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Separation of *E. coli* Fermentation Broths Using the CEPA LE and the Eppendorf® Centrifuge 5920 R Laboratory Systems

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Abstract

Continuous or conventional centrifuges are among the most used equipment for cell separation. In this typical bioprocess laboratory application, the performances of both a CEPA LE and an Eppendorf Centrifuge 5920 R were evaluated by their capacity to clarify *E. coli* fermentation broths, produced using BioFlo® benchtop fermentors in batch and fed-batch modes. Both centrifuges could successfully process around 4 L of two distinct batch fermentation broths in under 60 minutes, and the final *E. coli* weight collected was very similar – around 190 g. The Eppendorf Centrifuge 5920 R also effectively processed a high density fed-batch broth in the same timeframe and it collected 1,292.3 g of *E. coli* wet weight. The tests performed showed that both the Centrifuge 5920 R and the CEPA LE can be used to effectively process fermentation broths produced in benchtop fermentors.

Introduction

Cell separation or harvesting is the first step towards downstream bioprocess and is required for the recovery of either extra- or intracellular products. A few different equipments can be found in industrial practices, and centrifuges are among the most commonly used in fermentation applications. Conventional centrifuges are a widely chosen option. In fact, being commonplace in laboratories, the relative ease of use and other advantages such as temperature control and variable speed make them a great choice, mainly for small-scale processes. The volume of the bucket is usually a limitation for this type of centrifuge [1].
For larger scales, continuous centrifuges have been the instrument of choice. The tubular-cylinder continuous centrifuges, in particular, are separation instruments characterized by their ability to process many times the capacity of their cylinder total volume without interruption. This characteristic results from a design that allows continuous feeding of a solid-liquid mixture while simultaneously expelling the liquid component. In a typical bioprocess application, the solid, which is retained in the cylinder, is the cell mass. Clarified liquid is obtained from an exit port while the machine is running. Cell mass is taken from the tubular cylinder after the machine is stopped. A removable plastic cylinder liner is often used to simplify cell paste removal.
Ever since introducing its very first centrifuge in 1964, Eppendorf has been synonymous with innovative design, technology and performance, that stand the test of time.

Nowadays, Eppendorf offers a comprehensive line of centrifuges that serve the multiple applications you encounter in a lab. This widely renowned line of centrifuges is increasing with the addition of a new member – the Centrifuge 5920 R, which features extraordinary high capacity and performance. Its high capacity of up to 4 x 1 L makes it the ideal instrument for centrifugation of bench-scale bioreactor cell culture or clarification of fermentation broth for benchtop fermentors. As for continuous centrifugation, Eppendorf offers multiple options through the CEPA product line in different countries, with varying cylinder capacities, for applications from benchtop to pilot and production scale (shown in Table 1).

Eppendorf Centrifuge 5920 R

> Benchtop laboratory centrifuge
> High capacity up to 4 x 1 L Nalgene® bottles*
> The ideal instrument for high throughput applications such as clarification of fermentation broth for benchtop bioprocess applications
> High centrifugation speed up to 25,062 x *g* (13,700 rpm)

Figure 1: The new Eppendorf Centrifuge 5920 R

CEPA LE

> Continuous benchtop laboratory centrifuge
> Variable speed control
> Wide array of optional bowls for research, scale-up, and small-volume production
> Maximum throughput: 30 liters/hour. Typically used with 2 to 15 L cultures

More economical than filtration:
> No costly membranes or other disposables
> Faster
> No product waste
> Consistent performance (no clogging, membrane aging or lot variations)

Figure 2: CEPA Model LE

* Order no.: 3120-1010, 3122-1000, 3122-1010 (Thermo Fisher Scientific)

