

Single-use bioreactor for mammalian cell culture.

Piet van Hees¹, Judith van Creij-Meij¹, Arianne Bril-Huisman¹, Timo Keijzer², Hans van den Berg²

¹ Section SSC&D, Diosynth Biotechnology Europe, PO Box 20, 5340 BH, Oss, The Netherlands, ² Applikon Biotechnology B.V., P.O. Box 149, 3100 AC, Schiedam, The Netherlands.

Introduction

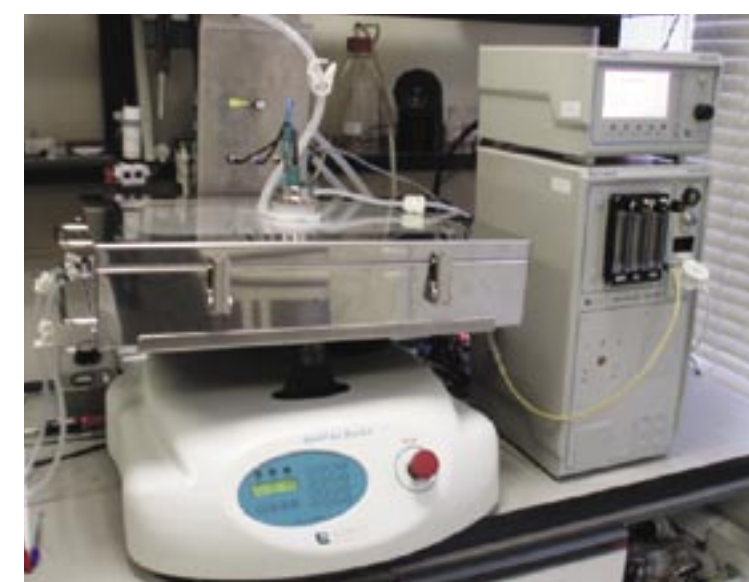
Single-use alternatives for the culture of mammalian cells in glass or stainless steel re-useable stirred tank reactors are increasingly being developed and marketed. Disposable bioreactors have the advantage of saving time and costs with regards to sterilization, cleaning and validation.

Using disposables reduces the risk for microbial contamination significantly due to the fewer open operations. For these reasons disposable bioreactors are implemented more and more in the (uncontrolled) pre-culture phase of large scale production processes. However, due to lack of control functions like pH and DO, the use of disposable bioreactors in the production phase is not likely. Recently a prototype of a disposable 10-L bioreactor was developed enabling the execution of fully controlled cell culture processes.

Physical experiments (mixing, K_a , heating) as well as a fed-batch cell culture experiment using a CHO cell line are described. Cell culture results obtained with the disposable bioreactor system are compared to the data obtained on the same fed-batch process performed in a conventional 10-L stirred bioreactor.

Materials and Methods

Disposable bioreactor system:



The single-use culture system tested consists of a rocker unit containing a box in which the disposable bag is placed. The disposable 20-L bag (working volume 7-12 liters) is made of a film commonly used in the pharmaceutical industry. Against the outside bottom wall, the heating blanket is positioned. The system is completed by a Bio Controller to control pH,

temperature and dissolved oxygen. Mixing conditions are set on the rocker unit. The system is tested for physical parameters like mixing performance, oxygen transfer capacity and heat transfer capacity. Subsequently the disposable bioreactor system is used to perform a routinely performed cell culture fed-batch process with CHO cells on 10-L scale.

Before the start of the culture process a disposable sensor set, consisting of a pH, dissolved oxygen and a temperature probe, must be installed aseptically in the 20-L culture bag.



- pH is controlled by adding either CO₂ or base (0.5 M NaOH). Excess of CO₂ is stripped from the culture by agitation and a constant flow of air in the head space.
- DO is controlled by adding air in the head space constantly and adding pure oxygen on demand.

- Temperature is controlled by supplying heat via a heating blanket positioned under the stainless steel container (no direct contact between heating blanket and culture bag).
- Agitation is performed using a rocking speed of 20 rpm combined with an elevation angle of 6°.

