Project #: CellTumbler (CF-C-10-42-008); WAVE Bioreactor, historic data (CF-C-09-19-008)

Project Name:

Stably transfected CHO-K1 culture in CellTumbler in 5 L scale

Purpose:

The aim of the project is to test the CerCell CellTumbler platform using wave bags from GE Healthcare and monitor growth performance for a batch culture of a stably transfected CHO-K1 culture line. Data from the CerCell CellTumbler platform is compared with historic data from the GE Healthcare WAVE Bioreactor System.

Materials and Methods:

The CerCell CellTumbler platform (Kit#13 from CerCell) was used in the tests and consisted of a twin platform with individual temperature control but common drive-unit. A WAVE Bioreactor Systems 2/10 (Base 2/10 EH) from GE Healthcare was used for the historic data. A Gas-Unit for air/CO_2 supply from GE Healthcare was used for oxygen supply and pH control. Wave bags with a working volume of 5 L were from GE Healthcare and aerated with a flow of 0.1-0.2 L/min with CO_2 % between 5 and 10%. Rocking speed was maintained at 22 rpm and the temperature was logged manually twice a day and was maintained at 36.8 $\pm$ 0.2°C.

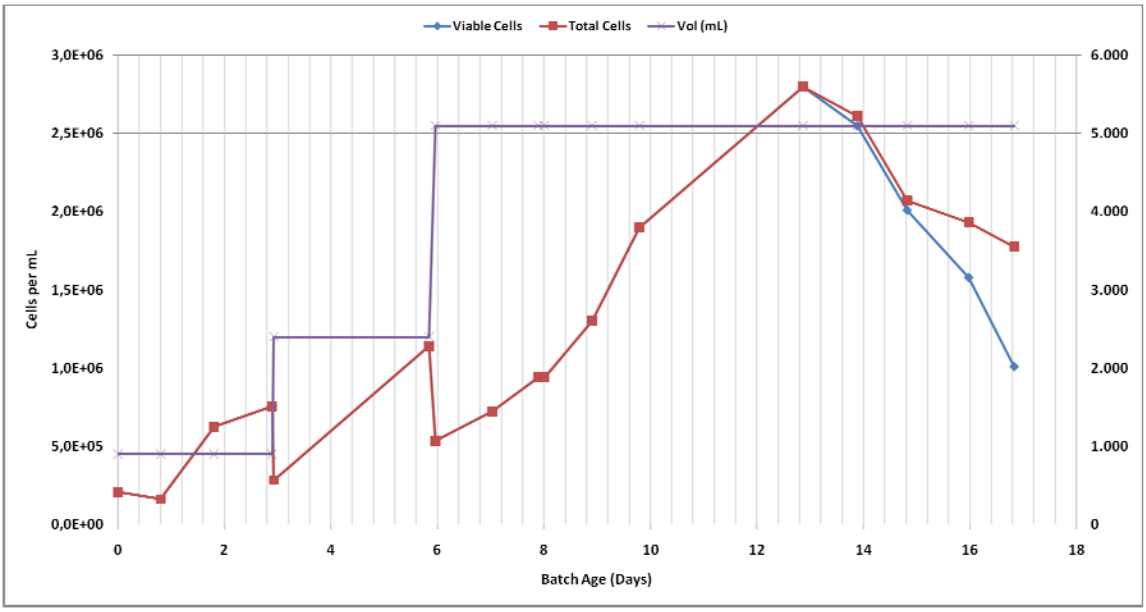
A stably transfected CHO-K1 cell line expressing a non-disclosed *in-house* human receptor fragment was adapted to grow in suspension in Hybridoma-SFM, 2 mM Glutamax; 100 U/ml penicillin/streptomycin ; 0,1 % pluronic acid (all from Invitrogen) using standard tissue culture techniques. Following adaptation the cells were expanded in culture flasks until enough cells were available to seed a wave bag (GE Healthcare) mounted on the CerCell CellTumbler platform. Growth performance was monitored by daily sampling and cell were expanded until a final culture volume of 5.1 L. Cell were stained with 0.4% Trypan Blue Stain and non-viable and viable cells counted in a hemocytometer under a microscope.

Results:

Following expansion in culture flasks the CHO-K1 seed culture of 100 mL was transferred into 800 mL preheated complete CD Hybridoma Medium to a final volume of 900 mL. At this point the cell viability was ~100% and had cell density of 2.09 x 10^5 cells/mL. During the expansion phase the cells were kept in log-phase and were diluted during the following days to maintain the cells in the log-phase (*Figure 1*).