Table 1: CEPA centrifuges models and specifications; ** Enclosed model

Specifications							
Model	**Cylinder Speed (rpm)**	**G-Force**	**Cylinder Capacity (Nominal Liters)**	**Running Load (Watts)**	**Dimensions (Overall) Width**	**Depth**	**Height**
LE	15	40	0.25 L	330	16.8" (42.6 cm)	16.8" (42.6 cm)	26.9" (68.3 cm)
GLE**	40	40	0.25 L	330	15.7" (40.0 cm)	20.3" (72.0 cm)	28.5" (72.5 cm)
Z41	20	17	2.0 L	900	16.1" (41.0 cm)	28.3" (51.5 cm)	46.0" (117.0 cm)
Z41G**	20	17	2.0 L	900	20.8" (53.0 cm)	33.7" (85.5 cm)	50.0" (127.0 cm)
Z61	17	17	6.0 L	1.500	24.2" (61.5 cm)	37.0" (94.0 cm)	61.0" (155.0 cm)
Z61G**	17	17	6.0 L	1.500	24.4" (62.0 cm)	37.4" (95.0 cm)	68.9" (175.0 cm)
Z81	16	18	8.0 L	2.200	19.7" (50.0 cm)	37.4" (95.0 cm)	61.0" (155.0 cm)
Z81G**	16	18	8.0 L	2.200	24.4" (62.0 cm)	37.4" (95.0 cm)	68.9" (175.0 cm)
Z101	14	15.5	10.0 L	2.200	19.7" (50.0 cm)	37.4" (95.0 cm)	63.0" (160.0 cm)
Z101**	14	15.5	10.0 L	2.200	24.4" (62.0 cm)	37.4" (95.0 cm)	68.9" (175.0 cm)

In this application, three *E. coli* fermentation broths were produced. One of them was produced using the BioFlo 3000, a previous generation BioFlo fermentor. The two others were produced using the BioFlo 320 bioprocess control station. The newest offering in the Eppendorf bioprocess portfolio, the BioFlo 320, was developed for both microbial fermentation and cell culture applications and seamlessly combines form and function in one state-of-the-art all-inclusive package.

BioFlo 320 – A premium choice in bench-scale bioprocess stations

> For both microbial fermentations and cell culture applications
> New industrial design
> Flexibility between autoclavable and single-use vessels
> Intelligent sensors
> Ethernet connectivity
> Enhanced software capabilities

Figure 3: BioFlo 320 bioprocess control station with water-jacketed (left) and stainless steel dish-bottom (right) vessels

Materials and Methods

Fermentation

Three *E. coli* K12 (ATCC)® fermentations were carried out in BioFlo benchtop fermenters for the purpose of evaluating both the CEPA LE High-speed Centrifuge and the Eppendorf Centrifuge 5920 R in a typical separation. Table 2 shows general information about each of the experiments. More details on procedures for fermentation can be found in Eppendorf application notes.

Table 2: Experiments conditions and results

Fermentation Conditions	A	B	C
Centrifuge	**CEPA LE**	**Eppendorf 5920 R**	**Eppendorf 5920 R**
Cultivation mode	Batch	Batch	Fed-batch
Initial working volume (L)	5.0	3.6	3.0
Final working volume (L)	5.0	3.8	3.8
Final cell concentration (OD_{600})	N/A	12.84	157.7
Culture wet weight before centrifugation (g/L)	45.7	52.0	340.4

Setup and Operation

When Fermentation A was completed, a peristaltic pump was used to transfer the broth from the fermentor to the centrifuge. A length of silicone flexible tubing was attached to a dip tube in the fermentor vessel, fed through the pump head, and connected to the centrifuge inlet nozzle. A second length of tubing was run from the centrifuge supernatant outlet port into a collection vessel.
The fermentor was set to maintain temperature at 19 °C. After starting the centrifuge and waiting for it to attain full speed, broth was pumped to the CEPA LE at a rate of 190 mL/ min (11.4 L/h). This value was arbitrarily selected and is near the low end of the systems range (the CEPA LE has throughput capability up to 30 L/h). The fermentor agitation was set to a low speed during the transfer to prevent settling and to help maintain temperature uniformity.

The centrifuge was configured with a clarifying cylinder, and a 2 mm inlet nozzle, and it was operating at full speed (40,000 rpm). The centrifuge and pump operation continued until the liquid in the fermentor fell below the dip tube level. Six 10 mL samples of supernatant were taken at 4 minute intervals during the separation process, and the optical density at 600 nm (OD_{600}) was measured off-line.
The 10 mL samples were spun down in a laboratory centrifuge for 10 minutes at 2,500 rpm to get a visual measure of residual cell mass.

