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Dynamic macroscopic model of dengue viral amplification in vero cell cultures

T. Abbate * **L. Dewasme** * **A. Vande Wouwer** *

* *Automatic Control Laboratory, University of Mons, 31, Boulevard Dolez , 7000 Mons, Belgium (e-mail: Thomas.Abbate@umons.ac.be).*

Abstract: In this work, a dynamic model of infection and virus amplification in vero cell cultures targeting vaccine production is proposed. In contrast with previous works, the model describes the process dynamics as functions of the whole living (uninfected and infected) biomass. The dynamic model is based on a slow-fast approximation where the infected biomass is considered as evolving faster than other variables. The resulting model contains unknown parameters that are inferred from datasets collected from an actual vaccine production process. Parameter identification is complemented by a sensitivity analysis and the determination of confidence intervals for the parameters and predicted trajectories. Results are in general in good agreement with the experimental data.

Keywords: Mathematical modeling, identification, sensitivity analysis, bioprocesses, vero cells, virus amplification.

1. INTRODUCTION

Dengue is a pandemic-prone viral infection that overall develops in tropical and sub-tropical regions of the globe. According to the World Health Organization more than half of the world population is at risks over 128 countries (cite who's website). Furthermore, with 390 million new infections every year (96 million effectively diagnosed) (Bhatt et al. (2013)), dengue is the fastest spreading mosquito-borne virus worldwide. Nevertheless, no specific treatment is currently available to protect against dengue. The elaboration of an effective vaccine, requiring cultures of animal cell as host strain, would then constitute an important breakthrough. To obtain more comprehension of the virus pathogenic mechanisms as well as the culture kinetics that take place during the vaccine production process is essential in order to reach this objective. Since mathematical models are helpful tools to explain, predict or even control complex biological processes, this work aims at modeling the critical dynamics of the dengue virus amplification within Vero cell cultures.

Different models are available in the literature to describe virus amplification dynamics of cell culture-based vaccine production. Influenza virus has been largely studied in this way: Möhler et al. (2005) model the infection dynamics of influenza virus as functions of the infected cell population, Schulze-Horsel et al. (2009) describe these dynamics with regards to the infection status and apoptotic levels of cells while Müller et al. (2013) provide a partial derivative equation model based on the infection level of cells. The baculovirus spreading dynamics in insect cell cultures based on population and mass balances have been studied in Licari and Bailey (1992) as well as in Power and Nielsen (1996). A general approach of the viral expansion in cell cultures is also provided by Haseltine et al. (2005). The latter models both extra and intracellular events occuring during viral infections. Finally, Ursache et al. (2015) furnish an interesting study of vero cell-based poliovirus vaccine production using population and mass balances.

Other examples can be found in the literature but, to our knowledge, all these models describe the virus dynamics as functions of the infected cells. Even though this method is obviously the most efficient way to define the virus amplification, measurements of the infected cell population require the existence of a measurement protocol which might be complicated to establish. The present study therefore proposes a model describing the viral dynamics as a function of the whole living biomass. The proposed model is deduced from a general population balance model assuming that the infection dynamics are faster than the others, allowing to study the steady-state equation of the infected cell population. Interestingly, these assumptions lead to the establishment of Monod and inhibition kinetics.

The identification process described in this work is based on experimental datasets from an industrial process presenting low variability in the experimental conditions.

This paper is therefore organized as follows: section 2 presents macroscopic modeling of vero cell cultures infected by dengue. Section 3 is dedicated to the experimental operating conditions and the description of the provided datasets. In section 4, an identification procedure is suggested with a related parametric sensitivity analysis. Section 5 discusses the results while conclusions are finally drawn in section 6.

2. MODEL DESCRIPTION

Based on several published models [Müller et al. (2013), Möhler et al. (2005), Ursache et al. (2015), Schulze-Horsel et al. (2009)], a model describing the evolution of uninfected biomass (X_u), infected biomass (X_i) and infectious virus particles (Vir) is proposed. The resulting reaction scheme is as follows:

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- Uninfected biomass growth consuming the substrates S and producing the metabolites P, namely consumption of glucose lead to lactate production while glutamine uptake produces ammonium ions.

$$\mathrm{Y_{S/Xu}} \cdot \mathrm{S} \xrightarrow{\phi_g} \mathrm{X_u} + \mathrm{Y_{P/Xu}} \cdot \mathrm{P} \quad (1)$$

- Spontaneous degradation of the glutamine Gln into ammonium ions NH_4 under heat conditions [Möhler et al. (2008), Ursache et al. (2015), Genzel et al. (2005)]

$$\mathrm{Gln} \xrightarrow{\phi_{dk,Gln}} \mathrm{NH_4} \quad (2)$$

- Biomass infection

$$\mathrm{X_u} \xrightarrow{\phi_{inf}} \mathrm{X_i} \quad (3)$$

- Virus amplification including replication and release of new virions in the culture medium. This process requires the consumption of substrates for energy production and therefore generates metabolic products.

