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Single-Use Fermentor Process Optimization and Scale-up of Microbial Cultures

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Figure 1. 300 L and 30 L HyPerforma™ S.U.F.

Abstract

Recent innovations in Single-Use technologies (SUT) have allowed traditional microbial fermentation processes to quickly capture the established benefits that have been proven over the past decade with animal cell culture processes when using disposable processing equipment. The Thermo Scientific™ HyPerforma™ Single-Use Fermentor (S.U.F.) is designed to deliver equivalent performance to stainless steel SIP/CIP reactors for research and pilot scale microbial bioproduction at 30 L and 300 L liquid working volume. To demonstrate feasibility, several studies were performed in 2014 for the purpose of determining if key process demand aspects of modern recombinant microbial strains like *E. coli* and *P. pastoris* could be met in the S.U.F. To this end, prior work did confirm the feasibility of meeting two critical benchmarks - high oxygen uptake demands (k_La of >600 per hour) and being able to monitor and control foam generation inside the disposable bag. Still there have been further questions from academia and industry as to if these new process systems have the ability to support broader and perhaps more aggressive applications. For example, being able to operate at an elevated process temperatures, rapid temperature shifts (heat shock induction), near precision exponential nutrient feed delivery, and cascade type oxygen sparge mass transfer control integrations are critical topics that merit further investigation. In this poster, new data presents the benefits and technical challenges of configuring a system to deliver enhanced recombinant plasmid production via induction using rapid temperature shifts, precise minimum O_2 control, and various culture protocols. This work serves to highlight that with proper guidance and relevant experience, SUT end users may successfully compensate for the constraint limitations of both disposable bags and compact temperature control units that are commonly employed when using modern single-use products.

Introduction

Advances in single-use bioprocess technology have lead to the rapid adoption of single-use bioreactors (SUB) across a broad range of applications within the biotechnology industry. However, the aggressive performance demands of industrial microbiology have limited the conversion of traditional fermentation processes into single-use systems. In order to address the unique needs of microbial applications, a dedicated team of scientists and engineers created the Thermo Scientific HyPerforma Single-Use Fermentor.

Built for scale-up and scale-out: Offered in both 30 L and 300 L working volumes (Figure 1). The S.U.F. was designed to meet the unique requirements of microbial fermentation instead of being modified from a cell culture bioreactor. The S.U.F. provides the following benefits:

- **Traditional configuration**–utilizes three Rushton-type impellers along with vessel geometric proportions, spacing, and baffling that are well proven in industrial biotechnology.
- **Vigorous mixing performance**–the agitator can sustain an agitation rate of 375–600 rpm and both systems target a maximum mixing power ratio of 11 hp/1,000 gal (2.27 W/L), offering capacity beyond systems that use a magnetically coupled impeller.
- **Powerful mass transfer performance**–many aggressive microbial cultures require systems that can produce an oxygen transfer coefficient (k_La) exceeding 350 h^{-1} (as measured without supplementing oxygen). These high k_La values are achieved in this system by supporting gas flows of two vessel volumes per minute (vvm).
- **Single-use and conventional sensors**–support critical process parameters of pH, dissolved oxygen (DO), temperature, pressure, foam level, cell mass, vessel mass, and agitation rates.
- **Reliability**–because there is no reliance on conventional mechanical (SIP/CIP) valves and actuators, the system requires nearly zero downtime and only minimal routine maintenance.
- **Validation**–constructed of the industry-leading options of Thermo Scientific™ CX5-14 or Aegis™ 5-14 films, these flexible products are pre-sterilized and offer the highest level of integrity and purity, as well as eliminate the possibility of contamination from previous culture residuals.
- **Modular design**–the S.U.F. impeller drive train, tank baffles, port locations, line sets, and sensor configurations can be customized to meet specific culture or facility needs.

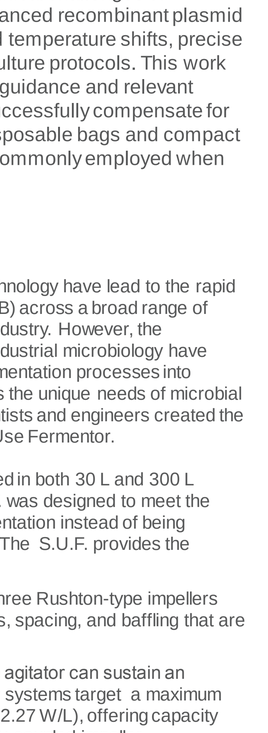
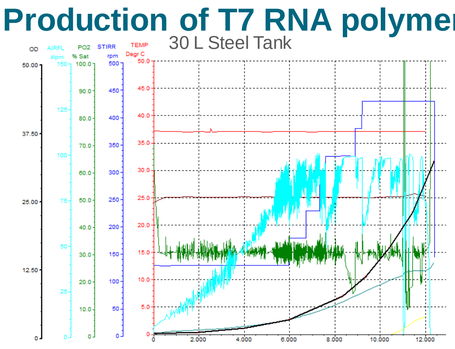
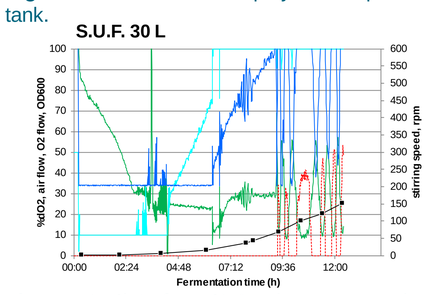


