Engineering characterization of Thomson Optimum Growth™ shake flasks with optimized geometry

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BIOCHEMICAL ENGINEERING AND CELL CULTIVATION TECHNIQUE

Introduction

The evolution of shaken flasks for biotechnological applications began in the 1940s with the increased interest in the microbial production of antibiotics [1]. The Nobel Prize awarded production of streptomycin by *Streptomyces* actinobacteria showed the main disadvantage of static cultures: slow growth. Hence, shaking platforms were constructed and an already established, readily available vessel was used: the Erlenmeyer flask [2]. Nowadays, the usage of shaking flasks is widespread due to their easy handling and thus, those reactors are preferred for use used in upstream processing [3].

In recent years, numerous articles about shake flask, e.g. about general engineering aspects [4], power consumption [3, 5-6], or gas exchange [7-8], have been published, underlining the importance of shake flask in biotechnology. However, the design hasn't changed significantly. Erlenmeyer and Fernbach style shake flask are still the predominant designs [2], despite their disadvantages (e.g. the relatively low filling volumes [1]). Consequently, the Thomson Instrument Company introduced a new shake flask design for cell cultures (Optimum Growth™), claiming higher yield on the same shaker footprint [9]. This poster gives an overview of different procedural parameters for the 500 mL and 5 L Optimum Growth™ flasks, enabling scientist to compare and evaluate the new design and to choose suitable cultivation conditions.

Methods and Results

Characterizing the mixing time

- The determination of the mixing time was accomplished with the Iodide-Thiosulfate decolorization method, according to the guideline of the DECHEMA expert group single-use technology [10].
- The mixing time was determined for 500 mL and 5 L flasks with filling volumes from 20 to 60 % and shaking rates from 60 to 250 rpm on Infors Multitron shakers with a 25 and 50 mm throw.
- Best results (mixing time below 3 s) were generated for high shaking rates (220 rpm for a 25 mm and 140 rpm for a 50 mm throw) and low to medium filling volumes (20 to 40 % filling volume, Fig. 1).

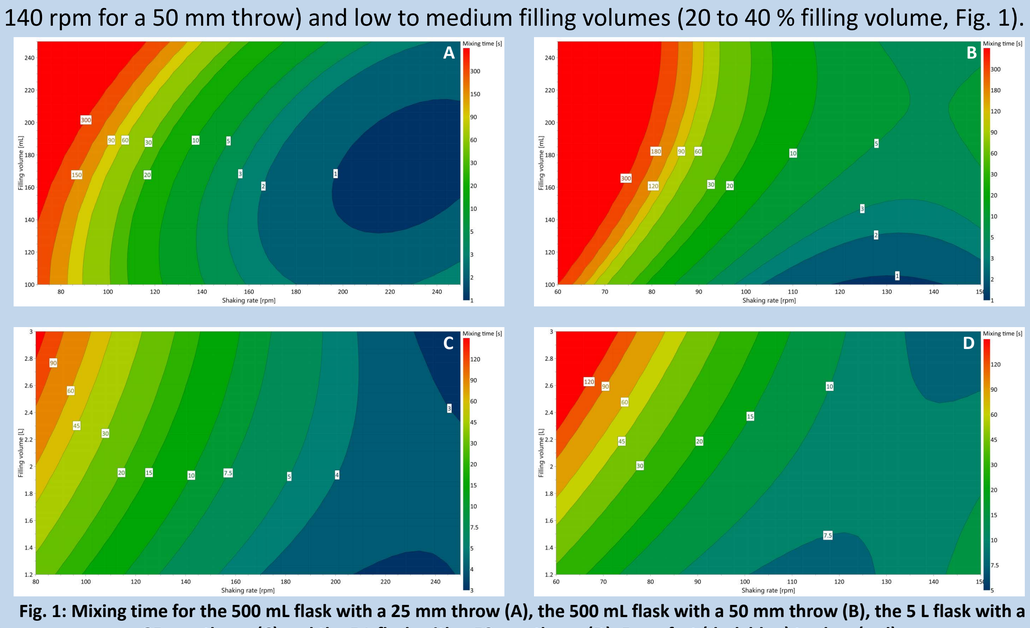

Fig. 1: Mixing time for the 500 mL flask with a 25 mm throw (A), the 500 mL flask with a 50 mm throw (B), the 5 L flask with a 25 mm throw (C) and the 5 L flask with a 50 mm throw (D). From fast (dark blue) to slow (red). Experiments were performed in triplicates.

