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Application Note

April 2019

Keywords or phrases:
Cell Expansion, Cell Passaging, Cell Culture

Effect of Shaker Speed on Media Foaming and Culture Growth Rates in Erlenmeyer Shake Flasks during Cell Expansion

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Abstract

The Mycap® CCX Cell Expansion System makes passaging outside a biosafety cabinet possible during cell expansion. Mycap® CCX allows passages outside a biosafety cabinet through innovative design; the cap combines tubing for aseptic fluid transfer and a filter cartridge for gas exchange in the cap, pre-assembled on plastic Erlenmeyer shake flasks. Thus, Mycap® CCX has the potential: (1) to reduce contamination risks (2) lessen or eliminate reliance on a biosafety cabinet and (3) lower costs by improving process and facility operations.

Sartorius scientists performed an experiment to study the effect of Mycap® CCX's unique cap design on critical parameters of cell expansion: cell growth, cell density, and cell viability. Additionally, foaming of the culture was measured. Experimental variables included two shaking speeds at 100 RPM and 120 RPM during cell growth for all but the 3 L flasks which were shaken at 75 RPM and 120 RPM.

We compared cell growth rates and foam scores in flasks with Mycap® CCX caps and traditional caps. Based on our conclusions, we recommend shaking speeds of less than or equal to 100 RPM during cell expansion processes using Mycap® CCX. Growth rates with Mycap® CCX were statistically equivalent with those in traditional flasks, suggesting that a cell-line specific validation study is worthwhile.

Find out more: www.sartorius.com/mycap-ccx

Introduction

Cell Culture Expansion from vial to bioreactor is normally done by passaging the cell culture through successively larger Erlenmeyer shake flasks. During each passage fresh media is added and the cells are transferred to the next flask. The caps of traditional Erlenmeyer flasks are opened at each passage to allow for fluid transfer.

Opening a culture flask is generally understood and widely accepted to present a risk for contamination. Contamination is the leading cause of batch failures, and poor aseptic technique during passaging is a primary concern for contamination in facility operations (Langer, 2008).

Mycap® CCX allows fluid transfer without having to remove the cap because tubing is integrated in the closure. Weldable tubing or aseptic connectors enable aseptic transfer of media and culture between flasks. Aseptic disconnections are made quick and easy with Quickseal® aseptic disconnect. In addition, the Mycap® CCX closure includes a novel gas exchange cartridge, which is specially designed to support passive exchange of gases during cell growth in the incubator.

Thus, use of Mycap® CCX flasks eliminates a significant risk of contamination and batch failure because the closure need not be opened, removed, or changed during passaging. Expansion processes using Mycap® CCX are done outside a biosafety cabinet (BSC), making a significant positive impact on process efficiency, facility utilization, and operation costs.

However, Mycap® CCX does introduce changes to an expansion process. For instance, the cap's integrated tubes extend to the bottom of the flask and are immersed in the culture media. Some constituents in media, such as serum, are known to foam when the culture is agitated. Concerns emerged that the immersed tubes could generate foam or adversely affect cell growth and viability.

Therefore, Sartorius performed an experiment comparing growth rates and culture conditions between traditional flasks and Mycap® CCX flasks. Our intent is to describe similarities and differences between the two systems at different shaking speeds and advise best practices for cell expansion with Mycap® CCX.

