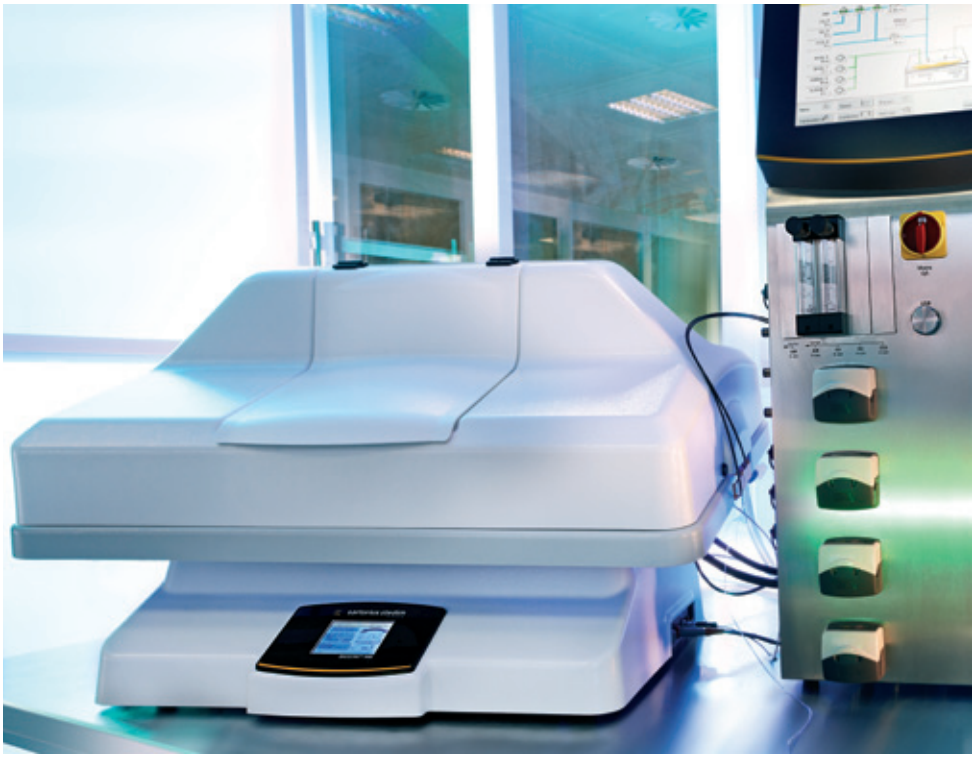
sartorius stedim biotech

CHO cultivation in next generation rocking motion single-use bioreactor



Application Note

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turning science into solutions

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Introduction
Single-use bioreactors are widely accepted as a tool for cell cultivation. Several technologies exist in the market, including stirred single use bioreactors, airlift bioreactors, paddle mixed bioreactors and others.

The rocking motion or wave-induced motion (WIM) systems are amongst the most frequently used systems and are available since more than 10 years.
The original Wave Bioreactor is the BioWave from Wave Biotech AG, Switzerland, which was marketed under the name BIOSTAT® RM (figure 1, left) from 2007 on, when Wave Biotech was taken over by Sartorius Stedim Biotech GmbH, Germany.

Recently, the second generation of the rocking motion bioreactor, the BIOSTAT® RM II (figure 1, right) was introduced into the market. The equipment features many new functions and an improved drive system and temperature control.

Werthenstein Biopharma conducted a beta-test of the equipment, to evaluate the performance.

Werthenstein BioPharma is a part of the global scientific healthcare company Merck & Co., Inc., USA. Outside of USA and Canada the company is named MSD. Werthenstein BioPharma plays an important role for the development of new drugs.

The biotechnology department produces active pharmaceutical ingredients for clinical studies phase I to III. The production occurs with cell culture within a pilot benchmark.

The cell culture inoculums (starter culture) is manufactured by using single use equipment and disposable materials.
We are working with Wave Bioreactors of 10 L up to 300 L working volume.

Figure 1:
Left: Image of first generation BIOSTAT® RM (previously marketed under the name BioWave 20 SPS). Right: Image of second generation BIOSTAT® RM II.

Aim of the study
The second generation BIOSTAT®RM II is equipped with a new drive system, which features a servo motor coupled to the rocking platform via a toothed belt. This enables an electronic angle control and less maintenance effort. The motor controller is programmed to enable an identical movement of the rocking platform compared to the precursor version. This allows a seamless transfer of the process from the first to the second generation rocking motion bioreactor and vice versa. Furthermore, in contrast to the precursor model, the new version contains an integrated gassing module for air and CO_2 mixing.

Werthenstein Biopharma conducted cultivation experiments with mammalian CHO cells in SAFC standard culture media. The same process parameters as with the BioWave 20 SPS are used for the new machine, in order to compare the performance of the second generation bioreactor to its precursor.

Material
- Raw Materials
  - CHO K1 cell line
  - SAFC CHO Standard Medium
  - Supplements such as 200 mM L-Glutamine Solution etc.
- Single Use Bioreactor Systems
  - Sartorius Stedim Biotech BioWave 20SPS
  - Sartorius Stedim Biotech BIOSTAT® RM II 20 basic;
- Disposable Bioreactor Bag
  - Sartorius Stedim Biotech CultiBag RM 10 L basic and RM 20 L basic;
- Analytical Devices for Inprocess-Control:
  - Cedex HiRes (Roche Diagnostic) Cell Counting
  - ABL-5 (Radiometer) pH, pCO_2, pO_2
  - Bioprofile Flex (Nova) Glucose, Lactate, Glutamine, Glutamate, Ammonia
- Disposable Shake Flask (Corning)
- Biosafety Cabinets
- CO_2 Shaker Incubators (Kühner)

Methods
Preculture: The CHO seed culture was grown and scaled up in disposable shake flasks.

