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RESEARCH

Kinetic modeling of NS0 cell line growth impairment in perfusion

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ABSTRACT

Continuous culture with cell recirculation is a system of high operational complexity, which allows high productivity. Previous investigations performed with the NS0 cell line in the industrial scale of monoclonal antibodies have shown an affectation of cell growth in this mode of operation. The lack of a model to explain the kinetics of the NS0 cell line in the production of monoclonal antibodies, as well as the absence of experimental information to know the causes that affect the growth of this cell line, suggest the use of mathematical modeling as a first approach. The aim of this work was to obtain a mathematical model to explain the possible causes that affect cell growth in the industrial scale processes of perfusion with NS0 cells for the production of the monoclonal antibody TKN. Different mathematical models were evaluated on the basis of experimental data, considering the cell growth limitation by nutrient concentration, its inhibition by the formation of a metabolic toxic product and mechanical stress within the rotofilter. The best model describing the growth of the NS0 cell line comprised the Monod equations, the balances for substrate and biomass, with the inclusion of a term representing the effect of mechanical stress on cell growth within the rotofilter. The obtained model is able to predict a cell concentration in the pseudo-steady state equal to 21.3 × 10^6 cells/mL, similar to the experimental value of 20.7 × 10^6 cells/mL.

Keywords: Cell lines, cell growth, mathematical model, NS0 cell line, continuous mode, perfusion

RESUMEN

Modelación cinética de la afectación del crecimiento de la línea celular NS0 en perfusión. El cultivo continuo con recirculación celular es un sistema de alta complejidad operacional, que permite una alta productividad. Investigaciones previas realizadas con la línea celular NS0 en la escala industrial de anticuerpos monoclonales han mostrado una afectación del crecimiento celular en este modo de operación. La inexistencia de un modelo para explicar la cinética de la línea celular NS0 en la producción de anticuerpos monoclonales, así como la ausencia de información experimental para conocer las causas que afectan el crecimiento de esta línea celular, sugieren el uso de la modelación matemática como una primera aproximación. El presente trabajo tuvo como objetivo obtener un modelo matemático que explique las causas posibles que provocan la afectación del crecimiento celular de perfusión con células NS0 durante los procesos para la producción del anticuerpo monoclonal TKN a escala industrial. A partir de datos experimentales, se evaluaron diferentes modelos matemáticos que consideran la limitación por concentración de nutrientes, la inhibición por formación de productos tóxicos y el estrés mecánico en el rotofiltro. El modelo que mejor describe el crecimiento de la línea celular NS0 está constituido por las ecuaciones de Monod, los balances para sustrato y biomasa con la inclusión del término γ que representa la afectación del crecimiento celular por estrés mecánico en el rotofiltro. El modelo predice una concentración celular en el estado pseudoestacionario igual a 21,3 × 10^6 células/mL, similar al valor experimental de 20,7 × 10^6 células/mL.

Palabras clave: líneas celulares, crecimiento celular, modelo matemático, línea celular NS0, perfusión

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Introduction

Animal cell culture is one of the biotechnological areas of great interest, originally developed to produce viral vaccines and a limited number of natural proteins. This technology is expensive but unique, providing glycosylated proteins which biological activity is compromised when produced in cells of lower-order organisms, and accounting for 70 % of pharmaceutical products available in the market [1, 2]. In this sense, the Cuban Biotechnology Industry employs animal cell culture to produce novel biotechnological products against cancer, monoclonal antibodies (mAbs) among them, commonly using Chinese hamster ovary (CHO) [3] and non-secreting zero (NS0) cells as appropriate hosts [4].

During mAbs production, cells are commonly grown in stirred-tank bioreactors, which can be operated under different modes, such as: batch, fed-batch, continuous and continuous with cell recirculation (CCR). This last renders the highest productivity with the highest operational complexity, supporting cellular densities above 10^8 cells/mL and operating at high dilution rates. Other advantages of the CCR operational

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mode comprise keeping low the levels of inhibitors of the cellular metabolism and shortening the residence time of the product, thereby contributing to preserve the integrity of the protein of interest [5-8]. However, previous research using the NS0 cell line to produce mAbs at the Center of Molecular Immunology (CIM, Havana Cuba), have shown low cell concentrations and shorter operation times during fermentation under CCR mode than previously reported [9, 10]. That could had been caused by low cell nutrient concentrations in the culture medium or due to mechanical stress by the cell recirculation system employed.

