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Application note

Sf9 Insect Cell Culture in the Multitron Cell with ShakerBag option

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1. Introduction

Sf9 insect cells are a clone of the Sf21 cell line that originates from ovarial tissue of the moth, *Spodoptera frugiperda*. The significance of Sf9 in the production of recombinant proteins is increasing steadily, especially in combination with the baculovirus expression vector system (BEVS). BEVS can be used to infect cells with baculovirus, which is a harmless virus to humans that transfers the gene sequence of the protein to the cell and makes the cell produce the protein of interest. The cell growth of Sf9 can be enhanced by a high glucose concentration such that fed-batch cultivation is recommended.

The Sf9 cell line is easy to cultivate. Like mammalian cells, insect cells show more tolerance with respect to degradation products and respond more sensitive to shearing stress, which makes them optimal to cultivate by gentle orbital shaking in disposable culture bags.

The following example demonstrates the cultivation of the Sf9 cell line in 2 L disposable culture bags in the Multitron Cell incubation shaker with ShakerBag option (INFORS HT, CH-Bottmingen).

2. Technical specifications of the Multitron Cell

- ShakerBag option
- 50 mm shaker throw
- Direct aeration with air
- Cooling
- Different trays available (for disposable bags, with «Sticky Stuff», with flask clamps for shaker flasks, etc.)

3. Experimental specifications

The Sf9 insect cells were cultivated in disposable bags of 2 L total volume (1 L working volume/WV). The inoculation concentration came within the range of 1.0 x 10^6 cells/mL. The minimum volume for fed-batch cultures in large disposable bags amounted to 25% of the maximum working volume. The culture conditions were selected with a temperature of 27°C and a pH about 6. The Sf9 cells were supplies with air using the gassing station.

4. Fed-batch cultures

a) Media

The cultivation in the 2 L disposable bag was set up with a correspondingly adapted feeding strategy of GIBCO Sf-900 II SFM (Invitrogen) serum-free and already mixed with Pluronic F-68 and glutamine media. The media is exclusively developed for insect cell cultivation and features a high glucose concentration.

b) 2 L disposable bag

The inoculation of the 2 L disposable bag takes place with a cell concentration of 1 x 10^6 cells per mL with a viability of > 75% in 300 mL SF-900 II SFM medium. Cultivation was carried out over a period of ten days altogether. On the first day, feeding with 200 mL took place and so leading to an increase in the final volume to 500 mL. Additionally, on the second and fifth day 200 mL were added and on the sixth day a further 100 mL of the Sf-900 II SFM medium was introduced to reach the working volume of 1 L.

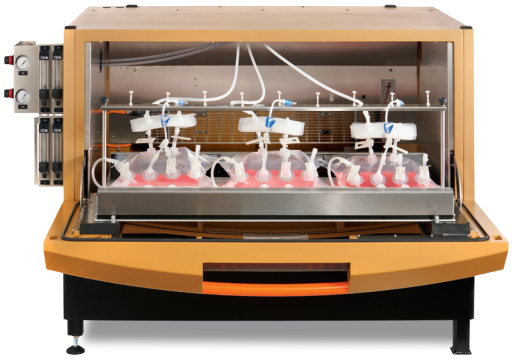

Fig. 1: Multitron Cell with ShakerBag option for 3 x 2 L disposable bags

For the supply of oxygen, air was gassed up to 1 vvm directly in to the disposable bag and thus the cells were continuously provided with gas.

The insect cells were shaken at a speed of 35 rpm.

5. Analysis

a) Parameter analysis

The daily determination of the viable cell concentration was performed using the Cedex (Innovatis). The analysis of growth and production substrates was accomplished using the Bioprofile Analyser 100 (Nova Biomedical).

b) Formulae

For calculation of the maximum growth rate μ_{max} and the doubling time t_d the formulae shown below were used.

$$\mu_{max} = \frac{\ln(x2) - \ln(x1)}{t2 - t1} \left[\frac{1}{h}\right] \qquad t_d = \frac{\ln(2)}{\mu_{max}} [h]$$

6. Analysis of results

For the process development of the cultivation, Sf9 insect cells were incubated in 2 L disposable bags for several days in the Multitron Cell incubation shaker with ShakerBag option. While the cultivation of Sf9 insect cells took place, a daily sample made measurements possible for the optimum comparison of cell concentrations and substrate consumption as well as the formation of metabolites.

