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# Evaluation of BIOSTAT® RM for Plant Cell Culture

Application Note

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## Introduction

Plants are major sources of natural or secondary products, pharmaceuticals, dyes, or flavours. Over the last decade, plant cell culture was developed as a technique for studying plant metabolism, physiology and development. This new biotechnological technology has reached maturity at industrial scale to offer new perspectives for the production of plant derived active ingredients.

Plant cells were first grown in bioreactors in the 1960s using various systems adapted from animal cell cultures. First cultures were performed in stirred-tank reactor. As shear stress was identified as a critical point for growth, cultures were run afterwards at low mixing. Indeed, plant cells are tolerant or sensitive to mechanical mixing used in classical bioreactors generating high shearing stress that may damage the cells. With the introduction of airlift bioreactor, particularly for biomass production, an alternative to the stirred tank reactor was proposed to overcome the shear stress limitation challenge.

Compared to microbial cells, growth and oxygen demand of plant cells are relatively low, requiring a low Kla value. Therefore to lead a plant cell culture in bioreactor, mixing has to be efficient with low shear conditions and without excessive aeration.

In general, plant cell cultures in suspensions form aggregates, therefore cells settle rapidly if mixing is stopped. Moreover the sampling could be difficult and non-reproducible. This could be accentuated at the end of growth when the plant cell cultures become viscous forming a kind of meringue. Sampling system has to be adapted to avoid loss of biomass and heterogeneity of samples.

Another characteristic of plant cell cultures, particularly in airlift bioreactors, is foam production. It is a point to consider because it can block air outlet. Antifoam has to be added but it may affect the growth performances of the culture.

Because of the low growth performances of the plant cells and therefore a long period of the culture process there is an increasing contamination risk, hence the control of sterility is an important parameter as well.

All those elements are relevant and in favour to find alternative bioreactor systems for the cultivation of plant cells at industrial scale.

In this application note, BIOSTAT® RM bioreactors were evaluated for the cultivation of plant cells. For this evaluation a suspended cell culture, belonging to the family of the Plantaginaceae was grown in the dark in order to produce cosmetic active ingredients, intended for the Cosmetic industry. We developed a unique range of biochemical actives, with substantiated efficacy for sebum regulation ('adjusting' dry or oily skin).

Our objectives were i) to manage cultivation of plant cells in new devices from small to large scale volume using different BIOSTAT® RM systems and ii) to maintain growth performances and productivity at good level throughout scale-up, as it can be measured in flask cultures.

## Materials and methods

### Plant cell cultures

Cells were cultivated in Gamborg medium at 25°C. Media were prepared according to internal protocols and added by sterilizing filtration.

### Bioreactors

- BIOSTAT® RM20/50
- BIOSTAT® RM50
- BIOSTAT® RM200
- BIOSTAT® RM600

### Monitoring

- Growth was characterized by Packed Cell Volume measurement (PCV %)
- Dissolved Oxygen (DO) was monitored by a single use optical sensor installed in the bag
- pH was measured by an external device temperature was set-up and regulated at 25°C

### Process scheme

- Feed transfer via connexion bag to the bioreactor bag by using a Biosealer and Biowelder.
- Add fresh MEDIA by filtration (sterilization of filters by autoclaving and sterile connection to the bag)
- Sampling: Connexion to the bag of a sterile vial, filling of the vial realized using a peristaltic pump
- Gas supplied AIR only

This experiment was performed using BIOSTAT® RM20/50, BIOSTAT® RM50, BIOSTAT® RM200 and BIOSTAT® RM600 bioreactors. The two objectives of this study were to demonstrate that we could cultivate the plant cells of interest in these bioreactors and to validate the scale-up. The parameters applied during the different cultures are shown in table 1.

| | Erlenmeyerflasks | RM20/50 | RM50 | RM200 | RM600 |
|---|---|---|---|---|---|
| Culture volume (L) | 1 | 5 | 25 | 100 | 300 |
| Aeration (lpm) | NA | 0.6 | 2 | 5 | 10 |
| Shaking (rpm) | 100 | 20 – 22 | 22 | 9 – 18 | 13 |
| Angle (°) | NA | 8 | 9 | 8 – 9 | 9 |

**Table 1:**
Process parameters for plant cell cultures

The culture cycles were led during 2 to 4 weeks.