In order to circumvent these limitations, it was proposed to apply mathematical modelling, to try to optimize the process under study. Process analytics using mathematical modelling is a new trend which saves experimentation time, improves process performance, and increases the cost-effectiveness of the research process by decreasing the requirements for raw materials. In fact, mathematical modelling predicts process performance through the analysis of key variables of the system under analysis. Any mathematical model implies a simplification of the actual phenomenon, led by the aims of the research and the availability of experimental data. Hence, this type of models is subjected to considerations aimed to simplify its resolution, with lower estimation errors. Previously, Hernández *et al*. [11] conducted a first approximation to the mathematical modeling of the NS0 cell line for mAb production at pilot scale, at CIM. Nevertheless, the model proposed did not consider the dependence of the specific rate on the substrate, the metabolic products, or both. The lack of a mathematical model explaining the growth kinetics of the NS0 cell line for mAb production and the insufficient experimental data to explain the effect seen were significant difficulties remaining.

Therefore, this work was aimed to propose a mathematical model, which could help to explain the elements causing the low NS0 cell growth with perfusion at industrial scale, during the production of the TKN monoclonal antibody.

Materials and methods

Cell line and bioreactor

The NS0 cell line, a murine myeloma transfected with the therapeutic TKN antibody, was used. The dry weight of a NS0 cell was estimated as 302×10^{-12} g and 25.3 g/mol normalized molar weight, according to Fernández [12]. A commercial, protein- and serum-free cell culture medium was used, as reported by the same source [9], named F3.

Working data for cell counts were obtained from two runs of a fully implemented, 2000-L bioreactor (Bioengineering, Switzerland). It was operated in fed-batch mode during the first stage, and changed to continuous mode with or without cell recirculation (perfusion), once reaching the working volume. Perfusion was done by using a 50-L cell retention device, named rotofilter, which is a rotating cylinder spinning on its own axis and recirculating cells into the bioreactor. The design of the stirred-tank bioreactor in continuous mode with perfusion is depicted in figure 1.

The volumetric feed flow (F_{in}) was established as the derivate of the volume versus time during the fed-batch mode stage, and as the multiple of the working volume and the dilution rate at start of operating under continuous mode. The volumetric flow of the harvesting main stream (F_{out}) was defined in the same way as Fin for the continuous mode operation, to keep constant the stage volume.

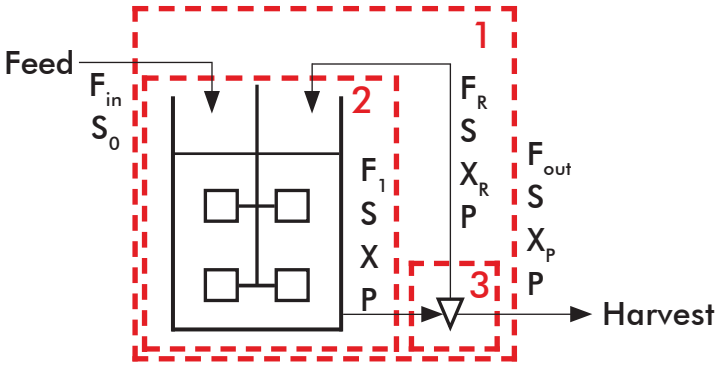

Figure 1. Diagram of the fermentation process for the production of the TKM monoclonal antibody in NS0 cells, by using a cell culture system of fermentation in a bioreactor operated with perfusion. The numbers indicate the parts of the system: 1, full system; 2, bioreactor; 3, cell retention device. F_{in}, F_1, F_R, F_{out}: volumetric flow (L/h) in the inlet, the outlet, the recirculation flow and the harverst of the bioreactor, respectively; S_0, S: substrate concentration (g/L) in the feed and the bioreactor, respectively. P: concentration of the extracellular product in the bioreactor (g/L).

