Engineering characterization and cell culture application examples for two dimensional orbitally shaken single-use bags

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BIOCHEMICAL ENGINEERING AND CELL CULTIVATION TECHNIQUE

Introduction

Engineering characterization is essential for successful scaling-up, in particular for geometrically dissimilar cultivations systems. The study provides an initial engineering characterization of orbitally shaken single-use bags with square footprints, using traditional methods, and is compared with geometrically dissimilar tubular and conical cultivation vessels such as TubeSpin and shake flasks. As an additional tool, Computational Fluid Dynamics (CFD) was used to gain a deeper insight and increase knowledge of fluid flow behaviour.

The results of the engineering characterization were validated by cultivating plant suspension cultures and insect cell cultures. Both, plant and insect suspension cells can be used for the production of bioactive substances such as secondary metabolites and recombinant proteins having importance in pharmacy and cosmetics. Thus, the option to easily scale-up processes using orbitally shaken bags shows great promise.

Figure 1: INFORS HT Multitron Cell with ShakerBag Option

Procedures

- Classical engineering characterization
  - Mixing time, both with de-colorization and conductivity method
  - Oxygen mass transfer coefficient k_La with sulphite method
- Numerical methods
  - CAD geometry: either by measuring (TubeSpin and shake flasks) or by reverse engineering after laser scanning method of gypsum model (single-use bags)
  - Complete solution by open-source software: CAD (OpenSalome), Meshing (gmsh), CFD (OpenFOAM), Post-processing (Paraview)
  - Models: ~300-500k cells, k-w SST turbulence model, transient (until ~20s)
- Biological experiments
  - Plant cells: *Nicotiana tabacum* BY-2, MSMO+ medium, 25°C
  - Insect cells: *Spodoptera frugiperda* subclon 9, SFM-900 medium, 27°C, Baculovirus Expression Vector System (BEVS)

Classical engineering characterization

- Mixing time
  - Sufficient in all cultivation vessels (below 30s)
  - Demixing at certain shaking frequencies due to "out-of-phase" phenomena (10L filling volume between 70-90 rpm)
- Oxygen mass transfer coefficient k_La
  - Sufficient in all cultivation vessels (see Table 1)
  - Results obtained at maximal filling level
  - Low oxygen transfer within demixing range

