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Cultivation of Sf9 cells in the single use bioreactor BIOSTAT® RM 20 | 50 optical system

Application Note

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turning science into solutions

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Introduction

Insect cells like mammalian cells are able to produce eukaryotic proteins (i.e. posses the ability of post-translational modifications, protein folding etc). The most commonly used insect cell line is Sf9 (Spodoptera frugiperda ovary cells). These cells can be growth in suspension culture or adherent as monolayer. A variety of recombinant proteins can be obtained by utilizing the baculovirus expression system.
The BIOSTAT® RM optical (figure 1) is the most advanced single use bioreactor using rocking motion technology. The pillow shaped cultivation chamber is rocked back and forth, creating waves which provide mixing with low shear. The liquid surface is constantly renewed, thereby enabling efficient mass transfer between head space and media. The cultivation chamber itself is a single use bag composed of a multilayer film with pharmaceutical grade ethyl vinyl acetate (EVA) as the contact layer. This single use bags are easy to use, reduce validation costs, and remove the need for cleaning, sterilizing, and provide stress free convenient culturing.

In this application notes we tried to demonstrate the use of single use Cultibag RM system in cultivation of Sf9 cell line.

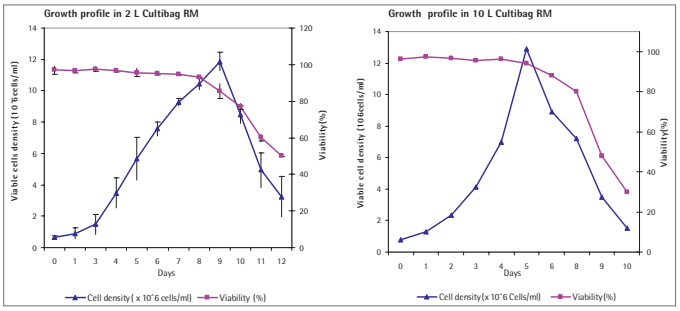
Figure 1:
BIOSTAT® RM optical single use bioreactor

Material & Methods

Spodoptera frugiperda (Sf9) cell line was procured from Invitrogen and cultivated in Serum free sf-900-II SFM media (GIBCO, 10902). The Cultibag RM 2 L Optical bag (DBO002L) was filled with 800 ml of media under aseptic condition and the bag was inflated with air. Thereafter DO and pH sensors were inserted in respective ports and equilibrated for 2 hrs at 8 rocks per minutes. DO and pH sensors were calibrated as per manufacturer instructions. The pre culture was seeded in the Cultibag at seeding density of 0.7 x 10^6 cells/ml. Temperature was set to 28°C, DO to 60%, pH to 6.2. Rocking was varied from 18 to 25 rocks/minutes and an angle of 6°.
5 ml of sample was collected every day to determine viable cell density by trypan blue exclusion method and glucose and lactate estimation in the spent media. (Enzyme based assay kit from Chema diagnostic kit for Glucose assay and Radiant kit for Lactate assay was used).

Results

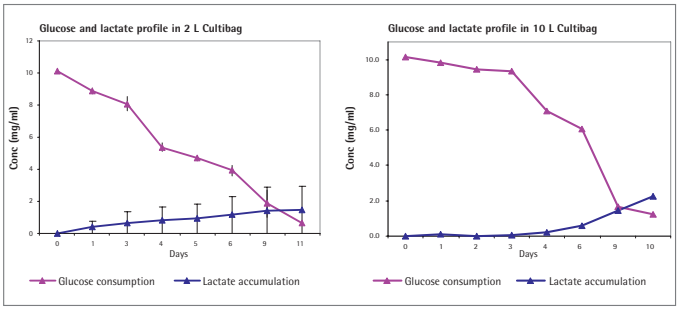
The maximum cell density obtained was 11.87 × 10^6 cells/ml at 9th day of cultivation (average of three consecutive trials). Cell viability decreased from 9th day of cultivation and dropped to 50% till day 12. Glucose was consumed from 10.2 gm/l to 4 gm/l during cultivation and lactate accumulation was 3 mg/ml at the end of cultivation. Temperature was maintained at 28°C constantly and pH around 6.2. Further the process was scaled up and cultivation was done in 5 L media in 10 L Cultibag RM. The peak cell density was 12.9 × 10^6 cells/ml similar to 2 L Cultibag RM.