$$\mathrm{Y_{S/Xi}} \cdot \mathrm{S} + \mathrm{X_i} \xrightarrow{\phi_{rel}} \mathrm{Xi} + \mathrm{Y_{vir/Xi}} \cdot \mathrm{Vir} + \mathrm{Y_{P/Xi}} \cdot \mathrm{P} \quad (4)$$

- Infected cell death

$$\mathrm{X_i} \xrightarrow{\phi_{d,vir}} \mathrm{X_d} \quad (5)$$

- Virion degradation

$$\mathrm{Vir} \xrightarrow{\phi_{dk,vir}} \mathrm{Vir_d} \quad (6)$$

Obviously, biomass growth and virus amplification are sustained by cell metabolism. The corresponding reduced metabolic network is represented in figure 1 and is assumed to be identical for both populations. Once infected, it is assumed that biomass does not grow any more but uses all the energy for virus production.

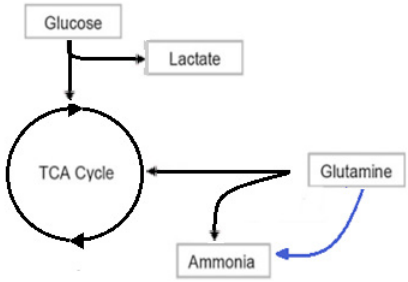

Fig. 1. Reduced cell metabolic network. Black arrows symbolize all the fluxes generated for energy production. Glucose and glutamine consumptions lead to the respective formations of lactate and ammonium

Growth and virus amplification kinetics are given by:

$$\phi_g = \mu_x.X_u = \mu_{x,max}.\mu_{met}.X_u \quad (7)$$
$$\phi_{rel} = \mu_{vir}.X_i.\sigma(t) = \mu_{vir,max}.\mu_{met}.X_i.\sigma(t) \quad (8)$$

with μ_x the specific growth rate, μ_{vir} the specific virion production rate, $\mu_{x,max}$ and $\mu_{x,max}$ the maximum specific replication rates of biomass and virus respectively, μ_{met} the cell metabolism, function of the limiting substrates and the inhibitors, and $\sigma(t)$ is a Heavyside function used to model the lag phase, which represents the time required by the virus to penetrate the cell after infection, to be replicated and to be finally released in the extracellular medium, as well as to avoid the use of a delayed function. Virus-induced death is assumed to start alongside with the virus replication.

$$\sigma(t) = \begin{cases} 0 \text{ if } t < TOI + \tau \\ 1 \text{ if } t >= TOI + \tau \end{cases} \quad (9)$$

where TOI represents the time of infection. Cell metabolism μ is assumed as likely to be saturated by glucose and glutamine (following Monod activation terms) [Möhler et al. (2008), De Tremblay et al. (1992)] and inhibited by, namely, lactate and ammonium [Möhler et al. (2008)] as well as the virus itself. A last constraint is imposed under the form of a logistic term describing the limited spreading capacity of biomass induced by the use of microcarriers.

$$\mu_{met} = \frac{Glc}{Glc + K_{Glc}} \frac{Gln}{Gln + K_{Gln}} \frac{K_{Lac}}{Lac + K_{Lac}} \frac{K_{NH_4}}{NH_4 + K_{NH_4}} \frac{K_{v,m}}{Vir + K_{v,m}} \frac{X_{max} - X}{X_{max}} \quad (10)$$

Infection kinetics are proportional to the uninfected biomass and virus concentrations:

$$\phi_{inf} = k_i.X_u.Vir \quad (11)$$

The death rate and the virion degradation rate are both proportional to biomass but the virus-induced death only starts after the lag-phase.

$$\phi_{d,vir} = k_{d,vir}.X_i.\sigma(t) \quad (12)$$
$$\phi_{dk,vir} = k_{dk,vir}.X_i \quad (13)$$

Applying mass balances to the reaction scheme, a nonlinear state-space model is obtained for the particular case of batch cultures:

$$\frac{d\bar{\xi}}{dt} = K\bar{\varphi}\left(\bar{\xi}\right) \quad (14)$$

where the product $K\varphi(\xi)$ represents the biochemical reaction dynamics that take place during the process. $\bar{\xi}$ is the macroscopic state concentration vector, K is the matrix of the time-invariant yield coefficients and $\varphi(\xi)$ is the vector of reaction rates, functions of the state variables. Consequently, with regards to equation 14, the population dynamics are given by:

$$\frac{dX_u}{dt} = \mu_x.X_u - k_i.X_u.Vir \quad (15)$$
$$\frac{dX_i}{dt} = k_i.X_u.Vir - k_{d,vir}.X_i.\sigma(t) \quad (16)$$
$$\frac{dVir}{dt} = Y_{Vir/X_i}.\mu.X_i.\sigma(t) - k_{vir,dk}.Vir \quad (17)$$

It is therefore possible to obtain the whole living biomass dynamics by summing equations 15 and 16, which gives:

$$\frac{dX}{dt} = \mu_x X_u - k_{d,vir} X_i(t - \tau) \quad (18)$$