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BIOSTAT® CultiBag RM
Culturing Convenience

Application Note

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Evaluation of the BIOSTAT® CultiBag RM for Microbial seed stage Fermentation

turning science into solutions

Terry Burns*, Gerhard Greller**, Millie Ullah***, Thorsten Adams**

*Wyeth Vaccines, 4300 Oak Park, Sanford, NC 27330; **Sartorius Stedim Biotech GmbH, August-Spindler-Str.11, 37079 Goettingen, Germany; ***Sartorius Stedim North America Inc. 131 Heartland Blvd. Edgewood, NY 11717.

1. Background

The BIOSTAT® CultiBag RM is the new generation of disposable bioreactors utilizing rocking motion for mixing with low shear. The combination of a new generation of Sartorius Stedim Biotech engineered control capabilities launches the disposable bioreactor system into a new era of cultivation.

BIOSTAT® CultiBag RM uses a rocking platform for Disposable Bag Bioreactors. The rocking technology utilizes mechanical energy to ensure homogeneous mixing with low shear. Energy input is affected by rocking the CultiBag RM back and forth, generating a fluid movement in the cell culture medium. In this way the surface of the medium is continuously renewed enabling mass transfer between the headspace and the medium.

Single-use bags reduce validation costs; remove the need for cleaning, sterilizing, and provide stress free convenience culturing. Easy to use, it is applicable to all cell types, including mammalian cells, plant cells, insect cells and microbial cells. A comprehensive validation guide and extractables report is available for the bags.

The measurement and control capabilities supplied by Sartorius Stedim Biotech are second to none. Utilizing proven technology and expert engineering, we have developed our existing in-house systems to bring powerful control capabilities to the disposable market. The BIOSTAT® Control system presents an easy-to use touch screen control system with integrated measurement and control hardware, pumps, temperature and gassing systems, for excellent process control. Application-driven, configured packages for basic, optical and perfusion are available, providing everything needed to get started immediately. The BIOSTAT® CultiBag RM is available with scalable working volumes from 0.1 L to 100 L. Just select the size that meets your needs today. Each basic, optical and perfusion package also includes our BioPAT® MFCS|DA software package for data collection and analysis.

Whilst the headspace aeration together with the rocking motion supports an optimal growth of mammalian cells, growth of cultures requiring high oxygen input is limited by the mass transfer. Nonetheless, the CultiBag can be successfully employed for microbial cultivation. The kLa values determined herein prove that the oxygen transfer is sufficient to promote growth even of microbial cultures.

In this application note, we show the successful utilization of the BIOSTAT® CultiBag RM for microbial seed stage fermentation in the production of recombinant proteins and vaccines. This is exemplified by the cultivation of *Escherichia coli* and *Corynebacterium diphtheriae*.

Furthermore, we evaluate the performance of the BIOSTAT® CultiBag RM in comparison to a stirred tank bioreactor.

2. Determination of k_{La} values
The k_{La}-values for the CultiBag RM were determined by the gassing-out method for typical rocking speeds, angles and gas flow rates. Ambient air and pure oxygen were used as process gasses.

The maximum k_{La}-values at full rocking speed, angle and gas flow using air were 22.0 h^{-1} for the 2 L system and 6.0 h^{-1} for the 20 L system. Using pure oxygen as process gas, the k_{La}-values could be raised to 43.2 h^{-1} and 12.9 h^{-1}, respectively.

