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BIOSTAT® CultiBag RM
Culturing Convenience

Application Note

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Comparison of single use bioreactor BIOSTAT® CultiBag RM and reusable STR for cultivation of CHO cells in serum free media

turning science into solutions

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1. Background

The BIOSTAT® CultiBag RM is the most advanced single use bioreactor using rocking motion. The pillow shaped cultivation chamber is rocked back and forth, creating waves which provide mixing with low shear. The liquid surface is constantly renewed, thereby enabling efficient mass transfer between head space and media. The cultivation chamber itself is a single use bag composed of a multilayer film with pharmaceutical grade low density polyethylene (LPDE) as the contact layer.

Single-use bags reduce validation costs, remove the need for cleaning, sterilizing, and provide stress free convenient culturing. A comprehensive validation guide and extractables report is offered for the bags. CultiBags with available cultivation volumes of 0.1 – 300 L are suitable for R&D, process development and small scale production.

The BIOSTAT® CultiBag RM features full process automation using optical probes for pH and DO measurement. The control system presents an easy-to use touch screen control system with integrated measurement and control hardware, pumps, temperature and gassing systems. Easy to use, it is applicable to all cell types, including mammalian cells, plant cells, insect cells and microbial cells.

Still today, CHO (Chinese Hamster Ovary) is the most widely used mammalian cell line for the production of recombinant proteins in commercial scale. Nearly 70 % of all recombinant protein therapeutics manufactured today are made in CHO cells, including blockbuster products such as Avastin™, Humira™, Heceptin™ and Enbrel™.

The original CHO cell line was established in 1957 by Theodore Puck in Colorado. It soon became evident that this cell line was well suited for in-vitro cultivation and had relatively fast generation times. The emergence of DFHR-CHO cell lines in the 1980's and the DHFR expression system for vector mediated gene transfer cleared the way for industrial scale production of recombinant proteins.

For commercial processes, nowadays serum free media (SFM) is routinely used. The use of serum in culture may pose several problems such as batch to batch variations, high protein content, the potential health risk due to the presence of contaminants and their transfer to the end product, high costs of fetal calf serum etc. Thus, the use of serum containing media is becoming less desirable in industrial large scale processes. Chemically defined media are based on the knowledge of required components and their respective concentrations. Supplements such as hormones, growth factors, carrier proteins and hydrolysates are added to the media according to the requirements of the specific cell lines. In addition to the advantages described above, the use of chemically defined media may also lead to enhanced growth characteristics compared to traditional media.

In this application note, we present a study of the cultivation of suspension adapted CHO-S and CHO-K1 cells in the single use bioreactor BIOSTAT® CultiBag RM compared to the BIOSTAT® B-DCU® with stirred glass vessels. Serum free chemically defined media was used for the propagation of cells. Growth characteristics and basic metabolic profiles were investigated.

2. Material

- Media: PowerCHO-2 CD (Lonza), + 0,1% Pluronic + 6mM L-glutamine
- Reusable Stirred Tank Bioreactor: Sartorius Stedim Biotech BIOSTAT® B-DCU Twin
- 10 L UniVessel® (Sartorius Stedim Biotech) with pitched 3-blade impellers and ring sparger
- Single-Use Bioreactor: Sartorius Stedim Biotech BIOSTAT® CultiBag RM 20 optical
- CultiBag RM 20L optical (Sartorius Stedim Biotech) single use bags. Maximum working volume 10 L.
- Laminar flow cabinet
- CO_2 Incubator, Hereaus
- Beckman Coulter CellView XR
- Glucose/Lactate Analyzer YSI 7100

3. Methods

Two different CHO sub clones, CHO-S and CHO-K1, were compared for their growth characteristics and metabolic profiles. The cells were cultured in repeated batch mode in the reusable bioreactor as well as in the single use CultiBag RM 20L. The CHO-S cultivations were run in a head-to-head comparison, while for the comparison of CHO-K1, two similar bioreactor runs were assessed.

The CHO-S seed culture was grown in a stirred tank bioreactor. 2000 ml of the seed were used to inoculate the BIOSTAT® B-DCU reusable stirred tank bioreactor as well as the BIOSTAT® CultiBag RM. The final volume inside both bioreactors was 10 L of Power-CHO 2 (0.1 % Pluronic, 6 mM L-glutamine) media. The initial cell density was ~1x10^6/mL.

The cultivation was started and the process parameters according to the following table were set.

CHO-S	B-DCU STR	CultiBag RM20
pH	7.0	7.0
DO	40 %	40 %
Temperature	36 °C	36 °C
Gasflow	0.3 L min^{-1} (via ring sparger)	0.2 L min^{-1}
Stirrer Speed	200 rpm	n.a.
Rocking Rate	n.a.	22 rpm
Angle	n.a.	5 °

After 72 h of cultivation, the culture was split 1:5 in the same cultivation vessel, i.e. a repeated batch process was carried out. To this end, 8 L of media containing the cell suspension were harvested and replaced with 8 L of fresh media. Samples were taken in regular intervals and the viable cell number was determined. Lactate and glucose levels were measured using the YSI 7100 analyzer.

The cultivation of CHO-K1 was carried out in a similar manner. The only difference was that the B-DCU STR and the CultiBag RM were inoculated from individual seed cultures to an initial cell density of 5 × 10^5/mL in the STR and 1 × 10^6/mL in the CultiBag RM, respectively. The temperature in the CultiBag RM was set to 37 °C.

