End-user evaluation of 30 L and 300 L HyPerforma Single-Use Fermentors and scale-down model

Summary

In moving processes from bench to pilot and ultimately production fermentors, it is critical to have a reliable scaledown model. The purpose of a scale-down model is to demonstrate at the benchtop level what can be achieved in production-scale fermentors. Scale-down models can vary depending on the media, strain, and process conditions. In this case, a customer wanted a scale-down model of the Thermo Scientific™ HyPerforma™ Single-Use Fermentor (S.U.F.) in order to establish what fermentation processes could be scaled into the S.U.F., in particular for the production of plasmid DNA. The 300 L S.U.F. exceeded the customer's expectations. The customer anticipated that the test process using *E. coli* with high oxygen consumption would reach a final OD of ~80 and the HyPerforma S.U.F. exceeded expectations by reaching an OD of 147.

This application also was a successful demonstration of media preparation through culture harvest, using a jacketed 50 L Thermo Scientific™ HyPerforma™ Single-Use Mixer (S.U.M.) (60 L working volume and heated to 67°C), 200 L Thermo Scientific™ HyPerforma™ DS 300 Single-Use Mixer (S.U.M.), Thermo Scientific™ Powdertainer™ BioProcess Container (BPC) system, 2 L—50 L Thermo Scientific™ 2D Labtainer™ BPC systems, pre-irradiated filters, sterile funnel BPC, seed BPC, 30 L and 300 L HyPerforma S.U.F.s, and Thermo Scientific™ CentriPAK™ harvest BPCs.

After evaluating additional leading single-use fermentors from two other suppliers, the customer purchased the Thermo Scientific 300 L HyPerforma S.U.F..

Purpose

To evaluate the 300 L HyPerforma S.U.F. for GMP plasmid production process using a customer's standard evaluation procedure and test strain.

Procedure

The *E. coli* culture was maintained at 37°C ± 0.1, pH 7 ± 0.05, maximum pressure of 0.55 psi, and a minimum dissolved oxygen (DO) of 30%. DO control cascaded at 200–375 rpm, 200–500 slpm air, and 0–400 slpm of oxygen as needed to maintain the minimum set point of 30% DO.

After scaling up the model procedure to the 300 L S.U.F., the power-to-volume input, k_La, and gassing rates were scaled down into the 1 L glass fermentors. The same original procedure was then followed but using the scaled-down power-to-volume input, k_La, and gassing rates for S.U.F. comparison. The scale-down model could then be used for estimating performance with all production strains for this customer.

The procedure was then repeated with the 30 L S.U.F. and showed scalability within the HyPerforma product line.

E. coli cells were collected with the Sorvall BIOS 16 centrifuge using CentriPAK BPC singles with quick connect at 5,373 x g for 15 minutes in 15 L batches. The 30 L S.U.F. was harvested as closed system in two batches using CentriPAK manifolds. The 300 L S.U.F. was harvested within 3 hours using two BIOS 16 centrifuges and CentriPAK singles filled to about 1.87 L each.

During this feasibility study, the off-gas analyses of the cultures were compared. It was noticed that the off-gas trend was comparable. The off-gas analyzer sample line to each S.U.F. can be placed inside the exhaust filter exit cavity or connected to a small sterile filter connected to the S.U.F.'s filter chamber.

Equipment and supplies

Strain:

- HMS174(DE3)

Chemicals:

- Base
- Acid
- Antifoam C8840 (New London Chemicals)
- 60 L nitrogen source, 30% w/v feed solution
- 50 L carbon source, 50% w/v feed solution
- Batch medium

Bioreactor setup

The S.U.F. systems were set up according to the user guide and controlled by Thermo Scientific™ TruBio™ Software, powered by powered by the DeltaV™ Distributed Control Platform from Emerson, utilizing the Thermo Scientific™ TruFluor DO single-use sensors and Hamilton or Mettler Toledo single-use pH sensors. Operating parameters are listed in Table 2.

Bioreactor inoculum, cultivation, and scale-up

The inoculum was cultured in an incubator at 37°C, 250 rpm, 1 inch arc, and for 16 hr.

Table 1. Equipment and materials.

Description	Cat. No.
30 L S.U.F. Hardware	S.U.F.0030.AAA.BAAABB0C00
30 L S.U.F. BPC	SH3B11722.01
300 L S.U.F. Hardware	S.U.F.0300.AAA.DAAABB0C00
300 L S.U.F. BPC	SH3B11861.01
100 L S.U.M., jacketed with touchscreen console	SUM0100.9002
200 L plastic drum	SH30959.03
Drum dolly	SH30958.01
Nalgene™ polyethylene 5 gal tank liner	11100-0005
Nalgene cylindrical 5 gal tank	43050-0005
1.5 L funnel	SH3B14865.01
PowderFill or funnel stand	129752
HQ incubator shaker	11-676-235
Seed BPC (3 L working volume)	SH3B9830.01
Seed BPC clip	122554
0.5, 1, 2, 5, 10, and 50 L Labtainer BioProcess Container (BPCs)	SH30712.01-.02 and SH30963.01-.03
Powdertainer BPCs	SH30737.01 and SH30737.02
HyPerforma™ G3 Controllers	NA
Prima™ BT off-gas analyzer	NC1256292
Bios 16 Sorvall Bioprocessing Centrifuge	2 x L85007685
CentriPAK BPC Adapter 2 L bucket liner	4 x 75003873
CentriPAK BPC 6 x 1.7 L harvest manifold	2 x 75003880
CentriPAK BPC Single with Quick connect	14 x 75003891

Table 2. 30 L and 300 L S.U.F. operating conditions.

