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Insect Cell Culture Using the New Brunswick™ BioFlo®/CelliGen® 115 Benchtop Fermentor/Bioreactor with Spin Filter Assembly

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Abstract

This application report presents a simple protocol for achieving high-density culture of *Spodoptera frugiperda* (Sf9) cells using a New Brunswick bench-top, autoclavable stirred-tank reactor with a spin-filter assembly. Factors such as substrate concentration and metabolite buildup can be limiting for culture growth and viability at high densities. Using the spin filter in a 2 L vessel (0.8 – 2.2 L working volume) attached to the BioFlo/CelliGen 115 cabinet, a cell density of 18.24 x 10^6 cells with viability over 90 % was achieved, outperforming the batch or fed-batch process.

Introduction

Stirred-tank bioreactors are widely used for research and industrial applications for cultivating a wide variety of cells types, including insect cells, hybridoma, CHO, BHK21, HEK293, and others; these cultures manufacture viral vaccines and monoclonal antibodies, blood clotting factors, etc.

The BioFlo/CelliGen 115 features an easy-to-use control station with color touch-screen monitor and built-in capability to operate in either fermentation or cell-culture modes. Switching between the operating modes automatically adjusts the control settings. Three fixed-speed pumps, temperature and agitation control, and one rotameter with choice of gas flow ranges are available in the BioFlo/CelliGen 115 systems. Pre-packaged kits for basic or advanced fermentation and advanced cell-culture simplify ordering. All kits include options for direct-drive or magnetic-drive agitation as well as water-jacketed or heat-blanketed vessels in 1, 2, 5, and 10 L sizes. pH/DO and foam/level controllers can be included depending on the selected kit or can be added individually as options. Options for additional rotameters or thermal mass flow controllers (TMFC) are also available.

Spodoptera frugiperda, also known as the *Fall Armyworm* or Sf9, are insect cells commonly used for the production of proteins of interest in pharmaceutical research due to their unique ability to replicate mammalian post-translational modifications such as glycosylation. Insect cells produce a variety of proteins utilizing the Baculovirus Expression Vector System (BEVS). Cell lines such as Sf9, Hi-5, Sf21, etc., are proven to express high levels of end products.

Insect cell culture can be achieved by using batch, fed batch or perfusion methods. For this study, the perfusion method was used in conjunction with New Brunswick spin filter assembly.

The spin filter allows for the removal of exhausted media without removing the cells in suspension, making room for fresh media addition, thus achieving and maintaining the highest culture densities possible.

Figure 1: BioFlo/CelliGen 115 systems feature a compact control station capable of either fermentation or cell culture operating modes to accommodate growth of a wide variety of cell types. A built in color touch-screen interface facilitates setpoint control and monitoring. The BioFlo/CelliGen 115 system (left) is equipped with a 2 L water-jacketed vessel with pitched-blade impeller and four rotameters.

Figure 2: Eppendorf impellers: pitched blade, left; spin filter with marine blade, right.

Materials and Methods

Bioreactor
For this application, a standard 2 L BioFlo/CelliGen 115 advanced cell culture kit with a magnetic drive and water-jacketed vessel was used. A Suspension-Cell Spin Filter with 10µm screen was used to grow the insect cells in a continuous, high flow rate perfusion mode. In addition, BioCommand® Batch Control software was used to monitor the system and control the feeding schedule.

Medium
This application used an animal component-free chemically-defined ESF-921 medium from Expression Systems (Woodland, CA).

Inoculum
The cell stock used was Sf9 cells derived from ATCC® CRL-1711 adapted to a serum free environment, obtained from Expression Systems (Woodland, CA). The inoculum was cultivated in an Eppendorf shaker (New Brunswick Innova® 40, order no.).

Controller setpoints
Calibrate pH probe prior to autoclaving. Enter controller set points prior to inoculation and allow the media equilibrate to prior to proceeding. The DO may remain high after calibration and before inoculation due to the absence of cells consuming it. An initial DO value of > 95 % is acceptable; it will decrease as the cells start to metabolize it. Normal setpoints for *Spodoptera* cells are controlled by the Primary Control Unit (PCU) and are as follows:

Parameter	Setpoint
Temperature	28°C
Dissolved oxygen	50 %
pH	6.3
Agitation	100 rpm
Gas control	4-gas mode
Inoculum	4.1 x 10^5 cells/mL

DO calibration
The DO probe is calibrated using a standard two-point calibration method: 0 % (often referred to as the zero point) and 100 % (often referred to as span). The zero can be achieved by either disconnecting the DO cable (the electronic zero; used in this process) or by sparging N_2 into the media to achieve a level stable near zero. The 100 % calibration point is achieved by bringing the vessel filled with medium to all of the operational setpoints, i.e. agitation, temperature, etc. DO should be calibrated post-autoclave and pre-inoculation after a six hour polarization period. After calibration, the DO may remain around 100 % until after inoculation.

pH calibration
The pH probe was calibrated prior to the autoclave cycle outside the vessel using a two-point calibration method with standard pH buffers. The pH 7.0 buffer is used to zero the probe and the pH 4.0 or 10.0 can be used as the span (Refer to the BioFlo 115 operating manual).

pH control
The pH for insect cells normally does not drift much from setpoint, but at higher culture densities the pH may drop. The pH parameters are maintained by the addition of CO_2 to lower the pH or an 8 % sodium bicarbonate solution to raise the pH. The dead band was set to 0.1 for this run.

