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Evaluation of the Biostat STR® Microbial Bioreactor in a High Cell Density *E. coli* Exponential Fed-Batch Cultivation

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Abstract

Single-use tools offer a variety of benefits for biopharmaceutical processes. However, their widespread and successful implementation requires that they perform comparably to established, multi-use systems.

Novel modalities (such as mRNA therapeutics) mean microbial systems are increasingly employed in modern biopharmaceutical processes. Microbial culture comes with its own operational challenges due to the high cell density at which cells are grown. Therefore, before implementing single-use technologies, it is essential to confirm that these systems can handle the increased demands.

In this application note, we test the capabilities and robustness of the new single-use fermenter Biostat STR® Microbial in a fast, exponentially growing, high cell density *Escherichia coli (E. coli)* process.

Introduction

Today, single-use bioreactors are the preferred cultivation equipment for the production of biopharmaceutical drugs derived from mammalian cell cultures. The equipment's flexibility and easy installation speed up facility setups. The opportunity for high batch-to-batch turnover rates and the suitability for commercial manufacturing accelerates the development of new drugs and increases productivity.

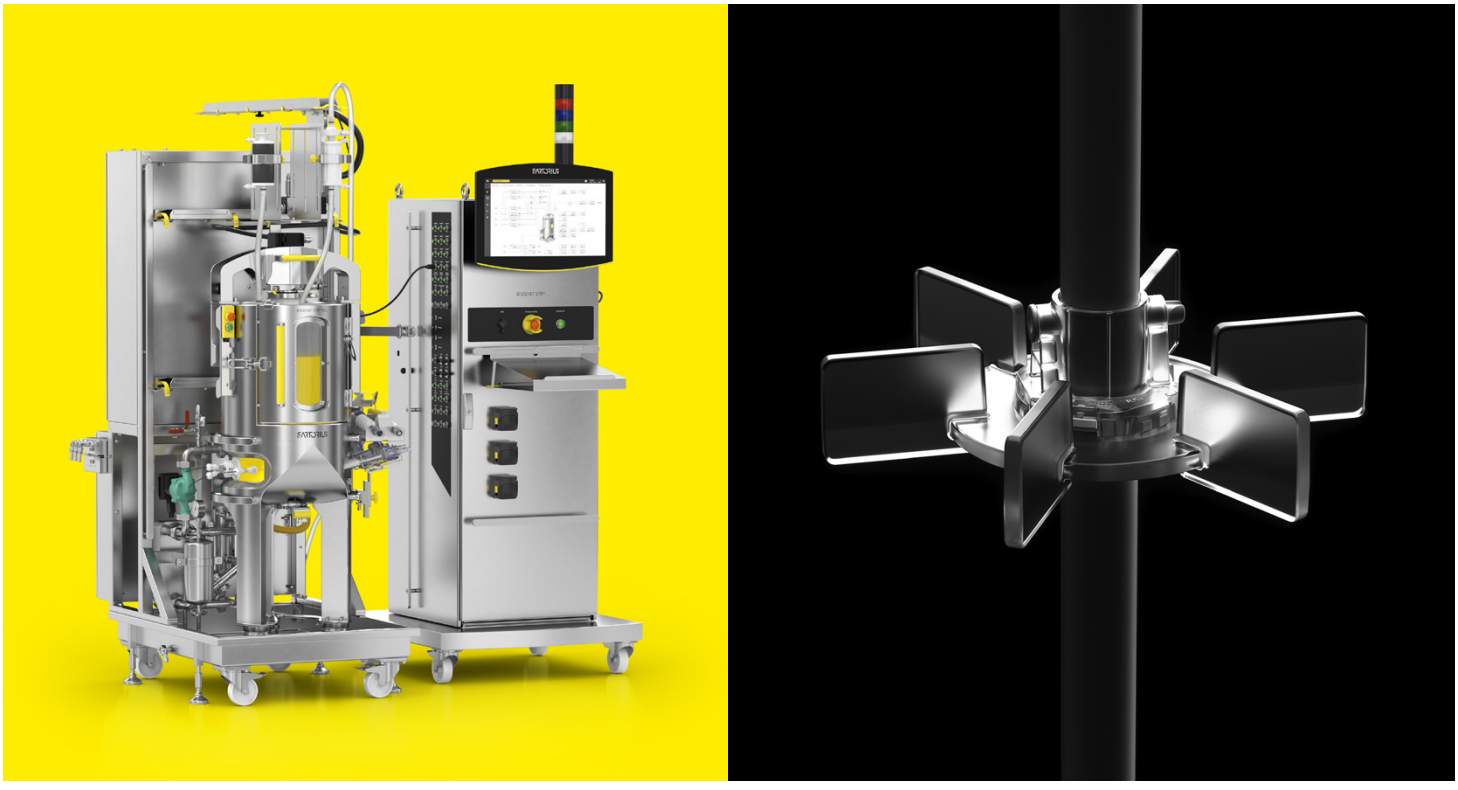
There is rising interest in reaping the benefits of these features in microbial cultivations. Besides flourishing microbial drug modalities — such as therapeutic plasmid DNA (pDNA), which plays a crucial role in the development of next-generation cell and gene therapies and vaccines — there is a trend towards the displacement of traditional mammalian-manufactured monoclonal antibodies for antibody fragments and other often microbially-manufactured antibody-like agents.[1]

Escherichia coli (E. coli) remains the main microbial expression system in the biopharmaceutical industry.[2] Its cultivation processes are well established in stainless steel in situ fermenters, which set the benchmark for microbial applications in single-use upstream equipment.

