SARTORIUS

Application Note

March 09, 2020

Keywords or phrases:
Keywords or phrases: 4Cell® XtraCHO Media System, FlexSafe® bags, cell growth, shelf life

Consistent Performance of 4Cell® XtraCHO Media System in Flexsafe® Bags

Adrien Lugari
Sartorius Stedim FMT (SAS), 510 avenue de Jouques, 13400 Aubagne

* Correspondence
E-Mail: Info_CellCultureMedia@sartorius.com

Abstract

Selecting optimal growth media and reproducible process parameters is important for the generation of high-yield, high-quality biotherapeutics.

Current industry standard to produce these drugs is to use mammalian cells such as clonal CHO suspension cell lines and to support their culture so as to produce high cell densities, protein titers and critical quality attributes in the final product.

In this context, achieving consistent cell growth is critically important to assure both reproducible quality and the desired characteristics of the final product.

To support these efforts, we examined the maintenance of high cell growth with the 4Cell® XtraCHO Media System in single-use FlexSafe® bags. We carried out time-course studies for up to 36 months with single-use FlexSafe® bags and confirmed equally high and consistent cell growth results.

Introduction

The uptake of single-use bags in upstream processing from R&D to cGMP clinical and commercial production requires superior and consistent cell growth performance. Multiple factors of single-use bags, including the resin and additive formulations, the extrusion parameters and gamma sterilization, can affect the release of leachables into the cell culture medium that can inhibit cell growth and performance. Reproducible cell growth can only be obtained with complete control of the entire process, from resins and film extrusion to the final bag assembly.

Achieving consistent cell growth is critically important to the biotech industry. To optimize the final drug product, current industry trends point toward higher cell densities and protein titers while maintaining reproducible outcomes.

With the increasing use of single-use bags in cell culture processes, some manufacturers have reported inconsistent cell growth. Hammond & al.1 identified a degradation product, the bis (2,4-di-tert-butylphenyl) phosphate (bDtBPP), derived from a commonly used antioxidant in polyethylene films. However, such antioxidants are necessary to keep films stable. They protect the polymer from oxidation during film extrusion and during gamma irradiation. Therefore, it is extremely important to optimize and closely control the concentration of these additives.

Sartorius has successfully addressed this challenge by showing consistent cell growth performance with the 4Cell® XtraCHO Media System in single-use bags of our Flexsafe® platform.

The excellent cell growth performance is the result of optimized antioxidant packages. For the Flexsafe® bags, we minimized these and carefully controlled their concentration. Comparable performance to glass containers is demonstrated here by cell growth and shelf life studies, as well as lot-to-lot consistency.

The excellent cell growth performance in Flexsafe® bags is the result of optimized antioxidant packages. We have minimized and carefully control their concentration.

Materials and methods

Standardized media extraction and CHO cell growth assays in 4Cell® XtraCHO Media System have been developed internally and applied to Flexsafe® bags to demonstrate reproducible cell growth performances across their entire lifecycle. The cell growth test involves a media incubation in 0.8 L irradiated bags (~50 kGy).

Bags are filled with 200 mL of 4Cell® XtraCHO Media System and incubated at 37°C. The control medium is incubated in a glass bottle to serve as a reference.

The extraction media are transferred to six-well-plates for cell growth tests in triplicate using a recombinant CHO DG44 production cell line (Cellca) at 2 × 105 cells/mL. A CERTOMAT® .CTplus shaking incubator was used for agitation at 160 rpm @ 37°C. Cultivation was performed with 10 mL/well for three days. VCD (Viable Cell Density) and CV (Cell Viability) cell count was performed with a Nucleocounter (Chemometec).

We developed a targeted extractable method to detect trace amounts of the bDtBPP compound in films. Extraction was performed in water and ethanol, and we analyzed extracts by the most sensitive HPLC-UV methodology. The analytical methods for detecting bDtBPP with HPLC-UV uses the bDtBPP standard developed by Amgen. Extractable data for Flexsafe® to demonstrate the absence of bDtBPP were performed in water and ethanol in worst-case extraction scenarios.

Design of Experiments (DoE) design space and lot-to-lot consistency of Flexsafe® extrusion and bag production are shown in Figures 1 and 2.

