Enhanced single-use fermentor for improved cooling and oxygen mass transfer

Introduction

The Thermo Scientific™ HyPerforma™ Single-Use Fermentor (S.U.F.) has proven to a be a powerful process development and manufacturing platform for drug and enzyme production for biotechnology. It is important for us to continue product improvement to enable the achievement of higher cell mass or product titer in less space. Here in Logan, Utah, we develop various single-use bioprocessing systems that help enable pharmaceutical companies to quickly bring products such as vaccines, therapeutics, and other medicines to market with shorter scale-up times and lower capital costs. HyPerforma™ single-use products also reduce downtime and waste volume, because deep cleaning is not necessary as is the standard process for stainless-steel vessels.

As users of HyPerforma S.U.F.s continually push to increase production levels in their fermentors, we continue to increase our product capabilities to meet their needs. Here we share how we have further enhanced the HyPerforma S.U.F. to meet higher cooling requirements of exponentially growing, high oxygen–consuming cultures. First, we illustrate how the jacketed surface area was enlarged to increase cooling capacity of the reused support vessel. We discuss loading of Thermo Scientific™ BioProcess Containers (BPCs) to achieve optimal cooling performance. We show how we developed larger single-use parabolic turbine impellers with greater oxygen delivery capabilities and lower power consumption than equivalently scaled Rushton impellers on the original HyPerforma S.U.F. Customers need improved oxygen delivery with reduced oxygen consumption, comparable to or beyond the abilities of stainless-steel vessels.

Features of the molded impellers were designed to be shipping-friendly, with improved integrity of the final assembled HyPerforma S.U.F. while the modified impellers were enlarged. These improvements have led to the HyPerforma enhanced S.U.F. (eS.U.F.) with performance comparable to the abilities of stainless-steel vessels (Figure 1).

Figure 1. Original 300 L HyPerforma S.U.F. (left) and 300 L HyPerforma eS.U.F. (right).

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Development results

The design of the 300 L HyPerforma S.U.F. left the bottom and the door unjacketed for the sake of simplicity and cost savings. By jacketing these two areas, the heat transfer area of the jacketed area increased by 25%. The jacket was also extended up the hardware walls to include the gas holdup volume for an additional 10% jacketed surface area. Compared to the original HyPerforma S.U.F., a total of 35% cooling surface area was added to the HyPerforma eS.U.F. Most customer feedback has shown the standard jacket to be adequate, but in some cases it has become necessary for the customer to reduce their exponential feed rate or end the culture early to avoid elevated process temperature conditions. Actual tests using a 24 kW Thermo Scientific™ NESLAB™ temperature control unit (TCU) (460 VAC, 60 Hz, 3-phase, 50 A; Cat. No. SV50239.08) with a setpoint range from –5°C to 55°C with the 300 L HyPerforma eS.U.F. hardware showed that the 35% increased jacket area reduced the time for full-volume heating (from 10°C to 42°C) by 16.5 minutes, a 23% improvement over the original HyPerforma S.U.F. Similarly, the time for cooling from 42°C to 10°C was reduced by 20 minutes with the 300 L HyPerforma eS.U.F., for a 25% improvement.

To achieve optimal cooling, jacket contact, and consistent mixing performance, it is important to pre-fit the BPC to the baffles during installation. To achieve this, the BPC should be installed and then inflated with air to 0.3–0.5 psi before adding liquid to the chamber. When working at a 5:1 liquid volume, the air inflation step and pressurizing the BPC is especially important in allowing the film to conform properly to the baffles before liquid filling. This procedure is necessary in order to achieve the heating and cooling times listed in the user guide. The use of this method on a routine basis will improve run-to-run consistency as it improves contact and fit around the baffles and water jacket, resulting in the best possible heat transfer and superior mixing performance as needed for optimal oxygen delivery.

A variety of impellers were investigated in developing the HyPerforma eS.U.F. A total of 15 iterations of impellers were tested using computational fluid dynamics (CFD) analysis for theoretical performance in comparison to the original Rushton impellers in the HyPerforma S.U.F.

These options were narrowed down, and 5 of these options were further tested in liquid testing for power consumption and k_La performance. Two of the optimal impellers were compared head to head with the Rushton impellers in worst-case *E. coli* cultures. The first option, while larger than the Rushton impeller in diameter and blade height, had power usage equal to that of the Rushton impeller while mixing in ungassed water at room temperature. The second, larger enhanced impeller option used about 50% more energy (watts) than Rushton impellers in ungassed water. Ultimately the second, larger option was chosen for production because the power usage still fit within the limits of the motor and provided better performance (Figure 2).

