Production of antibody fragments by *S. cerevisiae* in the HyPerforma Single-Use Fermentor

Comparison of production efficiency with stainless steel tank fermentor

Introduction

Advances in single-use bioprocess technology have led to the development of the Thermo Scientific™ HyPerforma™ Single-Use Fermentor (S.U.F.), the first specifically designed fermentor of its kind. This S.U.F. equipment has since been integrated into our cGMP production facility in Lithuania. This study was undertaken to demonstrate the feasibility of technical transfer of procedures for routine production of an anti–human IgG antibody fragment in *Saccharomyces cerevisiae,* from a stainless steel vessel to the HyPerforma S.U.F. Procedural timing for the culture remained unchanged, and only small adjustments were made to agitation and gas-flow settings. For simplicity, the S.U.F. nutrient medium was sterile-filtered and the stainless steel vessel with medium was steam-sterilized. Results showed that production of the native antibody fragment and titers in the S.U.F. were comparable to those of the stainless steel fermentor.

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Methods

Recombinant *S. cerevisiae* was cultivated for 3.5 days in the 30 L HyPerforma S.U.F. and also in the 10 L stainless steel fermentor. A starting volume of 15 L of a semisynthetic medium containing 10 g/L glucose as the batch-phase carbon source was used in the HyPerforma S.U.F., and 5 L of the same medium was used in the steel-tank fermentor. The inoculation was performed by adding 10% inocula from 48-hour seed cultures (1.5 L and 0.5 L for the 30 L HyPerforma S.U.F. and the 10 L stainless steel fermentor, respectively). At approximately 12 hours post-inoculation, the batch glucose and most of the ethanol were metabolized. The cultures were then fed by simultaneous addition of ethanol and a concentrated nutrient solution. The initial rate of both feeds was 2 g/L per hour (g/L/hr), and it was gradually increased at a constant rate over 48 hours to 8 g/L/hr at the final stage of the fermentation. The feeds were stopped after 85 hours post-inoculation, and the temperature of the cultures was reduced to 15°C for harvesting (Table 1).

Table 1. Culture conditions for *S. cerevisiae*.

Parameter	Value
pH	5.00 ± 0.05
Temperature at different time points	
Hour 1–12	30°C
Hour 12–85	21°C
Hour 85–88	15°C
Dissolved oxygen (DO)	30%
Airflow (vvm)*	
30 L S.U.F.	0.5–1.7**
10 L stainless steel fermentor	0.3–3.0**
Oxygen (vvm)*	
30 L S.U.F.	0–0.17**
Agitation (rpm)	200–600**
Feeds	
Ethanol	
Hours 12–60	2–8 (g/L/hr)
Hours 60–85	8 (g/L/hr)
Nutrient	
Hours 12–60	2–8 (g/L/hr)
Hours 60–85	8 (g/L/hr)

* Airflow and oxygen vessel volumes per minute (vvm) are based on recommended maximum liquid volume for the vessel.

** Airflow, oxygen, and agitation rates were cascaded to maintain the DO set point.

An auto-inducible system was used for production of the antibody fragment. The *S. cerevisiae* culture was grown in the semisynthetic medium supplied with 10 g/L of glucose and 1 g/L of galactose. The expression was controlled by the galactose-inducible GAL7 promoter and was repressed while the culture was growing on glucose. When the glucose present in the starting medium was consumed (around 8 hours post-inoculation), the GAL7 promoter was derepressed, and the protein production was automatically induced by galactose. Since the *S. cerevisiae* strain possessed a *Δgal1* genotype and was unable to metabolize galactose, the concentration of galactose remained constant with continuous protein expression throughout the fermentation process. The amount of the target protein was monitored by taking samples of the culture at various time points and analyzing them by SDS-PAGE. The concentration of the target protein in the final samples was estimated densitometrically by running samples from the culture medium at different dilutions alongside bovine serum albumin (BSA) standards of known concentrations on a gel, and comparing the sizes of the bands. The bands were quantified using TotalLab Quant software.