Development of the mathematical model

Monod equation was used, being the most common mathematical equation for modeling biological systems and because it explains a wide array of conditions at steady state. It has been modified frequently to adequately represent experimental data, due to the lack of good approximations of the equation for inhibition by substrate, product, or both [5]. Therefore, due to the complexity of cell growth and product formation from reactions occurring within the cell, the complex system was modeled through dimensionless expressions, the respective dimensionless variables were defined by means of the following equations:

$$Q = \frac{S}{K_s} \quad [1]$$

$$T = \frac{K_i}{K_s} \quad [2]$$

$$W = \frac{P}{K_s} \quad [3]$$

where:
Q: substrate concentration ratio.
S: limiting substrate (g/L).
K_s: substrate saturation constant (g/L).
T: inhibition to saturation ratio.
K_i: inhibition constant of the toxic product (g/L).
P: concentration of the toxic product (g/L).
W: toxic product concentration ratio.

The kinetic parameters used in the mathematical models of maximum specific growth rate, substrate-biomass yield and the maintenance coefficient, were determined by the least squares method from the simultaneous solution of Monod equation and that of the mass balance equations for substrate, cell and

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metabolic products concentrations. The fit was done during the exponential growth phase.

The model fit was verified by the χ^2 test, to evaluate the model's goodness of fit with respect to the performance predicted by with the data from the real run of the fermentation under continuous mode with perfusion. Besides, a continuous mode run was used to corroborate the kinetic parameters of the model, by determining the relative error of cell concentration both at real pseudostationary state and as predicted by the model. For this, relative error values lower than 20 % were accepted, as cut-off of permissible error for the cell counting method [13]. Different physiological conditions affecting cell growth were tested: growth limitation by nutrient concentration in the culture medium, growth inhibition by toxic metabolic products and cell death within the cell retention system.

Modeling cell growth limitation by nutrient concentration in the culture medium

The cell growth limitation by the nutrient concentration in the cell culture medium was modeled by simultaneously solving the balance equations for biomass (eq. 4), Monod (eq. 5) and the substrate balance (eq. 6), the last two as a function of the concentration ratio. In this case, the concentration of toxic metabolic products was regarded as irrelevant.

The effect of the cell retention device was modeled by eq. 7, its design based on the functioning as a combination of rotational filters and filtering centrifuges [9]. The initial resolution efficiency started from a maximum value and progressively decreased during operation, as described by Hernández *et al*. [11]. This model works at 75-95 % efficiency, these conditions been practically proven and recurrent during the operation.

Therefore, a set of equations was obtained as follows:

$$\frac{dX}{dt} = \left[\mu - \frac{1}{V}\cdot\frac{dV}{dt} - \phi\cdot\frac{F_{out}}{V}\right]\cdot X \qquad [4]$$

$$\mu = \mu_{max}\cdot\frac{Q}{Q+1} \qquad [5]$$

$$\frac{dQ}{dt} = -\left[Y_{Q/X}\cdot\mu + m_Q\right]\cdot X + \frac{F_{in}}{V}\cdot Q_0 - \frac{F_{out}}{V}\cdot Q_0 - \frac{1}{V}\cdot\frac{dV}{dt}\cdot Q \qquad [6]$$

$$\phi = 1 - \left(0.95 - \frac{0.2\cdot\tau}{24}\right) \qquad [7]$$

where:

X: concentration of live cells in the bioreactor (cells/mL)

μ_{max}: maximum specific growth rate (h^{-1})

$Y_{Q/X}$: generic substrate-biomass yield (L/gDW)

τ: operation time of the cell retention device (h)

m_Q: maintenance ratio (L/gDW·h)

ϕ: recirculation factor

Q_0: substrate concentration ratio in the feed.

Modeling cell growth inhibition by the formation of a toxic metabolic product

Cell growth inhibition by the generation of toxic metabolic products, such as lactate and ammonium, was also analyzed, its accumulation in the culture medium resulting from substrate consumption, it changes pH and interferes with cell growth [14-16]. It was set through the equations for biomass balance, Monod for product inhibition (eq. 8), the substrate mass balance (eq. 6) and the product mass balance (eq. 9) as a function of Q, W and T ratios. The kinetic performance of the system was explained by using a generic inhibitory metabolite and the generic substrate defined by the variables Q, T and W.

$$\mu = \mu_{max}\cdot\frac{Q}{Q+1}\cdot\frac{T}{W+T} \qquad [8]$$

$$\frac{dW}{dt} = -\left[Y_{W/X}\cdot\mu\right]\cdot X - \frac{F_{out}}{V}\cdot W - \frac{1}{V}\cdot\frac{dV}{dt}\cdot W \qquad [9]$$

