

A Benchmark Comparison

Superior 3D Cultivation of CHO Cells using a novel bubble-free 10 L bionic Bioreactor

Introduction

Production systems for cell lines producing Advanced Therapy Medicinal Products (ATMPs) or antibodies need optimal cultivation conditions. The novel membrane stirrer (*MemStir*) developed by BioThrust, is designed for use in Stirred-Tank Bioreactors (STRs), provides lower shear stress, maximal oxygen supply by bubble-free diffusion, and eliminates foam formation [1].

The application note is focusing on 3D cultivation experiments with CHO cells conducted to establish a benchmark run comparing the BioThrust *MemStir* with a conventional STR. The *MemStir* showed prolonged growth stability for 12 days, enhanced oxygen supply and continuous CO₂-stripping, resulting in reduced cell stress and a viable cell concentration (VCC) of > 20 million cells/mL. These findings highlight the *MemStir*'s potential for economic efficiency on an industrial scale.

Objective

- Proof-of-Concept (POC) for a high-density cultivation of CHO Cells over a 12-Day period using BioThrust's *MemStir*.
- Determination of the oxygen supply requirements through diffusion to achieve optimal mass transfer rates.
- Evaluation of the impact of reduced shear stress on cell growth.

Key Results

1. >20 Mio viable cells/mL in a first POC Run.
2. Enhanced Oxygen supply and CO₂ stripping.
3. Lower shear stress in comparison to reference.
4. No Antifoam needed.

Abbreviations

ATMP = Advanced Therapy Medicinal Products
MemStir = Membrane Stirrer
 STR = Stirred-tank bioreactor
 CHO = Chinese hamster ovary
 Vvm = volume of air sparged per working volume per minute

VCC = Viable cell concentration
 Vb = Cell viability
 Glc = Glucose
 Lac = Lactate
 Gln = Glutamine
 b = Osmolality

Materials & Methods

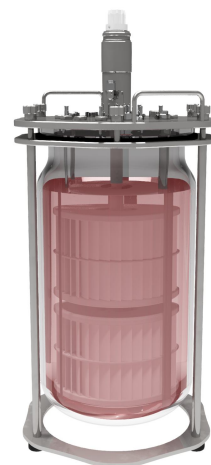


Figure 1: Front view of the 10 L *MemStir* bioreactor vessel.

Specifications

- Production bioreactor: 10 L; Working volume.: 6.5 L.
- 1 x STR (reference) vs. 1 x *MemStir* 10 L (top image).
- Cultivation method: Fed-Batch.
- Seeding VCC: 1.28E+06 cells/mL, 99.8 % Viability (Vb).
- Process time: 12 days, Glucose & Base addition: Day 2.

Parameter	<i>MemStir</i>	STR Reference
Stirrer	93 rpm	134 rpm (2 x pitch blade impellor)
DO	60 %	60 %
pH	7.0 ± 0.2 (base and CO ₂ adjusted)	7.0 ± 0.2 (base and CO ₂ adjusted)
Headspace gassing	-	Constant air flow
Sparger gassing	0.75 L/min constant air flow; flow of 10 mL/min CO ₂ on demand.	O ₂ and CO ₂ on demand.
Transmembrane pressure (TMP)	100 mbar from day 6	N/A
Antifoam	-	8 mL

[1] – Bongartz et al. (2023). A novel membrane stirrer system enables foam-free biosurfactant production. *Biotechnology & Bioengineering*. 120(5), pp. 1269 – 1287. DOI: 10.1002/cite.202255100.

Results

Cellular Growth

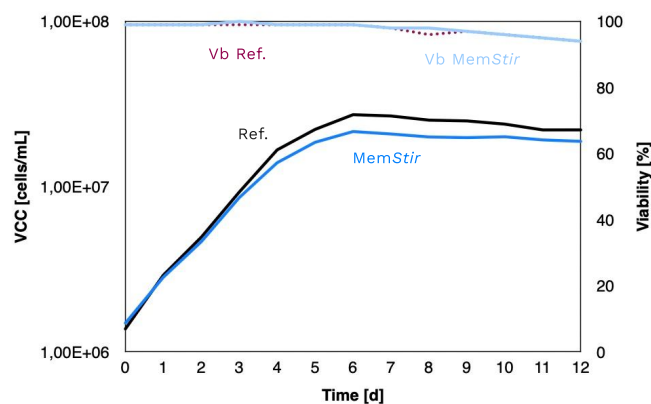


Figure 2 CHO cell growth and viability during a 12-day fed batch cultivation. Benchmark run of BioThrust MemStir in comparison to reference STR.

