Mass transfer of single-use fermentors to stirred glass bioreactors

Abstract

The Thermo Scientific™ HyPerforma™ Single-Use Fermentor (S.U.F.) has been purposefully engineered to best match traditional stirred tank reactor (STR) design features. The S.U.F. delivers performance equivalent to that of stainless steel steam-in-place/clean-in-place (SIP/CIP) reactors for research- and pilot-scale microbial bioproduction at both 30 L and 300 L liquid working volumes. However, due to the implementation of single-use film wall, sparge, and agitation, there can be limitations when moving aggressively growing, dense cultures from steel tanks to single-use reactors. To characterize the limitations of available single-use bioreactors, a rigorous comparison was undertaken in collaboration with multiple vendors. To evaluate the oxygen transfer coefficient (k_La), a procedure with a 10 g/L sodium chloride (NaCl) solution and single-use optical dissolved oxygen (DO) probes was used in all vessels to obtain comparable results. The various reactors were tested at preset maximum rpm and air flow rates without any oxygen supplementation allowed. The results were supplied to reactor vendors without disclosing the suppliers' names. Here we present the performance of 30 L and 300 L S.U.F.s in comparison to other unnamed vendor options.

Introduction

Advances in single-use bioprocess technology have led to the rapid adoption of single-use bioreactors 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.

To address the unique needs of microbial applications, a dedicated team of scientists and engineers created the S.U.F, which has the following features:

- **Built for scale-up and scale-out**—Both 30 L and 300 L S.U.F.s are designed for scale-up and scale-out, allowing operation from 5:1 (20%) volume up to full (100%) volume. Thus, actual liquid working volumes are 6 L—30 L and 60 L—300 L, respectively. The total volume of S.U.F. systems (43 L and 435 L, respectively) allows for normal volume increase from gas bubble hold-up and foam accumulation with gas flows of 2 vessel volumes per minute (VVM). The S.U.F. design meets the unique requirements of microbial fermentation instead of being modified from a cell culture bioreactor. The traditional configuration utilizes three Rushton-type impellers along with vessel geometric proportions, spacing, and baffling that are well proven in industrial biotechnology.

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- **Vigorous mixing performance**—The agitator can sustain mixing rates of up to 600 rpm for 30 L and 375 rpm for 300 L. Both system sizes 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 aggressively growing microbial cultures require systems that can produce k_La exceeding 600 hr^{-1} (as measured without supplementing oxygen). These high k_La values are achieved in this system by supporting gas flows of 2 VVM.
- **Single-use and conventional sensors**—The sensors support critical process parameters of pH, DO, temperature, pressure, foam level, cell mass, vessel mass, and agitation rates.
- **Reliability**—Since there is no reliance on conventional mechanical (SIP/CIP) valves and actuators, the system requires nearly zero downtime and only minimal routine maintenance. The S.U.F. has a pinch clamp back-up exhaust filter option that extends the life of the Thermo Scientific™ BioProcess Container (BPC) for instances of filter fouling due to foam or debris carried in the gas flow from dense media formulations (Figures 1 and 2).
- **Validation**—Constructed for the industry-leading Thermo Scientific™ CX5-14 and Aegis™ 5-14 films, flexible BPC products are presterilized and offer the highest level of integrity and purity. In addition, they eliminate the possibility of contamination from previous culture residuals. At full volume (30 L or 300 L, respectively), the S.U.F. system is validated to run at 0.5 psi and 2 VVM (60 slpm or 600 slpm, respectively) for 14 days.
- **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.