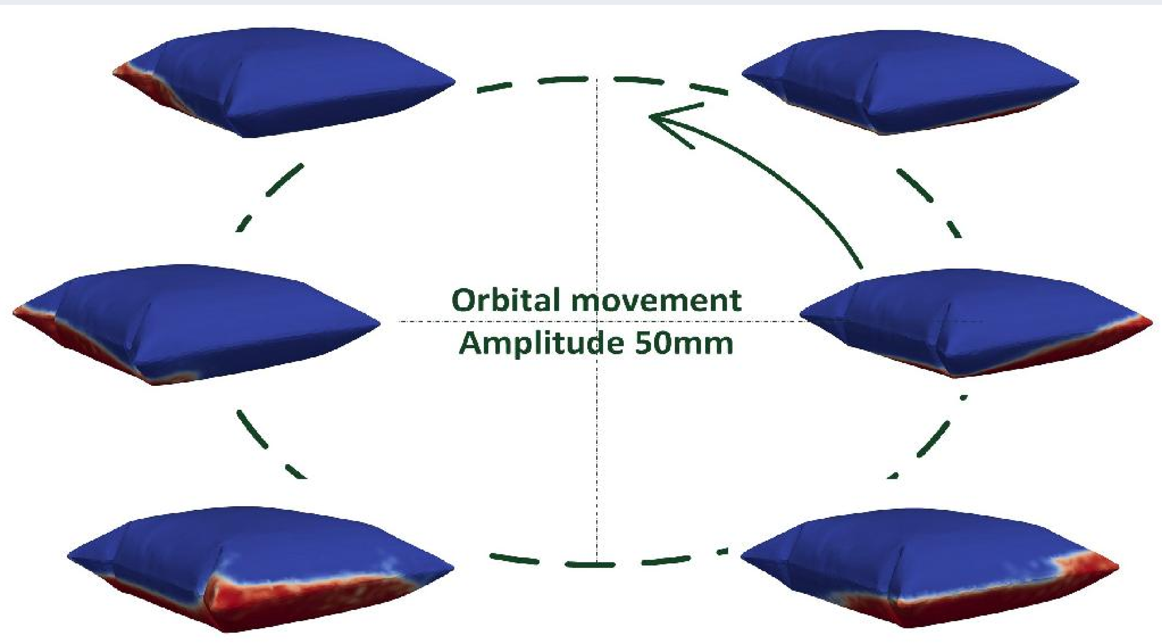

Figure 2: Time course for one period of a orbitally shaken bag

Numerical investigations

- Model
  - CFD model for 3D orbitally shaking systems with dynamic mesh, free surfaces (VOF model) und turbulence (see Figure 2 and Figure 3)
- Surface calculation
  - Calculation of the free surface (see Figure 4)
  - Aim: Calculation of oxygen transfer in next models
- Turbulence parameters
  - Spatial distribution of turbulence within the vessels over the whole volume (see Figure 5)
  - Might be used as a new scale-up and transfer parameter

Cultivation experiments

- Plant cells
  - Comparable growth up to high cell densities of about 80% packed cell volume (PCV) in all cultivation vessels (see Figure 6)
- Insect cells
  - Comparable growth characteristics both in growth and production phase in all cultivation vessels (see Figure 7)
- Proof-of-concept successful