However, the demands placed on single-use components are significantly higher for microbial applications than mammalian cultivations due to the higher stirring and gassing rates as well as heat transfer required to cope with the cells' oxygen consumption and metabolic heat generation. In order to establish single-use fermenters as a real alternative to in situ bioreactor technology at pilot scale, the most critical parameters of robustness and comparable performance must be demonstrated.

This application note demonstrates the capabilities and robustness of the new single-use bioreactor Biostat STR® Microbial (Figure 1). First, process engineering parameters were characterized before a fast, exponentially growing, high cell density *E. coli* process was executed. This process uses our in-house model, which is based on the DECHEMA guidelines for comparability and has been used to characterize Sartorius' microbial fermenters for decades. Designed to push the fermenter to its technical limits, it demonstrates the performance and makes it comparable to in-situ cultivation systems.[3,4]

Figure 1: *The Biostat STR® Microbial Bioreactor*



Materials

Table 1: *Equipment Used in This Study*

Equipment	Part No.
Biostat STR® Microbial	BIOSTATSTR3-R2
Flexsafe STR® bag	FRS312255 (prototype)
Biostat® RM 20 | 50 rocker	BIOSTATCBRM
Single-Use exhaust cooler	DS001K—EC
Transfer line	FBT312258
Exhaust filter lines	DS001K-SBFLO
Sartofluor® Midicap exhaust filter (0.45 m²)	5185307T0
Flexsafe® RM 10 L optical	DFO010L
Biowelder® TC	605.001
Biosealer® TC	WN-3S

Methods

Process Engineering and Cooling Time

The methods for determination of the volumetric mass coefficient ($k_L a$-value — gassing out method, using a fast-responding DO probe (probe body by Hamilton (OIM 225 / PG13.5) with integrated optical fiber (IMP-PSt7) from Presens), the mixing time (θ-decolorization method), and volumetric power input/Newton number (P/V and Ne) of a fermenter system were conducted according to the DECHEMA guidelines.[5]

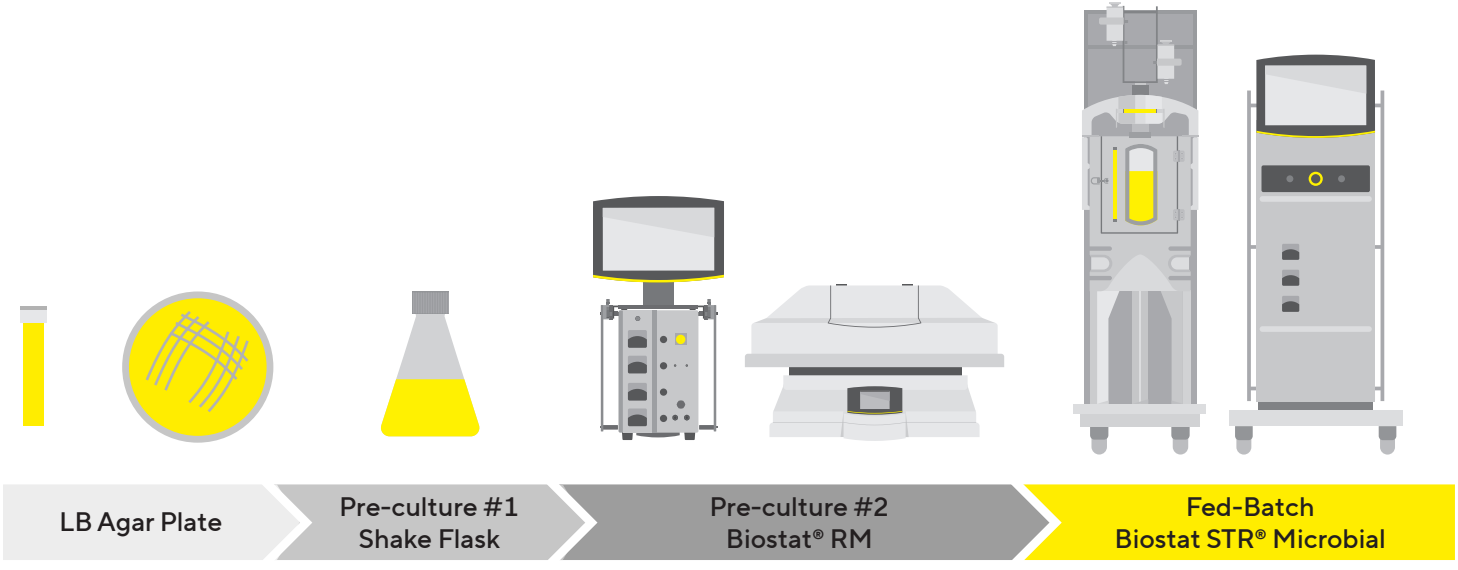
To determine the cooling time, a Lauda Chiller UC 3 (3.4 kW at 5 °C Setpoint, 4.1 kW at 10 °C Setpoint with water as the coolant) was connected to the heat exchanger of the Biostat STR® Microbial. Temperature curves between 37 °C to 20 °C were selected as the most relevant and used to calculate the respective cooling rate.