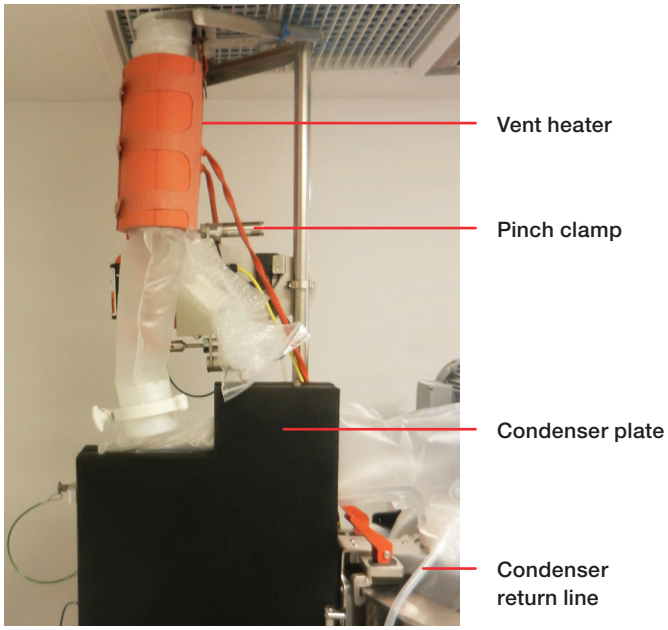

Figure 1. Exhaust filters, pinch clamp, and condenser on the 300 L S.U.F.

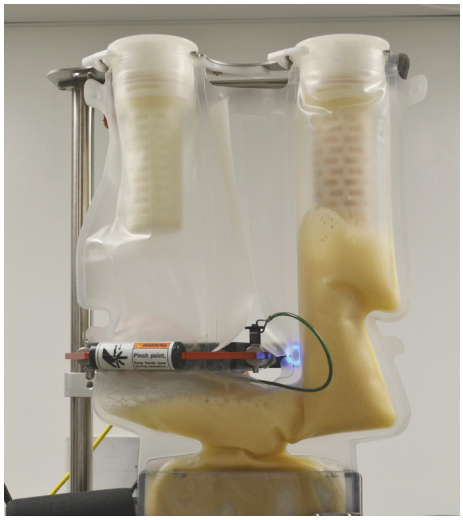

Figure 2. Pinch clamp preventing fouling of the back-up exhaust filter in case of accidental foam-out. The exhaust filter shown was intentionally foamed out to validate the effectiveness of the pinch clamp.

Mass transfer studies

To evaluate the S.U.F. and compare it to other available options, mass transfer studies were performed using the standard dynamic method [1]. The transfer rate of oxygen from the gas to liquid phase is represented by the following equation, in which k_La is the volumetric mass transfer coefficient, C_{O_2} is the concentration of dissolved oxygen, and $C_{O^*_2}$ is the saturation concentration of dissolved oxygen.

$$\frac{dC_{O_2}}{dt} = k_L a \cdot (C^*_{O_2} - C_{O_2})$$

Oxygen mass transfer was measured as the k_La or rate of oxygen from sparged air transferring into a nitrogen-saturated solution.

DECHEMA mass transfer studies

Oxygen mass transfer studies of S.U.F.s at maximum agitation with 0.1 up to 2 VVM were completed using the DECHEMA procedure to show how the S.U.F.s perform at the full range of air flow rates (Figure 3).

More recent testing has shown that inflating the chamber to 0.2–0.5 psi is important for achieving the best fit for reproducible best mixing and oxygen mass transfer, though the data used were prior to including full inflation in the procedure. The DECHEMA testing with water simulant showed that the 300 L S.U.F. mass transfer efficiency gains plateau around 1.7 VVM, due to a combination of impeller flooding and larger Sauter bubble size.. It should be noted that in actual fermentation cultures, the liquid viscosity is much higher, and the impeller flooding effect is unsubstantial up to 2 VVM.

