Production of therapeutic plasmid DNA in single-use fermentors

Introduction

The manufacturing of plasmid DNA is becoming a supply bottleneck due to rapidly growing demand for various gene-targeting biotherapeutics. Many producers and end users are moving towards expanding or implementing single-use production facilities to efficiently meet the demand. Here we will review three procedures for production of therapeutic plasmid DNA in Thermo Scientific™ HyPerforma™ Single-Use Fermentors (S.U.F.s) at 6—30 L working volumes. With all three procedures, equivalent yields per gram of cell paste were achieved in comparison to heat-sterilized vessels.

Modern S.U.F. technologies enable production facilities to achieve equivalent yields in the same time frame (or better) as traditional stainless steel fermentor vessels. Typically, vessel setup time and startup costs are significantly less, as the S.U.F. hardware requires less stainless steel for manufacture and no clean-in-place (CIP) skid, steam equipment, or validation process. Each single-use Thermo Scientific™ BioProcess Container (BPC) ships sterile and validated, reducing downtime between cultures and allowing for greater production volume in less space. With 6—300 L working volumes, the S.U.F.s provide a closed system that eliminates the chance of cross-contamination while providing rapid scale-up for therapeutic plasmid DNA production.

Procedure

Three procedures for production of therapeutic plasmid DNA were compared in a 30 L S.U.F.: 6 L batch culture, 30 L fed-batch culture, and 30 L fed-batch culture with heat induction. The three procedures were first performed in heat-sterilized vessels, and the yields per liter were compared.

The first procedure is a simple tech transfer batch culture in the S.U.F. with Terrific Broth (TB). The goal was to replicate previous CIP 20 L culture runs while evaluating capabilities of a 5:1 turndown ratio (20% of maximum working volume). The medium was 6 L of TB. Control parameters were pH 7.0 ± 0.05 (with 30% ammonium hydroxide and 42.5% phosphoric acid), 30% dissolved oxygen (DO) with 300–600 rpm and gas flow up to 12 slpm, and temperature 37°C. Harvest was at 18 hr, and the cells were pelleted in a Thermo Scientific™ Sorvall™ BIOS 16 centrifuge in 15 min at 5,373 x *g* with Thermo Scientific™ CentriPAK™ BPC singles.

For the second procedure, the prior procedure was modified to an exponential fed-batch culture in the S.U.F. starting at 26 L of TB. The feed was 4 L of 1:1 glycerol to TB with 1% $MgSO_4$ and 1 mL of 1,000X trace elements solution per liter of culture, to bring the final volume to 30 L. The goal was to try to optimize the 20 L culture procedure in one run. Control parameters were the same as in the first procedure except with the addition of the exponential feed rate based on the growth rate, and the gas flow was cascaded up to 60 slpm (2 vvm,) or twice the maximum liquid working volume. Harvest was at 16 hr, and the culture pellet was collected as a closed system with a CentriPAK manifold and each BPC filled to about 1.87 L and Sorvall BIOS 16 centrifuge at 5,373 x *g* for 15 min in two batches of 15 L each.

The third procedure was a process replicated from a CIP/sterilization-in-place (SIP) stainless steel 30 L vessel. glycerol-based synthetic medium was used.

The DO control was set to maintain 30% with a cascade strategy that ramped from 200 to 600 rpm, then air from 6 to 30 slpm, and then oxygen from 0 to 30 slpm. The pH was controlled at 7 ± 0.05. When the optical density (OD) reached about 10 after 14 hr, a nutrient-rich glycerol feed (1/3 of the total culture volume) was initiated. The temperature was reduced from 37° to 32°C at an OD of about 20. The plasmid was induced with heat shock by shifting the temperature from 32° to 43°C once an OD of 60 was reached. The culture was held at 42°C for 2 hr before harvesting.

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
100 L S.U.M.	SH31052.02
300 L S.U.M. DS 300	SUMDS300.9001
200 L drum	SH30959.03
Drum dolly	SH30958.01
200 L DS 300 S.U.M. (custom)	SH30973.02
Nalgene™ polyethylene 5 gal tank liner	11100-0005
Nalgene cylindrical 5 gal tank	43050-0005
1.5 L funnel	SH3B14865.01
PowderFilll 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 Containers (BPCs)	SH30662.14 and SH30662.15 SH30712.01–.05
Gibco™ Bacto™ Yeast Extract and Phytone™ Peptone	212730 and 211906
Powdertainer™ BPCs	SH30737.01 and SH30737.02
G3 Controllers	NA
Bios 16 Sorvall Bioprocessing Centrifuge	L85007685 (fishersci.com)
CentriPAK BPC 6 x 1.7L harvest manifold	2 x 75003880
CentriPAK BPC Single	2x 75003885

Bioreactor setup

The inocula were cultured in an incubator at 37°C, 250 rpm, 1 inch arc, for 8–16 hr. The S.U.F. systems were set up according to the user's guide and controlled by a Thermo Scientific™ TruBio™ software system, powered by the Emerson DeltaV™ platform, utilizing Thermo Scientific™ TruFluor™ single-use DO sensors and Hamilton™ or Mettler Toledo™ single-use pH sensors.

