INFORS HT

Application note

ShakerBag Option: Recommendations for setting the shaking speed

1. Introduction

The ShakerBag Option is an innovative disposable culturing system for expanding mammalian, insect, and plant cells. For this purpose, the cell cultures are cultured in orbitally shaken disposable culturing bags with a working volume in the range of 0.2 L to 10 L in an INFORS HT Multitron Cell. A comprehensive characterisation of the ShakerBag Option system in terms of its process engineering by means of classical and modern methods provided a specification of the most important settings for optimal utilisation of the system. Using exemplary culturing of animal and plant cell types the suitable shaking speeds were verified, which are comparable with other cell cultivation systems (shown in separate cultivation protocols).

2. Technical specifications

Multitron Cell incubator shaker (50 mm) with ShakerBag Option

- Trays for various sizes of disposable culturing bags (2 L, 10 L, 20 L)
- Direct gas feed (air or air/CO_2; 20–2000 mL/min)
- CO_2 control 0–20 %
- Feed-through for cables, measuring instruments and gas line

3. Process engineering methods

The process engineering analysis of the ShakerBag Option (INFORS HT) for 2 L, 10 L, and 20 L orbitally shaken disposable culturing bags Cultibag RM (Sartorius Stedim Biotech) included an assessment of the mixing time θ_M and of the volumetric oxygen transport coefficient k_La.

The mixing time θ_M is the crucial factor for the compensation of concentration and temperature differences within a bioreactor system. The determination of θ_M was done using the classical process engineering method, i.e. the iodine-thiosulfate discolouration method, in ultra-pure water without the presence of a biological system.

The presence of dissolved oxygen in the liquid phase is absolutely essential for the growth of aerobic micro-organisms or cells. The volumetric oxygen transport coefficient k_La was therefore used to analyse the transport of oxygen from the gas to the liquid phase. The analysis was performed using the sulfite method at 25 °C and at a gas flow rate of 0.2 vvm. The oxygen concentration was measured using optical sensor spots (PreSens, Germany).

Modern numerical fluid mechanics applying the computational fluid dynamics (CFD) system allows location- and time-dependent parameters to be determined, such as, for example, the calculation of the surface area for estimation of k_La values. In addition, the volume-related distribution of turbulent energy dissipation and turbulent kinetic energy within the systems can be assessed. Accordingly, the flow simulation allows geometrically similar and non-similar culturing systems to be compared, which are a challenge specifically in the case of disposable culturing bags.

The result of the process engineering analysis of the ShakerBag Option leads to recommendations concerning shaking parameters which would afford a good supply of oxygen and nutrients to animal cell cultures, but also for plant cell cultures as well as some micro-organisms.

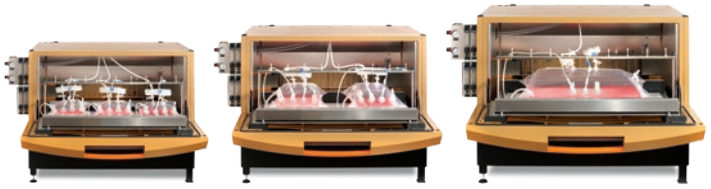

Fig. 1: Multitron Cell featuring the ShakerBag Option 2 L, 10 L, and 20 L

4. Results

The results of the process engineering characterisation of the mixing times and oxygen transport, supported by a CFD analysis, are shown, in exemplary manner, in the contour plots xy for the following application example: 10 L disposable culturing bag characterised in a 50 mm Multitron Cell with ShakerBag Option.