To determine whether the expressed antibody fragment was correctly folded and functional (i.e., able to bind to human IgG), the sample was:

1. Exchanged into TST buffer (50 mM Tris-HCl, pH 7.6, 150 mM NaCl, and 0.05% Tween™ 20 detergent): 0.5 mL of the sample was concentrated 5-fold using a Thermo Scientific™ Pierce™ Protein Concentrator of 3K molecular weight cutoff (MWCO) (Cat. No. 88512) and then brought up to the starting volume (0.5 mL) with TST buffer; the process was repeated 5 times in total;
2. Loaded onto a Thermo Scientific™ Pierce™ Screw Cap Spin Column (Cat. No. 69705) containing 0.2 mL IgG Sepharose™ 6 Fast Flow resin (GE Healthcare), following the supplier's manual; sample volume loaded was 0.2 mL;
3. Washed with 2 mL (10 resin volumes) of TST buffer;
4. Eluted using 0.2 mL of 0.1 M glycine buffer, pH 2.5.

Flow-through, wash, and elution fractions were visualized by SDS-PAGE.

Results

Optical density (OD) and wet cell weight (WCW) were plotted for comparison of the cultures (Figure 1). Samples from the culture supernatants were run on a gel to visualize the accumulation of the recombinant antibody fragment, with 7.5 µL loaded in each lane (Figure 2). The concentrations of the recombinant antibody fragment in the supernatant at the final sampling point (88 hr), analyzed with the TotalLab Quant device, were 0.5 g/L in the 10 L stainless stainless steel fermentor and 0.9 g/L in the 30 L HyPerforma S.U.F.

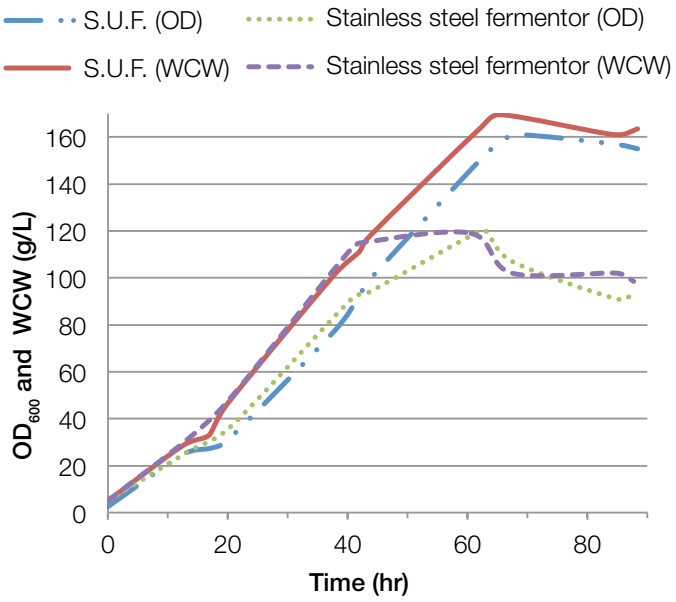

Figure 1. Comparison of OD and WCW of *S. cerevisiae* cultures in the HyPerforma S.U.F. versus a stainless steel fermentor, for a 3.5-day process of fed-batch production of a recombinant antibody fragment.