Cultivation #1
Cell culture process over 4 days with a final culture volume of 8 L. A single CultiBag RM 20 L basic (maximum working volume 10 L) was installed on the BIOSTAT® RM II 20 basic system and inoculated with 2 L of cell inoculum with a seeding density of approx. 4.5×10^5 cells/mL. The process was started and the following process parameters were set:
- Aeration 5 % CO_2/air gasmix, controlled by the bioreactor
- Gas flow: 100 mL/min
- Temperature: 37.0 °C
- Rocking angle: 8°
- Rocking speed: 11 – 12 rpm

After 2 days of cultivation, a filling up step with fresh media and supplements to a final culture volume of 8 L has been performed (1:4 dilution). Daily samples were taken to determine pH, metabolites, cell density and viability. The cells were cultivated over 96 hours.

Cultivation #2
Duplicate cell culture process over 4 days with 2 cultibags simultaneously with a final culture volume of 5 L

Two single CultiBags RM 10 L basic (maximum working volume 5 L) were installed on the same bag holder of the BIOSTAT® RM II 20 basic and inoculated with 2 L of cell inoculum with a seeding density of approx. 5×10^5 cells/mL. The process was started and the same process parameters were set as used for cultivation #1.

After 2 days of cultivation a filling up step with fresh media and supplements to a final culture volume of 5 L has been performed (1:2.5 dilution).

Daily samples were taken to analyze pH, metabolites, cell density and viability. The cells were cultivated over 96 hours.

Cultivation #control batch using the previous generation bioreactor

Batch culture of 4 days duration with a final volume of 8 L
A single CultiBag RM 20 L basic (maximum working volume 10 L) was installed on the BioWave 20 SPS system (first generation) and inoculated with 2 L of cell inoculum with a seeding density of approx. 4×10^5 cells/mL.

The process was started and the same process parameters were set as used for cultivation #1 and #2.

After 2 days of cultivation a filling up step with fresh media and supplements to a final culture volume of 8 L has been performed (1:4 dilution).

Daily samples were taken to analyze pH, metabolites, cell density and viability. The cells were cultivated over 96 hours.

Results

The aim of these experiments was to demonstrate that the next generation rocking motion bioreactor BIOSTAT® RM II gives equivalent cultivation results.

Two sets of experiments, in total three cultivations, were performed.

Graphics

Figure 1 shows the viable cell density according to the time of cultivation runs #1, #2 and # control batch. As can be seen from the graph, the cell culture performance of all the runs in the BIOSTAT® RM II are very comparable to the control run in the BioWave 20 SPS. Small differences are related to a different seeding densities.

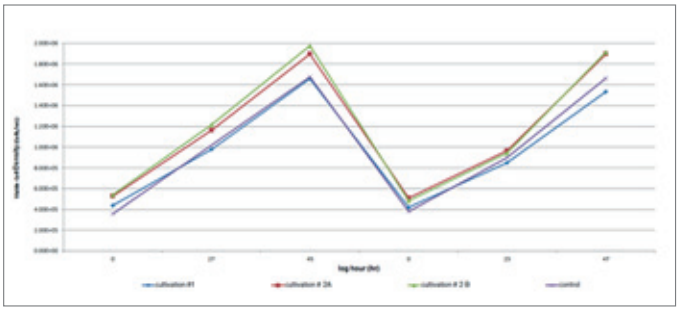

Figure 1:
Viable cell density vs. time. Cultivation #1 final volume 8 L. Cultivation #2A and #2B are duplicate runs with 2 × 5 L on the same rocker platform. After media fill, the logged time is reset to 0. The total length of the run is 96 hrs.

Figure 2 shows the glucose and lactate concentration according to the time of cultivation runs #1 and #2 and # control batch. As can be seen from the graph, the cell culture performance of all the runs in the BIOSTAT® RM II are very comparable to the control run in the BioWave 20 SPS.

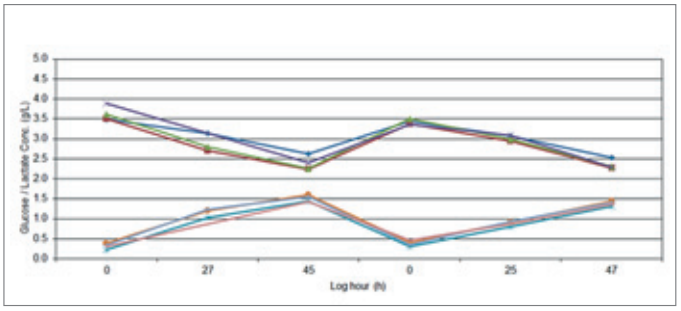

Figure 2:
Glucose and lactate concentration vs. time. Cultivation #1 final volume 8 L. Cultivation #2A and #2B are duplicate runs with 2 × 5 L on the same rocker platform. After media fill, the logged time is reset to 0. The total length of the run is 96 hrs.

Conclusion

Combining ease of use, reliability and minimum workload BIOSTAT® RM is ideally suited for cultivation of mammalian cells. The handling and the functionality of the BIOSTAT® RM is very comfortable by using a touch screen panel. The new feature, the integrated CO_2 gasmix unit ensures an independence from external gasmix systems or gas supply and the CO_2 controlling is very accurate.

In this note, we could demonstrate that the growth performance and reaching cell densities in the BIOSTAT® RM is comparable to its precursor the BioWave 20 SPS. The process parameters can be directly applied to the BIOSTAT® RM without any adaptation.


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