nucleo™ Application note

Tests performed by Henogen

1. Scale-up of a cell culture (preclinical and clinical lots) from a 20L working volume (NBS bioreactor) to an 80L working volume (Nucleo™-200 disposable bioreactor) - Additional tests on the bioreactor with a maximum working volume.

2. Test on a preculture at low & high densities in batch & perfusion mode (with ATF4 system) with a minimum working volume.

Abstract

Production of recombinant proteins with a CHO-based cell line using the Nucleo™-200 disposable bioreactor is compared to production with a classical stainless steel bioreactor. The results are very similar in the configuration of tests carried out. Furthermore, the performance of the Nucleo-200 disposable bioreactor, assessed first in batch mode, and then in perfusion mode during preculture is very satisfactory. The different parameters and results described in this application note quantify the obtained performances.

Objectives

Run 1: The tests first involved comparison of culture results at a 20L working volume with those obtained at a scale of 80L, respectly performed with a New Brunswick Scientific stainless steel bioreactor and the Nucleo-200 disposable bioreactor.

The next objective was to study the performance of the Nucleo disposable bioreactor with a working volume taken up to 180L.

Run 2: In certain applications, the perfusion cell culture proves to have various benefits compared to traditional methods. Moreover, the type of process equipment also significantly affects efficiency. In an attempt to optimize performance, the Nucleo-200 disposable bioreactor created by ATMI® LifeSciences and Pierre Guerin Technologies® represents an interesting alternative. Consequently, tests were carried out to assess the reaction of the Nucleo-200 disposable bioreactor, particularly with a minimum volume, at low and high densities in batch and perfusion mode.

NUCLEO™ Disposable Bioreactor
ATMI® LifeSciences &
Pierre Guerin Technologies®

Materials & Methods

- Test performed on preclinical and clinical lots
- CHO-based cell line

New Brunswick Scientific stainless steel **NBS** bioreactor
Culture scale 20L

- Stirred by a water propeller
- Diffuser underneath the propeller
- Temperature controlled by the bioreactor regulator

NUCLEO-200 Disposable Bioreactor ATMI LifeSciences - Pierre Guerin Technologies
Culture scale 80L and 180L

- 200L cubic bag housed in a jacketed container (control through circulating water)
- Temperature control outside the bioreactor (independent cryostat)
- Paddle system agitation – rotational speed: 45 rpm – tilt angle: 12.5°
- Sparger device mounted on the paddle (dispersion with motion-tracking)
- Dissolved Oxygen set point: 35%
- Continuous surface air flow: 20-50L/h
- Run 1: Oxygen flow = max 300L/h – Run 2: Oxygen flow = max 50L/h
- Pressure in bag: less than 0.05 bars

For run 2: perfusion with ATF4 system (Alternating Tangential Flow) from Refine Technology LLC

Experimental: run 1

Figure 1 shows the change in viable cell density over time for both reactors. During the first four days, the working volume was 20L for the NBS reactor and 80L for the Nucleo disposable bioreactor. The viable cell density growth obtained with each of the two bioreactors is very similar.

After day 4, the test is continued with the Nucleo disposable bioreactor only, taking the working volume up to 180L. Through a dilution effect, the value starts low and consistently increases over 3 days. During this second phase, the rate of increase may be compared to the increase initially obtained at the 20L scale. The viable cell density then continues to progress more moderately. Figure 2 illustrates the cumulated PDL variation for each of the two bioreactors. The two curves can almost be superimposed. As of day 4, the results obtained with the Nucleo disposable bioreactor at the 180L scale continue to evolve in the same way. The graph in figure 3 traces the viability rate expressed in percentage terms. As

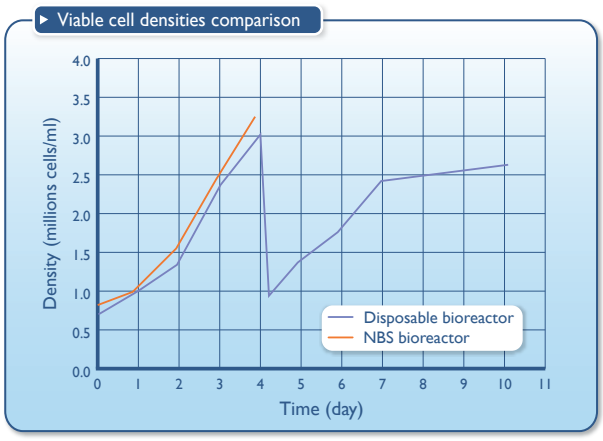

Figure 1.

