A combined computational-fluid-dynamics model and control strategies for perfusion bioreactor systems in tissue engineering

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Abstract: This work sets the foundations for the design of control algorithms to facilitate manufacturing of a cell growth process using a continuous perfusion bioreactor. The algorithms are designed to work with different types of cell cultures and deal with major disturbances that might appear in the process. Different types of control strategies are designed, implemented and tested. First, a comprehensive mathematical model of convection and diffusion in a perfusion bioreactor, combined with cell growth kinetics, is developed and implemented using Computational Fluid Dynamics. The model describes the spatio-temporal evolution of glucose concentration and cell density within a 3D polymeric scaffold. Since such a model is too complex to be used directly for control studies, a simplified version is used for the design of the controllers. Finally, the performances of the control strategies are validated against the original high-fidelity CFD model, thus closing the loop. The simulations show good performances and satisfactory behavior.

Keywords: tissue engineering, perfusion bioreactor, CFD modeling, PID.

1. INTRODUCTION

Tissue engineering (TE) is an emerging field focused on growing cells with adequate functionality that are used for in vivo implantation. For the cultivation of cells, bioreactor systems need to use tissue-engineered grafts having uniform viability, cell distribution and growth in a reproducible way. The application of bioreactor systems gives rise to improved tissue quality compared to static cultivation by using suitable cultivation conditions that will mimic an in vivo environment (Schmid, Schwarz et al. 2018). To determine these suitable cultivation conditions as well as the reproducible generation of tissue engineered grafts, a bioreactor system is beneficial. This bioreactor system includes the control of critical cultivation parameters, i.e. flow rate and nutrient concentration, in bioreactors. Using perfusion bioreactors enables even cell distributions on stable scaffolds and allows for an optimal feed of nutrients as well as successfully removing the toxic metabolites from the cell culture (Coletti, Macchietto et al. 2006).

Glucose concentration are generally higher than the concentrations needed by cells to produce energy and assimilate biomass. This excess of glucose will induce an elevated uptake of the nutrients, leading to the production of inhibitory levels of waste metabolites. The accumulation of these inhibitory metabolites such as lactate and ammonia, poses a limitation on the maximum obtainable and product yields. There has been extensive work done on how to use dynamic nutrient feeding to keep the glucose at low levels in fed-batch cultures. This approach has proved to decrease the overflow of glucose metabolism as well as shift cell metabolism to an efficient state and having reduced waste metabolites production that will lead to a higher cell density for enhanced productivity. Using control for the low glucose level in the culture by estimating the glucose consumption rate from the online measurements of the oxygen uptake rate will result in an enhancement in cell density as well as antibody production (Lee, Yap et al. 2003). So far, the common practice in the literature is to estimate offline the optimal feeding using high fidelity mathematical models without closing the loop. The optimal feeding profile is determined considering a base calculation on the cells need for glucose (Kiparissides, Koutinas et al. 2011). The goal of this work and in general from a process engineering perspective, the objective of any modelling attempt is to close the loop. Even though there have been various studies in literature presenting the potential of using model-based optimization (de Tremblay, Perrier et al. 1993, Zhou, Rehm et al. 1997, Frahm, Lane et al. 2003) and control strategies on bioprocesses, there is still a lot of work to be done in developing and implementing these control strategies.

This work sets the foundations for the design of several control algorithms to facilitate manufacturing for any type of cell culture using a continuous perfusion bioreactor. Different types of control strategies are designed, implemented and tested. As Computational Fluid Dynamics (CFD) has the capability of describing the interplay between the flow field in a perfusion bioreactor and the cell growth kinetics this tool will be used to develop a comprehensive high-fidelity mathematical model. Usually, the mathematical model for

such process is too complex to be used directly for control studies and therefore, a simplified version is approximated. The reduced model is then used to facilitate the implementation of the control strategies. Finally, the performance of the control strategies is validated against the original high-fidelity CFD model. This work illustrates how using model-based control approaches greatly improves the time and resource utilization during bioreactor operation. It will reduce or even eliminate the need for Design of Experiments (DoE) to design new processes. By standardizing and automating tissue manufacturing, production costs and time could be reduced when using closed-loop controlled bioreactors systems. This will facilitate a wider use of engineered tissues, it can assure consistency of product quality and of the time spent producing the product which will bring great benefits from a scheduling point of view.

