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Production of the probiotic *Lactobacillus rhamnosus* GG under microaerophilic conditions in a single-use bioreactor using the Bionet Control System

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Abstract

Recent studies demonstrate that our microbiome can affect our health, not only with gastrointestinal diseases but also with other illnesses, such as asthma or psychological diseases (1,2). For this reason, the interest in the production of probiotics for therapeutic applications is increasing. The standards and requirements of the bioprocesses industry for high added value products, such as probiotics, are increasingly demanding, including reducing the preparation and cleaning times and the contamination risks, as well as a high level of automation and versatile and flexible control systems, to be adapted to different needs and requirements of processes. The probiotic *Lactobacillus rhamnosus GG*, which has raised increasing interest for its beneficial effects in human health (3-6), is a Gram-positive, non-sporeforming, facultatively anaerobic or microaerophilic bacteria. Herein, the production of this bacteria is presented, using a 3-liter single-use bioreactor (SUB) coupled with the Bionet Control System for bench-top bioreactors, and compared to a classical glass vessel to demonstrate the feasibility of using the Bionet Control System for control and monitorization of a fermentation process with a SUB. The profiles of pH and DO under microaerobic conditions with a reverse cascade were similar in both systems, with a good control temperature using only a heating blanket.

Objective

To produce the probiotic bacteria *Lactobacillus rhamnosus GG* in a single-use bioreactor at bench-top scale under microaerobic conditions to demonstrate the feasibility to use the Bionet's Bioreactor Control Unit (BCU) with Bionet´s bioprocess automation SW, ROSITA, for control and monitoring of the single use bioreactor.

Materials & Methods

Media

The composition of the medium used for both the inoculum and the fermentation was: 20 g/L glucose, 10 g/L Yeast Extract, 15 g/L Peptone, 0.2 g/L MgSO4x7H2O, 0.045 g/L MnCl2x4H2O, 1% Tween80 and 0.1M phosphate buffer pH 6.5. For the fermentation stage, 0.25 mL/L Acepol-69 were added to control the foam formation.