Modeling of the mechanical stress caused by the cell retention device

A mass balance analysis was done for the biomass for the recirculation device (eq. 10), considering the lack of accumulation of cells and liquid within the rotofilter, as well as the mechanical stress on cells within the recirculation system. Then, γ was defined as the specific cell death rate associated to mechanical stress.

$$F_1\cdot X_1 = F_R\cdot X_R + F_{out}\cdot X_{out} - X_1\cdot\gamma\cdot V_R \qquad [10]$$

Subsequently, eq. 10 was transformed into eq. 11, using the recirculation factor (ϕ = Xp / X):

$$F_R\cdot X_R = F_1\cdot X_1 - \phi\cdot F_{out}\cdot X_1 - X_1\cdot\gamma\cdot V_R \qquad [11]$$

where:

γ: specific cell death rate within the recirculation system (h^{-1})

X_p- concentration of live cells into the harvest stream (cells/mL)

V_R- volume of the cell retention system (L).

Consequently, the mass balance equation for cell concentration (eq. 4) was expressed by eq. 12. The final equation system describing the entire system comprises equations 5, 6, 7 and 12 as follows:

$$\frac{dX}{dt} = \left[\mu - \frac{1}{V}\cdot\frac{dV}{dt} - \phi\cdot\frac{F_{out}}{V} - \frac{\gamma\cdot V_R}{V}\right]\cdot X \qquad [12]$$

Results

Modeling cell growth limitation by nutrients concentration in the culture medium

All the working parameters were adjusted according to the considerations proposed, for the cell growth limitation by nutrients in the F3 culture medium for the NS0 cell line. At 310.0 ± 0.5 K temperature and pH 6.8 ± 0.20, they were: 0.029 h^{-1} μ_{max}, 5.483 L/g_{DW} $Y_{Q/X}$, 0.012 $L/g_{DW}\cdot h$ mQ and 10.706 Q_0. Coincidently, the estimated maximum growth rate was the typical described for mammalian cells, as estimated by the model, according to previous reports for similar cell lines [17, 18]. As expected, the Q_0 value indicates that the culture starts without substrate limitation.

The analysis of the cell growth limitation by nutrients concentration in the culture medium (figure 2) showed a good correlation ($R^2 = 0.99$; $\chi^2 = 0.04$) among experimental data and the model performance at the

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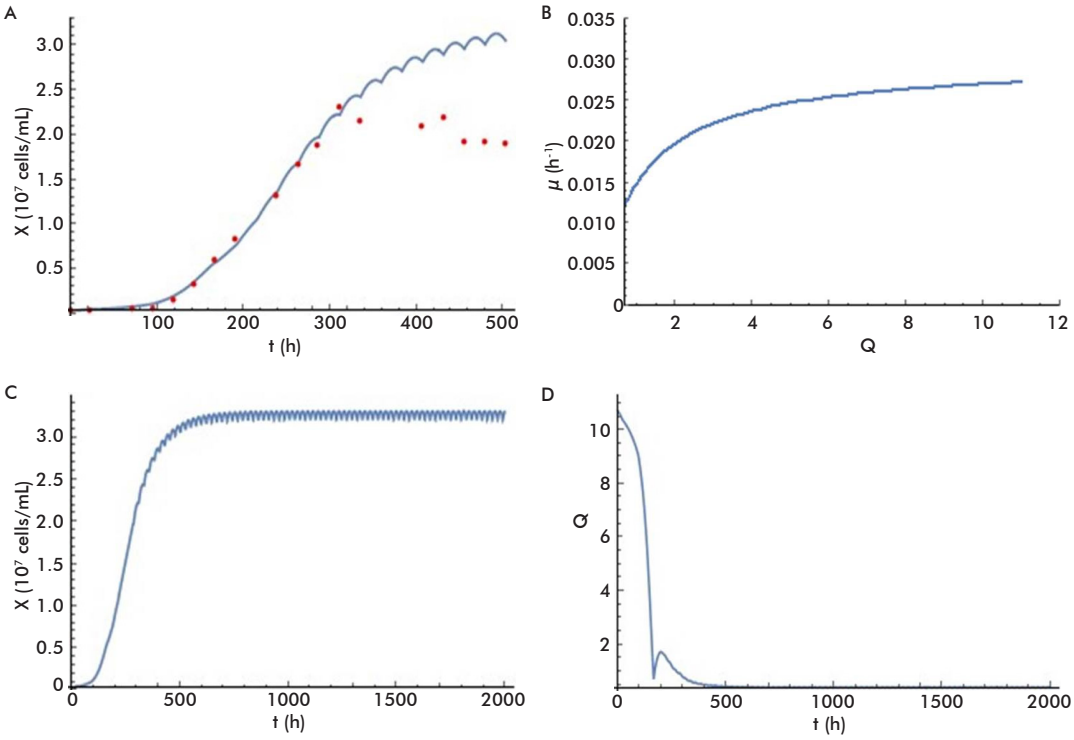

