

Scale-up and tech transfer from 100 L SIP/CIP vessel to the 300 L S.U.F.

High-density and high-yield recombinant *E. coli* expression in S.U.F.

Abstract

With proven expertise and process knowledge, we effectively support product demonstrations in the field. A collaborative study with a new customer was undertaken with the objective of taking an existing, smaller preclinical *E. coli* process previously performed in stainless steel vessels and scaling it up quickly using the Thermo Scientific™ HyPerforma™ 300 L Single-Use Fermentor (S.U.F.) (Figure 1). The following were key objectives for the study:

1. Demonstrate equivalent growth and product yield at a larger scale.
2. Identify any process steps or parameters that must be changed to accommodate tech transfer using single-use products.
3. Demonstrate that various online analytics, such as pH, dissolved oxygen (DO), mass, foam, and mass spectrometer (MS) off-gas analysis, can be beneficial to workflows for disposables.
4. Demonstrate that expanded data quality may improve outcomes and success in the decision-making process.



Figure 1. 300 L HyPerforma S.U.F. with Thermo Scientific™ HyPerforma™ G3Lite™ controller and Prima BT off-gas analyzer.

Procedure

The standard procedure began with transfer of a 16-hour seed culture into the production culture medium. The fed-batch phase began at 5.5 hours, when changes in substrate metabolism are evident. The feeding was continued until harvest. IPTG was used to induce expression of the target protein at 25.5 hours, and harvest occurred 20 hours after induction.

With a doubling time of ~1 hour, the final harvest's optical density (measured at 600 nm) is typically 210, with a dry cell weight (DCW) of 60 g/L and a product yield of 8 g/L. Initial culture volume was 46.67% of the working volume (WV) and was brought to 100% WV by the end of the culture with nutrient feed. An incubation temperature of $37^{\circ}\text{C} \pm 0.1$ was maintained, and pH was controlled at 7 ± 0.05 by addition of acid or base as needed. Dissolved oxygen (DO) was controlled to $p\text{O}_2$ of $>20\%$ with 1 vvm of gas flow, and supplemented with oxygen as needed. For the DO cascade control strategy, the stirrer was used in the range of 30%–100% of the maximum revolutions per minute (rpm). The stirrer speed ranges used were: 200–600 rpm for the 30 L S.U.F., 100–700 rpm for the 100 L stainless steel vessel, and 125–375 rpm for the 300 L S.U.F. For the S.U.F. cultures, conditions were controlled using the Thermo Scientific™ G3Lite™ Delta-V™ controller and NesLab™ ThermoFlex™ temperature control unit (TCU), with a nominal rating of 33.3 W/L cooling capacity at 20°C .

Results and discussion

To show feasibility, the procedure was first tech-transferred to the 30 L S.U.F. and compared to a 5 L steam-in-place/clean-in-place (SIP/CIP) fermentor. The standard procedure for preclinical expression in *E. coli* in a 100 L SIP/CIP was used as technical transfer to the S.U.F. Culture conditions in the 30 L S.U.F. are seen in Figure 2. The procedure was then scaled up to the 300 L S.U.F., following the same procedure for the 100 L SIP/CIP. Culture conditions in the 300 L S.U.F. are seen in Figure 4.

During the first 300 L S.U.F. demo, the customer's procedure called for sparging 1 vvm of gas flow overnight. This procedure worked in the 30 L S.U.F., as the high-flow 5-inch filters were sufficiently oversized. In the 300 L S.U.F., the filter life tests with a high-density culture showed that a single high-flow 10-inch filter was good for 6 days. However, this medium formulation contained significant hydrolysate levels, and the prescribed 20 hours of 1 vvm of air flow prior to inoculation carried significant amounts of media debris into the filter prior to inoculation. This pre-fouling of the filter caused pressure to exceed the maximum recommended operating pressure of 0.5 psi at mid-culture. In this case, the pinch clamp opened automatically with the preprogrammed criteria of the pressure reaching 0.6 psi and no foam being detected. The redundant backup exhaust filter isolation clamp between the filters was then automatically actuated through the DeltaV controller and safely utilized for the first time. As a result, the culture continued without incident.