The paper is organized as follows: the perfusion bioreactor, the CFD model as well as the control strategies are described in Section II. Section III presents the results of the simulations for both the CFD mathematical model as well as the designed strategies. Finally, Section V summarizes the main outcome of this paper.

2. THEORETICAL BACKGROUND

2.1 Perfusion Bioreactor

Bioreactors are usually defined as devices where biological and/or biochemical processes develop under tightly controlled and closely monitored operating and environmental conditions such as temperature, pH, pressure, waste removal and nutrient supply. They can be used to aid the in-vitro development of new tissue by providing biochemical and physical regulatory signals.

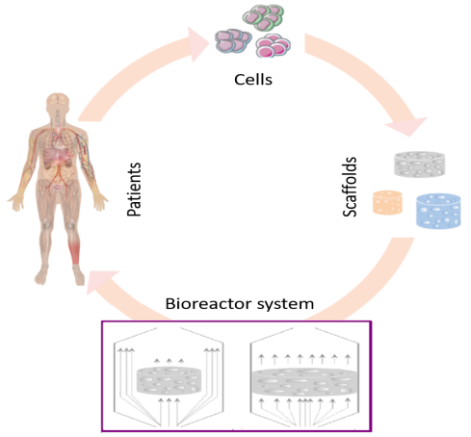

Fig. 1. Tissue engineering grafts bioreactor systems.

Bioreactors used in tissue engineering applications should: (i) provide as well as maintain the physiological requirements of the cell such as growth factors, nutrients and oxygen; (ii) enable uniform cell distribution; (iii) expose cells to physical stimuli; (iv) increase mass transport using the mixing systems of the culture medium; and (v) facilitate reproducibility, monitoring, control and automation. (Martin, Wendt et al. 2004) (Figure 1).

Perfusion bioreactors are culture systems composed of various key elements (Figure 2): (i) one or more perfusion chambers for the cell/scaffold constructs; (ii) a reservoir for the medium; (iii) a pump for mass transport of oxygen and nutrients throughout the perfusion chamber; and (iv) a tubing circuit. The scaffold is placed in position across the flow path of the bioreactor and media is perfused through the scaffold, enhancing fluid transport. Perfusion bioreactors can be generally classified into direct or indirect systems. This classification depends on the way the culture medium is perfused throughout or around the cell/scaffold constructs. Culture using perfusion bioreactors provide more homogeneous cell distribution through the scaffold and have shown to be the best for fluid transport.

In the indirect perfusion systems, as presented in Figure 2A, the scaffold that is connected to the cassette is not tightly sealed. This will enable the medium to follow the path of least resistance around the scaffold. For this reason, flow-derived shear stress may not reach the cells that are found in the construct interior.

In direct perfusion bioreactors, presented in Figure 2B the scaffold is placed inside the perfusion chamber in a press-fit manner such as the culture medium will be forced to pass through the centre of the samples. This type of bioreactor exerts biophysical forces by fluid flow in the interior of the so cultivated cell/ scaffold constructs and allows the reduction of internal mass transfer limitations (Bancroft, Sikavitsas et al. 2003). Systems using direct perfusion have proven to enhance cell density in the scaffold centre (Warren, Sailon et al. 2009), cell proliferation and differentiation.

The easiest manipulated operating degree of freedom in a glucose exchange unit is flow rate of the medium. The cell density profiles and the glucose concentration inside the scaffold will increase with higher velocities, especially in the deeper sections of the scaffold. Alas, the shear stress generated in the scaffold will give an upper bound for the flow rate. If it becomes too high, the cells will detach from the porous surface. An upper velocity value of 4 ml/min is used in this paper.