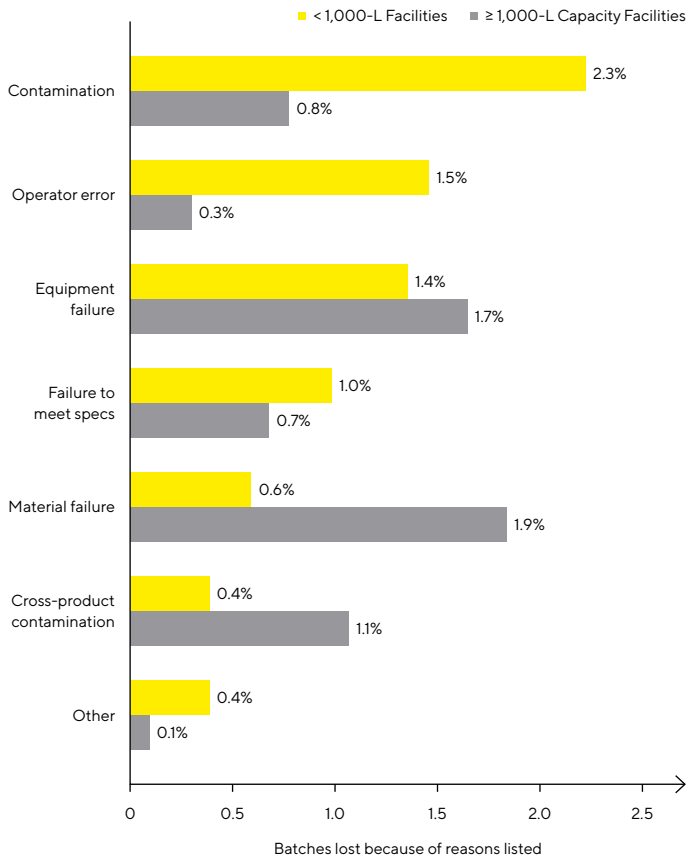
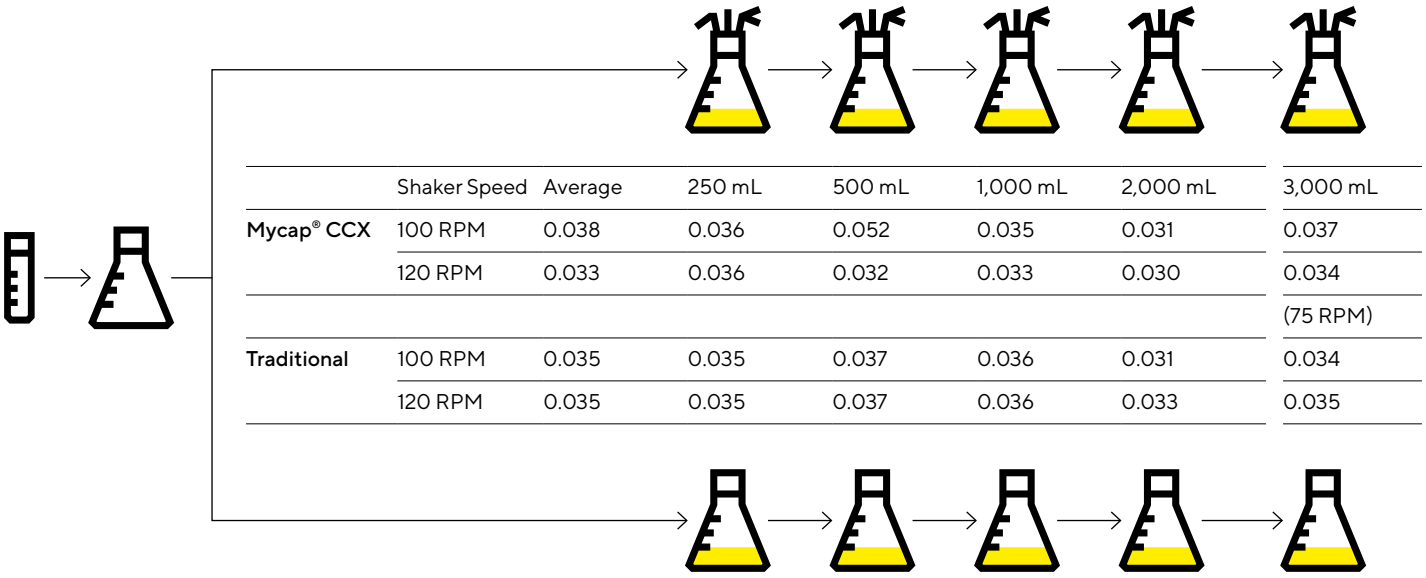


Figure 1: Fifth Annual Report and Survey of Biopharmaceutical Manufacturing Capacity and Production. Langer E, Ed. BioPlan Associates: Rockville, MD, 2008.

