Global Sensitivity Analysis for a perfusion bioreactor system in tissue engineering

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Abstract: This work presents a global sensitivity analysis and simulations of a perfusion bioreactor process using the method of high-dimensional model representation (HDMR). This method was developed to express the input–output relationships of a complex model with a high dimensional input space. The comprehensive mathematical model of convection and diffusion in a perfusion bioreactor, combined with cell growth kinetics, is developed and implemented using Computational Fluid Dynamics (with the commercial software COMSOL Multiphysics v5.5). The model describes the spatio-temporal evolution of glucose concentration, oxygen concentration, lactate concentration and cell density within a 3D polymeric scaffold. A quantitative analysis of the complex kinetic mechanisms using recent development of advanced mathematical approaches to global sensitivity and uncertainty analysis through HDMR can be exploited to investigate the important features of the perfusion bioreactor process as well as possible factors underlying qualitative discrepancies.

Keywords: tissue engineering, perfusion bioreactor, CFD modeling, high-dimensional model representation, global sensitivity analysis

1. INTRODUCTION

Tissue engineering (TE) is a complex field that combines chemical and material engineering, biology, and medicine. It implies growing cells within supporting scaffolds to attain structures for in vivo implantation with adequate functionality. To cultivate cells, bioreactor systems need tissue-engineered grafts that have uniform cell distribution, growth, and viability in a reproducible way. By providing proper cultivation conditions that mimic an in vivo environment, (the application of) bioreactor systems can provide improved tissue quality in comparison with the static cultivation. Using perfusion bioreactors allows for an optimal supply with nutrients, while effectively removing the toxic metabolites from the cell culture and enables even cell distributions on stable scaffolds (Coletti et al. 2006).

The use of sensitivity analysis in the biomedical field has been very beneficial especially for the assessment of the robustness of complex biological and biomedical models and in uncertainty quantification (Kiparissides et al. 2009). Assessing the impact of the lack of knowledge regarding the model inputs on the predicted outputs of the model is an important step. This is usually performed using sensitivity analysis (SA) and uncertainty analysis. The main scope of SA is to estimate the effects of each model input, either in isolation or through combined effects, on the model output and to determine the main contributors to the output uncertainty. Local sensitivity approaches are usually used at parameter values close to the nominal ones. If large uncertainties are present, significant changes in sensitivity can occur across the input parameter range for which local methods do not account for. Due to its capability of detecting parameter interactions, and providing more insight for nonlinear models, global sensitivity analysis has gained more attention compared to local sensitivity analysis approaches (Saltelli et al. 2004). Variance based method, such as Sobol' method of sensitivity indices (Sobol 2001) are an important class of global sensitivity analysis techniques (Saltelli et al. 2004). However, due to the computational cost of the models and their nonlinearity, traditional methods for sensitivity analysis are often not suitable (Ziehn and Tomlin 2009, Chen and Yang 2011). To express the input–output relationship of such complex models with high-dimensional input spaces, the high-dimensional model representation (HDMR) method was developed. A fully functional surrogate model (meta-model) that uses relatively simpler models to emulate the dynamic behaviour of the original computationally intensive model are used. This can be easily employed within global sensitivity analysis and it provides an importance ranking for the input parameters and it explores the influence of parameter interactions.

In terms of quantitative analysis of complex kinetic mechanisms, the recent development of advanced mathematical approaches to global sensitivity and uncertainty analysis through HDMR can be exploited to investigate the important features of the perfusion bioreactor process. Perfusion bioreactors for tissue engineering is not a process that is yet fully understood in literature and information about the model is scarce. Therefore, the model provides an interesting case study for the application of global sensitivity methods, since there is a lack of consensus and information in the literature as to the nominal values and uncertainty ranges for certain of its input parameters.

The paper is organized as follows: the perfusion bioreactor, the CFD model as well as the mathematical fundamental of HDMR and Global Analysis using HDMR is presented in Section 2. The methodologies are applied on the perfusion bioreactor process and the results are presented in Section 3. Finally Section 4 summarizes the main outcome of this paper.

