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Introduction to CHO Culture in a Stirred-Tank Bioreactor

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Abstract

This application report presents a simple protocol for achieving high-density culture of Chinese Hamster Ovary (CHO) cells using a New Brunswick™ benchtop, autoclavable stirred-tank bioreactor.

Introduction

Chinese Hamster Ovary cells, or CHO cells, are commonly used in biotechnology for protein production in the growing sector of mammalian cell culture. Mammalian cell culture has become increasingly popular due to the ability of Eukaryotic cells to achieve more complex post-translational modification of proteins.

Materials and Methods

Medium

Many pre-defined media are available for mammalian CHO cultures from companies such as Hyclone®, GIBCO®, JRH, Sigma. While options exist for media containing serum, the use of animal-component-free chemically-defined media is quickly becoming the industry standard.

Batch/Fed-Batch/Continuous

There are three ways which CHO cells are commonly grown. In a **batch method**, all the necessary medium and nutrients for the run are added to the vessel after sterilization and the vessel is inoculated by adding cells. The cells are allowed to grow until there is no more available carbon/nitrogen source and they begin to die. At this point the cells would either be transferred or harvested.

Fed-batch starts out similar to a batch method but the vessel is only partially filled with medium and nutrients, leaving room to add additional components. When the cells have consumed all available carbon/nitrogen sources, they are fed a supplement and they continue to grow. This is sometimes repeated several times.

In a **continuous system** cells are allowed to grow up to a certain density at which point the medium is removed and an equal amount of fresh medium is added. Continuous systems can often operate for very long runs, sometimes over a month.

Typical Control Setpoints for CHO

Setpoints for CHO should be entered prior to inoculation and, except for dissolved oxygen (DO) which often remains high, the medium should be allowed to equilibrate prior to inoculation. DO remains high because it takes time for the O_2 to permeate out of the medium as it is not yet being utilized by the culture. An initial DO value of approximately 100 % air saturation is acceptable; it will decrease as the cells metabolize it. Setpoints are commonly controlled in automatic mode.

Parameter	Setpoint
Temperature	37° C
pH	7.2
Dissolved Oxygen	35 - 50 %
Agitation	70 - 130 rpm
Gas Control	3 or 4 Gas
Inoculum	1.0 - 4.0 x 10^5

*Agitation ranges will vary depending on the vessel size and the type of impeller used.

pH/DO Control

Dissolved Oxygen (DO) Control

DO control is manually or automatically accomplished by adding various gasses into the medium to either raise or lower the DO. Air supplementation is most commonly used to maintain and raise DO. O_2 is sometimes supplemented if air is not capable of control alone. N_2 may be used to reduce the DO level of the medium.

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 medium 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 in animal cell culture is typically accomplished through a combination of liquid and gas additions. CO_2 is usually sparged into the medium as a means of lowering the pH, and addition of an alkaline solution, typically sodium bicarbonate solution, is often made to increase the pH. pH deadbands of 0.1 - 0.2 may also be used. These will reduce the volume of base being added.

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.

Impeller Choices

Many mammalian cell lines, and CHO in particular, can be grown using a wide variety of different impellers. Pitched blade and marine impellers are popular when growing suspension and anchorage-dependent cells in batch, fed-batch, or perfusion. By design, these impellers deliver excellent mixing while producing minimal shear. Filter or screen impellers such as the New Brunswick Spin Filter and Cell Lift are useful when growing cultures where cell-free medium needs to be removed, as either harvest or waste, while cells are retained within the medium. Our Packed-Bed Basket impellers use Fibra-Cel Disks as the cell attachment matrix and can produce very high cell densities, as cells grow tightly in or on the fibrous-bed material. This method is most commonly used when secretedproteins or viral particles are the desired product.

Culture Type	Process	Average Cell Density (cells/mL)
Suspension	Batch	2 - 4 x 10^6
	Fed-Batch	3 - 6 x 10^6
	Perfusion	10 - 15 x 10
Microcarrier	Batch	2 - 3 x 10^6
	Perfusion	
Packed-Bed Fibra-Cel Disks	Perfusion	

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