Figure 2. The enhanced impellers in the HyPerforma eS.U.F.s provide better performance than the original Rushton impellers in the HyPerforma S.U.F.s.

As the impellers of the HyPerforma eS.U.F.s are larger than the original Rushton impellers in HyPerforma S.U.F.s, the variable frequency drive (VFD) settings were adjusted to help ensure the power delivery during the process did not exceed the motor rating. Previously, the VFD of the HyPerforma S.U.F. was set to shut off the motor if the amperage limit was exceeded. If this limit was met and the motor shut down during the peak of a bacterial or yeast culture, the process would most likely be compromised. The setting in the HyPerforma eS.U.F. was changed so that the VFD on the 300 L system will limit impeller speed, if necessary, while delivering the maximum allowed power input without shutting down. With the change in VFD settings, it was observed that even during a worst-case culture test, the maximum rpm of the 300 L HyPerforma eS.U.F. reached 334 ± 5 rpm.

Due to the enhanced impellers being larger on the HyPerforma eS.U.F., the temperature probe well was moved from the bottom probe belt to the second or higher probe belt. Customer feedback has indicated that most S.U.F. customers working with aggressive bacteria start the culture well above a third of the rated volume. As such, for custom HyPerforma eS.U.F. BPCs, the resistance temperature detector (RTD) probe should also be placed in the second or upper probe belt. If a customer wants to run at 5:1 volume, the user needs to ensure the RTD probe is in contact with the liquid. To ensure the RTD is submerged in the liquid at 5:1 volume, the agitator speed needs to be above 500 rpm for the 30 L HyPerforma eS.U.F. and above 200 rpm for the 300 L HyPerforma eS.U.F.

Reliability testing was performed on both the 30 L HyPerforma eS.U.F. and 300 L HyPerforma S.U.F. using the standard drive shaft with the enhanced agitator features. No defects were identified, and the study was conducted at full volume and maximum agitator output in triplicate for both systems. As a precaution, due to the high mechanical stress loads placed on the agitation system, users of the HyPerforma eS.U.F. must replace the drive shaft after 6 months of cumulative use, or on an annual use basis, whichever comes first. This will help prevent a shaft failure, to avoid the loss of the batch and unplanned system downtime.

Culture results

For comparison, all experiments were replicated exactly except for the change in the impeller. The improvement in performance of the enhanced impellers is demonstrated by the decrease in the amount of oxygen supplemented throughout the culture. The feed started when the carbon source was depleted in the culture, and the automated feed started once a dissolved oxygen (DO) spike was detected.

The data comparing the 30 L and 300 L HyPerforma eS.U.F.s to the 30 L HyPerforma S.U.F. with the Rushton impeller can be seen in Figure 3. The data of the 300 L HyPerforma eS.U.F. were shifted by about an hour to match up feed start times to those of the datasets of the 30 L HyPerforma S.U.F., which matched up densities. A reduction in dissolved oxygen demand automatically triggers the start of the feed program.

Reduced oxygen requirements were observed in the HyPerforma eS.U.F. runs, and similar vessel volumes per minute (vvm) of oxygen use were observed between the 30 L and 300 L HyPerforma eS.U.F.s (Figure 3). Compared to the HyPerforma eS.U.F. with enhanced impellers, the original HyPerforma S.U.F. with a Rushton impeller used 7 times as much oxygen supplementation.

The increase in noise observed in oxygen supplementation near the end of the run on the HyPerforma S.U.F. was due to increased addition of an antifoam agent, in which less antifoam was used by the enhanced impellers. Also, during the last 1.25 hours of culture in the 300 L vessel of the original HyPerforma S.U.F., there was an increase in temperature above the 37°C setpoint caused by the use of the original HyPerforma S.U.F. jacket with a thermo-circulator (9 kW heater, house glycol chiller). A larger TCU or pump in the TCU could reduce or prevent this noise, but the noise demonstrated the need for the additional 35% jacket surface area of HyPerforma eS.U.F. in more demanding culture processes. In a replicate run with the same TCU but with the 300 L HyPerforma eS.U.F. vessel with 35% more jacketed area, the temperature did not vary more than 0.2°C above the 37°C setpoint, even when the OD reached 210, which was 70 higher than when the temperature began to rise above the setpoint in the original 300 L HyPerforma S.U.F. in Figure 3.

