BIOTHRUST

A Benchmark Comparison

Enhanced Growth Stability in CHO Cell Cultures using 2 L BioThrust MemStir

Introduction

Production systems using mammalian cell lines need optimal cultivation conditions. The novel membrane stirrer (Mem*Stir*) developed by BioThrust, is designed for use in Stirred Tank Bioreactors (STRs), provides adjustable low shear rates, maximal oxygen supply by bubble-free diffusion, and eliminates foam formation [1]. Cultivation experiments with CHO cells were conducted as a duplicate to establish a benchmark run comparing the Mem*Stir* with a conventional STR. The Mem*Stir* showed prolonged growth stability for 11 days with superior oxygen availability, resulting in a viable cell concentration (VCC) of more than 20 million cells/mL while maintaining a high viability (Vb). These findings highlight the Mem*Stir*'s potential as an improved system for industrial processes.

Objective

Aligning cultivation conditions of STR and Mem*Stir* based on critical process parameters [2], [3]:

1. Volumetric power input P/VL
2. Volumetric aeration rate Qtotal.

Operation of two phases:

1. Batch: direct comparison in a simplified system.
2. Spike: observing long-term culture stability by replenishing the primary carbon source.

Quantifying and evaluating system performances via key parameters:

1. Cellular growth: VCC, Vb, Physiology
2. Respiration activity: Gas transfer rates (GTR) of oxygen (OTR) and carbon dioxide (CTR)
3. Metabolic activity: concentrations of Glucose (Glc), Lactate (Lac) and Glutamine (Gln)
4. Osmotic pressure: Osmolality, pH

Key Results

1. Improved viable cell concentration of up to 24 mio cells/mL.
2. Advanced product conversion rate: + 40 % compared to reference.
3. Higher respiratory activity with faster substrate depletion rates.
4. Simplified cell handling: No Foam & No oxygen limitations.

Materials & Methods

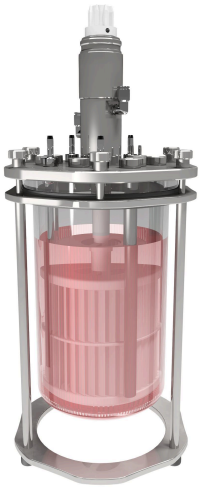

Figure 1: Front view of the 2 L Mem*Stir* bioreactor vessel.

Specifications

- Laboratory bioreactor: Total vol.: 2.5 L; working vol.: 2 L
- 2 x STR with two-stage 2- blade impeller (Reference) & 2 x Mem*Stir* (see Fig. 1)
- Cultivation method: Batch
- VCC = 5E+5 cells/mL,
- Vb = 97.0 %
- Process time: 11 days, Glucose Feed: Day 6, Base addition: constant

Parameter	Mem*Stir*	STR Reference
Stirrer	70 rpm	150 – 250 rpm
DO	50 %	50 %
pH	7.3 ± 0.05 (base and CO2 adjusted)	7.3 ± 0.05 (base and CO2 adjusted)
Headspace gassing	Open-loop off-gas circulation	Constant air flow
Aeration volume flow	0.5 L/min (0.2 vvm) 5 ± 0.05 % CO2	0.25 – 0.75 L/min (0.125 – 0.375 vvm) 5 ± 0.05 % CO2
Antifoam	-	12 mL

Abbreviations

Mem*Stir* = Membrane stirrer
STR = Stirred tank bioreactor
CHO = Chinese hamster ovary
P/VL = Volumetric power input
Qtotal = Volumetric aeration rate
Vvm = volume of air sparged per working volume per minute
VCC = Viable cell concentration
Vb = Cell viability
GTR = Gas transfer rate
OTR = O2 transfer rate
CTR = CO2 transfer rate
Glc = Glucose
Lac = Lactate
Gln = Glutamine
b = Osmolality

Results

Cellular Growth

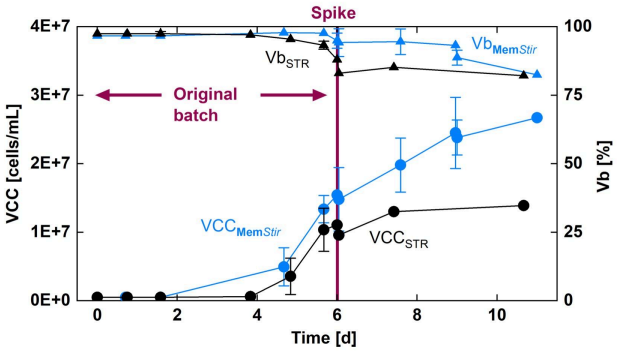

Figure 2: VCC and Vb over the cultivation time of STR (n = 1 – 2) and Mem*Stir* (n = 2) process cultures. The purple vertical line represents the time of introducing a high-concentrated substrate solution (Spike).

Off-line sampling revealed a comparable VCC at around 12 million cells/mL in both systems at the end of the batch phase, while the spiking phase shows 13 million cells/mL (STR) and 24 million cells/mL (Mem*Stir*), respectively (see Fig. 2). Cell viability seemed to be more stable throughout the spiking phase in the Mem*Stir* process, indicating that long-term cultivations with continuous feeds could profit from the Mem*Stir* approach.