Process Overview and Parameters

A fast, exponentially growing, high cell density *E. coli* process was performed in order to test the capabilities of the Biostat STR® Microbial. This Sartorius in-house non-product forming process is designed to push the fermenter to its technical limits, particularly oxygen transfer and cooling power, to demonstrate the highest fermenter performance and bag robustness. This procedure is a standard process conducted on different scales and in all Sartorius glass and stainless steel multi-use fermenters and is also ideally suited to show scalability and transferability for single-use fermenters.[3,4]

E. coli K-12 W3110 (DSMZ: 5911, Braunschweig, Germany) was used for fed-batch cultivation (Figure 2). Secure and sterile transfer of cells was ensured using the Biowelder® TC for connecting tubes and the Biosealer® TC for disconnection.

Figure 2: *Process Steps for the Expansion of E. coli Required for the Fed-Batch Culture in the Biostat STR® Microbial*



LB Agar Plate and Shake Flask (Pre-culture #1)
Cells were thawed and distributed on an LB agar plate to create single colonies. The cells were incubated for 24 hours (h) at 37 °C. A single colony was then transferred into a 500 mL disposable baffled shake flask filled with 150 mL pre-warmed LB medium. The cells were incubated in a shaking incubator at 37 °C, 180 rpm, and 50 mm orbital diameter for 8 h. The final OD_{600} was measured to calculate the required inoculation volume for the subsequent pre-culture.

Biostat® RM 20 | 50 (Pre-culture #2)
The Biostat® RM 20 | 50 rocker (Figure 3) was used with a Flexsafe® RM 10 L optical bag. Using modified Biener pre-culture medium, the Biostat® RM was inoculated at OD_{600}= 0.1. The cells were expanded for 16 h at 30 °C and in 35% dissolved oxygen (DO), reaching a final OD_{600} of around 12.

The temperature setpoint of 30 °C was controlled using heating mats, and the DO setpoint of 35% was controlled using the cascade using default PID parameters. The pH was not controlled but monitored and recorded to mimic cultivation in shake flasks. The batch processes were monitored and recorded with BioPAT® MFCS to ensure reliability and comparability.

Biostat STR® Microbial Fermenter (Fed-Batch Cultivation)
The fed-batch processes were conducted in Biostat STR® Microbial stainless steel bag holder using the single-use Flexsafe STR® Microbial bag at 50 L scale (Figure 2).
The Biostat STR® Microbial is powered by the automation platform Biobrain®. Using modified Biener fed-batch medium, the Biostat STR® Microbial was inoculated at OD_{600}=1. After an initial batch phase, exponential feeding was started with feed 1 at a feeding rate of μ_{set} = 0.15 h^{-1}. At an OD_{600} of 150 ± 10, a bolus of 250 mL feed 2 was administered.[6]

The process was inoculated from the pre-culture with a starting volume of 24 L. The temperature was set to 37 °C and controlled with Biobrain® via the jacket temperature of the double-walled bag holder using an external thermocirculator (Lauda Chiller UC-3). The DO setpoint of 35% was controlled using a PID-based control cascade. DO was measured using the single-use optical DO sensor. The single-use Flexsafe STR® Microbial bag is equipped with two 6-blade impellers (Figure 1) and a ring sparger with upward-facing holes (5 holes with 0.8 mm). Additionally, two baffles were installed into the Biostat STR® Microbial stainless steel bag holder.

Figure 3: *The Biostat® RM 20 | 50 Rocker*

The pH setpoint of 6.8 was controlled using a PID-based control cascade with base (20% ammonia). pH was measured using a multi-use pH probe.

The exhaust gas was cooled using the single-use exhaust cooler with condensate return. The outlet of the exhaust cooler was connected via a transfer set with two exhaust filter lines equipped with a Sartofluor® Midicap filter. The second filter line was used if needed due to pressure build-up.

The fed-batch processes were operated based on an automated BioPAT® MFCS recipe. The initial batch phase lasted until the initial glucose in the batch medium (20 gL^{-1}) was depleted, indicated by a temporary peak in the DO profile. This peak initiates the onset of the exponential feeding of feed 1 (according to equation 1) with parameters given in Table 2. Feed 2 (250 mL) was supplied as a bolus at an OD_{600} of 150 ± 10.

$$F = \frac{\mu \times c_{x,\,Batch,\,end} \times V_R}{Y_{x/s} \times c_{s,\,Feed}} \times e^{\mu \times \Delta t_{FB,i}}$$

Table 2: *Parameters for the Calculation of the Flow Rate of Feed 1*

Symbol	Parameter	Value | Unit
F	Flow rate of feed 1	calculated ($L \times h^{-1}$)
μ	Growth rate	0.15 h^{-1}
$c_{x,\,Batch,\,end}$	Biomass concentration (DCW) at batch end	9.3 gL^{-1}
V_R	Initial reactor volume	24 L
$Y_{x/s}$	Biomass (DCW) yield on substrate (glucose)	0.3843 gL^{-1}
$\Delta t_{FB,i}$	Time from feed start at $t = i$	Increasing variable (h)
$c_{s,\,Feed}$	Glucose concentration in feed 1	655.3 gL^{-1}

Results

Process Engineering Characterization

In the first step, the process engineering parameters of the Biostat STR® Microbial fermenter were characterized. At 40 L maximum working volume with 500 rpm and 1.5 vvm (air) gassing (60 Lpm) as the highest parameter, a volumetric oxygen mass transfer coefficient (k_La) of 680 h^{-1} was obtained (Figure 4A). Mixing time, determined in an ungassed state according to DECHEMA guidelines, was at 40 L and 500 rpm for 1.5 s.