Results and discussion

Batch procedure at 5:1 (6 L)

The plasmid production process was scaled up from processes in an SIP 20 L fermentor where the average peak density was an OD_{600} of 20. The customer hoped to achieve the same results in the S.U.F. Since the growth medium was simply a TB formulation, the culture reached about OD 20 as is typically the maximum density with *E. coli* in TB in a fermentor. The results were duplicated in the S.U.F. at 5:1 working volume (6 L), achieving the same density and expression per liter of medium (Figure 1).

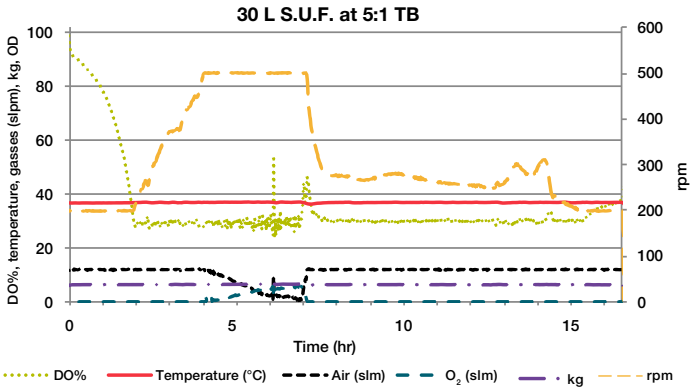

Figure 1. Trial batch culture conditions for the 30 L S.U.F. (5:1 turndown ratio).

Batch procedure (26 L with 4 L feed)

Simple modifications of the batch procedure with a feed would greatly increase the yields. Further optimization of the media and procedure could further improve the yields to levels seen in most any SIP fermentor procedure. This batch procedure was modified to a fed-batch procedure by adding a simple feed solution consisting of one part glycerol to one part TB. In previous work it had been noted that if $MgSO_4$ and trace elements solution were left out the could become static at an OD of 30; hence these components were added to the feed, bringing the concentration to 1% $MgSO_4$ and 1X trace elements.

The simple fed-batch culture achieved 4 times as much cell mass per liter with a final OD_{600} of 80 (Figures 2 and 3). Compared to a previous SIP 20 L vessel, the same plasmid yield per gram of cell paste was achieved while the total yield of plasmid was increased 5 fold.

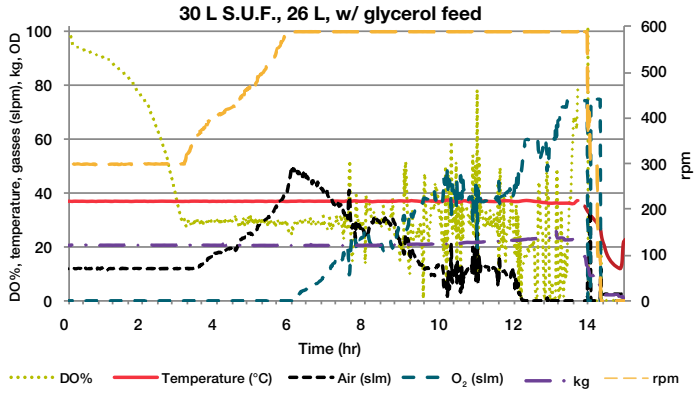

Figure 2. Plot of trial fed-batch culture conditions for the 30 L S.U.F. There is some noted DO% noise around 12 hr of culture, which is expected due to the simple tech transfer. By adjusting the control strategies, future cultures can easily be smoothed out.

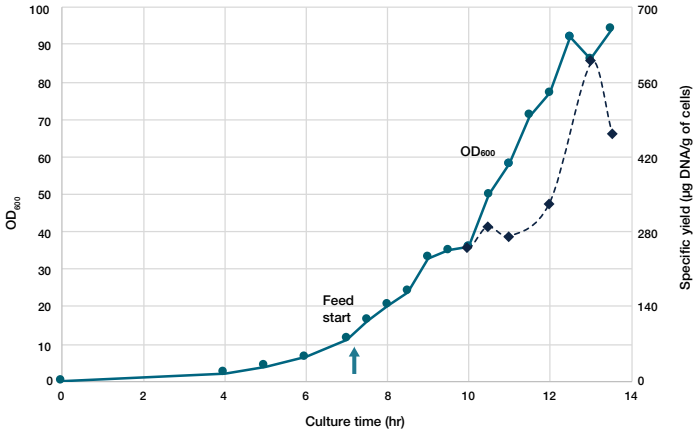

Figure 3. Growth in comparison to plasmid production yields. The customer noted that the yields per gram of cell paste were equivalent to that of a previous SIP 20 L vessel, though with added production yields as the cell mass was 4 times greater per liter.