The broths produced at Fermentations B and C were collected and distributed into 4 x 1 L Nalgene bottles with:

> 900 g (approx. 0.95 L) of a 52 g/L culture per bottle (Fermentation B)
> 950 g (approx. 0.95 L) of a 340.4 g/L culture per bottle (Fermentation C)

For both experiments, the Eppendorf Centrifuge 5920 R was tested at 3,700 rpm at room temperature (22 °C) and different centrifugation times. The broth from Fermentation B was centrifuged 10 minutes four consecutive times, while the one from Fermentation C was centrifuged 20 minutes three consecutive times. After each centrifugation, the supernatants OD_{600} and the remaining wet weights were analyzed.

Results and Discussion

A total volume of 4.2 L from Fermentation A was processed through the centrifuge in 22 minutes yielding 193 grams of wet cellular paste in the CEPA cylinder. The 250 mL cylinder was approximately 75 % full of paste at the point the processing was complete.
Predictably, the supernatant OD_{600} increased as the separation progressed, but even the last sample showed less than 0.1 % wet cell volume. Visually, this was a barely perceptible amount of cells in the supernatant, which could have been reduced further, either by feeding more slowly, or by exchanging the partially filled rotor for an empty one during the harvesting process.
In addition to separation efficiency, it was noted that the time required to carry out procedures was very short, and handling the system during operation was very easy. The separation itself took approximately 22 minutes.
We determined the LE model to be easy to use, as depicted from the short times for setup and clean up. The ease of handling is partly due to its small size, and partly because of its accessible design.
For the tests performed with the Eppendorf Centrifuge 5920 R, different centrifugation times were used while maintaining speed. The results are shown in Tables 3 and 4 for Fermentations B (batch) and C (fed-batch), respectively.

Table 3: Centrifugation results obtained with the Eppendorf 5920 R at different spin times with the broth from Fermentation B.

Time (min.)	Supernatant OD_{600}	Remaining Wet weight (g/L)	% completion based on supernatant wet weight
10	4.37±0.11	32.5±2.1	37.5 %
20*	1.79±0.04	17.8±0.3	65.8 %
30*	0.97±0.03	16.0±6.0	69.2 %
40*	0.62±0.01	10.2±4.4	80.4 %

* Represents multiple 10 min. runs.

Table 4: Centrifugation results obtained with the Eppendorf 5920 R at different spin down times with the broth from Fermentation C.

Time (min.)	Supernatant OD_{600}	Remaining Wet weight (g/L)	% completion based on supernatant wet weight
20	21.44±0.75	62.7±7.6	81.6 %
40*	12.08±1.25	47.2±3.6	86.2 %
60*	11.01±0.18	46.7±9.1	86.3 %

* Represents multiple 20 min. runs.

The wet weights collected on CEPA LE collection cylinder from Fermentation A and with the Eppendorf Centrifuge 5920 R from Fermentation B were 193 g and 187.2 g, respectively. It shows that both centrifuges are able to effectively process around 4 L for a batch fermentation that typically produces at most 250 g of wet weight. The procedures to spin down the broths from Fermentations A and B took less than 60 minutes for both centrifuges. On the other hand, the wet weight of 1,292.3 g produced Fermentation C would require five CEPA LE centrifugation runs, while with the Centrifuge 5920 R, one run within one hour was enough. It must be noted that all tests performed with the Centrifuge 5920 R were in multiple intervals and, therefore, uninterrupted runs may produce even better results.
These tests showed that both the CEPA LE and the new Eppendorf Centrifuge 5920 R efficiently and conveniently harvested and clarified *E. coli* broths, making them highly effective instruments for fermentation applications.
Tests under various operating conditions could be used to develop a protocol that results in the optimum compromise between process time and supernatant clarity for a specific application. Acceptable residual cell mass depends on several factors, including whether the desired product is in the supernatant or the cells, as well as the post-centrifuge filtration and downstream purification processes, if any. Although not explored here, more complex protocols could be established to optimize the process for the user.

Conclusion

The tests performed in this work demonstrated the successful utilization of both the CEPA LE and the Eppendorf Centrifuge 5920 R to process fermentation broths produced in batch and fed-batch modes using a benchtop fermentor.

Literature

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[2] Li B, Siddiquee K and Sha M. The Eppendorf BioFlo® 320 Bioprocess Control Station: An Advanced System for High Density *Escherichia coli* Fermentation. Eppendorf Application Note No. 340, 2015.

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