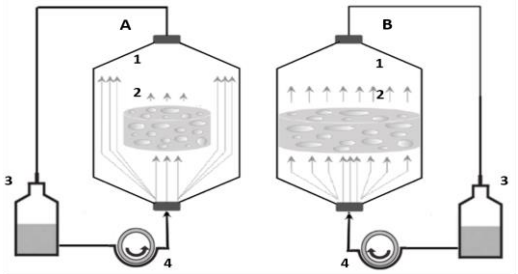

Fig. 2. The indirect (A) and the direct (B) perfusion bioreactor. (1) - culture chambers, (2) the cell/scaffold constructs, (3) the culture medium reservoirs, (4) the peristaltic pumps and (5) - the tubing systems .

2.2 High Fidelity CFD Model

The first step of the framework is the development of a high-fidelity model of the process. This model will most commonly feature non-linear (partial) differential as well as algebraic equations ((P)DAEs) that can be developed in a

high-level modelling environment. For the development of the mathematical model several works related to different types of bioreactors have been studied: batch bioreactor (Kiparissides, Koutinas et al. 2011, Lin, Lin et al. 2011) and perfusion bioreactor ((Chung, Yang et al. 2006, Coletti, Macchietto et al. 2006, Hossain, Bergstrom et al. 2015, Paim, Cardozo et al. 2019)). The model used in this study was developed using the commercial finite element method code COMSOL Multiphysics v.5.4.

The computational domain was divided in two zones: the scaffold, defined as a porous medium, and the surroundings. The momentum conservation equation modified with Darcy's law was solved in both domains

$$\rho\frac{\partial\vec{v}}{\partial t} = -\nabla P - \frac{\mu}{K}\vec{v} + \mu\nabla^2\vec{v} + \rho\vec{g}, \tag{1}$$

which combined with the transport equation gives

$$\frac{\partial c_i}{\partial t} = -(\nabla\cdot)\nabla P - \frac{\mu}{K}\vec{v} + \mu\nabla^2\vec{v} + \rho\vec{g} + R_i, \tag{2}$$

where $\vec{v}$ is the velocity and R_i is a mass source term that accounts for the creation/consumption of product/nutrients. R_i is only defined in the scaffold (R_i is the generation rate of species i (here due to reactions only) and is a spatial-temporal functions of the cell concentration). In this work we only consider one species, which is glucose, hence, i = g (glucose).

When cells grow and proliferate, they occupy some of the void space so the scaffold porosity ε decreases from its initial value ε_0 as the cell density increases. The porosity of the scaffold was set as a function of the number of cells density :

$$\varepsilon = \varepsilon_0 - V_{cell}\rho_{cell}. \tag{3}$$

For the permeability K, the functional form of Koponen [3] was used

$$K = \frac{\varepsilon^3}{q\tau^2 s^2}, \tag{4}$$

where s represents the pore surface area per unit volume of scaffold and q is a structural scaffold parameter. The consumption of the nutrient and the production of the product was modelled as a mass source term R_g in the transport equation. The reaction was only defined in the scaffold domain.

Within the scaffold, glucose is consumed (Hossain, Bergstrom et al. 2015) according to the Michaelis–Menten kinetics as

$$R_g = \rho_{cell}\frac{Q_m C_g}{C_m + C_g} \tag{5}$$

where Q_m is the glucose maximum consumption rate, C_m is the concentration of the substrate at which the reaction occurs at half of the maximum rate and ρ_{cell} is the cell concentration (cells per unit volume in the scaffold). The inclusion of the cell density in the Michaelis-Menten equation ensures appropriate intertwining between all the physics involved in this problem. Cell growth in the scaffold was modelled by introducing the Contois equation, to be solved only in the scaffold subdomain. It was chosen over other typical equations because it accounts well for contact inhibition (Galban and Locke 1999).