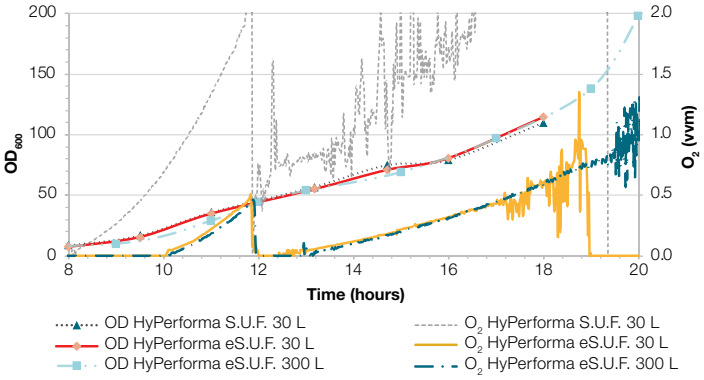

Figure 3. Comparison of process parameters for optical density (OD) and oxygen (O_2) supplementation between runs on a 30 L original HyPerforma S.U.F. with Rushton impellers, and runs on 30 L and 300 L HyPerforma eS.U.F.s with enhanced impellers. Duplicate runs were performed similarly in the three vessels.

Customer verification results

Two customers were selected for early-access evaluation of the HyPerforma eS.U.F. concept. As these customers were both users of existing hardware, the scope of improvement was limited to testing of the impeller improvements of the HyPerforma eS.U.F. BPC design. The early customer evaluation program included early concept (preliminary prototypes) and late verification (equivalent to final design prototypes) versions of the new parabolic turbines for the HyPerforma eS.U.F.

The first external verification was performed by Synlogic Inc., based in Cambridge, Massachusetts, USA (synlogictx.com). Synlogic is a clinical-stage biotechnology company bringing the transformative potential of synthetic biology to medicine. They have been using the HyPerforma S.U.F. as a process development and scale-up tool capable of GMP manufacturing. Certain fermentation processes at Synlogic require effectively 5% DO control for part of the culture. The original 30 L and 300 L HyPerforma S.U.F.s did not produce the necessary 5% DO required for an adequate microaerobic environment for microbial growth. This resulted in having to run the process at 10% DO with the original HyPerforma S.U.F. Running the fermentation at 10% DO was not an optimal outcome as it increased oxygen supplementation, which resulted in a maximum O_2 flow rate of 92.5% of total fermentation gas flow. However, switching to the HyPerforma eS.U.F. BPC design allowed for precise 5% DO that provided ample microaerobic environment for microbial growth. Synlogic found that the 30 L HyPerforma eS.U.F. decreased the maximum oxygen flow rate to only 7% output of the total fermentation flow rate (Figure 4). This was a 93% reduction of oxygen supplementation necessary to run the process in the 30 L HyPerforma eS.U.F. Additionally, the 300 L HyPerforma eS.U.F. was tested to evaluate scale-up. The 300 L HyPerforma eS.U.F. produced similar results, and the maximum oxygen supplemented was about 20 SLPM, compared to 2 SLPM for the 30 L model. The observed reduction in oxygen supplementation flow rates has given confidence for future development of their processes. With the successful results, the customer is now implementing the 30 L and 300 L HyPerforma eS.U.F.s in their GMP production process.