Characterizing the oxygen mass transfer rate (k_La)

- The oxygen mass transfer rate was determined using the dynamic gassing out method, recommended by the guideline of the DECHEMA expert group single-use technology [10].
- Firstly, oxygen was stripped from the media with pure nitrogen. Afterwards, the saturation function was determined, using pressurized air and an optical dissolved oxygen sensor.
- Shaking rates and filling volumes were varied similar to the mixing time experiments (Fig. 2).
- The highest k_La (up to 50 h^{-1}) values were achieved with high shaking rates and low filling volumes

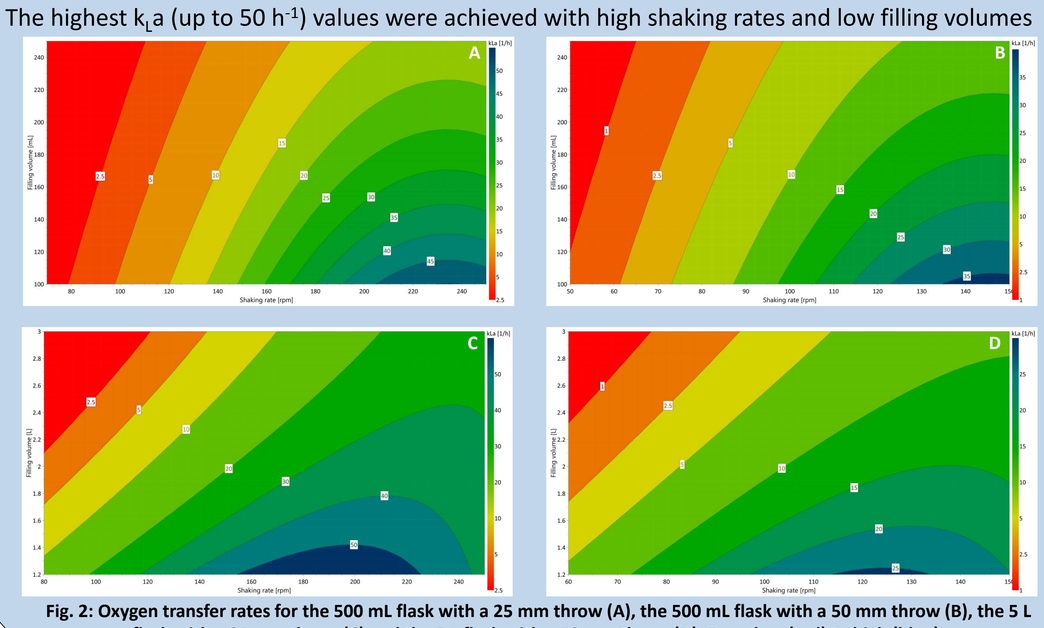

Fig. 2: Oxygen transfer rates for the 500 mL flask with a 25 mm throw (A), the 500 mL flask with a 50 mm throw (B), the 5 L flask with a 25 mm throw (C) and the 5 L flask with a 50 mm throw (D). From low (red) to high (blue). Experiments were performed in triplicates.

Estimating the specific power input

- The flask Reynolds number, modified Newton Number, and the volumetric power input were calculated using a model developed by Büchs et al. [3]. Furthermore, the energy dissipation rate was estimated using the analogy of the maximum stable drop diameters proposed by Peter et al. [11].
- The fluid behavior was simulated using the open source computational fluid dynamics software OpenFOAM with the interDyMFoam solver and the dynamic mesh method (Fig 3.)
- A specific power consumption of up to 300 W·kg^{-1} for the 5 L flask or up to 600 W·kg^{-1} for the 500 mL flask, respectively, was calculated.

