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Introduction to Sf-9 Insect Culture in a Stirred-Tank Bioreactor

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Abstract

This applications report presents a simple protocol for achieving high-density culture of Sf-9 Insect cells using a New Brunswick benchtop, autoclavable stirred-tank bioreactor.

Introduction

Sf-9 cells are derived from pupal ovarian tissue of Spodoptera frugiperda, the Fall Armyworm. Sf-9 cells are suspension cultures which are commonly used to isolate and propagate recombinant Baculoviral stocks and to produce recombinant proteins. A key factor in the popularity of Sf-9 and other insect culture is the ability to produce large quantities of proteins that show post-translational modification typically exclusive to Eukaryotes.

Materials and Methods

Medium
Sf-900 II serum-free medium from GIBCO® or SFX-Insect from Hyclone® can be used. The medium is similar to mammalian cell culture medium in that it is temperature-sensitive and should be added after sterilization.Inoculum Inoculum is prepared using LB broth at 25 g/L.

Inoculum
Inoculum should be grown to a density of 4.0 - 5.0 x 10^5 per mL in a non-humidified, non-gas-regulated environment.

Batch/Fed-Batch/Continuous
Sf-9 Insect cells can be grown using any of the three common culture methods, batch, fed-batch, or continuous, but they are usually grown using fed-batch or continuous culture because of the increased yields.

The **fed-batch** method uses either a low-shear marine blade impeller or a low-shear pitched blade impeller to provide uniform mixing throughout the culture. When using a fed-batch method, a percentage of the medium's total working volume is intentionally left out and feed supplements or additional medium are added as necessary. Feeds are often based on cell densities or off-line measurement of metabolites.

The **continuous** mode employs an ultra-low-shear spin filter that allows cell-fee medium, typically with product, to be harvested while supplying the culture with an equal amount of fresh medium. The continuous culture method allows for very high cell densities because the cells are retained within the vessel and continuously supplied with fresh nutrients.

Typical Control Setpoints for Sf-9

Common setpoints for Sf-9 should be entered and achieved prior to inoculation and, except for DO which often remains high, the medium should be allowed to equilibrate prior to inoculation. DO remains high because it takes time for O_2 to permeate out of the medium as it is not yet being utilized by the cultures. An initial DO value of approximately 100 % is acceptable; it will decrease as the culture metabolizes it. Some studies have indicated that monitoring the level dCO_2 in the culture and adding a continuous flow of air by means of an overlay can increase cell densities of insect culture as well as some mammalian cell cultures.

Parameter	Setpoint
Temperature	28 °C
pH	6.2
Dissolved Oxygen	40 %
Agitation	70 - 100 rpm
Gas Control	4 Gas
Overlay	300 mL/Air

pH/DO Control

Dissolved Oxygen (DO) Control

DO control often includes Air, O_2 and N_2 alone or in combination to maintain the selected setpoint. Cascades allow the controller to maintain setpoints by automatically adjusting other process loops. Insect cells generally have a high demand for oxygen, so the inclusion of oxygen in a cascade may help to increase the overall culture density.

DO Calibration

The DO probes are calibrated using a standard two-point calibration method: 0 % (often referred to as Zero) and 100 % (commonly referred to as Span). The 0 % calibration can be achieved two separate ways: either with an electronic Zero, obtained by briefly disconnecting the cable from the control station, or by sparging nitrogen into the media at approximately 1 VVM (vessel volume per minute) until the value stabilizes near zero. The 100 % calibration is achieved by increasing agitation to approximately 200 - 400 rpm and increasing airflow to 0.5 - 1 VVM. These values may need to be adjusted to fit the operational specifications of your controller. DO should be calibrated after autoclaving and the probe should be allowed to polarize for approximately 6 hours after being connected to the controller. After calibration, DO may remain at approximately 100 % until after inoculation.

pH Control

pH control often uses the addition of liquid acid or gaseous CO_2 to lower the pH and the addition of liquid base into the solution to raise it, but for insect cell cultures there generally is little need to regulate using these methods.

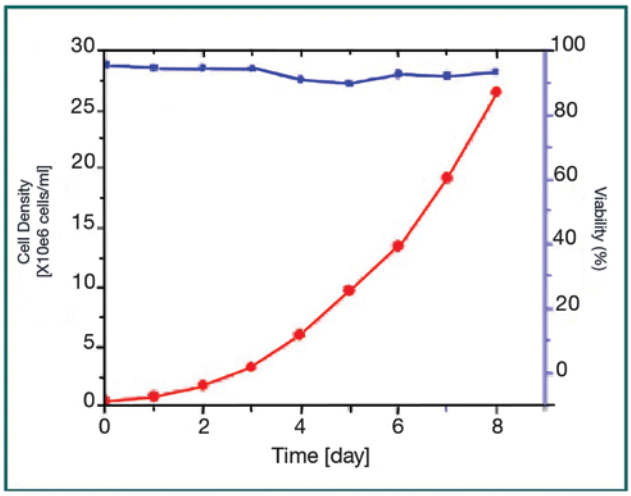
pH Calibration

pH calibration is usually done outside the vessel using a two-point calibration method and standard buffers. Buffer 7.0 is commonly used for the Zero and either 4.0 or 10.0 is commonly used for the Span. pH is calibrated prior to autoclaving.

Results

Depending on the process used and parameters selected, high density cultures can be achieved in as little time as 6 days. Typical cell densities are between 6 x 10^6 and 12 x 10^6 using a fed-batch method and up to 2.5 x 10^7 when using a continuous method.

The results shown below are from an 8-day culture using a spin filter and a continuous-culture method.



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