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Simplifying Progress

Biostat STR® Microbial for Highly Demanding Fermentation Processes

Marco Leupold[1] , Lara Nascimento[2], Ty Ulrich[3], Jens Rupprecht[1]

[1]Sartorius Stedim Biotech, August-Spindler-Str. 11, · 37079 Goettingen, Germany, [2]Sartorius, York Way, Royston, SG8 5WY, UK, [3]Sartorius Stedim North America Inc., 565 Johnson Ave, Bohemia, NY 11716

Introduction

Microbial hosts offer advantages over cell cultures systems in terms of growth rate, protein production, media costs and process robustness, but until recently they have not been able to take advantage of the benefits of single use fermenters.

Large consistent, scalable, single-use fermenter solutions are needed to meet the obstacles of modern microbial process development and pilot manufacturing. Challenges such as mass transfer, heat transfer, mixing, foam formation, etc. are all amplified by the increased growth and production rates of microbial organisms, and often demonstrate the limitations of single-use fermenters in such applications.

We will explain the technical challenges to overcome in the development of these systems,, such as foam detection, pH measurement, scalability, heat transfer, mixing, and mass transfer. Consistency was demonstrated between scales by measurement with standard guidelines for engineering characterization principles.

We will also discuss the benefits of microbial process development using consistent scalable single-use fermenter solutions, such as the Sartorius single-use Biostat STR® Microbial. We will present the capabilities of system, including comparison of characterization data with existing systems like the Sartorius Ambr® and an industrial case study highlighting the scalability to existing systems (9)(10)(11)

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) (3)

Assessment of Bioreactor Performance

MODDE®

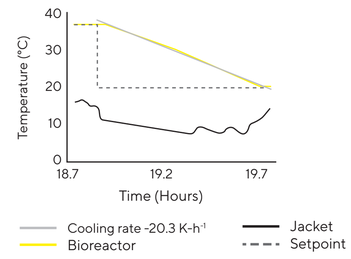
These characterizations can benefit significantly from MODDE® - Umetrics' suite for enhanced DOE investigations.

Process Engineering Characterization

- 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 (1)

Figure 2

Cooling Capacity with Water: 34 K/h



Note. In process cooling capacity with high cell density *E. coli* culture (incl. metabolic heat): 21 k/h

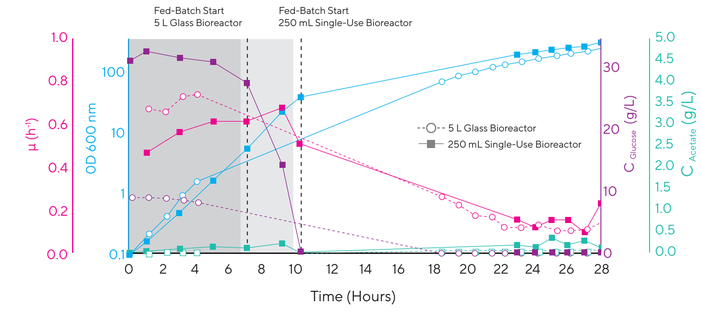
Ambr® Platform for Enhanced Screening and Optimization

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® and larger scale volumes Batch Model graph not shown however batch growth data is shown in Table 2 and 3

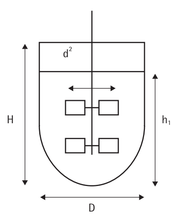
Figure 3

Fed-Batch Model E. coli Cultivation in an Ambr® 250 Modular System (Squares) Compared with a Univessel® Glass 5 L Bioreactor (Circles)



Note. Shown are optical density at 600 nm (blue), growth rate (dark pink), acetate (teal), and glucose concentration (purple). Dashed line indicates feed (6).

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 mini bioreactor vessels.

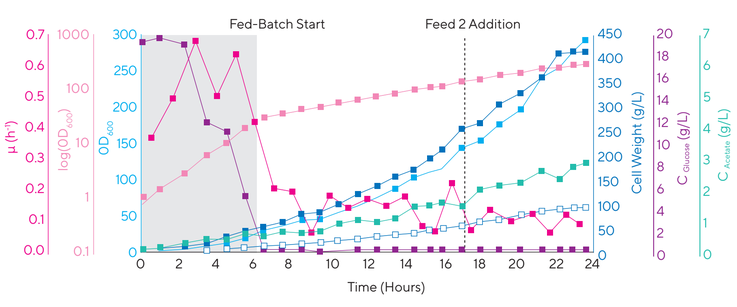
- Microbial Strain Screening
- Media Optimization

Reliable Scale-Up With the Biostat STR® 50 L System featured by Biobrain® Automation Platform for Commercial Operationalization

Key biological characterization results on Biostat STR® Microbial in Figure 4. 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 2 and 3.