Conventional stirred bioreactor system:

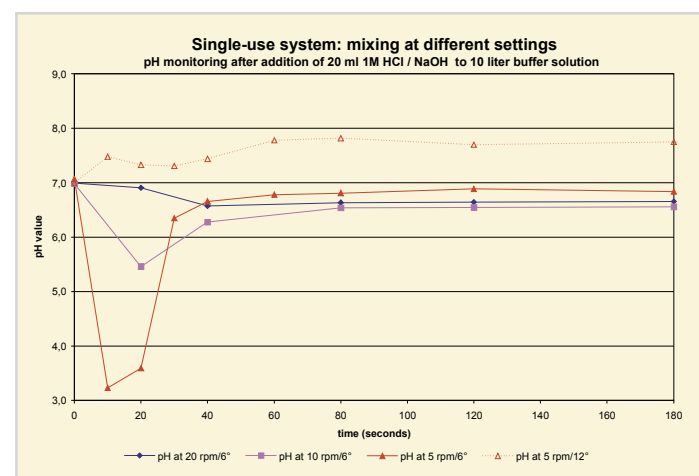


The reference fed-batch cell culture process is performed in a 15-L autoclavable bioreactor. The jacketed glass vessel is equipped with a stirrer assembly, pH, DO and temperature sensors. Pure oxygen is supplied via a sparger. At 10-L scale working volume the Height/Diameter ratio is 1.2.

- pH is controlled by adding either CO₂ or base (0.5 M NaOH). Excess of CO₂ is stripped from the culture by agitation combined with a constant air flow in the head space.
- DO is controlled by adding air in the head space constantly and adding pure oxygen on demand directly into the culture broth via a sparger.
- Mixing is performed using 2 marine impellers stirring at 150 rpm.

Results and Discussion

Mixing characteristics



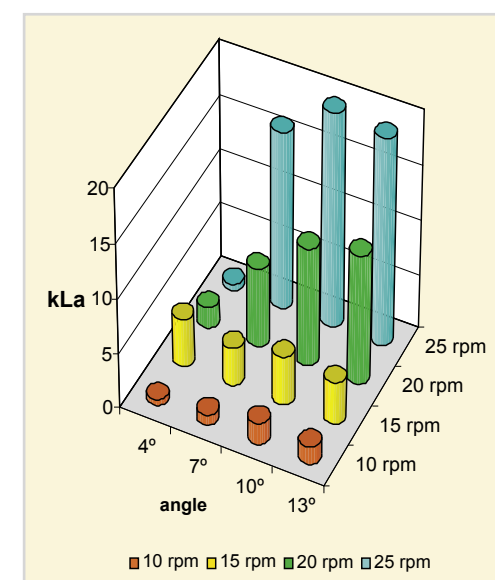
Mammalian cells are very sensitive to strong changes in pH. The addition of NaOH to adjust the pH of the cell culture creates high pH regions (pH > 10). The residence time of cells within these regions should be as short as possible. To reduce the risk for cell damage caused by large pH a good mixing performance is very important.

Mixing performance deviations or excursions is measured under various shaking conditions by adding either NaOH 1M or HCl 1M to a phosphate buffered saline solution. pH is monitored in-line in the culture bag.

Conclusions:

- Mixing performance is inversely proportional to the rocker speed.
- A setting of 20 rpm at 6° results in a mixing time of about < 40 seconds, which is comparable to a conventional 10-L stirred bioreactor system

