BIOTHRUST

BioThrust's 2 L ComfyCell: Technical data & Simulations

Introduction

3D production systems using procaryotic- or eucaryotic cell lines, yeast and plant cells need optimal cultivation conditions especially adapted to their needs. Conventional stirred tank bioreactors (STRs) are one of the most commonly used systems due to their versatile application, but struggle with high shearing, insufficient gas transfer and foam formation. To overcome these challenges, BioThrust developed a novel membrane stirrer (Mem*Stir*), which is used for both mixing and aeration of STRs. Aeration is provided through bubble-free diffusion at the membrane-liquid interphase. The depletion of gas-bubbles provides lower shear stress, maximal oxygen supply, fast gas-transfer rates and eliminates foam formation [1]. In the following, technical properties and simulated performance of the Mem*Stir* are displayed.

Objective

- Design & 3D printing of a robust Mem*Stir*.
- Investigation and simulation of different process modalities (oxygen, shear rate and fluidics) at small to laboratory scales.
- Observation of critical process parameters in both digital and real reactors during operation.
- Facilitating cell and gene therapy product scalability.

Key Results

1. Highly adjustable gas transfer (kLa) for effective cultivation conditions.
2. Superior oxygen supply of up to 180 mmol/L×h.
3. Designed to achieve near-absolute mixing homogeneity.
4. Shear rates significantly reduced to 25 % of conventional stirrers.
5. No foam formation!

The Mem*Stir*

The Mem*Stir* consists of multiple 3D-printed medical-grade parts that are assembled to a hollow module housing (see Fig. 1). Vertically inserted dense membranes are connected with the hollow space to establish an open-loop tangential gas flow direction (see Fig. 1A, B). Gas is introduced into the system via membrane diffusion, avoiding bubble and hence foam formation. Prior to application, it is mounted onto a modified stirrer shaft allowing module rotation during aeration. By this, it generates a radial flow profile, displacing the fluid towards the reactor wall and subsequently drawing it in from the intermediate space (see Fig. 1A). Due to the cyclone shape of the Mem*Stir* (see Fig. 1C), stirring the module generates lower local shear than conventional stirrers [2]. The design was optimized to be linearly scalable from 250 mL to 200 L (see Fig. 2). The Mem*Stir* is registered as a patent application under the international patent law treaty PTC/EP2021/ 052162 publication number W0 2021/152128 [3].

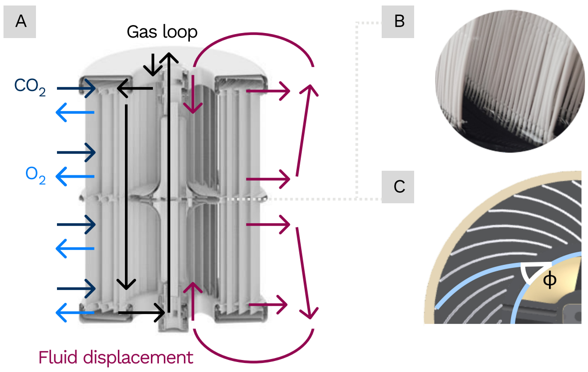

Figure 1: Schematic images of the Mem*Stir*. A. Side cross section portraying the open-loop gas flow (blue and black arrows) and the fluid displacement (purple arrows). B. Close-up of Mem*Stir* blade consisting of fixed hollow-fibers. C. Top-down perspective of a Mem*Stir* section, which shows the placement angle ϕ of the vertical membranes.

Scales

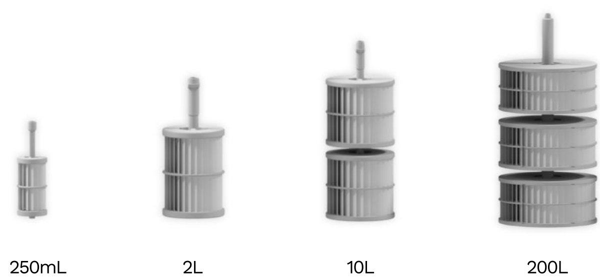

Figure 2: Current Mem*Stir* sizes for different process modalities.