$$\mu_{cell} = \mu_{cell}^{\max}\frac{C_g}{K_c\cdot V_{cell}\cdot\rho_{cell}\cdot\rho_c + C_g} \tag{6}$$

The parameters in eq (6) are defined as $\mu_{cell}^{\max}$, the maximum cell growth rate; K_c is the Contois parameter; ρ_c and V_{cell}, represent the single cell density and volume, respectively. The cell density variation with respect to time is given by the following differential equation:

$$\frac{\partial\rho_{cell}}{\partial t} = \left(\frac{\mu_{cell}^{\max} C_g}{K_c\cdot V_{cell}\cdot\rho_{cell}\cdot\rho_c + C_g} - k_d\right)\rho_{cell} \tag{7}$$

where k_d is the cells death kinetic parameter.

2.3 Control Design

This section describes the implementation of a PID control strategy for the output glucose concentration in the bioreactor. The main point of this control strategy is to reveal that even a simple closed loop control based on a PID controller can boost the performance of the bioreactor. A more complex control strategy, with increased performances will be the subject of a future study.

The PID controller transfer function can be written as:

$$H_c(s) = k_p\left(1 + \frac{1}{T_i\cdot s} + T_d\cdot s\right) \tag{8}$$

where k_p is the proportional gain of the controller, T_i and T_d are the integral and derivative time constants, respectively and s is the Laplace operator. The three PID controller parameters, k_p, T_i and T_d, are tuned based on the process model and desired closed loop performance.

3. RESULTS

The simulations using the mathematical model presented in Section 2 are aimed at gaining a more in-depth understanding of the process, analyse the influence of the flowrate and concentration inputs, compare the different types of perfusion bioreactors and determine the controlled/manipulated variables for the design of the controller. Starting from this, different types of control strategies are designed, implemented and tested.

The model proposed in Section II includes several parameters. Some of them depend on the growth kinetics and the type of cells, some of them are properties of the reactor itself, and finally a few of them depend on the way the bioreactor is prepared and operated. The values of the most important parameters used in this paper are: μ_{cell}^{max}=0.30562e-5 [1/sec], K_c=0.006 [mol/m^3], V_{cell}=2.5e-18 [m^3], k_d=0.1025e-5 [1/sec], Q_m= 1.86e-17 [mol/cells·s], C_m=1.86e-17 [mol/m^3], V_{sc}=125e-9 [m^3], ρ_c= 1020 [kg/ m^3], ρ_{cell}^{in} =1e12 [1/ m^3].

3.1 CFD Model

The high-fidelity CFD model of both the indirect and direct Perfusion Bioreactor used the values of the parameters presented above.

Figure 3 depicts the glucose concentration at the output of the scaffold for both indirect perfusion bioreactor and direct perfusion bioreactor for 3 cases: (i) a nominal case where the input glucose concentration is 0.476 mol/m^3 and the input flowrate is 1ml/min (blue line); (ii) a case where the glucose concentration input is decreased but the flowrate is maintained as per nominal case (red line); (iii) a case where the flowrate is decreased and the concentration is maintained as per nominal case. The purpose of these simulations was to analyze the influence of the flowrate and concentration inputs on the output of the process, compare the two types of perfusion bioreactors and determine how best to control the process.

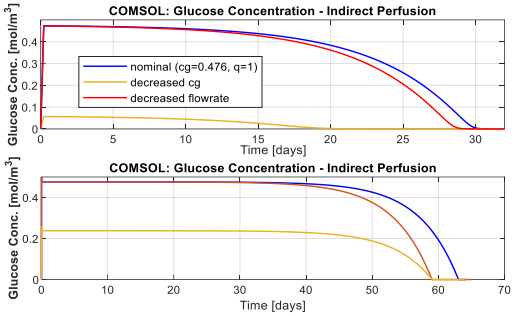

Fig. 3: Glucose concentration of Indirect Perfusion vs Direct

For the Indirect Perfusion Bioreactor the medium follows the path of least resistance around the scaffold, changes on the input flowrate will have very little effect on the output (upper plot, blue line compared with red line) and changes in input glucose concentration (upper plot, blue line compared with yellow line) will significantly affect the output. For this case the controlled variable should be the glucose concentration since from the simulations we observed that changing the flowrate does not affect the output of the process considerably.