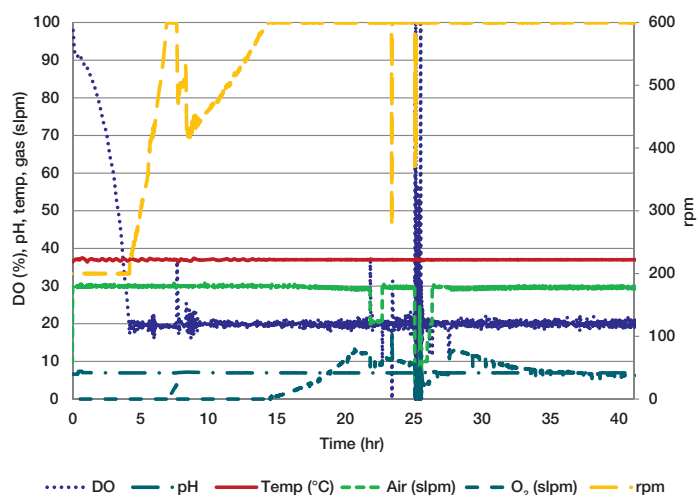


Figure 2. Conditions during a 30 L S.U.F. culture, in which $\text{OD}_{600} = 216$ with 62 g/L DCW and 7.8 g/L product were achieved. Production was comparable that of standard 100 L SIP/CIP fermentations.

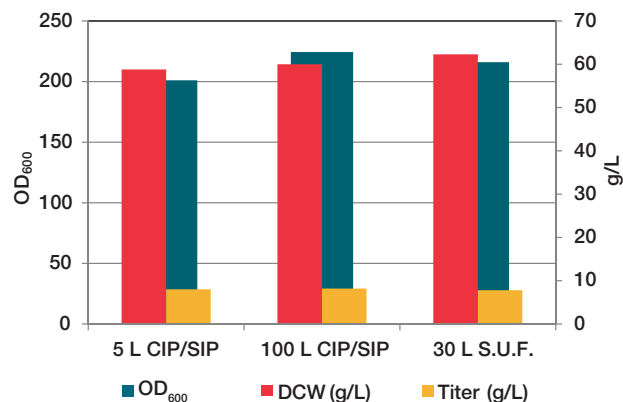


Figure 3. Comparison of 5 L benchtop (Sartorius™ BIOSTAT™ Cplus), 100 L SIP/CIP (Sartorius BIOSTAT D-DCU), and 30 L S.U.F. for product titer, DCW, and OD_{600} . The 30 L S.U.F. yield was comparable to those of the 5 L SIP/CIP and 100 L fermentor, using the same procedure. The product titer of the 30 L S.U.F. was 7.8 g/L (0.4 g/L less than that of the 100 L SIP/CIP).

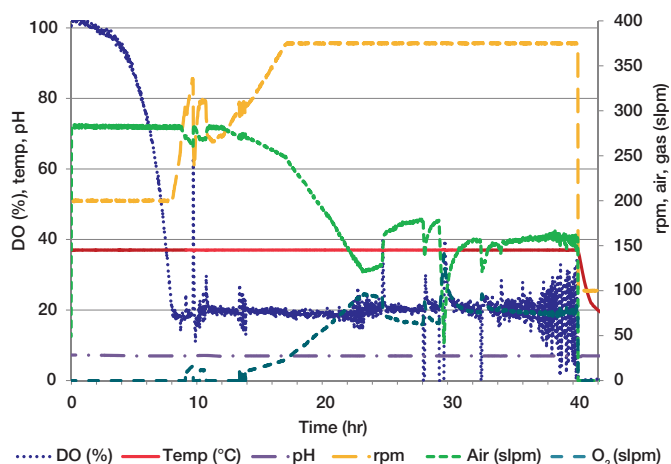


Figure 4. Conditions during a 300 L S.U.F. culture, in which $\text{OD}_{600} = 240$ with 63 g/L DCW and 9.9 g/L product were achieved. Production was comparable to that of standard 100 L SIP/CIP fermentations.

It is possible that the condenser or vent heaters were off or set to a nonoptimal temperature, as this was the first culture with this controller/vessel unit. Thus, it is important to test the vent heater's proportional integral derivative (PID) and condenser system prior to actual culture to ensure that the settings keep the vent heaters at 50°C and the condenser plates close to 5°C.

The first run in each S.U.F. size was used to adjust PID and stabilize control. The S.U.F. performed adequately for production by the second run in both sizes, and performed at production levels equivalent to SIP/CIP fermentors by the third run in both sizes.

