IOCBE 2024 Conference
The 1st International Online Conference on Bioengineering
16–18 October 2024 | Online
MDPI

Transfected *Nicotiana tabacum BY-2* cell cultivation in a bioreactor with wave-induced agitation

Grzegorz Karpiński[1], Kamil Wierzchowski[1],*, Mateusz Bartczak[1], Mateusz Kawka[2], Antonina Kawka[2], Jakub Kornatowski[2], Katarzyna Sykłowska-Baranek[2], Maciej Pilarek[1]

1. Faculty of Chemical and Process Engineering, Warsaw University of Technology, Warsaw, Poland

2. Faculty of Pharmacy, Medical University of Warsaw, Warsaw, Poland

***kamil.wierzchowski@pw.edu.pl**

INTRODUCTION & AIM

Single-use, wave-agitated bioreactors are widely utilized in bioprocesses with shear-sensitive biomass, regardless of its plant, mammalian, fungal, or bacterial origin [1,2]. In previous studies, it was shown that in the case of *Nicotiana tabacum* BY-2 cells the oxygen concentration is the main growth-limiting factor. In a 10-day bioprocess, the aforementioned cells begin to degenerate after 6 days. A simple increase in agitation does not provide a satisfying solution, as shear-sensitive biomass disintegrates. In the presented study, modified vessels were used to cultivate BY-2 cells. The proposed modification consisted of passive, 3D-printed elements glued to the bottom part of the culture bag. In previous experiments, the introduced modification was assessed by means of CFD simulations, and its experimental values of t_{95} and k_La were determined and compared with those of unmodified vessels. The aim of this study was to conduct a bioprocess validation of the abovementioned culture bag modification by *Nicotiana tabacum* BY-2 cells cultivation and culture parameters comparison.

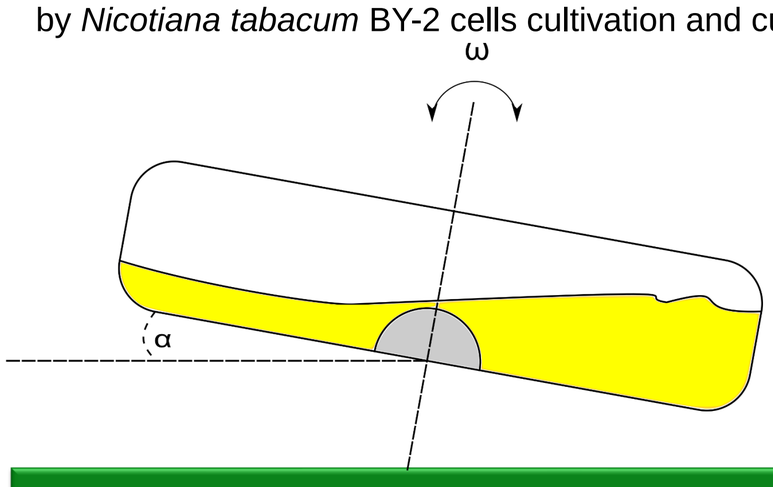

Fig. 1 Diagram of a modified culture bag used in the presented study.

METHOD

Reactor: The ReadyToProcess WAVE™ 25 system with Cellbag™ 2 L culture bags. The bioreactor system was controlled with dedicated UNICORN™ software and consisted of the rocking platform with heating elements, DO and pH sensors, and the airflow control unit.

Modified culture bag : The 2-liter Cellbags were modified by fixing a row of seven half-spheres (Fig. 2), which were 11 mm in diameter, in the middle of the bottom of the bag, perpendicularly to the main direction of liquid flow (Figure 2).