Gas control
The BioFlo/CelliGen 115 was set to the 4-gas mode to maintain the DO and pH setpoints automatically. The cascade in 4-gas mode was set to gas flow and the O_2 control was set to 4-gas mode.

Continuous feed (perfusion)
All pumps were calibrated using the standard, supplied tubing to track liquid quantities entering and exiting the vessel. Samples were taken several times a day to measure the density of the culture as well as nutrient consumption.

Controller setup	
Pump 1	Base addition dependent on pH accomplished through the tri-port adaptor in the vessel head plate.
Pump 2	Harvest of spent media accomplished with a level sensor configured to a predetermined level with a dip tube to the interior of the spin-filter. This allows for the extraction of media without the loss of cell density.
Pump 3	Perfusion in of fresh media at a predetermined rate.

Control program
For this study, a BioFlo/CelliGen 115 for the control of the pH, DO, level sensor, and pumps was used; BioCommand Plus software was also used to monitor the culture parameters.

Results and Discussion

Insect cells generally have a high demand for oxygen during protein production. Maximum growth rate and high cell densities are achieved by keeping the DO at a constant set point.

Factors such as substrate concentration and metabolite buildup can be limiting factors; these were made more controllable through the abilities of the BioFlo/CelliGen 115 bioreactor with the Advanced Cell Culture Kit coupled with a spin filter kit.

The BioFlo/CelliGen 115 system allowed for the growth of insect cells to a final density of 18.24 x 10^6 cells/mL. The inclusion of the TMFCs (thermal mass flow controller) provided the ability to mix the four gases according to culture needs and further enhanced the final culture density.

Conclusion
Considering the above results, we can view the viable cell density of 18.24 x 10^6 as proof of the fundamental capabilities of the BioFlo/CelliGen 115 system.

The temperature of the system remained steady and was controlled by using un-chilled tap water as the coolant.

DO and pH control remained stable and consistent throughout the experiment.

Overall, the BioFlo/CelliGen 115 system performed extremely well. The BioFlo/CelliGen 115 advanced cell culture system with spin filter assembly is recommended for insect cell culture to achieve high cell densities.

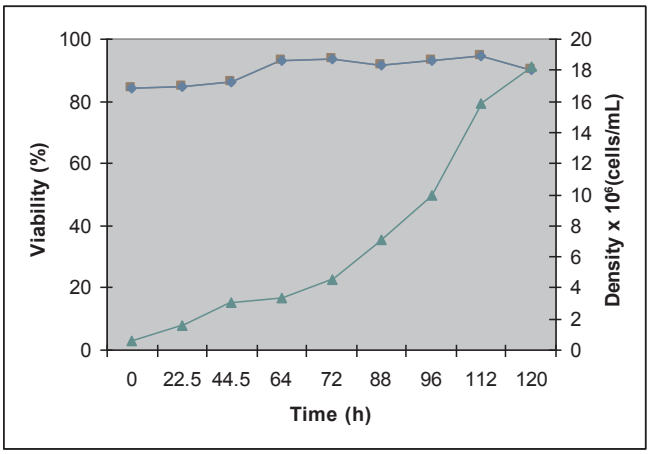

Figure 3: Insect cell Sf9 perfusion culture in a 2 L BioFlo/CelliGen 115 bioreactor with spin filter impeller. The cells were inoculated from 1000 mL shaker flask culture. The inoculum cell density was 4.9 x 10^5 cells/mL. After two days of the batch process, medium perfusion was started at the rate of 0.5 – 2 L working volumes per day.

Ordering information	Order no.
New Brunswick™ BioFlo®/CelliGen® 115 Advanced Cell Culture Kit	
2 L, 100 - 120 V, Water Jacket, Direct Drive	M1369-1112
2 L, 200 - 240 V, Water Jacket, Direct Drive	M1369-1162
Impeller	
2 L Spin Filter Impeller - Suspension	M1273-3202
Software	
BioCommand® Batch Control	M1326-0010
New Brunswick™ Innova® 40	
230 V/50 Hz, orbit diameter 1.9 cm	M1299-0082
230 V/50 Hz, orbit diameter 2.5 cm	M1299-0092

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