4. Results

The viable cell densities of the CHO-S and CHO-K1 cells were determined in the BIOSTAT® B-DCU STR and the BIOSTAT® CultiBag RM (figure 1). The growth characteristics are very comparable, both cell lines reaching comparable levels in both types of bioreactor. The CHO-S showed a better growth, reaching higher cell densities than the CHO-K1, probably reflecting the better adoption to suspension culture.

Figure 2 shows the glucose and lactate profile of the CHO-S cultivation. In this instance, the glucose consumption of the cells grown in the B-DCU STR was higher, consequently, also the lactate build up was higher. This observation is congruent with the slightly higher viable cell density reached in the B-DCU.

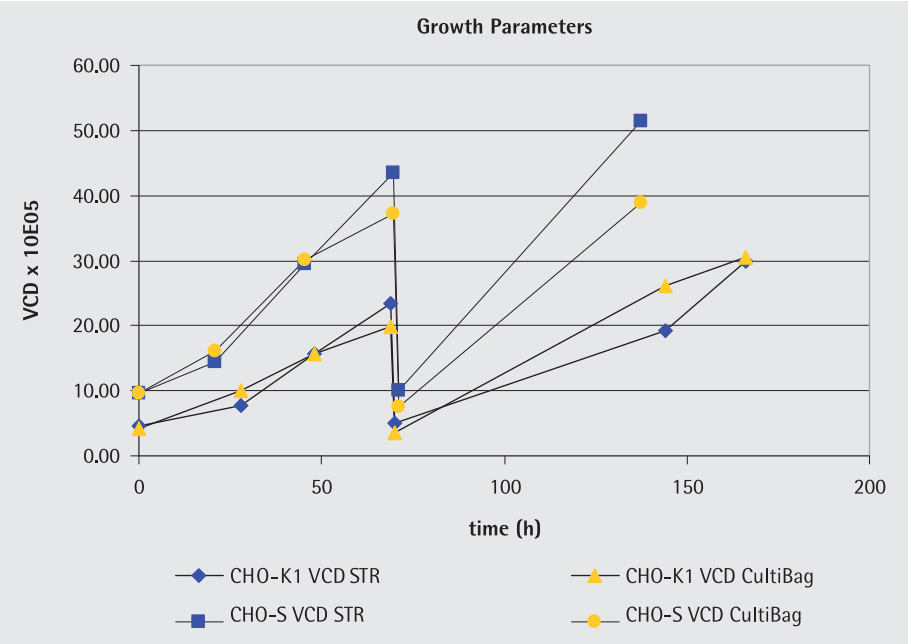

Figure 1: Growth parameters of CHO-S and CHO-K1

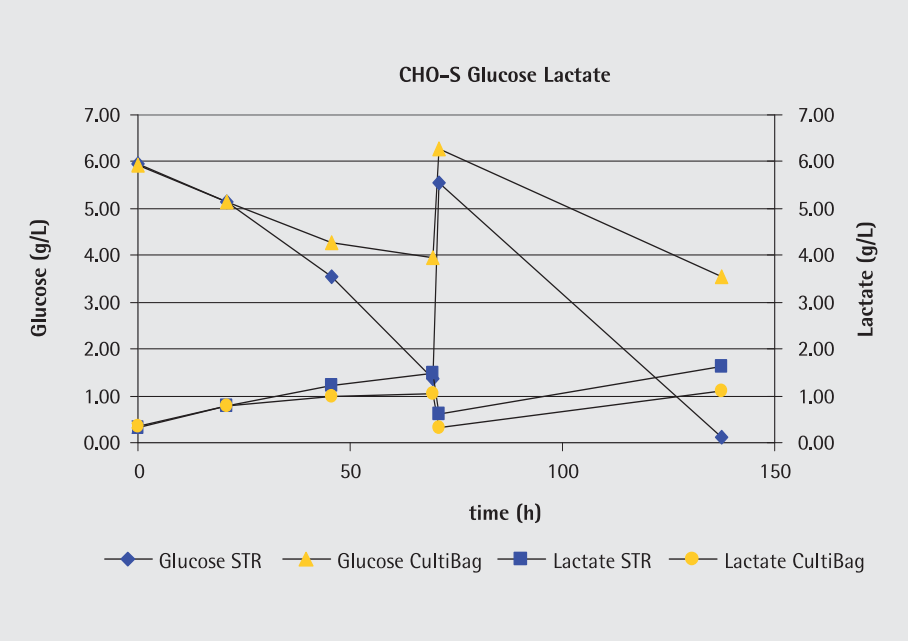

Figure 2: Glucose and lactate levels in CHO-S cultures.

5. Conclusion

In this note, we have demonstrated the ideal suitability of the single use bioreactor BIOSTAT® CultiBag 20 RM optical for the cultivation of CHO cell lines in serum free chemically defined media, making the instrument the preferred single use bioreactor in today's cutting edge applications in R&D, process development and small scale production.

Having compared the single use bioreactor against the high end BIOSTAT® B-DCU stirred tank reactor, we were able to show that the optical sensor technology used in the CultiBag delivers a reliable performance for process control leading to high performance in CHO cell culture.

Every part, including the sensors for pH and DO, that is in contact with product is designed as disposable, therefore removing the need for cleaning validation, keeping maintenance to a minimum and providing maximum operator safety.

The BIOSTAT® CultiBag RM is a safe, reliable and convenient tool for the cultivation of all kinds of organisms. With the available comprehensive validation guide and extractable analysis, in conjunction with full qualification and validation support including FAT and SAT, the BIOSTAT® CultiBag RM is perfectly suited for use in a GMP regulated environment.

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