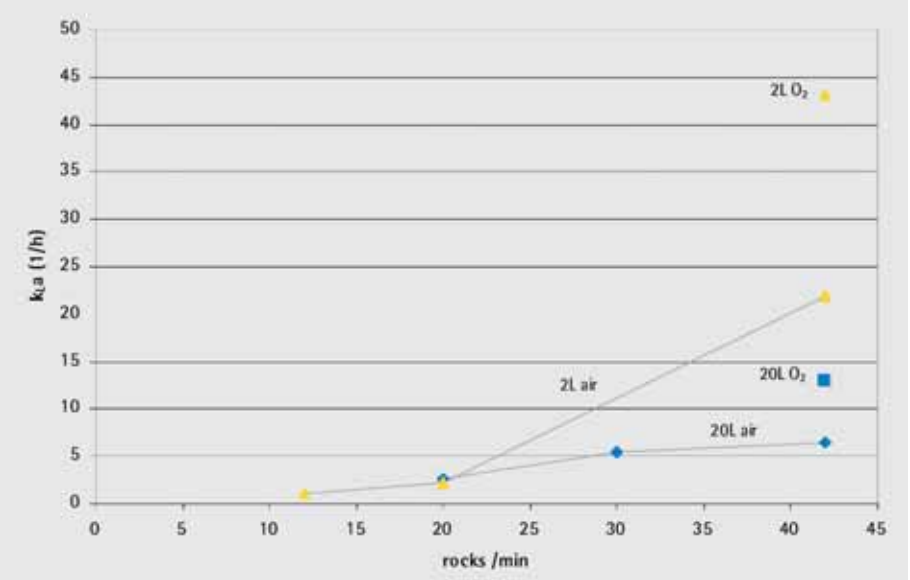

Fig 1: k_{La} values

3. Cultivation of *Escherichia Coli*

a. Material

- BIOSTAT® CultiBag RM 2 basic (Sartorius Stedim Biotech DH-002-L-B-RM-2)
- CultiBag RM 2L basic (Sartorius-Stedim Biotech DBB002L2)
- CultiFlask 50 disposable bioreactor (Sartorius-Stedim Biotech DF-050MB-SSH---4)
- Baffled Erlenmeyer flask
- Incubator Sartorius Stedim Biotech Certomat
- LB-media (10 g/L tryptone, 5 g/L yeast extract, 5 g/l NaCL)
- *E. coli* BL21(DE3)

b. Methods

For preparation of a seed culture, *E. coli* BL21 (DE3) streaked out on LB agar plates were used to inoculate two Sartorius Stedim Biotech CultiFlask 50 disposable bioreactors each filled with 20 mL LB medium. The seed culture was grown over night at 37°C and 150 rpm in an incubator. The CultiBag was filled with 1 L of LB medium, pre-heated to 37°C and inoculated with the pre-culture to reach an optical density (OD_{600}) of 0.15.

Cultivation was started with the following process parameters: temperature 37 °C, rocking speed 42 rpm, rocking angle 10° and airflow 0.5 lpm. Ambient air was used for oxygen supply. Growth was monitored by measuring the optical density in regular intervals.

For comparison of the growth characteristics, a baffled Erlenmeyer flask filled with 200 mL sterile LB medium was inoculated with *E. Coli* BL21 (DE3) to a final OD_{600} of 0.15 and incubated at 37 °C and 150 rpm in an incubator.

c. Results

The optical density of the culture was recorded and specific growth rate was calculated $\mu = \ln(OD_{t2}/OD_{t1})/(t_2-t_1)$. In the CultiBag, the maximal specific growth rate was 18 % higher than in the Erlenmeyer flask (1.67 h^{-1} vs. 1.38 h^{-1}). The final cell density was comparable in both cultivation devices.

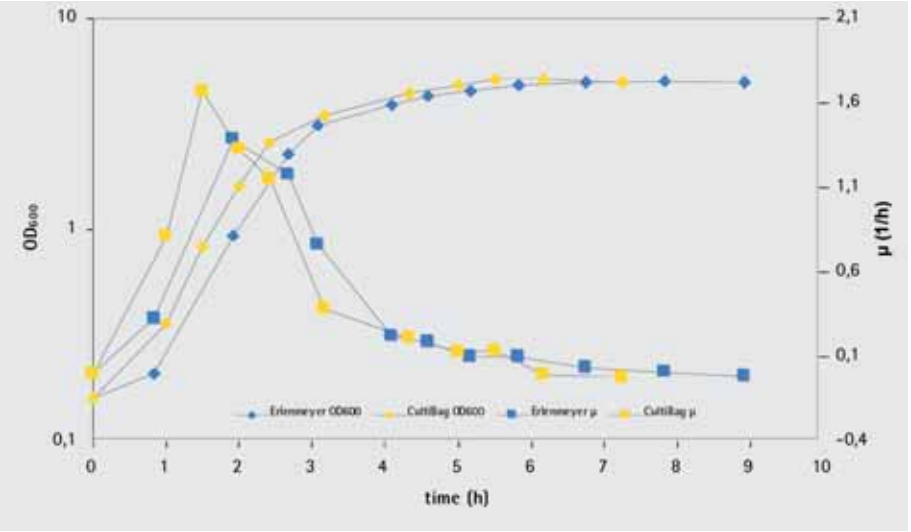

Fig 2: Comparison of growth characteristics in CultiBags and baffled Erlenmeyer Flasks

4. Cultivation of *Corynebacterium diphtheriae* in the CultiBag RM vs. Stirred Tank Bioreactor

The BIOSTAT CultiBag RM 20 is used for seed stage fermentation in vaccine production.