The 2 L ComfyCell - CFD Simulations [50-500 rpm]

The Mem*Stir*-driven bioreactor ComfyCell was initially designed for use at a 2 L scale, a common size in process development for bioreactor research. This scale is particularly suitable for evaluating potential applications of microbial bioprocesses using bacteria, yeast or fungi [1]. The focus of these studies was to analyze critical process variables, including oxygen supply, fluid dynamics, and mechanical shear using process modalities of microbial cultivations.

Oxygen supply

A critical requirement for effective bioprocessing is the adequate supply of oxygen. To evaluate the suitability of the Mem*Stir* under the desired process conditions, the kLa value was experimentally determined using the sulfite oxidation method [4]. The amount of gas transferred into the system depends on the stirring rate, gas flow rate and the transmembrane pressure (TMP) applied to the Mem*Stir*. All three parameters were systematically varied in the experimental series. Increasing the TMP facilitated greater gas entry into the module, enhancing the concentration gradient between the hollow fiber space and the liquid, thereby improving gas transfer efficiency. Measured kLa values of the Mem*Stir* without an applied TMP reached up to 36 1/h, outperforming conventional systems (see Fig. 3). Conventional systems of renowned companies on the market reached maximal kLa's of 21, 17 and 6.6 1/h, respectively [5–7]. Furthermore, using the off-gas method to calculate the kLa, defined by DECHEMA, Society for Chemical Engineering and Biotechnology e.V. (2020), at low to moderate aeration rates (0,1 - 2 vvm) yielded superior kLa values of up to 60 1/h (data not shown) [9]. Additionally, both abiotic and biotic experiments proved that the Mem*Stir* kLa values can correlate with maximum oxygen transfer rates of up to 180 mmol/L/h (for more details please refer to our publication [1] as well as application note of our *P. putida* fermentation).

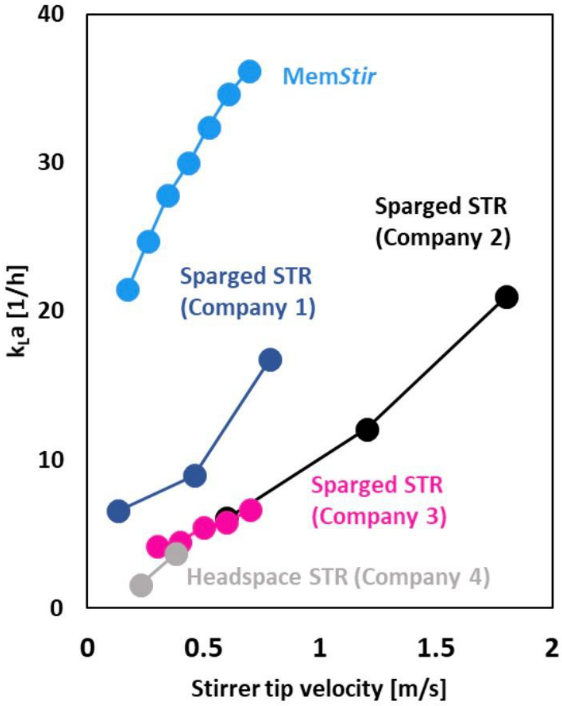

Figure 3: Determined kLa values in 1/h for bioreactors on the market (1.5 – 3 L) using the sulfite oxidation method at physiological conditions (37°C, pH 7, 0.1 vvm, tip speeds: 0,2 – 2 m/s: 50 – 650 rpm).

Simulation – Fluid Velocity

To investigate the fluid flow velocity of the Mem*Stir* a CFD simulation of fluid velocity distribution in a 2 L scale was performed using the simulation software COMSOL® Multiphysics 5.4 (see Fig. 4). Both mixing efficiency and cellular stress caused by operation conditions, fluid velocity and shearing rates were included.

At a stirring rate of 500 rpm within the 2 L Mem*Stir* reactor system fluid velocities of 0.3 m/s on average were reached, with fluid tip velocities of up to 1.4 m/s (see Fig. 4A). With an increase of the rpm from 200 to 500 rpm (see Fig. 4B), fluid velocities gradually increased from less than 0.1 m/s at 200 rpm, 1 m/s at 300 rpm, 1.2 m/s at 400 rpm and 1.4 m/s at 500 rpm, respectively. Mechanical efficiency of the Mem*Stir* for mixing is therefore demonstrated. Thus, when an adaptation of a conventional impeller-based STR to the Mem*Stir* process is planned, lower rotational speeds will be necessary to reach similar mixing efficiencies.

