Simplifying Progress

Evaluation of a Stirred Small Scale Single-Use Bioreactor for Microbial Application

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Introduction

For a successful process transfer, as well as for bioprocess development and optimization a suitable small scale approach is essential to reduce time and costs. The traditional approach are flasks and bioreactors made of glass or stainless steel (Figure 2a.).

To further increase the efficiency of these transfers, single-use technology is very attractive (Figure 2b.). Cleaning and sterilization efforts with faster set-up times as well as contamination risks are reduced, the time-to-market can be accelerated and the invest-ments are lower than for conventional multi-use bioreactors [1]. Unfortunately, small scale single-use systems often cannot mimic the conditions of established bioreactors, e.g. in terms of oxygen transfer and heat removal, especially, for microbial applications. The Ambr® 250 Modular (Figure 1) was developed for mentioned applications with all features of multi-use bioreactors to achieve high cell density cultures. The approach of the systems characterization was:

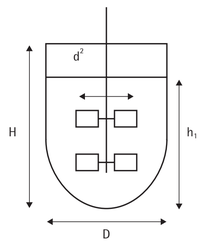
1. Process engineering characterization
2. Cultivation and comparison of the results with a Univessel® Glass 5 L.

The Ambr® 250 Modular

Features of the Ambr® 250 Modular:
- 1-8 parallel bioreactors per system
- Automated with all features of common multi-use bioreactor systems
- Peristaltic pumps and offgas-measurement for every vessel
- Fast and easy installation
- Chilled feed reservio

Figure 1.
Ambr® 250 Modular system

Process Engineering



For the process engineering characterization of both systems (Table 1.) the geometries, k_La values [2] as well as the power input were determined. Results indicated a large similarity and with the same cultivation conditions, similar cultivation results were expected.

Table 1: Process engineering characterization

Criteria	Univessel® Glass 5 L	Ambr® 250 Modular	Conclusion
Geometric similarity	H/D = 2.2 h1/D = 1.63 d2/D = 0.40	H/D = 2 h1/D = 1.44 d2/D = 0.42	Similar
Constant kLa	310 – 410 h^{-1} (Gassing out Method)	250 – 430 h^{-1} (Gassing out Method	Similar within the same stirrer tip speed range
Constant power input	– 1,900 W/m3 at 2 m/s – 6,400 W/m3 at 3 m/s – 15,300 W/m3 at 4 m/s	– 2,600 W/m3 at 2 m/s – 7,650 W/m3 at 3 m/s – 16,900 W/m3 at 4 m/s	Similar for similar stirrer tip speeds

Cultivation Conditions

To benchmark the Ambr® 250 Modular a *Escherichia coli* W3110 (DSM: 5911) high cell density fed-batch cultivation was conducted and compared to data derived from a Univessel® Glass 5 L cultivation. The cultivation parameters were:
- Medium: Biener et al., 2010 [3] – defined mineral salt medium
- Temperature: 37°C
- pHset: controlled at 6.8 with 20% ammonia solution
- pO_{2set}: 20% (with air and pure oxygen)
- Vessel geometry: 2 × 6 blade disc impeller (Ambr® 250 Modular: up to 4,400 rpm, tip speed: 4.6 m/s; Univessel® Glass 5 L: up to 1500 rpm, tip speed: 5.0 m/s) and baffles
- Maximal gassing rate: 1 vvm
- μ_{set}: 0.15 h^{-1}

Results

The fed-batch cultivation in the Ambr® 250 Modular achieved after 28 h a final optical density (OD_{600}) of 335 and a final dry cell weight (DCW) of 120 g/L, which is comparable to the cell densities achieved in the Univessel® Glass 5 L (Figure 3a.). A maximal OUR of 600 mmol/L/h was measured and in contrast to the k_La value determined during process engineering characterization (Tab. 1), a process k_La value of 870 h^{-1} was calculated. The reason for the increased k_La is the high response time of the pO_2 sensor in a buffer system compared to a cultivation. All other cultivation conditions were comparable within the two systems and were in the desired range. Consequently, even at high cell densities no limitations occured, especially regarding oxygen transfer and heat generation (Figure 3b.).

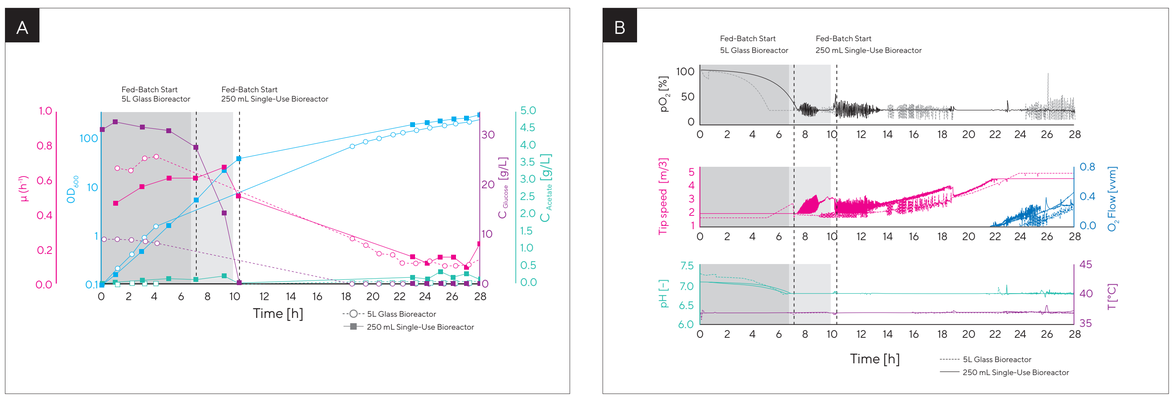


Figure 2.
a) Traditional Process development approach with shake flasks, glass bioreactors (e.g. Univessel® Glass) and stainless steel bioreactors (e.g. Biostat® D-DCU)
b) Single-Use Process development approach with the Ambr® 250 Modular and the Biostat STR®. The Ambr® 250 Modular system can be used for both screening, and process development..

Figure 3.
a) Overview of the cultivation in the Ambr® 250 Modular in comparison to the process performed in the Univessel® Glass 5 L. Shown is the optical density at 600 nm (blue), growth rate (magenta), acetate (teal) and glucose concentration (purple). The start of the feed is indicated by a dashed line.
b) Process control of both cultivations. Shown is the pO_2, which was controlled by changing the tip speed and supplementing oxygen. Additionally, pH and temperature was controlled.

Conclusions

- The Ambr® 250 Modular is a fully integrated to sensors as well as reagent bottles and automated system ideal for single-use small scale approaches.
- It accelerates process development, transfer and optimization by integrating most liquid and sensor operations.
- High cell density *E. coli* cultivations with dry cell weight >100 g/L possible with the same performance like in established multi-use bioreactors (Univessel® Glass 5 L).

References

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