GE Healthcare
Life Sciences

# High Performing Fed-Batch Culture in a WAVE Bioreactor™ system using the ActiCHO Media System

*Thomas Falkman, Eric Fäldt and Anita Vitina*

*GE Healthcare Bio-Sciences AB, Björkgatan 30, SE-751 84 Uppsala, Sweden*

## Introduction

Higher yields, more potent compounds, smaller batch sizes and the cost pressure on R&D budgets push the production of biopharmaceuticals towards single use bioreactors. Especially simple cultivation systems like the WAVE Bioreactor can be an attractive platform for the production of e.g. monoclonal antibodies by high cell density fed-batch processes.

We have developed a high cell density fed-batch process based on an IgG1 producing CHO cell line and the ActiCHO Media System (PAA) platform in conventional stainless steel bioreactors up to 100-L cultivation volume. The process yielded in maximum viable cell densities in the range of 20 MVC/ml and product titers in the range of 4 g/L. The process was then transferred to a WAVE Bioreactor system (Fig. 1 and Fig. 2) and the process performance was evaluated in both 10-L and 100-L scale.

**Fig 1.** WAVE Bioreactor 20/50 with WAVEPOD II.

**Fig 2.** WAVE Bioreactor 200.

## Materials and Methods

WAVE Bioreactor 20/50 and 200 systems (GE Healthcare)

WAVEPOD™ II (GE Healthcare)

Cellbag™ 20L and 200L with optical pH sensor (GE Healthcare)

100-L stainless steal STR Bioreactor (Belach Bioteknik AB)

CHO-DG44 cell line expressing monoclonal antibody IgG1

ActiCHO SM medium (PAA) supplemented with 6 mM L-Glutamine

ActiCHO P medium (PAA) supplemented with 6 mM L-Glutamine

ActiCHO Feed A and Feed B medium (PAA)

UNICORN™ DAQ 1.0 software

Nutrients and physical parameters were measured off-line using the Bioprofile Flex (Nova Biomedical). Cell density and viability were measured using CASY Cell Counter and Analyzer (Roche) IgG titers were determined with MabSelect SuRe™ analytical chromatography and Biacore™.

- Cells were inoculated at 0.3×10e6 cells/ml in production medium (ActiCHO P).
- Feeding of Feed A and Feed B started at day 3 post-inoculation with 43.2 g/L/day and 4.32 g/L/day, respectively.
- Feeding of glucose started at day 6 post-inoculation and was carried out to maintain glucose level at 6 g/L.

## Bioreactor settings

| Unit | WAVE Bioreactor 20/50 | WAVE Bioreactor 200 | Stainless steal STR 100 L |
|---|---|---|---|
| Culture volume | 6-10 L | 60-100 L | 60-100 L |
| Mixing | 22-29 rpm and 6° | 12-19 rpm and 6° | 50-62 rpm |
| Gasflow overlay | 0.15-0.3 L/min | 3-4 L/min | 2 L/min |
| Gasflow sparger | N/A | N/A | 0-5 L/min |
| pH (control by $CO_2$) | 7.0 | 7.0 | 7.0 |
| DO | 60 % air sat. | 60 % air sat. | 60 % air sat. |
| Temperature | 36.8 °C | 36.8 °C | 36.8 °C |
| DO control | $O_2$ enriched air | $O_2$ enriched air | Air, nitrogen and $O_2$ |

## Results

Three fed-batch runs were started to show the stability of the process and the rigidity of the medium platform. In Fig. 3 it is clearly shown that cell growth and viability were comparable between the three sequential runs (red). The IgG production levels were also very consistent and were continuously increasing to as high as 5.7 g/L. It became apparent, that cell growth and viability were higher in the WAVE Bioreactor 20/50 than compared to the STR Bioreactor. IgG production was, however, unaffected by the type of process unit being used.

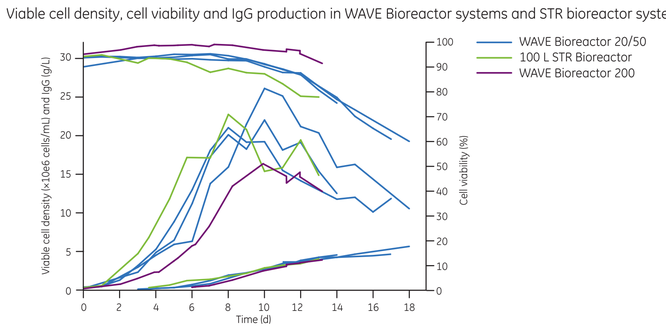


**Fig 3.** Graph illustrating cell growth, cell viability and IgG titers of mAb5 producing cell line over time in WAVE Bioreactor 20/50, WAVE Bioreactor 200 as well as in 100 L STR bioreactor. The growth and production charactheristics between the different runs were close to comparable except for a notable increase in cell viability in the WAVE Bioreactor systems.

The process was successfully transferred to a WAVE Bioreactor 200 system showing comparable viability to WAVE Bioreactor 20/50 but slightly lower cell growth. The IgG production rate in the WAVE Bioreactor 200 was comparable to the WAVE Bioreactor 20/50 although the final yield was lower due to the shorter production time. The overall process control in WAVE Bioreactor 20/50 was stable and the rocking rate was adjusted to manage the increasing oxygen demand and the accumulation of carbon dioxide (Fig. 4). It was also clear that the accuracy of the optical pH sensor was within ±0.05 pH units throughout the culture after just two offset calibrations (Fig. 4).

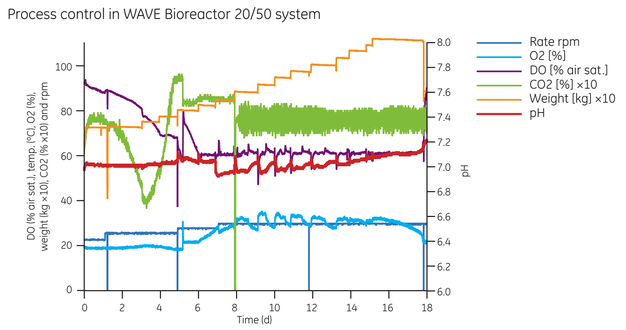


**Fig 4.** Graph illustrating the process performance in WAVE Bioreactor 20/50 system over time. Data extracted from UNICORN DAQ

## Discussion

Consistent process performance was achieved across the evaluated bioreactor systems, however in the 100 L STR bioreactor large amount of foam was accumulated in the headspace due to the high sparger flow rate. That was resolved by adding antifoam when needed.

$CO_2$ was being accumulated in the WAVE Bioreactor systems (not shown here) as a result of the absence of a sparger. That was resolved to some extent by increasing the rocking rate as well as the airflow rate.

The IgG production in the WAVE Bioreactor 200 system would most likely have been comparable to what was seen in the WAVE Bioreactor 20/50 system should it have been prolonged a few more days.

No antifoam additions were needed for any of the WAVE Bioreactor runs.

## Conclusions

- Comparable process performance between all cell culture runs with respect to cell growth and IgG production reaching in the range of 20 MVC/mL and 4 g/L, respectively.
- Improved viability in WAVE Bioreactor systems due to the absence of sparging and the need for antifoam.
- The fed-batch process could also be scaled up to a WAVE Bioreactor 200 system.
- Excellent process control and accuracy of the optical pH sensor.
- In conclusion, it is demonstrated that production of monoclonal antibodies in WAVE Bioreactor systems is a very competitive process option compared to stirred tank bioreactors.



29-0312-58 AB 10/2012