More oxygen was used in the S.U.F. than in the 100 L SIP/CIP fermentor. If this is potentially a concern, an alternative involves increasing the air cascade limit from 1 vvm to 1.7 vvm. This would, in turn, reduce the amount of oxygen needed in the second half of the culture. It is also important to inflate the S.U.F. BioProcess Container (BPC) with sterile air to 0.2–0.5 psi prior to filling it with medium. Inflating the BPC helps the film fit around the four baffles, which greatly improves heat transfer and oxygen mass transfer rates, especially at the 30 L scale. For these tests performed by the customer, the vessels were not fully inflated to 0.2 psi.

During this feasibility study, off-gas analysis of the cultures was compared. The off-gas trend was comparable and flows were consistent. In this comparison, the off-gas was sampled from the S.U.F. through a small disk filter located after the condenser chamber, and before the exhaust filters and vent heater. This allowed the analyzer to pull from the BPC regardless of which filter is used. If not heated, this disk filter can become fouled with moisture before the normal exhaust filter. In the 30 L culture, the initial vessel pressure was too low for sufficient flow to the off-gas analyzer.

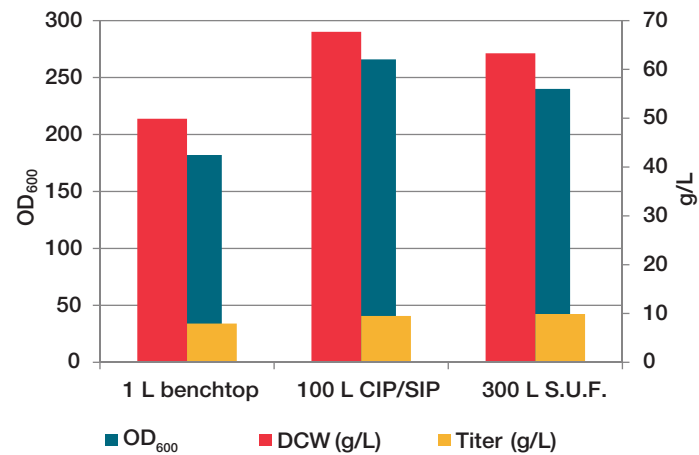


Figure 5. Comparison of 1 L benchtop (Sartorius BIOSTAT Qplus), 100 L SIP/CIP (Sartorius BIOSTAT D-DCU), and 300 L S.U.F. for product titer, DCW, and OD₆₀₀. Growth and product yield of the 300 L S.U.F., as seen in Figure 5, were comparable to those of the 1 L and 100 L SIP/CIP. The product titer of the 300 L S.U.F. was 9.9 g/L, which was higher than that of the 100 L SIP/CIP by 0.4 g/L.