2. THEORETICAL BACKGROUND

2.1 Perfusion Bioreactor

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

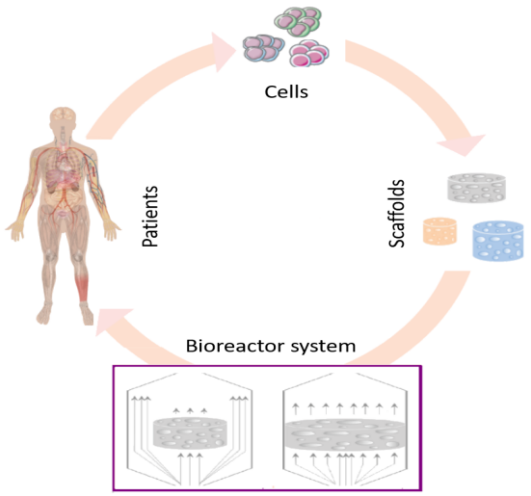

Fig. 1. Tissue engineering grafts bioreactor systems.

A bioreactor for tissue engineering applications should: (i) facilitate uniform cell distribution; (ii) provide and maintain the physiological requirements of the cell (e.g., nutrients, oxygen, growth factors); (iii) increase mass transport both by diffusion and convection using mixing systems of culture medium; (iv) expose cells to physical stimuli; and (v) enable reproducibility, control, monitoring and automation. (Martin et al. 2004) (Fig. 1).

Perfusion bioreactors are culture systems composed of several key elements, including one or more perfusion chambers where the cell/scaffold constructs are placed, a medium reservoir, a tubing circuit and a pump enabling mass transport of nutrients and oxygen throughout the perfusion chamber (Fig. 1). The scaffold is kept in position across the flow path of the device and media is perfused through the scaffold, thus enhancing fluid transport. Culture using perfusion bioreactors have been shown to provide more homogeneous cell distribution through the scaffold and have proved to be the best for fluid transport.

In this work a direct perfusion bioreactor is used where the cell/scaffold constructs are placed in the perfusion chamber in a press-fit fashion so that the culture medium is forced to pass through the centre of the samples. Systems using direct perfusion have been shown to enhance cell density in the scaffold centre, cell proliferation and differentiation.

2.2 High Fidelity CFD Model

For the development of the mathematical model several works related to different types of bioreactors have been studied: batch bioreactor (Kiparissides et al. 2011) and perfusion bioreactor (Coletti et al. 2006, Chung et al. 2006, Hossain et al. 2015, Paim 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 were 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 convective term vector 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 consider two species, glucose and oxygen; hence, i = g (glucose), o (oxygen).

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 (Koponen et al. 1996) was used

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

where s is the pore surface area per unit volume of scaffold and r 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 and oxygen are consumed (Hossain et al. 2015) according to the Michaelis–Menten kinetics as

$$R_i = \rho_{cell}\frac{Q_{m,i}C_i}{C_{m,i}+C_i} \tag{5}$$

where $Q_{m,i}$ is the maximum consumption rate of species i, $C_{m,i}$ is the substrate concentration 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 an extra equation, the Contois equation, to be solved only in the scaffold subdomain. It was chosen in preference to other typical equations as it accounts well for contact inhibition (Galban and Locke 1999).

$$\mu_{cell}(t) = \mu_{cell}^{\max}\left(\frac{C_g(t)}{K_{eq}^{-1}\cdot K_c\cdot\rho_c\cdot V_{cell}\cdot\rho_{cell}(t)+C_g(t)}\right)\cdot \left(\frac{C_o(t)}{K_c\cdot\rho_c\cdot V_{cell}\cdot\rho_{cell}(t)+C_o(t)}\right)\cdot\left(\frac{K_L}{K_L+C_L(t)}\right) \quad (6)$$

The parameters in eq (6) are defined as $\mu_{cell}^{\max}$, the maximum cell growth rate; K_c, the Contois parameter; ρ_c and V_{cell}, the single cell density and volume, respectively; K_L, the lactate Michaelis-Menten growth constant and C_g, C_o and C_L are the glucose, the oxygen and the lactate concertation, respectively. The cell density variation with respect to time is given by the following differential equation:

$$\frac{\partial\rho_{cell}(t)}{\partial t} = \left(\mu_{cell}(t) - k_d\right)\rho_{cell}(t) \quad (7)$$

where k_d is the cells death kinetic parameter.