Figure 2. Representation of the model of cell growth limitation of the NS0 cell line by nutrients concentration in the F3 culture medium, the system operated under perfusion at 310.0 ± 0.5 K T and pH 6.8 ± 0.2. A) Cell density in the bioreactor during the entire operation time. The curve depicts the prediction of the models and dost stand for experimental data. B) Specific growth rate (μ) versus concentration ratio (Q). C) Cell density in the bioreactor for 2000 h. D) Substrate concentration ratio for 2000 h.

exponential growth phase (311.5 h; figure 2A). At the end of that phase, the culture enters in a pseudo-steady state, such a performance not described by the model.

The specific growth rate was close to the maximum growth rate possible for values of the concentration ratio close to and higher than 10 (Figure 2B), since there were no limitations for substrate consumption. In fact, the substrate concentration ratio and the specific growth rate decrease with the increase in the cell concentration, until reaching the pseudo-steady state.

The model was able to predict a pseudo-steady state after 500 h of operation (Figure 2C), at a 32.7 × 10^6 cells/mL concentration, higher than the one obtained in the experimental run (20.7 × 10^6 cells/mL).

At the start of the simulation, the substrate concentration ratio decreases below 1.0 (Figure 2D), due to a high substrate consumption rate at the fed-batch culture phase. Then, the ratio recovers to values slightly higher than 1.0 when starting the continuous operational mode with biomass recirculation, until the value of the substrate concentration ratio ultimately tend to zero.

Therefore, these results indicate the absence of metabolic limitation of cell growth for the NS0 cell line when using a cell growth-limiting substrate, since cell concentrations higher than those of the experimental data were predicted.

Modeling cell growth inhibition caused by the formation of a toxic metabolic product

The adjusted parameters for the model of cell growth inhibition by the formation of a toxic metabolic product, under the conditions tested were: 0.032 h^{-1} μ_{max}, 6.466 K T, 1.833 L/g_{DW} $Y_{Q/X}$, 0.041 L/g_{DW}·h, 8.294 Q_0 and 1.075 $Y_{W/X}$ (L/g_{DW}). The maximum specific growth rate adjusted by the model was the usual one described for mammalian cells, in agreement with previous reports for similar cell lines [17-19]. The Q_0 value obtained indicated that the culture is affected by nutrient limitation since the very start of the operation, the growth limitation by toxic metabolic products being tested by using the parameters shown in table 2 (Figure 3). A good correlation was obtained between the experimental data and the performance described by the model in the exponential cell growth phase (R^2 = 0.99, χ^2 = 0.06). Otherwise, the pseudo-steady state was reached at a cell density of 20.7 × 10^6 cells/mL with actual data, lower than the cell concentration values of 25.2 × 10^6 cells/mL (7.60 g_{DW}/L) predicted by the model (Figure 3B). There was a 21.7 % relative error between these concentrations, very close to the expected cell count error (approximately 20 %).

Meanwhile, the substrate concentration ratio (Q) rapidly decreased to 167 h, due to a high substrate consumption rate during the phase of fed-batch culture. This performance was less marked at the start of the continuous culture with cell recirculation, and the substrate concentration ratio finally tended to zero.