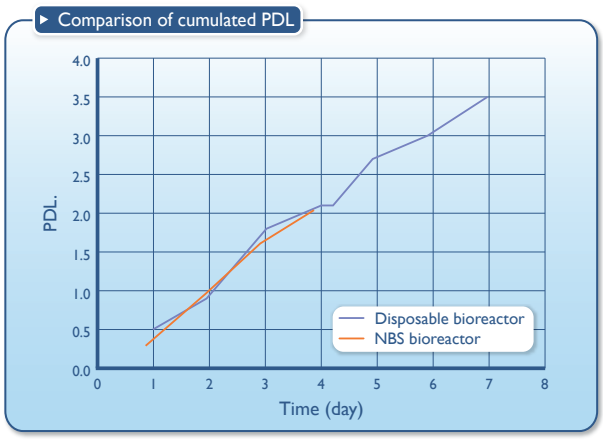

Figure 2.

was the case before, similar results are obtained for both systems during the first four days. For the next period, the values fluctuate slightly within a rather narrow interval, to decrease in a linear manner as of day 7. At inoculation, the cellular density was identical in both reactors. The NBS bioreactor kept the culture at a constant temperature of 33° C. It can be noted that the temperature of the Nucleo disposable bioreactor could have been more efficiently controlled with a better capacity adaptation of the thermostatic bath used for these tests.

Figure 4 consolidates the evolutive curves of the metabolite levels for the Nucleo disposable bioreactor.

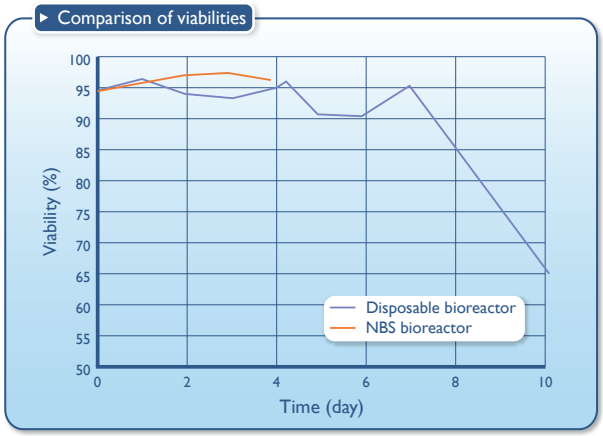

Figure 3.

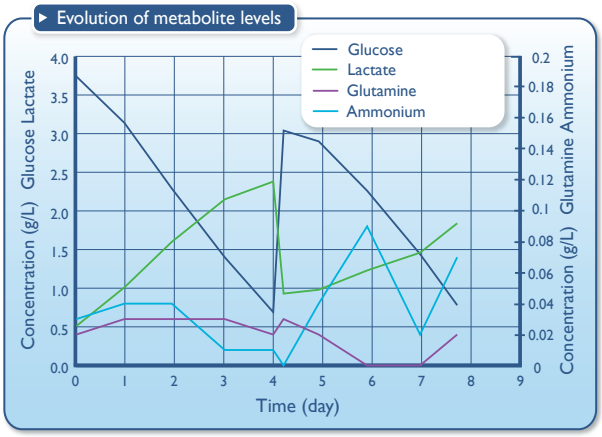

Figure 4.

Figure 5 illustrates the growth of the protein production level from day 3 to day 4. The curves evolve in a similar manner for both reactors. As of day 4, the dilution effect involved by the increase in volume reduced the concentration rate. From this value, the production level of the Nucleo disposable bioreactor, taken up to the 180L scale, triggers an increase that is maintained over several days. This progression rises sharply after day 8 to reach 155 µg/mL.

Thus, growth during the first four-day phase following inoculation and the production level achieved with both bioreactors are equivalent.