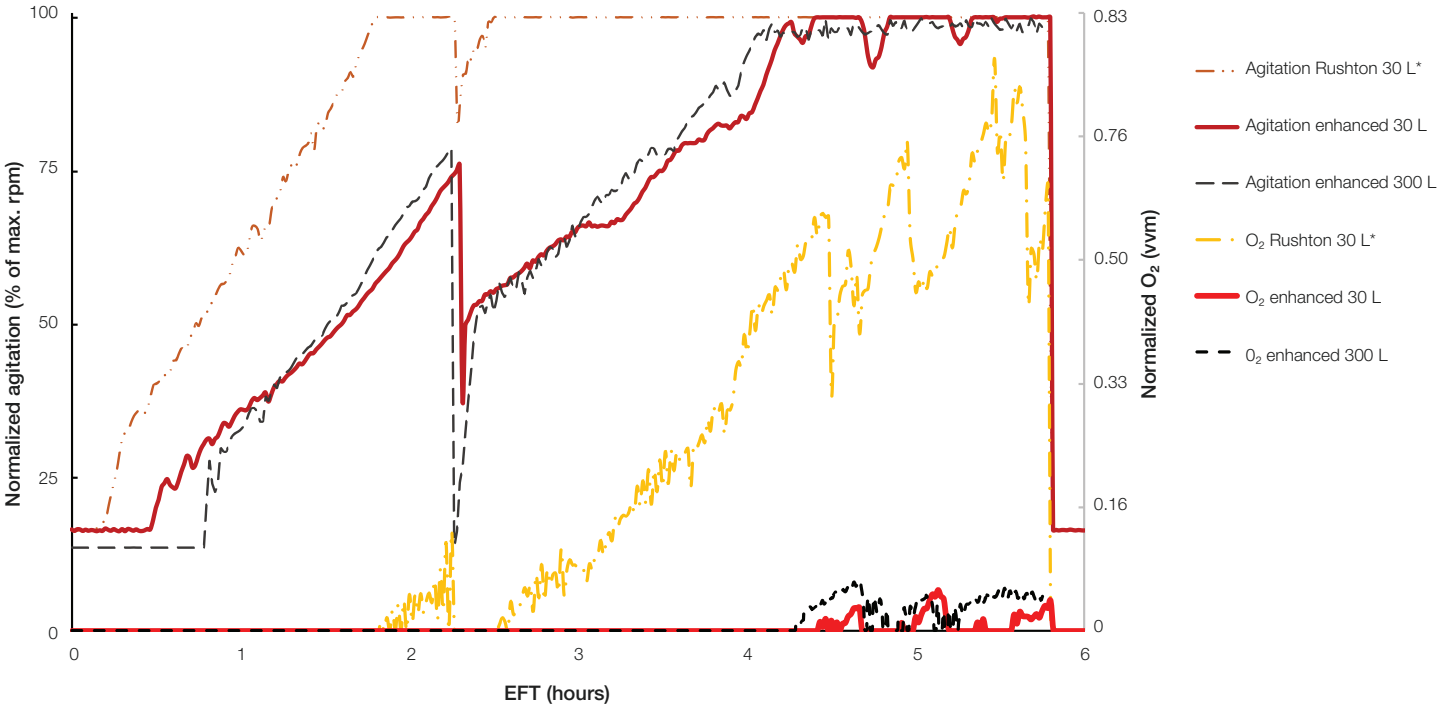

Figure 4. Data showing a comparison of oxygen (O_2) supplementation between the enhanced impeller, at 30 L and 300 L scales, compared to the original Rushton impeller of a 30 L HyPerforma S.U.F. The HyPerforma S.U.F. with Rushton impeller was set to 10% DO, but with the HyPerforma eS.U.F., the DO control was more precise and could be set to 5% DO. The enhanced impeller in the HyPerforma eS.U.F. reduced the oxygen supplementation by 93%.

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The second customer is a CDMO in Asia with challenges that are similar to other S.U.F. retrofits; the capacity of the existing facility's gas hard piping is limited, and thus it is very expensive and time-consuming to upgrade. The HyPerforma eS.U.F. proved to be a viable and low-risk solution, because the dramatic reduction in oxygen demand made it an easy-to-implement solution that avoids the needs for costly facility upgrades or the cost burden and safety concerns of using large quantities of high-pressure oxygen gas cylinders. This customer demonstrated a similar 7-fold reduction in oxygen supplementation when they switched from 30 L with Rushton impellers to 30 L enhanced SUF impellers. In this case, the customer had previously experienced problems with their process consuming high volumes of oxygen, which caused issues because (1) their facility supply was limited to 40 SLPM of oxygen, and (2) the demand for multiple high-pressure gas cylinder replacements was expensive, labor-intensive, and a persistent logistical challenges they wanted to eliminate. Their test showed that the original HyPerforma S.U.F. with the Rushton impeller required a supply limit of 40 SLPM, with multiple cylinders of oxygen used. In contrast, the HyPerforma eS.U.F. configuration required using a maximum of 6 SLPM of oxygen, with less than a single cylinder to complete the run. This customer was pleased with the performance of the HyPerforma eS.U.F., which they indicated was similar to a stainless-steel fermentor. Plans are underway for this customer to continue to use the 30 L HyPerforma eS.U.F. and scale up to 300 L in both process development and GMP production.

Conclusions

The HyPerforma eS.U.F. design significantly improved cooling capacity and reduces oxygen supplementation requirements. To achieve these improvements, the design increased jacketed surface area by 35% in the 300 L HyPerforma eS.U.F., along with the inclusion of larger, more efficient parabolic impellers in the bag design. Testing of the enhanced jacket verified shortened temperature shifts of 23% for heating and 25% for cooling. The HyPerforma eS.U.F. parabolic impellers distributed sparged gases more efficiently throughout the culture, which reduced the oxygen supplementation by at least 80% compared to the Rushton impeller design, while using the same motor in both the 30 L and 300 L sizes. The HyPerforma eS.U.F. further enables cultivation of worst-case bacteria and yeast cultures to higher densities and with performance similar to stainless-steel vessels.

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