Once a high concentration of the toxic metabolic product is reached, the product concentration ratio spikes during the phase of increased-batch operational mode. Despite, this ratio starts to decrease at the beginning of the continuous mode with cell

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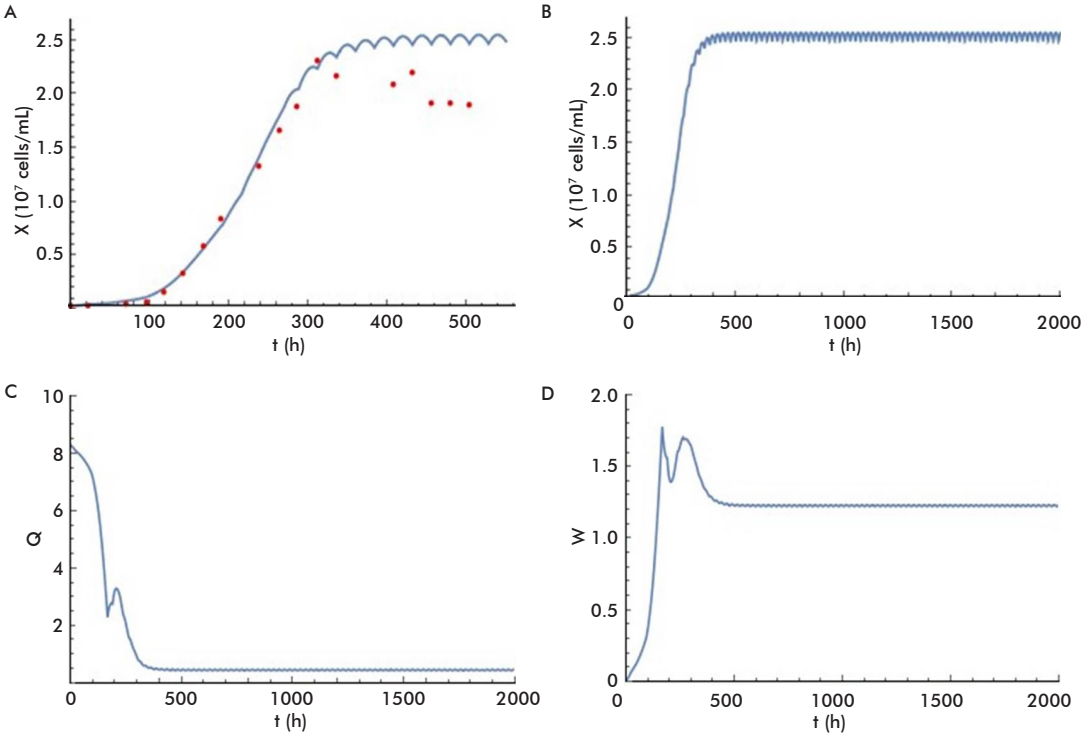

Figure 3. Representation of the model of cell growth limitation of the NS0 cell line by the formation of toxic metabolic products nutrients concentration in the F3 culture medium, the system operated under perfusion at 310.0 ± 0.5 K T and pH 6.8 ± 0.2. A) Cell density in the bioreactor during the entire operation time. The curve depicts the prediction of the models and dost stand for experimental data. B) Cell density in the bioreactor for 2000 h. C) Substrate concentration ratio for 2000 h. D) Toxic product concentration ratio for 2000 h.

recirculation, in the bioreactor with exhausted medium, subsequently stabilizing at a 1.22 value (Figure 3D).

Ultimately, it was demonstrated that the cell growth inhibition by the accumulation of the toxic metabolic product does not account for the performance seen during the entire experimental run, despite causing a drop in the cell density below the one predicted by the model of cell growth limitation by the concentration of nutrients in the culture medium.

Modeling the mechanical stress caused by the cell retention device

The two proposed conditions were compared according to the previously adjusted parameters, for an industrial scale culture in continuous mode (Figure 4).

As shown, both models support a cell growth during the exponential growth phase, faster than that demonstrated by experimental data. The model of growth limitation by nutrients concentration showed quite similar results for the experimental data of the cell line NS0 culture and those estimated by the simulation, with slight differences at the end of the run, showing dilution rate values of 0.55 d^{-1} (Figure 4B). There was a 9.0 % relative error for concentration values of the actual pseudo-steady state, as predicted by the model. Despite, the mathematical model for toxic effects under conditions causing an increase up to 0.50 d^{-1} in the dilution, showed a lower performance of cell density at real values, with a 11.6 % relative error of the real cellular concentration for this dilution rate and the one predicted by the model. In fact, the relative error increased to

