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Simplifying Progress

Unveiling Process Solutions For Plasmid DNA Fermentation Across Upstream Scales

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Introduction

Developing pDNA processes depend 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 Process Solutions portfolio it is possible to accelerate development timelines and ensure process success.

That is the reason why Sartorius and JAFRAL partnered together for plasmid DNA production. JAFRAL is a world's leading CMO and CRO for production of bacteriophages and other biomolecules as pDNA. JAFRAL delivers non-GMP and GMP pDNA for various applications, including gene therapy and cell therapy. JAFRAL's GMP facilities enable short turn-around times and best production prices.

The aim of this joint poster with JAFRAL is to provide evidence to demonstrate the benefits of a pDNA process developed using Sartorius scalable solutions. The method chosen to showcase the bioreactor consistency is based on the DECHEMA Guidelines for Engineering Characterization principles. Alongside a strong bioreactor process characterization for pDNA, Sartorius analytical solutions provide the accurate and relevant in-process knowledge for all pDNA production needs.

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)

DECHEMA
Gesellschaft für Chemische Technik und Biotechnologie e.V.

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 (1)

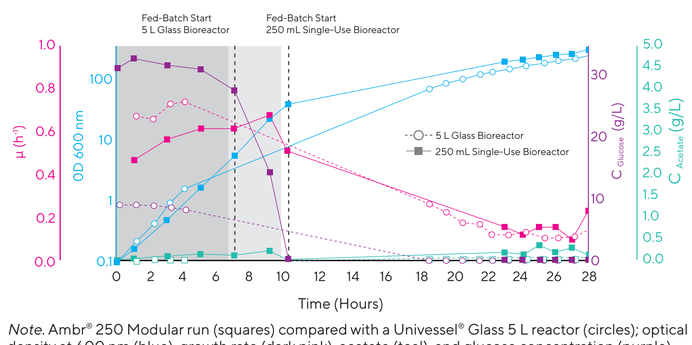
Ambr® Platform for Enhanced Optimization

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 (Table 1)

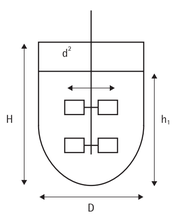
Figure 2

E. Coli Cultivation in an Ambr® 250 Modular System



Note. Ambr® 250 Modular run (squares) compared with a Univessel® Glass 5 L reactor (circles); optical density at 600 nm (blue), growth rate (dark pink), acetate (teal), and glucose concentration (purple). Dashed line indicates feed

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

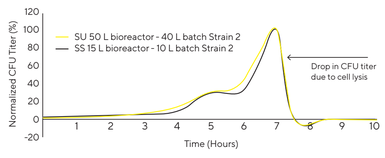
Reliable Scale-Up at JAFRAL with Biostat STR® Microbial System

JAFRAL
passion for biosolutions

- Good consistency demonstrated between 15 L stainless steel (SS) and 40 L single-use (SU) and systems
- Rapid scale-up with reduced risk to project timelines

Figure 3A

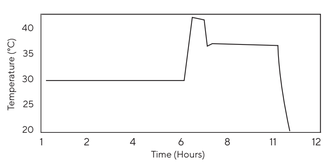
Bacterial Growth Comparison



Yield of the process was as expected and comparable with stainless steel fermenter systems.

Figure 3B

Temperature Control Profile

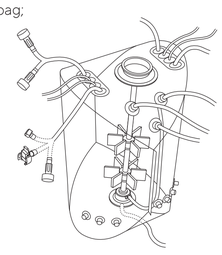


Process required precision temperature control and high stir speed.

Fast temperature rise in the bag followed by fast temperature drop were required to achieve optimal conditions for induction of product synthesis in the fermentation broth.

Temperature control provided was excellent. An increase to 42 °C was achieved within 15 min.

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.

From Sartorius process engineering investigations (1)(3):

- Power input increased exponentially with stir speed
- Similar kLa values (gassing out method) were measured for the highest specific power input in both systems (Table 1).

- Process Development and Optimization
- Process Characterization

Streamlined Analytical Capabilities

pDNA characterization with fast and innovative analytics to maximize process productivity:

- CIMac™ pDNA analytical columns are based on the monolith technology, especially developed for pDNA applications and ideal in the context of process analytical technology (PAT).
- The PATfix™ HPLC system gives you "at-line" analysis for the control of impurities and critical quality components using HPLC fingerprinting
- These technologies allow you to achieve rapid, high-resolution and flow-independent separations in a matter of minutes.