2.3 High Dimensional Model Representation (HDMR)

The high dimensional model representation (HDMR) method is a set of tools (explored by (Rabitz and Aliş 1999)) used to define the input–output relationship of complex models that have a large number of input variables. The mapping between the input variables $x_1, ., x_n$ and the output variables $f(x) = f(x_1,\ldots,x_n)$ in the domain R^n can be written in the following form (Sobol 2001):

$$f(x) = f_0 + \sum_{i=1}^{n} f_i(x_i) + \sum_{1\le i<j\le n} f_{ij}(x_i,x_j) + \ldots + f_{12\ldots n}(x_1,x_2,\ldots,x_n) \quad (8)$$

Where f_0 is the mean effect (zeroth order), which is a constant. The function $f_i(x_i)$ is a first order term that represents the effect of variable x_i acting independently (generally non-linearly) on the output $f(x)$. The function $f_{ij}(x_i, x_j)$ represents a second order term that describes the cooperative effects of the variables xi and xj on the output $f(x)$. The higher order terms reflect the cooperative effects of increasing numbers of input variables that are acting together to influence the output $f(x)$. In the case where there is no interaction between the input variables, the zeroth order term f_0 and the first order terms $f_i(x_i)$ will appear in the HDMR expansion.

If higher order input variable correlations are weak and can therefore be neglected, the HDMR expansion is computationally very efficient. For a lot of systems a HDMR expression up to second order already provides satisfactory results and a good approximation of $f(x)$.

A particular way of deriving an HDMR representation through monte-carlo sampling is the Random Sampling HDMR technique (RS-HDMR). Since the computation of multidimensional integrals may become prohibitive (Sobol 2001), an alternative technique based on the use of interpolation has been introduced by Li and co-workers (Li et al. 2002).

In the RS-HDMR approach (Li et al. 2002), a set of random sample points N over the entire domain R^n is used. The zeroth order term f_0 can be approximated by the average value of $f(x)$. To determine the higher order component functions, the approximation of the component functions by orthonormal basis functions is used:

$$f_i(\mathrm{x}_i) \approx \sum_{r=1}^{k}\alpha_r^i\varphi_r(\mathrm{x}_i)$$

$$f_{ij}(\mathrm{x}_i,\mathrm{x}_j) \approx \sum_{p=1}^{l}\sum_{q=1}^{l'}\beta_{pq}^{ij}\varphi_p(\mathrm{x}_i)\varphi_q(\mathrm{x}_j) \quad (9)$$

...

where k, l, l' represent the order of the polynomial expansion, α_r^i and β_{pq}^{ij} are constant coefficients to be determined, and $\varphi_r(x_i)$, $\varphi_p(x_i)$ and $\varphi_q(x_j)$ are the orthonormal basis functions. Note that only one set of random samples N is necessary in order to determine all RS-HDMR component functions (Li et al. 2002).

2.4 Global Sensitivity Analysis using HDMR

A common method used in global SA is the method of Sobol' (Sobol 2001), which has the same concept as the RS-HDMR approach. In statistics the decomposition of $f(x)$ into summands of increasing dimensionality (see equation (8)) is called Analysis of Variance (ANOVA) decomposition, a member of the high dimensional model representations known as ANOVA–HDMR (Rabitz and Aliş 1999). However, the estimation procedure is not the same, and the aim is to calculate the total and partial variances instead of the HDMR component functions.

The total variance D can be obtained by:

$$D = \int_{K^n} f^2(\mathrm{x})\,\mathrm{dx} - f_0^2 \quad (10)$$

where the partial variances $D_{i_1,\ldots,i_s}$ can be determined from equation (8):

$$D_i = \int_0^1 f_i^2(\mathrm{x}_i)dx_i$$

$$D_{ij} = \int_0^1\int_0^1 f_i^2(\mathrm{x}_i,\mathrm{x}_j)dx_i dx_j \quad (11)$$

...

Monte Carlo (MC) integration can be used to approximate the integrals in equations (11). However, for a full characterisation of the model, the calculation of the partial variances requires the evaluation of 2^n Monte Carlo integrals. Therefore, a separate MC integral is needed for the computation of each of the partial variances, leading to a computationally expensive method.

The calculation of the partial variances on the basis of the RS-HDMR function expansion provides a much more efficient approach, because the RS-HDMR expansion already gives the ANOVA decomposition (Rabitz and Aliş 1999).