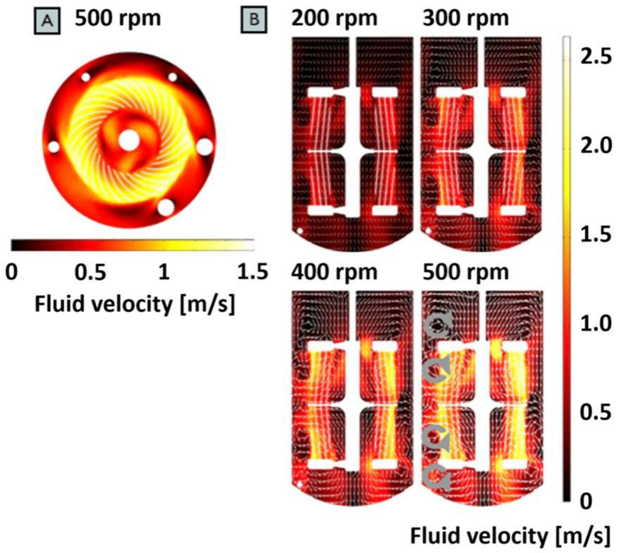

Figure 4: CFD-simulated fluid flow velocity distributions of the 2 L Mem*Stir*. A. Horizontal cross-section of the local fluid velocity at a stirring rate of 500 rpm. B. Vertical cross-sections of four different exemplary stirring rates from 200, 300, 400 and 500 rpm. The grey arrows indicate the flow direction.

Simulation – Shear rates

The average shear rate of all membrane surfaces was linear in the examined stirrer rate of 200 rpm (see Fig. 5, 3D model of the 2 L Mem*Stir*) and 300 rpm (Fig. 5 detailed blade view), ranging between 50 and 500 min−1, easily undercutting the damage threshold of shear-sensitive cells at 1800 1/s [1,8]. As shear forces are induced only by the stirrer in the Mem*Stir* reactor (no bubble rupture), stirrer tip speeds can be used as an indicator for the intensity of shear forces.

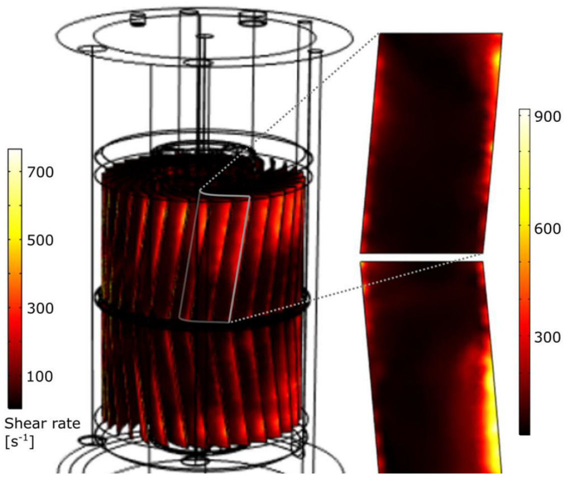

Figure 5: CFD-simulation of local Mem*Stir* shearing rates in operation mode. On the left a 3D model of the Mem*Stir* is shown with indicated shear rates on the membrane-mesh blades at a stirring rate of 200 min^{-1}. On the right, shear rate distribution on a single membrane-mesh blade at a stirring rate of 300 min^{-1} is indicated. CFD, computational fluid dynamics.