Fermenter

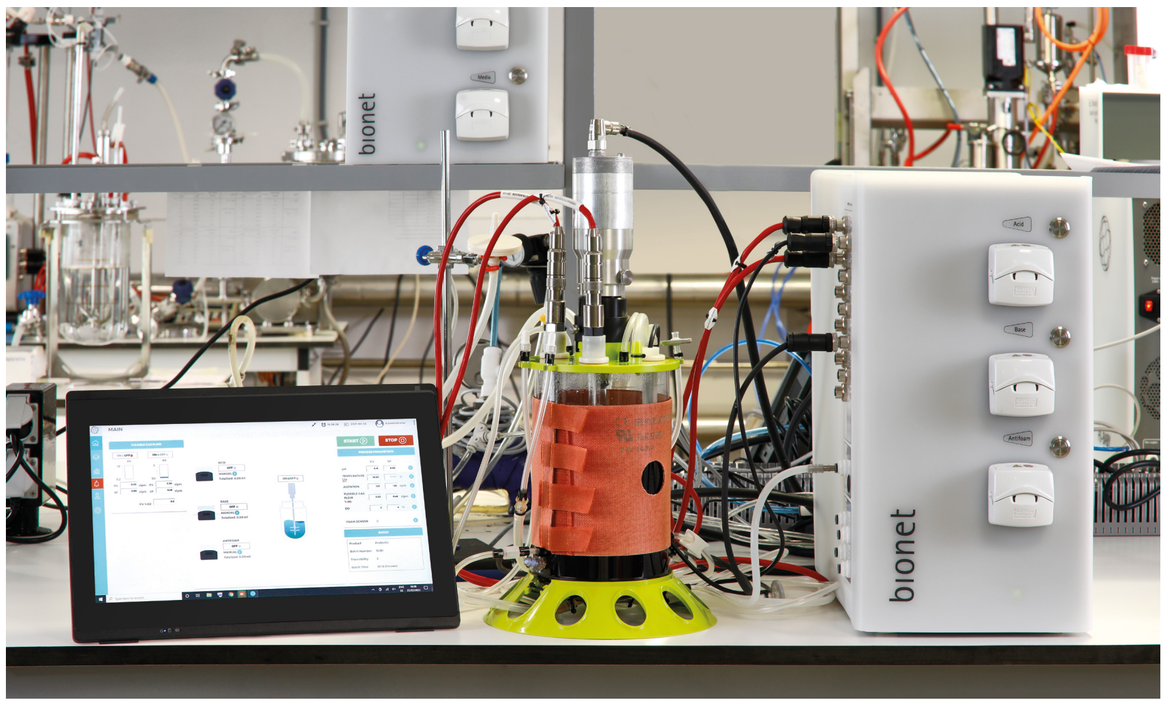

Fig.1: The set-up of the fermentation system at bench-top scale with a single-use bioreactor (SUB) coupled with the Bionet Control System

Table 1: Bioreactor technical specifications

ELEMENT	GLASS VESSEL	SINGLE-USE BIOREACTOR
Main Application	Microbial (MB)	MB & Cell Culture (CC)
Model	Bionet F0-BABY 3-MB; non-jacketed glass vessel. Geometry H:D 3:1	Mobius® CellReady 3-Lnon-jacketed polycarbonate vessel. Geometry H:D 2:1
Bioreactor working volume	3 L	2,4 L
Cooling finger	None	None
Heating Blanket	140 W	140 W
Gas sparging	Ring-sparger (pore Ø 1mm)	Sintered micro–sparger (pore Ø 15-20 µm)
Impeller	Rushton type turbines; 2 UDS. Ratio d/D=0.4	Marine type; 1 UD. Ratio d/D=0.4
Accessories	Condenser at exhaust gas	Without condenser

Table 2: Bioreactor Control Unit - technical specifications.

ELEMENT	GLASS VESSEL	SINGLE-USE BIOREACTOR
Gas supply	• Gas1: Air @ 2 bar — Flow range 0.2–10 slpm • Gas2: N_2 @ 2 bar — Flow range 0.1–5 slpm	
Agitation	80–1800 rpm Standard connection	50–500 rpm Adapted Stirrer Backpack
Probes	pH, DO_2 Length 225	

Fermentation

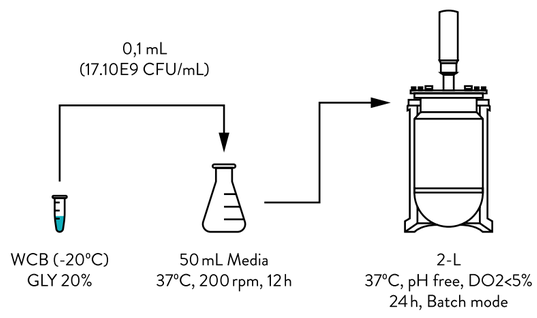

Fig.2: Main steps of the fermentation process at bench-top scale

Table 3. Summary of process parameter Set Points (SP) applied

PARAMETER	SP VALUE
Working Volume (Culture)	2L
pH	Free, not controlled
Temp	37±0.1°C
Agitation	150 rpm
DO2 (reverse cascade)	4±1% controlled by sparged air and N_2. Gas Flow rate: 0–0,25 vvm

