BIOSTAT® CultiBag RM
Culturing Convenience

Application Note

Comparison of single use bioreactor
BIOSTAT® CultiBag RM and reusable STR for cultivation of CHO cells in serum free media
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
- CO₂ 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.

<table>
<thead>
<tr>
<th>CHO-S</th>
<th>B-DCU STR</th>
<th>CultiBag RM20</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>7.0</td>
<td>7.0</td>
</tr>
<tr>
<td>DO</td>
<td>40 %</td>
<td>40 %</td>
</tr>
<tr>
<td>Temperature</td>
<td>36 °C</td>
<td>36 °C</td>
</tr>
<tr>
<td>Gasflow</td>
<td>0.3 L min^-1 (via ring sparger)</td>
<td>0.2 L min^-1</td>
</tr>
<tr>
<td>Stirrer Speed</td>
<td>200 rpm</td>
<td>n.a.</td>
</tr>
<tr>
<td>Rocking Rate</td>
<td>n.a.</td>
<td>22 rpm</td>
</tr>
<tr>
<td>Angle</td>
<td>n.a.</td>
<td>5 °</td>
</tr>
</tbody>
</table>

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 x 10^5/mL in the STR and 1 x 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.
Sales and Service Contacts
For further contacts, visit www.sartorius-stedim.com

Europe

Germany
Sartorius Stedim Biotech GmbH
August-Spindler-Strasse 11
37079 Gottingen
Phone +49.551.308.0
Fax +49.551.308.3289
www.sartorius-stedim.com

Sartorius Stedim Systems GmbH
Schwarzenberger Weg 73–79
34212 Melsungen
Phone +49.5661.71.3400
Fax +49.5661.71.3702
www.sartorius-stedim.com

France
Sartorius Stedim Biotech S.A.
Z.I. des Paluds
Avenue de Jouques – BP 1051
13781 Aubagne Cedex
Phone +33.442.845600
Fax +33.442.845619

Sartorius Stedim France
4, rue Emile Baudot
91127 Palaiseau Cedex
Phone +33.1.6920.0922
Fax +33.1.6920.0922

Austria
Sartorius Stedim Austria GmbH
Franzosenengraben 12
A-1030 Vienna
Phone +43.1.7965763.18
Fax +43.1.796576344

Belgium
Sartorius Stedim Belgium N.V.
Leuvensesteenweg, 248/B
1800 Vilvoorde
Phone +32.2.756.06.80
Fax +32.2.756.06.81

Denmark
Sartorius Stedim Nordic A/S
Hoerskaetten 6D, 1.
DK-2630 Taastrup
Phone +45.7023.4400
Fax +45.4630.4030

Netherlands
Sartorius Stedim Netherlands B.V.
Edisonbaan 24
3439 MN Nieuwegein
Phone +31.30.6025080
Fax +31.30.6025090

Spain
Sartorius Stedim Spain SA
C/Isabel Colbrand 10–12,
Planta 4, Oficina 121
Poligono Industrial de Fuencarral
28050 Madrid
Phone +34.91.3586102
Fax +34.91.3588804

Switzerland
Sartorius Stedim Switzerland GmbH
Lerzenstrasse 21
8953 Dietikon
Phone +41.44.741.05.00
Fax +41.44.741.05.09

U.K.
Sartorius Stedim UK Limited
Longmead Business Park
Blenheim Road, Epsom
Surrey KT19 9 QQ
Phone +44.1372.737159
Fax +44.1372.726171

America

USA
Sartorius Stedim North America Inc.
131 Heartland Blvd.
Edgewood, NY 11717
Toll-Free +1.800.368.7178
Fax +1.631.254.4253

Sartorius Stedim SUS Inc.
1910 Mark Court
Concord, CA 94520
Phone +1.925.689.6650
Toll Free +1.800.914.6644
Fax +1.925.689.6988

Sartorius Stedim Systems Inc.
201 South Ingram Mill Road
Springfield, MO 65802
Phone +1.417.873.9275
Fax +1.417.873.9275

Argentina
Sartorius Argentina S.A.
Int. A. Avalos 4251
B1605ECS Munro
Buenos Aires
Phone +54.11.4721.0505
Fax +54.11.4762.2333

Brazil
Sartorius do Brasil Ltda
Av. Dom Pedro I, 241
Bairro Vila Pires
Santo André
São Paulo
Cep 09110-001
Phone +55.11.4451.6226
Fax +55.11.4451.4369

Mexico
Sartorius de México S.A. de C.V.
Circuito Circunvalación Poniente No. 149
Ciudad Satélite
53100 Naucalpan, Estado de México
Phone +52.5555.62.1102
Fax +52.5555.62.2942

Asia|Pacific

China
Sartorius Stedim Beijing
Representative Office
No. 33, Yu’an Road,
Airport Industrial Zone B, Shunyi District
Beijing 101300
Phone +86.10.80426516
Fax +86.10.80426580

Sartorius Stedim Shanghai
Representative Office
Room 618, Tower 1, German Centre,
Shanghai, PRC., 201203
Phone +86.21.28986393
Fax +86.21.28986392.11

Sartorius Stedim Guangzhou Office
Room 704, Broadway Plaza,
No. 233–234 Dong Feng West Road
Guangzhou 510180
Phone +86.20.8351.7921
Fax +86.20.8351.7931

India
Sartorius Stedim India Pvt. Ltd.
10, 6th Main, 3rd Phase Peenya
KIADB Industrial Area
Bangalore – 560 058
Phone +91.80.2839.1046
Fax +91.80.2839.1046

Japan
Sartorius Stedim Japan K.K.
KY Building, 8–11
Kita Shinagawa 1-chome
Shinagawa-ku
Tokyo 140-0001
Phone +81.3.3740.5407
Fax +81.3.3740.5406

Malaysia
Sartorius Stedim Malaysia Sdn. Bhd.
Lot L3–E-3B, Enterprise 4
Technology Park Malaysia
Bukit Jalil
57000 Kuala Lumpur
Phone +60.3.8996.0622
Fax +60.3.8996.0755

Singapore
Sartorius Stedim Singapore Pte. Ltd.
10, Science Park Road, The Alpha
#02–25, Singapore Science Park 2
Singapore 117684
Phone +65.6872.3966
Fax +65.6778.2494

Australia
Sartorius Stedim Australia Pty. Ltd.
Unit 17/104 Ferntree Gully Road
Waverley Business Park
East Oakleigh, Victoria 3166
Phone +61.3.9590.8800
Fax +61.3.9590.8828