INFORS HT

Application note

Mass proliferation of a *Nicotiana tabacum* Bright yellow 2 suspension cell line

1. Introduction

The plant cell line *Nicotiana tabacum* Bright yellow 2 has been isolated from a tobacco plant and is one of the few fully characterised plant cells cell. The genetically modified BY-2 cell line established at Fraunhofer IME in Aachen [1] expresses a monoclonal antibody.

The *Nicotania tabacum* BY-2 cell culture is often used in fundamental research and offers advantages in terms of the production of cell mass due to its high growth rate, short generation times, and ease of handling. The significance of plant cells is on a steady rise due to their ingredients, secondary metabolites, and commercially relevant proteins. Plant cells are expedient mainly in the pharmaceutical, cosmetics and food industries, which utilise their ingredients and active substances. The mass proliferation of the BY-2 suspension cell line was optimised through a comparison of the proliferation in different culturing systems.

The supply of oxygen is a particularly important factor for the culturing of plant cell suspensions. For comparison of cultivation systems the k_La (specific oxygen transfer coefficient) was selected as the transmission parameters for appropriate settings, which was determined in previous process engineering characterisation.

2. Technical specifications of the Multitron Cell

Multitron Cell incubator shaker (50 mm) with ShakerBag Option (INFORS HT, Switzerland)

- Trays: «StickyStuff» tray and ShakerBag Option tray for 2 L, 10 L, 20 L disposable bags
- Direct gas feed (air or air/CO_2; 20–2000 mL/min)
- CO_2 control 0–20 %
- Mobile Pt100

3. Materials and methods

The proliferation experiments were accomplished in 2 L orbitally shaken disposable culturing bags (CultiBag RM) with the ShakerBag Option in the Multitron Cell (INFORS HT) and in 2 L wave-mixed disposable culturing bags (CultiBag RM) with the Biostat CultiBag RM. For reference, cultivations were accomplished with 500 mL shake flask (Corning) with the Multitron Cell.

The BY-2 cells were cultured in Murashige & Skoog medium with minimal organics plus (MSMO+). The "plus" indicates the addition of kanamycin.

All experiments were carried out as batch culture at a temperature of 25 °C in the dark. The inoculation density for all cell cultures was in the range of 10–15 % packed cell volume (pcv).

500 mL shaking flask

100 mL BY-2 cell suspension were cultured in a 500 mL shake flask at a shaking frequency of 120 rpm in the Multitron Cell incubator shaker. The experiment was started at a pcv of 10 %, which corresponded to a fresh weight concentration of 11 g L^{-1}. Passive aeration was provided by means of an air-permeable membrane in the lid.

Fig. 1: Cultivation of the BY-2 cells in the single-use bag

Cultibag RM in the Biostat CultiBag RM

1000 mL BY-2 cell suspension were cultured in a 2 L Cultibag RM in a Biostat CultiBag RM and aerated actively at 0.5 vvm. The experiment was started at a pcv of 15 %. This corresponded to a fresh weight concentration of 52 g L^{-1}. The drive parameters (tilt rate and tilt angle) were adapted to the varying viscosity during culturing:

Start: 30 rpm/8° > day 1: 32 rpm/8° > day 2: 34 rpm/9° > day 3: 36 rpm/9° > day 4: 38 rpm/9° > day 5–7: 42 rpm/10°

ShakerBag Option with orbitally shaken disposable bag

The 1000 mL BY-2 cell suspension was cultured in a Cultibag RM in a Multitron Cell incubator shaker with ShakerBag Option and aerated actively at 0.5 vvm. The pcv fraction started at 12 %, which corresponded to a fresh weight concentration of 39 g l^{-1}. The shaking speed were adapted during culturing:

Start: 40 rpm > day 1: 43 rpm > day 2: 46 rpm > day 3: 49 rpm > day 4: 52 rpm > day 5: 57 rpm > day 6–7: 67 rpm

The selected transfer criterion was a k_La value > 15 h^{-1}. Moreover, the flow status was comparable, which was ensured by earlier simulations.

For process control and in order to determine a growth curve, the packed cell volume (pcv) and the fresh weight concentration (fw) were determined daily. Moreover, the morphology, viability, pH value, and the conductivity were determined as well. The nutrient profile of sucrose, fructose, glucose, ammonium, and nitrate was determined by means of HPLC (data partly shown).

4. Results

Pcv and fw

500 mL shake flask

The maximal pcv attained in the shaking flask was 65 %, which corresponded to a fresh weight concentration of 316 g L^{-1}. The biomass productivity was determined over the entire period of time and was 43.6 g L^{-1} d^{-1}.

The maximal growth rate of 0.022 h^{-1} was determined at the steepest slope of the pcv, therefore between 70 h and 94 h. The resulting doubling time was 32 h.

