Application Note:
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Full Scale of Yeast Using Thermo Scientific Contifuge Stratos Continuous Flow Centrifuge

KEY WORDS

- Continuous Flow Centrifuge
- Yeast
- *Saccharomyces Cerevisiae*
- Recombinant Protein Expression
- Large Batch

Summary

In the area of scalability, the process of developing recombinant protein expression requires a great deal of thought. There are many stages in the scale-up process before a product reaches full commercial production. To minimize overall processing time while managing the cost of production, efficiency has always been a key factor in the process. This is why it is so important to be able to scale up from small (downstream) to mid or large batch sizes (upstream) with minimal change in product yields and quality while maintaining an efficient process time.

In this application note, the scalability of yeast processing from development to full scale production without any reduction in separation performance is described. The processing times were documented after using the Thermo Scientific Contifuge Stratos tabletop continuous flow centrifuge for the required centrifugation step in the process. With this unit 20 L of sample was processed at a flow rate of 300 mL/min in less than 2.5 hrs for mid to large scale production.

Figure 1. Thermo Scientific Contifuge Stratos centrifuge and its continuous flow rotor on cart with castors and pump secured in its compartment

Introduction

The term ''Yeast'' usually refers to the budding yeast *Saccharomyces cerevisiae (S. cerevisiae)*. It is an ideal eukaryotic micro-organism for biological studies[1, 2]. Properties that make yeast suitable for biological studies include its ability for rapid growth, dispersion of cells, the ease of manipulation, the ease of mutant isolation and replica plating, and the versatility of the DNA and protein expression systems, permitting the convenient production of numerous altered protein forms. The modern age for yeast undoubtedly arrived with the development of methods for the transformation of *S.cerevisiae* with plasmid DNA[3, 4].

Achieving the same separation performance throughout the scale-up process is very important for consistency. Considering that the protein of interest is secreted in the supernatant, it is imperative in maintaining a supernatant that is free of particulates, reducing the risk of introducing more debris into the process which can cause further downstream processing and more production time.

Figure 2. Thermo Scientific titanium continuous flow rotor

In this particular study using yeast suspension, the percentage of clearance was obtained using batch and continuous flow centrifugal separations to demonstrate scalability. Several Thermo Scientific centrifuge systems are available for each stage of process development. For this study the Thermo Scientific Sorvall Evolution RC superspeed centrifuge and the Contifuge Stratos continuous flow centrifuge was used. At the developmental scale, the Sorvall® Evolution RC superspeed centrifuge was chosen and was equipped with the Thermo Scientific Fiberlite F21-8x50 (8 x 50 mL tubes) and Fiberlite® F8-6x1000y (6 x 1 L bottles) rotors for small scale production (from 500 mL to 6 L). The Contifuge Stratos continuous flow centrifuge (Figure 1), with a solids rotor capacity of 400 mL and a maximum flow rate of 300 mL/min, was chosen for small to medium-scale production of a 20 L batch.

10% Yeast Cell Results				
Unit Tested	Sorvall Evolution RC		Contifuge Stratos	
Rotors Tested	Fiberlite F21-8x50	Fiberlite F8-6x1000y	Titanium Continuous Flow Rotor	
RCF (x g)	15,810	15,810	15,810	20,000
Flow rate (mL/min)/Time(min)	9 min	9 min	300 mL/min	300 mL/min
Solids Capacity (mL)	100	1500	400	400
Sample Volume (mL)	500	6,000	20,000	20,000
# Cycles	2	1	13	13
Cycle Time (min)	14.0	14.0	10.3	10.3
Total Run Time (hrs)	0.5	0.2	2.2	2.2
% Clearance	**99.1**	**99.6**	**98.3**	**98.4**

Table 1: Centrifuge conditions for Sorvall Evolution RC unit and Contifuge Stratos continuous flow unit and data of clearance (%) and total run time (hr).