As another important scaling criterion, the specific power input (P/V) at different stirring speeds (50 – 500 rpm) and gas flow rates (0 – 60 Lpm) was obtained by measuring the torque on the stirrer shaft (Figure 4B). A wide range of specific power inputs (10 – 3,500 $W \times m^{-3}$) can be provided in the operating range of 50 – 500 rpm and up to 1.5 vvm (60 Lpm) gassing.

Temperature measurements in water were used to quantify the cooling rate of the Biostat STR® Microbial. Cooling from 37 to 20 °C was achieved in 0.5 h, i. e. 34 Kh^{-1} (Figure 4C).

Based on these high-performance process engineering results — in particular, the k_La of 680 h^{-1} – a culture with an optical density OD_{600} > 300 (>90 gL^{-1} cell dry weight) representing high cell density can be achieved (translated to oxygen transfer rate (OTR) and corresponding oxygen uptake rate (OUR) (oxygen uptake rate) in an exponential fed-batch process of μ_{set} = 0.15 h^{-1}).[7]

Based on the achieved parameters and according to modeling, the highest process parameters were transferred and set to the *E. coli* model process. This allowed us to match process engineering with bioprocess results and confirm the ability of the Biostat STR® Microbial fermenter to achieve high cell-density cultures. In this model process, appropriate oxygen supply was ensured by utilizing a dedicated gassing cascade for the DO control with max. k_La of 680 h^{-1} at 100% control output (Table 3).

Table 3: *Polygons of the Gassing Cascade for DO Control Used for the E. coli Fed-Batch Process Operated in the Biostat STR® Microbial*

Control Output [%]	0	20	40	60	80	100
Air [Lpm]	30	45	60	40	20	0
O_2 [Lpm]	0	0	0	20	40	60
Stir Speed [rpm]	250	375	500	500	500	500

Microbial Seed Culture in the Biostat® RM

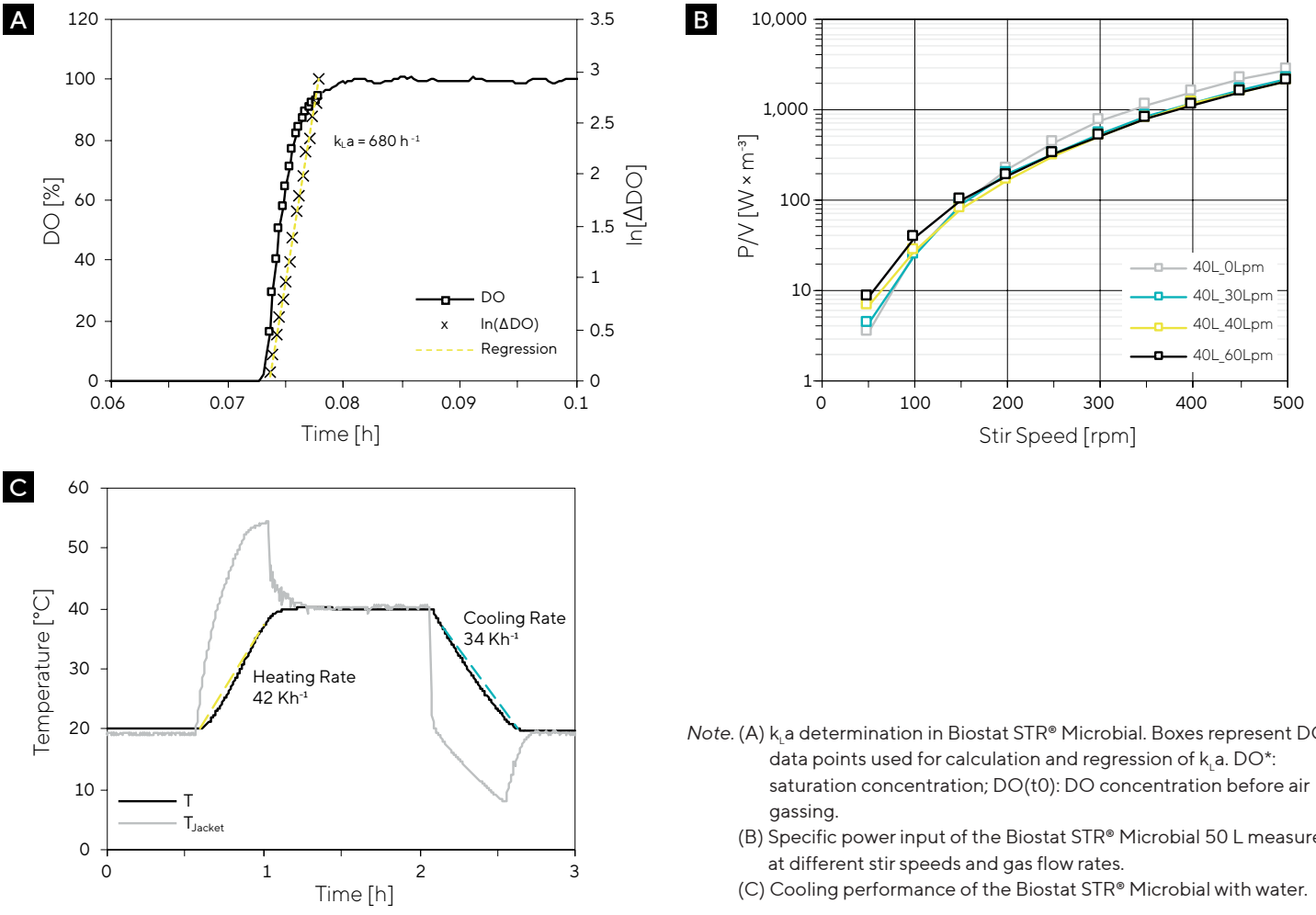
The Biostat® RM was used as a pre-culture step for the inoculation of the fed-batch process performed in the Biostat STR® Microbial fermenter. The process parameters DO, pH and temperature were measured throughout the cultivation.[5]

DO was maintained accurately at 35% once cells grew to a sufficient density high after about 10 h (Figure 5A). The DO controller replaced air with O_2 (to max. 2.5 Lpm) and increased the rocks (to max. 36 rpm), enhancing mass transfer.