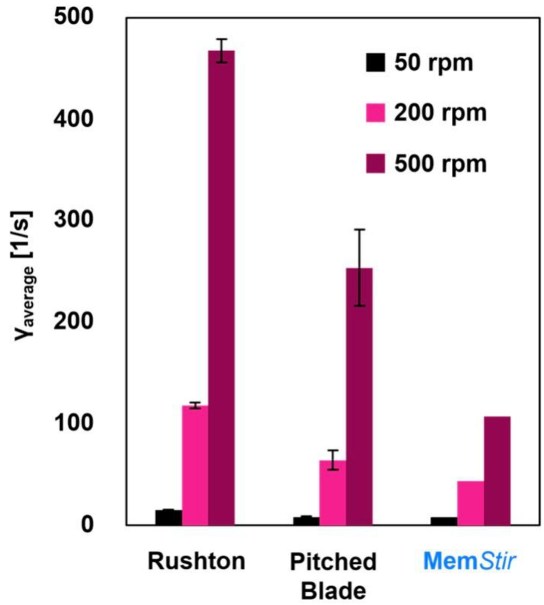

Figure 6: Comparison of average shear rates $\gamma_{average}$ for different reactor systems at 50, 200 and 500 rpm. $\gamma_{average}$ values for both Rushton and Pitched Blade were calculated by procedures of Muralidharan (2023), whereas values for the Mem*Stir* are a CFD simulated.

With a circumferential speed of the outer membrane at 2.4 m/s, mechanical shearing of the Mem*Stir* lies at the lower end of stirrer tip speeds in microbial processes ranging between 2 and 5 m/s, respectively [10].

Figure 6 presents the simulated average shear rates ($\gamma_{average}$) of the Mem*Stir*, compared to those of stirred tank reactor (STR) systems equipped with either a Rushton or a conventional pitched-blade impeller. The Rushton impeller generates the highest average shear rates, ranging from 15 s^{-1} at 50 rpm to over 470 s^{-1} at 500 rpm. In contrast, the pitched-blade impeller produces lower shear rates, from 8 s^{-1} at 50 rpm to over 250 s^{-1} at 500 rpm. The Mem*Stir* impeller exhibits the lowest shear rates, with values of 8 s^{-1} at 50 rpm and approximately 110 s^{-1} at 500 rpm, resulting in a shear stress reduction of up to 77 % at 500 rpm.

Application fields

Cell types/-Catalysators

iPSCs | MSCs | Organoids | Insect Cells | CHO | NK-Cells | Bacteria | Enzymes | HEK | T-Cells | Macrophages | Spheroids Fungi | Yeast | Plant Cells

Products

In vitro study material | Tissue regeneration | Immune modulation mRNA products | Antibodies | Platform chemicals | Enzymes | Viral vectors | Proteins | extracellular Vesicles

Process modalities

Adherent cells | Suspension cells | Differentiation | Cell clusters

Discussion

Cell cultivations in conventional stirred tank bioreactors face various limitations such as foam and shear stress from bubble-aeration and mechanical agitation, insufficient oxygen transfer, nutrient gradients, and scalability challenges, all negatively impacting cell viability and productivity. BioThrust's bionic ComfyCell with integrated Mem*Stir* addresses these issues by minimizing shear forces, eliminating foam, reducing culture time, and enhancing medium efficiency. Real process scalability is achieved by geometric analogy, enabling linear scale-up from 250 mL to 200 L. The Mem*Stir* ensures low shear forces, uniform mixing, and precise process control for consistent product quality.

The initial experiments conducted with the Mem*Stir* bioreactor yielded promising results at a scale of 2 L. In particular, the achieved kLa values within operation range indicate an overall improved oxygen supply, with even higher potential when applying a TMP. It is also noteworthy that lower stirring speeds in the Mem*Stir* resulted in enhanced mixing efficiencies relative to conventional STRs. The Mem*Stir* geometry is designed by CFD simulations for homogeneous mixing and maximum oxygen supply while maintaining the mechanical shear forces below the damage threshold for shear-sensitive cells of 1800 1/s [1].

All aspects indicate that the Mem*Stir* offers a promising solution to enhanced bioprocess efficiency. Considering shear sensitivity, but also oxygen supply, the Mem*Stir* poses as a perfectly fitting alternative for not only microbial but also eukaryotic processes in pharmaceutical production.

References

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Abbreviations

STR = Stirred tank reactor
Mem*stir* = Membrane stirrer
$k_L a$ = volumetric mass transfer
iPSCs = induced pluripotent stem cells
CHO = Chinese hamster ovary
NK = Natural killer
HEK = Human embryonic kidney
mRNA = messenger Ribonucleic acid
MSC = mesenchymal stem cells
OTR = Oxygen transfer rate
TMP = Transmembrane pressure
rpm = Rotations per minute
CFD = Computational fluid dynamics
$\gamma_{average}$ = Average shear rate in 1/s

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