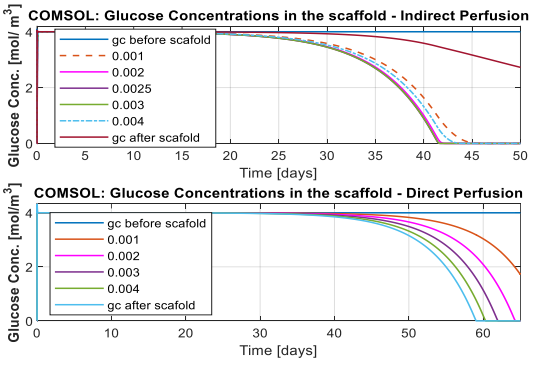

Fig. 4: Indirect Perfusion vs Direct Perfusion Glucose concentration at different points within the scaffold

For the case of the Direct Perfusion Bioreactor, the culture medium is forced to pass through the scaffold. From Figure 3 it can be observed that the case where the flowrate is decreased and the case where the input concentration is decreased will have a similar effect on the output glucose concentration (lower plot, starting from different values for glucose concentration will reach 0 at the same time). Therefore, we will be able to use both the input flowrate and the concentration as a manipulated variable for the process.

To have a better understanding of what is happening inside the scaffold a simulation was run where several probes are placed inside and outside the scaffold. The dimensions of the scaffold were 5x5 mm and the probes were placed at every 1 mm inside the scaffold and an extra one in the middle of the scaffold (at 2.5 mm). The results are presented in Figure 4 for both the indirect and direct perfusion bioreactor. It can be observed in the perfusion bioreactor due to the culture medium being forced to pass through the scaffold we have a more homogenous glucose concentration distribution compared to the indirect perfusion bioreactor.

5.2 Control of the Direct Perfusion Bioreactor

To analyze the performances of the control system only the case of the direct perfusion bioreactor is considered in this paper. To design the controllers, a simplified nonlinear version of the direct perfusion bioreactor model was implemented in Matlab Simulink where the high fidelity CFD model was used for calibration as well as for testing purposes. For the direct perfusion bioreactor, as presented in Section A, both the input flowrate and the input glucose concentration can be used as manipulated variables. The PID controller using the concentration as a manipulated variable is more difficult to implement in an in vitro environment since the changing of input glucose concentration is more challenging. Therefore, for this paper only the PID controller using the flowrate as a manipulated variable is presented.

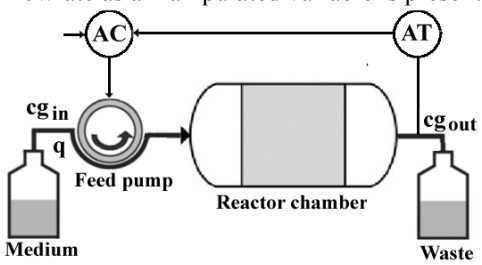

Fig. 5: Perfusion bioreactor control –input flowrate as manipulated variable

The first control scheme analysed is the classic variant, the controlled variable is the output concentration cg_{out}, the manipulated variable is the glucose inlet flowrate q and the inlet glucose concentration cg_{in} is considered constant. The flowrate will be controlled through the peristaltic pump as presented in Figure 5. AT represents the glucose concentration transmitter and AC the concentration controller.

The closed loop response with a setpoint step change from 0.05 to 0.02 mol/m^3 at t=15 days for the controlled output glucose concentration and manipulated variable are presented in Figure 6. It can be observed that the controller shows good performances, no overshoot or undershoot and very fast response to step changes. Due to saturation of the manipulated variable, the maximum value of the flowrate being limited to 4ml/min after t=30 days the control system is no longer able to maintain the value of the setpoint at the output.

