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ENGINEERING

Monoclonal Antibody Production:

Characterization of the batch and fed batch production of Trastuzumab Therapeutic mAb by CHO cells using BIONET's F0-BABY bioreactor

ABSTRACT

The core of the technological infrastructure required for biopharmaceutical manufacturing is the bioreactor, used in all stages from R&D to biomanufacturing. The F0-BABY bioreactor line by BIONET is comprised of robust and user-friendly laboratory bioreactor models that can be configured for different cell expression systems like microbial cultures or cell cultures.

In the present work, the common characterization phase required in monoclonal antibody production is presented by using a CHO-S strain and BIONET's F0-BABY 2CC bioreactor. This bioreactor has a working volume of 2L and it is equipped with the required process analytical technologies, a variable speed pump for fed batch, and an Advanced Gas Module for the control of up to 5 gas supplies. It also includes BIONET's proprietary bioprocess software ROSITA®, for the visualization, control and data registration of all bioprocess parameters. The growth and viability of the mammalian cell culture in shake-flasks and in the bioreactor in both batch and fed batch modes were compared, as well as the production of the mAb Trastuzumab as a target product of the strain was checked. The Bionet's F0-BABY 2CC bioreactor turned out to be a reliable and easy device to use for the growth of CHO-S cells, providing an environment that assures the lack of contamination and the most optimal cell culture conditions.

KEYWORDS

Upstream, laboratory bioreactor, cell culture, mammalian cells, biopharmaceuticals, monoclonal antibody production, batch, fed batch, CHO cells, Trastuzumab, stirred-bioreactor

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1 INTRODUCTION

Biotechnology is the most suitable platform for biopharmaceuticals manufacturing and one of its main tools are bioreactors (link a: https://bionet.com/technologies/upstream-bioprocesing/). These devices are systems in which biological processes are carried out, providing an sterile environment and the suitable cell culture conditions in which to conduct efficient operational strategies to optimize the production of the product of interest (biomolecule or cell) and to enhance productivity [1,2].

The main objective of this work was the characterization of the production of the monoclonal antibody against the epidermal growth factor receptor-2 (HER2) Trastuzumab using CHO-S cells as expression system in a the F0-BABY laboratory wbioreactor. For this purpose, both cell culture operation modes batch and fed batch were carried out (link a: https://bionet.com/technology/f0-baby-bioreactor/). In parallel, a process was performed in Erlenmeyer shake-flasks to compare results.

2 MATERIALS AND METHODS

Cell line and culture conditions

CHO-S cells were provided by Cobra Biologics (Keele, Staffordshire, UK). Cells were cultured in suspension in chemically defined medium (CDFortiCHO, GIBCO, Billing, USA) supplemented with 8mM GlutaMAX (Invitrogen, Paisley, United Kingdom) and 10mM of sodium hypoxanthine mixed with 1.6mM thymidine (HT Supplement, GIBCO, Billing, USA). Cells were kept in exponential growth phase by passaging them every 2-3 days (0.2x106-0.5 x106 cells/mL) with viabilities over 90% in 125ml disposable polycarbonate Erlenmeyer flasks (Corning, New York, USA) and amplified in 1L disposable polycarbonate Erlenmeyer flasks (Corning, New York, USA). The flasks were disposed of in incubating orbital shakers (Kuhner, Birsfelden, Switzerland) at 130 rpm, 37°C and 5% CO2.

Methods of inoculation and medium preparation

The cells from Erlenmeyer shake-flask were transferred aseptically to a bottle for the propagation. The bioreactor used was a F0-BABY 2CC bioreactor, figure 1 (Bionet, Murcia, Spain). The bioreactor was inoculated aseptically inside a class II laminar flux biological cabinet with cells at 0.3x106 cells/mL. For an initial volume of 1L, the first 800mL of the medium is poured in. When the medium was warmed up at 37°C to minimize cellular stress, approximately 100mL with the cell inoculum were fed in the bioreactor. In parallel to the bioreactor culture, Erlenmeyer flasks culture was run in triplicate, using the same initial cell density.

