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WAVE BIOREACTOR CHARACTERIZATION: RESIDENCE TIME DISTRIBUTION DETERMINATION

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INTRODUCTION

In recent years, therapeutic antibodies have assumed an increasing importance to modern medicine. Associated with the high dose requirements of these antibodies, this has led to a rising interest in the development of technologies that aim to improve their biomanufacturing capacity. Most of these technologies focus on mammalian cell culture, due to their ability to perform post-translational modifications that are necessary to obtain biologically functional antibodies. Most of the developments observed in the biomanufacturing field are associated with the improvement of bioreactor performance. Indeed, several bioreactors are currently available either for adherent or suspension culture. Among them, disposable reactors have been attracting increased attention in the last years, with Wave bioreactor being one of the most interesting. In this system, an undulation movement is induced to the cell culture, ensuring good mixing and oxygen transfer without causing shear damage to the cells. Furthermore, this reactor does not require any process of cleaning or sterilization, therefore providing ease of operation and protection against cross-contamination. However, the innovative mechanism of rocking of the Wave bioreactor, associated with its recentness, results in the need for a better characterization of the reactor operation. This will allow the know-how concerning Wave to become comparable to that of more common bioreactors, such as the stirred tank reactor (STR). One of the most important and widely used tools for this characterization is the measurement and analysis of residence time distribution (RTD). Briefly, the RTD function, E(t), measures the time that the various fractions of "fluid element" (small volume of fluid where continuous properties, such as concentration, can still be defined) reside inside the reactor. By comparison with the RTD of ideal reactors, such as the continuous stirred tank reactor (CSTR, where the inlet is perfectly mixed into the bulk of the reactor) and the plug-flow reactor (PFR, where there is no mixing and the fluid elements leave in the same order they arrived) it is possible to evaluate the mixing in the real reactor and to identify the nature of possible deviations from the ideal behavior which are the cause of many operating problems.

Objectives

To evaluate the residence time distribution (RTD) in a Wave reactor, in order to characterize its mixing and flow and to compare its behavior with ideal models and a commercial STR available for mammalian cell culture.

MATERIAL AND METHODS

RTD was determined using methylene blue with a pulse input methodology, at three flow rates, usually identified as low (L: 3.3×10^{-5} m^3/h), intermediate (I: 7.9×10^{-5} m^3/h), and high (H: 1.25×10^{-4} m^3/h) for mammalian cell culture. Samples were taken periodically and the absorbance was read at 660 nm.

RESULTS

The results obtained show that the behavior of Wave approximates the ideal and experimental continuous

STR at flow rate L. For flows I and H, the least square fitting of data from Wave bioreactor indicates a considerable deviation from the ideal models.
Additionally, the comparison of the average residence time (*tr*) with the time of passage (τ) of both Wave and STR provided a possible explanation for the non-ideality of their behavior. For STR, *tr* was lower than τ for all the flow rates tested, indicating the development of dead zones inside the reactor. The same was observed at flow rate H in Wave bioreactor. However, for flows L and I, the deviation from the ideal behavior can be a result of the development of short-circuits inside the reactor, as indicated by a *tr* higher than τ.

CONCLUSIONS

The present study demonstrated that the choice of the flow rate will strongly influence the behavior of the Wave bioreactor. The use of a low flow rate seems to be a choice that provides a behavior closer to an ideal model (CSTR).

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M. ELISA RODRIGUES went to the University of Minho, where she studied Biomedical Engineering, completing the Master Integrated cycle in Clinical Engineering in 2007. She trained for three months at Hospital de São Marcos de Braga, before starting working on a collaboration project between University of Minho, the biopharmaceutical company Biotecnol and the Institute of Molecular Pathology and Immunology of the University of Porto, in 2008. Now at University of Minho she is working on Professor Rosário Oliveira's group, performing her PhD on Optimization of Monoclonal Antibodies Production in Wave and Stirred Tank Reactors. Her e-mail address is elisarodrigues@deb.uminho.pt and her web page is http://www.ceb.uminho.pt/pessoas/pid.aspx?id=292.

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