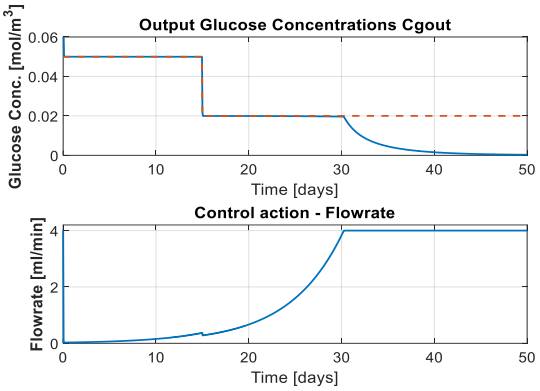

Fig. 6: Closed loop response– setpoint step change

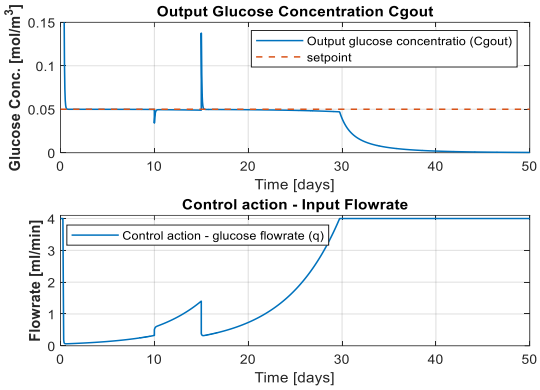

Fig. 7. Closed loop response– Disturbance on glucose concentration input

To further assess the performances of the controller we test it for a decreasing step disturbance of 40% at t=10 and an increasing step disturbance of 120% at t=15 on the input glucose concentration and the results are shown in Figure 7. It can be observed that the controller is able to deal with disturbances and bring the system back to its setpoint values. By changing the tuning parameters, we can design a more aggressive or less aggressive controller.

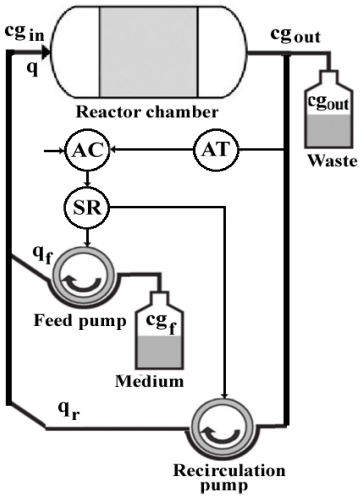

Fig. 8. Perfusion bioreactor – Recirculation

The second control scheme analyzed is the variant including medium recirculation system (as presented in Figure 8). The controlled variable is the output glucose concentration cg_{out}, the manipulated variable are the feed and recirculation flowrates. The input concentration cg_{in} is obtained by combining glucose recirculation (concentration cg_{out} and flow q_r) and feed medium (concentration cg_f and flow q_f). In this case the input flow q for the bioreactor (the sum of feed and recirculation flow) is kept constant but any variation shape can be set. The input flow q is constant while input concentration cg_{in} varies. AT represents the glucose concentration transmitter, AC the concentration controller and SR the split-range device (split the controller output to control the booth feed and recirculation pump).

