



## BIOSTAT® CultiBag RM Culturing Convenience



#9

Application  
Note

#1

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

#2

#3

#4

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## 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  $22.0 \text{ h}^{-1}$  for the 2 L system and  $6.0 \text{ h}^{-1}$  for the 20 L system. Using pure oxygen as process gas, the  $k_{La}$ -values could be raised to  $43.2 \text{ h}^{-1}$  and  $12.9 \text{ h}^{-1}$ , respectively.

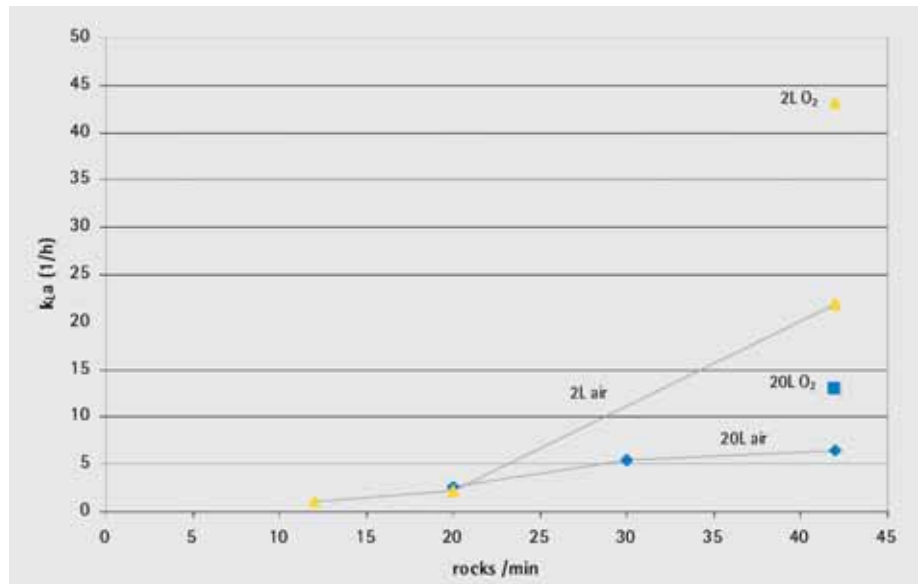


Fig 1:  $k_{La}$  values

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

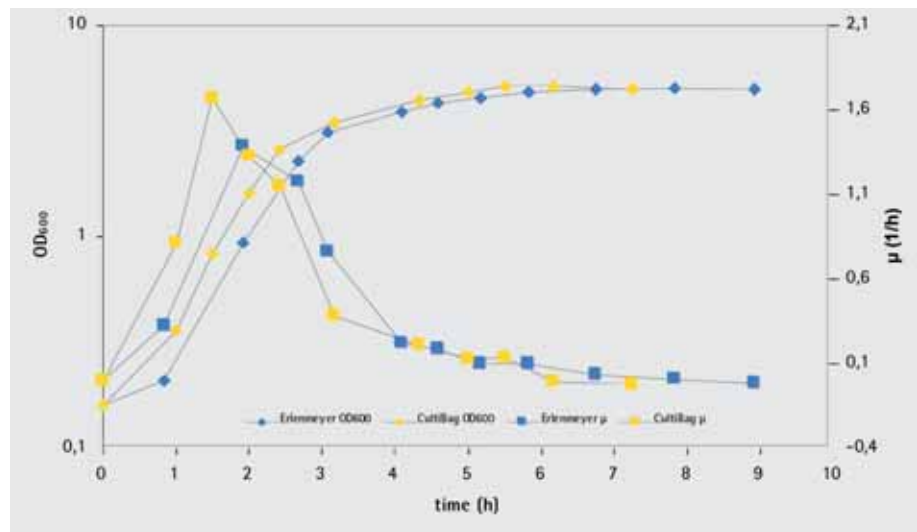


Fig 2: Comparison of growth characteristics in CultiBags and baffled Erlenmeyer Flasks

#### 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. diphtheriae*

##### 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<sub>590</sub> of 8.66 resulting in an optical density (OD<sub>590</sub>) 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 OD<sub>590</sub> of 8.66 resulting in an optical density (OD<sub>590</sub>) 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<sub>590</sub> 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. diphtheriae*. *C. diphtheriae* was successfully cultured in the CultiBag RM for preparation of a seed inoculum.

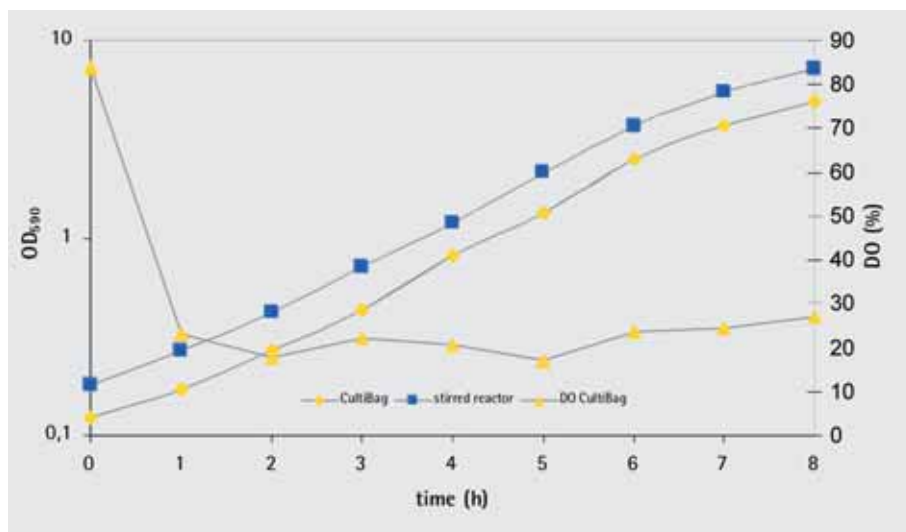


Fig 3: Growth comparison of *C. diphtheriae* in CultiBags and Stirred Tank Bioreactor

#### 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<sup>-1</sup>, 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. diphtheriae* 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.

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