Graph 1:
Growth curve of Sf9 cell line in 2 L and 10 L Cultibag RM

Discussion

DO was maintained at 60% through out cultivation period. The variation in DO was ± 5%. DO was maintained by using nitrogen, gas flow and oxygen in cascade mode. DO was maintained primarily by air. Oxygen was required to maintain DO after the cell reaches the exponential log phase. The evaporation of media can also be curtailed by intermittent supply of oxygen instead of continuous supply of air. The pH was maintained at 6.2 by using 0.1 N alkalis ($NaHCo_3$) and CO_2 in cascade mode. CO_2 is not necessary at the start of the cultivation but can be used in toward the end of cultivation when pH tends to rise as result of toxic accumulation.



Graph 2:
Glucose and lactate profile of Sf9 cell line in 2 L and 10 L Cultibag RM

Rocking Speed

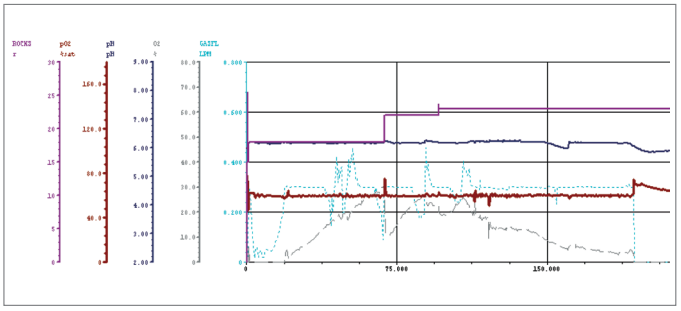
The rocking speed is dependent on both the culture volume and cell density. At low volumes i.e. 20 – 30% of the Cultibag working volume, an initial rocks of 15 is sufficient for mixing. It would be necessary to increase the number of rocks to 18 – 25 at 100% of Cultibag working volume or at high cell density. It is advisable to monitor the oxygen demand and adjust the rocks and rocking angle as needed. Rocking speed of 18 to 23 was suitable for cultivation of Sf9 in 2 L and 10 L Cultibag RM systems.

Rocking Angle

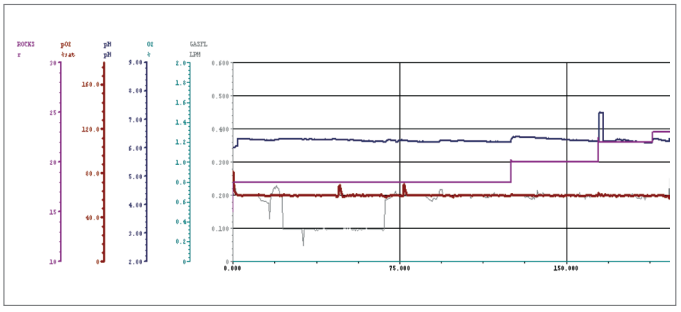
An initial angle of 6 degrees is sufficient. In general the angle is increased as the oxygen demand increases. When the Cultibag is at 100% of working volume, an angle of 6 – 10 degrees may be needed. An angle of 6° was suitable for cultivation in 2 L and 10 L Cultibag RM. The angle of rocking can be reduced when excessive foaming is observed.

Aeration Rate

DO is controlled by using nitrogen, air and oxygen in cascade mode. The controller for air flow was set maximum of 30% and minimum of 5%, corresponding to flow rates between 50 and 300 ml/min. Nitrogen and oxygen controller limits were set to 100%. The required set point of 60% is maintained through out the cultivation run.



Graph 3:
History plot of Cultivation of Sf9 cell line in 2 L Cultibag RM



Graph 4:
History plot of Cultivation of Sf9 cell line in 10 L Cultibag RM

Conclusion

In this note, we have demonstrated that the single use bioreactor BIOSTAT® Cultibag 20 RM is suitable for the cultivation of sf9 cell lines in serum free chemically defined media. Insect cell lines have higher oxygen demand compared to mammalian cell lines and Cultibag RM is able to support their growth. Single use bioreactors are preferred in today's cutting edge applications in R&D, process development and small scale production. Single use bioreactors remove the requirement for cleaning validation and require minimum maintenance while providing maximum operator ease of use.

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