Aseptic fluid transfer to growth in the incubator

Study Overview



	Shaker Speed	Average	250 mL	500 mL	1,000 mL	2,000 mL	3,000 mL
Mycap® CCX	100 RPM	0.038	0.036	0.052	0.035	0.031	0.037
	120 RPM	0.033	0.036	0.032	0.033	0.030	0.034
							(75 RPM)
Traditional	100 RPM	0.035	0.035	0.037	0.036	0.031	0.034
	120 RPM	0.035	0.035	0.037	0.036	0.033	0.035

Figure 2: Illustration of experimental design and growth rate per hour. Experiment done in triplicate. Drawings not to scale.

A vial of cells from Sartorius's CHO DG44 cell line was thawed in a 250 mL flask. The culture was then split into two trains; one train expanded from 250 mL to 3,000 mL in Mycap® CCX and the other in traditional flasks.

We used chemically defined, animal origin free media in both trains. Incubator conditions were set at 36.8° C and 7.5% CO_2 concentration.

We ran each train in triplicate at two shaking speeds: 100 RPM and 120 RPM. The 3,000 mL flasks were shaken at 75 RPM and 120 RPM.

Samples were collected at the beginning and end of each growth phase, and analyzed for cell density and viability using Cedex HiRes Analyzer (Roche Diagnostics). Samples were collected from traditional flasks in the biosafety cabinet using a serological pipette. Samples were collected from Mycap® CCX outside the biosafety cabinet via the luer-activated access site. The access site was sanitized with 70 % IPA and allowed to dry. A syringe with male-luer lock fitting was attached to the access site for sample collection. Residual liquid in the sampling line was purged into a separate syringe and discarded prior to sample collection. Purge volumes were 1 mL on 250 mL and 500 mL flasks and 2 mL on 1 L, 2 L and 3 L flasks. Viable cell density (VCD) and growth rate per hour was calculated for each passage using the following formula:

Growth Rate = $\ln(VCD_N / VCD_0)$ / hours

We observed the culture at the end of each passage and assigned a foam score according to Sartorius's foam score chart (Table 1).

Foam Score	Conditions
0	No foam
1	Single layer of foam, 25% coverage
2	Single layer of foam, 50% coverage
3	⅛" multi layer of foam, 50% coverage
4	⅛" multi layer of foam, 75% coverage
5	Full surface coverage of multi layer foam

Table 1: Sartorius foam score chart

Growth Rate, Foaming & Shaking Speed

Figure 3 shows bar graphs comparing the average growth rates across the expansion process under the two shaker speeds.

The first two pairs of bars show average growth rate across all passages in Mycap® CCX flasks (gold) compared to traditional flasks (grey) at 100 RPM and 120 RPM, respectively. The middle two pairs of bars are alternate pair-wise comparisons of average growth rate across all passages in Mycap® CCX flasks at 100 RPM vs. traditional flasks at 120 RPM and Mycap® CCX flasks at 120 RPM vs. traditional flasks at 100 RPM.

The last two pairs of bars show comparison of average growth rate at both 120 RPM and 100 RPM in Mycap® CCX vs. traditional flasks. The error bars represent standard error (SE) for each data set.

Using the Student's T-test, we found no statistically significant differences in the average growth rate across all passages for the paired data, with the exception of Mycap® CCX at 100 RPM vs. Mycap® CCX 120 RPM ($p = 0.046$).

Notably, growth rate in Mycap® CCX at 100 RPM was the highest of all test replicates. Our analysis suggests that when using Mycap® CCX flasks during cell culture expansion, a shaker speed of 100 RPM (75 RPM for 3 L Flask) is preferred because growth is both equivalent to that in traditional flasks and may be slightly improved compared to Mycap® CCX at 120 RPM.

Figure 4 shows average foam score across triplicates for all experimental conditions in flasks with Mycap® CCX caps shaken at 100 RPM (grey bars) and 120 RPM (gold bars). We observed foam in all Mycap® CCX capped flasks when shaken at 120 RPM but only in the 2,000 mL Mycap® CCX capped flask when shaken at 100 RPM.

Figure 5 shows average foam score in flasks with traditional caps. We observed no quantifiable foam in traditional flasks, except for cultures grown in 3,000 mL flasks shaken at 120 RPM.