Until reaching the maximum working volume of 1 L, the daily gradual feeding with fresh medium permitted cell growth of the Sf9 cells with a maximum viable cell concentration of 1.3 x 10^7 cells per mL and a viability of > 85% by the seventh day of cultivation. Glucose consumption was observed compared to cell growth and reached a limited concentration on day nine. Altogether, the cultivation was finished on day 10, after the consumption of the substrates and a cell viability below 70% (Fig. 2).

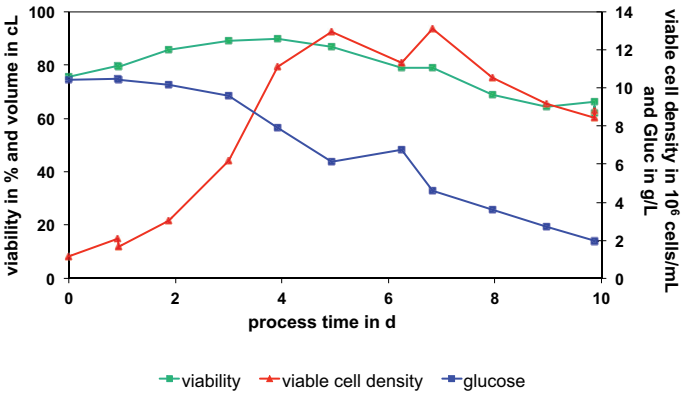

Fig. 2: Comparison viable cell density and viability, glucose and volume

The maximum growth rate μ_{max}, which could be achieved, was 0.6038 per day and the doubling time t_d was 27.55 h. The maximum cell concentration was measured at 1.3 x 10^7 per mL and had a viability of > 85% (Fig.3).

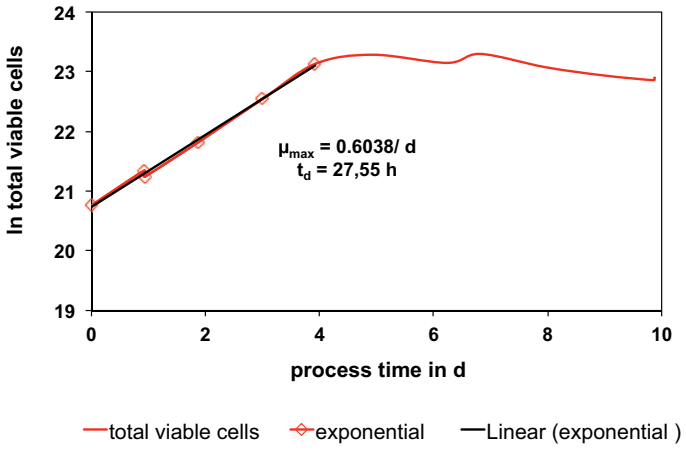

Fig. 3: Growth rate and doubling time

Glucose and glutamine concentration are shown in Figure 4. Both substrates serve as energy sources for the Sf9 insect cells and are consumed during growth. Parallel to the decrease of glucose and glutamine, the metabolites, ammonium and glutamine, will be produced by the cell. With increased concentration both, ammonia and glutamate, might get toxic for the cells. In presence of ammonia, glutamine can be build, which can explain the decreased ammonia and the static glutamin concentration (Fig. 4).

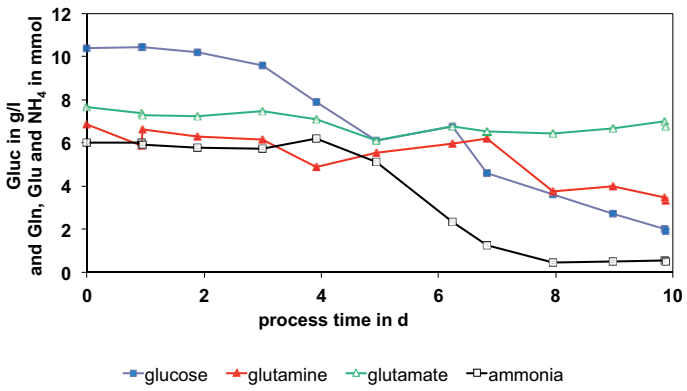

Fig. 4: Metabolites during Sf9 cell cultivation

7. Summary

- The maximum cell concentration achieved of 1.3 x 10^7 per mL with a viability of > 85% was found in the 2 L disposable bag with 1 L working volume.
- An optimised substrate supply can be achieved with a suitable feeding strategy.
- The shaker throw of 50 mm permits a gentle shaking of the cell culture.
- A single unit of Multitron Cell can be used for cultures varying from 10 mL to 10 L working volume. Thus, expansion from the starter cultures to small-scale production in only one equipment becomes possible.

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