a. Material

- BIOSTAT® CultiBag RM 20 (Sartorius Stedim Biotech DH-020-L-0-RM-1)
- CultiBag RM 20L optical (Sartorius Stedim Biotech DBO020L)
- CY (casamino/yeast) -media
- C. diphteriae

b. Methods

The CultiBag RM 20L was filled with 10 L of CY-media and inoculated with approximately 130 ml of a *C. diphtheriae* culture grown in an aspirator bottle to an OD_{590} of 8.66 resulting in an optical density (OD_{590}) of 0.123 at the start of fermentation. Cultivation was started using following process parameters: temperature 32 °C, rocking speed 12 rpm, rocking angle 5.9° airflow 0.299 lpm. During the course of the cultivation, the rocking rate was raised to 42 rpm, the angle to 10° and the airflow to 0.55 lpm to enable high mass transfer. Ambient air was used for oxygen supply. The optical density was measured in regular intervals throughout the process. For comparison, a stainless steel reactor filled with 20 L of medium was inoculated with approximately 270 ml of a *C. diphtheriae* culture grown in an aspirator bottle to an OD590 of 8.66 resulting in an optical density (OD_{590}) of 0.179 at the start of fermentation.

c. Results

The optical densities of the cultures were measured and the dissolved oxygen (DO) was monitored in the CultiBag RM using the disposable optical DO probes. During the course of the cultivation, the dissolved oxygen drops to around 25% due to the limited oxygen transfer by headspace aeration. However, the culture reached an OD_{590} of 5 in the CultiBag RM and 7.3 in the stainless steel fermentor after an 8 h cultivation period, indicating that the BIOSTAT CultiBag RM is well suited for cultivation of C. diphteriae. C. diphteriae was successfully cultured in the CultiBag RM for preparation of a seed inoculum.

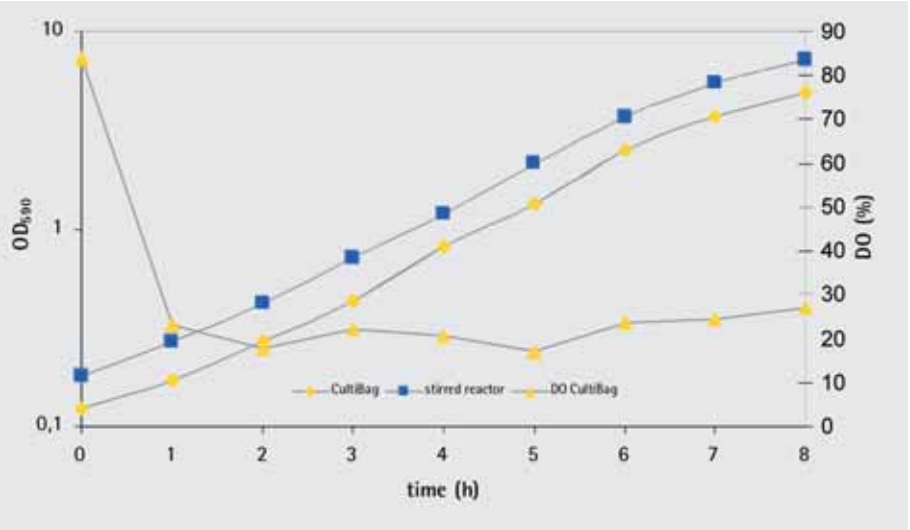

Fig 3: Growth comparison of *C.diphtheriae* in CultiBags and Stirred Tank Bioreactor

5. Conclusion

Combining ease of use, reliability and minimum workload BIOSTAT® CultiBag RM is ideally suited for fermentation of microbial organisms. Reaching K_{La} values of max. 43.2 h^{-1}, the reactor easily provides oxygen transfer rates sufficient for medium cell densities in microbial fermentation. 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.

In this note, we could demonstrate that C. diphteriae can be cultivated in the BIOSTAT® CultiBag RM reaching cell densities comparable to stainless steel fermentors. This makes the reactor ideally suited as a seed stage fermentor in vaccine production. Similarly, *E. coli*, still the preferred microbial host for recombinant protein production, was successfully cultivated to medium cell densities.

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 Goettingen

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 SAS
ZI des Paluds
Avenue de Jouques – CS 71058
13781 Aubagne Cedex

Phone +33.442.845600
Fax +33.442.846545

Austria
Sartorius Stedim Austria GmbH
Franzosengraben 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

Italy
Sartorius Stedim Italy S.p.A.
Via dell'Antella, 76/A
50012 Antella-Bagno a Ripoli (FI)

Phone +39.055.63.40.41
Fax +39.055.63.40.526

Netherlands
Sartorius Stedim Netherlands B.V.
Edisonbaan 24
3439 MN Nieuwegein

Phone +31.30.6025080
Fax +31.30.6025099

Spain
Sartorius Stedim Spain SA
C/Isabel Colbrand 10–12,
Planta 4, Oficina 121
Polígono 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 +1.417.873.9636
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
Represantative 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 +91.80.2839.1963|0461
Fax +91.80.2839.8262

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

Specifications subject to change without notice. Printed in Germany on paper that has been bleached without any use of chlorine. W/sart-000 · G.
Publication No.: SBT1001-e08102 · Order No.: 85034-535-59