Title: **Growth of CHO cells in wave bags**

1. Aim

Batch experiment – growth of CHO cells in shakers, and two wave bag systems: 1) A commercially available widely used Wave system and 2) CerCell CellTumbler in order to do:

- Comparison between two wave bag systems
- Comparison between wave bag systems and shaker culture

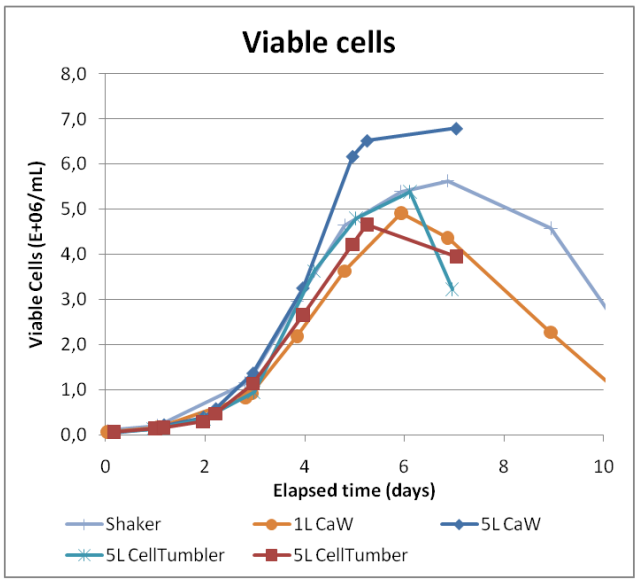
2. RESULTS

Batch cultivations using CHO cells were carried out in:

- Shaker (90 ml working volume)
- 1L bag using a Commercially available cell culture Wave system (1L CaW)
- 5L bag using a Commercially available cell culture Wave system(5L CaW) and
- Two 5L bags using CerCell's Cell Tumbler platform

All cultivations in wave systems were done using GE Cell bags.

The results of the viable cell density measurements from the experiments (Figure 1) show that the cells grow quite similarly in the different systems with respect to growth rate, peak viable cell density and cultivation length. This shows that the growth properties are very similar in the different systems. The high cell density measured in 5L CaW from day 5 and forward was not observed in other runs and most likely represents an analysis outlier.



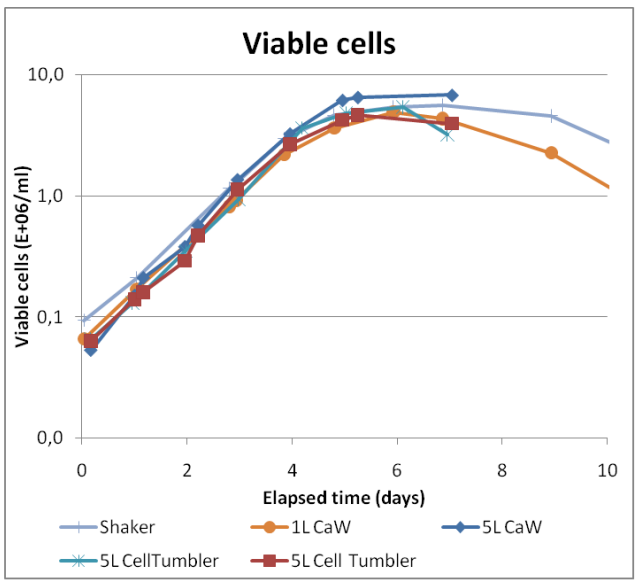


Figure 1. Viable cell density. Maximum viable cell densities are similar (left). Logarithmic curve (right) show similar growth rate.

Viability curves (Figure 2, left) are very similar in all systems showing that the systems have similar effect on the cells. The increase in NH_4^+ in the cultures with time is also similar (Figure 2, right) as well as other parameters (glucose, L-glutamine, lactate and osmolarity profiles; data not shown).