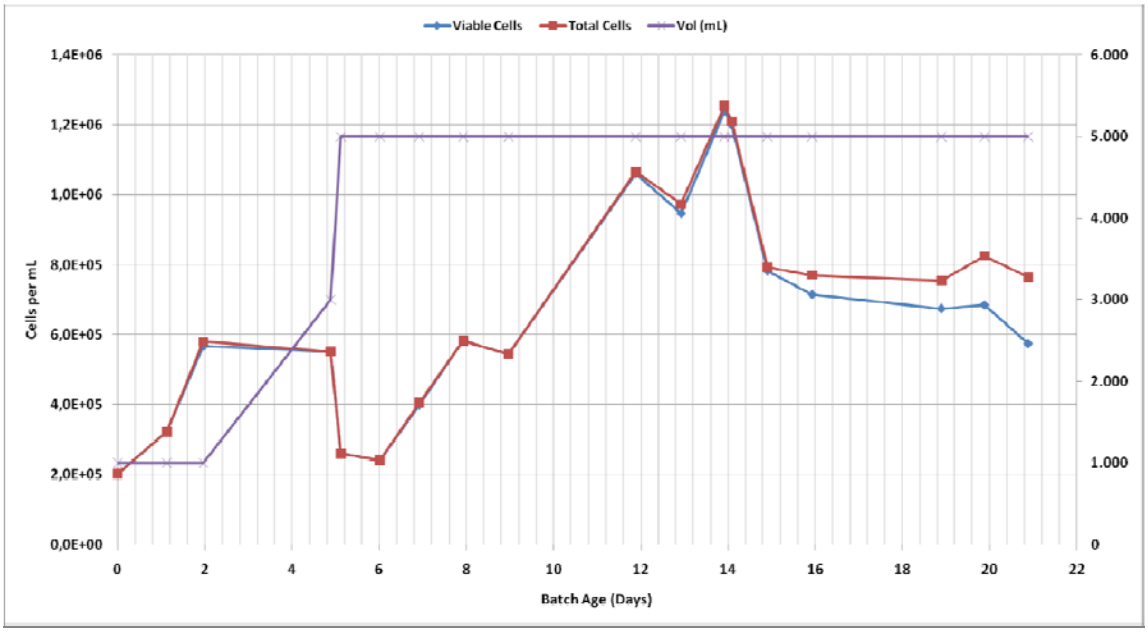
After 6 days the final volume of 5.1 L was reached after which the culture was maintained in batch mode until the viability has decreased below 60%. At this point the titer of the receptor fragment is usually at its highest without cell death affecting the product quality (data not shown).

Figure 1: Cell expansion plot (CellTumbler)



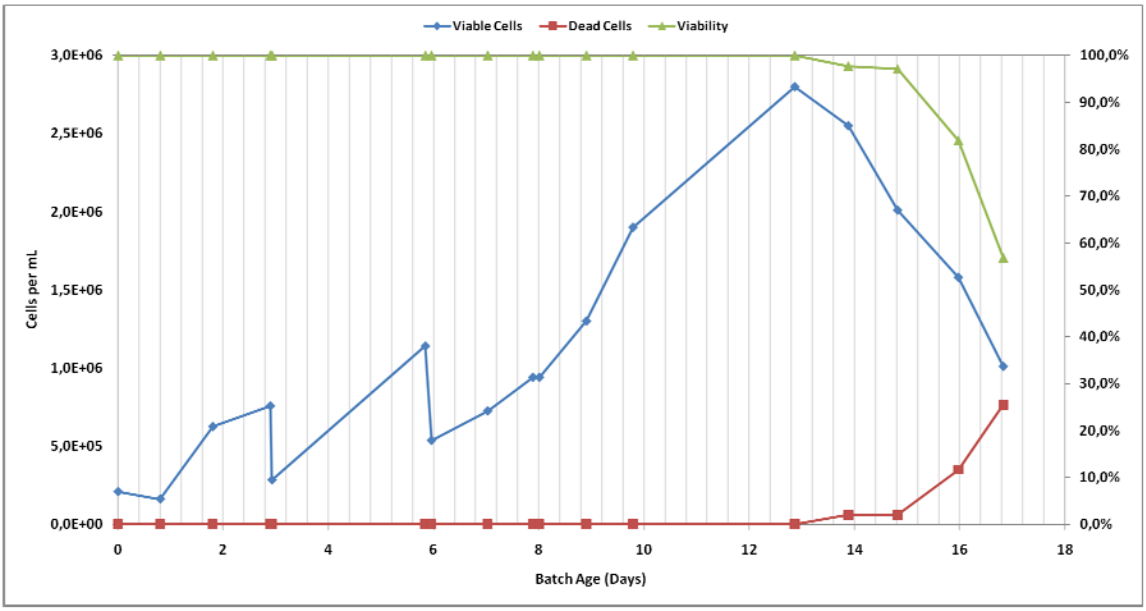
Below in figure 2 data from a similar run on a WAVE Bioreactor system from GE Healthcare using the same cell line and expansion plan. At the seeding point the cell viability was ~100% and had cell density of 2.02 x 10^5 cells/mL. During the expansion phase the cells were kept in log-phase and were diluted during the following days to maintain the cells in the log-phase (*Figure 2*). However, unlike the Cell Tumbler run above a single injection of a glucose feed was done at day 14.