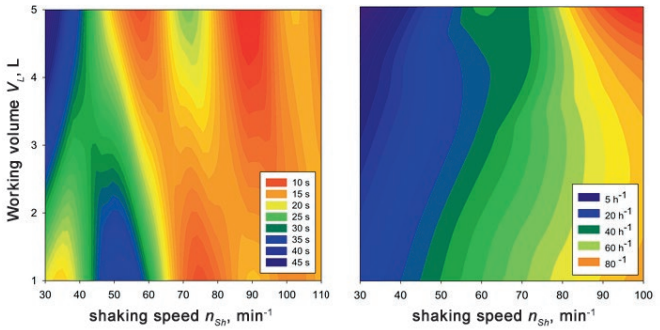

Fig. 2: Comparison of mixing time θ_M and volumetric substance transport coefficient k_La of the CB 10 L at a 50 mm amplitude (left: θ_M; right: k_La). The two contour plots show good correlation. In the ranges, in which low mixing times result, the k_La values are correspondingly higher, and vice versa.

Recommended shaking speeds for the ShakerBag Option in the Multitron Cell

The following tables summarise which shaking speeds can be set for a defined working volume in order to attain the desired mixing times and k_La ranges as recommended for animal and plant cell cultures.

This concerns recommended shaking frequencies which take into account at least a k_La value > 10 h^{-1} or a k_La value > 25 h^{-1}.

For some working volumes, the maximal k_La value determined is given together with the corresponding shaking speed. This value is important especially for applications involving some micro-organisms which have an elevated oxygen consumption. In the scope of the investigations, a maximal k_La value of 97 h^{-1} was determined for the 2 L culturing bag.

In addition, shaking speed ranges are being considered, in which a maximal mixing time of 35 s is not exceeded. The minimal mixing time of 5 s was observed with the 2 L disposable culturing bag.

ShakerBag Option 20 L

Cultibag 20 L	Working volume, L	Shaking speed, min^{-1} (range for $k_La > 10\ h^{-1}$)	Shaking speed, min^{-1} (range for $k_La > 25\ h^{-1}$)	Max. k_La, h^{-1} (rpm)	Shaking speed, min^{-1} (range for selected mixing time*)	*Mixing time, s
CB 20 L	2	30–70	40–70	33 (60)	55–100	15–25
	4	30–70	35–70	–	55–70	15–25
	6	30–70	50–60	33 (50)	35–70	20–30
	8	30–60	45–55	–	35–65	15–30
	10	35–60	40–60	41 (60)	35–65	15–35

ShakerBag Option 10 L

Cultibag 10 L	Working volume, L	Shaking speed, min^{-1} (range for $k_La > 10\ h^{-1}$)	Shaking speed, min^{-1} (range for $k_La > 25\ h^{-1}$)	Max. k_La, h^{-1} (rpm)	Shaking speed, min^{-1} (range for selected mixing time*)	*Mixing time, s
CB 10 L	1	35–100	40–100	69 (90)	40–110	13–38
	2	35–100	40–100	75 (100)	–	–
	3	40–100	45–100	70 (100)	–	–
	4	40–100	45–100	77 (100)	–	–
	5	40–100	45–100	63 (80)	40–110	7–33

ShakerBag Option 2 L

Cultibag 2 L	Working volume, L	Shaking speed, min^{-1} (range for $k_La > 10\ h^{-1}$)	Shaking speed, min^{-1} (range for $k_La > 25\ h^{-1}$)	Max. k_La, h^{-1} (rpm)	Shaking speed, min^{-1} (range for selected mixing time*)	*Mixing time, s
CB 2 L	0.2	35–100	40–100	97 (90)	30–120	5–17
	0.4	40–100	45–100	>90 (70)	–	–
	0.6	40–100	45–100	70–88 (60–70)	–	–
	0.8	40–100	45–100	60–75 (60–100)	–	–
	1	40–100	45–100	68 (70)	40–120	7–12

5. Summary

The result of the process engineering analysis of the ShakerBag Option can be used to define shaking parameters that ensure a good supply of oxygen and nutrients. In this context, different working volumes of disposable culturing bags of 2 L, 10 L, and 20 L were considered more closely. The recommended ranges apply mainly to applications for animal cell cultures and plant cell cultures.

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• Werner et al.: An Approach for Scale-Up of Geometrically Dissimilar Orbitally Shaken Single-Use Bioreactors. Chemie Ingenieur Technik 2013, 85, No. 1–2, 118–126; DOI: 10.1002/cite.201200153
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