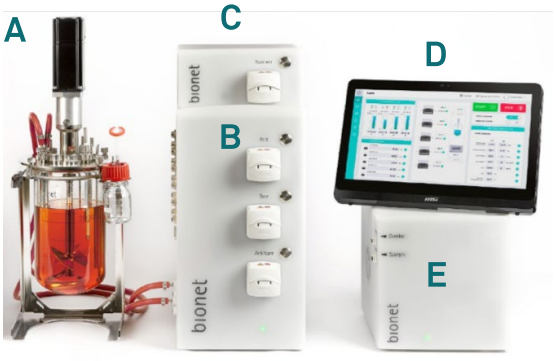

Fig.1. (A) Bionet F0-BABY 2CC bioreactor for cell culture application (link a: https://bionet.com/technology/f0-baby-bioreactor/), (B) Bioreactor Control Unit (BCU), (C) Variable Speed Pump (VSP), (D) ROSITA® SW for control, data visualization and data acquisition, (D) Advanced Gas Module (AGM) (link a: https://bionet.com/technology/f0-baby-bioreactor/)

Production in Bioreactor

CHO-S cells from steps in Erlenmeyer shake flasks were seeded into 2 litre working volume in the F0-BABY 2 CC Bioreactor (link a: https://bionet.com/technology/f0-baby-bioreactor/), (Bionet, Murcia, Spain) as was explained in the inoculation step. The main specifications of the bioreactor are shown in table 1. The main cell culture conditions were set as follow and are also summarized in table 2:

CHO cultivation in batch culture

For batch culture, all the medium components were loaded into the bioreactor at the beginning of the process. The bioreactor was inoculated with cells, with no further additions (except those related to pH control). Cell growth and product formation took place over time until the depletion of limiting substrate.

CHO cultivation in fed batch

For the fed batch, CDEfficientFeed C AGT (GIBCO, Billing, USA) was prepared following the supplier's instructions. It was added during the fed-batch experiments at 2X concentration and 5% of the volume of both the bioreactor and the parallel Erlenmeyer shake-flasks.

Cell counting and viability

These assays were performed using the NucleoCounter® NC-3000 automatic cell counter (Chemometec, Allerod, Denmark) according to manufacturer's instructions.

Glucose and lactate determination

Samples were centrifuged for 5 minutes at 1000xg separating pellet and supernatant. Pellet was discarded while supernatant was kept in order to analyse glucose and lactate using YSI 2900 automatic analyser (Yellow Sprong Instruments, Yellow Springs, CA, USA).

Table 1:
Biorreactor configuration

Element	Description	
Application	Cell Culture (CC)	
Model	Bionet F0-BABY single, single wall glass vessel Geometry H:D 2:1	
Heating Blanket	140 W	
Cooling Finger	Cool water supply @ 6°C / 0.6 barg	
Bioreactor working volume	2 litres	
Gas supply Advanced Gas module	Air @ 2 bar	Flow range 20-750sccm
	O2 @ 2 bar	Flow range 20-750sccm
	N2 @ 2 bar	Flow range 20-750sccm
	CO2 @2 bar	Flow range 20-750sccm
Sparger	Ring-sparger (pore ∅ 1mm)	
Agitation	Max speed 500 rpm	
Impeller	Marine turbine; 1 unit Ratio d/D=0.4	
Variable speed pump	Speed 4 rpm Flow rate: 0.1-3.4 ml/min Tube bore ID: 1.6-4.8 mm	
Accesories	**Condenser at exhaust gas** **Dip curve tube for harvesting** **Dip tube for sampling (variable height)**	
Roughness (in parts in contact with the product)	**Ra < 0,8**	

Table 2
Biorreactor configuration Summary of process parameters set points applied

Parameter	SP Value
Working Volume	1 L
pH	7.1±0.1
Temp	37°C
Agitation	200 rpm
DO (cascade)	40% controlled by sparged air. Aeration rate: max 0.1 vvm

3 RESULTS AND DISCUSSION

BATCH CULTURE

The figure 2, represents the time line of the experiments in batch mode performed by triplicate. The F0-BABY bioreactor enabled to perform a CHO-S cell culture successfully without any contamination issue and good performance according variations in media colour and visual check under microscope. The average cell density achieved in the bioreactor was 56% higher than Erlenmeyer shake-flask (fig.2.B). In the experiments in the bioreactor, the death phase was started later than Erlenmeyer shake-flask, with 54% more viability at 120 h in the bioreactor, maintaining the culture viable for a longer period and in better production conditions than in the shake-flask. The specific growth rate (μ) in the exponential phase according the duplication time were 0.05 h-1 and 0.04 h-1, in the bioreactor and Erlenmeyer shake-flask respectively. This means an average of specific growth rate 16% higher in the bioreactor. Glucose was consumed as lactate was generated as a secondary metabolite. When glucose was completely depleted, lactate started being consumed (fig.2.C)