Figure 2: Cell expansion plot (WAVE Bioreactor, historic data)



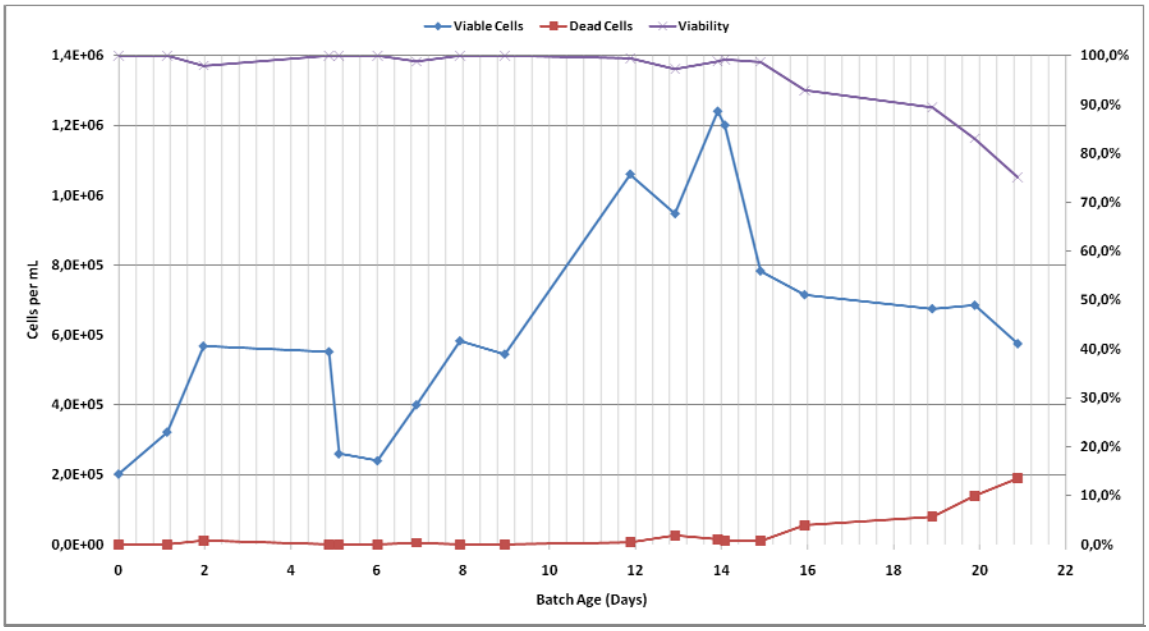
The viability of the CHO-K1 cell culture is maintained above 98% for 14 days where after the medium becomes exhausted and the cell density start to decrease. (*Figure 3*)

Figure 3: Viability plot (CellTumbler)



The viability of the WAVE bioreactor system CHO-K1 cell culture is maintained above 98% for 15 days where after the medium becomes exhausted and the cell density start to decrease. However the effect of the glucose injection at day 14 prolongs the death-phase until day 21 (*Figure 4*)

Figure 4: Viability plot (WAVE Bioreactor, historic data)



In comparison there is little difference between the two runs until day 14, after which they differ due to the addition of glucose to the WAVE bioreactor system.