Respiratoy activity

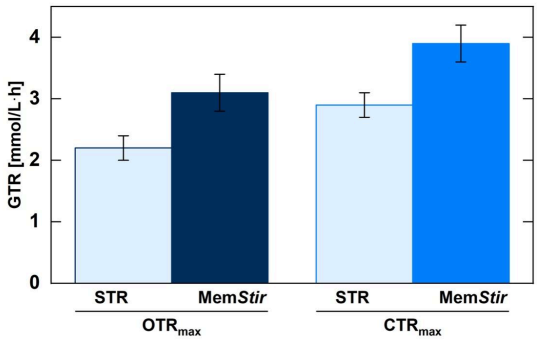

Figure 3: Maximum GTR values for STR and Mem*Stir* processes. Error bars represent minima and maxima of the performed duplicates including error propagation caused by measuring noise.

During CHO processes, off-gas measurements of OTR and CTR showed an overall higher respiration in Mem*Stir* system cultures (see Fig. 3). Maximum values of 2.2 mmol/L·h (STR) and 3.1 mmol/L·h (Mem*Stir*), increasing activity to around 40 %. From this, a higher product conversion might be achieved in induced processes.

Handling of operation

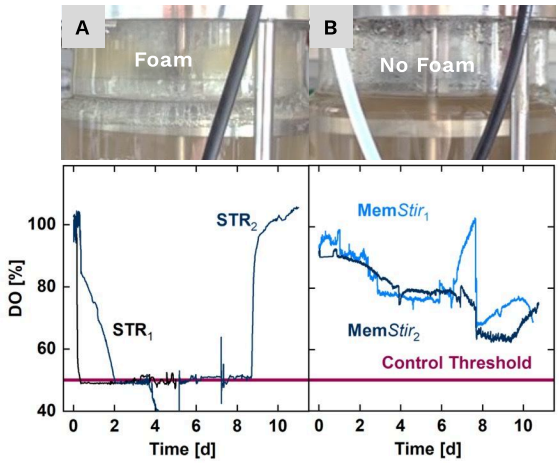

Figure 4: Dissolved oxygen trends for STR (A, n = 1 – 2) and Mem*Stir* processes (B, n = 2). Pictures above were taken on day 5 and display the different degrees of foaming. The control threshold acts as a minimum value under which CHO cells are not supplied sufficiently with O2.

Over the course of the cultivation, STR operation struggled with foam formation while keeping a sufficient oxygen availability[4] (control threshold, see Fig. 4). Under given conditions, Mem*Stir* cultures did not face any oxygen limitations. This displays an easier reactor handling.

Metabolic activity

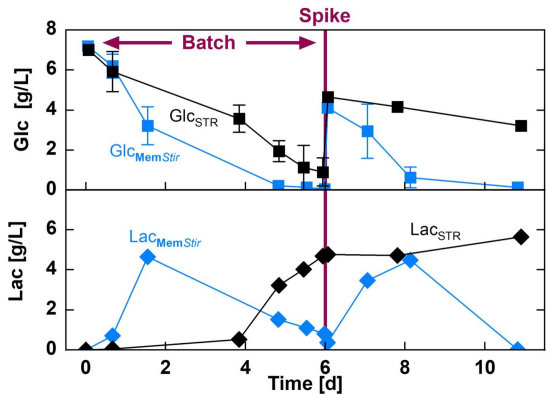

Figure 5: Metabolite concentrations of Glc and Lac over the cultivation time of one representative STR and Mem*Stir* process each. The purple vertical line represents the time of spiking.

Chromatography analysis displays a higher Glc consumption during batch and spiking phase in the Mem*Stir* system. Gln shows a similar trend. The intermediate product Lac is produced earlier in the Mem*Stir* system, but also subsequently metabolized during glucose depletion (see Fig. 5). This substrate metabolization suggests a higher metabolic activity.

Osmotic stress

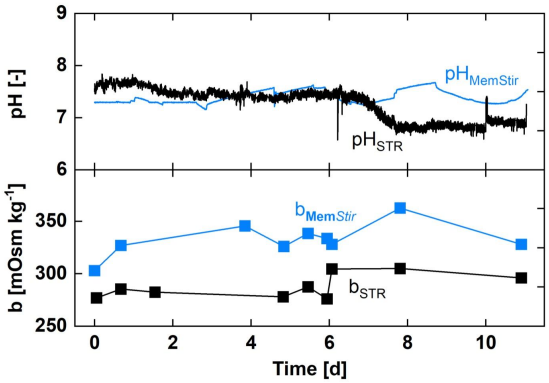

Figure 6: Values for b and pH over the cultivation time of one representative STR and Mem*Stir* process each. Samples were analyzed as technical triplicates.

During STR and Mem*Stir* cultivation, the pH of both processes do not drop below growth-limiting conditions. At the same time, both osmolality values stay within a recommended range of cell culture media (see Fig. 6).

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.

References

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[3] – Mandenius (2006). Bioreactors: Design, operation and novel applications. Wiley-VCH Verlag BmbH & Co. KGaA, pp. 323 – 354. DOI: 10.1002/9781118067147.ch13.

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