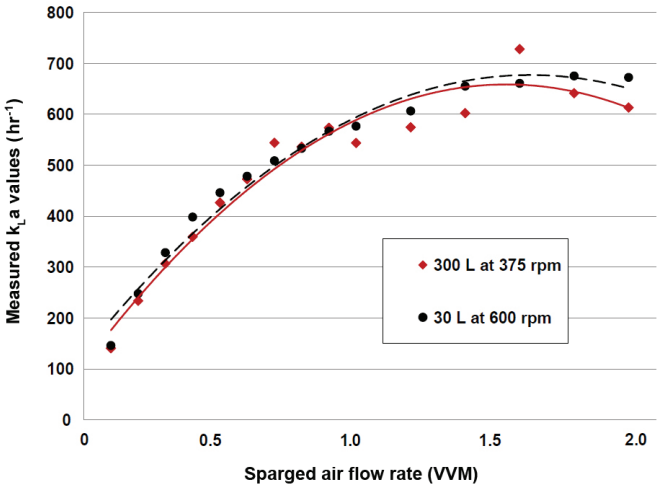

Figure 3. Oxygen delivery in the 30 L and 300 L S.U.F.s was about 650 ± 25 hr^{-1} at maximum agitation rpm and air flow rates of 2 VVM in 10 g/L NaCl in DI H_2O test solution.

Competitor k_La comparison

A collaborative comparison of available single-use reactors was conducted by competing manufacturers using a DECHEMA standardized procedure. The test solution consisted of 10 g/L NaCl at air saturation. Tests were performed by single-use manufacturers at maximum agitation and air flow rates. For the S.U.F., this is 2,250 W/m^3 mixing with 2 VVM air flow rate through the drilled-hole sparger (DHS).

The data in the k_La publication were obtained from the bioreactor manufacturers when using the maximum possible specific power input and aeration rate at 37°C. The standard operating procedure (SOP) used and the results were categorized into two scales: laboratory scale (below 30 L working volume), and pilot scale. The bioreactor from manufacturer 1 was a stirred glass bioreactor used for a comparison against single-use bioreactors. The 30 L and 300 L S.U.F. data show results for single-use reactors 12 and 13 based on the k_La (672.6 hr^{-1} and 613.4 hr^{-1}) and standard deviation (43.1 and 91.6 respectively), although the working volume of 30 L would designate laboratory scale in the plot description. The S.U.F. outperformed all vessels in the comparisons.

The variance noted in the plot from the DECHEMA k_La publication [1] is due to the sensitivity of the fast-responding single-use sensors. As noted with the larger reactors, all had more variance due to the volume. It should be noted that the variability in the k_La data ranged from 10 to 20 percent, which is significantly higher than originally anticipated. This is primarily due to the high sensitivity of the optical probes in large vessels. If loaded properly and pressurized to 0.2–0.5 psi before filling, the 30 L and 300 L S.U.F.s perform more closely to the high range shown in the DECHEMA k_La plot [1]. It was determined that the HyPerforma S.U.F. was the most scalable system.

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Testing summary

- The 30 L S.U.F. outperformed all other single-use reactors of 50 L or less.
- The 300 L S.U.F. outperformed all but one of the single-use reactors of larger than 50 L, although that reactor was of lower volume.
- Properly loading and pressurizing the S.U.F. to 0.2–0.5 psi before filling with medium allows performance closer to the higher range shown in Figure 4.
- The HyPerforma S.U.F. was deemed the most scalable system out of all the options.

Conclusion

Results from this collaboration comparison confirm our internal k_La testing, which shows scalability of the S.U.F. for fermentation processes from benchtop reactors. This comparison confirms culture results that have also shown scalability and reproducibility from benchtop to 30 and 300 L S.U.F.s in comparison to stainless steel fermentors. The HyPerforma S.U.F. performs similarly to stainless steel SIP/CIP fermentors, making it the best option against other single-use reactors that fall below the mark. Fermentation processes in the HyPerforma S.U.F. are scalable and reproducible—between both batches and processes.

References

1. Meusel W et al. (2016) Recommendations for process engineering characterizations of single-use bioreactors and mixing systems by using experimental methods. DECHEMA ISBN: 978-3-89746-171-0.
2. Doran PM (2013) “Mass Transfer.” In *Bioprocess Engineering Principles*, 2nd ed., 416-425. Waltham, MA: Elsevier.

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