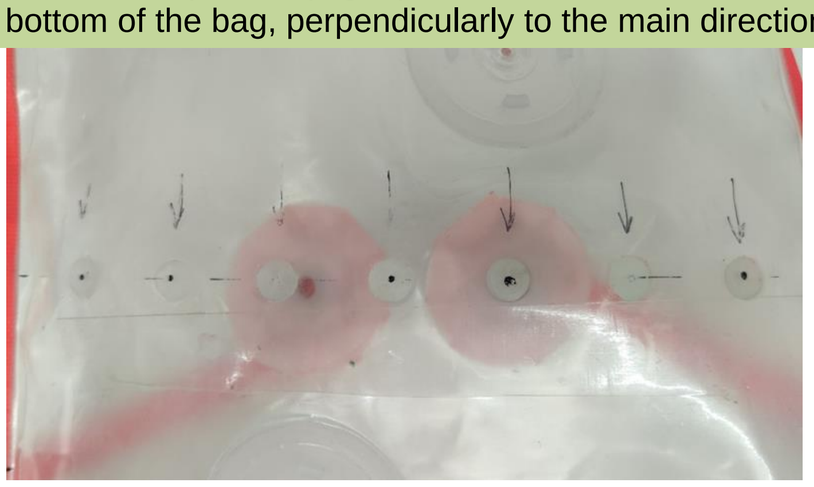

Fig. 2 Perpendicularly to the flow, a row of the modifying elements.

Cell cultures: Submerged batch cultures of *Nicotiana tabacum* BY-2 cells modified genetically to produce GFP were performed in the modified Cellbags. As a reference, an unmodified Cellbag was used. The cultures were carried out for 10 days in a modified MS medium at pH 5.8, 26°C in darkness. The working volume of the bioreactor was 1 L and the aeration rate was 0.5 L/min. The initial biomass concentration was 12.5 g/L. DO and pH were monitored online during the process. Mixing parameters are specified in Table 1 [3].

Table 1. Wave mixing parameters

Cultivation time [h]	Rocking speed [rpm]	Angle [°]
0-48	20	6
48-120	26	8
120-240	30	12

RESULTS & DISCUSSION

Cell cultures: With the progression of the culture, as the biomass concentration increased, an increase in broth viscosity was observed, which disturbed the wave formation significantly. DO and pH were monitored during the cultures in both unmodified (Fig. 3) and modified (Fig. 4) vessels. After each increase in agitation, a further decrease in DO level is especially visible. The DO decrease in each case was approximated by a linear equation, which allowed to compare the angular coefficients to determine whether the DO level decrease speed in a modified culture bag varied from the reference culture (Table 2). The final fresh biomass concentration increased from 474 g/L in a reference vessel to 505 g/L in a modified bag.

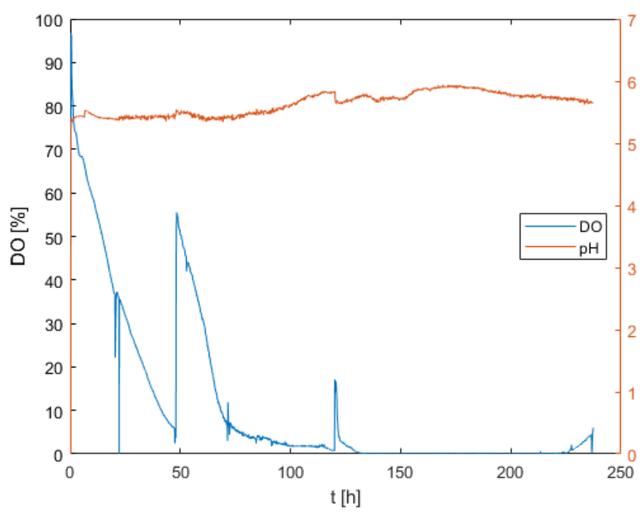

Fig. 3 The values of DO and pH for BY-2 cell cultures in the unmodified bag.

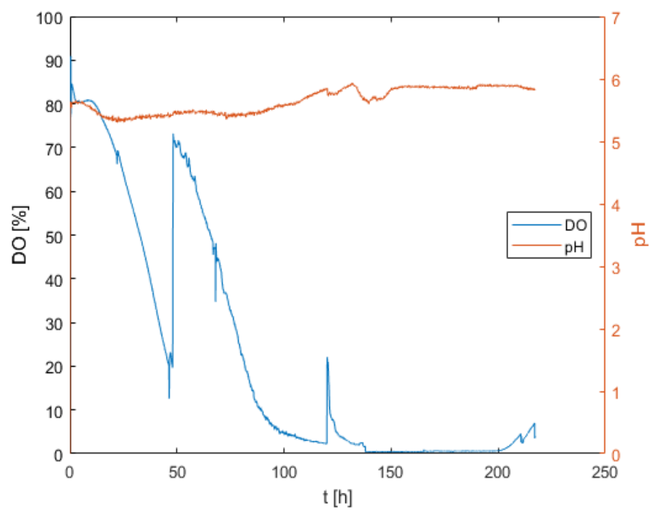

Fig. 4 The values of DO and pH for BY-2 cell cultures in the modified bag.