During the 12-day CHO-cell fed-batch run a maximal VCC of $2.15E+07$ cells/mL in the BioThrust MemStir was achieved with a viability > 94 % (Fig. 2).

Metabolic/ Chemical Parameters

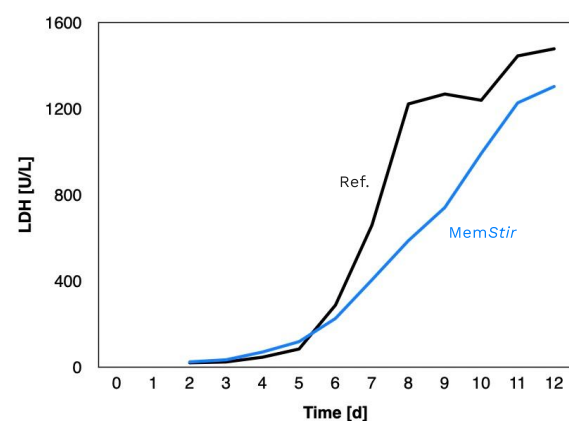


Figure 3 Lactate dehydrogenase production during a 12-day fed-batch cultivation. Benchmark run of BioThrust MemStir in comparison to reference STR.

The lactate dehydrogenase (LDH) concentration within the BioThrust MemStir remained below the reference during the stationary phase of the cell growth (Fig. 3). The metabolic and chemical profile of the MemStir were within the same range as within the reference STR (Fig. 4-9).

Ammonium

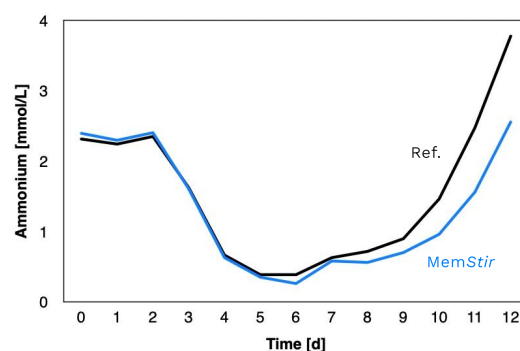


Figure 4 Ammonium concentrations measured in reference STR (Ref.) and BioThrusts Membrane stirrer (MemStir). During a 12-day fed-batch CHO cell cultivation daily cell-free samples were taken and analyzed using Cedex® Bio analyzer.

Glucose

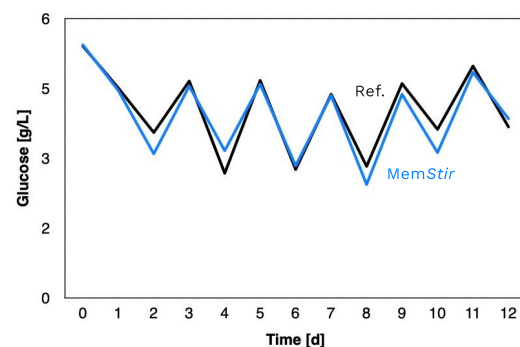


Figure 5 Glucose concentrations measured in reference STR (Ref.) and BioThrusts Membrane stirrer (MemStir). During a 12-day fed-batch CHO cell cultivation daily cell-free samples were taken and analyzed using Cedex® Bio analyzer.