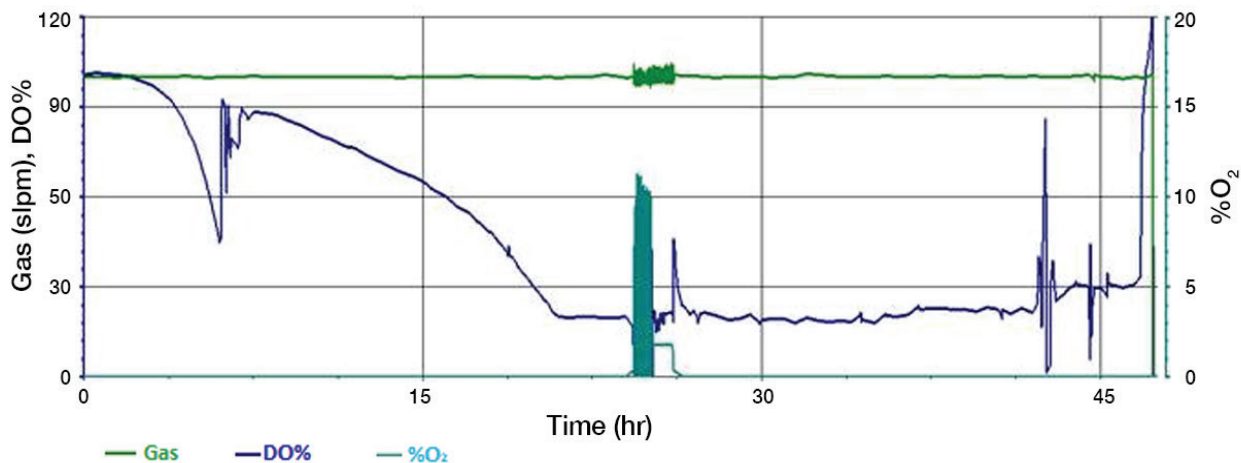


Figure 6. Representative data for conditions during routine 100 L SIP/CIP (Sartorius BIOSTAT D-DCU) production culture. In this comparison, OD₆₀₀ = 266 with 67.7 g/L DCW and 9.5 g/L product were achieved. The excessive ramp rate of the D-DCU controller causes the spiking seen when agitation was ramped up, and again when oxygen addition became necessary.

Later in the culture the pressure increased to 0.2 psi, and the off-gas analysis was comparable to that of the SIP/CIP. After significant trial and error, our final recommendation is to use a sample pump on the analyzer exhaust exit, and to place the sample line into the opening of the S.U.F. exhaust filter(s) (Figure 8). A rotameter should be used on the sample line from sparge gas and exhaust sample lines to ensure consistent and similar flow rates of about 0.05–0.2 slpm.

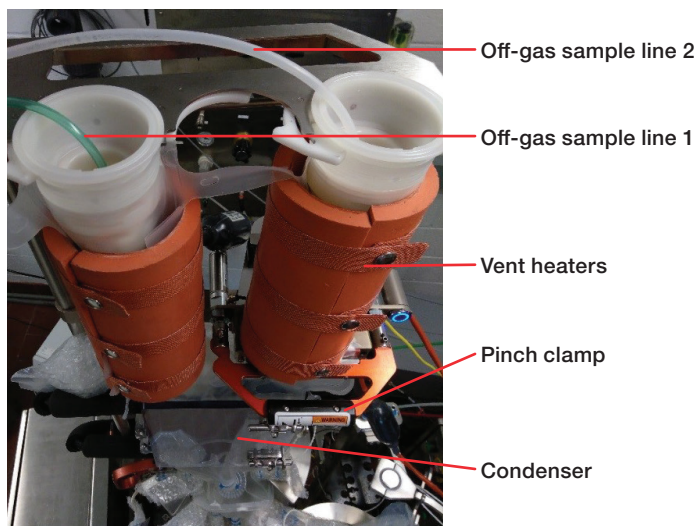


Figure 8. Exhaust filters with a sample tube inserted inside the exit for off-gas analysis. Noted in the picture are the vent heaters, pinch clamp, and 30 L condenser plate. The vent heaters for both 30 L and 300 L S.U.F.s are the same size. The pinch clamp is also the same for both, except the mounting positions differ by 90°, so the 30 L unit has an additional part for mounting. If desired, the pinch clamp can be released manually by pressing the blue-lit button (shown) on the clamp. The Thermo Scientific™ Prima™ BT off-gas analyzer can run samples from 16 sources through mass spectrometry; usually the sparge gas is compared to the vessel exhaust.

Conclusion

The tech-transfer to the 30 L and scale-up 300 L S.U.F.s were successful, and showed that the same growth characteristics and high product yields can be achieved as in SIP/CIP vessels. The customer expressed satisfaction with the HyPerforma S.U.F.'s ability to reproduce their titer and procedure timeline in single-use fermentors, exceeding their expectations (based upon issues working with an alternative system). Because the S.U.F. is designed to meet the unique requirements of microbial fermentation instead of being modified from a cell culture bioreactor, customers are able to perform simple tech transfer while achieving yields equal to or greater than those of SIP/CIP vessels.

The following operating conditions are recommended:

- Though the high-flow filters are validated for 6 days of culture (depending on media composition), it is important to reduce conditioning gas flow, except during the DO calibration oxygen saturation step.
- The flow should be increased to desired rates for culture prior to inoculation. This helps reduce the chance of foaming out due to operator error or debris carried in media, and extends the filter life for actual culture time.
- Media formulations vary significantly. As noted in this and previous culture comparisons, during culture, the cells seem to keep the debris of rich media in solution, extending filter life. For incidences of operator error and unexpected incidents of filter fouling, we recommend use of the S.U.F. redundant exhaust filter pinch clamp.

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