We conclude that foam does not adversely affect cell growth because average cell growth rate in Mycap® CCX capped flasks is equivalent to that in traditional flasks, regardless of average foam score. We do acknowledge that many prefer minimal to no foam in their cell expansion process, and this can be achieved using Mycap® CCX at 100 RPM with high growth rates and minimal foam. We therefore recommend shaking speeds of less than or equal to 100 RPM during a cell expansion process using Mycap® CCX.

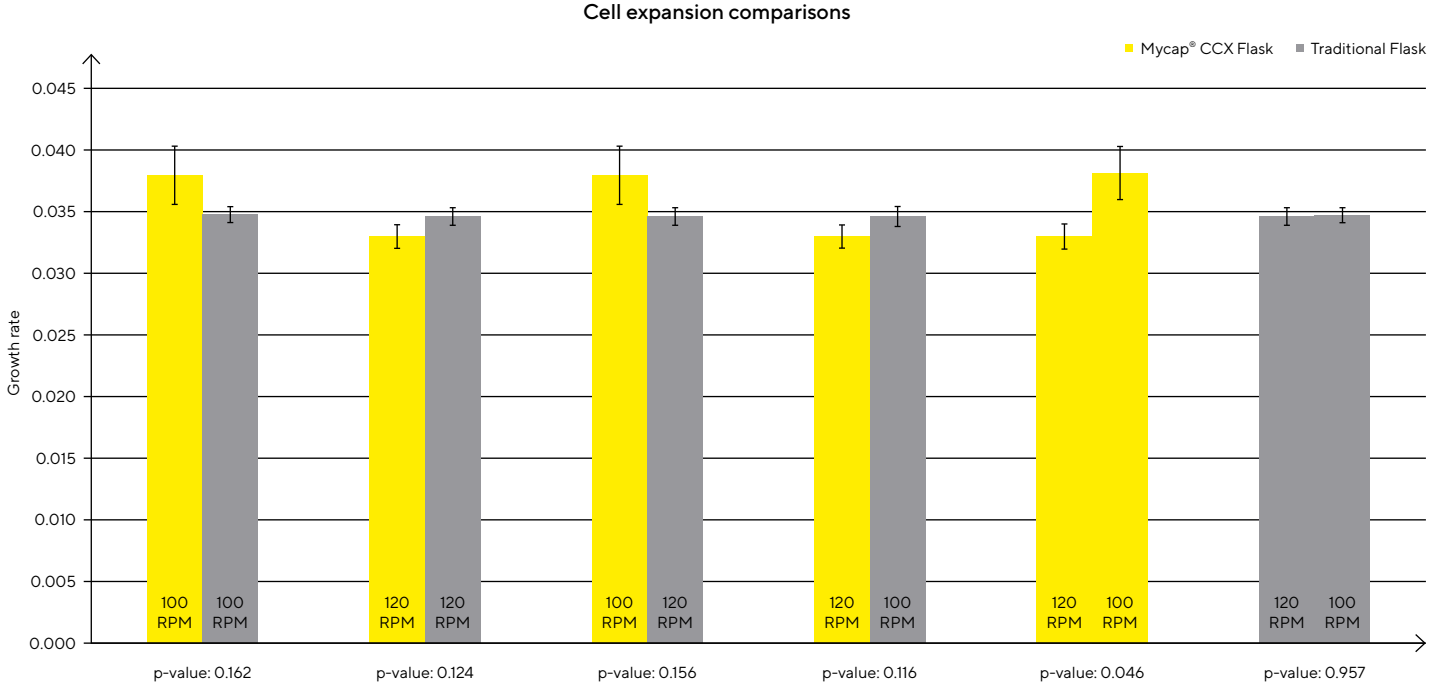


Figure 3: Paired growth rate graph

Discussion and Conclusions

The aims of continuous improvement in biopharmaceutical operations include increased yields, reduced costs, or elimination of contamination. Continuous improvement almost always requires some change in equipment, processes, or materials. According to Good Manufacturing Practices (GMP), changes are best managed through a change control process, which advances through three stages: change rationale, impact assessment, and change implementation.