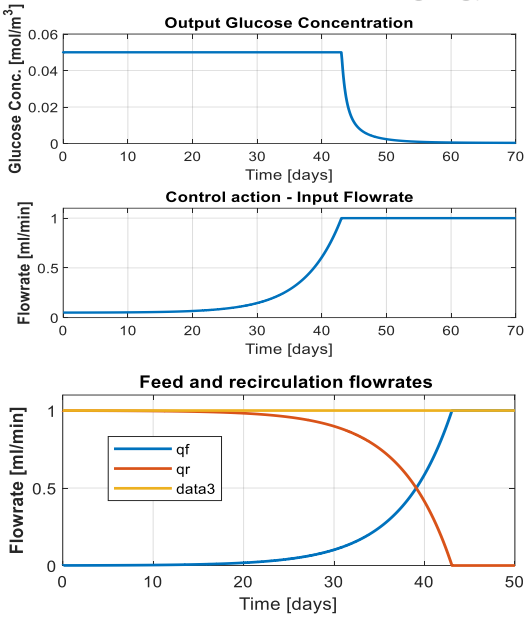

Fig. 9. Recirculation – Closed loop response

The closed loop response of the PID controller with recirculation for the manipulated variable and control actions are presented in Figure 9 where the input concentration is 1 mol/m^3 and the setpoint is 0.05 mol/m^3. It can be observed that the controller shows good performances, no overshoot or undershoot. Flowrate q will have a constant value, therefore when the flowrate q_r from the recirculation starts to decrease due to the fact that the cells will be consuming more nutrients as they grow and multiply, this will be compensated using the flowrate q_f. The constant value of the flow through the scaffold assures a constant pressure on the cells on the duration of the entire simulation.

The optimization common practice in the field literature is to estimate offline the optimal feeding using high fidelity mathematical models without closing the loop. In this work the optimal feeding is obtained by closing the loop. For a better understanding of these concepts, two cases are designed and simulated: (i) no control implemented, the inlet glucose flowrate and concentration are set at constant values of 4 ml/min and 2.38 mol/m^3 respectively; and (ii) the previous designed PID controller is implemented to calculate and give the optimal flowrate of glucose while the feed glucose concentration is kept constant at 2.38 mol/m^3. Figure 10 presents a comparison between these two cases depicting the concentration of glucose at the output, the input flowrate and the cell density. It can be observed that for the case where the optimal feeding is determined by offline optimization (Kiparissides, Koutinas et al. 2011) with no

controller implemented, the resulting optimal feeding profile is similar with the one obtained in the PID control simulations and presented in Figure 10 (middle plot). The response of the culture cell density is similar in both cases, without control and with constant setpoint control, but using closed loop control, the controller optimizes the medium feeding quantity for the bioreactor. Using hierarchic control can lead to the optimization of the response of the culture density, the setpoint values can be calculated at the optimization level. Some of the most important advantages of using a controller is that it is able to deal with model uncertainties as well as rejecting disturbances and taking the process back to the desired setpoint values.

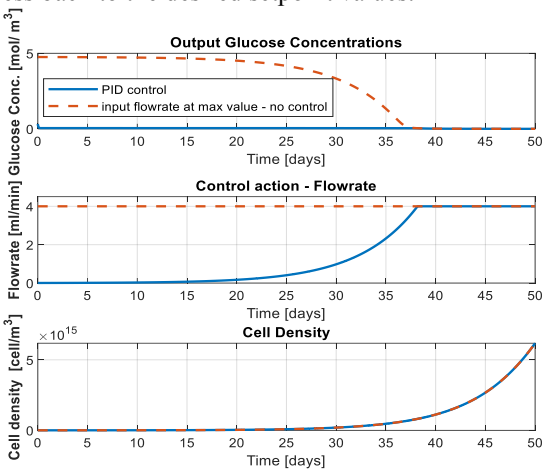

Fig. 10. Comparison of the bioreactor process with and without control

4. CONCLUSIONS

In this paper, we develop a comprehensive mathematical model of convection and diffusion in a perfusion bioreactor, combined with cell growth kinetics. The model describes the spatio-temporal evolution of glucose concentration and cell density within a 3D polymeric scaffold and is implemented using Computational Fluid Dynamics (with the commercial software COMSOL Multiphysics v5.5). Usually, the mathematical model for such process is too complex to be used directly for control studies and therefore, a simplified version is developed that will be further used for the design of the controllers. Different types of control strategies based on PID controllers are designed, implemented and tested. These strategies are designed to be able to work with different manipulated and controlled variables combination, depending on the needs of the process. The designed strategies show good performances with no significant undershoot or overshoot and fast settling time. Moreover, the controllers are able to maintain the desired setpoint while rejecting disturbances.