Results

Figure 2 shows the comparison of the vessels, plotting the values of pH, dissolved oxygen (DO), and temperature. pH, starting at 6.2, was not controlled and the profiles for the SUB and glass vessel system were similar, obtaining similar pH lowering rates, which were translated in the graphs to 3 phases of different rates, a slower one corresponding to the lag phase during the first 3 hours of cultivation, followed by an exponential growth phase with a higher acidification rate with a duration of 4 hours approximately and finally a stabilization phase of the pH to a value of 3.6. The microaerophilic conditions require low oxygen concentration because in some cases high concentrations of oxygen can be detrimental for the lactic bacteria and the production of organic compounds of interest, obtaining a better growth rate than aerobic conditions in some cases (7, 8). With the Bionet system used, the DO was controlled in both vessels and maintained in the range of 4±1%, optimally using the reverse cascade control and alternating air and N_2 with a maximum gas flow rate of 0.5 slpm. The temperature was controlled with a low deviation in the range of ±0.1°C without a cooling system and only using a heating blanket. The yield of cells obtained was 30% higher in the SUB compared to the glass vessel, probably due to an improvement of gas transference by the small bubbles from the sintered–sparger compared with the sparger with 1mm of diameter used in the glass vessel. This difference in gas transfer was the main difference between these systems, achieving a more homogeneous behaviour of the DO cascade control with the sintered sparger.

Fig.2: Comparison of the evolution of pH (light-blue line), DO2 (dark-blue line), and temperature control (green line) using the Comparative tool for graphics in ROSITA SW, to compare the experiments in glass vessel and SUB.

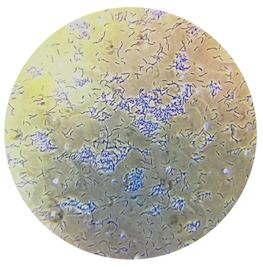

Fig.3: Aspect of the final culture under microscope at 40X from the SUB, both vessels showed similar morphology aspect and high purity.

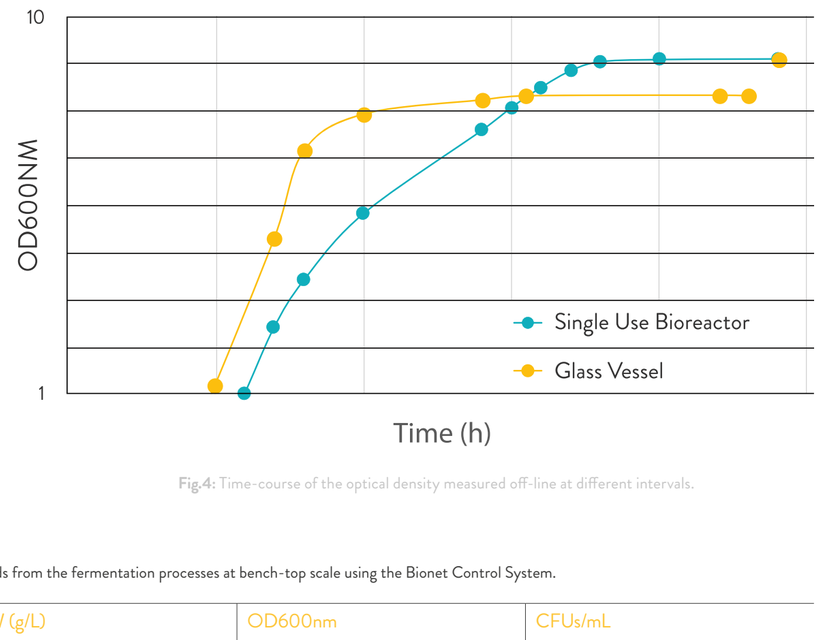

Fig.4: Time-course of the optical density measured off-line at different intervals.

Table 4: Yields from the fermentation processes at bench-top scale using the Bionet Control System.

	DCW (g/L)	OD600nm	CFUs/mL
Glass Vessel	3,6	6	8.10^8
Single-Use	4,8	7,7	$1,02.10^9$

Conclusions

In the present work, the possibility to use the Bionet Control System for bench–top bioreactors with a single–use bioreactor (SUB) for the production of a probiotic under microaerophilic conditions was demonstrated, maintaining a DO_2 of under 5%, reaching values of 1.10^9 CFU/mL and around 4 g/L of dry cell weight (DCW), for a non-optimized process in batch mode.

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