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Simplifying Progress

Upstream Microbial Process Characterization with Single-Use Bioreactors from 250 mL to 50 L

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Introduction

Developing biopharmaceuticals derived from microbial fermentation relies upon performant bioreactors to allow a rapid scale up to commercial batches. For this it is relevant to minimize any possible risks while developing a process that fits the industry quality standards. The choice of a well characterized system plays an important role from R&D through to production stages. With the Sartorius Upstream portfolio it is possible to accelerate development timelines and ensure process success.

The aim of this poster is to provide evidence to demonstrate the benefits of a microbial process developed using Sartorius scalable solutions. The method chosen to showcase this consistency is based on the DECHEMA Guidelines for Engineering Characterization principles which include a set of standard conditions for bioreactor characterization.

DECHEMA
Gesellschaft für Chemische Technik und Biotechnologie e.V.

Process Characterization with the DECHEMA Guidelines

Figure 1. DECHEMA Characterization principles

Process Engineering Characterization
kLa (Gassing out Method), Mixing Time, P/V

DECHEMA 2016 Experimental Methods guidelines defined for Bioreactors (Single-use and Reusable systems) [1]

+

Microbial/Biological Characterization
kLa (Oxygen Balance Method)

DECHEMA Standardized Batch Fermentation Biological Model based on E. coli W3110 culture [2] [11]

Assessment of Bioreactor Performance

MODDE®

These characterizations can benefit significantly from MODDE® - Umetrics' suite for enhanced DoE investigations.

Process Engineering Characterization
Define the best parameters to work with [1]:
- Stir speed
- Maximum working volume
- Mixing times via conductivity/ decolourization
- Power input based on the vessel and motor geometry/torque
- pO_2 with good sensor response time (11s)
- kLa via gassing-out method [6]

Biological Characterization
- Based on process engineering characterization parameters [11]
- tip speed and gassing rates are defined according to Table 3
- kLa measurement method via exhaust gas composition (Balancing method) [4] [11]
- Batch settings:
  - Initial OD^{600} = 1
  - Glucose concentration at 80g/L
  - pH and foam controlled
  - pO_2 is not controlled, process terminates at pO_2 <5%

Ambr® Platform for Enhanced Screening and Optimization

Key biological characterization results on Ambr® 250 Modular
- Excellent OTR and mixing support a range of high density cultures
- Comparability proven to 5 L benchtop Univessel® [9] and larger scale volumes (Table 2)
- Batch Model graph not shown however batch growth data is shown in Table 2

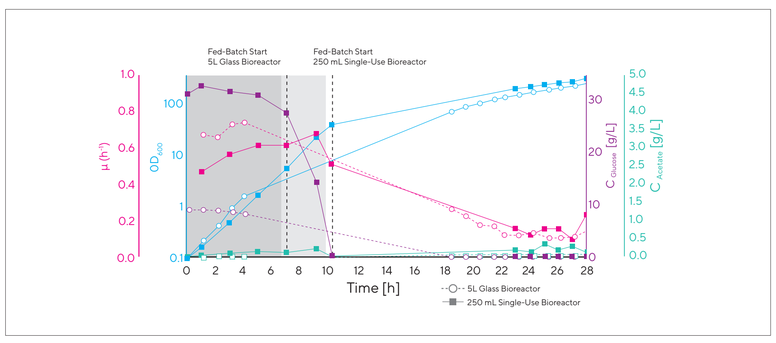
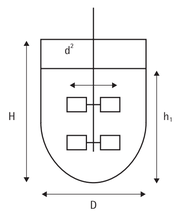


Figure 2. Fed-batch Model E. coli cultivation in an Ambr® 250 Modular system (two runs, diamonds and squares) compared with a Univessel® Glass 5 L bioreactor (circles); Shown are optical density at 600 nm (blue), growth rate (dark pink), acetate (teal), and glucose concentration (purple). Dashed line indicates feed [9].

Ambr® 250 bioreactor dimensions:
H/D = 2.0;
h1/D = 1.44;
d2/D = 0.42



Ambr® 250 Modular system: comprising control module and bioreactor stations. Shown here with 2, the system is available with stations for up to 8 minibioreacor vessels.

- Microbial Strain Screening
- Media Optimization

Reliable Scale-Up With the Biostat STR® 50L System

Key biological characterization results on Biostat STR® 50L Prototype in Figure 3. Shaded area shows the batch part before fed-batch start. The vertical dashed line indicates the one-time addition of feed 2 (grey). Batch Model graph not shown however batch growth data is shown in Table 3