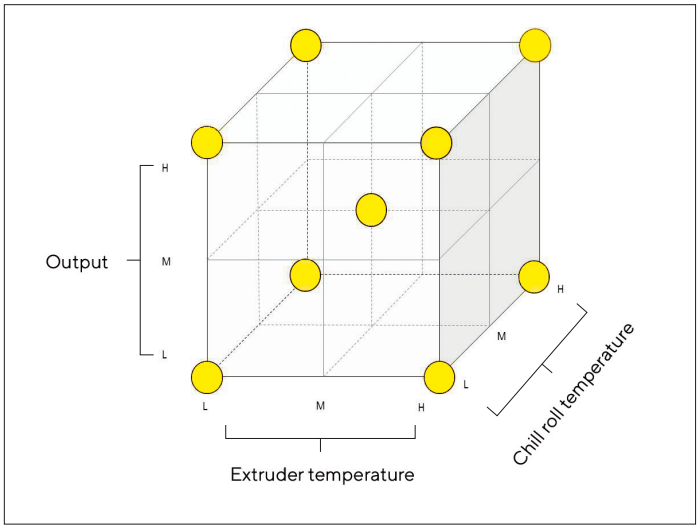

Figure 1: DoE extrusion design space for production of Flexsafe® bags

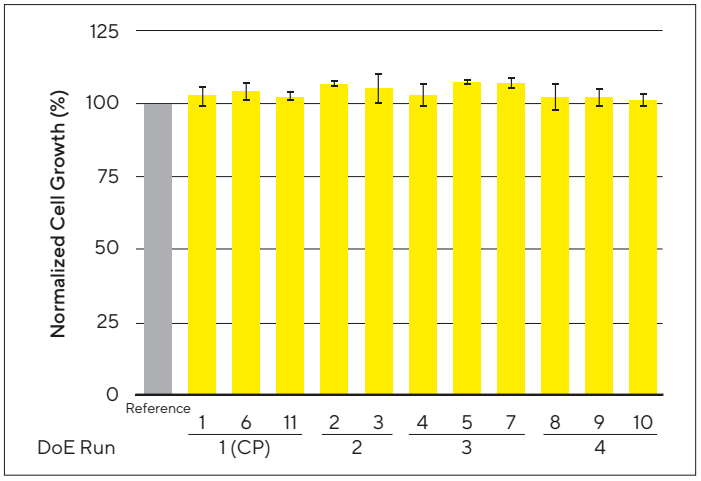

Figure 2: Excellent and reproducible cell growth in different Flexsafe® production lots in the production design space

Results and discussion

Cell growth performance of 4Cell® XtraCHO Media System in Flexsafe® during nine months of media storage

A prolonged media extraction trial was conducted to evaluate potential migration of toxic leachables from Flexsafe® bags into cell culture media during normal media storage conditions.

We conducted the test according to the standardized cell growth method described above at each due date at 4 – 6°C for up to nine months of storage.

Cell growth performance was comparable between the reference (glass bottle) and the results obtained in bags at each due date of media storage. The results also show that cell growth performance was consistent for media storage from time = 0 up to nine months.

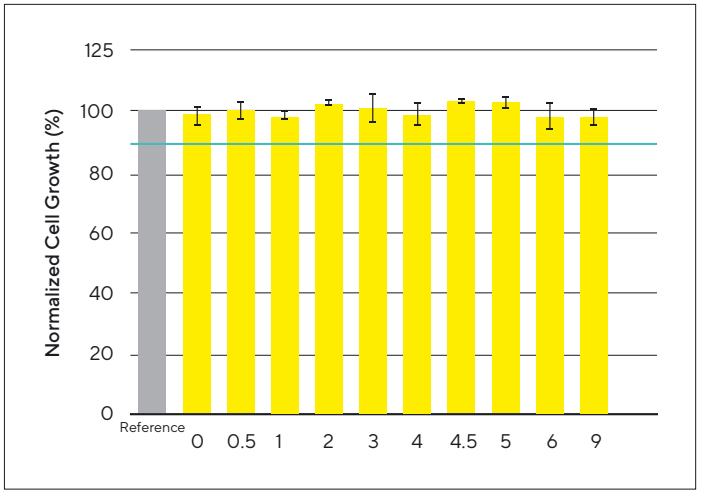

Figure 3: Cell growth testing of 4Cell® XtraCHO Media System in Flexsafe® single-use bags against glass bottle reference showed consistent levels of CHO cell growth with the media stored at 4-6°C up to the nine-month testing period

We examined the impact of polymer aging on the biological performance of the Flexsafe® film using an accelerated aging study.

For these experiments, Flexsafe® bags were stored up to an equivalent of 24 months according to ASTM F1980, 75 percent humidity before being filled with 4Cell® XtraCHO Media System. We conducted the tests according to the standardized cell growth method at each due date as described above.

