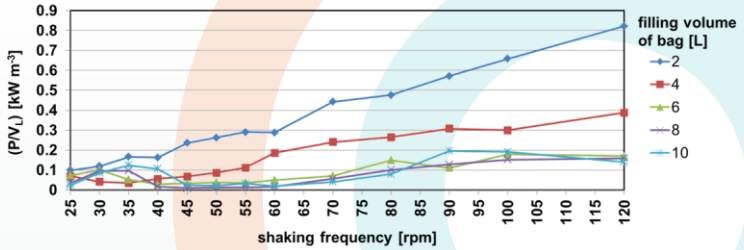
**Volumetric mass transfer coefficient $k_La$ [h$^{-1}$]**
Dynamic gassing out method in ambient temperature with air and nitrogen using a non-invasive optical oxygen sensor (PreSens)

## Results and discussion

For the measurements, the Cultibag RM 20 L from Sartorius Stedim was used on the LS-X and the new created F-2D-Bag-Tray.

F-2D-Bag-Tray

**Volumetric electric power input $P/V_L$**

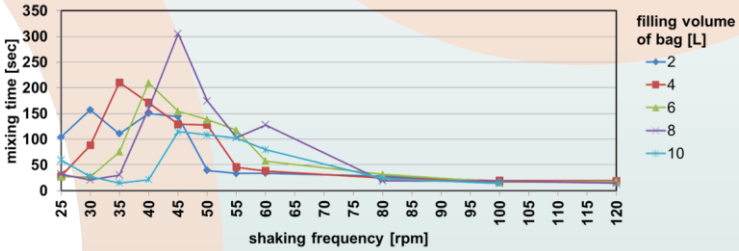



- Up to 60 rpm the curves show peaks
- Above 60 rpm slightly increasing $P/V_L$ with increasing shaking frequency,
- Increasing $P/V_L$ with decreasing filling volume from 2 L to 6 L, but further to 10 L no shifts (more or less stable $P/V_L$)
- $P/V_L$ between 0.0095 kW m$^{-3}$ (8 L, 45 rpm) and 0.8218 kW m$^{-3}$ (2 L, 120 rpm)

**Mixing time $\theta_{100}$**

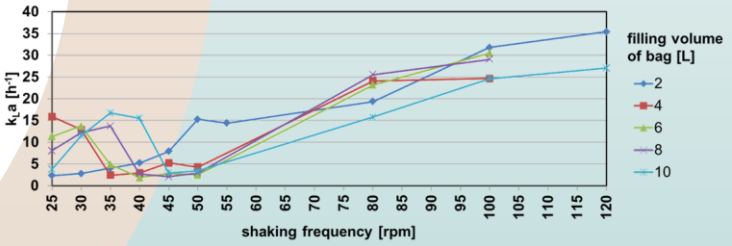
| | shaking frequency [rpm] | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| filling vol. [L] | 25 | 30 | 35 | 40 | 45 | 50 | 55 | 60 | 80 | 100 | 120 |
| 2 | 104 | 157 | 111 | 151 | 144 | 40 | 34 | 34 | 28 | 19 | |
| 4 | 28 | 88 | 211 | 171 | 129 | 128 | 46 | 39 | 25 | 20 | 19 |
| 6 | 27 | 27 | 76 | 210 | 155 | 138 | 118 | 58 | 32 | 17 | 18 |
| 8 | 32 | 21 | 31 | 159 | 305 | 175 | 103 | 128 | 19 | 18 | 15 |
| 10 | 60 | 28 | 15 | 22 | 115 | 109 | 103 | 80 | 24 | 14 | |

- The mixing times (in seconds) coloured from red (long mixing time) to green (short mixing time) show a diagonal pattern




- Up to 60 rpm irregular mixing times with several peaks
- Above 60 rpm the mixing times are shorter with higher shaking frequency
- Mixing times between 14 sec (10 L, 100 rpm) and 305 sec (8 L, 45 rpm)

**Volumetric mass transfer coefficient $k_La$ [h$^{-1}$]**




- Up to 60 rpm irregular $k_La$ values with several peaks
- Above 60 rpm increasing $k_La$ with increasing shaking frequency
- $k_La$ between 1.884 (6 L, 40 rpm) and 35.4 (2 L, 120 rpm)

## Conclusion

The investigated scale-up parameters of the RSB show that this system is suitable for the cultivation of shear sensitive cells. Therefore the RSB could be an alternative to wave induced mixing bioreactors. The new F-2D-Bag-Tray is adjustable for several 2D-bag brands and usable in Kuhner shakers (LS-X, ISF1-X)

## Literature

[1] Eibl R. *et* Eibl D. (2007). Disposable bioreactors for cell culture-based bioprocessing. *ACHEMA worldwide News* 2: 8 - 10
[2] Löffelholz C., Husemann U., Greller, G. *et al.* (2013). Bioengineering parameters for single-use bioreactors: overview and evaluation of suitable methods. *Chemie Ingenieur Technik* 85 (1-2): 1 - 18

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