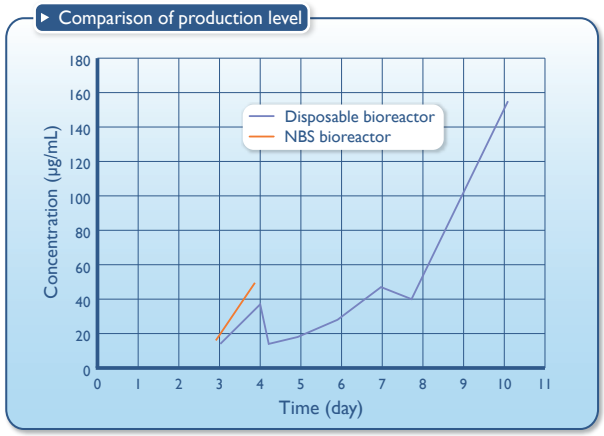

Figure 5.

Experimental: run 2

The second test was carried out in two phases. First, the cells were cultured in batch mode over 2 days. Then the process was continued in perfusion mode for the next three days, using the ATF4 system. The working volume of the Nucleo disposable bioreactor used at inoculation was 70L and the test was carried out at a temperature of 37° C.

ATF™ System (Alternating Tangential Flow) from Refine Technology LLC

A low Shear System

> Low shear diaphragm pump (instead of recirculation pump) involves a low shear action which does not damage the cells

>The system is simple to operate and directly scales for manufacturing

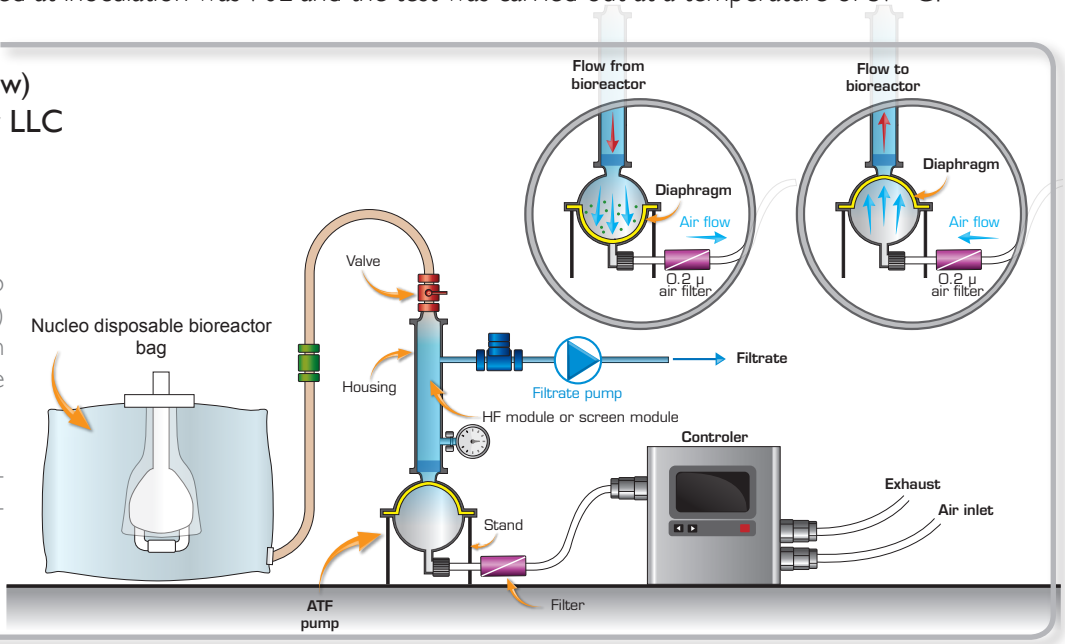

figure 6 illustrates the curved progression of the viable cell density and the associated increase in perfusion rate over time (in ratios of one, one and a half and finally two volumes). The perfusion step allowed increasing the cell density to over 12 million cells/mL. The graph in figure 7 shows that the cell viability percentage remains above 92% during all of the tests. The continuous evolution of the cumulated PDL is practically rectilinear. Finally, figure 9 shows that lactate production achieves 2 g/L during the perfusion mode phase (the volume, affected by the perfusion, fluctuated between 70L and 90L., the density is summarized by the graphs without any volume correction).

Consequently, it is clear that the Nucleo disposable bioreactor reacts perfectly during preculture at minimum volume, be it at low or high density.