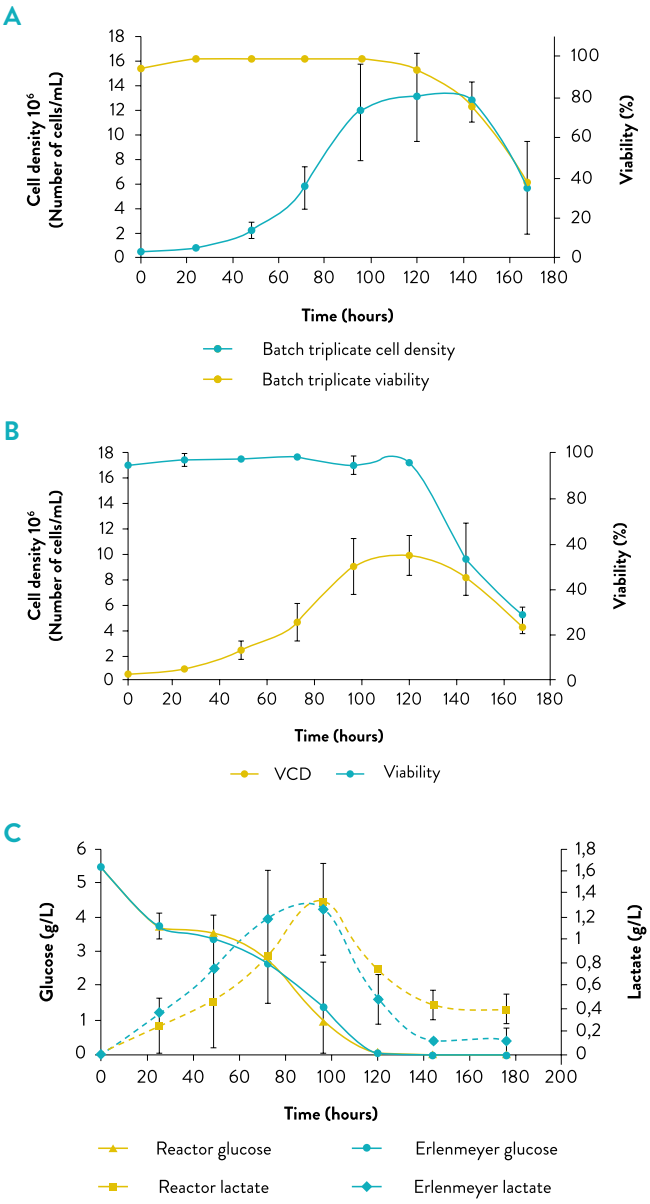

Fig.2. Time-course of the cell density and viability of the experiments in batch mode with CHO-S cells in the (A) Bionet F0-BABY bioreactor, and (B) Erlenmeyer shake-flask. (C) Glucose and lactate consumption for CHO-S culture batch. Being R de bioreactor and P the Erlenmeyer flasks running in parallel.

FED-BATCH

The additions in the fed batch culture were carried out following a pulse feeding strategy. The averages of the cell density and viability of the experiments 1 and 2 throughout time are represented in the figure 3.A, where the additions are marked with arrows. The culture time was 7 days longer than in batch mode, and the highest cell density peak reached in the bioreactor was 18·106 cell/mL, which decreased to 8·106 cell/mL afterwards. In the shake-flasks the cells entered in death phase after reaching a density peak of 11·106 cells/mL. The glucose concentration changed from growth to death phase in the bioreactor corresponding to the depletion of glucose in the medium and re-metabolization of lactate between 150 and 200 hours.

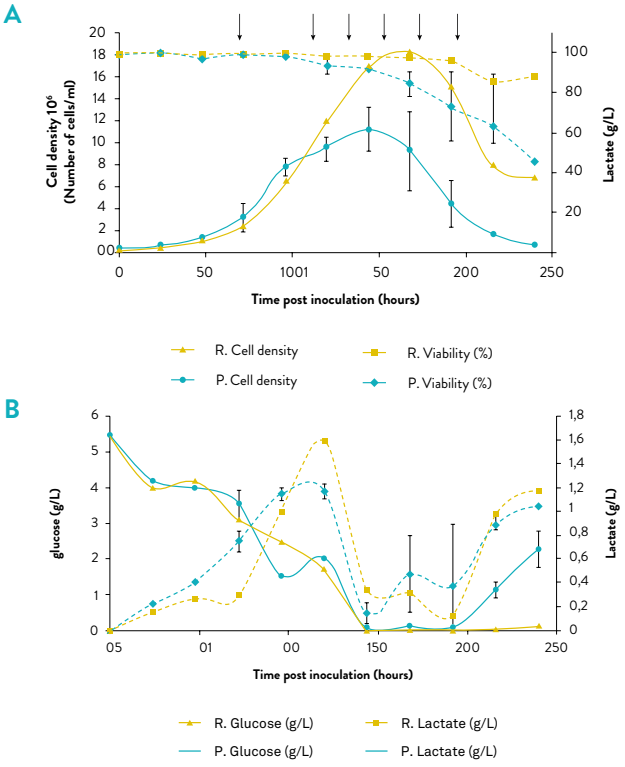

Figure 3. (A) Cell density and viability of a CHO-S fed-batch culture where the signposts mean addition of feed. Being R de bioreactor and P the Erlenmeyer flasks running in parallel. (B) Glucose and lactate consumption of a fed-batch CHO-S culture.

