Production of Proteinase K

Comparison of single-use versus stainless steel for microbial fermentation

Introduction

The Thermo Scientific™ HyPerforma™ Single-Use Fermentor (S.U.F.) is a unique and rigorous solution for microbial fermentation applications, offering the flexibility, ease of use, and efficiency found in single-use systems. This comparison of the HyPerforma S.U.F.s versus stainless steel fermentors demonstrates equivalent results, with the additional benefit of no fouling of the exhaust filter.

Goal

In this study, the native production and titer activity for secreted Proteinase K from *Tritirachium album* (*T. album*) during the course of an 11-day culture were compared between the S.U.F. and stainless steel fermentor systems.

Materials and methods

The *T. album* (fungus, wild-type) was cultured for 11 days using the conditions listed in Table 1 for the following sizes:

- S.U.F.: 30 L and 300 L
- Stainless steel fermentor: 10 L and 350 L

To maintain continual growth and production, the culture was fed a yeast extract nutrient solution that was started 24 hours post-inoculation, when the initial carbon source (glucose, 10 g/L of culture) was consumed [1].

Culture samples were taken regularly to estimate the accumulation of biomass (wet and dry cell weight) and Proteinase K in the culture. The culture was harvested 11 days post-inoculation. Proteinase K activity in the culture samples was calculated using a stop-point assay of hemoglobin degradation by a target protease [2].

Table 1. Culture conditions.

Parameters	Values
pH	5.9 ± 0.025
Dissolved oxygen (DO)	30%
Temperature	27°C
Airflow (SLPM)	• 10 L: 6 • 30 L: 8.3 • 300 L: 50 • 350 L: 20–120*
Agitation (RPM)	• 10 L: 200–600* • 30 L: 200–600* • 300 L: 200–375* • 350 L: 200

* Cascade to maintain DO setpoint.

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Results

Operations

Reported HyPerforma S.U.F. and stainless steel fermentor *T. album* culture operation values are shown in Figures 1–4. The 300 L S.U.F. shows a small spike in agitation with consistent airflow rate (Figure 2). The 10 L stainless steel fermentor (Figure 3) and 30 L S.U.F. (Figure 1) shows a spike in agitation at the beginning of the culture with a rise of agitation toward the end of the culture while keeping the airflow consistent. The 350 L stainless steel fermentor shows a spike in airflow at the beginning of the culture (20 hours) with a rise of airflow toward the end of the culture while keeping the agitation consistent (Figure 4).

Figure 1. 30 L S.U.F. operation values for agitation, airflow, and DO during the *T. album* culture.

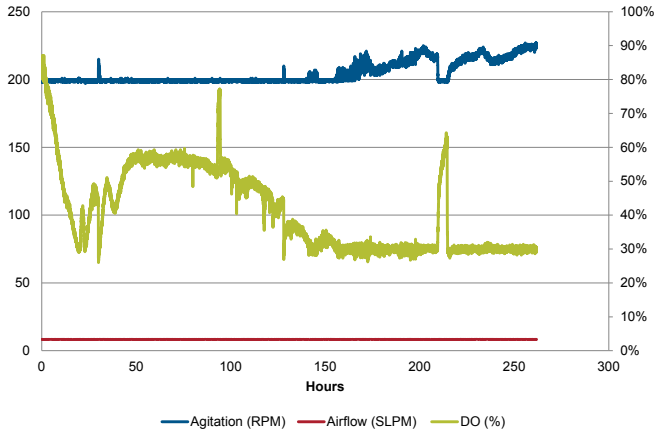


Figure 2. 300 L S.U.F. operation values for agitation, airflow, and DO during the *T. album* culture.

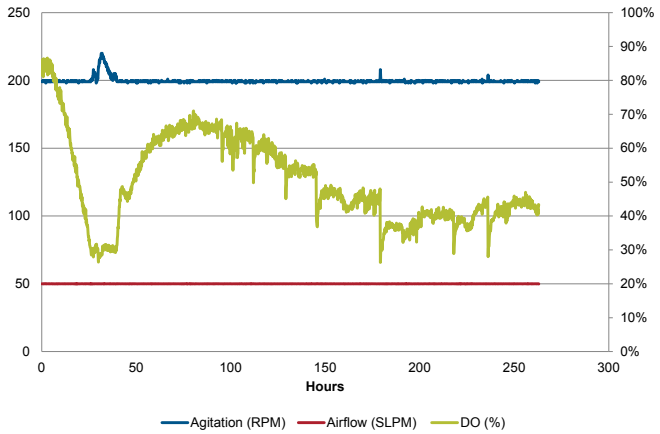


Figure 3. 10 L stainless steel fermentor operation values for agitation, airflow, and DO during the *T. album* culture.