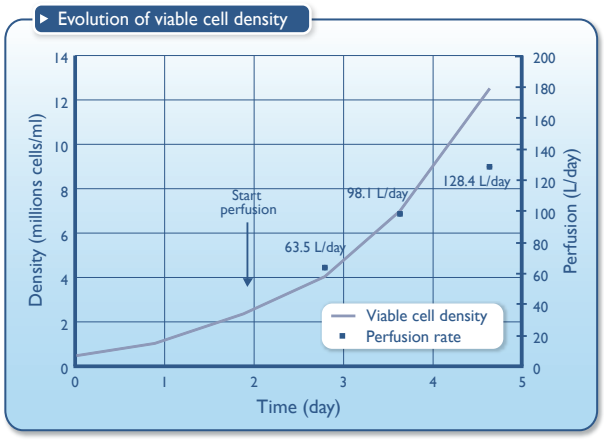

Figure 6.

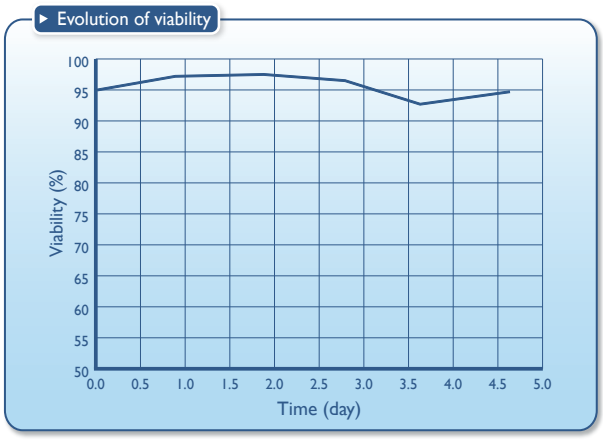

Figure 7.

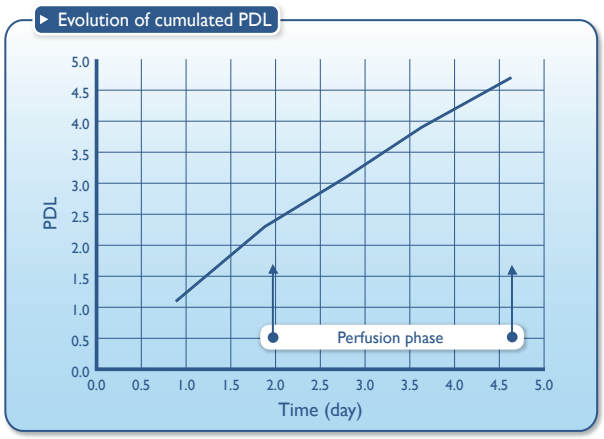

Figure 8.

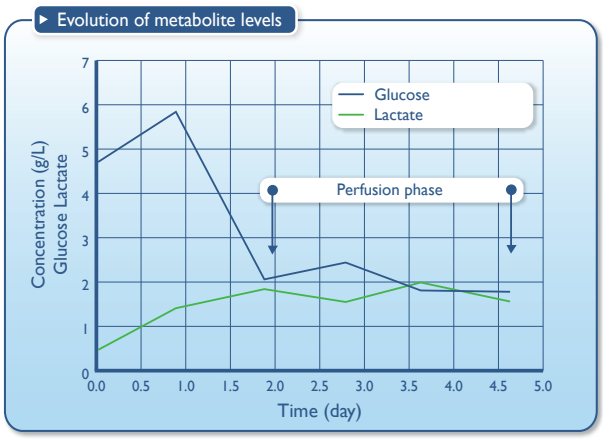

Figure 9.

Conclusion

The Nucleo disposable bioreactor, when used to produce recombinant proteins with a CHO-based cell line, achieves results that are comparable to those obtained through the use of a traditional stainless steel reactor, while providing the technical and economic advantages of a single-use system. The scale-up is also very conclusive and demonstrates the flexibility of the system. The resulting performance is essentially based on the inherent characteristics of the bioreactor, such as its unique agitation system that incorporates a sparger. These various tests, carried out in practical examples, demonstrate the performance of the Nucleo disposable bioreactor, which was tested in different configurations to meet various existing and future needs...

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