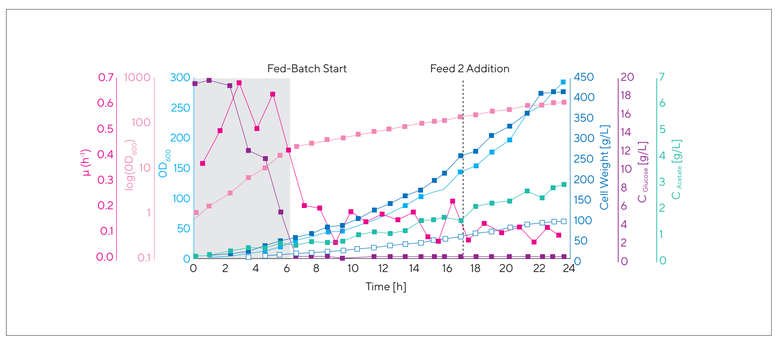


Figure 3. Fed-batch Model E. coli cultivation in the Biostat STR® 50 L MO prototype. Shown are logarithmic optical density log(OD600) with exponential fit for batch phase y = 0.71 e0.60 t with R = 0.998 and for fed-batch phase y = 16 e0.13 t with R = 0.995 (light pink). Moreover, optical density OD_{600} (blue), specific growth rate μ (dark pink), wet cell weight (dark blue filled squares), dry cell weight (dark blue empty squares), glucose concentration 'C glucose' (purple) and acetate concentration 'C acetate' (teal) are shown.

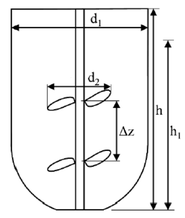
Table 2. Fed-batch parameters on Biostat STR® 50 L

	fed-batch
Specific growth rate μ_{set} [1/h]	0.15
Filling volume start [L]	24.0 (60%)
Filling volume (max) [L]	40
Gas flow rate [vvm] (Lpm)	1.5 (60)
Tip speed start [m/s] (stirrer speed [rpm])	1.1 (150)
Tip speed maximum [m/s] (stirrer speed [rpm])	3.4 (450])
Temperature [°C]	37
pH[-]	6.8
pO_2 [%]	35

Figure 3.

STR® 50 L bag dimensions

Total volume [L]	68
Working volume [L]	12.5–40
Vessel diameter d_i [mm]	370
Vessel height h [mm]	666
High-to-diameter ratio h/d_i [-]	1.8
Filling height maximum h_i [mm]	480
High-to-diameter ratio h_i/d_i [-]	1.3
Impeller diameter $d_{2,\ 3\text{-blade or }6\text{-blade}}$ [mm]	143
Ratio d_2/d_i[-]	0.38
Distance between impeller Δz [mm]	186



Biostat STR® system: comprising control unit, bioreactor unit that holds Flexsafe® bioreactor bag.

- Process Development and Optimization
- Process Characterization
- Process Development
- Pilot Scale

Well Characterized Platform Enabling Biological Consistency

Table 3. Results on Ambr® 250 Modular and Biostat STR® 50L

	Volume [L]	Tip Speed [m/s]	Gas Flow rate [vvm]	k_La-value [h^{-1}] Gassing-Out Method [1]	Mixing Times (s)	Batch Growth rate[3] (μ) [h^{-1}]	k_La-Value [h^{-1}] Oxygen Balance Method [11]	Final Batch DCW weight (no pO_2 regulation) [g/l]	Fed-Batch Growth Rate (μ) [h^{-1}]	Final Fed-Batch OD 600 nm	References
Biostat STR® 50L	40	3.4	1.5	735	~2	0.4	500-707*	~6	0.15	~300 (*E. coli*W3110)	Internal data
Ambr® 250 Modular[4]	0.25	4.4	1.0	400 ± 7 [1] 1488 ± 40 [2]	< 2	0.40	782 ± 27	12	0.15	~335 (*E. coli*W3110)	[12] and Internal data

1 Head Space Exchange term (HSE) not included.
2 Head Space Exchange term (HSE) included.
3 Up to ~6hours of culture (when pO2>5%)
4 System parameters assumed to be similar to Ambr® 250 high throughput
5 3xRT = 3 Rushton turbines, 2xRT= 2 Rushton turbines.

Discussion

- Single-use bioreactors can face hurdles when being used for a microbial processes in terms of gassing and torque demands as well as temperature control. However, Sartorius single-use bioreactors are based on the classic stainless steel stirring impeller design, and have proven mixing times and kLa values being relevant for microbial bioreactors used in industrial processes [2] [5] [9]
- The studies show the reliability and consistency when scaling between the Ambr® and Biostat® single-use platforms [10]
- Ambr® and Biostat® platforms provided biological kLa of minimum of ~ 500 h–1 across all scales
- Growth data (μ) and maximum produced biomass (OD_{600} nm) are reproducible for both batch and fed-batch modes across scales allowing a fast and optimal process development for cell lines screening as well as reliability when developing a production platform.
- Higher yields per process can be achieved in a standardized manner.
- Therefore a biological model initially developed with the Ambr® platform can easily be transferred to the larger scale Biostat STR® 50L MO including intensified processes with high cell density and requiring higher gassing exchanges.

Literature and References

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Conclusion

- The Ambr® platform brings a high throughput strategy for multi-parallel experiments with state-of-the-art automation, enabled by innovative process analytical tools, fast set-up and high performance process controls and automatic sampling especially for R&D and Process Development applications
- The Biostat STR® 50L Prototype is a reliable single-use bioreactor to scale up processes that meet reproducible results at high industry standards, and it enables increased automation via the latest process analytical tools innovations from Sartorius BioPAT® platform
- Both Ambr® and Biostat® platforms contribute for well characterized processes and reduced risk during scale up and tech transfer stages.

Sartorius brings added value to customers seeking robustness and flexibility in fast paced environments.