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REFERENCES

Bancroft, G. N., V. I. Sikavitsas and A. G. Mikos (2003). "Design of a flow perfusion bioreactor system for bone tissue-engineering applications." Tissue Engineering **9**(3): 549-554.

Chung, C. A., C. W. Yang and C. W. Chen (2006). "Analysis of cell growth and diffusion in a scaffold for cartilage tissue engineering." Biotechnology and Bioengineering **94**(6): 1138-1146.

Coletti, F., S. Macchietto and N. Nlvassore (2006). Mathematical modelling of three-dimensional cell cultures in perfusion bioreactors. Part II. Computer Aided Chemical Engineering., Elsevier. **21:** 1699-1704.

de Tremblay, M., M. Perrier, C. Chavarie and J. Archambault (1993). "Fed-batch culture of hybridoma cells: comparison of optimal control approach and closed loop strategies." Bioprocess Engineering **9**(1): 13-21.

Frahm, B., P. Lane, H. Märkl and R. Pörtner (2003). "Improvement of a mammalian cell culture process by adaptive, model-based dialysis fed-batch cultivation and suppression of apoptosis." Bioprocess and Biosystems Engineering **26**(1): 1-10.

Galban, C. J. and B. R. Locke (1999). "Analysis of cell growth kinetics and substrate diffusion in a polymer scaffold." Biotechnology and Bioengineering **65**(2): 121-132.

Hossain, M. S., D. J. Bergstrom and X. B. Chen (2015). "Modelling and simulation of the chondrocyte cell growth, glucose consumption and lactate production within a porous tissue scaffold inside a perfusion bioreactor." Biotechnology Reports **5**(1): 55-62.

Kiparissides, A., M. Koutinas, C. Kontoravdi, A. Mantalaris and E. N. Pistikopoulos (2011). "'Closing the loop' in biological systems modeling — From the in silico to the in vitro." Automatica **47**(6): 1147-1155.

Lee, Y. Y., M. Yap, W.-S. Hu and K. Wong (2003). "Low-Glutamine Fed-Batch Cultures of 293-HEK Serum-Free Suspension Cells for Adenovirus Production." Biotechnology progress **19**: 501-509.

Lin, T. H., C. H. Lin and C. A. Chung (2011). "Comutational study of oxygen and glucose transport in engineered cartilage constructs." Journal of Mechanics **27**(3): 337-346.

Martin, I., D. Wendt and M. Heberer (2004). "The role of bioreactors in tissue engineering." Trends in Biotechnology **22**(2): 80-86.

Paim, Á., N. Cardozo, P. Pranke and I. Tessaro (2019). "SENSITIVITY ANALYSIS FOR MODEL COMPARISON AND SELECTION IN TISSUE ENGINEERING." Brazilian Journal of Chemical Engineering **36**: 383-391.

Schmid, J., S. Schwarz, R. Meier-Staude, S. Sudhop, H. Clausen-Schaumann, M. Schieker and R. Huber (2018). "A Perfusion Bioreactor System for Cell Seeding and Oxygen-Controlled Cultivation of Three-Dimensional Cell Cultures." Tissue Eng Part C Methods **24**(10): 585-595.

Warren, S. M., A. M. Sailon, A. C. Allori, E. H. Davidson, D. D. Reformat and R. J. Allen Jr (2009). "A novel flow-perfusion bioreactor supports 3D dynamic cell culture." Journal of Biomedicine and Biotechnology **2009**.

Zhou, W., J. Rehm, A. Europa and W. S. Hu (1997). "Alteration of mammalian cell metabolism by dynamic nutrient feeding." Cytotechnology **24**(2): 99-108.