The downstream process has been optimized to recover the active ingredient but is not part of this application note.

## Results

Plant cell growth profiles according to the type of bioreactor used are shown in figure 1.

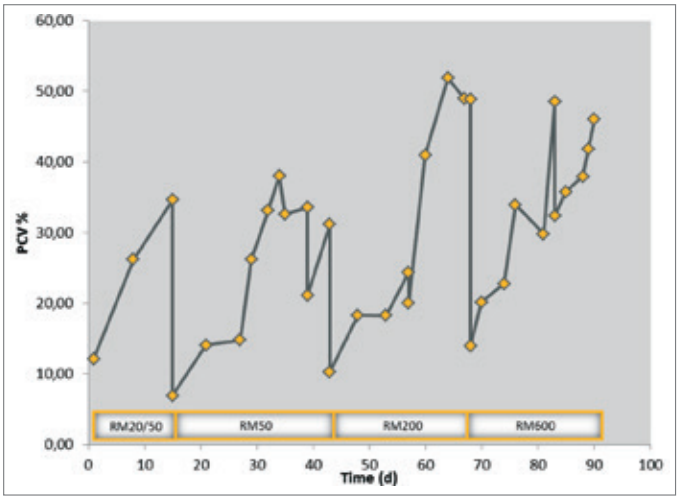


**Figure 1:**
Growth performances for plant cells production in BIOSTAT® CultiBag bioreactors

A 5 L culture was carried out during 2 weeks in the BIOSTAT® RM20/50, biomass concentration increased from 12% PCV to 35% final PCV value which is in line with flask culture performances (PCV value up to 50% in 2–3 weeks).

Biomass content for 25L culture reached a similar PCV value as in the 5 L culture (38% PCV). For both 5L and 25L cultures, pH values were around 6.3–6.8 and dissolved oxygen (DO) values were between 20 and 30%.

To continue the scale-up, a 100L culture was performed in a BIOSTAT® RM 200. Growth performances reached 50% PCV and pH and DO values were respectively around 6.5 and 10%.

These results were confirmed in 300 L culture which showed satisfactory growth performances of 50% PCV. pH data were collected with an external device and the profile showed values around 6.2–6.4. DO data were collected via the single use optical sensor and remained stable around 10%.

## Conclusion

The objective of this study was to demonstrate the ability to cultivate plant cells in BIOSTAT® RM bioreactors and to manage scale-up from Erlenmeyerflasks to industrial production scale.

These objectives were reached and a satisfactory biomass production was achieved of 45–50% PCV during a scale-up of the process from flasks to the BIOSTAT® RM 600 via the BIOSTAT® RM 20, BIOSTAT® RM 50 and BIOSTAT® RM 200. As the evaluation of the active ingredient didn't fall under the scope of this evaluation, further tests are required to also evaluate this important factor of the process.

In terms of practicality, sampling and transfers of the culture were made either under laminar flow hood for the small volume or with Bunsen burner. However using routinely the Biosealer and Biowelder for making the tubing connections can significantly improve handling and eliminate the need for the mentioned alternatives. Even while cells were in aggregates, sampling was rather easy and no losses of biomass was noted.

Thanks to Magella Drouet, Marion Thibault and Frédéric de Baene (Research Manager) for their great work and participation for this study.

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Specifications subject to change without notice. Printed in Germany on paper that has been bleached without any use of chlorine. | W
Publication No.: SBT1019-e13061 · Order No.: 85037-541-55 · Ver. 06 | 2013