Glutamine

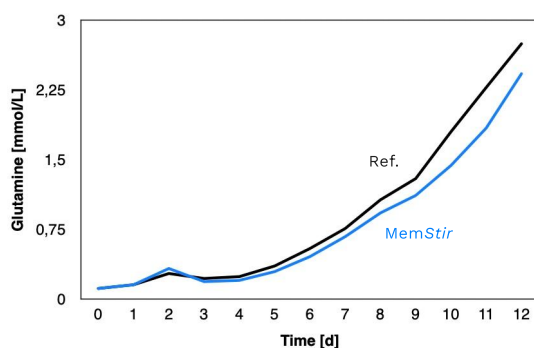


Figure 6 Glutamine concentrations measured in reference STR (Ref.) and BioThrusts Membrane stirrer (MemStir). During a 12-day fed-batch CHO cell cultivation daily cell-free samples were taken and analyzed using Cedex® Bio analyzer.

Outcome

- VCC of >20 Mio/mL was reached in a first POC run.
- Improved and very efficient gas transitions. Small adjustments lead to fast conversion rates.
- Very efficient air transfer rates achieved and thus no pure oxygen needed, enabling high cell-density cultivations in industrial scale in the future!
- Lower shear-stress than the reference indicated by lower LDH, as well as remaining DNA und Host cell protein (not shown) levels.
- Metabolic and chemical parameters within physiological range and comparable with the reference.
- No Antifoam needed within the MemStir.

Discussion

Mammalian cell cultivations in conventional stirred-tank bioreactors face various limitations such as foam and shear stress from bubble-aeration as well as mechanical agitation, insufficient oxygen transfer, nutrient gradients, and scalability challenges, all negatively impacting cell viability and productivity. BioThrust's bionic MemStir 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 100 mL to 200 L. The MemStir ensures low shear forces, uniform mixing, and precise process control for consistent product quality especially suitable for mammalian cell cultivations as shown in this work using CHO cells in 3D.

Osmolality

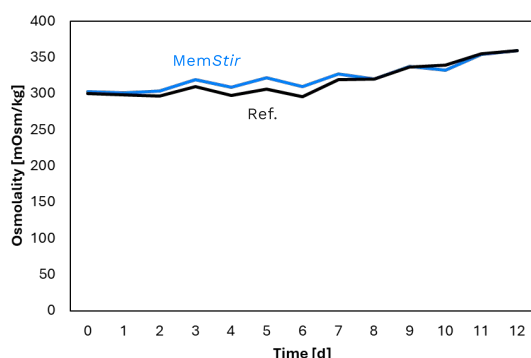


Figure 7 Osmolality measured in reference STR (Ref.) and BioThrusts Membrane stirrer (MemStir). During a 12-day fed-batch CHO cell cultivation daily cell-free samples were taken and analyzed using Cedex® Bio analyzer.

pCO₂

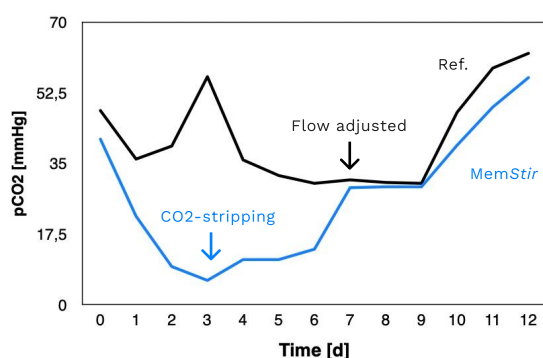


Figure 8 Partial pressure of carbon dioxide measured in reference STR (Ref.) and BioThrusts Membrane stirrer (MemStir) during a 12-day fed-batch CHO cell cultivation.

pH

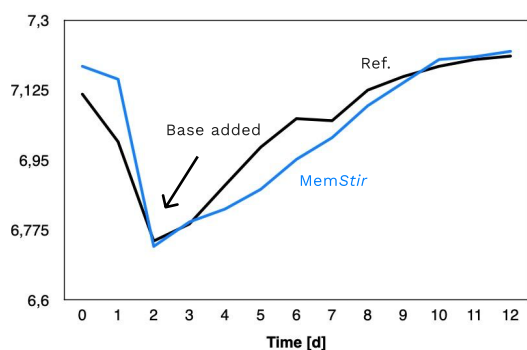


Figure 9 PH measured in reference STR (Ref.) and BioThrusts Membrane stirrer (MemStir) during a 12-day fed-batch CHO cell cultivation. At the lower deadband 30 mL base was added up to day 3.



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