Mathematical Biosciences 289 (2017) 40–50

Contents lists available at ScienceDirect

Mathematical Biosciences

journal homepage: www.elsevier.com/locate/mbs

Washout and non-washout solutions of a system describing microbial fermentation process under the influence of growth inhibitions and maximal concentration of yeast cells

Kasbawati[a,b], Agus Yodi Gunawan[a,*], Kuntjoro Adjie Sidarto[a]

[a] Industrial & Financial Mathematics Research Group, Department of Mathematics, Institut Teknologi Bandung, Jl. Ganesa 10 Bandung 40132, Indonesia
[b] Department of Mathematics, Faculty of Mathematics and Natural Sciences, Hasanuddin University, Jl. Perintis Kemerdekaan Km. 10 Makassar 90245, Indonesia

ARTICLE INFO

Article history:
Received 16 August 2016
Revised 16 April 2017
Accepted 17 April 2017
Available online 18 April 2017

MSC:
34A34
92C45
34C23

Keywords:
Unstructured fermentation model
Growth inhibition
(Non-)washout solution
Bifurcation analysis

ABSTRACT

An unstructured model for the growth of yeast cell on glucose due to growth inhibitions by substrate, products, and cell density is discussed. The proposed model describes the dynamical behavior of fermentation system that shows multiple steady states for a certain regime of operating parameters such as inlet glucose and dilution rate. Two types of steady state solutions are found, namely washout and non-washout solutions. Furthermore, different numerical impositions to the two parameters put in evidence three results regarding non-washout solution: a unique locally stable non-washout solution, a unique locally stable non-washout solution towards which other nearby solutions exhibit damped oscillations, and multiple non-washout solutions where one is locally stable while the other is unstable. It is also found an optimal inlet glucose which produces the highest cell and ethanol concentration.

1. Introduction

Fermentation is a microbial process that can be viewed as a complex dynamical system. It consists of intracellular and extracellular systems whose properties are uniquely determined by the yeast engineered in the process. *Saccharomyces cerevisiae*, the well-known ethanol producer, is one of the typical yeasts used in fermentation processes. It has been studied comprehensively using modeling approaches that spin around its optimal operating conditions. For the interest in some more details we refer to Astudillo and Alzate [2] who presented an excellent review about such optimal operating conditions. Given empirical measurement data, it is always of particular interest to perform calculations over the optimal conditions under which a *Saccharomyces cerevisiae* engineered fermentation operates well.

Mathematical modeling is one of the useful tools that can be applied to determine optimal conditions of a fermentation system. In modeling biochemical processes, the advances can be classified into unstructured and structured models. An unstructured model captures the dynamics of yeast cells from time to time in the concentration unit–the group of the cells can thus be viewed as a single species in a solution. It has been developed to study an extracellular fermentation system without considering the metabolic process within a cell [5,8,13]. A structured model focuses more on the metabolic structure of a yeast cell including the chemical processes (enzymatic reactions) within the cell [6,7,12,14,16–18,20,28].

When looking more at an unstructured model, the dynamical behavior of yeasts, products, and substrate in a fermentation process is an important feature to be explored. It amounts to provide important information regarding the transient as well as the asymptotic behavior of the system that possibly becomes a guidance for experimentalists. As a matter of observation, every stable steady state solution is characterized by a unique experimental condition. From the economic point of view, finding one steady state solution is usually more desirable [29]. Related to this, bifurcation analysis can be a powerful tool to reveal the asymptotic behavior of the solution of a mathematical model based on a complex system such as fermentation process (see e.g. [11,24] for the details about bifurcation analysis of models based on ordinary differential equations). It predicts the response of the model system

* Corresponding author.
E-mail addresses: kasbawati@unhas.ac.id (Kasbawati), aygunawan@math.itb.ac.id (A.Y. Gunawan).

http://dx.doi.org/10.1016/j.mbs.2017.04.003
0025-5564/

in the long run under small changes in the values of some parameters. In practice, the prediction can be utilized as a guidance to formulate an optimal regulation that improves the growth of the yeast cells.

Some researchers have come up with continuous unstructured models for yeast cells whose solutions exhibit unusual dynamical behavior such as damped oscillations or converging to multiple steady state solutions [2,6,8,12,20,29–31]. This behavior, mainly influenced by inhibitions from the substrate (glucose) and products (ethanol and acetate), directly affects the ethanol production and culture operability. Furthermore, it has also been shown that the concentration of yeast cells becomes another inhibitor when an extremely high concentration is reached [4,19,25]. New line, among the aforementioned modeling studies, the relatively high concentration of yeast cells has not yet been considered as another inhibitor beside the substrate and products. Here we introduce the notion of carrying capacity for the concentration of yeast cells in our model to study its inhibiting effects to the growth of the cells and products formation. We then take into consideration which ranges of some parameters give which asymptotical behavior of the model solution in the long run. This theoretical exploration can be considered as a guidance in selecting the optimal condition towards which a real experiment can be designed and controlled to produce an optimal yield of the products.

We organize the rest of the paper as follows. In Section 2, we present several assumptions underlying the formulation of our model. In Section 3, we discuss the conditions for the existence of multiple steady state solutions, the stability and bifurcation analysis of the overall steady state solutions, and effects of increasing the cells' carrying capacity and substrate efficiency on the yield of the products. A summary and some concluding remarks are presented in Section 4.