Figure 2. 300 L culture at 600 slpm gas flow and 375 rpm.

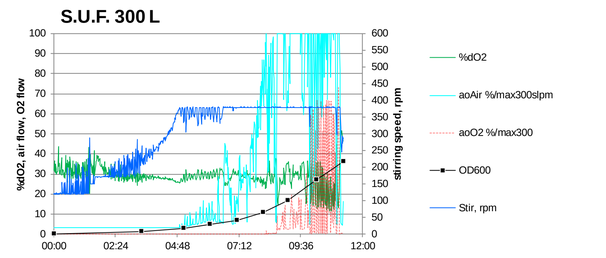
Production of T7 RNA polymerase



Figures 3. Plot of T7 RNA polymerase production in 30 L steel tank.



Figures 4. Plot of T7 RNA polymerase production in 30 L S.U.F.



Figures 5. Plot of T7 RNA polymerase production in 300 L S.U.F.

Enzyme accumulation profile

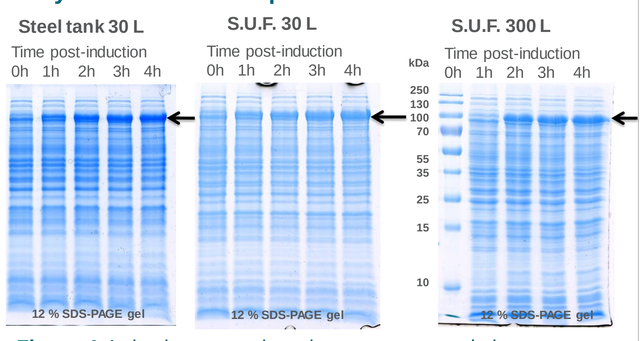


Figure 4. Induction strength and enzyme accumulation patterns are identical between the fermentations in S.U.F. and steel tank.

T7 RNA polymerase folding *E. coli* cytoplasmic space

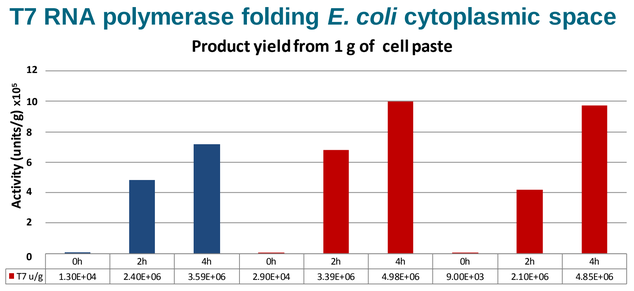


Figure 5. Fermentations in S.U.F. yielded higher DNA modifying enzyme activity from 1g of cell paste compared to steel tank system.

E. coli heat induction

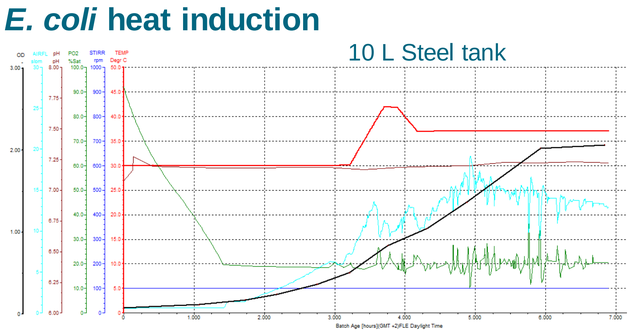


Figure 6. *E. coli*, LB medium 8 L, heat-shock: 30°C → 10 min. 42° C → 37° C (the controller was programmed to gradually increase and then decrease temperature) post induction: 3 hr.