The temperature setpoint of 30 °C was accurately maintained throughout the entire process (Figure 5B). Starting at pH 7.1, after inoculation with an initial OD_{600} of 0.1, the cells grew and acidified the media with increasing culture duration due to cellular respiration such that the pH decreased (Figure 4B).

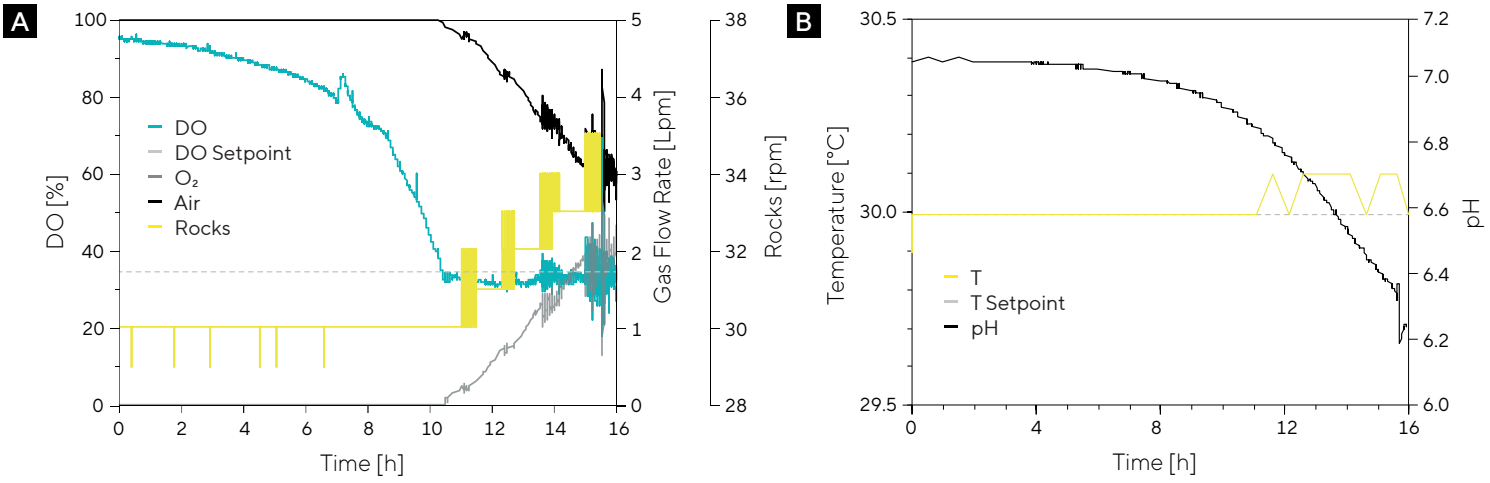
The final pH reached around pH 6.3, demonstrating the reliability and reproducibility of the seed culture for the inoculation of the subsequent fed-batch process. With configurable oxygen flow rates and a technical maximum rocking rate of 42 rpm, the Biostat® RM can accommodate more demanding pre-culture processes compared to shake flasks, where DO limitations might occur.

Figure 4: *Characterization of Process Engineering Parameters of the Biostat STR® Microbial Fermenter*



Note. (A) k_La determination in Biostat STR® Microbial. Boxes represent DO data points used for calculation and regression of k_La. DO*: saturation concentration; DO(t0): DO concentration before air gassing.
(B) Specific power input of the Biostat STR® Microbial 50 L measured at different stir speeds and gas flow rates.
(C) Cooling performance of the Biostat STR® Microbial with water.

Figure 5: *Microbial Seed Culture in the Biostat® RM*



Note. A) DO control
B) Temperature and pH

Fed-Batch Cultivation in Biostat STR® Microbial

Growth Performance

The growth performance of the Biostat STR® Microbial is shown in Figure 6. The initial batch phase with a very high unlimited growth rate of about 0.7 h^{-1} (Figure 6A) lasted for 5.3 h until glucose was depleted (Figure 6C), indicated by a characteristic peak in the DO profile (Figure 7). The high growth rate is also demonstrated by increasing biomass (Figure 6B). From 5.3 h onwards, feed 1 was provided exponentially (μ = 0.15 h^{-1}; glucose was immediately consumed, thus remaining at about 0 gL^{-1}), and feed 2 was added as a bolus at an OD_{600} of 140±10 (13.2 h). OD_{600} reached 269 (Figure 6A), and DCW reached 80 gL^{-1} (WCW = 393 gL^{-1}) at the end of the experiment (Figure 6B).

