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Continuous Separation of *E. coli* Fermentation Broth Using a CEPA® LE Laboratory Centrifuge System

Y. Chen, J. Gerber, G. Hart and J. Capone, Eppendorf Inc., Enfield, CT, U.S.A.

Abstract

In this bioprocess laboratory application, 4.2 liters of *E. coli* fermentation broth containing 6 % solids by volume were separated by CEPA LE in 22 minutes. The cellular paste that was collected amounted to 193 grams. Supernatant clarity was excellent, with all samples containing less than our visual detection limit of 0.1 % solids.

Introduction

The CEPA LE Model, a tubular-bowl continuous centrifuge, is one of a family of separation instruments characterized by their ability to process many times the capacity of their bowl 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. The solids, in this application, are the cell mass, and are retained in the bowl.

Clarified liquid is obtained from an exit port while the machine is running. Cell mass is taken from the tubular bowl after the machine is stopped. A removable plastic bowl liner is often used to simplify cell paste removal.

Figure 1: The CEPA LE is a benchtop laboratory centrifuge, featuring variable speed contol as standard and a wide array of optional bowls for research, scaleup experiments, and small volume production. The LE is typically used with 2 to 15 liter cultures. Maximum throughput is 30 liters/hour.

Materials and Methods

Fermentation

A five liter fermentation was carried out in a New Brunswick BioFlo® benchtop fermentor for the purpose of evaluating the CEPA LE High Speed Centrifuge in a typical *E. coli* separation. The fermentation broth was determined to contain approximately 6 % wet solids by volume by spinning down a small sample in a laboratory batch centrifuge operated at 2500 rpm for 10 minutes. Dry cell weight was 11.48 grams per liter.

Setup and Operation

When the fermentation was completed, a Watson-Marlow® 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's inlet nozzle. A second length of tubing was run from the centrifuge's 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/hr). This value was arbitrarily selected and is near the low end of the system's range — the CEPA LE has throughput capability up to 30 L/hr. 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 type clarifying cylinder, and a 2 mm inlet nozzle. It was operating at full speed (40,000 rpm) which produces a radial acceleration or G-force of 45,000. The centrifuge and pump operation continued until the liquid in the fermentor fell below the dip tube level.

Clarity Measurements

Six 10 mL samples of supernatant were taken at 4 minute intervals during the separation process, and the 600 nanometer optical density was measured off-line. The 10 mL samples were spun down in a laboratory centrifuge for 10 minutes at 2500 rpm to get a visual measure of residual cell mass.

Results and Discussion

A total volume of 4.2 L was processed through the centrifuge in 22 minutes yielding 193 grams of wet cellular paste in the CEPA bowl. The 250 mL bowl was approximately 75 % full of paste at the point the processing was complete.

Sample	OD	Visual
1	0.162	< 0.1%
2	0.203	< 0.1%
3	0.226	< 0.1%
4	0.249	< 0.1%
5	0.300	< 0.1%
6	0.392	< 0.1%

Table 1: Supernatant clarity as indicated by optical density (600 nm) and visual observation of sediment samples taken at four minute intervals.

In addition to separation efficiency, we noted that the time required to carry out the procedures was very short, and handling the system during operation was obvious.

The separation itself took approximately 22 minutes. The entire process from setup through cleaning took less than an hour.

CEPA LE Processing Time in Minutes	
Setup	5 min
Accelerate	2 min
Process 4.2 L	22 min
Shut down and allocate paste	15 min
Clean and reassemble	10 min

Table 2: Time taken for different processing steps for the CEPA® LE in 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.

Predictably, the supernatant OD 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.

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. Certainly, this particular process could have been run more quickly or more slowly with a change in clarity. Although not explored here, more complex protocols could be established to optimize the process for the user. One example would be to discharge a high feed rate initially, and then decrease it as the bowl fills to take advantage of the initially higher separation efficiency to improve either speed or clarity with no penalty in the harvest process.

This test showed that the smallest CEPA centrifuge efficiently and conveniently harvested and clarified *E. coli* broth, making it a highly effective instrument for fermentation applications.

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