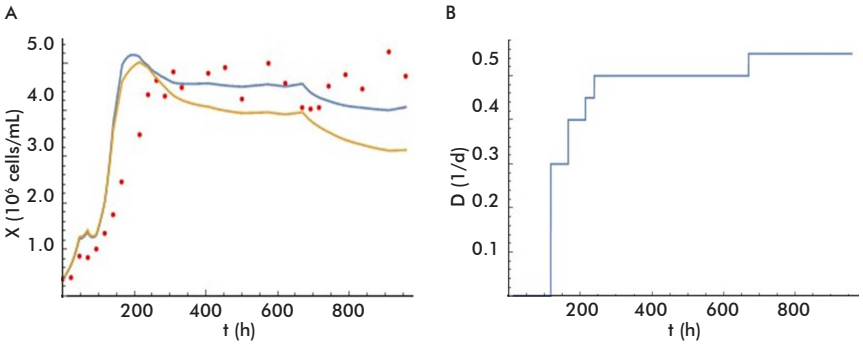

Figure 4. Representation of the model of cell growth limitation of the NS0 cell line, the system operated under continuous mode at 310.0 ± 0.5 K T and pH 6.8 ± 0.2. A) The curve in blue stand for the model of cell growth limitation by nutrients, the yellow curve for the model of cell growth limitation by mechanical stress, and dots for experimental data. B) Performance of the dilution rate during the experimental run.

26.6 % when the dilution rate raised to 0.55 d^{-1}. This indicated that the mathematical model for toxic effects is unable to describe the real performance of cell growth under this operational mode.

Modeling of the mechanical stress within the cell retention device

The previous analyses indicated the relevance of assessing the influence of the damage caused by the biomass recirculation system on cell growth. Then, the kinetics parameters of the model of growth limitation by substrate consumption were used to estimate its

deleterious effect. This was based on the capacity of that model to acceptably represent the operation under continuous mode for the cell line tested. Then, the fit of the biomass balance equation was obtained, from experimental data of the concentration of live cells, including the influence of the cell retention system (eq. 12) (Figure 5). A good correlation was obtained between experimental data and the expected performance described by the model ($R^2 = 0.95$; $\chi^2 = 0.08$). There was a decrease in the cell concentration, resulting from the specific cell death rate in the retention system (γ; 0,14 h^{-1}), higher than the specific growth rate in the bioreactor, as determined by the unfavorable conditions within the cell retention system.

The pseudo-steady state for this cell line predicts an approximate cell density of 21.1 × 10^6 cells/mL (6.37 g_{DW}/L) (Figure 5B), this value similar to the cell concentration obtained in the experimental run (20.7 × 10^6 cells/mL), with an approximate 2.0 % relative error. This demonstrates the unfavorable conditions for cell growth within the cell retention device, this experimental setting as the most limiting among the ones tested for the studied NS0 cell line.

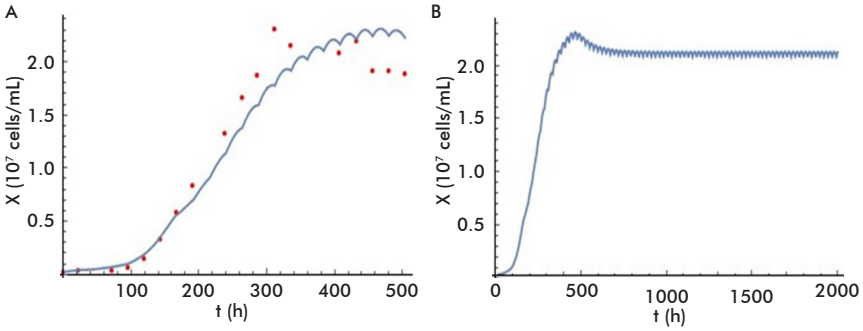

Figure 5. Representation of the model of cell growth limitation of the NS0 cell line within the cell retention device, the system operated under continuous mode at 310.0 ± 0.5 K T and pH 6.8 ± 0.2. A) The curve in blue stand for the model of cell growth limitation by nutrients, the yellow curve for the model of cell growth limitation by mechanical stress within the cell retention device, and dots for experimental data. B) Performance of the dilution rate during the experimental run.