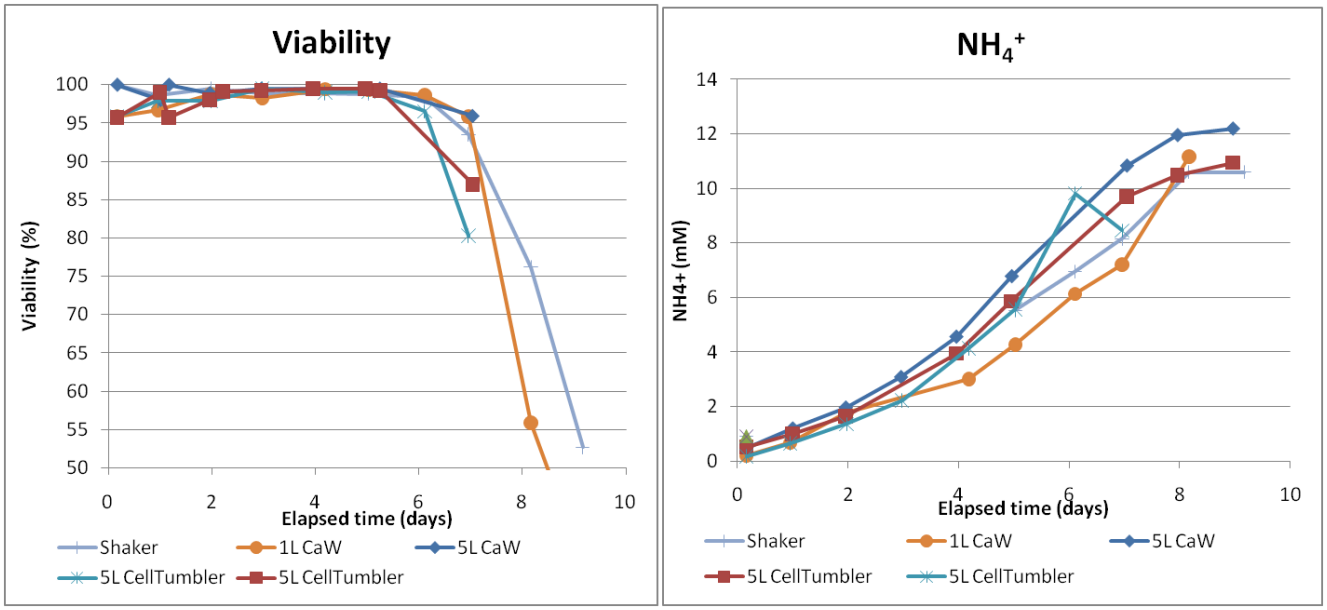


Figure 2. Viability and ammonium. Viability curves are very similar in all systems (left). NH_4^+ (right) measured by BioProfile.

3. PROCEDURE

3.1 Cultivation systems

Commercially available cell culture wave system (CaW) and CerCell's CellTumbler platform (Kit#13 on http://cercell.com/products/celltumbler/products/configurations) were used for the tests (CellTumbler, Figure 3). The CellTumbler platform consisted of: The aluminium frame fitted with two platforms; the Drive-Unit; The Heat-Control-Unit with independent channels for two platforms and a Gas-Unit for air/CO2 supply.

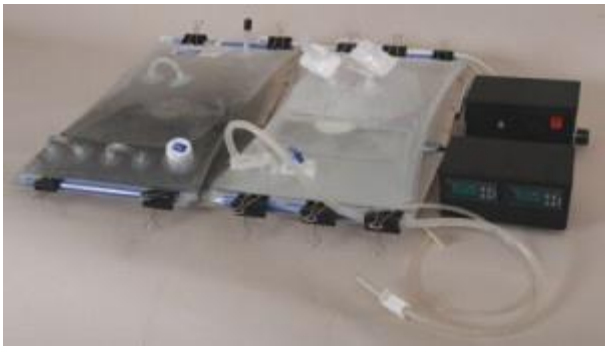


Figure 3. CellTumbler platform. CerCell's CellTumbler 2x5L fitted with Cell Bags (GE) used in the experiments.

3.2 Seed train

Parental untransfected CHO cells were expanded in growth medium in shaker flasks at 140 rpm in an incubator with 5% CO_2 at 37°C. Cells were passaged 3 times a week and seeded to 0.07E+06 viable cells/ml for 3-day passages and to 0.15E+06 viable cells/ml for 2-day passages.

Table 1. Growth medium.

Growth medium		Cat. No.	Lot no.	Mix ml	Working Conc.
Freestyle CHO medium	Invitrogen	12651-014	773409	1000	-
L-Glutamine	Invitrogen	25030-024	751507	40	8 mM

3.3 Setup in batch shaker

The 500 ml shaker was supplied with pre-heated growth medium and inoculated with cells to the inoculum viable cell concentration target. After this the shaker was moved to a shaker platform at 140 rpm in an incubator with 5% CO_2 at 37°C.

3.4 Setup in batch wave bags

1L and 5L wave bags were supplied with growth medium and then preheated and the gas flow was started at 0.1 L/min and 5% CO_2. After stable conditions the bags were inoculated with cells to the inoculum viable cell concentration target.

Table 2. Cultivation settings.

Parameter	**1L (CaW)**	**5L (CaW)**	**5L Cell Tumbler**	**500 ml shaker (90 ml volume)**
Rocks pr. min	37	35		N/A
Shaker angle	9°	9°		N/A
Aeration (lpm)	0.1	0.1		N/A
CO2 addition (%)	5%	5% - 0% day 5		5%
Temperature (°C)	37	37		37

Inoculum viable cell conc. target (E+06 viable cells/ml)	0.07	0.07	0.07
Rpm	N/A	N/A	140 rpm
Cultivation vessel	GE HealthCare Cell Bag: CB0002L10-02	GE HealthCare Cell Bag: CB0010L10-02	Corning shaker flask w. 0.2µm vent cap, 431145

3.5 Sampling

Cell counts were performed on a ViCell XR cell Counter, an ABL-5 was used for analysis of CO2, offline pH and O_2 and a BioProfile 100plus was used for metabolite measurements.