INFORS HT

Application note

Propagation of hairy roots delivering bioactive compounds using ShakerBag Option

N. Imseng and Prof. Dr. R. Eibl, ZHAW, IBT, CH-Wädenswil, and INFORS HT, CH-Bottmingen, www.infors-ht.com

1. Introduction

The aim of this project was the mass propagation of hairy roots in a commercially available incubation shaker (Multitron Cell with ShakerBag Option, INFORS HT, CH-Bottmingen) operating with the single-use bag CultiBag 2L. Experiments were carried out with modified Gamborg B5 medium, either in batch or fed-batch mode (feeding).

The inoculum required was prepared in petri dishes one week before inoculation in order to guarantee the root material was in exponential growth phase. The bag bioreactor was filled with 200 mL culture medium and inoculated with the root biomass (2 g WW L^{-1} for batch and 5 g WW L^{-1} for fed-batch experiments) before it was placed on the ShakerBag Option platform and inserted into the shaking incubator. Cultivation was initiated at 26°C and 30 rpm.

The experiments lasted between 21 and 28 days, and revealed dry biomass of up to 12.1 g DW L^{-1}. The results achieved in the orbitally shaken bag are comparable with those of wave-mixed bags (CultiBag 2L) operated on a BIOSTAT CultiBag RM 20.

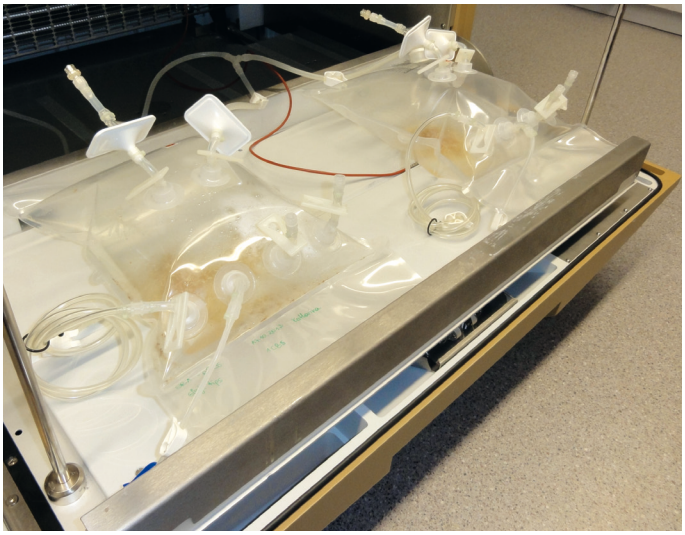

Fig. 1: Hairy roots in Multitron Cell with ShakerBag Option

2. Material and methods

2.1 Equipment and material

- Autoclave Systec 150
- CultiBag 2L D/O/S (Sartorius Stedim Biotech)
- Balance Ab 104-S (Mettler Toledo)
- Conductivity meter MC (Mettler Toledo)
- Drying oven
- Filtration system (Nalgene)
- Filtration unit (0.2 µm)
- Magnetic stirrer
- Petri dishes (Ø = 14 mm)
- pH meter FiveEasy (Mettler Toledo)
- Safety cabinet class II
- Sample vials
- Serological pipettes
- ShakerBag Option (INFORS HT)
- Incubation shaker Multitron Cell (INFORS HT)
- Sterile syringes 10 mL
- Vacuum pump
- Water bath
- Water treatment plant GenPure TKA

2.2 Overview of setting-up procedures

	Batch	Fed-batch 1	Fed-batch 2
Day 0	Filling of the bag bioreactor with 200 mL liquid medium and inoculation with:		
	2 g WW L^{-1}	5 g WW L^{-1}	5 g WW L^{-1}
Day 8	–	–	50 mL feeding and 40 rpm
Day 16	–	–	50 mL feeding and 55 rpm
Day 18	–	200 mL feeding	–
Day 21	Harvest and drying	–	–
Day 25	–	–	Harvest and drying
Day 28	–	Harvest and drying	–

2.3 Media

A modified Gamborg B5 medium was used for inoculum preparation and propagation experiments. The medium was prepared (pH 5.8) and sterilized using a bottle-top filter and autoclaved glass bottles (1 L). For the preparation of solid medium, 4.5 g Gelrite was added to 1 L liquid medium and autoclaved for 30 minutes at 121°C, and then transferred to petri dishes.

2.4 Inoculum preparation

One-week-old hairy roots were used for inoculation. In order to obtain the desired amount of biomass (0.4 to 1 g), 8 petri dishes were inoculated and cultivated at 26°C in the dark.

2.5 Bioreactor preparation

The ready-to-use CultiBag was unpacked in the safety cabinet and filled with 200 mL of the prewarmed medium (26°C) directly before inoculation.

2.6 Culture conditions

Culture volume:	200 mL, feeding up to 300 or 400 mL
Shaking rate:	30 rpm, increasing up to 40 and 55 rpm
Shaking diameter:	50 mm
Temperature:	26°C
pH:	5.8 (not regulated)
Aeration rate:	0.2 vvm
Duration:	21 to 28 days

2.7 Sampling and analysis

Samples were taken after the transfer of the bag into the safety cabinet by connecting a sterile syringe to the sampling port. Every second or third day, about 8 mL culture medium was taken and replaced by fresh liquid medium under sterile conditions. Conductivity, pH, sugars and inorganic metabolites were analysed for each sample. Wet and dry weight was determined in the beginning and at the end of the experiments.

3. Results

The final dry weight and growth parameters of the different experiments are summarized in Table 1. In the batch experiment, a final dry weight of 10.5 g L^{-1} was achieved. This is about 11% higher than in fed-batch 1 where a single addition of 200 mL medium resulted in a final dry weight of 9.3 g L^{-1}. In contrast, the highest final dry weight of 12.1 g L^{-1} was realized in fed-batch 2 (two feeding steps with 50 mL). This corresponds to an increase in biomass of 15% in comparison with the batch experiment.

	Batch	Fed-batch 1	Fed-batch 2
Final dry weight (g DW L^{-1})	10.5	9.3	12.1
Biomass productivity (g DW L^{-1} d^{-1})	0.49	0.31	0.47
Specific growth rate (d^{-1})	0.189	0.104	0.128

Table 1. Growth parameters of hairy roots in the orbitally shaken CultiBag

4. Conclusions

This application note outlines the suitability of orbitally shaken, two-dimensional bags for the cultivation of hairy roots. The results achieved in orbitally shaken CultiBags are comparable with those in wave-mixed cultivation bags when similar fluid flow and oxygen transfer are guaranteed [1].

The technique presented enables 270 g fresh, bioactive root biomass to be produced within 25 days when the cultivation is initiated with 5 g wet weight L^{-1} and operated in feeding mode. This corresponds to a 50-fold increase in biomass during the cultivation period. The biomass produced may be harvested under sterile conditions and applied as inoculum for scale-up experiments. Alternatively, desired bioactive ingredients for the pharmaceutical industry and cosmetics can be extracted from harvested root material or secreted and purified after root elicitations.

Acknowledgement

The research leading to these results has received funding from the European Union Seventh Framework Programme FP7/2007-2013 under grant agreement number 222716 – SMARTCELL.

Reference: ZHAW Zurich University of Applied Sciences, School of Life Sciences and Facility Management, IBT Institute for Biotechnology

[1] N. Imseng, Biomass and terpenoid production of hairy roots in single-use bioreactors, Master's thesis, Zurich University of Applied Sciences, Switzerland, 2011.

AP009_en_1112