A third fed batch was performed changing the fed batch feeding strategy as well as doubling the flow-rate of aeration in the bioreactor (0.2 L/min). The fed batch phase started at day 4 with a 2.5% reactor volume addition. From this point, the volume of addition was increased up to 5%, following an increasing feeding strategy, in order to maintain glucose and lactate levels. As shown in Figure 4.A, this fed batch reached a cell density peak of 25.10^6 cells/mL. However, cell viability dropped rapidly after reaching the maximum cell concentration probably due to glucose depletion (fig.4.B).

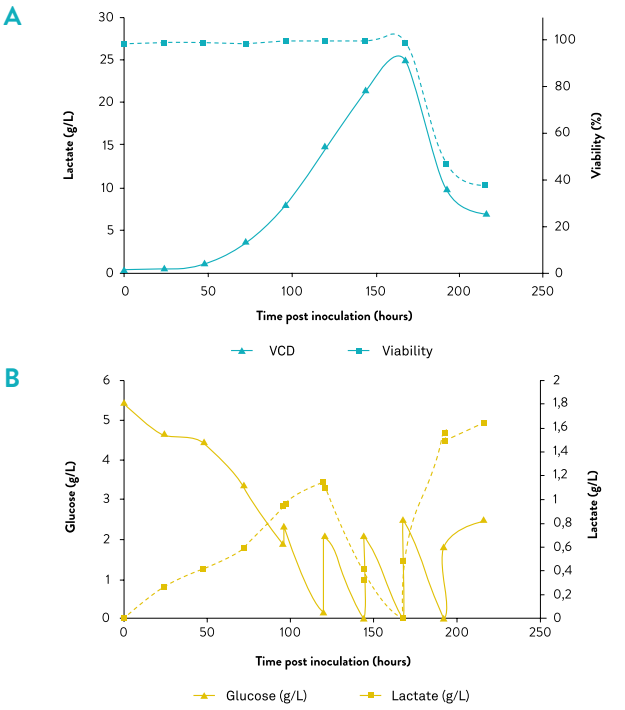

Fig.4. (A) Growth kinetics of a fed-batch of CHO-S cells. (B) Glucose and lactate profile of a CHO-S fed-batch

The cell doubling time was 17.6 hours, improving the performance of the previous fed batch. As observed in Figure 4.B, glucose was kept at low levels in order to avoid overfeeding and consequently cell growth inhibition by excess by-products generation. It can also be observed that cells dye abruptly, and that glucose is exhausted at different points of the growth curve.

Monoclonal Antibody production

The final average concentration of the mAb Trastuzumab was 2.6-fold higher in the experiments carried out in fed-batch mode in comparison to the batch mode, the productivity 3-fold higher (in terms of grams of mAb per litre and hour), and the specific productivity of pg of mAb per cell and day 72% higher.

CONCLUSIONS

The Bionet F0-BABY bioreactor has performed successfully in monoclonal antibody production (Trastuzumab in this particular case) with CHO cultures. The system allowed to maintain sterility and optimal control of mammalian cell culture conditions with high selective growth requirements. As expected, the cell densities- and cell viabilities- yields were higher by using a bioreactor to maintain the optimal cell culture conditions for growth in comparison to an Erlenmeyer shake-flask, reaching peaks of 17.10^6 cells/ml and 25.10^6 cells/ml in the F0-BABY bioreactor in batch and fed batch modes respectively. Regarding the experiments to compare both operation modes, although batch cultures enabled to obtain high cell densities, the fed batch culture provided better culture outputs, with higher cell densities and extended operation times at viabilities over 80%. In terms of productivity of the target product, the fed batch mode reached 3-fold productivity of mAb than batch mode, despite the fed batch feeding strategy not being optimized in this first proof of concept.

The results obtained so far make evident the additional benefits that using further Process Analytical Technologies (PATs) could bring. For example, an automatic feeding strategy according to real-time cell concentration measurements is possible upon the connection to the F0-BABY bioreactor of an optical density sensor. The Plug&Play concept under which BIONET's bioreactors are designed allows for the automatic upgrade in ROSITA software of visualization, control and registration possibilities for such measurements and the parameters they have impact on.

REFERENCES

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