Figure 4

Chromatogram Graph (In-House Data) Using CIMac™ pDNA Analytical Column

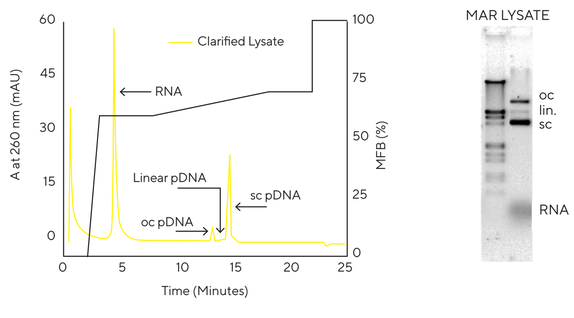


Figure 5A

PATfix® HPLC and CIMac™ Analytical Columns

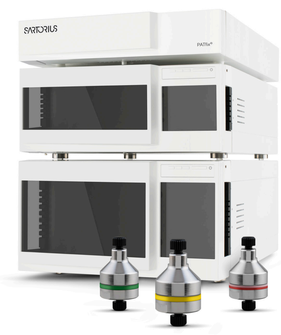


Figure 5B

CIMac™ Analytical Column for pDNA In-Process Control

- Process Development
- Pilot Scale

Well Characterized Platform Enabling Biological Consistency

Table 1

Results in Ambr® 250 Modular and Biostat STR® 50 L

	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 (3)	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	0.4 - 0.65	782 ± 27	0.15	~335 (*E. coli* W3110)	(4) and Internal data (3)

Note. Data sources from literature references, internal studies and data kindly shared from biopharma customers.

* Head Space Exchange term (HSE) not included.

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Discussion

- Sartorius single-use bioreactors are based on the classic stirring impeller design and have proven mixing times and kLa values being relevant for microbial bioreactors used in industrial processes (3)(4)(5)
- The studies show the reliability and consistency when scaling up with the Ambr® and Biostat® platforms.
- It has been demonstrated that the Biostat STR® Microbial is suitable for *E. coli* cultivation producing plasmid DNA. The process can be scaled-up from Ambr and transferred from stainless steel fermenters.
- Growth data (μ) and maximum produced biomass (OD 600 nm) are reproducible across scales for both batch and fed-batch modes allowing a fast and optimal process development for your cell line as well as flexibility when developing your own platform.
- Higher yields per process can be achieved in a standardized manner.
- Across all scales biological kLa is minimum of >675 h–1.
- Therefore a biological model initially developed with the Ambr® platform can easily be transferred to the larger scale Biostat STR® Microbial including intensified processes with high cell density and requiring higher gassing exchanges.
- The CIMac™ Analytical technology enables the separation of all three pDNA conformations and can monitor the removal of other impurities such as RNA (6)

Conclusion

- The Ambr® platform brings a high throughput strategy for multi-parallel experiments with state-of-the-art automation, fast set-up and high performance especially for R&D and Process Development (4)(5)
- The Biostat® STR Microbial based on the GMP ready stand-alone automation platform Biobrain® is a reliable option to scale up processes that meet reproducible results at high industry standards.
- Both Ambr® and Biostat® platforms contribute for well characterized processes and reduced risk during scale up and tech transfer stages alongside strong analytical capabilities with PATfix® HPLC and CIMac™ Analytical Columns.
- In the recent years plasmid DNA became a product of strongest interest. Sartorius bioreactors demonstrate excellent and easy scalability for this product type from fast process development towards small-scale production helping to enter the market with the highest pace.
- Sartorius brings added value to customers seeking robustness and flexibility in fast paced environments.

Literature and References

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4 M. Leupold, T. Dreher, M. Ngibuini, G. Greller. A Stirred, Single-Use, Small-Scale Process Development System, Evaluation for Microbial Cultivation. BioProcess International, November 2017.

5 Velez-Suberbie, M. L., Betts, J. P. J., Walker, K. L., Robinson, C., Zoro, B. and Keshavarz-Moore, E. (2017), High throughput automated microbial bioreactor system used for clone selection and rapid scale-down process optimization. Biotechnol Progress. DOI:10.1002/btpr.2534

6 Application note: In-Process Control of pDNA Production on CIMac™ pDNA Analytical Column