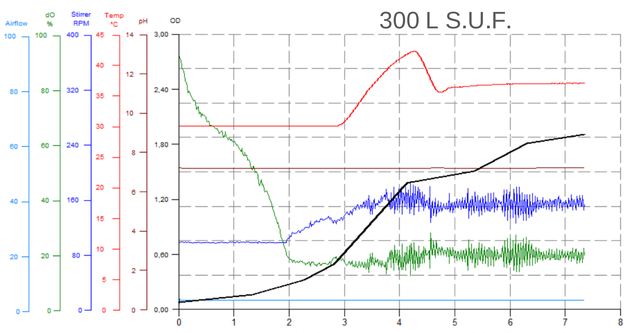
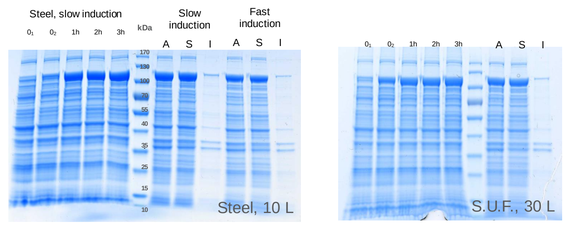


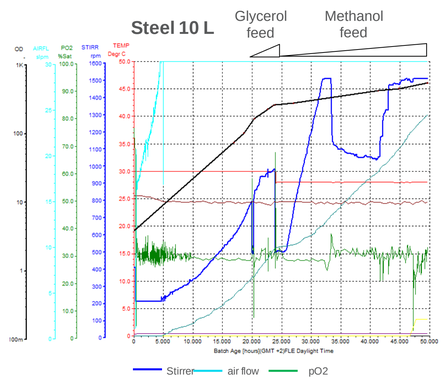
Figure 7. *E. coli*, LB medium 250 L, heat-shock: 30°C → 10 min. 42° C → 37° C,Ferm time post induction: 3 hr. Gas flow rates are shown as % from maximum, not as actual rates in slpm (as it says on the graph). Protein and biomass (based on the culture optical density) yields are identical in the steel fermenter and both size S.U.F.s.



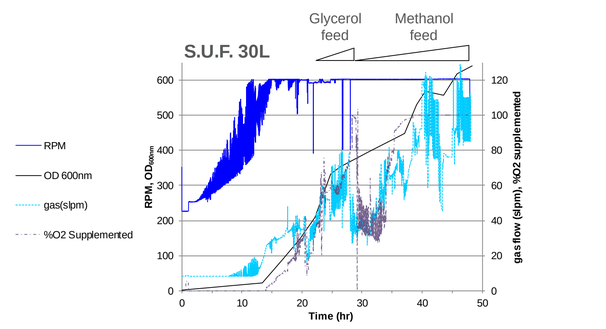
- 0_1: start of the temperature increase
- 0_2: reached 42° C
- 1 hr, 2 hr, 3 hr: time post-induction
- A: all proteins
- S: soluble
- I: insoluble

Figure 8. Protein expression from heat induction in the S.U.F. and steel tank fermentors produced identical target protein accumulation patterns – in total, soluble and insoluble fractions.

Phosphatase secretion by *Pichia* yeast

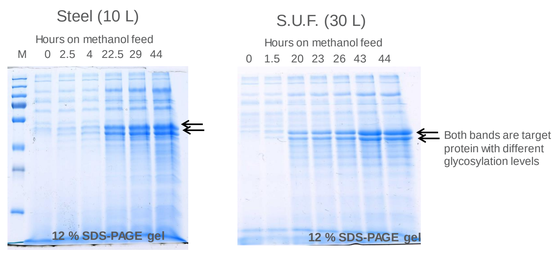


Figures 9. Plot of *Pichia pastoris* culture in 10 L steel fermentor.



Figures 10. Plot of *Pichia pastoris* culture in 30 L S.U.F.

Fermentation conditions:	
•Strain: *P. pastoris*	•Fed-batch (batch → glycerol feed → methanol feed)
•AOX promoter (induction with methanol)	•Pre induction temperature = 30° C
•Mineral salt media, glycerol based	•Post induction temperature = 28° C
	• pO_2 = 30 %



1 g/l

Figure 11. Extracellular accumulation of the target protein in culture media.