A glass bottle container was used as a reference

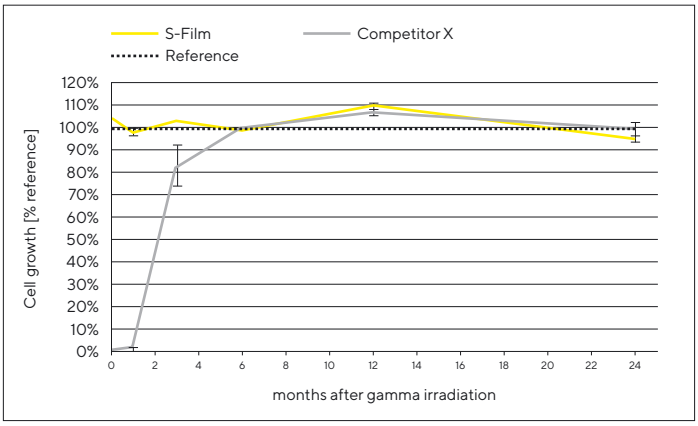

Figure 4: Cell growth testing of 4Cell® XtraCHO Media System in Flexsafe® single-use bags against glass bottle reference and bag competitor X

S-Film = Flexsafe® bag

The results showed that the age of bags can impact cell growth if the additive is not strictly controlled. We further conclude from Figure 4 that the cell growth performance in Flexsafe® bags is consistent from freshly irradiated bags up to 24 months of empty bag storage. Other readily available bags from different suppliers were also tested and demonstrated that cell growth performance can vary with storage time. These results also show that bags with no control of the additive packages can significantly impact cell growth and influence cell growth more so during their early stages of shelf life.

Reproducible cell growth performance of Flexsafe® bags established during qualification and routine production

Lot-to-lot reproducible cell growth in 4Cell® XtraCHO Media System in Flexsafe® bags was established during process qualification and product validation and was further controlled during bag production using continued internal cell growth tests. Typical results are shown in Figure 5.

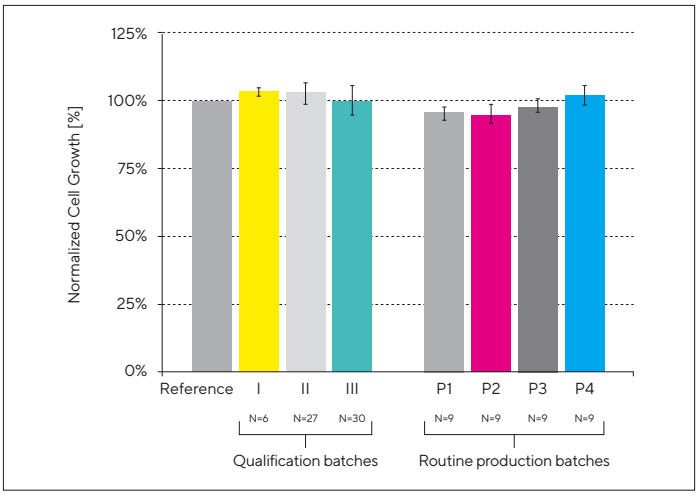

Figure 5: Reproducible cell growth in 4Cell® XtraCHO Media System compared against glass bottle reference in different batches of Flexsafe® bags

Conclusions

- Reproducible cell growth was ensured by the establishment of the design space and control of critical process parameters.
- Excellent cell growth performance and stability of 4Cell® XtraCHO Media System in FlexSafe® bags was confirmed.
- The results were obtained by optimizing the resin and additive package formulation in the FlexSafe® PE film contact layer, especially by limiting the concentration of antioxidant.
- Cell growth stability and consistency was demonstrated by testing CHO cell growth.
- Consistent performance is further ensured by routine cell growth testing during production of Flexsafe® bags as well as of the 4Cell® XtraCHO Media System.

References

[1] Matthew Hammond, Heather Nunn, Gary Rogers, et al., Identification of a Leachable Compound Detrimental to Cell Growth in Single-Use Bioprocess Containers, PDA J Pharm Sci and Tech 2013, 67 123-134.

Sales and Service Contacts

For further contacts, visit www.sartorius.com

Germany
Sartorius Stedim Biotech GmbH
August-Spindler-Strasse 11
37079 Goettingen
Phone +49 551 308 0

USA
Sartorius Stedim North America Inc.
565 Johnson Avenue
Bohemia, NY 11716
Toll-Free +1 800 368 7178

DIR: 2570828-000-00
Status: 04 | 2020