Discussion

Cell growth is mathematically modelled by experimental kinetics studies [5], which determine the respective concentrations of substrates and metabolites. It is impossible to discern which are the limiting compounds among those present in the culture medium, and which dominate over the kinetics of the process while the balance among cells, culture medium and environmental factors remain unchanged. Therefore, modelling is conducted dimensionless and without determining the limiting substrate, as reported by Westgate and Emery [20], and as established in growth kinetics and the analyses of microorganisms' multiplication in waste water, due to the myriads of compounds present [21]. Such an approach provided a proper description of the exponential growth phase of the fermentation process in continuous mode with cell recirculation, for all the conditions tested.

Our simulation lasted for 2000 h, as established by Hernández *et al*. [9], and successfully predicted a pseudo-steady state in the performance of the concentration of live cells, with just a slight variation associated to changes in the efficiency of the cell recirculation device (rotofilter).

The mathematical model of cell growth limitation by the concentration of nutrients in the culture medium, starting from establishing a generic substrate, allows to adequately simulate two conditions for cell culture with different metabolic limitations, *i.e*., continuous culture and continuous culture with cell recirculation at the exponential growth phase. The difference observed for the continuous culture could be caused by an operation time insufficient as to achieve culture stabilization, not reaching the steady state for neither actual nor simulated conditions. This was in agreement with observations by Kyparissidis [17], when providing a mathematical model for the NS0 cell line growth using glucose as the main substrate. Besides, the seudo-steady state is reached at concentration ratio values below the unit, further conditioning a greater control over cell culture due to the lower substrate concentrations. This leads to growth deceleration until reaching the pseudo-steady state, its specific growth rate decreasing to 0.008 h^{-1}.

Regarding the mathematical model of cell growth inhibition by concentration of toxic metabolic products, it was able to predict a cell density of 25.2 × 10^6 cells/mL at the pseudo-steady state, and, in that state, the concentration ratio stabilized at values below the unit, further supporting a better control of the cell culture. Also, the ratio of the toxic metabolic product concentration stabilized at values approximately 5.3-times lower than the inhibition-saturation ratio, indicating that the formation of toxic metabolic products should not lead to a marked inhibition of cell growth. Nevertheless, the model was unable to simulate the conditions occurring in continuous culture, thereby predicting cell density values below the real ones. Hence, the possible inhibitory effect should not be significant during fermentation of the NS0 cell line at industrial scale.

Lastly, the mechanical stress caused by the cell retention system significantly influences on the cell concentration. The specific cell death rate for the NS0 cell line in the cell retention system was higher than the maximum specific growth rate in the bioreactor. This is caused by the adverse environment of uncontrolled conditions within the cell retention system for cell culture variables as dioxygen concentration, nutrients, pH, temperature, and others, which ultimately compromise cell survival and proliferation [5, 4]. In this regard, He *et al*. [22] provided a quantitative analysis on the decrease of CHO cell viability in mini hydrocyclons in respect to the magnitude of the shear stress, making available a validated numerical model of this last variable as the main cause for the drop in cell viability. In this sense, Vallez-Chetreanu [23] stated that perfusion techniques external to bioreactors require pumping systems, those systems ultimately compromising cell integrity by shear stress and by subjecting cells to conditions different to those maintained within the bioreactor.

Conclusions

A mathematical model was successfully developed to describe the kinetics of the NS0 cell line culture. It describes the performance of the cell culture under

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continuous operational mode, and at the exponential phase during the continuous mode with cell recirculation. It comprises the biomass and substrate balance equations and the Monod equations, and includes a term representing the effect of mechanical stress on cell growth within the rotofilter. The introduction of the factors related to the cell growth limitation by the production of metabolic toxic products allows to simulate the exponential phase in continuous mode with cell recirculation, but is unable to properly predict the performance under continuous mode. This indicates that the inhibition by the metabolic product formation could be insignificant for this process at industrial scale. Furthermore, the definition of a kinetics constant for cell death and its inclusion in the mathematical model, allowed to adequately estimate the cell concentrations during the different operational runs at industrial scale.

Overall, the mathematical model effectively identifies the possible causes affecting cell growth of NS0 cells during perfusion at industrial scale, for the production of TKN monoclonal antibody, by including expressions describing substrate limitation and cell death within the cell retention device.

Conflicts of interest statement

The authors declare that there are no conflicts of interest.

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