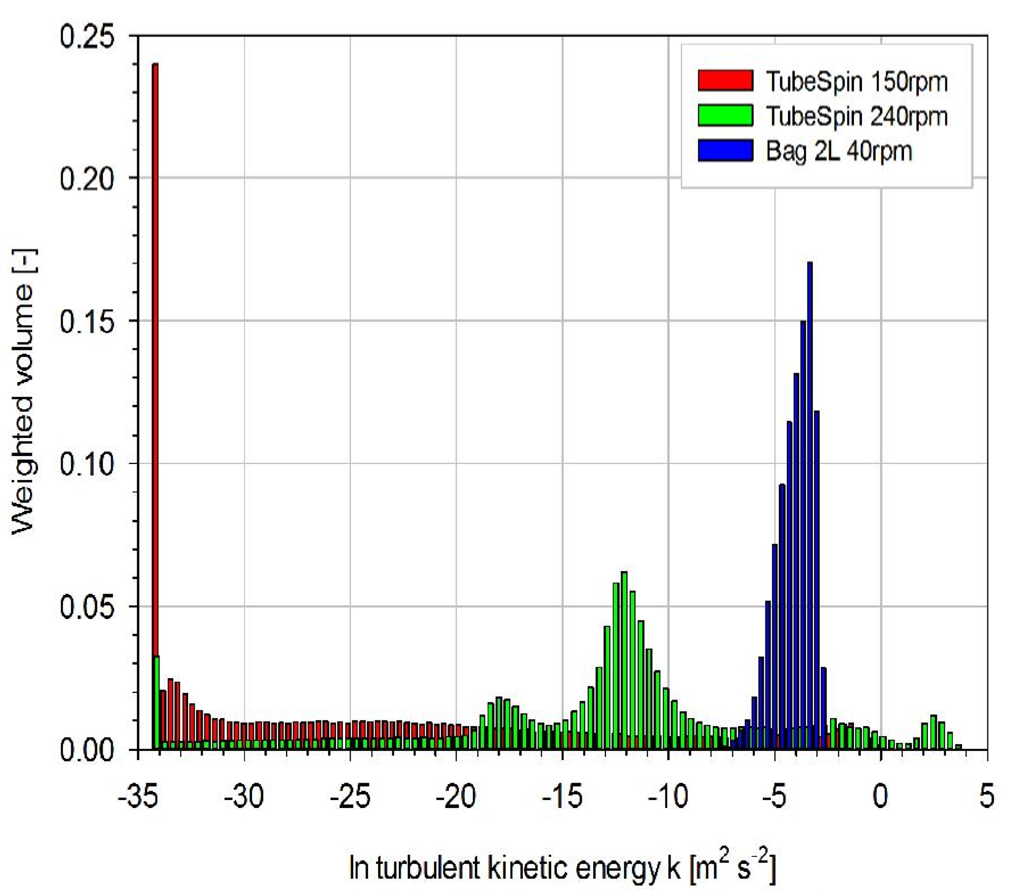

Figure 5: Volume-weighted turbulence parameters for all vessels

Table 1: k_La values at maximal filling volume in each cultivation vessel

Type	k_La [h^{-1}]	Parameter
TubeSpin	21	30mL, 275rpm, 50mm
Shake flask 500mL	30	200mL, 250rpm, 50mm
Bag 2L	68	1L, 70rpm, 50mm
Bag 20L	40	10L, 60rpm, 50mm

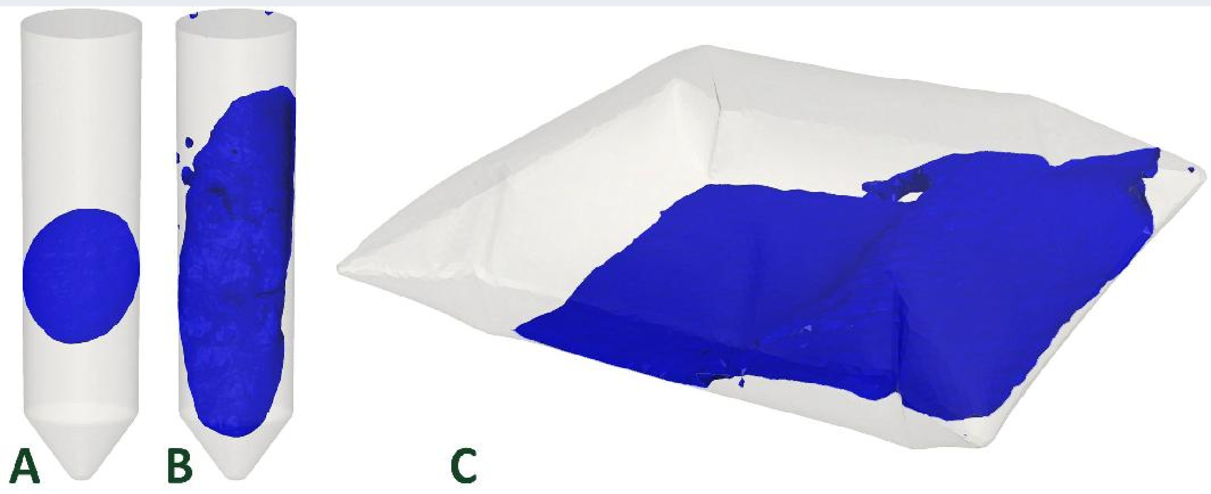

Figure 4: Surface of the TubeSpin with 20 mL filling volume, 50 mm amplitude, 150 rpm (A) and 240 rpm (B) and the orbitally shaken bag with 1 L filling volume, 50 mm amplitude, 40 rpm (C).