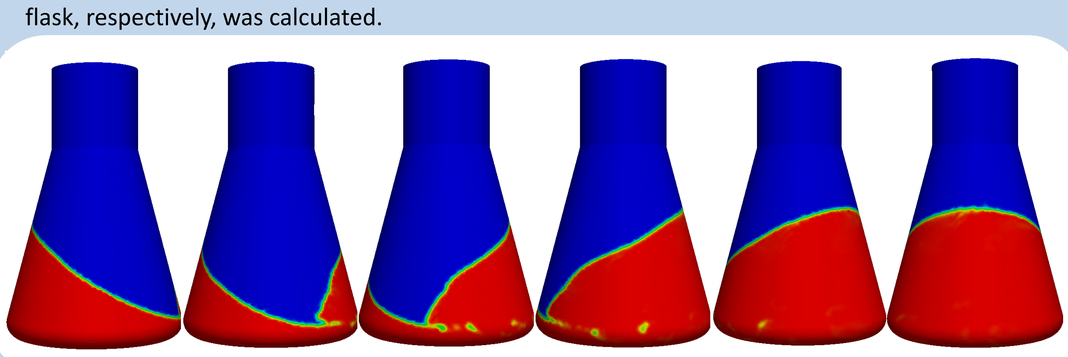

Fig. 3: Simulation of the movement of a 500 mL Thomson shake flask with a shaking speed of 150 rpm, a shaking throw of 50 mm, and a water like fluid (density of 997.05 kg m^{-3} and dynamic viscosity of 0.8927·10^{-6} Pa·s) with the computational fluid dynamics software OpenFOAM 2.4.1 (solver: interDyMFoam solver, method: dynamicMesh)

Determining favorable cultivation conditions

- Finding suitable parameter combinations for cell cultivations is in most cases a compromise between sufficient mixing, gas transfer, and suspension on the one hand and an appropriate, not too high hydromechanical stress on the other hand (represented by the volumetric power consumption or the energy dissipation rate).
- Hence, advantageous cultivation conditions ('sweet spots' as shown in Fig. 4) may be selected, e.g. an oxygen transfer rate of above 10 h^{-1}, a mixing time of below 10 s, and a specific power input of below 100 W·kg^{-1}.

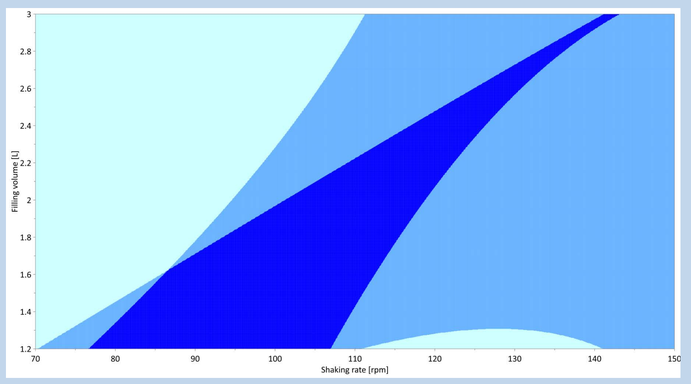

Fig. 4: Forecasting the sweet spot for the 5 L flask with a 50 mm throw: In the dark blue area, the requirements (mixing time below 10 s, k_La above 10 h^{-1} and average energy dissipation rate of below 100 W kg^{-1}) were met; in the medium blue area, two criteria were met; in the light blue area, one criterion was met

Conclusion and outlook

- Several procedural parameters for the 500 mL and 5 L Thomson Optimum Growth™ shake flasks (k_La values up to 50 h^{-1}, mixing times of under 1 s, specific power consumptions from 10 to 600 W ·kg^{-1}) were ascertained, enabling researchers to use this cultivation system expediently.
- An approach for the determination of beneficial cultivation conditions was proposed, adaptable to the needs and limitations of different cell culture cultivations.
- Further simulations with computational fluid dynamics are already in progress, increasing the range of applicability of the proposed correlations.
- Validation experiments for the proposed, favorable cultivation conditions with mammalian (Chinese hamster ovary) and plant (*Nicotiana tabaccum*) cell cultures are currently being carried out.

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