Fed-batch heat induction procedure (26 L with 4 L feed)

In the third case, a model process was tech transferred to the 30 L S.U.F. at the Thermo Fisher Scientific site in Vilnius, Lithuania. Heat induction was used to increase the plasmid yields from this particular strain (Figures 4 and 5).

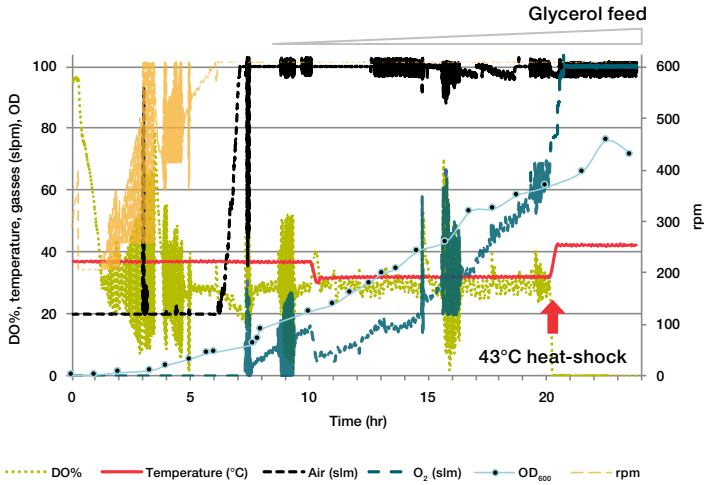

Figure 4. Conditions during fed-batch culture in the 30 L S.U.F. with heat induction. More noise than usual is present since another brand of controller was used for this experiment.

The controller used in this last study lacked many of the most powerful features of the HyPerforma G3 Controller with TruBio software used in the other S.U.F. described here. As a result, the control loop parameters had to be adjusted manually and, as such, the process lacked stability. However, this serves to highlight the robustness of the S.U.F. platform in that the most critical final results of yield and product quality were maintained and in many areas actually improved.

Manual shifts in rpm limits were made to reduce DO% noise during rpm cascade. The PID controller for DO had to be changed manually during the culture, because gain scheduling is not an option with other controller brands. Gain scheduling would have smoothed out DO and temperature control, reducing temperature shift overshoot to within 1°C and oscillation during control to ±0.05°C.

As noted in Figure 4, the final offline density measurement was OD_{600} of 70. The wet cell weight was 100 g per liter of culture. The 1.5 kW temperature control unit used increased the temperature from 32° to 43°C in about 20 min in the 30 L S.U.F. The choice of a higher heater element rating would reduce this response time.

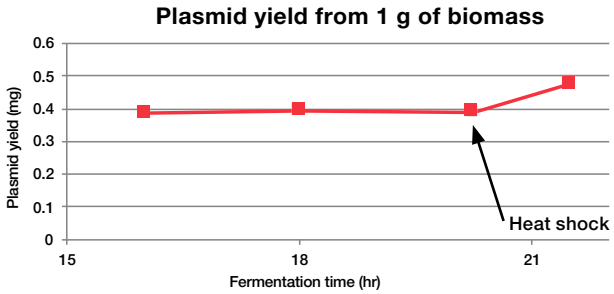

Figure 5. Plasmid yield with heat induction procedure. The graph shows plasmid yield per gram of cell paste. Heat shock increased the yields by 0.1 mg per gram of cell paste.

Conclusions

The yield of plasmid DNA was approximately the same per gram of cell mass in the 20 L SIP fermentor, the 30 L S.U.F. at a 5:1 turndown ratio, and the 30 L S.U.F. with fed-batch culture, though the cell mass of the fed-batch culture increased 4-fold per liter. Tech transfer into the S.U.F. achieved a 1.5x volume increase and 4x cell mass per liter with a simple trial feed strategy.

In the heat-induced cultures, the yield was also the same per gram of cell pellet as in SIP fermentors. The S.U.F. can be used for plasmid DNA production under high-density conditions in *E. coli* cultures and for production processes with temperature manipulations.

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