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Hybridoma culture using New Brunswick™ CelliGen® 310 with Packed-bed Fibra-Cel® Basket Impeller

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Abstract

The Fibra-Cel packed-bed basket technology has been established as an excellent method for the growth of suspension and anchorage-dependent cell lines. The three dimensional structure of the Fibra-Cel disk provides an excellent solid-support matrix for the entrapment or attachment of animal cells, allowing constant perfusion of nutrients in a low-shear environment. In this application note, we show that Hybridoma cells can be successfully cultivated in high densities in the 2.5 L packed-bed Fibra-Cel basket controlled by a CelliGen 310 bioreactor.

Introduction

Packed-bed bioreactor cell culture is generally accepted as one of the best methods to simulate the conditions of animal cell growth in vivo since cells are maintained in a low-shear environment with constant refreshment of nutrients and removal of waste. The growth of attachment-dependent cells on Fibra-Cel has been shown to increase both cell and product yields. In particular, Hybridoma cells are inherently sensitive to waste buildup and the implementation of packed-bed Fibra-Cel growth conditions in addition to perfusion production methods has greatly increased yields. To demonstrate that the CelliGen 310 2.5 L basket impeller bioreactor is capable of robust, reproducible high density Hybridoma culture under perfusion conditions, two independent trials were conducted using the suspension-adapted DA4.4 hybridoma cell line.

Figure 1. Left: The packed-bed basket impeller including Fibra-Cel disks. Right: The CelliGen 310 bioreactor with 2.5 L vessel.

Materials and Methods

Inoculum preparation

DA4.4 Hybridoma cells (ATCC® #HB-57; Manassas, VA) were grown in 1 L shake flasks at 37 °C with 5 % CO_2 and agitation set at 95 rpm. Culture medium was prepared using Gibco® Hybridoma-SFM complete DPM powder supplemented with 5 % Hyclone® Fetal Bovine Serum and 1 % Gibco liquid Pen/Strep before sterile filtration using a 0.2 µm Millipore® Millipak® gamma gold filter into sterile Hyclone bags (5 L and 10 L, as necessary). Medium was stored at 2 – 8 °C until use. The 1.75 L vessel working volume was inoculated with a target total of 4.1 x 10^8 cells. Actual viable cell numbers were 3.5 x 10^8 cells (2.2 x 10^5 cells/mL) for the first run and 4.8 x 10^8 cells (3 x 10^5 cells/mL) for the second run. The table below shows the origin of the materials used in this study.

Material	Supplier	Catalog #	Lot #
Hybridoma-SFM complete DPM powder	Gibco	12300-067	949234
Pen/Strep 100X liquid	Gibco	15140	1092590
Hyclone Fetal bovine serum	Hyclone	SH30070.03	AWC99936
D (+) - Glucose Hybri-Max powder	Sigma®	G5146-10k	071M01453V
45 % Glucose solution	Sigma	G8769	54K2371
Fibra-Cel	Eppendorf	M1292-9988	Trial 1: 78690 Trial 2: 1100081

Bioreactor conditions

For both runs, hybridoma cells were cultured in the same vessel, using the same CelliGen 310 cabinet for 9 consecutive days, using the basket impeller system packed with 75 g of Fibra-Cel disks.

CelliGen 310 Setpoints	
Agitation	80 rpm
Temperature	37 °C
pH	7.15 Dead band 0.04
DO	50 %
Gas supplied	4-gas mix control (N_2 off; CO_2 fo pH control)
Gas flow conditions	0.4 SLPM
Vessel	2.5 L glass water jacketed
Fibra-Cel	75 g

Perfusion was initiated for each bioreactor on day 3 and continued through day 9. Initially, the main objective was to increase the perfusion rate to maintain a glucose concentration above and near 1 g/L. For the second bioreactor experiment, the perfusion rate was adjusted to match the first bioreactor rate in order to make the two runs as identical as possible. The tables illustrate the experimental parameters and perfusion volumes for both trials.

Day	Perfusion volume (L)
3	0.73
4	1.81
5	4.25
6	5.5
7	4.25
8	4.75
9	5

Biochemistry analysis

Daily off-line measurements of glucose concentration were performed using a YSI® 2700 analyzer (YSI, Inc., Yellow Springs, OH). The glucose consumption was calculated for each time point and plotted as an average of the two independent trials. Error bars indicate standard error of the mean.

Results and Discussion

As presented in the graph below (Figure 2), the rate of glucose consumption across both trials is indicative of reproducible growth of hybridoma cells in this environment. We conclude that the use of Fibra-Cel in the basket impeller system on the CelliGen 310 is an excellent method for high density hybridoma culture. In a batch run with the CelliGen pitch blade bioreactor, hybridoma cells usually peak at approximately 5 g/day of glucose consumption. The packed-bed basket impeller system presents significantly higher productivity with glucose consumption peaking at, on average, 25 g/day. In addition, if growth conditions are maintained by continued fresh media perfusion and glucose concentration is never allowed to fall below 1 g/L, hybridoma can be continuously cultured in the basket many days after the 9 day window provided in this study; this further increases productivity and decreases overall antibody production costs. No optimization of growth conditions were attempted for either bioreactor run.

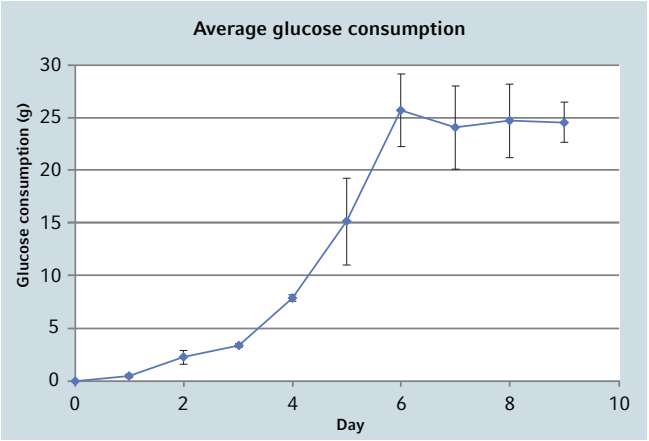

Figure 2. The glucose consumption was calculated daily for each bioreactor and the mean is presented. Error bars indicate standard error of the mean. Comparable consumption was observed across the two bioreactors.

Ordering information

Description	North America Order no.	International Order no,
New Brunswick™ CelliGen® 310 Bioreactor, 2.5 L System	M1287-1260 (100-120 V)	M1287-1264 (200-240 V)
Fibra-Cel® Disks, 250 grams	M1292-9988	M1292-9988
2.5 L Basket Impeller Kit	M1287-1140	M1287-1140

Your local distributor: www.eppendorf.com/contact
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