Parameter	30 L	300 L
Initial volume	24 L	240 L
Final volume	~30 L	~300 L
Temperature	37°C	37°C
pH	7.0 ± 0.05	7.0 ± 0.05
Agitation	300–600 RPM	200–375 RPM
DO setpoint	30%	30%
DO cascade	Cascade RPM, air, then supplement oxygen	Cascade RPM, air, then supplement oxygen
Gas flow	3–60 standard liter per min (slpm)	30–500 slpm
Antifoam	3 mL, more as foam detected	30 mL initially, more as foam detected

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Results

The standard procedure for expression in *E. coli* model was used for technical transfer to the S.U.F. The culture conditions in the 300 L S.U.F. are seen in Figure 1, showing feasibility of plasmid production in the 300 L S.U.F. as per the customer's standard procedure. The customer stopped the feed in 300 L S.U.F. to be able to harvest right away, where in the 30 L the feed ran out hence the 30 L reached a higher density.

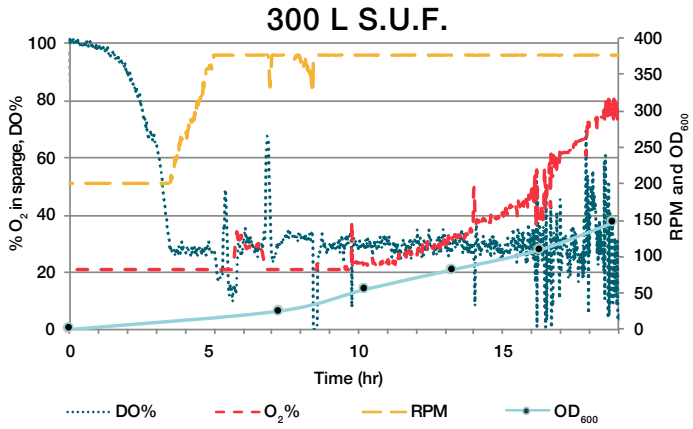

Figure 1. Data from 300 L S.U.F. (n = 1) after one trial scale-up following customer's procedure. The process in the HyPerforma S.U.F. reached an OD of 147 by 19 hr. Samples showed a wet cell weight (WCW) of 167 g/L was achieved.

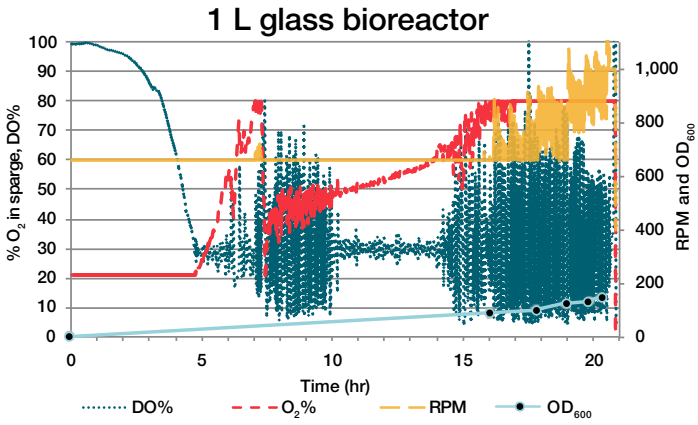

Figure 2. Data from 1 L glass vessel (n = 1) used as a working-volume model for the scale-up procedure. The WCW was 170 g/L for the final samples, same as that achieved in the 300 L S.U.F.

The power-to-volume inputs was calculated for reproducing the conditions from a 300 L S.U.F. to the customer's 1 L benchtop glass fermentors. The same original procedure was then followed, but using the scaled-down power-to-volume input, k_La, and gassing rates. Culture conditions of one of the two scale-down 1 L fermentors are shown in Figure 2. The procedure was then scaled to the 30 L S.U.F. (Figure 3).

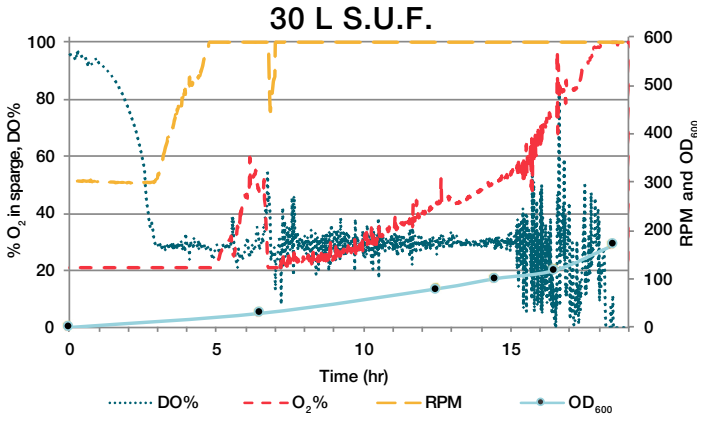

Figure 3. Data from 30 L S.U.F. (n = 1)—second scale-up following customer's procedure. In the 30 L S.U.F., an OD of 170 was reached in our offline measurements. The WCW was 221 g/L.

Conclusion

The HyPerforma 300 L S.U.F. exceeded the customer's expectations and reached a WCW of 167 g/L before feed was stopped. It was determined that the 300 L and 30 L S.U.F.s are adequate for production following the procedures for most of the customer's strains.

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