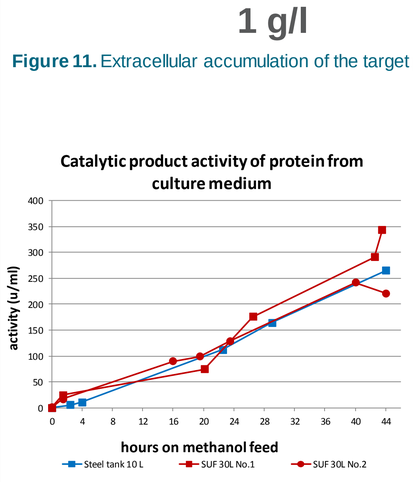
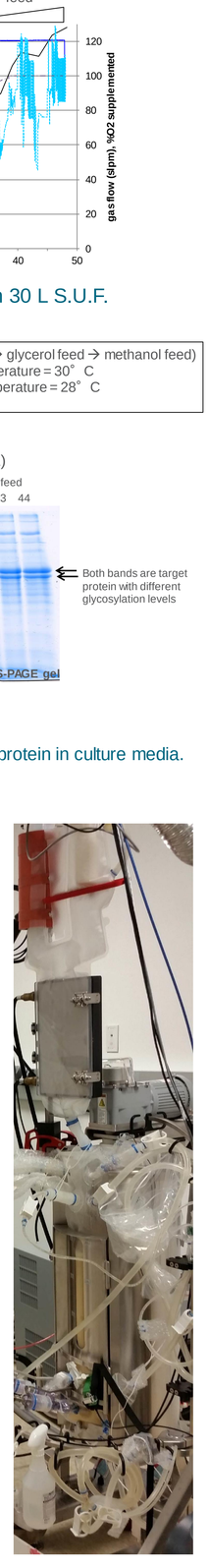


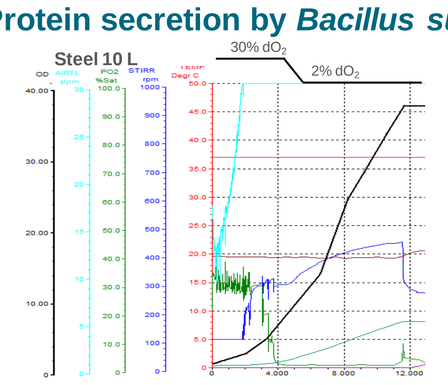
Figure 12. Identical phosphatase activity possessing target protein yield was obtained in S.U.F. and steel tank fermenters.

Target protein correctly folds and exhibits higher catalytic activity when expressed in S.U.F. compared to analogical process in the steel tank

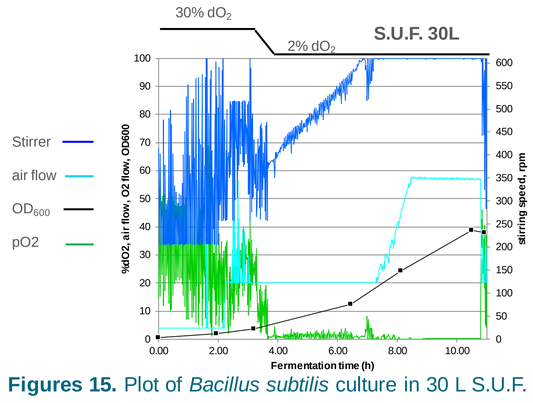
Figure 13.
30 L S.U.F. during dense cultivation at 60 slpm and 600 rpm.



Protein secretion by *Bacillus subtilis*



Figures 14. Plot of *Bacillus subtilis* culture in 10 L steel fermentor.



Figures 15. Plot of *Bacillus subtilis* culture in 30 L S.U.F.

Fermentation conditions:	
•Strain: *B. subtilis*	•Semi-synthetic medium
•Constitutive promoter, secreted protein	•Batch
	•Temperature = 37° C
	•pO_2 = 30 % for 3 hours, then reduced to and maintained at 2%

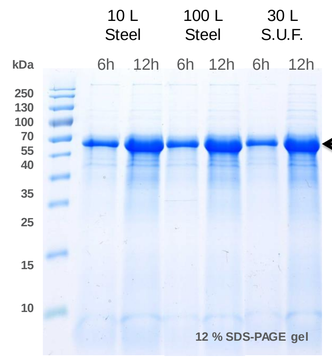


Figure 16.

- Extracellular accumulation of the target protein-affinity chromatography ligand.
- Target protein production levels in S.U.F. were achieved as good as in steel tank reactor.
- **~1.5 g/l target protein produced in all vessels**

End User Production Experience

- The hands-on time of S.U.F. preparation process is much shorter.
- Easy S.U.F. container preparation, installation and start of the fermentation.
- No reactor cleaning or vessel disassembly/assembly.
- Favorable conditions created in the S.U.F. vessels facilitates cellular productivity and thus creates conditions for product accumulation and effective folding.

Conclusions

- HyPerforma S.U.F. performs as well or better than steel tank reactors in terms of maintenance of conditions for propagation and folding of recombinant proteins and nucleic acids.
- HyPerforma S.U.F. can be used for high cell density cultivation of bacterial and yeast cells, for intracellular and extracellular expression of recombinant products.
- The configuration of the S.U.F. allows manipulation of the culture environment to ensure proper conditions for growth, folding, and accumulation of recombinant products.
- The fermentation process in S.U.F. is scalable and reproducible – from batch-to-batch, from process-to-process.

Reference

Pichia Fermentation Process Guidelines Ver. B, www.thermofisher.com

Acknowledgements

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