These results are slightly below the theoretical possible maximum derived from modeling but show the highest fermenter performance and can be interpreted as high cell density.[7]

Acetate concentrations of 0.7 gL^{-1} were obtained at the end of the batch phase (Figure 8C). The acetate concentration increased to around 5 gL^{-1} towards the process end and accelerated in the last hours of the process but remained under a critical growth inhibitory concentration.[8]

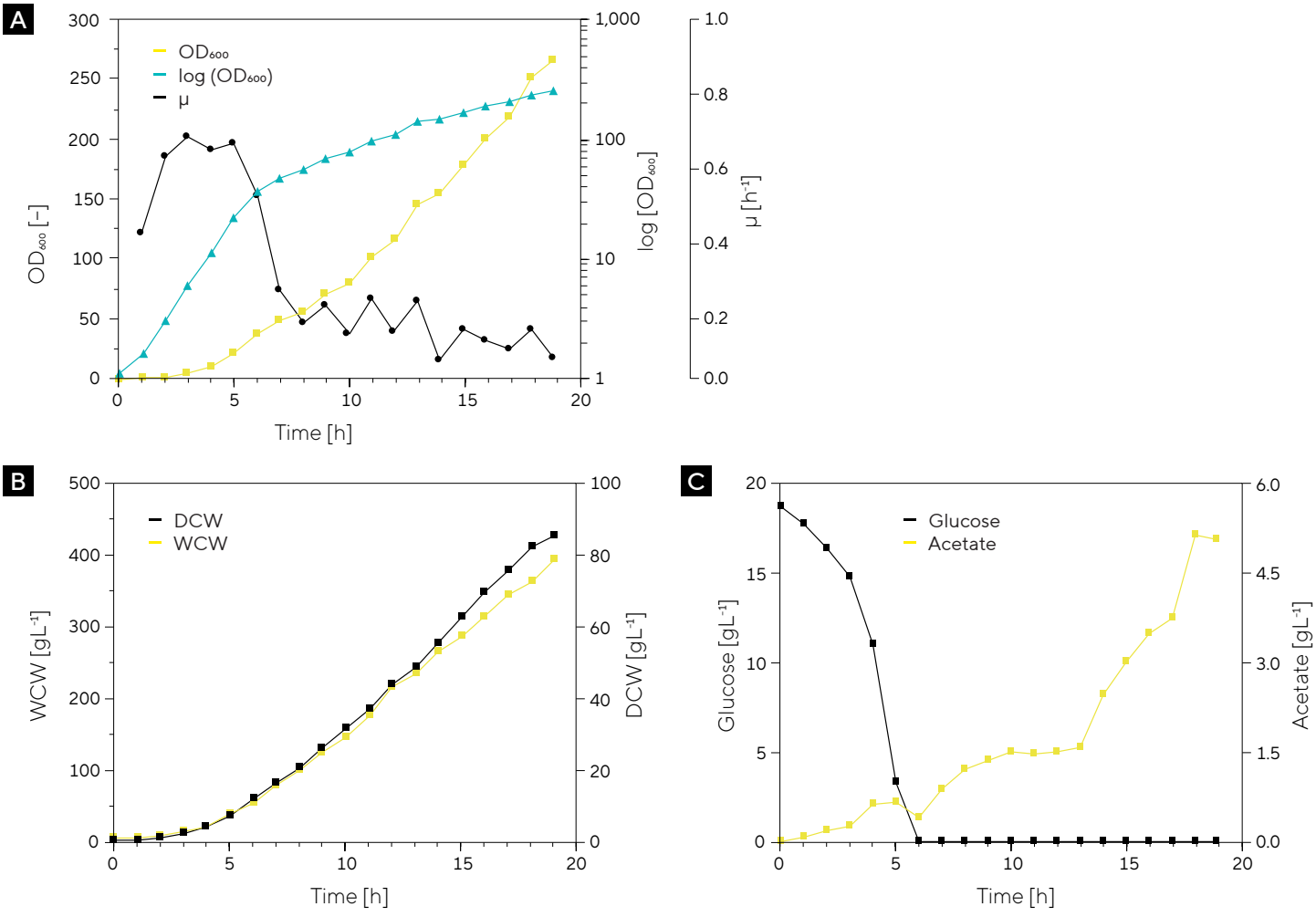
Process Performance

Aside from measuring the capability of the Biostat® STR Microbial to support microbial growth, we also sought to determine how well it controls and maintains process parameters (DO, pH, and temperature).

The DO during the initial batch phase was accurately controlled at 35% until it peaked, indicating glucose depletion and triggering the feed start (Figure 7). Maximum gas flow rates of 60 (air) Lpm were reached after about 4 hours during the initial batch phase. After that, air gassing was gradually replaced by pure oxygen addition supporting the high oxygen demand of the process.

