Propagation of hairy roots delivering bioactive compounds for the pharmaceutical industry and cosmetics in a 2 L orbitally shaken single-use 2D-bag

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BIOCHEMICAL ENGINEERING AND CELL CULTIVATION TECHNIQUE

Introduction

Hairy roots represent potential production organisms for bioactive compounds (secondary metabolites, recombinant proteins) having importance in pharmacy and in cosmetics [1]. Whereas their propagation at small-scale is mostly done in orbitally shaken Erlenmeyers, at larger scale mist reactors [1] and wave-mixed bag bioreactors [2] are preferred. Last called such as Sartorius Stedim's BIOSTAT CultiBag RM operate with a two-dimensional cultivation bag, which is made from plastics and moved on a rocker. Till this date, there are no references about suitability of two-dimensional cultivation bags which are orbitally shaken in commercially available shaking incubators for hairy root propagation. Thus, the aim of the investigations was the mass propagation of hairy roots in a commercially available shaking incubator (Multitron Cell from Infors HT with ShakerBag Option platform) operating with a single-use bag - the CultiBag RM 2L basic with screw cap (Sartorius Stedim Biotech). Experiments were carried out with modified Gamborg B5 medium, either in batch or fed-batch mode (feeding).

Medium and inoculum preparation

A modified Gamborg B5 medium was used for inoculum preparation and propagation experiments. The medium (pH 5.8) was prepared and sterilized by usage of a bottle-top filter (VacuCap® 90, 0.2 µm, Pall). 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. One-week old hairy roots were used for inoculation. In order to obtain the desired amount of biomass (0.4 to 1 g FW), 8 petri dishes were cultivated at 26 °C in the dark one week before inoculation.

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 with fresh liquid medium under sterile conditions. Conductivity, pH, sugars and inorganic metabolites were analyzed for each sample. Fresh and dry weight were determined in the beginning and at the end of the experiments.

Results

Selected results are summarized in Table 3. In the batch experiment, a final dry weight of 10.5 g L^{-1} was achieved. Interestingly, 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}. A final dry weight of 12.1 g L^{-1} was generated in fed-batch 2 (two feeding steps with 50 mL). This corresponds to an increase in biomass of 15 % in comparison with the orbitally shaken bag running in batch mode. The courses of conductivity and pH during the experiments are presented in Figure 2A. The corresponding curves are similar. pH values during the experiments ranged between 4.9 and 8.1. The initial augmentation in conductivity indicates an increase in dry weight of the hairy roots. Heterogeneous morphological structure of the hairy roots at the end of the experiments is shown in Figure 2B. Brownish cores of older tissue are covered with white root hairs representing dividing tip cells of the apical root meristem.

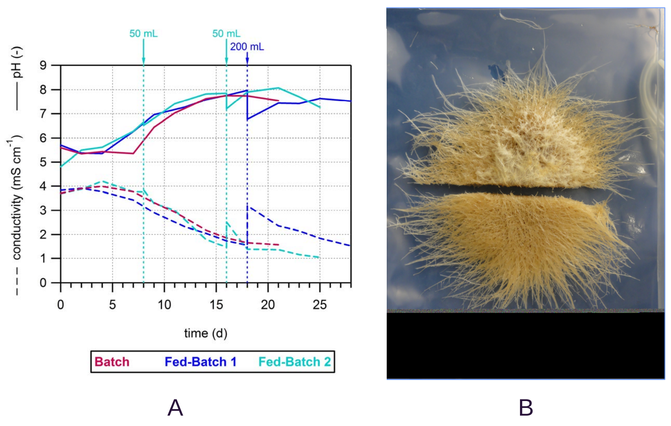

Figure 2: Hairy root growth in orbitally shaken CulitBag RM 2L. A: Courses of conductivity and pH during cultivation. B: Morphology of harvested root material.

Table 3: Growth parameters of hairy roots in the orbitally shaken CultiBag.

	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

Bag preparation and culture conditions

The ready-to-use CultiBag RM 2L basic with screw cap was unpacked in the safety cabinet and filled with 200 mL of the prewarmed medium (26 °C) directly before inoculation. A sterile forceps was used to harvest hairy roots from petri dishes and transfer them into the bag (via screw cap port). After the inoculation the bag was placed in the shaking incubator as shown in Figure 1.

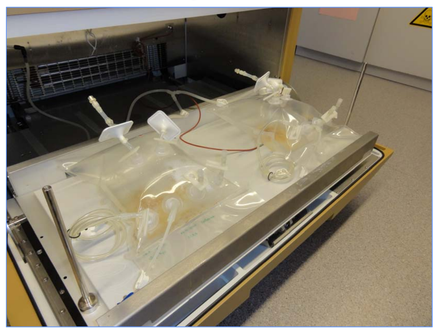

Figure 1: CultiBag RM 2L with tobacco hairy roots in the Infors Multitron ShakerBag Option.

An overview of culture conditions and setting-up procedures is given in Table 1 and Table 2.

Table 1: Summary of culture conditions.

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

Table 2: Overview of setting up procedures.

	Batch	Fed-Batch 1	Fed-Batch 2
Day 0:	Filling of the CultiBag RM 2L basic with screw cap with 200 mL liquid medium and inoculation with		
	2 g FW L^{-1}	5 g FW L^{-1}	5 g FW L^{-1}
Day 8:	-	-	50 mL feed & 40 rpm
Day 16:	-	-	50 mL feed & 55 rpm
Day 18:	-	200 mL feed	-
Day 21:	Harvest and drying	-	-
Day 25:		-	Harvest and drying
Day 28:		Harvest and drying	

Summary

The results outline the suitability of orbitally shaken, two-dimensional bags for the cultivation of hairy roots for the first time. Moreover, the results achieved in orbitally shaken CultiBags are comparable to those found in wave-mixed cultivation bags under similar fluid flow and oxygen transfer conditions [3]. The methodology presented enables 270 g fresh, bioactive root biomass to be produced within 25 days when the cultivation is initiated with 5 g fresh weight L^{-1}, and the bag is operated in feeding mode. It results in 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 can be extracted from harvested root material or secreted and purified after root elicitations. Based on these results, experiments in the orbitally shaken CultiBag RM 20L for the propagation of a geraniol expressing hairy root clone have been planned and carried out.

Acknowledgement

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Abbreviations: FW = fresh weight; DW = dry weight; RM = rocking motion; rpm = revolution per minute; vvm = volume per volume per minute

Reference

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