Oxygen mass transfer

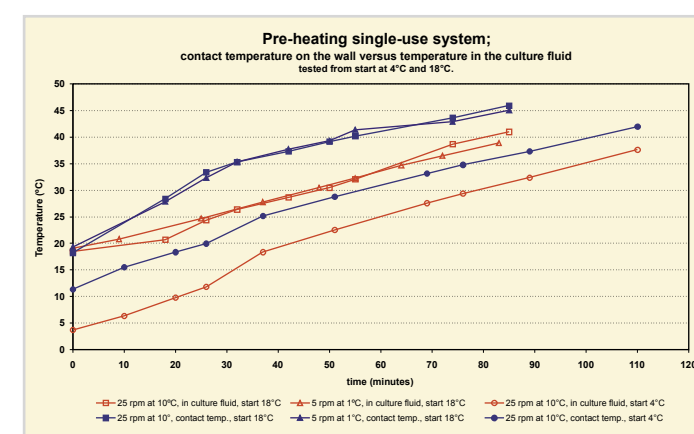


The culture of mammalian cells requires a sufficient oxygen transfer rate. Due to increasing cell densities in newly designed cell culture processes also the oxygen transfer capacity needs to be scaled up. The single-use bioreactor system is tested for its oxygen transfer capacity (K_a). A Pt100 sensor and a DO sensor are placed in the sensor holder of the bag. Experiments are performed using 10 liter demineralized water of 37°C. Before start the water is saturated with nitrogen to achieve a dissolved oxygen concentration of 0 %. An airflow rate of 0.5 liter per minute is set. The effect of the rocking speed at different angles on the transfer rate of oxygen is measured.

Conclusions:

- The k_a strongly depends on the rocker speed whereas the rocking angle affects the k_a to a lesser extent.

Heat transfer



The time needed to pre-heat 10 liter culture fluid in the culture bag from 4°C and 18°C respectively up to 37°C is measured. During the heating phase the contact temperature (temperature directly on the outside wall of the culture bag) is measured simultaneously with the temperature of the culture fluid.

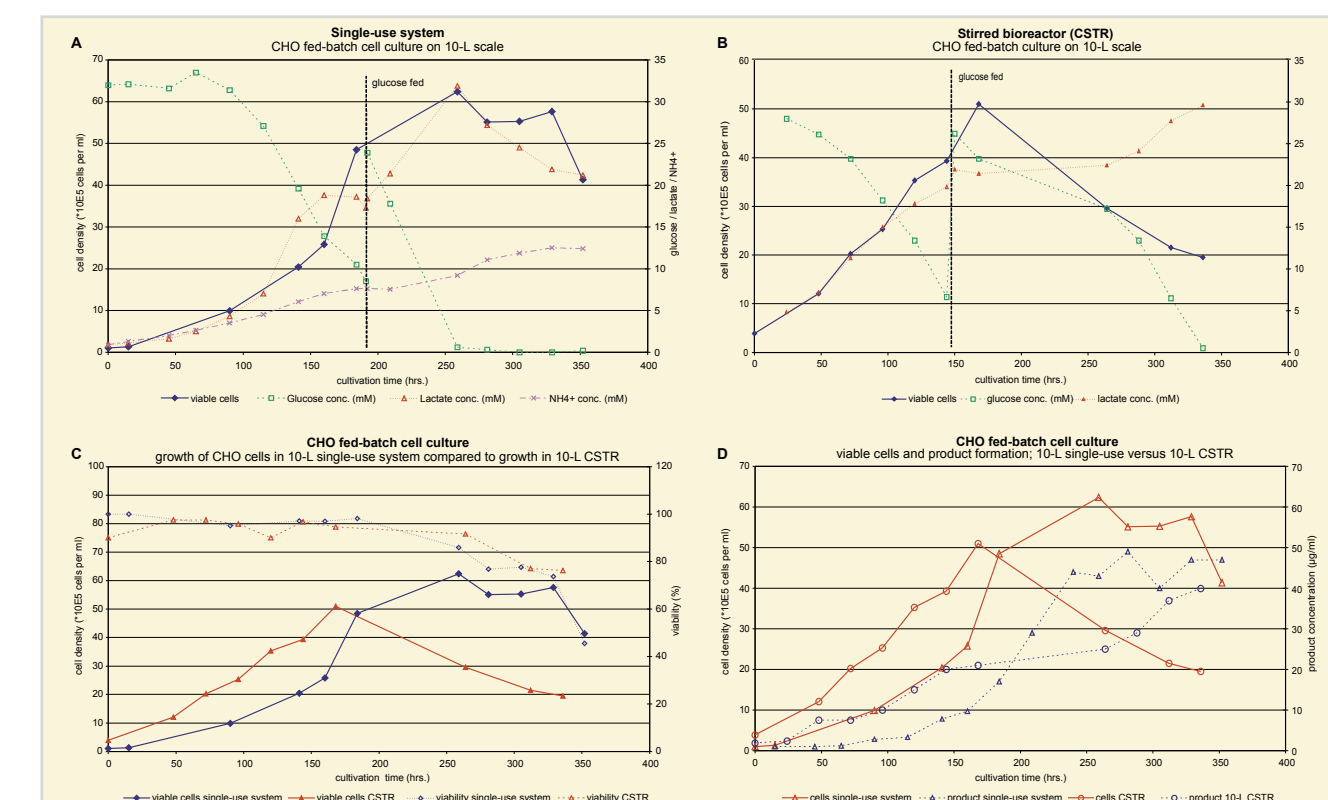
Conclusions:

- Starting from 4°C the culture fluid can be pre-heated to 37°C in about 110 minutes. Starting from room temperature it takes about 80 minutes.
- The contact temperature (temperature on the outside wall of the culture bag) never exceeds 45°C.

Cell culture

- Recombinant CHO cells expressing a protein of therapeutic interest are cultured on 10-L scale in a serum-free culture medium containing pluronic. pH is controlled at pH 6.8. Dissolved oxygen concentration is maintained at 50 % air saturation. at a temperature of 37°C.
- The pre-culture of the cells is performed in shake flasks at 37°C. The disposable bioreactor is inoculated at a cell density of 1x10⁵ viable cells per ml. The reference 10-L stirred bioreactor is inoculated at a cell density of 3.9x10⁵ cells per ml.
- Fed solution contains glucose 1M in culture medium.
- The extra-cellular product is measured using a product specific Enzyme Immuno assay (EIA).
- Cell density and viability are determined by performing the trypan blue exclusion test. Cells are counted using a haemocytometer.
- Glucose, L-lactate and NH₄⁺ concentrations are measured off line using the Bio-profile 100⁺ analyzer of NOVA biomedical.

	10-L fed-batch culture	
	Single-use system	Stirred bioreactor
Growth rate (hr ⁻¹)	0.016	0.015
Maximum cell density (x 10 ⁶ cells per ml)	6.2	5.1
Specific productivity (µg per 10 ⁶ cells per day)	0.69	0.59
Final product concentration (µg per ml)	47	40



Conclusions:

- It is shown that good cell growth can be obtained in the single-use 10-L system.
- The product concentration in the disposable bioreactor reached a maximum of 47 µg/ml on day 10. The stirred bioreactor showed an increase of product concentration up to a maximum of 40 µg/ml on day 14.

Overall conclusions and recommendations

- The mixing conditions set for the cell culture experiment turned out to be effective and supported cell growth.
- Oxygen supply in the disposable system proved to be sufficient to enable cell growth up to a cell density of 6.2x10⁶ viable cells per ml.
- Heating capacity and temperature control of the system fulfill the requirements for the culture of mammalian cells.
- The data presented in this poster show that the disposable bioreactor system performs comparable to a conventional stirred bioreactor on the same scale. Good cell growth and product yields could be obtained. Additional purification and characterization of the final product is necessary to show equivalent product quality.
- In contrast to most of the disposable culture systems on the market the single-use system tested here enables fully monitoring and control of the standard culture parameters (pH, DO and T).
- The 20-L bag requires a minimum culture volume of approximately 7 liters (due to the sensor set). A lower inoculum volume would be very advantageous because open handling in the pre-culture phase could be reduced.
- Before use the disposable bioreactor must be assembled by installing the pre-sterilized sensor set aseptically in the 20-L bag. We would recommend a system in which this type of aseptic handling can be avoided.
- In general it is concluded that the (pilot model) single-use system tested demonstrates suitability for the culture of mammalian cells under well controlled conditions.