Cultibag RM in a Biostat CultiBag RM

The fw was measured to be 388 g L^{-1} and corresponded to a pcv of 76 %. The viability of the cells was determined to be 99 % at the start and decreased only slightly to 95 % over the culturing period. The biomass productivity was determined over the entire period of time and was 49.7 g L^{-1} d^{-1}. The maximal growth rate of 0.022 h^{-1} was calculated at the steepest slope of the pcv, therefore between 70 h and 94 h. The resulting doubling time was 32 h.

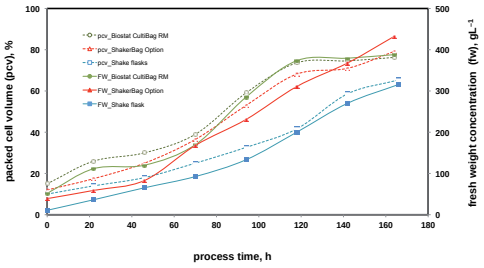

Fig. 2: Comparison of pcv and fresh weight concentration of BY-2 cell cultures in different culturing systems

ShakerBag Option with orbitally shaken disposable bag

The maximal pcv of almost 80 % was attained in the 2 L disposable bag and corresponded to a of 432 g L^{-1}. The viability varied between 96 % and 98 %. The biomass productivity was determined over the duration of the experiment of 164 h and was 56.2 g L^{-1} d^{-1}. The maximal growth rate was calculated between 46 h and 70 h in this case and was 0.029 h^{-1}. The doubling time was 23.9 h.

Nutrients

The profiles of the carbohydrate concentrations are shown in Figure 3 for the purpose of illustration. In all three cases, the sucrose was fully catabolised to glucose and fructose after 2 or 3 days. Referring to glucose, the maximal value was 9 g L^{-1} in the Biostat CultiBag RM, whereas it was approx. 6 g L^{-1} in the two other disposable reactors. The different inoculation volumes were the reason for the different starting volumes.

With regard to the fructose concentration, there were similarities observed between the shake flask and the ShakerBag Option. In both reactors, the maximal concentration of 10 g L^{-1} was approximately equal. In the Biostat CultiBag RM, a higher concentration of approx. 13.5 g L^{-1} was present in the medium for a longer period of time. The fructose was used up fully after 5 days in the orbitally shaken cultures and after 6 days in the Biostat CultiBag RM.

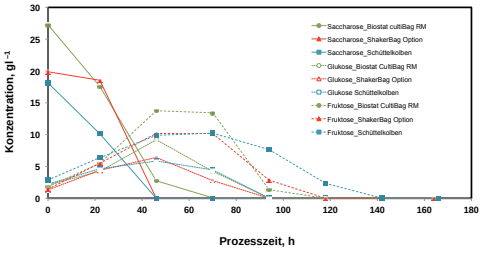

Fig. 3: Nutrient (sucrose and its metabolites, glucose and fructose)

Ion concentration

A comparison of the nitrate and ammonium ion concentrations showed a very similar profile for all culturing systems. After 5–6 days, a nitrate concentration of approx. 10 mg L^{-1} was attained. The ammonium concentration decreased below 2 mg L^{-1} after 4–5 days, but then increased again to 4–6 mg L^{-1} by the end of culturing.

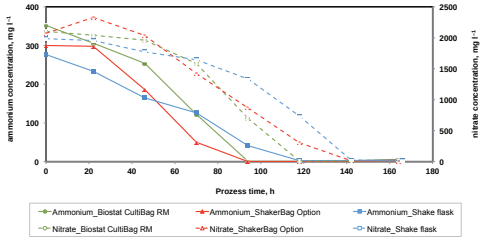

Fig. 4: Comparison of nitrate and ammonium concentrations

Summary

The growth of the *Nicotiana tabacum* BY-2 cell line is significantly comparable, despite of the deviation of the PCV in shake flasks, due to lower inoculation density, in wave-mixed and orbitally-shaken single-use cultivation bags. A maximal pcv of 80 % was attained with the ShakerBag Option and corresponded to a fresh weight concentration of 432 g L^{-1}. The highest biomass productivity determined was 56.2 g L^{-1} d^{-1}. The growth rate was 0.029 h^{-1} and thus corresponded to a doubling time of 23.9 h.

- [1] Raven, N., Schillberg, S., Kirchhoff, J., Brändli, J., Imseng, N., & Eibl, R. (2011). Growth of BY-2 suspension cells and plantibody production in single-use bioreactors. In R. Eibl & D. Eibl (Eds.), Single-use technology in biopharmaceutical manufacture (pp. 251–262). Wiley.
- Werner et al.: An Approach for Scale-Up of Geometrically Dissimilar Orbitally Shaken Single-Use Bioreactors. Chemie Ingenieur Technik 2013, 85, No. 1–2, 118–126; DOI: 10.1002/cite.201200153 ZHAW Zürcher Hochschule für Angewandte Wissenschaften, Life Sciences und Facility Management, IBT Institut für Biotechnologie

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Infors AG
Headoffice, Switzerland

Rittergasse 27
CH-4103 Bottmingen
T +41 (0)61 425 77 00
F +41 (0)61 425 77 01
headoffice@infors-ht.com