Once the partial variances are determined the sensitivity indices can be calculated as follows:

$$S_{i_1,\ldots,i_s} = \frac{D_{i_1,\ldots,i_s}}{D}, \quad 1\le i_1<\cdots<i_s\le n \quad (12)$$

so that all its terms add up to 1:

$$\sum_{i=1}^{n} S_i + \sum_{1\le i<j\le n} S_{ij} + \ldots + S_{1,2,\ldots,n} = 1 \qquad (13)$$

The first order sensitivity index S_i represents the main effect of the input variable x_i on the output, meaning that it is the fractional contribution of x_i to the variance of $f(x)$. The second order sensitivity index Sij is a measurement of the interaction effect of xi and xj on the output and so on. For a better understanding about the calculation of the sensitivity indices based on the RS-HDMR component functions more detailed information can be found in (Li et al. 2002).

3. RESULTS

In this section the above presented methodologies are applied to the perfusion bioreactor process described in Section 2.2.

One of the main issues with the perfusion bioreactor process is that so far all the mathematical models found in literature show discrepancies between the models as well as discrepancies between the values of the parameters. Moreover, for some of the parameters, no values could be found for the case of the perfusion reactor. For example, in the case of mammalian cells even though such models have been employed for many years in the production of biotherapeutics, information related to their kinetic parameters is scarce (López-Meza et al. 2016, Rodrigues et al. 2021).

Due to the lack of consensus in the literature regarding the nominal values as well as the uncertainty ranges for some of the input parameters, this model provides an interesting case study for the application of global sensitivity methods. Global sensitivity analysis provides an importance ranking for the input parameters and it explores the influence of parameter interactions. The first order sensitivity indices S_i representing the main effect of the considered parameters on the output are determined and analyzed. Even though the interaction between parameters has been calculated and analysed it is not presented in this paper.

Table 1. Model parameters and values

parameter	value	unit	ref
μ_{cell}^{max}	0.3056e-5	1/sec	(Chung et al. 2006)
K_c	0.006	mol/m^3	(Coletti et al. 2006)
V_{cell}	2.5e-18	m^3	(Coletti et al. 2006)
K_d	0.0285	1/h	(Chung et al. 2006)
$Q_{m,g}$	1.339e-12	kg/h	(Chung and Ho 2010)
$Q_{m,o}$	1.607e-13	mol/cells·s	(Coletti et al. 2006)
$C_{m,g}$	0.063	kg/ m^3	(Chung and Ho 2010)
$C_{m,o}$	0.006	mol/m^3	(Coletti et al. 2006)
K_l	3.87	kg/ m^3	(Osiecki et al. 2018)
V_{sc}	6.635e-7	m^3	calculated
ρ_c	182	kg/ m^3	(Chung et al. 2006)
ρ_{cell}^{in}	1.737e+10	1/ m^3	calculated

In this work we would like to explore the discrepancies between the models found in literature as well as the nominal parameter values and the uncertainty ranges. The input parameter considered are: K_c, K_l, $Q_{m,g}$, $C_{m,g}$, $Q_{m,o}$, $C_{m,o}$ and K_d. The selected target outputs are the output concentration of glucose, the output concentration of oxygen, the output concentration of lactate, cell density and cell growth.

The model proposed in this work includes a number of parameters and the most important ones (nominal values) are presented in Table 1. Some of these parameters depend on the type of cells and their growth kinetics, others are properties of the reactor itself, and a few depend on the way the bioreactor is prepared and operated.

The inputs considered for SA are part of the grow kinetics: K_c, K_l, $Q_{m,g}$, $C_{m,g}$, $Q_{m,o}$, $C_{m,o}$ and K_d. Since no information about the uncertainties of these parameters are available and for a better understanding of how different ranges will affect the sensitivity indices of the inputs with respect to the outputs, a set of simulations for different ranges of the parameter uncertainties has been performed. A comparison of the SA results for two different ranges is presented and analysed in Fig. 2 for the glucose concentration output. For the rest of the work, the uncertainties presented are in the interval [50% - 200%] of the nominal value.

The relative influence of the parameters uncertainty on the output concentration of glucose, the output concentration of oxygen, the output concentration of lactate, cell density and cell growth is investigated. The sensitivity index (SI) represents the relative influence of the parameter on the output at the given time. To perform SI analysis, using the mathematical models presented in Section II, a perfusion bioreactor experiment was simulated. A value of 10 [mol/m^3] has been considered for the input glucose concentration and 0.2 [mol/m^3] for the input oxygen concentration. The evolution of the output was investigated on an interval of 15 days. During simulations all parameters and variables were varied between their bounds.