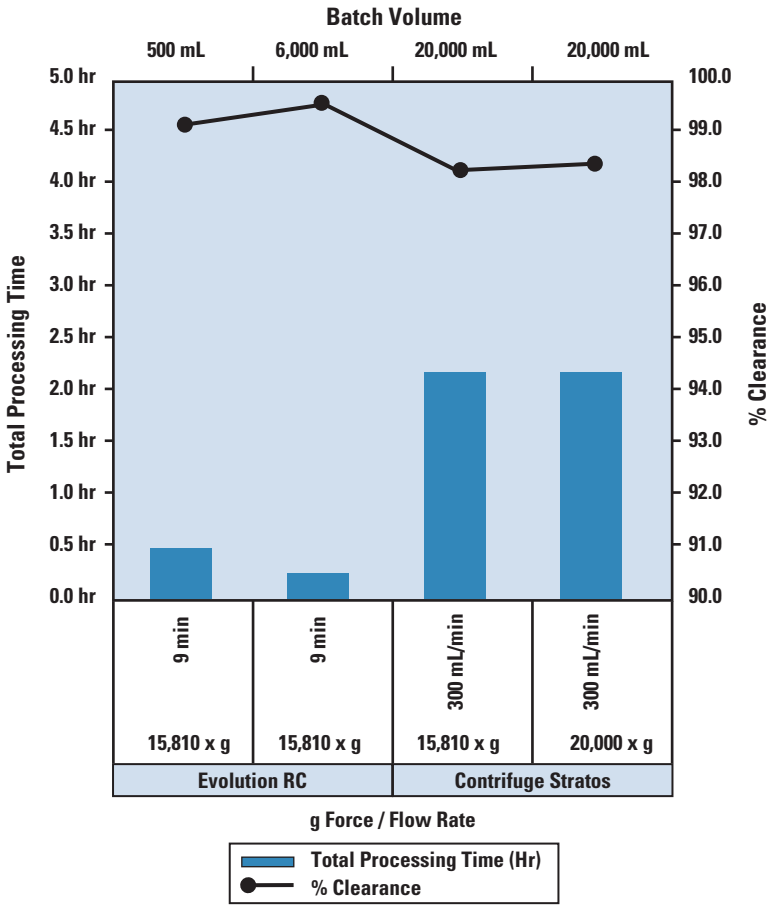

Figure 3: Separation performance versus run time
This plots data of clearance (%) against the total run time for each of the stages of scale-up

Material and Methods

Cell culture

25% *Saccharomyces Cerevisiae* baker's yeast in tap water (w/v) is used. This cell line is very robust and some metabolic activity is noted, bringing the wetted solids volume to 25%.

Methods

Performance was based on the percent clearance of the supernatant, which was determined by obtaining supernatant samples throughout the run and reading the absorbance on a spectrophotometer at a wavelength of 600 nm; water was used as a blank. Feed sample concentration was corrected for dilution. All runs were performed at a constant RCF of 15,810 x g, at a constant temperature of 4 °C. The number of cycles, total process time, and percent clearance of the supernatant was determined in each case. The volumes to replicate the stages of scale-up were 500 mL, 6 L and 20 L. The batch volumes of 500 mL and 6 L were centrifuged in the Sorvall Evolution RC for 9 mins and the 20 L batch was processed using the Contifuge Stratos continuous flow centrifuge at a flow rate of 300 mL/min. (See Table 1).

To minimize variability, a constant gravitational force and temperature of the scale-up process were consistent with both centrifuges. Once acceptable separation performance was achieved with all centrifuge conditions, the Contifuge Stratos was then spun at 20,000 x g to determine if a higher gravitational force would achieve better separation performance. The final goal was to scale up from 500 mL to 20 L while maintaining high separation performance and throughput.

Results

Runs performed on all centrifuge conditions maintained a separation performance in excess of 98% (See Figure 3) although the high percent of solids in the sample required more spin cycles on the continuous flow system. While more spin/scrape cycles were required with the continuous flow system, all runs were achievable at all batch sizes in a 2.5 hour time frame. This allowed adequate time for cleaning and further processing of the supernatant after the centrifugation step.

The Contifuge Stratos successfully processed a volume of 20 L at a flow rate of 300 mL/min in less than 2.5 hrs and an increase of the g force from 15,810 x g to 20,000 x g did not greatly improve the percent clearance of the supernatant (Figure 3). Therefore it was determined that the most efficient g-force to process the large sample volume using the Contifuge Stratos was 15,810 x g and adjustments in flow rates helped to determine the best clarity of the supernatant.

Conclusion

Yeast samples have demonstrated their usefulness as model systems for basic research.

The use of the Sorvall Evolution RC superspeed and Contifuge Stratos continuous flow centrifuges as scalable solutions for yeast processing has proved to be successful in processing up to 20 L of sample in less than 2.5 hour processing time while maintaining a more than 98% separation performance.

The Contifuge Stratos continuous flow system did efficiently process 20L at a flow rate of 300 mL/min, at 15,810 x g in less than 2.5 hrs. This allowed for a very rapid and reliable separation of the large volume of suspension with low solid matter content. With the Contifuge Stratos, a high speed titanium continuous flow rotor (cat. number: 75003049) (see Figure 2) was used to process the large volume of yeast, hence allowing for batch-to-batch coherence and less deviation in the process.

Applications such as harvesting of bacterial cultures[5] and human or animal cells can also be performed using the Contifuge Stratos continuous flow centrifuge and time savings of up to 80% can be possible when compared with batch centrifugation.

References

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Figure 4: Thermo Scientific Fiberlite F21 8x50y carbon fiber rotor

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