Table 2. The absolute value of the angular coefficient of the linear approximations of the DO decreases.

Time	Absolute value of the angular coefficient	
	Reference Cellbag	Modified Cellbag
0-48h	1,5947	1,4269
48-120h	1,9407	1,5396
120-240h	8,8593	11,3681

Molecular biology: The Western Blot method was used to determine on which day of the culture the BY-2 cells produced the highest amount of GFP in a reference cell bag (Fig. 5) and in a modified Cellbag (Fig. 6). The Western Blot electrophoresis also allows to determine the effect of vessels modification on which day the maximum protein production was observed.

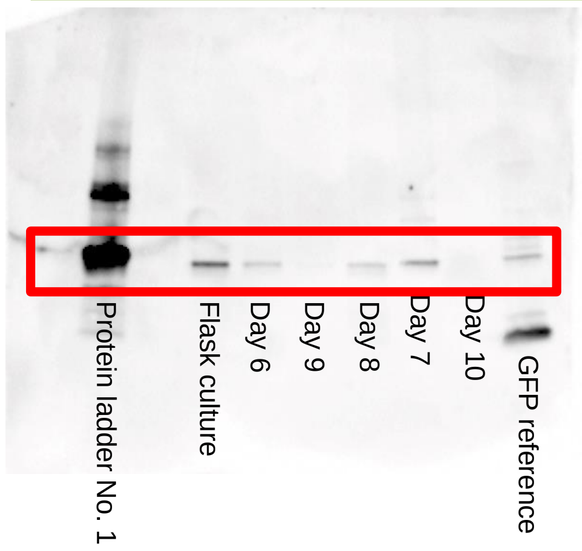

Fig. 5. Western blot, BY-2 cells in a reference Cellbag.

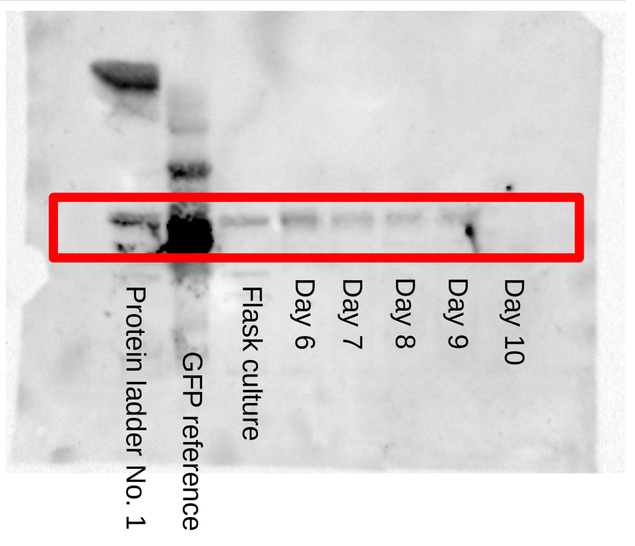

Fig. 6. Western blot, BY-2 cells in a modified Cellbag.

CONCLUSIONS

The presented results show a possibility of intensification cell cultures in wave-agitated bioreactors. Results of BY-2 cells cultures show an increase in the final biomass yield without increasing the agitation rate only due to the introduction of a Cellbag modification. Those results may improve after inserting more modifying elements to the bag in the future. It is also shown that the maximal protein product retention was quickened by a full day. Further improvements of the modification are required, were it to be introduced at a larger scale.

REFERENCES

[1] K. Wierzchowski, M. Pilarek, 2023, Disposable rocking bioreactors: recent applications and progressive perspectives, Trends in Biotechnology, doi:10.1016/j.tibtech.2023.09.003

[2] M. Bartczak, K. Wierzchowski, M. Pilarek, 2022, Mixing performance in a litre-scale rocking disposable bioreactor: DoE-based investigation of mixing time dependence on operational parameters. Chemical Engineering Journal, doi:10.1016/j.cej.2021.133288

[3] Cytiva, *Cultivation of antibody producing fast-growing suspension tobacco plant cells in ReadyToProcess WAVE™ 25 bioreactor system,* 2016, application note 29203199 AA

This work has been funded by (POB Biotechnology and Biomedical Engineering) Warsaw University of Technology within the Excellence Initiative: Research University (IDUB) program (YOUNG PW I).