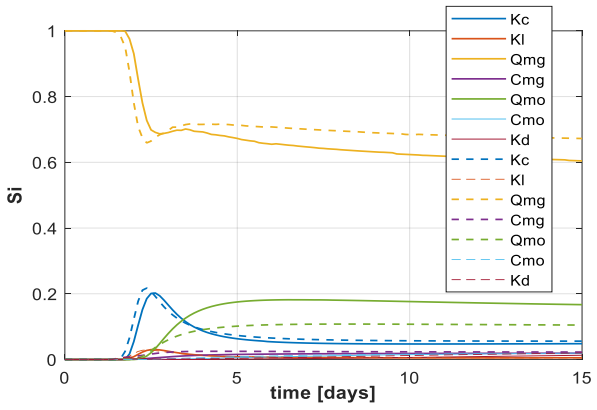

Fig. 2: Evolution of the sensitivity indices with respect to the glucose concentration output

Fig. 2 presents the evolution of the first order sensitivity indices at different sample points for the glucose concentration output for two different sets of input uncertainty ranges. For the parameters with solid line the range is in the interval [50% - 200%] and for the parameters with dashed line the range is in the interval [20% - 500%]. It

can be observed that there are no significant changes for different input ranges (solid line vs dashed line). The only parameter that have a noticeable change are the $Q_{m,g}$ and $Q_{m,o}$ and from these only $Q_{m,o}$ has a significant relative deviation. This comparison between ranges is only presented for the glucose concentration output. The rest of the outputs maintain similar behaviours in the dynamics. After doing the analysis on different ranges for all the outputs, we can say that the influence of the kinetic parameters $Q_{m,o}$ and $Q_{m,g}$ increases with the decrease of the uncertainty range interval while the sensitivity index for K_c decreases.

Analysing the sensitivities indices from Fig. 2, $Q_{m,g}$, the maximum uptake rate of glucose has the highest sensitivity index with respect to the glucose concentration output but it starts decreasing after ~1.75 days while K_c and $Q_{m,o}$ start gaining importance.

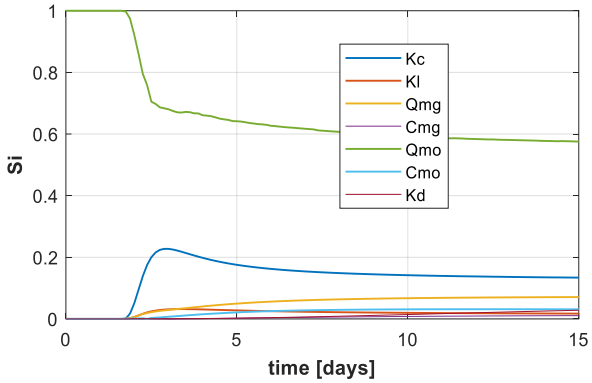

Fig. 3: Evolution of the sensitivity indices with respect to the oxygen concentration output

In Fig. 3 It can be observed that the sensitivity indices with respect to the oxygen concentration output has a very similar profile with the sensitivity indices with respect to the glucose concentration output (Fig. 2).

From Fig. 2 and Fig. 4 we can observe that the first order sensitivity indices for the lactate concentration output (Fig. 4) are almost identical to the sensitivity indices for the glucose concentration output. This makes sense since the output lactate concentration model is using the same reaction rate (R_g) as the model for glucose.

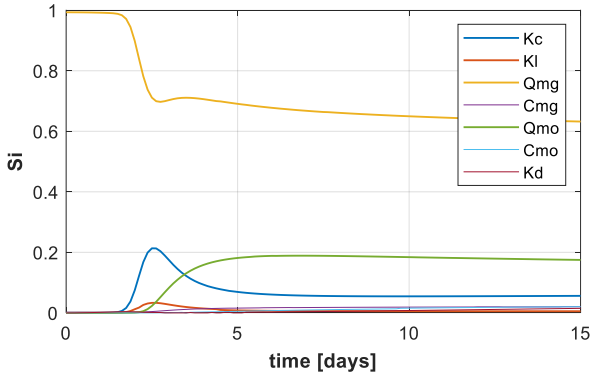

Fig. 4: Evolution of the sensitivity indices with respect to the lactate concentration output