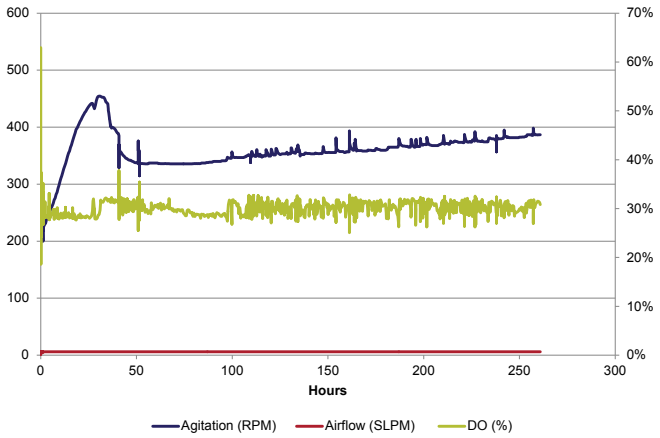
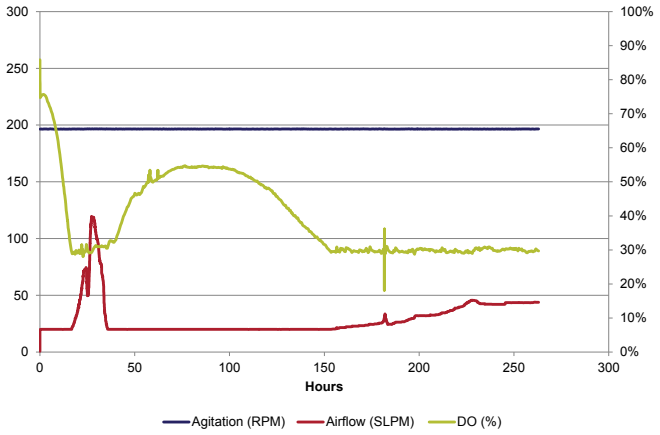


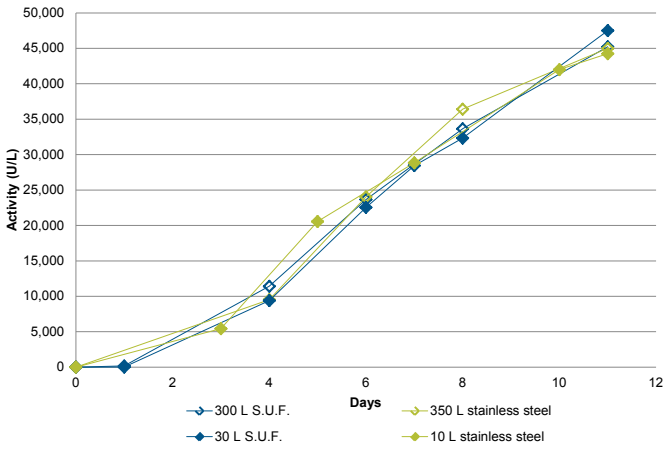
Figure 4. 350 L stainless steel fermentor operation values for agitation, airflow, and DO during the *T. album* culture.



Proteinase K activity

Proteinase K activity was found to be equivalent for all sizes as shown in Figure 5. The final Proteinase K yield was estimated to be 1.5 g of protein from 1 L of culture, based on the specific activity of 45 U/mL.

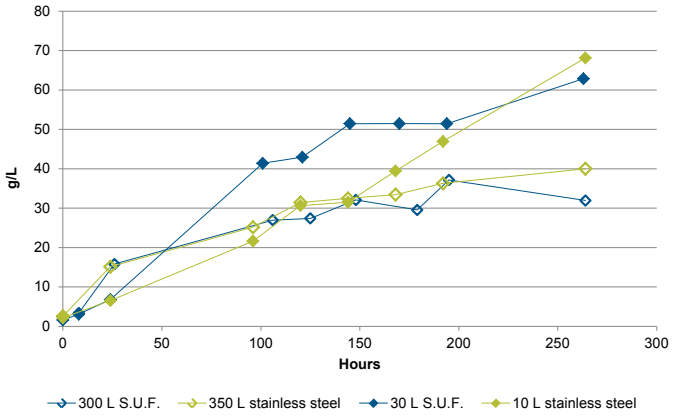
Figure 5. S.U.F. and stainless steel fermentor Proteinase K activity comparison.



Biomass

The *T. album* dry cell weight (DCW) for the S.U.F. and stainless steel fermentor systems are shown in Figure 6.

Figure 6. *T. album* dry cell weight (adjusted for evaporation) from the S.U.F. and stainless steel fermentor systems.



Discussion

T. album is a mycelium-forming organism capable of growing on surfaces. During fermentation, large deposits of biomass forms on the inner wall of the fermentors. Despite the extensive growth of biomass, the S.U.F. filters were not clogged or fouled during fermentation, which is usually a concern when this type of culture is grown in a stainless steel fermentor. A couple of factors may have contributed to this result:

- The order of the cascade was different:
  - The 350 L stainless steel fermentor increased the airflow while maintaining constant agitation (Figure 4)
  - The S.U.F.s and the 10 L stainless steel fermentor increased the agitation while maintaining constant airflow (Figures 1–3)
- The S.U.F. used filter heater blankets to keep the filters dry and to minimize humidity, which creates an environment within the filter that does not support the growth of *T. album*

It was observed that evaporation caused liquid loss to occur during the 11-day cultures. However, when adjusted by the evaporation, the product yield (1.5 g/L) and biomass growth (Figure 6) was found to be equivalent between systems.

The S.U.F. culturing process of mycelium fungus reached similar DCWs (Figure 6) while maintaining sufficient dynamic conditions to ensure an efficient target protease secretion equivalent to stainless steel fermentors.

Conclusion

The performance of the HyPerforma S.U.F.s were equivalent in product yield and activity per the amount of substrate consumed as compared to the stainless steel fermentors. The *T. album* production runs on the S.U.F.s were able to handle the extensive growth of biomass without fouling the exhaust filters, making their operation easy to use.

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References

1. Fortelius C and Markkanen P. 2000. Nutritional regulation of proteinase production in the fungus, *Tritirachium album*. *Journal of Industrial Microbiology and Biotechnology.* Volume 24, Issue 6: pages 369–373.
2. Anson, M. 1938. The estimation of pepsin, trypsin, papain, and cathepsin with hemoglobin. *J Gen Physiol.* Volume 22, Issue 1: pages 79–89.

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