2. Model formulation

Let $C(t)$, $G(t)$, $E(t)$, $A(t)$ respectively denote the concentrations of yeast cells, substrate (glucose), ethanol, and acetate in a culture at time t. All the concentrations are measured in gram per liter (gl^{-1}) and time is measured in hour (h). We preliminary assume that the growth rate of C is influenced by the availability of the substrate G. Therefore, the inflow in the concentration C from time to time based on this growth is given by

$$f_c = \mu C, \tag{1}$$

where μ denotes the specific growth rate of yeast cells,

$$\mu = \frac{\mu_{max} G}{G+\theta}. \tag{2}$$

The last specific formula for μ follows the Monod equation [22] that models the response of the growth rate of yeast cells to the glucose concentration as the life support. The parameters μ_{max} and θ respectively stand for the maximum specific growth rate for the yeast cells (in h^{-1}) to which μ tends to μ_{max} when the substrate G is too abundant (i.e. $G \gg \theta$) and the magnitude of G when μ/μ_{max} is proportional to 0.5 (in gl^{-1}).

The next assumption advocates the idea that the growth of yeast cells stops (completely inhibited) as the concentrations of the substrate and the two products equal to what we refer to as the *measurable saturated concentrations*, denoted by G_{crit}, E_{crit}, and A_{crit} (in gl^{-1}) with $0 \le G \le G_{crit}$, $0 \le E \le E_{crit}$, and $0 \le A \le A_{crit}$. The growth rate of yeast cells as in (2) can now be corrected as

$$\mu = \frac{\mu_{max} G}{G+\theta}\left(1-\frac{G}{G_{crit}}\right)\left(1-\frac{E}{E_{crit}}\right)\left(1-\frac{A}{A_{crit}}\right), \tag{3}$$

where all the aforementioned inhibition factors are lumped together. Here, we assume that the order of inhibitions is equal to one (linear decrease in rate constant as substrate and products build up, [21]). Note that the inhibition pattern as formulated in (3) is similar to the non-competitive enzyme inhibition as in [21,27].

Moreover, the notion of carrying capacity for the lone C is also taken into account in this modeling. This assumption is based on several experimental results, which found that the concentration of yeast cells itself can become another inhibitor for the growth when an extremely high level is reached [4,19,25]. This idea bears the use of another parameter C_{crit} denoting the carrying capacity of C with $0 \le C \le C_{crit}$, i.e. the maximum concentration level of yeast cells the observed culture can accommodate [4,15,27]. This assumption leads to the following correction to (1) in conjunction with (3):

$$f_c = \mu C\left(1-\frac{C}{C_{crit}}\right). \tag{4}$$

We assume that the only glucose is supplied to the system continuously. The rate of glucose supply is assumed to be constant per time unit, i.e.

$$f_s = \rho G_s, \tag{5}$$

where G_s denotes the *inlet glucose* defined in an interval $0 < G_s \le G_u$ given G_u the *maximum inlet glucose*, and ρ is the dilution rate of glucose in the culture. This parameter ρ merely is defined as the volumetric flow rate of glucose supplied to the culture divided by the volume of the culture, i.e. $0 < \rho < 1$. Experimentally, adjusting this parameter allows one to control the growth of yeast cells besides controlling the temperature, pH, or the oxygen level in the culture [20].

Furthermore, the rate of glucose consumption is given as follows:

$$f_g = \frac{1}{Y_{cg}} f_c + uC, \tag{6}$$

where Y_{cg} is the so-called *yield factor* describing the maximum possible yield of the yeast cells C on the given glucose G during the observation period, and u is the specific uptake rate of substrate. The taken substrate is then converted to become energy that is used, for instances, to repair damaged cellular components and to transfer some other nutrients and products into and out of the cells [27]. The rates of ethanol and acetate formations are respectively given by

$$f_e = Y_{ec} f_c \quad \text{and} \quad f_a = Y_{ac} f_c, \tag{7}$$

where Y_{ec} and Y_{ac} respectively denote the yield rate of ethanol and that of acetate from the yeast cells.

In its entirety, our model for a continuous fermentation process is governed by the following system of ordinary differential equations:

$$\begin{aligned} \frac{dC(t)}{dt} &= f_c - \rho C(t), \\ \frac{dG(t)}{dt} &= f_s - f_g - \rho G(t), \\ \frac{dE(t)}{dt} &= f_e - \rho E(t), \\ \frac{dA(t)}{dt} &= f_a - \rho A(t), \end{aligned} \tag{8}$$

supplemented by initial concentrations $C(0) = C_0 > 0$, $G(0) = G_0 > 0$, and $E(0) = A(0) = 0$. The outflows of the yeast cells, substrate, and products from the culture are assumed to be proportional to their own concentrations. For the sake of simplicity, we would attempt to work using the following dimensionless variables

$$X_1 = \frac{C}{C_{crit}},\ X_2 = \frac{G}{G_{crit}},\ X_3 = \frac{E}{E_{crit}},\ X_4 = \frac{A}{A_{crit}},\ \tilde{t} = \mu_{max} \cdot t. \tag{9}$$