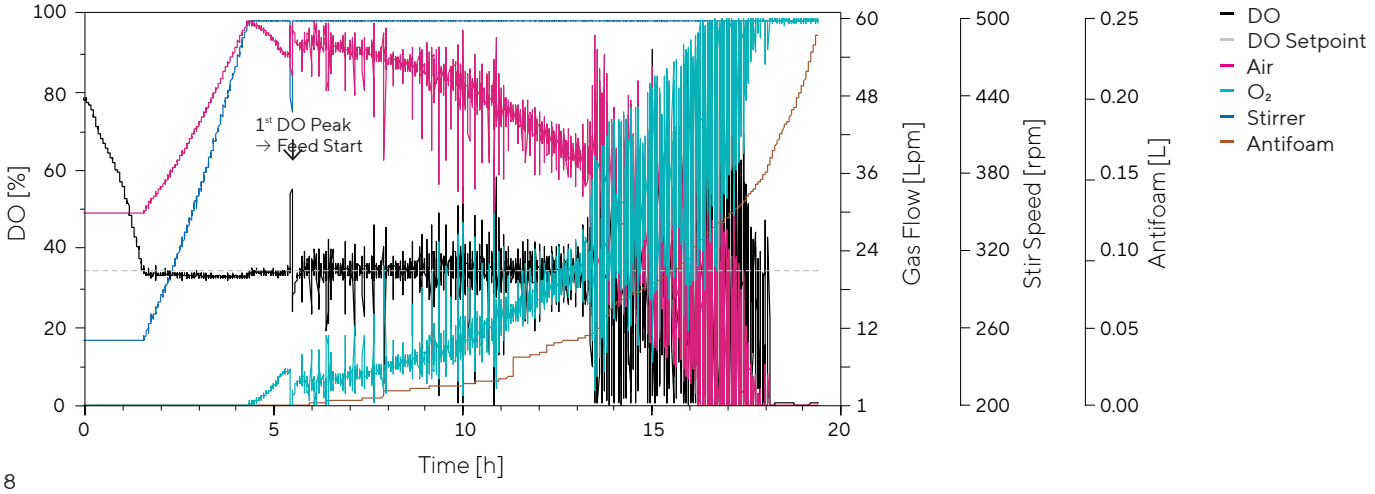
The addition of antifoam (around 0.2 L total) during the last four hours of the process affected the DO control, causing it to fluctuate. The gassing cascade for DO control was adjusted, controlling the DO at 35% and supporting aerobic cell growth over the whole process. Maximum parameters used were 500 rpm stirring and 60 Lpm (1.5 vvm) gassing determined during process engineering characterization (Table 3). High fluctuations in the DO level towards the end of the process are caused by the addition of antifoam for these challenging process conditions. The acetate concentrations reveal no oxygen limitation or inhibitory effect.[8]

Figure 6: *Characterization of E. coli Cultures Grown in the Biostat STR® Microbial*



Note. (A) Growth profiles of *E. Coli* fed-batch cultivation represented by OD_{600}, $log(OD_{600})$, and the respective growth rate.
(B) Growth profiles represented by wet and dry cell weight (WCW, DCW).
(C) Profile of glucose and acetate concentration.

Figure 7: *DO Control in Exponentially Growing E. Coli Fed-batch Process*



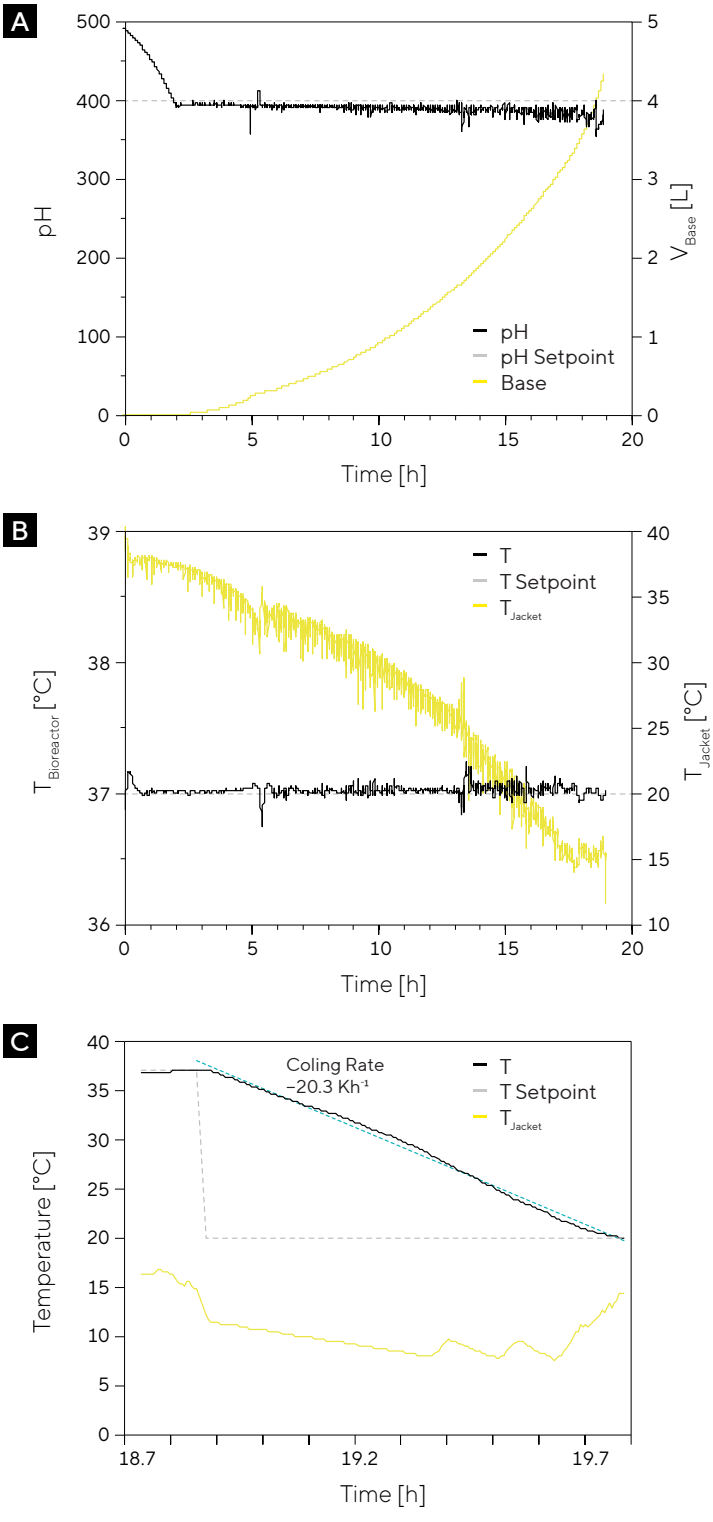
The pH profile (Figure 9A) shows that the setpoint of pH 6.8 was accurately maintained by an accumulated addition of 4.4 L ammonia (20%). The temperature setpoint of 37 °C was successfully maintained in the Biostat STR® (Figure 9B). The end of the batch phase and start of feed 1 was characterized by a small dip at 5.3 h. Once the process was finished, it was cooled to a temperature of 20 °C. However, metabolic heat remained, and the jacket temperature was decreased from 15 °C to minimally 8 °C. The respective cooling rate was −20.3 Kh^{-1} (Figure 9C).