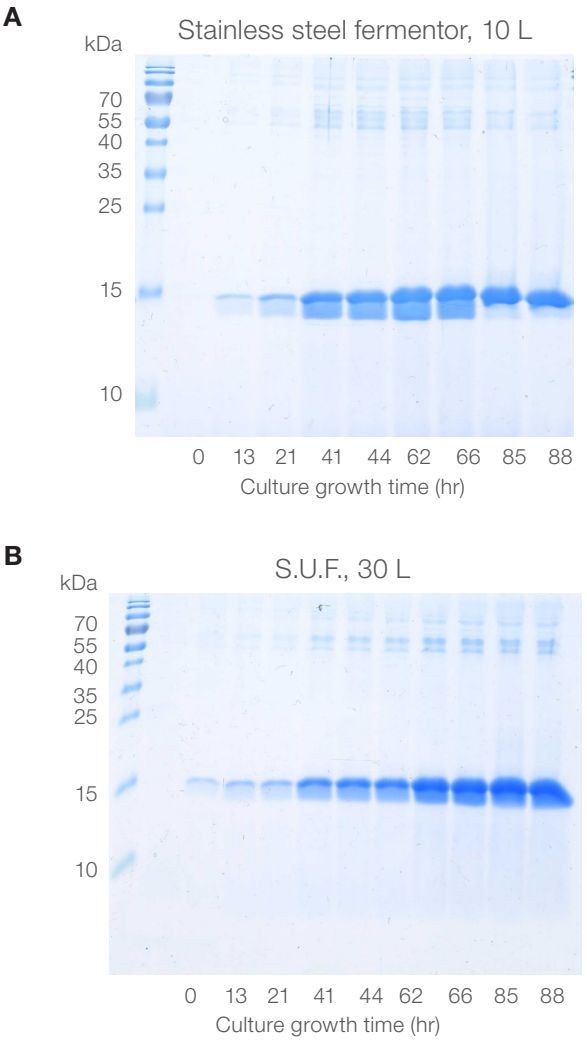

Figure 2. Accumulation of a recombinant antibody fragment in *S. cerevisiae* growth medium. SDS-PAGE (15% gels) shows accumulation of the antibody fragment in the growth medium during fermentation in **(A)** the 10 L stainless steel fermentor and **(B)** the 30 L S.U.F. Wells were loaded with 7.5 µL of undiluted medium taken from the cultures at the indicated time points. Expression starts around 8 hr post-inoculation when the entire amount of glucose in the culture medium has been consumed and galactose-inducible expression is derepressed.

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Discussion

The final cell densities of the *S. cerevisiae* cultures appeared to be 60% higher in the S.U.F. (cultures were very dense, OD_{600} >160). When compared to the conventional fermentation equipment, the yield was 80% greater in the S.U.F. (0.9 g/L vs. 0.5 g/L). The results of an IgG-binding assay revealed that the proteins expressed in both vessels during the fermentation process exhibited highly similar binding features and quality (Figure 3). Further studies could be performed to determine if differences in the media preparation procedures (sterile filtration for the S.U.F. and steam sterilization for the stainless reactor) contribute to the differences seen in the final results.

Conclusion

Extracellular production of an antibody fragment in *S. cerevisiae* culture was successfully reproduced in the 30 L HyPerforma S.U.F. The expressed antibody fragment produced in the S.U.F. was correctly folded and functional. Based on this study, the HyPerforma S.U.F. outperformed the stainless steel fermentor in production of the recombinant antibody fragment by *S. cerevisiae.*

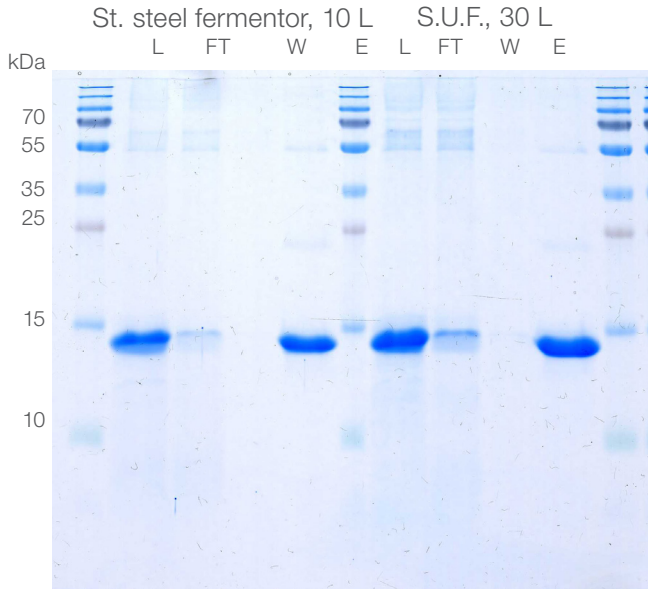

Figure 3. Results of an IgG-binding assay on proteins expressed in cultures grown in the stainless steel fermentor vs. the S.U.F.
With both systems, >95% of the expressed antibody fragment was able to bind to human IgG, as shown by this SDS-PAGE (15% gel) analysis. L: loading; FT: flow-through; W: wash; E: elution.

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