Mycap® CCX represents an advance in cell expansion processes. Mycap® CCX eliminates the need to remove caps from flasks during passaging and expansion, thus significantly decreasing the risk of contamination and subsequent batch failure. Passaging with Mycap® CCX is done outside the biosafety cabinet allowing for reduction in cost by avoiding hood and materials preparation time, hood maintenance and environmental monitoring. Working outside a BSC is generally accepted as more ergonomic and preferred by operators. Transferring media and cells by peristaltic pump instead of by serological pipette speeds passaging time, particularly on larger flasks.

Cell expansion is measured by viable cell density and cell growth rates. In a comparative study of growth rates between Mycap® CCX and traditional flasks, we found no statistically significant difference between the two systems and conclude that Mycap® CCX has no impact on the growth rates of cell expansion process. We also observed for media foaming during flask shaking in the expansion process.

We noted media foaming with shaker speed of 120 RPM, particularly in Mycap® CCX flasks. However, cell growth rates were not statistically different when compared to traditional flasks at 120 RPM or 100 RPM|75 RPM shaking speeds. We observed our highest cell growth rates in Mycap® CCX at 100 RPM|75 RPM with minimal foaming. Therefore, Sartorius recommends shaking speeds not exceed 100 RPM (75 RPM for 3 L flasks) for cell expansion with Mycap® CCX.

Our study therefore suggests that Mycap® CCX is an exceptional alternative to traditional Erlenmeyer flasks for cell expansion.

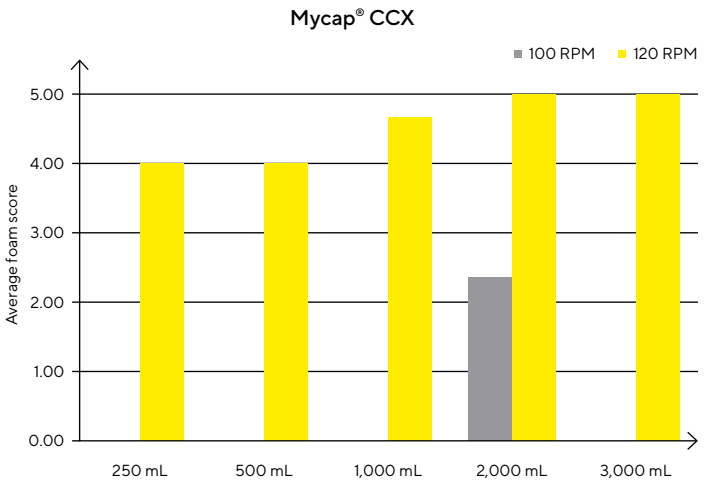


Figure 4: Average foam score – Mycap® CCX

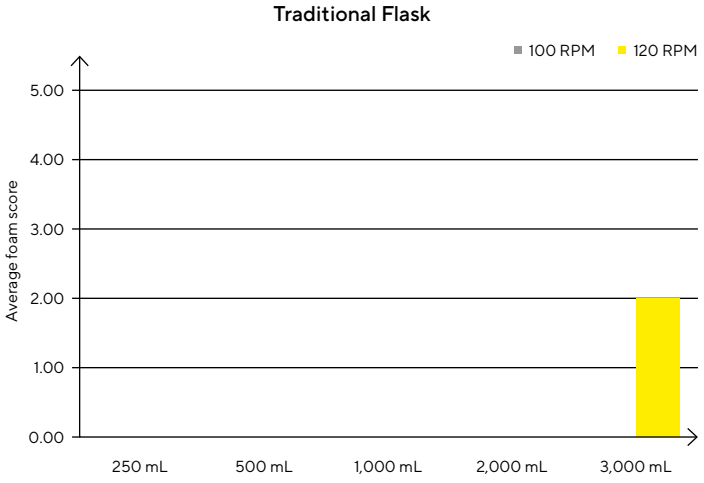


Figure 5: Average foam score – Traditional Flask

References

- Langer, E. (2008). Fifth Annual Report and Survey of Biopharmaceutical Manufacturing Capacity and Production. Rockville, MD: BioPlan Associates.

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Version 06 | 2020