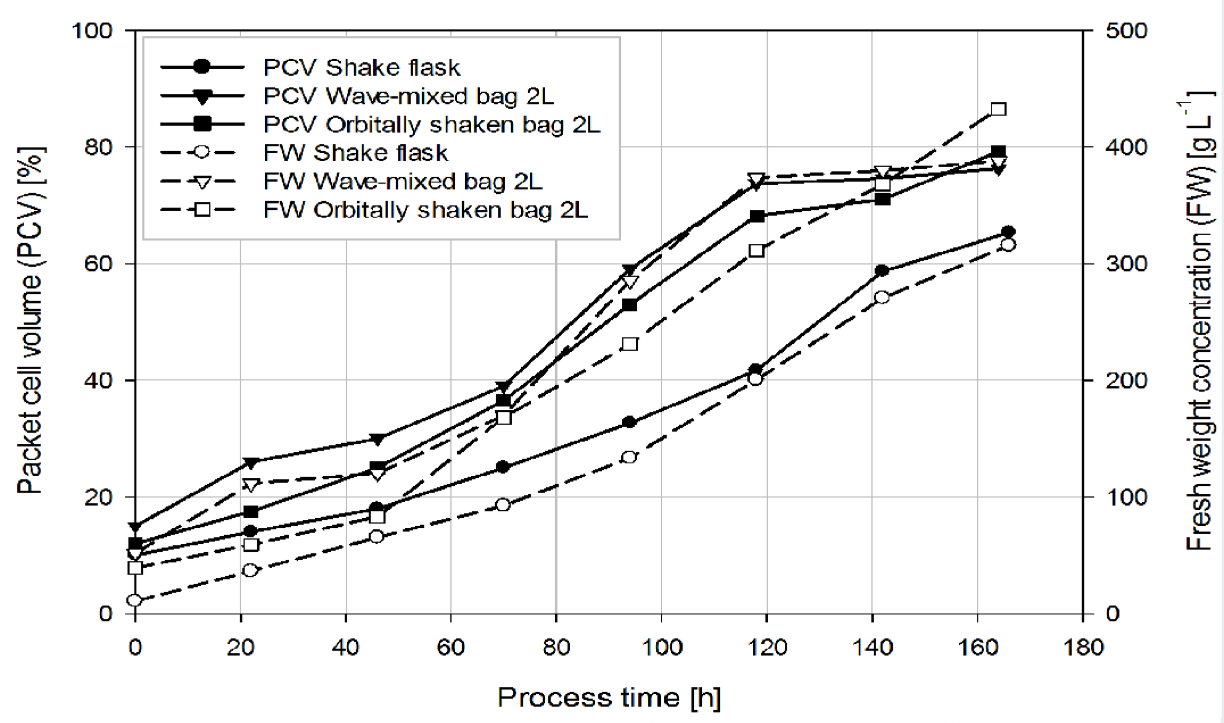

Figure 6: Packed cell volume (PCV) and fresh weight (FW) of BY-2 cells

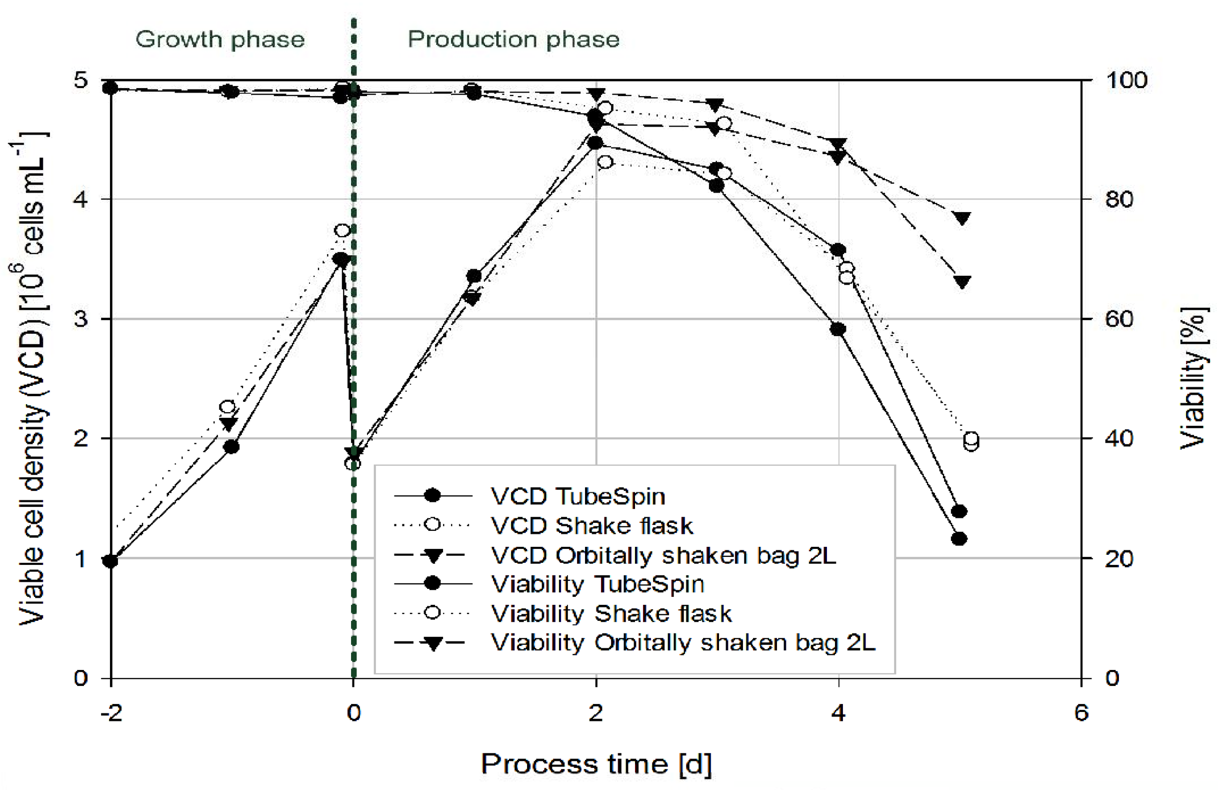

Figure 7: Viable cell density (VCD) and viability of *Sf*-9 cells

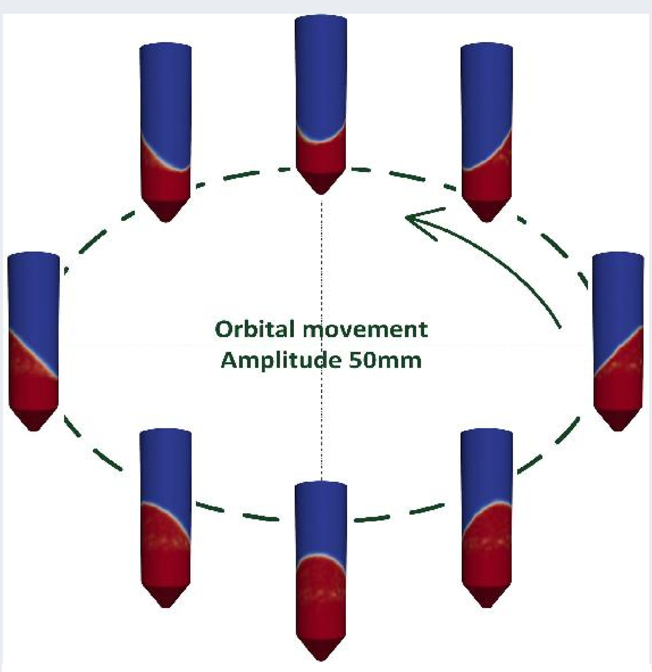

Figure 3: : Time course for one period of a TubeSpin bioreactor

Conclusion

The mixing time and the oxygen mass transfer rate was determined for all cultivation vessels by using DoE and thus, cultivation conditions can be estimated for a wide range of operating conditions.

The implementation of numerical models represents a basis for further investigations on scaling-up and process transfer for geometrically dissimilar cultivation vessels.

The cultivation of animal and plant suspension cells in the Multitron Cell with orbital shaken single use bags showed a good correlation to shake flask and wave-mixed single-use bags. The oxygen mass transfer rate was used as a basis for the transfer of process parameters.

Further investigations in CFD will be concerned with fluid flow conditions for mid to high viscosities, which occur especially in well-growing plant cell suspensions, and the calculation of the corresponding oxygen mass transfer rate.

Literature

[1] S. Werner, J. Olownia, D. Egger, D. Eibl (2013) An Approach for Scale-Up of Geometrically Dissimilar Orbitally Shaken Single-Use Bioreactors, Chemie Ingenieur Technik, 85 (1-2), 118 - 126

[2] C. Löffelholz, S.C. Kaiser, S. Werner, D. Eibl (2011) CFD as tool to characterize single-use bioreactors, *In: Single-use technology in biopharmaceutical manufacture*, R. Eibl, D. Eibl (eds), John Wiley & Sons, Hoboken, New Jesey, 264-279

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