Figure 4

Fed-Batch Model E. coli Cultivation in the Biostat STR® 50 L Microbial



Note. 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 OD600 (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

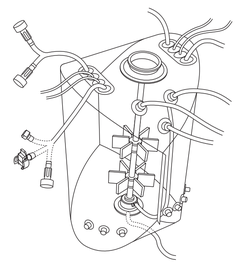
Fed-Batch Parameters on Biostat STR® 50 L

Fed-batch	
Specific growth rate μset (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
pO2 (%)	35

- Process Development and Optimization
- Process Characterization

Biobrain® Software	**Benefits of Scaling Up**
■ Modern & common user interface across Biostat® STR &RM ■ Industry Standards ■ Life-cycle ■ OPC-UA Connectivity ■ cGMP Standalone ready ■ Flexibility of configurations ■ Local Recipe execution and PAT ■ Secured Remote Access for service & Antivirus support	■ Decrease training effort and risk of errors ■ Increased reliability, maintenance and confidence ■ Future proof automation software Innovation & IT requirements ■ Enable Integration into customers Historian, DCS & BioPAT® MFCS ■ Short time-to-facility ■ Support fast & secure process changes configured vs. engineered ■ Process Automation, Advancedautomation & control ■ Increased system availability

cGMP	current Good Manufacturing Practice
DCS	Distributed Control System
OPC UA	Open platform communication; latest version UA – Unified Architecture
PAT	Process Analytical Tools



Graphical representation of the geometry of Biostat STR® Flexsafe bag;
H/D = 1.8;
h1/D = 1.2;
d2/D = 0.38

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

- 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)	Mixing Times (s)	Batch Growth rate[3] (μ) (h^{-1})	k_La-Value (h^{-1}) Oxygen Balance Method	Fed-Batch Growth Rate (μ) (h^{-1})	Final Fed-Batch OD 600 nm	References
Biostat STR® 50L	40	3.8	1.5	675	< 2	0.4 - 0.65	500 - 707*	0.15	~ 300 (*E. coli* W3110)	Internal data (8)
Ambr® 250 Modular	0.25	4.4	1.0	400 ± 7 * 1488 ± 40 **	< 2	0.40	782 ± 27	0.15	~335 (*E. coli* W3110)	(5) and Internal data (8)

* Head Space Exchange term (HSE) not included. **Head Space Exchange term (HSE) included.

Discussion

The foundation of for successful microbial fermentation processes are excellent oxygen supply through suitable stirring speed and gassing rates, resulting in high kLa values, and reliable temperature control. Those parameters have been proven excellent for both systems, Ambr® 250 and Biostat STR® Microbial, making these single-use instruments a real alternative to multi-use fermenters for the development and the commercialization of microbial cultivation in industrial processes. The studies show the reliability and consistency when scaling between the Ambr® and Biostat® single-use platforms.

Ambr® and Biostat® platforms provided biological kLa of minimum of >675 h–1 across all scales. Growth data (μ) and maximum produced biomass (OD600) are reproducible for both batch and fed-batch modes across scales allowing a fast and optimal process development for cell line 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 Biostat STR® Microbial. In terms of foam control both systems are scalable. Despite different foam measurement principles in the Ambr® (optical) and STR® (capacitance), both systems support a high cell density culture without a foam event and clogged exhaust filter. Geometrical similarity of Sartorius's single-use fermenters both being based on the classic stirring impeller design allow easy scaling as well as transferability between single-use vessels multi-use fermenter designs (2)(6)(6)(7)(10).

Conclusion

The Ambr® platform provides 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.

With the Biostat STR® Microbial, Sartorius is now offering a single-use fermenter, which is perfectly suited to scale-up from Ambr® 250 for even highly demanding processes, that meet reproducible results at high industry standards. The instrument enables increased automation via the latest process analytical tools innovations from Sartorius BioPAT® platform via the Biobrain® automation platform.

Both Ambr® and Biostat® platforms contribute to characterized processes and reduced risk during scale up and tech transfer stages.

Sartorius is now working on a comprehensive scaling story for single-use microbial instruments simply overcoming the boundaries between multi-use to single-use instruments.

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

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