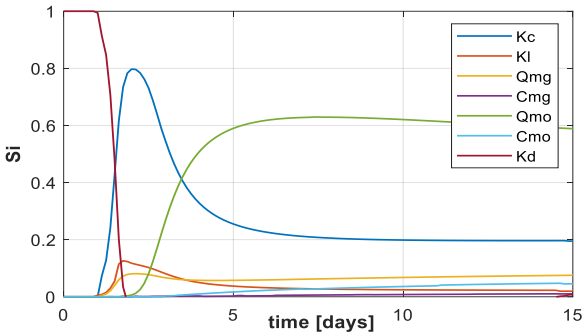

Fig. 5: Evolution of the sensitivity indices with respect to the cell density output

For the evolution of the sensitivity indices of the cell density output from Fig. 5, in the beginning of the process only the cell death rate, K_d has an effect on the output of the process. After 1.5 days, K_c has the highest sensitivity index, the highest value being at 2 days then it starts decreasing while the sensitivity index for Q_{mo} starts increasing. Some other important parameters here are Q_{mg} and K_l.

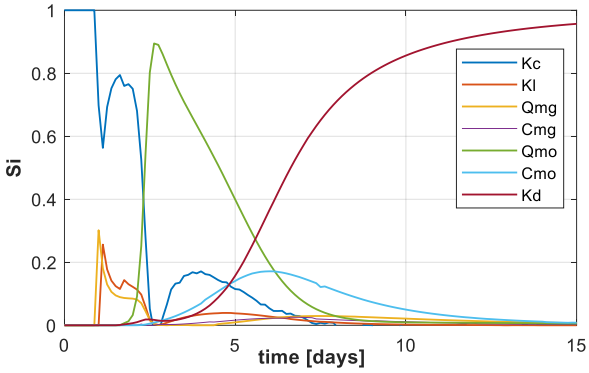

Fig. 6. Evolution of the sensitivity indices with respect to the cell growth output

Fig. 6 presents the evolution of the first order sensitivity indices for the cell growth output. Analysing the sensitivities indices, the most important parameters are K_c for the first 2.6 days of the process, then Q_{mo} and after 5.6 days K_d becomes the most important parameter. It can be observed that at around 2.6 days, the sensitivity index for K_c drops to 0 and then starts increasing again, meaning that around day 3 it will have no influence on the output. To understand this behaviour, Fig. 7 depicts the cell growth output for different values of K_c. It can be observed that by changing Kc we will have different outputs for the cell growth. Decreasing the value of K_c will make the cell growth more steep and increasing K_c less steep. But regardless the change in K_c all outputs for the cell growth will intersect around the same point and this is the same point where in Fig. 6 the SI for K_c drops to 0.01. Meaning that around that point Kc has almost no influence on the cell growth output since regardless of the K_c value they will all go through the same point.

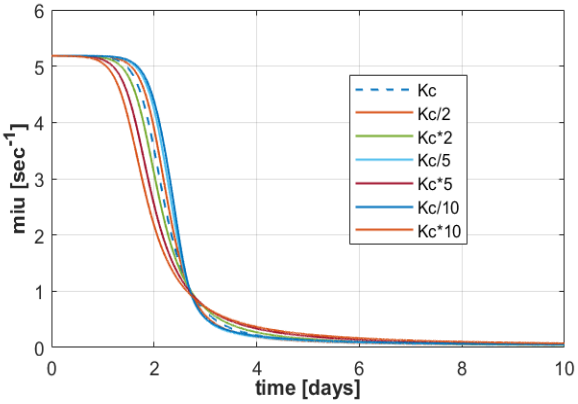

Fig. 7. the cell growth output for different values of Kc

4. CONCLUSIONS

Perfusion bioreactors for tissue engineering are complex processes that are not yet fully understood. Moreover, literature information about the model as well as the values of the parameter and ranges are scarce. This work presents a global sensitivity analysis of the perfusion bioreactor process using the method of high-dimensional model representation (HDMR). These methods have been shown to provide a straightforward approach that can explore the input–output mapping of the perfusion bioreactor model. The model used for simulations describes the spatio-temporal evolution of glucose, oxygen and lactic acid concentration and cell density within a 3D polymeric scaffold and is implemented using Computational Fluid Dynamics.

In this work the nominal parameter values and the uncertainty ranges are explored having as input parameters: K_c, K_l, Q_{mg}, C_{mg}, Q_{mo}, C_{mo} and K_d. The selected target outputs are the output concentration of glucose, the output concentration of oxygen, the output concentration of lactate, cell density and cell growth. The results are presented and analysed in detail.

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