These in-process cooling results combined with the process engineering results with water (Figure 4C) demonstrate the high cooling performance of the Biostat STR®, necessary to support high cell density processes with exponential growth.

The results described here show that processes with high cell density (OD_{600} > 280) can be operated robustly in the Biostat STR® Microbial. High-density cultures are supported by accurate and sufficient control of DO and pH, as well as the cooling capacity necessary for such demanding fermentation processes.

Figure 8: *Biostat® RM 20 | 50 Rocker*

Figure 9: *Process Parameter Control in the Biostat STR®*



Note. (A) pH profile controlled at the setpoint of pH 6.8. The pH controller relied on the addition of base (20% ammonia), in total, about 4 L were added.
(B) Temperature control.
(C) Cooling rate at process end.

Discussion

Speed, reliability, and productivity are crucial elements for the successful development of an efficient production process. *E. coli* cultures, which are fundamental for the production of various biotherapeutics, grow at high densities with high oxygen demand. Solutions that support the rapid expansion of bacterial cultures while maintaining tight process control and generating high yields are desired in the industry.

A comprehensive process engineering characterization is fundamental to understanding the capabilities of a fermenter in a cultivation process. To perform such characterizations, we used an *E. coli* in-house model process, which uses the same parameters as in situ equipment corresponding to industry standards[4] to test the capabilities of the Biostat STR® Microbial fermenter.

Our results demonstrate that the Biostat STR® Microbial can effectively accommodate challenging microbial processes, especially with respect to the requirements for oxygen transfer and cooling power. Additionally, the process can be operated securely and robustly thanks to the strength and flexibility of Flexsafe® bags and the tight process monitoring and control offered by biosensors.

The experimental set-up tested in this trial shows that processes with high cell density (OD_{600} > 280) can be reliably performed in the Biostat STR® Microbial. High-density cultures are supported by accurate and sufficient control of DO and pH, as well as sufficient cooling capacity necessary for such demanding fermentation processes.

Previously, industrial production of a model His-tagged protease inhibitor protein (which relies on expression by an *E. coli* pAVEway strain) was successfully transferred to our single-use system.[9] A simple method of scaling from a single-use 250 mL fermenter to the Biostat STR® Microbial pilot fermenter scale has also been demonstrated in another study.[3,10]

Finally, we also examined seed train expansion in our trial. Pre-cultures are often grown in shake flasks, resulting in poorly supervised cultivation and varying quality cultures used to inoculate the final fermenter, leading to inconsistent behavior. The Flexsafe® RM bags permit a fast transfer of cells to inoculate larger production fermenter volumes. Its setup requires minimal operator interaction, and key parameters, such as DO and pH, can be accurately monitored and controlled (N.B., in this case, we controlled DO but not pH). Overall, the seed train monitoring is significantly improved with excellent batch-to-batch comparability.

In summary, the Biostat STR® Microbial supports the production of highly viable, high-density microbial cultures, even where processes are dynamic and challenging.

Conclusion

Increasing interest in single-use technology for microbial cultivations is reflected in a > 18% evaluation rate of such equipment in the coming years.[1] Given that most biopharmaceutical companies do not possess a microbial expression platform, this is a remarkable number and emphasizes its meaning for future biopharmaceutical strategies. Clearly, the industry seeks to reap the benefits of single-use solutions in the microbial world as they have before for mammalian cell culture applications.

The COVID-19 pandemic demonstrated how quickly and unexpectedly new drugs can be required, demanding a rapid and agile reaction from the biopharmaceutical industry. The pandemic also paved the way for mRNA vaccines and pDNA therapeutics,[11,12] an advance that means drugs derived from microbial expression hosts are becoming increasingly relevant.

When comparing single-use with in situ fermenter setups, easy installation and less effort for lab and production room preparations are obvious benefits. As well as saving time and costs, single-use equipment also promises significantly higher batch-to-batch turnover. This reduces the time to market during the development phase and increases productivity in commercial manufacturing. Finally, single-use solutions offer superior product and operator safety by minimizing the risks of contamination and product exposure.

Besides the general advantages of single-use fermenter technology, its feasibility depends strongly on the performance of such systems. This application note demonstrates the high performance of the Biostat STR® Microbial, including k_La values and heat transfer capabilities comparable with an established in situ fermenter. These findings demonstrate that biopharmaceutical manufacturers can take advantage of single-use technologies for microbial processes without compromising performance.

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